University of Southampton

Faculty of Environmental and Life Sciences

Biological Sciences

C. elegans social behaviour as a functional approach to investigate the genetic determinants of autism spectrum disorder

by

Helena Rawsthorne-Manning

ORCID ID 0000-0002-2284-8112

Thesis for the degree of Doctor of Philosophy

June 2021

University of Southampton

Abstract

Faculty of Environmental and Life Sciences

Biological Sciences

Doctor of Philosophy

C. elegans social behaviour as a functional approach to investigate the genetic determinants of autism spectrum disorder

by

Helena Rawsthorne-Manning

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which is clinically characterised by core behavioural deficits including disruption to social behaviour. ASD has a clear genetic underpinning and hundreds of genes, with varying penetrance, have been implicated in its aetiology. Here I use the model organism *C. elegans* as an experimental platform to investigate the effect of genetic mutation on social behaviour.

To do this I utilised a social paradigm in *C. elegans* that is based on observing a complex, sensory-integrative, food leaving behaviour. Adult worms increasingly leave an otherwise replete food source as the number of progeny populating the environment increases. This behaviour is mediated by a progeny-derived social cue and hence represent an inter-organismal social interaction. In this thesis I first designed a bioinformatic pipeline to filter high risk ASD-associated genes to select those that could be investigated using the progeny-induced food leaving assay. I identified several candidate human genes by defining *C. elegans* orthologues which when mutated result in selective deficits in social behaviour. This work highlights that genetic determinants within synaptic, cell signalling, epigenetic modification and phospholipid metabolism functional domains have a role in the co-ordination of *C. elegans* social behaviour. Using a null mutant, I show that *C. elegans* social behaviour is dependent on the *nlg-1* gene. I refine this approach by generating a mutation in *C. elegans* which is synonymous to a highly penetrant ASD-associated variant identified in humans. In doing this I used and optimised a CRISPR/Cas9 technique to precisely edit *C. elegans* genes.

Overall, the combined use of social behaviour analysis and genetic intervention in *C. elegans* provides a useful model to investigate the genetic determinants of autism. ASD is a complex disorder in which disruption within the biological system can influence different levels of biological organisation. Therefore, this thesis provides an avenue for future research to probe the effect of genetic disruption at different levels of the biological system to further understand the emergence of disrupted social behaviour.

Table of Contents

Table of Contents ..... **i**

Table of Tables ..... **vii**

Table of Figures ..... **ix**

Research Thesis: Declaration of Authorship ..... **xiii**

Acknowledgements ..... **xv**

Definitions and Abbreviations ..... **xvii**

Chapter 1 General introduction **1**

1.1 Autism spectrum disorder 1

1.1.1 Introduction to autism spectrum disorder 1

1.1.2 ASD as a brain disorder 2

1.1.3 Sensory processing impairment in ASD 3

1.1.4 Excitatory/Inhibitory imbalance in ASD 4

1.1.5 Comorbidity in ASD 4

1.2 Genetics in the aetiology of ASD 5

1.2.1 Rare, highly penetrant, genetic variants in ASD 7

1.2.1.1 Copy Number Variants 7

1.2.1.2 Rare de novo and inherited variants 7

1.2.2 Common, low penetrant variants in ASD 8

1.2.3 Complex interplay between rare and common variants 9

1.3 Convergence of ASD genes on biological domains 10

1.3.1 Functional diversity of ASD-risk genes 10

1.3.2 ASD-risk genes in interaction networks 11

1.3.3 Biological domains associated with ASD 13

1.3.3.1 Synaptic and neuronal 13

1.3.3.2 Cell signalling 14

1.3.3.3 Epigenetics 15

1.3.3.4 Epigenetics and environmental risk factors 15

1.3.3.5 Gene transcription 16

1.3.3.6 Metabolic pathways 16

1.3.4 Genetic overlap of ASD with other psychiatric disorders 17
1.3.5 SFARI gene database 17
1.4 Pre-clinical investigation of ASD-candidate genes 22
1.4.1 Mice as a model of autism 22
1.4.2 Rats as a model of autism 24
1.4.3 Voles as a model of autism 24
1.4.4 *Drosophila* as a model of autism 25
1.5 *Caenorhabditis elegans* 26
1.5.1 Introduction to *Caenorhabditis elegans* 26
1.5.2 *C. elegans* lifecycle and development 27
1.5.3 *C. elegans* nervous system 29
1.5.4 *C. elegans* genome 30
1.5.5 *C. elegans* as a model of autism 31
1.5.5.1 Using *C. elegans* behaviour to investigate autism 31
1.5.5.2 Using the *C. elegans* nervous system to investigate autism 32
1.5.5.3 Defining neuronal circuits and the use of sub-behaviours to investigate autism 33
1.6 *C. elegans* social behaviour 34
1.6.1 Worm social behaviour 34
1.6.1.1 Ascarosides as a chemosensory cue 34
1.6.1.2 Food as a chemosensory and mechanosensory cue 36
1.6.2 Food leaving behaviour 37
1.6.2.1 Progeny induced food leaving behaviour 38
1.7 CRISPR/Cas9 39
1.7.1 The origin of CRISPR/Cas9 39
1.7.2 CRISPR/Cas9 as a genome editing technique 40
1.7.3 Problems associated with CRISPR/Cas9 41
1.7.4 CRISPR/Cas9 in *C. elegans* 42
1.7.4.1 Methods to increase the efficiency of CRISPR/Cas9 in *C. elegans* 43
1.7.4.2 Methods for identifying positive CRISPR mutant strains 45

1.7.5 Using CRISPR/Cas9 to investigate ASD-risk genes 46

1.8 Aims of the project 47

Chapter 2 Investigating autism associated genes in *C. elegans* reveals candidates with a role in social behaviour 49

2.1 Abstract 50

2.2 Introduction 50

2.3 Materials and Methods 52

2.3.1 Prioritising ASD-associated genes for study in *C. elegans* 52

2.3.2 *C. elegans* culturing and strains used 64

2.3.3 Food leaving assay 64

2.3.4 Pre-conditioned food leaving assay 64

2.3.5 Pharyngeal pumping 65

2.3.6 Thrashing 65

2.3.7 Statistical analysis 65

2.4 Results 65

2.4.1 Selection of human ASD-associated genes for study using *C. elegans* social behaviour 65

2.4.2 Screening mutants using food leaving behaviour identifies ASD-associated genetic determinants of social behaviour 68

2.4.3 Impaired social behaviour of mutants is likely a selective response to progeny derived social cues 70

2.4.4 Exposure to N2 progeny selectively modulates social behaviour in a number of mutants 75

2.5 Discussion 81

Chapter 3 Neuroligin dependence of social behaviour in *C. elegans* provides a model to investigate an autism associated gene 87

3.1 Abstract 88

3.2 Introduction 88

3.3 Materials and Methods 90

3.3.1 *C. elegans* culturing and strains used 90

3.3.2 Rescue construct and transgenic methods 90
3.3.3 Behavioural assays 91
3.3.3.1 Food leaving 91
3.3.3.2 Body bends 91
3.3.4 Genome editing 91
3.3.5 Protein sequence alignment 92
3.4 Results 92
3.4.1 The importance of *nlg-1* in modulating social communication in adult worms 92
3.4.2 *C. elegans* carrying R433C mutation phenocopy the social impairment of *nlg-1* null 97
3.5 Discussion 99

Chapter 4 Confounds of using the *unc-58* selection marker highlights the importance of genotyping co-CRISPR genes 103

4.1 Abstract 103
4.2 Introduction 104
4.3 Materials and Methods 107
4.3.1 *C. elegans* culturing and strains used 107
4.3.2 CRISPR/Cas9 genome editing 107
4.3.3 Molecular screening and sequencing 107
4.3.4 Removal of *unc-58* background mutation 108
4.3.5 Food leaving assay 108
4.3.6 Thrashing assay 108
4.3.7 UNC-58 protein sequence analysis 108
4.4 Results 109
4.4.1 The use of co-CRISPR genes facilitated the generation of a precise *nlg-1* edit 109
4.4.2 *unc-58* CRISPR mutation is predicted to result in a non-functional ion channel protein 111

4.4.3 Phenotypic analysis of *unc-58* CRISPR mutant identifies it is LOF and effects worm motility 114

4.5 Discussion 116

Chapter 5 General discussion 119

5.1 Principal findings 119

5.2 The *nlg-1* dependence of social behaviour 120

5.3 Sub-behaviour analysis 121

5.4 Circuit level understanding of the co-ordination of social behaviour 125

5.5 *C. elegans* as a model for genetic interaction in ASD 126

5.6 Changes to the SFARI gene database 128

5.6.1 Changes to the gene scoring criteria 128

5.6.2 Re-categorisation of genes in the SFARI Gene database 128

5.7 Selecting genes for future study 132

5.7.1 Genes excluded during the bioinformatic filtering process 132

5.7.2 Prioritising the remaining SFARI ASD-risk genes for study 134

5.7.3 Mapping gene interaction to identify cellular pathways that may have a role in social behaviour 135

5.8 Investigating the function of genes that are less well studied for their association with ASD 137

5.8.1 Investigating the function of the *jmjd-3.1* gene 137

5.8.2 Investigating the function of the *mboa-7* gene 138

5.8.2.1 Confirming the food leaving phenotype of *mboa-7* mutants 138

5.8.2.2 Probing the function of *mboa-7* in *C. elegans* social behaviour 140

5.8.3 Mapping ASD mutations onto *C. elegans* proteins for future CRISPR analysis 140

5.9 Summary 141

List of References 143

Table of Tables

Table 1.1 Summary of the ASD-risk genes investigated in this project.....18

Table 2.1 Summary of human genes prioritised for study in *C. elegans* mutant strains 54

Table 5.1 The re-categorisation of genes investigated in this thesis by SFARI Gene database 129

Table 5.2 Two genes excluded during bioinformatic filtering of interest for future study133

Table 5.3 Prioritising SFARI ASD-risk genes for future analysis by analysing genetic interactions.....134

Table of Figures

Figure 1.1 Overview of the different categories of genetic variants associated with ASD.6

Figure 1.2 Cartoon outlining the complex interplay between common and rare genetic variants in ASD. 10

Figure 1.3 Cartoon outlining the complex effects that genetic variants can have on genes within interaction networks. 12

Figure 1.4 Cartoon outlining some of the major functional categories of ASD-risk genes at the synapse. 14

Figure 1.5 Representative image of *C. elegans* under a light microscope. 27

Figure 1.6 A diagram of the gross anatomical features of *C. elegans*. 27

Figure 1.7 Schematic of wild type *C. elegans* lifecycle. 28

Figure 1.8 Cartoon outlining the role of CRISPR/Cas in bacterial immunity. 39

Figure 1.9 Cartoon outlining the two methods of DNA repair that result from cleavage by the CRISPR/Cas9 system. 41

Figure 1.10 Cartoon outlining a two-step CRISPR procedure that creates scarless DNA...44

Figure 2.1 Prioritisation and categorization of the *C. elegans* orthologues of prioritised human ASD-associated genes.....67

Figure 2.2 Food leaving behaviour of *C. elegans* mutants after 24 hours on food to investigate human ASD-associated genes. 69

Figure 2.3 Pharyngeal pump rate for *C. elegans* mutants. 71

Figure 2.4 Percent total eggs and progeny produced by *C. elegans* mutants at 24 hours.73

Figure 2.5 Proportion of eggs and progeny produced by *C. elegans* mutants after 24 hours. 74

Figure 2.6 Correlation between food leaving behaviour of *C. elegans* mutants and progeny production during food leaving assay. 75

Figure 2.7 Food leaving behaviour of *C. elegans* mutants in the absence of progeny and exposure to N2 progeny. 76

Table of Figures

Figure 2.8 Thrashing behaviour of *C. elegans* mutants compared to N2..... 78

Figure 2.9 The number of eggs laid is unchanged in the presence of progeny. 80

Figure 3.1 *nlg-1(ok259)* adults show deficient food leaving in response to progeny. 93

Figure 3.2 *nlg-1(ok259)* show a deficit in social interaction when exposed to N2 progeny. 95

Figure 3.3 Parental *nlg-1* is required for recognition and/or processing of progeny derived social cues..... 96

Figure 3.4 R433C mutation in *nlg-1* phenocopies social impairment of *nlg-1* null. 98

Figure 3.5 CRISPR generated *nlg-1(qa3780)* nucleotide edit. 98

Figure 4.1 co-CRISPR genes used to facilitate the generation of a *nlg-1* CRISPR mutant.110

Figure 4.2 CRISPR mediated mutation to the co-CRISPR gene *unc-58* is predicted to result in a non-functional ion channel protein. 112

Figure 4.3 Chromatograms showing partial *nlg-1* and *unc-58* DNA sequence. 113

Figure 4.4 *unc-58* CRISPR mutant phenocopies *unc-58(bln223)* null mutant and is likely to be a LOF mutant. 115

Figure 5.1 Summary of the main findings of this thesis and factors that remain unknown. 122

Figure 5.2 Progeny-enhanced food leaving shows inverted relationship between food leaving and partial food leaving. 123

Figure 5.3 N2 *C. elegans* positioning on the food lawn is similar in the absence and presence of progeny..... 124

Figure 5.4 Interaction map of the 14 genes identified as having a role in social behaviour. 127

Figure 5.5 Epigenetic and synaptic genes are the dominant functional categories before and after the bioinformatic filtering process. 131

Figure 5.6 Interaction of *mboa-7* with other *C. elegans* genes. 136

Figure 5.7 Summary of the phenotypic data for *mboa-7* mutants. 139

Figure 5.8 Mapping human MBOAT7 missense mutations onto *C. elegans mboa-7* protein. 141

Research Thesis: Declaration of Authorship

Print name: HELENA RAWSTHORNE-MANNING

Title of thesis: *C. elegans* social behaviour as a functional approach to investigate the genetic determinants of autism spectrum disorder

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Rawsthorne, H., Calahorro, F., Feist, E., Holden-Dye, L., O'Connor, V. and Dillon, J. (2020) 'Neuroligin dependence of social behaviour in *C. elegans* provides a model to investigate an autism associated gene', *Human Molecular Genetics,* 29(21), pp. 3546-3553.

Rawsthorne, H., Calahorro, F., Holden-Dye, L., O'Connor, V. and Dillon, J. (2021) 'Investigating autism associated genes in *C. elegans* reveals candidates with a role in social behaviour', *PLOS ONE,* 16(5), pp. e0243121.

Rawsthorne-Manning, H., Calahorro F., Izquierdo P. G., Holden-Dye, L., O'Connor, V. and Dillon, J. (2021) 'Confounds of using the *unc-58* selection marker highlights the importance of genotyping co-CRISPR genes', *biorxiv*, pp. 2021.05.26.44785.

Signature: Date:17.06.2021

Acknowledgements

I would first like to thank my supervisors, Dr James Dillon, Professor Vincent O'Connor and Professor Lindy Holden-Dye for their guidance and feedback throughout my PhD. I would also like to thank all members of the WormLab for their help and encouragement along the way. In particular, I would like to say a special thank you to Dr Fernando Calahorro and Patricia Gonzalez for their guidance, support and continued friendship. I am very grateful to Fernando Calahorro for all the time, guidance and advice he gave me during this project. In particular, I would like to thank him for the support he gave me with the molecular aspects of this project and the generation of the CRISPR line. I would like to thank Nicole Hubbard for her work during her lab project, some of her results are presented in chapter 5. I am very grateful to the The Gerald Kerkut Charitable Trust for their financial support. Finally, my love and gratitude go to Joe, my parents and parents-in-law and the rest of my family for their constant love and support, without which none of this would have been possible.

Definitions and Abbreviations

AA.....Amino acid

ADHD.....Attention deficit hyperactivity disorder

ASD.....Autism spectrum disorder

CAM.....Cell adhesion molecule

C. elegans.....*Caenorhabditis elegans*

CGC.....*Caenorhabditis* Genetics Centre

CRISPR.....Clustered regularly interspaced palindromic repeat

CNV.....Copy number variant

DD.....Developmental disorder

DSM.....Diagnostic and Statistical Manual of Mental Disorders

EP.....Epilepsy

GI.....Gastrointestinal disorder

GOF.....Gain-of-function

GPCR.....G-protein coupled receptor

gRNA.....Guide RNA

GWAS.....Genome wide association study

HDR.....Homology directed repair

ID.....Intellectual disability

IDT.....Integrated DNA Technologies

K2P.....Two-pore domain potassium channel

LNS.....Laminin-neurexin-sex hormone-binding globulin

LOF.....Loss-of-function

NBRP.....National BioResource Project

NEB.....New England BioLabs

NGM.....Nematode growth medium

NHEJ.....Non-homologous end joining

Definitions and Abbreviations

NLGN ..... Neuroligin

NT ..... Nucleotide

OCD ..... Obsessive-compulsive disorder

PAM ..... Protospacer adjacent motif

SCZ ..... Schizophrenia

SFARI ..... Simons Foundation Autism Research Initiative

sgRNA ..... Single guide RNA

SNP ..... Single nucleotide polymorphism

STRING ..... Search Tool for the retrieval of Interacting Genes/Proteins

TMD ..... Transmembrane domain

Chapter 1 General introduction

1.1 Autism spectrum disorder

1.1.1 Introduction to autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental behavioural disorder (Sharma, Gonda and Tarazi, 2018) that was first described in 1943 in children who lacked interest in others (Kanner, 1943). The umbrella term, ASD, encompasses a group of pervasive developmental disorders including Asperger's syndrome. ASD is most often referred to as having a triad of behavioural phenotypes, these being impaired verbal communication including language delay, repetitive behaviours and impaired social interactions. More recently, in the latest version of the Diagnostic and Statistical Manual of Mental Disorders-5 (DSMV), ASD is defined as having two core domain deficits in social communication/interaction and repetitive behaviour. Before the DSMV nonverbal communication such as hand gestures, facial expressions and forms of social communication during play were being arbitrarily classified under either the social or communication domains (Lord and Jones, 2012). Also, it was shown that the majority of children with language delay don't meet the other criteria of ASD, therefore in the DSMV the verbal communication domain was removed. Instead, nonverbal and social communication deficits were added under the social domain and repetitive language was added under the repetitive behaviour domain with the aim to improve the reliability of the DSM as a diagnostic tool of ASD (Lord and Jones, 2012; Lai *et al.*, 2013).

ASD is typically diagnosed before 3-4 years of age and in the last 10 years prevalence rates have steadily increased (Centers for Disease Control and Prevention, 2020). It was estimated that 1 in 150 children had ASD in the year 2000 but increased awareness and understanding of the disorder is thought to have contributed to the increase in prevalence (Neggers, 2014), which is now thought to be as high as 1 in 36 children (Zablotsky, Black and Blumberg, 2017). As set out by the DSMV, for an individual to be diagnosed with ASD they must demonstrate at least three symptoms in the social domain and at least two symptoms in the repetitive behaviour domain. The social domain describes criteria that contribute to the persistent deficit in social communication and interaction. These criteria include failure to initiate and respond to social interaction and a lack of interest in sharing. Also, in terms of nonverbal communication, the criteria include a poor understanding of communication using gestures, body language and eye contact. Behaviours that make up the restricted behaviour domain include repetitive speech, motor movements or use of objects, for example lining up toys or turning on and off lights. Also,

individuals may often adhere to strict routines, finding change to routines difficult and may display fixation on certain interest or objects. Finally, the DSMV outlines the hyper or hypo-sensitivity that may be displayed in response to sensory stimuli (American Psychiatric, 2002).

ASD is a pervasive disorder meaning it effects an individual throughout their life. It also has a pervasive impact on caregivers, family and the wider community with estimates suggesting that 85% of individuals diagnosed with ASD are unable to live completely independently (Karst and Van Hecke, 2012). In fact, the DSMV stresses the impact that ASD has on carers as it defines levels of ASD severity from level 1 defined as 'requiring support' through to level 3 which is defined as 'requiring very substantial support' (American Psychiatric, 2002). It has also been estimated that the cost of supporting one individual with ASD over their lifespan is £1.5 million (Buescher *et al.*, 2014). Currently, there are no targeted therapeutics to treat the core symptoms of ASD. Overall, the high incidence rate, economic burden and poor prognosis of ASD highlights the need for a better understanding of this disorder in order to improve the quality of life of individuals living with ASD.

1.1.2 ASD as a brain disorder

ASD is associated with abnormalities in brain development, neuroanatomy, function and connectivity compared to neurotypical individuals who have typical development and cognitive abilities (Park *et al.*, 2016; Maximo, Cadena and Kana, 2014). Soon after ASD was first described it was observed that autistic behaviours were similar to the behaviour of patients with brain damage to the frontal lobe (Damasio and Maurer, 1978). Early studies also suggested that brain activity may be altered (Horwitz *et al.*, 1988) and macrocephaly (enlargement of the brain) is associated with autism (Shen and Piven, 2017). Since then neuroimaging studies have identified multiple brain regions that control high-order cognition and social processing (Wood and Grafman, 2003). Task based neuroimaging studies, where brain function is monitored during memory, social and emotional tasks, have identified both abnormally strong and weak connectivity between brain regions in ASD patients and have correlated this with changes in behavioural output (Maximo, Cadena and Kana, 2014). Post mortem analysis has shown differences in neuron size and axon lengths in ASD brains suggesting that neuron physiology is likely a key contributing factor in ASD (Hashem *et al.*, 2020).

The human brain is made up of 100 billion neurons. Together they control information processing and ultimately underpin higher-order processes such as memory, cognition and the co-ordination of behaviours (Purves *et al.*, 2001). Clusters of neurons that carry out information processing in a given brain region are called microcircuits (Shepherd, 2011). Communication between

microcircuits form larger, more complex, neural circuits that integrate vast amounts of information across different regions of the brain (Tau and Peterson, 2010). It is understood how neural circuits control some simple motor reflexes (Purves *et al.*, 2001) but how the nervous system controls more complex behavioural outputs is not as well defined. Human social behaviour is an example of a complex behaviour that involves the detection and response to many different cues, including speech and body language. Therefore, the detection, processing and regulation of behavioural outcome to coordinate behaviours like social behaviour is likely to be extremely complex, requiring crosstalk between multiple circuits in different brain regions. Some human social circuits are beginning to be put together, including aggression and parenting behaviours. However, to date this includes only the identification of important brain regions for these behaviours whilst the understanding of the microcircuits which may underpin behaviour are not clear (Chen and Hong, 2018). In the case of ASD, where it has been shown that neuronal physiology and brain connectivity is atypical, it is hypothesised that abnormalities in the brain's ability to process information may result in dysregulation of circuits that underpin behaviours and that this can then emerge as a behavioural phenotype presented by a patient (Maximo, Cadena and Kana, 2014).

1.1.3 Sensory processing impairment in ASD

A clinical feature of ASD is hyper- or hypo-reactivity to sensory input which includes indifference to pain and temperature and adverse response to certain sounds and textures (American Psychiatric, 2002). Sensory processing is the recognition and organisation of information that allows a person to interact with their environment (Blanche and Gunter, 2020). During complex behaviours, like social interaction, a person is required to integrate information from multiple sensory channels, including visual, auditory and chemical social cues (Chen and Hong, 2018). Social communication by pheromone signalling in mammals is a good example of a chemo-sensory cue that can modify another animals behavioural output to promote mating and aggression (Chen and Hong, 2018). Recently it has been shown that individuals with ASD produce altered responses to social chemo-signals in comparison to controls (Endevelt-Shapira et al., 2018). Also, it has been shown that multisensory processing impairments exist in ASD with a study showing that individuals with ASD have an impaired ability to integrate audio and visual cues into a perceptual whole (Stevenson *et al.*, 2014b). Interestingly, the literature suggests that sensory processing impairments correlate with ASD symptom severity (Brandwein *et al.*, 2015; Mayer, 2017; Horder *et al.*, 2014). This has been used to theorise that suboptimal processing of sensory information could have cascading effects that converge on neural circuits that control behavioural

output (Robertson and Baron-Cohen, 2017), the consequence of this being the production of atypical behaviour.

1.1.4 Excitatory/Inhibitory imbalance in ASD

Activity of neural circuits is primarily controlled by the activity of excitatory glutamatergic and inhibitory GABAergic neurotransmission in the brain (Purves *et al.*, 2001). Decreased GABAergic signalling has been identified as a common feature of the ASD brain (Hussman, 2001). This led to the excitatory/inhibitory imbalance theory of ASD which is now widely accepted as a model in the aetiology of autism (Rubenstein and Merzenich, 2003; Sohal and Rubenstein, 2019). As well as decreased inhibitory signalling, increased levels of glutamate in the blood plasma of ASD patients (Zheng *et al.*, 2016) and increased expression of glutamate receptors have been observed (Fatemi *et al.*, 2011). Both increased excitation and decreased inhibition contribute to the hyperexcitability of neural circuits. This has been suggested to create an imbalance in the signal to noise ratio in the brain and ultimately impede the function of circuits which process information and control behaviour (Dinstein *et al.*, 2012; Robertson and Baron-Cohen, 2017; Rubenstein and Merzenich, 2003). Interestingly, experiments of excitatory/inhibitory imbalance in mice have shown that increased excitatory signalling in the brain results in behaviour impairment, including in the social domain (Yizhar *et al.*, 2011). This provides key insight into the circuit level dysfunction that may be underpinning alterations in human social behaviour.

1.1.5 Comorbidity in ASD

The complex circuit disruption identified in ASD likely underpins a range of comorbidities. The simultaneous presentation of two or more disorders occurs in 70% of ASD cases (Sztainberg and Zoghbi, 2016). Epilepsy is more common in individuals with ASD than it is in the general population (Giovanardi Rossi, Posar and Parmeggiani, 2000; Besag, 2017). Like ASD, epilepsy is associated with excitatory/inhibitory imbalance of signalling in the brain which leads to hyperexcitability of neural circuits and therefore the increased probability of seizures (Frye *et al.*, 2016). Comorbidities with autism also include gastrointestinal disorders. The gut-brain axis describes the bidirectional communication between the two organs and new research suggests a link between gut microbiota and ASD behavioural phenotypes (Wasilewska and Klukowski, 2015). Although the mechanism by which this occurs is not known it is hypothesised that the gut microbiome can influence the composition of immune cells and metabolites in the body and that this may contribute to brain dysfunction (Wasilewska and Klukowski, 2015; Srikantha and Mohajeri, 2019). As well as this, psychiatric disorders which are often comorbid with ASD include intellectual disability (Bilder *et al.*, 2013) attention deficit hyperactivity disorder (ADHD),

obsessive-compulsive disorder (OCD), anxiety (Zaboski and Storch, 2018) and schizophrenia (SCZ) (Stahlberg *et al.*, 2004). These disorders share some clinical features with ASD, for example SCZ, another neurodevelopmental disorder (Owen *et al.*, 2011), is also associated with language impairments and social deficits (Hommer and Swedo, 2015). The overlap of symptom presentation in ASD and other neuropsychiatric disorders makes the diagnosis of these disorders complicated (Trevisan *et al.*, 2020; Eack *et al.*, 2013).

Although comorbidities complicate the diagnostic process, they can provide clues to the complexity of ASD and may help in the dissection of the neural basis of autism. The psychiatric disorders which often co-occur with autism are also associated with abnormalities in brain structure and function. Neuroimaging studies have highlighted various brain areas disrupted in these disorders (Martin *et al.*, 2009; Mikita *et al.*, 2016; Gehricke *et al.*, 2017; Zhang *et al.*, 2018; Huey *et al.*, 2008; Karlsgodt, Sun and Cannon, 2010). It is becoming increasingly clear that the brain circuitries that underpin these disorders can be both distinct and shared between the disorders (Tye *et al.*, 2019; Mikita *et al.*, 2016; Taurines *et al.*, 2012). Comparison of the circuitries that are thought to be involved in ASD and its comorbidities suggest that they are likely to be highly interconnected (Tye *et al.*, 2019). This forms the basis of the theory that disruption within one circuit can have cascading effects through other shared pathways such that someone with a psychiatric illness is likely to have more than one disorder (Tye *et al.*, 2019). A better understanding of how variability in neural networks can lead to different symptom profiles in ASD is needed. To facilitate this, further investigation of distinct circuits underling symptoms is needed to disentangle causal pathways (Albajara Sáenz *et al.*, 2020). Equally, understanding common pathways that are shared between comorbid disorders will likely aid the development of therapeutics for individuals with complex diagnoses (Doshi-Velez, Ge and Kohane, 2014).

1.2 Genetics in the aetiology of ASD

ASD is a heterogeneous disorder with a strong genetic component and environmental risk factors in its aetiology (Chaste and Leboyer, 2012; Geschwind, 2011). Early genetic studies identified higher concordance rates of autism among monozygotic twins compared to dizygotic twins (Folstein and Rutter, 1977; Ritvo *et al.*, 1985; Steffenburg *et al.*, 1989; Bailey *et al.*, 1995). Over the years numerous twin studies have been conducted, identifying monozygotic concordance rates between 36-93% (Ronald and Hoekstra, 2011). Additional family studies have shown that the closer in relation a sibling is to the affected proband the more at risk they are for having ASD. For example, full siblings are more at risk of both being diagnosed with ASD than half-siblings or cousins (Sandin *et al.*, 2014). Overall, this early work provided strong evidence for the heritability of ASD.

Since the discovery of the genetic component of ASD there has been a lot of research investigating the genetic architecture of autism. ASD can be classified into syndromic and non-syndromic autism. Syndromic ASD describes a genetic disorder in which ASD like symptoms are displayed along with other phenotypes associated with the given disorder. In most cases the aetiology of these syndromic disorders is known, for example Fragile X syndrome is a single gene disorder which is the most common monogenic form of syndromic ASD (Sztainberg and Zoghbi, 2016). In contrast, non-syndromic or idiopathic autism has been shown to result from a single gene in less than 5% of cases (Jiang *et al.*, 2014) pointing to the complex polygenic nature of idiopathic ASD. In fact there are currently over 1000 genes that have been associated with increasing the risk for autism (https://gene.sfari.org/) and different types of genetic variation can impact these genes. These genetic variants can be broadly split into two categories, these being rare, highly penetrant variants and common, low penetrant variants (Devlin and Scherer, 2012; Huguet, Ey and Bourgeron, 2013; Iakoucheva, Muotri and Sebat, 2019). The contribution of thousands of ASD-risk genes and various genetic variants makes for a complex genetic landscape in autism (Figure 1.1).

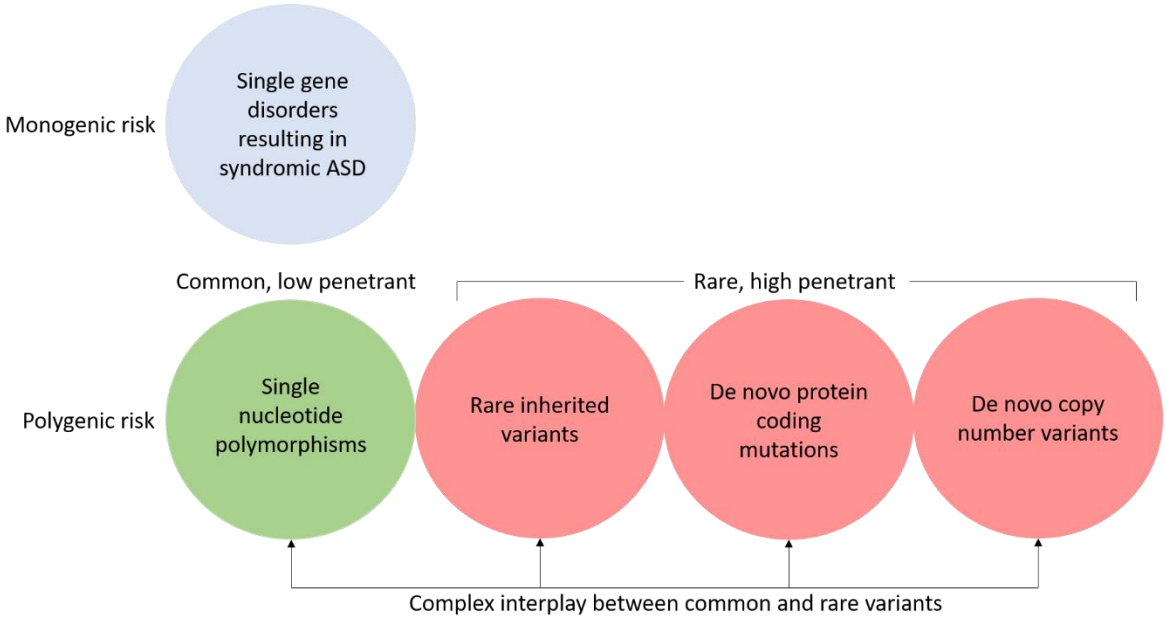


Figure 1.1 Overview of the different categories of genetic variants associated with ASD. Monogenic risk for ASD like symptoms comes from single gene disorders that cause syndromic forms of autism. Polygenic risk in ASD is more complicated as it can be influenced by different types of genetic variants that can be broadly split into common, low penetrance, variants and rare, high penetrant variants. There is also a complex interplay that exists between common variants and various types of rare variants that contributes to the extreme heterogenic nature of autism.

1.2.1 Rare, highly penetrant, genetic variants in ASD

1.2.1.1 Copy Number Variants

Whole genome and exome sequencing has facilitated the understanding of the genetic landscape of ASD (Szego and Zawati, 2016). Comparing the genetic makeup of individuals with ASD to neurotypical controls allows for the identification of genetic variation that may underlie autism (Jiang *et al.*, 2014; Devlin and Scherer, 2012). Sequencing studies have shown that the ASD genome is often enriched with rare copy number variants (CNVs) (Pinto *et al.*, 2010; Sebat *et al.*, 2007). Copy number variants are regions of DNA sequence larger than 1kb that have a variable number compared to the human reference genome. These variants can be caused by duplications, deletions or more complex losses and gains of sequence within the genome (Redon *et al.*, 2006). Interestingly, whilst these variants are known to be enriched in ASD they are rare (Figure 1.1), occurring in less than 5% of the human population (Bourgeron, 2015). For example, it has been shown that in sporadic cases of ASD, in which neuro-atypical children are born into a family with no history of autism, 6.6% of children with ASD had at least one rare de novo CNV in comparison to 1.4% of unaffected siblings or 1.9% of control cases (Huguet, Ey and Bourgeron, 2013).

Understanding the enrichment of CNVs in ASD has facilitated the understanding of which chromosomal loci, and therefore which genes, may be risk factors for autism. A study of 17 children with ASD identified that only 2 of the 17 carried a second CNV within their genome, providing evidence that single CNVs can be highly penetrant in ASD (Schaaf *et al.*, 2012; Jiang *et al.*, 2014). As well as being highly penetrant, CNVs in individuals with ASD can be large. In fact in comparison to controls, CNVs associated with ASD impact 16 times more genes therefore contributing to the large number of potential risk genes that have been identified (Sanders *et al.*, 2011).

1.2.1.2 Rare de novo and inherited variants

As well as CNVs, whole genome and exome sequencing studies have identified a number of protein coding sequence mutations, for example missense, splice site and frameshift variants that are associated with ASD (Huguet, Ey and Bourgeron, 2013). Like CNVs these coding sequence variants are rare within the population but enriched and highly penetrant in individuals with ASD (Figure 1.1) (Bourgeron, 2015). Both CNVs and coding sequence variants can be de novo or inherited from parents. A de novo mutation is a mutation that occurs in the germline of the parent, most often from the father, and are thought to occur in 30% of ASD cases (Iakoucheva, Muotri and Sebat, 2019). An inherited variant is one that is passed from the parent to the child. In

most cases the parent themselves has no or mild ASD symptoms whilst the child has diagnosed autism (Sebat *et al.*, 2007). Incomplete penetrance describes a variant which is present in both affected and non-affected family members and suggests there must be an added level of complexity that contributes to the genetic vulnerability of ASD. Also contributing to the genetic complexity of autism is the fact that most rare gene mutations are specific to the individual, with a single ASD-associated gene mutation accounting for less than 1% of ASD cases suggesting the genetic makeup of each individual with ASD is highly variable (Huguet, Ey and Bourgeron, 2013; Yoo, 2015).

1.2.2 Common, low penetrant variants in ASD

A single nucleotide polymorphism (SNP) is a genetic variation involving a single DNA base pair (Robert and Pelletier, 2018). It is estimated that one individual in the general population carries, on average, three million SNPs when compared to the human reference genome (Bourgeron, 2015). The majority of these variants are common to the human population, occurring in 5% or more of the population (Bourgeron, 2016). Genome wide association studies (GWAS) identify if a SNP is more frequently associated with a disorder relative to controls (Barešić *et al.*, 2020). Various GWAS in ASD using this study design have identified over 1,000 SNPs associated with ASD. However, most GWAS to date have been too underpowered to conclude which SNPs are causative of ASD (Bourgeron, 2016). For example, GWAS of ~3,000 families was not enough to identify even a single variant with genome wide significance (Anney *et al.*, 2012).

The study of SNPs is complicated by the fact that each are usually of low penetrance, having weak effect on the heritability of ASD (Figure 1.1) (Anney *et al.*, 2012). Therefore the contribution of different SNPs at multiple loci is thought to be additive (Dudbridge, 2013). Indeed, it has been shown that inheritance of common variants fits an additive heritability model where parents transmit many SNPs, each with small effect, that additively contribute to the liability of ASD in the child (Klei *et al.*, 2012). Both the low penetrance and the low power of previous GWAS means that most of the understanding of the genetic underpinnings in ASD has come from the identification of rare de novo variants. In contrast, whilst common variants are now understood to make up part of the genetic landscape of ASD they are less well understood. However, recently a large genome wide association meta-analysis using 18,381 ASD cases and 27,969 controls identified five genome wide significant loci (Grove *et al.*, 2019), paving the way for a more robust understanding of the polygenic risk that common variants attribute in ASD.

1.2.3 Complex interplay between rare and common variants

The genetic landscape of ASD can range from monogenic risk from a single, rare de novo variant through to polygenic risk from various common variants (Figure 1.1) (Bourgeron, 2015). Interestingly, individuals with a rare de novo variant in ASD are more likely to have a high burden of common SNPs (Weiner *et al.*, 2017; Niemi *et al.*, 2018), suggesting that a combinatorial effect of rare and common variants can contribute to the polygenic risk of ASD (Figure 1.2). Common SNPs make up a person's genetic background and therefore can influence their genetic buffering capability. A strong genetic buffer can compensate for the effect of a penetrant rare variant. Each person carries two copies of each autosomal gene meaning that when one is mutated the other may be able to compensate for its dysfunction or loss. Likewise, a gene with overlapping function may be able to buffer the effect of the mutation. In contrast, a weak genetic buffer can make a person more susceptible to the effects of a rare mutation (Bourgeron, 2015). The complex effects of genetic buffering on autism susceptibility might help to explain why a child with ASD can inherit a variant from a parent who themselves does not have autism (1.2.1.2) (Figure 1.2). Different combinations and different genetic burdens of rare and common variants means that the genetic makeup of any one individual with ASD is very diverse (Figure 1.2).

Similarly, other neuropsychiatric disorders such as SCZ, OCD, ADHD, epilepsy, depression and anxiety are polygenic in nature. All of these disorders have a complex genetic landscape, often with hundreds of genes implicated as risk factors. For most of these disorders, both rare and common genetic variants have been, and are continuing to be, identified (Dunn *et al.*, 2015; McIntosh, Sullivan and Lewis, 2019; Thapar, 2018; Koeleman, 2018; Purty *et al.*, 2019; International Obsessive Compulsive Disorder Foundation Genetics and Studies, 2018; Purves *et al.*, 2020; Gottschalk and Domschke, 2017; Avramopoulos, 2018) suggesting that these disorders have a similar architecture at the level of circuit dysfunction (1.1.5) and also genetic complexity. Whilst the genetic complexity is understood in these disorders, the complex genetic interplay that

can occur between different genes and different genetic variants is best well understood in ASD and SCZ (Bergen *et al.*, 2019; Weiner *et al.*, 2017; Niemi *et al.*, 2018).

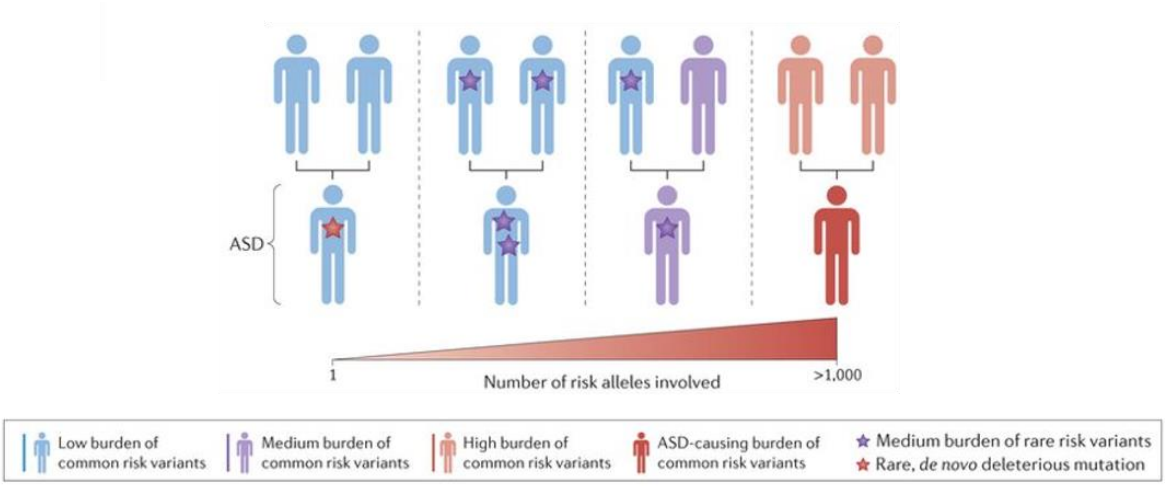


Figure 1.2 Cartoon outlining the complex interplay between common and rare genetic variants in ASD. The cartoon depicts a range of paths for ASD transmission from monogenic risk from a single rare de novo variant through to polygenic risk from various common variants. It also illustrates how rare and common variants inherited from parents can combine in children with autism. Cartoon modified from (Bourgeron, 2015).

1.3 Convergence of ASD genes on biological domains

1.3.1 Functional diversity of ASD-risk genes

Identification of the different genetic variants that have been associated with ASD (1.2) has led to a large number of ASD-candidate genes. The largest database of ASD-risk genes called the Simons Foundation Autism Research Initiative (SFARI) currently houses over 1,000 genes associated with autism (https://gene.sfari.org/ version 3.0). It is clear from the gene function information collated by SFARI and from genetic studies that ASD-risk genes are functionally diverse (Pinto *et al.*, 2014; De Rubeis *et al.*, 2014). There is no single category of ASD-risk genes, instead they encode proteins which span a diverse range of biological functions. However, ASD-candidate genes seem to converge on two main biological processes, these being synaptic transmission and gene expression (Iakoucheva, Muotri and Sebat, 2019). The function of genes to control these processes and how they can impinge on autism is described below.

1.3.2 ASD-risk genes in interaction networks

Analysis of protein-protein interactions between ASD-associated genes has predicted a high level of interconnectivity between risk genes by direct interaction (Corominas *et al.*, 2014; Pinto *et al.*, 2014). This inferred molecular connectivity adds potential for another layer of complexity in the genetic aetiology of ASD because it means that ASD-risk genes, even if they are not directly mutated in an individual, can be dysregulated by an interconnected variant causing widespread disruption across the genome (Figure 1.3 A,B). The breadth of disruption becomes even more widespread if you consider the effect of multiple polygenic risk loci, that will have the ability to impact multiple interaction networks (Figure 1.3C) (Iakoucheva, Muotri and Sebat, 2019). Understanding how proteins interact in networks has helped to resolve common biological pathways that ASD susceptibility genes converge on. These studies have built on the analysis of interaction networks to build proteomic interactomes and have identified synaptic transmission, cell adhesion, cell signalling, transcriptional and translational regulation as important determinants within biological domains that mutated ASD genes converge (Pinto *et al.*, 2014; De Rubeis *et al.*, 2014; Berg and Geschwind, 2012; Iakoucheva, Muotri and Sebat, 2019).

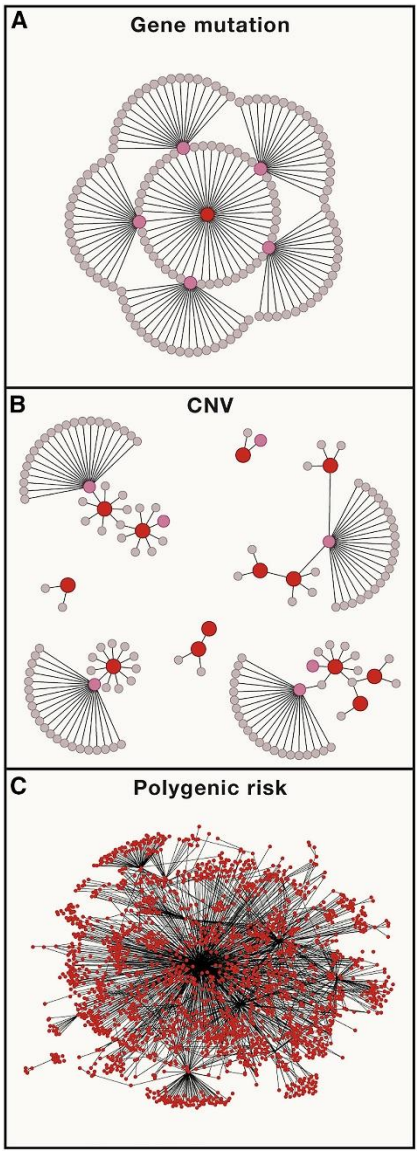


Figure 1.3 Cartoon outlining the complex effects that genetic variants can have on genes within interaction networks. Red circles represent an ASD-risk gene affected by a genetic variant. Pink circles represent other ASD-risk genes that are not affected by a genetic variant but interact in a network with a gene that is. (A) A single risk variant in a gene can have effects that fan out through other ASD-risk genes within an interaction network. (B) Large CNVs can impact multiple genes on a chromosome meaning the variant will impact a wider range of networks within the genome. (C) Polygenic risk from common variants in multiple loci have very complex and widespread effects across the genome. Cartoon taken from (Iakoucheva, Muotri and Sebat, 2019).

1.3.3 Biological domains associated with ASD

1.3.3.1 Synaptic and neuronal

Synaptic signalling within neural circuits is fundamental for higher cognitive functions including the control of behavioural output (1.1.2). Of the genes implicated in ASD those that function at the synapse and in neurons are some of the most well studied (Guang *et al.*, 2018; Zoghbi and Bear, 2012; Giovedi *et al.*, 2014). This is consistent with the behavioural and neuronal dysfunction identified in ASD clinical cohorts (1.1). At the synapse, genes encoding cell adhesion molecules (CAMs), scaffold proteins, neurotransmitter receptors and ion channels have all been implicated in ASD (Sudhof, 2008; Chen *et al.*, 2014; Koberstein *et al.*, 2018; Daghsni *et al.*, 2018) (Figure 1.4) (Table 1.1). CAMs are proteins that adhere to one another to bridge the synaptic cleft and are important for the stability, function and development of the nervous system (Missler, Südhof and Biederer, 2012). CAMs interact with scaffold proteins in the post synaptic density which interact directly and indirectly with receptors and ion channels to recruit the necessary components for synaptic transmission to the post-synaptic membrane (Meyer *et al.*, 2004). Genetic variants identified in CAMs and scaffold proteins have been shown to cause alterations in synaptic signalling and alterations to the number and strength of synaptic connections (Baudouin *et al.*, 2012; Shcheglovitov *et al.*, 2013; Sudhof, 2008; Culotta and Penzes, 2020). Also, changes to the regulation of ion flux has been associated with mutation in ion channels associated with ASD (Daghsni *et al.*, 2018). In most cases, dysfunction in these classes of synaptic proteins are thought to contribute to the imbalance of synaptic signalling described by the excitatory/inhibitory imbalance theory of autism (1.1.4) and are likely to result in circuit dysfunction that underpins ASD behavioural characteristics (Nelson and Valakh, 2015).

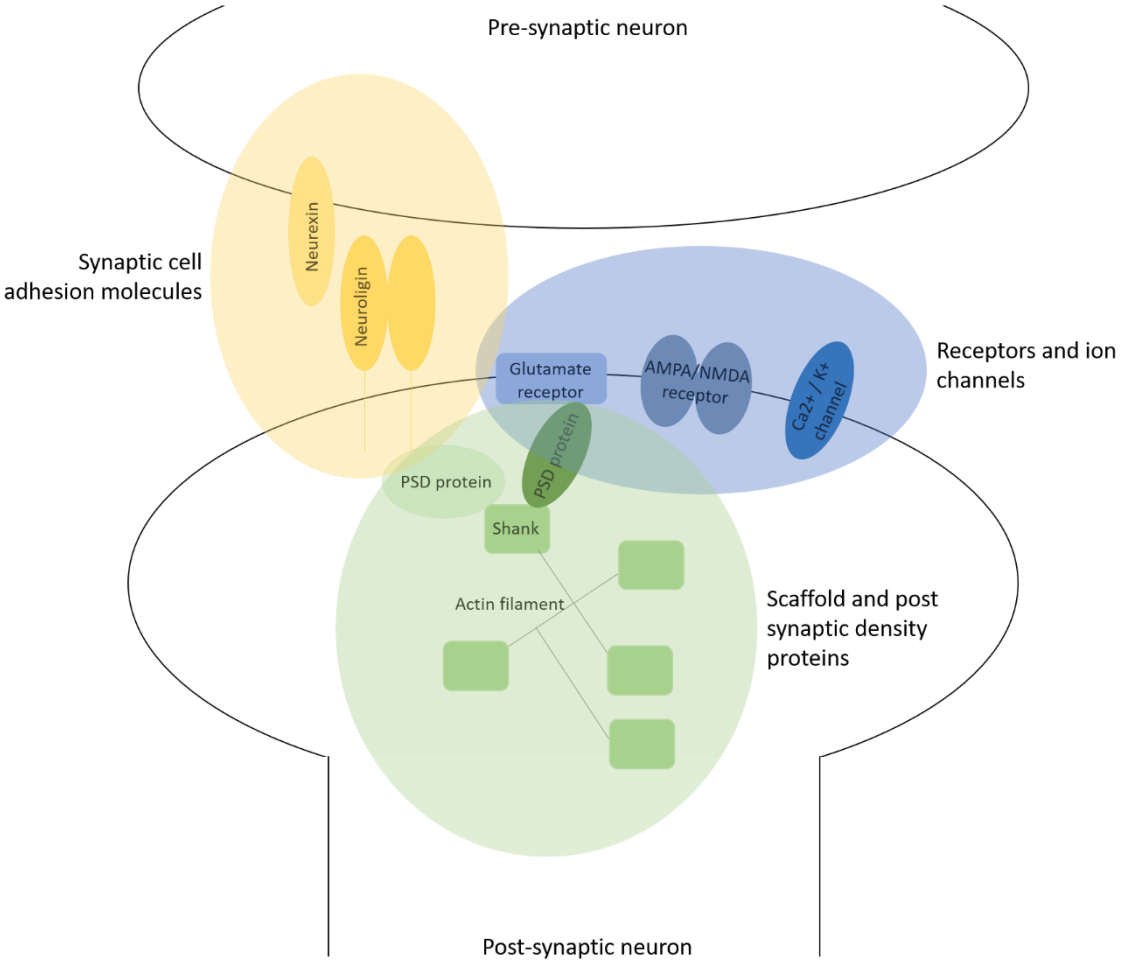


Figure 1.4 Cartoon outlining some of the major functional categories of ASD-risk genes at the synapse. Genes involved in synapse structure and function have been implicated in increasing the risk of autism. Synaptic cell adhesion molecules (yellow) bridge the gap between the pre- and post-synapse and are important for the stability, development and proper functioning of a synapse. Scaffolds and post-synaptic density proteins (green) are important for stabilising and anchoring receptors and ion channels (blue) which are required for synaptic transmission. The cartoon represents a simplified overview of some synaptic ASD-risk genes and is not a comprehensive list.

1.3.3.2 Cell signalling

Cell signalling pathways that have been associated with ASD include those that control aspects of brain development, synaptogenesis and synapse function (Ebert and Greenberg, 2013; Baranova *et al.*, 2021; Doll and Broadie, 2014; Kumar *et al.*, 2019). Many of the implicated pathways are synapse activity-dependent signalling cascades. Activation of a receptor or ion flux at the synaptic

membrane can initiate various signalling pathways that control gene expression; hence these pathways provide a route of communication between the synapse and the nucleus. Regulating the expression of genes that are crucial for synapse function means neural activity can shape the development and strength of neural circuits (Ebert and Greenberg, 2013). This allows circuits to respond dynamically to different stimuli and to be modified by experience. Experimental manipulation of a signalling pathway important for regulating synaptic plasticity showed that inhibition of the pathway led to alterations in the expression of a CAM and increased excitatory transmission (Gkogkas *et al.*, 2013). Considering the hyperexcitability of neural circuits that has been associated with ASD it appears that dysregulation of signalling pathways may contribute to the pathogenesis of ASD (Table 1.1).

1.3.3.3 Epigenetics

Epigenetics is the modification of gene expression without changing the DNA sequence. Epigenetic modifications edited by mechanisms such as DNA methylation and histone acetylation impact how tightly DNA is packaged in the nucleus and therefore influences gene expression (Waye and Cheng, 2018). DNA methylation and histone acetylation patterns have been shown to differ in ASD epigenomes compared to neurotypical controls suggesting epigenetics may contribute to the pathogenesis of ASD (Ladd-Acosta *et al.*, 2014; Waye and Cheng, 2018). Analysis of post mortem brain samples has indicated that ASD brains share common epigenetic signatures and that this likely affects the expression of genes involved in processes like synapse function and neuronal excitability (Sun *et al.*, 2016). In support of this are gene expression studies from neuronal cells cultured from ASD cohorts that have shown that synaptic genes such as ion channels are differentially expressed (Marchetto *et al.*, 2017). Many ASD-candidate genes have been identified to be involved in chromatin remodelling (Lasalle, 2013) (Table 1.1). It is possible that alterations to the epigenome are leading to disruptions within core biological domains, such as synapse function, and hence contributing to the aetiology of autism (Eshraghi *et al.*, 2018; Loke, Hannan and Craig, 2015).

1.3.3.4 Epigenetics and environmental risk factors

Epigenetics provides a route by which environmental factors can influence autism. Environmental influences such as diet and stress have been linked to increasing the risk of ASD (Kubota and Mochizuki, 2016). Studies in mice have shown that protein restricted diet leads to decreased methylation of DNA in offspring (Lillycrop *et al.*, 2008). Parental imprinting describes the maintenance of epigenetic modifications from parent to offspring (Fedoriw, Mugford and Magnuson, 2012). Investigation of this in rodents has shown that stress can alter the methylation pattern of DNA in germline cells and consequently alter the epigenetic markers that are passed

down to the next generation (Franklin *et al.*, 2010). With this in mind, transgenerational epigenetic inheritance could be another reason for the high heritability of ASD and shows how environmental factors can influence the risk of autism (Choi *et al.*, 2016; Eshraghi *et al.*, 2018).

1.3.3.5 Gene transcription

Transcription factors which bind to DNA to facilitate the activation or repression of gene expression (Mitsis, 2020) have also been implicated in ASD (Ben-David and Shifman, 2013; De Rubeis *et al.*, 2014). Knockdown of ASD-associated transcription factor genes in mice studies have shown that it can result in defective neuronal migration and development (Li *et al.*, 2015). The mice model both brain and behavioural alterations seen in human cohorts as they display macrocephaly (Bernier *et al.*, 2014), repetitive behaviours and impaired social interactions (Platt *et al.*, 2017). Interestingly, knockdown of a single transcription factor can alter the expression of hundreds of genes, including other ASD-risk genes which has been shown to result in the up/downregulation of multiple ASD-candidate genes during development (Sugathan *et al.*, 2014; Cotney *et al.*, 2015; Wilkinson *et al.*, 2015). Therefore, the disruption to a single transcription factor is thought to have wide reaching effects on numerous downstream networks that may converge on brain development and behavioural output (Ayhan and Konopka, 2019).

1.3.3.6 Metabolic pathways

A diverse range of metabolic pathways have been associated with ASD. Measuring the intensity of metabolites in the urine, blood and brain tissue of ASD patients identified differences compared to controls (Yap *et al.*, 2010; Kurochkin *et al.*, 2019) suggesting alterations within metabolic pathways including those involved in oxidative stress, TCA cycle and metabolism of carbohydrates and sugars (Bitar *et al.*, 2018). Emerging literature in this field also suggests an association between autism and abnormalities in the metabolism of cell membrane lipids and fatty acids (Table 1.1) (Wang *et al.*, 2016a; Tamiji and Crawford, 2010; Wong and Crawford, 2014). ASD-risk genes identified as having a role in cell membrane metabolism are thought to disrupt the synthesis of fatty acids and the cycling of fatty acids in and out of the phospholipid bilayer (Johansen *et al.*, 2016; Wong and Crawford, 2014; Tamiji and Crawford, 2010). Fatty acids are important for membrane integrity, brain development and function of the nervous system. Their composition in the membrane helps to modulate ion channels, enzyme and receptor activity (Tamiji and Crawford, 2010). It has also been shown that individuals with ASD can have an imbalance in the ratio of different classes of fatty acids (Thomas *et al.*, 2010) and supplementation to remedy this has been shown to improve some ASD-associated behaviours (Meguid *et al.*, 2008). This suggests that metabolic control of pathways that regulate nervous system function are an important factor in the pathology of autism.

1.3.4 Genetic overlap of ASD with other psychiatric disorders

As the above illustrates disruption to different biological domains converges on the development, stability and functioning of the nervous system with circuit dysfunction and dysregulated electrical signalling at the synapse likely contributing to the emergence of ASD. Specificity in terms of disease outcome in the face of gene mutation is not clear and overlap between distinct psychiatric disorders seems to extend beyond comorbidity of clinical symptoms (1.1.5) to overlapping genetic underpinnings.

Autism associated SNPs and CNVs have also been shown to confer risk for other psychiatric disorders including SCZ, ADHD, OCD, intellectual disability and anxiety (Doherty and Owen, 2014) (Table 1.1). Large genetic overlap has been identified between ASD and SCZ, with a meta-analysis of GWAS studies indicating that 40% of risk genes are enriched in both clinical cohorts (Autism Spectrum Disorders Working Group of The Psychiatric Genomics, 2017). Overlapping risk genes can be grouped into biological domains including neurodevelopment, chromatin remodelling, lipid metabolism and synapse function (McCarthy *et al.*, 2014; Rylaarsdam and Guemez-Gamboa, 2019; Doherty and Owen, 2014). This suggests that points of biological convergence in ASD are also likely to be disrupted in other disorders. Genetic similarities will likely help in the identification and investigation of common pathways across disorders to facilitate the understanding of the aetiology and development of treatment. However, the overlap of disorders at both a clinical and genetic level makes diagnosis of different disorders difficult. It has been suggested in the literature that the distinct categorisation of psychiatric disorders is arbitrary. Instead disorders might be better defined as a gradient where the severity and load of genetic and environmental factors act together to determine neuro-atypical trajectory through development which emerges as different groupings of clinical symptoms that are common to multiple psychiatric disorders (Doherty and Owen, 2014).

1.3.5 SFARI gene database

SFARI gene is a publicly available database which curates ASD-candidate genes (https://gene.sfari.org/). SFARI gene aims to compile a comprehensive list of the candidate genes that have been identified in published literature and reviewed by autism geneticists (Banerjee-Basu and Packer, 2010). Within the database is the so called ‘gene scoring module’ which is a list of criteria that genes are reviewed against in order to evaluate the strength of evidence that associates a gene with autism (Abrahams *et al.*, 2013). At the time of this project the gene scoring module had six categories for risk genes (1-6) https://gene-archive.sfari.org/, version 3.0, accessed October 2018). For genes to be ranked in category 1 that gene must have genome-wide

statistical significance and the evidence linking this gene to ASD must have been replicated in an independent genome-wide study. For genes in category 2 the gene must also have genome-wide statistical significance, but the evidence does not have to be independently replicated. For genes ranked 3-6 the ranking criteria included genes that had been identified in small, replicated human studies that had not produced genome-wide significance and animal model studies that implicated a gene without confirmation from human studies. In this project, genes in categories 1 and 2 were selected to enter a bioinformatic filtering pipeline to select genes for investigation (Table 1.1). Interestingly, the genes in these categories contain representatives from the main biological domains implicated in autism, including synaptic and neuronal, cell signalling, epigenetics and metabolism.

Table 1.1 Summary of the ASD-risk genes investigated in this project.

Human gene	**Protein function**	**SFARI gene category**	**Rare and/or common variants identified**	**Associated psychiatric disorders**	**References**
Synaptic					
GRIA1	Glutamate receptor	2	Rare[1]	SCZ[2]	[1] (De Rubeis *et al.*, 2014) [2] (Ripke *et al.*, 2013)
GRIN2B	NMDA receptor	1	Rare[1] and common[2]	SCZ[3], ID[4], EPS[5]	[1] (O'Roak *et al.*, 2011) [2] (Pan *et al.*, 2015) [3] (Myers *et al.*, 2011) [4] (Dimassi *et al.*, 2013) [5] (Platzer *et al.*, 2017)
NLGN3	Synaptic adhesion	1	Rare[1] and common[2]	ID[3], SCZ[4]	[1] (Jamain *et al.*, 2003) [2] (Yu *et al.*, 2011) [3] (Redin *et al.*, 2014) [4] (Hu *et al.*, 2020)

Human gene	**Protein function**	**SFARI gene category**	**Rare and/or common variants identified**	**Associated psychiatric disorders**	**References**
NRXN1	Synaptic adhesion	1	Rare[1] and common[2]	SCZ[3], ID[4], EP[4], ADHD[5]	[1] (Feng *et al.*, 2006) [2] (Szatmari *et al.*, 2007) [3] (Voineskos *et al.*, 2011) [4] (Schaaf *et al.*, 2012) [5] (Viñas-Jornet *et al.*, 2014)
PTCHD1	Synaptic receptor	1	Rare[1] and common[2]	ID[2], EP[3], ADHD[4]	[1] (Marshall *et al.*, 2008) [2] (Torrico *et al.*, 2015) [3] (Rochtus *et al.*, 2020) [4] (Zarrei *et al.*, 2019)
SHANK2	Synaptic scaffold	1	Rare[1] and common[2]	ID[3], SCZ[4], OCD[5]	[1] (Berkel *et al.*, 2010) [2] (Bai *et al.*, 2018) [3] (Leblond *et al.*, 2012) [4] (Peykov *et al.*, 2015) [5] (Lu *et al.*, 2018)
SHANK3	Synaptic scaffold	1	Rare[1] and common[2]	ID[3], SCZ[4], ID[5], GI[5], EP[5]	[1] (Durand *et al.*, 2007) [2] (Boccuto *et al.*, 2013) [3](Hamdan *et al.*, 2011b) [4] (Gauthier *et al.*, 2010) [5] (Soorya *et al.*, 2013)

Human gene	**Protein function**	**SFARI gene category**	**Rare and/or common variants identified**	**Associated psychiatric disorders**	**References**
SLC6A1	GABA transporter	1	Rare[1] and common[2]	ADHD[2], EPS[3], ID[4], SCZ[5]	[1] (Sanders *et al.*, 2012) [2] (Yuan *et al.*, 2017) [3] (Carvill *et al.*, 2015) [4] (Borlot *et al.*, 2019) [5] (Rees *et al.*, 2020)
SYNGAP1	Ras GTPase activating protein	1	Rare[1]	ID[1], EP[1]	[1] (Hamdan *et al.*, 2011a)
Neuronal					
CACNA1H	Calcium channel	2	Rare[1]	EP[2], ADHD[3], GI[3]	[1] (Splawski *et al.*, 2006) [2] (Heron *et al.*, 2007) [3] (Chourasia *et al.*, 2019)
CNTN4	Axonal adhesion	2	Rare[1] and common[2]	DD[3], SCZ[4]	[1] (Roohi *et al.*, 2009) [2] (Liu *et al.*, 2016) [3] (Fernandez *et al.*, 2004) [4] (Fromer *et al.*, 2016)
Cell signalling					
DYRK1A	Protein kinase	1	Rare [1]	ID[2], EP[2]	[1] (O'Roak *et al.*, 2012) [2] (Courcet *et al.*, 2012)

Human gene	**Protein function**	**SFARI gene category**	**Rare and/or common variants identified**	**Associated psychiatric disorders**	**References**
PTEN	Protein phosphatase	1	Rare[1]	EP[2], DD[3]	[1] (De Rubeis *et al.*, 2014) [2] (Epi *et al.*, 2013) [3] (McBride *et al.*, 2010)
Epigenetic modifiers					
CHD8	Transcription factor	1	Rare[1]	ID[2], ADHD[2], GI[2], SCZ[3]	[1] (O'Roak *et al.*, 2012) [2] (Bernier *et al.*, 2014) [3] (Merner *et al.*, 2016)
FOXP1	Transcription factor	1	Rare[1] and common[2]	ID[3], anxiety[3], OCD[3], GI[3], EP[4], SCZ[5]	[1] (O'Roak *et al.*, 2011) [2] (Pardiñas *et al.*, 2018) [3] (Siper *et al.*, 2017) [4] (Jay *et al.*, 2019) [5](Levchenko *et al.*, 2021)
IRF2BPL	Transcription factor	1	Rare[1]	EP[2]	[1] (Sanders *et al.*, 2015) [2] (Tran Mau-Them *et al.*, 2019)
KDM6A	Histone demethylase	2	Rare[1]	-	[1] (C Yuen *et al.*, 2017)
KMT5B	Transcription factor	1	Rare[1]	EP[2]	[1] (Sanders *et al.*, 2012) [2] (Faundes *et al.*, 2018)
SETD2	Transcriptional regulation	1	Rare[1]	ID[2], EP[2]	[1] (O'Roak *et al.*, 2012)

Human gene	Protein function	SFARI gene category	Rare and/or common variants identified	Associated psychiatric disorders	References
					[2] (Lumish *et al.*, 2015)
SETD5	Transcriptional regulation	1	Rare[1]	ID[2], OCD[2], ADHD[3]	[1] (De Rubeis *et al.*, 2014) [2] (Grozeva *et al.*, 2014) [3] (Powis *et al.*, 2018)
Phospholipid metabolism					
MBOAT7	Acyltransferase	1	Rare[1]	ID[1], EP[1], anxiety[2], ADHD[2]	[1] (Johansen *et al.*, 2016) [2] (Jacher *et al.*, 2019)

The table groups the ASD-risk genes investigated in this project into five biological functional domains. Abbreviations are: SCZ – schizophrenia. EP – epilepsy. ID – intellectual disability. ADHD – attention deficit hyperactivity disorder. OCD – obsessive compulsive disorder. GI – gastrointestinal disorder. DD – developmental disorder. References are indicated using numbers. A comprehensive list of the rare and common variants identified for these genes and the references can be found at https://gene.sfari.org/database/human-gene/. A rare variant occurs in less than 5% of the human population and a common variant occurs in 5% or more of the human population (Bourgeron, 2015).

1.4 Pre-clinical investigation of ASD-candidate genes

1.4.1 Mice as a model of autism

In order to try to understand the genetic underpinnings of ASD symptoms such as impaired social behaviour, animal models can be used. Mice are the most prevalent model organism used in biomedical research (Ellenbroek and Youn, 2016). Mice lend themselves to the study of ASD because they can be genetically manipulated and they exhibit a number of social behaviours that can be investigated (Crawley, 2012). In this way, mice offer a translational tool to probe the biological mechanisms underlying behavioural phenotypes associated with autism. There is a high

conservation of genes in mice and human genomes (Chinwalla *et al.*, 2002) meaning genetic orthologues of human ASD-associated genes can be investigated in this animal. Also, a number of different genetic techniques that are well developed in mice allow for various gene mutations to be created, including missense, frameshift, loss and gain-of-function mutations (Wang *et al.*, 2020; Jin *et al.*, 2020; Tabuchi *et al.*, 2007; Platt *et al.*, 2017). Generating precise mutations such as these has allowed genetic variants that have been identified in human ASD to be modelled in mice to investigate their effect on the physiology and behaviour of the animal.

The aim in any model organism is to investigate an animal that displays behaviours that closely relate to the symptoms of a human disorder (van der Staay, 2006). Mice display a number of social behaviours and behavioural assays have been developed and standardised to measure social interaction, social communication and repetitive behaviours that relate to ASD. Such tests include scoring social interaction when a mouse is introduced to a novel mouse by measuring parameters such as physical contact and nose-to-nose sniffing behaviours (Crawley, 2012). In this way it has been shown that in response to genetic manipulation of a number of ASD-associated genes mice can model impaired social behaviour (Silverman *et al.*, 2010). Therefore, mice models of ASD have been used to elucidate which genes may co-ordinate social behaviours, how those genes may function in neural circuits that underpin social behaviours (Golden, Buxbaum and Rubeis, 2018) and possible therapeutic targets that could alleviate ASD-related symptoms (Kazdoba *et al.*, 2016; Burket *et al.*, 2011).

At a circuit level, mice have been used to investigate morphological changes such as increased brain size (Clipperton-Allen and Page, 2014) and abnormalities in dendritic branching (Aceti *et al.*, 2015) that occur upon mutation to various ASD-risk genes. Electrophysiology and brain imaging techniques have also allowed neural activity to be measured in brain tissue samples and brain activity to be measured in euthanised animals. This has helped to identify hyperexcitability of circuits during development and also increased activity of circuits that may underpin hypersensitivity of the animal to sensory stimuli (Gonçalves *et al.*, 2013; Rotschafer and Razak, 2013; Gogolla *et al.*, 2014). Understanding how mutations to risk genes may disrupt the activity of neural circuits has provided insights into the circuit level dysfunction in the aetiology of autism (Golden, Buxbaum and Rubeis, 2018). Methods utilising the implantation of electrodes into mice brains has been developed to monitor brain activity in freely moving animals and has identified circuits that are hyperactive during impaired social behaviours (Wang *et al.*, 2016b). This facilitates the dissection of circuits that may be involved in the production of atypical behavioural output.

A limitation in the use of mouse models is the fact that there are a number of extraneous variables that are known to affect behavioural phenotypes. A handful of mice strains are commonly used for testing in the laboratory and their genetic background appears to influence behavioural differences between these strains (Sultana, Ogundele and Lee, 2019). In fact, the same genetic mutation made in different strains of mice can alter the behavioural readout suggesting that different genetic backgrounds can confound the assessment of behaviour (Paradee *et al.*, 1999; Fertan *et al.*, 2021). It has also been shown that differences in the laboratory environment, apparatus used and differences in test protocols can also impact the behaviour of mice (Crabbe, Wahlsten and Dudek, 1999). Whilst effort has been made to standardise behavioural analysis in mice, different strains used in different studies can make it difficult to properly interpret results across the field of literature.

1.4.2 Rats as a model of autism

Rats are another rodent model organism used in the study of autism. Similar to mice, rats are used to investigate human disease and disorders because of the anatomical, biochemical and physiological similarities between them and the human system (Crawley, 2012). Historically, mice have dominated as the model organism of choice for biomedical research because of the well-established genetic toolbox that allows for the manipulation of mice genes (Ellenbroek and Youn, 2016). However, advances in genome engineering in rats means they are now being utilised in ASD research (Hamilton *et al.*, 2014). Rats have a similar social behaviour repertoire to mice; however rats are less stressed by human handling and they are more likely to engage in social interaction. Given that social interaction deficits are a core symptom of ASD it has been argued rats are a better model of this disorder (Ellenbroek and Youn, 2016). Unlike mice, rats display social play behaviours that includes play fighting and chasing. This behaviour is being used to try and understand the neurobiology that underpins the production of different types of social behaviours (Vanderschuren, Niesink and Van Ree, 1997; Vanderschuren, Achterberg and Trezza, 2016). Also, rat models have contributed to the understanding of how prenatal drug exposure may impact the risk of autism and its influence on social behaviours (Schneider and Przewłocki, 2005). However, similar to the disadvantages of mice models, it has been shown that extraneous variables such as the genetic background of the rat being investigated can influence behavioural outcome (Ku *et al.*, 2016).

1.4.3 Voles as a model of autism

The prairie vole is another rodent model organism that can be easily reared in the lab and used to investigate social behaviours. An advantage of using the vole over other models like mice and rats

is the fact that they form long lasting monogamous social attachments between mates (McGraw and Young, 2010). This is therefore beneficial when trying to recapitulate complex social cognition in humans. Social attachment between voles can be quantified using partner preference assays in which partnered voles spend more time interacting with each other than a novel stranger vole (Williams, Catania and Carter, 1992). Interestingly, different species of vole do not show this social bonding behaviour meaning intraspecies comparisons can be made to try and understand the neurobiology and genetics that underpin social attachment (Young and Wang, 2004). In this way the vole has facilitated the understanding of the brain regions and circuitries involved in social cognition (McGraw and Young, 2010). In particular, the vole has been used to identify the importance of the neuropeptides, vasopressin and oxytocin, and the neurotransmitter, dopamine, in regulating social behaviour (Lee and Beery, 2019). Understanding how and where these signalling molecules act in the vole brain has advanced the understanding of how they could be used in therapeutic interventions (Modi and Young, 2012; Hammock and Young, 2006; Williams *et al.*, 1994). Currently, the genomic tools available to investigate the vole are less advanced than those used in mice and rats (Lee and Beery, 2019). However, the vole has huge potential as a rodent model for understanding the neurobiological and genetic mechanisms underpinning social behaviour.

1.4.4 *Drosophila* as a model of autism

Drosophila melanogaster are a type of fruit fly that are commonly used as an invertebrate model organism. Although less anatomically conserved to humans than rodent models, *Drosophila* offer key similarities in neurobiological mechanisms such as nervous system formation and function (Tian, Zhang and Han, 2017). Similar to the benefits provided by rodent model organisms, *Drosophila* encode orthologues of 75% of human disease genes (Reiter *et al.*, 2001) and an array of genome editing tools available in the fly means complex genetic disorder like ASD can be investigated (Coll-Tané *et al.*, 2019). An advantage of *Drosophila* is that their nervous system contains only 100,000 neurons, far fewer than that of rodents and humans (Scheffer *et al.*, 2020). Although simplified, their nervous system uses similar classes of neurotransmitters compared to the human system (Coll-Tané *et al.*, 2019). This has been key in understanding structural and functional deficits in the nervous system of the fly following mutation to the gene that is responsible for Fragile X Syndrome (Drozd, Bardoni and Capovilla, 2018; Tessier and Broadie, 2012). In fact, one of the main contributions that *Drosophila* have made in ASD research is on the understanding of the function of the genetic and neural underpinnings of Fragile X (1.2) (Tessier and Broadie, 2012). *Drosophila* have a short life cycle, produce many progeny and are cheap to culture. This makes them amenable to high throughput drug screens (Chang *et al.*, 2008) which

has helped to elucidate pharmacological targets for treating Fragile X which have gone to clinical trial (Berry-Kravis *et al.*, 2009).

Drosophila display a range of behaviours such as circadian rhythms and learning and memory which have been widely used to investigate human disorders (Pandey and Nichols, 2011). In comparison, there is less literature using behavioural analysis to study neuropsychiatric disorders like ASD in the fly, likely because social assays only emerged around 10 years ago (Bolduc *et al.*, 2010; Simon *et al.*, 2012). *Drosophila* do show social behaviours like courtship, aggregating in a group and collective feeding (Rooke *et al.*, 2020). When in a group flies regulate their social space between themselves and other flies. This behaviour has been shown to be an adaptive and context dependent behaviour that when assayed can provide insight into social interaction and avoidance in flies (Simon *et al.*, 2012). The social space assay has been used in a number of studies, which mainly focus on a single ASD-associated CAM (neuroligin), to investigate the genetic underpinnings of fly social behaviour (Chen *et al.*, 2019; Yost *et al.*, 2020; Hahn *et al.*, 2013; Corthals *et al.*, 2017). Mutants where the CAM has been knocked down or knocked out show alterations to fly social interaction in the form of increased social space and suggests that this gene has a prominent role in regulating fly social behaviour (Yost *et al.*, 2020).

1.5 *Caenorhabditis elegans*

1.5.1 Introduction to *Caenorhabditis elegans*

Caenorhabditis elegans (*C. elegans*) are a species of nematode that belong to the Nematoda phylum (Blaxter *et al.*, 1998). A *C. elegans* strain called 'Bristol N2' were originally isolated from soil and were defined as the wild type reference strain (Brenner, 1974). In the lab, *C. elegans* are cultured on agar plates and are provided with a bacterial food source. Adult *C. elegans* are approximately 1mm in length and are therefore observed under a microscope where they can be clearly seen to have a head and tail region and a sinusoidal body posture (Figure 1.5). *C. elegans* are transparent and at high magnification their main anatomical features can be observed (Figure 1.6).

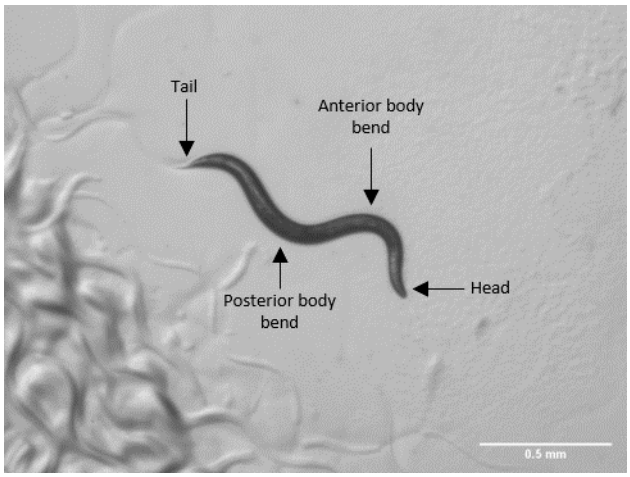


Figure 1.5 Representative image of *C. elegans* under a light microscope. Image of a one-day old adult N2 *C. elegans*. The head and tail of the worm are indicated as well as the anterior and posterior body bends giving the worm a sinusoidal body posture. Image taken at 30X magnification.

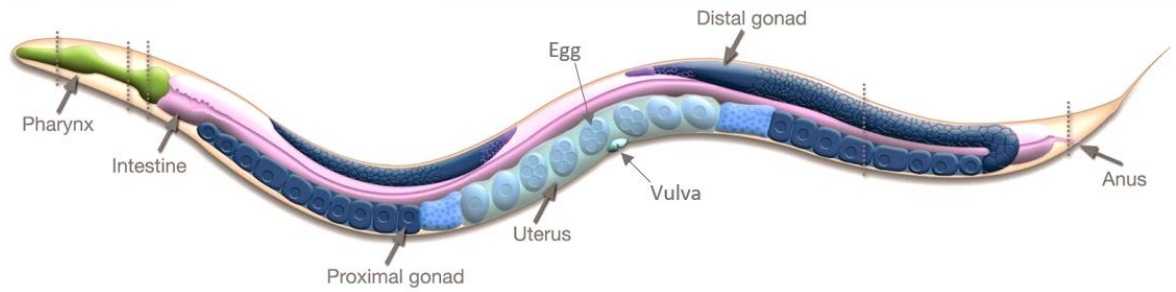


Figure 1.6 A diagram of the gross anatomical features of *C. elegans*. A cartoon image highlighting the major anatomical features of *C. elegans.* Cartoon modified from WormAtlas, (Altun and Hall, 2009).

1.5.2 *C. elegans* lifecycle and development

Wild type *C. elegans* have a short lifecycle lasting approximately three days under optimal conditions. *C. elegans* exist as either self-fertilising hermaphrodites or males, which spontaneously occur infrequently within a population. After self-fertilisation or fertilisation by a male, embryonic development occurs first in utero before an egg is laid. Approximately 9 hours following the egg being laid a worm hatches in the L1 larval stage (Figure 1.7). Post embryonic development is triggered by feeding and in the presence of food worms develop through four larval stages (L1-L4) before reaching adulthood (Figure 1.7). At the end of each larval stage the animal moults in which it sheds its old cuticle and synthesises a new one. Before each moult worms show developmentally timed quiescent behaviour (Raizen *et al.*, 2008). At each new larval stage the worm increases in size and therefore size is a good indicator of worm development. A

worm in the L4 larval stage has a white crescent moon shape that appears half way along the body which is generated by the development of the vulva. This marking can be easily seen down the microscope and is therefore used to identify worms in this larval stage. Following this, worms develop into gravid adults that are capable of laying eggs and hence the lifecycle begins once again (Figure 1.7).

Temperature can alter the *C. elegans* lifecycle. At 20˚C the lifecycle takes ~65 hours. The time taken for a complete lifecycle increases at 16˚C to ~90 hours and decreases at 25˚C to ~47 hours. At 20˚C adult *C. elegans* typically lay about 9 eggs per hour and lay a total of ~280 eggs in their adult life. As well as temperature, other environmental conditions, such as crowding and starvation can alter the progression of the lifecycle. In the absence of food, L1 larva can arrest their development and survive for 6-10 days with no food (Figure 1.7). Once development has begun if environmental conditions are unfavourable worms in the late L1 phase can arrest and produce a dauer larva. Dauer larva are non-feeding and non-aging worms that can survive for several months in unfavourable conditions (Figure 1.7) (Altun and Hall, 2009).

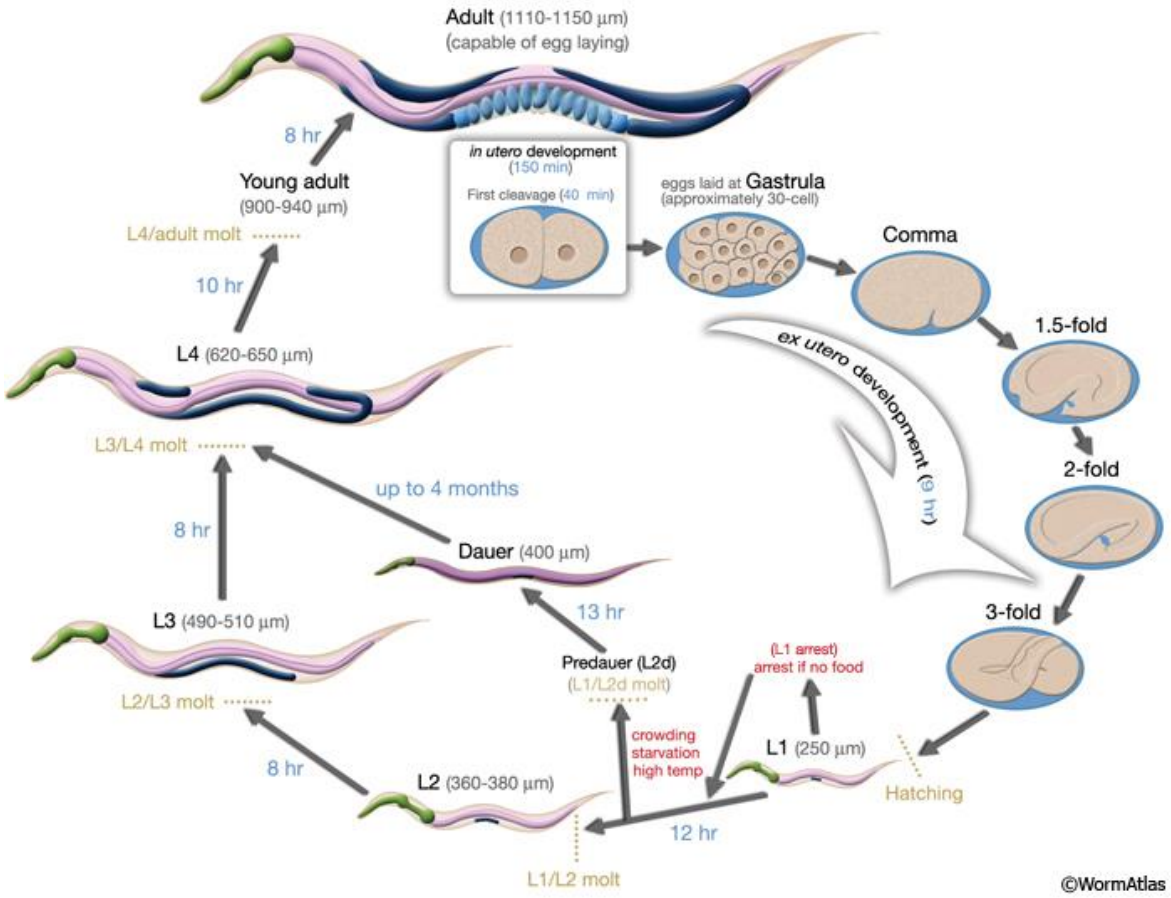


Figure 1.7 Schematic of wild type *C. elegans* lifecycle. Cartoon outlining the lifecycle of N2 *C. elegans* at 22˚C. Time 0 is at fertilisation; developmental timings are shown in blue. The stage in the lifecycle at which the four moults occur is indicated in yellow. The

length of the worms at each stage is indicated in micrometres. Cartoon taken from WormAtlas, (Altun and Hall, 2009).

1.5.3 *C. elegans* nervous system

The hermaphroditic *C. elegans* nervous system is comprised of 302 neurons whereas the male nervous system contains 385 (Lints and Hall, 2009). Early in post embryonic development hermaphrodite/male differences in neural precursor proliferation and cell fate specification results in sex specific differences in the nervous system. Hermaphrodites retain cells that become neurons required for egg laying, whereas these are lost in males. Males develop 87 sex specific neurons, most of which function in the tail to regulate mating behaviours (Fagan and Portman, 2014).

In the hermaphrodite nervous system, neuronal cell bodies cluster in the head and tail region with ventral and dorsal nerve cords sending nerve processes longitudinally along the worm body (Schafer, 2016). *C. elegans* neurons can be grouped into four categories. Motor neurons synapse onto muscle cells and regulate worm movement. Sensory neurons detect sensory stimuli in the form of mechanosensation, chemosensation, thermosensation and nociception. Interneurons receive information from incoming synapses and form outgoing synapses with other neurons. Finally, polymodal neurons are those that can perform more than one of these functions. Within the nervous system, worms contain ~7,000 chemical synapses and ~1,500 neuromuscular junctions (Altun and Hall, 2009). Synaptic transmission is highly conserved between *C. elegans* and humans. *C. elegans* utilise similar classes of neurotransmitters such as dopamine, acetylcholine, GABA and glutamate and vesicles of neurotransmitter dock at active zones in pre-synaptic terminals (Schafer, 2016; Altun and Hall, 2009). Also the worm uses similar classes of neurotransmitter receptors and ion channels which can cluster post-synaptically with the aid of PDZ containing scaffold proteins (Hobert, 2013). *C. elegans* were the first animal to have its nervous system completely mapped out (White *et al.*, 1986; Cook *et al.*, 2019). Overall, the key similarities in synapses structure and function, in comparison to humans, and the connectome data provides a high-resolution view of a relatively simple nervous system which is fundamental in trying to understand nervous system function and dysfunction.

The relatively simple and well characterised nervous system of *C. elegans* has allowed for the identification of individual neurons, and circuits of multiple neurons, that are important for the regulation of different behavioural states that are further underpinned by sub-behaviours (Schafer, 2005). Adaptive behaviours that are key for survival in *C. elegans,* including looking for food in the environment, are under the control of the nervous system and require constant

modulation depending on environmental cues (Bargmann and Mori, 1997). Cell ablation and genetic studies are well established and used to dissect the neural circuitries involved in complex behaviours (Oranth *et al.*, 2018; Luo *et al.*, 2014; Gray, Hill and Bargmann, 2005; Milward *et al.*, 2011). Such behaviours will often require worms to integrate multiple sensory cues and it has been shown that worms are able to do this via the use of integrative neurons (Metaxakis, Petratou and Tavernarakis, 2018; Iino and Yoshida, 2009; Kocabas *et al.*, 2012; Hilliard, Bargmann and Bazzicalupo, 2002). A single interneuron has been shown to be capable of modulating a worms locomotory response to a noxious stimulus depending on the context of its environment. In the absence of food, worms reverse away from and avoid a noxious chemical stimulant. However, in the presence of food, the worms' response to the same noxious stimulant is to immediately reverse and then carry on forward to continue feeding. It appears that the two sensory stimuli converge on the interneuron, providing evidence for the role of this interneuron in the sensory-motor control of behaviour (Summers *et al.*, 2015). Multi-sensory processing is impaired in ASD (1.1.3), so the ability of *C. elegans* to integrate multiple sensory cues across their nervous system makes them an attractive model to decode the neural basis of behaviour.

1.5.4 *C. elegans* genome

The *C. elegans* genome has been fully sequenced ('Genome sequence of the nematode *C. elegans*: a platform for investigating biology,' 1998) and consists of five autosomal chromosomes and, in the case of hermaphrodites, a pair of sex chromosomes (Hodgkin, 2005). The genome encodes ~20,000 protein-coding genes and interestingly only a few of those (11%) are nematode specific (Lai *et al.*, 2000). In fact, the *C. elegans* genome shows genome wide orthology with the human genome, with 53% of human protein-coding genes having an orthologue in the worm (Kim *et al.*, 2018). In addition, 50% of human disease related genes have been predicted to have an orthologue in *C. elegans* making them an excellent model for probing the genetic basis of disease (Culetto and Sattelle, 2000). Importantly, *C. elegans* gene orthologues have been shown to have structural (Wheelan *et al.*, 1999) and functional conservation to the human counterparts (Duerr *et al.*, 1999; Haerty *et al.*, 2008). Some of this work has demonstrated that expression of a human gene in a mutant worm can rescue behavioural deficits (Calahorro and Ruiz-Rubio, 2012; Levitan *et al.*, 1996; Solari *et al.*, 2005), providing evidence that human and *C. elegans* genes encode proteins with a similar function.

Analysis of genes in *C. elegans* is aided by a number of genome editing techniques that are well established in the worm (Sugi, 2016). *C. elegans* allow for relatively quick and simple genome editing, in comparison to rodent models, due to the fact that they are hermaphrodites. Self-fertilisation by hermaphrodites quickly generates progeny that are clones of the adult worm. This

allows for easy maintenance of homozygous genetic mutations in the lab without the need for crossing with males (Corsi, 2006). Another benefit of *C. elegans* over rodent models is the use of the Bristol N2 strain as a standardised control across laboratories, negating the issue of genetic background as an extraneous variable, which can be problematic when working with mice and rats (1.4.1, 1.4.2).

1.5.5 *C. elegans* as a model of autism

C. elegans are commonly used as a model organism in the investigation of neuropsychiatric disorders such as SCZ, intellectual disability, epilepsy and ASD (Bessa, Maciel and Rodrigues, 2013). Although many autism risk genes have now been identified, the understanding of how these genes function and how genetic variants impact autism related behaviours is lacking. *C. elegans* have been used as a model to try and understand the genetic and neural basis of ASD because of their simple, well characterised nervous system and their genetic tractability (Calahorro and Ruiz-Rubio, 2011). At first the use of *C. elegans* in ASD research was controversial because the simplicity of the organism, compared to rodent models, can be considered a limitation when trying to model a complex disorder like ASD (Schmeisser and Parker, 2018). However, the simplicity that *C. elegans* offers has provided a powerful platform to dissect the fundamental role of genes, and gene dysfunction, on the nervous system and behaviour which can be complex, time consuming and expensive in rodent models (Schmeisser and Parker, 2018).

1.5.5.1 Using *C. elegans* behaviour to investigate autism

C. elegans possess an array of behaviours, from simple to more complex, adaptive, behaviours that are under the control of the nervous system (1.5.3). Assaying behavioural phenotypes in *C. elegans* in response to mutation of an ASD-associated genetic orthologue has contributed to the understanding of how ASD-candidate genes function to increase the risk of the disorder (Bessa, Maciel and Rodrigues, 2013; Schmeisser and Parker, 2018; Calahorro and Ruiz-Rubio, 2011). Sensory processing deficits are a clinical feature of ASD (1.1.3) and this can be modelled in *C. elegans* because they are able to sense different stimuli in their environment and coordinate their behavioural output accordingly (Bessa, Maciel and Rodrigues, 2013). One of the first sensory assays used in *C. elegans* to model ASD involved the use of an osmotic barrier. When confronted with a high concentration fructose barrier in its environment, wild type worms reverse away from the stimulus. This assay was used to investigate the worm orthologue of the human neuroligin genes, CAMs which have been strongly implicated in autism (Sudhof, 2008; Chen *et al.*, 2014; Jamain *et al.*, 2003). The worm orthologue, *nlg-1*, has a similar structure and function (Hunter *et al.*, 2010; Calahorro and Ruiz-Rubio, 2012) to that in humans and when knocked out in the worm

results in sensory processing deficits such that the worms crawl through the fructose barrier as if they cannot sense it (Calahorro, Alejandre and Ruiz-Rubio, 2009).

Another ASD-risk gene called shank (Chen *et al.*, 2014), a synaptic scaffold protein, has also been studied using *C. elegans* behavioural assays. *shn-1,* shank orthologue, mutants have been utilised to show that the PDZ domain of the protein is crucial for receptor binding and regulation of defecation and feeding behaviours (Jee *et al.*, 2004; Oh *et al.*, 2011). This highlights how *C. elegans* have been used to help functionally characterise ASD-risk gene orthologues and shed light on which protein domains are important for function. In fact, the high throughput nature of *C. elegans* means they have been used to screen genotype-phenotype relationships by looking at behaviours like motility and habituation learning to try and characterise which ASD-risk gene may be involved in these behaviours (Wong *et al.*, 2019; Schmeisser, Fardghassemi and Parker, 2017; McDiarmid *et al.*, 2019). Similar behaviours have also been used in drug screens to prioritise compounds for future research that are able to reverse behavioural phenotypes associated with some risk genes (Schmeisser, Fardghassemi and Parker, 2017). In the last decade the research surrounding *C. elegans* behaviour as a way to investigate ASD-risk genes has been dominated by studies looking at CAMs *nlg-1* and neurexin (*nrx-1*) and *shn-1*. However, recent papers looking at genes encoding proteins involved in cell signalling and protein biosynthesis have used *C. elegans* and their behaviours to model ASD upon mutation to genes other than those involved directly at the synapse (Post *et al.*, 2020; Wong *et al.*, 2021b). These studies are also using a more targeted approach to investigate these genes. As opposed to investigating a gene knock out mutant, these studies have used genome editing to investigate the behavioural effect when a gene is mutated with a missense or nonsense mutation that more closely resembles the genetic variants identified in human autism.

1.5.5.2 Using the *C. elegans* nervous system to investigate autism

The transparency of *C. elegans* along with molecular and genetic techniques for labelling neurons and receptors allows the *C. elegans* nervous system function and dysfunction to be interrogated (Yan and Jin, 2011). Studies of *C. elegans* nervous system function in response to the mutation of CAMS, neuroligin and neurexin and synaptic scaffold gene, shank, have shown that these genes play an important role in the regulation of receptor composition at the synapse (Tu *et al.*, 2015; Tong *et al.*, 2015; Pym *et al.*, 2017). Adhesion of NLG-1 and NRX-1 proteins at the worm neuromuscular junction has been shown to regulate synaptic transmission by inhibiting neurotransmitter release (Hu *et al.*, 2012; Tong *et al.*, 2017). The regulation of excitatory and inhibitory signalling is thought to be impaired in ASD (1.1.4) hence the *C. elegans* nervous system is a good model of nervous system dysfunction that is relevant in ASD.

As well as synaptic signalling, orthologues of ASD-risk genes have also been shown to be important for nervous system development and the development of synaptic connections to shape the worm nervous system (Oliver, Alexander and Francis, 2018; Philbrook *et al.*, 2018; Christensen *et al.*, 2020). Studying the development and architecture of the nervous system in the worm is starting to pinpoint how genetic variants in ASD may be altering circuit formation and how this links to behavioural deficits (Buddell *et al.*, 2019). A recent screen which investigated a number of ASD-candidate genes and their impact on dendritic branching showed that 16% of the genes they investigated were essential for dendritic development (Aguirre-Chen *et al.*, 2020). Overall, investigation of the *C. elegans* nervous system is helping to understand the impact of genetic variation on the development and formation of circuits within the nervous system. Continuation of this work to correlate the effects of gene mutation on mechanisms that alter nervous system formation and function will likely be very informative as to how these mechanisms underpin behaviour change.

1.5.5.3 Defining neuronal circuits and the use of sub-behaviours to investigate autism

An aim when modelling autism in *C. elegans* is to try and dissect the neural circuits that are altered in ASD and how they modify behavioural output. *C. elegans* display a number of complex behaviours that can be broken down into simpler 'sub-behaviours' that act as behavioural units that combine to form a complex behaviour. Sub-behaviours include reversals, changes in direction (turns) and speed of movement (Thill and Pearce, 2007; Liu and Sternberg, 1995). Sub-behaviours have been used in *C. elegans* research to decipher the neural circuits that underpin them and how they interact in networks to control complex behaviours like foraging (López-Cruz *et al.*, 2019; Macosko *et al.*, 2009; Chalasani *et al.*, 2016). When first removed from food worms display a foraging behaviour called area restricted search where they search for food in the local vicinity (Wakabayashi, Kitagawa and Shingai, 2004; Gray, Hill and Bargmann, 2005). Quantification of sub-behaviours underlying this foraging strategy showed that worms perform a high frequency of turns and reversals in order to restrict their search area. Further dissection of such sub-behaviours has helped to understand them at a circuit level, for example the interneuron AIY is now understood to be a key neuron involved in regulating reversal behaviour (Gray, Hill and Bargmann, 2005; Chalasani *et al.*, 2016). In this way sub-behaviours represent a useful tool to simplify the investigation of complex *C. elegans* behaviours and facilitate the investigation of behaviour at a circuit level.

In regard to autism research in the worm, the literature is limited to the understanding of which neurotransmitters may act in circuits affected by ASD-risk genes (Calahorro and Ruiz-Rubio, 2013; Izquierdo, Calahorro and Ruiz-Rubio, 2013). However, circuits controlling simple behaviours in *C.*

elegans such as crawling locomotion (Thapliyal and Babu, 2018; Zhen and Samuel, 2015), egg laying (Zhang, Schafer and Breitling, 2010; Zhang *et al.*, 2008) and pharyngeal pumping (feeding behaviour) (Trojanowski and Raizen, 2015; Bhatla *et al.*, 2015) are being increasingly well defined. In addition, thrashing (swimming behaviour) is beginning to be understood in terms of the neurons that control it (Pierce-Shimomura *et al.*, 2008; Refai and Blakely, 2019). Phenotypic profiling of *C. elegans* is starting to prioritise the types of sub-behaviours that are impaired in ASD-associated mutants (McDiarmid *et al.*, 2019). Therefore, it is likely that investigation of sub-behaviours and further molecular and genetic detailing of circuits that underly them will be key in understanding the neuronal control of behaviour and how dysfunction of this can be used to further understand autism.

1.6 *C. elegans* social behaviour

1.6.1 Worm social behaviour

C. elegans display a number of social behaviours including mating, aggregation, population density sensing and group feeding (Bessa, Maciel and Rodrigues, 2013; Schmeisser and Parker, 2018; Ardiel and Rankin, 2009). One of the most well studied social behaviours in *C. elegans* is the social feeding behaviour that exists in some *C. elegans* strains. The laboratory N2 strain of *C. elegans* is described as a solitary feeder, which disperse on a bacterial food lawn and feed separately. However, other wild isolate strains of *C. elegans* have been shown to aggregate in groups and feed together (de Bono and Bargmann, 1998). The molecular and neural basis of this behaviour has been dissected and has identified a number of receptors as important determinants of the behaviour (de Bono and Bargmann, 1998; Macosko *et al.*, 2009). Another social behaviour in *C. elegans* is the ability of worms to sense the population density in their environment. Unfavourable conditions, such as overcrowding can modulate a worm's behaviour. For example, overcrowding can affect a worms development and lifespan and also prevent adult worms from laying eggs in an environment where food is likely to be scarce. *C. elegans* social behaviour can be modulated by a range of sensory cues, including chemosensory cues which allow worms to communicate with one another (Ludewig *et al.*, 2017; Wong *et al.*, 2020). The major classes of sensory cues that affect social behaviour are described below.

1.6.1.1 Ascarosides as a chemosensory cue

The first studies that provided evidence for a chemical cue that allowed for inter-organismal signalling between *C. elegans* suggested that pheromones released by worms could signal to other worms in the environment (Golden and Riddle, 1982). Making crude purifications of

pheromones and exposing them to young *C. elegans* showed that the chemical cue could modulate the developmental progression of worms and induce dauer formation (1.5.2) (Golden and Riddle, 1982; Golden and Riddle, 1984b; Golden and Riddle, 1984a). It was subsequently shown that dauer pheromone is released by *C. elegans* during their lifecycle meaning that as the population density of worms in the environment increases the concentration of pheromones also increase. Dauer pheromone is therefore a measure of population density and signals the nature of the environment to young worms (Butcher *et al.*, 2008). High population density and overcrowding of a food source is likely to mean that food is limited. Therefore, induction of young worms to form dauer larva increases their chance of survival in harsh environments (Wolkow and Hall, 2015).

Mass spectrometry and nuclear magnetic resonance spectrometry studies which analysed the dauer pheromone identified that it contained small molecules called ascarosides (Jeong *et al.*, 2005; Butcher *et al.*, 2007). Ascarosides are based on a sugar molecule linked to a fatty acid that can be decorated with amino acids and other metabolites derived from various metabolic pathways (Ludewig and Schroeder, 2013). *daf-22* is a *C. elegans* gene that is crucial for the biosynthesis of ascarosides (Butcher *et al.*, 2009). *daf-22* loss-of-function mutants are defective in pheromone production and were first identified because they are unable to induce dauer formation in nearby worms (Golden and Riddle, 1985). The inability of *daf-22* mutants to produce ascarosides influences the population density sensing behaviour of other worms (Matsuura, Sato and Shingai, 2005; Scott *et al.*, 2017). A chemotaxis assay, which measured the attraction of *C. elegans* to a chemical stimulant, showed that wild type worms will move towards the attractive location unless it is overcrowded with other wild type worms. Interestingly, overcrowding of the attractive location with *daf-22* mutants meant that wild type worms moved towards the chemical stimulant instead of avoiding it, as if they were unable to sense the *daf-22* mutant worms (Matsuura, Sato and Shingai, 2005). This experiment provided evidence that ascaroside communication is important for sensing other worms in the environment and modulating behaviour in response to chemosensory cues.

To date, more than 200 ascarosides have been identified and have been shown to regulate more than just dauer formation (Muirhead and Srinivasan, 2020). Ascaroside signalling can mediate both attractive and repulsive behaviours (Srinivasan *et al.*, 2012) and therefore can regulate social behaviours including mating, aggregation and feeding strategies (Muirhead and Srinivasan, 2020). The regulation of a diverse range of behaviours is facilitated by the complexity of ascaroside signalling in *C. elegans*. Ascarosides can act synergistically and different cocktails of ascaroside molecules are released depending on the developmental stage and physiological condition of the worm (Artyukhin *et al.*, 2013; Kaplan *et al.*, 2011). In addition, small changes in the chemical

structure or concentration of ascarosides can have dramatic effects on the behavioural response they elicit (Srinivasan *et al.*, 2012).

Ascarosides can be used by worms to communicate the environmental conditions and affect the foraging strategies of nearby worms (Greene *et al.*, 2016a). For example, adverse conditions such as lack of food can be signalled via the release of ascarosides from starved worms, which consequently repels other worms from foraging in that area (Artyukhin *et al.*, 2013). Exposure of worms to ascarosides has also been linked to changes in locomotory behaviour, including speed, the number of reversals a worm makes and how they explore the environment (Greene *et al.*, 2016a; Srinivasan *et al.*, 2012; Muirhead and Srinivasan, 2020). The modulation of these behaviours provides a clear link between chemical signalling and the locomotory behavioural output of a worm.

Studies have shown that the recognition of ascarosides is mediated by G-protein coupled receptors (GPCRs) (Kim *et al.*, 2009; Park *et al.*, 2012). Genetic mutant studies, cell ablation and calcium imaging in *C. elegans* has identified a number of GPCRs, chemosensory neurons and interneurons that are important for ascaroside recognition (Srinivasan *et al.*, 2012; Greene *et al.*, 2016a; Park *et al.*, 2012; Chute *et al.*, 2019). However, neuronal signalling downstream of interneurons to regulate behavioural change upon recognition of ascarosides is not as well understood. Overall, the use of chemosensory signalling molecules mediates a complex chemical language in *C. elegans* that can regulate the development and locomotory behaviour of nearby worms.

1.6.1.2 Food as a chemosensory and mechanosensory cue

Bacterial food is a gustatory chemosensory cue for *C. elegans* (Bargmann, 2006). Initial chemotaxis studies identified that worms can sense a lawn of bacteria and will move towards it (Grewal and Wright, 1992) and that this is mediated by olfactory sensory neurons (Bargmann, Hartwieg and Horvitz, 1993). It has also been shown that worms will avoid pathogenic bacteria (Zhang, Lu and Bargmann, 2005) and can distinguish the nutritional value of food (Shtonda and Avery, 2006). In fact, it has been shown that worms can show preference towards food that better supports their growth (Shtonda and Avery, 2006). Various studies of the neural circuits and signalling molecules have shown that serotonin signalling is important for olfactory learning (Nuttley, Atkinson-Leadbeater and van der Kooy, 2002) whilst glutamate neurotransmission and neuropeptides are important for recognition and food preference respectively (Harris *et al.*, 2014; Chalasani *et al.*, 2016).

Food also provides a mechanosensory cue and *C. elegans* will change their behaviour depending on whether they are on or off food (Chao *et al.*, 2004; McCloskey *et al.*, 2017). When worms encounter a bacterial food lawn they perform a behaviour called enhanced slowing response in which they slow their locomotion (Sawin, Ranganathan and Horvitz, 2000) and increase their pharyngeal pumping rate in order to feed (Dallière *et al.*, 2016). Mechanosensory neurons that project cilia out through the worms cuticle signal the presence of food by releasing dopamine (Kindt *et al.*, 2007; Ezcurra *et al.*, 2011). Dopamine release has been shown to signal to interneurons which then regulates locomotory control neurons (Bhattacharya *et al.*, 2014) and inhibits signalling in neural circuits that control 'off food' behaviours (Suo, Culotti and Van Tol, 2009). Both neural circuits involved in on food and off food behaviours have been well characterised in terms of the neurons and signalling molecules used (Bhattacharya *et al.*, 2014; Gray, Hill and Bargmann, 2005; Calhoun *et al.*, 2015).

Both chemo-sensation and mechano-sensation of food are key for modulating *C. elegans* foraging strategies. Worms display a number of stereotyped behaviours when foraging for food. In the presence of food worms predominantly display dwelling behaviour in which they move slowly and reverse frequently in order to keep them in the vicinity of the food (Wakabayashi, Kitagawa and Shingai, 2004; Gray, Hill and Bargmann, 2005). Upon removal from food worms display local search behaviour in which they make a high number of turns which is assumed to represent an attempt to stay close to the food source (Gray, Hill and Bargmann, 2005). Then, in the absence of food they begin to disperse in which they move quickly and execute long forward runs to look for a new food source (Fujiwara, Sengupta and McIntire, 2002). It's clear that the regulation of these foraging behaviours is dependent on the context of food and how long the worms have been off food (Harris *et al.*, 2011). In fact, worms impaired in chemo-sensation of food display defective foraging behaviour (Cohen *et al.*, 2009). More recently, food dependent behaviours are being investigated to see how the integration of both external food cues and internal gustatory cues affect behaviour (Ben Arous, Laffont and Chatenay, 2009; Lee and Mylonakis, 2017). Internal signals from the gut that signal food intake have been shown to contribute to the slowing of worm locomotion whilst they feed (Rhoades *et al.*, 2019).

1.6.2 Food leaving behaviour

Optimal foraging requires worms to make a decision as to whether to exploit their current food source or leave in search of another (Ackley, 2019). A paradigm designed to investigate optimal foraging involves quantifying the food leaving rate at which *C. elegans* leave a small patch of bacterial food (Bendesky *et al.*, 2011). Analysis of this behaviour has identified a number of genes that appear to be important in controlling the decision to leave a food lawn (Bendesky *et al.*,

2011; Zhu *et al.*, 2015; Wolf, Perez and Harris, 2020). N2 *C. elegans* rarely leave a dense food lawn (Bendesky *et al.*, 2011; Shtonda and Avery, 2006) but environmental cues that provide the worm with information about the quality of food (Shtonda and Avery, 2006), food depletion (Milward *et al.*, 2011) and population density (Scott *et al.*, 2017) are known to regulate food leaving and dispersal behaviours. Food leaving is a complex behaviour (Ackley, 2019) and the decision to leave food can be influenced by multisensory processing of multiple sensory cues. In an experiment where a chemical repellent was dropped near to worms feeding on a bacterial food lawn, worms left the food lawn in search of a new food source. Interestingly, the extent to which the worms left the food lawn was dependent on the concentration of the repellent. Worms exposed to the lowest concentration of repellent moved to the edge of the food lawn furthest away from the repellent before continuing to eat, presumably because the attractive food cue outweighed the repellent cue. Increasing concentrations of repellent resulted in increasing proportions of worms leaving the food lawn. This experiment provides insight into the type of multisensory integration that can occur to modify food leaving behaviour (Harris *et al.*, 2019).

1.6.2.1 Progeny induced food leaving behaviour

C. elegans are capable of sensing the population density in the environment (1.6.1.1) and this has been shown to be another factor involved in the regulation of food leaving behaviour (Scott *et al.*, 2017). A study that measured the food leaving behaviour of seven adult worms over 24 hours showed that over time the food leaving rate of the adult worms significantly increased. Over the time course of the assay the adult worms laid eggs which hatched into young *C. elegans* progeny meaning that after 24 hours the food lawn contained a mixed population of eggs, L1, L2 progeny and adult worms. The authors use this to suggest that the increasing numbers of progeny populating the food lawn may be modulating adult food leaving behaviour. To test this, they conditioned a food lawn with young *C. elegans* progeny and quantified adult *C. elegans* food leaving behaviour in response to the food lawn populated with progeny in comparison to a naive (no progeny) control food lawn. Conditioning food lawns with 20-70 progeny caused a dose-dependent increase in adult food leaving events that plateaued after 140 progeny. Additional experiments concluded that conditioning a food lawn with eggs or older L4 stage progeny did not induce the enhanced food leaving response, suggesting that this response is likely due to chemosensory cues released by early stage progeny.

Conditioning food lawns with *daf-22* progeny, which do not produce pheromone signals, also did not induce enhanced food leaving response in adults. This further supported the hypothesis that food leaving behaviour can be modulated by progeny released pheromone cues. Interestingly, adult mutants deficient in a peptide called nematocin and its receptors did not enhance their food

leaving behaviour in response to wild type progeny. Nematocin is the *C. elegans* orthologue of the human hormone oxytocin, which has been shown to be important in human social behaviours (Garrison *et al.*, 2012). The impaired behavioural response of adults deficient in nematocin signalling suggests that it is important for the recognition and/or response to ascaroside based signals released by progeny. In this way the study provided evidence for an offspring-parent interaction between *C. elegans* that modulates food leaving behaviour. Also, the oxytocin dependence of this interaction provides a novel route by which to investigate *C. elegans* social behaviour.

1.7 CRISPR/Cas9

1.7.1 The origin of CRISPR/Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR) were first identified as part of the adaptive immune response in prokaryotic cells. CRISPR/Cas works to prevent the conjugation of foreign DNA into a host by cleaving phage DNA using the endonuclease, Cas (Marraffini and Sontheimer, 2008; Garneau *et al.*, 2010). Cleavage of foreign DNA by Cas creates a novel spacer, a short piece of DNA which is incorporated into the cell's CRISPR array. Spacers can be transcribed and cleaved into short crRNA sequences, often called guide RNA (gRNA) that target the Cas enzyme to the complementary phage DNA upon its re-entry (Brouns *et al.*, 2008). Following this, Cas cleaves the phage DNA in order to inactive it, hence protecting the cell from invading pathogens (Gasiunas *et al.*, 2012; Jinek *et al.*, 2012) (Figure 1.8).

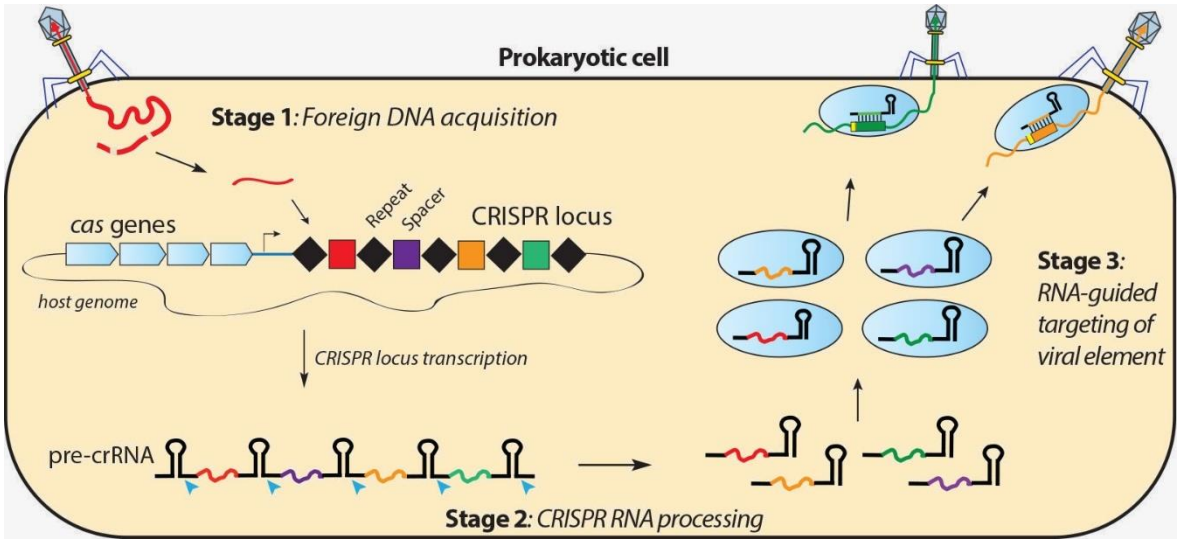


Figure 1.8 Cartoon outlining the role of CRISPR/Cas in bacterial immunity.Stage 1, entry of double stranded viral DNA into a prokaryotic cell results in cleavage of that DNA by the enzyme Cas. This creates a novel spacer piece of DNA which is incorporated as an

interspacing sequence in the CRISPR array. Stage 2, upon re-entry of viral DNA the CRISPR array is transcribed and cleaved to generate gRNA. Stage 3, the gRNA and viral DNA have complementary sequences which enables the gRNA to guide Cas to the viral DNA and bind to it before Cas cleaves the DNA, inactivating it. Cartoon taken from https://doudnalab.org/research_areas/crispr-systems/

1.7.2 CRISPR/Cas9 as a genome editing technique

CRISPR/Cas9 emerged as a genome editing technique in 2012 (Gupta and Musunuru, 2014; Jinek *et al.*, 2012). Before then, two technologies used for gene editing included the use of zinc finger nucleases and transcription activator-like effector nucleases. Both work by designing a protein to bind to DNA, close to the target site, which then allows an endonuclease to cleave DNA and mutate it. However, both technologies require large (1-3kb) cDNA sequences to encode the proteins making it difficult for non-experts to engineer. Also, target regions of DNA that these proteins could bind were limited meaning specific mutations, like knock ins, were difficult to make (Gaj, Gersbach and Barbas, 2013; Gupta and Musunuru, 2014).

CRISPR/Cas9 is now a popular gene editing tool due to its relative ease of use, compared to previous methods, and the precision editing that it allows for (Chira *et al.*, 2017). To edit a sequence of interest a single gRNA (sgRNA) of approximately 20nt is designed with a complementary sequence to the target DNA of interest (Kiani *et al.*, 2015) (Figure 1.9). The sgRNA is then bound to Cas9 and will guide Cas9 to the target sequence (Cong *et al.*, 2013). The DNA sequence of interest must be followed by a 2-6bp protospacer adjacent motif (PAM) for the sgRNA/Cas9 complex to bind. Once bound, Cas9 cleaves the DNA which initiates one of two repair pathways that function to repair the DNA break (Jiang and Doudna, 2017). The non-homologous end joining (NHEJ) pathway creates insertions and/or deletions in the DNA sequence in order to re-join the broken ends (Figure 1.9). This repair pathway often results in frameshift mutations and therefore is often used to knock out genes. In contrast, the homology directed repair (HDR) pathway involves the use of a homologous strand of DNA (also called donor DNA or repair template) which is inserted between the break. By designing donor DNA that contains a specific mutation of interest the HDR pathway allows for precise and customisable gene editing (Sansbury, Hewes and Kmiec, 2019) (Figure 1.9). Overall, the relative simplicity and precision that CRISPR/Cas9 offers has revolutionised the ability to study the effect of gene mutation down to the level of single point mutations.

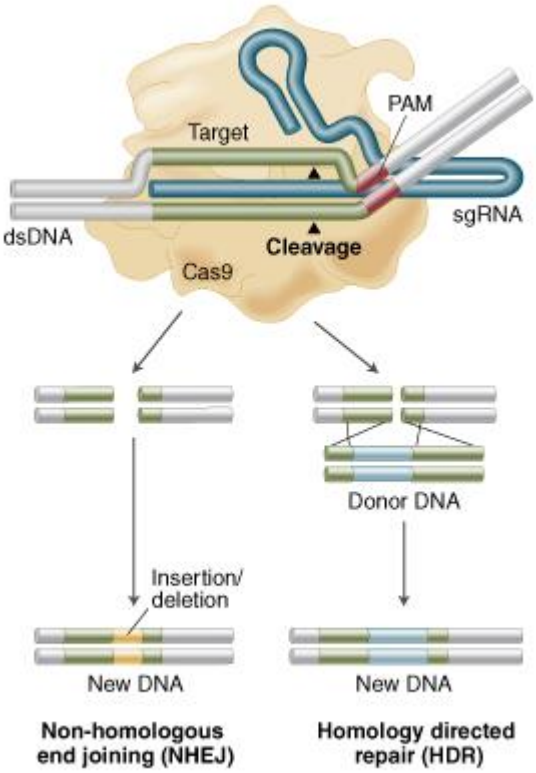


Figure 1.9 Cartoon outlining the two methods of DNA repair that result from cleavage by the CRISPR/Cas9 system. Cas9 is guided to the target DNA sequence (green), adjacent to a PAM sequence (red), by a sgRNA. Cleavage by Cas9 results in one of two DNA repair pathways. NHEJ creates insertions and/or deletions (yellow) in the gene in order to repair the DNA break. HDR uses a homologous donor piece of DNA which can be designed to carry a mutation of interest (blue) which is then inserted between the DNA break. Cartoon taken from https://international.neb.com/applications/genome-editing.

1.7.3 Problems associated with CRISPR/Cas9

Although CRISPR/Cas9 represents a revolutionary genome editing tool the technique has encountered some problems (Peng, Lin and Li, 2016). Cas9 first scans the DNA looking for a PAM sequence before the sgRNA can bind and Cas9 can cleave the DNA (Sternberg *et al.*, 2014). Therefore, CRISPR is limited to DNA in close proximity to a PAM site which can leave some of the genome inaccessible to editing (Tang, 2020). However, in response to this, new Cas enzymes have been developed which recognise different PAM sites, increasing the scope of CRISPR in humans and model organisms (Kleinstiver *et al.*, 2015; Bell, Fu and Fire, 2016). Another problem involves the variable efficiency of sgRNAs, whilst some act with high efficiency others can be completely inactive. Therefore, the design of sgRNAs needs to be well considered including the length and sequence (Wang *et al.*, 2014; Xu *et al.*, 2015; Moreno-Mateos *et al.*, 2015). Computational tools

now exist to aid the design of sgRNAs. Nevertheless, the design can be time consuming and often multiple sgRNAs need to be designed and screened to predict efficiency before they can be used (Peng, Lin and Li, 2016).

Another issue that can drastically decrease the precision of CRISPR editing is off-target effects. The sgRNA/Cas9 complex can recognise sequences similar to the target that have up to five mismatches, making it possible for Cas9 to cleave a DNA sequence which is not the intended target (Fu *et al.*, 2013). Again, sgRNA design has been shown to be important in minimising off-target effects and computer software's can be used to predict off-target sites (Lin *et al.*, 2014; Peng, Lin and Li, 2016). This can then inform the selection of the best sgRNA and highlight sequences that could be sequenced following CRISPR editing to check for imprecise mutations. Recently, it has been discovered that on-target deleterious CRISPR mutations are also possible (Weisheit *et al.*, 2020). This is when the region of the genome that was intended to be mutated becomes mutated during the CRISPR process but the mutation that occurs is not the one that was desired. On-target mutations are proving to be an issue because in certain situations where the on-target mutation is heterozygous the mutation can escape recognition by generic PCR and Sanger sequencing, although can be detected by more sensitive methods. It has been predicted that up to 40% of human pluripotent stem cell lines may have on-target mutations (Weisheit *et al.*, 2020), highlighting the importance of carefully validating CRISPR mutations following editing.

1.7.4 CRISPR/Cas9 in *C. elegans*

Before CRISPR/Cas9, methods for introducing mutations into *C. elegans*, for example the use of mutagens, were relatively imprecise and inefficient (Sugi, 2016). This, and the fact that CRISPR had been shown to be effective in mammalian cells and other model organisms (Ma and Liu, 2015) led to the experimentation of CRISPR in *C. elegans*. The first study of CRISPR in worms showed that microinjection of expression vectors encoding a sgRNA and Cas9 into the gonad of a worm led to the successful expression of these components (Friedland *et al.*, 2013). CRISPR mediated mutations, that occurred in the germline cells of the injected worm, could then be screened for in the progeny (Vicencio and Cerón, 2021). Co-injection of a fluorescent marker meant fluorescent progeny could be screened for CRISPR mutations. This study reported a mutation success rate of up to 80% making CRISPR/Cas9 a desirable genome editing technique in *C. elegans* (Friedland *et al.*, 2013). Whilst this first study provided evidence that it is possible to perform CRISPR in worms, the mutations generated were random and out of the control of the editor. Studies then sought to increase the specificity of CRISPR editing in *C. elegans*. Designing and injecting a donor piece of DNA (also called a repair template) that encodes the mutation of interest along with the sgRNA and Cas9 meant that HDR of the gene would facilitate the

incorporation of a specific mutation (Dickinson *et al.*, 2013; Paix *et al.*, 2014; Zhao *et al.*, 2014) (Figure 1.9). Using this technique a variety of gene edits can be made to a specific target locus in *C. elegans*, including knock-outs, knock-ins, point mutations and fluorescent tagging (Dickinson and Goldstein, 2016).

1.7.4.1 Methods to increase the efficiency of CRISPR/Cas9 in *C. elegans*

Since the first studies of CRIPSR in *C. elegans* various methods to increase the efficiency of gene editing have been developed. Multiple methods to increase the efficiency surround the design of CRISPR reagents. It has been shown that repair templates designed with short homology arms between 30-60bp in length are more efficient than longer sequences for recombination of DNA during repair (Paix *et al.*, 2014). Also, sgRNAs approximately 20-30bp (Ran *et al.*, 2013) in length designed to contain a GG motif at the 3' end increases the efficiency of binding to target DNA and therefore leads to an increased rate of CRISPR success (Farboud and Meyer, 2015).

One of the major issues surrounding the early use of CRISPR in *C. elegans* was the inability to inactivate Cas9 following its first DNA cleavage. When Cas9 recognises a PAM sequence it can bind to and cleave DNA (Sternberg *et al.*, 2014). Therefore, if the PAM sequence remains intact following the desired CRISPR mediated mutation to a gene, Cas9 will continue to bind and cut the target DNA. Continually cutting by Cas9 often leads to the insertion of random errors in the DNA sequence by the NHEJ repair pathway (Zhao *et al.*, 2014). Failure to prevent re-cutting by Cas9 can decrease the efficiency of the desired HDR mechanism to 0% (Kim *et al.*, 2014). A technique designed to negate this issue involves mutating the PAM sequence at the same time as generating the mutation of interest. Mutating just one nucleotide of the 3bp PAM sequence is enough to prevent Cas9 re-cutting and therefore increase the reliability and efficiency of the CRISPR procedure. PAM sequence mutations are extraneous to the mutation of interest and therefore it is recommended to introduce a silent mutation, where the mutation of a DNA nucleotide results in the same amino acid in the resulting protein (Paquet *et al.*, 2016).

Although mutating the PAM sequence is a quick way to prevent continual Cas9 cutting, the introduction of a mutation which is extraneous to the mutation of interest is said to 'scar' the DNA (El Mouridi *et al.*, 2017). Even silent mutations can have unwanted consequences on protein synthesis and function (Bali and Bebok, 2015) meaning interpretation of the effect of the mutation of interest could be confounded. A two-step CRISPR procedure was developed to create 'scarless' DNA without the need to mutate the PAM sequence (Figure 1.10). In the first step, target DNA is cleaved before a d10 sequence is inserted into the gene. A d10 sequence is a short sequence, including PAM, which is derived from an endogenous *C. elegans* gene called *dpy-10*. Recombination of this sequence into the target gene means Cas9 will not recognise the novel

PAM sequence and therefore cannot re-cut the target sequence. Then in the second step the d10 sequence is replaced by a repair template which encodes the wildtype PAM sequence and the specific mutation of interest (El Mouridi *et al.*, 2017) (Figure 1.10). This results in scarless DNA which contains only the mutation of interest. As well as this, the use of the d10 sequence has multiple other advantages. The *dpy-10* gene was chosen as the template for the d10 sequence because the sgRNAs that target Cas9 to this gene have been shown to be one of the most efficient in *C. elegans*. Hence, the insertion of a d10 sequence into the gene of interest renders it more amenable to CRISPR editing (El Mouridi *et al.*, 2017) and has therefore proven a popular editing technique (Wang *et al.*, 2018a; Schreier *et al.*, 2020).

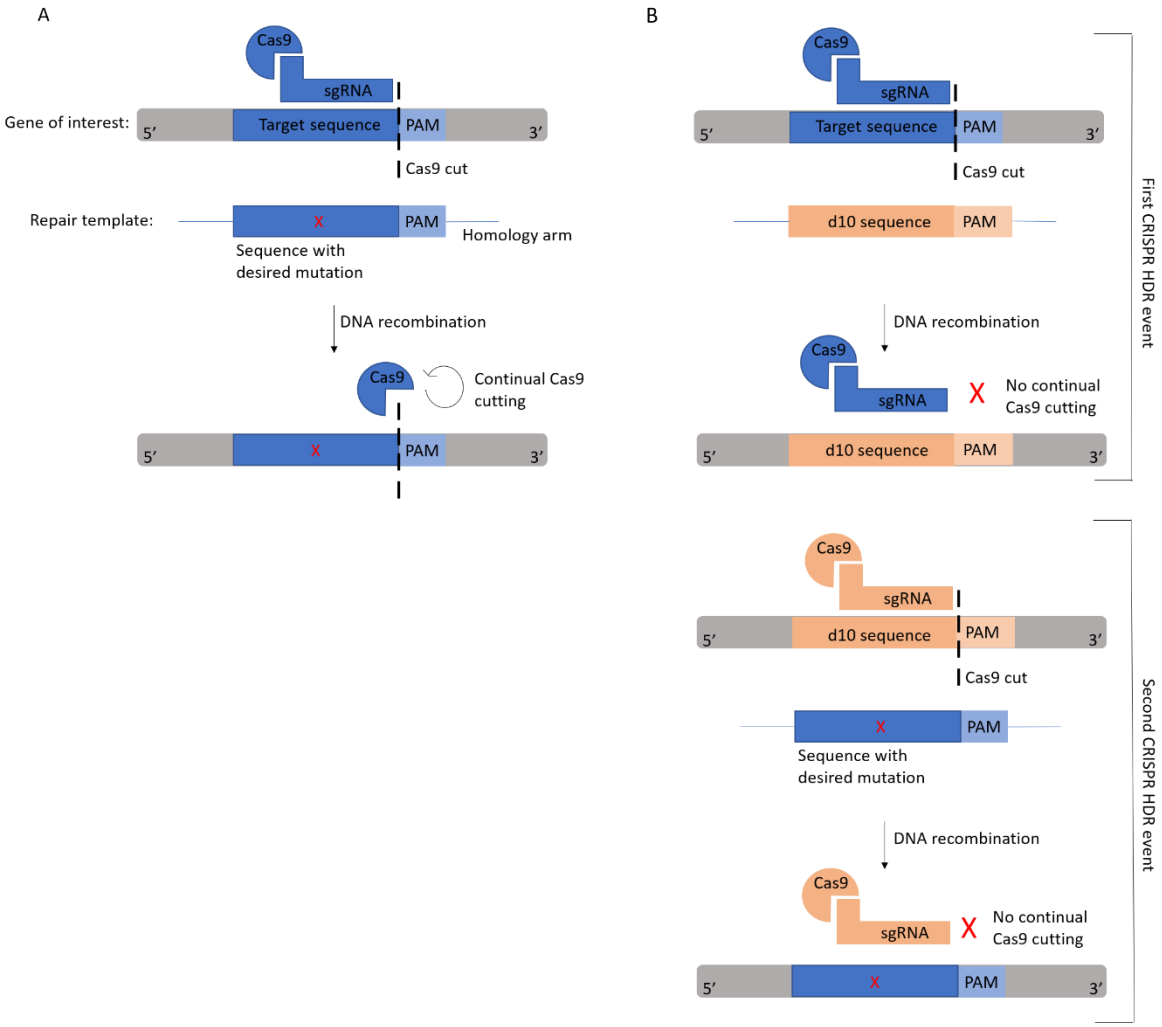


Figure 1.10 Cartoon outlining a two-step CRISPR procedure that creates scarless DNA. (A) The sgRNA/Cas9 complex (dark blue) is designed to recognise and bind to the wild type target sequence (dark blue) followed by a PAM (light blue). The repair template DNA sequence is almost identical to the target sequence and PAM, except for a single nucleotide (red X) which is mutated. HDR facilitated recombination of the repair template into the gene of interest creates a gene that carries the desired mutation. However, Cas9 may continue to recognise the PAM (light blue) and continually cut

the gene of interest reducing CRISPR efficiency. Silent mutations can be introduced into the PAM, however these mutations can have unpredictable consequences on the protein. (B) A two-step procedure designed to create scarless DNA without the need to mutate the PAM. The sgRNA/Cas9 complex (dark blue) recognises and binds to the wild type sequence in the gene of interest (dark blue with light blue PAM). The repair template is designed to encode a 'd10 sequence' which is a short piece of DNA, including PAM, taken from an endogenous *C. elegans* gene called *dpy-10*. HDR facilitates the recombination of the d10 sequence into the gene of interest. The sgRNA/Cas9 complex is unable to recognise the novel d10 sequence therefore Cas9 cannot continually re-cut the target gene. In a second round of CRISPR editing the d10 sequence can be replaced by the mutation of interest. Another sgRNA/Cas9 complex (orange) is designed to recognise the d10 sequence (orange). The repair template is designed to encode the mutation of interest (red X) as well as the wild type PAM. HDR facilitated recombination of the repair template into the gene of interest leaves the gene with a precise mutation and wild type PAM. The sgRNA/Cas9 complex (orange) cannot recognise the novel sequence (dark blue) and hence prevents continual Cas9 recutting without the need to introduce mutations into the wild type PAM sequence. The resulting CRISPR edited gene is scarless, containing only the mutation of interest.

1.7.4.2 Methods for identifying positive CRISPR mutant strains

Fluorescent markers are a common selection marker used when generating transgenic *C. elegans* (Rieckher and Tavernarakis, 2017). Co-injection of a plasmid that drives expression of a fluorescent protein in the body or pharynx of *C. elegans* was the selection method of choice in early CRISPR studies (Dickinson *et al.*, 2013; Prior *et al.*, 2017; Friedland *et al.*, 2013). Screening progeny of the injected parent for fluorescent expression is a good visual indicator that the injection procedure has been successful and that the injection reagents have been incorporated into the worm's genome. Fluorescent progeny are then checked for the CRISPR mutation using PCR. Using this method the percentage of fluorescent progeny that carried the correct CRISPR mutation ranged from >1-25% (Friedland *et al.*, 2013).

For a successful CRISPR event to occur there are a complex series of events that follow the injection of reagents. Firstly, the plasmid DNA needs to be transcribed and translated. Cas9 and the sgRNA then need to form a complex before locating, binding to and cutting the target DNA. Cas9 then needs to cleave the DNA and correct DNA recombination needs to occur (Lino *et al.*, 2018). Therefore, a caveat of using fluorescent markers is that they do not indicate if the CRISPR

reagents are functional or if CRISPR events are occurring successfully in the transgenic worm (Arribere *et al.*, 2014; Kim *et al.*, 2014).

A method was developed that aimed to create a selection maker that was not only an indicator for injection success but also indicates that CRISPR reagents are functional. This method involves generating a second CRISPR mutation in a co-CRISPR gene at the same time as mutating the gene of interest. A co-CRISPR gene, when mutated, results in a worm which displays an easily recognisable phenotype which can be distinguished from wildtype progeny (Arribere *et al.*, 2014; Kim *et al.*, 2014). The criteria developed for co-CRISPR genes were (1) the mutant phenotype must be easily recognisable even if the mutation is heterozygous, (2) the mutant phenotype must be different from an animal with a null mutation so that precise HDR mediated CRISPR events can be discriminated from a null mutation and (3) the mutant phenotype should rarely occur spontaneously to reduce false positives. Using these criteria, a screen of potential co-CRISPR genes identified a handful that could be utilised. This included genes that when mutated result in worms displaying defective locomotion or an obvious change to physical appearance from wildtype (Arribere *et al.*, 2014). A study has shown that 14-84% of progeny displaying a co-CRISPR phenotype also carried the CRISPR mutation of interest (Arribere *et al.*, 2014). Whilst this figure is variable it shows that the use of a Co-CRISPR gene is a more efficient marker for use in CRISPR protocols than fluorescence.

1.7.5 Using CRISPR/Cas9 to investigate ASD-risk genes

CRISPR/Cas9 is being used to investigate genes implicated in ASD using both vertebrate and invertebrate model organisms. Studies have used CRISPR to knock-out ASD-risk genes and study the effect this has on phenotypes such as development, repetitive behaviours and aggression (Wong *et al.*, 2019; de Brouwer *et al.*, 2018; Wei *et al.*, 2020). The precision gene editing that CRISPR allows for has also been exploited to generate specific mutations that have been identified in human ASD. Mimicking missense and point mutations that have been identified in human ASD cohorts has allowed the effect of specific genetic variants to be modelled. *C. elegans* and mice experiments have characterised the effect that such mutations have on the pathology of the brain, synaptic transmission and behaviour (Wong *et al.*, 2019; Mariappa, Ferenbach and van Aalten, 2018; Rogers *et al.*, 2019; Wong *et al.*, 2021a). CRISPR gene editing in *C. elegans* has seen the development of a technique that allows the expression of ASD-risk genes to be switched on and off and has identified genes that appear to play an important role in nervous system development (McDiarmid *et al.*, 2019; Aguirre-Chen *et al.*, 2020). As well as this CRISPR/Cas9 can also be used to investigate therapeutic strategies in ASD. Injection of CRISPR reagents into the brains of adult mice has been used to edit an ASD-associated gene and consequently rescue

behavioural deficits (Lee *et al.*, 2018), providing evidence for the potential of CRISPR in therapeutics (Yu *et al.*, 2020).

A *C. elegans* study which utilised data from the SAFRI gene database (1.3.5) was able to filter ASD-risk genes to select candidates that could be studied using CRISPR/Cas9 in worms. Prioritisation of risk genes where a missense mutation had been associated with ASD allowed the authors to model the effect of the variants on *C. elegans* behavioural phenotypes and identified deficits in neurodevelopment and locomotion in worms (Wong *et al.*, 2019). In comparison, CRISPR/Cas9 based genotype-phenotype studies in rodent models have focused on studying the effects that genetic variants have on social behaviours, facilitating the understanding of which ASD-associated genetic variants may underlie impaired social behaviour (Gonatopoulos-Pournatzis *et al.*, 2020; Wong *et al.*, 2021a; Wei *et al.*, 2020; Rogers *et al.*, 2019). Considering the fact that social impairment is a core deficit in ASD (1.1.1) precision gene editing by CRISPR/Cas9 in *C. elegans* will likely aid the understating of the genetic and neural basis underlying their social behaviour and how impairment to this behaviour could be used to model autism.

1.8 Aims of the project

ASD is a neurodevelopmental disorder which is clinically characterised by core behavioural deficits, including disruption to social behaviour (Sharma, Gonda and Tarazi, 2018). It is clear that ASD has a strong genetic underpinning with hundreds of genes and genetic variants, with varying degrees of penetrance, having been implicated in its aetiology (Iakoucheva, Muotri and Sebat, 2019). For many of these genes it is still unclear how they function within the biological system to regulate behavioural outputs. Also, how single penetrant genetic variants, and interaction of common variants, impact gene function to disrupt the activity of neural circuits that control behaviour is incompletely understood (Iakoucheva, Muotri and Sebat, 2019) (1.2).

C. elegans can be used to investigate the function and dysfunction of ASD-risk gene orthologues. Clinical databases that collate information about ASD-risk genes (1.3.5) provide a platform on which to base *C. elegans* autism research. SFARI gene database sorts ASD-candidate genes based on the evidence that associates a gene with autism and assigns it a risk score. This has been used to guide *C. elegans* studies and helped to prioritise ASD-risk genes and risk variants for study in worms (Schmeisser, Fardghassemi and Parker, 2017; Wong *et al.*, 2019; McDiarmid *et al.*, 2019). *C. elegans* behavioural impairment has been used to model ASD and has provided insight into the function of ASD-risk genes (Calahorro, Alejandre and Ruiz-Rubio, 2009; Hunter *et al.*, 2010; Jee *et al.*, 2004; Wong *et al.*, 2019; Schmeisser, Fardghassemi and Parker, 2017; McDiarmid *et al.*, 2019). However, the behavioural repertoire investigated in these studies is largely limited to

morphological and locomotory readouts. Social communication between adult *C. elegans* and progeny has been described (Scott *et al.*, 2017). The communication was shown to be mediated by a progeny-derived social cue that modulates *C. elegans* food leaving behaviour. The development of this social paradigm provides a platform to quantify *C. elegans* social behaviour and therefore investigate the genetic determinants that regulate the production of this behaviour. The recent developments in genome engineering have led to an increasing number of CRISPR/Cas9 strategies to increase the speed and precision of gene editing in worms (Kim *et al.*, 2014; Arribere *et al.*, 2014; El Mouridi *et al.*, 2017). Custom edits are being used to investigate ASD-associated genes in *C. elegans* (Wong *et al.*, 2019; McDiarmid *et al.*, 2019) but there is a lack of understanding of the effect precise genetic variants have on the social domain of the worm.

This project aimed to use *C. elegans* as a model of ASD to investigate the genetic determinants underpinning social behaviour. To do this the project aimed to:

- Design a bioinformatic pipeline to strategically filter ASD-candidate genes to identify high risk genes that can be investigated in a *C. elegans* social paradigm
- Use behavioural parameters, including an existing social paradigm, to investigate the prioritised ASD-risk genes to gain further insight into their function in *C. elegans* and their potential role in coordinating social behaviour
- Use CRISPR/Cas9 to create a *C. elegans* strain that encodes a highly penetrant human mutation associated with ASD

Here I present work to address these aims in the format of three papers, each one presented as a separate results chapter, for the submission of a Three Paper Thesis.

Chapter 2 Investigating autism associated genes in *C. elegans* reveals candidates with a role in social behaviour

Helena Rawsthorne[1], Fernando Calahorro[1], Lindy Holden-Dye[1], Vincent O'Connor[1], James Dillon[1*]

1 School of Biological Sciences, Highfield Campus, University of Southampton, Southampton, United Kingdom

*Corresponding author

Email: j.c.dillon@soton.ac.uk (JD)

Author contributions. Helena Rawsthorne: Conceptualisation, investigation (all experiments), methodology, formal analysis, writing-original draft preparation and editing. Fernando Calahorro: Conceptualisation, resources, methodology, supervision, writing- review and editing. Lindy Holden-Dye: Conceptualisation, methodology, supervision, writing- review and editing. Vincent O'Connor: Conceptualisation, methodology, supervision, writing- review and editing. James Dillon: Conceptualisation, methodology, supervision, writing- review and editing.

The content of this chapter is that of the paper published in PLOS ONE (Rawsthorne *et al.*, 2021). Any corrections made to the original publication are listed below:

The text in section 2.3.4 'The plates were then incubated at 20°C for 2 hours, this time course and pre-conditioning is equivalent to a 24 hour naive incubation. Leaving events were then observed for 30 minutes as described above' has been changed to 'The plates were then incubated at 20°C for 2 hours. Leaving events were then observed for 30 minutes as described above. The pre-conditioning of progeny is equivalent to a 24 hour naive incubation, with the exception that in a naïve assay adults are L4+2 days old and adults in a pre-conditioned assay are L4+1 day old when food leaving events are observed.'

Supplementary figure 'S1 Fig' in the published paper is Figure 2.5 in this thesis.

Supplementary figure 'S2 Fig' in the published paper is Figure 2.9 in this thesis.

2.1 Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by a triad of behavioural impairments and includes disruption in social behaviour. ASD has a clear genetic underpinning and hundreds of genes are implicated in its aetiology. However, how single penetrant genes disrupt activity of neural circuits which lead to affected behaviours is only beginning to be understood and less is known about how low penetrant genes interact to disrupt emergent behaviours. Investigations are well served by experimental approaches that allow tractable investigation of the underpinning genetic basis of circuits that control behaviours that operate in the biological domains that are neuro-atypical in autism. The model organism *C. elegans* provides an experimental platform to investigate the effect of genetic mutations on behavioural outputs including those that impact social biology. Here we use progeny-derived social cues that modulate *C. elegans* food leaving to assay genetic determinants of social behaviour. We used the SAFRI Gene database to identify *C. elegans* orthologues of human ASD-associated genes. We identified a number of mutants that displayed selective deficits in response to progeny. The genetic determinants of this complex social behaviour highlight the important contribution of synaptopathy and implicates genes within cell signalling, epigenetics and phospholipid metabolism functional domains. The approach overlaps with a growing number of studies that investigate potential molecular determinants of autism in *C. elegans*. However, our use of a complex, sensory integrative, emergent behaviour provides routes to enrich new or underexplored biology with the identification of novel candidate genes with a definable role in social behaviour.

2.2 Introduction

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental behavioural disorder. ASD is characterised by a triad of behavioural impairments, these being repetitive behaviours and impairment to verbal and social communication (Faras, Al Ateeqi and Tidmarsh, 2010). Neuro-atypical individuals have been shown to produce altered behavioural outputs in response to a range of sensory cues (Balasco, Provenzano and Bozzi, 2020), including chemosensory cues that drive social behaviours (Endevelt-Shapira *et al.*, 2018). Impairment within the integration of sensory stimuli is thought to underlie the altered perception of such cues (Stevenson *et al.*, 2014a). This highlights the importance of neural circuits in the processing of sensory information to coordinate a behavioural output in the social domain (Marco *et al.*, 2011).

It is well established that there is a strong genetic contribution in autism (Huguet, Ey and Bourgeron, 2013). The genetic architecture of ASD is complex with hundreds of genes of varying

penetrance implicated in its aetiology (De Rubeis and Buxbaum, 2015). This is complicated further by the interplay between genetic variants in the form of rare, highly penetrant, and common low penetrant variants (Iakoucheva, Muotri and Sebat, 2019; Bourgeron, 2015). Common variants attribute polygenic risk in ASD with mutations to multiple loci having additive effects on a given phenotype (Iakoucheva, Muotri and Sebat, 2019). The burden of common variants in an individual's genetic background can influence the degree of risk a rare variant can impose (Bourgeron, 2015). The combinatorial effect of rare and common variants contributes to the spectrum of phenotypes displayed across autism cases (Weiner *et al.*, 2017).

ASD-associated genes span across a range of biological functions, for example synaptic, cell signalling and epigenetic modification (De Rubeis *et al.*, 2014). Evidence suggests that although ASD genes are functionally diverse they are connected through protein interaction networks (Corominas *et al.*, 2014) and control processes such as neuronal morphology and synaptic function that modulate the activity state of neural networks (Iakoucheva, Muotri and Sebat, 2019; Krumm *et al.*, 2014; Aguirre-Chen *et al.*, 2020). This means that the consequence of even a single genetic variant can be widespread through inter-connecting gene networks and have emergent effects on neural circuits (Iakoucheva, Muotri and Sebat, 2019). For many ASD-associated genes it is still unclear how they function in neural networks which underpin behavioural phenotypes (Geschwind, 2011), such as disrupted social and motor behaviour. However, investigating determinants of defined neural circuits underpinning autism associated neuro-atypical behaviour is providing traction for discrete investigation of complex traits. Studies of distinct behaviours in mice has begun to unpick the effect of genetic disruption on molecular circuits and synapse function (Kim, Lim and Kaang, 2016; Golden, Buxbaum and Rubeis, 2018; Lee, Lee and Kim, 2017). Additionally, the impact of genetic variation on a number of synaptic genes has been extensively studied in various animal models (Yoo *et al.*, 2014; Verma *et al.*, 2019; Rabaneda *et al.*, 2014; Hunter *et al.*, 2010; Calahorro and Ruiz-Rubio, 2012; Calahorro and Ruiz-Rubio, 2013).

Animal models highlight the value of using orthologues to understand the function of ASD-associated genes in behavioural domains associated with autism (Schroeder *et al.*, 2017; Crawley, 2012; Ueoka *et al.*, 2019; Tang *et al.*, 2020). *C. elegans* provides a tractable system that allows for the high throughput of genetic determinants to be investigated in a simple nervous system (Sengupta and Samuel, 2009). Conservation of genes involved in synapse function and the use of integrative neurons highlights the utility of *C. elegans* neuronal function and how it co-ordinates complex sensory integrative behaviours that model the disruptions that are expressed through genetic mutations associated with autism (Bargmann, 1998; Metaxakis, Petratou and Tavernarakis, 2018). The genetic homology between the *C. elegans* and mammalian genome (Lai *et al.*, 2000), and the fact that mutant strains are widely accessible, means that the *C. elegans*

model lends itself to the systems level analysis of disease associated genes. This has led to a plethora of studies using single gene analysis to investigate the impact of genetic mutation to ASD-associated gene orthologues on behavioural output (Schmeisser and Parker, 2018). As well as this, *C. elegans* have been utilised in multiple high-throughput screens which have largely used morphological and locomotory readouts to screen for behavioural deficit (Schmeisser, Fardghassemi and Parker, 2017; Wong *et al.*, 2019; McDiarmid *et al.*, 2019).

Behavioural output in response to integration of sensory cues can be assayed in *C. elegans* by way of food leaving behaviour. The propensity of a worm to leave a lawn of bacterial food can be modulated by multiple sensory cues (Shtonda and Avery, 2006). It has been shown that in the presence of increasing numbers of progeny, adult worms will leave an otherwise replete food lawn in a dose-dependent manor. This progeny-dependent food leaving behaviour is the result of inter-organismal communication and is thought to be underpinned by a novel social circuit (Scott *et al.*, 2017). The utility of this social paradigm to probe autism related dysfunction was demonstrated by showing that when a penetrant mutation of human neuroligin is introduced into the worm orthologue, *nlg-1*, it results in disrupted progeny induced food leaving behaviour (Rawsthorne *et al.*, 2020).

We have used this bona fide social paradigm to investigate genetic determinants associated with human ASD. Investigation of *C. elegans* orthologues in a subset of candidate genes identified a number that disrupt a social behavioural paradigm in the worm. Furthermore, we show that whilst a large proportion of mutants displayed behavioural deficit in the social domain, there was limited disruption to the other phenotypes investigated suggesting a selective behavioural deficit. Identification of novel candidate genes in this way has also highlighted key biological functional domains that appear to play an important role in social behaviour, therefore shedding light on the functional contribution ASD-associated genes may have on the disrupted phenotypes associated with this disorder.

2.3 Materials and Methods

2.3.1 Prioritising ASD-associated genes for study in *C. elegans*

To identify genes associated with ASD we used SFARI Gene Archive (http://gene-archive.sfari.org/, version 3.0). Within this database the Human Gene Module (https://gene-archive.sfari.org/database/human-gene/) ranks genes from 1-6 based on the evidence supporting the gene's association with ASD. Genes in category 1-High confidence and category 2-Strong candidate were selected for analysis due to the fact that data implicating those genes in

ASD reach genome wide significance and there is evidence for the variant having a functional effect in humans. Orthologous genes in *C. elegans* were identified by searching the human gene name in WormBase (https://wormbase.org/, version WS264) and using the human gene Ensembl ID in OrthoList (http://www.greenwaldlab.org/ortholist/). *C. elegans* strains available for order from the *Caenorhabditis* Genetics Centre (CGC) and/or the National BioResource Project (NBRP) were prioritised for investigation. Using information gathered from WormBase, CGC, NBRP and a literature review, mutants were excluded if they were lethal, sterile or uncoordinated. Thus, we filtered for candidates best suited to investigation in the food lawn based assay. The prioritised *C. elegans* mutant strains for study can be found in Table 2.1. Genes were ascribed to one of five functional categories: synaptic, neuronal, cell signalling, epigenetic modifiers and phospholipid metabolism based on their function described by UniProtKB (https://www.uniprot.org/uniprot/). Genes described as having a role in synaptic transmission, structure, activity or plasticity were categorised as 'synaptic'. Genes with a role in neuronal excitability or adhesion were categorised as 'neuronal'. Genes described as having a predominant role in cell signalling pathways were categorised as 'cell signalling'. Genes with a role in transcriptional regulation or chromatin remodelling were categorised as 'epigenetic modifier'. The gene MBOAT7 is described as functioning in phospholipid metabolism and so was categorised as 'phospholipid metabolism'.

Table 2.1 Summary of human genes prioritised for study in *C. elegans* mutant strains

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
Synaptic										
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1	Glutamate receptor	*glr-1*	*n2461*	KP4	4	Nonsense mutation in codon 807 (Hart, Sims and Kaplan, 1995)	LOF (Hart, Sims and Kaplan, 1995)	Defective local search behaviour (Chalasani *et al.*, 2016)	AVA,AVB, AVD,AVE, PVC,AIB,RMD, RIM,SMD,AVG PVQ,URY, RIS, AVJ, DVC, RME, RIG (Hart, Sims and Kaplan, 1995)
			glr-2	*tm669*	FX00669	0	Complex substitution[1]	Unpublished	Enhanced gustatory plasticity (Hukema, Rademakers and Jansen, 2008)	AVA,AVD,AVE PVC,RMDV, RMDD,AIA, AIB,AVG,RIG, RIA,M1 (Brockie *et al.*, 2001)
			glr-2	*ok2342*	RB1808	0	Deletion[1]	Unpublished	Unknown	

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
GRIN2B	Glutamate Ionotropic Receptor NMDA Type Subunit 2B	NMDA receptor	*nmr-2*	*ok3324*	VC2623	1	Deletion[1]	LOF (Schmeisser, Fardghassemi and Parker, 2017)	Reduced swimming locomotion (Schmeisser, Fardghassemi and Parker, 2017)	AVA,AVD,AVE, RIM,AVG, PVC (Brockie *et al.*, 2001)
			nmr-2	*tm3785*	FX03785	0	Deletion[1]	LOF (Lemieux *et al.*, 2015)	Impaired learning (Vohra *et al.*, 2017)	
NLGN3	Neuroligin 3	Synaptic adhesion	*nlg-1*	*ok259*	VC228	6	Deletion to half of cholinesterase-like domain and TMD (Hunter *et al.*, 2010)	Null (Hunter *et al.*, 2010)	Reduced spontaneous reversals (Hunter *et al.*, 2010) Reduced pharyngeal pumping (Calahorro *et al.*, 2019)	VA,DA,AIY, URB,URA, PVD,HSN,ADE,URX, AVJ, ALA (Hunter *et al.*, 2010; Calahorro *et al.*, 2019)

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
NRXN1	Neurexin 1	Synaptic adhesion	*nrx-1*	*ds1*	SG1	3	Deletion in the long *nrx-1* isoform (Philbrook *et al.*, 2018)	Unpublished	Unknown	Nervous system, GABAergic neurons (Maro *et al.*, 2015; Haklai-Topper *et al.*, 2011)
			nrx-1	*tm1961*	FX01961	0	Deletion in the long *nrx-1* isoform (Philbrook *et al.*, 2018)	Truncated protein (Calahorro and Ruiz-Rubio, 2013)	Deficient gentle touch response (Calahorro and Ruiz-Rubio, 2013)	
PTCHD1	Patched domain containing 1	Synaptic receptor	*ptr-5*	*gk472*	VC1067	0	Deletion[1]	Unpublished	Unknown	Unknown
SHANK2/3	SH3 and multiple ankyrin repeat domains	Synaptic scaffold	*shn-1*	*ok1241*	RB1196	0	Deletion covering PDZ domain and proline rich motif (Oh *et al.*, 2011)	LOF (Oh *et al.*, 2011)	None reported (Oh *et al.*, 2011)	Widely expressed (Jee *et al.*, 2004)
			shn-1	*gk181*	VC376	0	Deletion covering most of ANK repeat and entire PDZ domain (Oh *et al.*, 2011)	LOF (Oh *et al.*, 2011)	Unknown	

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
SLC6A1	Solute carrier family 6 member 1	GABA transporter	*snf-11*	*ok156*	RM2710	6	Deletion (Mullen *et al.*, 2006)	Putative null (Mullen *et al.*, 2006)	None reported (Mullen *et al.*, 2006)	AVL,RIBR,ALA, RIBL,GLRV, RME,AVF,EF1, EF2,EF3,EF4, Body wall muscle (Gendrel, Atlas and Hobert, 2016)
			snf-11	*tm625*	FX00625	0	Deletion and insertion (Mullen *et al.*, 2006)	Putative null (Mullen *et al.*, 2006)	Unknown	
SYNGAP1	Synaptic Ras GTPase activating protein 1	Ras GTPase activating protein	*gap-2*	*tm748*	JN147	0	Complex substitution[1]	LOF (Gyurko *et al.*, 2015)	No effect on body bends (Gyurko *et al.*, 2015)	Widely expressed (Gyurko *et al.*, 2015)
			gap-2	*ok1001*	VC680	0	Complex substitution[1]	Unpublished	Unknown	
Neuronal										
CACNA1H	Calcium voltage-gated channel subunit Alpha1 H	Calcium channel	*cca-1*	*ad1650*	JD21	7	Deletion (Steger *et al.*, 2005)	LOF (Steger *et al.*, 2005)	Reduced pharyngeal pumping (Steger *et al.*, 2005)	Pharyngeal muscle, neurons in pharynx and VNC (Steger *et al.*, 2005)

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
CNTN4	Contactin 4	Axonal adhesion	*rig-6*	*ok1589*	VC1125	0	Deletion (Katidou, Tavernarakis and Karagogeos, 2013)	Hypo-morphic (Katidou, Tavernarakis and Karagogeos, 2013)	None reported (Katidou, Tavernarakis and Karagogeos, 2013)	Widely expressed in nervous system (Schwarz *et al.*, 2009)
			rig-6	*gk376*	VC884	0	Deletion- knocks down expression of isoform a only (Katidou, Tavernarakis and Karagogeos, 2013)	Hypo-morphic (Katidou, Tavernarakis and Karagogeos, 2013)	Unknown	
Cell signalling										
DYRK1A	Dual specificity tyrosine phosphorylation regulated kinase 1A	Protein kinase	*hpk-1*	*pk1393*	EK273	6	Deletion to most of kinase domain (Das *et al.*, 2017)	Null (Das *et al.*, 2017)	Reduced lifespan (Das *et al.*, 2017)	Gonad, nervous system – not otherwise specified (Das *et al.*, 2017)

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
			mbk-1	*pk1389*	EK228	6	Deletion to most of kinase domain (Mack *et al.*, 2017)	Putative null (Mack *et al.*, 2017)	Reduced lifespan (Mack *et al.*, 2017)	Somatic tissue, not otherwise specified (Raich *et al.*, 2003)
			mbk-1	*ok402*	RB677	0	Unknown	Unknown	Reduced swimming locomotion (Schmeisser, Fardghassemi and Parker, 2017)	
PTEN	Phosphatase and tensin homolog	Protein phosphatase	*daf-18*	*e1375*	CB1375	0	Insertion (Ogg and Ruvkun, 1998)	Reduction of function (Ogg and Ruvkun, 1998)	Chemotaxis deficit (Adachi *et al.*, 2010)	Widely expressed (Masse *et al.*, 2005)
			daf-18	*ok480*	RB712	0	Deletion (Brisbin *et al.*, 2009)	Putative null (Brisbin *et al.*, 2009)	Abnormal mitotic arrest in dauer (Fukuyama, Rougvie and Rothman, 2006)	
Epigenetic modifiers										

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
CHD8	Chromodomain helicase DNA binding protein 8	Transcription factor	*chd-7*	*gk290*	VC606	0	Deletion[1]	Unpublished	Reduced swimming locomotion (Schmeisser, Fardghassemi and Parker, 2017)	Unknown
			chd-7	*gk306*	VC676	0	Deletion[1]	Unpublished	Impaired habituation (McDiarmid *et al.*, 2019)	
FOXP1	Forkhead box P1	Transcription factor	*fkh-7*	*gk793*	VC1646	0	Deletion[1]	Unpublished	Unknown	Widely expressed (Feng, Craig and Hope, 2012)
IRF2BPL	Interferon regulatory factor 2 binding protein like	Transcription factor	*tag-260*	*ok1339*	VC812	0	Insertion[1]	Putative null (De Arras *et al.*, 2013)	Unknown	Unknown
KDM6A	Lysine-specific demethylase 6A	Histone demethylase	*jmjd-3.1*	*gk387*	VC912	0	Deletion[1]	Unpublished	Unknown	PDA motor neuron and Y cell (Zuryn *et al.*, 2014)
			jmjd-3.1	*gk384*	VC936	0	Insertion[1]	Null (Labbadia and Morimoto, 2015)	Unknown	

Human Gene	**Gene name**	**Protein function**	***C. elegans* orthologue**	**Allele**	**Strain name**	**Outcrossed**	**Mutation**	**Mutation effect**	**Behavioural phenotype**	**Gene expression**
KMT5B	Lysine methyltransferase 5B	Transcription factor	*set-4*	*n4600*	MT14911	2	Deletion (Delaney *et al.*, 2017)	LOF (Delaney *et al.*, 2017)	Deficient dauer arrest (Delaney *et al.*, 2017)	Nervous system, not otherwise specified (Delaney *et al.*, 2017)
			set-4	*ok1481*	VC997	0	Deletion (Delaney *et al.*, 2017)	LOF (Delaney *et al.*, 2017)	Deficient dauer arrest (Delaney *et al.*, 2017)	
SETD2	Histone-lysine N-methyltransferase SETD2	Transcriptional regulation	*met-1*	*n4337*	MT16973	4	Deletion (Kreher *et al.*, 2018)	LOF (Pu *et al.*, 2015)	Sterility at 25◦C (Kreher *et al.*, 2018)	Broadly expressed (Engert *et al.*, 2018)
			met-1	*tm1738*	FX01738	0	Deletion (Kreher *et al.*, 2018)	Putative null (Kreher *et al.*, 2018)	Sterility at 25◦C (Kreher *et al.*, 2018)	

Human Gene	**Gene name**	**Protein function**	***C. elegans* orthologue**	**Allele**	**Strain name**	**Outcrossed**	**Mutation**	**Mutation effect**	**Behavioural phenotype**	**Gene expression**
SETD5	Histone-lysine N-methyltransferase SETD5	Transcriptional regulation	*set-26*	*tm3526*	FX03526	0	Deletion[1]	Putative null (Greer *et al.*, 2014)	None reported (Greer *et al.*, 2014)	Widely expressed (Wang *et al.*, 2018b)
			set-24	*n4909*	MT16133	0	Unknown	Unknown	None reported (Andersen and Horvitz, 2007)	Germline specific (Engert *et al.*, 2018)
			set-9	*n4949*	MT16426	1	Deletion[1]	Putative null (Greer *et al.*, 2014)	None reported (Andersen and Horvitz, 2007)	Germline specific (Wang *et al.*, 2018b)

Human Gene	Gene name	Protein function	*C. elegans* orthologue	Allele	Strain name	Outcrossed	Mutation	Mutation effect	Behavioural phenotype	Gene expression
Phospholipid metabolism										
MBOAT7	Membrane bound O-acyltransferase domain containing 7	Acetyl transferase	*mboa-7*	*ok1028*	RB1071	0	Deletion (Lee *et al.*, 2012b)	LOF (Lee *et al.*, 2012b)	Unknown	Muscle, vulva, intestine (Lee *et al.*, 2008)
			mboa-7	*gk399*	VC942	0	Deletion (Lee *et al.*, 2012b)	LOF (Lee *et al.*, 2012b)	Egg laying deficit (Lee *et al.*, 2008)	
			mboa-7	*tm3536*	FX03536	0	Deletion (Lee *et al.*, 2012b)	LOF (Lee *et al.*, 2012b)	Developmental defects (Lee *et al.*, 2012b)	
			mboa-7	*tm3645*	FX03645	0	Deletion (Lee *et al.*, 2012b)	LOF (Lee *et al.*, 2012b)	Unknown	

Human genes were used to ascribe functional domains. For each human gene the *C. elegans* orthologue used for investigation is listed and the mutant allele, known phenotypes and expression of the gene indicated. LOF stands for loss-of-function. AA stands for amino acids. TMD stands for transmembrane domain. LNS stands for laminin-neurexin-sex hormone-binding globulin. References are indicated. [1] http://www.wormbase.org,release WS264, date 19.01.18.

2.3.2 *C. elegans* culturing and strains used

All *C. elegans* strains were maintained using standard conditions (Brenner, 1974). *C. elegans* were age synchronised by picking L4 hermaphrodites onto a new plate 18 hours prior to behavioural assays. Bristol N2 were used as wild-type control. All other strains used can be found in Table 2.1. Strains were obtained from either the *Caenorhabditis* Genetics Center or National BioResource Project.

2.3.3 Food leaving assay

5cm NGM (nematode growth medium) plates were prepared using a standard protocol (Brenner, 1974). 50µl of OP50 *E.coli* at OD_{600} of 0.8 was gently spotted on the middle of an unseeded plate. Approximately 18 hours following this, seven L4+1 day old hermaphrodites were picked onto the centre of the bacterial lawn. Plates were then incubated at 20°C for 24 hours, during this time the seven assay worms lay eggs which produces a progeny laden lawn. In all food leaving assays the number of food leaving events were counted manually during a 30 minute observation period using a binocular dissecting microscope (Nikon SMZ800; X10). A food leaving event was defined as when the whole of the worm's body came off the bacterial food lawn, as previously described (Rawsthorne *et al.*, 2020). Following each food leaving assay the % proportion of eggs, L1 and L2 progeny on the plate was calculated. For all food leaving assays N2 and *nlg-1(ok259)* animals were analysed in parallel to other mutant cohorts. Investigators were blind to the genotypes being observed.

2.3.4 Pre-conditioned food leaving assay

NGM plates were prepared and seeded as described above. 18 hours after seeding assay plates, half were pre-conditioned with progeny using the protocol described previously (Scott *et al.*, 2017) and the remaining plates were used as matched unconditioned controls. In the preconditioned plates 10 gravid adults were picked onto the centre of the bacterial lawn and left to lay 150-200 eggs before being picked off. 18 hours after this, for each mutant under investigation, seven L4+1 day old hermaphrodites were picked onto the centre of a naïve bacterial food lawn. This acts as a matched unconditioned control. Another seven L4+1 day old hermaphrodites were picked onto a pre-conditioned bacterial food lawn in which 150-200 eggs had developed for 18 hours. The plates were then incubated at 20°C for 2 hours. Leaving events were then observed for 30 minutes as described above. The pre-conditioning of progeny is equivalent to a 24 hour naive incubation, with the exception that in a naïve assay adults are L4+2

days old and adults in a pre-conditioned assay are L4+1 day old when food leaving events are observed.

2.3.5 Pharyngeal pumping

Following the measurement of food leaving at the 24 hour time point, feeding behaviour was quantified by counting the pharyngeal pumping of three of the seven worms. The worms selected for these measurements were on food for the observation period. One pharyngeal pump was defined as one cycle of contraction-relaxation of the terminal bulb of the pharyngeal muscle. This behaviour was measured for 1 minute using a binocular dissecting microscope (Nikon SMZ800; X63) and the pumps per minute for each worm recorded from a single observation (Dallière *et al.*, 2016).

2.3.6 Thrashing

Thrashing analysis was performed on the *C. elegans* mutants that were investigated in the pre-conditioned food leaving assay. Using a 24 well plate, 6-7 N2 or mutant worms were picked per well containing 500µl of M9 with 0.1% bovine serum albumin and left for 5-10 minutes before thrashing was observed. For each worm thrashing was counted for 30 seconds. Each thrash was defined as a complete movement through the midpoint of the worm's body and back. For each mutant under investigation N2 control worms were analysed in parallel and at least two separate assays were performed. Investigators were blind to the genotypes being investigated.

2.3.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software. Data are expressed as mean or mean ±SEM as indicated in the figure legend. Statistical tests and post-hoc analysis is indicated in the figure legends. Significance level was set to $P<0.05$.

2.4 Results

2.4.1 Selection of human ASD-associated genes for study using *C. elegans* social behaviour

The genetic architecture of autism is complex with over 1,000 genes currently implicated in the disorder (De Rubeis *et al.*, 2014). Furthermore, the functional contribution that many of these genes make to the behavioural domains implicated in ASD remains unclear. We have created a pipeline (Figure 2.1) to select *C. elegans* orthologues of human ASD-associated genes and that can be investigated in a paradigm of social behaviour in the worm.

We used SFARI Gene, a growing database which categorises ASD-risk genes based on the strength of evidence supporting the association. We prioritised 91 genes ranked by SFARI Gene Archive (accessed October 2018) as category 1-high confidence and category 2-strong candidate. Of these 91 genes, 84% (76/91) had at least one orthologue in *C. elegans.* A mutant strain was available for 84% (64/76) of the orthologous genes using the criteria that the mutant strain was available from the CGC and/or NBRP. Of these, 43 genes had available mutants that were either lethal, sterile or uncoordinated (Figure 2.1). We considered that such phenotypes rendered these mutants unsuitable for investigation in the social behaviour assay. On this basis we selected 40 *C. elegans* mutants spanning 21 human ASD-associated genes for further investigation (Table 2.1). The human ASD-associated genes were each assigned to a group based upon the functional description of the encoded protein in UniProtKB database. The functional groupings were: synaptic, neuronal, cell signalling, epigenetic modifiers and phospholipid metabolism. This led to a distribution of candidates highlighting 43% as synaptic genes, 33% as epigenetic modifiers, 10% as cell signalling, 9% neuronal and 5% phospholipid metabolism (Figure 2.1).

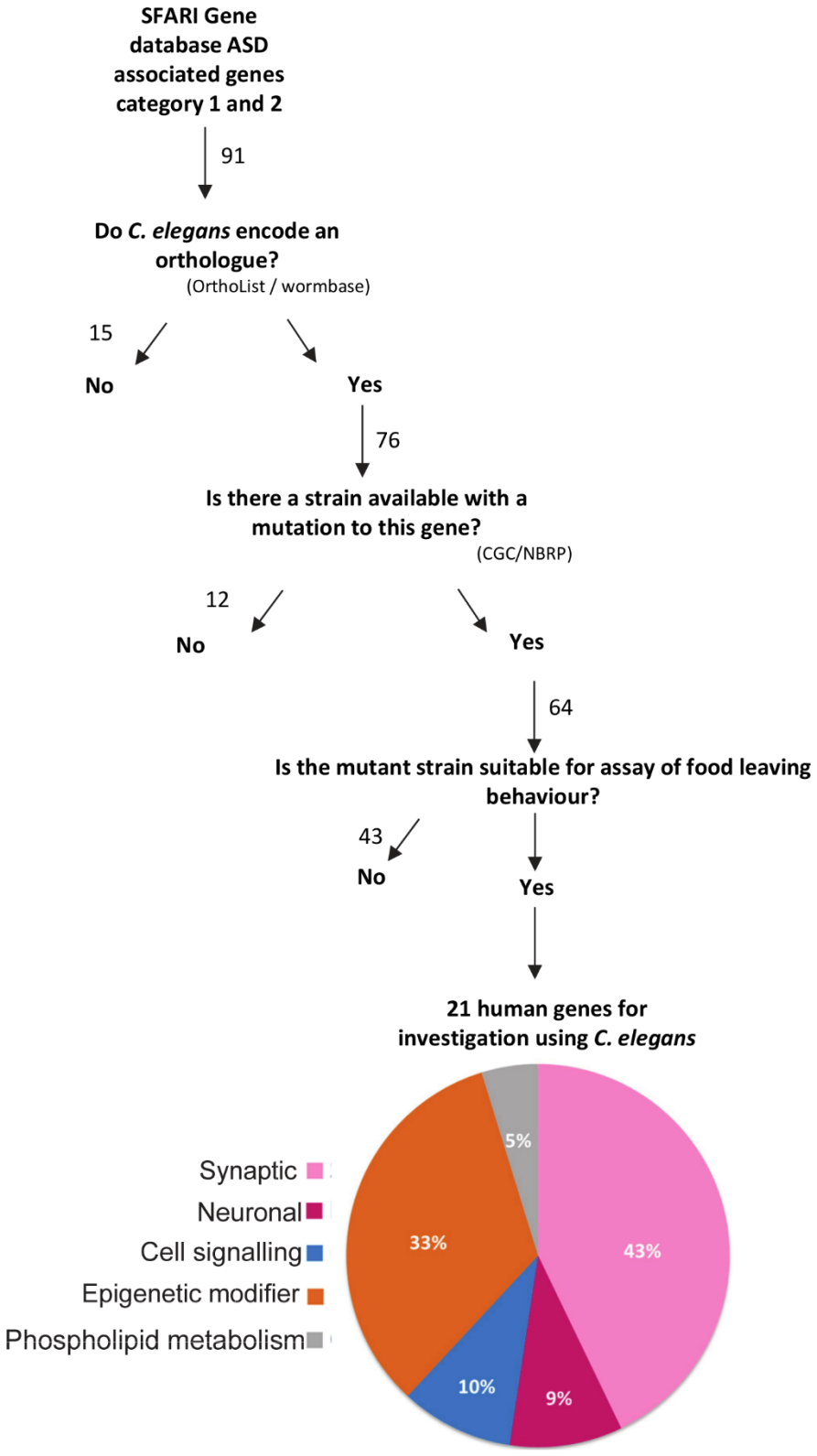


Figure 2.1 Prioritisation and categorization of the *C. elegans* orthologues of prioritised human ASD-associated genes. High confidence ASD-associated genes in category 1 and 2 in SFARI Gene Archive were input. The pipeline selects human genes which have an orthologue in *C. elegans* which can be studied in an available mutant strain which is neither lethal, sterile or uncoordinated. In brackets are the resources used for analysis. CGC – Caenorhabditis Genetics Center. NBRP – National BioResource

Project. The number of genes analysed using SFARI Gene Archive (https://gene-archive.sfari.org/, accessed October 2018) are stated. The pie chart indicates the percent of the 21 human genes that were placed into five functional groupings.

2.4.2 Screening mutants using food leaving behaviour identifies ASD-associated genetic determinants of social behaviour

To investigate food leaving behaviour, mutants were picked onto the centre of a bacterial lawn and food leaving events were measured after 24 hours. During the 24 hour incubation period the adult worms lay eggs which hatch into *C. elegans* progeny. It has been previously shown that progeny-derived social cues mediate a progeny-dependent increase in adult food leaving behaviour (Scott *et al.*, 2017). In accordance with previous findings we observed that N2 worms left the food lawn after 24 hours at a rate of approximately 0.088 leaving events/worm/minute (Figure 2.2). We had previously established a blunted food leaving response for the *nlg-1(ok259)* mutant (Rawsthorne *et al.*, 2020) and this was used as an internal measure in the current assays (Figure 2.2).

Against this backdrop, N2 and *nlg-1(ok259)* were investigated alongside the selected mutants we filtered through following initial selection from the SAFRI Gene database. This comparison showed that 23 of the 39 *C. elegans* mutants showed a mean food leaving rate lower than that of *nlg-1(ok259)* suggesting food leaving impairment (Figure 2.2). Mutants with a reduced food leaving phenotype were distributed across the five functional categories we defined suggesting genetic disruption within a range of molecular determinants from distinct biological domains may contribute to the emergence of *C. elegans* social behaviour.

As part of the investigation, where possible, we analysed two or more mutant alleles for a single gene (Figure 2.2). For some mutants, for example for *gap-2* and *rig-6* mutants, the two mutants phenocopied one another and showed a food leaving rate similar to that of N2. Interestingly, we found two loss-of-function *nmr-2* mutants which also phenocopied one another but showed significant food leaving impairment (Figure 2.2). In contrast, there were also instances where mutant alleles did not phenocopy each other. For example *nrx-1* and *chd-7* mutants showed one mutant allele with impaired food leaving and one with a behavioural response to progeny similar to N2 (Figure 2.2).

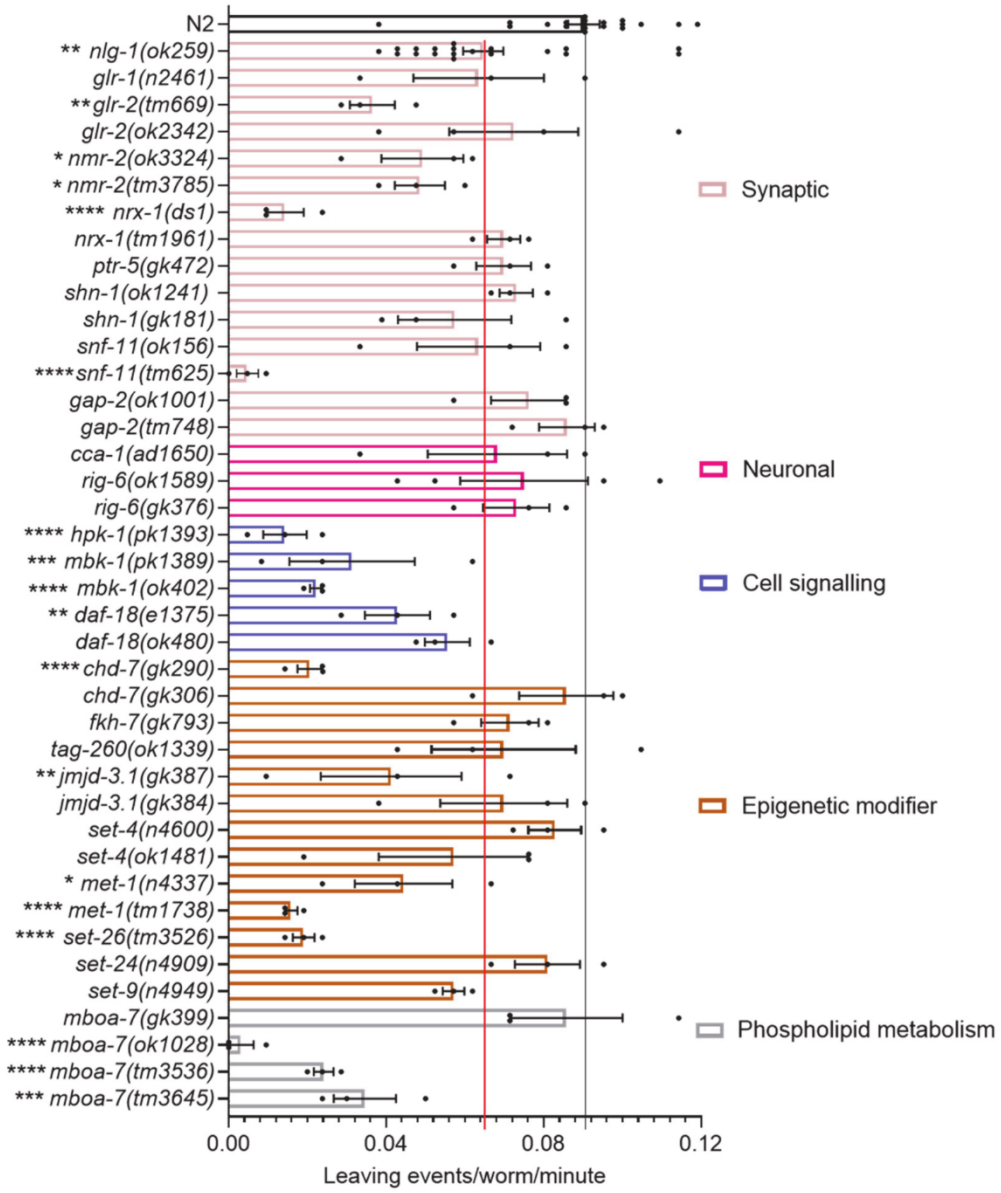


Figure 2.2 Food leaving behaviour of *C. elegans* mutants after 24 hours on food to investigate human ASD-associated genes. A food leaving assay was performed with N2*, nlg-1(ok259)* and 39 other *C. elegans* mutants. Genes are categorised and colour coded into different functional domains. The black line indicates the number of leaving events/worm/minute for N2 control. The red line indicates the food leaving rate of *nlg-1(ok259)* control. N2 and *nlg-1(ok259)* n=19. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. All data shown as mean ±SEM. Statistical analysis performed using a one-way ANOVA and Dunnetts's multiple comparison test; ns, p>0.05; *, p<0.05; **, p p≤0.01; ***, p≤0.001; ****, p≤0.0001. All significance relates to a comparison with N2 control.

2.4.3 Impaired social behaviour of mutants is likely a selective response to progeny derived social cues

Previous work has identified the value of investigating additional behaviours that can be scored in the observational arena (McDiarmid *et al.*, 2019). In this respect the food leaving assay allows for multi-tracking phenotypic analysis including pharyngeal pumping, development and egg laying. In the case of pharyngeal pumping and egg laying, this reflects the output of a defined neuromodulation and the possible consequence progeny exposure might have on this. In the case of development, this provides insight into whether the mutations perturb gross development, a useful consideration in a neurodevelopmental disorder.

After each food leaving assay we quantified the pharyngeal pump rate of the mutants. Pharyngeal pumping is modulated via external sensory cues such as food (Li *et al.*, 2012). Therefore, we wanted to test whether another sensory regulated behaviour was affected in these mutants. 87% of mutants showed no pumping phenotype (Figure 2.3). In fact, the majority of mutants with impaired social behaviour (Figure 2.2) had a pumping rate similar to N2 (Figure 2.3). This shows that most mutants with reduced food leaving behaviour are largely capable of responding to food-dependent sensory cues and co-ordinating normal feeding behaviour. In addition, the *cca-1(ad1650)* mutant which showed the most deficient pumping phenotype (Figure 2.3) did not show a food leaving phenotype (Figure 2.2), further suggesting that deficits in feeding behaviour are unlikely to explain differences in food leaving behaviour.

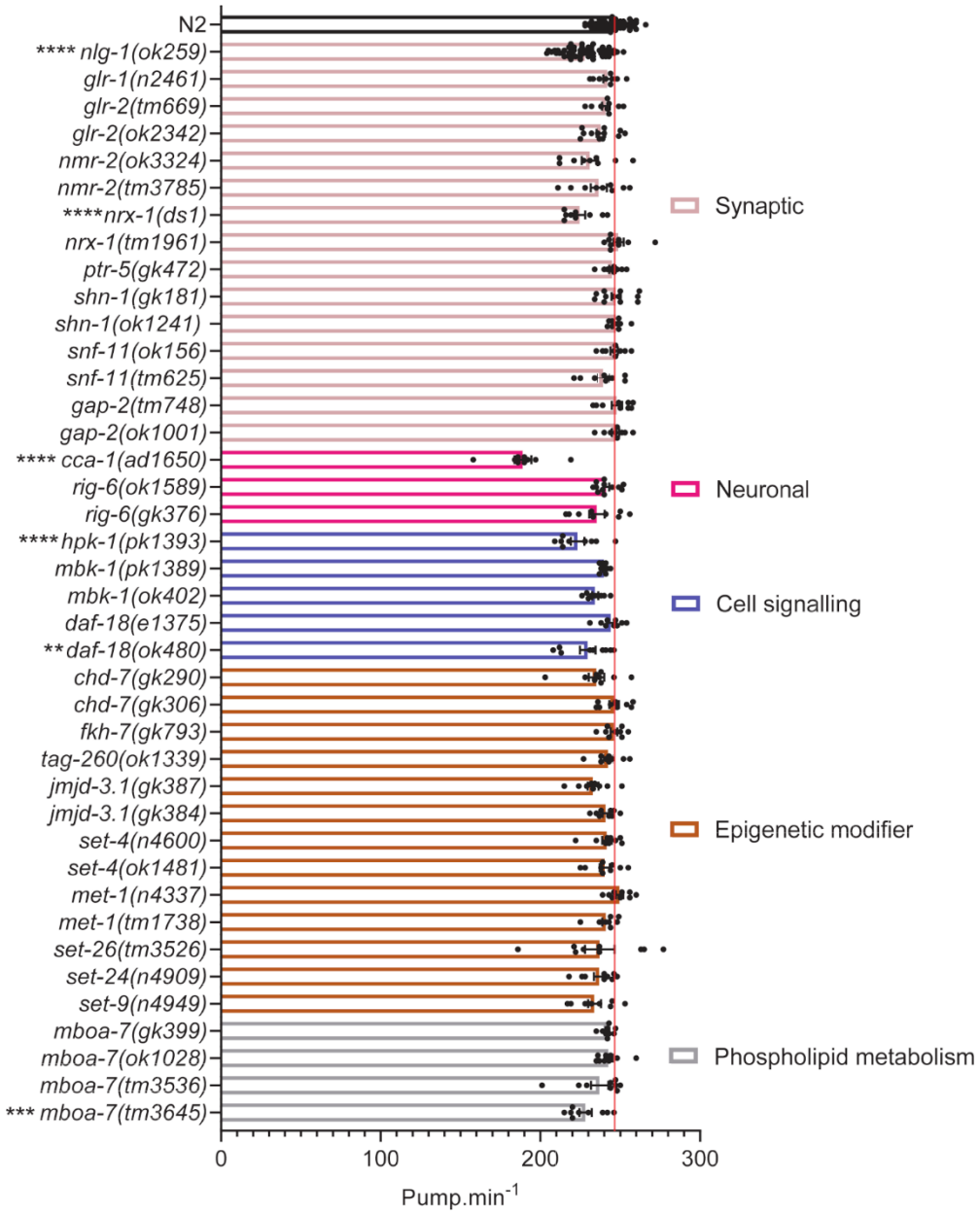


Figure 2.3 Pharyngeal pump rate for *C. elegans* mutants. After a food leaving assay at 24 hours, three worms were chosen at random and their pharyngeal pump rate was counted per minute. N2 and *nlg-1(ok259)* n=57. All other mutants n=9-12, where n refers to the number of individual animals investigated. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. The red line indicates pumps per minute for N2 control. All data shown as mean ±SEM. Statistical analysis performed using a one-way ANOVA and Dunnetts's multiple comparison test; ns, $p>0.05$; *, $p<0.05$; **, $p\leq0.01$; ***, $p\leq0.001$; ****, $p\leq0.0001$. All significance relates to a comparison with N2 control.

Next, we measured early development by quantifying the proportion of total progeny that were eggs, L1 and L2 progeny 24 hours after introducing 7 L4+1. We used % proportion to normalise for

observed variation in the total number of eggs laid. 75% of mutants developed at a similar rate to N2 showing that there is no gross early developmental delay (Figure 2.4). Interestingly, whilst early development seems to be largely unaffected we noted a larger variation in the egg laying of distinct mutants when compared to N2 controls (Figure 2.5). The number of eggs laid by a mutant is an important consideration for this assay because the density of progeny populating a food lawn is known to influence the food leaving rate of adult worms (Scott *et al.*, 2017). We plotted the relationship between the number of progeny produced by a mutant and the food leaving behaviour and showed that the two were correlated (Figure 2.6). Interestingly, this applies to the *nrx-1* and *chd-7* mutants for which the two alleles tested resulted in distinct social phenotypes (Figure 2.2). In each case the mutant that showed impaired social behaviour (Figure 2.2) also produced fewer progeny (Figure 2.5). Producing fewer progeny means adult worms were exposed to fewer progeny-derived social cues (Scott *et al.*, 2017) and could explain the low food leaving rate seen. The correlation between social behaviour and progeny exposure, and the limited disruption seen to the other phenotypes tested, implies that progeny-derived social cues selectively affect social behaviour and therefore progeny exposure is an important consideration in this type of investigation.

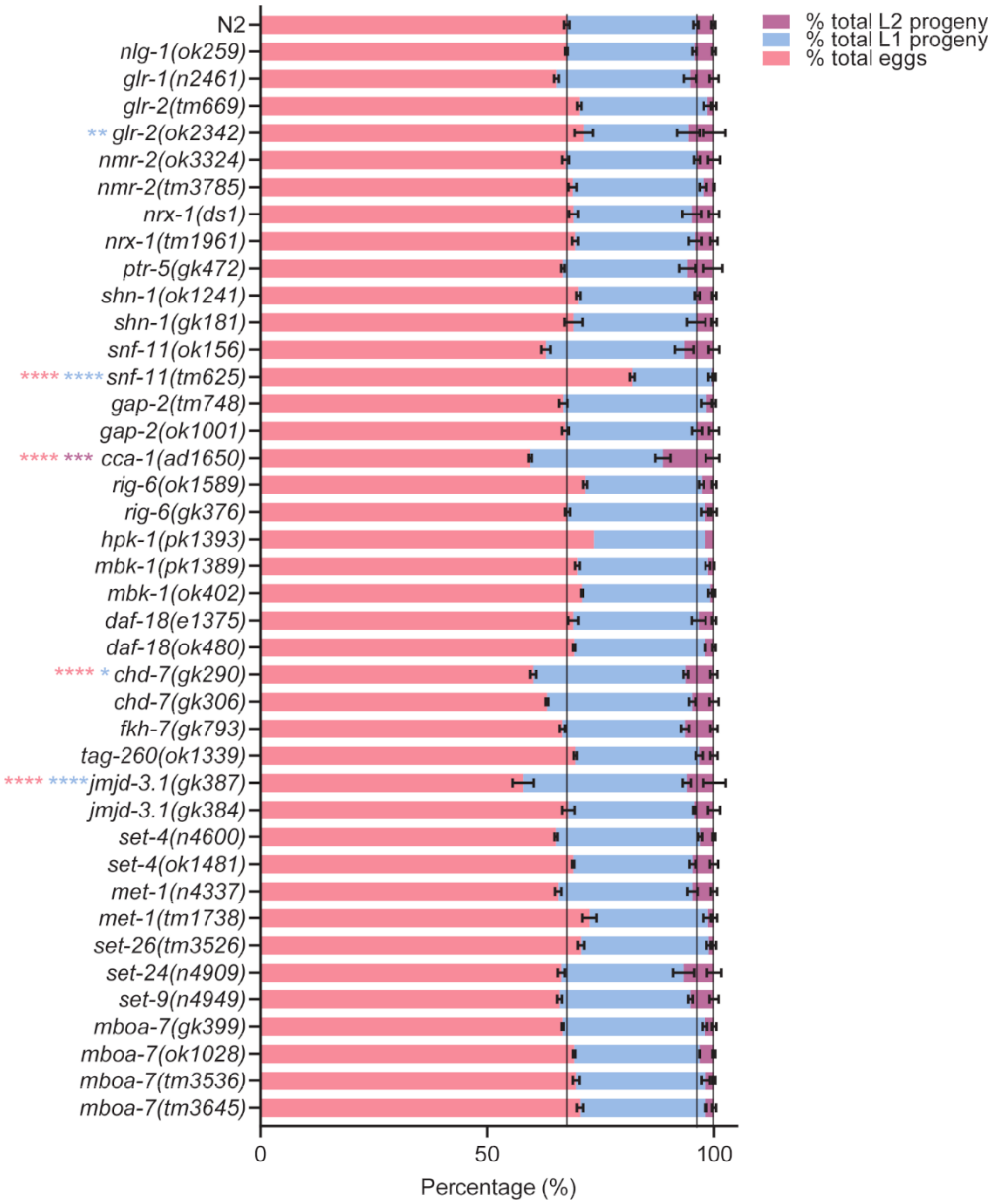


Figure 2.4 Percent total eggs and progeny produced by *C. elegans* mutants at 24 hours. After a food leaving assay from naïve food lawns occupied by 7 L4+1, the percent total offspring that were eggs, L1 and L2 progeny were quantified. N2 and *nlg-1(ok259)* n=19. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. The black lines indicate % total eggs, % total L1 progeny and % total L2 progeny for N2 control. Pink asterisks indicate statistical difference between mutant and N2 for % total eggs. Blue asterisks indicate statistical difference between mutant and N2 for % total L1 progeny. Purple asterisks indicate statistical difference between mutant and N2 for % total L2 progeny. All data shown as mean ±SEM. Statistical analysis performed using a two-way ANOVA and Tukey's

multiple comparison test; ns, p>0.05; *, p<0.05; **, p≤0.01; ***, p≤0.001; ****, p≤0.0001.

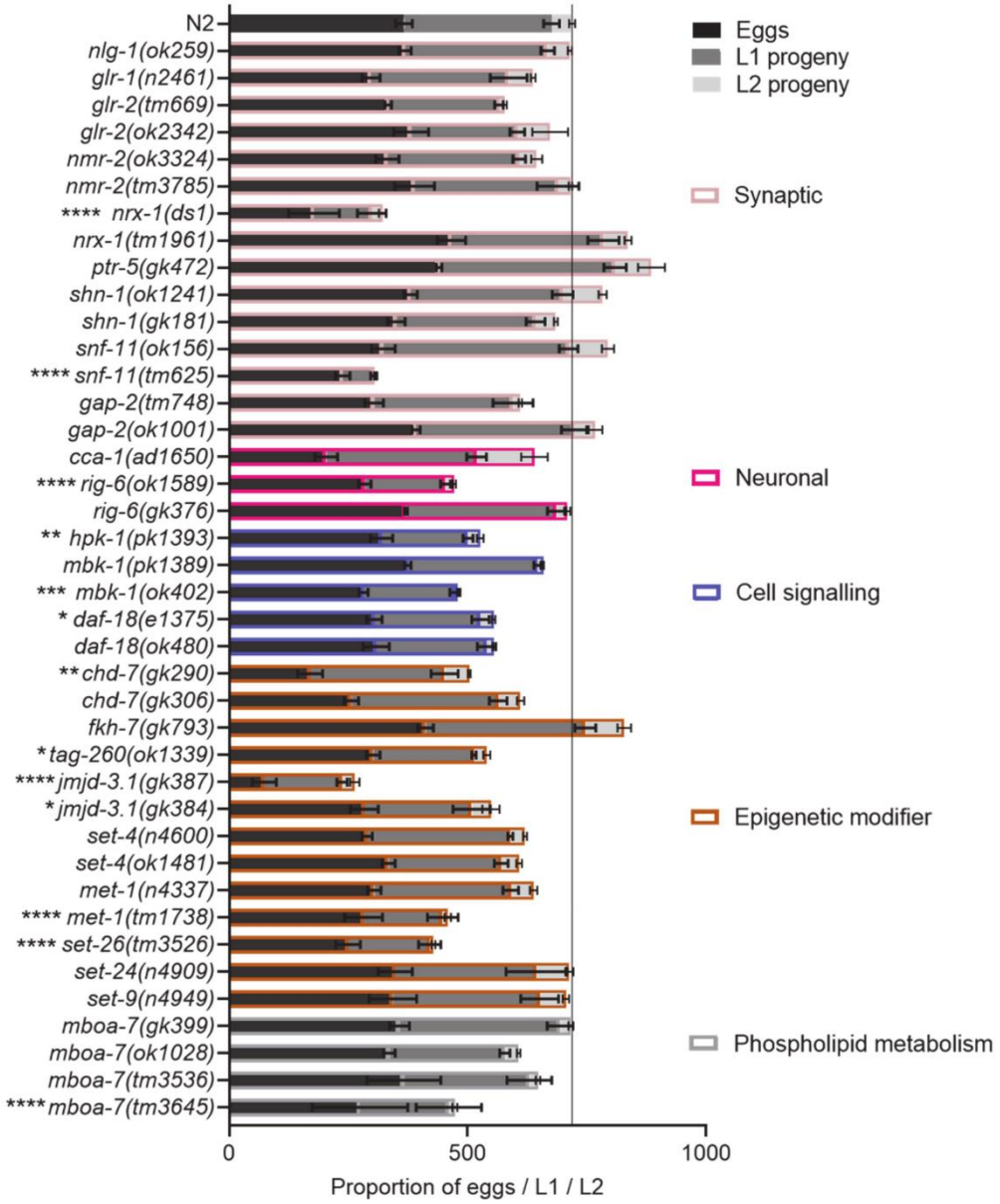


Figure 2.5 Proportion of eggs and progeny produced by *C. elegans* mutants after 24 hours. After a food leaving assay the number of eggs and progeny produced after 24 hours was counted. N2 and *nlg-1(ok259)* n=19. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. The black line indicates the total number of eggs and progeny produced by N2 control. All data shown as mean ±SEM. Statistical analysis performed using a two-way ANOVA and Dunnetts's multiple comparison test; ns, p>0.05; *, p<0.05; **, p≤0.01; ***, p≤0.001; ****, p≤0.0001. All

significance relates to the total number of eggs and progeny produced in comparison with N2 control.

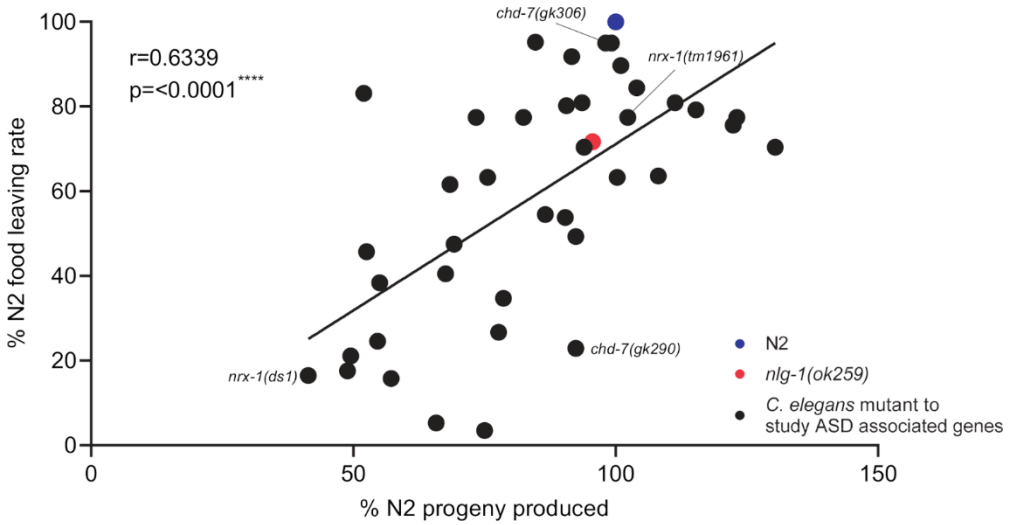


Figure 2.6 Correlation between food leaving behaviour of *C. elegans* mutants and progeny production during food leaving assay. The percent food leaving rate and progeny produced for each *C. elegans* mutant was calculated in comparison to N2. N2 and *nlg-1(ok259)* n=19. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. All data shown as mean. Statistical analysis performed using Pearson correlation coefficient.

2.4.4 Exposure to N2 progeny selectively modulates social behaviour in a number of mutants

Those strains which produced few progeny confound the assessment of the reduced food leaving behaviour as the response is dependent on the density of progeny populating the food lawn (Scott *et al.*, 2017). This was addressed by testing the food leaving behaviour of 30 mutants in response to an experimentally controlled number of N2 progeny. This used a pre-conditioning approach in which N2 progeny pre-populate the lawn and precondition them by mimicking the progeny population that emerge in the first food leaving assay. These assays allow the acute effect of progeny exposure on food leaving behaviour to be investigated. This secondary screen focussed on mutants that showed a mean food leaving rate lower than that of *nlg-1(ok259)* in at least one allele tested (Figure 2.2). Thus, we directly tested the veracity of mutants that emerge from the first screen and explicitly address the potential confound of reduced progeny number. For each mutant we performed a paired experiment in which mutant food leaving was measured on a naïve, unmatched control, plate containing OP50 and a preconditioned plate that incubated 140-200 eggs for 24 hours before introducing 7 L4+1 adults. In accordance with previous findings,

N2 adults showed enhanced food leaving in response to progeny and this response was blunted in the *nlg-1(ok259)* adults exposed to pre-loaded N2 progeny (Figure 2.7).

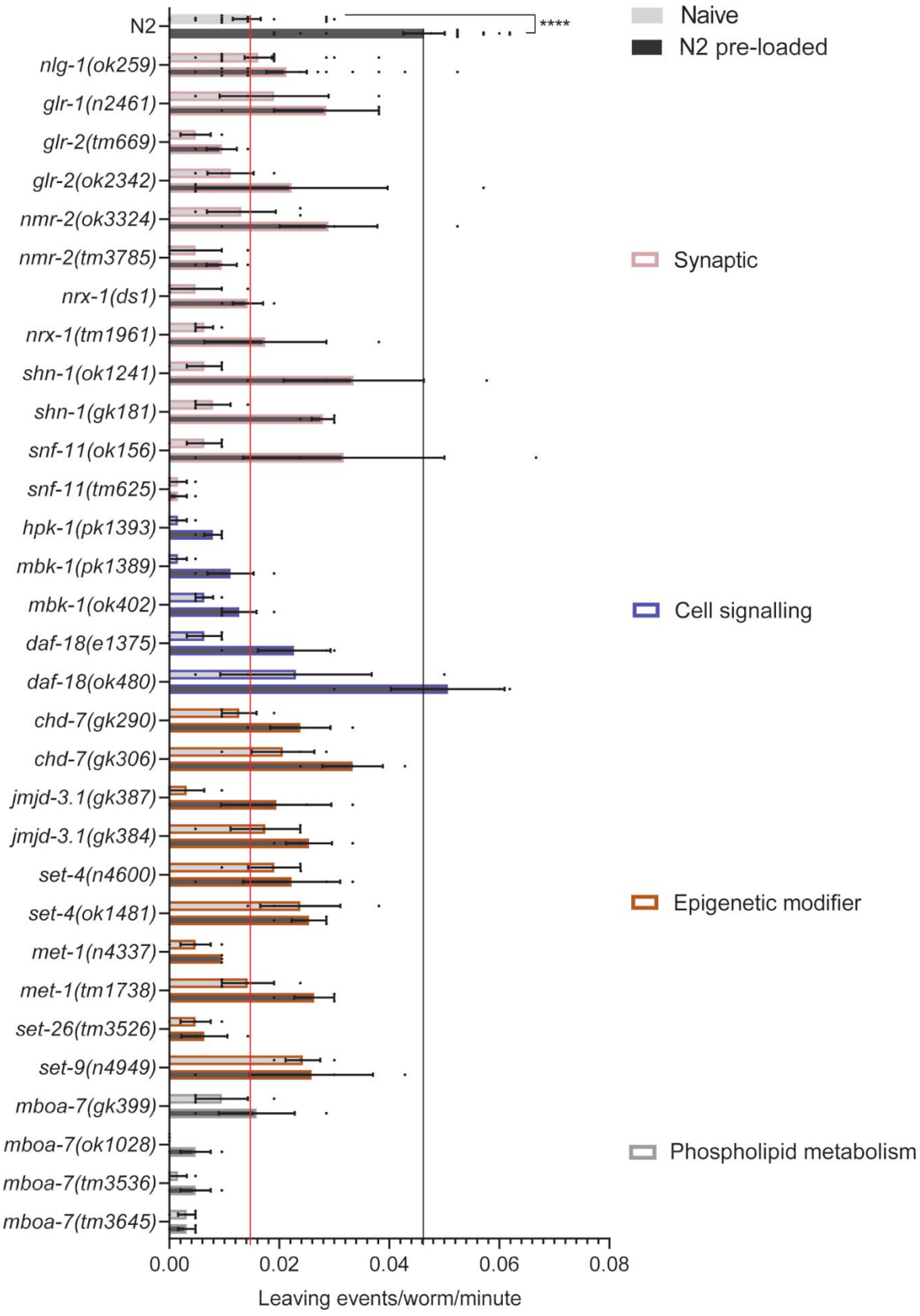


Figure 2.7 Food leaving behaviour of *C. elegans* mutants in the absence of progeny and exposure to N2 progeny. A food leaving assay was performed with N2, *nlg-1(ok259)*

and 30 other *C. elegans* mutants on naïve and pre-conditioned food lawns. A naïve lawn contains no progeny whereas a pre-conditioned food lawn contains ~150-200 N2 progeny. The red line indicates the food leaving rate of N2 naïve control. The black line indicates the food leaving rate of the N2 pre-loaded control. Data shown as mean ±SEM. N2 and *nlg-1(ok259)* n=16. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. Statistical analysis performed using a two-way ANOVA and Sidak's multiple comparison test; ns, $p>0.05$; $p \leq 0.001$****.

Analysis of mutants in response to pre-loaded N2 progeny revealed a number of mutants which left infrequently on both naïve and pre-conditioned food lawns, showing little progeny-enhanced food leaving (Figure 2.7). We reasoned that the low food leaving rate of these mutants could be explained by locomotory deficits. To address this, we performed a thrashing assay to assess the innate movement ability of the mutants. 12 of the 31 mutants tested showed minor disruption to thrashing behaviour (Figure 2.8A). For the majority of mutants thrashing did not predict food leaving phenotype. For example, *nlg-1(ok259)* and *set-4* mutants showed deficits in food leaving behaviour (Figure 2.7) without any impairment to thrashing (Figure 2.8A). Furthermore, four mutants of the *mboa-7* gene all showed impaired progeny-induced food leaving behaviour with only one of these mutants having reduced thrashing (Figure 2.8A). The bioinformatic pipeline filtered out mutant strains deficient in gross motility. In addition, we investigated the association between thrashing and food leaving behaviour and identified that there is a correlation between these behaviours (Figure 2.8B). This suggests that, whilst there are examples of mutants that have impaired food leaving in the absence of a thrashing phenotype, we cannot discount more subtle motility defects contributing to the summed food leaving response. The social impairment we observed in mutants suggests that a variety of genes may act as molecular determinants of social behaviour. Interestingly, these genes were part of synaptic, cell signalling, epigenetic modifier and phospholipid metabolism categories. This highlights that molecular determinants from these biological domains may be important for the emergence of social behaviour.

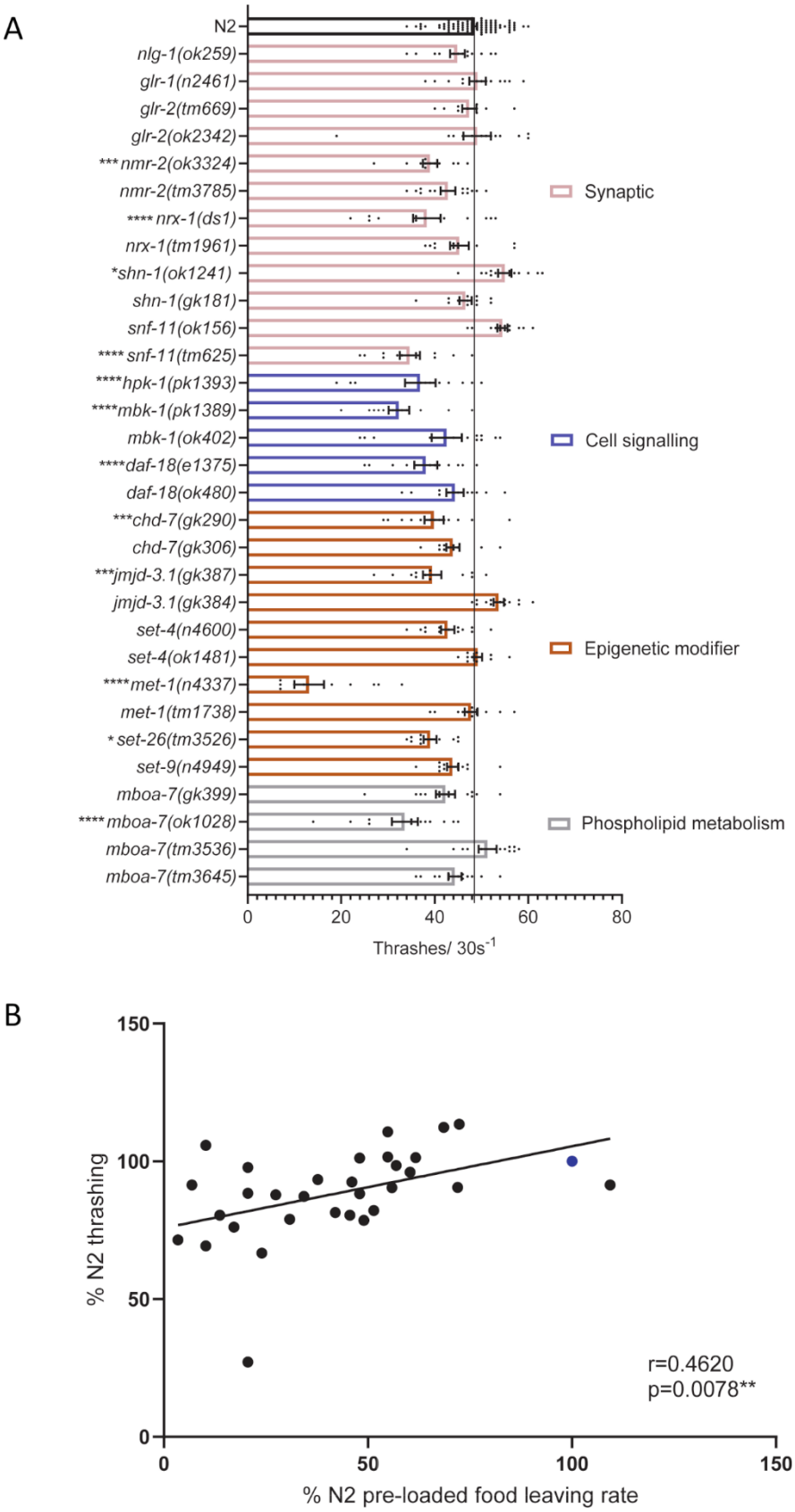


Figure 2.8 Thrashing behaviour of *C. elegans* mutants compared to N2. (A) 5-10 minutes after being picked into liquid medium, *C. elegans* thrashing behaviour was measured for 30 seconds per worm. The black line indicates the thrashes/30s of N2 control. All data shown as mean ±SEM. Statistical analysis performed using a one-way ANOVA

and Dunnetts's multiple comparison test; ns, p>0.05; *, p<0.05; ***, p≤0.001; ****, p≤0.0001. All significance relates to a comparison with N2 control. (B) Correlation between thrashing (Figure 2.8A) and food leaving behaviour on N2 pre-loaded food lawns (Figure 2.7). The percent thrashing rate and food leaving rate on N2 pre-loaded food lawns for *C. elegans* mutants was calculated in comparison to N2. N2 is indicated by the datapoint coloured in blue. All data shown as mean. Statistical analysis performed using Pearson correlation coefficient. For thrashing experiments N2 n=88. All other mutants n=10-13, where n refers to the number of individual animals investigated. Strains were screened in batches across different days. Each batch consisted of 3-8 mutant strains and a paired wild-type control. For food leaving assays N2 and *nlg-1(ok259)* n=16. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control.

The comparison of behaviour on naïve and pre-conditioned lawns also allowed for the analysis of egg laying behaviour in response to progeny. We quantified the number of eggs laid by each mutant on naive and pre-conditioned food lawns after each food leaving assay. Interestingly, all mutants laid the same number of eggs on naïve and pre-conditioned food lawns (Figure 2.9). This shows that progeny exposure modulates food leaving behaviour and not egg laying. This therefore suggests that the circuit which integrates progeny cues to sculpt food leaving motility is independent of egg laying behaviour which is modulated by other environmental cues.

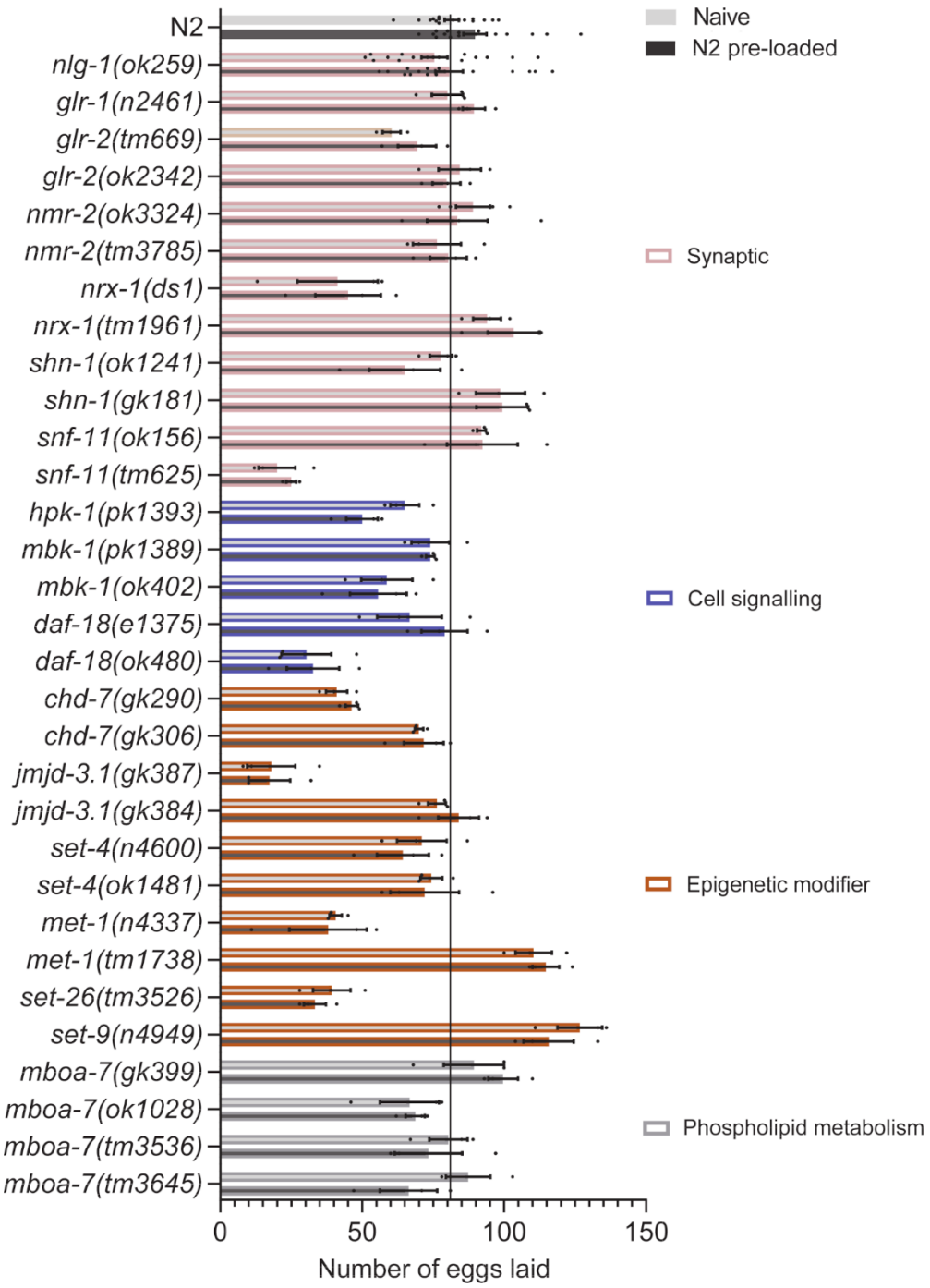


Figure 2.9 The number of eggs laid is unchanged in the presence of progeny. After a food leaving assay the number of eggs laid was quantified. The black line indicates the number of eggs laid by N2 control. All data shown as mean ±SEM. N2 and *nlg-1(ok259)* n=16. All other mutants n=3-4, where n refers to the number of replicates of an individual experiment. Strains were screened in batches across different days. Each batch consisted of 4-6 mutant strains and a paired wild-type control. Data plotted includes all wild-type controls. Statistical analysis performed using a two-way ANOVA and sidak's multiple comparison test. No significance was identified when comparing eggs laid on a naïve and pre-loaded food lawn for each strain.

Overall, starting with 91 human ASD-associated genes we used criteria based filtering to define 21 candidate genes for analysis using a *C. elegans* social paradigm. An initial screen of social behaviour in response to progeny produced over 24 hours indicated 23 mutants with a reduced food leaving phenotype. We then confirmed the veracity of this phenotype using a progeny pre-conditioned food leaving approach and identified mutants that showed a socially impaired phenotype. The limited disruption in other phenotypes tested for these mutants suggests that reduced food leaving is a selective social impairment in response to progeny-derived social cues. Identification of these mutants highlights genetic determinants that appear to play a role in social behaviour and also suggests that a number of biological domains (synaptic, cell signalling, epigenetic modification and phospholipid metabolism) are important for the co-ordination of social behaviour.

2.5 Discussion

ASD is characterised by a triad of behavioural impairments including neuro-atypical behaviour in the social domain (Faras, Al Ateeqi and Tidmarsh, 2010). Individuals with ASD have also been shown to produce altered behavioural responses to a range of chemosensory cues such as olfactory, tactile and gustatory cues (Marco *et al.*, 2011; Bennetto, Kuschner and Hyman, 2007). Multi-sensory processing deficits identified in ASD highlights the importance of sensory integration at a circuit level (Stevenson *et al.*, 2014b) however, it is still unclear how disruption within neural circuits evoke a modified behavioural output. Recent experiments have highlighted the value of investigating molecular determinants of ASD in the context of defined integrative circuits to try and understand more precisely how disruption within these circuits underpins the phenotypes associated with ASD (Kim, Lim and Kaang, 2016; Golden, Buxbaum and Rubeis, 2018; McDiarmid *et al.*, 2019; Calahorro *et al.*, 2019). Approaches, such as these, that better resolve the underlying mechanisms should facilitate pharmacological treatment of ASD and other neuropsychiatric disorders (Sahin and Sur, 2015).

ASD is known to have a complex genetic architecture, with hundreds of genes with varying penetrance implicated in its aetiology (De Rubeis and Buxbaum, 2015). Although the genetic basis is well documented the functional contribution which many of the genes make to the behavioural domains associated with ASD is unclear. Animal models have begun to understand genetic contribution in autism (Crawley, 2012). The analysis of single, high penetrant, variants is becoming increasingly well refined with the use of animal social behaviours. Use of social behaviours underpinned by discrete neural circuits has helped establish the role of some ASD-associated genes in the social domain (Rawsthorne *et al.*, 2020; Silverman *et al.*, 2010). However, the analysis of common, low penetrant, variants is more complex. Additive effects from polygenic interaction

of multiple common variants contributes to widespread disruption at distinct levels of the biological system which is expressed as an emergent behaviour (Iakoucheva, Muotri and Sebat, 2019).

C. elegans have been used in targeted single gene approaches and in screens of ASD-associated genes to provide valuable insight into the role of some genes in sensory processing, development and learning phenotypes (McDiarmid *et al.*, 2019; Calahorro, Alejandre and Ruiz-Rubio, 2009). Recently we have shown the utility of using a social behavioural paradigm in *C. elegans* to investigate a single ASD-associated gene (Rawsthorne *et al.*, 2020). This paradigm is based on inter-organismal signalling by use of chemosensory social cues which results in a progeny-induced food leaving phenotype (Scott *et al.*, 2017). In this study we have used this social paradigm in a screen of ASD-associated genes and identified gene candidates with a role in *C. elegans* social behaviour.

We created a pipeline to prioritise human genes for investigation using *C. elegans* social behaviour. The pipeline identified 84% of the ASD genes ranked in categories 1 and 2 by SFARI have an orthologue in *C. elegans*. In general, the rate of conservation between the human and *C. elegans* genomes is 53% (Shaye and Greenwald, 2011) and suggests an enriched conservation of ASD genes between humans and *C. elegans*. A large proportion of the orthologous genes had known developmental phenotypes and were not selected for further analysis in this study. The analysis of such genes has the potential to provide broader insight into other neuropsychiatric disorders, such as schizophrenia given the overlap of risk genes between this disorder and ASD and the shared neurodevelopmental component of these two conditions. We selected 21 human genes for investigation using 40 *C. elegans* mutant orthologues. Similarities between our prioritisation strategy and those used in previous *C. elegans* studies resulted in the iterative selection of some well-studied ASD-associated genes such as neuroligin and neurexin (Schmeisser, Fardghassemi and Parker, 2017; McDiarmid *et al.*, 2019). Our study is distinct from others because we biased our gene filtering approach to select for *C. elegans* mutants that were appropriate for analysis of social behaviour using a pre-conditioned food leaving approach. We were selective in choosing mutants appropriate for our behavioural analysis, for example the exclusion of overt locomotory mutants due to their possible confounding effect on food leaving motility.

We used a single point analysis focused on progeny induced food leaving from which we also analysed pharyngeal pumping, early development and egg laying capabilities in response to progeny derived social cues. We identified a number of mutants with an altered behavioural response to progeny populating a food lawn providing evidence that *C. elegans* are capable of

modelling disruption to an emergent behaviour in response to mutation to an ASD-associated gene. Where possible more than a single variant for a particular gene of interest was tested. Whilst in the majority of cases there was concordance in the behavioural output of variants, there were some exceptions and this may reflect differences in the number of times strains were outcrossed and potential differences in genetic backgrounds. Refined parametrization of the sub-behaviour that underpins the food leaving behaviour, combined with precise genetic lesion will facilitate the systematic testing of alleles with equivalence to human mutations (Rawsthorne *et al.*, 2020).

Movement in liquid has been used in other studies to screen ASD-associated genes (Schmeisser, Fardghassemi and Parker, 2017). Our analysis of thrashing in mutants identified that this type of locomotory assay does not accurately predict an impaired food leaving behaviour and does not serve as a surrogate for the more complex integrative progeny-induced social behaviour phenotype. This highlights our behavioural screen as a unique platform which is selectively tuned to identify genetic determinants with a role in social circuits, that when disrupted could appear phenotypically normal in thrashing behaviour.

The candidates that we identified as having a role in social behaviour are orthologues of human genes that range in function including synaptic, cell signalling and epigenetic modification. Genes disrupted in each of these domains are known to contribute to ASD (Guang *et al.*, 2018; Rylaarsdam and Guemez-Gamboa, 2019). Therefore, the genes that emerge from our screen are representative of the main functional domains disrupted in autism. Our screen has therefore produced a diverse list of candidate genes that can be used to interrogate the systems level disruption that evokes modified behavioural output in ASD. Previous work has focused largely on locomotory and morphological readouts of altered behavioural phenotypes (Schmeisser, Fardghassemi and Parker, 2017; Wong *et al.*, 2019; McDiarmid *et al.*, 2019) whereas our approach facilitates the identification of candidate genes with a role in a more complex, sensory regulated, emergent behaviour which more closely resembles the social domain disrupted in autism. Identification of candidates using this approach therefore provides a benchmark from which the social circuit can be further dissected (Macosko *et al.*, 2009).

We identified five synaptic genes, *nlg-1*, *nrx-1*, *shn-1*, *glr-1* and *nmr-2,* with a role in coordinating progeny-induced social behaviour. In the mammalian nervous system, NLGN, NRXN and SHANK's interaction at the synapse is well established and dysfunction to all three genes has been widely implicated in ASD (Sudhof, 2008). Synaptic scaffolds including SHANK are known to interact with receptors such as AMPA and NMDA to help regulate the ion channel composition at the synapse (Sheng and Kim, 2011). This provides evidence that this assay for social interaction identifies

behavioural disruption in orthologues of genes that function together at mammalian synapses. The role of these mammalian genes in nervous system function and/or synaptic transmission are functionally conserved in *C. elegans* (Hart, Sims and Kaplan, 1995; Maro *et al.*, 2015; Oh *et al.*, 2011; Tong *et al.*, 2017; Maricq *et al.*, 1995; Kano *et al.*, 2008). This means that we can resolve singular determinants with the potential to unpick genes that encode dysfunctional interactions. This raises the opportunity to model the polygenic nature of ASD (Iakoucheva, Muotri and Sebat, 2019; McDiarmid *et al.*, 2019; Buddell *et al.*, 2019; Genç *et al.*, 2020; Sledziowska, Kalbassi and Baudouin, 2020).

Previous scaled use of assays to investigate ASD-associated genes in *C. elegans* used strategies to prioritise genes before behavioural analysis (Schmeisser, Fardghassemi and Parker, 2017; Wong *et al.*, 2019; McDiarmid *et al.*, 2019). The outcome of these studies resulted in an incomplete overlap of some genes investigated in our study. We made a comparison between *C. elegans* mutants that emerged from our study with an impaired social phenotype to mutants that have emerged from previous studies as having impaired movement and habituation phenotypes (Schmeisser, Fardghassemi and Parker, 2017; McDiarmid *et al.*, 2019). However, the vast majority of mutants that we identified with behavioural impairment are unique to this study. For example, *shn-1(gk181)* and *set-9(n4949)* show impaired social behaviour in response to progeny, whilst appearing grossly wild-type for the other phenotypes we tested. In addition these mutants do not show a behavioural phenotype in movement or habituation behaviour when investigated in previous studies (Schmeisser, Fardghassemi and Parker, 2017; McDiarmid *et al.*, 2019). This highlights that the emergent behaviour we have used reveals genes that are missed when they emerge from the bioinformatic pipeline. This makes the case that applying a lower throughput observer based assay will refine previous efforts to model the functional impact of genes implicated in ASD.

The emergent behaviour that we have used is a complex, sensory integrative behaviour. Habituation learning is another complex behaviour in *C. elegans* that has been investigated in a previous screen of ASD-associated genes (McDiarmid *et al.*, 2019). Therefore, we wanted to identify whether there was overlap in mutants with behavioural impairment in two distinct complex behavioural phenotypes. We made a comparison of mutants that we had identified as having a social impairment to mutants that have been shown to have a habituation phenotype (McDiarmid *et al.*, 2019). We identified four synaptic mutants, *nlg-1(ok259)*, *nrx-1(ds1)*, *glr-1(n2461)* and *nmr-2(ok3324)*, which have impaired social behaviour and have also been shown to have a habituation phenotype. This suggests that these genes may have a role in coordinating more than one complex sensory-regulated behaviour in *C. elegans*. This may also suggest that a key role of synaptic genes is in coordinating higher behaviours in *C. elegans*. With this in mind it

would be interesting to extend the analysis of mutants with habituation impairment and screen them for social deficits. Our approach lends itself to the identification of complex behavioural deficits and so would be valuable in this analysis to further understand if there is an over-representation of synaptic genes in complex sensory integrative phenotypes.

In addition to highlighting the important contribution of synaptic, cell signalling and epigenetic genes, we identify a gene involved in phospholipid metabolism that appears to play a role in progeny-induced social behaviour. *mboa-7* is a lysoPI acetyltransferase which is important in the regulation of phospholipid membranes (Lee *et al.*, 2008) and cell signalling (Lee et al., 2012b) in *C. elegans*. In mammals, the regulation of membrane composition is important for cellular processes, signalling and nervous system function (Volpatti *et al.*, 2019; Raghu *et al.*, 2019). Studies of the MBOAT7 orthologue in mice suggest it may function in brain development (Lee *et al.*, 2012a), however this gene is comparatively less well studied than other ASD-associated genes for its functional contribution to the disorder. Therefore, the identification of this gene with a role in progeny-induced social behaviour highlights how this study enriches the understanding of the molecular determinants of social behaviour from underrepresented genes in autism.

In conclusion, investigation of ASD-associated orthologues in *C. elegans* identified genes from a number of candidates implicated in ASD that disrupt social behaviour in the worm. Identification of these genes highlights how this assay might be used in quantitative approaches that can probe the single (Rawsthorne *et al.*, 2020) and polygenic nature of ASD and its underpinning genetic architecture (McDiarmid *et al.*, 2019). The robust nature of this assay provokes a better detailing of the cellular and circuit dependence of this social interaction. Guided by the cellular determinants of behaviour, investigation can extend to probe the polygenic nature of ASD and take a similar approach in other psychiatric diseases that have significant consequences for behavioural traits in the social domain (St Pourcain *et al.*, 2018; Barak and Feng, 2016).

Chapter 3 Neuroligin dependence of social behaviour in *C. elegans* provides a model to investigate an autism associated gene

Helena Rawsthorne[1], Fernando Calahorro[1], Emily Feist[1], Lindy Holden-Dye[1], Vincent O'Connor[1], James Dillon[1,2,*]

[1] School of Biological Sciences, Highfield Campus, University of Southampton, Southampton, SO17 1BJ, UK

[2] Corresponding author

[*] Correspondence: j.c.dillon@soton.ac.uk

Author contributions. Helena Rawsthorne: Conceptualisation, investigation (all experiments, except those specified), methodology, formal analysis, writing-original draft preparation and editing. Fernando Calahorro: Conceptualisation, investigation (injection of *C. elegans* for CRISPR/Cas9), methodology, resources, supervision, writing- review and editing. Emily Feist: Investigation (collection of data for Figure 3.2D). Lindy Holden-Dye: Conceptualisation, methodology, supervision, writing- review and editing. Vincent O'Connor: Conceptualisation, methodology, supervision, writing- review and editing. James Dillon: Conceptualisation, methodology, supervision, writing- review and editing.

The content of this chapter is that of the paper published in Human Molecular Genetics (Rawsthorne *et al.*, 2020). Any corrections made to the original publication are listed below:

The statement '*nlg-1* Δ#14 represents an isoform of *C. elegans nlg-1* gene that lacks exon 14. This isoform has been shown to be the dominantly expressed isoform in adult worms (Calahorro, Holden-Dye and O'Connor, 2015), hence we chose this isoform for our rescue experiments in which adult worm behaviour would be investigated' has been added to section 3.3.2.

In Figure 3.1A the legend which reads 'N2 naïve' and '*nlg-1(ok259)* naïve' in the published paper has been changed to read 'N2' and '*nlg-1(ok259)*' in this thesis.

The statement 'Data collected by me and Emily Feist' has been added to Figure 3.2 figure legend.

Supplementary Figure 1 in the published paper is Figure 3.5 in this thesis.

3.1 Abstract

Autism spectrum disorder (ASD) is characterised by a triad of behavioural impairments including social behaviour. Neuroligin, a trans-synaptic adhesion molecule, has emerged as a penetrant genetic determinant of behavioural traits that signature the neuro-atypical behaviours of autism. However, the function of neuroligin in social circuitry and the impact of genetic variation to this gene is not fully understood. Indeed, in animal studies designed to model autism there remains controversy regarding the role of neuroligin dysfunction in the expression of disrupted social behaviours. The model organism, *C. elegans,* offers an informative experimental platform to investigate the impact of genetic variants on social behaviour. In a number of paradigms it has been shown that inter-organismal communication by chemical cues regulates *C. elegans* social behaviour. We utilise this social behaviour to investigate the effect of autism associated genetic variants within the social domain of the research domain criteria. We have identified neuroligin as an important regulator of social behaviour and segregate the importance of this gene to the recognition and/or processing of social cues. We also use CRISPR/Cas9 to edit an R-C mutation that mimics a highly penetrant human mutation associated with autism. *C. elegans* carrying this mutation phenocopy the behavioural dysfunction of a *C. elegans* neuroligin null mutant, thus confirming its significance in the regulation of animal social biology. This highlights that quantitative behaviour and precision genetic intervention can be used to manipulate discrete social circuits of the worm to provide further insight to complex social behaviour.

3.2 Introduction

Autism spectrum disorder (ASD) is a pervasive developmental disorder (Baio *et al.*, 2018). It is clinically characterised by a triad of neuro-atypical behaviours that include impaired verbal communication, repetitive behaviours and impaired social interactions. Sensory processing deficits span across the autism spectrum (Sharma, Gonda and Tarazi, 2018). For example, recognition of social cues and multi-sensory integration of those cues are often impaired (Sharma, Gonda and Tarazi, 2018; Endevelt-Shapira *et al.*, 2018). There is a strong genetic association in ASD with hundreds of genes implicated in the disorder (De Rubeis *et al.*, 2014). Many of these genes encode synaptic proteins (Bourgeron, 2015; Giovedi *et al.*, 2014; Pinto *et al.*, 2014), suggesting that synaptic dysfunction underpins the expression of ASD-associated neuro-atypical behaviours (Bourgeron, 2015). However, it is still unclear how genetic variants lead to changes in neural circuits that result in the spectrum of characteristics that are represented in individuals with a recognizable ASD diagnosis. The role of individual genes and the wider complex interaction between polygenic loci make the genetic architecture of ASD complex. This complexity requires

investigation that will ultimately delineate the weight of contribution in genetic backgrounds that range from a single penetrant gene to ones with multiple common variants at multiple loci (Huguet, Ey and Bourgeron, 2013).

One of the synaptic proteins that have been implicated in ASD is neuroligin (NLGN). NLGN is a post-synaptic cell adhesion protein which aids the stabilisation of synaptic function (Sudhof, 2008). One highly penetrant variation which has been shown to affect the NLGN3 gene of autistic individuals is the R451C mutation (Jamain *et al.*, 2003). This missense variant causes a substitution of arginine for cysteine in the extracellular domain of NLGN3 which leads to its misfolding and failure to traffic to the cell surface. It has been suggested that the R451C mutation may result in a loss-of-function and disruption of synaptic function and plasticity within a number of central neural circuits (Etherton *et al.*, 2011). Despite broad investigation it remains unclear as to how penetrant this mutation and further neuroligin mutants are in disrupting social behaviour in animal models. There have been differing reports of disrupted social behaviour in mice where the mutation has been introduced into strains with different genetic backgrounds (Chadman *et al.*, 2008; Tabuchi *et al.*, 2007).

The model organism, *Caenorhabditis elegans,* is a good experimental platform to investigate the effect of ASD-associated genetic variants. *C. elegans* facilitate systems level analysis due to their genetic tractability, simple nervous system and gene homology to humans (Kaletta and Hengartner, 2006; Sonnhammer and Durbin, 1997). Included within this is the conservation of genes involved in synapse maturation and function (Hobert, 2013; Bargmann, 1998; Cherra and Jin, 2015). *C. elegans* encode a single orthologue of mammalian NLGN3 called *nlg-1*, which has been shown to share key structural and functional domains with the human protein (Calahorro and Ruiz-Rubio, 2012; Hunter *et al.*, 2010). This conservation is reinforced by observations showing human NLGN is able to provide functional rescue of a *nlg-1* deficiency in *C. elegans* (Calahorro and Ruiz-Rubio, 2012).

In order to investigate the potential function of *nlg-1* within the social domain, we have utilised a paradigm of inter-organismal communication between *C. elegans* and progeny. The effect of chemosensory stimuli on worm behaviour can be investigated using an assay in which the propensity of an adult worm to leave its food source, a bacterial lawn, is monitored over time. This food leaving assay scores food leaving events in which each event is defined as an occasion when the whole of the worm's body comes off food. This is a behavioural output which can be modulated in response to different cues (Shtonda and Avery, 2006). For example, when exposed to ad libitum source of food, *C. elegans* will remain on the food lawn and perform infrequent food leaving events (Shtonda and Avery, 2006). However, an increase in the number of progeny

populating an otherwise replete food lawn causes a population-dependent increase in food leaving events of adult worms. This progeny induced leaving is not observed in a *daf-22* loss-of-function mutant (Scott *et al.*, 2017) showing that offspring produced ascaroside pheromones modulate adult behaviour. This suggests that inter-organismal signalling from progeny to parents on food replete lawns provides a quantifiable behaviour to investigate the genetic determinants of social circuits (Scott *et al.*, 2017).

We use this assay to show that the ASD-associated gene, NLGN, is an important determinant in regulating *C. elegans* social circuitry. We identify that a genetic change that models the human R451C phenocopies the functional null. In doing this, we facilitate further insight into the genetic underpinnings of social behaviour, a key diagnostic Research Domain Criteria in autism and other psychiatric disorders (https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc/index.shtml).

3.3 Materials and Methods

3.3.1 *C. elegans* culturing and strains used

All *C. elegans* strains were maintained using standard conditions (Brenner, 1974). *C. elegans* were age synchronised by picking L4 day old hermaphrodites onto a new plate 18 hours prior to the behavioural assay. Strains used: Bristol N2; VC228 *nlg-1(ok259) X* (x6 outcrossed); *nlg-1(ok259) X, Ex* [WRM0610dD09 ; P*myo-3*::*gfp*] ; *nlg-1(ok259) X, Ex* [pPD95.77 (P*nlg-1::nlg-1*Δ#14); P*myo-3::gfp*]; *nlg-1(ok259) X, Ex* [pPD95.77; P*myo-3::gfp*]; CRISPR/Cas9 edited XA3780 *nlg-1(qa3780)* (x2 outcrossed). For transgenic animals, stable lines were selected for behavioural analysis.

3.3.2 Rescue construct and transgenic methods

The *nlg-1* fosmid, WRM0610dD09, was provided by SourceBioScience. The *nlg-1* cDNA rescue construct was designed as previously described (Calahorro *et al.*, 2019). Briefly, 2.5kb P*nlg-1* was cloned into the pPD95.77 vector. Subsequently, *nlg-1* Δ#14 cDNA sequence was fused to P*nlg-1*. *nlg-1* Δ#14 represents an isoform of *C. elegans nlg-1* gene that lacks exon 14. This isoform has been shown to be the dominantly expressed isoform in adult worms (Calahorro, Holden-Dye and O'Connor, 2015), hence we chose this isoform for our rescue experiments in which adult worm behaviour would be investigated.

nlg-1(ok259) L4+1 day old worms were microinjected with *nlg-1* fosmid WRM0610dD09 (0.3ng/µl) or *nlg-1* cDNA rescue plasmid (50ng/µl) and the marker plasmid P*myo-3::gfp* (30ng/µl). Control lines were microinjected with marker plasmid P*myo-3::gfp* (30ng/µl).

3.3.3 Behavioural assays

3.3.3.1 Food leaving

5cm NGM plates were prepared using a standard protocol (Brenner, 1974). Plates were seeded with OP50 *E.coli* as described previously (Scott *et al.*, 2017). 50µl of OP50 *E. coli* at OD_{600} of 0.8 was gently spotted on the middle of an unseeded plate the day prior to the assay. For a naive food leaving assay, seven age synchronised L4+1 day old worms were gently picked onto the centre of the bacterial lawn on the assay plate. To pre-condition the food lawn, progeny were loaded onto the bacterial lawn as previously described (Scott *et al.*, 2017). 10 gravid adults were picked onto the bacterial lawn and left to lay 140-200 eggs before being picked off. Approximately 18-hours following this, seven L4+1 day old worms were picked onto the centre of the bacterial lawn. In all food leaving assays the number of food leaving events were counted during 30-minute observations at 2-hours only or 2 and 24-hours. A food leaving event is defined as when the whole of the worm's body comes off the bacterial lawn. For all assays N2 animals were systematically observed in parallel with the strain under investigation. For all behavioural analysis investigators were blind to the genotypes being observed.

3.3.3.2 Body bends

Following a food leaving assay 5-7 worms were selected for body bend quantification. Each worm was observed for 1 minute on food. A body bend was defined as a muscle contraction that resulted in a dorsal or ventral bend of the worm's body.

3.3.4 Genome editing

CRISPR/Cas9 editing was generated using a previously described method (El Mouridi *et al.*, 2017). N2 L4+1 day old hermaphrodites were microinjected with expression vectors for Cas9 and sgRNA's targeting *nlg-1*, *unc-58* and *dpy-10*. The sequence of the *nlg-1* sgRNA, synthesised by Integrated DNA Technologies (IDT) was: 5'-GATTTCGAATTGATTTCGGGTGG-3'. The sequence for *unc-58* and *dpy-10* sgRNA were as previously described (Arribere *et al.*, 2014). The repair templates used for co-CRISPR genes *unc-58* and *dpy-10* were as previously described (El Mouridi *et al.*, 2017). The repair template targeted to *nlg-1* was: 5'-ACGCCTCAAAAATTAAAGGTAGAACATTTATTTCATCATTATAGGACCACCCGAAATCAATTTGCAATGGAGTTCTGAATGCTCTTAGCGACGTACTTTACACCGCACCTCTCATTGAAACATTGCGAAG-3'. The mutated codon is underlined. Repair templates were purchased from IDT. All injection reagents were diluted in water and injected at a final concentration of 50ng/µl. Worms were screened using the co-CRISPR phenotype then recombination of the repair template was screened using restriction

digest and finally recombinant worms were sequenced over the targeted region to identify the mutation. The CRISPR edited line *nlg-1(qa3780)* was outcrossed against the wild-type background used in paired behavioural assays twice.

3.3.5 Protein sequence alignment

Multiple protein sequence alignment of *C. elegans* NLG-1 and human NLGN1-4 was performed using the Clustal W method. *C. elegans* NLG-1 sequence was downloaded from WormBase version WS274. The longest NLG-1 isoform (C40C9.5e) was used for analysis. Human NLGN1-4 sequences were downloaded from NCBI. Accession numbers for the sequences used are as follows: NLGN1, NP_001352856; NLGN2, XP_005256801; NLGN3, NP_061850; NLGN4, AAQ88925.

3.4 Results

3.4.1 The importance of *nlg-1* in modulating social communication in adult worms

Two of the major characteristics of ASD are impaired social behaviour and deficits to multi-sensory integration (Sharma, Gonda and Tarazi, 2018). It has been previously shown that *C. elegans* elicit a social response to progeny populating a food lawn (Scott *et al.*, 2017). More specifically, in response to increasing numbers of progeny on food, wild-type (N2) worms show a population-dependent increase in food leaving in the presence of an otherwise replete food lawn. This progeny enhanced food leaving behaviour was shown to be the result of chemosensory social signalling between progeny and adult worms (Scott *et al.*, 2017). We have used this social behaviour to investigate the autism associated gene, NLGN3. To investigate the behaviour of *nlg-1(ok259)* null mutants in response to progeny, worms were picked onto the centre of a bacterial lawn and food leaving events were counted at 2 and 24 hours. At 2 hours no progeny are present on the food lawn. In comparison, at 24 hours progeny will have accumulated on the food lawn. Therefore, quantifying food leaving events at these time points allows for a direct comparison of food leaving behaviour in the absence and presence of progeny.

Observing food leaving in N2 adults showed an increase in leaving events over time. After 2 hours, when no progeny were present, worms remained on the food lawn (Figure 3.1A). At 24 hours, when the food lawn is populated with progeny, N2 display progeny enhanced food leaving behaviour, in which they show a 12-fold increase in the number of food leaving events (Figure 3.1A). *nlg-1(ok259)* mutants remained on food, similar to N2, after 2 hours on the food lawn (Figure 3.1A). However, at 24 hours, when the food lawn is dense with progeny, *nlg-1(ok259)* leaves the food patch less than N2 (Figure 3.1A). *nlg-1(ok259)* did not show the same degree of

progeny enhanced food leaving as N2, suggesting that their social interaction with progeny may be impaired.

We next wanted to understand if the reduced food leaving behaviour of *nlg-1(ok259)* was due to impaired social interaction with progeny. We reasoned that reduced food leaving could also result if *nlg-1(ok259)* had an egg laying deficiency. Previous studies had shown that the amount of food leaving was directly related to the number of progeny, with a reduced progeny accumulation giving a reduced food leaving (Scott *et al.*, 2017). To test this, the number of eggs laid and the number of progeny present on the food lawn was counted after each food leaving assay. For both N2 and *nlg-1(ok259)* the total number of eggs laid (Figure 3.1B) during the assay, and number of progeny present at 24-hours were counted (Figure 3.1C) and there was no difference between the strains. These data show the reduced food leaving of *nlg-1(ok259)* cannot be explained by reduced progeny available to drive adult food leaving. Furthering this, no difference was seen in the number of body bends for *nlg-1(ok259)* (Figure 3.1D) indicating that locomotion per se does not underlie the reduced food leaving of *nlg-1(ok259)*. Thus, the deficit in progeny enhanced food leaving behaviour of *nlg-1(ok259)* appears due to impaired social interaction of the mutant with progeny.

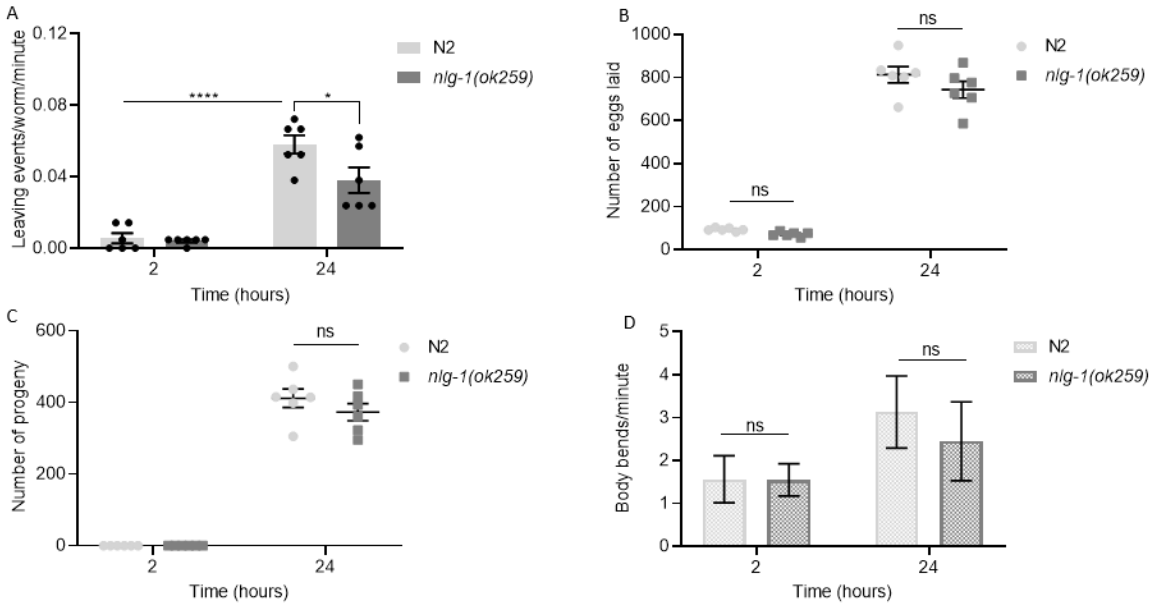


Figure 3.1 *nlg-1(ok259)* adults show deficient food leaving in response to progeny. (A) A food leaving assay was performed with N2 and *nlg-1(ok259)* null mutant. N2 and *nlg-1(ok259)* adults were picked onto the centre of a bacterial lawn before food leaving events were counted at 2 and 24 hours. N2 and *nlg-1(ok259)* n=6. (B and C) the number of eggs (B) and progeny (C) for N2 and *nlg-1(ok259)*. N2 and *nlg-1(ok259)* n=6. (D) The number of body bends per minute performed by N2 and *nlg-1(ok259)* worms on food at 2 and 24-hours. N2 n=6. *nlg-1(ok259)* n=4. All data shown as mean

±SEM. Statistical analysis performed using Two-way ANOVA with Sidak's multiple comparison test; ns P≥0.05, * P<0.05, **** P≤0.0001.

Next, we wanted to investigate whether the reduced food leaving described above for *nlg-1(ok259)* was due to an impaired social interaction with progeny. To this end, we used a pre-conditioned food lawn, as previously described (Scott *et al.*, 2017). Pre-loading progeny onto a food lawn before assaying the adult food leaving events preconditions the lawn with chemosensory cues released by progeny. This recapitulates the progeny dense conditions worms are exposed to after 24-hours on the food lawn but the assayed adults undergo more acute exposure to the progeny (Figure 3.2A).

The pre-conditioning with N2 progeny results in enhanced food leaving in N2 adults compared to the naive control at 2 hours (Figure 3.2B). This is consistent with previous findings which showed that social interaction of adult worms and progeny on pre-conditioned food lawns results in enhanced food leaving in N2 *C. elegans* (Scott et al., 2017). In comparison, *nlg-1(ok259)* adults when exposed to plates preconditioned with N2 progeny did not show enhanced food leaving. They left infrequently on both naïve and pre-conditioned food lawns at 2-hours (Figure 3.2B). This confirms that *nlg-1(ok259)* have reduced food leaving in response to progeny compared to N2. Therefore, this suggests that NLG-1 may be an important regulator of chemosensory driven social interaction in *C. elegans*. To confirm the importance of NLG-1 in the social circuit of the worm we generated two transgenic rescue lines expressing either *nlg-1* gDNA or *nlg-1* cDNA in the *nlg-1(ok259)* background. In response to pre-loaded N2 progeny both the rescue lines expressing *nlg-1* gDNA and cDNA showed progeny enhanced food leaving, similar to that of N2 (Figure 3.2 C and D). This shows that expression of either *nlg-1* in its genomic or cDNA form can rescue the reduced food leaving of *nlg-1(ok259)* mutants in response to progeny. Together, these data suggest that *nlg-1(ok259)* mutants are impaired in their ability to modulate food leaving behaviour in the presence of progeny and that NLG-1 may play an important role in regulating this social behaviour.

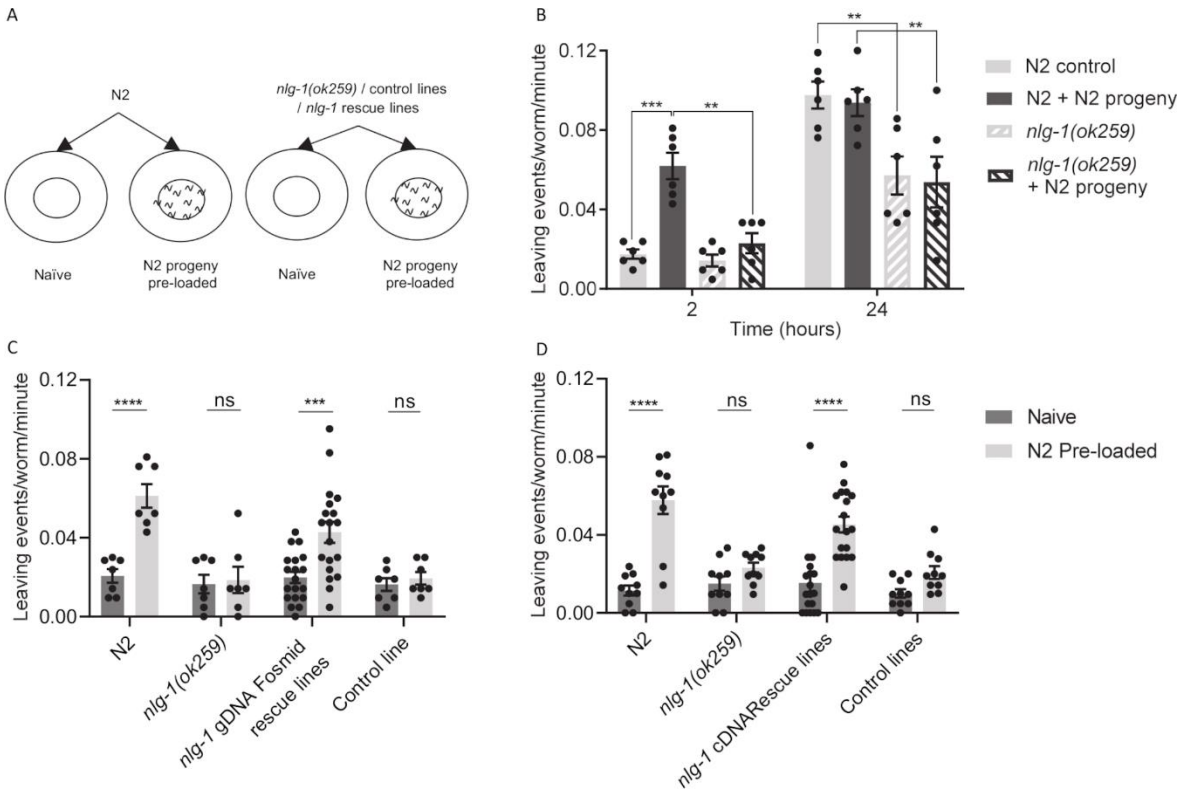


Figure 3.2 *nlg-1(ok259)* show a deficit in social interaction when exposed to N2 progeny. (A) Cartoon showing the principles of the pre-conditioned food leaving assay. In order to test the effect of progeny on food leaving behaviour, *C. elegans* adults were picked onto either a naïve or pre-conditioned food lawn. A naïve lawn contains no progeny whereas a pre-conditioned food lawn contains ~140 N2 progeny. (B) N2 and *nlg-1(ok259)* were picked onto naïve and pre-conditioned food lawns and their food leaving behaviour observed at 2 and 24 hours. N2 and *nlg-1(ok259)* n=6. Two-way ANOVA with Tukey's multiple comparison test; ** P≤0.01, *** P≤0.001. (C) The number of food leaving events of N2, *nlg-1(ok259)*, *nlg-1* gDNA fosmid rescue *nlg-1(ok259) X, Ex* [WRM0610dD09 ; P*myo-3::gfp*] and *nlg-1(ok259) X, Ex* [pPD95.77;P*myo-3::gfp*] control adults on naïve and pre-conditioned food lawns. N2, *nlg-1(ok259)* and *nlg-1(ok259) X, Ex* [pPD95.77; P*myo-3::gfp*] control line n=7, *nlg-1(ok259) X, Ex* [WRM0610dD09 ; P*myo-3::gfp*] n=19. Two-way ANOVA with Sidak's multiple comparison test; ns P≥0.05, *** P≤0.001, **** P≤0.0001. (D) The number of food leaving events of N2, *nlg-1(ok259)*, *nlg-1* cDNA rescue *nlg-1(ok259) X, Ex* [pPD95.77 (P*nlg-1::nlg-1 Δ#14*); P*myo-3::gfp*] and *nlg-1(ok259) X, Ex* [pPD95.77; P*myo-3::gfp*] control line on naïve and pre-conditioned food lawns. N2, *nlg-1(ok259)* and *nlg-1(ok259) X, Ex* [pPD95.77; P*myo-3::gfp*] control line n=10. *nlg-1(ok259) X, Ex* [pPD95.77 (P*nlg-1::nlg-1 Δ#14*); P*myo-3::gfp*] n=18. Data collected by me and Emily Feist. Conditioning assays were performed as paired experiments and data obtained

from independent replicas is presented. Two-way ANOVA with Tukey's multiple comparison test; ns P≥0.05, **** P≤0.0001. All data shown as mean ±SEM.

We next wanted to segregate whether NLG-1 is important for the production and/or release of social cues from progeny or the recognition and/or integration of the social cue in the adult worm. To do this we pre-conditioned food lawns with either N2 or *nlg-1(ok259)* progeny. Food leaving behaviour was then observed for N2 and *nlg-1(ok259)* adults in response to either N2 or *nlg-1 progeny* (Figure 3.3A). In this way we were able to investigate whether *nlg-1(ok259)* mutant progeny are capable of driving enhanced food leaving behaviour in adult worms, hence informing on their ability to produce / release chemical social cues.

N2 adults showed enhanced food leaving behaviour in the presence of both N2 and *nlg-1(ok259)* progeny (Figure 3.3B). This suggests that both N2 and *nlg-1(ok259)* progeny are capable of driving enhanced food leaving behaviour in N2 adults. In turn, this suggests that *nlg-1(ok259)* mutant progeny are capable of producing and releasing chemical social cues in order to stimulate enhanced food leaving in adults. In comparison, *nlg-1(ok259)* adults do not show enhanced food leaving in response to either progeny relative to the naïve control at 2-hours (Figure 3.3B). Taken together, these results suggest that N2 and *nlg-1(ok259)* progeny are not impaired in the production and release of social cues. Furthermore, these results are consistent with the hypothesis that *nlg-1(ok259)* adults have impaired social communication with progeny which may involve deficits in recognition and/or integration of progeny derived social cues.

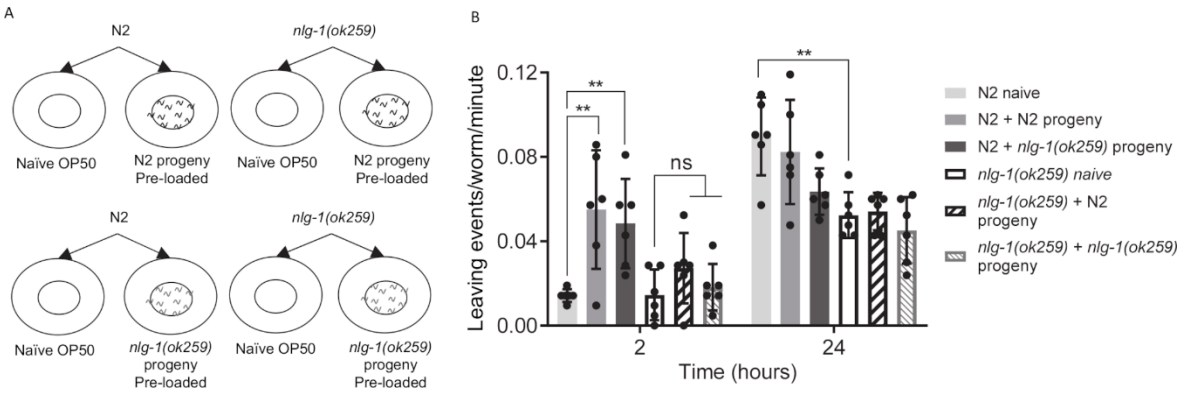


Figure 3.3 Parental *nlg-1* is required for recognition and/or processing of progeny derived social cues. (A) Experimental setup. N2 and *nlg-1(ok259)* were placed onto a naïve OP50 bacterial lawn or a lawn preconditioned with N2 or *nlg-1(ok259)* progeny. (B) The number of food leaving events of N2 and *nlg-1(ok259)* adults on naïve and pre-conditioned food lawns. Data are mean ±SEM. N2 and *nlg-1(ok259)* n=6. Conditioning assays were performed as paired experiments and data obtained from independent replicas is presented. Two-way ANOVA with Tukey's multiple comparison test; ns P≥0.05, ** P≤0.01.

3.4.2 *C. elegans* carrying R433C mutation phenocopy the social impairment of *nlg-1* null

So far we have shown that the *nlg-1(ok259)* null mutant does not show progeny driven enhanced food leaving behaviour and we hypothesise that this behavioural deficit is specific to the recognition and/or integration of social cues in the adult worm. In this way, *nlg-1(ok259)* mutants are modelling neuro-atypical behaviour in the social domain. Considering this, we next wanted to know whether *C. elegans* could model the same social impairment but in response to a specific human genetic variation implicated in autism. We investigated a penetrant missense variant which has been identified in the NLGN3 gene of individuals on the Autistic spectrum. The R451C mutation results in an R-C amino acid substitution within the extracellular cholinesterase-like domain of NLGN3 (Jamain *et al.*, 2003). *C. elegans* encode a conserved arginine within the cholinesterase-like domain of NLG-1 which is present in human NLGN1-4 (Figure 3.4A). Using CRISPR/Cas9 the R451C mutation was generated in *C. elegans* by editing an R-C substitution at position 433 within the cholinesterase-like domain of NLG-1 (Figure 3.4A). The bona fide nature of this CRISPR event was confirmed by genomic sequencing which identified that the CGA codon of the wild type was converted to TGC encoding cysteine in the mutant line (Figure 3.5). The conservation in sequence suggests that the same disruption in NLGN structure that arises in the human protein with this mutation at position 451 will be replicated in *C. elegans* with the orthologous mutation at 433 (based on NLG-1 isoform C40C9.5e). The social behaviour of the CRISPR generated line, *nlg-1(qa3780),* was investigated using lawns that were pre-conditioned with N2 progeny in order to compare the response of CRISPR generated *nlg-1(qa3780)* to *nlg-1(ok259)* null mutant in response to N2 derived social cues.

Consistent with our previous findings, N2 adults show enhanced food leaving in response to progeny on pre-conditioned food lawns (Figure 3.4B). Furthermore, *nlg-1(ok259)* do not show enhanced food leaving in the presence of progeny (Figure 3.4B). The response of *nlg-1(qa3780)* mutants, carrying the R433C mutation, is very similar to that of *nlg-1(ok259)* null mutants. *nlg-1(qa3780)* showed no enhanced food leaving when exposed to progeny (Figure 3.4B). This suggests that the single R433C missense variant to *nlg-1* results in social impairment which phenocopies that of the *nlg-1* null mutant. Furthering this, these results suggest that *C. elegans* can model social impairment in response to autism associated human genetic variants.

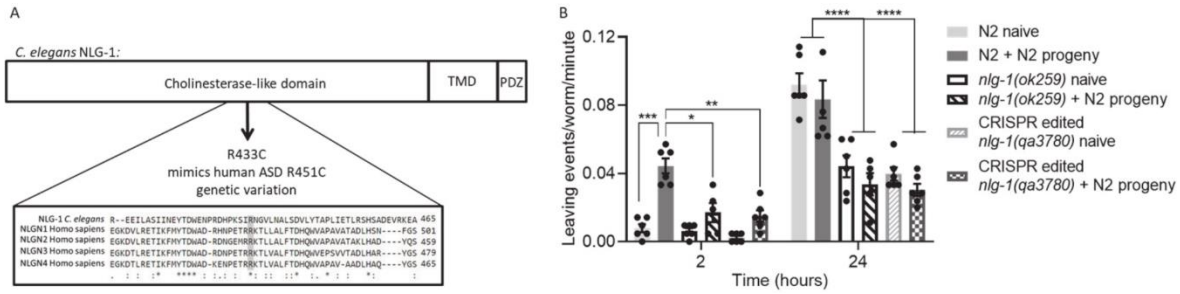


Figure 3.4 R433C mutation in *nlg-1* phenocopies social impairment of *nlg-1* null. (A) Domain structure of NLG-1 indicating the arginine to cysteine (R-C) amino acid substitution at position 433 generated within the cholinesterase like domain following CRISPR/Cas9. Protein sequence alignment of *C. elegans* NLG-1 and human NLGN1-4 indicates the arginine residue involved in ASD and its conservation in *C. elegans* (shaded in grey). '*' indicates conservation of a single amino acid residue, ':' indicates conservation between amino acid groups with similar properties and '.' indicates conservation between amino acid groups with weakly similar properties. (B) The number of food leaving events were counted for N2, *nlg-1(ok259)* and CRISPR generated *nlg-1(qa3780)* on naïve and N2 progeny conditioned food lawns. Data are mean ±SEM. N2, *nlg-1(ok259)* and *nlg-1(qa3780)* n=6. Two-way ANOVA with Tukey's multiple comparison test; * P<0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

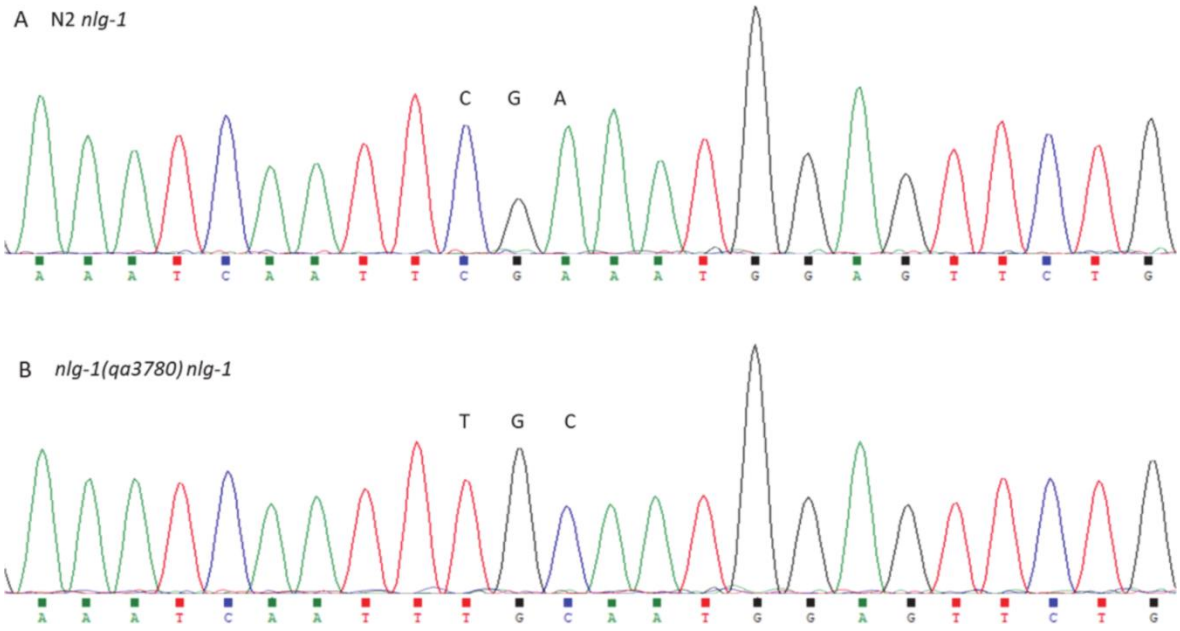


Figure 3.5 CRISPR generated *nlg-1(qa3780)* nucleotide edit. (A) Chromatogram showing partial N2 *nlg-1* DNA sequence. The codon of interest, CGA, to be edited by CRISPR is indicated. (B) Chromatogram showing partial *nlg-1(qa3780) nlg-1* DNA sequence. The edited codon, TGC, is indicated. Sequence is displayed in the 5' to 3' orientation.

3.5 Discussion

ASD causes neuro-atypical social behaviour and deficits in sensory processing (Sharma, Gonda and Tarazi, 2018). ASD has a well characterised genetic dependence, with the underlying determinants becoming increasingly well resolved with the use of quantitative genetics. There is a range of determinants that imply polygenic interaction between common variation of the genome. In addition, there are a number of more penetrant single gene mutations that are identified as contributing to the expression of behavioural traits that signature neuro-atypical behaviour of ASD. Many of these penetrant genes encode synaptic proteins (De Rubeis *et al.*, 2014) highlighting synaptic dysfunction as orchestrating the phenotypes associated with ASD. In the case of penetrant genes, animal experiments utilizing functional nulls or engineered mutants designed to mimic human mutations have facilitated investigation of the cellular circuit and system level mechanisms that disrupt behavioural domains that model ASD-associated behaviour (Crawley, 2012). In this study we have shown that *C. elegans* can be used to model social impairment in response to genetic models of the human variants identified in individuals with an ASD diagnosis. This is highlighted by our investigation and comparison of the single neuroligin gene in *C. elegans* that has high conservation with the 5 human neuroligin genes, particularly the NLGN3 gene that has been strongly implicated in ASD (Calahorro and Ruiz-Rubio, 2012; Calahorro, Alejandre and Ruiz-Rubio, 2009).

NLGNs are a family of synaptic adhesion proteins required for synaptic maturation through interaction with synaptic adhesion partners. Of these, the most studied interaction is between neuroligin and neurexin which can co-ordinate the localization of receptors at inhibitory synapses (Graf *et al.*, 2004). It has been shown in *C. elegans* that this interaction and its function in the organisation of receptors is conserved at the neuromuscular junction of the worm (Maro *et al.*, 2015). The neuroligin functional null used in our study has been previously reported as having defects in synaptic transmission at the neuromuscular junction, in the absence of an obvious locomotory phenotype (Maro *et al.*, 2015). We reached a similar finding, that the neuroligin null mutation did not impair locomotion. It has previously been shown that the *nlg-1* knockout has a hypo-reversal phenotype (Hunter *et al.*, 2010). Hence, we do not think that the decreased food leaving behaviour observed in the *nlg-1* mutants in our study can be explained by a hyper-reversal phenotype or excessive dwelling. We propose that the difference in food leaving behaviour of the null cannot be explained by impaired motor control but are mindful that there may be differences in the relative profile of more subtle locomotory sub-behaviours that underpin the food leaving behaviour in response to social cues.

The clinical data for the NLGN3 R451C mutation suggests it causes severe autism and Asperger's syndrome in a sibling carrying the mutation (Jamain *et al.*, 2003). This motivated animal experiments in which the functional null and R451C mutation were compared. These mutants exhibit cellular changes selective for inhibitory synapses that disrupt plasticity and cause changes in learned behaviours associated with social interaction and motor control (Chadman *et al.*, 2008; Tabuchi *et al.*, 2007; Rothwell *et al.*, 2014). In the former case there is a controversy as to how penetrant the mutations that mimic the human mutation are in causing a mutation dependent change in social interaction. The differential conclusions derived from mice strains raised in distinct genetic backgrounds suggests that background modifiers can affect the phenotypic output of the genetic mutation being investigated (Chadman *et al.*, 2008; Tabuchi *et al.*, 2007). In this study we used a neuroligin null mutant and a CRISPR/Cas9 line in the same wild-type N2 genetic background to compare the effects of the mutation on social behaviour.

Distinct cues and neural circuits regulate social behaviour in humans, both in terms of their nature and complexity (Bergan, 2015). The behavioural observations reported in this study are underpinned by a previously characterised progeny dependent inter-organismal communication that drives changes in adult behaviour and provides a basis for understanding the molecular determinants of a social behaviour in *C. elegans* (Scott et al., 2017). Our results show that *nlg-1(ok259)* adults have impaired food leaving, caused by impaired social communication with progeny. We are aware that food leaving is a complex behaviour and consider that the reduced food leaving response observed in our experimental system is not defined by deficient responses to other environmental sensory cues described elsewhere (Shtonda and Avery, 2006; Milward *et al.*, 2011). This deficit in *nlg-1(ok259)* shows NLGN's synaptic organising function may play a key role in the neural circuits that underlie social interaction. We provide evidence that neuroligin-dependent signalling is required in the adult for their ability to respond to the chemical cues generated by the progeny. This assay thus provides a quantitative measure for neuroligin function in the adult. We used this to probe the integrity of the circuits driving this output utilizing a CRISPR/Cas9 mutant that models the penetrant human ASD mutation R451C. Our data show that this mutation phenocopies the deficit in food leaving seen in the null mutation which is consistent with the widely held view that this mutation is a loss-of-function (Singh and Eroglu, 2013). Taken together our data imply that *nlg-1* plays an important role in the circuits that organise social interaction in *C. elegans*. This would reinforce a conserved role for this class of adhesion molecule in organizing circuits that underpin social behaviour. Previous work has identified *nlg-1* function in the animal's response to a number of environmental cues including food, chemicals and temperature. Interestingly, disruption at the input level of sensory cues is an important emerging theme in human autism and future work will be required to address if the sensitivity to

pheromones per se is the major determinant of the observed disruption. Equally, work in *C. elegans* has implicated changes in the balance of excitation and inhibition down stream of sensory inputs generating behavioural disruption in *C. elegans* so the results here may arise through an essential role for neuroligin at several levels of the social circuit responsible for the adult response to progeny induced food leaving (Zhou *et al.*, 2017; Chalasani *et al.*, 2016).

Previous studies have used *C. elegans* morphology and locomotion as readouts of disrupted behaviour in response to mutation to ASD-associated genes (McDiarmid *et al.*, 2019; Wong *et al.*, 2019). We have extended this analysis to inform on the effect of a neuroligin variant on a phenotype that relates to one of the triad of impairments that make up the diagnostic criteria of ASD. The social interaction assay used in this study allows for quantification of ASD-associated variants and their effect on social behaviour. The gene homology of *C. elegans* with mammalian systems, conservation of synaptic architecture and requirement for sensory-motor integration in the face of environmental cues provides a tractable paradigm for these investigations. Overall, the assay of social interaction in *C. elegans* has the potential to provide crucial insight into the neural circuits that underpin social behaviour and how genetic variants impact on those circuitries. This suggests it provides a robust platform to screen other genes implicated in autism and complex psychiatric disorders that exhibit underpinning disruption of the social domain.

Chapter 4 Confounds of using the *unc-58* selection marker highlights the importance of genotyping co-CRISPR genes

Helena Rawsthorne-Manning[1], Fernando Calahorro[1], Patricia Gonzalez Izquierdo[1], Lindy Holden-Dye[1], Vincent O'Connor[1], James Dillon[1*]

[1] School of Biological Sciences, Highfield Campus, University of Southampton, Southampton, SO17 1JB, United Kingdom

[*] Corresponding author: j.c.dillon@soton.ac.uk

Author contributions. Helena Rawsthorne: Conceptualisation, investigation (all experiments), methodology, formal analysis, writing-original draft preparation and editing. Fernando Calahorro: Conceptualisation, investigation (design of *nlg-1* gRNA, supervision in the design of CRISPR/cas9 reagents and injection of *C. elegans* for CRISPR/Cas9), methodology, resources, supervision, writing- review and editing. Patricia Gonzalez Izquierdo: Methodology, writing- review and editing. Lindy Holden-Dye: Conceptualisation, methodology, supervision, writing- review and editing. Vincent O'Connor: Conceptualisation, methodology, supervision, writing- review and editing. James Dillon: Conceptualisation, methodology, supervision, writing- review and editing.

The content of this chapter is that of the pre-print manuscript available on bioRxiv (Rawsthorne-Manning *et al.*, 2021). Any corrections made to this pre-print manuscript are listed below:

Supplementary figure 'Figure S1' in the pre-print manuscript is Figure 4.3 in this thesis.

4.1 Abstract

Multiple advances have been made to increase the efficiency of CRISPR/Cas9 editing using the model genetic organism *Caenorhabditis elegans* (*C. elegans*). Here we report on the use of co-CRISPR 'marker' genes: worms in which co-CRISPR events have occurred have overt, visible phenotypes which facilitates the selection of worms that harbour CRISPR events in the target gene. Mutation in the co-CRISPR gene is then removed by outcrossing to wild type but this can be challenging if the CRISPR and co-CRISPR gene are hard to segregate. However, outcrossing can be avoided by selecting worms of wild type appearance from a 'jackpot' brood. These are broods in which a high proportion of the progeny of a single injected worm display the co-CRISPR phenotype suggesting high CRISPR efficiency. This can deliver worms that harbour the desired

mutation in the target gene locus without the co-CRISPR mutation. We have successfully generated a discrete mutation in the *C. elegans nlg-1* gene using this method. However, in the process of sequencing to authenticate editing in the *nlg-1* gene we discovered genomic rearrangements that arise at the co-CRISPR gene *unc-58* that by visual observation were phenotypically silent but nonetheless resulted in a significant reduction in motility scored by thrashing behaviour. This highlights that careful consideration of the hidden consequences of co-CRISPR mediated genetic changes should be taken before downstream analysis of gene function. Given this, we suggest sequencing of co-CRISPR genes following CRISPR procedures that utilise phenotypic selection as part of the pipeline.

4.2 Introduction

Originally identified as a mechanism of bacterial immunity (Mojica *et al.*, 2005), CRISPR/Cas9 is now a widely used genome editing technique that allows for precise and customisable DNA modification (Jinek *et al.*, 2012). Cas9 endonuclease cleavage of DNA results in a double strand break which can then be repaired via homology directed repair (HDR) or non-homologous end joining (NHEJ) pathways. HDR can be utilised to generate precise DNA edits because it allows for the integration of an exogenous repair template into the gene of interest (Adli, 2018). In contrast, NHEJ can create noncontrolled insertions and deletions into a sequence and is therefore often used to generate disruptive genetic mutations that aim to knockout gene function (Hsu, Lander and Zhang, 2014). The ability to precisely manipulate DNA in this way has seen CRISPR/Cas9 facilitate insight into gene function in multiple model organisms (Ma and Liu, 2015).

C. elegans is an attractive model organism for the application of CRISPR/Cas9 because their genetic tractability and short life cycle allows for the rapid generation of progeny with cloned genetic identity (Kaletta and Hengartner, 2006). Systems level analysis of gene function in *C. elegans* means CRISPR mediated modifications can be investigated at a molecular and behavioural level (Rawsthorne *et al.*, 2020; McDiarmid *et al.*, 2019; Wong *et al.*, 2019; Greene *et al.*, 2016b; López-Cruz *et al.*, 2019). Furthermore, due to the genetic homology of mammalian and *C. elegans* genomes (Sonnhammer and Durbin, 1997), CRISPR/Cas9 has been used to investigate specific human disease related mutations (Rawsthorne *et al.*, 2020; Wong *et al.*, 2019; McDiarmid *et al.*, 2019).

Despite its advantages, the efficiency of CRISPR/Cas9 can be limited by a number of factors. For example, the interaction of the single guide RNA (sgRNA) Cas9 complex with target DNA requires Cas9 to bind to a protospacer adjacent motif (PAM) (Dickinson and Goldstein, 2016). It has been shown that the closer the PAM to the target site the better the editing efficiency (Paix *et al.*,

2014). However, the prevalence of PAM sites has been shown to be scarce within some *C. elegans* genes (El Mouridi *et al.*, 2017) thus limiting the regions of the genome that can be targeted efficiently. A method developed to try and negate this issue involves the transplantation of a d10 sequence into the gene of interest prior to generating the desired edit. The d10 sequence contains a protospacer and PAM from the endogenous *dpy-10 C. elegans* gene. Transplantation of the necessary motifs for efficient CRISPR/Cas9 therefore renders the gene of interest more amenable to editing and hence enhances the scope of CRISPR in *C. elegans* (El Mouridi *et al.*, 2017).

Another technique used to increase the efficiency of the CRISPR process in *C. elegans* is the use of co-CRISPR genes (Arribere *et al.*, 2014; Kim *et al.*, 2014). A co-CRISPR gene is a gene which is subjected to gene editing simultaneously with the target gene of interest. Upon mutation, co-CRISPR target genes result in a visibly obvious, marker phenotype which can be easily distinguished from worms with a wild-type appearance. The marker phenotype provides a visual representation of CRISPR efficiency and therefore minimises the number of progeny that need to be sequenced to identify the desired edit. Multiple co-CRISPR genes have been identified, including *dpy-10, rol-6, sqt-1* and *unc-58* (Arribere et al., 2014; Kim et al., 2014). Mutations in *dpy-10* result in dumpy, roller or dumpy roller phenotypes, depending on the mutant genotype. Mutations in *rol-6* and *sqt-1* result in roller phenotypes causing the worms to move in a circular pattern (Arribere *et al.*, 2014). *unc-58* encodes a two-pore domain potassium channel (K2P) in *C. elegans* (Salkoff, 2005) which plays an important role in maintaining neuronal and muscle cell excitability (Salkoff *et al.*, 2001; Reiner, Weinshenker and Thomas, 1995). A prime function of the protein encoded by *unc-58* is to act as a leak channel that is important in setting the resting membrane potential (Kasap *et al.*, 2018). A gain-of-function (GOF) mutation in *unc-58(e665)* fourth transmembrane helix results in a hypercontracted and uncoordinated phenotype which leaves the worm immobile (Brenner, 1974; Park and Horvitz, 1986). Interestingly, it is hypothesised that this hypercontraction results from a change in the selectivity of the channel from K^+ to Na^+ (Kasap *et al.*, 2018). This pronounced behavioural change provides a simple binary readout of the integrity of the *unc-58* locus. In particular, the switch from immobile to motile provides an obvious measure of reversion from GOF to WT. The easily recognisable phenotype of *unc-58* GOF mutants has led to its successful widespread use in technologies that are aided by phenotypic markers (Kasap and Dwyer, 2020; Huumonen *et al.*, 2012; Hartman *et al.*, 2014) and this has recently been extended to co-CRISPR techniques (Arribere *et al.*, 2014; El Mouridi *et al.*, 2017; Wang *et al.*, 2018a).

Easily recognisable co-CRISPR phenotypes can also be used to identify so called jackpot broods. Jackpot broods are a population of progeny derived from a single parent, injected with Cas9,

sgRNAs and repair templates to facilitate CRISPR at both a target gene and co-CRISPR gene, in which a high percentage of the population displays the co-CRISPR marker phenotype (Paix *et al.*, 2015). A large number of co-CRISPR marked progeny provides a visual representation of high efficiency CRISPR editing at the co-CRISPR gene and is therefore indicative of high CRISPR efficiency at the target gene of interest (Arribere et al., 2014; Kim et al., 2014). co-CRISPR marked worms from jackpot broods are therefore selected for sequencing for the mutation of interest. Within jackpot broods there are also siblings that do not display the easily recognisable co-CRISPR phenotype. Whilst it can be assumed, based on the phenotype, that these unmarked siblings are WT for the co-CRISPR loci they can still carry the desired CRISPR mutation in the gene of interest, albeit at a lower frequency than in the progeny that show the co-CRISPR phenotype (Paix *et al.*, 2014). It can therefore be beneficial to sequence some apparently wild type siblings from high efficiency jackpot broods for the mutation of interest. For example, it has been suggested that if the gene of interest is located on the same chromosome as the co-CRISPR gene then unmarked siblings should be selected for sequencing to avoid the need to backcross the co-CRISPR mutation, which would be complicated for genes that are chromosomally linked (El Mouridi *et al.*, 2017). It is noteworthy that when selecting marked and unmarked progeny, the integrity of the co-CRISPR gene is assumed based on the worms' phenotype and therefore is rarely sequenced as part of the CRISPR pipeline (El Mouridi *et al.*, 2017; Wang *et al.*, 2018a; Schreier *et al.*, 2020).

Recently, a method of CRISPR/Cas9 has been developed that combines multiple techniques to increase the efficiency of CRISPR and allows for highly targeted editing in *C. elegans*. The method encompasses a two-step procedure which uses a d10 sequence to facilitate the generation of an edit in a precise location within the gene of interest and uses co-CRISPR marker genes for rapid phenotypic screening (El Mouridi *et al.*, 2017). We utilised this method with the aim of mimicking an autism associated missense variant, R451C in neuroligin (Jamain *et al.*, 2003), in order to investigate the mutant in a motility based food leaving assay that scores social behaviour (Rawsthorne *et al.*, 2020). This first involved transplantation of a d10 sequence into the *C. elegans nlg-1* gene. Simultaneously, the co-CRISPR gene *unc-58* was edited to generate a GOF mutation that results in an uncoordinated phenotypic marker for visual screening of CRISPR efficiency. Following this, in a second round of CRISPR editing, the d10 sequence in *nlg-1* was replaced by a repair template containing the sequence for the desired R451C mutation. Again, simultaneously another co-CRISPR gene, *dpy-10*, was edited to generate *dpy-10(cn64)* GOF mutant for the purpose of phenotypic screening.

In this study, we describe an unexpected CRISPR mediated mutation that occurred within the co-CRISPR gene *unc-58*. The resulting mutants did not display the hypercontraction characteristic of the *unc-58* GOF but rather resulted in a subtle loss-of-function (LOF) not discernible by visual

inspection of gross locomotion. We use this to suggest that the phenotypic appearance of *C. elegans* is not a robust way to determine the genotype of co-CRISPR genes. Given this, we suggest routine sequencing of co-CRISPR genes following CRISPR procedures that utilise phenotypic selection before downstream analysis.

4.3 Materials and Methods

4.3.1 *C. elegans* culturing and strains used

All *C. elegans* strains were maintained using standard conditions (Brenner, 1974). Strains used: Bristol N2 (wild-type), VC228 *nlg-1(ok259) X* (x6 outcrossed), CB665 *unc-58(e665) X,* TN64 *dpy-10(cn64) II,* provided by *Caenorhabditis* Genetics Center (CGC). XA3780 *nlg-1(qa3780) X* (x2 outcrossed), XA3773 *unc-58(qa3788) X; nlg-1(qa3780) X* (x1 outcrossed), XA3788 *unc-58(qa3788) X* (x2 outcrossed), generated in this study. JIP1154 *unc-58(bln223) X*, provided by Thomas Boulin.

4.3.2 CRISPR/Cas9 genome editing

The previously reported method by El Mouridi et al was followed for CRSIPR/Cas9 editing (El Mouridi *et al.*, 2017). L4+1 day old hermaphrodites were microinjected with expression vectors for Cas9 and sgRNAs targeting *nlg-1* and *unc-58* or *dpy-10* for the first and second round of CRISPR, respectively. The design of the sgRNAs and repair templates to edit *unc-58, dpy-10* and *nlg-1* genes were as previously reported (Rawsthorne *et al.*, 2020; Arribere *et al.*, 2014). sgRNAs and repair templates were purchased from Integrated DNA Technologies (IDT™ – Integrated DNA Technologies). All injection reagents were diluted in molecular grade water to a final concentration of 50ng/µl in the CRISPR mix.

4.3.3 Molecular screening and sequencing

nlg-1 primers, forward 5'- ATGAGTATACAGATTGGGAAAATCCC-3' and reverse 5'-ACTGTTTGGTTGCTCTTGGCTCCAAG-3', were used to amplify the CRISPR targeted region of *nlg-1* using a single worm PCR protocol (He, 2011). A BanI site was used to screen PCR amplicons from individual worms for the incorporation or loss of the d10 sequence in *nlg-1.* The primers used for sequencing of the target regions of *unc-58* and *dpy-10* were: forward 5'-GACTCGGAGATATCGTTGTGACTG-3', reverse 5'- CGCGGAGTTCGTTATCCAGGAAG-3' and forward 5'- ACTAATTCAGAGTCATCATCTCGCC-3', reverse 5'- CATCAATTCCCTTAAGTCCTGGTGG-3' respectively.

4.3.4 Removal of *unc-58* background mutation

unc-58(qa3788); nlg-1(qa3780) double CRISPR mutant strain was backcrossed with N2 males once to generate heterozygous F1 progeny. These progeny were cloned by picking a single F1 onto individual plates before being left for 2-3 days to self-fertilise and F2 progeny were screened using a single worm PCR protocol (He, 2011) and restriction digest. Restriction digest of *unc-58 and nlg-1* PCR amplicons were performed using restriction enzymes BsiWI and BstBI respectively to screen for the presence or absence of CRISPR mediated edits. Restriction enzymes were supplied by New England BioLabs (NEB) and used according to manufacturer instructions.

4.3.5 Food leaving assay

Food leaving assays were carried out as previously described (Rawsthorne *et al.*, 2020). Briefly, 50µl of OP50 *E. coli* at OD_{600} of 0.8 was gently spotted on to the middle of an unseeded plate the day prior to the assay. Seven age synchronised L4+1 day old hermaphrodites were gently picked onto the centre of the bacterial lawn on the assay plate. At 2 and 24 hours food leaving events were counted visually using a Nikon SMZ800 microscope (X10 magnification) during 30 minute observations. A food leaving event was defined as when the whole of the worm's body exited the bacterial lawn. N2 animals were used as a paired control, run in parallel with the strain under investigation and the investigator was blind to the genotypes being observed.

4.3.6 Thrashing assay

Using a 24 well plate, a single worm was picked per well containing 500µl of M9 with 0.1% bovine serum albumin and left for 5 minutes before thrashing was measured. For each worm, thrashing events were visually counted under a Nikon SMZ800 microscope (X30 magnification) for a period of 30 seconds. This was repeated three consecutive times and the mean was calculated. Each thrash was defined as a complete movement through the midpoint of the worm's body and back. N2 animals were used as a paired control, run in parallel with the strain under investigation and the investigator was blind to the genotypes.

4.3.7 UNC-58 protein sequence analysis

UNC-58, isoform b protein sequence was downloaded from WormBase version WS278. The wild-type or CRISPR mutant protein sequence was entered into the membrane topology prediction tool TMHMM (v.2.0) (Sonnhammer, von Heijne and Krogh, 1998; Krogh *et al.*, 2001) to determine the predicted transmembrane topology.

4.4 Results

4.4.1 The use of co-CRISPR genes facilitated the generation of a precise *nlg-1* edit

We used co-CRISPR genes *unc-58* and *dpy-10* to facilitate the generation of a precise edit in *C. elegans nlg-1* gene. 84 N2 animals were injected with plasmids encoding Cas9 and a sgRNA and repair template designed to transplant a d10 sequence (El Mouridi *et al.*, 2017) into the gene of interest, *nlg-1.* In addition, the CRISPR injection mix included a sgRNA and repair template designed to generate a GOF mutation in the co-CRISPR gene *unc-58* (Figure 4.1A). This was used as a CRISPR efficiency marker based on the hypercontraction and uncoordinated phenotype that results from the mutation. 7 of the 84 parental injected worms produced F1 broods containing progeny that displayed an uncoordinated phenotype, supporting the notion that the *unc-58* gene had been successfully edited (Figure 4.1B). 2 of these 7 broods were identified as jackpot broods, containing more than 30 progeny with an *unc-58* paralysis phenotype. In total, 72 *unc-58* marked progeny were selected for molecular screening and sequencing of the target region of the *nlg-1* gene (Figure 4.1A). We also selected 100 unmarked, apparently wild type, siblings from these jackpot broods for screening because *nlg-1* and *unc-58* are both located on the same chromosome (X) and approximately 3.5mb apart. Identifying the *nlg-1* edit in an unmarked worm can be beneficial because it avoids the need for backcrossing to separate two chromosomally linked edits. This resulted in the identification of a single *nlg-1* d10 entry strain in an unmarked F1 worm from a jackpot brood which displayed no obvious *unc-58* marked phenotype. Consistent with other studies, the lack of *unc-58* marked phenotype was used to assume that the *nlg-1* d10 entry strain was WT for the *unc-58* co-CRISPR loci (El Mouridi *et al.*, 2017; Wang *et al.*, 2018a; Schreier *et al.*, 2020).

Next, 14 d10 entry siblings, from the identified *nlg-1* d10 entry strain, were injected with plasmids encoding Cas9, a sgRNA and a repair template designed to replace the *nlg-1* d10 sequence with the R451C mutation of interest. In addition, the CRISPR injection mix included a sgRNA and repair template designed to generate a GOF mutation in the co-CRISPR gene *dpy-10* (Figure 4.1A). Four of the injected parental worms produced *dpy-10* marked F1 progeny, displaying a mixture of dumpy (Figure 4.1C), roller and dumpy roller phenotypes. Due to the high efficiency of the *dpy-10* sgRNA previously reported (El Mouridi *et al.*, 2017), the frequency of *dpy-10* marked progeny was higher than that of *unc-58* marked progeny (El Mouridi *et al.*, 2017). We identified three plates containing 9-15 *dpy-10* marked progeny each and one plate with 56 marked progeny. Unlike *unc-58, dpy-10* is not located on the same chromosome as *nlg-1* so we selected only *dpy-10* marked progeny for molecular screening. In total, 95 *dpy-10* marked progeny were selected for molecular analysis and sequencing of the target region of the *nlg-1* gene. We identified one dumpy

phenotype worm that contained the R451C mutation in *nlg-1*. The aim for the *nlg-1* CRISPR edited strain was to test their behaviour in a motility based assay (Rawsthorne *et al.*, 2020). Given that the dumpy phenotype would confound this assessment, we backcrossed the *nlg-1* CRISPR strain with the N2 strain and screened for progeny that retained the *nlg-1* CRISPR mutation and visually lost the dumpy phenotype, appearing phenotypically similar to wild-type in terms of body length and motility (Figure 4.1D). Sanger sequencing was performed and confirmed the generation of the R451C mutation in *nlg-1* (Figure 4.1E).

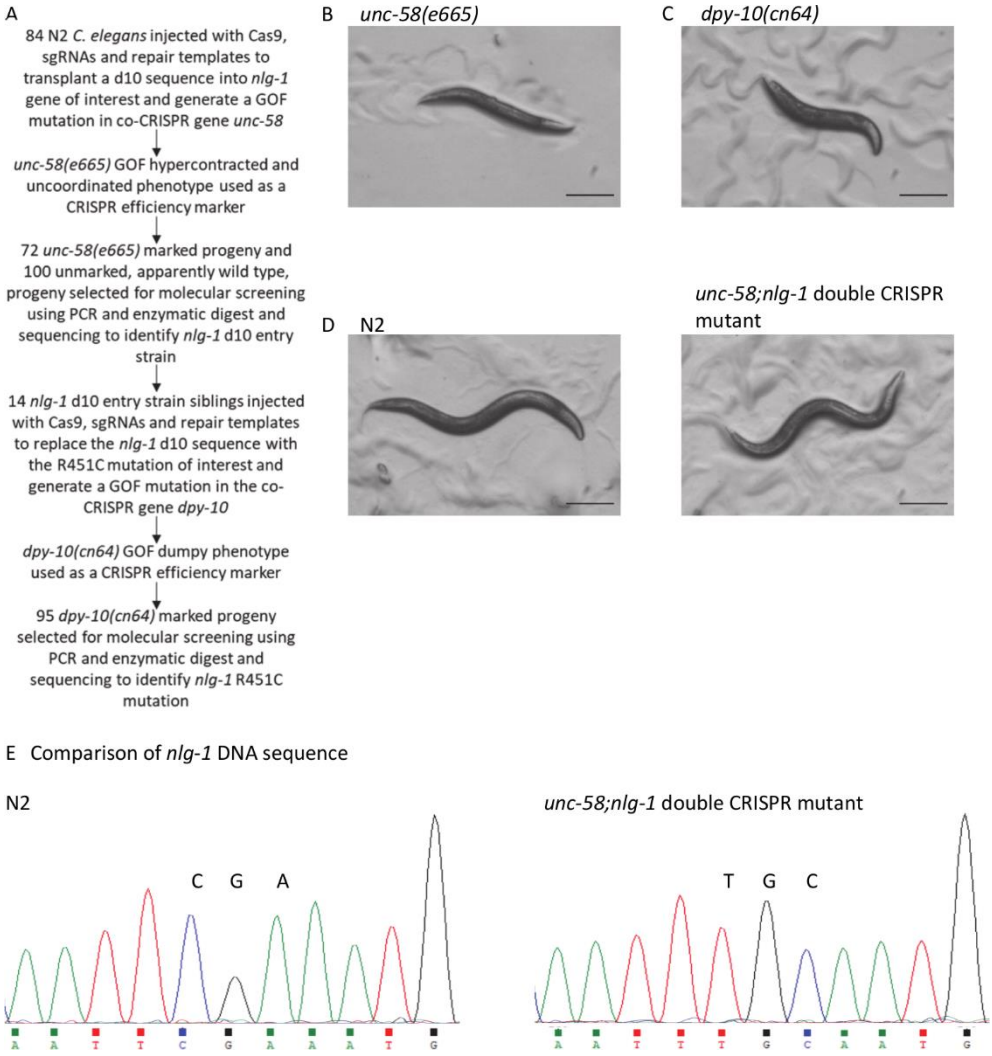


Figure 4.1 co-CRISPR genes used to facilitate the generation of a *nlg-1* CRISPR mutant. (A) Flow diagram summarising the steps taken to generate a CRISPR/Cas9 mutant which utilised two co-CRISPR genes. (B) Representative image of an *unc-58(e665)* GOF *C. elegans* mutant strain which has an uncoordinated phenotype with severe motility deficits which result in sluggish movement. (C) Representative image of a *dpy-10(cn64)* GOF *C. elegans* mutant strain displaying a dumpy phenotype. (D) Representative images of N2 wild-type and *unc-58(qa3788);nlg-1(qa3780)* double CRISPR mutant strain that highlight the gross phenotypic similarities between the

two strains. (B-D) All images show L4+1 day old hermaphrodites and were taken at 30x magnification. All scale bars represent 0.2mm. (E) Chromatograms showing partial *nlg-1* DNA sequences from N2 and *unc-58(qa3788);nlg-1(qa3780)* double CRISPR mutant strain. The codons of interest, CGA and TGC are indicated. TGC encodes the desired *nlg-1* R451C mutation. DNA sequences are shown in the 5' to 3' orientation.

4.4.2 *unc-58* CRISPR mutation is predicted to result in a non-functional ion channel protein

As well as HDR, CRISPR/Cas9 can also result in NHEJ which can introduce random errors into the DNA sequence in order to repair it (Pannunzio, Watanabe and Lieber, 2018). With this in mind we sequenced the regions surrounding the targeted co-CRISPR loci in order to confirm if the sequences were wild-type as suggested by the lack of *unc-58* or *dpy-10* phenotypes displayed by the CRISPR edited strain (Figure 4.1D). Thus, we confirmed that the *dpy-10* targeted sequence was wild-type (Figure 4.2A). However, the targeted *unc-58* sequence was mutated (Figure 4.2B). We observed a two nucleotide (nt) deletion followed by a 15nt insertion (Figure 4.2B and C) at the predicted Cas9 cut site (Arribere *et al.*, 2014). The disrupted *unc-58* gene causes a frame shift that generates a premature stop codon at position 467, deleting approximately 70% of the predicted C-terminal domain (Figure 4.2D). Protein sequence analysis also showed that the frameshift mutation alters the amino acid sequence from position 431, which in the WT protein is predicted to be within the fourth transmembrane domain of the potassium channel subunit (Figure 4.2D) and predicts the mutant UNC-58 protein may have an incomplete fourth transmembrane domain. Overall, this suggests that the mutant *unc-58* gene identified in this study is unlikely to encode a functional protein.

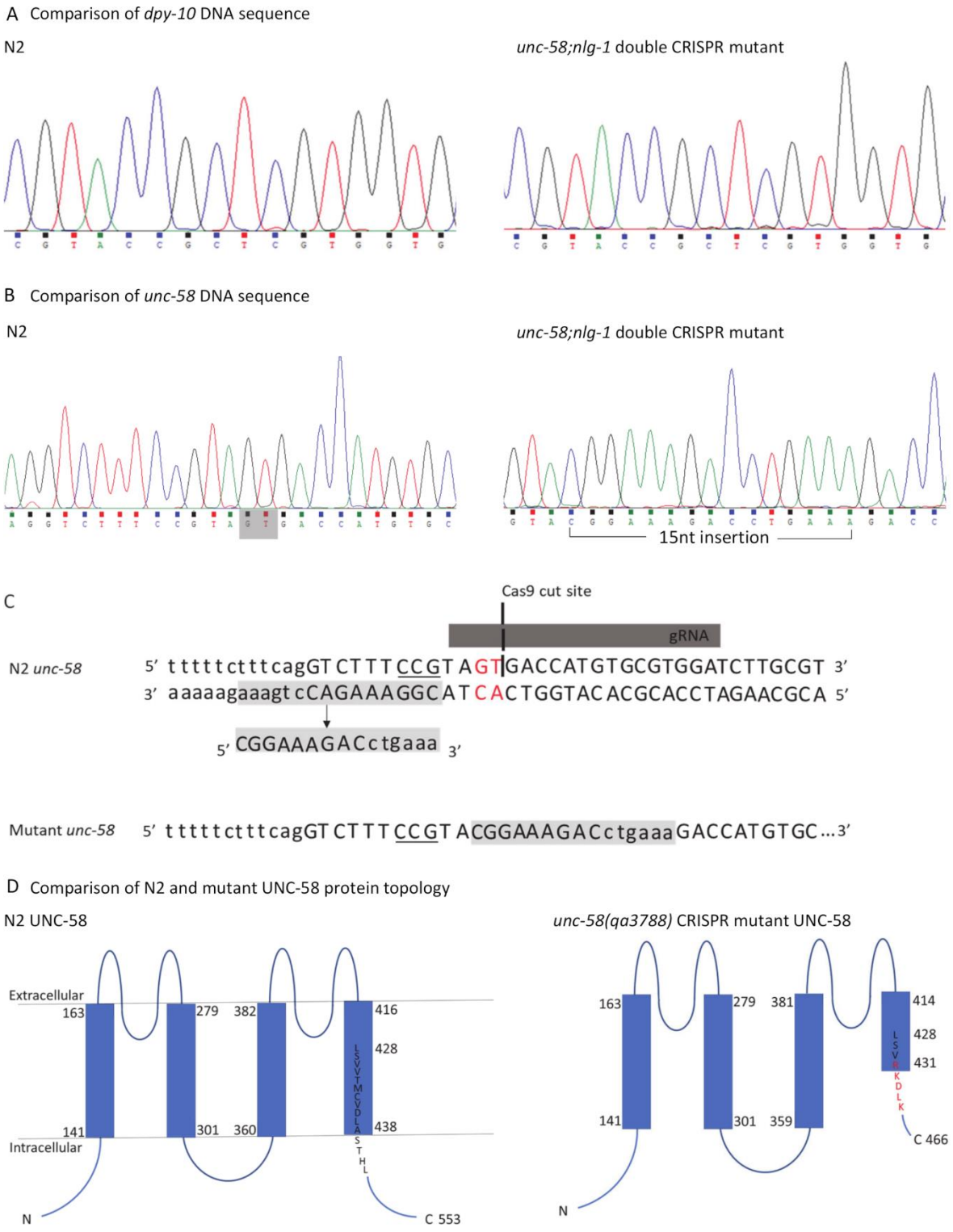


Figure 4.2 CRISPR mediated mutation to the co-CRISPR gene *unc-58* is predicted to result in a non-functional ion channel protein. (A) Chromatograms comparing partial *dpy-10* DNA sequence from N2 and *unc-58(qa3788);nlg-1(qa3780)* double CRISPR mutant confirming the *dpy-10* sequence is wild-type in both. (B) Chromatograms comparing partial *unc-58* DNA sequence from N2 and *unc-58(qa3788);nlg-1(qa3780)* double CRISPR mutant. Two NTs, GT, that exist in the WT sequence but absent in the mutated *unc-58* sequence are shaded in grey. A 15nt sequence found in the *unc-*

58(qa3788);nlg-1(qa3780) double CRISPR mutant is highlighted. All DNA sequences are shown in the 5' to 3' orientation. (C) Diagram illustrating the possible origin of the 15nt insert found in the CRISPR mutant. Double stranded wild-type *unc-58* DNA sequence is shown, with the DNA sequence orientation indicated. The gRNA binding site and Cas9 cut site are shown. The PAM sequence is underlined. The two NTs coloured in red indicate the NTs that are deleted as part of the mutation. On the *unc-58* wild-type antisense strand, 15nts are shaded in grey. These NTs correspond to the 15nt insert when read in the 5' to 3' orientation. Single stranded mutant *unc-58* sequence containing the 15nt insert is shown. (D) Comparison of wild-type and *unc-58(qa3788)* mutant UNC-58 protein predicted transmembrane domain topology. Wild-type amino acid sequence is shown in black and the mutated amino acid sequence is shown in red.

Discovery of the *unc-58* mutation meant that our CRISPR edited strain, which displayed no obvious *unc-58* marked phenotype, was in fact an *unc-58(qa3788);nlg-1(qa3780)* double mutant strain. Genetic crosses, combined with PCR amplification, enzymatic digest and sequencing allowed the identification of a single strain that retained the *nlg-1* CRISPR mutation and had lost the *unc-58* CRISPR mutation (Figure 4.3). In the process we also identified strains that retained the *unc-58* CRISPR mutation and lost the *nlg-1* CRISPR mutation.

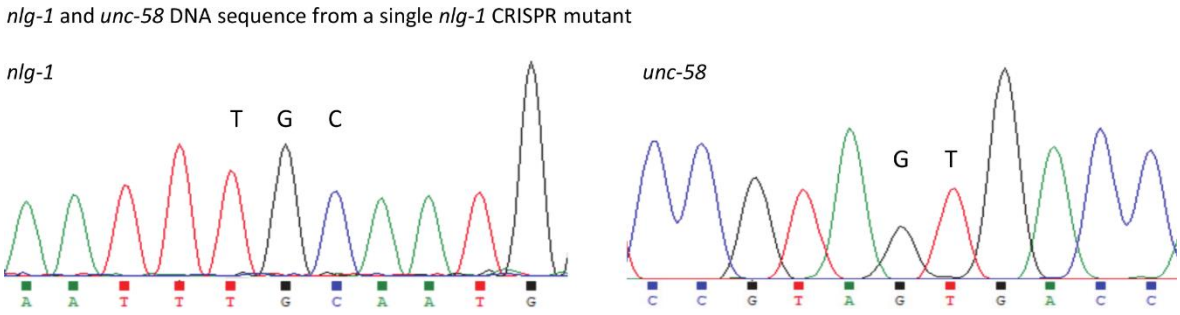


Figure 4.3 Chromatograms showing partial *nlg-1* and *unc-58* DNA sequence. Chromatograms confirm that the *nlg-1(qa3780)* CRISPR mutant carries the R451C mutation as well as wild-type *unc-58* sequence. The TGC codon that encodes the R451C mutation is indicated. The wild-type GT NTs that were deleted as part of the *unc-58* CRISPR mutation are indicated. DNA sequences are shown in the 5' to 3' orientation.

4.4.3 Phenotypic analysis of *unc-58* CRISPR mutant identifies it is LOF and effects worm motility

The overarching aim for generating the *nlg-1* CRISPR edited strain was to investigate its effect on the social biology of the worm using a food leaving assay as a paradigm (Rawsthorne *et al.*, 2020). Given this, we were interested to understand if the *unc-58* CRISPR mutation, when contemporary to the *nlg-1* CRISPR mutation, would have confounded the assessment of food leaving behaviour. To investigate this, we performed a food leaving assay in which worms were picked onto the centre of a bacterial lawn before food leaving events were quantified at 2 and 24 hours. We have previously shown that N2 worms increase their food leaving rate over 24 hours producing a rate of approximately 0.08 leaving events/worm/minute (Rawsthorne *et al.*, 2020). In comparison, both a *nlg-1* null mutant and the *nlg-1* CRISPR mutant showed significantly reduced food leaving rate at 24 hours (Figure 4.4A) (Rawsthorne *et al.*, 2020). Interestingly, the *unc-58;nlg-1* double mutant also showed reduced food leaving behaviour (Figure 4.4A). In contrast, both the *unc-58* CRISPR mutant and the *unc-58* null mutant showed food leaving behaviour which was similar to the N2 control (Figure 4.4A). The *unc-58* GOF mutant displays an uncoordinated phenotype (Figure 4.1B) and we were unable to get a readout of food leaving behaviour (Figure 4.4A). Taken together, these data show that the *unc-58* CRISPR mutation does not produce a food leaving phenotype and did not confound the assessment of the *nlg-1* CRISPR mutant food leaving behaviour.

Given the functional importance of the UNC-58 potassium channel subunit for locomotion (Kasap *et al.*, 2018), we wanted to test the intrinsic locomotion of our *unc-58* CRISPR edited strain. To do this we picked worms into liquid medium and observed their thrashing behaviour. We observed that both the *nlg-1* null mutant and the *nlg-1* CRISPR mutant had no thrashing phenotype, thrashing at a similar rate to the N2 control (Figure 4.4B). In comparison, the *unc-58;nlg-1* double CRISPR mutant, *unc-58* CRISPR mutant and *unc-58* null mutant all showed a similar thrash rate which was significantly lower compared to the N2 paired control (Figure 4.4B). Furthermore, the *unc-58* GOF mutant did not thrash due to its uncoordinated phenotype (Figure 4.4B). Taken together, these data show that the *unc-58* CRISPR mutant phenocopies the *unc-58(bln223)* null mutant, suggesting that the CRISPR mediated mutation results in loss-of-function to the *unc-58* gene. These data also show that the loss-of-function to *unc-58* in the *unc-58;nlg-1* double mutant results in a reduced thrashing rate which is not displayed in the *nlg-1* CRISPR mutant.

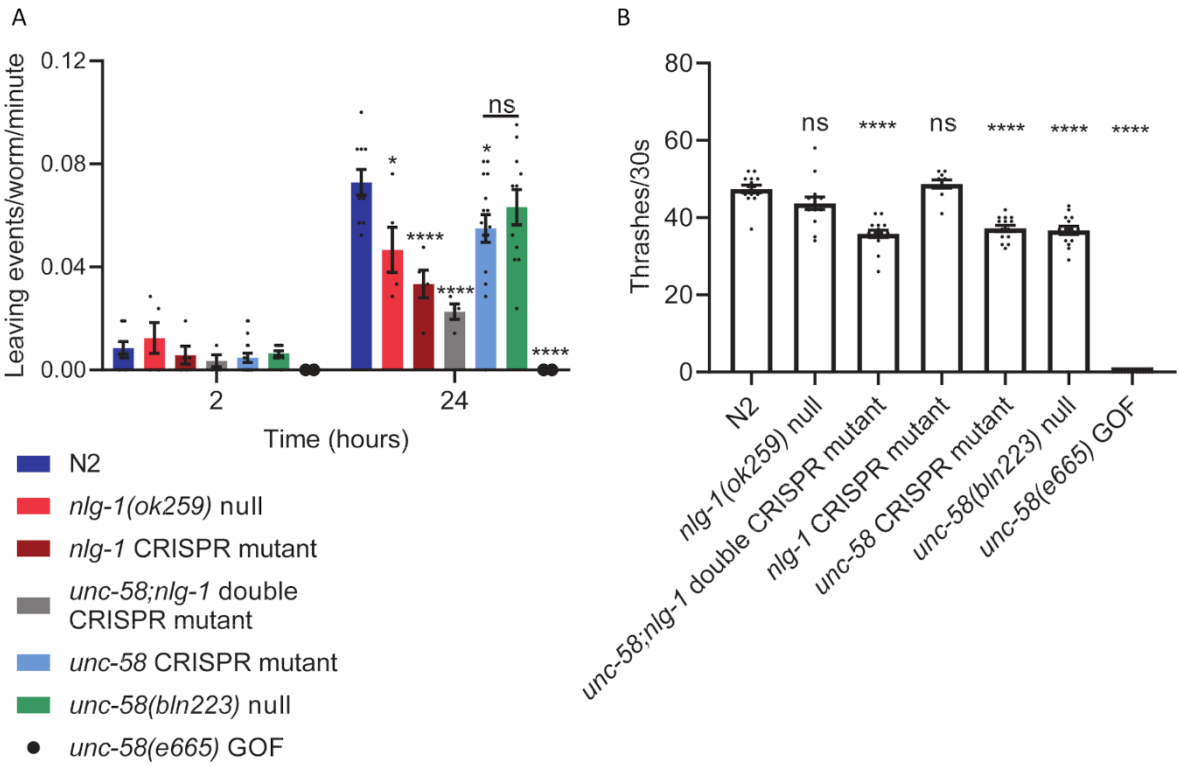


Figure 4.4 *unc-58* CRISPR mutant phenocopies *unc-58(bln223)* null mutant and is likely to be a LOF mutant. (A) A food leaving assay was performed by picking N2 or mutant worms onto the centre of a bacterial lawn before food leaving events were counted at 2 and 24 hours. *unc-58(qa3788)* CRISPR mutant phenocopies the food leaving behaviour of *unc-58(bln223)* null mutant and N2 at 24 hours. N2 n=10, *nlg-1(ok259)* null n= 5, *nlg-1(qa3780)* CRISPR mutant n=5, *unc-58(qa3788);nlg-1(qa3780)* double mutant (x2 independent lines) n=4, *unc-58(qa3788)* CRISPR mutant (x2 independent lines) n= 16, *unc-58(bln223)* null n= 11, *unc-58(e665)* GOF n=2. Statistical analysis performed using a Two-way ANOVA with Tukey's multiple comparison test; ns P≥0.05, * P<0.05, **** P≤0.0001. (B) The number of thrashes/30 seconds was counted for N2 and mutant *C. elegans* in liquid medium. The *unc-58(qa3788)* CRISPR mutant shows reduced thrashing behaviour, phenocopying that of *unc-58(bln223)* null mutant. N2 n= 14, *nlg-1(ok259)* n= 14, *unc-58(qa3788);nlg-1(qa378)* double mutant n= 15, *nlg-1(qa3780)* CRISPR mutant n= 10, *unc-58(qa3788)* CRISPR mutant n= 14, *unc-58(bln223)* null n=14, *unc-58(e665)* GOF n=14. Statistical analysis performed using a one-way ANOVA and Dunnett's multiple comparison test; ns, p>0.05; ****, p≤0.0001. All significance relates to a comparison with N2 control. Data are mean ±SEM.

Overall, in the process of CRISPR editing the *nlg-1* gene we unexpectedly created a loss-of-function mutation within the *unc-58* gene as a consequence of targeting it with reagents designed to generate a gain-of-function phenotype. Analysis of the *unc-58* CRISPR mutant demonstrates

that it has a predicted protein sequence that would result in a severe loss of channel function. We show that this loss-of-function mutation in *unc-58* results in a subtle, but significant effect on motility consistent with its established role in regulation of neuronal membrane potential (Kasap *et al.*, 2018).

4.5 Discussion

We demonstrate the use of a previously described method of CRISPR/Cas9 that relies upon the use of co-CRISPR genes (El Mouridi *et al.*, 2017) to facilitate screening of transformed worms on the basis of a clearly observable phenotype. The first step in this process involved the transplantation of a d10 sequence in *nlg-1* and utilised the *unc-58* GOF phenotype to facilitate the screening for CRISPR events. The efficiency of d10 transplantation into the gene of interest, *nlg-1*, was lower than that previously reported (El Mouridi *et al.*, 2017). The high efficiency of the *dpy-10* sgRNA (El Mouridi *et al.*, 2017) meant that fewer worms needed to be injected to produce *dpy-10* marked progeny. However, within the pool of marked progeny the edit efficiency at *nlg-1* was approximately 1%, which is lower than that reported in other genes (El Mouridi *et al.*, 2017). This likely reflects that the efficiency varies depending on the gene being targeted.

Our use of *unc-58* as a co-CRISPR gene led to the creation of a novel *unc-58* mutant strain and we have provided evidence that the *unc-58* CRISPR strain phenocopies an *unc-58* null mutant suggesting it is LOF. K2P LOF mutants do not display an easily recognisable phenotype, unlike GOF mutants and are therefore comparatively less well studied (Kasap *et al.*, 2018). *unc-58* is expressed widely in interneurons and motor neurons (Salkoff *et al.*, 2001). It is hypothesised that GOF to this channel results in loss of ion selectivity and therefore allows the passage of sodium ions. This may then facilitate the depolarisation of motor neurons and may explain the uncoordinated phenotype (Kasap *et al.*, 2018) and the hypercontracted state seen in our study and reported more widely (Park and Horvitz, 1986). To our knowledge there is no electrophysiological data to support this hypothesis.

In comparison, it has been suggested that loss-of-function *unc-58* mutants display a less severe phenotype because there is functional redundancy with other K2P channels and this may compensate for the effect of the loss of UNC-58 on resting membrane potential (Kasap *et al.*, 2018). Of the few K2P LOF mutants that have been investigated it was shown that the *twk-7* null has moderately enhanced motility (Lüersen, Gottschling and Döring, 2016). This, combined with our findings that the CRISPR mutated *unc-58* is required for normal thrashing behaviour demonstrates that K2P LOF mutants can display locomotory phenotypes, albeit less severe than for GOF mutants. This is consistent with K2Ps playing an important role in co-ordinating normal

functioning of the nervous system and highlights how recombination events within this gene could impact downstream analysis of complex integrative behaviours that are dependent upon the worms being able to execute locomotory behaviour.

We show that in the *unc-58* CRISPR LOF mutant the amino acid sequence is mutated in the second half of the fourth transmembrane domain and the majority of the intracellular C-terminus is deleted. This suggests that these domains are likely to play a crucial role in *unc-58* channel functioning. In mammalian K2Ps the C-terminus is important for channel gating (Piechotta *et al.*, 2011). Studies are beginning to define the mechanism of gating in *C. elegans* K2Ps (Ben Soussia *et al.*, 2019) however further analysis is needed to elucidate if there is a conserved role of the C-terminus in channel gating in *C. elegans.*

The caveats of using *unc-58* as a co-CRISPR gene have been previously discussed (Arribere *et al.*, 2014). Unlike *unc-58,* other more favourable co-CRISPR genes, such as *dpy-10* and *sqt-1* produce easily recognisable phenotypes in response to both GOF and LOF (Arribere *et al.*, 2014). For example, mutation to *dpy-10* can result in dumpy, roller or dumpy roller phenotypes depending on the mutant genotype (Arribere *et al.*, 2014). Despite this limitation, *unc-58* continues to be used in co-CRISPR approaches (El Mouridi *et al.*, 2017; Wang *et al.*, 2018a; Schreier *et al.*, 2020). We have shown that when using *unc-58* as a co-CRISPR gene, relying solely on the gross phenotypic appearance of the worm is not a robust way to assume genotype. Therefore, we suggest that sequencing of co-CRISPR genes, particularly *unc-58*, is important following their use.

In conclusion, the visibly obvious hypercontraction in the viable adult *C. elegans* that is generated by a GOF mutation in *unc-58* provides an excellent marker for screening for CRISPR events in target genes of interest. In using this approach we unexpectedly generated a novel *unc-58* mutation and provided evidence that this LOF impaired motility in liquid. This will facilitate the understanding of K2Ps in *C. elegans* and the behaviours they regulate. However, it is clear that if utilised to generate mutants for investigation of more complex behaviours or readouts that depend on neuronal excitability, the spurious re-arrangements at the *unc-58* marker locus can generate more subtle but not inconsequential LOF mutations. Based on our experience of using *unc-58* in co-CRISPR strategies destined to target gene loci that impact discrete determinants of integrative behaviour, we conclude that this requires routine sequencing of the co-CRISPR gene to avoid potential confounds of unpredicted CRISPR events.

Chapter 5 General discussion

5.1 Principal findings

The main aim of this thesis was to use *C. elegans* behaviour as a platform to investigate genetic determinants of autism and develop bioinformatic and genetic approaches to validate the use of *C. elegans* as a model of ASD.

The main findings were:

- 14 ASD-associated gene orthologues investigated for their role in *C. elegans* social behaviour have selective impairment in progeny-induced food leaving behaviour
- Genes from synaptic, cell signalling, epigenetic modification and phospholipid metabolism functional domains have a role in *C. elegans* social behaviour
- Social behaviour is dependent on the *nlg-1* gene and its function is required in adult worms for proper co-ordination of the behaviour
- *C. elegans* can be used to model disruption within the social domain upon precision genetic manipulation to mimic a human ASD genetic variant
- Optimisation of a CRISPR/Cas9 technique to facilitate accurate gene editing in *C. elegans*

The findings from this thesis provide evidence for the utility of *C. elegans* as a model of ASD. Social behaviours like mating and group feeding have been investigated in *C. elegans* and their genetic and neural determinants have been dissected (1.6.1). However, the social paradigm used here is the first social assay, to my knowledge, that represents a quantifiable social communication between progeny and adult worms. Previous ASD research in *C. elegans* has focused on simple measurements of morphology, such as length of the worm, and quantification of motility, like thrashing and body bends (2.5). The novel approach used here, that utilised a bioinformatic filtering pipeline to select genes for assay using the social paradigm allowed genes with a role in a more complex, sensory integrative, behaviour to be highlighted (2.5). This work has generated a diverse list of genes and highlighted biological functional domains that are less well studied in the ASD field which can now be prioritised for future research to dissect their functional role in ASD.

Results within this thesis demonstrate that the *nlg-1* R451C mutation, when modelled in *C. elegans,* encodes a LOF mutation. However, within the wider literature there is some discrepancy as to whether this mutation is GOF or LOF (3.5). The R451C mutation has been shown to cause the retention of *nlg-1* in the endoplasmic reticulum resulting in only a small fraction of the gene being

correctly transported to the synapse membrane (Comoletti *et al.*, 2004; De Jaco *et al.*, 2010). A paper that showed increased GABAergic inhibition in a R451C mouse model suggested that the mutation was likely GOF due to the small fraction of *nlg-1* at the synapse being capable of increasing signalling in the mutant (Tabuchi *et al.*, 2007). However, results from this study were unable to be repeated (Chadman *et al.*, 2008). Also, it has been discussed that the increased GABAergic signalling that was seen could also be explained by the R451C mutation causing LOF to *nlg-1* in a cell specific manner which consequently disrupts the abundance of *nlg-1* across circuits and produces widespread disruption and excitatory/inhibitory imbalance within the nervous system (Singh and Eroglu, 2013). Evidence for this theory showed that R451C mice had both increased excitatory and inhibitory signalling within different brain areas, indicative of widespread signalling disruption (Etherton *et al.*, 2011). Therefore, the results obtained in this thesis that are consistent with the R451C mutation being LOF helps to clarify the role of this mutation and it's possible role in the balance of neuronal signalling.

Overall, the use of *C. elegans* as a model of ASD has helped to identify genes with a role in co-ordinating social behaviour. Future work to dissect the role of these genes in circuits that control this behaviour will provide insight into the fundamental mechanisms required for the production and modulation of social behaviours. This information could ultimately be translated into more complex rodent and primate models with the goal of more closely modelling the function and dysfunction of genes in social behaviour. This will facilitate understanding of the genetic and neural underpinnings of social behaviour in humans and is likely to help in the identification of drug targets and therapeutic approaches for ASD and related disorders.

Discussion of results obtained in this thesis and future directions are provided in the sections that follow.

5.2 The *nlg-1* dependence of social behaviour

Two *nlg-1* transgenic rescue strains were generated, one using *nlg-1* cDNA and the other using gDNA (3.3.2). These strains were used to show the dependence of *C. elegans* social behaviour on the *nlg-1* gene based on the partial rescue of food leaving behaviour upon re-expression of *nlg-1* into mutant animals (3.4.1). Reasons for observing partial, and not full, rescue may include the length of the *nlg-1* promoter used in the cDNA rescue construct. The non-coding promoter region of *nlg-1* extends ~8.7kb upstream before the next protein-coding gene on the X chromosome. The cDNA rescue construct generated contained 2.5kb of this promoter region. Different studies have previously used different lengths of *nlg-1* promoter including 2.5kb, 3.5kb and 6.5kb and have produced both partial and full rescue depending on the phenotype being investigated (Calahorro

and Ruiz-Rubio, 2012; Calahorro *et al.*, 2019; Hunter *et al.*, 2010; Tong *et al.*, 2017). The 2.5kb promoter region used in this thesis was chosen because it has been successfully used to study *nlg-1* expression pattern and has been shown to fully rescue multiple phenotypes (Calahorro *et al.*, 2019; Calahorro and Ruiz-Rubio, 2012). However, it is possible that regulatory regions outside of the 2.5kb promoter region used are missed in the cDNA rescue construct and that this may have confounding effects on the expression of *nlg-1* and hence may be contributing to the partial rescue seen.

A disadvantage of the cDNA rescue construct is the fact that it only contains a single isoform of *nlg-1*. The Δ#14 isoform was chosen because it is the dominantly expressed isoform in adult worms (Calahorro, Holden-Dye and O'Connor, 2015). However, other isoforms, that have been suggested to play a role in development (Calahorro, Holden-Dye and O'Connor, 2015), will not be expressed in the transgenic strain. Therefore, a fosmid rescue approach was also used because the ~33kb construct covers all *nlg-1* isoforms. It also contains 26kb of DNA sequence upstream of *nlg-1* and therefore encapsulates the entire promoter region. However, this fosmid also encodes four other protein-coding genes, these being *twk-20*, *acr-9* and two genes with unknown function, C40C9.3 and C40C9.4. *twk-20* is a two-pore domain potassium channel (Ben Soussia *et al.*, 2019) and *acr-9* encodes an acetylcholine receptor subunit (Barbagallo *et al.*, 2010). Both are neuronally expressed and known to have roles in worm body posture and motility (Ben Soussia *et al.*, 2019; Yemini *et al.*, 2013). The overexpression of these genes in the transgenic rescue strain may have had confounding effects on motility and therefore influenced the rescue of food leaving behaviour.

As well as the limitations of each rescue construct, rescue experiments in *C. elegans* are affected by mosaic expression of the transgene. Transgenes are extrachromosomal and are therefore expressed in a variable number of cells (Yochem and Herman, 2005). Therefore, it is likely that the expression pattern of *nlg-1* in the rescue strains was different from that in wildtype animals and this may have also contributed to the partial rescue observed. Extrachromosomal arrays can be integrated into the genome to result in non-mosaic expression (Mariol *et al.*, 2013). This method can be time consuming but could be used to validate the *nlg-1* dependence of social behaviour.

5.3 Sub-behaviour analysis

The progeny-induced food leaving is distinct from other food leaving behaviours and represents a novel social circuit in *C. elegans* (Scott *et al.*, 2017). However, it is currently unclear what underpins this social behaviour at a cellular, circuit or sub-behavioural level (Figure 5.1). It is likely that progeny induce a change in the locomotor output of the adult worm which allows it to leave

the food more often (1.6.1.1). However, it is still unclear what underlying locomotory changes might occur to result in the overall emergence of increased food leaving behaviour.

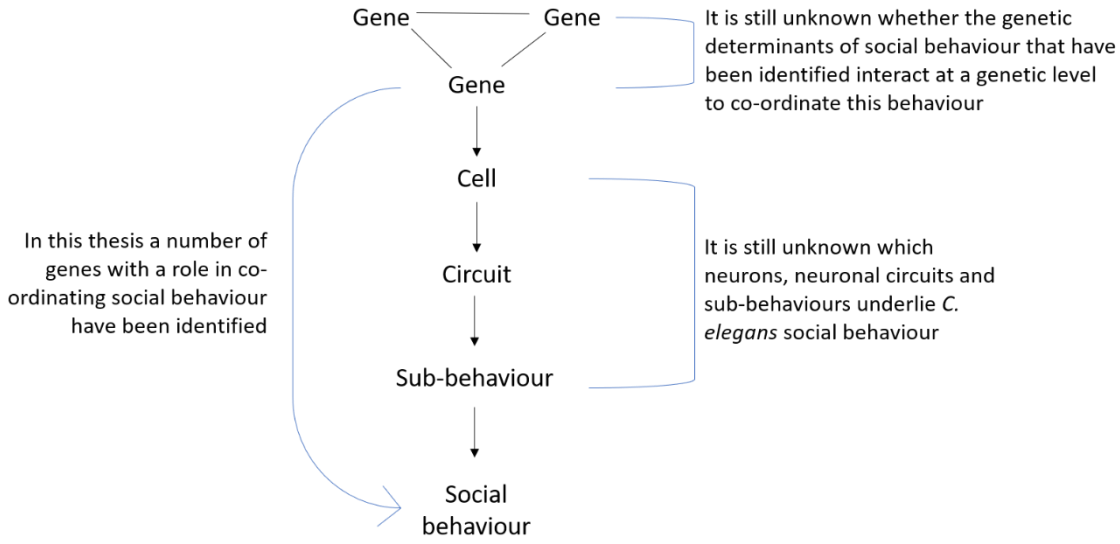


Figure 5.1 Summary of the main findings of this thesis and factors that remain unknown. The flow diagram outlines the major determinants of a biological system to highlight the flow of information from a genetic level through to social behaviour output.

To better understand the behavioural changes that occur in the presence of progeny I have carried out analysis of sub-behaviours with a master's student that analysed the data I provided. The aim of this ongoing work is to investigate the frequency of sub-behaviours performed by wild-type worms which underly progeny-induced food leaving. Sub-behaviours have been invaluable in dissecting more complex behaviours like foraging (Wakabayashi, Kitagawa and Shingai, 2004; Gray, Hill and Bargmann, 2005; Fujiwara, Sengupta and McIntire, 2002). However, these studies have focused on behavioural changes that occur in the presence and absence of food but have not investigated the effect of progeny on these behaviours.

Video recordings were made of seven wild type worms two hours after being placed on a naïve or progeny-loaded food lawn. Videos were captured for 30 minutes (at 25 frames/second) and subsequently analysed for differences in sub-behaviours. Pre-liminary analysis of reversals, speed and omega turns has been undertaken. These behaviours have been previously used to understand complex foraging behaviour and led to the dissection of the circuits and cells that underlie these behaviours (Gray, Hill and Bargmann, 2005) (1.5.5.3). Therefore, understanding if these sub-behaviours are modified by the presence of progeny-derived social cues will likely provide a clue as to the types of circuits and neurons that have a role in the co-ordination of social behaviour. Analysis of sub-behaviours led to the observation of an interesting behaviour which has been termed a partial food leaving event. A partial food leaving event is defined as when the

worm's head leaves the food lawn before the worm makes a reversal so that the whole of its body is back on the food lawn. Food leaving assays were analysed to quantify the frequency of partial food leaving events to investigate whether this behaviour changed depending on the presence or absence of progeny. Interestingly, worms performed fewer partial food leaving events in the presence of progeny (Figure 5.2A). I have already shown that the food leaving rate of worms increases in the presence of progeny (2.4.2, 3.4.1) and this result has been replicated by analysing video recordings (Figure 5.2B). Therefore, taken together these results suggest that in the context of progeny, adult worms change their food leaving behaviour in which the number of partial food leaving events decreases and the number of full food leaving events increases.

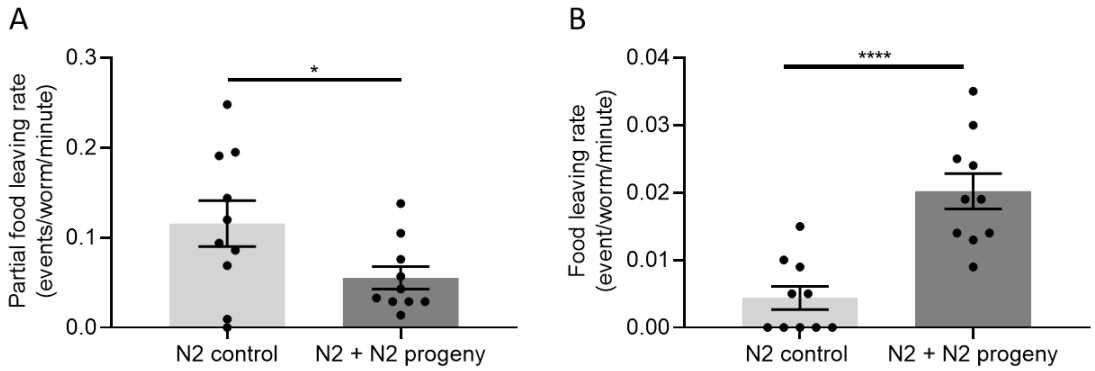


Figure 5.2 Progeny-enhanced food leaving shows inverted relationship between food leaving and partial food leaving. (A) Partial food leaving rate of N2 *C. elegans* in the absence and presence of progeny. A partial food leaving event is defined as when the worm's head leaves the food lawn before the worm makes a reversal so that the whole of its body is back on the food lawn. Statistical analysis performed using an Unpaired Student's t-test; P≤0.1, *. N=10. (B) Food leaving rate of N2 *C. elegans* in the absence and presence of N2 progeny. A food leaving event is defined as when the whole of the worm's body comes off the food lawn. Statistical analysis performed using an Unpaired Student's t-test; P≤0.0001, ****. N=10. Data are mean ±SEM. Videos were captured 2 hours after adult worms were placed on the centre of the food lawn. Data collected by me and analysed by me and Nicole Hubbard.

For a partial food leaving event to take place a worm must come into contact with the border of the food lawn. Therefore, understanding how often worms contact the border and whether this changes in the context of progeny will be useful. Preliminary analysis of worm positioning on the food lawn during a food leaving assay showed that on both naïve and progeny-loaded food lawns worms spend the majority of the time in close proximity with the border (Figure 5.3). However, further analysis is required to quantify the number of times worms in each condition contact the border to understand if the frequency of partial food leaving events is a product of changes to

border exposure during the food leaving assay. Partial food leaving events, by definition, require the production of a reversal. Reversals are a type of sub-behaviour that have been used to gain insight into other complex *C. elegans* behaviours (Gray, Hill and Bargmann, 2005; Flavell *et al.*, 2013). Therefore, it would be interesting to extend the preliminary analysis to include all reversals at the border, not just those that meet the definition of a partial food leaving event. This will help to better define sub-behavioural changes underlying the progeny-induced food leaving behaviour.

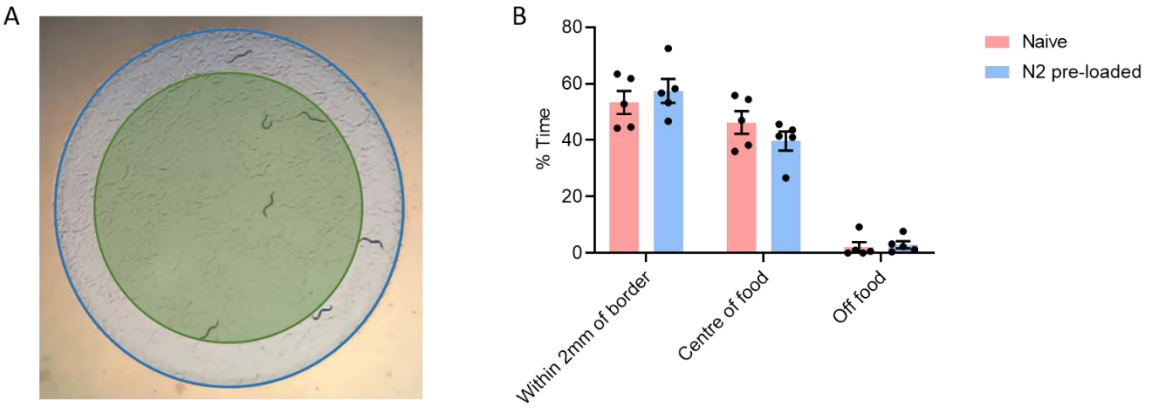


Figure 5.3 N2 *C. elegans* positioning on the food lawn is similar in the absence and presence of progeny. (A) Example plate showing how worm positioning was quantified. Green shading indicates the centre of the food lawn. Blue shading indicates the area of the food lawn which is within 2mm of the food border (thick blue line). Off food is not coloured. (B) The percent time N2 *C. elegans* spend within close proximity of the border (within 2mm), in the centre of the lawn and off food is similar in the absence and presence of progeny. Statistical analysis performed using a Two-way ANOVA with Sidak's multiple comparison test; ns $P \geq 0.05$. No significant difference was found between naïve and N2 pre-loaded conditions. N=5. Data are mean ±SEM. 30-minute video recordings were analysed. Videos were captured 2 hours after adult worms were placed on the centre of the food lawn. Data collected by me and analysed by Nicole Hubbard.

Identification of impaired sub-behaviour has the potential to provide insight into the circuitry underlying the social behaviour. This will pave the way for future investigations into the impaired food leaving behaviour of *C. elegans* ASD-associated mutants. Testing for sub-behavioural changes in these mutants may help to understand if sub-behavioural disruption is distinct across the different mutants or if there are any common sub-behavioural disruptions that could provide a clue as to the types of cells and circuits that are important for the proper co-ordination of social behaviour.

5.4 Circuit level understanding of the co-ordination of social behaviour

Multi-sensory processing is impaired in ASD (1.1.3). The use of interneurons by *C. elegans* to integrate multiple sensory cues to co-ordinate complex behaviours mean they have been used to model multi-sensory integration (1.5.3). Based on the partial food leaving data (5.3) it can be hypothesised that one reason for progeny-enhanced food leaving may be the suppression of reversals at the food border (Figure 5.2) which leads to more food leaving events. At the food border the worm receives multiple mechanosensory and chemosensory cues that are representative of the 'on food' context and if the worm begins to transition off the food lawn it will receive cues representative of the 'off food' environment (Pradhan *et al.*, 2019). Hence, at the border the worm will need to integrate multiple sensory modalities in order to decide whether to stay on the food lawn or leave. An interneuron in *C. elegans* called AIY has been shown to be a hub for processing information to control complex behaviours (Kimata *et al.*, 2012; Ryu and Samuel, 2002; Tsalik and Hobert, 2003; Gray, Hill and Bargmann, 2005; Shtonda and Avery, 2006) and is functionally coupled to and downstream of sensory neuron AWC which responds to food cues (Gray, Hill and Bargmann, 2005). AIY is also known to be important for supressing reversal behaviour when worms are off food (Gray, Hill and Bargmann, 2005; Chalasani *et al.*, 2016) however its control of reversals has not been investigated in relation to the food border. Therefore, this neuron represents an interesting candidate to investigate its possible role in social behaviour by controlling sub-behaviours at the food border.

It can be hypothesised that if AIY were important for supressing reversals at the border and this neuron's function was impaired, it could lead to more frequent reversals and hence result in an abnormally low food leaving rate. *nlg-1* mutants have impaired progeny-induced food leaving (3.4.1) and this gene is expressed in 11 neurons (Table 1.1) including AIY interneurons (Hunter *et al.*, 2010; Calahorro *et al.*, 2019). An experiment which is currently ongoing is to test if *nlg-1* function is required in AIY for *C. elegans* social behaviour by performing cell specific rescue of *nlg-1* into AIY. The frequency of reversals performed at the border by wild type worms, *nlg-1* null and *nlg-1* R451C mutants could be quantified and compared to a rescue strain expressing *nlg-1* exclusively in AIY. This would facilitate insight into whether this neuron plays a role in reversal behaviours at the food border and if *nlg-1* is important for its function in the co-ordination of social behaviour.

Cellular activity is intrinsic to the function of interneurons for sensory integration (Macosko *et al.*, 2009; Kocabas *et al.*, 2012). Calcium imaging allows for the investigation of the dynamics of signal integration by monitoring neuronal activity and in *C. elegans* this can be achieved for a single neuron in freely behaving animals (Li *et al.*, 2014). Using this technique, AIY activity could be

monitored in wild type animals on naïve and progeny-loaded food lawns. This would allow AIY activity to be compared in the absence and presence of progeny to help understand if it is important for the integration of cues in the context of progeny to regulate enhanced food leaving behaviour. Following this, if AIY appeared to have a role in progeny-induced food leaving behaviour then the effect of genetic changes to *nlg-1* could be investigated. Comparing AIY activity in wild type and *nlg-1* mutants that have been shown to have social behaviour impairment would help to gain insight into any neural activity changes that may occur when the function of *nlg-1* is disrupted. This would help to elucidate if altered neuronal signalling has a part to play in impaired social behaviour and if so would help to model a clinical feature in autism which is the imbalance of signalling in the nervous system (1.1.4).

5.5 *C. elegans* as a model for genetic interaction in ASD

ASD has a complex polygenic architecture with multiple genes of varying penetrance additively contributing to an individual's risk for the disorder (1.2). In this thesis, orthologues of ASD-risk genes were investigated in *C. elegans* using single gene mutants (2.4). In doing so, 14 genes were identified as having a role in co-ordinating worm social behaviour. However, it is still unknown whether these genes interact at a genetic level to co-ordinate this behaviour (Figure 5.1).

Performing STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis on the 14 genes with a role in social behaviour identified known and predicted interactions between proteins encoded by these genes. The majority of the genetic interaction is between genes within the same biological domain, with interaction identified or predicted between synaptic and epigenetic modifying genes (Figure 5.4). For example, *nlg-1*, *nrx-1* and *shn-1* are predicted to interact in *C. elegans* (Figure 5.4). The interaction of neuroligin, neurexin and shank is well understood at the human synapse (Chen *et al.*, 2014) and the functional interaction of *nlg-1* and *nrx-1* in *C. elegans* has been suggested in multiple studies (Calahorro, Alejandre and Ruiz-Rubio, 2009; Hu *et al.*, 2012). Interestingly, the analysis also predicts possible crosstalk between epigenetic and cell signalling domains through the interaction of *chd-7* and *mbk-1* (Figure 5.4). Interaction of genes in distinct biological domains has been discussed in ASD (1.3.3) therefore it would be interesting to understand if these genes function together in the co-ordination of social behaviour.

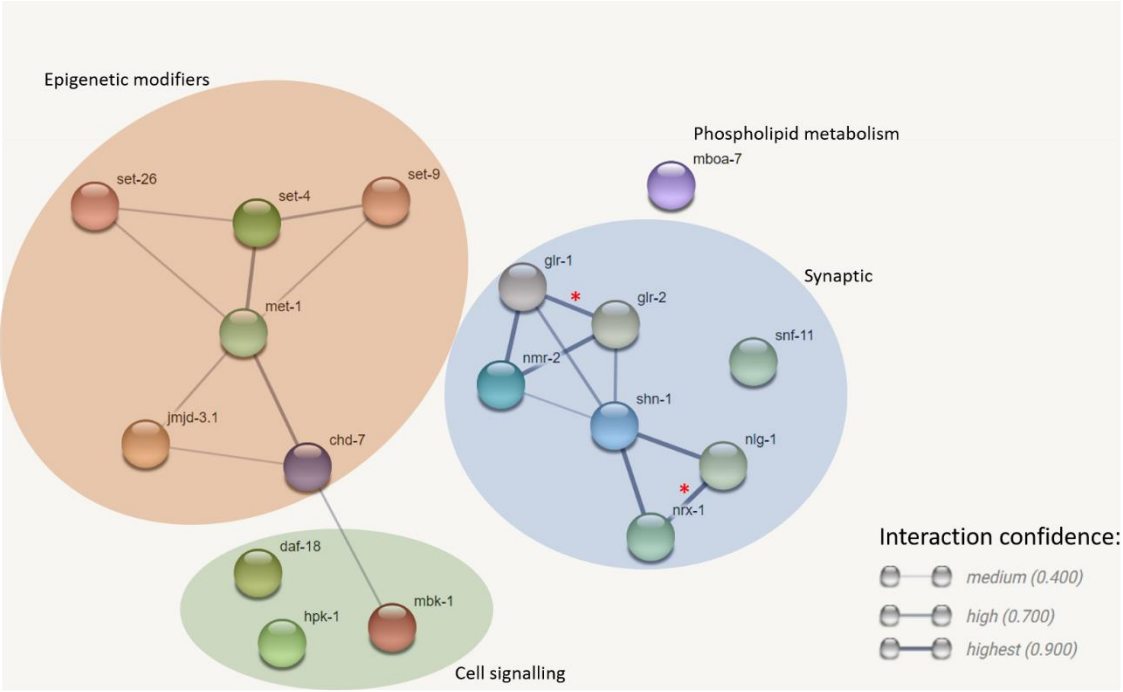


Figure 5.4 Interaction map of the 14 genes identified as having a role in social behaviour. Protein-protein interaction network showing the linkage between 14 genes in the STRING database. Genes are represented as different coloured nodes. Shaded circles group genes into their biological domains. STRING is a database of known and predicted protein-protein interactions that can be direct (physical) or indirect (functional) interactions. Interaction is represented by grey bars, the thickness of which represent the confidence of evidence for protein-protein interaction. STRING confidence scores are set on a scale of 0-1 where 1 represents the highest confidence in the evidence for protein-protein interaction (Szklarczyk *et al.*, 2020). STRING parameters were set as follows: active interaction sources included textmining, experiments, databases, co-expression, neighbourhood, gene fusion and co-occurrence; minimum required interaction score set at 'medium confidence (0.400)'. https://string-db.org/, Version 11.0. Red asterisks highlight two protein-protein interactions where a literature search identified experimental evidence from *C. elegans* studies to suggest the two genes functionally interact. For *glr-1-glr-2*, experimental evidence from: (Baidya *et al.*, 2014). For *nlg-1-nrx-1*, experimental evidence from: (Calahorro, Alejandre and Ruiz-Rubio, 2009; Hu *et al.*, 2012).

The bioinformatic predictions (Figure 5.4) combined with the genetic tractability of the model system *C. elegans*, would enable genetic pathways and interactions that contribute to social behaviour to begin to be dissected. This approach has the potential to characterise novel functional interactions between biological domains described in this thesis and to further understand the polygenic nature of ASD.

5.6 Changes to the SFARI gene database

5.6.1 Changes to the gene scoring criteria

Since my original investigation of genes in SFARI categories 1 and 2, SFARI gene database has changed the way it categorises ASD-risk genes. For category 1 (high confidence) genes, now in the archived version of SFARI (https://gene-archive.sfari.org/, accessed October 2018), gene variants had to meet genome wide statistical significance and be independently replicated in more than one human study. A similar standard needed to be met by genes in category 2 (strong candidate) except that no replication was needed. In the revised version of the SFARI gene database (https://gene.sfari.org/, accessed May 2021) for a gene to meet the standards of category 1 there must be at least three de novo likely-gene-disrupting mutations that have been associated with autism and the gene must meet a threshold of false discovery rate <0.1 but does not need to meet genome wide significance. For genes in category 2 at least two de novo mutations must have been identified with accompanying evidence that the variant has an effect on gene function (https://gene.sfari.org/about-gene-scoring/, version Q4, accessed May 2021). Also, genes that used to be spread across six different categories are now grouped into just three. Overall, this means that the number of genes in category 1 has increased from 25 to 207 and the number of genes in category 2 has increased from 66 to 211.

The lowering of statistical thresholds appears to have resulted in lower confidence genes being shifted up into categories 1 and 2. Interestingly, no genes in the SFARI gene database have moved down to a lower confidence category, all have remained the same or moved up therefore resulting in a larger number of genes in high confidence categories 1 and 2. However, the reason for this increase appears to be multifactorial, not only due to the alterations made to the gene scoring criteria. The addition of 200 novel genes into the database in the last two years and the accumulation of evidence for existing genes has also contributed to the changes seen within the database.

5.6.2 Re-categorisation of genes in the SFARI Gene database

Recently, SFARI have developed a new autism research initiative, recruiting 50,000 participants for autism research (https://www.sfari.org/resource/spark/). Access to this large cohort and the recent publication of the largest exome sequencing study to date (Satterstrom *et al.*, 2020) has resulted in the rapid accumulation of evidence for ASD-risk genes. This accumulation of evidence has contributed to the re-categorisation of genes. An extreme example of this is the re-categorisation of two category 5 genes into category 1. Category 5 in SFARI gene archive used to

list genes that were 'hypothetically' associated with ASD. This category was for genes where there was evidence from model organisms to suggest the gene was a risk factor for autism but no human studies to confirm this. The two genes are CREBBP, a histone acetyltransferase, and DLG4, a synaptic scaffold. For the DLG4 gene, the primary evidence that placed this gene into category 5 was a study that modelled multiple autistic phenotypes in mice upon knockout of this gene (Feyder *et al.*, 2010). Since then, the identification of these genes in the large human cohort exome sequencing study (Satterstrom *et al.*, 2020) has provided evidence justifying their re-categorisation. This suggests that the use of behavioural analysis in animal models is capable of identifying high risk ASD-associated genes before human evidence emerges. Therefore, the social behaviour used in this thesis and the ongoing analysis of underlying sub-behaviours could be used to investigate low confidence genes remaining in SFARI. Also, *C. elegans* behaviours can be used to investigate other neuropsychiatric disorders, such as SCZ (Dwyer, 2018). Ten of the human genes investigated in this thesis are thought to overlap with a risk for SCZ (Table 1.1). Also, one gene KDM6A has no associated psychiatric disorder listed on the SFARI database (Table 1.1). Therefore, it would be interesting to use *C. elegans* to investigate a link between these genes and other psychiatric disorders because it appears that model organisms can predict neuropsychiatric risk genes, the significance of which is currently underestimated in SFARI.

The re-categorisation has also affected the genes investigated in this thesis. At the time of analysis the majority of genes that entered the bioinformatic pipeline were in category 2 (2.4.1). In comparison, after the re-categorisation most genes investigated in this thesis are now in category 1 (Table 5.1). Therefore, this highlights that whilst the distribution of genes in the SFARI gene database has changed since my analysis, the genes that have been investigated in this thesis are still representative of those where there is a high level of confidence in the evidence associating them with ASD.

Table 5.1 The re-categorisation of genes investigated in this thesis by SFARI Gene database

Human gene name	SFARI Gene archive category	SFARI Gene category
GRIA1	2	2
GRIN2B	1	1
NLGN3	2	1
NRXN1	2	1

Human gene name	SFARI Gene archive category	SFARI Gene category
PTCHD1	2	1
SHANK2	2	1
SHANK3	1	1
SLC6A1	2	1
SYNGAP1	1	1
CACNA1H	2	2
CNTN4	2	2
DYRK1A	1	1
PTEN	1	1
CHD8	1	1
FOXP1	2	1
IRF2BPL	2	1
KDM6A	2	2
KMT5B	1	1
SETD2	2	1
SETD5	1	1
MBOAT7	2	1

At the beginning of the bioinformatic filtering process the 91 genes in categories 1 and 2 on SFARI Gene Archive could be classified into 12 different categories based on function. A large proportion of those genes (41%) were either epigenetic or synaptic in function (Figure 5.5). This is similar for

the genes investigated in this thesis following the bioinformatic filtering process (2.4.1) (Figure 5.5). Interestingly, re-analysis of genes in the latest version of SFARI showed that genes in categories 1 and 2 are still dominated by synaptic and epigenetic modifying genes. This shows that even though the genetic landscape in SFARI has changed dramatically since my analysis, the genes that have been investigated in this thesis are still representative of the functional domains which continue to be highly represented in both category 1 and 2.

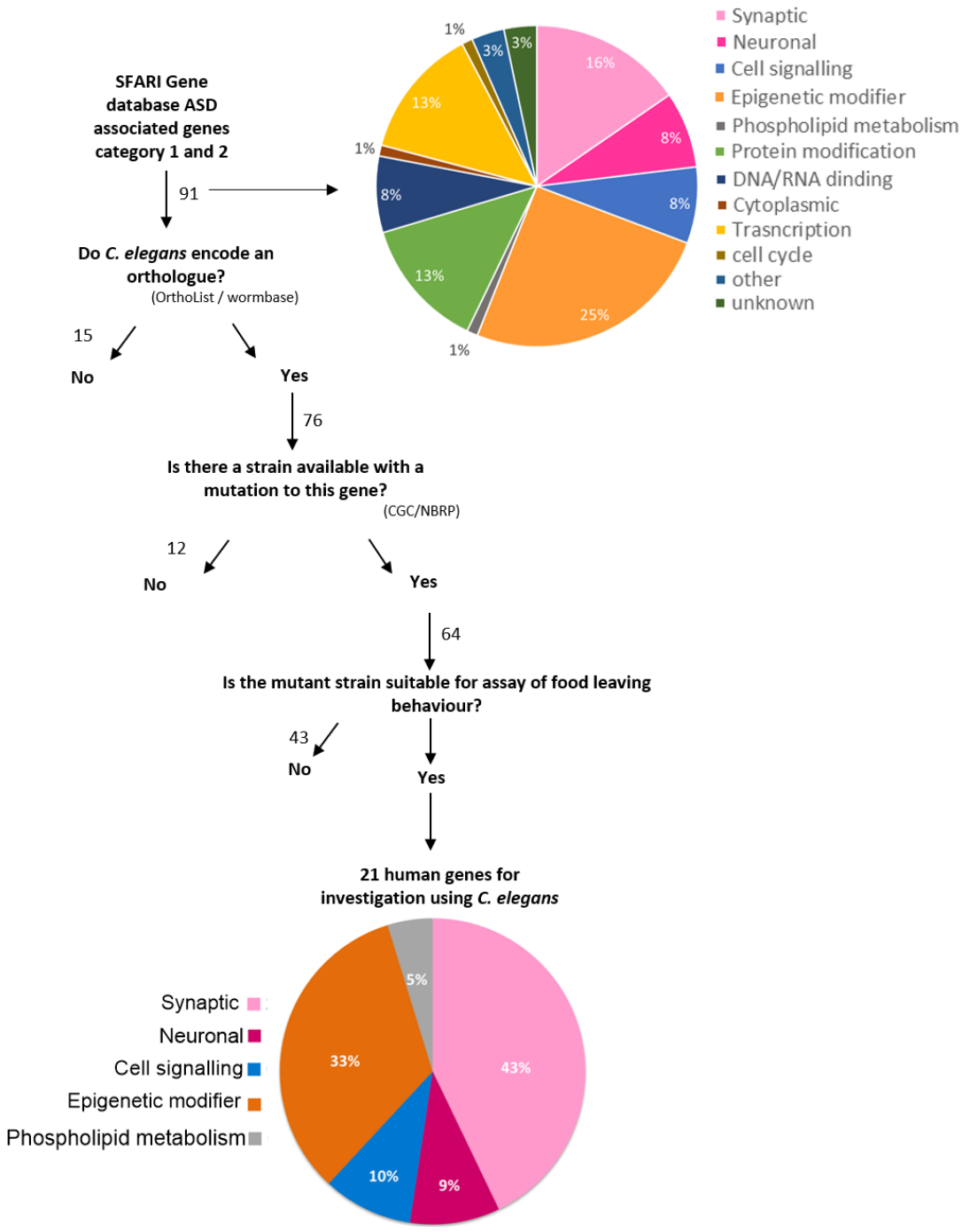


Figure 5.5 Epigenetic and synaptic genes are the dominant functional categories before and after the bioinformatic filtering process. High confidence ASD-associated genes in category 1 and 2 in SFARI Gene Archive were input. The pie chart shows the

percentage of the 91 genes that are placed into 12 different functional categories. The pipeline selects human genes which have an orthologue in *C. elegans* which can be studied in an available mutant strain which is neither lethal, sterile or uncoordinated. In brackets are the resources used for analysis. CGC – Caenorhabditis Genetics Center. NBRP – National BioResource Project. The number of genes analysed using SFARI Gene Archive (https://gene-archive.sfari.org/, accessed October 2018) are stated. The pie chart indicates the percent of the 21 human genes that were placed into five functional groupings. This figure is based on Figure 2.1.

5.7 Selecting genes for future study

5.7.1 Genes excluded during the bioinformatic filtering process

The bioinformatic pipeline developed in this thesis (2.4.1) led to the exclusion of human ASD-associated genes that could not be investigated using a *C. elegans* orthologue gene, because the mutant was lethal or sterile, or the available mutant was inappropriate for behavioural analysis. As well as genes excluded for these reasons, 12 human genes were excluded because there was no homozygous *C. elegans* mutant available for study. For the majority of the excluded genes RNAi analysis has shown that silencing of the *C. elegans* orthologue causes early larval lethality (Vandamme *et al.*, 2012; Maeda *et al.*, 2001) hence there was no stable homozygous mutant line for investigation. I chose to focus on homozygous, non-lethal, mutants to simplify the maintenance of strains and maximise the number of genes that could be investigated in this project. Furthermore, heterozygous genotypes have the potential to give rise to subtle behavioural effects, which could be challenging to characterise given the intrinsic variability within the food leaving assay and the low number of food leaving events (on average 4 leaving events in 30 minutes on a naïve food lawn and 13 on a pre-loaded lawn (Figure 3.2B). Sub-behaviours, like those previously described (5.3) may allow a more refined discrimination and identification of subtle behavioural differences within the food leaving assay and facilitate the investigation of heterozygous strains. With that in mind two of the excluded genes, one where no *C. elegans* mutant was available and one heterozygous mutant strain would be of interest for future study (Table 5.2).

Table 5.2 Two genes excluded during bioinformatic filtering of interest for future study

Excluded human gene	**CNTNAP2**	**ILF2**
Function	Neuron development (Rodenas-Cuadrado, Ho and Vernes, 2014).	Transcription factor (Marcoulatos *et al.*, 1996)
Reason for exclusion	No homozygous *C. elegans* mutant	No *C. elegans* mutant available from CGC or NBRP
***C. elegans* gene orthologue**	*nlr-1*	R11H6.5
Reason for interest in gene	Part of the neurexin family of genes. Important for gap junction formation and neuron function (Meng and Yan, 2020)	RNAi of R11H6.5 produces worms with abnormal fat content suggesting a role in metabolism (Ashrafi *et al.*, 2003)
How the gene can be investigated in the future	Use of quantifiable sub-behaviour to investigate heterozygous *nlr-1* strain	CRISPR to knock out or edit a precise lesion, for example point mutation Arg185Ter identified in association with autism (De Rubeis *et al.*, 2014)

Despite its relation to the well-known ASD-associated gene, neurexin, *nlr-1* is not well studied in *C. elegans.* I have shown that *nrx-1* mutants have an impaired social behaviour phenotype (2.4.4). Therefore, investigating *nlr-1* heterozygous mutants in the social behaviour assay may help understand if the role of the neurexin family of genes is broadly important for coordinating social behaviour in *C. elegans* as this seems to be the case in humans based on its association with autism and other psychiatric diseases (Kasem, Kurihara and Tabuchi, 2018). As well as this, R11H6.5 would be of interest for future study because of its possible role in *C. elegans* metabolism (Ashrafi *et al.*, 2003). Metabolism represents an underexplored area of biology in autism research (2.5). Therefore, understanding R11H6.5 in terms of its function may help to develop routes to investigate metabolic function in autism (Cheng, Rho and Masino, 2017). Investigating this gene's impact on social behaviour would also help to elucidate if there are more metabolic genetic determinants for impaired *C. elegans* social behaviour and therefore could add gravitas to my current findings that metabolism plays a key role in regulating social behaviour.

5.7.2 Prioritising the remaining SFARI ASD-risk genes for study

903 human genes remain in the SFARI Gene database that haven't been investigated for a role in social behaviour (190 in category 1, 207 in category 2 and 506 in category 3). An approach that could be taken to prioritise the remaining genes is to use interaction analysis to identify possible interactions between the genes identified in this thesis and the remaining SFARI genes. As an example of how this approach could be used, a single human gene from each biological domain identified in this thesis has been preliminarily analysed for protein-protein interaction using the STRING database (Table 5.3). From this, interactions between a gene of interest and other ASD-risk genes can be identified. For example, this analysis highlighted interaction between neuroligin, neurexin and shank, thus confirming the interest to investigate the possible interaction of these genes in *C. elegans* social behaviour (5.5). The analysis also highlights genes that haven't been investigated for a role in social behaviour and the *C. elegans* orthologues that could be used in future studies (Table 5.3). This preliminary analysis is not exhaustive. Future interaction analysis of genes could include looking more widely at the interactomes of each of the genes identified in this thesis. This will not only help to prioritise future genes for study but will also likely facilitate an understanding of which ASD-associated genes may interact within networks to control social behaviour in *C. elegans*.

Table 5.3 Prioritising SFARI ASD-risk genes for future analysis by analysing genetic interactions

Human gene of interest (*C. elegans* orthologue)	**Predicted protein-protein interaction of human gene of interest with other SFARI ASD-risk genes**	***C. elegans* orthologue**
NLGN3 (*nlg-1*)	SHANK1 SHANK2	*shn-1*
	NRXN1 NRXN2 NRXN3	*nrx-1*
	DLG2 DLG4	*dlg-1*
	DLGAP2	W03A5.6

Human gene of interest (*C. elegans* orthologue)	**Predicted protein-protein interaction of human gene of interest with other SFARI ASD-risk genes**	***C. elegans* orthologue**
	GRM5	*mgl-2*
PTEN (*daf-18*)	USP7	*math-33*
	PIK3R2	*aap-1*
CHD8 (*chd-7*)	KANSL1	None
	TAF1	*taf-1*
	KMT2A	None
	CTNNB1	*hmp-2, bar-1*
MBOAT7 (*mboa-7*)	None	-

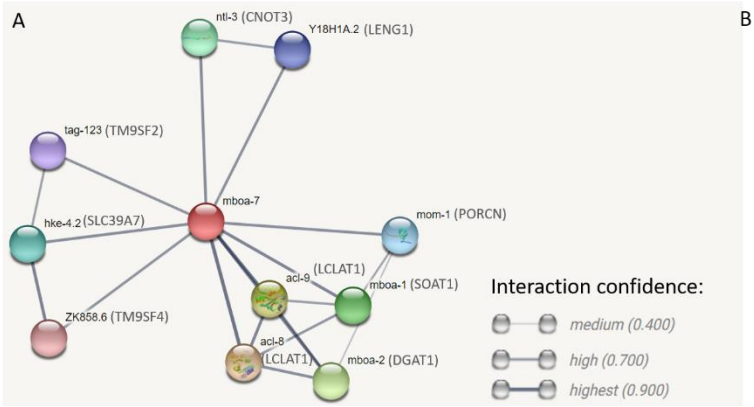
5.7.3 Mapping gene interaction to identify cellular pathways that may have a role in social behaviour

A key theory in the aetiology of autism, and other neuropsychiatric disorders, is that diverse cellular processes converge to disrupt nervous system formation and function (1.3). Therefore, understanding the types of cellular pathways that the genes identified in this thesis may have a role in will help to elucidate pathways that contribute to social behaviour and also highlight pathways for interrogation in other disorders. For example, MBOAT7 encodes an enzyme involved in phospholipid metabolism with a role in recycling lipids in and out of the cell membrane (Caddeo *et al.*, 2019). This gene has been associated with serval other psychiatric disorders as well as autism (Table 1.1) however its functional role in these disorders is not well understood.

Analysis of genetic interaction between ASD-risk genes showed that the MBOAT7 human gene had no interaction with other SFARI genes (Table 5.3) suggesting that its interactions with other ASD-risk genes may be limited or remain to be identified. To identify possible pathways that MBOAT7 may function in I analysed gene interactions of the *C. elegans* ortholog, *mboa-7* with other *C. elegans* genes without limiting the analysis to genes that have been associated with ASD.

This showed interactions of *mboa-7* with multiple genes with a similar role in lipid metabolism as well as genes with other functions, such as cell signalling (Figure 5.6). One of the predicted interactions of *mboa-7* is with the gene *mom-1* (Figure 5.6). *mom-1* is involved in the Wnt signalling pathway in *C. elegans* as it is thought to modify lipids to synthesise Wnt signalling proteins (Sawa and Korswagen, 2013). The predicted interaction of *mboa-7* with *mom-1* suggests that *mboa-7* may have functional interaction with a key regulator of the Wnt cell signalling pathway. Wnt signalling is important for various processes in *C. elegans* including development, aging and synapse formation (Sawa and Korswagen, 2013). The identification that MBOAT7 is associated with a number of psychiatric disorders (Table 1.1) suggests its dysfunction may impact pathways that are common to autism and other comorbid disorders, like ID and anxiety which also have disruption in the social domain (1.1.5). This highlights how the mapping of functional interactions opens the possibility to identify candidate pathways that may underpin social behaviour and other psychiatric disorders.

A



B

Interacting gene	Function
ntl-3	Transcription regulator
Y18H1A.2	Unknown
mom-1	Cell signalling
mboa-1	Lipid metabolism
mboa-2	Lipid metabolism
acl-8	Lipid metabolism
acl-9	Lipid metabolism
ZK858.6	Unknown
hke-4.2	Zinc transporter
tag-123	Unknown

Figure 5.6 Interaction of *mboa-7* with other *C. elegans* genes. (A) Protein-protein interaction network showing predicted interaction of *mboa-7* with ten *C. elegans* genes in the STRING database. Genes are represented as different coloured nodes. Human orthologues are indicated in brackets. STRING is a database of known and predicted protein-protein interactions that can be direct (physical) or indirect (functional) interactions. Interaction is represented by grey bars, the thickness of which represent the confidence of evidence for protein-protein interaction. STRING confidence scores are set on a scale of 0-1 where 1 represents the highest confidence in the evidence for protein-protein interaction (Szklarczyk *et al.*, 2020). STRING parameters were set as follows: active interaction sources included textmining, experiments, databases, co-expression, neighbourhood, gene fusion and co-occurrence; minimum required interaction score set at 'medium confidence (0.400)'. https://string-db.org/, Version 11.0. For this preliminary interaction analysis the number of interacting genes was capped at ten, further interactome analysis is needed to investigate the function of

other genes interacting with *mboa-7*. (B) Table of the ten *mboa-7* interacting genes and their functions.

5.8 Investigating the function of genes that are less well studied for their association with ASD

Of the genes that have been shown to have a role in social behaviour there are two which, to the best of my knowledge, haven't been investigated in *C. elegans* before in terms of how they impact an autism relevant behavioural phenotype. These genes are KDM6A and MBOAT7, the *C. elegans* orthologues of which are *jmjd-3.1* and *mboa-7* respectively. Both genes have been functionally characterised in *C. elegans* and have a similar proposed function to their human orthologue. *jmjd-3.1* has been shown to function as a histone methylase to regulate gene expression (Merkwirth *et al.*, 2016; Vandamme *et al.*, 2012) whilst *mboa-7* is an enzyme that functions to recycle phospholipids (Lee *et al.*, 2012b). How the biological function of these genes contributes to the properties of neuronal circuits that control social behaviour is of particular interest and can be addressed in the model organism *C. elegans* as outlined in the subsections that follow. Prior to any future experiments it will be important to confirm the phenotype observed is due to the genetic lesions in the gene of interest. This can be done by outcrossing mutant lines where necessary and conducting rescue experiments.

5.8.1 Investigating the function of the *jmjd-3.1* gene

Overexpression of *jmjd-3.1* in *C. elegans* has been shown to result in the differential expression of up to 7,000 genes, including genes involved in development, growth and translation (Merkwirth *et al.*, 2016). Currently, there is a limited understanding of the expression pattern of *jmjd-3.1* in *C. elegans* (Smith *et al.*, 2010). Using a transcriptional reporter would allow a precise characterisation of tissue expression of this gene in *C. elegans*. Combined with circuit level understanding from sub-behavioural analysis (5.4) these two approaches would begin to unpick which neurons and circuits *jmjd-3.1* functions within to co-ordinate social behaviour. The same construct would also allow for investigation of gene expression throughout development and may help elucidate if there is a developmentally timed role for this gene. The morphology of the nervous system in *C. elegans* is well characterised (1.5.3) and in conjunction with the investigations outlined above, morphological comparisons of candidate cells between wild type and mutant lines may help to establish if *jmjd-3.1* contributes to the development of the nervous system and therefore might provide insight into how disruption to this gene contributes to the impaired social behavioural phenotype.

5.8.2 Investigating the function of the *mboa-7* gene

5.8.2.1 Confirming the food leaving phenotype of *mboa-7* mutants

The impaired food leaving response to progeny seen in three of the four *mboa-7* mutant alleles highlighted this gene as a candidate for a role in *C. elegans* social behaviour. However, one of the mutants (*mboa-7(gk399)*) showed a food leaving response that was higher than the other mutants tested in both the 24 hour naïve food leaving assay and the pre-conditioned food leaving assay (Figure 5.7). For other genes tested as part of the same screen (2.4), differences in food leaving behaviour between different mutant alleles could be explained by differences in the other phenotypes investigated, for example egg laying deficiencies (2.4.3). However, in the case of the *mboa-7* mutants the other phenotypes investigated look similar between the four mutants (Figure 5.7), offering no obvious explanation as to why the food leaving rate of *mboa-7(gk399)* was higher than the others.

Biochemical assay of the four *mboa-7* deletion mutants showed that all had almost no acetyltransferase activity (Lee *et al.*, 2012b). This was used to suggest that all four mutants are strong loss-of-function. In this study all mutants were outcrossed at least five times prior to investigation (Lee *et al.*, 2012b). In contrast, when these mutants were assayed for food leaving behaviour they had not been outcrossed (Table 2.1). Therefore, it is possible that the food leaving phenotypes are confounded by extraneous mutations in the genetic background of these mutants. The fact that three of the four mutants phenocopied a food leaving deficit (Figure 5.7) suggests that the identification of this genes as a candidate for a role in *C. elegans* social behaviour is robust. However, to help confirm this suggestion, mutants should be outcrossed with N2 and food leaving behaviour should be re-tested and compared to the non-outcrossed strains. This will help to understand if the food leaving response of *mboa-7(gk399)* differed from the others due to background mutation effect. Furthering this, generation of a rescue construct to re-express *mboa-7* back into the mutants would allow the role of *mboa-7* on food leaving behaviour to be validated.

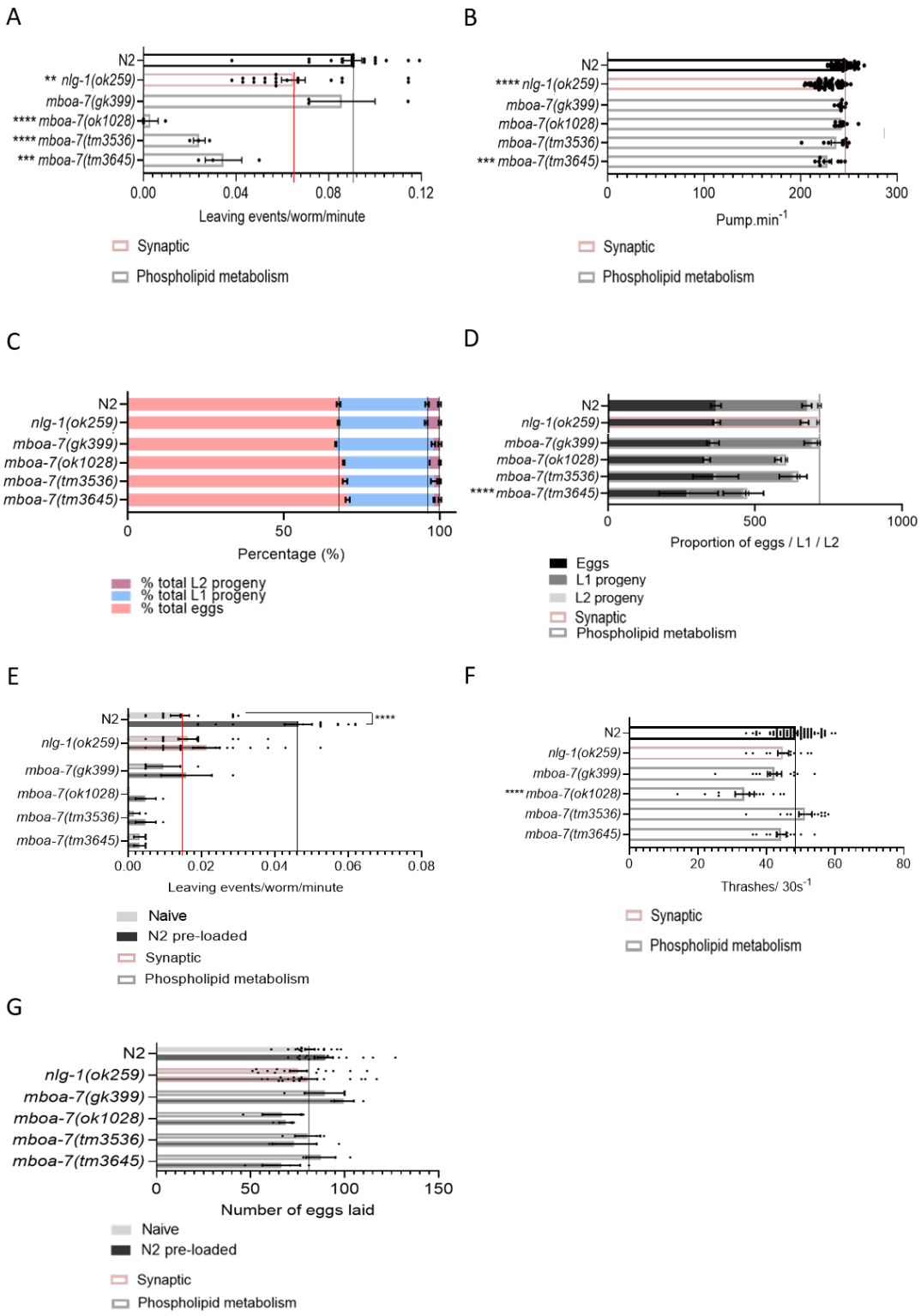


Figure 5.7 Summary of the phenotypic data for *mboa-7* mutants. (A) Food leaving rate of N2, *nlg-1(ok259)* and *mboa-7* mutants 24 hours after being placed onto a naïve food lawn. This data can be found in Figure 2.2. (B) Pharyngeal pump rate for N2, *nlg-1(ok259)* and *mboa-7* mutants counted after a food leaving assay at 24 hours. This data can be found in Figure 2.3. (C) Percent total eggs and progeny produced by N2, *nlg-1(ok259)* and *mboa-7* mutants at 24 hours. This data can be found in Figure 2.4. (D) Proportion of eggs and progeny produced by N2, *nlg-1(ok259)* and *mboa-7*

mutants after 24 hours. This data can be found in Figure 2.5. (E) Food leaving rate of N2, *nlg-1(ok259)* and *mboa-7* mutants in the absence of progeny and exposure to N2 progeny. This data can be found in Figure 2.7. (F) Thrashing behaviour of N2, *nlg-1(ok259)* and *mboa-7* mutants in liquid. This data can be found in Figure 2.8. (G) The number of eggs laid by N2, *nlg-1(ok259)* and *mboa-7* mutants in the absence and presence of progeny. This data can be found in Figure 2.9. For more information relating to this data see the relevant figure legends.

5.8.2.2 Probing the function of *mboa-7* in *C. elegans* social behaviour

Expression analysis of the *mboa-7* gene in *C. elegans* has shown that it is expressed ubiquitously during development and in the adult worm (Lee *et al.*, 2012b). Study of this gene in *C. elegans* and other model organisms is sparse, however it has been shown that it is important for proper brain development in mice (Lee *et al.*, 2012a). Given this, it would be interesting to understand if this gene is important for nervous system development in *C. elegans*. I have shown that for all *mboa-7* mutants tested their developmental progression from egg to L2 was not affected, suggesting the gene is not crucial for early development (2.4.3). However, these data only inform on the developmental timings of the worms and do not inform on potential developmental defects within the nervous system that might impair the ability to co-ordinate social behaviour (Hobert, 2010). Methods have been developed in *C. elegans* to selectively induce the degradation of a gene, allowing gene expression to be turned on and off during development (Zhang *et al.*, 2015; McDiarmid *et al.*, 2019). Similar techniques have been used to show that re-expression of a gene in late development can revert the effects of early development disruption and rescue behavioural phenotypes associated with another neurodevelopmental disorder (Ure *et al.*, 2016; Lamonica *et al.*, 2017). Therefore, investigating the effect of selectively expressing *mboa-7* during development would facilitate insight into whether this gene is required during development for the proper functioning of the nervous system and control of social behaviour. Also, it may provide insight into whether selective expression of *C. elegans* genes is a potential avenue for therapeutic research.

5.8.3 Mapping ASD mutations onto *C. elegans* proteins for future CRISPR analysis

Taking a similar approach as that used for the R451C mutation, the remaining *C. elegans* genes identified as having a role in social behaviour could be analysed for conservation of amino acids mutated in autism. This information would guide future CRISPR experiments to mimic human genetic variants and investigate the effect this has on social behaviour. As an example of how human variants could be selected for this approach, three missense variants identified in the

human MBOAT7 protein have been mapped onto the *C. elegans* MBOA-7 protein (Figure 5.8). Sequence alignment shows that the amino acid sequences are 29% identical and 48% similar. From this it can be seen that two of the three amino acids mutated by missense variants are conserved in *C. elegans* MBOA-7 and hence are candidates for future study by CRISPR.

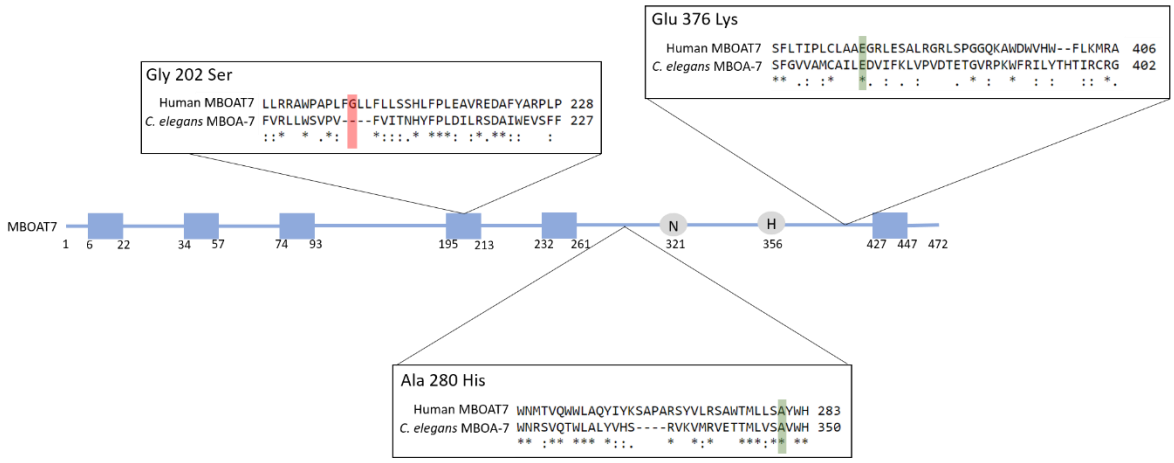


Figure 5.8 Mapping human MBOAT7 missense mutations onto *C. elegans mboa-7* protein. Cartoon showing human MBOAT7 protein (isoform 1). Amino acid positions indicated. Blue squares represent the parts of the protein sequence which is predicted to be transmembranous (Caddeo *et al.*, 2019). Asparagine (N) and histidine (H) residues are highlighted, these residues are predicted to be a catalytic dyad important for MBOAT7 activity (Caddeo *et al.*, 2019) and are conserved in *C. elegans*. Three human missense variants are shown in boxes. Partial sequence alignment between MBOAT7 and *C. elegans mboa-7* protein sequences are shown. Shaded in green are conserved amino acids that are candidates for CRISPR editing to mimic the human variant in *C. elegans*. Shaded in red is an amino acid that is not conserved in *C. elegans*. '*' indicates conservation of a single amino acid residue, ':' indicates conservation between amino acid groups with similar properties and '.' indicates conservation between amino acid groups with weakly similar properties. For Glu376Lys and Gly202Ser alignments MBOAT7 isoform 1 protein sequence was downloaded from UniProtKB, accession number: Q96N66. For Ala280His MBOAT7 alignment isoform 2 protein sequence was downloaded from UniProtKB, accession number: Q96N66. For all alignments the MBOA-7 (F14F3.3) sequence was downloaded from WormBase version WS280.

5.9 Summary

In summary, this thesis has provided evidence that *C. elegans* can be used to identify genetic determinants with a role in social behaviour. Additionally, I have shown the dependence of social

behaviour on the function of the *nlg-1* gene and that CRISPR editing can be used to probe the function of this gene in response to a human ASD-associated missense variant. The future directions discussed in this chapter highlight how my findings will facilitate the understanding of social behaviour at a level of genetic interaction, circuits and sub-behaviours. Continued understanding of the function of the genetic determinants I have identified at distinct levels of the biological system will facilitate the future aim to unpick the social circuit underlying social behaviour. An increased systems level understanding of *C. elegans* social behaviour will help to model systems level disruption associated with ASD and other brain disorders such as SCZ and epilepsy (1.3.4). This will ultimately help to understand how gene dysfunction impedes distinct levels of the biological system to result in the emergence of disrupted social behaviour and hopefully lead to the translation of these findings into the human system.

List of References

Abrahams, B. S., Arking, D. E., Campbell, D. B., Mefford, H. C., Morrow, E. M., Weiss, L. A., Menashe, I., Wadkins, T., Banerjee-Basu, S. and Packer, A. (2013) 'SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs)', *Molecular Autism,* 4(1), https://doi.org/10.1186/2040-2392-4-36.

Aceti, M., Creson, T. K., Vaissiere, T., Rojas, C., Huang, W.-C., Wang, Y.-X., Petralia, R. S., Page, D. T., Miller, C. A. and Rumbaugh, G. (2015) 'Syngap1 haploinsufficiency damages a postnatal critical period of pyramidal cell structural maturation linked to cortical circuit assembly', *Biological psychiatry,* 77(9), pp. 805-815.

Ackley, B. D. (2019) 'Behavior: Should I Stay or Should I Go?', *Current Biology,* 29(17), pp. 842-844.

Adachi, T., Kunitomo, H., Tomioka, M., Ohno, H., Okochi, Y., Mori, I. and Iino, Y. (2010) 'Reversal of Salt Preference Is Directed by the Insulin/PI3K and G(q)/PKC Signaling in Caenorhabditis elegans', *Genetics,* 186(4), pp. 1309-1383.

Adli, M. (2018) 'The CRISPR tool kit for genome editing and beyond', *Nat Commun,* 9(1), https://doi.org/10.1038/s41467-018-04252-2.

Aguirre-Chen, C., Stec, N., Ramos, O. M., Kim, N., Kramer, M., McCarthy, S., Gillis, J., McCombie, W. R. and Hammell, C. M. (2020) 'A Caenorhabditis elegans Model for Integrating the Functions of Neuropsychiatric Risk Genes Identifies Components Required for Normal Dendritic Morphology', *G3-Genes Genomes Genetics,* 10(5), pp. 1617-1628.

Albajara Sáenz, A., Septier, M., Van Schuerbeek, P., Baijot, S., Deconinck, N., Defresne, P., Delvenne, V., Passeri, G., Raeymaekers, H., Salvesen, L., Victoor, L., Villemonteix, T., Willaye, E., Peigneux, P. and Massat, I. (2020) 'ADHD and ASD: distinct brain patterns of inhibition-related activation?', *Translational Psychiatry,* 10(1), https://doi.org/10.1038/s41398-020-0707-z.

Altun, Z.F and Hall, D.H. 2009. Introduction. In *WormAtlas.* doi:10.3908/wormatlas.1.1

American Psychiatric, A. (2002) 'DSM-5 Development', *http://www.dsm5.org*.

Andersen, E. C. and Horvitz, H. R. (2007) 'Two C-elegans histone methyltransferases repress lin-3 EGF transcription to inhibit vulval development', *Development,* 134(16), pp. 2991-2999.

Anney, R. and Klei, L. and Pinto, D. and Almeida, J. and Bacchelli, E. and Baird, G. and Bolshakova, N. and Bölte, S. and Bolton, P. F. and Bourgeron, T. and Brennan, S. and Brian, J. and Casey, J. and Conroy, J. and Correia, C. and Corsello, C. and Crawford, E. L. and de Jonge, M. and Delorme, R. and Duketis, E. and Duque, F. and Estes, A. and Farrar, P. and Fernandez, B. A. and Folstein, S. E. and Fombonne, E. and Gilbert, J. and Gillberg, C. and Glessner, J. T. and Green, A. and Green, J. and Guter, S. J. and Heron, E. A. and Holt, R. and Howe, J. L. and Hughes, G. and Hus, V. and Igliozzi, R. and Jacob, S. and Kenny, G. P. and Kim, C. and Kolevzon, A. and Kustanovich, V. and Lajonchere, C. M. and Lamb, J. A. and Law-Smith, M. and Leboyer, M. and Le Couteur, A. and Leventhal, B. L. and Liu, X.-Q. and Lombard, F. and Lord, C. and Lotspeich, L. and Lund, S. C. and Magalhaes, T. R. and Mantoulan, C. and McDougle, C. J. and Melhem, N. M. and Merikangas, A. and Minshew, N. J. and Mirza, G. K. and Munson, J. and Noakes, C. and Nygren, G. and Papanikolaou, K. and Pagnamenta, A. T. and Parrini, B. and Paton, T. and Pickles, A. and Posey, D. J. and Poustka, F. and Ragoussis, J. and Regan, R. and Roberts, W. and Roeder, K. and Roge, B. and Rutter, M. L. and Schlitt, S. and Shah, N. and Sheffield, V. C. and Soorya, L. and Sousa, I. and Stoppioni, V. and Sykes, N. and Tancredi, R. and Thompson, A. P. and Thomson, S. and Tryfon, A. and Tsiantis, J. and Van Engeland, H. and Vincent, J. B. and Volkmar, F. and Vorstman, J. A. S. and

Wallace, S. and Wing, K. and Wittemeyer, K. and Wood, S. and Zurawiecki, D. and Zwaigenbaum, L. and Bailey, A. J. and Battaglia, A. and Cantor, R. M. and Coon, H. and Cuccaro, M. L. and Dawson, G. and Ennis, S. and Freitag, C. M. and Geschwind, D. H. and Haines, J. L. and Klauck, S. M. and McMahon, W. M. and Maestrini, E. and Miller, J. and Monaco, A. P. and Nelson, S. F. and Nurnberger, J. I., Jr. and Oliveira, G. and Parr, J. R. and Pericak-Vance, M. A. and Piven, J. and Schellenberg, G. D. and Scherer, S. W. and Vicente, A. M. and Wassink, T. H. and Wijsman, E. M. and Betancur, C. and Buxbaum, J. D. and Cook, E. H. and Gallagher, L. and Gill, M. and Hallmayer, J. and Paterson, A. D. and Sutcliffe, J. S. and Szatmari, P. and Vieland, V. J. and Hakonarson, H. and Devlin, B. (2012) 'Individual common variants exert weak effects on the risk for autism spectrum disorders', *Human molecular genetics,* 21(21), pp. 4781-4792.

Ardiel, E. L. and Rankin, C. H. (2009) 'Chapter 1 - C. elegans: Social Interactions in a “Nonsocial” Animal', *Advances in Genetics*: Academic Press, pp. 1-22.

Arribere, J. A., Bell, R. T., Fu, B. X. H., Artiles, K. L., Hartman, P. S. and Fire, A. Z. (2014) 'Efficient Marker-Free Recovery of Custom Genetic Modifications with CRISPR/Cas9 in Caenorhabditis elegans', *Genetics,* 198(3), pp. 837-842.

Artyukhin, A. B., Yim, J. J., Srinivasan, J., Izrayelit, Y., Bose, N., von Reuss, S. H., Jo, Y., Jordan, J. M., Baugh, L. R., Cheong, M., Sternberg, P. W., Avery, L. and Schroeder, F. C. (2013) 'Succinylated Octopamine Ascarosides and a New Pathway of Biogenic Amine Metabolism in Caenorhabditis elegans', *Journal of Biological Chemistry,* 288(26), pp. 18778-18783.

Ashrafi, K., Chang, F. Y., Watts, J. L., Fraser, A. G., Kamath, R. S., Ahringer, J. and Ruvkun, G. (2003) 'Genome-wide RNAi analysis of Caenorhabditis elegans fat regulatory genes', *Nature,* 421(6920), pp. 268-72.

Autism Spectrum Disorders Working Group of The Psychiatric Genomics, C. (2017) 'Meta-analysis of GWAS of over 16,000 individuals with autism spectrum disorder highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia', *Molecular autism,* 8, https://doi.org/10.1186/s13229-017-0137-9.

Avramopoulos, D. (2018) 'Recent Advances in the Genetics of Schizophrenia', *Molecular neuropsychiatry,* 4(1), pp. 35-51.

Ayhan, F. and Konopka, G. (2019) 'Regulatory genes and pathways disrupted in autism spectrum disorders', *Progress in neuro-psychopharmacology & biological psychiatry,* 89, pp. 57-64.

Bai, Y., Qiu, S., Li, Y., Li, Y., Zhong, W., Shi, M., Zhu, X., Jiang, H., Yu, Y., Cheng, Y. and Liu, Y. (2018) 'Genetic association between SHANK2 polymorphisms and susceptibility to autism spectrum disorder', *IUBMB Life,* 70(8), pp. 763-776.

Baidya, M., Genovez, M., Torres, M. and Chao, M. Y. (2014) 'Dopamine modulation of avoidance behavior in Caenorhabditis elegans requires the NMDA receptor NMR-1', *PloS one,* 9(8), pp. e102958-e102958.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E. and Rutter, M. (1995) 'Autism as a strongly genetic disorder: evidence from a British twin study', *Psychol Med,* 25(1), pp. 63-77.

Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., Kurzius-Spencer, M., Zahorodny, W., Rosenberg, C. R., White, T., Durkin, M. S., Imm, P., Nikolaou, L., Yeargin-Allsopp, M., Lee, L. C., Harrington, R., Lopez, M., Fitzgerald, R. T., Hewitt, A., Pettygrove, S., Constantino, J. N., Vehorn, A., Shenouda, J., Hall-Lande, J., Braun, K. V. and Dowling, N. F. (2018) 'Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism Developmental Disabilities

Monitoring Network, 11 Sites, United States, 2014', *Mmwr-Morbidity and Mortality Weekly Report,* 67(45), pp. 1-23.

Balasco, L., Provenzano, G. and Bozzi, Y. (2020) 'Sensory Abnormalities in Autism Spectrum Disorders: A Focus on the Tactile Domain, From Genetic Mouse Models to the Clinic', *Frontiers in Psychiatry,* 10, https://doi.org/10.3389/fpsyt.2019.01016.

Bali, V. and Bebok, Z. (2015) 'Decoding mechanisms by which silent codon changes influence protein biogenesis and function', *The international journal of biochemistry & cell biology,* 64, pp. 58-74.

Banerjee-Basu, S. and Packer, A. (2010) 'SFARI Gene: an evolving database for the autism research community', *Disease Models & Mechanisms,* 3(3-4), pp. 133-135.

Barak, B. and Feng, G. (2016) 'Neurobiology of social behavior abnormalities in autism and Williams syndrome', *Nature neuroscience,* 19(6), pp. 647-655.

Baranova, J., Dragunas, G., Botellho, M. C. S., Ayub, A. L. P., Bueno-Alves, R., Alencar, R. R., Papaiz, D. D., Sogayar, M. C., Ulrich, H. and Correa, R. G. (2021) 'Autism Spectrum Disorder: Signaling Pathways and Prospective Therapeutic Targets', *Cellular and Molecular Neurobiology,* 41(4), pp. 619-649.

Barbagallo, B., Prescott, H. A., Boyle, P., Climer, J. and Francis, M. M. (2010) 'A dominant mutation in a neuronal acetylcholine receptor subunit leads to motor neuron degeneration in Caenorhabditis elegans', *J Neurosci,* 30(42), pp. 13932-42.

Barešić, A., Nash, A. J., Dahoun, T., Howes, O. and Lenhard, B. (2020) 'Understanding the genetics of neuropsychiatric disorders: the potential role of genomic regulatory blocks', *Molecular Psychiatry,* 25(1), pp. 6-18.

Bargmann, C. I. (1998) 'Neurobiology of the Caenorhabditis elegans genome', *Science,* 282(5396), pp. 2028-2033.

Bargmann, C.I. Chemosensation in *C. elegans* (October 25, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.123.1, http://www.wormbook.org

Bargmann, C. I., Hartwieg, E. and Horvitz, H. R. (1993) 'Odorant-selective genes and neurons mediate olfaction in C. elegans', *Cell,* 74(3), pp. 515-27.

Bargmann CI, Mori I. Chemotaxis and Thermotaxis. In: Riddle DL, Blumenthal T, Meyer BJ, et al., editors. C. elegans II. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Chapter 25. Available from: https://www.ncbi.nlm.nih.gov/books/NBK19978/

Baudouin, S. J., Gaudias, J., Gerharz, S., Hatstatt, L., Zhou, K., Punnakkal, P., Tanaka, K. F., Spooren, W., Hen, R., De Zeeuw, C. I., Vogt, K. and Scheiffele, P. (2012) 'Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism', *Science,* 338(6103), pp. 128-32.

Bell, R. T., Fu, B. X. H. and Fire, A. Z. (2016) 'Cas9 Variants Expand the Target Repertoire in Caenorhabditis elegans', *Genetics,* 202(2), pp. 381-388.

Ben-David, E. and Shifman, S. (2013) 'Combined analysis of exome sequencing points toward a major role for transcription regulation during brain development in autism', *Molecular Psychiatry,* 18(10), pp. 1054-1056.

Ben Arous, J., Laffont, S. and Chatenay, D. (2009) 'Molecular and Sensory Basis of a Food Related Two-State Behavior in C. elegans', *Plos One,* 4(10), pp. e7584.

Ben Soussia, I., El Mouridi, S., Kang, D., Leclercq-Blondel, A., Khoubza, L., Tardy, P., Zariohi, N., Gendrel, M., Lesage, F., Kim, E.-J., Bichet, D., Andrini, O. and Boulin, T. (2019) 'Mutation of a single residue promotes gating of vertebrate and invertebrate two-pore domain potassium channels', *Nature Communications,* 10(1), doi: 10.1038/s41467-019-08710-3.

Bendesky, A., Tsunozaki, M., Rockman, M. V., Kruglyak, L. and Bargmann, C. I. (2011) 'Catecholamine receptor polymorphisms affect decision-making in C. elegans', *Nature,* 472(7343), pp. 313-318.

Bennetto, L., Kuschner, E. S. and Hyman, S. L. (2007) 'Olfaction and taste processing in autism', *Biological psychiatry,* 62(9), pp. 1015-1021.

Berg, J. M. and Geschwind, D. H. (2012) 'Autism genetics: searching for specificity and convergence', *Genome biology,* 13(7), pp. 247-247.

Bergan, J. F. (2015) 'Neural Computation and Neuromodulation Underlying Social Behavior', *Integrative and Comparative Biology,* 55(2), pp. 268-280.

Bergen, S. E., Ploner, A., Howrigan, D., O'Donovan, M. C., Smoller, J. W., Sullivan, P. F., Sebat, J., Neale, B. and Kendler, K. S. (2019) 'Joint Contributions of Rare Copy Number Variants and Common SNPs to Risk for Schizophrenia', *Am J Psychiatry,* 176(1), pp. 29-35.

Berkel, S., Marshall, C. R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D., Bonin, M., Riess, A., Engels, H., Sprengel, R., Scherer, S. W. and Rappold, G. A. (2010) 'Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation', *Nat Genet,* 42(6), pp. 489-91.

Bernier, R., Golzio, C., Xiong, B., Stessman, H. A., Coe, B. P., Penn, O., Witherspoon, K., Gerdts, J., Baker, C., Vulto-van Silfhout, A. T., Schuurs-Hoeijmakers, J. H., Fichera, M., Bosco, P., Buono, S., Alberti, A., Failla, P., Peeters, H., Steyaert, J., Vissers, L., Francescatto, L., Mefford, H. C., Rosenfeld, J. A., Bakken, T., O'Roak, B. J., Pawlus, M., Moon, R., Shendure, J., Amaral, D. G., Lein, E., Rankin, J., Romano, C., de Vries, B. B. A., Katsanis, N. and Eichler, E. E. (2014) 'Disruptive CHD8 Mutations Define a Subtype of Autism Early in Development', *Cell,* 158(2), pp. 263-276.

Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., Hutchison, J., Snape, M., Tranfaglia, M., Nguyen, D. V. and Hagerman, R. (2009) 'A pilot open label, single dose trial of fenobam in adults with fragile X syndrome', *Journal of medical genetics,* 46(4), pp. 266-271.

Besag, F. M. (2017) 'Epilepsy in patients with autism: links, risks and treatment challenges', *Neuropsychiatric disease and treatment,* 14, pp. 1-10.

Bessa, C., Maciel, P. and Rodrigues, A. J. (2013) 'Using C. elegans to decipher the cellular and molecular mechanisms underlying neurodevelopmental disorders', *Mol Neurobiol,* 48(3), pp. 465-89.

Bhatla, N., Droste, R., Sando, S. R., Huang, A. and Horvitz, H. R. (2015) 'Distinct Neural Circuits Control Rhythm Inhibition and Spitting by the Myogenic Pharynx of C. elegans', *Curr Biol,* 25(16), pp. 2075-89.

Bhattacharya, R., Touroutine, D., Barbagallo, B., Climer, J., Lambert, C. M., Clark, C. M., Alkema, M. J. and Francis, M. M. (2014) 'A conserved dopamine-cholecystokinin signaling pathway shapes context-dependent Caenorhabditis elegans behavior', *PLoS Genet,* 10(8), pp. e1004584.

Bilder, D., Botts, E. L., Smith, K. R., Pimentel, R., Farley, M., Viskochil, J., McMahon, W. M., Block, H., Ritvo, E., Ritvo, R.-A. and Coon, H. (2013) 'Excess mortality and causes of death in autism spectrum disorders: a follow up of the 1980s Utah/UCLA autism epidemiologic study', *Journal of autism and developmental disorders,* 43(5), pp. 1196-1204.

Bitar, T., Mavel, S., Emond, P., Nadal-Desbarats, L., Lefèvre, A., Mattar, H., Soufia, M., Blasco, H., Vourc'h, P., Hleihel, W. and Andres, C. R. (2018) 'Identification of metabolic pathway disturbances using multimodal metabolomics in autistic disorders in a Middle Eastern population', *J Pharm Biomed Anal,* 152, pp. 57-65.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K. (1998) 'A molecular evolutionary framework for the phylum Nematoda', *Nature,* 392(6671), pp. 71-75.

Boccuto, L., Lauri, M., Sarasua, S. M., Skinner, C. D., Buccella, D., Dwivedi, A., Orteschi, D., Collins, J. S., Zollino, M., Visconti, P., Dupont, B., Tiziano, D., Schroer, R. J., Neri, G., Stevenson, R. E., Gurrieri, F. and Schwartz, C. E. (2013) 'Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders', *Eur J Hum Genet,* 21(3), pp. 310-316.

Bolduc, F. V., Valente, D., Nguyen, A. T., Mitra, P. P. and Tully, T. (2010) 'An assay for social interaction in Drosophila fragile X mutants', *Fly,* 4(3), pp. 216-225.

Borlot, F., de Almeida, B. I., Combe, S. L., Andrade, D. M., Filloux, F. M. and Myers, K. A. (2019) 'Clinical utility of multigene panel testing in adults with epilepsy and intellectual disability', *Epilepsia,* 60(8), pp. 1661-1669.

Bourgeron, T. (2015) 'From the genetic architecture to synaptic plasticity in autism spectrum disorder', *Nature Reviews Neuroscience,* 16(9), pp. 551-563.

Bourgeron, T. (2016) 'Current knowledge on the genetics of autism and propositions for future research', *Comptes Rendus Biologies,* 339(7), pp. 300-307.

Brandwein, A. B., Foxe, J. J., Butler, J. S., Frey, H.-P., Bates, J. C., Shulman, L. H. and Molholm, S. (2015) 'Neurophysiological Indices of Atypical Auditory Processing and Multisensory Integration are Associated with Symptom Severity in Autism', *Journal of Autism and Developmental Disorders,* 45(1), pp. 230-244.

Brenner, S. (1974) 'The genetics of Caenorhabditis elegans', *Genetics,* 77(1), pp. 71-94.

Brisbin, S., Liu, J., Boudreau, J., Peng, J., Evangelista, M. and Chin-Sang, I. (2009) 'A Role for C. elegans Eph RTK Signaling in PTEN Regulation', *Developmental Cell,* 17(4), pp. 459-469.

Brockie, P. J., Madsen, D. M., Zheng, Y., Mellem, J. and Maricq, A. V. (2001) 'Differential expression of glutamate receptor subunits in the nervous system of Caenorhabditis elegans and their regulation by the homeodomain protein UNC-42', *Journal of Neuroscience,* 21(5), pp. 1510-1522.

Brouns, S. J. J., Jore, M. M., Lundgren, M., Westra, E. R., Slijkhuis, R. J. H., Snijders, A. P. L., Dickman, M. J., Makarova, K. S., Koonin, E. V. and van der Oost, J. (2008) 'Small CRISPR RNAs guide antiviral defense in prokaryotes', *Science,* 321(5891), pp. 960-964.

Buddell, T., Friedman, V., Drozd, C. J. and Quinn, C. C. (2019) 'An autism-causing calcium channel variant functions with selective autophagy to alter axon targeting and behavior', *PLoS genetics,* 15(12), pp. e1008488-e1008488.

Buescher, A. V. S., Cidav, Z., Knapp, M. and Mandell, D. S. (2014) 'Costs of Autism Spectrum Disorders in the United Kingdom and the United States', *Jama Pediatrics,* 168(8), pp. 721-728.

Burket, J. A., Herndon, A. L., Winebarger, E. E., Jacome, L. F. and Deutsch, S. I. (2011) 'Complex effects of mGluR5 antagonism on sociability and stereotypic behaviors in mice: Possible implications for the pharmacotherapy of autism spectrum disorders', *Brain Research Bulletin,* 86(3), pp. 152-158.

Butcher, R. A., Fujita, M., Schroeder, F. C. and Clardy, J. (2007) 'Small-molecule pheromones that control dauer development in Caenorhabditis elegans', *Nat Chem Biol,* 3(7), pp. 420-422.

Butcher, R. A., Ragains, J. R., Kim, E. and Clardy, J. (2008) 'A potent dauer pheromone component in Caenorhabditis elegans that acts synergistically with other components', *Proceedings of the National Academy of Sciences of the United States of America,* 105(38), pp. 14288-14292.

Butcher, R. A., Ragains, J. R., Li, W., Ruvkun, G., Clardy, J. and Mak, H. Y. (2009) 'Biosynthesis of the Caenorhabditis elegans dauer pheromone', *Proceedings of the National Academy of Sciences,* 106(6), pp. 1875-1879.

C Yuen, R. K., Merico, D., Bookman, M., L Howe, J., Thiruvahindrapuram, B., Patel, R. V., Whitney, J., Deflaux, N., Bingham, J., Wang, Z., Pellecchia, G., Buchanan, J. A., Walker, S., Marshall, C. R., Uddin, M., Zarrei, M., Deneault, E., D'Abate, L., Chan, A. J. S., Koyanagi, S., Paton, T., Pereira, S. L., Hoang, N., Engchuan, W., Higginbotham, E. J., Ho, K., Lamoureux, S., Li, W., MacDonald, J. R., Nalpathamkalam, T., Sung, W. W. L., Tsoi, F. J., Wei, J., Xu, L., Tasse, A.-M., Kirby, E., Van Etten, W., Twigger, S., Roberts, W., Drmic, I., Jilderda, S., Modi, B. M., Kellam, B., Szego, M., Cytrynbaum, C., Weksberg, R., Zwaigenbaum, L., Woodbury-Smith, M., Brian, J., Senman, L., Iaboni, A., Doyle-Thomas, K., Thompson, A., Chrysler, C., Leef, J., Savion-Lemieux, T., Smith, I. M., Liu, X., Nicolson, R., Seifer, V., Fedele, A., Cook, E. H., Dager, S., Estes, A., Gallagher, L., Malow, B. A., Parr, J. R., Spence, S. J., Vorstman, J., Frey, B. J., Robinson, J. T., Strug, L. J., Fernandez, B. A., Elsabbagh, M., Carter, M. T., Hallmayer, J., Knoppers, B. M., Anagnostou, E., Szatmari, P., Ring, R. H., Glazer, D., Pletcher, M. T. and Scherer, S. W. (2017) 'Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder', *Nature neuroscience,* 20(4), pp. 602-611.

Caddeo, A., Jamialahmadi, O., Solinas, G., Pujia, A., Mancina, R. M., Pingitore, P. and Romeo, S. (2019) 'MBOAT7 is anchored to endomembranes by six transmembrane domains', *Journal of Structural Biology,* 206(3), pp. 349-360.

Calahorro, F., Alejandre, E. and Ruiz-Rubio, M. (2009) 'Osmotic avoidance in Caenorhabditis elegans: synaptic function of two genes, orthologues of human NRXN1 and NLGN1, as candidates for autism', *Journal of visualized experiments : JoVE,* (34) https://doi.org/10.3791/1616.

Calahorro, F., Holden-Dye, L. and O'Connor, V. (2015) 'Analysis of splice variants for the C. elegans orthologue of human neuroligin reveals a developmentally regulated transcript', *Gene Expression Patterns,* 17(2), pp. 69-78.

Calahorro, F., Keefe, F., Dillon, J., Holden-Dye, L. and O'Connor, V. (2019) 'Neuroligin tuning of pharyngeal pumping reveals extrapharyngeal modulation of feeding in Caenorhabditis elegans', *Journal of Experimental Biology,* 222(3), jeb189423.

Calahorro, F. and Ruiz-Rubio, M. (2011) 'Caenorhabditis elegans as an experimental tool for the study of complex neurological diseases: Parkinson's disease, Alzheimer's disease and autism spectrum disorder', *Invert Neurosci,* 11(2), pp. 73-83.

Calahorro, F. and Ruiz-Rubio, M. (2012) 'Functional Phenotypic Rescue of Caenorhabditis elegans Neuroligin-Deficient Mutants by the Human and Rat NLGN1 Genes', *Plos One,* 7(6), pp. e39277.

Calahorro, F. and Ruiz-Rubio, M. (2013) 'Human alpha- and beta-NRXN1 isoforms rescue behavioral impairments of Caenorhabditis elegans neurexin-deficient mutants', *Genes Brain and Behavior,* 12(4), pp. 453-464.

Calhoun, A. J., Tong, A., Pokala, N., Fitzpatrick, J. A., Sharpee, T. O. and Chalasani, S. H. (2015) 'Neural Mechanisms for Evaluating Environmental Variability in Caenorhabditis elegans', *Neuron,* 86(2), pp. 428-41.

Carvill, G. L., McMahon, J. M., Schneider, A., Zemel, M., Myers, C. T., Saykally, J., Nguyen, J., Robbiano, A., Zara, F., Specchio, N., Mecarelli, O., Smith, R. L., Leventer, R. J., Møller, R. S., Nikanorova, M., Dimova, P., Jordanova, A., Petrou, S., Helbig, I., Striano, P., Weckhuysen, S., Berkovic, S. F., Scheffer, I. E. and Mefford, H. C. (2015) 'Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures', *Am J Hum Genet,* 96(5), pp. 808-15.

Centers for Disease Control and Prevention (2020) *Autism Spectrum Disorder (ASD)*: Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/ncbddd/autism/data.html (Accessed: 6th April 2021).

Chadman, K. K., Gong, S. C., Scattoni, M. L., Boltuck, S. E., Gandhy, S. U., Heintz, N. and Crawley, J. N. (2008) 'Minimal Aberrant Behavioral Phenotypes of Neuroligin-3 R451C Knockin Mice', *Autism Research,* 1(3), pp. 147-158.

Chalasani, S. H., Chronis, N., Tsunozaki, M., Gray, J. M., Ramot, D., Goodman, M. B. and Bargmann, C. I. (2016) 'Correction: Corrigendum: Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans', *Nature,* 533(7601), pp. 130-130.

Chang, S., Bray, S. M., Li, Z., Zarnescu, D. C., He, C., Jin, P. and Warren, S. T. (2008) 'Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila', *Nat Chem Biol,* 4(4), pp. 256-63.

Chao, M. Y., Komatsu, H., Fukuto, H. S., Dionne, H. M. and Hart, A. C. (2004) 'Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit', *Proceedings of the National Academy of Sciences of the United States of America,* 101(43), pp. 15512-15517.

Chaste, P. and Leboyer, M. (2012) 'Autism risk factors: genes, environment, and gene-environment interactions', *Dialogues in clinical neuroscience,* 14(3), pp. 281-292.

Chen, J., Yu, S., Fu, Y. and Li, X. (2014) 'Synaptic proteins and receptors defects in autism spectrum disorders', *Frontiers in cellular neuroscience,* 8, pp. 276-276.

Chen, K., Luan, X., Liu, Q., Wang, J., Chang, X., Snijders, A. M., Mao, J.-H., Secombe, J., Dan, Z., Chen, J.-H., Wang, Z., Dong, X., Qiu, C., Chang, X., Zhang, D., Celniker, S. E. and Liu, X. (2019) 'Drosophila Histone Demethylase KDM5 Regulates Social Behavior through Immune Control and Gut Microbiota Maintenance', *Cell host & microbe,* 25(4), pp. 537-552.

Chen, P. and Hong, W. (2018) 'Neural Circuit Mechanisms of Social Behavior', *Neuron,* 98(1), pp. 16-30.

Cheng, N., Rho, J. M. and Masino, S. A. (2017) 'Metabolic Dysfunction Underlying Autism Spectrum Disorder and Potential Treatment Approaches', *Frontiers in molecular neuroscience,* 10, doi:10.3389/fnmol.2017.00034.

Cherra, S. J. and Jin, Y. S. (2015) 'Advances in synapse formation: forging connections in the worm', *Wiley Interdisciplinary Reviews-Developmental Biology,* 4(2), pp. 85-97.

Chinwalla, A. T. and Cook, L. L. and Delehaunty, K. D. and Fewell, G. A. and Fulton, L. A. and Fulton, R. S. and Graves, T. A. and Hillier, L. W. and Mardis, E. R. and McPherson, J. D. and Miner, T. L. and Nash, W. E. and Nelson, J. O. and Nhan, M. N. and Pepin, K. H. and Pohl, C. S. and Ponce, T. C. and Schultz, B. and Thompson, J. and Trevaskis, E. and Waterston, R. H. and Wendl, M. C. and Wilson, R. K. and Yang, S.-P. and An, P. and Berry, E. and Birren, B. and Bloom, T. and Brown, D. G. and Butler, J. and Daly, M. and David, R. and Deri, J. and Dodge, S. and Foley, K. and Gage, D. and Gnerre, S. and Holzer, T. and Jaffe, D. B. and Kamal, M. and Karlsson, E. K. and Kells, C. and Kirby, A. and Kulbokas, E. J. and Lander, E. S. and Landers, T. and Leger, J. P. and Levine, R. and Lindblad-Toh, K. and Mauceli, E. and Mayer, J. H. and McCarthy, M. and Meldrim, J. and Meldrim, J. and

Mesirov, J. P. and Nicol, R. and Nusbaum, C. and Seaman, S. and Sharpe, T. and Sheridan, A. and Singer, J. B. and Santos, R. and Spencer, B. and Stange-Thomann, N. and Vinson, J. P. and Wade, C. M. and Wierzbowski, J. and Wyman, D. and Zody, M. C. and Birney, E. and Goldman, N. and Kasprzyk, A. and Mongin, E. and Rust, A. G. and Slater, G. and Stabenau, A. and Ureta-Vidal, A. and Whelan, S. and Ainscough, R. and Attwood, J. and Bailey, J. and Barlow, K. and Beck, S. and Burton, J. and Clamp, M. and Clee, C. and Coulson, A. and Cuff, J. and Curwen, V. and Cutts, T. and Davies, J. and Eyras, E. and Grafham, D. and Gregory, S. and Hubbard, T. and Hunt, A. and Jones, M. and Joy, A. and Leonard, S. and Lloyd, C. and Matthews, L. and McLaren, S. and McLay, K. and Meredith, B. and Mullikin, J. C. and Ning, Z. and Oliver, K. and Overton-Larty, E. and Plumb, R. and Potter, S. and Quail, M. and Rogers, J. and Scott, C. and Searle, S. and Shownkeen, R. and Sims, S. and Wall, M. and West, A. P. and Willey, D. and Williams, S. and Abril, J. F. and Guigó, R. and Parra, G. and Agarwal, P. and Agarwala, R. and Church, D. M. and Hlavina, W. and Maglott, D. R. and Sapojnikov, V. and Alexandersson, M. and Pachter, L. and Antonarakis, S. E. and Dermitzakis, E. T. and Reymond, A. and Ucla, C. and Baertsch, R. and Diekhans, M. and Furey, T. S. and Hinrichs, A. and Hsu, F. and Karolchik, D. and Kent, W. J. and Roskin, K. M. and Schwartz, M. S. and Sugnet, C. and Weber, R. J. and Bork, P. and Letunic, I. and Suyama, M. and Torrents, D. and Zdobnov, E. M. and Botcherby, M. and Brown, S. D. and Campbell, R. D. and Jackson, I. and Bray, N. and Couronne, O. and Dubchak, I. and Poliakov, A. and Rubin, E. M. and Brent, M. R. and Flicek, P. and Keibler, E. and Korf, I. and Batalov, S. and Bult, C. and Frankel, W. N. and Carninci, P. and Hayashizaki, Y. and Kawai, J. and Okazaki, Y. and Cawley, S. and Kulp, D. and Wheeler, R. and Chiaromonte, F. and Collins, F. S. and Felsenfeld, A. and Guyer, M. and Peterson, J. and Wetterstrand, K. and Copley, R. R. and Mott, R. and Dewey, C. and Dickens, N. J. and Emes, R. D. and Goodstadt, L. and Ponting, C. P. and Winter, E. and Dunn, D. M. and von Niederhausern, A. C. and Weiss, R. B. and Eddy, S. R. and Johnson, L. S. and Jones, T. A. and Elnitski, L. and Kolbe, D. L. and Eswara, P. and Miller, W. and O'Connor, M. J. and Schwartz, S. and Gibbs, R. A. and Muzny, D. M. and Glusman, G. and Smit, A. and Green, E. D. and Hardison, R. C. and Yang, S. and Haussler, D. and Hua, A. and Roe, B. A. and Kucherlapati, R. S. and Montgomery, K. T. and Li, J. and Li, M. and Lucas, S. and Ma, B. and McCombie, W. R. and Morgan, M. and Pevzner, P. and Tesler, G. and Schultz, J. and Smith, D. R. and Tromp, J. and Worley, K. C. and Lander, E. S. and Abril, J. F. and Agarwal, P. and Alexandersson, M. and Antonarakis, S. E. and Baertsch, R. and Berry, E. and Birney, E. and Bork, P. and Bray, N. and Brent, M. R. and Brown, D. G. and Butler, J. and Bult, C. and Chiaromonte, F. and Chinwalla, A. T. and Church, D. M. and Clamp, M. and Collins, F. S. and Copley, R. R. and Couronne, O. and Cawley, S. and Cuff, J. and Curwen, V. and Cutts, T. and Daly, M. and Dermitzakis, E. T. and Dewey, C. and Mouse Genome Sequencing, C. and Genome Sequencing, C. and Whitehead Institute, M. I. T. C. f. G. R. and European Bioinformatics, I. and Wellcome Trust Sanger, I. and Research Group in Biomedical, I. and Bioinformatics and National Center for Biotechnology, I. and Department of, M. and Division of Medical, G. and Center for Biomolecular, S. and Engineering and Embl and Consortium, U. M. M. S. and Lawrence Berkeley National, L. and Department of Computer, S. and School of Computer, S. and The Jackson, L. and Laboratory for Genome, E. and Affymetrix, I. and Departments of, S. and Health Evaluation, S. and National Human Genome Research, I. and Wellcome Trust Centre for Human, G. and Department of Electrical, E. and Department of Human, A. and Genetics and Department of Human, G. and Howard Hughes Medical, I. and Department of, G. and Departments of, B. and Molecular, B. and Computer, S. and Engineering and Department of Computer, S. and Engineering and Baylor College of, M. and The Institute for Systems, B. and Department of, B. and Molecular, B. and Howard Hughes Medical, I. and Department of, C. and Biochemistry and Departments of, G. and Medicine and Harvard-Partners Center for, G. and Genomics and Department of, S. and Institute, U. D. J. G. and Cold Spring Harbor, L. and Wellcome, T. and Max Planck Institute for Molecular, G. and Genome Therapeutics, C. and Bioinformatics Solutions, I. and Department of, M. and Human, G. and Department of, B. and Members of the Mouse Genome Analysis, G. (2002) 'Initial sequencing and comparative analysis of the mouse genome', *Nature,* 420(6915), pp. 520-562.

Chira, S., Gulei, D., Hajitou, A., Zimta, A.-A., Cordelier, P. and Berindan-Neagoe, I. (2017) 'CRISPR/Cas9: Transcending the Reality of Genome Editing', *Molecular Therapy - Nucleic Acids,* 7, pp. 211-222.

Choi, C. S., Gonzales, E. L., Kim, K. C., Yang, S. M., Kim, J.-W., Mabunga, D. F., Cheong, J. H., Han, S.-H., Bahn, G. H. and Shin, C. Y. (2016) 'The transgenerational inheritance of autism-like phenotypes in mice exposed to valproic acid during pregnancy', *Scientific Reports,* 6(1), pp. 36250.

Chourasia, N., Ossó-Rivera, H., Ghosh, A., Von Allmen, G. and Koenig, M. K. (2019) 'Expanding the Phenotypic Spectrum of CACNA1H Mutations', *Pediatric Neurology,* 93, pp. 50-55.

Christensen, E. L., Beasley, A., Radchuk, J., Mielko, Z. E., Preston, E., Stuckett, S., Murray, J. I. and Hudson, M. L. (2020) 'ngn-1/neurogenin Activates Transcription of Multiple Terminal Selector Transcription Factors in the Caenorhabditis elegans Nervous System', *G3 (Bethesda),* 10(6), pp. 1949-1962.

Chute, C. D., DiLoreto, E. M., Zhang, Y. K., Reilly, D. K., Rayes, D., Coyle, V. L., Choi, H. J., Alkema, M. J., Schroeder, F. C. and Srinivasan, J. (2019) 'Co-option of neurotransmitter signaling for inter-organismal communication in C. elegans', *Nature Communications,* 10(1), https://doi.org/10.1038/s41467-019-11240-7.

Clipperton-Allen, A. E. and Page, D. T. (2014) 'Pten haploinsufficient mice show broad brain overgrowth but selective impairments in autism-relevant behavioral tests', *Hum Mol Genet,* 23(13), pp. 3490-505.

Cohen, M., Reale, V., Olofsson, B., Knights, A., Evans, P. and de Bono, M. (2009) 'Coordinated regulation of foraging and metabolism in C. elegans by RFamide neuropeptide signaling', *Cell Metab,* 9(4), pp. 375-85.

Coll-Tané, M., Krebbers, A., Castells-Nobau, A., Zweier, C. and Schenck, A. (2019) 'Intellectual disability and autism spectrum disorders 'on the fly': insights from Drosophila', *Dis Model Mech,* 12(5), doi:10.1242/dmm.039180.

Comoletti, D., De Jaco, A., Jennings, L. L., Flynn, R. E., Gaietta, G., Tsigelny, I., Ellisman, M. H. and Taylor, P. (2004) 'The Arg451Cys-neuroligin-3 mutation associated with autism reveals a defect in protein processing', *J Neurosci,* 24(20), pp. 4889-93.

Cong, L., Ran, F. A., Cox, D., Lin, S. L., Barretto, R., Habib, N., Hsu, P. D., Wu, X. B., Jiang, W. Y., Marraffini, L. A. and Zhang, F. (2013) 'Multiplex Genome Engineering Using CRISPR/Cas Systems', *Science,* 339(6121), pp. 819-823.

Cook, S. J., Jarrell, T. A., Brittin, C. A., Wang, Y., Bloniarz, A. E., Yakovlev, M. A., Nguyen, K. C. Q., Tang, L. T. H., Bayer, E. A., Duerr, J. S., Bülow, H. E., Hobert, O., Hall, D. H. and Emmons, S. W. (2019) 'Whole-animal connectomes of both Caenorhabditis elegans sexes', *Nature,* 571(7763), pp. 63-71.

Corominas, R., Yang, X. P., Lin, G. N., Kang, S. L., Shen, Y., Ghamsari, L., Broly, M., Rodriguez, M., Tam, S., Trigg, S. A., Fan, C. Y., Yi, S., Tasan, M., Lemmens, I., Kuang, X. Y., Zhao, N., Malhotra, D., Michaelson, J. J., Vacic, V., Calderwood, M. A., Roth, F. P., Tavernier, J., Horvath, S., Salehi-Ashtiani, K., Korkin, D., Sebat, J., Hill, D. E., Hao, T., Vidal, M. and Iakoucheva, L. M. (2014) 'Protein interaction network of alternatively spliced isoforms from brain links genetic risk factors for autism', *Nature Communications,* 5, doi: 10.1038/ncomms4650.

Corsi, A. K. (2006) 'A biochemist's guide to Caenorhabditis elegans', *Analytical biochemistry,* 359(1), pp. 1-17.

Corthals, K., Heukamp, A. S., Kossen, R., Großhennig, I., Hahn, N., Gras, H., Göpfert, M. C., Heinrich, R. and Geurten, B. R. H. (2017) 'Neuroligins Nlg2 and Nlg4 Affect Social Behavior in Drosophila melanogaster', *Frontiers in psychiatry,* 8, doi:10.3389/fpsyt.2017.00113.

Cotney, J., Muhle, R. A., Sanders, S. J., Liu, L., Willsey, A. J., Niu, W., Liu, W. Z., Klei, L., Lei, J., Yin, J., Reilly, S. K., Tebbenkamp, A. T., Bichsel, C., Pletikos, M., Sestan, N., Roeder, K., State, M. W., Devlin, B. and Noonan, J. P. (2015) 'The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment', *Nature Communications,* 6, https://doi.org/10.1038/ncomms7404.

Courcet, J. B., Faivre, L., Malzac, P., Masurel-Paulet, A., Lopez, E., Callier, P., Lambert, L., Lemesle, M., Thevenon, J., Gigot, N., Duplomb, L., Ragon, C., Marle, N., Mosca-Boidron, A. L., Huet, F., Philippe, C., Moncla, A. and Thauvin-Robinet, C. (2012) 'The DYRK1A gene is a cause of syndromic intellectual disability with severe microcephaly and epilepsy', *J Med Genet,* 49(12), pp. 731-736.

Crabbe, J. C., Wahlsten, D. and Dudek, B. C. (1999) 'Genetics of mouse behavior: interactions with laboratory environment', *Science,* 284(5420), pp. 1670-1672.

Crawley, J. N. (2012) 'Translational animal models of autism and neurodevelopmental disorders', *Dialogues in clinical neuroscience,* 14(3), pp. 293-305.

Culetto, E. and Sattelle, D. B. (2000) 'A role for Caenorhabditis elegans in understanding the function and interactions of human disease genes', *Human Molecular Genetics,* 9(6), pp. 869-877.

Culotta, L. and Penzes, P. (2020) 'Exploring the mechanisms underlying excitation/inhibition imbalance in human iPSC-derived models of ASD', *Molecular Autism,* 11(1), https://doi.org/10.1186/s13229-020-00339-0.

Daghsni, M., Rima, M., Fajloun, Z., Ronjat, M., Brusés, J. L., M'Rad, R. and De Waard, M. (2018) 'Autism throughout genetics: Perusal of the implication of ion channels', *Brain and behavior,* 8(8), pp. e00978.

Dallière, N., Bhatla, N., Luedtke, Z., Ma, D. K., Woolman, J., Walker, R. J., Holden-Dye, L. and O'Connor, V. (2016) 'Multiple excitatory and inhibitory neural signals converge to fine-tune Caenorhabditis elegans feeding to food availability', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* 30(2), pp. 836-848.

Damasio, A. R. and Maurer, R. G. (1978) 'A neurological model for childhood autism', *Arch Neurol,* 35(12), pp. 777-86.

Das, R., Melo, J. A., Thondamal, M., Morton, E. A., Cornwell, A. B., Crick, B., Kim, J. H., Swartz, E. W., Lamitina, T., Douglas, P. M. and Samuelson, A. V. (2017) 'The homeodomain-interacting protein kinase HPK-1 preserves protein homeostasis and longevity through master regulatory control of the HSF-1 chaperone network and TORC1restricted autophagy in Caenorhabditis elegans', *Plos Genetics,* 13(10), pp. e1007038.

De Arras, L., Seng, A., Lackford, B., Keikhaee, M. R., Bowerman, B., Freedman, J. H., Schwartz, D. A. and Alper, S. (2013) 'An Evolutionarily Conserved Innate Immunity Protein Interaction Network', *Journal of Biological Chemistry,* 288(3), pp. 1967-1978.

de Bono, M. and Bargmann, C. I. (1998) 'Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in C-elegans', *Cell,* 94(5), pp. 679-689.

de Brouwer, A. P. M., Abou Jamra, R., Körtel, N., Soyris, C., Polla, D. L., Safra, M., Zisso, A., Powell, C. A., Rebelo-Guiomar, P., Dinges, N., Morin, V., Stock, M., Hussain, M., Shahzad, M., Riazuddin, S., Ahmed, Z. M., Pfundt, R., Schwarz, F., de Boer, L., Reis, A., Grozeva, D., Raymond, F. L., Riazuddin, S., Koolen, D. A., Minczuk, M., Roignant, J. Y., van Bokhoven, H. and Schwartz, S. (2018) 'Variants

in PUS7 Cause Intellectual Disability with Speech Delay, Microcephaly, Short Stature, and Aggressive Behavior', *Am J Hum Genet,* 103(6), pp. 1045-1052.

De Jaco, A., Lin, M. Z., Dubi, N., Comoletti, D., Miller, M. T., Camp, S., Ellisman, M., Butko, M. T., Tsien, R. Y. and Taylor, P. (2010) 'Neuroligin Trafficking Deficiencies Arising from Mutations in the alpha/beta-Hydrolase Fold Protein Family', *Journal of Biological Chemistry,* 285(37), pp. 28674-28682.

De Rubeis, S. and Buxbaum, J. D. (2015) 'Genetics and genomics of autism spectrum disorder: embracing complexity', *Human Molecular Genetics,* 24, pp. 24-31.

De Rubeis, S., He, X., Goldberg, A. P., Poultney, C. S., Samocha, K., Cicek, A. E., Kou, Y., Liu, L., Fromer, M., Walker, S., Singh, T., Klei, L., Kosmicki, J., Fu, S. C., Aleksic, B., Biscaldi, M., Bolton, P. F., Brownfeld, J. M., Cai, J. L., Campbell, N. G., Carracedo, A., Chahrour, M. H., Chiocchetti, A. G., Coon, H., Crawford, E. L., Crooks, L., Curran, S. R., Dawson, G., Duketis, E., Fernandez, B. A., Gallagher, L., Geller, E., Guter, S. J., Hill, R. S., Ionita-Laza, I., Gonzalez, P. J., Kilpinen, H., Klauck, S. M., Kolevzon, A., Lee, I., Lei, J., Lehtimaki, T., Lin, C. F., Ma'ayan, A., Marshall, C. R., McInnes, A. L., Neale, B., Owen, M. J., Ozaki, N., Parellada, M., Parr, J. R., Purcell, S., Puura, K., Rajagopalan, D., Rehnstrom, K., Reichenberg, A., Sabo, A., Sachse, M., Sanders, S. J., Schafer, C., Schulte-Ruther, M., Skuse, D., Stevens, C., Szatmari, P., Tammimies, K., Valladares, O., Voran, A., Wang, L. S., Weiss, L. A., Willsey, A. J., Yu, T. W., Yuen, R. K. C., Cook, E. H., Freitag, C. M., Gill, M., Hultman, C. M., Lehner, T., Palotie, A., Schellenberg, G. D., Skiar, P., State, M. W., Sutcliffe, J. S., Walsh, C. A., Scherer, S. W., Zwick, M. E., Barrett, J. C., Cutler, D. J., Roeder, K., Devlin, B., Daly, M. J., Buxbaum, J. D., Study, D. D. D., Homozygosity Mapping, C., Consortium, U. K. and Autism Sequencing, C. (2014) 'Synaptic, transcriptional and chromatin genes disrupted in autism', *Nature,* 515(7526), pp. 209-215.

Delaney, C. E., Chen, A. T., Graniel, J. V., Dumas, K. J. and Hu, P. J. (2017) 'A histone H4 lysine 20 methyltransferase couples environmental cues to sensory neuron control of developmental plasticity', *Development,* 144(7), pp. 1273-1282.

Devlin, B. and Scherer, S. W. (2012) 'Genetic architecture in autism spectrum disorder', *Current Opinion in Genetics & Development,* 22(3), pp. 229-237.

Dickinson, D. J. and Goldstein, B. (2016) 'CRISPR-Based Methods for Caenorhabditis elegans Genome Engineering', *Genetics,* 202(3), pp. 885-901.

Dickinson, D. J., Ward, J. D., Reiner, D. J. and Goldstein, B. (2013) 'Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination', *Nature Methods,* 10(10), pp. 1028-1034.

Dimassi, S., Andrieux, J., Labalme, A., Lesca, G., Cordier, M.-P., Boute, O., Neut, D., Edery, P., Sanlaville, D. and Schluth-Bolard, C. (2013) 'Interstitial 12p13.1 deletion involving GRIN2B in three patients with intellectual disability', *American Journal of Medical Genetics Part A,* 161(10), pp. 2564-2569.

Dinstein, I., Heeger, D. J., Lorenzi, L., Minshew, N. J., Malach, R. and Behrmann, M. (2012) 'Unreliable evoked responses in autism', *Neuron,* 75(6), pp. 981-991.

Doherty, J. L. and Owen, M. J. (2014) 'Genomic insights into the overlap between psychiatric disorders: implications for research and clinical practice', *Genome medicine,* 6(4), doi: 10.1186/gm546.

Doll, C. and Broadie, K. (2014) 'Impaired activity-dependent neural circuit assembly and refinement in autism spectrum disorder genetic models', *Frontiers in Cellular Neuroscience,* 8(30), doi:10.3389/fncel.2014.00030.

Doshi-Velez, F., Ge, Y. and Kohane, I. (2014) 'Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis', *Pediatrics,* 133(1), pp. e54-e63.

Drozd, M., Bardoni, B. and Capovilla, M. (2018) 'Modeling Fragile X Syndrome in Drosophila', *Frontiers in molecular neuroscience,* 11, doi:10.3389/fnmol.2018.00124.

Dudbridge, F. (2013) 'Power and predictive accuracy of polygenic risk scores', *PLoS genetics,* 9(3), pp. e1003348-e1003348.

Duerr, J. S., Frisby, D. L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L. E. and Rand, J. B. (1999) 'The cat-1 gene of Caenorhabditis elegans encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors', *J Neurosci,* 19(1), pp. 72-84.

Dunn, E. C., Brown, R. C., Dai, Y., Rosand, J., Nugent, N. R., Amstadter, A. B. and Smoller, J. W. (2015) 'Genetic determinants of depression: recent findings and future directions', *Harvard review of psychiatry,* 23(1), pp. 1-18.

Durand, C. M., Betancur, C., Boeckers, T. M., Bockmann, J., Chaste, P., Fauchereau, F., Nygren, G., Rastam, M., Gillberg, I. C., Anckarsater, H., Sponheim, E., Goubran-Botros, H., Delorme, R., Chabane, N., Mouren-Simeoni, M. C., de Mas, P., Bieth, E., Roge, B., Heron, D., Burglen, L., Gillberg, C., Leboyer, M. and Bourgeron, T. (2007) 'Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders', *Nature Genetics,* 39(1), pp. 25-27.

Dwyer, D. S. (2018) 'Crossing the Worm-Brain Barrier by Using Caenorhabditis elegans to Explore Fundamentals of Human Psychiatric Illness', *Molecular neuropsychiatry,* 3(3), pp. 170-179.

Eack, S. M., Bahorik, A. L., McKnight, S. A. F., Hogarty, S. S., Greenwald, D. P., Newhill, C. E., Phillips, M. L., Keshavan, M. S. and Minshew, N. J. (2013) 'Commonalities in social and non-social cognitive impairments in adults with autism spectrum disorder and schizophrenia', *Schizophrenia research,* 148(1-3), pp. 24-28.

Ebert, D. H. and Greenberg, M. E. (2013) 'Activity-dependent neuronal signalling and autism spectrum disorder', *Nature,* 493(7432), pp. 327-337.

El Mouridi, S., Lecroisey, C., Tardy, P., Mercier, M., Leclercq-Blondel, A., Zariohi, N. and Boulin, T. (2017) 'Reliable CRISPR/Cas9 Genome Engineering in Caenorhabditis elegans Using a Single Efficient sgRNA and an Easily Recognizable Phenotype', *G3-Genes Genomes Genetics,* 7(5), pp. 1429-1437.

Ellenbroek, B. and Youn, J. (2016) 'Rodent models in neuroscience research: is it a rat race?', *Disease Models & Mechanisms,* 9(10), pp. 1079-1087.

Endevelt-Shapira, Y., Perl, O., Ravia, A., Amir, D., Eisen, A., Bezalel, V., Rozenkrantz, L., Mishor, E., Pinchover, L., Soroka, T., Honigstein, D. and Sobel, N. (2018) 'Altered responses to social chemosignals in autism spectrum disorder', *Nature Neuroscience,* 21(1), pp. 111-119.

Engert, C. G., Droste, R., van Oudenaarden, A. and Horvitz, H. R. (2018) 'A C. elegans protein with a PRDM9-like SET domain localizes to chromatin-associated foci and promotes spermatocyte gene expression, sperm production and fertility', *Plos Genetics,* 14(4), pp. e1007295.

Epi, K. C., Epilepsy Phenome/Genome, P., Allen, A. S., Berkovic, S. F., Cossette, P., Delanty, N., Dlugos, D., Eichler, E. E., Epstein, M. P., Glauser, T., Goldstein, D. B., Han, Y., Heinzen, E. L., Hitomi, Y., Howell, K. B., Johnson, M. R., Kuzniecky, R., Lowenstein, D. H., Lu, Y.-F., Madou, M. R. Z., Marson, A. G., Mefford, H. C., Esmaeeli Nieh, S., O'Brien, T. J., Ottman, R., Petrovski, S., Poduri, A., Ruzzo, E. K., Scheffer, I. E., Sherr, E. H., Yuskaitis, C. J., Abou-Khalil, B., Alldredge, B. K., Bautista, J. F., Berkovic, S. F., Boro, A., Cascino, G. D., Consalvo, D., Crumrine, P., Devinsky, O., Dlugos, D.,

Epstein, M. P., Fiol, M., Fountain, N. B., French, J., Friedman, D., Geller, E. B., Glauser, T., Glynn, S., Haut, S. R., Hayward, J., Helmers, S. L., Joshi, S., Kanner, A., Kirsch, H. E., Knowlton, R. C., Kossoff, E. H., Kuperman, R., Kuzniecky, R., Lowenstein, D. H., McGuire, S. M., Motika, P. V., Novotny, E. J., Ottman, R., Paolicchi, J. M., Parent, J. M., Park, K., Poduri, A., Scheffer, I. E., Shellhaas, R. A., Sherr, E. H., Shih, J. J., Singh, R., Sirven, J., Smith, M. C., Sullivan, J., Lin Thio, L., Venkat, A., Vining, E. P. G., Von Allmen, G. K., Weisenberg, J. L., Widdess-Walsh, P. and Winawer, M. R. (2013) 'De novo mutations in epileptic encephalopathies', *Nature,* 501(7466), pp. 217-221.

Eshraghi, A. A., Liu, G., Kay, S. I. S., Eshraghi, R. S., Mittal, J., Moshiree, B. and Mittal, R. (2018) 'Epigenetics and Autism Spectrum Disorder: Is There a Correlation?', *Frontiers in Cellular Neuroscience,* 12, doi:10.3389/fncel.2018.00078.

Etherton, M., Foldy, C., Sharma, M., Tabuchi, K., Liu, X. R., Shamloo, M., Malenka, R. C. and Sudhof, T. C. (2011) 'Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function', *Proceedings of the National Academy of Sciences of the United States of America,* 108(33), pp. 13764-13769.

Ezcurra, M., Tanizawa, Y., Swoboda, P. and Schafer, W. R. (2011) 'Food sensitizes C. elegans avoidance behaviours through acute dopamine signalling', *The EMBO journal,* 30(6), pp. 1110-1122.

Fagan, K. A. and Portman, D. S. (2014) 'Sexual modulation of neural circuits and behavior in Caenorhabditis elegans', *Seminars in cell & developmental biology,* 33, pp. 3-9.

Faras, H., Al Ateeqi, N. and Tidmarsh, L. (2010) 'Autism spectrum disorders', *Annals of Saudi Medicine,* 30(4), pp. 295-300.

Farboud, B. and Meyer, B. J. (2015) 'Dramatic Enhancement of Genome Editing by CRISPR/Cas9 Through Improved Guide RNA Design', *Genetics,* 199(4), pp. 959-971.

Fatemi, S. H., Folsom, T. D., Kneeland, R. E. and Liesch, S. B. (2011) 'Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism', *Anat Rec (Hoboken),* 294(10), pp. 1635-45.

Faundes, V., Newman, W. G., Bernardini, L., Canham, N., Clayton-Smith, J., Dallapiccola, B., Davies, S. J., Demos, M. K., Goldman, A., Gill, H., Horton, R., Kerr, B., Kumar, D., Lehman, A., McKee, S., Morton, J., Parker, M. J., Rankin, J., Robertson, L., Temple, I. K., Clinical Assessment of the Utility of, S., Evaluation as a Service, S., Deciphering Developmental Disorders, S. and Banka, S. (2018) 'Histone Lysine Methylases and Demethylases in the Landscape of Human Developmental Disorders', *American journal of human genetics,* 102(1), pp. 175-187.

Fedoriw, A., Mugford, J. and Magnuson, T. (2012) 'Genomic Imprinting and Epigenetic Control of Development', *Cold Spring Harbor Perspectives in Biology,* 4(7), pp. a008136.

Feng, H. Y., Craig, H. L. and Hope, I. A. (2012) 'Expression Pattern Analysis of Regulatory Transcription Factors in Caenorhabditis elegans', in Deplancke, B. and Gheldof, N. (eds.) *Gene Regulatory Networks: Methods and Protocols Methods in Molecular Biology*. Totowa: Humana Press Inc, pp. 21-50.

Feng, J. N., Schroer, R., Yan, J., Song, W. J., Yang, C. M., Bockholt, A., Cook, E. H., Skinner, C., Schwartz, C. E. and Sommer, S. S. (2006) 'High frequency of neurexin 1 beta signal peptide structural variants in patients with autism', *Neuroscience Letters,* 409(1), pp. 10-13.

Fernandez, T., Morgan, T., Davis, N., Klin, A., Morris, A., Farhi, A., Lifton, R. P. and State, M. W. (2004) 'Disruption of contactin 4 (CNTN4) results in developmental delay and other features of 3p deletion syndrome', *American journal of human genetics,* 74(6), pp. 1286-1293.

Fertan, E., Wong, A. A., Purdon, M. K., Weaver, I. C. G. and Brown, R. E. (2021) 'The effect of background strain on the behavioral phenotypes of the MDGA2(+/-) mouse model of autism spectrum disorder', *Genes Brain Behav,* 20(3), pp. e12696.

Feyder, M., Karlsson, R. M., Mathur, P., Lyman, M., Bock, R., Momenan, R., Munasinghe, J., Scattoni, M. L., Ihne, J., Camp, M., Graybeal, C., Strathdee, D., Begg, A., Alvarez, V. A., Kirsch, P., Rietschel, M., Cichon, S., Walter, H., Meyer-Lindenberg, A., Grant, S. G. and Holmes, A. (2010) 'Association of mouse Dlg4 (PSD-95) gene deletion and human DLG4 gene variation with phenotypes relevant to autism spectrum disorders and Williams' syndrome', *Am J Psychiatry,* 167(12), pp. 1508-17.

Flavell, S. W., Pokala, N., Macosko, E. Z., Albrecht, D. R., Larsch, J. and Bargmann, C. I. (2013) 'Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans', *Cell,* 154(5), pp. 1023-1035.

Folstein, S. and Rutter, M. (1977) 'Genetic influences and infantile autism', *Nature,* 265(5596), pp. 726-728.

Franklin, T. B., Russig, H., Weiss, I. C., Graff, J., Linder, N., Michalon, A., Vizi, S. and Mansuy, I. M. (2010) 'Epigenetic Transmission of the Impact of Early Stress Across Generations', *Biological Psychiatry,* 68(5), pp. 408-415.

Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiacovo, M. P., Church, G. M. and Calarco, J. A. (2013) 'Heritable genome editing in C. elegans via a CRISPR-Cas9 system', *Nature Methods,* 10(8), pp. 741-743.

Fromer, M., Roussos, P., Sieberts, S. K., Johnson, J. S., Kavanagh, D. H., Perumal, T. M., Ruderfer, D. M., Oh, E. C., Topol, A., Shah, H. R., Klei, L. L., Kramer, R., Pinto, D., Gümüş, Z. H., Cicek, A. E., Dang, K. K., Browne, A., Lu, C., Xie, L., Readhead, B., Stahl, E. A., Xiao, J., Parvizi, M., Hamamsy, T., Fullard, J. F., Wang, Y.-C., Mahajan, M. C., Derry, J. M. J., Dudley, J. T., Hemby, S. E., Logsdon, B. A., Talbot, K., Raj, T., Bennett, D. A., De Jager, P. L., Zhu, J., Zhang, B., Sullivan, P. F., Chess, A., Purcell, S. M., Shinobu, L. A., Mangravite, L. M., Toyoshiba, H., Gur, R. E., Hahn, C.-G., Lewis, D. A., Haroutunian, V., Peters, M. A., Lipska, B. K., Buxbaum, J. D., Schadt, E. E., Hirai, K., Roeder, K., Brennand, K. J., Katsanis, N., Domenici, E., Devlin, B. and Sklar, P. (2016) 'Gene expression elucidates functional impact of polygenic risk for schizophrenia', *Nature neuroscience,* 19(11), pp. 1442-1453.

Frye, R. E., Casanova, M. F., Fatemi, S. H., Folsom, T. D., Reutiman, T. J., Brown, G. L., Edelson, S. M., Slattery, J. C. and Adams, J. B. (2016) 'Neuropathological Mechanisms of Seizures in Autism Spectrum Disorder', *Frontiers in Neuroscience,* 10(192), doi:10.3389/fnins.2016.00192.

Fu, Y., Foden, J. A., Khayter, C., Maeder, M. L., Reyon, D., Joung, J. K. and Sander, J. D. (2013) 'High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells', *Nature Biotechnology,* 31(9), pp. 822-826.

Fujiwara, M., Sengupta, P. and McIntire, S. L. (2002) 'Regulation of body size and behavioral state of C. elegans by sensory perception and the EGL-4 cGMP-dependent protein kinase', *Neuron,* 36(6), pp. 1091-1102.

Fukuyama, M., Rougvie, A. E. and Rothman, J. H. (2006) 'C-elegans DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline', *Current Biology,* 16(8), pp. 773-779.

Gaj, T., Gersbach, C. A. and Barbas, C. F., 3rd (2013) 'ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering', *Trends in biotechnology,* 31(7), pp. 397-405.

Garneau, J. E., Dupuis, M. E., Villion, M., Romero, D. A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadan, A. H. and Moineau, S. (2010) 'The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA', *Nature,* 468(7320), pp. 67-71.

Garrison, J. L., Macosko, E. Z., Bernstein, S., Pokala, N., Albrecht, D. R. and Bargmann, C. I. (2012) 'Oxytocin/Vasopressin-Related Peptides Have an Ancient Role in Reproductive Behavior', *Science,* 338(6106), pp. 540-543.

Gasiunas, G., Barrangou, R., Horvath, P. and Siksnys, V. (2012) 'Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria', *Proceedings of the National Academy of Sciences of the United States of America,* 109(39), pp. E2579-E2586.

Gauthier, J., Champagne, N., Lafrenière, R. G., Xiong, L., Spiegelman, D., Brustein, E., Lapointe, M., Peng, H., Côté, M., Noreau, A., Hamdan, F. F., Addington, A. M., Rapoport, J. L., Delisi, L. E., Krebs, M.-O., Joober, R., Fathalli, F., Mouaffak, F., Haghighi, A. P., Néri, C., Dubé, M.-P., Samuels, M. E., Marineau, C., Stone, E. A., Awadalla, P., Barker, P. A., Carbonetto, S., Drapeau, P., Rouleau, G. A. and Team, S. D. (2010) 'De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia', *Proceedings of the National Academy of Sciences of the United States of America,* 107(17), pp. 7863-7868.

Gehricke, J.-G., Kruggel, F., Thampipop, T., Alejo, S. D., Tatos, E., Fallon, J. and Muftuler, L. T. (2017) 'The brain anatomy of attention-deficit/hyperactivity disorder in young adults - a magnetic resonance imaging study', *PloS one,* 12(4), pp. e0175433-e0175433.

Genç, Ö., An, J.-Y., Fetter, R. D., Kulik, Y., Zunino, G., Sanders, S. J. and Davis, G. W. (2020) 'Homeostatic plasticity fails at the intersection of autism-gene mutations and a novel class of common genetic modifiers', *eLife,* 9, pp. e55775.

Gendrel, M., Atlas, E. G. and Hobert, O. (2016) 'A cellular and regulatory map of the GABAergic nervous system of C. elegans', *Elife,* 5, pp. e17686.

'Genome sequence of the nematode C. elegans: a platform for investigating biology', (1998) *Science,* 282(5396), pp. 2012-2018.

Geschwind, D. H. (2011) 'Genetics of autism spectrum disorders', *Trends in cognitive sciences,* 15(9), pp. 409-416.

Giovanardi Rossi, P., Posar, A. and Parmeggiani, A. (2000) 'Epilepsy in adolescents and young adults with autistic disorder', *Brain Dev,* 22(2), pp. 102-106.

Giovedi, S., Corradi, A., Fassio, A. and Benfenati, F. (2014) 'Involvement of synaptic genes in the pathogenesis of autism spectrum disorders: the case of synapsins', *Frontiers in Pediatrics,* 4(2), doi: 10.3389/fped.2014.00094.

Gkogkas, C. G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D. B., Vasuta, C., Yee, S., Truitt, M., Dallaire, P., Major, F., Lasko, P., Ruggero, D., Nader, K., Lacaille, J.-C. and Sonenberg, N. (2013) 'Autism-related deficits via dysregulated eIF4E-dependent translational control', *Nature,* 493(7432), pp. 371-377.

Gogolla, N., Takesian, A. E., Feng, G., Fagiolini, M. and Hensch, T. K. (2014) 'Sensory integration in mouse insular cortex reflects GABA circuit maturation', *Neuron,* 83(4), pp. 894-905.

Golden, C. E. M., Buxbaum, J. D. and Rubeis, S. (2018) 'Disrupted circuits in mouse models of autism spectrum disorder and intellectual disability', *Current Opinion in Neurobiology,* 48, pp. 106-112.

Golden, J. W. and Riddle, D. L. (1982) 'A pheromone influences larval development in the nematode Caenorhabditis elegans', *Science,* 218(4572), pp. 578-80.

Golden, J. W. and Riddle, D. L. (1984a) 'ACaenorhabditis elegans dauer-inducing pheromone and an antagonistic component of the food supply', *J Chem Ecol,* 10(8), pp. 1265-1280.

Golden, J. W. and Riddle, D. L. (1984b) 'The Caenorhabditis elegans dauer larva: developmental effects of pheromone, food, and temperature', *Dev Biol,* 102(2), pp. 368-378.

Golden, J. W. and Riddle, D. L. (1985) 'A gene affecting production of the Caenorhabditis elegans dauer-inducing pheromone', *Mol Gen Genet,* 198(3), pp. 534-536.

Gonatopoulos-Pournatzis, T., Niibori, R., Salter, E. W., Weatheritt, R. J., Tsang, B., Farhangmehr, S., Liang, X., Braunschweig, U., Roth, J., Zhang, S., Henderson, T., Sharma, E., Quesnel-Vallières, M., Permanyer, J., Maier, S., Georgiou, J., Irimia, M., Sonenberg, N., Forman-Kay, J. D., Gingras, A. C., Collingridge, G. L., Woodin, M. A., Cordes, S. P. and Blencowe, B. J. (2020) 'Autism-Misregulated eIF4G Microexons Control Synaptic Translation and Higher Order Cognitive Functions', *Mol Cell,* 77(6), pp. 1176-1192.e16.

Gonçalves, J. T., Anstey, J. E., Golshani, P. and Portera-Cailliau, C. (2013) 'Circuit level defects in the developing neocortex of Fragile X mice', *Nature neuroscience,* 16(7), pp. 903-909.

Gottschalk, M. G. and Domschke, K. (2017) 'Genetics of generalized anxiety disorder and related traits', *Dialogues in clinical neuroscience,* 19(2), pp. 159-168.

Graf, E. R., Zhang, X. Z., Jin, S. X., Linhoff, M. W. and Craig, A. M. (2004) 'Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins', *Cell,* 119(7), pp. 1013-1026.

Gray, J. M., Hill, J. J. and Bargmann, C. I. (2005) 'A circuit for navigation in Caenorhabditis elegans', *Proceedings of the National Academy of Sciences of the United States of America,* 102(9), pp. 3184-3191.

Greene, J. S., Brown, M., Dobosiewicz, M., Ishida, I. G., Macosko, E. Z., Zhang, X., Butcher, R. A., Cline, D. J., McGrath, P. T. and Bargmann, C. I. (2016a) 'Balancing selection shapes density-dependent foraging behaviour', *Nature,* 539(7628), pp. 254-258.

Greene, J. S., Dobosiewicz, M., Butcher, R. A., McGrath, P. T. and Bargmann, C. I. (2016b) 'Regulatory changes in two chemoreceptor genes contribute to a Caenorhabditis elegans QTL for foraging behavior', *Elife,* 28(5), doi: 10.7554/eLife.21454..

Greer, E. L., Beese-Sims, S. E., Brookes, E., Spadafora, R., Zhu, Y., Rothbart, S. B., Aristizabal-Corrales, D., Chen, S. Z., Badeaux, A. I., Jin, Q. Y., Wang, W., Strahl, B. D., Colaiacovo, M. P. and Shi, Y. (2014) 'A Histone Methylation Network Regulates Transgenerational Epigenetic Memory in C-elegans', *Cell Reports,* 7(1), pp. 113-126.

Grewal, P. S. and Wright, D. J. (1992) 'Migration of Caenorhabditis elegans (Nematoda : Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus', *Fundamental and applied nematology,* 15, pp. 159-166.

Grove, J. and Ripke, S. and Als, T. D. and Mattheisen, M. and Walters, R. K. and Won, H. and Pallesen, J. and Agerbo, E. and Andreassen, O. A. and Anney, R. and Awashti, S. and Belliveau, R. and Bettella, F. and Buxbaum, J. D. and Bybjerg-Grauholm, J. and Baekvad-Hansen, M. and Cerrato, F. and Chambert, K. and Christensen, J. H. and Churchhouse, C. and Dellenvall, K. and Demontis, D. and De Rubeis, S. and Devlin, B. and Djurovic, S. and Dumont, A. L. and Goldstein, J. I. and Hansen, C. S. and Hauberg, M. E. and Hollegaard, M. V. and Hope, S. and Howrigan, D. P. and Huang, H. and Hultman, C. M. and Klei, L. and Maller, J. and Martin, J. and Martin, A. R. and

Moran, J. L. and Nyegaard, M. and Naeland, T. and Palmer, D. S. and Palotie, A. and Pedersen, C. B. and Pedersen, M. G. and dPoterba, T. and Poulsen, J. B. and St Pourcain, B. and Qvist, P. and Rehnstrom, K. and Reichenberg, A. and Reichert, J. and Robinson, E. B. and Roeder, K. and Roussos, P. and Saemundsen, E. and Sandin, S. and Satterstrom, F. K. and Smith, G. D. and Stefansson, H. and Steinberg, S. and Stevens, C. R. and Sullivan, P. F. and Turley, P. and Walters, G. B. and Xu, X. Y. and Stefansson, K. and Geschwind, D. H. and Nordentoft, M. and Hougaard, D. M. and Werge, T. and Mors, O. and Mortensen, P. B. and Neale, B. M. and Daly, M. J. and Borglum, A. D. and Wray, N. R. and Trzaskowski, M. and Byrne, E. M. and Abdellaoui, A. and Adams, M. J. and Air, T. M. and Andlauer, T. F. M. and Bacanu, S. A. and Beekman, A. T. F. and Bigdeli, T. B. and Binder, E. B. and Blackwood, D. H. R. and Bryois, J. and Buttenschon, H. N. and Cai, N. and Castelao, E. and Clarke, T. K. and Coleman, J. R. I. and Colodro-Conde, L. and Couvy-Duchesne, B. and Craddock, N. and Crawford, G. E. and Davies, G. and Deary, I. J. and Degenhardt, F. and Derks, E. M. and Direk, N. and Dolan, C. V. and Dunn, E. C. and Eley, T. C. and Escott-Price, V. and Kiadeh, F. F. H. and Finucane, H. K. and Forstner, A. J. and Frank, J. and Gaspar, H. A. and Gill, M. and Goes, F. S. and Gordon, S. D. and Hall, L. S. and Hansen, T. F. and Herms, S. and Hickie, I. B. and Hoffmann, P. and Homuth, G. and Horn, C. and Hottenga, J. J. and Ising, M. and Jansen, R. and Jorgenson, E. and Knowles, J. A. and Kohane, I. S. and Kraft, J. and Kretzschmar, W. W. and Krogh, J. and Kutalik, Z. and Li, Y. and Lind, P. A. and MacIntyre, D. J. and MacKinnon, D. F. and Maier, R. M. and Maier, W. and Marchini, J. and Mbarek, H. and McGrath, P. and McGuffin, P. and Medland, S. E. and Mehta, D. and Middeldorp, C. M. and Mihailov, E. and Milaneschi, Y. and Milani, L. and Mondimore, F. M. and Montgomery, G. W. and Mostafavi, S. and Mullins, N. and Nauck, M. and Ng, B. and Nivard, M. G. and Nyholt, D. R. and O'Reilly, P. F. and Oskarsson, H. and Owen, M. J. and Painter, J. N. and Peterson, R. E. and Pettersson, E. and Peyrot, W. J. and Pistis, G. and Posthuma, D. and Quiroz, J. A. and Rice, J. P. and Riley, B. P. and Rivera, M. and Mirza, S. S. and Schoevers, R. and Schulte, E. C. and Shen, L. and Shi, J. X. and Shyn, S. I. and Sigurdsson, E. and Sinnamon, G. C. B. and Smit, J. H. and Smith, D. J. and Streit, F. and Strohmaier, J. and Tansey, K. E. and Teismann, H. and Teumer, A. and Thompson, W. and Thomson, P. A. and Thorgeirsson, T. E. and Traylor, M. and Treutlein, J. and Trubetskoy, V. and Uitterlinden, A. G. and Umbricht, D. and Van Der Auwera, S. and van Hemert, A. M. and Viktorin, A. and Visscher, P. M. and Wang, Y. P. and Webb, B. T. and Weinsheimer, S. M. and Wellmann, J. and Willemsen, G. and Witt, S. H. and Wu, Y. and Xi, H. S. and Yang, J. and Zhang, F. T. and Arolt, V. and Baune, B. T. and Berger, K. and Boomsma, D. I. and Cichon, S. and Dannlowski, U. and de Geus, E. J. C. and DePaulo, J. R. and Domenici, E. and Domschke, K. and Esko, T. and Grabe, H. J. and Hamilton, S. P. and Hayward, C. and Heath, A. C. and Kendler, K. S. and Kloiber, S. and Lewis, G. and Li, Q. S. and Lucae, S. and Madden, P. A. F. and Magnusson, P. K. and Martin, N. G. and McIntosh, A. M. and Metspalu, A. and Muller-Myhsok, B. and Nothen, M. M. and O'Donovan, M. C. and Paciga, S. A. and Pedersen, N. L. and Penninx, B. and Perlis, R. H. and Porteous, D. J. and Potash, J. B. and Preisig, M. and Rietschel, M. and Schaefer, C. and Schulze, T. G. and Smoller, J. W. and Tiemeier, H. and Uher, R. and Volzke, H. and Weissman, M. M. and Lewis, C. M. and Levinson, D. F. and Breen, G. and Agee, M. and Alipanahi, B. and Auton, A. and Bell, R. K. and Bryc, K. and Elson, S. L. and Fontanillas, P. and Furlotte, N. A. and Hromatka, B. S. and Huber, K. E. and Kleinman, A. and Litterman, N. K. and McIntyre, M. H. and Mountain, J. L. and Noblin, E. S. and Northover, C. A. M. and Pitts, S. J. and Sathirapongsasuti, J. F. and Sazonova, O. V. and Shelton, J. F. and Shringarpure, S. and Tung, J. Y. and Vacic, V. and Wilson, C. H. and Psychiat Genomics, C. and Bupgen and andMe Res, T. (2019) 'Identification of common genetic risk variants for autism spectrum disorder', *Nature Genetics,* 51(3), pp. 431-444.

Grozeva, D., Carss, K., Spasic-Boskovic, O., Parker, M. J., Archer, H., Firth, H. V., Park, S.-M., Canham, N., Holder, S. E., Wilson, M., Hackett, A., Field, M., Floyd, J. A. B., Consortium, U. K., Hurles, M. and Raymond, F. L. (2014) 'De novo loss-of-function mutations in SETD5, encoding a methyltransferase in a 3p25 microdeletion syndrome critical region, cause intellectual disability', *American journal of human genetics,* 94(4), pp. 618-624.

Guang, S., Pang, N., Deng, X., Yang, L., He, F., Wu, L., Chen, C., Yin, F. and Peng, J. (2018) 'Synaptopathology Involved in Autism Spectrum Disorder', *Frontiers in cellular neuroscience,* 12, pp. 470-470.

Gupta, R. M. and Musunuru, K. (2014) 'Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9', *The Journal of clinical investigation,* 124(10), pp. 4154-4161.

Gyurko, M. D., Csermely, P., Soti, C. and Stetak, A. (2015) 'Distinct roles of the RasGAP family proteins in C. elegans associative learning and memory', *Scientific Reports,* 5, https://doi.org/10.1038/srep15084.

Haerty, W., Artieri, C., Khezri, N., Singh, R. S. and Gupta, B. P. (2008) 'Comparative analysis of function and interaction of transcription factors in nematodes: Extensive conservation of orthology coupled to rapid sequence evolution', *BMC Genomics,* 9(1), https://doi.org/10.1186/1471-2164-9-399.

Hahn, N., Geurten, B., Gurvich, A., Piepenbrock, D., Kästner, A., Zanini, D., Xing, G., Xie, W., Göpfert, M. C., Ehrenreich, H. and Heinrich, R. (2013) 'Monogenic heritable autism gene neuroligin impacts Drosophila social behaviour', *Behav Brain Res,* 252, pp. 450-457.

Haklai-Topper, L., Soutschek, J., Sabanay, H., Scheel, J., Hobert, O. and Peles, E. (2011) 'The neurexin superfamily of Caenorhabditis elegans', *Gene Expression Patterns,* 11(1-2), pp. 144-150.

Hamdan, F. F., Daoud, H., Piton, A., Gauthier, J., Dobrzeniecka, S., Krebs, M. O., Joober, R., Lacaille, J. C., Nadeau, A., Milunsky, J. M., Wang, Z., Carmant, L., Mottron, L., Beauchamp, M. H., Rouleau, G. A. and Michaud, J. L. (2011a) 'De novo SYNGAP1 mutations in nonsyndromic intellectual disability and autism', *Biol Psychiatry,* 69(9), pp. 898-901.

Hamdan, F. F., Gauthier, J., Araki, Y., Lin, D.-T., Yoshizawa, Y., Higashi, K., Park, A. R., Spiegelman, D., Dobrzeniecka, S., Piton, A., Tomitori, H., Daoud, H., Massicotte, C., Henrion, E., Diallo, O., Group, S. D., Shekarabi, M., Marineau, C., Shevell, M., Maranda, B., Mitchell, G., Nadeau, A., D'Anjou, G., Vanasse, M., Srour, M., Lafrenière, R. G., Drapeau, P., Lacaille, J. C., Kim, E., Lee, J.-R., Igarashi, K., Huganir, R. L., Rouleau, G. A. and Michaud, J. L. (2011b) 'Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability', *American journal of human genetics,* 88(3), pp. 306-316.

Hamilton, S. M., Green, J. R., Veeraragavan, S., Yuva, L., McCoy, A., Wu, Y., Warren, J., Little, L., Ji, D., Cui, X., Weinstein, E. and Paylor, R. (2014) 'Fmr1 and Nlgn3 knockout rats: novel tools for investigating autism spectrum disorders', *Behav Neurosci,* 128(2), pp. 103-109.

Hammock, E. A. and Young, L. J. (2006) 'Oxytocin, vasopressin and pair bonding: implications for autism', *Philos Trans R Soc Lond B Biol Sci,* 361(1476), pp. 2187-2198.

Harris, G., Korchnak, A., Summers, P., Hapiak, V., Law, W. J., Stein, A. M., Komuniecki, P. and Komuniecki, R. (2011) 'Dissecting the serotonergic food signal stimulating sensory-mediated aversive behavior in C. elegans', *PLoS One,* 6(7), pp. e21897.

Harris, G., Shen, Y., Ha, H., Donato, A., Wallis, S., Zhang, X. and Zhang, Y. (2014) 'Dissecting the signaling mechanisms underlying recognition and preference of food odors', *The Journal of neuroscience : the official journal of the Society for Neuroscience,* 34(28), pp. 9389-9403.

Harris, G., Wu, T., Linfield, G., Choi, M. K., Liu, H. and Zhang, Y. (2019) 'Molecular and cellular modulators for multisensory integration in C. elegans', *PLoS Genet,* 15(3), pp. e1007706.

Hart, A. C., Sims, S. and Kaplan, J. M. (1995) 'SYNAPTIC CODE FOR SENSORY MODALITIES REVEALED BY C-ELEGANS GLR-1 GLUTAMATE-RECEPTOR', *Nature,* 378(6552), pp. 82-85.

Hartman, P. S., Barry, J., Finstad, W., Khan, N., Tanaka, M., Yasuda, K. and Ishii, N. (2014) 'Ethyl methanesulfonate induces mutations in Caenorhabditis elegans embryos at a high frequency', *Mutat Res,* 766-767, pp. 44-8.

Hashem, S., Nisar, S., Bhat, A. A., Yadav, S. K., Azeem, M. W., Bagga, P., Fakhro, K., Reddy, R., Frenneaux, M. P. and Haris, M. (2020) 'Genetics of structural and functional brain changes in autism spectrum disorder', *Translational Psychiatry,* 10(1), https://doi.org/10.1038/s41398-020-00921-3.

He, F. (2011) 'Single Worm PCR', *Bio-protocol,* 1(8), pp. e60.

Heron, S. E., Khosravani, H., Varela, D., Bladen, C., Williams, T. C., Newman, M. R., Scheffer, I. E., Berkovic, S. F., Mulley, J. C. and Zamponi, G. W. (2007) 'Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants', *Ann Neurol,* 62(6), pp. 560-568.

Hilliard, M. A., Bargmann, C. I. and Bazzicalupo, P. (2002) 'C-elegans responds to chemical repellents by integrating sensory inputs from the head and the tail', *Current Biology,* 12(9), pp. 730-734.

Hobert, O. Neurogenesis in the nematode *Caenorhabditis elegans* (October 4, 2010), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.12.2, http://www.wormbook.org.

Hobert O. The neuronal genome of *Caenorhabditis elegans* (August 13, 2013), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.161.1, http://www.wormbook.org.

Hodgkin, J., Karyotype, ploidy and gene dosage (June 25, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.3.1, http://www.wormbook.org.

Hommer, R. E. and Swedo, S. E. (2015) 'Schizophrenia and autism-related disorders', *Schizophrenia bulletin,* 41(2), pp. 313-314.

Horder, J., Wilson, C. E., Mendez, M. A. and Murphy, D. G. (2014) 'Autistic traits and abnormal sensory experiences in adults', *Journal of autism and developmental disorders,* 44(6), pp. 1461-1469.

Horwitz, B., Rumsey, J. M., Grady, C. L. and Rapoport, S. I. (1988) 'The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization', *Arch Neurol,* 45(7), pp. 749-55.

Hsu, P. D., Lander, E. S. and Zhang, F. (2014) 'Development and applications of CRISPR-Cas9 for genome engineering', *Cell,* 157(6), pp. 1262-1278.

Hu, T.-M., Wang, Y.-C., Wu, C.-L., Hsu, S.-H., Tsai, H.-Y. and Cheng, M.-C. (2020) 'Multiple Rare Risk Coding Variants in Postsynaptic Density-Related Genes Associated With Schizophrenia Susceptibility', *Frontiers in Genetics,* 11(1517), doi: 10.3389/fgene.2020.524258.

Hu, Z. T., Hom, S., Kudze, T., Tong, X. J., Choi, S., Aramuni, G., Zhang, W. Q. and Kaplan, J. M. (2012) 'Neurexin and Neuroligin Mediate Retrograde Synaptic Inhibition in C. elegans', *Science,* 337(6097), pp. 980-984.

Huey, E. D., Zahn, R., Krueger, F., Moll, J., Kapogiannis, D., Wassermann, E. M. and Grafman, J. (2008) 'A psychological and neuroanatomical model of obsessive-compulsive disorder', *The Journal of neuropsychiatry and clinical neurosciences,* 20(4), pp. 390-408.

Huguet, G., Ey, E. and Bourgeron, T. (2013) 'The Genetic Landscapes of Autism Spectrum Disorders', *Annual Review of Genomics and Human Genetics,* 14, pp. 191-213.

Hukema, R. K., Rademakers, S. and Jansen, G. (2008) 'Gustatory plasticity in C-elegans involves integration of negative cues and NaCl taste mediated by serotonin, dopamine, and glutamate', *Learning & Memory,* 15(11), pp. 829-836.

Hunter, J. W., Mullen, G. P., McManus, J. R., Heatherly, J. M., Duke, A. and Rand, J. B. (2010) 'Neuroligin-deficient mutants of C. elegans have sensory processing deficits and are hypersensitive to oxidative stress and mercury toxicity', *Disease Models & Mechanisms,* 3(5-6), pp. 366-376.

Hussman, J. P. (2001) 'Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism', *J Autism Dev Disord,* 31(2), pp. 247-248.

Huumonen, K., Immonen, H. K., Baverstock, K., Hiltunen, M., Korkalainen, M., Lahtinen, T., Parviainen, J., Viluksela, M., Wong, G., Naarala, J. and Juutilainen, J. (2012) 'Radiation-induced genomic instability in Caenorhabditis elegans', *Mutat Res,* 748(1-2), pp. 36-41.

Iakoucheva, L. M., Muotri, A. R. and Sebat, J. (2019) 'Getting to the Cores of Autism', *Cell,* 178(6), pp. 1287-1298.

Iino, Y. and Yoshida, K. (2009) 'Parallel Use of Two Behavioral Mechanisms for Chemotaxis in Caenorhabditis elegans', *The Journal of Neuroscience,* 29(17), pp. 5370-5380.

International Obsessive Compulsive Disorder Foundation Genetics, C. and Studies, O. C. D. C. G. A. (2018) 'Revealing the complex genetic architecture of obsessive-compulsive disorder using meta-analysis', *Molecular psychiatry,* 23(5), pp. 1181-1188.

Izquierdo, P. G., Calahorro, F. and Ruiz-Rubio, M. (2013) 'Neuroligin modulates the locomotory dopaminergic and serotonergic neuronal pathways of C. elegans', *Neurogenetics,* 14(3-4), pp. 233-242.

Jacher, J. E., Roy, N., Ghaziuddin, M. and Innis, J. W. (2019) 'Expanding the phenotypic spectrum of MBOAT7-related intellectual disability', *Am J Med Genet B Neuropsychiatr Genet,* 180(7), pp. 483-487.

Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I. C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., Bourgeron, T. and Paris Autism Res Int Sibpair, S. (2003) 'Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism', *Nature Genetics,* 34(1), pp. 27-29.

Jay, K., Mitra, A., Harding, T., Matthes, D. and Van Ness, B. (2019) 'Identification of a de novo FOXP1 mutation and incidental discovery of inherited genetic variants contributing to a case of autism spectrum disorder and epilepsy', *Mol Genet Genomic Med,* 7(7), pp. e00751.

Jee, C., Lee, J., Lee, J. I., Lee, W. H., Park, B. J., Yu, J. R., Park, E., Kim, E. and Ahnn, J. (2004) 'SHN-1, a Shank homologue in C-elegans, affects defecation rhythm via the inositol-1,4,5-trisphosphate receptor', *Febs Letters,* 561(1-3), pp. 29-36.

Jeong, P.-Y., Jung, M., Yim, Y.-H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y. H., Kim, K. and Paik, Y.-K. (2005) 'Chemical structure and biological activity of the Caenorhabditis elegans dauer-inducing pheromone', *Nature,* 433(7025), pp. 541-545.

Jiang, F. and Doudna, J. A. (2017) 'CRISPR-Cas9 Structures and Mechanisms', *Annu Rev Biophys,* 46, pp. 505-529.

Jiang, Y.-H., Wang, Y., Xiu, X., Choy, K. W., Pursley, A. N. and Cheung, S. W. (2014) 'Genetic diagnosis of autism spectrum disorders: the opportunity and challenge in the genomics era', *Critical reviews in clinical laboratory sciences,* 51(5), pp. 249-262.

Jin, X., Simmons, S. K., Guo, A., Shetty, A. S., Ko, M., Nguyen, L., Jokhi, V., Robinson, E., Oyler, P., Curry, N., Deangeli, G., Lodato, S., Levin, J. Z., Regev, A., Zhang, F. and Arlotta, P. (2020) 'In vivo Perturb-Seq reveals neuronal and glial abnormalities associated with autism risk genes', *Science,* 27(370), eaaz6063.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A. and Charpentier, E. (2012) 'A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity', *Science,* 337(6096), pp. 816-821.

Johansen, A., Rosti, R. O., Musaev, D., Sticca, E., Harripaul, R., Zaki, M., Çağlayan, A. O., Azam, M., Sultan, T., Froukh, T., Reis, A., Popp, B., Ahmed, I., John, P., Ayub, M., Ben-Omran, T., Vincent, J. B., Gleeson, J. G. and Abou Jamra, R. (2016) 'Mutations in MBOAT7, Encoding Lysophosphatidylinositol Acyltransferase I, Lead to Intellectual Disability Accompanied by Epilepsy and Autistic Features', *American journal of human genetics,* 99(4), pp. 912-916.

Kaletta, T. and Hengartner, M. O. (2006) 'Finding function in novel targets: C-elegans as a model organism', *Nature Reviews Drug Discovery,* 5(5), pp. 387-398.

Kanner, L. (1943) 'Autistic disturbances of affective contact', *Nervous child,* 2(3), pp. 217-250.

Kano, T., Brockie, P. J., Sassa, T., Fujimoto, H., Kawahara, Y., Iino, Y., Mellem, J. E., Madsen, D. M., Hosono, R. and Maricq, A. V. (2008) 'Memory in Caenorhabditis elegans is mediated by NMDA-type ionotropic glutamate receptors', *Current biology : CB,* 18(13), pp. 1010-1015.

Kaplan, F., Srinivasan, J., Mahanti, P., Ajredini, R., Durak, O., Nimalendran, R., Sternberg, P. W., Teal, P. E., Schroeder, F. C., Edison, A. S. and Alborn, H. T. (2011) 'Ascaroside expression in Caenorhabditis elegans is strongly dependent on diet and developmental stage', *PLoS One,* 6(3), pp. e17804.

Karlsgodt, K. H., Sun, D. and Cannon, T. D. (2010) 'Structural and Functional Brain Abnormalities in Schizophrenia', *Current directions in psychological science,* 19(4), pp. 226-231.

Karst, J. S. and Van Hecke, A. V. (2012) 'Parent and Family Impact of Autism Spectrum Disorders: A Review and Proposed Model for Intervention Evaluation', *Clinical Child and Family Psychology Review,* 15(3), pp. 247-277.

Kasap, M. and Dwyer, D. S. (2020) 'Clozapine, nimodipine and endosulfan differentially suppress behavioral defects caused by gain-of-function mutations in a two-pore domain K(+) channel (UNC-58)', *Neurosci Res*, 15(20), doi: 10.1016/j.neures.2020.07.006.

Kasap, M., Weeks, K., Aamodt, E. J. and Dwyer, D. S. (2018) 'Gain-of-function mutations in a two-pore domain K+ channel (unc-58) cause developmental, motor and feeding defects in C. elegans modified by temperature and a channel inhibitor, loratadine', *Current Neurobiology,* 9(3), pp. 84-91.

Kasem, E., Kurihara, T. and Tabuchi, K. (2018) 'Neurexins and neuropsychiatric disorders', *Neuroscience Research,* 127, pp. 53-60.

Katidou, M., Tavernarakis, N. and Karagogeos, D. (2013) 'The contactin RIG-6 mediates neuronal and non-neuronal cell migration in Caenorhabditis elegans', *Developmental Biology,* 373(1), pp. 184-195.

Kazdoba, T. M., Leach, P. T., Yang, M., Silverman, J. L., Solomon, M. and Crawley, J. N. (2016) 'Translational Mouse Models of Autism: Advancing Toward Pharmacological Therapeutics', *Current topics in behavioral neurosciences,* 28, pp. 1-52.

Kiani, S., Chavez, A., Tuttle, M., Hal, R. N., Chari, R., Ter-Ovanesyan, D., Qian, J., Pruitt, B. W., Beal, J., Vora, S., Buchthal, J., Kowal, E. J. K., Ebrahimkhani, M. R., Collins, J. J., Weiss, R. and Church, G. (2015) 'Cas9 gRNA engineering for genome editing, activation and repression', *Nature Methods,* 12(11), pp. 1051-1054.

Kim, H., Ishidate, T., Ghanta, K. S., Seth, M., Conte, D., Shirayama, M. and Mello, C. C. (2014) 'A Co-CRISPR Strategy for Efficient Genome Editing in Caenorhabditis elegans', *Genetics,* 197(4), pp. 1069-1037.

Kim, H., Lim, C. S. and Kaang, B. K. (2016) 'Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder', *Behavioral and Brain Functions,* 12(1), doi: 10.1186/s12993-016-0087-y.

Kim, K., Sato, K., Shibuya, M., Zeiger, D. M., Butcher, R. A., Ragains, J. R., Clardy, J., Touhara, K. and Sengupta, P. (2009) 'Two Chemoreceptors Mediate Developmental Effects of Dauer Pheromone in C-elegans', *Science,* 326(5955), pp. 994-998.

Kim, W., Underwood, R. S., Greenwald, I. and Shaye, D. D. (2018) 'OrthoList 2: A New Comparative Genomic Analysis of Human and Caenorhabditis elegans Genes', *Genetics,* 210(2), pp. 445-461.

Kimata, T., Sasakura, H., Ohnishi, N., Nishio, N. and Mori, I. (2012) 'Thermotaxis of C. elegans as a model for temperature perception, neural information processing and neural plasticity', *Worm,* 1(1), pp. 31-41.

Kindt, K. S., Quast, K. B., Giles, A. C., De, S., Hendrey, D., Nicastro, I., Rankin, C. H. and Schafer, W. R. (2007) 'Dopamine mediates context-dependent modulation of sensory plasticity in C. elegans', *Neuron,* 55(4), pp. 662-76.

Klei, L., Sanders, S. J., Murtha, M. T., Hus, V., Lowe, J. K., Willsey, A. J., Moreno-De-Luca, D., Yu, T. W., Fombonne, E., Geschwind, D., Grice, D. E., Ledbetter, D. H., Lord, C., Mane, S. M., Martin, C. L., Martin, D. M., Morrow, E. M., Walsh, C. A., Melhem, N. M., Chaste, P., Sutcliffe, J. S., State, M. W., Cook, E. H., Roeder, K. and Devlin, B. (2012) 'Common genetic variants, acting additively, are a major source of risk for autism', *Molecular Autism,* 15(1), doi: 10.1186/2040-2392-3-9.

Kleinstiver, B. P., Prew, M. S., Tsai, S. Q., Topkar, V. V., Nguyen, N. T., Zheng, Z. L., Gonzales, A. P. W., Li, Z. Y., Peterson, R. T., Yeh, J. R. J., Aryee, M. J. and Joung, J. K. (2015) 'Engineered CRISPR-Cas9 nucleases with altered PAM specificities', *Nature,* 523(7561), pp. 481-485.

Koberstein, J. N., Poplawski, S. G., Wimmer, M. E., Porcari, G., Kao, C., Gomes, B., Risso, D., Hakonarson, H., Zhang, N. R., Schultz, R. T., Abel, T. and Peixoto, L. (2018) 'Learning-dependent chromatin remodeling highlights noncoding regulatory regions linked to autism', *Sci Signal,* 11(513), eaan6500.

Kocabas, A., Shen, C.-H., Guo, Z. V. and Ramanathan, S. (2012) 'Controlling interneuron activity in Caenorhabditis elegans to evoke chemotactic behaviour', *Nature,* 490(7419), pp. 273-277.

Koeleman, B. P. C. (2018) 'What do genetic studies tell us about the heritable basis of common epilepsy? Polygenic or complex epilepsy?', *Neuroscience Letters,* 667, pp. 10-16.

Kreher, J., Takasaki, T., Cockrum, C., Sidoli, S., Garcia, B. A., Jensen, O. N. and Strome, S. (2018) 'Distinct Roles of Two Histone Methyltransferases in Transmitting H3K36me3-Based Epigenetic Memory Across Generations in Caenorhabditis elegans', *Genetics,* 210(3), pp. 969-982.

Krogh, A., Larsson, B., von Heijne, G. and Sonnhammer, E. L. (2001) 'Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes', *J Mol Biol,* 305(3), pp. 567-80.

Krumm, N., O'Roak, B. J., Shendure, J. and Eichler, E. E. (2014) 'A de novo convergence of autism genetics and molecular neuroscience', *Trends in Neurosciences,* 37(2), pp. 95-105.

Ku, K. M., Weir, R. K., Silverman, J. L., Berman, R. F. and Bauman, M. D. (2016) 'Behavioral Phenotyping of Juvenile Long-Evans and Sprague-Dawley Rats: Implications for Preclinical Models of Autism Spectrum Disorders', *PLoS One,* 11(6), pp. e0158150.

Kubota, T. and Mochizuki, K. (2016) 'Epigenetic Effect of Environmental Factors on Autism Spectrum Disorders', *International journal of environmental research and public health,* 13(5), doi: 10.3390/ijerph13050504.

Kumar, S., Reynolds, K., Ji, Y., Gu, R., Rai, S. and Zhou, C. J. (2019) 'Impaired neurodevelopmental pathways in autism spectrum disorder: a review of signaling mechanisms and crosstalk', *Journal of Neurodevelopmental Disorders,* 11(1), https://doi.org/10.1186/s11689-019-9268-y.

Kurochkin, I., Khrameeva, E., Tkachev, A., Stepanova, V., Vanyushkina, A., Stekolshchikova, E., Li, Q., Zubkov, D., Shichkova, P., Halene, T., Willmitzer, L., Giavalisco, P., Akbarian, S. and Khaitovich, P. (2019) 'Metabolome signature of autism in the human prefrontal cortex', *Communications Biology,* 2(1), https://doi.org/10.1038/s42003-019-0485-4.

Labbadia, J. and Morimoto, R. I. (2015) 'Repression of the Heat Shock Response Is a Programmed Event at the Onset of Reproduction', *Molecular Cell,* 59(4), pp. 639-650.

Ladd-Acosta, C., Hansen, K. D., Briem, E., Fallin, M. D., Kaufmann, W. E. and Feinberg, A. P. (2014) 'Common DNA methylation alterations in multiple brain regions in autism', *Molecular Psychiatry,* 19(8), pp. 862-871.

Lai, C. H., Chou, C. Y., Ch'ang, L. Y., Liu, C. S. and Lin, W. (2000) 'Identification of novel human genes evolutionarily conserved in Caenorhabditis elegans by comparative proteomics', *Genome research,* 10(5), pp. 703-713.

Lai, M.-C., Lombardo, M. V., Chakrabarti, B. and Baron-Cohen, S. (2013) 'Subgrouping the autism "spectrum": reflections on DSM-5', *PLoS biology,* 11(4), pp. e1001544-e1001544.

Lamonica, J. M., Kwon, D. Y., Goffin, D., Fenik, P., Johnson, B. S., Cui, Y., Guo, H., Veasey, S. and Zhou, Z. (2017) 'Elevating expression of MeCP2 T158M rescues DNA binding and Rett syndrome-like phenotypes', *J Clin Invest,* 127(5), pp. 1889-1904.

Lasalle, J. M. (2013) 'Autism genes keep turning up chromatin', *OA autism,* 1(2), doi:10.13172/2052-7810-1-2-610.

Leblond, C. S., Heinrich, J., Delorme, R., Proepper, C., Betancur, C., Huguet, G., Konyukh, M., Chaste, P., Ey, E., Rastam, M., Anckarsäter, H., Nygren, G., Gillberg, I. C., Melke, J., Toro, R., Regnault, B., Fauchereau, F., Mercati, O., Lemière, N., Skuse, D., Poot, M., Holt, R., Monaco, A. P., Järvelä, I., Kantojärvi, K., Vanhala, R., Curran, S., Collier, D. A., Bolton, P., Chiocchetti, A., Klauck, S. M., Poustka, F., Freitag, C. M., Waltes, R., Kopp, M., Duketis, E., Bacchelli, E., Minopoli, F., Ruta, L., Battaglia, A., Mazzone, L., Maestrini, E., Sequeira, A. F., Oliveira, B., Vicente, A., Oliveira, G., Pinto, D., Scherer, S. W., Zelenika, D., Delepine, M., Lathrop, M., Bonneau, D., Guinchat, V., Devillard, F., Assouline, B., Mouren, M.-C., Leboyer, M., Gillberg, C., Boeckers, T. M. and Bourgeron, T. (2012)

'Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders', *PLoS genetics,* 8(2), pp. e1002521-e1002521.

Lee, B., Lee, K., Panda, S., Gonzales-Rojas, R., Chong, A., Bugay, V., Park, H. M., Brenner, R., Murthy, N. and Lee, H. Y. (2018) 'Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours', *Nat Biomed Eng,* 2(7), pp. 497-507.

Lee, E., Lee, J. and Kim, E. (2017) 'Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders', *Biological Psychiatry,* 81(10), pp. 838-847.

Lee, H.C., Inoue, T., Imae, R., Kono, N., Shirae, S., Matsuda, S., Gengyo-Ando, K., Mitani, S. and Arai, H. (2008) 'Caenorhabditis elegans mboa-7, a member of the MBOAT family, is required for selective incorporation of polyunsaturated fatty acids into phosphatidylinositol', *Molecular biology of the cell,* 19(3), pp. 1174-1184.

Lee, H.-C., Inoue, T., Sasaki, J., Kubo, T., Matsuda, S., Nakasaki, Y., Hattori, M., Tanaka, F., Udagawa, O., Kono, N., Itoh, T., Ogiso, H., Taguchi, R., Arita, M., Sasaki, T. and Arai, H. (2012a) 'LPIAT1 regulates arachidonic acid content in phosphatidylinositol and is required for cortical lamination in mice', *Molecular biology of the cell,* 23(24), pp. 4689-4700.

Lee, H. C., Kubo, T., Kono, N., Kage-Nakadai, E., Gengyo-Ando, K., Mitani, S., Inoue, T. and Arai, H. (2012b) 'Depletion of mboa-7, an enzyme that incorporates polyunsaturated fatty acids into phosphatidylinositol (PI), impairs PI 3-phosphate signaling in Caenorhabditis elegans', *Genes to Cells,* 17(9), pp. 748-757.

Lee, K. and Mylonakis, E. (2017) 'An Intestine-Derived Neuropeptide Controls Avoidance Behavior in Caenorhabditis elegans', *Cell Rep,* 20(10), pp. 2501-2512.

Lee, N. S. and Beery, A. K. (2019) 'Neural Circuits Underlying Rodent Sociality: A Comparative Approach', *Curr Top Behav Neurosci,* 43, pp. 211-238.

Lemieux, G. A., Cunningham, K. A., Lin, L., Mayer, F., Werb, Z. and Ashrafi, K. (2015) 'Kynurenic Acid Is a Nutritional Cue that Enables Behavioral Plasticity', *Cell,* 160(1-2), pp. 119-131.

Levchenko, A., Kanapin, A., Samsonova, A., Fedorenko, O. Y., Kornetova, E. G., Nurgaliev, T., Mazo, G. E., Semke, A. V., Kibitov, A. O., Bokhan, N. A., Gainetdinov, R. R. and Ivanova, S. A. (2021) 'A genome-wide association study identifies a gene network associated with paranoid schizophrenia and antipsychotics-induced tardive dyskinesia', *Prog Neuropsychopharmacol Biol Psychiatry,* 105(105), doi: 10.1016/j.pnpbp.2020.110134.

Levitan, D., Doyle, T. G., Brousseau, D., Lee, M. K., Thinakaran, G., Slunt, H. H., Sisodia, S. S. and Greenwald, I. (1996) 'Assessment of normal and mutant human presenilin function in Caenorhabditis elegans', *Proc Natl Acad Sci U S A,* 93(25), pp. 14940-1494.

Li, X., Xiao, J., Fröhlich, H., Tu, X., Li, L., Xu, Y., Cao, H., Qu, J., Rappold, G. A. and Chen, J.-G. (2015) 'Foxp1 regulates cortical radial migration and neuronal morphogenesis in developing cerebral cortex', *PloS one,* 10(5), pp. e0127671-e0127671.

Li, Z., Liu, J., Zheng, M. and Xu, X. Z. (2014) 'Encoding of both analog- and digital-like behavioral outputs by one C. elegans interneuron', *Cell,* 159(4), pp. 751-765.

Li, Z. Y., Li, Y. D., Yi, Y. L., Huang, W. M., Yang, S., Niu, W. P., Zhang, L., Xu, Z. J., Qu, A. L., Wu, Z. X. and Xu, T. (2012) 'Dissecting a central flip-flop circuit that integrates contradictory sensory cues in C. elegans feeding regulation', *Nature Communications,* 3, https://doi.org/10.1038/ncomms1780.

Lillycrop, K. A., Phillips, E. S., Torrens, C., Hanson, M. A., Jackson, A. A. and Burdge, G. C. (2008) 'Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring', *British Journal of Nutrition,* 100(2), pp. 278-282.

Lin, Y., Cradick, T. J., Brown, M. T., Deshmukh, H., Ranjan, P., Sarode, N., Wile, B. M., Vertino, P. M., Stewart, F. J. and Bao, G. (2014) 'CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences', *Nucleic acids research,* 42(11), pp. 7473-7485.

Lino, C. A., Harper, J. C., Carney, J. P. and Timlin, J. A. (2018) 'Delivering CRISPR: a review of the challenges and approaches', *Drug delivery,* 25(1), pp. 1234-1257.

Lints, R. and Hall, D. H. 2009. Male neuronal support cells, overview. In *WormAtlas*. doi:10.3908/wormatlas.2.9

Liu, K. S. and Sternberg, P. W. (1995) 'Sensory regulation of male mating behavior in caenorhabditis elegans', *Neuron,* 14(1), pp. 79-89.

Liu, X., Shimada, T., Otowa, T., Wu, Y. Y., Kawamura, Y., Tochigi, M., Iwata, Y., Umekage, T., Toyota, T., Maekawa, M., Iwayama, Y., Suzuki, K., Kakiuchi, C., Kuwabara, H., Kano, Y., Nishida, H., Sugiyama, T., Kato, N., Chen, C. H., Mori, N., Yamada, K., Yoshikawa, T., Kasai, K., Tokunaga, K., Sasaki, T. and Gau, S. S. (2016) 'Genome-wide Association Study of Autism Spectrum Disorder in the East Asian Populations', *Autism Res,* 9(3), pp. 340-9.

Loke, Y. J., Hannan, A. J. and Craig, J. M. (2015) 'The role of epigenetic change in autism spectrum disorders', *Frontiers in Neurology,* 26(6), doi: 10.3389/fneur.2015.00107.

López-Cruz, A., Sordillo, A., Pokala, N., Liu, Q., McGrath, P. T. and Bargmann, C. I. (2019) 'Parallel Multimodal Circuits Control an Innate Foraging Behavior', *Neuron,* 102(2), pp. 407-419.

Lord, C. and Jones, R. M. (2012) 'Annual research review: re-thinking the classification of autism spectrum disorders', *Journal of child psychology and psychiatry, and allied disciplines,* 53(5), pp. 490-509.

Lu, Z. A., Mu, W., Osborne, L. M. and Cordner, Z. A. (2018) 'Eighteen-year-old man with autism, obsessive compulsive disorder and a SHANK2 variant presents with severe anorexia that responds to high-dose fluoxetine', *BMJ Case Rep,* bcr2018225119. doi: 10.1136/bcr-2018-225119.

Ludewig, A. H., Gimond, C., Judkins, J. C., Thornton, S., Pulido, D. C., Micikas, R. J., Döring, F., Antebi, A., Braendle, C. and Schroeder, F. C. (2017) 'Larval crowding accelerates C. elegans development and reduces lifespan', *PLoS Genet,* 13(4), pp. e1006717.

Ludewig AH. And Schroeder FC. Ascaroside signalling in *C. elegans* (January 18, 2013), *WormBook*,

ed. The *C. elegans* Research Community, WormBook,

doi/10.1895/wormbook.1.155.1, http://www.wormbook.org.

Lüersen, K., Gottschling, D.-C. and Döring, F. (2016) 'Complex Locomotion Behavior Changes Are Induced in Caenorhabditis elegans by the Lack of the Regulatory Leak K+ Channel TWK-7', *Genetics,* 204(2), pp. 683-701.

Lumish, H. S., Wynn, J., Devinsky, O. and Chung, W. K. (2015) 'Brief Report: SETD2 Mutation in a Child with Autism, Intellectual Disabilities and Epilepsy', *J Autism Dev Disord,* 45(11), pp. 3764-3770.

Luo, L., Wen, Q., Ren, J., Hendricks, M., Gershow, M., Qin, Y., Greenwood, J., Soucy, E. R., Klein, M., Smith-Parker, H. K., Calvo, A. C., Colón-Ramos, D. A., Samuel, A. D. T. and Zhang, Y. (2014) 'Dynamic encoding of perception, memory, and movement in a C. elegans chemotaxis circuit', *Neuron,* 82(5), pp. 1115-1128.

Ma, D. and Liu, F. (2015) 'Genome Editing and Its Applications in Model Organisms', *Genomics, proteomics & bioinformatics,* 13(6), pp. 336-344.

Mack, H. I. D., Zhang, P. C., Fonslow, B. R. and Yates, J. R. (2017) 'The protein kinase MBK-1 contributes to lifespan extension in daf-2 mutant and germline-deficient Caenorhabditis elegans', *Aging-Us,* 9(5), pp. 1414-1432.

Macosko, E. Z., Pokala, N., Feinberg, E. H., Chalasani, S. H., Butcher, R. A., Clardy, J. and Bargmann, C. I. (2009) 'A hub-and-spoke circuit drives pheromone attraction and social behaviour in C-elegans', *Nature,* 458(7242), pp. 1171-1175.

Maeda, I., Kohara, Y., Yamamoto, M. and Sugimoto, A. (2001) 'Large-scale analysis of gene function in Caenorhabditis elegans by high-throughput RNAi', *Curr Biol,* 11(3), pp. 171-176.

Marchetto, M. C., Belinson, H., Tian, Y., Freitas, B. C., Fu, C., Vadodaria, K., Beltrao-Braga, P., Trujillo, C. A., Mendes, A. P. D., Padmanabhan, K., Nunez, Y., Ou, J., Ghosh, H., Wright, R., Brennand, K., Pierce, K., Eichenfield, L., Pramparo, T., Eyler, L., Barnes, C. C., Courchesne, E., Geschwind, D. H., Gage, F. H., Wynshaw-Boris, A. and Muotri, A. R. (2017) 'Altered proliferation and networks in neural cells derived from idiopathic autistic individuals', *Molecular psychiatry,* 22(6), pp. 820-835.

Marco, E. J., Hinkley, L. B. N., Hill, S. S. and Nagarajan, S. S. (2011) 'Sensory processing in autism: a review of neurophysiologic findings', *Pediatric research,* 69(5 Pt 2), pp. 48-54.

Marcoulatos, P., Koussidis, G., Mamuris, Z., Velissariou, V. and Vamvakopoulos, N. C. (1996) 'Mapping interleukin enhancer binding factor 2 gene (ILF2) to human chromosome 1 (1q11-qter and 1p11-p12) by polymerase chain reaction amplification of human-rodent somatic cell hybrid DNA templates', *J Interferon Cytokine Res,* 16(12), pp. 1035-1038.

Mariappa, D., Ferenbach, A. T. and van Aalten, D. M. F. (2018) 'Effects of hypo-O-GlcNAcylation on Drosophila development', *J Biol Chem,* 293(19), pp. 7209-7221.

Maricq, A. V., Peckol, E., Driscoll, M. and Bargmann, C. I. (1995) 'Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor', *Nature,* 378(6552), pp. 78-81.

Mariol, M. C., Walter, L., Bellemin, S. and Gieseler, K. (2013) 'A rapid protocol for integrating extrachromosomal arrays with high transmission rate into the C. elegans genome', *J Vis Exp,* (82), pp. e50773.

Maro, G. S., Gao, S. B., Olechwier, A. M., Hung, W. L., Liu, M., Ozkan, E., Zhen, M. and Shen, K. (2015) 'MADD-4/Punctin and Neurexin Organize C. elegans GABAergic Postsynapses through Neuroligin', *Neuron,* 86(6), pp. 1420-1432.

Marraffini, L. A. and Sontheimer, E. J. (2008) 'CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA', *Science,* 322(5909), pp. 1843-1845.

Marshall, C. R., Noor, A., Vincent, J. B., Lionel, A. C., Feuk, L., Skaug, J., Shago, M., Moessner, R., Pinto, D., Ren, Y., Thiruvahindrapduram, B., Fiebig, A., Schreiber, S., Friedman, J., Ketelaars, C. E., Vos, Y. J., Ficicioglu, C., Kirkpatrick, S., Nicolson, R., Sloman, L., Summers, A., Gibbons, C. A., Teebi, A., Chitayat, D., Weksberg, R., Thompson, A., Vardy, C., Crosbie, V., Luscombe, S., Baatjes, R., Zwaigenbaum, L., Roberts, W., Fernandez, B., Szatmari, P. and Scherer, S. W. (2008) 'Structural variation of chromosomes in autism spectrum disorder', *Am J Hum Genet,* 82(2), pp. 477-488.

Martin, E. I., Ressler, K. J., Binder, E. and Nemeroff, C. B. (2009) 'The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology', *The Psychiatric clinics of North America,* 32(3), pp. 549-575.

Masse, I., Molin, L., Billaud, M. and Solari, F. (2005) 'Lifespan and dauer regulation by tissue-specific activities of Caenorhabditis elegans DAF-18', *Developmental Biology,* 286(1), pp. 91-101.

Matsuura, T., Sato, T. and Shingai, R. (2005) 'Interactions between Caenorhabditis elegans individuals during chemotactic response', *Zoolog Sci,* 22(10), pp. 1095-1103.

Maximo, J. O., Cadena, E. J. and Kana, R. K. (2014) 'The implications of brain connectivity in the neuropsychology of autism', *Neuropsychology review,* 24(1), pp. 16-31.

Mayer, J. L. (2017) 'The Relationship Between Autistic Traits and Atypical Sensory Functioning in Neurotypical and ASD Adults: A Spectrum Approach', *J Autism Dev Disord,* 47(2), pp. 316-327.

McBride, K. L., Varga, E. A., Pastore, M. T., Prior, T. W., Manickam, K., Atkin, J. F. and Herman, G. E. (2010) 'Confirmation study of PTEN mutations among individuals with autism or developmental delays/mental retardation and macrocephaly', *Autism Research,* 3(3), pp. 137-141.

McCarthy, S. E., Gillis, J., Kramer, M., Lihm, J., Yoon, S., Berstein, Y., Mistry, M., Pavlidis, P., Solomon, R., Ghiban, E., Antoniou, E., Kelleher, E., O'Brien, C., Donohoe, G., Gill, M., Morris, D. W., McCombie, W. R. and Corvin, A. (2014) 'De novo mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability', *Molecular psychiatry,* 19(6), pp. 652-658.

McCloskey, R. J., Fouad, A. D., Churgin, M. A. and Fang-Yen, C. (2017) 'Food responsiveness regulates episodic behavioral states in Caenorhabditis elegans', *Journal of Neurophysiology,* 117(5), pp. 1911-1934.

McDiarmid, T. A., Belmadani, M., Liang, J., Meili, F., Mathews, E. A., Mullen, G. P., Hendi, A., Wong, W.-R., Rand, J. B., Mizumoto, K., Haas, K., Pavlidis, P. and Rankin, C. H. (2019) 'Systematic phenomics analysis of autism-associated genes reveals parallel networks underlying reversible impairments in habituation', *Proceedings of the National Academy of Sciences*, 117(1), pp. 656-667.

McGraw, L. A. and Young, L. J. (2010) 'The prairie vole: an emerging model organism for understanding the social brain', *Trends in neurosciences,* 33(2), pp. 103-109.

McIntosh, A. M., Sullivan, P. F. and Lewis, C. M. (2019) 'Uncovering the Genetic Architecture of Major Depression', *Neuron,* 102(1), pp. 91-103.

Meguid, N. A., Atta, H. M., Gouda, A. S. and Khalil, R. O. (2008) 'Role of polyunsaturated fatty acids in the management of Egyptian children with autism', *Clinical biochemistry,* 41(13), pp. 1044-1048.

Meng, L. and Yan, D. (2020) 'NLR-1/CASPR Anchors F-Actin to Promote Gap Junction Formation', *Developmental Cell,* 55(5), pp. 574-587.

Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Jordan, S. D., Quiros, P. M., Steffen, K. K., Williams, E. G., Mouchiroud, L., Tronnes, S. U., Murillo, V., Wolff, S. C., Shaw, R. J., Auwerx, J. and Dillin, A. (2016) 'Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity', *Cell,* 165(5), pp. 1209-1223.

Merner, N., Forgeot d'Arc, B., Bell, S. C., Maussion, G., Peng, H., Gauthier, J., Crapper, L., Hamdan, F. F., Michaud, J. L., Mottron, L., Rouleau, G. A. and Ernst, C. (2016) 'A de novo frameshift

mutation in chromodomain helicase DNA-binding domain 8 (CHD8): A case report and literature review', *American Journal of Medical Genetics Part A,* 170(5), pp. 1225-1235.

Metaxakis, A., Petratou, D. and Tavernarakis, N. (2018) 'Multimodal sensory processing in Caenorhabditis elegans', *Open Biology,* 8(6), doi: 10.1098/rsob.180049.

Meyer, G., Varoqueaux, F., Neeb, A., Oschlies, M. and Brose, N. (2004) 'The complexity of PDZ domain-mediated interactions at glutamatergic synapses: a case study on neuroligin', *Neuropharmacology,* 47(5), pp. 724-733.

Mikita, N., Simonoff, E., Pine, D. S., Goodman, R., Artiges, E., Banaschewski, T., Bokde, A. L., Bromberg, U., Büchel, C., Cattrell, A., Conrod, P. J., Desrivières, S., Flor, H., Frouin, V., Gallinat, J., Garavan, H., Heinz, A., Ittermann, B., Jurk, S., Martinot, J. L., Paillère Martinot, M. L., Nees, F., Papadopoulos Orfanos, D., Paus, T., Poustka, L., Smolka, M. N., Walter, H., Whelan, R., Schumann, G. and Stringaris, A. (2016) 'Disentangling the autism–anxiety overlap: fMRI of reward processing in a community-based longitudinal study', *Translational Psychiatry,* 6(6), pp. e845-e845.

Milward, K., Busch, K. E., Murphy, R. J., de Bono, M. and Olofsson, B. (2011) 'Neuronal and molecular substrates for optimal foraging in Caenorhabditis elegans', *Proceedings of the National Academy of Sciences of the United States of America,* 108(51), pp. 20672-20677.

Missler, M., Südhof, T. C. and Biederer, T. (2012) 'Synaptic cell adhesion', *Cold Spring Harbor perspectives in biology,* 4(4), pp. a005694-a005694.

Mitsis, T., Efthimiadou, A., Bacopoulou, F., Vlachakis, D., Chrousos, G.P., & Eliopoulos, E. (2020) 'Transcription factors and evolution: An integral part of gene expression (Review)', *World Acad Sci J,* 2, pp. 3-8.

Modi, M. E. and Young, L. J. (2012) 'The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies', *Hormones and behavior,* 61(3), pp. 340-350.

Mojica, F. J., Díez-Villaseñor, C., García-Martínez, J. and Soria, E. (2005) 'Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements', *J Mol Evol,* 60(2), pp. 174-82.

Moreno-Mateos, M. A., Vejnar, C. E., Beaudoin, J. D., Fernandez, J. P., Mis, E. K., Khokha, M. K. and Giraldez, A. J. (2015) 'CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo', *Nat Methods,* 12(10), pp. 982-988.

Muirhead, C. S. and Srinivasan, J. (2020) 'Small molecule signals mediate social behaviors in C. elegans', *Journal of neurogenetics,* 34(3-4), pp. 395-403.

Mullen, G. P., Mathews, E. A., Saxena, P., Fields, S. D., McManus, J. R., Moulder, G., Barstead, R. J., Quick, M. W. and Rand, J. B. (2006) 'The Caenorhabditis elegans snf-11 gene encodes a sodium-dependent GABA transporter required for clearance of synaptic GABA', *Molecular Biology of the Cell,* 17(7), pp. 3021-3030.

Myers, R. A., Casals, F., Gauthier, J., Hamdan, F. F., Keebler, J., Boyko, A. R., Bustamante, C. D., Piton, A. M., Spiegelman, D., Henrion, E., Zilversmit, M., Hussin, J., Quinlan, J., Yang, Y., Lafrenière, R. G., Griffing, A. R., Stone, E. A., Rouleau, G. A. and Awadalla, P. (2011) 'A population genetic approach to mapping neurological disorder genes using deep resequencing', *PLoS Genet,* 7(2), pp. e1001318.

Neggers, Y. H. (2014) 'Increasing Prevalence, Changes in Diagnostic Criteria, and Nutritional Risk Factors for Autism Spectrum Disorders', *ISRN Nutrition,* 2014, pp. 514026.

Nelson, S. B. and Valakh, V. (2015) 'Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders', *Neuron,* 87(4), pp. 684-698.

Niemi, M. E. K., Martin, H. C., Rice, D. L., Gallone, G., Gordon, S., Kelemen, M., McAloney, K., McRae, J., Radford, E. J., Yu, S., Gecz, J., Martin, N. G., Wright, C. F., Fitzpatrick, D. R., Firth, H. V., Hurles, M. E. and Barrett, J. C. (2018) 'Common genetic variants contribute to risk of rare severe neurodevelopmental disorders', *Nature,* 562(7726), pp. 268-271.

Nuttley, W. M., Atkinson-Leadbeater, K. P. and van der Kooy, D. (2002) 'Serotonin mediates food-odor associative learning in the nematode Caenorhabditis elegans', *Proceedings of the National Academy of Sciences,* 99(19), pp. 12449-12454.

O'Roak, B. J., Deriziotis, P., Lee, C., Vives, L., Schwartz, J. J., Girirajan, S., Karakoc, E., Mackenzie, A. P., Ng, S. B., Baker, C., Rieder, M. J., Nickerson, D. A., Bernier, R., Fisher, S. E., Shendure, J. and Eichler, E. E. (2011) 'Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations', *Nature genetics,* 43(6), pp. 585-589.

O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P., Levy, R., Ko, A., Lee, C., Smith, J. D., Turner, E. H., Stanaway, I. B., Vernot, B., Malig, M., Baker, C., Reilly, B., Akey, J. M., Borenstein, E., Rieder, M. J., Nickerson, D. A., Bernier, R., Shendure, J. and Eichler, E. E. (2012) 'Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations', *Nature,* 485(7397), pp. 246-250.

Ogg, S. and Ruvkun, G. (1998) 'The C-elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway', *Molecular Cell,* 2(6), pp. 887-893.

Oh, W. C., Song, H. O., Cho, J. H. and Park, B. J. (2011) 'ANK repeat-domain of SHN-1 Is indispensable for in vivo SHN-1 function in C. elegans', *Molecules and Cells,* 31(1), pp. 79-84.

Oliver, D., Alexander, K. and Francis, M. M. (2018) 'Molecular Mechanisms Directing Spine Outgrowth and Synaptic Partner Selection in Caenorhabditis elegans', *J Exp Neurosci,* 12, pp. 1179069518816088.

Oranth, A., Schultheis, C., Tolstenkov, O., Erbguth, K., Nagpal, J., Hain, D., Brauner, M., Wabnig, S., Steuer Costa, W., McWhirter, R. D., Zels, S., Palumbos, S., Miller Iii, D. M., Beets, I. and Gottschalk, A. (2018) 'Food Sensation Modulates Locomotion by Dopamine and Neuropeptide Signaling in a Distributed Neuronal Network', *Neuron,* 100(6), pp. 1414-1428.

Owen, M. J., O'Donovan, M. C., Thapar, A. and Craddock, N. (2011) 'Neurodevelopmental hypothesis of schizophrenia', *British Journal of Psychiatry,* 198(3), pp. 173-175.

Paix, A., Folkmann, A., Rasoloson, D. and Seydoux, G. (2015) 'High Efficiency, Homology-Directed Genome Editing in Caenorhabditis elegans Using CRISPR-Cas9 Ribonucleoprotein Complexes', *Genetics,* 201(1), pp. 47-54.

Paix, A., Wang, Y. M., Smith, H. E., Lee, C. Y. S., Calidas, D., Lu, T., Smith, J., Schmidt, H., Krause, M. W. and Seydoux, G. (2014) 'Scalable and Versatile Genome Editing Using Linear DNAs with Microhomology to Cas9 Sites in Caenorhabditis elegans', *Genetics,* 198(4), pp. 1347-1356.

Pan, Y., Chen, J., Guo, H., Ou, J., Peng, Y., Liu, Q., Shen, Y., Shi, L., Liu, Y., Xiong, Z., Zhu, T., Luo, S., Hu, Z., Zhao, J. and Xia, K. (2015) 'Association of genetic variants of GRIN2B with autism', *Sci Rep,* 5, https://doi.org/10.1038/srep08296.

Pandey, U. B. and Nichols, C. D. (2011) 'Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery', *Pharmacological reviews,* 63(2), pp. 411-436.

Pannunzio, N. R., Watanabe, G. and Lieber, M. R. (2018) 'Nonhomologous DNA end-joining for repair of DNA double-strand breaks', *The Journal of biological chemistry,* 293(27), pp. 10512-10523.

Paquet, D., Kwart, D., Chen, A., Sproul, A., Jacob, S., Teo, S., Olsen, K. M., Gregg, A., Noggle, S. and Tessier-Lavigne, M. (2016) 'Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9', *Nature,* 533(7601), pp. 125-129.

Paradee, W., Melikian, H. E., Rasmussen, D. L., Kenneson, A., Conn, P. J. and Warren, S. T. (1999) 'Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function', *Neuroscience,* 94(1), pp. 185-192.

Pardiñas, A. F. and Holmans, P. and Pocklington, A. J. and Escott-Price, V. and Ripke, S. and Carrera, N. and Legge, S. E. and Bishop, S. and Cameron, D. and Hamshere, M. L. and Han, J. and Hubbard, L. and Lynham, A. and Mantripragada, K. and Rees, E. and MacCabe, J. H. and McCarroll, S. A. and Baune, B. T. and Breen, G. and Byrne, E. M. and Dannlowski, U. and Eley, T. C. and Hayward, C. and Martin, N. G. and McIntosh, A. M. and Plomin, R. and Porteous, D. J. and Wray, N. R. and Caballero, A. and Geschwind, D. H. and Huckins, L. M. and Ruderfer, D. M. and Santiago, E. and Sklar, P. and Stahl, E. A. and Won, H. and Agerbo, E. and Als, T. D. and Andreassen, O. A. and Bækvad-Hansen, M. and Mortensen, P. B. and Pedersen, C. B. and Børglum, A. D. and Bybjerg-Grauholm, J. and Djurovic, S. and Durmishi, N. and Pedersen, M. G. and Golimbet, V. and Grove, J. and Hougaard, D. M. and Mattheisen, M. and Molden, E. and Mors, O. and Nordentoft, M. and Pejovic-Milovancevic, M. and Sigurdsson, E. and Silagadze, T. and Hansen, C. S. and Stefansson, K. and Stefansson, H. and Steinberg, S. and Tosato, S. and Werge, T. and Harold, D. and Sims, R. and Gerrish, A. and Chapman, J. and Escott-Price, V. and Abraham, R. and Hollingworth, P. and Pahwa, J. and Denning, N. and Thomas, C. and Taylor, S. and Powell, J. and Proitsi, P. and Lupton, M. and Lovestone, S. and Passmore, P. and Craig, D. and McGuinness, B. and Johnston, J. and Todd, S. and Maier, W. and Jessen, F. and Heun, R. and Schurmann, B. and Ramirez, A. and Becker, T. and Herold, C. and Lacour, A. and Drichel, D. and Nothen, M. and Goate, A. and Cruchaga, C. and Nowotny, P. and Morris, J. C. and Mayo, K. and Holmans, P. and O'Donovan, M. and Owen, M. and Williams, J. and Achilla, E. and Agerbo, E. and Barr, C. L. and Böttger, T. W. and Breen, G. and Cohen, D. and Collier, D. A. and Curran, S. and Dempster, E. and Dima, D. and Sabes-Figuera, R. and Flanagan, R. J. and Frangou, S. and Frank, J. and Gasse, C. and Gaughran, F. and Giegling, I. and Grove, J. and Hannon, E. and Hartmann, A. M. and Heißerer, B. and Helthuis, M. and Horsdal, H. T. and Ingimarsson, O. and Jollie, K. and Kennedy, J. L. and Köhler, O. and Konte, B. and Lang, M. and Legge, S. E. and Lewis, C. and MacCabe, J. and Malhotra, A. K. and McCrone, P. and Meier, S. M. and Mill, J. and Mors, O. and Mortensen, P. B. and Nöthen, M. M. and O'Donovan, M. C. and Owen, M. J. and Pardiñas, A. F. and Pedersen, C. B. and Rietschel, M. and Rujescu, D. and Schwalber, A. and Sigurdsson, E. and Sørensen, H. J. and Spencer, B. and Stefansson, H. and Støvring, H. and Strohmaier, J. and Sullivan, P. and Vassos, E. and Verbelen, M. and Walters, J. T. R. and Werge, T. and Collier, D. A. and Rujescu, D. and Kirov, G. and Owen, M. J. and O'Donovan, M. C. and Walters, J. T. R. and Consortium, G. and Consortium, C. (2018) 'Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection', *Nature Genetics,* 50(3), pp. 381-389.

Park, D., O'Doherty, I., Somvanshi, R. K., Bethke, A., Schroeder, F. C., Kumar, U. and Riddle, D. L. (2012) 'Interaction of structure-specific and promiscuous G-protein-coupled receptors mediates small-molecule signaling in Caenorhabditis elegans', *Proc Natl Acad Sci U S A,* 109(25), pp. 9917-9922.

Park, E. C. and Horvitz, H. R. (1986) 'Mutations with dominant effects on the behavior and morphology of the nematode Caenorhabditis elegans', *Genetics,* 113(4), pp. 821-852.

Park, H. R., Lee, J. M., Moon, H. E., Lee, D. S., Kim, B.-N., Kim, J., Kim, D. G. and Paek, S. H. (2016) 'A Short Review on the Current Understanding of Autism Spectrum Disorders', *Experimental neurobiology,* 25(1), pp. 1-13.

Peng, R., Lin, G. and Li, J. (2016) 'Potential pitfalls of CRISPR/Cas9-mediated genome editing', *The FEBS Journal,* 283(7), pp. 1218-1231.

Peykov, S., Berkel, S., Schoen, M., Weiss, K., Degenhardt, F., Strohmaier, J., Weiss, B., Proepper, C., Schratt, G., Nöthen, M. M., Boeckers, T. M., Rietschel, M. and Rappold, G. A. (2015) 'Identification and functional characterization of rare SHANK2 variants in schizophrenia', *Mol Psychiatry,* 20(12), pp. 1489-1498.

Philbrook, A., Ramachandran, S., Lambert, C. M., Oliver, D., Florman, J., Alkema, M. J., Lemons, M. and Francis, M. M. (2018) 'Neurexin directs partner-specific synaptic connectivity in C-elegans', *Elife,* 7, e35692.

Piechotta, P. L., Rapedius, M., Stansfeld, P. J., Bollepalli, M. K., Ehrlich, G., Andres-Enguix, I., Fritzenschaft, H., Decher, N., Sansom, M. S. P., Tucker, S. J. and Baukrowitz, T. (2011) 'The pore structure and gating mechanism of K2P channels', *The EMBO journal,* 30(17), pp. 3607-3619.

Pierce-Shimomura, J. T., Chen, B. L., Mun, J. J., Ho, R., Sarkis, R. and McIntire, S. L. (2008) 'Genetic analysis of crawling and swimming locomotory patterns in C. elegans', *Proceedings of the National Academy of Sciences of the United States of America,* 105(52), pp. 20982-20987.

Pinto, D. and Delaby, E. and Merico, D. and Barbosa, M. and Merikangas, A. and Klei, L. and Thiruvahindrapuram, B. and Xu, X. and Ziman, R. and Wang, Z. Z. and Vorstman, J. A. S. and Thompson, A. and Regan, R. and Pilorge, M. and Pellecchia, G. and Pagnamenta, A. T. and Oliveira, B. and Marshall, C. R. and Magalhaes, T. R. and Lowe, J. K. and Howe, J. L. and Griswold, A. J. and Gilbert, J. and Duketis, E. and Dombroski, B. A. and De Jonge, M. V. and Cuccaro, M. and Crawford, E. L. and Correia, C. T. and Conroy, J. and Conceicao, I. C. and Chiocchetti, A. G. and Casey, J. P. and Cai, G. and Cabrol, C. and Bolshakova, N. and Bacchelli, E. and Anney, R. and Gallinger, S. and Cotterchio, M. and Casey, G. and Zwaigenbaum, L. and Wittemeyer, K. and Wing, K. and Wallace, S. and van Engeland, H. and Tryfon, A. and Thomson, S. and Soorya, L. and Roge, B. and Roberts, W. and Poustka, F. and Mouga, S. and Minshew, N. and McInnes, L. A. and McGrew, S. G. and Lord, C. and Leboyer, M. and Le Couteur, A. S. and Kolevzon, A. and Gonzalez, P. J. and Jacob, S. and Holt, R. and Guter, S. and Green, J. and Green, A. and Gillberg, C. and Fernandez, B. A. and Duque, F. and Delorme, R. and Dawson, G. and Chaste, P. and Cafe, C. and Brennan, S. and Bourgeron, T. and Bolton, P. F. and Bolte, S. and Bernier, R. and Baird, G. and Bailey, A. J. and Anagnostou, E. and Almeida, J. and Wijsman, E. M. and Vieland, V. J. and Vicente, A. M. and Schellenberg, G. D. and Pericak-Vance, M. and Paterson, A. D. and Parr, J. R. and Oliveira, G. and Nurnberger, J. I. and Monaco, A. P. and Maestrini, E. and Klauck, S. M. and Hakonarson, H. and Haines, J. L. and Geschwind, D. H. and Freitag, C. M. and Folstein, S. E. and Ennis, S. and Coon, H. and Battaglia, A. and Szatmari, P. and Sutcliffe, J. S. and Hallmayer, J. and Gill, M. and Cook, E. H. and Buxbaum, J. D. and Devlin, B. and Gallagher, L. and Betancur, C. and Scherer, S. W. (2014) 'Convergence of Genes and Cellular Pathways Dysregulated in Autism Spectrum Disorders', *American Journal of Human Genetics,* 94(5), pp. 677-694.

Pinto, D. and Pagnamenta, A. T. and Klei, L. and Anney, R. and Merico, D. and Regan, R. and Conroy, J. and Magalhaes, T. R. and Correia, C. and Abrahams, B. S. and Almeida, J. and Bacchelli, E. and Bader, G. D. and Bailey, A. J. and Baird, G. and Battaglia, A. and Berney, T. and Bolshakova, N. and Bölte, S. and Bolton, P. F. and Bourgeron, T. and Brennan, S. and Brian, J. and Bryson, S. E. and Carson, A. R. and Casallo, G. and Casey, J. and Chung, B. H. Y. and Cochrane, L. and Corsello, C. and Crawford, E. L. and Crossett, A. and Cytrynbaum, C. and Dawson, G. and de Jonge, M. and Delorme, R. and Drmic, I. and Duketis, E. and Duque, F. and Estes, A. and Farrar, P. and Fernandez, B. A. and Folstein, S. E. and Fombonne, E. and Freitag, C. M. and Gilbert, J. and Gillberg, C. and

Glessner, J. T. and Goldberg, J. and Green, A. and Green, J. and Guter, S. J. and Hakonarson, H. and Heron, E. A. and Hill, M. and Holt, R. and Howe, J. L. and Hughes, G. and Hus, V. and Igliozzi, R. and Kim, C. and Klauck, S. M. and Kolevzon, A. and Korvatska, O. and Kustanovich, V. and Lajonchere, C. M. and Lamb, J. A. and Laskawiec, M. and Leboyer, M. and Le Couteur, A. and Leventhal, B. L. and Lionel, A. C. and Liu, X.-Q. and Lord, C. and Lotspeich, L. and Lund, S. C. and Maestrini, E. and Mahoney, W. and Mantoulan, C. and Marshall, C. R. and McConachie, H. and McDougle, C. J. and McGrath, J. and McMahon, W. M. and Merikangas, A. and Migita, O. and Minshew, N. J. and Mirza, G. K. and Munson, J. and Nelson, S. F. and Noakes, C. and Noor, A. and Nygren, G. and Oliveira, G. and Papanikolaou, K. and Parr, J. R. and Parrini, B. and Paton, T. and Pickles, A. and Pilorge, M. and Piven, J. and Ponting, C. P. and Posey, D. J. and Poustka, A. and Poustka, F. and Prasad, A. and Ragoussis, J. and Renshaw, K. and Rickaby, J. and Roberts, W. and Roeder, K. and Roge, B. and Rutter, M. L. and Bierut, L. J. and Rice, J. P. and Salt, J. and Sansom, K. and Sato, D. and Segurado, R. and Sequeira, A. F. and Senman, L. and Shah, N. and Sheffield, V. C. and Soorya, L. and Sousa, I. and Stein, O. and Sykes, N. and Stoppioni, V. and Strawbridge, C. and Tancredi, R. and Tansey, K. and Thiruvahindrapduram, B. and Thompson, A. P. and Thomson, S. and Tryfon, A. and Tsiantis, J. and Van Engeland, H. and Vincent, J. B. and Volkmar, F. and Wallace, S. and Wang, K. and Wang, Z. and Wassink, T. H. and Webber, C. and Weksberg, R. and Wing, K. and Wittemeyer, K. and Wood, S. and Wu, J. and Yaspan, B. L. and Zurawiecki, D. and Zwaigenbaum, L. and Buxbaum, J. D. and Cantor, R. M. and Cook, E. H. and Coon, H. and Cuccaro, M. L. and Devlin, B. and Ennis, S. and Gallagher, L. and Geschwind, D. H. and Gill, M. and Haines, J. L. and Hallmayer, J. and Miller, J. and Monaco, A. P. and Nurnberger Jr, J. I. and Paterson, A. D. and Pericak-Vance, M. A. and Schellenberg, G. D. and Szatmari, P. and Vicente, A. M. and Vieland, V. J. and Wijsman, E. M. and Scherer, S. W. and Sutcliffe, J. S. and Betancur, C. (2010) 'Functional impact of global rare copy number variation in autism spectrum disorders', *Nature,* 466(7304), pp. 368-372.

Platt, R. J., Zhou, Y., Slaymaker, I. M., Shetty, A. S., Weisbach, N. R., Kim, J. A., Sharma, J., Desai, M., Sood, S., Kempton, H. R., Crabtree, G. R., Feng, G. and Zhang, F. (2017) 'Chd8 Mutation Leads to Autistic-like Behaviors and Impaired Striatal Circuits', *Cell Rep,* 19(2), pp. 335-350.

Platzer, K., Yuan, H., Schütz, H., Winschel, A., Chen, W., Hu, C., Kusumoto, H., Heyne, H. O., Helbig, K. L., Tang, S., Willing, M. C., Tinkle, B. T., Adams, D. J., Depienne, C., Keren, B., Mignot, C., Frengen, E., Strømme, P., Biskup, S., Döcker, D., Strom, T. M., Mefford, H. C., Myers, C. T., Muir, A. M., LaCroix, A., Sadleir, L., Scheffer, I. E., Brilstra, E., van Haelst, M. M., van der Smagt, J. J., Bok, L. A., Møller, R. S., Jensen, U. B., Millichap, J. J., Berg, A. T., Goldberg, E. M., De Bie, I., Fox, S., Major, P., Jones, J. R., Zackai, E. H., Abou Jamra, R., Rolfs, A., Leventer, R. J., Lawson, J. A., Roscioli, T., Jansen, F. E., Ranza, E., Korff, C. M., Lehesjoki, A. E., Courage, C., Linnankivi, T., Smith, D. R., Stanley, C., Mintz, M., McKnight, D., Decker, A., Tan, W. H., Tarnopolsky, M. A., Brady, L. I., Wolff, M., Dondit, L., Pedro, H. F., Parisotto, S. E., Jones, K. L., Patel, A. D., Franz, D. N., Vanzo, R., Marco, E., Ranells, J. D., Di Donato, N., Dobyns, W. B., Laube, B., Traynelis, S. F. and Lemke, J. R. (2017) 'GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects', *J Med Genet,* 54(7), pp. 460-470.

Post, K. L., Belmadani, M., Ganguly, P., Meili, F., Dingwall, R., McDiarmid, T. A., Meyers, W. M., Herrington, C., Young, B. P., Callaghan, D. B., Rogic, S., Edwards, M., Niciforovic, A., Cau, A., Rankin, C. H., O'Connor, T. P., Bamji, S. X., Loewen, C. J. R., Allan, D. W., Pavlidis, P. and Haas, K. (2020) 'Multi-model functionalization of disease-associated PTEN missense mutations identifies multiple molecular mechanisms underlying protein dysfunction', *Nat Commun,* 11(1), https://doi.org/10.1038/s41467-020-15943-0.

Powis, Z., Farwell Hagman, K. D., Mroske, C., McWalter, K., Cohen, J. S., Colombo, R., Serretti, A., Fatemi, A., David, K. L., Reynolds, J., Immken, L., Nagakura, H., Cunniff, C. M., Payne, K., Barbaro-Dieber, T., Gripp, K. W., Baker, L., Stamper, T., Aleck, K. A., Jordan, E. S., Hersh, J. H., Burton, J., Wentzensen, I. M., Guillen Sacoto, M. J., Willaert, R., Cho, M. T., Petrik, I., Huether, R. and Tang, S. (2018) 'Expansion and further delineation of the SETD5 phenotype leading to global

developmental delay, variable dysmorphic features, and reduced penetrance', *Clinical Genetics,* 93(4), pp. 752-761.

Pradhan, S., Quilez, S., Homer, K. and Hendricks, M. (2019) 'Environmental Programming of Adult Foraging Behavior in C. elegans', *Current Biology,* 29(17), pp. 2867-2879.

Prior, H., Jawad, A. K., MacConnachie, L. and Beg, A. A. (2017) 'Highly Efficient, Rapid and Co-CRISPR-Independent Genome Editing in Caenorhabditis elegans', *G3-Genes Genomes Genetics,* 7(11), pp. 3693-3698.

Pu, M. T., Ni, Z. Y., Wang, M. H., Wang, X. J., Wood, J. G., Helfand, S. L., Yu, H. Y. and Lee, S. S. (2015) 'Trimethylation of Lys36 on H3 restricts gene expression change during aging and impacts life span', *Genes & Development,* 29(7), pp. 718-731.

Purty, A., Nestadt, G., Samuels, J. F. and Viswanath, B. (2019) 'Genetics of obsessive-compulsive disorder', *Indian J Psychiatry,* 61(Suppl 1), pp. 37-42.

Purves, D., Augustine, G., Fitzpatrick, D, et al. (2001) *Neuroscience. 2nd edition.* Sunderland (MA): Sinauer Associates.

Purves, K. L., Coleman, J. R. I., Meier, S. M., Rayner, C., Davis, K. A. S., Cheesman, R., Bækvad-Hansen, M., Børglum, A. D., Wan Cho, S., Jürgen Deckert, J., Gaspar, H. A., Bybjerg-Grauholm, J., Hettema, J. M., Hotopf, M., Hougaard, D., Hübel, C., Kan, C., McIntosh, A. M., Mors, O., Bo Mortensen, P., Nordentoft, M., Werge, T., Nicodemus, K. K., Mattheisen, M., Breen, G. and Eley, T. C. (2020) 'A major role for common genetic variation in anxiety disorders', *Molecular Psychiatry,* 25(12), pp. 3292-3303.

Pym, E., Sasidharan, N., Thompson-Peer, K. L., Simon, D. J., Anselmo, A., Sadreyev, R., Hall, Q., Nurrish, S. and Kaplan, J. M. (2017) 'Shank is a dose-dependent regulator of Ca(v)1 calcium current and CREB target expression', *Elife,* 6(6), e18931.

Rabaneda, L. G., Robles-Lanuza, E., Nieto-Gonzalez, J. L. and Scholl, F. G. (2014) 'Neurexin Dysfunction in Adult Neurons Results in Autistic-like Behavior in Mice', *Cell Reports,* 8(2), pp. 337-345.

Raghu, P., Joseph, A., Krishnan, H., Singh, P. and Saha, S. (2019) 'Phosphoinositides: Regulators of Nervous System Function in Health and Disease', *Frontiers in molecular neuroscience,* 12, doi:10.3389/fnmol.2019.00208.

Raich, W. B., Moorman, C., Lacefield, C. O., Lehrer, J., Bartsch, D., Plasterk, R. H. A., Kandel, E. R. and Hobert, O. (2003) 'Characterization of Caenorhabditis elegans homologs of the Down syndrome candidate gene DYRK1A', *Genetics,* 163(2), pp. 571-580.

Raizen, D. M., Zimmerman, J. E., Maycock, M. H., Ta, U. D., You, Y.-j., Sundaram, M. V. and Pack, A. I. (2008) 'Lethargus is a Caenorhabditis elegans sleep-like state', *Nature,* 451(7178), pp. 569-572.

Ran, F. A., Hsu, P. D., Lin, C.-Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., Scott, D. A., Inoue, A., Matoba, S., Zhang, Y. and Zhang, F. (2013) 'Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity', *Cell,* 154(6), pp. 1380-1389.

Rawsthorne-Manning, H., Calahorro, F., Izquierdo, P. G., Holden-Dye, L., O'Connor, V. and Dillon, J. (2021) 'Confounds of using the unc-58 selection marker highlights the importance of genotyping co-CRISPR genes', *bioRxiv*, pp. 2021.05.26.445785.

Rawsthorne, H., Calahorro, F., Feist, E., Holden-Dye, L., O'Connor, V. and Dillon, J. (2020) 'Neuroligin dependence of social behaviour in C. elegans provides a model to investigate an autism associated gene', *Human Molecular Genetics,* 29(21), 3546-3553.

Rawsthorne, H., Calahorro, F., Holden-Dye, L., O' Connor, V. and Dillon, J. (2021) 'Investigating autism associated genes in C. elegans reveals candidates with a role in social behaviour', *PLOS ONE,* 16(5), pp. e0243121.

Redin, C., Gérard, B., Lauer, J., Herenger, Y., Muller, J., Quartier, A., Masurel-Paulet, A., Willems, M., Lesca, G., El-Chehadeh, S., Le Gras, S., Vicaire, S., Philipps, M., Dumas, M., Geoffroy, V., Feger, C., Haumesser, N., Alembik, Y., Barth, M., Bonneau, D., Colin, E., Dollfus, H., Doray, B., Delrue, M. A., Drouin-Garraud, V., Flori, E., Fradin, M., Francannet, C., Goldenberg, A., Lumbroso, S., Mathieu-Dramard, M., Martin-Coignard, D., Lacombe, D., Morin, G., Polge, A., Sukno, S., Thauvin-Robinet, C., Thevenon, J., Doco-Fenzy, M., Genevieve, D., Sarda, P., Edery, P., Isidor, B., Jost, B., Olivier-Faivre, L., Mandel, J. L. and Piton, A. (2014) 'Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing', *J Med Genet,* 51(11), pp. 724-36.

Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D., Fiegler, H., Shapero, M. H., Carson, A. R., Chen, W., Cho, E. K., Dallaire, S., Freeman, J. L., González, J. R., Gratacòs, M., Huang, J., Kalaitzopoulos, D., Komura, D., MacDonald, J. R., Marshall, C. R., Mei, R., Montgomery, L., Nishimura, K., Okamura, K., Shen, F., Somerville, M. J., Tchinda, J., Valsesia, A., Woodwark, C., Yang, F., Zhang, J., Zerjal, T., Zhang, J., Armengol, L., Conrad, D. F., Estivill, X., Tyler-Smith, C., Carter, N. P., Aburatani, H., Lee, C., Jones, K. W., Scherer, S. W. and Hurles, M. E. (2006) 'Global variation in copy number in the human genome', *Nature,* 444(7118), pp. 444-454.

Rees, E., Han, J., Morgan, J., Carrera, N., Escott-Price, V., Pocklington, A. J., Duffield, M., Hall, L. S., Legge, S. E., Pardiñas, A. F., Richards, A. L., Roth, J., Lezheiko, T., Kondratyev, N., Kaleda, V., Golimbet, V., Parellada, M., González-Peñas, J., Arango, C., Gawlik, M., Kirov, G., Walters, J. T. R., Holmans, P., O'Donovan, M. C. and Owen, M. J. (2020) 'De novo mutations identified by exome sequencing implicate rare missense variants in SLC6A1 in schizophrenia', *Nat Neurosci,* 23(2), pp. 179-184.

Refai, O. and Blakely, R. D. (2019) 'Blockade and reversal of swimming-induced paralysis in C. elegans by the antipsychotic and D2-type dopamine receptor antagonist azaperone', *Neurochem Int,* 123, pp. 59-68.

Reiner, D. J., Weinshenker, D. and Thomas, J. H. (1995) 'Analysis of dominant mutations affecting muscle excitation in Caenorhabditis elegans', *Genetics,* 141(3), pp. 961-976.

Reiter, L. T., Potocki, L., Chien, S., Gribskov, M. and Bier, E. (2001) 'A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster', *Genome Res,* 11(6), pp. 1114-1125.

Rhoades, J. L., Nelson, J. C., Nwabudike, I., Yu, S. K., McLachlan, I. G., Madan, G. K., Abebe, E., Powers, J. R., Colón-Ramos, D. A. and Flavell, S. W. (2019) 'ASICs Mediate Food Responses in an Enteric Serotonergic Neuron that Controls Foraging Behaviors', *Cell,* 176(1-2), pp. 85-97.

Rieckher, M. and Tavernarakis, N. (2017) 'Caenorhabditis elegans Microinjection', *Bio-protocol,* 7(19), pp. e2565.

Ripke, S. and O'Dushlaine, C. and Chambert, K. and Moran, J. L. and Kähler, A. K. and Akterin, S. and Bergen, S. E. and Collins, A. L. and Crowley, J. J. and Fromer, M. and Kim, Y. and Lee, S. H. and Magnusson, P. K. E. and Sanchez, N. and Stahl, E. A. and Williams, S. and Wray, N. R. and Xia, K. and Bettella, F. and Borglum, A. D. and Bulik-Sullivan, B. K. and Cormican, P. and Craddock, N. and de Leeuw, C. and Durmishi, N. and Gill, M. and Golimbet, V. and Hamshere, M. L. and Holmans, P. and Hougaard, D. M. and Kendler, K. S. and Lin, K. and Morris, D. W. and Mors, O. and Mortensen, P. B. and Neale, B. M. and O'Neill, F. A. and Owen, M. J. and Milovancevic, M. P. and Posthuma, D. and Powell, J. and Richards, A. L. and Riley, B. P. and Ruderfer, D. and Rujescu, D. and Sigurdsson, E. and Silagadze, T. and Smit, A. B. and Stefansson, H. and Steinberg, S. and Suvisaari, J. and

Tosato, S. and Verhage, M. and Walters, J. T. and Multicenter Genetic Studies of Schizophrenia, C. and Levinson, D. F. and Gejman, P. V. and Kendler, K. S. and Laurent, C. and Mowry, B. J. and O'Donovan, M. C. and Owen, M. J. and Pulver, A. E. and Riley, B. P. and Schwab, S. G. and Wildenauer, D. B. and Dudbridge, F. and Holmans, P. and Shi, J. and Albus, M. and Alexander, M. and Campion, D. and Cohen, D. and Dikeos, D. and Duan, J. and Eichhammer, P. and Godard, S. and Hansen, M. and Lerer, F. B. and Liang, K.-Y. and Maier, W. and Mallet, J. and Nertney, D. A. and Nestadt, G. and Norton, N. and O'Neill, F. A. and Papadimitriou, G. N. and Ribble, R. and Sanders, A. R. and Silverman, J. M. and Walsh, D. and Williams, N. M. and Wormley, B. and Psychosis Endophenotypes International, C. and Arranz, M. J. and Bakker, S. and Bender, S. and Bramon, E. and Collier, D. and Crespo-Facorro, B. and Hall, J. and Iyegbe, C. and Jablensky, A. and Kahn, R. S. and Kalaydjieva, L. and Lawrie, S. and Lewis, C. M. and Lin, K. and Linszen, D. H. and Mata, I. and McIntosh, A. and Murray, R. M. and Ophoff, R. A. and Powell, J. and Rujescu, D. and Van Os, J. and Walshe, M. and Weisbrod, M. and Wiersma, D. and Wellcome Trust Case Control, C. and Donnelly, P. and Barroso, I. and Blackwell, J. M. and Bramon, E. and Brown, M. A. and Casas, J. P. and Corvin, A. P. and Deloukas, P. and Duncanson, A. and Jankowski, J. and Markus, H. S. and Mathew, C. G. and Palmer, C. N. A. and Plomin, R. and Rautanen, A. and Sawcer, S. J. and Trembath, R. C. and Viswanathan, A. C. and Wood, N. W. and Spencer, C. C. A. and Band, G. and Bellenguez, C. and Freeman, C. and Hellenthal, G. and Giannoulatou, E. and Pirinen, M. and Pearson, R. D. and Strange, A. and Su, Z. and Vukcevic, D. and Donnelly, P. and Langford, C. and Hunt, S. E. and Edkins, S. and Gwilliam, R. and Blackburn, H. and Bumpstead, S. J. and Dronov, S. and Gillman, M. and Gray, E. and Hammond, N. and Jayakumar, A. and McCann, O. T. and Liddle, J. and Potter, S. C. and Ravindrarajah, R. and Ricketts, M. and Tashakkori-Ghanbaria, A. and Waller, M. J. and Weston, P. and Widaa, S. and Whittaker, P. and Barroso, I. and Deloukas, P. and Mathew, C. G. and Blackwell, J. M. and Brown, M. A. and Corvin, A. P. and McCarthy, M. I. and Spencer, C. C. A. and Bramon, E. and Corvin, A. P. and O'Donovan, M. C. and Stefansson, K. and Scolnick, E. and Purcell, S. and McCarroll, S. A. and Sklar, P. and Hultman, C. M. and Sullivan, P. F. (2013) 'Genome-wide association analysis identifies 13 new risk loci for schizophrenia', *Nature genetics,* 45(10), pp. 1150-1159.

Ritvo, E. R., Freeman, B. J., Mason-Brothers, A., Mo, A. and Ritvo, A. M. (1985) 'Concordance for the syndrome of autism in 40 pairs of afflicted twins', *Am J Psychiatry,* 142(1), pp. 74-77.

Robert, F. and Pelletier, J. (2018) 'Exploring the Impact of Single-Nucleotide Polymorphisms on Translation', *Frontiers in Genetics,* 9(507), doi:10.3389/fgene.2018.00507.

Robertson, C. E. and Baron-Cohen, S. (2017) 'Sensory perception in autism', *Nature Reviews Neuroscience,* 18(11), pp. 671-684.

Rochtus, A., Olson, H. E., Smith, L., Keith, L. G., El Achkar, C., Taylor, A., Mahida, S., Park, M., Kelly, M., Shain, C., Rockowitz, S., Rosen Sheidley, B. and Poduri, A. (2020) 'Genetic diagnoses in epilepsy: The impact of dynamic exome analysis in a pediatric cohort', *Epilepsia,* 61(2), pp. 249-258.

Rodenas-Cuadrado, P., Ho, J. and Vernes, S. C. (2014) 'Shining a light on CNTNAP2: complex functions to complex disorders', *European Journal of Human Genetics,* 22(2), pp. 171-178.

Rogers, E. J., Jada, R., Schragenheim-Rozales, K., Sah, M., Cortes, M., Florence, M., Levy, N. S., Moss, R., Walikonis, R. S., Palty, R., Shalgi, R., Lichtman, D., Kavushansky, A., Gerges, N. Z., Kahn, I., Umanah, G. K. E. and Levy, A. P. (2019) 'An IQSEC2 Mutation Associated With Intellectual Disability and Autism Results in Decreased Surface AMPA Receptors', *Frontiers in Molecular Neuroscience,* 12(43), doi: 10.3389/fnmol.2019.00043.

Ronald, A. and Hoekstra, R. A. (2011) 'Autism Spectrum Disorders and Autistic Traits: A Decade of New Twin Studies', *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics,* 156B(3), pp. 255-274.

Roohi, J., Montagna, C., Tegay, D. H., Palmer, L. E., DeVincent, C., Pomeroy, J. C., Christian, S. L., Nowak, N. and Hatchwell, E. (2009) 'Disruption of contactin 4 in three subjects with autism spectrum disorder', *J Med Genet,* 46(3), pp. 176-182.

Rooke, R., Rasool, A., Schneider, J. and Levine, J. D. (2020) 'Drosophila melanogaster behaviour changes in different social environments based on group size and density', *Communications Biology,* 3(1), https://doi.org/10.1038/s42003-020-1024-z.

Rothwell, P. E., Fuccillo, M. V., Maxeiner, S., Hayton, S. J., Gokce, O., Lim, B. K., Fowler, S. C., Malenka, R. C. and Sudhof, T. C. (2014) 'Autism-Associated Neuroligin-3 Mutations Commonly Impair Striatal Circuits to Boost Repetitive Behaviors', *Cell,* 158(1), pp. 198-212.

Rotschafer, S. and Razak, K. (2013) 'Altered auditory processing in a mouse model of fragile X syndrome', *Brain Res,* 1506, pp. 12-24.

Rubenstein, J. L. R. and Merzenich, M. M. (2003) 'Model of autism: increased ratio of excitation/inhibition in key neural systems', *Genes, brain, and behavior,* 2(5), pp. 255-267.

Rylaarsdam, L. and Guemez-Gamboa, A. (2019) 'Genetic Causes and Modifiers of Autism Spectrum Disorder', *Frontiers in Cellular Neuroscience,* 13, doi:10.3389/fncel.2019.00385.

Ryu, W. S. and Samuel, A. D. (2002) 'Thermotaxis in Caenorhabditis elegans analyzed by measuring responses to defined Thermal stimuli', *J Neurosci,* 22(13), pp. 5727-5733.

Sahin, M. and Sur, M. (2015) 'Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders', *Science,* 350(6263), doi: 10.1126/science.aab3897.

Salkoff, et al. Potassium channels in *C. elegans* (December 30, 2005), *WormBook*, ed. The *C.*

elegans Research Community, WormBook,

doi/10.1895/wormbook.1.42.1, http://www.wormbook.org.

Salkoff, L., Butler, A., Fawcett, G., Kunkel, M., McArdle, C., Paz-y-Mino, G., Nonet, M., Walton, N., Wang, Z. W., Yuan, A. and Wei, A. (2001) 'Evolution tunes the excitability of individual neurons', *Neuroscience,* 103(4), pp. 853-859.

Sanders, S. J., Ercan-Sencicek, A. G., Hus, V., Luo, R., Murtha, M. T., Moreno-De-Luca, D., Chu, S. H., Moreau, M. P., Gupta, A. R., Thomson, S. A., Mason, C. E., Bilguvar, K., Celestino-Soper, P. B. S., Choi, M., Crawford, E. L., Davis, L., Wright, N. R. D., Dhodapkar, R. M., DiCola, M., DiLullo, N. M., Fernandez, T. V., Fielding-Singh, V., Fishman, D. O., Frahm, S., Garagaloyan, R., Goh, G. S., Kammela, S., Klei, L., Lowe, J. K., Lund, S. C., McGrew, A. D., Meyer, K. A., Moffat, W. J., Murdoch, J. D., O'Roak, B. J., Ober, G. T., Pottenger, R. S., Raubeson, M. J., Song, Y., Wang, Q., Yaspan, B. L., Yu, T. W., Yurkiewicz, I. R., Beaudet, A. L., Cantor, R. M., Curland, M., Grice, D. E., Günel, M., Lifton, R. P., Mane, S. M., Martin, D. M., Shaw, C. A., Sheldon, M., Tischfield, J. A., Walsh, C. A., Morrow, E. M., Ledbetter, D. H., Fombonne, E., Lord, C., Martin, C. L., Brooks, A. I., Sutcliffe, J. S., Cook, E. H., Jr., Geschwind, D., Roeder, K., Devlin, B. and State, M. W. (2011) 'Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism', *Neuron,* 70(5), pp. 863-885.

Sanders, S. J., He, X., Willsey, A. J., Ercan-Sencicek, A. G., Samocha, K. E., Cicek, A. E., Murtha, M. T., Bal, V. H., Bishop, S. L., Dong, S., Goldberg, A. P., Jinlu, C., Keaney, J. F., 3rd, Klei, L., Mandell, J. D., Moreno-De-Luca, D., Poultney, C. S., Robinson, E. B., Smith, L., Solli-Nowlan, T., Su, M. Y., Teran, N. A., Walker, M. F., Werling, D. M., Beaudet, A. L., Cantor, R. M., Fombonne, E., Geschwind, D. H., Grice, D. E., Lord, C., Lowe, J. K., Mane, S. M., Martin, D. M., Morrow, E. M., Talkowski, M. E., Sutcliffe, J. S., Walsh, C. A., Yu, T. W., Ledbetter, D. H., Martin, C. L., Cook, E. H., Buxbaum, J. D., Daly, M. J., Devlin, B., Roeder, K. and State, M. W. (2015) 'Insights into Autism

Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci', *Neuron,* 87(6), pp. 1215-1233.

Sanders, S. J., Murtha, M. T., Gupta, A. R., Murdoch, J. D., Raubeson, M. J., Willsey, A. J., Ercan-Sencicek, A. G., DiLullo, N. M., Parikshak, N. N., Stein, J. L., Walker, M. F., Ober, G. T., Teran, N. A., Song, Y., El-Fishawy, P., Murtha, R. C., Choi, M., Overton, J. D., Bjornson, R. D., Carriero, N. J., Meyer, K. A., Bilguvar, K., Mane, S. M., Šestan, N., Lifton, R. P., Günel, M., Roeder, K., Geschwind, D. H., Devlin, B. and State, M. W. (2012) 'De novo mutations revealed by whole-exome sequencing are strongly associated with autism', *Nature,* 485(7397), pp. 237-241.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M. and Reichenberg, A. (2014) 'The familial risk of autism', *Jama,* 311(17), pp. 1770-7.

Sansbury, B. M., Hewes, A. M. and Kmiec, E. B. (2019) 'Understanding the diversity of genetic outcomes from CRISPR-Cas generated homology-directed repair', *Communications Biology,* 2(1), https://doi.org/10.1038/s42003-019-0705-y.

Satterstrom, F. K., Kosmicki, J. A., Wang, J., Breen, M. S., De Rubeis, S., An, J. Y., Peng, M., Collins, R., Grove, J., Klei, L., Stevens, C., Reichert, J., Mulhern, M. S., Artomov, M., Gerges, S., Sheppard, B., Xu, X., Bhaduri, A., Norman, U., Brand, H., Schwartz, G., Nguyen, R., Guerrero, E. E., Dias, C., Betancur, C., Cook, E. H., Gallagher, L., Gill, M., Sutcliffe, J. S., Thurm, A., Zwick, M. E., Børglum, A. D., State, M. W., Cicek, A. E., Talkowski, M. E., Cutler, D. J., Devlin, B., Sanders, S. J., Roeder, K., Daly, M. J. and Buxbaum, J. D. (2020) 'Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism', *Cell,* 180(3), pp. 568-584.

Sawa H., Korswagen H. C. Wnt signalling in *C. elegans* (December 9, 2013), *WormBook*, ed. The *C.*

elegans Research Community, WormBook,

doi/10.1895/wormbook.1.7.2, http://www.wormbook.org.

Sawin, E. R., Ranganathan, R. and Horvitz, H. R. (2000) 'C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway', *Neuron,* 26(3), pp. 619-631.

Schaaf, C. P., Boone, P. M., Sampath, S., Williams, C., Bader, P. I., Mueller, J. M., Shchelochkov, O. A., Brown, C. W., Crawford, H. P., Phalen, J. A., Tartaglia, N. R., Evans, P., Campbell, W. M., Tsai, A. C.-H., Parsley, L., Grayson, S. W., Scheuerle, A., Luzzi, C. D., Thomas, S. K., Eng, P. A., Kang, S.-H. L., Patel, A., Stankiewicz, P. and Cheung, S. W. (2012) 'Phenotypic spectrum and genotype-phenotype correlations of NRXN1 exon deletions', *European journal of human genetics : EJHG,* 20(12), pp. 1240-1247.

Schafer, W. (2016) 'Nematode nervous systems', *Current Biology,* 26(20), pp. 955-959.

Schafer, W. R. (2005) 'Deciphering the neural and molecular mechanisms of C. elegans behavior', *Curr Biol,* 15(17), pp. 723-729.

Scheffer, L. K. and Xu, C. S. and Januszewski, M. and Lu, Z. and Takemura, S. Y. and Hayworth, K. J. and Huang, G. B. and Shinomiya, K. and Maitlin-Shepard, J. and Berg, S. and Clements, J. and Hubbard, P. M. and Katz, W. T. and Umayam, L. and Zhao, T. and Ackerman, D. and Blakely, T. and Bogovic, J. and Dolafi, T. and Kainmueller, D. and Kawase, T. and Khairy, K. A. and Leavitt, L. and Li, P. H. and Lindsey, L. and Neubarth, N. and Olbris, D. J. and Otsuna, H. and Trautman, E. T. and Ito, M. and Bates, A. S. and Goldammer, J. and Wolff, T. and Svirskas, R. and Schlegel, P. and Neace, E. and Knecht, C. J. and Alvarado, C. X. and Bailey, D. A. and Ballinger, S. and Borycz, J. A. and Canino, B. S. and Cheatham, N. and Cook, M. and Dreher, M. and Duclos, O. and Eubanks, B. and Fairbanks, K. and Finley, S. and Forknall, N. and Francis, A. and Hopkins, G. P. and Joyce, E. M. and Kim, S. and Kirk, N. A. and Kovalyak, J. and Lauchie, S. A. and Lohff, A. and Maldonado, C. and

Manley, E. A. and McLin, S. and Mooney, C. and Ndama, M. and Ogundeyi, O. and Okeoma, N. and Ordish, C. and Padilla, N. and Patrick, C. M. and Paterson, T. and Phillips, E. E. and Phillips, E. M. and Rampally, N. and Ribeiro, C. and Robertson, M. K. and Rymer, J. T. and Ryan, S. M. and Sammons, M. and Scott, A. K. and Scott, A. L. and Shinomiya, A. and Smith, C. and Smith, K. and Smith, N. L. and Sobeski, M. A. and Suleiman, A. and Swift, J. and Takemura, S. and Talebi, I. and Tarnogorska, D. and Tenshaw, E. and Tokhi, T. and Walsh, J. J. and Yang, T. and Horne, J. A. and Li, F. and Parekh, R. and Rivlin, P. K. and Jayaraman, V. and Costa, M. and Jefferis, G. S. and Ito, K. and Saalfeld, S. and George, R. and Meinertzhagen, I. A. and Rubin, G. M. and Hess, H. F. and Jain, V. and Plaza, S. M. (2020) 'A connectome and analysis of the adult Drosophila central brain', *Elife,* 7(9), e57443.

Schmeisser, K., Fardghassemi, Y. and Parker, J. A. (2017) 'A rapid chemical-genetic screen utilizing impaired movement phenotypes in C.elegans: Input into genetics of neurodevelopmental disorders', *Experimental Neurology,* 293, pp. 101-114.

Schmeisser, K. and Parker, J. A. (2018) 'Worms on the spectrum - C-elegans models in autism research', *Experimental Neurology,* 299, pp. 199-206.

Schneider, T. and Przewłocki, R. (2005) 'Behavioral Alterations in Rats Prenatally Exposed to Valproic Acid: Animal Model of Autism', *Neuropsychopharmacology,* 30(1), pp. 80-89.

Schreier, J., Dietz, S., de Jesus Domingues, A. M., Seistrup, A.-S., Nguyen, D. A. H., Gleason, E. J., Ling, H., L'Hernault, S. W., Phillips, C. M., Butter, F. and Ketting, R. F. (2020) 'A membrane-associated condensate drives paternal epigenetic inheritance in <em>C. elegans</em&gt', *bioRxiv*, pp. 2020.12.10.417311.

Schroeder, J. C., Reim, D., Boeckers, T. M. and Schmeisser, M. J. (2017) 'Genetic Animal Models for Autism Spectrum Disorder', in Wohr, M. and Krach, S. (eds.) *Social Behavior from Rodents to Humans: Neural Foundations and Clinical Implications Current Topics in Behavioral Neurosciences*. Cham: Springer International Publishing Ag, pp. 311-324.

Schwarz, V., Pan, J., Voltmer-Irsch, S. and Hutter, H. (2009) 'IgCAMs redundantly control axon navigation in Caenorhabditis elegans', *Neural Development,* 4(13), doi: 10.1186/1749-8104-4-13.

Scott, E., Hudson, A., Feist, E., Calahorro, F., Dillon, J., de Freitas, R., Wand, M., Schoofs, L., O'Connor, V. and Holden-Dye, L. (2017) 'An oxytocin-dependent social interaction between larvae and adult C. elegans', *Scientific Reports,* 7(1), pp. 10122.

Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., Yamrom, B., Yoon, S., Krasnitz, A., Kendall, J., Leotta, A., Pai, D., Zhang, R., Lee, Y. H., Hicks, J., Spence, S. J., Lee, A. T., Puura, K., Lehtimaki, T., Ledbetter, D., Gregersen, P. K., Bregman, J., Sutcliffe, J. S., Jobanputra, V., Chung, W., Warburton, D., King, M. C., Skuse, D., Geschwind, D. H., Gilliam, T. C., Ye, K. and Wigler, M. (2007) 'Strong association of de novo copy number mutations with autism', *Science,* 316(5823), pp. 445-449.

Sengupta, P. and Samuel, A. D. T. (2009) 'Caenorhabditis elegans: a model system for systems neuroscience', *Current Opinion in Neurobiology,* 19(6), pp. 637-643.

Sharma, S. R., Gonda, X. and Tarazi, F. I. (2018) 'Autism Spectrum Disorder: Classification, diagnosis and therapy', *Pharmacol. Ther.,* 190, pp. 91-104.

Shaye, D. D. and Greenwald, I. (2011) 'OrthoList: A Compendium of C. elegans Genes with Human Orthologs', *Plos One,* 6(5), e20085.

Shcheglovitov, A., Shcheglovitova, O., Yazawa, M., Portmann, T., Shu, R., Sebastiano, V., Krawisz, A., Froehlich, W., Bernstein, J. A., Hallmayer, J. F. and Dolmetsch, R. E. (2013) 'SHANK3 and IGF1

restore synaptic deficits in neurons from 22q13 deletion syndrome patients', *Nature,* 503(7475), pp. 267-71.

Shen, M. D. and Piven, J. (2017) 'Brain and behavior development in autism from birth through infancy', *Dialogues in clinical neuroscience,* 19(4), pp. 325-333.

Sheng, M. and Kim, E. (2011) 'The Postsynaptic Organization of Synapses', *Cold Spring Harbor Perspectives in Biology,* 3(12), a005678.

Shepherd, G. (2011) 'The Microcircuit Concept Applied to Cortical Evolution: from Three-Layer to Six-Layer Cortex', *Frontiers in Neuroanatomy,* 5(30), doi: 10.3389/fnana.2011.00030.

Shtonda, B. B. and Avery, L. (2006) 'Dietary choice behavior in Caenorhabditis elegans', *Journal of Experimental Biology,* 209(1), pp. 89-102.

Silverman, J. L., Yang, M., Lord, C. and Crawley, J. N. (2010) 'Behavioural phenotyping assays for mouse models of autism', *Nature reviews. Neuroscience,* 11(7), pp. 490-502.

Simon, A. F., Chou, M. T., Salazar, E. D., Nicholson, T., Saini, N., Metchev, S. and Krantz, D. E. (2012) 'A simple assay to study social behavior in Drosophila: measurement of social space within a group', *Genes, brain, and behavior,* 11(2), pp. 243-252.

Singh, S. K. and Eroglu, C. (2013) 'Neuroligins provide molecular links between syndromic and nonsyndromic autism', *Science signaling,* 6(283), doi: 10.1126/scisignal.2004102.

Siper, P. M., De Rubeis, S., Trelles, M. D. P., Durkin, A., Di Marino, D., Muratet, F., Frank, Y., Lozano, R., Eichler, E. E., Kelly, M., Beighley, J., Gerdts, J., Wallace, A. S., Mefford, H. C., Bernier, R. A., Kolevzon, A. and Buxbaum, J. D. (2017) 'Prospective investigation of FOXP1 syndrome', *Mol Autism,* 8(57), doi: 10.1186/s13229-017-0172-6.

Sledziowska, M., Kalbassi, S. and Baudouin, S. J. (2020) 'Complex Interactions between Genes and Social Environment Cause Phenotypes Associated with Autism Spectrum Disorders in Mice', *eNeuro,* 7(4), pp. ENEURO.0124-20.2020.

Smith, C. J., Watson, J. D., Spencer, W. C., O'Brien, T., Cha, B., Albeg, A., Treinin, M. and Miller, D. M., 3rd (2010) 'Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in C. elegans', *Dev Biol,* 345(1), pp. 18-33.

Sohal, V. S. and Rubenstein, J. L. R. (2019) 'Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders', *Molecular psychiatry,* 24(9), pp. 1248-1257.

Solari, F., Bourbon-Piffaut, A., Masse, I., Payrastre, B., Chan, A. M. and Billaud, M. (2005) 'The human tumour suppressor PTEN regulates longevity and dauer formation in Caenorhabditis elegans', *Oncogene,* 24(1), pp. 20-7.

Sonnhammer, E. L., von Heijne, G. and Krogh, A. (1998) 'A hidden Markov model for predicting transmembrane helices in protein sequences', *Proc Int Conf Intell Syst Mol Biol,* 6, pp. 175-82.

Sonnhammer, E. L. L. and Durbin, R. (1997) 'Analysis of protein domain families in Caenorhabditis elegans', *Genomics,* 46(2), pp. 200-216.

Soorya, L., Kolevzon, A., Zweifach, J., Lim, T., Dobry, Y., Schwartz, L., Frank, Y., Wang, A. T., Cai, G., Parkhomenko, E., Halpern, D., Grodberg, D., Angarita, B., Willner, J. P., Yang, A., Canitano, R., Chaplin, W., Betancur, C. and Buxbaum, J. D. (2013) 'Prospective investigation of autism and

genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency', *Mol Autism,* 4(1), doi: 10.1186/2040-2392-4-18.

Splawski, I., Yoo, D. S., Stotz, S. C., Cherry, A., Clapham, D. E. and Keating, M. T. (2006) 'CACNA1H mutations in autism spectrum disorders', *J Biol Chem,* 281(31), pp. 22085-22091.

Srikantha, P. and Mohajeri, M. H. (2019) 'The Possible Role of the Microbiota-Gut-Brain-Axis in Autism Spectrum Disorder', *International journal of molecular sciences,* 20(9), doi: 10.3390/ijms20092115.

Srinivasan, J., von Reuss, S. H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M. C., O'Doherty, O. G., Edison, A. S., Sternberg, P. W. and Schroeder, F. C. (2012) 'A modular library of small molecule signals regulates social behaviors in Caenorhabditis elegans', *PLoS Biol,* 10(1), pp. e1001237.

St Pourcain, B., Robinson, E. B., Anttila, V., Sullivan, B. B., Maller, J., Golding, J., Skuse, D., Ring, S., Evans, D. M., Zammit, S., Fisher, S. E., Neale, B. M., Anney, R. J. L., Ripke, S., Hollegaard, M. V., Werge, T., i, P.-S. S. I. B. A. G., Ronald, A., Grove, J., Hougaard, D. M., Børglum, A. D., Mortensen, P. B., Daly, M. J. and Davey Smith, G. (2018) 'ASD and schizophrenia show distinct developmental profiles in common genetic overlap with population-based social communication difficulties', *Molecular psychiatry,* 23(2), pp. 263-270.

Stahlberg, O., Soderstrom, H., Rastam, M. and Gillberg, C. (2004) 'Bipolar disorder, schizophrenia, and other psychotic disorders in adults with childhood onset AD/HD and/or autism spectrum disorders', *J Neural Transm (Vienna),* 111(7), pp. 891-902.

Steffenburg, S., Gillberg, C., Hellgren, L., Andersson, L., Gillberg, I. C., Jakobsson, G. and Bohman, M. (1989) 'A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden', *J Child Psychol Psychiatry,* 30(3), pp. 405-16.

Steger, K. A., Shtonda, B. B., Thacker, C., Snutch, T. P. and Avery, L. (2005) 'The C. elegans T-type calcium channel CCA-1 boosts neuromuscular transmission', *Journal of Experimental Biology,* 208(11), pp. 2191-2203.

Sternberg, S. H., Redding, S., Jinek, M., Greene, E. C. and Doudna, J. A. (2014) 'DNA interrogation by the CRISPR RNA-guided endonuclease Cas9', *Nature,* 507(7490), pp. 62-67.

Stevenson, R. A., Siemann, J. K., Schneider, B. C., Eberly, H. E., Woynaroski, T. G., Camarata, S. M. and Wallace, M. T. (2014a) 'Multisensory Temporal Integration in Autism Spectrum Disorders', *Journal of Neuroscience,* 34(3), pp. 691-697.

Stevenson, R. A., Siemann, J. K., Woynaroski, T. G., Schneider, B. C., Eberly, H. E., Camarata, S. M. and Wallace, M. T. (2014b) 'Evidence for diminished multisensory integration in autism spectrum disorders', *Journal of autism and developmental disorders,* 44(12), pp. 3161-3167.

Sudhof, T. C. (2008) 'Neuroligins and neurexins link synaptic function to cognitive disease', *Nature,* 455(7215), pp. 903-911.

Sugathan, A., Biagioli, M., Golzio, C., Erdin, S., Blumenthal, I., Manavalan, P., Ragavendran, A., Brand, H., Lucente, D., Miles, J., Sheridan, S. D., Stortchevoi, A., Kellis, M., Haggarty, S. J., Katsanis, N., Gusella, J. F. and Talkowski, M. E. (2014) 'CHD8 regulates neurodevelopmental pathways associated with autism spectrum disorder in neural progenitors', *Proc Natl Acad Sci U S A,* 111(42), pp. E4468-77.

Sugi, T. (2016) 'Genome Editing in C. elegans and Other Nematode Species', *International Journal of Molecular Sciences,* 17(3), doi:10.3390/ijms17030295.

Sultana, R., Ogundele, O. M. and Lee, C. C. (2019) 'Contrasting characteristic behaviours among common laboratory mouse strains', *R Soc Open Sci,* 6(6), pp. 190574.

Summers, P. J., Layne, R. M., Ortega, A. C., Harris, G. P., Bamber, B. A. and Komuniecki, R. W. (2015) 'Multiple Sensory Inputs Are Extensively Integrated to Modulate Nociception in C. elegans', *Journal of Neuroscience,* 35(28), pp. 10331-10342.

Sun, W. J., Poschmann, J., del Rosario, R. C. H., Parikshak, N. N., Hajan, H. S., Kumar, V., Ramasamy, R., Belgard, T. G., Elanggovan, B., Wong, C. C. Y., Mill, J., Geschwind, D. H. and Prabhakar, S. (2016) 'Histone Acetylome-wide Association Study of Autism Spectrum Disorder', *Cell,* 167(5), pp. 1385-1397.

Suo, S., Culotti, J. G. and Van Tol, H. H. (2009) 'Dopamine counteracts octopamine signalling in a neural circuit mediating food response in C. elegans', *Embo j,* 28(16), pp. 2437-2448.

Szatmari, P. and Paterson, A. D. and Zwaigenbaum, L. and Roberts, W. and Brian, J. and Liu, X. Q. and Vincent, J. B. and Skaug, J. L. and Thompson, A. P. and Senman, L. and Feuk, L. and Qian, C. and Bryson, S. E. and Jones, M. B. and Marshall, C. R. and Scherer, S. W. and Vieland, V. J. and Bartlett, C. and Mangin, L. V. and Goedken, R. and Segre, A. and Pericak-Vance, M. A. and Cuccaro, M. L. and Gilbert, J. R. and Wright, H. H. and Abramson, R. K. and Betancur, C. and Bourgeron, T. and Gillberg, C. and Leboyer, M. and Buxbaum, J. D. and Davis, K. L. and Hollander, E. and Silverman, J. M. and Hallmayer, J. and Lotspeich, L. and Sutcliffe, J. S. and Haines, J. L. and Folstein, S. E. and Piven, J. and Wassink, T. H. and Sheffield, V. and Geschwind, D. H. and Bucan, M. and Brown, W. T. and Cantor, R. M. and Constantino, J. N. and Gilliam, T. C. and Herbert, M. and Lajonchere, C. and Ledbetter, D. H. and Lese-Martin, C. and Miller, J. and Nelson, S. and Samango-Sprouse, C. A. and Spence, S. and State, M. and Tanzi, R. E. and Coon, H. and Dawson, G. and Devlin, B. and Estes, A. and Flodman, P. and Klei, L. and McMahon, W. M. and Minshew, N. and Munson, J. and Korvatska, E. and Rodier, P. M. and Schellenberg, G. D. and Smith, M. and Spence, M. A. and Stodgell, C. and Tepper, P. G. and Wijsman, E. M. and Yu, C. E. and Rogé, B. and Mantoulan, C. and Wittemeyer, K. and Poustka, A. and Felder, B. and Klauck, S. M. and Schuster, C. and Poustka, F. and Bölte, S. and Feineis-Matthews, S. and Herbrecht, E. and Schmötzer, G. and Tsiantis, J. and Papanikolaou, K. and Maestrini, E. and Bacchelli, E. and Blasi, F. and Carone, S. and Toma, C. and Van Engeland, H. and de Jonge, M. and Kemner, C. and Koop, F. and Langemeijer, M. and Hijmans, C. and Staal, W. G. and Baird, G. and Bolton, P. F. and Rutter, M. L. and Weisblatt, E. and Green, J. and Aldred, C. and Wilkinson, J. A. and Pickles, A. and Le Couteur, A. and Berney, T. and McConachie, H. and Bailey, A. J. and Francis, K. and Honeyman, G. and Hutchinson, A. and Parr, J. R. and Wallace, S. and Monaco, A. P. and Barnby, G. and Kobayashi, K. and Lamb, J. A. and Sousa, I. and Sykes, N. and Cook, E. H. and Guter, S. J. and Leventhal, B. L. and Salt, J. and Lord, C. and Corsello, C. and Hus, V. and Weeks, D. E. and Volkmar, F. and Tauber, M. and Fombonne, E. and Shih, A. and Meyer, K. J. (2007) 'Mapping autism risk loci using genetic linkage and chromosomal rearrangements', *Nat Genet,* 39(3), pp. 319-28.

Szego, M. J. and Zawati, M. n. H. (2016) 'Whole Genome Sequencing as a Genetic Test for Autism Spectrum Disorder: From Bench to Bedside and then Back Again', *Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie canadienne de psychiatrie de l'enfant et de l'adolescent,* 25(2), pp. 116-121.

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J. and von Mering, C. (2020) 'The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets', *Nucleic Acids Research,* 49(D1), pp. 605-612.

Sztainberg, Y. and Zoghbi, H. Y. (2016) 'Lessons learned from studying syndromic autism spectrum disorders', *Nat Neurosci,* 19(11), pp. 1408-1417.

Tabuchi, K., Blundell, J., Etherton, M. R., Hammer, R. E., Liu, X. R., Powell, C. M. and Sudhof, T. C. (2007) 'Neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice', *Science,* 318(5847), pp. 71-76.

Tamiji, J. and Crawford, D. A. (2010) 'The neurobiology of lipid metabolism in autism spectrum disorders', *Neurosignals,* 18(2), pp. 98-112.

Tang, L. (2020) 'PAM-less is more', *Nature Methods,* 17(6), https://doi.org/10.1038/s41592-020-0861-5.

Tang, W., Davidson, J. D., Zhang, G., Conen, K. E., Fang, J., Serluca, F., Li, J., Xiong, X., Coble, M., Tsai, T., Molind, G., Fawcett, C. H., Sanchez, E., Zhu, P., Couzin, I. D. and Fishman, M. C. (2020) 'Genetic Control of Collective Behavior in Zebrafish', *iScience,* 23(3), pp. 100942.

Tau, G. Z. and Peterson, B. S. (2010) 'Normal Development of Brain Circuits', *Neuropsychopharmacology,* 35(1), pp. 147-168.

Taurines, R., Schwenck, C., Westerwald, E., Sachse, M., Siniatchkin, M. and Freitag, C. (2012) 'ADHD and autism: differential diagnosis or overlapping traits? A selective review', *ADHD Attention Deficit and Hyperactivity Disorders,* 4(3), pp. 115-139.

Tessier, C. R. and Broadie, K. (2012) 'Molecular and genetic analysis of the Drosophila model of fragile X syndrome', *Results and problems in cell differentiation,* 54, pp. 119-156.

Thapar, A. (2018) 'Discoveries on the Genetics of ADHD in the 21st Century: New Findings and Their Implications', *Am J Psychiatry,* 175(10), pp. 943-950.

Thapliyal, S. and Babu, K. (2018) 'C. elegans Locomotion: Finding Balance in Imbalance', *Adv Exp Med Biol,* 1112, pp. 185-196.

Thill, S. and Pearce, T. C. (2007) 'Understanding complex behaviors by analyzing optimized models: C. elegans gradient navigation', *HFSP journal,* 1(4), pp. 263-273.

Thomas, R. H., Foley, K. A., Mepham, J. R., Tichenoff, L. J., Possmayer, F. and MacFabe, D. F. (2010) 'Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders', *J Neurochem,* 113(2), pp. 515-29.

Tian, Y., Zhang, Z. C. and Han, J. (2017) 'Drosophila Studies on Autism Spectrum Disorders', *Neuroscience bulletin,* 33(6), pp. 737-746.

Tong, X.-J., Hu, Z., Liu, Y., Anderson, D. and Kaplan, J. M. (2015) 'A network of autism linked genes stabilizes two pools of synaptic GABA(A) receptors', *eLife,* 4, pp. e09648-e09648.

Tong, X. J., López-Soto, E. J., Li, L., Liu, H., Nedelcu, D., Lipscombe, D., Hu, Z. and Kaplan, J. M. (2017) 'Retrograde Synaptic Inhibition Is Mediated by α-Neurexin Binding to the α2δ Subunits of N-Type Calcium Channels', *Neuron,* 95(2), pp. 326-340.

Torrico, B., Fernàndez-Castillo, N., Hervás, A., Milà, M., Salgado, M., Rueda, I., Buitelaar, J. K., Rommelse, N., Oerlemans, A. M., Bralten, J., Freitag, C. M., Reif, A., Battaglia, A., Mazzone, L., Maestrini, E., Cormand, B. and Toma, C. (2015) 'Contribution of common and rare variants of the PTCHD1 gene to autism spectrum disorders and intellectual disability', *Eur J Hum Genet,* 23(12), pp. 1694-701.

Tran Mau-Them, F., Guibaud, L., Duplomb, L., Keren, B., Lindstrom, K., Marey, I., Mochel, F., van den Boogaard, M. J., Oegema, R., Nava, C., Masurel, A., Jouan, T., Jansen, F. E., Au, M., Chen, A. H., Cho, M., Duffourd, Y., Lozier, E., Konovalov, F., Sharkov, A., Korostelev, S., Urteaga, B., Dickson, P.,

Vera, M., Martínez-Agosto, J. A., Begemann, A., Zweier, M., Schmitt-Mechelke, T., Rauch, A., Philippe, C., van Gassen, K., Nelson, S., Graham, J. M., Jr., Friedman, J., Faivre, L., Lin, H. J., Thauvin-Robinet, C. and Vitobello, A. (2019) 'De novo truncating variants in the intronless IRF2BPL are responsible for developmental epileptic encephalopathy', *Genet Med,* 21(4), pp. 1008-1014.

Trevisan, D. A., Foss-Feig, J. H., Naples, A. J., Srihari, V., Anticevic, A. and McPartland, J. C. (2020) 'Autism Spectrum Disorder and Schizophrenia Are Better Differentiated by Positive Symptoms Than Negative Symptoms', *Frontiers in Psychiatry,* 11(548), doi:10.3389/fpsyt.2020.00548.

Trojanowski, Nicholas F. and Raizen, David M. (2015) 'Neural Circuits: From Structure to Function and Back', *Current Biology,* 25(16), pp. 711-713.

Tsalik, E. L. and Hobert, O. (2003) 'Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans', *J Neurobiol,* 56(2), pp. 178-97.

Tu, H. J., Pinan-Lucarre, B., Ji, T. T., Jospin, M. and Bessereau, J. L. (2015) 'C. elegans Punctin Clusters GABA(A) Receptors via Neuroligin Binding and UNC-40/DCC Recruitment', *Neuron,* 86(6), pp. 1407-1419.

Tye, C., Runicles, A. K., Whitehouse, A. J. O. and Alvares, G. A. (2019) 'Characterizing the Interplay Between Autism Spectrum Disorder and Comorbid Medical Conditions: An Integrative Review', *Frontiers in psychiatry,* 9, doi:10.3389/fpsyt.2018.00751.

Ueoka, I., Pham, H. T. N., Matsumoto, K. and Yamaguchi, M. (2019) 'Autism Spectrum Disorder-Related Syndromes: Modeling with Drosophila and Rodents', *International Journal of Molecular Sciences,* 20(17), doi: 10.3390/ijms20174071.

Ure, K., Lu, H., Wang, W., Ito-Ishida, A., Wu, Z., He, L.-J., Sztainberg, Y., Chen, W., Tang, J. and Zoghbi, H. Y. (2016) 'Restoration of Mecp2 expression in GABAergic neurons is sufficient to rescue multiple disease features in a mouse model of Rett syndrome', *eLife,* 5, pp. e14198.

van der Staay, F. J. (2006) 'Animal models of behavioral dysfunctions: Basic concepts and classifications, and an evaluation strategy', *Brain Research Reviews,* 52(1), pp. 131-159.

Vandamme, J., Lettier, G., Sidoli, S., Di Schiavi, E., Nørregaard Jensen, O. and Salcini, A. E. (2012) 'The C. elegans H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity', *PLoS Genet,* 8(5), pp. e1002647.

Vanderschuren, L. J., Niesink, R. J. and Van Ree, J. M. (1997) 'The neurobiology of social play behavior in rats', *Neurosci Biobehav Rev,* 21(3), pp. 309-26.

Vanderschuren, L. J. M. J., Achterberg, E. J. M. and Trezza, V. (2016) 'The neurobiology of social play and its rewarding value in rats', *Neuroscience and biobehavioral reviews,* 70, pp. 86-105.

Verma, V., Paul, A., Vishwanath, A. A., Vaidya, B. and Clement, J. P. (2019) 'Understanding intellectual disability and autism spectrum disorders from common mouse models: synapses to behaviour', *Open Biology,* 9(6), doi: 10.1098/rsob.180265.

Vicencio, J. and Cerón, J. (2021) 'A Living Organism in your CRISPR Toolbox: Caenorhabditis elegans Is a Rapid and Efficient Model for Developing CRISPR-Cas Technologies', *Crispr j,* 4(1), pp. 32-42.

Viñas-Jornet, M., Esteba-Castillo, S., Gabau, E., Ribas-Vidal, N., Baena, N., San, J., Ruiz, A., Coll, M. D., Novell, R. and Guitart, M. (2014) 'A common cognitive, psychiatric, and dysmorphic phenotype in carriers of NRXN1 deletion', *Molecular genetics & genomic medicine,* 2(6), pp. 512-521.

Vohra, M., Lemieux, G. A., Lin, L. and Ashrafi, K. (2017) 'The beneficial effects of dietary restriction on learning are distinct from its effects on longevity and mediated by depletion of a neuroinhibitory metabolite', *Plos Biology,* 15(8), pp. e2002032.

Voineskos, A. N., Lett, T. A., Lerch, J. P., Tiwari, A. K., Ameis, S. H., Rajji, T. K., Müller, D. J., Mulsant, B. H. and Kennedy, J. L. (2011) 'Neurexin-1 and frontal lobe white matter: an overlapping intermediate phenotype for schizophrenia and autism spectrum disorders', *PLoS One,* 6(6), pp. e20982.

Volpatti, J. R., Al-Maawali, A., Smith, L., Al-Hashim, A., Brill, J. A. and Dowling, J. J. (2019) 'The expanding spectrum of neurological disorders of phosphoinositide metabolism', *Dis Model Mech,* 12(8). doi: 10.1242/dmm.038174.

Wakabayashi, T., Kitagawa, I. and Shingai, R. (2004) 'Neurons regulating the duration of forward locomotion in Caenorhabditis elegans', *Neuroscience Research,* 50(1), pp. 103-111.

Wang, H., Liang, S., Wang, M., Gao, J., Sun, C., Wang, J., Xia, W., Wu, S., Sumner, S. J., Zhang, F., Sun, C. and Wu, L. (2016a) 'Potential serum biomarkers from a metabolomics study of autism', *J Psychiatry Neurosci,* 41(1), pp. 27-37.

Wang, H., Park, H., Liu, J. and Sternberg, P. W. (2018a) 'An Efficient Genome Editing Strategy To Generate Putative Null Mutants in Caenorhabditis elegans Using CRISPR/Cas9', *G3 (Bethesda, Md.),* 8(11), pp. 3607-3616.

Wang, L., Pang, K., Han, K., Adamski, C. J., Wang, W., He, L., Lai, J. K., Bondar, V. V., Duman, J. G., Richman, R., Tolias, K. F., Barth, P., Palzkill, T., Liu, Z., Holder, J. L., Jr. and Zoghbi, H. Y. (2020) 'An autism-linked missense mutation in SHANK3 reveals the modularity of Shank3 function', *Mol Psychiatry,* 25(10), pp. 2534-2555.

Wang, T., Wei, J. J., Sabatini, D. M. and Lander, E. S. (2014) 'Genetic screens in human cells using the CRISPR-Cas9 system', *Science (New York, N.Y.),* 343(6166), pp. 80-84.

Wang, W. K., Chaturbedi, A., Wang, M. H., An, S., Velayudhan, S. S. and Lee, S. S. (2018b) 'SET-9 and SET-26 are H3K4me3 readers and play critical roles in germline development and longevity', *Elife,* 7, pp. e34970.

Wang, X., Bey, A. L., Katz, B. M., Badea, A., Kim, N., David, L. K., Duffney, L. J., Kumar, S., Mague, S. D., Hulbert, S. W., Dutta, N., Hayrapetyan, V., Yu, C., Gaidis, E., Zhao, S., Ding, J.-D., Xu, Q., Chung, L., Rodriguiz, R. M., Wang, F., Weinberg, R. J., Wetsel, W. C., Dzirasa, K., Yin, H. and Jiang, Y.-H. (2016b) 'Altered mGluR5-Homer scaffolds and corticostriatal connectivity in a Shank3 complete knockout model of autism', *Nature communications,* 7, pp. 11459-11459.

Wasilewska, J. and Klukowski, M. (2015) 'Gastrointestinal symptoms and autism spectrum disorder: links and risks - a possible new overlap syndrome', *Pediatric health, medicine and therapeutics,* 6, pp. 153-166.

Waye, M. M. Y. and Cheng, H. Y. (2018) 'Genetics and epigenetics of autism: A Review', *Psychiatry Clin Neurosci,* 72(4), pp. 228-244.

Wei, H., Wang, M., Lv, N., Yang, H., Zhao, M., Huang, B. and Li, R. (2020) 'Increased repetitive self-grooming occurs in Pax2 mutant mice generated using CRISPR/Cas9', *Behav Brain Res,* 393, pp. 112803.

Weiner, D. J., Wigdor, E. M., Ripke, S., Walters, R. K., Kosmicki, J. A., Grove, J., Samocha, K. E., Goldstein, J. I., Okbay, A., Bybjerg-Grauholm, J., Werge, T., Hougaard, D. M., Taylor, J., i, P.-B. A. G., Psychiatric Genomics Consortium Autism, G., Skuse, D., Devlin, B., Anney, R., Sanders, S. J., Bishop, S., Mortensen, P. B., Børglum, A. D., Smith, G. D., Daly, M. J. and Robinson, E. B. (2017)

'Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders', *Nature genetics,* 49(7), pp. 978-985.

Weisheit, I., Kroeger, J. A., Malik, R., Klimmt, J., Crusius, D., Dannert, A., Dichgans, M. and Paquet, D. (2020) 'Detection of Deleterious On-Target Effects after HDR-Mediated CRISPR Editing', *Cell Reports,* 31(8), pp. 107689.

Wheelan, S. J., Boguski, M. S., Duret, L. and Makałowski, W. (1999) 'Human and nematode orthologs--lessons from the analysis of 1800 human genes and the proteome of Caenorhabditis elegans', *Gene,* 238(1), pp. 163-70.

White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1986) 'THE STRUCTURE OF THE NERVOUS-SYSTEM OF THE NEMATODE CAENORHABDITIS-ELEGANS', *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 314(1165), pp. 1-340.

Wilkinson, B., Grepo, N., Thompson, B. L., Kim, J., Wang, K., Evgrafov, O. V., Lu, W., Knowles, J. A. and Campbell, D. B. (2015) 'The autism-associated gene chromodomain helicase DNA-binding protein 8 (CHD8) regulates noncoding RNAs and autism-related genes', *Transl Psychiatry,* 5(5), pp. e568.

Williams, J. R., Catania, K. C. and Carter, C. S. (1992) 'Development of partner preferences in female prairie voles (Microtus ochrogaster): the role of social and sexual experience', *Horm Behav,* 26(3), pp. 339-49.

Williams, J. R., Insel, T. R., Harbaugh, C. R. and Carter, C. S. (1994) 'Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster)', *J Neuroendocrinol,* 6(3), pp. 247-50.

Wolf, T., Perez, A. and Harris, G. (2020) 'Glutamatergic transmission regulates locomotory behavior on a food patch in C. elegans', *microPublication biology,* 2020, pp. 10.17912/micropub.biology.000332.

Wolkow, C.A. and Hall, D.H. 2015. Introduction to the Dauer Larva, Overview. In *WormAtlas*.

doi:10.3908/wormatlas.3.10.

Wong, C. and Crawford, D. A. (2014) 'Lipid Signalling in the Pathology of Autism Spectrum Disorders', in Patel, V.B., Preedy, V.R. and Martin, C.R. (eds.) *Comprehensive Guide to Autism*. New York, NY: Springer New York, pp. 1259-1283.

Wong, J. C., Grieco, S. F., Dutt, K., Chen, L., Thelin, J. T., Inglis, G. A. S., Parvin, S., Garraway, S. M., Xu, X., Goldin, A. L. and Escayg, A. (2021a) 'Autistic-like behavior, spontaneous seizures, and increased neuronal excitability in a Scn8a mouse model', *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-021-00985-9.

Wong, S. S., Yu, J., Schroeder, F. C. and Kim, D. H. (2020) 'Population Density Modulates the Duration of Reproduction of C. elegans', *Current Biology,* 30(13), pp. 2602-2607.e2.

Wong, W.-R., Maher, S., Oh, J. Y., Brugman, K. I., Gharib, S. and Sternberg, P. W. (2021b) 'Conserved missense variant in ALDH1A3 ortholog impairs fecundity in C. elegans', *microPublication biology,* 2021, pp. 10.17912/micropub.biology.000357.

Wong, W. R., Brugman, K. I., Maher, S., Oh, J. Y., Howe, K., Kato, M. and Sternberg, P. W. (2019) 'Autism-associated missense genetic variants impact locomotion and neurodevelopment in Caenorhabditis elegans', *Human Molecular Genetics,* 28(13), pp. 2271-2281.

Wood, J. N. and Grafman, J. (2003) 'Human prefrontal cortex: processing and representational perspectives', *Nature Reviews Neuroscience,* 4(2), pp. 139-147.

Xu, H., Xiao, T., Chen, C. H., Li, W., Meyer, C. A., Wu, Q., Wu, D., Cong, L., Zhang, F., Liu, J. S., Brown, M. and Liu, X. S. (2015) 'Sequence determinants of improved CRISPR sgRNA design', *Genome Res,* 25(8), pp. 1147-57.

Yan, D. and Jin, Y. (2011) 'Chapter 15 - Molecular and Genetic Approaches for the Analysis of C. elegans Neuronal Development', in Rothman, J.H. and Singson, A. (eds.) *Methods in Cell Biology*: Academic Press, pp. 413-443.

Yap, I. K., Angley, M., Veselkov, K. A., Holmes, E., Lindon, J. C. and Nicholson, J. K. (2010) 'Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls', *J Proteome Res,* 9(6), pp. 2996-3004.

Yemini, E., Jucikas, T., Grundy, L. J., Brown, A. E. X. and Schafer, W. R. (2013) 'A database of Caenorhabditis elegans behavioral phenotypes', *Nature Methods,* 10(9), pp. 877-879.

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., Sohal, V. S., Goshen, I., Finkelstein, J., Paz, J. T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J. R., Hegemann, P. and Deisseroth, K. (2011) 'Neocortical excitation/inhibition balance in information processing and social dysfunction', *Nature,* 477(7363), pp. 171-178.

Yochem, J. and Herman, R. K. (2005) *Genetic mosaics.* In: WormBook: The Online Review of C. elegans Biology.

Yoo, H. (2015) 'Genetics of Autism Spectrum Disorder: Current Status and Possible Clinical Applications', *Experimental Neurobiology,* 24(4), pp. 257-272.

Yoo, J., Bakes, J., Bradley, C., Collingridge, G. L. and Kaang, B. K. (2014) 'Shank mutant mice as an animal model of autism', *Philosophical Transactions of the Royal Society B-Biological Sciences,* 369(1633), doi:10.1098/rstb.2013.0143.

Yost, R. T., Robinson, J. W., Baxter, C. M., Scott, A. M., Brown, L. P., Aletta, M. S., Hakimjavadi, R., Lone, A., Cumming, R. C., Dukas, R., Mozer, B. and Simon, A. F. (2020) 'Abnormal Social Interactions in a Drosophila Mutant of an Autism Candidate Gene: Neuroligin 3', *Int J Mol Sci,* 21(13), doi: 10.3390/ijms21134601.

Young, L. J. and Wang, Z. (2004) 'The neurobiology of pair bonding', *Nature Neuroscience,* 7(10), pp. 1048-1054.

Yu, B., Yuan, B., Dai, J. K., Cheng, T. L., Xia, S. N., He, L. J., Yuan, Y. T., Zhang, Y. F., Xu, H. T., Xu, F. Q., Liang, Z. F. and Qiu, Z. L. (2020) 'Reversal of Social Recognition Deficit in Adult Mice with MECP2 Duplication via Normalization of MeCP2 in the Medial Prefrontal Cortex', *Neurosci Bull,* 36(6), pp. 570-584.

Yu, J., He, X., Yao, D., Li, Z., Li, H. and Zhao, Z. (2011) 'A sex-specific association of common variants of neuroligin genes (NLGN3 and NLGN4X) with autism spectrum disorders in a Chinese Han cohort', *Behavioral and brain functions : BBF,* 7, pp. 13-13.

Yuan, F. F., Gu, X., Huang, X., Zhong, Y. and Wu, J. (2017) 'SLC6A1 gene involvement in susceptibility to attention-deficit/hyperactivity disorder: A case-control study and gene-environment interaction', *Prog Neuropsychopharmacol Biol Psychiatry,* 77, pp. 202-208.

Zablotsky, B., Black, L. I. and Blumberg, S. J. (2017) 'Estimated Prevalence of Children With Diagnosed Developmental Disabilities in the United States, 2014-2016', *NCHS Data Brief,* (291), pp. 1-8.

Zaboski, B. A. and Storch, E. A. (2018) 'Comorbid autism spectrum disorder and anxiety disorders: a brief review', *Future neurology,* 13(1), pp. 31-37.

Zarrei, M., Burton, C. L., Engchuan, W., Young, E. J., Higginbotham, E. J., MacDonald, J. R., Trost, B., Chan, A. J. S., Walker, S., Lamoureux, S., Heung, T., Mojarad, B. A., Kellam, B., Paton, T., Faheem, M., Miron, K., Lu, C., Wang, T., Samler, K., Wang, X., Costain, G., Hoang, N., Pellecchia, G., Wei, J., Patel, R. V., Thiruvahindrapuram, B., Roifman, M., Merico, D., Goodale, T., Drmic, I., Speevak, M., Howe, J. L., Yuen, R. K. C., Buchanan, J. A., Vorstman, J. A. S., Marshall, C. R., Wintle, R. F., Rosenberg, D. R., Hanna, G. L., Woodbury-Smith, M., Cytrynbaum, C., Zwaigenbaum, L., Elsabbagh, M., Flanagan, J., Fernandez, B. A., Carter, M. T., Szatmari, P., Roberts, W., Lerch, J., Liu, X., Nicolson, R., Georgiades, S., Weksberg, R., Arnold, P. D., Bassett, A. S., Crosbie, J., Schachar, R., Stavropoulos, D. J., Anagnostou, E. and Scherer, S. W. (2019) 'A large data resource of genomic copy number variation across neurodevelopmental disorders', *NPJ genomic medicine,* 4(26), https://doi.org/10.1038/s41525-019-0098-3.

Zhang, F.-F., Peng, W., Sweeney, J. A., Jia, Z.-Y. and Gong, Q.-Y. (2018) 'Brain structure alterations in depression: Psychoradiological evidence', *CNS neuroscience & therapeutics,* 24(11), pp. 994-1003.

Zhang, L., Ward, J. D., Cheng, Z. and Dernburg, A. F. (2015) 'The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in C. elegans', *Development,* 142(24), pp. 4374-84.

Zhang, M., Chung, S. H., Fang-Yen, C., Craig, C., Kerr, R. A., Suzuki, H., Samuel, A. D., Mazur, E. and Schafer, W. R. (2008) 'A self-regulating feed-forward circuit controlling C. elegans egg-laying behavior', *Curr Biol,* 18(19), pp. 1445-55.

Zhang, M., Schafer, W. R. and Breitling, R. (2010) 'A circuit model of the temporal pattern generator of Caenorhabditis egg-laying behavior', *BMC Syst Biol,* 4(81), doi: 10.1186/1752-0509-4-81.

Zhang, Y., Lu, H. and Bargmann, C. I. (2005) 'Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans', *Nature,* 438(7065), pp. 179-184.

Zhao, P., Zhang, Z., Ke, H. M., Yue, Y. R. and Xue, D. (2014) 'Oligonucleotide-based targeted gene editing in C. elegans via the CRISPR/Cas9 system', *Cell Research,* 24(2), pp. 247-250.

Zhen, M. and Samuel, A. D. (2015) 'C. elegans locomotion: small circuits, complex functions', *Curr Opin Neurobiol,* 33, pp. 117-26.

Zheng, Z., Zhu, T., Qu, Y. and Mu, D. (2016) 'Blood Glutamate Levels in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis', *PLoS One,* 11(7), pp. e0158688.

Zhou, K. M., Cherra, S. J., Goncharov, A. and Jin, Y. S. (2017) 'Asynchronous Cholinergic Drive Correlates with Excitation-Inhibition Imbalance via a Neuronal Ca2+ Sensor Protein', *Cell Reports,* 19(6), pp. 1117-1129.

Zhu, Z., Lu, Q., Wang, J. and Huang, S. (2015) 'Collective effects of common SNPs in foraging decisions in Caenorhabditis elegans and an integrative method of identification of candidate genes', *Sci Rep,* 5, pp. 16904.

Zoghbi, H. Y. and Bear, M. F. (2012) 'Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities', *Cold Spring Harbor perspectives in biology,* 4(3), pp. a009886.

Zuryn, S., Ahier, A., Portoso, M., White, E. R., Morin, M. C., Margueron, R. and Jarriault, S. (2014) 'Sequential histone-modifying activities determine the robustness of transdifferentiation', *Science,* 345(6198), pp. 826-829.