

Mutation spectrum of *PRPF31*, genotype-phenotype correlation in retinitis pigmentosa, and opportunities for therapy

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Abstract

Pathogenic variants in pre-messenger RNA (pre-mRNA) splicing factor 31, *PRPF31*, are the second most common genetic cause of autosomal dominant retinitis pigmentosa (adRP) in most populations. This remains a completely untreatable and incurable form of blindness, and it can be difficult to predict the clinical course of disease. In order to design appropriate targeted therapies, a thorough understanding of the genetics and molecular mechanism of this disease is required. Here, we present the structure of the *PRPF31* gene and PRPF31 protein, current understanding of PRPF31 protein function and the full spectrum of all reported clinically relevant variants in *PRPF31*. We delineate the correlation between specific *PRPF31* genotype and RP phenotype, suggesting that, except in cases of complete gene deletion or large-scale deletions, dominant negative effects contribute to phenotype as well as haploinsufficiency. This has important impacts on design of targeted therapies, particularly the feasibility of gene augmentation as a broad approach for treatment of *PRPF31*-associated RP. We discuss other opportunities for therapy, including antisense oligonucleotide therapy and gene-independent approaches and offer future perspectives on treatment of this form of RP.

Introduction

Pre-mRNA splicing

Human pre-mRNA splicing factor 31 (PRPF31) is a component of the spliceosome, the huge macromolecular ribonucleoprotein (RNP) complex which catalyses the splicing of pre-messenger RNAs (pre-mRNAs) to remove introns and produce mature mRNAs.(Will and Luhrmann 2011)

Pre-mRNA splicing is a core function in all eukaryotic cells. The vast majority of genes have multiple exons and introns, and around 95% of these multiexon genes undergo alternative splicing.(Pan et al. 2008) Alternative splicing allows increased organism complexity without increasing genome size, and helps to explain the c-value paradox; the observation that phenotypic complexity in the eukaryotic domain is not proportional to genome size.

The spliceosome is composed of 5 small nuclear RNAs (snRNAs), U1-U5, and many proteins, together making 5 snRNPs. In the process of splicing, U1snRNP recognises and binds the splice donor site (the 5' splice site), and promotes the binding of U2snRNP to the branch site. Independently of this, the U4/U6.U5 tri-snRNP forms in the cell, and is recruited to the pre-mRNA, where U6snRNP replaces U1snRNP. This forms the catalytically active spliceosome, which cuts away the intron and joins the exons through two transesterification reactions (**Figure 1**).

PRPF31, splicing and retinal disease

The *S.cerevisiae* yeast homologue of PRPF31, Prp31, was cloned and identified as a key splicing factor in 1996(Weidenhammer et al. 1996), and later was shown to be essential for the association of the U4/U6.U5 tri-snRNP with pre-spliceosomes.(Weidenhammer, Ruiz-Noriega and Woolford 1997) It was subsequently found to play a role in both splicing and meiosis in *S.pombe*.(Bishop et al. 2000) Unexpectedly, in 2001, it was discovered that heterozygous pathogenic variants in *PRPF31* are associated with retinitis pigmentosa (RP), an inherited retinal dystrophy affecting 1:2000 to 1:3500 people worldwide.(Vithana et al. 2001) This was surprising because pre-mRNA splicing factors are highly conserved from yeast to man with a core function in all cells. Intuitively, it would be expected that a defect in a core spliceosomal protein should have an impact on all cells, not just retinal cells.

The original paper described seven different pathogenic variants in four families and three simplex cases. These included mutations in the region of the splice site, leading to inactivation of a splice acceptor site, inactivation of a splice donor site, two missense changes, three frameshift variants and an in-frame duplication.(Vithana et al. 2001)

Since then, and particularly since the advent of massively parallel sequencing technologies, it has become clear that pathogenic variants in *PRPF31* are a major cause of autosomal dominant RP (adRP). Indeed they are the second most common genetic cause of adRP in most populations, accounting for 6% of US cases,(Sullivan et al. 2013b) 8% of Spanish, French and French-Canadian cases,(Martin-Merida et al. 2018, Audo et al. 2010, Coussa et al. 2015) 8.9% of cases in North America,(Daiger et al. 2014) 10-11.1% of Chinese cases(Lim et al. 2009, Xu et al. 2012) and 10.5% of Belgian cases.(Van Cauwenbergh et al. 2017)

However, this is likely to be an underestimate due to non-penetrance of this form of RP (Rose and Bhattacharya 2016). It is common to see very variable severity of eye disease in different members of the same family with the same pathogenic *PRPF31* variant. Furthermore, obligate carriers may be totally asymptomatic, showing complete non-penetrance. This complicates attempts to co-segregate *PRPF31* variants with clinical disease and makes genetic diagnosis difficult, likely contributing to an underestimation of the prevalence of RP associated with *PRPF31* variants.

The genetic mechanism controlling incomplete penetrance remains unclear, but a fairly consistent observation of correlation between expression level of the non-mutant copy of *PRPF31* and disease severity has been reported.(Rio Frio et al. 2008b, Rio Frio et al. 2009, Rivolta et al. 2006).

This varied expression can be explained by a number of factors including:

- expression quantitative trait loci (eQTLs) (on ch.14q21-23) in trans with *PRPF31*(Rio Frio et al. 2008a)
- variable level of expression of *CNOT3*, a trans-acting epistatic factor which is genetically linked to *PRPF31* and regulates expression of *PRPF31*. *CNOT3* encodes a subunit of the Ccr4-not transcription complex, which binds to the promoter of *PRPF31* and represses transcription of *PRPF31*. An intronic variant in *CNOT3* determines its level of expression and thus how efficiently *PRPF31* expression is downregulated. The alleles of *CNOT3* inherited determine the expression of non-mutant *PRPF31* and thus whether a person will be affected by the disease.(Venturini et al. 2012, Rose et al. 2014)
- the number of minisatellite repeat elements (MSR1) adjacent to the *PRPF31* core promoter, which determines the level of transcriptional repression of the non-mutant *PRPF31*. 4 MSR1 copies are associated with higher non-mutant *PRPF31* expression and are found in non-symptomatic carriers only.(Rose et al. 2016)

On the basis of these observations, the mechanism of incomplete penetrance in this form of RP has been described as 'variant haploinsufficiency', in which the absence of a second wild-type *PRPF31* allele is sometimes sufficient to produce disease, and sometimes is not, depending on the nature of the mutant allele inherited *and* the nature of the wild-type allele inherited. So the severity of the resultant disease depends on both the type of mutant allele inherited (ie complete loss-of-function, gain-of-function or hypomorphic), the level at which this allele is expressed, and the level at which the wild-type allele is expressed (Rose and Bhattacharya 2016). This form of variant haploinsufficiency has only been described in a very few Mendelian disorders, making the mechanism of variable penetrance in this disease quite unique (Rose and Bhattacharya 2016).

***PRPF31* gene and *PRPF31* protein structure**

PRPF31 is a 16.3kb gene on chromosome 19 which encodes 9 different transcripts, 6 of which are protein coding. The largest, most widely expressed transcript consists of 14 exons; 1 non-coding and 13 coding, which produces a 499 amino acid protein of 55kDa in size, pre-mRNA splicing factor 31, *PRPF31*.

PRPF31 contains several important functional domains; the flexible loop, Nop domain, coiled-coil domain and tip. Recent advances in spectroscopy and microscopy methods such as NMR and cryo-electron microscopy have allowed accurate resolution of the crystal structure of proteins of the spliceosome, including *PRPF31*, in their native conformations at different points during splicing.(Agafonov et al. 2016, Bertram et al. 2017b, Bertram et al. 2017a, Haselbach et al. 2018) These studies have revealed that *PRPF31* contains a conserved Nop domain (residues 222-254 and 278-307), with regions for binding protein and RNA.(Liu et al. 2007) This Nop domain has relaxed sequence conservation in *PRPF31*, but it retains high specificity for binding U4 or U4atac and 15.5K protein. (Liu et al. 2007) The flexible loop (residues 256 – 265) protects the exposed C4' atoms of residues 37 and 38 from attack by free radicals, to protect the RNA without directly contacting it.(Liu et al. 2007) The protein also has several phosphorylation sites, clustered in the C-terminus.(Liu et al. 2007) *PRPF31* contains a nuclear localisation sequence, NLS, which allows it to be targeted to the nucleus after translation (**Figure 2**).

***PRPF31* protein function**

PRPF31 is required for tri-snRNP assembly in human cells.(Makarova et al. 2002) With *PRPF6*, *PRPF31* forms an essential connection between the U4/U6 and U5 snRNPs. siRNA knockdown of *PRPF31*

results in inhibition of tri-snRNP formation and nuclear accumulation of U5 mono-snRNPs and U4/U6 di-snRNPs containing U4/U6 proteins and the U4/U6 recycling factor p110.(Schaffert et al. 2004)

The specific function of PRPF31 in retinal cells remains less clear. It remains unclear whether the photoreceptor cells are the primary affected cells in RP associated with PRPF31, with a number of studies suggesting that the RPE is the primary affected tissue.(Farkas et al. 2014, Hamieh and Nandrot 2019, Valdés-Sánchez et al. 2019) Retinal cells are highly metabolically active, with a high demand for ATP and protein anabolism as around 10% of protein from photoreceptor outer segments is shed every day. Rates of metabolism in photoreceptors are similar to dividing tumour cells, and undergo extensive anaerobic glycolysis rather than oxidative phosphorylation to produce energy, in what is termed the 'Warburg effect'.(Ng et al. 2015, Rajala et al. 2016) The reliance on glycolysis seems to promote efficient protein anabolism in photoreceptors.(Chinchore et al. 2017) However, the photoreceptors still require mitochondria to produce a proportion of their ATP via oxidative phosphorylation.(Grenell et al. 2019) It has been postulated that photoreceptor cells have a greater demand for pre-mRNA splicing factors to meet this metabolic demand, but evidence to support this hypothesis is inconsistent. Some studies have reported higher levels of PRPF31 expression in retina than in other tissues(Cao et al. 2011) but other studies show a consistent level of expression in all tissues, with no significantly higher expression in retina or any other tissue.(Yuan et al. 2005)

Related to this elevated rate of oxidative phosphorylation, retinal cells are subject to much higher rates of oxidative damage, including UV-induced photooxidative damage, which may explain the retinal-specific phenotype of RP associated with pre-mRNA splicing factor mutations.(Comitato et al. 2007, Shinde et al. 2016, Jin et al. 2011, Schmidt-Kastner et al. 2008). In patients expressing mutant forms of pre-mRNA splicing factors, it has been shown that proteins have reduced solubility, which can lead to formation of protein aggregates, and it has been suggested that the environment of UV-induced photooxidative damage in the photoreceptors makes these cells specifically prone to degeneration.(Wheway et al. 2019, Valdés-Sánchez et al. 2019, Wilkie et al. 2006, Yin et al. 2011, Bryant et al. 2018) This splicing-independent disease mechanism is appealing because there is inconsistent evidence of splicing defects in cells carrying PRPF31 mutations. Studies seem to suggest that expression of mutant *PRPF31* affects splicing of some transcripts but not others.

Immunoprecipitation of splicing complexes from *PRPF31* mutant retinal cells showed that mutant PRPF31 proteins significantly inhibited pre-mRNA splicing of intron 3 in the rhodopsin (*RHO*) gene.(Yuan et al. 2005) In primary retinal cell cultures, expression of the mutant PRPF31 proteins reduced total *RHO* expression and caused apoptosis of rhodopsin-positive retinal cells.(Alagramam et al. 2001) In a study of patient lymphoblastoid cell lines, splicing efficiency of *RPGR* intron 9 was significantly decreased in *PRPF31* mutant cell lines but no consistent decrease in the splicing efficiency of U12 and noncanonical U2 introns was seen in *PRPF31* mutant cells.(Ivings et al. 2008) In a minigene study, assays using the *RHO* intron 3 minigene template revealed a direct negative effect on splicing efficiency of mutant PRPF31. However, no effect of the mutation on splicing efficiency could be detected using the longer *GNAT1* minigene template or using a full-length *RHO* transcript, splicing of which had an efficiency of 100%. Similarly, no unspliced *RHO* transcripts could be detected in RNA from human retina.(Wilkie et al. 2008)

Using novel stem cell technologies, recent studies in retinal organoids and retinal pigment epithelium (RPE) derived from induced pluripotent stem cells (iPSCs) from patients with *PRPF31* mutations show decreased efficiency of splicing of E1A minigene.(Buskin et al. 2018) RPE from patient iPSCs also show a substantial downregulation of *SART1*, a U5 snRNP protein important for the formation of the pre-catalytic spliceosome, but no changes in the expression of the U5 protein PRPF8 or the U4/U6

protein PRPF4.(Buskin et al. 2018) In both RPE and retinal organoids derived from *PRPF31* patients, the most significantly mis-spliced genes were genes involved in pre-mRNA and alternative mRNA splicing via the spliceosome.(Buskin et al. 2018)

Alongside these findings, it was observed that retinal organoids from patients showed differential expression of actin cytoskeleton, ciliary membrane, primary cilium, photoreceptor inner and outer segment, axon terminal and phototransduction proteins. Furthermore, patient organoids showed an enrichment of mis-spliced centriole and microtubule organisation genes. This suggests that centriole and ciliogenesis and cilium function are all regulated by alternative splicing in the retina, and this is defective in patients carrying *PRPF31* mutations.(Buskin et al. 2018) These findings were confirmed in independent studies of splicing in *PRPF31* siRNA-treated human organotypic retinal cultures.(Azizzadeh Pormehr et al. 2019) This is in keeping with earlier studies from ourselves, and others, which showed that siRNA knockdown of pre-mRNA splicing factors including *PRPF31* has a specific and significant effect on ciliogenesis.(Wheway et al. 2015, Kim et al. 2016) Further investigation showed that these proteins localise to the base of the photoreceptor cilium, classifying these conditions as retinal ciliopathies.(Wheway et al. 2015) Recent work developing *PRPF31* gene augmentation therapy has shown rescue of ciliogenesis in *PRPF31*+/- RPE cells derived from human patient iPSCs after expression of wild-type *PRPF31* delivered by an AAV vector, further suggesting that *PRPF31* plays a key role in regulating ciliogenesis in patients.(Brydon et al. 2019)

Further work is needed to understand the nature of the splicing factors' involvement in ciliogenesis and cilium function in the retina, and this work is ongoing. It is possible that *PRPF31* and other splicing factors have roles beyond splicing. Many proteins involved in splicing have multiple functions in the cell, such as the proteins of the PRP19 complex which have roles in ubiquitination (Vander Kooi et al. 2006), in DNA damage sensing (Grey et al. 1996, Marechal et al. 2014), DNA damage repair (Zhang et al. 2005), mRNA export (Chanarat, Seizl and Strasser 2011) and in mitotic spindle assembly (Hofmann et al. 2013). *PRPF31* has been shown to perform splicing-independent functions in mitotic chromosome segregation, although this would not explain disease phenotype in the post-mitotic retina. With deeper understanding of the molecular mechanism of *PRPF31* disease arise greater opportunities for developing effective targeted therapies.

***PRPF31* mutation spectrum**

In order to fully understand the molecular mechanism of RP associated with *PRPF31* variants, it is necessary to fully understand the genetics of this condition. This will aid accurate diagnostics, prognostics and development of targeted therapies. To this end, we have reviewed the literature and the major clinical variant database ClinVar to summarise all reported pathogenic variants in *PRPF31* (Table 1). Mutations are spread throughout the gene, but are most common in exons 6-10, particularly exons 7 and 8 (Figure 3).

The majority of reported mutations in *PRPF31* are presumed loss-of-function variants including frameshift (51 different variants reported in 70 different families), splice site (30 variants in 52 families), nonsense (30 variants in 40 families) or large-scale insertions or deletions (25 variants in 32 families), which are predicted to lead to complete loss of expression of protein from the affected allele. *PRPF31* is highly intolerant to loss-of-function with a probability of being loss-of-function intolerant (pLI) score of 0.98 (Lek et al. 2016). A pLI score of >0.9 indicates that a gene is intolerant of protein-truncating variation (Lek et al. 2016) and thus loss-of-function variants in *PRPF31* are highly likely to cause disease through a haploinsufficiency disease mechanism (discussed in more detail later). However, it is important to note that whilst frameshift, consensus splice site, nonsense and

large indel variants are often assumed to cause loss-of-function, this is not always the case, particularly when frameshift or nonsense variants are found in the final exon or C-terminal portion of the penultimate exon; transcripts from genes with such variants are likely to evade nonsense mediated decay (Ziegler et al. 2019). At least 3 frameshift or nonsense mutations in the final two exons of *PRPF31* have been reported as pathogenic, but functional study is required to confirm pathogenicity (Martin-Merida et al. 2018, Huang et al. 2015). Similarly, consensus splice site mutations are often also assumed to cause complete loss of wild-type protein expression from the affected allele, when in fact the complex mechanisms of alternative splicing may lead to production of a truncated protein, particularly if the splicing change produces an in-frame transcript. In several cases where mutations are assumed to be causing loss-of-function through haploinsufficiency, in addition to presumed loss-of-function variants, at least 19 missense variants have been reported in *PRPF31* as being pathogenic. Gene constraint metrics, which provide quantitative measures of the extent to which a gene can tolerate change, indicate that *PRPF31* gene is highly intolerant to missense variants ($Z = 3.27$) (Samocha et al. 2014, Lek et al. 2016). Missense mutations in *PRPF31* tend to reduce the solubility of protein so it does not translocate into nucleus efficiently after being translated in the cytoplasm (Deery et al. 2002, Bryant et al. 2018, Wheway et al. 2019), effectively leading to a loss of this protein. However, only 4 missense variants have been functionally studied *in vitro*, and a comprehensive study of reported missense variants is required to confirm the functional effect of pathogenic variants, and indeed the pathogenicity of reported variants. At least one variant originally described as a missense variant was later confirmed to be affecting splicing (Rio Frio et al. 2008b) and it is possible that other variants classified as missense, both recognised pathogenic and those currently considered non-pathogenic, may in fact be impacting upon splicing of *PRPF31*. Furthermore, non-synonymous rare variants may impact on splicing. It is therefore likely that the rate of pathogenic variants affecting splicing in *PRPF31* is underestimated.

Genotype-phenotype correlation

We reviewed the literature and recorded the age of onset of first symptoms, and age of diagnosis, where it was reported alongside specific genetic variants. Age of onset of first symptoms (usually night-blindness) is lowest in patients with nonsense, frameshift or indel variants, with median age of onset between 8 and 12 years of age. Patients with large deletions or splice variants tend to show first symptoms at a slightly later median age of 20-24. Patients with in-frame duplications, insertions or missense variants show the latest median age of onset of first symptoms, around 27 years of age (**Figure 4a**). The difference in age of onset between the different types of mutation is statistically significant (one-way ANOVA $p=5.76 \times 10^{-5}$).

We also recorded the age of diagnosis where it was reported alongside specific *PRPF31* genetic variants. In this case, patients with nonsense, frameshift or splice variants were diagnosed at a median age of 20-30 years (usually because of loss of peripheral vision alongside night blindness), whereas patients with missense variants, in-frame deletions or large deletions tended to be diagnosed between the ages of 45 and 50 (**Figure 4b**). The difference in age of diagnosis between the different types of mutation is statistically significant (one-way ANOVA $p=0.030$).

There is no significant correlation between location of the variant in the gene and age of onset of symptoms or age of diagnosis.

It is an interesting observation, made in several studies and confirmed here, that patients with large-scale deletions, including multi-exon and whole gene deletions have the latest age of diagnosis. There is a clear difference in age of diagnosis of patients with large-scale deletions compared to patients with nonsense mutations or splice mutations although this is not statistically significant after correction for multiple testing (two-tailed unpaired t-test $p=0.016$ and $p=0.032$ respectively, $p=0.24$ and $p=0.48$ respectively after Bonferroni correction) (**Figure 4b**). It could be postulated that there is an element of dominant negative effect at play in cases of nonsense, frameshift, indel, in-frame and

missense variants compared to large deletions. This is a feature of the disease which should be considered when designing targeted therapies. The abundance of loss-of-function mutations, including complete gene deletions, in *PRPF31* patients has led to a consensus view that haploinsufficiency is the disease mechanism in this form of RP.(Abu-Safieh et al. 2006, Rio Frio et al. 2008b, Rose and Bhattacharya 2016) This has influenced approaches for targeted therapies, namely gene augmentation approaches, which involve replacing a wild-type copy of the coding sequence of *PRPF31* into the subretinal space of patients. This may not be fully effective in patients with genetic variants which have a dominant negative effect as well as a haploinsufficiency effect, and as a result other approaches for treatment may need to be investigated. These findings are supported by other recent work which also proposes a combined haploinsufficiency and dominant-negative disease mechanism in disease associated with *PRPF31* mutations.(Valdés-Sánchez et al. 2019) Study of the *Prpf31*^{p.A216P/+} mouse has shown that heterozygous missense mutations in *Prpf31* lead to aggregation of both wild-type and mutant protein in the cytoplasm of the RPE cells of mice, leading to overexpression of HSP70 family proteins.(Valdés-Sánchez et al. 2019) This work suggests that overexpression of these HSP70 proteins may be a target for therapy in *PRPF31* patients, rather than targeting *PRPF31* itself.(Valdés-Sánchez et al. 2019)

Opportunities for therapies

- Gene augmentation therapy

As a result of the abundance of loss-of-function variants in *PRPF31* gene augmentation has been postulated as a potential therapeutic approach to treat this form of RP.(Hafler et al. 2016) The coding sequence of *PRPF31* is only 1.5kb, well within the limits of current gene therapy vectors, and a *PRPF31* heterozygous knockout mouse is available for study, although it only develops very late onset retinal degeneration which may be more characteristic of age-related macular degeneration than RP.(Farkas et al. 2014) Researchers have begun preparatory work to define pre-treatment characteristics of RP associated with *PRPF31* mutations in order to be able to assess the effectiveness of AAV-mediated *PRPF31* gene augmentation therapy.(Hafler et al. 2016) These researchers have also patented *PRPF31* gene therapy by AAV2 delivery (International Publication Number WO2016144892A1) and, shown rescue of key cellular disease phenotypes including phagocytosis, ciliogenesis, cell morphology and barrier function in mutant *PRPF31*^{+/-} RPE derived from patient iPSCs after deliver of *PRPF31*.(Brydon et al. 2019)

- Antisense oligonucleotide therapy

If the majority of genetic variants have some dominant negative effect, it is important to consider other potential therapeutic approaches. These include antisense oligonucleotides (ASOs) which can bind pre-mRNA or mRNA and modulate splicing of *PRPF31* pre-mRNA or inhibit translation of the mRNA. In addition, siRNAs, shRNAs or gapmer-style ASOs can be used to completely silence a gene, which when combined with gene augmentation could potentially correct a disease with dominant negative effects. This approach has been successfully applied to the treatment of RP associated with dominant negative *RHO* mutations.(Cideciyan et al. 2018) Splice-switching ASOs can be used to bind and mask deep intronic variants which introduce novel splice sites (such as the deep intronic variant in intron 13 reported in Rio Frio et al., 2009.(Rio Frio et al. 2009) Alternatively, they can be used to induce exon skipping of an in-frame exon (ie an exon with a multiple of 3 base pairs) carrying a frameshift or null variant, in order to remove this variant and restore the reading frame. Three of the fourteen exons in *PRPF31* have multiples of 3 base pairs; exons 3, 11 and 14 (Figure 2, 3). These are also relatively small exons, and do not encode functional important domains of the protein (Figure 2) so they could be targeted for skipping without removing large or functionally important regions of the protein. This could have the effect of reverting a severe, early-onset frameshift or nonsense variant

into a less severe splice or in-frame deletion variant, although the exon skipping would affect both alleles, mutant and normal, so the effect may be like having a homozygous exon deletion. According to the genotype-phenotype data in this study, this could delay age of onset from 8-10 years of age to 25 years of age or later. If this exon skipping approach led to a disease more like in-frame deletions, this could delay age of diagnosis (taken as a proxy for loss of peripheral vision) from 25-30 years of age to 47 years of age. This could potentially preserve vision in the working age of these individuals. This is a promising approach in theory, and such drugs are already being developed for a range of previously untreatable genetic conditions.(Scoles and Pulst 2019, Levin 2019, Khan et al. 2019). A clinically available splice-switching ASO drug (nusinersen) based on 2'-O-methoxyethyl phosphorothioate chemistry has been successfully developed for the treatment of the neurodegenerative disease spinal muscular atrophy (approved by NICE) and a similar type of drug (eteplirsen) utilising phosphorodiamidate morpholino chemistry has been developed for treatment of certain forms of Duchenne muscular dystrophy.(Finkel et al. 2017, Mendell et al. 2016) Intraocularly delivered ASO drugs are also currently undergoing clinical trials for a specific form of Leber congenital amaurosis caused by a *CEP290* deep intronic mutation (ClinicalTrials.gov NCT03140969). ASOs are highly versatile drugs, being sequence-specific in their action, titratable in dosage, and in the setting of a well-defined and enclosed target organ such as the eye, straightforward to deliver by direct intravitreal or subretinal injection. However, there are limited numbers of affected individuals who could be treated by targeting these regions of *PRPF31* (around 27 families).

- Gene independent approaches

As RP associated with *PRPF31* is so genetically diverse, (172 different reported variants in 240 different families or simplex cases) gene independent approaches are extremely attractive alternatives to gene therapies. These include stem cell therapies and bionic retinal implants. Stem cell therapies are both gene and disease-agnostic, and can replace lost retinal cells, whereas gene therapies can only recover function of intact cells. Stem cell therapies are closest to being effective in replacement of the retinal pigment epithelium (RPE), which has no neural connection. It is more challenging to regenerate functional neural retina. Recent studies have shown promising results in stem cell replacement of RPE for treatment of age-related macular degeneration.(da Cruz et al. 2018, Kashani et al. 2018) Bioinetic retinæ such as the Argus II(Finn, Grewal and Vajzovic 2018) are able to restore limited light and shape perception in people with end-stage retinal disease and limited to no remaining retinal function.

Conclusions and future perspective

Gene therapy offers real potential for treatment of a range of currently untreatable inherited retinal degenerations. As the second most common cause of adRP, and a relatively small gene, *PRPF31* is becoming a focus for gene augmentation therapy.(Brydon et al. 2019) This approach assumes a disease mechanism of haploinsufficiency, of which there is considerable evidence. However, new data presented here supports the recently proposed theory that, except in cases of complete exon or gene deletion, dominant negative effects may contribute to disease progression in RP associated with *PRPF31* variants, (Valdés-Sánchez et al. 2019) and that gene augmentation therapy may not be as effective in patients with missense, nonsense or splice mutations compared to whole exon or whole gene deletions. Whilst it is important to pursue these studies, data from knockout mice must be interpreted with caution when translating into human studies, and alternatively approaches must also be investigated. These include antisense oligonucleotide therapy targeting suitable exons, and gene-independent approaches. With several potential therapeutic approaches under investigation, there is real hope that treatment options for this disorder will be available to the next generation of patients.

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Author Contributions

GW undertook the literature review, collected and tabulated genotype and phenotype data and prepared figures. EG performed statistical analysis of data. GW, AD and DB wrote the manuscript.

References

- Abdulridha-Aboud, W., U. Kjellstrom, S. Andreasson & V. Ponjavic (2016) Characterization of macular structure and function in two Swedish families with genetically identified autosomal dominant retinitis pigmentosa. *Mol Vis*, 22, 362-73.
- Abu-Safieh, L., E. N. Vithana, I. Mantel, G. E. Holder, L. Pelosini, A. C. Bird & S. S. Bhattacharya (2006) A large deletion in the adRP gene PRPF31: evidence that haploinsufficiency is the cause of disease. *Molecular vision*, 12, 384-388.
- Agafonov, D. E., B. Kastner, O. Dybkov, R. V. Hofele, W. T. Liu, H. Urlaub, R. Luhrmann & H. Stark (2016) Molecular architecture of the human U4/U6.U5 tri-snRNP. *Science (New York, N.Y.)*, 351, 1416-1420.
- Alagramam, K. N., H. Yuan, M. H. Kuehn, C. L. Murcia, S. Wayne, C. R. Srisailpathy, R. B. Lowry, R. Knaus, L. Van Laer, F. P. Bernier, S. Schwartz, C. Lee, C. C. Morton, R. F. Mullins, A. Ramesh, G. Van Camp, G. S. Hageman, R. P. Woychik & R. J. Smith (2001) Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. *Hum Mol Genet*, 10, 1709-18.
- Aleman, T. S., B. L. Lam, A. V. Cideciyan, A. Sumaroka, E. A. Windsor, A. J. Roman, S. B. Schwartz, E. M. Stone & S. G. Jacobson (2009) Genetic heterogeneity in autosomal dominant retinitis pigmentosa with low-frequency damped electroretinographic wavelets. *Eye (Lond)*, 23, 230-3.
- Almoguera, B., J. Li, P. Fernandez-San Jose, Y. Liu, M. March, R. Pellegrino, R. Golhar, M. Corton, F. Blanco-Kelly, M. I. Lopez-Molina, B. Garcia-Sandoval, Y. Guo, L. Tian, X. Liu, L. Guan, J. Zhang, B. Keating, X. Xu, H. Hakonarson & C. Ayuso (2015) Application of Whole Exome Sequencing in Six Families with an Initial Diagnosis of Autosomal Dominant Retinitis Pigmentosa: Lessons Learned. *PLoS One*, 10, e0133624.
- Audo, I., K. Bujakowska, S. Mohand-Said, M. E. Lancelot, V. Moskova-Doumanova, N. H. Waseem, A. Antonio, J. A. Sahel, S. S. Bhattacharya & C. Zeitz (2010) Prevalence and novelty of PRPF31 mutations in French autosomal dominant rod-cone dystrophy patients and a review of published reports. *BMC medical genetics*, 11, 145-2350-11-145.
- Azizzadeh Pormehr, L., S. Ahmadian, N. Daftarian, S. A. Mousavi & M. Shafiezhadeh (2019) PRPF31 reduction causes mis-splicing of the phototransduction genes in human organotypic retinal culture. *Eur J Hum Genet*.
- Bertram, K., D. E. Agafonov, O. Dybkov, D. Haselbach, M. N. Leelaram, C. L. Will, H. Urlaub, B. Kastner, R. Luhrmann & H. Stark (2017a) Cryo-EM Structure of a Pre-catalytic Human Spliceosome Primed for Activation. *Cell*, 170, 701-713.e11.
- Bertram, K., D. E. Agafonov, W. T. Liu, O. Dybkov, C. L. Will, K. Hartmuth, H. Urlaub, B. Kastner, H. Stark & R. Luhrmann (2017b) Cryo-EM structure of a human spliceosome activated for step 2 of splicing. *Nature*, 542, 318-323.

- Bhatia, S., S. Goyal, I. R. Singh, D. Singh & V. Vanita (2018) A novel mutation in the PRPF31 in a North Indian adRP family with incomplete penetrance. *Documenta ophthalmologica. Advances in ophthalmology*.
- Birtel, J., T. Eisenberger, M. Gliem, P. L. Muller, P. Herrmann, C. Betz, D. Zahnleiter, C. Neuhaus, S. Lenzner, F. G. Holz, E. Mangold, H. J. Bolz & P. Charbel Issa (2018a) Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci Rep*, 8, 4824.
- Birtel, J., M. Gliem, E. Mangold, P. L. Muller, F. G. Holz, C. Neuhaus, S. Lenzner, D. Zahnleiter, C. Betz, T. Eisenberger, H. J. Bolz & P. Charbel Issa (2018b) Next-generation sequencing identifies unexpected genotype-phenotype correlations in patients with retinitis pigmentosa. *PLoS One*, 13, e0207958.
- Bishop, D. T., W. H. McDonald, K. L. Gould & S. L. Forsburg (2000) Isolation of an essential *Schizosaccharomyces pombe* gene, *prp31(+)*, that links splicing and meiosis. *Nucleic Acids Res*, 28, 2214-20.
- Bowne, S. J., L. S. Sullivan, D. C. Koboldt, L. Ding, R. Fulton, R. M. Abbott, E. J. Sodergren, D. G. Birch, D. H. Wheaton, J. R. Heckenlively, Q. Liu, E. A. Pierce, G. M. Weinstock & S. P. Daiger (2011) Identification of disease-causing mutations in autosomal dominant retinitis pigmentosa (adRP) using next-generation DNA sequencing. *Invest Ophthalmol Vis Sci*, 52, 494-503.
- Bryant, L., O. Lozynska, A. Marsh, T. E. Papp, L. van Gorder, L. W. Serrano, X. Gai, M. D. Maguire Am, T. S. Aleman & J. Bennett (2018) Identification of a novel pathogenic missense mutation in PRPF31 using whole exome sequencing: a case report. *The British journal of ophthalmology*.
- Brydon, E. M., R. Bronstein, A. Buskin, M. Lako, E. A. Pierce & R. Fernandez-Godino (2019) AAV-Mediated Gene Augmentation Therapy Restores Critical Functions in Mutant PRPF31. *Mol Ther Methods Clin Dev*, 15, 392-402.
- Buskin, A., L. Zhu, V. Chichagova, B. Basu, S. Mozaffari-Jovin, D. Dolan, A. Droop, J. Collin, R. Bronstein, S. Mehrotra, M. Farkas, G. Hilgen, K. White, K. T. Pan, A. Treumann, D. Hallam, K. Bialas, G. Chung, C. Mellough, Y. Ding, N. Krasnogor, S. Przyborski, S. Zwolinski, J. Al-Aama, S. Alharthi, Y. Xu, G. Whewey, K. Szymanska, M. McKibbin, C. F. Inglehearn, D. J. Elliott, S. Lindsay, R. R. Ali, D. H. Steel, L. Armstrong, E. Sernagor, H. Urlaub, E. Pierce, R. Luhrmann, S. N. Grellscheid, C. A. Johnson & M. Lako (2018) Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nat Commun*, 9, 4234.
- Cao, H., J. Wu, S. Lam, R. Duan, C. Newnham, R. S. Molday, J. J. Graziotto, E. A. Pierce & J. Hu (2011) Temporal and tissue specific regulation of RP-associated splicing factor genes PRPF3, PRPF31 and PRPC8--implications in the pathogenesis of RP. *PloS one*, 6, e15860.
- Carss, K. J., G. Arno, M. Erwood, J. Stephens, A. Sanchis-Juan, S. Hull, K. Megy, D. Grozeva, E. Dewhurst, S. Malka, V. Plagnol, C. Penkett, K. Stirrups, R. Rizzo, G. Wright, D. Josifova, M. Bitner-Glindzicz, R. H. Scott, E. Clement, L. Allen, R. Armstrong, A. F. Brady, J. Carmichael, M. Chitre, R. H. H. Henderson, J. Hurst, R. E. MacLaren, E. Murphy, J. Paterson, E. Rosser, D. A. Thompson, E. Wakeling, W. H. Ouwehand, M. Michaelides, A. T. Moore, A. R. Webster & F. L. Raymond (2017) Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. *Am J Hum Genet*, 100, 75-90.
- Chakarova, C. F., S. Cherninkova, I. Tournev, N. Waseem, R. Kaneva, A. Jordanova, B. K. Veraitch, B. Gill, T. Colclough, A. Nakova, A. Oscar, V. Mihaylova, A. Nikolova-Hill, A. F. Wright, G. C. Black, S. Ramsden, I. Kremensky & S. S. Bhattacharya (2006) Molecular genetics of retinitis pigmentosa in two Romani (Gypsy) families. *Mol Vis*, 12, 909-14.
- Chanarat, S., M. Seizl & K. Strasser (2011) The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes. *Genes & development*, 25, 1147-1158.
- Chinchore, Y., T. Begaj, D. Wu, E. Drokhlyansky & C. L. Cepko (2017) Glycolytic reliance promotes anabolism in photoreceptors. *Elife*, 6.
- Cideciyan, A. V., R. Sudharsan, V. L. Dufour, M. T. Massengill, S. Iwabe, M. Swider, B. Lisi, A. Sumaroka, L. F. Marinho, T. Appelbaum, B. Rossmiller, W. W. Hauswirth, S. G. Jacobson, A. S. Lewin, G. D.

- Aguirre & W. A. Beltran (2018) Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc Natl Acad Sci U S A*, 115, E8547-e8556.
- Comitato, A., C. Spampinato, C. Chakarova, D. Sanges, S. S. Bhattacharya & V. Marigo (2007) Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells. *Human molecular genetics*, 16, 1699-1707.
- Coussa, R. G., C. Chakarova, R. Ajlan, M. Taha, C. Kavalec, J. Gomolin, A. Khan, I. Lopez, H. Ren, N. Waseem, K. Kamenarova, S. S. Bhattacharya & R. K. Koenekeop (2015) Genotype and Phenotype Studies in Autosomal Dominant Retinitis Pigmentosa (adRP) of the French Canadian Founder Population. *Investigative ophthalmology & visual science*, 56, 8297-8305.
- da Cruz, L., K. Fynes, O. Georgiadis, J. Kerby, Y. H. Luo, A. Ahmado, A. Vernon, J. T. Daniels, B. Nommiste, S. M. Hasan, S. B. Gooljar, A. F. Carr, A. Vugler, C. M. Ramsden, M. Bictash, M. Fenster, J. Steer, T. Harbinson, A. Wilbrey, A. Tufail, G. Feng, M. Whitlock, A. G. Robson, G. E. Holder, M. S. Sagoo, P. T. Loudon, P. Whiting & P. J. Coffey (2018) Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. *Nat Biotechnol*, 36, 328-337.
- Daiger, S. P., S. J. Bowne, L. S. Sullivan, S. H. Blanton, G. M. Weinstock, D. C. Koboldt, R. S. Fulton, D. Larsen, P. Humphries, M. M. Humphries, E. A. Pierce, R. Chen & Y. Li (2014) Application of next-generation sequencing to identify genes and mutations causing autosomal dominant retinitis pigmentosa (adRP). *Adv Exp Med Biol*, 801, 123-9.
- de la Cerda, B., A. Diez-Lloret, B. Ponte, L. Valles-Saiz, S. M. Calado, E. Rodriguez-Bocanegra, A. B. Garcia-Delgado, M. Moya-Molina, S. S. Bhattacharya & F. J. Diaz-Corrales (2019) Generation and characterization of the human iPSC line CABI001-A from a patient with retinitis pigmentosa caused by a novel mutation in PRPF31 gene. *Stem Cell Res*, 36, 101426.
- de Sousa Dias, M., I. Hernan, B. Pascual, E. Borrás, B. Mane, M. J. Gamundi & M. Carballo (2013) Detection of novel mutations that cause autosomal dominant retinitis pigmentosa in candidate genes by long-range PCR amplification and next-generation sequencing. *Mol Vis*, 19, 654-64.
- Deery, E. C., E. N. Vithana, R. J. Newbold, V. A. Gallon, S. S. Bhattacharya, M. J. Warren, D. M. Hunt & S. E. Wilkie (2002) Disease mechanism for retinitis pigmentosa (RP11) caused by mutations in the splicing factor gene PRPF31. *Human molecular genetics*, 11, 3209-3219.
- Dong, B., J. Chen, X. Zhang, Z. Pan, F. Bai & Y. Li (2013) Two novel PRP31 premessenger ribonucleic acid processing factor 31 homolog mutations including a complex insertion-deletion identified in Chinese families with retinitis pigmentosa. *Mol Vis*, 19, 2426-35.
- Eisenberger, T., C. Neuhaus, A. O. Khan, C. Decker, M. N. Preising, C. Friedburg, A. Bieg, M. Gliem, P. Charbel Issa, F. G. Holz, S. M. Baig, Y. Hellenbroich, A. Galvez, K. Platzer, B. Wollnik, N. Laddach, S. R. Ghaffari, M. Rafati, E. Botzenhart, S. Tinschert, D. Borger, A. Bohring, J. Schreml, S. Kortge-Jung, C. Schell-Apacik, K. Bakur, J. Y. Al-Aama, T. Neuhann, P. Herkenrath, G. Nurnberg, P. Nurnberg, J. S. Davis, A. Gal, C. Bergmann, B. Lorenz & H. J. Bolz (2013) Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One*, 8, e78496.
- Ellingford, J. M., S. Barton, S. Bhaskar, J. O'Sullivan, S. G. Williams, J. A. Lamb, B. Panda, P. I. Sergouniotis, R. L. Gillespie, S. P. Daiger, G. Hall, T. Gale, I. C. Lloyd, P. N. Bishop, S. C. Ramsden & G. C. M. Black (2016a) Molecular findings from 537 individuals with inherited retinal disease. *J Med Genet*, 53, 761-767.
- Ellingford, J. M., S. Barton, S. Bhaskar, S. G. Williams, P. I. Sergouniotis, J. O'Sullivan, J. A. Lamb, R. Perveen, G. Hall, W. G. Newman, P. N. Bishop, S. A. Roberts, R. Leach, R. Tearle, S. Bayliss, S. C. Ramsden, A. H. Nemeth & G. C. Black (2016b) Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. *Ophthalmology*, 123, 1143-50.

- Farkas, M. H., D. S. Lew, M. E. Sousa, K. Bujakowska, J. Chatagnon, S. S. Bhattacharya, E. A. Pierce & E. F. Nandrot (2014) Mutations in pre-mRNA processing factors 3, 8, and 31 cause dysfunction of the retinal pigment epithelium. *The American journal of pathology*, 184, 2641-2652.
- Fernandez-San Jose, P., M. Corton, F. Blanco-Kelly, A. Avila-Fernandez, M. A. Lopez-Martinez, I. Sanchez-Navarro, R. Sanchez-Alcudia, R. Perez-Carro, O. Zurita, N. Sanchez-Bolivar, M. I. Lopez-Molina, B. Garcia-Sandoval, R. Riveiro-Alvarez & C. Ayuso (2015) Targeted Next-Generation Sequencing Improves the Diagnosis of Autosomal Dominant Retinitis Pigmentosa in Spanish Patients. *Invest Ophthalmol Vis Sci*, 56, 2173-82.
- Finkel, R. S., E. Mercuri, B. T. Darras, A. M. Connolly, N. L. Kuntz, J. Kirschner, C. A. Chiriboga, K. Saito, L. Servais, E. Tizzano, H. Topaloglu, M. Tulinius, J. Montes, A. M. Glanzman, K. Bishop, Z. J. Zhong, S. Gheuens, C. F. Bennett, E. Schneider, W. Farwell & D. C. De Vivo (2017) Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med*, 377, 1723-1732.
- Finn, A. P., D. S. Grewal & L. Vajzovic (2018) Argus II retinal prosthesis system: a review of patient selection criteria, surgical considerations, and post-operative outcomes. *Clin Ophthalmol*, 12, 1089-1097.
- Gandra, M., V. Anandula, V. Authiappan, S. Sundaramurthy, R. Raman, S. Bhattacharya & K. Govindasamy (2008) Retinitis pigmentosa: mutation analysis of RHO, PRPF31, RP1, and IMPDH1 genes in patients from India. *Mol Vis*, 14, 1105-13.
- Ghazawy, S., K. Springell, V. Gauba, M. A. McKibbin & C. F. Inglehearn (2007) Dominant retinitis pigmentosa phenotype associated with a new mutation in the splicing factor PRPF31. *Br J Ophthalmol*, 91, 1411-3.
- Glockle, N., S. Kohl, J. Mohr, T. Scheurenbrand, A. Sprecher, N. Weisschuh, A. Bernd, G. Rudolph, M. Schubach, C. Poloschek, E. Zrenner, S. Biskup, W. Berger, B. Wissinger & J. Neidhardt (2014) Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. *Eur J Hum Genet*, 22, 99-104.
- Golovleva, I., L. Kohn, M. Burstedt, S. Daiger & O. Sandgren (2010) Mutation spectra in autosomal dominant and recessive retinitis pigmentosa in northern Sweden. *Adv Exp Med Biol*, 664, 255-62.
- Grenell, A., Y. Wang, M. Yam, A. Swarup, T. L. Dilan, A. Hauer, J. D. Linton, N. J. Philp, E. Gregor, S. Zhu, Q. Shi, J. Murphy, T. Guan, D. Lohner, S. Kolandaivelu, V. Ramamurthy, A. F. X. Goldberg, J. B. Hurley & J. Du (2019) Loss of MPC1 reprograms retinal metabolism to impair visual function. *Proc Natl Acad Sci U S A*, 116, 3530-3535.
- Grey, M., A. Dusterhoft, J. A. Henriques & M. Brendel (1996) Allelism of PSO4 and PRP19 links pre-mRNA processing with recombination and error-prone DNA repair in *Saccharomyces cerevisiae*. *Nucleic acids research*, 24, 4009-4014.
- Hafler, B. P., J. Comander, C. Weigel DiFranco, E. M. Place & E. A. Pierce (2016) Course of Ocular Function in PRPF31 Retinitis Pigmentosa. *Semin Ophthalmol*, 31, 49-52.
- Hamieh, A. & E. F. Nandrot (2019) Retinal Pigment Epithelial Cells: The Unveiled Component in the Etiology of Prpf Splicing Factor-Associated Retinitis Pigmentosa. *Adv Exp Med Biol*, 1185, 227-231.
- Hariri, A. H., W. Gui, G. A. Datto O'Keefe, M. S. Ip, S. R. Sadda & M. B. Gorin (2018) Ultra-Widefield Fundus Autofluorescence Imaging of Patients with Retinitis Pigmentosa: A Standardized Grading System in Different Genotypes. *Ophthalmol Retina*, 2, 735-745.
- Haselbach, D., I. Komarov, D. E. Agafonov, K. Hartmuth, B. Graf, O. Dybkov, H. Urlaub, B. Kastner, R. Luhrmann & H. Stark (2018) Structure and Conformational Dynamics of the Human Spliceosomal B(act) Complex. *Cell*, 172, 454-464.e11.
- Hofmann, J. C., J. Tegha-Dunghu, S. Drager, C. L. Will, R. Luhrmann & O. J. Gruss (2013) The Prp19 complex directly functions in mitotic spindle assembly. *PLoS one*, 8, e74851.

- Huang, X. F., F. Huang, K. C. Wu, J. Wu, J. Chen, C. P. Pang, F. Lu, J. Qu & Z. B. Jin (2015) Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med*, 17, 271-8.
- Ivings, L., K. V. Towns, M. A. Martin, C. Taylor, F. Ponchel, R. J. Grainger, R. S. Ramesar, D. A. Mackey & C. F. Inglehearn (2008) Evaluation of splicing efficiency in lymphoblastoid cell lines from patients with splicing-factor retinitis pigmentosa. *Molecular vision*, 14, 2357-2366.
- Jespersgaard, C., M. Fang, M. Bertelsen, X. Dang, H. Jensen, Y. Chen, N. Bech, L. Dai, T. Rosenberg, J. Zhang, L. B. Moller, Z. Tumer, K. Brondum-Nielsen & K. Gronskov (2019) Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. *Sci Rep*, 9, 1219.
- Jin, Z. B., S. Okamoto, F. Osakada, K. Homma, J. Assawachananont, Y. Hirami, T. Iwata & M. Takahashi (2011) Modeling retinal degeneration using patient-specific induced pluripotent stem cells. *PloS one*, 6, e17084.
- Kashani, A. H., J. S. Lebkowski, F. M. Rahhal, R. L. Avery, H. Salehi-Had, W. Dang, C. M. Lin, D. Mitra, D. Zhu, B. B. Thomas, S. T. Hikita, B. O. Pennington, L. V. Johnson, D. O. Clegg, D. R. Hinton & M. S. Humayun (2018) A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration. *Sci Transl Med*, 10.
- Khan, N., H. Eliopoulos, L. Han, T. B. Kinane, L. P. Lowes, J. R. Mendell, H. Gordish-Dressman, E. K. Henricson & C. M. McDonald (2019) Eteplirsen Treatment Attenuates Respiratory Decline in Ambulatory and Non-Ambulatory Patients with Duchenne Muscular Dystrophy. *J Neuromuscul Dis*, 6, 213-225.
- Kim, J. H., S. M. Ki, J. G. Joung, E. Scott, S. Heynen-Genel, P. Aza-Blanc, C. H. Kwon, J. Kim, J. G. Gleeson & J. E. Lee (2016) Genome-wide screen identifies novel machineries required for both ciliogenesis and cell cycle arrest upon serum starvation. *Biochim Biophys Acta*, 1863, 1307-18.
- Kiser, K., K. D. Webb-Jones, S. J. Bowne, L. S. Sullivan, S. P. Daiger & D. G. Birch (2019) Time Course of Disease Progression of PRPF31-mediated Retinitis Pigmentosa. *Am J Ophthalmol*, 200, 76-84.
- Kohn, L., S. J. Bowne, S. S. L., S. P. Daiger, M. S. Burstedt, K. Kadzhaev, O. Sandgren & I. Golovleva (2009) Breakpoint characterization of a novel approximately 59 kb genomic deletion on 19q13.42 in autosomal-dominant retinitis pigmentosa with incomplete penetrance. *Eur J Hum Genet*, 17, 651-5.
- Koyanagi, Y., M. Akiyama, K. M. Nishiguchi, Y. Momozawa, Y. Kamatani, S. Takata, C. Inai, Y. Iwasaki, M. Kumano, Y. Murakami, K. Omodaka, T. Abe, S. Komori, D. Gao, T. Hirakata, K. Kurata, K. Hosono, S. Ueno, Y. Hotta, A. Murakami, H. Terasaki, Y. Wada, T. Nakazawa, T. Ishibashi, Y. Ikeda, M. Kubo & K. H. Sonoda (2019) Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. *J Med Genet*, 56, 662-670.
- Kurata, K., K. Hosono & Y. Hotta (2018) Long-term clinical course of 2 Japanese patients with PRPF31-related retinitis pigmentosa. *Jpn J Ophthalmol*, 62, 186-193.
- Lek, M., K. J. Karczewski, E. V. Minikel, K. E. Samocha, E. Banks, T. Fennell, A. H. O'Donnell-Luria, J. S. Ware, A. J. Hill, B. B. Cummings, T. Tukiainen, D. P. Birnbaum, J. A. Kosmicki, L. E. Duncan, K. Estrada, F. Zhao, J. Zou, E. Pierce-Hoffman, J. Berghout, D. N. Cooper, N. Deflaux, M. DePristo, R. Do, J. Flannick, M. Fromer, L. Gauthier, J. Goldstein, N. Gupta, D. Howrigan, A. Kiezun, M. I. Kurki, A. L. Moonshine, P. Natarajan, L. Orozco, G. M. Peloso, R. Poplin, M. A. Rivas, V. Ruano-Rubio, S. A. Rose, D. M. Ruderfer, K. Shakir, P. D. Stenson, C. Stevens, B. P. Thomas, G. Tiao, M. T. Tusie-Luna, B. Weisburd, H.-H. Won, D. Yu, D. M. Altshuler, D. Ardissino, M. Boehnke, J. Danesh, S. Donnelly, R. Elosua, J. C. Florez, S. B. Gabriel, G. Getz, S. J. Glatt, C. M. Hultman, S. Kathiresan, M. Laakso, S. McCarroll, M. I. McCarthy, D. McGovern, R. McPherson, B. M. Neale, A. Palotie, S. M. Purcell, D. Saleheen, J. M. Scharf, P. Sklar, P. F. Sullivan, J. Tuomilehto, M. T. Tsuang, H. C. Watkins, J. G. Wilson, M. J. Daly, D. G. MacArthur & C. Exome Aggregation (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, 536, 285-291.
- Levin, A. A. (2019) Treating Disease at the RNA Level with Oligonucleotides. *N Engl J Med*, 380, 57-70.

- Lim, K. P., S. P. Yip, S. C. Cheung, K. W. Leung, S. T. Lam & C. H. To (2009) Novel PRPF31 and PRPH2 mutations and co-occurrence of PRPF31 and RHO mutations in Chinese patients with retinitis pigmentosa. *Arch Ophthalmol*, 127, 784-90.
- Liu, J. Y., X. Dai, J. Sheng, X. Cui, X. Wang, X. Jiang, X. Tu, Z. Tang, Y. Bai, M. Liu & Q. K. Wang (2008) Identification and functional characterization of a novel splicing mutation in RP gene PRPF31. *Biochem Biophys Res Commun*, 367, 420-6.
- Liu, S., P. Li, O. Dybkov, S. Nottrott, K. Hartmuth, R. Luhrmann, T. Carlomagno & M. C. Wahl (2007) Binding of the human Prp31 Nop domain to a composite RNA-protein platform in U4 snRNP. *Science (New York, N.Y.)*, 316, 115-120.
- Lu, F., L. Huang, C. Lei, G. Sha, H. Zheng, X. Liu, J. Yang, Y. Shi, Y. Lin, B. Gong, X. Zhu, S. Ma, L. Qiao, H. Lin, J. Cheng & Z. Yang (2013) A novel PRPF31 mutation in a large Chinese family with autosomal dominant retinitis pigmentosa and macular degeneration. *PLoS One*, 8, e78274.
- Lu, S. S., C. Zhao, Y. Cui, N. D. Li, X. M. Zhang & K. X. Zhao (2005) [Novel splice-site mutation in the pre-mRNA splicing gene PRPF31 in a Chinese family with autosomal dominant retinitis pigmentosa]. *Zhonghua Yan Ke Za Zhi*, 41, 305-11.
- Makarova, O. V., E. M. Makarov, S. Liu, H. P. Vornlocher & R. Luhrmann (2002) Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6*U5 tri-snRNP formation and pre-mRNA splicing. *The EMBO journal*, 21, 1148-1157.
- Marechal, A., J. M. Li, X. Y. Ji, C. S. Wu, S. A. Yazinski, H. D. Nguyen, S. Liu, A. E. Jimenez, J. Jin & L. Zou (2014) PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR activation via a ubiquitin-mediated circuitry. *Molecular cell*, 53, 235-246.
- Martin-Merida, I., D. Aguilera-Garcia, P. Fernandez-San Jose, F. Blanco-Kelly, O. Zurita, B. Almoguera, B. Garcia-Sandoval, A. Avila-Fernandez, A. Arteche, P. Minguez, M. Carballo, M. Corton & C. Ayuso (2018) Toward the Mutational Landscape of Autosomal Dominant Retinitis Pigmentosa: A Comprehensive Analysis of 258 Spanish Families. *Investigative ophthalmology & visual science*, 59, 2345-2354.
- Martinez-Gimeno, M., M. J. Gamundi, I. Hernan, M. Maseras, E. Milla, C. Ayuso, B. Garcia-Sandoval, M. Beneyto, C. Vilela, M. Baiget, G. Antinolo & M. Carballo (2003) Mutations in the pre-mRNA splicing-factor genes PRPF3, PRPF8, and PRPF31 in Spanish families with autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 44, 2171-7.
- McLennan, S., D. Zhang, X. Zhang, S. C. Chen, T. Lamey, J. A. Thompson, T. McLaren, J. N. De Roach, S. Fletcher & F. K. Chen (2019) Generation of two induced pluripotent stem cell lines from a patient with dominant PRPF31 mutation and a related non-penetrant carrier. *Stem Cell Res*, 34, 101357.
- Mendell, J. R., N. Goemans, L. P. Lowes, L. N. Alfano, K. Berry, J. Shao, E. M. Kaye & E. Mercuri (2016) Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol*, 79, 257-71.
- Neveling, K., R. W. Collin, C. Gilissen, R. A. van Huet, L. Visser, M. P. Kwint, S. J. Gijzen, M. N. Zonneveld, N. Wieskamp, J. de Ligt, A. M. Siemiatkowska, L. H. Hoefsloot, M. F. Buckley, U. Kellner, K. E. Branham, A. I. den Hollander, A. Hoischen, C. Hoyng, B. J. Klevering, L. I. van den Born, J. A. Veltman, F. P. Cremers & H. Scheffer (2012) Next-generation genetic testing for retinitis pigmentosa. *Hum Mutat*, 33, 963-72.
- Ng, S. K., J. P. Wood, G. Chidlow, G. Han, T. Kittipassorn, D. J. Peet & R. J. Casson (2015) Cancer-like metabolism of the mammalian retina. *Clin Exp Ophthalmol*, 43, 367-76.
- Pan, Q., O. Shai, L. J. Lee, B. J. Frey & B. J. Blencowe (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet*, 40, 1413-5.
- Pan, X., X. Chen, X. Liu, X. Gao, X. Kang, Q. Xu, X. Chen, K. Zhao, X. Zhang, Q. Chu, X. Wang & C. Zhao (2014) Mutation analysis of pre-mRNA splicing genes in Chinese families with retinitis pigmentosa. *Mol Vis*, 20, 770-9.

- Pomares, E., M. Riera, J. Permanyer, P. Mendez, J. Castro-Navarro, A. Andres-Gutierrez, G. Marfany & R. Gonzalez-Duarte (2010) Comprehensive SNP-chip for retinitis pigmentosa-Leber congenital amaurosis diagnosis: new mutations and detection of mutational founder effects. *Eur J Hum Genet*, 18, 118-24.
- Rajala, R. V., A. Rajala, C. Kooker, Y. Wang & R. E. Anderson (2016) The Warburg Effect Mediator Pyruvate Kinase M2 Expression and Regulation in the Retina. *Sci Rep*, 6, 37727.
- Rio Frio, T., N. Civic, A. Ransijn, J. S. Beckmann & C. Rivolta (2008a) Two trans-acting eQTLs modulate the penetrance of PRPF31 mutations. *Hum Mol Genet*, 17, 3154-65.
- Rio Frio, T., T. L. McGee, N. M. Wade, C. Iseli, J. S. Beckmann, E. L. Berson & C. Rivolta (2009) A single-base substitution within an intronic repetitive element causes dominant retinitis pigmentosa with reduced penetrance. *Hum Mutat*, 30, 1340-7.
- Rio Frio, T., N. M. Wade, A. Ransijn, E. L. Berson, J. S. Beckmann & C. Rivolta (2008b) Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. *The Journal of clinical investigation*, 118, 1519-1531.
- Rivolta, C., T. L. McGee, T. Rio Frio, R. V. Jensen, E. L. Berson & T. P. Dryja (2006) Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations. *Human mutation*, 27, 644-653.
- Roberts, L., R. Ratnapriya, M. du Plessis, V. Chaitankar, R. S. Ramesar & A. Swaroop (2016) Molecular Diagnosis of Inherited Retinal Diseases in Indigenous African Populations by Whole-Exome Sequencing. *Investigative ophthalmology & visual science*, 57, 6374-6381.
- Rose, A. M. & S. S. Bhattacharya (2016) Variant haploinsufficiency and phenotypic non-penetrance in PRPF31-associated retinitis pigmentosa. *Clin Genet*, 90, 118-26.
- Rose, A. M., R. Mukhopadhyay, A. R. Webster, S. S. Bhattacharya & N. H. Waseem (2011) A 112 kb deletion in chromosome 19q13.42 leads to retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 52, 6597-603.
- Rose, A. M., A. Z. Shah, G. Venturini, A. Krishna, A. Chakravarti, C. Rivolta & S. S. Bhattacharya (2016) Transcriptional regulation of PRPF31 gene expression by MSR1 repeat elements causes incomplete penetrance in retinitis pigmentosa. *Scientific reports*, 6, 19450.
- Rose, A. M., A. Z. Shah, G. Venturini, C. Rivolta, G. E. Rose & S. S. Bhattacharya (2014) Dominant PRPF31 mutations are hypostatic to a recessive CNOT3 polymorphism in retinitis pigmentosa: a novel phenomenon of "linked trans-acting epistasis". *Annals of Human Genetics*, 78, 62-71.
- Rose, A. M., A. Z. Shah, N. H. Waseem, C. F. Chakarova, G. Alfano, R. G. Coussa, R. Ajlan, R. K. Koenekoop & S. S. Bhattacharya (2012) Expression of PRPF31 and TFPT: regulation in health and retinal disease. *Hum Mol Genet*, 21, 4126-37.
- Saini, S., P. N. Robinson, J. R. Singh & V. Vanita (2012) A novel 7 bp deletion in PRPF31 associated with autosomal dominant retinitis pigmentosa with incomplete penetrance in an Indian family. *Exp Eye Res*, 104, 82-8.
- Samocha, K. E., E. B. Robinson, S. J. Sanders, C. Stevens, A. Sabo, L. M. McGrath, J. A. Kosmicki, K. Rehnström, S. Mallick, A. Kirby, D. P. Wall, D. G. MacArthur, S. B. Gabriel, M. DePristo, S. M. Purcell, A. Palotie, E. Boerwinkle, J. D. Buxbaum, E. H. Cook, R. A. Gibbs, G. D. Schellenberg, J. S. Sutcliffe, B. Devlin, K. Roeder, B. M. Neale & M. J. Daly (2014) A framework for the interpretation of de novo mutation in human disease. *Nature Genetics*, 46, 944-950.
- Sato, H., Y. Wada, T. Itabashi, M. Nakamura, M. Kawamura & M. Tamai (2005) Mutations in the pre-mRNA splicing gene, PRPF31, in Japanese families with autosomal dominant retinitis pigmentosa. *Am J Ophthalmol*, 140, 537-40.
- Schaffert, N., M. Hossbach, R. Heintzmann, T. Achsel & R. Luhrmann (2004) RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies. *The EMBO journal*, 23, 3000-3009.
- Schmidt-Kastner, R., H. Yamamoto, D. Hamasaki, H. Yamamoto, J. M. Parel, C. Schmitz, C. K. Dorey, J. C. Blanks & M. N. Preising (2008) Hypoxia-regulated components of the U4/U6.U5 tri-small

- nuclear riboprotein complex: possible role in autosomal dominant retinitis pigmentosa. *Molecular vision*, 14, 125-135.
- Scoles, D. R. & S. M. Pulst (2019) Antisense therapies for movement disorders. *Mov Disord*, 34, 1112-1119.
- Shinde, V., P. Kotla, C. Strang & M. Gorbatyuk (2016) Unfolded protein response-induced dysregulation of calcium homeostasis promotes retinal degeneration in rat models of autosomal dominant retinitis pigmentosa. *Cell death & disease*, 7, e2085.
- Sullivan, L. S., S. J. Bowne, D. G. Birch, D. Hughbanks-Wheaton, J. R. Heckenlively, R. A. Lewis, C. A. Garcia, R. S. Ruiz, S. H. Blanton, H. Northrup, A. I. Gire, R. Seaman, H. Duzkale, C. J. Spellicy, J. Zhu, S. P. Shankar & S. P. Daiger (2006a) Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. *Invest Ophthalmol Vis Sci*, 47, 3052-64.
- Sullivan, L. S., S. J. Bowne, M. J. Reeves, D. Blain, K. Goetz, V. Ndifor, S. Vitez, X. Wang, S. J. Tumminia & S. P. Daiger (2013a) Prevalence of mutations in eyeGENE probands with a diagnosis of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 54, 6255-61.
- Sullivan, L. S., S. J. Bowne, C. R. Seaman, S. H. Blanton, R. A. Lewis, J. R. Heckenlively, D. G. Birch, D. Hughbanks-Wheaton & S. P. Daiger (2006b) Genomic rearrangements of the PRPF31 gene account for 2.5% of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 47, 4579-88.
- Taira, K., M. Nakazawa & M. Sato (2007) Mutation c. 1142 del G in the PRPF31 gene in a family with autosomal dominant retinitis pigmentosa (RP11) and its implications. *Jpn J Ophthalmol*, 51, 45-8.
- Terray, A., V. Fort, A. Slembrouck, C. Nanteau, J. A. Sahel, S. Reichman, I. Audo & O. Goureau (2017) Establishment of an induced pluripotent stem (iPS) cell line from dermal fibroblasts of an asymptomatic patient with dominant PRPF31 mutation. *Stem Cell Res*, 25, 26-29.
- Tiwari, A., J. Lemke, J. Altmueller, H. Thiele, E. Glaus, J. Fleischhauer, P. Nurnberg, J. Neidhardt & W. Berger (2016) Identification of Novel and Recurrent Disease-Causing Mutations in Retinal Dystrophies Using Whole Exome Sequencing (WES): Benefits and Limitations. *PLoS One*, 11, e0158692.
- Utz, V. M., C. D. Beight, M. J. Marino, S. A. Hagstrom & E. I. Traboulsi (2013) Autosomal dominant retinitis pigmentosa secondary to pre-mRNA splicing-factor gene PRPF31 (RP11): review of disease mechanism and report of a family with a novel 3-base pair insertion. *Ophthalmic Genet*, 34, 183-8.
- Valdés-Sánchez, L., S. M. Calado, B. de la Cerda, A. Aramburu, A. B. García-Delgado, S. Massalini, A. Montero-Sánchez, V. Bhatia, E. Rodríguez-Bocanegra, A. Díez-Lloret, D. Rodríguez-Martínez, C. Chakarova, S. S. Bhattacharya & F. J. Díaz-Corrales (2019) Retinal pigment epithelium degeneration caused by aggregation of PRPF31 and the role of HSP70 family of proteins. *Mol Med*, 26, 1.
- Van Cauwenbergh, C., F. Coppieters, D. Roels, S. De Jaegere, H. Flipts, J. De Zaeytijd, S. Walraedt, C. Claes, E. Fransen, G. Van Camp, F. Depasse, I. Casteels, T. de Ravel, B. P. Leroy & E. De Baere (2017) Mutations in Splicing Factor Genes Are a Major Cause of Autosomal Dominant Retinitis Pigmentosa in Belgian Families. *PloS one*, 12, e0170038.
- van Huet, R. A., L. H. Pierrache, M. A. Meester-Smoor, C. C. Klaver, L. I. van den Born, C. B. Hoyng, I. J. de Wijs, R. W. Collin, L. H. Hoefsloot & B. J. Klevering (2015) The efficacy of microarray screening for autosomal recessive retinitis pigmentosa in routine clinical practice. *Mol Vis*, 21, 461-76.
- Vander Kooi, C. W., M. D. Ohi, J. A. Rosenberg, M. L. Oldham, M. E. Newcomer, K. L. Gould & W. J. Chazin (2006) The Prp19 U-box crystal structure suggests a common dimeric architecture for a class of oligomeric E3 ubiquitin ligases. *Biochemistry*, 45, 121-130.

- Venturini, G., A. M. Rose, A. Z. Shah, S. S. Bhattacharya & C. Rivolta (2012) CNOT3 is a modifier of PRPF31 mutations in retinitis pigmentosa with incomplete penetrance. *PLoS genetics*, 8, e1003040.
- Villanueva, A., J. R. Willer, J. Bryois, E. T. Dermitzakis, N. Katsanis & E. E. Davis (2014) Whole exome sequencing of a dominant retinitis pigmentosa family identifies a novel deletion in PRPF31. *Invest Ophthalmol Vis Sci*, 55, 2121-9.
- Vithana, E. N., L. Abu-Safieh, M. J. Allen, A. Carey, M. Papaioannou, C. Chakarova, M. Al-Magthteh, N. D. Ebenezer, C. Willis, A. T. Moore, A. C. Bird, D. M. Hunt & S. S. Bhattacharya (2001) A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). *Molecular cell*, 8, 375-381.
- Wang, F., H. Wang, H. F. Tuan, D. H. Nguyen, V. Sun, V. Keser, S. J. Bowne, L. S. Sullivan, H. Luo, L. Zhao, X. Wang, J. E. Zaneveld, J. S. Salvo, S. Siddiqui, L. Mao, D. K. Wheaton, D. G. Birch, K. E. Branham, J. R. Heckenlively, C. Wen, K. Flagg, H. Ferreyra, J. Pei, A. Khan, H. Ren, K. Wang, I. Lopez, R. Qamar, J. C. Zenteno, R. Ayala-Ramirez, B. Buentello-Volante, Q. Fu, D. A. Simpson, Y. Li, R. Sui, G. Silvestri, S. P. Daiger, R. K. Koenekoop, K. Zhang & R. Chen (2014) Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. *Hum Genet*, 133, 331-45.
- Wang, L., M. Ribaud, K. Zhao, N. Yu, Q. Chen, Q. Sun, L. Wang & Q. Wang (2003) Novel deletion in the pre-mRNA splicing gene PRPF31 causes autosomal dominant retinitis pigmentosa in a large Chinese family. *Am J Med Genet A*, 121a, 235-9.
- Waseem, N. H., V. Vaclavik, A. Webster, S. A. Jenkins, A. C. Bird & S. S. Bhattacharya (2007) Mutations in the gene coding for the pre-mRNA splicing factor, PRPF31, in patients with autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 48, 1330-4.
- Weidenhammer, E. M., M. Ruiz-Noriega & J. L. Woolford, Jr. (1997) Prp31p promotes the association of the U4/U6 x U5 tri-snRNP with pre-spliceosomes to form spliceosomes in *Saccharomyces cerevisiae*. *Mol Cell Biol*, 17, 3580-8.
- Weidenhammer, E. M., M. Singh, M. Ruiz-Noriega & J. L. Woolford, Jr. (1996) The PRP31 gene encodes a novel protein required for pre-mRNA splicing in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, 24, 1164-70.
- Weisschuh, N., A. K. Mayer, T. M. Strom, S. Kohl, N. Glockle, M. Schubach, S. Andreasson, A. Bernd, D. G. Birch, C. P. Hamel, J. R. Heckenlively, S. G. Jacobson, C. Kamme, U. Kellner, E. Kunstmann, P. Maffei, C. M. Reiff, K. Rohrschneider, T. Rosenberg, G. Rudolph, R. Vámos, B. Varsanyi, R. G. Weleber & B. Wissinger (2016) Mutation Detection in Patients with Retinal Dystrophies Using Targeted Next Generation Sequencing. *PLoS One*, 11, e0145951.
- Wheway, G., L. Nazlamova, N. Meshad, S. Hunt, N. Jackson & A. Churchill (2019) A Combined in silico, in vitro and Clinical Approach to Characterize Novel Pathogenic Missense Variants in PRPF31 in Retinitis Pigmentosa. *Front Genet*, 10, 248.
- Wheway, G., M. Schmidts, D. A. Mans, K. Szymanska, T. M. Nguyen, H. Racher, I. G. Phelps, G. Toedt, J. Kennedy, K. A. Wunderlich, N. Soroush, Z. A. Abdelhamed, S. Natarajan, W. Herridge, J. van Reeuwijk, N. Horn, K. Boldt, D. A. Parry, S. J. Letteboer, S. Roosing, M. Adams, S. M. Bell, J. Bond, J. Higgins, E. E. Morrison, D. C. Tomlinson, G. G. Slaats, T. J. van Dam, L. Huang, K. Kessler, A. Giessl, C. V. Logan, E. A. Boyle, J. Shendure, S. Anazi, M. Aldahmesh, S. Al Hazzaa, R. A. Hegele, C. Ober, P. Frosk, A. A. Mhanni, B. N. Chodirker, A. E. Chudley, R. Lamont, F. P. Bernier, C. L. Beaulieu, P. Gordon, R. T. Pon, C. Donahue, A. J. Barkovich, L. Wolf, C. Toomes, C. T. Thiel, K. M. Boycott, M. McKibbin, C. F. Inglehearn, U. K. Consortium, G. University of Washington Center for Mendelian, F. Stewart, H. Omran, M. A. Huynen, P. I. Sergouniotis, F. S. Alkuraya, J. S. Parboosingh, A. M. Innes, C. E. Willoughby, R. H. Giles, A. R. Webster, M. Ueffing, O. Blacque, J. G. Gleeson, U. Wolfrum, P. L. Beales, T. Gibson, D. Doherty, H. M. Mitchison, R. Roepman & C. A. Johnson (2015) An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. *Nature cell biology*, 17, 1074-1087.

- Wilkie, S. E., K. J. Morris, S. S. Bhattacharya, M. J. Warren & D. M. Hunt (2006) A study of the nuclear trafficking of the splicing factor protein PRPF31 linked to autosomal dominant retinitis pigmentosa (ADRP). *Biochimica et biophysica acta*, 1762, 304-311.
- Wilkie, S. E., V. Vaclavik, H. Wu, K. Bujakowska, C. F. Chakarova, S. S. Bhattacharya, M. J. Warren & D. M. Hunt (2008) Disease mechanism for retinitis pigmentosa (RP11) caused by missense mutations in the splicing factor gene PRPF31. *Molecular vision*, 14, 683-690.
- Will, C. L. & R. Luhrmann (2011) Spliceosome structure and function. *Cold Spring Harbor perspectives in biology*, 3, 10.1101/cshperspect.a003707.
- Wu, Z., M. Zhong, M. Li, H. Huang, J. Liao, A. Lu, K. Guo, N. Ma, J. Lin, J. Duan, L. Liu, F. Xu, Z. Zhong & J. Chen (2018) Mutation Analysis of Pre-mRNA Splicing Genes PRPF31, PRPF8, and SNRNP200 in Chinese Families with Autosomal Dominant Retinitis Pigmentosa. *Curr Mol Med*, 18, 287-294.
- Xi, X. H., D. Zheng, K. Xia, Q. Pan, L. Y. Lei, Z. Liu, C. Z. Tang, J. H. Xia, D. Y. Jiang & H. X. Deng (2005) [Splicing site mutation of D19S418 in PRPF-31 gene and its phenotypic characters with autosomal dominant retinitis pigmentosa]. *Zhonghua Yan Ke Za Zhi*, 41, 1020-6.
- Xia, K., D. Zheng, Q. Pan, Z. Liu, X. Xi, Z. Hu, H. Deng, X. Liu, D. Jiang, H. Deng & J. Xia (2004) A novel PRPF31 splice-site mutation in a Chinese family with autosomal dominant retinitis pigmentosa. *Mol Vis*, 10, 361-5.
- Xiao, X., Y. Cao, Z. Zhang, Y. Xu, Y. Zheng, L. J. Chen, C. P. Pang & H. Chen (2017) Novel Mutations in PRPF31 Causing Retinitis Pigmentosa Identified Using Whole-Exome Sequencing. *Investigative ophthalmology & visual science*, 58, 6342-6350.
- Xie, D., K. Peng, Q. Yi, W. Liu, Y. Yang, K. Sun, X. Zhu & F. Lu (2018) Targeted Next Generation Sequencing Revealed Novel PRPF31 Mutations in Autosomal Dominant Retinitis Pigmentosa. *Genet Test Mol Biomarkers*, 22, 425-432.
- Xu, F., R. Sui, X. Liang, H. Li, R. Jiang & F. Dong (2012) Novel PRPF31 mutations associated with Chinese autosomal dominant retinitis pigmentosa patients. *Molecular vision*, 18, 3021-xxx.
- Yang, L., X. Yin, L. Wu, N. Chen, H. Zhang, G. Li & Z. Ma (2013) Targeted exome capture and sequencing identifies novel PRPF31 mutations in autosomal dominant retinitis pigmentosa in Chinese families. *BMJ Open*, 3, e004030.
- Yang, Y., D. Tian, J. Lee, J. Zeng, H. Zhang, S. Chen, H. Guo, Z. Xiong, K. Xia, Z. Hu & J. Luo (2015) Clinical and genetic identification of a large chinese family with autosomal dominant retinitis pigmentosa. *Ophthalmic Genet*, 36, 64-9.
- Yin, J., J. Brocher, U. Fischer & C. Winkler (2011) Mutant Prpf31 causes pre-mRNA splicing defects and rod photoreceptor cell degeneration in a zebrafish model for Retinitis pigmentosa. *Molecular neurodegeneration*, 6, 56-1326-6-56.
- Yuan, L., M. Kawada, N. Havlioglu, H. Tang & J. Y. Wu (2005) Mutations in PRPF31 inhibit pre-mRNA splicing of rhodopsin gene and cause apoptosis of retinal cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25, 748-757.
- Zhang, N., R. Kaur, X. Lu, X. Shen, L. Li & R. J. Legerski (2005) The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *The Journal of biological chemistry*, 280, 40559-40567.
- Zhang, Q., M. Xu, J. D. Verriotto, Y. Li, H. Wang, L. Gan, B. L. Lam & R. Chen (2016) Next-generation sequencing-based molecular diagnosis of 35 Hispanic retinitis pigmentosa probands. *Scientific reports*, 6, 32792.
- Zhao, L., F. Wang, H. Wang, Y. Li, S. Alexander, K. Wang, C. E. Willoughby, J. E. Zaneveld, L. Jiang, Z. T. Soens, P. Earle, D. Simpson, G. Silvestri & R. Chen (2015) Next-generation sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Ireland. *Hum Genet*, 134, 217-30.
- Zheng, Y., H.-L. Wang, J.-K. Li, L. Xu, L. Tellier, X.-L. Li, X.-Y. Huang, W. Li, T.-T. Niu, H.-M. Yang, J.-G. Zhang & D.-N. Liu (2018) A novel mutation in PRPF31, causative of autosomal dominant

retinitis pigmentosa, using the BGISEQ-500 sequencer. *International journal of ophthalmology*, 11, 31-35.

Ziegler, A., E. Colin, D. Goudenège & D. Bonneau (2019) A snapshot of some pLI score pitfalls. *Human Mutation*, 40, 839-841.

Tables

exon	cDNA mutation	protein mutation	notes	Original references	Families (n)	Splicing	Frameshift	Nonsense	Missense	Inframe deletion	Inframe duplication	Indel	Large insertion or deletion	Age of onset	Age at diagnosis
exon 1 (non coding)															
intron 1	c.1-2481G>T		formerly: IVS1+1G>T	Liu et al., 2008(Liu et al., 2008)	1	1								3	20
exon 2 c.1-177	c.-3_7del	p.Met1?		Sullivan et al., 2013(Sullivan et al., 2013a); Kiser et al., 2019(Kiser et al., 2019)	2		2							10/17/29	10/58/62
	c.1A>T	p.Met1?		Carss et al., 2017(Carss et al., 2017)	1										
	c.18G>C	p.Glu6Asp		van Huet et al., 2015(van Huet et al., 2015)	1				1						
	c.19_20insA	p.Leu7Hisfs*4		Sullivan et al., 2013(Sullivan et al., 2013a)	1		1								
	c.34G>T	p.Glu12*		van Cauwenbergh et al., 2017(Van Cauwenbergh et al., 2017)	1		1								
	c.55del	p.Glu19Lysfs*46		Martin-Merida et al., 2018(Martin-Merida et al., 2018)	1		1								

	c.59_65del7	p.Gly20Alafs*43		Saini et al., 2012(Saini et al., 2012)	1		1										17
	c.79G>T	p.Glu27X		Waseem et al., 2007(Waseem et al., 2007)	1			1								15	43
	c.121C>G	p.Leu41Val	reported as cause of disease, but no functional studies	Ellingford et al., 2016(Ellingford et al., 2016a)	1				1								
	c.165G>A			de la Cerda et al., 2019(de la Cerda et al., 2019)	1												
	c.172A>T	p.Lys58X		Zheng et al., 2016(Zhang et al., 2016)	1												13
	c.196_197delAA	p.Lys66Aspfs*2		Xu et al., 2012(Xu et al., 2012)	1		1	1									24
intron 2	c.177+1G>A		formerly IVS2+1 G>A	Sullivan et al., 2006a(Sullivan et al., 2006a)	1	1											
	c.177+1delG			Rivolta et al., 2006(Rivolta et al., 2006)	1	1											
exon 3 c.178-238 (20aa)	c.217A>T	p.Lys73X		Eisenberger et al., 2013(Eisenberger et al., 2013)	1			1									
	c.220C>T	p.Gln74X		Sullivan et al., 2006a(Sullivan et al., 2006a); van Cauwenbergh et al., 2017(Van Cauwen	3			3								7	9

				bergh et al., 2017); Kiser et al., 2019(Kiser et al., 2019)													
intron 3																	
exon 4 239-322	c.267delA	p.Glu89Aspfs*11		Sullivan et al., 2013(Sullivan et al., 2013a)	1		1										
		p.Leu107del4 ctGAGT		Aleman et al., 2009(Aleman et al. 2009)	1		1									32	
	c.319C>G			Rivolta et al., 2006(Rivolta et al., 2006); Rio Frio et al., 2008(Rio Frio et al., 2008b)	2		2										
intron 4	c.322+4_322+7del	p.?	2 families in MM paper, 3 families in Zhang paper	Zhang et al., 2016(Zhang et al., 2016); Martin-Merida et al., 2018(Martin-Merida et al., 2018)	5		5									19/27	
	c.322+1G>A			Wu et al., 2018(Wu et al., 2018); Kiser et al., 2019(Kiser et al., 2019)	2		2										
	c.323-2A>G			Rivolta et al., 2006(Rivolta et al., 2006)	1		1										
exon 5 c.323-421	c.328_330del	p.Ile110del	Reported as p.Ile109del in de Sousa	de Sousa Dias et al., 2013(de Sousa	2						2						

	c.400delG	p.Asp134Ilefs		Ellingford et al., 2016(Ellingford et al. 2016a)	1	1											
	c.413C>A	Thr138Lys		Waseem et al, 2007(Waseem et al. 2007)	1				1							15,20	30
intron 5	c.421-2A>G			Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1	1											
	c.421-1G>A		formerly IVS5-1G>A	Xia et al., 2004(Xia et al. 2004); Xi et al., 2005(Xi et al. 2005)	2	2											
exon 6 c.421-527	c.421G>T	Glu141X		Sullivan et al., 2006a(Sullivan et al. 2006a)	1				1								
	c.433_434del	p.S145Pfs*8		Kurata et al., 2018(Kurata, Hosono and Hotta 2018)	1				1							7	7
	c.522_527+10del		Same family in these 2 papers	Ghazawy et al., 2007(Ghazawy et al. 2007); Buskin et al., 2018(Buskin et al. 2018)	1											30s	33
	c.525_526insAG			Kiser et al., 2019(Kiser et al. 2019)	1				1							16	47
intron 6	c.527+1G>A		Described as p.IVS 6+1G>T	Chakarova et al., 2006(Chakarova et al. 2006); Martin-Merida et al., 2018(Martin-Merida et al.	3	3										13/48/21	13/48

			2018); Abdulridha-Aboud et al., 2016(Abdulridha-Aboud et al., 2016)														
c.527+1G>T			Gandra et al., 2008(Gandra et al., 2008); Kiser et al., 2019(Kiser et al., 2019)	2	2												
c.527+2T>G			Wu et al., 2018(Wu et al., 2018)	1	1												
c.527+2T>C			Audo et al., 2010(Audo et al., 2010)	1	1												
c.527+3A>G		In 2 families in Waseem paper - Reported as IVS 6 +3 A>G in Ivings paper	Vithana et al., 2001(Vithana et al., 2001); Waseem et al., 2007(Waseem et al., 2007); Ivings et al., 2008(Ivings et al., 2008); Ellingford et al., 2016(Ellingford et al., 2016a); Xie et al., 2018(Xie et al., 2018); Kiser et al., 2019(Kiser et al., 2019)	7	7										20/29/28/27	52/30/70	
c.528 -3_45del			Vithana et al., 2001(Vithana et al., 2001);	2	2												

				Sato et al., 2005(Sato et al., 2005)													
	c.528-39_531del			Sullivan et al., 2013(Sullivan et al., 2013a)	1	1											
	c.528-1G>A			Waseem et al., 2007(Waseem et al., 2007); van Cauwenbergh et al., 2017(Van Cauwenbergh et al., 2017)	2	2											20
exon 7 c.528-697	c.541G>T	p.Glu181X	2 families in MM paper	Pomares et al., 2010(Pomares et al., 2010); van Cauwenbergh et al., 2017 (Van Cauwenbergh et al., 2017); Martin-Merida et al., 2018(Martin-Merida et al., 2018)	4			4									19, 23
	c.544_618del75bp	E182_E206del		Xu et al., 2012(Xu et al., 2012)	1					1							24
	c.547delG	p.E183fs		Xiao et al., 2017(Xiao et al., 2017)	1		1									5,6,7,8,10	
	c.548_580dup	p.Glu183_Met193dup		Tiwari et al., 2016(Tiwari et al., 2016)	1						1					24	
	c.550_552del	p.Leu184del		Kiser et al., 2019(Kiser et al., 2019)	1					1						71	71

c.553G>T	p.Glu185X	de novo in Neveling paper	Neveling et al., 2012(Neveling et al. 2012); van Huet et al., 2015(van Huet et al. 2015)	2			1										
c.562G>T	p.Glu188X		ClinVar (likely pathogenic)	1			1										
c.580_581delGC	p.Leu195GlyFs		ClinVar (likely pathogenic)	1		1											
c.580-581dup33bp			Vithana et al., 2001(Vithana et al. 2001)	1						1							
c.581C>A	Ala194Glu		Vithana et al., 2001(Vithana et al. 2001)	1				1									
c.590T>C	Leu197Pro		Bryant et al., 2018(Bryant et al. 2018); Wu et al., 2018(Wu et al. 2018)	2				2									
c.615C>A	p.Tyr205X		ClinVar (pathogenic)	1			1										
c.615C>G	p.Tyr205X		ClinVar (likely pathogenic)	1			1										
c.615delC	p.Y205X		Xu et al., 2012(Xu et al. 2012)	1			1										27
c.616G>T	p.Glu206X		Wang et al., 2014(Wang et al. 2014)	1			1										
c.629delC			Huang et al., 2015(Huang et al. 2015)	1		1											
c.636delG	p.Met212fs*238		Sullivan et al., 2006a(Sullivan et al.	3		3											

				2006a); Bowne et al., 2011(Bowne et al., 2011); Wang et al., 2014(Wang et al. 2014)													
	c.646G>C	Ala216Pro		Vithana et al., 2001(Vithana et al. 2001)	1				1								
	c.666_668del	p.Ile223del		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1				1								
	c.673del	p.Ala225Hisfs*14		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1		1										
intron 7	c.698-1G>A			Roberts et al., 2016(Roberts et al., 2016); Birtel et al., 2018(Birtel et al. 2018a)	2	2											
exon 8 c.698 - 855	c.709-734dup			Terray et al., 2017(Terray et al. 2017)	1				1								
	732-737delins20bp	M244fsX248		Martinez-Gimeno et al., 2003(Martinez-Gimeno et al. 2003)	1						1				6-20		
	c.736G>A	p.Ala246Thr		Xu et al., 2014(Xu et al., 2012); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	2				2								

c.741_742insA	p.Asn248Lys fs		ClinVar (likely pathogenic)	1		1										
c.758_767del	p.Gly253fs* 317		Sullivan et al., 2006a(S ullivan et al., 2006a); Kiser et al., 2019(Kis er et al., 2019)	2		2								19		31
c.763C>T	p.Gln255X		Wang et al., 2014(W ang et al. 2014)	1			1									
769-770insA	K257fs*277		Vithana et al., 2001(Vit hana et al., 2001); Martine z- Gimeno et al., 2003(M artinez- Gimeno et al., 2003)	2		2								10-18		
c.770dup	p.Thr258Asp fs*21		Vithana et al., 2001(Vit hana et al., 2001); Martin- Medira et al., 2018	2		2										
c.772_773del2ins CAACATGCAACAT CAT	p.(Thr258Gl nfs)		Zhao et al., 2015(Zh ao et al., 2015)	1		1										
c.781G>C	Gly261Arg		Xiao et al., 2017(Xia o et al., 2017)	1				1								
c.785delT	p.Phe262Ser fs*59		Lim et al., 2009(Li m et al., 2009)	1		1								<10		
c.804delG	p.L268fs		Xiao et al., 2017(Xia o et al., 2017)	1		1										
c.808_809insC	p.His270Prof s*8		Sullivan et al., 2013(Sul livan et	1		1										

				al. 2013a)													
	c.815G>T	p.Gly272Val	predi cted by Sulliv an to be benig n	Sullivan et la., 2006a(S ullivan et al., 2006a); Daiger et al., 2014(Da iger et al. 2014)	2					2							
	c.816_830delCTA CATCTACCACAG	p.Tyr273_Se r277del		Birtel et al., 2018(Bir tel et al. 2018b)	1					1							
	c.824_825insA	p.Y275X		Yang et al., 2013(Ya ng et al. 2013)	1			1								12	
	c.828_829del	p.His276Glnf s*2		Martine z- Gimeno et al., 2003(M artinez- Gimeno et al., 2003); Martin- Merida et al., 2018(M artin- Merida et al., 2018)	2		2									5-20	
	c.838_841dupGTG C	p.Gln281Arg fs*44		Carss et al., 2017(Ca rss et al. 2017)	1		1										
	c.839T>G	p.Val280Gl		Birtel et al., 2018(Bir tel et al. 2018b)	1					1							
intron 8	c.855+1G>C			Lu et al., 2005(Lu et al., 2005)	1	1											
	c.855+1G>T			Jespersg aard et al., 2019(Jes persgaar d et al., 2019)	1	1											
	c.855+1G>A			ClinVar (pathog enic)	1	1											

	c.856-2A>G			Rivolta et al., 2006(Rivolta et al. 2006)	1	1										
exon 9 c.856 - 945	c.862C>T	p.Arg288Trp		Coussa et al., 2015(Coussa et al. 2015)	1				1						66	68
	c.866_879delGGA AAGCGGCCCGG	p.R289Pfs*30		Villanueva et al., 2014(Villanueva et al. 2014); Zhang et al., 2016(Zhang et al. 2016)	2		2								2-16	7-63
	c.871G>C	Ala291Pro		Sullivan et al 2006a(Sullivan et al. 2006a)	1				1							
	c.877_910del	p.Arg293_Ar g304>Valfs* 17		Rivolta et al., 2006(Rivolta et al. 2006)	1		1									
	c.895T>C	Cys299Arg		Sullivan et al 2006a(Sullivan et al. 2006a); Xu et al., 2012(Xu et al. 2012); Martin-Merida et al., 2018(Martin-Merida et al. 2018); Kiser et al., 2019(Kiser et al. 2019)	4				4						21/27/ 41/63	27/44/ 63/65
	c.896G>A	p.Cys299Tyr		Bhatia et al., 2018(Bhatia et al. 2018)	1				1							
	c.910C>T	p.Arg304Cys		Huang et al., 2015(Huang et al. 2015); Hariri et al., 2018(Hariri et al. 2018)	2				2							45

	c.914_915insTGT	p.Val305_Asp306insVal		Utz et al., 2013(Utz et al., 2013)	1								1			30s	40s
	c.915_916insTGT	p.Val305_Asp306insCys		Sullivan et al., 2013(Sullivan et al., 2013a)	1								1				
	c.916G>A	p.Asp306Asn	reported as cause of disease, but functional studies not carried out	Ellingford et al., 2016(Ellingford et al., 2016a)	1					1							
		p.307fs*15		Lu et al., 2013(Lu et al., 2013)	1			1									
	c.939dup	p.Gly314Argfs*10		Fernandez-San Jose et al., 2015(Fernandez-San Jose et al., 2015); Martin-Merida et al., 2018(Martin-Merida et al., 2018)	2			2									
	c.940delG	p.Ala302Glnfs		ClinVar (pathogenic/likely pathogenic)	2			2									
intron 9	c.946-1 G>C			Bowne et al., 2011(Bowne et al., 2011); Daiger et al., 2014 (Daiger et al., 2014)	2		2										
exon 10 c.946 - 1073	c.950delG	p.Gly316Alafs*4		ClinVar (pathogenic)	1			1									

c.961A>T	p.Lys321X	Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1			1											
c.967G>T	p.Glu323X	Ellingford et al., 2016(Ellingford et al., 2016a)	1			1											
c.973G>T	Glu325X	Sullivan et al., 2006a(Sullivan et al., 2006a)	1			1											
c.978_982del	p.Lys327Argfs*146	van Cauwenbergh et al., 2017(Van Cauwenbergh et al., 2017)	1			1											
c.992G>A	p.Trp331X	ClinVar (pathogenic)	1			1											
c.994C>T	p.Gln332X	Ellingford et al., 2016(Ellingford et al., 2016a)	1			1											
c.997del	p.Glu333Serfs*5	Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1			1											
c.1015C>T	p.Q339X	Xie et al., 2018(Xie et al., 2018)	1			1											
c.1035_1036insGC	p.Pro346Argfs*18	Wu et al., 2018(Wu et al., 2018)	1			1											
c.1048C>T	p.Gln350X	Eisenberger et al., 2013(Eisenberger et al., 2013)	1			1											
c.1060C>T	p.Arg354X	Sullivan et al., 2013(Sullivan et al., 2013a); Ellingford et al.,	6			6										5/6/7/8/8/6/12	6/24

				2016(Ellingford et al. 2016a); Xiao et al., 2017(Xiao et al. 2017); Wu et al., 2018(Wu et al. 2018); Kurata et al., 2018(Kurata et al. 2018); Kiser et al., 2019(Kiser et al. 2019),												
intron 10	c.1067_1073+8del			Eisenberger et al., 2013(Eisenberger et al. 2013)	1	1										
	c.1073+1G>A			Sullivan et al., 2006a(Sullivan et al. 2006a); Kiser et al., 2019(Kiser et al. 2019)	2	2								28/40	9/12/14/40	
	c.1074-2 A>T	p.Tyr359Sfs* 29		Yang et al., 2013(Yang et al. 2013)	1	1								2-8		
	c.1074-1G>T	p.?		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1	1										
exon 11 c.1074-1146 (24aa)	c.1077C>A	p.Tyr359X		van Cauwenbergh et al., 2017(Van Cauwenbergh et al. 2017)	1			1								
	c.1084delA	p.Met362X		Sullivan et al., 2013(Sullivan et al. 2013a);	1			1						6	44	

				Kiser et al., 2019(Kiser et al., 2019)															
	c.1098delG	p.Leu366fs*1		Pan et al., 2014(Pan et al., 2014)	1		1									5		22	
	c.1115_1125del	p.Arg372Glnfs*99	Same family in Ivings and Buskin paper	Vithana et al., 2001(Vithana et al., 2001); Ivings et al., 2008(Ivings et al., 2008); Buskin et al., 2018(Buskin et al., 2018)	2		2											Severe at 47	
	c.1120C>T	p.Gln374X		Ellingford et al., 2016b(Ellingford et al., 2016b)	1			1											
	c.1129delC	p.Arg377Valfs*2		Carss et al., 2017(Carss et al., 2017)	1		1												
	c.1142delG	p.Gly381fs*30	in more than 4 Japanese families in Koya nagi paper	Sato et al., 2005(Sato et al., 2005); Taira et al., 2007(Taira, Nakazawa and Sato 2007); Koyanagi et al., 2019(Koyanagi et al., 2019)	6		6											30-45	
intron 11	c.1146+2T>C	p.?		Waseem et al., 2007(Waseem et al., 2007); Martin-Merida et al., 2018(Martin-Merida et al., 2018)	2	2												18, 20	

	c.1146+2T>A	p.?	Martin-Merida et al., 2018(Martin-Merida et al., 2018)	1	1												
exon 12 c.1147 - 1275	c.1155-1159delGGACG/in sAGGGATT	p.Asp386Gly fs*28	Sato et al., 2005(Sato et al., 2005); Sullivan et al., 2006a(Sullivan et al., 2006a)	2		1									20		45
	c.1190dup	p.His398Profs*77	Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1		1											
	c.1205C > A	p.Ser402X	McLennan et al., 2019(McLennan et al., 2019)	1			1										
	c.1215delG	p.G405fs*7	Dong et al., 2013(Dong et al., 2013)	1		1									9		9,22,73
	c.1222C>T	p.Arg408Trp	Xiao et al., 2017(Xiao et al., 2017)	1				1									
	c.1224dupG	p.Gln409Alafs*66	Wu et al., 2018(Wu et al., 2018)	1		1											
	c.1226_1227insA	p.Thr410Aspfs*65	Xie et al., 2018(Xie et al., 2018)	1													
	c.1234del	p.Val412X	Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1			1										
	c.1261_1262delTC	p.S421Qfs*53	Glockle et al., 2014(Glockle et al., 2014)	1		1											
	c.1273C>T	p.Gln425X	ClinVar (pathogenic)	1			1										

intron 12																			
exon 13 c.1276-1374	c.1291C>T	p.Gln431X		ClinVar (pathogenic)	1			1											
	c.1305T>A	p.Y435X		Huang et al., 2015(Huang et al. 2015)	1			1											
	c.1373A>T	p.Gln458Leu		Xiao et al. 2017(Xiao et al. 2017)	1				1										
intron 13	c.1374+654C>G		deep intronic	Rio Frio et al., 2009(Rio Frio et al. 2009)	1	1													
exon 14 c.1375-1492 (39aa)	c.1462_1472del	p.Lys488Argfs*75		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1		1												
Deletion	upstream			Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1										1				
Deletion	exons 1-14 (whole gene)			Ivings et al., 2008(Ivings et al. 2008); Bowne et al., 2011(Bowne et al. 2011); Eisenberger et al., 2013(Eisenberger et al. 2013); Almoguer et al., 2015(Almoguer et al. 2015); Hariri et al., 2018(Hariri et al. 2018); Martin-	6										6			18, severe at 65	

				Merida et al., 2018(Martin-Merida et al. 2018)												
30 kb deletion including putative promoter region of a novel gene OSCAR, the entire genomic content of genes NDUFA3, TFPT and most of the PRPF31 gene except for its terminal exon 14.				Abu-Safieh et al. 2006(Abu-Safieh et al. 2006)	1									1	6-30	
112 kb deletion encompassing over 90% of PRPF31 and five upstream genes: TFPT, OSCAR, NDUFA3, TARM-1, and VSTM-1				Rose et al., 2011(Rose et al. 2011)	1									1		

58.7kb deletion including TFPT, NDUFA3, OSCAR genes and 11 exons of the PRPF31		2 families in Sweden	Kohn et al., 2009(Kohn et al., 2009); Golovleva et al., 2010(Golovleva et al., 2010)	2										2		50s
Deletion	exon 1		Martin-Merida et al., 2018(Martin-Merida et al., 2018)	1										1		
Deletion	exons 1-3		Birtel et al., 2018(Birtel et al., 2018a)	1										1		
12kb deletion including exons 1-3 of PRPF31			Dong et al., 2013(Dong et al., 2013)	1										1		
Deletion	exons 1-5		Eisenberger et al., 2013(Eisenberger et al., 2013); Birtel et al., 2018(Birtel et al., 2018a)	2										2		
Deletion	Intron 1		Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1										1		
Deletion	exons 2-3		Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1										1		
Duplication	exons 2-5		Martin-Merida et al., 2018(Martin-Merida et al., 2018)	1										1		

Deletion	exons 2-5		Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1											1		
Deletion	exons 2-14		Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1											1		
Duplication	exons 4-5		Jespersgaard et al., 2019(Jespersgaard et al., 2019)	1											1		
Deletion	exons 4-13		Weisschuh et al., 2016(Weisschuh et al., 2016)	1											1		
Deletion	exon 9		Martin-Merida et al., 2018(Martin-Merida et al., 2018)	1											1		
Promoter mutation			Rose et al., 2012(Rose et al., 2012)	1											1		
Insertion/deletion	149 bp deleted/640 bp inserted	hg17 Deletion of 59,310,880-59,311,028/insertion of 59,292,594-59,291,955 reverse comp.	Sullivan et al., 2006b(Sullivan et al., 2006b)	1											1	14/16/25/46	37/46/50/52/77
Deletion	4.8 kb	hg 17 59,315,842-59,320,684	Sullivan et al., 2006b(Sullivan et al., 2006b)	1											1		
Deletion	11.3 kb	hg17 59,314,340-59,325,633	Sullivan et al., 2006b(Sullivan et al., 2006b)	1											1		
Deletion	32-42 kb	hg17 5' breakpoint: 59,290,949-59,295,848; ' breakpoint: 59,328,550-59,333,288	Sullivan et al., 2006b(Sullivan et al., 2006b)	1											1		

Deletion	>44.8 kb	hg17 5' breakpoint: <59,283,753 ; 3' breakpoint: 59,328,550–59,333,288		Sullivan et al., 2006b(Sullivan et al., 2006b)	1										1		
Deletion	19:54622548-54633842del11295			Carss et al., 2017(Carss et al., 2017)	1										1		
Deletion	19:54632279-54632481del203			Carss et al., 2017(Carss et al., 2017)	1										1		

Table 1. All reported pathogenic variants in *PRPF31* associated with adRP, from peer-reviewed publications and clinical variant database ClinVar (variants classified as pathogenic only). The location in cDNA, nature of the variant and impact on protein (if known) is included, alongside age of onset and age at diagnosis, where reported.

Figure legends

Figure 1. Schematic representation of the first four steps of pre-mRNA splicing by the major spliceosome, with *PRPF31* shown in red. In step 1, U1snRNP recognises and binds the splice donor site (the 5' splice site). In step 2, binding of U1snRNP to the splice donor site promotes the binding of U2snRNP to the branch site. Independently of this, the U4/U6.U5 tri-snRNP forms in the cell. In step 3, the U4/U6.U5 tri-snRNP is recruited to the pre-mRNA, where U6snRNP replaces U1snRNP. This forms the catalytically active spliceosome, which in step 4 cuts away the intron and joins the exons through two transesterification reactions.

Figure 2. Schematic representation of the protein and cDNA structure of *PRPF31*, showing major structural domains encoded by each exon.

Figure 3. Schematic representation *PRPF31* gene, with all reported pathogenic variants labelled above, and total numbers of variants in each intron and exon displayed as a bar chart below. This shows that exons 7 and 8 are most enriched for pathogenic variants.

Figure 4. (a) Box and whisker plots showing upper and lower limits, median and interquartile range of reported age of onset of RP patients with different types of variant in *PRPF31* (b) Box and whisker plots showing upper and lower limits, median and interquartile range of reported age of diagnosis of RP patients with different types of variant in *PRPF31*.

'Mutation spectrum of *PRPF31*, genotype-phenotype correlation in retinitis pigmentosa, and opportunities for therapy'

Highlights

- *PRPF31* is the second most common cause of autosomal dominant retinitis pigmentosa (adRP)
- *PRPF31* is under investigation as a target for gene therapy
- Here we present a comprehensive summary of all reported pathogenic variants in *PRPF31* to date, which is a useful resource for clinicians and diagnostic genetics labs, and researchers designing targeted therapies
- We delineate novel genotype-phenotype correlations which suggest that, except in cases of complete exon or complete gene deletion, dominant negative effects may contribute to the disease in addition to haploinsufficiency.
- This finding has important impacts on the suitability of gene augmentation approaches across all mutation types.
- This finding may aid prognosis of disease in *PRPF31*-associated RP patients

Mutation spectrum of *PRPF31*, genotype-phenotype correlation in retinitis pigmentosa, and opportunities for therapy

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Short title: *PRPF31* genetics, RP phenotype and therapy

Abstract

Pathogenic variants in pre-messenger RNA (pre-mRNA) splicing factor 31, *PRPF31*, are the second most common genetic cause of autosomal dominant retinitis pigmentosa (adRP) in most populations. This remains a completely untreatable and incurable form of blindness, and it can be difficult to predict the clinical course of disease. In order to design appropriate targeted therapies, a thorough understanding of the genetics and molecular mechanism of this disease is required. Here, we present the structure of the *PRPF31* gene and PRPF31 protein, current understanding of PRPF31 protein function and the full spectrum of all reported clinically relevant variants in *PRPF31*. We delineate the correlation between specific *PRPF31* genotype and RP phenotype, suggesting that, except in cases of complete gene deletion or large-scale deletions, dominant negative effects contribute to phenotype as well as haploinsufficiency. This has important impacts on design of targeted therapies, particularly the feasibility of gene augmentation as a broad approach for treatment of *PRPF31*-associated RP. We discuss other opportunities for therapy, including antisense oligonucleotide therapy and gene-independent approaches and offer future perspectives on treatment of this form of RP.

Introduction

Pre-mRNA splicing

Human pre-mRNA splicing factor 31 (PRPF31) is a component of the spliceosome, the huge macromolecular ribonucleoprotein (RNP) complex which catalyses the splicing of pre-messenger RNAs (pre-mRNAs) to remove introns and produce mature mRNAs.(Will and Luhrmann 2011)

Pre-mRNA splicing is a core function in all eukaryotic cells. The vast majority of genes have multiple exons and introns, and around 95% of these multiexon genes undergo alternative splicing.(Pan et al. 2008) Alternative splicing allows increased organism complexity without increasing genome size, and helps to explain the c-value paradox; the observation that phenotypic complexity in the eukaryotic domain is not proportional to genome size.

The spliceosome is composed of 5 small nuclear RNAs (snRNAs), U1-U5, and many proteins, together making 5 snRNPs. In the process of splicing, U1snRNP recognises and binds the splice donor site (the 5' splice site), and promotes the binding of U2snRNP to the branch site. Independently of this, the U4/U6.U5 tri-snRNP forms in the cell, and is recruited to the pre-mRNA, where U6snRNP replaces U1snRNP. This forms the catalytically active spliceosome, which cuts away the intron and joins the exons through two transesterification reactions (**Figure 1**).

PRPF31, splicing and retinal disease

The *S.cerevisiae* yeast homologue of PRPF31, Prp31, was cloned and identified as a key splicing factor in 1996(Weidenhammer et al. 1996), and later was shown to be essential for the association of the U4/U6.U5 tri-snRNP with pre-spliceosomes.(Weidenhammer, Ruiz-Noriega and Woolford 1997) It was subsequently found to play a role in both splicing and meiosis in *S.pombe*.(Bishop et al. 2000) Unexpectedly, in 2001, it was discovered that heterozygous pathogenic variants in *PRPF31* are associated with retinitis pigmentosa (RP), an inherited retinal dystrophy affecting 1:2000 to 1:3500 people worldwide.(Vithana et al. 2001) This was surprising because pre-mRNA splicing factors are highly conserved from yeast to man with a core function in all cells. Intuitively, it would be expected that a defect in a core spliceosomal protein should have an impact on all cells, not just retinal cells.

The original paper described seven different pathogenic variants in four families and three simplex cases. These included mutations in the region of the splice site, leading to inactivation of a splice acceptor site, inactivation of a splice donor site, two missense changes, three frameshift variants and an in-frame duplication.(Vithana et al. 2001)

Since then, and particularly since the advent of massively parallel sequencing technologies, it has become clear that pathogenic variants in *PRPF31* are a major cause of autosomal dominant RP (adRP). Indeed they are the second most common genetic cause of adRP in most populations, accounting for 6% of US cases,(Sullivan et al. 2013b) 8% of Spanish, French and French-Canadian cases,(Martin-Merida et al. 2018, Audo et al. 2010, Coussa et al. 2015) 8.9% of cases in North America,(Daiger et al. 2014) 10-11.1% of Chinese cases(Lim et al. 2009, Xu et al. 2012) and 10.5% of Belgian cases.(Van Cauwenbergh et al. 2017)

However, this is likely to be an underestimate due to non-penetrance of this form of RP (Rose and Bhattacharya 2016). It is common to see very variable severity of eye disease in different members of the same family with the same pathogenic *PRPF31* variant. Furthermore, obligate carriers may be totally asymptomatic, showing complete non-penetrance. This complicates attempts to co-segregate *PRPF31* variants with clinical disease and makes genetic diagnosis difficult, likely contributing to an underestimation of the prevalence of RP associated with *PRPF31* variants.

The genetic mechanism controlling incomplete penetrance remains unclear, but a fairly consistent observation of correlation between expression level of the non-mutant copy of *PRPF31* and disease severity has been reported.(Rio Frio et al. 2008b, Rio Frio et al. 2009, Rivolta et al. 2006).

This varied expression can be explained by a number of factors including:

- expression quantitative trait loci (eQTLs) (on ch.14q21-23) in trans with *PRPF31*(Rio Frio et al. 2008a)
- variable level of expression of *CNOT3*, a trans-acting epistatic factor which is genetically linked to *PRPF31* and regulates expression of *PRPF31*. *CNOT3* encodes a subunit of the Ccr4-not transcription complex, which binds to the promoter of *PRPF31* and represses transcription of *PRPF31*. An intronic variant in *CNOT3* determines its level of expression and thus how efficiently *PRPF31* expression is downregulated. The alleles of *CNOT3* inherited determine the expression of non-mutant *PRPF31* and thus whether a person will be affected by the disease.(Venturini et al. 2012, Rose et al. 2014)
- the number of minisatellite repeat elements (MSR1) adjacent to the *PRPF31* core promoter, which determines the level of transcriptional repression of the non-mutant *PRPF31*. 4 MSR1 copies are associated with higher non-mutant *PRPF31* expression and are found in non-symptomatic carriers only.(Rose et al. 2016)

On the basis of these observations, the mechanism of incomplete penetrance in this form of RP has been described as 'variant haploinsufficiency', in which the absence of a second wild-type *PRPF31* allele is sometimes sufficient to produce disease, and sometimes is not, depending on the nature of the mutant allele inherited *and* the nature of the wild-type allele inherited. So the severity of the resultant disease depends on both the type of mutant allele inherited (ie complete loss-of-function, gain-of-function or hypomorphic), the level at which this allele is expressed, and the level at which the wild-type allele is expressed (Rose and Bhattacharya 2016). This form of variant haploinsufficiency has only been described in a very few Mendelian disorders, making the mechanism of variable penetrance in this disease quite unique (Rose and Bhattacharya 2016).

***PRPF31* gene and *PRPF31* protein structure**

PRPF31 is a 16.3kb gene on chromosome 19 which encodes 9 different transcripts, 6 of which are protein coding. The largest, most widely expressed transcript consists of 14 exons; 1 non-coding and 13 coding, which produces a 499 amino acid protein of 55kDa in size, pre-mRNA splicing factor 31, *PRPF31*.

PRPF31 contains several important functional domains; the flexible loop, Nop domain, coiled-coil domain and tip. Recent advances in spectroscopy and microscopy methods such as NMR and cryo-electron microscopy have allowed accurate resolution of the crystal structure of proteins of the spliceosome, including *PRPF31*, in their native conformations at different points during splicing.(Agafonov et al. 2016, Bertram et al. 2017b, Bertram et al. 2017a, Haselbach et al. 2018) These studies have revealed that *PRPF31* contains a conserved Nop domain (residues 222-254 and 278-307), with regions for binding protein and RNA.(Liu et al. 2007) This Nop domain has relaxed sequence conservation in *PRPF31*, but it retains high specificity for binding U4 or U4atac and 15.5K protein. (Liu et al. 2007) The flexible loop (residues 256 – 265) protects the exposed C4' atoms of residues 37 and 38 from attack by free radicals, to protect the RNA without directly contacting it.(Liu et al. 2007) The protein also has several phosphorylation sites, clustered in the C-terminus.(Liu et al. 2007) *PRPF31* contains a nuclear localisation sequence, NLS, which allows it to be targeted to the nucleus after translation (**Figure 2**).

***PRPF31* protein function**

PRPF31 is required for tri-snRNP assembly in human cells.(Makarova et al. 2002) With *PRPF6*, *PRPF31* forms an essential connection between the U4/U6 and U5 snRNPs. siRNA knockdown of *PRPF31*

results in inhibition of tri-snRNP formation and nuclear accumulation of U5 mono-snRNPs and U4/U6 di-snRNPs containing U4/U6 proteins and the U4/U6 recycling factor p110.(Schaffert et al. 2004)

The specific function of PRPF31 in retinal cells remains less clear. It remains unclear whether the photoreceptor cells are the primary affected cells in RP associated with PRPF31, with a number of studies suggesting that the RPE is the primary affected tissue.(Farkas et al. 2014, Hamieh and Nandrot 2019, Valdés-Sánchez et al. 2019) Retinal cells are highly metabolically active, with a high demand for ATP and protein anabolism as around 10% of protein from photoreceptor outer segments is shed every day. Rates of metabolism in photoreceptors are similar to dividing tumour cells, and undergo extensive anaerobic glycolysis rather than oxidative phosphorylation to produce energy, in what is termed the 'Warburg effect'.(Ng et al. 2015, Rajala et al. 2016) The reliance on glycolysis seems to promote efficient protein anabolism in photoreceptors.(Chinchore et al. 2017) However, the photoreceptors still require mitochondria to produce a proportion of their ATP via oxidative phosphorylation.(Grenell et al. 2019) It has been postulated that photoreceptor cells have a greater demand for pre-mRNA splicing factors to meet this metabolic demand, but evidence to support this hypothesis is inconsistent. Some studies have reported higher levels of PRPF31 expression in retina than in other tissues(Cao et al. 2011) but other studies show a consistent level of expression in all tissues, with no significantly higher expression in retina or any other tissue.(Yuan et al. 2005)

Related to this elevated rate of oxidative phosphorylation, retinal cells are subject to much higher rates of oxidative damage, including UV-induced photooxidative damage, which may explain the retinal-specific phenotype of RP associated with pre-mRNA splicing factor mutations.(Comitato et al. 2007, Shinde et al. 2016, Jin et al. 2011, Schmidt-Kastner et al. 2008). In patients expressing mutant forms of pre-mRNA splicing factors, it has been shown that proteins have reduced solubility, which can lead to formation of protein aggregates, and it has been suggested that the environment of UV-induced photooxidative damage in the photoreceptors makes these cells specifically prone to degeneration.(Wheway et al. 2019, Valdés-Sánchez et al. 2019, Wilkie et al. 2006, Yin et al. 2011, Bryant et al. 2018) This splicing-independent disease mechanism is appealing because there is inconsistent evidence of splicing defects in cells carrying PRPF31 mutations. Studies seem to suggest that expression of mutant *PRPF31* affects splicing of some transcripts but not others.

Immunoprecipitation of splicing complexes from *PRPF31* mutant retinal cells showed that mutant PRPF31 proteins significantly inhibited pre-mRNA splicing of intron 3 in the rhodopsin (RHO) gene.(Yuan et al. 2005) In primary retinal cell cultures, expression of the mutant PRPF31 proteins reduced total RHO expression and caused apoptosis of rhodopsin-positive retinal cells.(Alagramam et al. 2001) In a study of patient lymphoblastoid cell lines, splicing efficiency of RPGR intron 9 was significantly decreased in *PRPF31* mutant cell lines but no consistent decrease in the splicing efficiency of U12 and noncanonical U2 introns was seen in *PRPF31* mutant cells.(Ivings et al. 2008) In a minigene study, assays using the RHO intron 3 minigene template revealed a direct negative effect on splicing efficiency of mutant PRPF31. However, no effect of the mutation on splicing efficiency could be detected using the longer GNAT1 minigene template or using a full-length RHO transcript, splicing of which had an efficiency of 100%. Similarly, no unspliced RHO transcripts could be detected in RNA from human retina.(Wilkie et al. 2008)

Using novel stem cell technologies, recent studies in retinal organoids and retinal pigment epithelium (RPE) derived from induced pluripotent stem cells (iPSCs) from patients with *PRPF31* mutations show decreased efficiency of splicing of E1A minigene.(Buskin et al. 2018) RPE from patient iPSCs also show a substantial downregulation of SART1, a U5 snRNP protein important for the formation of the pre-catalytic spliceosome, but no changes in the expression of the U5 protein PRPF8 or the U4/U6

protein PRPF4.(Buskin et al. 2018) In both RPE and retinal organoids derived from *PRPF31* patients, the most significantly mis-spliced genes were genes involved in pre-mRNA and alternative mRNA splicing via the spliceosome.(Buskin et al. 2018)

Alongside these findings, it was observed that retinal organoids from patients showed differential expression of actin cytoskeleton, ciliary membrane, primary cilium, photoreceptor inner and outer segment, axon terminal and phototransduction proteins. Furthermore, patient organoids showed an enrichment of mis-spliced centriole and microtubule organisation genes. This suggests that centriole and ciliogenesis and cilium function are all regulated by alternative splicing in the retina, and this is defective in patients carrying *PRPF31* mutations.(Buskin et al. 2018) These findings were confirmed in independent studies of splicing in *PRPF31* siRNA-treated human organotypic retinal cultures.(Azizzadeh Pormehr et al. 2019) This is in keeping with earlier studies from ourselves, and others, which showed that siRNA knockdown of pre-mRNA splicing factors including *PRPF31* has a specific and significant effect on ciliogenesis.(Wheway et al. 2015, Kim et al. 2016) Further investigation showed that these proteins localise to the base of the photoreceptor cilium, classifying these conditions as retinal ciliopathies.(Wheway et al. 2015) Recent work developing *PRPF31* gene augmentation therapy has shown rescue of ciliogenesis in *PRPF31*^{+/-} RPE cells derived from human patient iPSCs after expression of wild-type *PRPF31* delivered by an AAV vector, further suggesting that *PRPF31* plays a key role in regulating ciliogenesis in patients.(Brydon et al. 2019)

Further work is needed to understand the nature of the splicing factors' involvement in ciliogenesis and cilium function in the retina, and this work is ongoing. It is possible that *PRPF31* and other splicing factors have roles beyond splicing. Many proteins involved in splicing have multiple functions in the cell, such as the proteins of the PRP19 complex which have roles in ubiquitination (Vander Kooi et al. 2006), in DNA damage sensing (Grey et al. 1996, Marechal et al. 2014), DNA damage repair (Zhang et al. 2005), mRNA export (Chanarat, Seizl and Strasser 2011) and in mitotic spindle assembly (Hofmann et al. 2013). *PRPF31* has been shown to perform splicing-independent functions in mitotic chromosome segregation, although this would not explain disease phenotype in the post-mitotic retina. With deeper understanding of the molecular mechanism of *PRPF31* disease arise greater opportunities for developing effective targeted therapies.

***PRPF31* mutation spectrum**

In order to fully understand the molecular mechanism of RP associated with *PRPF31* variants, it is necessary to fully understand the genetics of this condition. This will aid accurate diagnostics, prognostics and development of targeted therapies. To this end, we have reviewed the literature and the major clinical variant database ClinVar to summarise all reported pathogenic variants in *PRPF31* (**Table 1**). Mutations are spread throughout the gene, but are most common in exons 6-10, particularly exons 7 and 8 (**Figure 3**).

The majority of reported mutations in *PRPF31* are presumed loss-of-function variants including frameshift (51 different variants reported in 70 different families), splice site (30 variants in 52 families), nonsense (30 variants in 40 families) or large-scale insertions or deletions (25 variants in 32 families), which are predicted to lead to complete loss of expression of protein from the affected allele. *PRPF31* is highly intolerant to loss-of-function with a probability of being loss-of-function intolerant (pLI) score of 0.98 (Lek et al. 2016). A pLI score of >0.9 indicates that a gene is intolerant of protein-truncating variation (Lek et al. 2016) and thus loss-of-function variants in *PRPF31* are highly likely to cause disease through a haploinsufficiency disease mechanism (discussed in more detail later). However, it is important to note that whilst frameshift, consensus splice site, nonsense and

large indel variants are often assumed to cause loss-of-function, this is not always the case, particularly when frameshift or nonsense variants are found in the final exon or C-terminal portion of the penultimate exon; transcripts from genes with such variants are likely to evade nonsense mediated decay (Ziegler et al. 2019). At least 3 frameshift or nonsense mutations in the final two exons of *PRPF31* have been reported as pathogenic, but functional study is required to confirm pathogenicity (Martin-Merida et al. 2018, Huang et al. 2015). Similarly, consensus splice site mutations are often also assumed to cause complete loss of wild-type protein expression from the affected allele, when in fact the complex mechanisms of alternative splicing may lead to production of a truncated protein, particularly if the splicing change produces an in-frame transcript. In several cases where mutations are assumed to be causing loss-of-function through haploinsufficiency, in addition to presumed loss-of-function variants, at least 19 missense variants have been reported in *PRPF31* as being pathogenic. Gene constraint metrics, which provide quantitative measures of the extent to which a gene can tolerate change, indicate that *PRPF31* gene is highly intolerant to missense variants ($Z = 3.27$) (Samocha et al. 2014, Lek et al. 2016). Missense mutations in *PRPF31* tend to reduce the solubility of protein so it does not translocate into nucleus efficiently after being translated in the cytoplasm (Deery et al. 2002, Bryant et al. 2018, Wheway et al. 2019), effectively leading to a loss of this protein. However, only 4 missense variants have been functionally studied *in vitro*, and a comprehensive study of reported missense variants is required to confirm the functional effect of pathogenic variants, and indeed the pathogenicity of reported variants. At least one variant originally described as a missense variant was later confirmed to be affecting splicing (Rio Frio et al. 2008b) and it is possible that other variants classified as missense, both recognised pathogenic and those currently considered non-pathogenic, may in fact be impacting upon splicing of *PRPF31*. Furthermore, non-synonymous rare variants may impact on splicing. It is therefore likely that the rate of pathogenic variants affecting splicing in *PRPF31* is underestimated.

Genotype-phenotype correlation

We reviewed the literature and recorded the age of onset of first symptoms, and age of diagnosis, where it was reported alongside specific genetic variants. Age of onset of first symptoms (usually night-blindness) is lowest in patients with nonsense, frameshift or indel variants, with median age of onset between 8 and 12 years of age. Patients with large deletions or splice variants tend to show first symptoms at a slightly later median age of 20-24. Patients with in-frame duplications, insertions or missense variants show the latest median age of onset of first symptoms, around 27 years of age (**Figure 4a**). The difference in age of onset between the different types of mutation is statistically significant (one-way ANOVA $p=5.76 \times 10^{-5}$).

We also recorded the age of diagnosis where it was reported alongside specific *PRPF31* genetic variants. In this case, patients with nonsense, frameshift or splice variants were diagnosed at a median age of 20-30 years (usually because of loss of peripheral vision alongside night blindness), whereas patients with missense variants, in-frame deletions or large deletions tended to be diagnosed between the ages of 45 and 50 (**Figure 4b**). The difference in age of diagnosis between the different types of mutation is statistically significant (one-way ANOVA $p=0.030$).

There is no significant correlation between location of the variant in the gene and age of onset of symptoms or age of diagnosis.

It is an interesting observation, made in several studies and confirmed here, that patients with large-scale deletions, including multi-exon and whole gene deletions have the latest age of diagnosis. There is a clear difference in age of diagnosis of patients with large-scale deletions compared to patients with nonsense mutations or splice mutations although this is not statistically significant after correction for multiple testing (two-tailed unpaired t-test $p=0.016$ and $p=0.032$ respectively, $p=0.24$ and $p=0.48$ respectively after Bonferroni correction) (**Figure 4b**). It could be postulated that there is an element of dominant negative effect at play in cases of nonsense, frameshift, indel, in-frame and

missense variants compared to large deletions. This is a feature of the disease which should be considered when designing targeted therapies. The abundance of loss-of-function mutations, including complete gene deletions, in *PRPF31* patients has led to a consensus view that haploinsufficiency is the disease mechanism in this form of RP.(Abu-Safieh et al. 2006, Rio Frio et al. 2008b, Rose and Bhattacharya 2016) This has influenced approaches for targeted therapies, namely gene augmentation approaches, which involve replacing a wild-type copy of the coding sequence of *PRPF31* into the subretinal space of patients. This may not be fully effective in patients with genetic variants which have a dominant negative effect as well as a haploinsufficiency effect, and as a result other approaches for treatment may need to be investigated. These findings are supported by other recent work which also proposes a combined haploinsufficiency and dominant-negative disease mechanism in disease associated with *PRPF31* mutations.(Valdés-Sánchez et al. 2019) Study of the *Prpf31*^{p.A216P/+} mouse has shown that heterozygous missense mutations in *Prpf31* lead to aggregation of both wild-type and mutant protein in the cytoplasm of the RPE cells of mice, leading to overexpression of HSP70 family proteins.(Valdés-Sánchez et al. 2019) This work suggests that overexpression of these HSP70 proteins may be a target for therapy in *PRPF31* patients, rather than targeting *PRPF31* itself.(Valdés-Sánchez et al. 2019)

Opportunities for therapies

- Gene augmentation therapy

As a result of the abundance of loss-of-function variants in *PRPF31* gene augmentation has been postulated as a potential therapeutic approach to treat this form of RP.(Hafler et al. 2016) The coding sequence of *PRPF31* is only 1.5kb, well within the limits of current gene therapy vectors, and a *PRPF31* heterozygous knockout mouse is available for study, although it only develops very late onset retinal degeneration which may be more characteristic of age-related macular degeneration than RP.(Farkas et al. 2014) Researchers have begun preparatory work to define pre-treatment characteristics of RP associated with *PRPF31* mutations in order to be able to assess the effectiveness of AAV-mediated *PRPF31* gene augmentation therapy.(Hafler et al. 2016) These researchers have also patented *PRPF31* gene therapy by AAV2 delivery (International Publication Number WO2016144892A1) and, shown rescue of key cellular disease phenotypes including phagocytosis, ciliogenesis, cell morphology and barrier function in mutant *PRPF31*^{+/-} RPE derived from patient iPSCs after deliver of *PRPF31*.(Brydon et al. 2019)

- Antisense oligonucleotide therapy

If the majority of genetic variants have some dominant negative effect, it is important to consider other potential therapeutic approaches. These include antisense oligonucleotides (ASOs) which can bind pre-mRNA or mRNA and modulate splicing of *PRPF31* pre-mRNA or inhibit translation of the mRNA. In addition, siRNAs, shRNAs or gapmer-style ASOs can be used to completely silence a gene, which when combined with gene augmentation could potentially correct a disease with dominant negative effects. This approach has been successfully applied to the treatment of RP associated with dominant negative *RHO* mutations.(Cideciyan et al. 2018) Splice-switching ASOs can be used to bind and mask deep intronic variants which introduce novel splice sites (such as the deep intronic variant in intron 13 reported in Rio Frio et al., 2009.(Rio Frio et al. 2009) Alternatively, they can be used to induce exon skipping of an in-frame exon (ie an exon with a multiple of 3 base pairs) carrying a frameshift or null variant, in order to remove this variant and restore the reading frame. Three of the fourteen exons in *PRPF31* have multiples of 3 base pairs; exons 3, 11 and 14 (**Figure 2, 3**). These are also relatively small exons, and do not encode functional important domains of the protein (**Figure 2**) so they could be targeted for skipping without removing large or functionally important regions of the protein. This could have the effect of reverting a severe, early-onset frameshift or nonsense variant

into a less severe splice or in-frame deletion variant, although the exon skipping would affect both alleles, mutant and normal, so the effect may be like having a homozygous exon deletion. According to the genotype-phenotype data in this study, this could delay age of onset from 8-10 years of age to 25 years of age or later. If this exon skipping approach led to a disease more like in-frame deletions, this could delay age of diagnosis (taken as a proxy for loss of peripheral vision) from 25-30 years of age to 47 years of age. This could potentially preserve vision in the working age of these individuals. This is a promising approach in theory, and such drugs are already being developed for a range of previously untreatable genetic conditions.(Scoles and Pulst 2019, Levin 2019, Khan et al. 2019). A clinically available splice-switching ASO drug (nusinersen) based on 2'-O-methoxyethyl phosphorothioate chemistry has been successfully developed for the treatment of the neurodegenerative disease spinal muscular atrophy (approved by NICE) and a similar type of drug (eteplirsen) utilising phosphorodiamidate morpholino chemistry has been developed for treatment of certain forms of Duchenne muscular dystrophy.(Finkel et al. 2017, Mendell et al. 2016) Intraocularly delivered ASO drugs are also currently undergoing clinical trials for a specific form of Leber congenital amaurosis caused by a *CEP290* deep intronic mutation (ClinicalTrials.gov NCT03140969). ASOs are highly versatile drugs, being sequence-specific in their action, titratable in dosage, and in the setting of a well-defined and enclosed target organ such as the eye, straightforward to deliver by direct intravitreal or subretinal injection. However, there are limited numbers of affected individuals who could be treated by targeting these regions of *PRPF31* (around 27 families).

- Gene independent approaches

As RP associated with *PRPF31* is so genetically diverse, (172 different reported variants in 240 different families or simplex cases) gene independent approaches are extremely attractive alternatives to gene therapies. These include stem cell therapies and bionic retinal implants. Stem cell therapies are both gene and disease-agnostic, and can replace lost retinal cells, whereas gene therapies can only recover function of intact cells. Stem cell therapies are closest to being effective in replacement of the retinal pigment epithelium (RPE), which has no neural connection. It is more challenging to regenerate functional neural retina. Recent studies have shown promising results in stem cell replacement of RPE for treatment of age-related macular degeneration.(da Cruz et al. 2018, Kashani et al. 2018) Bioinetic retinæ such as the Argus II(Finn, Grewal and Vajzovic 2018) are able to restore limited light and shape perception in people with end-stage retinal disease and limited to no remaining retinal function.

Conclusions and future perspective

Gene therapy offers real potential for treatment of a range of currently untreatable inherited retinal degenerations. As the second most common cause of adRP, and a relatively small gene, *PRPF31* is becoming a focus for gene augmentation therapy.(Brydon et al. 2019) This approach assumes a disease mechanism of haploinsufficiency, of which there is considerable evidence. However, new data presented here supports the recently proposed theory that, except in cases of complete exon or gene deletion, dominant negative effects may contribute to disease progression in RP associated with *PRPF31* variants, (Valdés-Sánchez et al. 2019) and that gene augmentation therapy may not be as effective in patients with missense, nonsense or splice mutations compared to whole exon or whole gene deletions. Whilst it is important to pursue these studies, data from knockout mice must be interpreted with caution when translating into human studies, and alternatively approaches must also be investigated. These include antisense oligonucleotide therapy targeting suitable exons, and gene-independent approaches. With several potential therapeutic approaches under investigation, there is real hope that treatment options for this disorder will be available to the next generation of patients.

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Author Contributions

GW undertook the literature review, collected and tabulated genotype and phenotype data and prepared figures. EG performed statistical analysis of data. GW, AD and DB wrote the manuscript.

References

- Abdulridha-Aboud, W., U. Kjellstrom, S. Andreasson & V. Ponjavic (2016) Characterization of macular structure and function in two Swedish families with genetically identified autosomal dominant retinitis pigmentosa. *Mol Vis*, 22, 362-73.
- Abu-Safieh, L., E. N. Vithana, I. Mantel, G. E. Holder, L. Pelosini, A. C. Bird & S. S. Bhattacharya (2006) A large deletion in the adRP gene PRPF31: evidence that haploinsufficiency is the cause of disease. *Molecular vision*, 12, 384-388.
- Agafonov, D. E., B. Kastner, O. Dybkov, R. V. Hofele, W. T. Liu, H. Urlaub, R. Luhrmann & H. Stark (2016) Molecular architecture of the human U4/U6.U5 tri-snRNP. *Science (New York, N.Y.)*, 351, 1416-1420.
- Alagramam, K. N., H. Yuan, M. H. Kuehn, C. L. Murcia, S. Wayne, C. R. Srisailpathy, R. B. Lowry, R. Knaus, L. Van Laer, F. P. Bernier, S. Schwartz, C. Lee, C. C. Morton, R. F. Mullins, A. Ramesh, G. Van Camp, G. S. Hageman, R. P. Woychik & R. J. Smith (2001) Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. *Hum Mol Genet*, 10, 1709-18.
- Aleman, T. S., B. L. Lam, A. V. Cideciyan, A. Sumaroka, E. A. Windsor, A. J. Roman, S. B. Schwartz, E. M. Stone & S. G. Jacobson (2009) Genetic heterogeneity in autosomal dominant retinitis pigmentosa with low-frequency damped electroretinographic wavelets. *Eye (Lond)*, 23, 230-3.
- Almoguera, B., J. Li, P. Fernandez-San Jose, Y. Liu, M. March, R. Pellegrino, R. Golhar, M. Corton, F. Blanco-Kelly, M. I. Lopez-Molina, B. Garcia-Sandoval, Y. Guo, L. Tian, X. Liu, L. Guan, J. Zhang, B. Keating, X. Xu, H. Hakonarson & C. Ayuso (2015) Application of Whole Exome Sequencing in Six Families with an Initial Diagnosis of Autosomal Dominant Retinitis Pigmentosa: Lessons Learned. *PLoS One*, 10, e0133624.
- Audo, I., K. Bujakowska, S. Mohand-Said, M. E. Lancelot, V. Moskova-Doumanova, N. H. Waseem, A. Antonio, J. A. Sahel, S. S. Bhattacharya & C. Zeitz (2010) Prevalence and novelty of PRPF31 mutations in French autosomal dominant rod-cone dystrophy patients and a review of published reports. *BMC medical genetics*, 11, 145-2350-11-145.
- Azizzadeh Pormehr, L., S. Ahmadian, N. Daftarian, S. A. Mousavi & M. Shafiezhadeh (2019) PRPF31 reduction causes mis-splicing of the phototransduction genes in human organotypic retinal culture. *Eur J Hum Genet*.
- Bertram, K., D. E. Agafonov, O. Dybkov, D. Haselbach, M. N. Leelaram, C. L. Will, H. Urlaub, B. Kastner, R. Luhrmann & H. Stark (2017a) Cryo-EM Structure of a Pre-catalytic Human Spliceosome Primed for Activation. *Cell*, 170, 701-713.e11.
- Bertram, K., D. E. Agafonov, W. T. Liu, O. Dybkov, C. L. Will, K. Hartmuth, H. Urlaub, B. Kastner, H. Stark & R. Luhrmann (2017b) Cryo-EM structure of a human spliceosome activated for step 2 of splicing. *Nature*, 542, 318-323.

- Bhatia, S., S. Goyal, I. R. Singh, D. Singh & V. Vanita (2018) A novel mutation in the PRPF31 in a North Indian adRP family with incomplete penetrance. *Documenta ophthalmologica. Advances in ophthalmology*.
- Birtel, J., T. Eisenberger, M. Gliem, P. L. Muller, P. Herrmann, C. Betz, D. Zahnleiter, C. Neuhaus, S. Lenzner, F. G. Holz, E. Mangold, H. J. Bolz & P. Charbel Issa (2018a) Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci Rep*, 8, 4824.
- Birtel, J., M. Gliem, E. Mangold, P. L. Muller, F. G. Holz, C. Neuhaus, S. Lenzner, D. Zahnleiter, C. Betz, T. Eisenberger, H. J. Bolz & P. Charbel Issa (2018b) Next-generation sequencing identifies unexpected genotype-phenotype correlations in patients with retinitis pigmentosa. *PLoS One*, 13, e0207958.
- Bishop, D. T., W. H. McDonald, K. L. Gould & S. L. Forsburg (2000) Isolation of an essential *Schizosaccharomyces pombe* gene, *prp31(+)*, that links splicing and meiosis. *Nucleic Acids Res*, 28, 2214-20.
- Bowne, S. J., L. S. Sullivan, D. C. Koboldt, L. Ding, R. Fulton, R. M. Abbott, E. J. Sodergren, D. G. Birch, D. H. Wheaton, J. R. Heckenlively, Q. Liu, E. A. Pierce, G. M. Weinstock & S. P. Daiger (2011) Identification of disease-causing mutations in autosomal dominant retinitis pigmentosa (adRP) using next-generation DNA sequencing. *Invest Ophthalmol Vis Sci*, 52, 494-503.
- Bryant, L., O. Lozynska, A. Marsh, T. E. Papp, L. van Gorder, L. W. Serrano, X. Gai, M. D. Maguire Am, T. S. Aleman & J. Bennett (2018) Identification of a novel pathogenic missense mutation in PRPF31 using whole exome sequencing: a case report. *The British journal of ophthalmology*.
- Brydon, E. M., R. Bronstein, A. Buskin, M. Lako, E. A. Pierce & R. Fernandez-Godino (2019) AAV-Mediated Gene Augmentation Therapy Restores Critical Functions in Mutant PRPF31. *Mol Ther Methods Clin Dev*, 15, 392-402.
- Buskin, A., L. Zhu, V. Chichagova, B. Basu, S. Mozaffari-Jovin, D. Dolan, A. Droop, J. Collin, R. Bronstein, S. Mehrotra, M. Farkas, G. Hilgen, K. White, K. T. Pan, A. Treumann, D. Hallam, K. Bialas, G. Chung, C. Mellough, Y. Ding, N. Krasnogor, S. Przyborski, S. Zwolinski, J. Al-Aama, S. Alharthi, Y. Xu, G. Whewey, K. Szymanska, M. McKibbin, C. F. Inglehearn, D. J. Elliott, S. Lindsay, R. R. Ali, D. H. Steel, L. Armstrong, E. Sernagor, H. Urlaub, E. Pierce, R. Luhrmann, S. N. Grellscheid, C. A. Johnson & M. Lako (2018) Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nat Commun*, 9, 4234.
- Cao, H., J. Wu, S. Lam, R. Duan, C. Newnham, R. S. Molday, J. J. Graziotto, E. A. Pierce & J. Hu (2011) Temporal and tissue specific regulation of RP-associated splicing factor genes PRPF3, PRPF31 and PRPC8--implications in the pathogenesis of RP. *PloS one*, 6, e15860.
- Carss, K. J., G. Arno, M. Erwood, J. Stephens, A. Sanchis-Juan, S. Hull, K. Megy, D. Grozeva, E. Dewhurst, S. Malka, V. Plagnol, C. Penkett, K. Stirrups, R. Rizzo, G. Wright, D. Josifova, M. Bitner-Glindzicz, R. H. Scott, E. Clement, L. Allen, R. Armstrong, A. F. Brady, J. Carmichael, M. Chitre, R. H. H. Henderson, J. Hurst, R. E. McLaren, E. Murphy, J. Paterson, E. Rosser, D. A. Thompson, E. Wakeling, W. H. Ouwehand, M. Michaelides, A. T. Moore, A. R. Webster & F. L. Raymond (2017) Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. *Am J Hum Genet*, 100, 75-90.
- Chakarova, C. F., S. Cherninkova, I. Tournev, N. Waseem, R. Kaneva, A. Jordanova, B. K. Veraitch, B. Gill, T. Colclough, A. Nakova, A. Oscar, V. Mihaylova, A. Nikolova-Hill, A. F. Wright, G. C. Black, S. Ramsden, I. Kremensky & S. S. Bhattacharya (2006) Molecular genetics of retinitis pigmentosa in two Romani (Gypsy) families. *Mol Vis*, 12, 909-14.
- Chanarat, S., M. Seizl & K. Strasser (2011) The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes. *Genes & development*, 25, 1147-1158.
- Chinchore, Y., T. Begaj, D. Wu, E. Drokhlyansky & C. L. Cepko (2017) Glycolytic reliance promotes anabolism in photoreceptors. *Elife*, 6.
- Cideciyan, A. V., R. Sudharsan, V. L. Dufour, M. T. Massengill, S. Iwabe, M. Swider, B. Lisi, A. Sumaroka, L. F. Marinho, T. Appelbaum, B. Rossmiller, W. W. Hauswirth, S. G. Jacobson, A. S. Lewin, G. D.

- Aguirre & W. A. Beltran (2018) Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc Natl Acad Sci U S A*, 115, E8547-e8556.
- Comitato, A., C. Spampinato, C. Chakarova, D. Sanges, S. S. Bhattacharya & V. Marigo (2007) Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells. *Human molecular genetics*, 16, 1699-1707.
- Coussa, R. G., C. Chakarova, R. Ajlan, M. Taha, C. Kavalec, J. Gomolin, A. Khan, I. Lopez, H. Ren, N. Waseem, K. Kamenarova, S. S. Bhattacharya & R. K. Koeneke (2015) Genotype and Phenotype Studies in Autosomal Dominant Retinitis Pigmentosa (adRP) of the French Canadian Founder Population. *Investigative ophthalmology & visual science*, 56, 8297-8305.
- da Cruz, L., K. Fynes, O. Georgiadis, J. Kerby, Y. H. Luo, A. Ahmado, A. Vernon, J. T. Daniels, B. Nommiste, S. M. Hasan, S. B. Gooljar, A. F. Carr, A. Vugler, C. M. Ramsden, M. Bictash, M. Fenster, J. Steer, T. Harbinson, A. Wilbrey, A. Tufail, G. Feng, M. Whitlock, A. G. Robson, G. E. Holder, M. S. Sagoo, P. T. Loudon, P. Whiting & P. J. Coffey (2018) Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. *Nat Biotechnol*, 36, 328-337.
- Daiger, S. P., S. J. Bowne, L. S. Sullivan, S. H. Blanton, G. M. Weinstock, D. C. Koboldt, R. S. Fulton, D. Larsen, P. Humphries, M. M. Humphries, E. A. Pierce, R. Chen & Y. Li (2014) Application of next-generation sequencing to identify genes and mutations causing autosomal dominant retinitis pigmentosa (adRP). *Adv Exp Med Biol*, 801, 123-9.
- de la Cerda, B., A. Diez-Lloret, B. Ponte, L. Valles-Saiz, S. M. Calado, E. Rodriguez-Bocanegra, A. B. Garcia-Delgado, M. Moya-Molina, S. S. Bhattacharya & F. J. Diaz-Corrales (2019) Generation and characterization of the human iPSC line CABI001-A from a patient with retinitis pigmentosa caused by a novel mutation in PRPF31 gene. *Stem Cell Res*, 36, 101426.
- de Sousa Dias, M., I. Hernan, B. Pascual, E. Borrás, B. Mane, M. J. Gamundi & M. Carballo (2013) Detection of novel mutations that cause autosomal dominant retinitis pigmentosa in candidate genes by long-range PCR amplification and next-generation sequencing. *Mol Vis*, 19, 654-64.
- Deery, E. C., E. N. Vithana, R. J. Newbold, V. A. Gallon, S. S. Bhattacharya, M. J. Warren, D. M. Hunt & S. E. Wilkie (2002) Disease mechanism for retinitis pigmentosa (RP11) caused by mutations in the splicing factor gene PRPF31. *Human molecular genetics*, 11, 3209-3219.
- Dong, B., J. Chen, X. Zhang, Z. Pan, F. Bai & Y. Li (2013) Two novel PRP31 premessenger ribonucleic acid processing factor 31 homolog mutations including a complex insertion-deletion identified in Chinese families with retinitis pigmentosa. *Mol Vis*, 19, 2426-35.
- Eisenberger, T., C. Neuhaus, A. O. Khan, C. Decker, M. N. Preising, C. Friedburg, A. Bieg, M. Gliem, P. Charbel Issa, F. G. Holz, S. M. Baig, Y. Hellenbroich, A. Galvez, K. Platzer, B. Wollnik, N. Laddach, S. R. Ghaffari, M. Rafati, E. Botzenhart, S. Tinschert, D. Borger, A. Bohring, J. Schreml, S. Kortge-Jung, C. Schell-Apacik, K. Bakur, J. Y. Al-Aama, T. Neuhann, P. Herkenrath, G. Nurnberg, P. Nurnberg, J. S. Davis, A. Gal, C. Bergmann, B. Lorenz & H. J. Bolz (2013) Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One*, 8, e78496.
- Ellingford, J. M., S. Barton, S. Bhaskar, J. O'Sullivan, S. G. Williams, J. A. Lamb, B. Panda, P. I. Sergouniotis, R. L. Gillespie, S. P. Daiger, G. Hall, T. Gale, I. C. Lloyd, P. N. Bishop, S. C. Ramsden & G. C. M. Black (2016a) Molecular findings from 537 individuals with inherited retinal disease. *J Med Genet*, 53, 761-767.
- Ellingford, J. M., S. Barton, S. Bhaskar, S. G. Williams, P. I. Sergouniotis, J. O'Sullivan, J. A. Lamb, R. Perveen, G. Hall, W. G. Newman, P. N. Bishop, S. A. Roberts, R. Leach, R. Tearle, S. Bayliss, S. C. Ramsden, A. H. Nemeth & G. C. Black (2016b) Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. *Ophthalmology*, 123, 1143-50.

- Farkas, M. H., D. S. Lew, M. E. Sousa, K. Bujakowska, J. Chatagnon, S. S. Bhattacharya, E. A. Pierce & E. F. Nandrot (2014) Mutations in pre-mRNA processing factors 3, 8, and 31 cause dysfunction of the retinal pigment epithelium. *The American journal of pathology*, 184, 2641-2652.
- Fernandez-San Jose, P., M. Corton, F. Blanco-Kelly, A. Avila-Fernandez, M. A. Lopez-Martinez, I. Sanchez-Navarro, R. Sanchez-Alcudia, R. Perez-Carro, O. Zurita, N. Sanchez-Bolivar, M. I. Lopez-Molina, B. Garcia-Sandoval, R. Riveiro-Alvarez & C. Ayuso (2015) Targeted Next-Generation Sequencing Improves the Diagnosis of Autosomal Dominant Retinitis Pigmentosa in Spanish Patients. *Invest Ophthalmol Vis Sci*, 56, 2173-82.
- Finkel, R. S., E. Mercuri, B. T. Darras, A. M. Connolly, N. L. Kuntz, J. Kirschner, C. A. Chiriboga, K. Saito, L. Servais, E. Tizzano, H. Topaloglu, M. Tulinius, J. Montes, A. M. Glanzman, K. Bishop, Z. J. Zhong, S. Gheuens, C. F. Bennett, E. Schneider, W. Farwell & D. C. De Vivo (2017) Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med*, 377, 1723-1732.
- Finn, A. P., D. S. Grewal & L. Vajzovic (2018) Argus II retinal prosthesis system: a review of patient selection criteria, surgical considerations, and post-operative outcomes. *Clin Ophthalmol*, 12, 1089-1097.
- Gandra, M., V. Anandula, V. Authiappan, S. Sundaramurthy, R. Raman, S. Bhattacharya & K. Govindasamy (2008) Retinitis pigmentosa: mutation analysis of RHO, PRPF31, RP1, and IMPDH1 genes in patients from India. *Mol Vis*, 14, 1105-13.
- Ghazawy, S., K. Springell, V. Gauba, M. A. McKibbin & C. F. Inglehearn (2007) Dominant retinitis pigmentosa phenotype associated with a new mutation in the splicing factor PRPF31. *Br J Ophthalmol*, 91, 1411-3.
- Glockle, N., S. Kohl, J. Mohr, T. Scheurenbrand, A. Sprecher, N. Weisschuh, A. Bernd, G. Rudolph, M. Schubach, C. Poloschek, E. Zrenner, S. Biskup, W. Berger, B. Wissinger & J. Neidhardt (2014) Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. *Eur J Hum Genet*, 22, 99-104.
- Golovleva, I., L. Kohn, M. Burstedt, S. Daiger & O. Sandgren (2010) Mutation spectra in autosomal dominant and recessive retinitis pigmentosa in northern Sweden. *Adv Exp Med Biol*, 664, 255-62.
- Grenell, A., Y. Wang, M. Yam, A. Swarup, T. L. Dilan, A. Hauer, J. D. Linton, N. J. Philp, E. Gregor, S. Zhu, Q. Shi, J. Murphy, T. Guan, D. Lohner, S. Kolandaivelu, V. Ramamurthy, A. F. X. Goldberg, J. B. Hurley & J. Du (2019) Loss of MPC1 reprograms retinal metabolism to impair visual function. *Proc Natl Acad Sci U S A*, 116, 3530-3535.
- Grey, M., A. Dusterhoft, J. A. Henriques & M. Brendel (1996) Allelism of PSO4 and PRP19 links pre-mRNA processing with recombination and error-prone DNA repair in *Saccharomyces cerevisiae*. *Nucleic acids research*, 24, 4009-4014.
- Hafler, B. P., J. Comander, C. Weigel DiFranco, E. M. Place & E. A. Pierce (2016) Course of Ocular Function in PRPF31 Retinitis Pigmentosa. *Semin Ophthalmol*, 31, 49-52.
- Hamieh, A. & E. F. Nandrot (2019) Retinal Pigment Epithelial Cells: The Unveiled Component in the Etiology of Prpf Splicing Factor-Associated Retinitis Pigmentosa. *Adv Exp Med Biol*, 1185, 227-231.
- Hariri, A. H., W. Gui, G. A. Datto O'Keefe, M. S. Ip, S. R. Sadda & M. B. Gorin (2018) Ultra-Widefield Fundus Autofluorescence Imaging of Patients with Retinitis Pigmentosa: A Standardized Grading System in Different Genotypes. *Ophthalmol Retina*, 2, 735-745.
- Haselbach, D., I. Komarov, D. E. Agafonov, K. Hartmuth, B. Graf, O. Dybkov, H. Urlaub, B. Kastner, R. Luhrmann & H. Stark (2018) Structure and Conformational Dynamics of the Human Spliceosomal B(act) Complex. *Cell*, 172, 454-464.e11.
- Hofmann, J. C., J. Tegha-Dunghu, S. Drager, C. L. Will, R. Luhrmann & O. J. Gruss (2013) The Prp19 complex directly functions in mitotic spindle assembly. *PLoS one*, 8, e74851.

- Huang, X. F., F. Huang, K. C. Wu, J. Wu, J. Chen, C. P. Pang, F. Lu, J. Qu & Z. B. Jin (2015) Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med*, 17, 271-8.
- Ivings, L., K. V. Towns, M. A. Martin, C. Taylor, F. Ponchel, R. J. Grainger, R. S. Ramesar, D. A. Mackey & C. F. Inglehearn (2008) Evaluation of splicing efficiency in lymphoblastoid cell lines from patients with splicing-factor retinitis pigmentosa. *Molecular vision*, 14, 2357-2366.
- Jespersgaard, C., M. Fang, M. Bertelsen, X. Dang, H. Jensen, Y. Chen, N. Bech, L. Dai, T. Rosenberg, J. Zhang, L. B. Moller, Z. Tumer, K. Brondum-Nielsen & K. Gronskov (2019) Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. *Sci Rep*, 9, 1219.
- Jin, Z. B., S. Okamoto, F. Osakada, K. Homma, J. Assawachananont, Y. Hirami, T. Iwata & M. Takahashi (2011) Modeling retinal degeneration using patient-specific induced pluripotent stem cells. *PloS one*, 6, e17084.
- Kashani, A. H., J. S. Lebkowski, F. M. Rahhal, R. L. Avery, H. Salehi-Had, W. Dang, C. M. Lin, D. Mitra, D. Zhu, B. B. Thomas, S. T. Hikita, B. O. Pennington, L. V. Johnson, D. O. Clegg, D. R. Hinton & M. S. Humayun (2018) A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration. *Sci Transl Med*, 10.
- Khan, N., H. Eliopoulos, L. Han, T. B. Kinane, L. P. Lowes, J. R. Mendell, H. Gordish-Dressman, E. K. Henricson & C. M. McDonald (2019) Eteplirsen Treatment Attenuates Respiratory Decline in Ambulatory and Non-Ambulatory Patients with Duchenne Muscular Dystrophy. *J Neuromuscul Dis*, 6, 213-225.
- Kim, J. H., S. M. Ki, J. G. Joung, E. Scott, S. Heynen-Genel, P. Aza-Blanc, C. H. Kwon, J. Kim, J. G. Gleeson & J. E. Lee (2016) Genome-wide screen identifies novel machineries required for both ciliogenesis and cell cycle arrest upon serum starvation. *Biochim Biophys Acta*, 1863, 1307-18.
- Kiser, K., K. D. Webb-Jones, S. J. Bowne, L. S. Sullivan, S. P. Daiger & D. G. Birch (2019) Time Course of Disease Progression of PRPF31-mediated Retinitis Pigmentosa. *Am J Ophthalmol*, 200, 76-84.
- Kohn, L., S. J. Bowne, S. S. L, S. P. Daiger, M. S. Burstedt, K. Kadzhaev, O. Sandgren & I. Golovleva (2009) Breakpoint characterization of a novel approximately 59 kb genomic deletion on 19q13.42 in autosomal-dominant retinitis pigmentosa with incomplete penetrance. *Eur J Hum Genet*, 17, 651-5.
- Koyanagi, Y., M. Akiyama, K. M. Nishiguchi, Y. Momozawa, Y. Kamatani, S. Takata, C. Inai, Y. Iwasaki, M. Kumano, Y. Murakami, K. Omodaka, T. Abe, S. Komori, D. Gao, T. Hirakata, K. Kurata, K. Hosono, S. Ueno, Y. Hotta, A. Murakami, H. Terasaki, Y. Wada, T. Nakazawa, T. Ishibashi, Y. Ikeda, M. Kubo & K. H. Sonoda (2019) Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. *J Med Genet*, 56, 662-670.
- Kurata, K., K. Hosono & Y. Hotta (2018) Long-term clinical course of 2 Japanese patients with PRPF31-related retinitis pigmentosa. *Jpn J Ophthalmol*, 62, 186-193.
- Lek, M., K. J. Karczewski, E. V. Minikel, K. E. Samocha, E. Banks, T. Fennell, A. H. O'Donnell-Luria, J. S. Ware, A. J. Hill, B. B. Cummings, T. Tukiainen, D. P. Birnbaum, J. A. Kosmicki, L. E. Duncan, K. Estrada, F. Zhao, J. Zou, E. Pierce-Hoffman, J. Berghout, D. N. Cooper, N. Deflaux, M. DePristo, R. Do, J. Flannick, M. Fromer, L. Gauthier, J. Goldstein, N. Gupta, D. Howrigan, A. Kiezun, M. I. Kurki, A. L. Moonshine, P. Natarajan, L. Orozco, G. M. Peloso, R. Poplin, M. A. Rivas, V. Ruano-Rubio, S. A. Rose, D. M. Ruderfer, K. Shakir, P. D. Stenson, C. Stevens, B. P. Thomas, G. Tiao, M. T. Tusie-Luna, B. Weisburd, H.-H. Won, D. Yu, D. M. Altshuler, D. Ardissino, M. Boehnke, J. Danesh, S. Donnelly, R. Elosua, J. C. Florez, S. B. Gabriel, G. Getz, S. J. Glatt, C. M. Hultman, S. Kathiresan, M. Laakso, S. McCarroll, M. I. McCarthy, D. McGovern, R. McPherson, B. M. Neale, A. Palotie, S. M. Purcell, D. Saleheen, J. M. Scharf, P. Sklar, P. F. Sullivan, J. Tuomilehto, M. T. Tsuang, H. C. Watkins, J. G. Wilson, M. J. Daly, D. G. MacArthur & C. Exome Aggregation (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, 536, 285-291.
- Levin, A. A. (2019) Treating Disease at the RNA Level with Oligonucleotides. *N Engl J Med*, 380, 57-70.

- Lim, K. P., S. P. Yip, S. C. Cheung, K. W. Leung, S. T. Lam & C. H. To (2009) Novel PRPF31 and PRPH2 mutations and co-occurrence of PRPF31 and RHO mutations in Chinese patients with retinitis pigmentosa. *Arch Ophthalmol*, 127, 784-90.
- Liu, J. Y., X. Dai, J. Sheng, X. Cui, X. Wang, X. Jiang, X. Tu, Z. Tang, Y. Bai, M. Liu & Q. K. Wang (2008) Identification and functional characterization of a novel splicing mutation in RP gene PRPF31. *Biochem Biophys Res Commun*, 367, 420-6.
- Liu, S., P. Li, O. Dybkov, S. Nottrott, K. Hartmuth, R. Luhrmann, T. Carlomagno & M. C. Wahl (2007) Binding of the human Prp31 Nop domain to a composite RNA-protein platform in U4 snRNP. *Science (New York, N.Y.)*, 316, 115-120.
- Lu, F., L. Huang, C. Lei, G. Sha, H. Zheng, X. Liu, J. Yang, Y. Shi, Y. Lin, B. Gong, X. Zhu, S. Ma, L. Qiao, H. Lin, J. Cheng & Z. Yang (2013) A novel PRPF31 mutation in a large Chinese family with autosomal dominant retinitis pigmentosa and macular degeneration. *PLoS One*, 8, e78274.
- Lu, S. S., C. Zhao, Y. Cui, N. D. Li, X. M. Zhang & K. X. Zhao (2005) [Novel splice-site mutation in the pre-mRNA splicing gene PRPF31 in a Chinese family with autosomal dominant retinitis pigmentosa]. *Zhonghua Yan Ke Za Zhi*, 41, 305-11.
- Makarova, O. V., E. M. Makarov, S. Liu, H. P. Vornlocher & R. Luhrmann (2002) Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6*U5 tri-snRNP formation and pre-mRNA splicing. *The EMBO journal*, 21, 1148-1157.
- Marechal, A., J. M. Li, X. Y. Ji, C. S. Wu, S. A. Yazinski, H. D. Nguyen, S. Liu, A. E. Jimenez, J. Jin & L. Zou (2014) PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR activation via a ubiquitin-mediated circuitry. *Molecular cell*, 53, 235-246.
- Martin-Merida, I., D. Aguilera-Garcia, P. Fernandez-San Jose, F. Blanco-Kelly, O. Zurita, B. Almoguera, B. Garcia-Sandoval, A. Avila-Fernandez, A. Arteche, P. Minguez, M. Carballo, M. Corton & C. Ayuso (2018) Toward the Mutational Landscape of Autosomal Dominant Retinitis Pigmentosa: A Comprehensive Analysis of 258 Spanish Families. *Investigative ophthalmology & visual science*, 59, 2345-2354.
- Martinez-Gimeno, M., M. J. Gamundi, I. Hernan, M. Maseras, E. Milla, C. Ayuso, B. Garcia-Sandoval, M. Beneyto, C. Vilela, M. Baiget, G. Antinolo & M. Carballo (2003) Mutations in the pre-mRNA splicing-factor genes PRPF3, PRPF8, and PRPF31 in Spanish families with autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 44, 2171-7.
- McLennan, S., D. Zhang, X. Zhang, S. C. Chen, T. Lamey, J. A. Thompson, T. McLaren, J. N. De Roach, S. Fletcher & F. K. Chen (2019) Generation of two induced pluripotent stem cell lines from a patient with dominant PRPF31 mutation and a related non-penetrant carrier. *Stem Cell Res*, 34, 101357.
- Mendell, J. R., N. Goemans, L. P. Lowes, L. N. Alfano, K. Berry, J. Shao, E. M. Kaye & E. Mercuri (2016) Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol*, 79, 257-71.
- Neveling, K., R. W. Collin, C. Gilissen, R. A. van Huet, L. Visser, M. P. Kwint, S. J. Gijzen, M. N. Zonneveld, N. Wieskamp, J. de Ligt, A. M. Siemiatkowska, L. H. Hoefsloot, M. F. Buckley, U. Kellner, K. E. Branham, A. I. den Hollander, A. Hoischen, C. Hoyng, B. J. Klevering, L. I. van den Born, J. A. Veltman, F. P. Cremers & H. Scheffer (2012) Next-generation genetic testing for retinitis pigmentosa. *Hum Mutat*, 33, 963-72.
- Ng, S. K., J. P. Wood, G. Chidlow, G. Han, T. Kittipassorn, D. J. Peet & R. J. Casson (2015) Cancer-like metabolism of the mammalian retina. *Clin Exp Ophthalmol*, 43, 367-76.
- Pan, Q., O. Shai, L. J. Lee, B. J. Frey & B. J. Blencowe (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet*, 40, 1413-5.
- Pan, X., X. Chen, X. Liu, X. Gao, X. Kang, Q. Xu, X. Chen, K. Zhao, X. Zhang, Q. Chu, X. Wang & C. Zhao (2014) Mutation analysis of pre-mRNA splicing genes in Chinese families with retinitis pigmentosa. *Mol Vis*, 20, 770-9.

- Pomares, E., M. Riera, J. Permanyer, P. Mendez, J. Castro-Navarro, A. Andres-Gutierrez, G. Marfany & R. Gonzalez-Duarte (2010) Comprehensive SNP-chip for retinitis pigmentosa-Leber congenital amaurosis diagnosis: new mutations and detection of mutational founder effects. *Eur J Hum Genet*, 18, 118-24.
- Rajala, R. V., A. Rajala, C. Kooker, Y. Wang & R. E. Anderson (2016) The Warburg Effect Mediator Pyruvate Kinase M2 Expression and Regulation in the Retina. *Sci Rep*, 6, 37727.
- Rio Frio, T., N. Civic, A. Ransijn, J. S. Beckmann & C. Rivolta (2008a) Two trans-acting eQTLs modulate the penetrance of PRPF31 mutations. *Hum Mol Genet*, 17, 3154-65.
- Rio Frio, T., T. L. McGee, N. M. Wade, C. Iseli, J. S. Beckmann, E. L. Berson & C. Rivolta (2009) A single-base substitution within an intronic repetitive element causes dominant retinitis pigmentosa with reduced penetrance. *Hum Mutat*, 30, 1340-7.
- Rio Frio, T., N. M. Wade, A. Ransijn, E. L. Berson, J. S. Beckmann & C. Rivolta (2008b) Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. *The Journal of clinical investigation*, 118, 1519-1531.
- Rivolta, C., T. L. McGee, T. Rio Frio, R. V. Jensen, E. L. Berson & T. P. Dryja (2006) Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations. *Human mutation*, 27, 644-653.
- Roberts, L., R. Ratnapriya, M. du Plessis, V. Chaitankar, R. S. Ramesar & A. Swaroop (2016) Molecular Diagnosis of Inherited Retinal Diseases in Indigenous African Populations by Whole-Exome Sequencing. *Investigative ophthalmology & visual science*, 57, 6374-6381.
- Rose, A. M. & S. S. Bhattacharya (2016) Variant haploinsufficiency and phenotypic non-penetrance in PRPF31-associated retinitis pigmentosa. *Clin Genet*, 90, 118-26.
- Rose, A. M., R. Mukhopadhyay, A. R. Webster, S. S. Bhattacharya & N. H. Waseem (2011) A 112 kb deletion in chromosome 19q13.42 leads to retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 52, 6597-603.
- Rose, A. M., A. Z. Shah, G. Venturini, A. Krishna, A. Chakravarti, C. Rivolta & S. S. Bhattacharya (2016) Transcriptional regulation of PRPF31 gene expression by MSR1 repeat elements causes incomplete penetrance in retinitis pigmentosa. *Scientific reports*, 6, 19450.
- Rose, A. M., A. Z. Shah, G. Venturini, C. Rivolta, G. E. Rose & S. S. Bhattacharya (2014) Dominant PRPF31 mutations are hypostatic to a recessive CNOT3 polymorphism in retinitis pigmentosa: a novel phenomenon of "linked trans-acting epistasis". *Annals of Human Genetics*, 78, 62-71.
- Rose, A. M., A. Z. Shah, N. H. Waseem, C. F. Chakarova, G. Alfano, R. G. Coussa, R. Ajlan, R. K. Koenekoop & S. S. Bhattacharya (2012) Expression of PRPF31 and TFPT: regulation in health and retinal disease. *Hum Mol Genet*, 21, 4126-37.
- Saini, S., P. N. Robinson, J. R. Singh & V. Vanita (2012) A novel 7 bp deletion in PRPF31 associated with autosomal dominant retinitis pigmentosa with incomplete penetrance in an Indian family. *Exp Eye Res*, 104, 82-8.
- Samocha, K. E., E. B. Robinson, S. J. Sanders, C. Stevens, A. Sabo, L. M. McGrath, J. A. Kosmicki, K. Rehnström, S. Mallick, A. Kirby, D. P. Wall, D. G. MacArthur, S. B. Gabriel, M. DePristo, S. M. Purcell, A. Palotie, E. Boerwinkle, J. D. Buxbaum, E. H. Cook, R. A. Gibbs, G. D. Schellenberg, J. S. Sutcliffe, B. Devlin, K. Roeder, B. M. Neale & M. J. Daly (2014) A framework for the interpretation of de novo mutation in human disease. *Nature Genetics*, 46, 944-950.
- Sato, H., Y. Wada, T. Itabashi, M. Nakamura, M. Kawamura & M. Tamai (2005) Mutations in the pre-mRNA splicing gene, PRPF31, in Japanese families with autosomal dominant retinitis pigmentosa. *Am J Ophthalmol*, 140, 537-40.
- Schaffert, N., M. Hossbach, R. Heintzmann, T. Achsel & R. Luhrmann (2004) RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies. *The EMBO journal*, 23, 3000-3009.
- Schmidt-Kastner, R., H. Yamamoto, D. Hamasaki, H. Yamamoto, J. M. Parel, C. Schmitz, C. K. Dorey, J. C. Blanks & M. N. Preising (2008) Hypoxia-regulated components of the U4/U6.U5 tri-small

- nuclear riboprotein complex: possible role in autosomal dominant retinitis pigmentosa. *Molecular vision*, 14, 125-135.
- Scoles, D. R. & S. M. Pulst (2019) Antisense therapies for movement disorders. *Mov Disord*, 34, 1112-1119.
- Shinde, V., P. Kotla, C. Strang & M. Gorbatyuk (2016) Unfolded protein response-induced dysregulation of calcium homeostasis promotes retinal degeneration in rat models of autosomal dominant retinitis pigmentosa. *Cell death & disease*, 7, e2085.
- Sullivan, L. S., S. J. Bowne, D. G. Birch, D. Hughbanks-Wheaton, J. R. Heckenlively, R. A. Lewis, C. A. Garcia, R. S. Ruiz, S. H. Blanton, H. Northrup, A. I. Gire, R. Seaman, H. Duzkale, C. J. Spellicy, J. Zhu, S. P. Shankar & S. P. Daiger (2006a) Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. *Invest Ophthalmol Vis Sci*, 47, 3052-64.
- Sullivan, L. S., S. J. Bowne, M. J. Reeves, D. Blain, K. Goetz, V. Ndifor, S. Vitez, X. Wang, S. J. Tumminia & S. P. Daiger (2013a) Prevalence of mutations in eyeGENE probands with a diagnosis of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 54, 6255-61.
- Sullivan, L. S., S. J. Bowne, C. R. Seaman, S. H. Blanton, R. A. Lewis, J. R. Heckenlively, D. G. Birch, D. Hughbanks-Wheaton & S. P. Daiger (2006b) Genomic rearrangements of the PRPF31 gene account for 2.5% of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 47, 4579-88.
- Taira, K., M. Nakazawa & M. Sato (2007) Mutation c. 1142 del G in the PRPF31 gene in a family with autosomal dominant retinitis pigmentosa (RP11) and its implications. *Jpn J Ophthalmol*, 51, 45-8.
- Terray, A., V. Fort, A. Slembrouck, C. Nanteau, J. A. Sahel, S. Reichman, I. Audo & O. Goureau (2017) Establishment of an induced pluripotent stem (iPS) cell line from dermal fibroblasts of an asymptomatic patient with dominant PRPF31 mutation. *Stem Cell Res*, 25, 26-29.
- Tiwari, A., J. Lemke, J. Altmueller, H. Thiele, E. Glaus, J. Fleischhauer, P. Nurnberg, J. Neidhardt & W. Berger (2016) Identification of Novel and Recurrent Disease-Causing Mutations in Retinal Dystrophies Using Whole Exome Sequencing (WES): Benefits and Limitations. *PLoS One*, 11, e0158692.
- Utz, V. M., C. D. Beight, M. J. Marino, S. A. Hagstrom & E. I. Traboulsi (2013) Autosomal dominant retinitis pigmentosa secondary to pre-mRNA splicing-factor gene PRPF31 (RP11): review of disease mechanism and report of a family with a novel 3-base pair insertion. *Ophthalmic Genet*, 34, 183-8.
- Valdés-Sánchez, L., S. M. Calado, B. de la Cerda, A. Aramburu, A. B. García-Delgado, S. Massalini, A. Montero-Sánchez, V. Bhatia, E. Rodríguez-Bocanegra, A. Díez-Lloret, D. Rodríguez-Martínez, C. Chakarova, S. S. Bhattacharya & F. J. Díaz-Corrales (2019) Retinal pigment epithelium degeneration caused by aggregation of PRPF31 and the role of HSP70 family of proteins. *Mol Med*, 26, 1.
- Van Cauwenbergh, C., F. Coppieters, D. Roels, S. De Jaegere, H. Flipts, J. De Zaeytijd, S. Walraedt, C. Claes, E. Fransen, G. Van Camp, F. Depasse, I. Casteels, T. de Ravel, B. P. Leroy & E. De Baere (2017) Mutations in Splicing Factor Genes Are a Major Cause of Autosomal Dominant Retinitis Pigmentosa in Belgian Families. *PloS one*, 12, e0170038.
- van Huet, R. A., L. H. Pierrache, M. A. Meester-Smoor, C. C. Klaver, L. I. van den Born, C. B. Hoyng, I. J. de Wijs, R. W. Collin, L. H. Hoefsloot & B. J. Klevering (2015) The efficacy of microarray screening for autosomal recessive retinitis pigmentosa in routine clinical practice. *Mol Vis*, 21, 461-76.
- Vander Kooi, C. W., M. D. Ohi, J. A. Rosenberg, M. L. Oldham, M. E. Newcomer, K. L. Gould & W. J. Chazin (2006) The Prp19 U-box crystal structure suggests a common dimeric architecture for a class of oligomeric E3 ubiquitin ligases. *Biochemistry*, 45, 121-130.

- Venturini, G., A. M. Rose, A. Z. Shah, S. S. Bhattacharya & C. Rivolta (2012) CNOT3 is a modifier of PRPF31 mutations in retinitis pigmentosa with incomplete penetrance. *PLoS genetics*, 8, e1003040.
- Villanueva, A., J. R. Willer, J. Bryois, E. T. Dermitzakis, N. Katsanis & E. E. Davis (2014) Whole exome sequencing of a dominant retinitis pigmentosa family identifies a novel deletion in PRPF31. *Invest Ophthalmol Vis Sci*, 55, 2121-9.
- Vithana, E. N., L. Abu-Safieh, M. J. Allen, A. Carey, M. Papaioannou, C. Chakarova, M. Al-Magthteh, N. D. Ebenezer, C. Willis, A. T. Moore, A. C. Bird, D. M. Hunt & S. S. Bhattacharya (2001) A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). *Molecular cell*, 8, 375-381.
- Wang, F., H. Wang, H. F. Tuan, D. H. Nguyen, V. Sun, V. Keser, S. J. Bowne, L. S. Sullivan, H. Luo, L. Zhao, X. Wang, J. E. Zaneveld, J. S. Salvo, S. Siddiqui, L. Mao, D. K. Wheaton, D. G. Birch, K. E. Branham, J. R. Heckenlively, C. Wen, K. Flagg, H. Ferreyra, J. Pei, A. Khan, H. Ren, K. Wang, I. Lopez, R. Qamar, J. C. Zenteno, R. Ayala-Ramirez, B. Buentello-Volante, Q. Fu, D. A. Simpson, Y. Li, R. Sui, G. Silvestri, S. P. Daiger, R. K. Koenekoop, K. Zhang & R. Chen (2014) Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. *Hum Genet*, 133, 331-45.
- Wang, L., M. Ribaud, K. Zhao, N. Yu, Q. Chen, Q. Sun, L. Wang & Q. Wang (2003) Novel deletion in the pre-mRNA splicing gene PRPF31 causes autosomal dominant retinitis pigmentosa in a large Chinese family. *Am J Med Genet A*, 121a, 235-9.
- Waseem, N. H., V. Vaclavik, A. Webster, S. A. Jenkins, A. C. Bird & S. S. Bhattacharya (2007) Mutations in the gene coding for the pre-mRNA splicing factor, PRPF31, in patients with autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, 48, 1330-4.
- Weidenhammer, E. M., M. Ruiz-Noriega & J. L. Woolford, Jr. (1997) Prp31p promotes the association of the U4/U6 x U5 tri-snRNP with prespliceosomes to form spliceosomes in *Saccharomyces cerevisiae*. *Mol Cell Biol*, 17, 3580-8.
- Weidenhammer, E. M., M. Singh, M. Ruiz-Noriega & J. L. Woolford, Jr. (1996) The PRP31 gene encodes a novel protein required for pre-mRNA splicing in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, 24, 1164-70.
- Weisschuh, N., A. K. Mayer, T. M. Strom, S. Kohl, N. Glockle, M. Schubach, S. Andreasson, A. Bernd, D. G. Birch, C. P. Hamel, J. R. Heckenlively, S. G. Jacobson, C. Kamme, U. Kellner, E. Kunstmann, P. Maffei, C. M. Reiff, K. Rohrschneider, T. Rosenberg, G. Rudolph, R. Vámos, B. Varsanyi, R. G. Weleber & B. Wissinger (2016) Mutation Detection in Patients with Retinal Dystrophies Using Targeted Next Generation Sequencing. *PLoS One*, 11, e0145951.
- Wheway, G., L. Nazlamova, N. Meshad, S. Hunt, N. Jackson & A. Churchill (2019) A Combined in silico, in vitro and Clinical Approach to Characterize Novel Pathogenic Missense Variants in PRPF31 in Retinitis Pigmentosa. *Front Genet*, 10, 248.
- Wheway, G., M. Schmidts, D. A. Mans, K. Szymanska, T. M. Nguyen, H. Racher, I. G. Phelps, G. Toedt, J. Kennedy, K. A. Wunderlich, N. Soroush, Z. A. Abdelhamed, S. Natarajan, W. Herridge, J. van Reeuwijk, N. Horn, K. Boldt, D. A. Parry, S. J. Letteboer, S. Roosing, M. Adams, S. M. Bell, J. Bond, J. Higgins, E. E. Morrison, D. C. Tomlinson, G. G. Slaats, T. J. van Dam, L. Huang, K. Kessler, A. Giessl, C. V. Logan, E. A. Boyle, J. Shendure, S. Anazi, M. Aldahmesh, S. Al Hazzaa, R. A. Hegele, C. Ober, P. Frosk, A. A. Mhanni, B. N. Chodirker, A. E. Chudley, R. Lamont, F. P. Bernier, C. L. Beaulieu, P. Gordon, R. T. Pon, C. Donahue, A. J. Barkovich, L. Wolf, C. Toomes, C. T. Thiel, K. M. Boycott, M. McKibbin, C. F. Inglehearn, U. K. Consortium, G. University of Washington Center for Mendelian, F. Stewart, H. Omran, M. A. Huynen, P. I. Sergouniotis, F. S. Alkuraya, J. S. Parboosingh, A. M. Innes, C. E. Willoughby, R. H. Giles, A. R. Webster, M. Ueffing, O. Blacque, J. G. Gleeson, U. Wolfrum, P. L. Beales, T. Gibson, D. Doherty, H. M. Mitchison, R. Roepman & C. A. Johnson (2015) An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. *Nature cell biology*, 17, 1074-1087.

- Wilkie, S. E., K. J. Morris, S. S. Bhattacharya, M. J. Warren & D. M. Hunt (2006) A study of the nuclear trafficking of the splicing factor protein PRPF31 linked to autosomal dominant retinitis pigmentosa (ADRP). *Biochimica et biophysica acta*, 1762, 304-311.
- Wilkie, S. E., V. Vaclavik, H. Wu, K. Bujakowska, C. F. Chakarova, S. S. Bhattacharya, M. J. Warren & D. M. Hunt (2008) Disease mechanism for retinitis pigmentosa (RP11) caused by missense mutations in the splicing factor gene PRPF31. *Molecular vision*, 14, 683-690.
- Will, C. L. & R. Luhrmann (2011) Spliceosome structure and function. *Cold Spring Harbor perspectives in biology*, 3, 10.1101/cshperspect.a003707.
- Wu, Z., M. Zhong, M. Li, H. Huang, J. Liao, A. Lu, K. Guo, N. Ma, J. Lin, J. Duan, L. Liu, F. Xu, Z. Zhong & J. Chen (2018) Mutation Analysis of Pre-mRNA Splicing Genes PRPF31, PRPF8, and SNRNP200 in Chinese Families with Autosomal Dominant Retinitis Pigmentosa. *Curr Mol Med*, 18, 287-294.
- Xi, X. H., D. Zheng, K. Xia, Q. Pan, L. Y. Lei, Z. Liu, C. Z. Tang, J. H. Xia, D. Y. Jiang & H. X. Deng (2005) [Splicing site mutation of D19S418 in PRPF-31 gene and its phenotypic characters with autosomal dominant retinitis pigmentosa]. *Zhonghua Yan Ke Za Zhi*, 41, 1020-6.
- Xia, K., D. Zheng, Q. Pan, Z. Liu, X. Xi, Z. Hu, H. Deng, X. Liu, D. Jiang, H. Deng & J. Xia (2004) A novel PRPF31 splice-site mutation in a Chinese family with autosomal dominant retinitis pigmentosa. *Mol Vis*, 10, 361-5.
- Xiao, X., Y. Cao, Z. Zhang, Y. Xu, Y. Zheng, L. J. Chen, C. P. Pang & H. Chen (2017) Novel Mutations in PRPF31 Causing Retinitis Pigmentosa Identified Using Whole-Exome Sequencing. *Investigative ophthalmology & visual science*, 58, 6342-6350.
- Xie, D., K. Peng, Q. Yi, W. Liu, Y. Yang, K. Sun, X. Zhu & F. Lu (2018) Targeted Next Generation Sequencing Revealed Novel PRPF31 Mutations in Autosomal Dominant Retinitis Pigmentosa. *Genet Test Mol Biomarkers*, 22, 425-432.
- Xu, F., R. Sui, X. Liang, H. Li, R. Jiang & F. Dong (2012) Novel PRPF31 mutations associated with Chinese autosomal dominant retinitis pigmentosa patients. *Molecular vision*, 18, 3021-xxx.
- Yang, L., X. Yin, L. Wu, N. Chen, H. Zhang, G. Li & Z. Ma (2013) Targeted exome capture and sequencing identifies novel PRPF31 mutations in autosomal dominant retinitis pigmentosa in Chinese families. *BMJ Open*, 3, e004030.
- Yang, Y., D. Tian, J. Lee, J. Zeng, H. Zhang, S. Chen, H. Guo, Z. Xiong, K. Xia, Z. Hu & J. Luo (2015) Clinical and genetic identification of a large chinese family with autosomal dominant retinitis pigmentosa. *Ophthalmic Genet*, 36, 64-9.
- Yin, J., J. Brocher, U. Fischer & C. Winkler (2011) Mutant Prpf31 causes pre-mRNA splicing defects and rod photoreceptor cell degeneration in a zebrafish model for Retinitis pigmentosa. *Molecular neurodegeneration*, 6, 56-1326-6-56.
- Yuan, L., M. Kawada, N. Havlioglu, H. Tang & J. Y. Wu (2005) Mutations in PRPF31 inhibit pre-mRNA splicing of rhodopsin gene and cause apoptosis of retinal cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25, 748-757.
- Zhang, N., R. Kaur, X. Lu, X. Shen, L. Li & R. J. Legerski (2005) The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *The Journal of biological chemistry*, 280, 40559-40567.
- Zhang, Q., M. Xu, J. D. Verriotto, Y. Li, H. Wang, L. Gan, B. L. Lam & R. Chen (2016) Next-generation sequencing-based molecular diagnosis of 35 Hispanic retinitis pigmentosa probands. *Scientific reports*, 6, 32792.
- Zhao, L., F. Wang, H. Wang, Y. Li, S. Alexander, K. Wang, C. E. Willoughby, J. E. Zaneveld, L. Jiang, Z. T. Soens, P. Earle, D. Simpson, G. Silvestri & R. Chen (2015) Next-generation sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Ireland. *Hum Genet*, 134, 217-30.
- Zheng, Y., H.-L. Wang, J.-K. Li, L. Xu, L. Tellier, X.-L. Li, X.-Y. Huang, W. Li, T.-T. Niu, H.-M. Yang, J.-G. Zhang & D.-N. Liu (2018) A novel mutation in PRPF31, causative of autosomal dominant

retinitis pigmentosa, using the BGISEQ-500 sequencer. *International journal of ophthalmology*, 11, 31-35.

Ziegler, A., E. Colin, D. Goudenège & D. Bonneau (2019) A snapshot of some pLI score pitfalls. *Human Mutation*, 40, 839-841.

Tables

exon	cDNA mutation	protein mutation	notes	Original references	Families (n)	Splicing	Frameshift	Nonsense	Missense	Inframe deletion	Inframe duplication	Indel	Large insertion or deletion	Age of onset	Age at diagnosis
exon 1 (non coding)															
intron 1	c.1-2481G>T		formerly: IVS1+1G>T	Liu et al., 2008(Liu et al. 2008)	1	1								3	20
exon 2 c.1-177	c.-3_7del	p.Met1?		Sullivan et al., 2013(Sullivan et al. 2013a); Kiser et al., 2019(Kiser et al. 2019)	2		2							10/17/29	10/58/62
	c.1A>T	p.Met1?		Carss et al., 2017(Carss et al. 2017)	1										
	c.18G>C	p.Glu6Asp		van Huet et al., 2015(van Huet et al. 2015)	1				1						
	c.19_20insA	p.Leu7Hisfs*4		Sullivan et al., 2013(Sullivan et al. 2013a)	1		1								
	c.34G>T	p.Glu12*		van Cauwenbergh et al., 2017(Van Cauwenbergh et al. 2017)	1		1								
	c.55del	p.Glu19Lysfs*46		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1		1								

	c.59_65del7	p.Gly20Alafs*43		Saini et al., 2012(Saini et al. 2012)	1		1										17
	c.79G>T	p.Glu27X		Waseem et al., 2007(Waseem et al. 2007)	1			1								15	43
	c.121C>G	p.Leu41Val	reported as cause of disease, but no functional studies	Ellingford et al., 2016(Ellingford et al. 2016a)	1				1								
	c.165G>A			de la Cerda et al., 2019(de la Cerda et al. 2019)	1												
	c.172A>T	p.Lys58X		Zheng et al., 2016(Zhang et al. 2016)	1												13
	c.196_197delAA	p.Lys66Aspfs*2		Xu et al., 2012(Xu et al. 2012)	1		1	1									24
intron 2	c.177+1G>A		formerly IVS2+1 G>A	Sullivan et al., 2006a(Sullivan et al. 2006a)	1	1											
	c.177+1delG			Rivolta et al., 2006(Rivolta et al. 2006)	1	1											
exon 3 c.178-238 (20aa)	c.217A>T	p.Lys73X		Eisenberger et al., 2013(Eisenberger et al. 2013)	1			1									
	c.220C>T	p.Gln74X		Sullivan et al., 2006a(Sullivan et al. 2006a); van Cauwenbergh et al., 2017(Van Cauwen	3			3								7	9

				bergh et al. 2017); Kiser et al., 2019(Kiser et al. 2019)													
intron 3																	
exon 4 239-322	c.267delA	p.Glu89Aspfs*11		Sullivan et al., 2013(Sullivan et al. 2013a)	1		1										
		p.Leu107del4 ctGAGT		Aleman et al., 2009(Aleman et al. 2009)	1		1									32	
	c.319C>G			Rivolta et al., 2006(Rivolta et al. 2006); Rio Frio et al., 2008(Rio Frio et al. 2008b)	2		2										
intron 4	c.322+4_322+7del	p.?	2 families in MM paper, 3 families in Zhang paper	Zhang et al., 2016(Zhang et al. 2016); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	5		5									19/27	
	c.322+1G>A			Wu et al., 2018(Wu et al. 2018); Kiser et al., 2019(Kiser et al. 2019)	2		2										
	c.323-2A>G			Rivolta et al., 2006 (Rivolta et al. 2006)	1		1										
exon 5 c.323-421	c.328_330del	p.Ile110del	Reported as p.Ile109del in de Sousa	de Sousa Dias et al., 2013(de Sousa	2						2						

	c.400delG	p.Asp134Ilefs		Ellingford et al., 2016(Ellingford et al. 2016a)	1		1										
	c.413C>A	Thr138Lys		Waseem et al, 2007(Waseem et al. 2007)	1				1						15,20	30	
intron 5	c.421-2A>G			Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1	1											
	c.421-1G>A		formerly IVS5-1G>A	Xia et al., 2004(Xia et al. 2004); Xi et al., 2005(Xi et al. 2005)	2	2											
exon 6 c.421-527	c.421G>T	Glu141X		Sullivan et al., 2006a(Sullivan et al. 2006a)	1			1									
	c.433_434del	p.S145Pfs*8		Kurata et al., 2018(Kurata, Hosono and Hotta 2018)	1		1								7	7	
	c.522_527+10del		Same family in these 2 papers	Ghazawy et al., 2007(Ghazawy et al. 2007); Buskin et al., 2018(Buskin et al. 2018)	1										30s	33	
	c.525_526insAG			Kiser et al., 2019(Kiser et al. 2019)	1		1								16	47	
intron 6	c.527+1G>A		Described as p.IVS 6+1G>T	Chakarova et al., 2006(Chakarova et al. 2006); Martin-Merida et al., 2018(Martin-Merida et al.	3	3									13/48/21	13/48	

			2018); Abdulridha-Aboud et al., 2016(Abdulridha-Aboud et al. 2016)														
c.527+1G>T			Gandra et al., 2008(Gandra et al. 2008); Kiser et al., 2019(Kiser et al. 2019)	2	2												
c.527+2T>G			Wu et al, 2018(Wu et al. 2018)	1	1												
c.527+2T>C			Audo et al., 2010(Audo et al. 2010)	1	1												
c.527+3A>G		In 2 families in Waseem paper . Reported as IVS 6 +3 A>G in Ivings paper	Vithana et al., 2001(Vithana et al. 2001); Waseem et al., 2007(Waseem et al. 2007); Ivings et al., 2008(Ivings et al. 2008); Ellingford et al., 2016(Ellingford et al. 2016a); Xie et al., 2018(Xie et al. 2018); Kiser et al., 2019(Kiser et al. 2019)	7	7										20/29/28/27	52/30/70	
c.528 -3_45del			Vithana et al., 2001(Vithana et al. 2001);	2	2												

				Sato et al., 2005(Sato et al. 2005)													
	c.528-39_531del			Sullivan et al., 2013(Sullivan et al. 2013a)	1	1											
	c.528-1G>A			Waseem et al., 2007(Waseem et al. 2007); van Cauwenbergh et al., 2017(Van Cauwenbergh et al. 2017)	2	2											20
exon 7 c.528-697	c.541G>T	p.Glu181X	2 families in MM paper	Pomares et al., 2010(Pomares et al. 2010); van Cauwenbergh et al., 2017 (Van Cauwenbergh et al. 2017); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	4			4									19, 23
	c.544_618del75bp	E182_E206del		Xu et al., 2012(Xu et al. 2012)	1					1							24
	c.547delG	p.E183fs		Xiao et al. 2017(Xiao et al. 2017)	1		1									5,6,7,8,10	
	c.548_580dup	p.Glu183_Met193dup		Tiwari et al., 2016(Tiwari et al. 2016)	1						1					24	
	c.550_552del	p.Leu184del		Kiser et al., 2019(Kiser et al. 2019)	1					1						71	71

c.553G>T	p.Glu185X	de novo in Neveling paper	Neveling et al., 2012(Neveling et al. 2012); van Huet et al., 2015(van Huet et al. 2015)	2			1										
c.562G>T	p.Glu188X		ClinVar (likely pathogenic)	1			1										
c.580_581delGC	p.Leu195GlyFs		ClinVar (likely pathogenic)	1		1											
c.580-581dup33bp			Vithana et al., 2001(Vithana et al. 2001)	1						1							
c.581C>A	Ala194Glu		Vithana et al., 2001(Vithana et al. 2001)	1				1									
c.590T>C	Leu197Pro		Bryant et al., 2018(Bryant et al. 2018); Wu et al., 2018(Wu et al. 2018)	2				2									
c.615C>A	p.Tyr205X		ClinVar (pathogenic)	1			1										
c.615C>G	p.Tyr205X		ClinVar (likely pathogenic)	1			1										
c.615delC	p.Y205X		Xu et al., 2012(Xu et al. 2012)	1			1										27
c.616G>T	p.Glu206X		Wang et al., 2014(Wang et al. 2014)	1			1										
c.629delC			Huang et al., 2015(Huang et al. 2015)	1		1											
c.636delG	p.Met212fs*238		Sullivan et al., 2006a(Sullivan et al.	3		3											

				2006a); Bowne et al., 2011(Bowne et al. 2011); Wang et al., 2014(Wang et al. 2014)													
	c.646G>C	Ala216Pro		Vithana et al, 2001(Vithana et al. 2001)	1				1								
	c.666_668del	p.Ile223del		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1				1								
	c.673del	p.Ala225Hisfs*14		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1		1										
intron 7	c.698-1G>A			Roberts et al., 2016(Roberts et al. 2016); Birtel et al., 2018(Birtel et al. 2018a)	2	2											
exon 8 c.698 - 855	c.709-734dup			Terray et al., 2017(Terray et al. 2017)	1					1							
	732-737delins20bp	M244fsX248		Martinez-Gimeno et al., 2003(Martinez-Gimeno et al. 2003)	1						1				6-20		
	c.736G>A	p.Ala246Thr		Xu et al., 2014(Xu et al. 2012); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	2				2								

c.741_742insA	p.Asn248Lysfs		ClinVar (likely pathogenic)	1		1											
c.758_767del	p.Gly253fs*317		Sullivan et al., 2006a(Sullivan et al. 2006a); Kiser et al., 2019(Kiser et al. 2019)	2		2									19		31
c.763C>T	p.Gln255X		Wang et al., 2014(Wang et al. 2014)	1			1										
769-770insA	K257fs*277		Vithana et al., 2001(Vithana et al. 2001); Martinez-Gimeno et al., 2003(Martinez-Gimeno et al. 2003)	2		2									10-18		
c.770dup	p.Thr258Aspfs*21		Vithana et al., 2001(Vithana et al. 2001); Martin-Medira et al., 2018	2		2											
c.772_773del2insCAACATGCAACATCAT	p.(Thr258Glnfs)		Zhao et al., 2015(Zhao et al. 2015)	1		1											
c.781G>C	Gly261Arg		Xiao et al. 2017(Xiao et al. 2017)	1				1									
c.785delT	p.Phe262Serfs*59		Lim et al., 2009(Lim et al. 2009)	1		1									<10		
c.804delG	p.L268fs		Xiao et al. 2017(Xiao et al. 2017)	1		1											
c.808_809insC	p.His270Profs*8		Sullivan et al., 2013(Sullivan et	1		1											

				al. 2013a)														
	c.815G>T	p.Gly272Val	predi cted by Sulliv an to be benig n	Sullivan et la., 2006a(S ullivan et al. 2006a); Daiger et al., 2014(Da iger et al. 2014)	2					2								
	c.816_830delCTA CATCTACCACAG	p.Tyr273_Se r277del		Birtel et al., 2018(Bir tel et al. 2018b)	1					1								
	c.824_825insA	p.Y275X		Yang et al., 2013(Ya ng et al. 2013)	1			1								12		
	c.828_829del	p.His276Glnf s*2		Martine z- Gimeno et al., 2003(M artinez- Gimeno et al. 2003); Martin- Merida et al., 2018(M artin- Merida et al. 2018)	2		2									5-20		
	c.838_841dupGTG C	p.Gln281Arg fs*44		Carss et al., 2017(Ca rss et al. 2017)	1		1											
	c.839T>G	p.Val280Gl		Birtel et al., 2018(Bir tel et al. 2018b)	1					1								
intron 8	c.855+1G>C			Lu et al., 2005(Lu et al. 2005)	1	1												
	c.855+1G>T			Jespersg aard et al., 2019(Jes persgaar d et al. 2019)	1	1												
	c.855+1G>A			ClinVar (pathog enic)	1	1												

	c.856-2A>G			Rivolta et al., 2006(Rivolta et al. 2006)	1	1										
exon 9 c.856 - 945	c.862C>T	p.Arg288Trp		Coussa et al., 2015(Coussa et al. 2015)	1				1						66	68
	c.866_879delGGA AAGCGGCCCGG	p.R289Pfs*30		Villanueva et al., 2014(Villanueva et al. 2014); Zhang et al., 2016(Zhang et al. 2016)	2		2								2-16	7-63
	c.871G>C	Ala291Pro		Sullivan et al 2006a(Sullivan et al. 2006a)	1				1							
	c.877_910del	p.Arg293_Arg304>Valfs*17		Rivolta et al., 2006(Rivolta et al. 2006)	1		1									
	c.895T>C	Cys299Arg		Sullivan et al 2006a(Sullivan et al. 2006a); Xu et al., 2012(Xu et al. 2012); Martin-Merida et al., 2018(Martin-Merida et al. 2018); Kiser et al., 2019(Kiser et al. 2019)	4				4						21/27/41/63	27/44/63/65
	c.896G>A	p.Cys299Tyr		Bhatia et al., 2018(Bhatia et al. 2018)	1				1							
	c.910C>T	p.Arg304Cys		Huang et al., 2015(Huang et al. 2015); Hariri et al., 2018(Hariri et al. 2018)	2				2							45

	c.914_915insTGT	p.Val305_Asp306insVal		Utz et al., 2013(Utz et al. 2013)	1							1			30s	40s
	c.915_916insTGT	p.Val305_Asp306insCys		Sullivan et al., 2013(Sullivan et al. 2013a)	1							1				
	c.916G>A	p.Asp306Asn	reported as cause of disease, but functional studies not carried out	Ellingford et al., 2016(Ellingford et al. 2016a)	1				1							
		p.307fs*15		Lu et al., 2013(Lu et al. 2013)	1		1									
	c.939dup	p.Gly314Argfs*10		Fernandez-San Jose et al., 2015(Fernandez-San Jose et al. 2015); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	2		2									
	c.940delG	p.Ala302Glnfs		ClinVar (pathogenic/likely pathogenic)	2		2									
intron 9	c.946-1 G>C			Bowne et al., 2011(Bowne et al. 2011); Daiger et al., 2014 (Daiger et al. 2014)	2	2										
exon 10 c.946 - 1073	c.950delG	p.Gly316Alafs*4		ClinVar (pathogenic)	1		1									

c.961A>T	p.Lys321X	Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1			1										
c.967G>T	p.Glu323X	Ellingford et al., 2016(Ellingford et al. 2016a)	1			1										
c.973G>T	Glu325X	Sullivan et al., 2006a(Sullivan et al. 2006a)	1			1										
c.978_982del	p.Lys327Argfs*146	van Cauwenbergh et al., 2017(Van Cauwenbergh et al. 2017)	1		1											
c.992G>A	p.Trp331X	ClinVar (pathogenic)	1			1										
c.994C>T	p.Gln332X	Ellingford et al., 2016(Ellingford et al. 2016a)	1			1										
c.997del	p.Glu333Serfs*5	Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1		1											
c.1015C>T	p.Q339X	Xie et al., 2018(Xie et al. 2018)	1			1										
c.1035_1036insGC	p.Pro346Argfs*18	Wu et al, 2018(Wu et al. 2018)	1		1											
c.1048C>T	p.Gln350X	Eisenberger et al., 2013(Eisenberger et al. 2013)	1			1										
c.1060C>T	p.Arg354X	Sullivan et al., 2013(Sullivan et al. 2013a); Ellingford et al.,	6			6								5/6/7/8/8/6/12	6/24	

				2016(Ellingford et al. 2016a); Xiao et al., 2017(Xiao et al. 2017); Wu et al, 2018(Wu et al. 2018); Kurata et al., 2018(Kurata et al. 2018); Kiser et al., 2019(Kiser et al. 2019),												
intron 10	c.1067_1073+8del			Eisenberger et al., 2013(Eisenberger et al. 2013)	1	1										
	c.1073+1G>A			Sullivan et al., 2006a(Sullivan et al. 2006a); Kiser et al., 2019(Kiser et al. 2019)	2	2								28/40	9/12/14/40	
	c.1074-2 A>T	p.Tyr359Sfs* 29		Yang et al., 2013(Yang et al. 2013)	1	1								2-8		
	c.1074-1G>T	p.?		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1	1										
exon 11 c.1074-1146 (24aa)	c.1077C>A	p.Tyr359X		van Cauwenbergh et al., 2017(Van Cauwenbergh et al. 2017)	1			1								
	c.1084delA	p.Met362X		Sullivan et al., 2013(Sullivan et al. 2013a):	1			1						6	44	

				Kiser et al., 2019(Kiser et al. 2019)															
	c.1098delG	p.Leu366fs*1		Pan et al., 2014(Pan et al. 2014)	1		1									5		22	
	c.1115_1125del	p.Arg372Glnfs*99	Same family in Ivings and Buskin paper	Vithana et al., 2001(Vithana et al. 2001); Ivings et al., 2008(Ivings et al. 2008); Buskin et al., 2018(Buskin et al. 2018)	2		2											Severe at 47	
	c.1120C>T	p.Gln374X		Ellingford et al., 2016b(Ellingford et al. 2016b)	1			1											
	c.1129delC	p.Arg377Valfs*2		Carss et al., 2017(Carss et al. 2017)	1		1												
	c.1142delG	p.Gly381fs*30	in more than 4 Japanese families in Koyanagi paper	Sato et al., 2005(Sato et al. 2005); Taira et al., 2007(Taira, Nakazawa and Sato 2007); Koyanagi et al., 2019(Koyanagi et al. 2019)	6		6											30-45	
intron 11	c.1146+2T>C	p.?		Waseem et al., 2007(Waseem et al. 2007); Martin-Merida et al., 2018(Martin-Merida et al. 2018)	2	2												18, 20	

	c.1146+2T>A	p.?		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1	1										
exon 12 c.1147 - 1275	c.1155-1159delGGACG/insAGGGATT	p.Asp386Glyfs*28		Sato et al., 2005(Sato et al. 2005); Sullivan et al., 2006a(Sullivan et al. 2006a)	2		1								20	45
	c.1190dup	p.His398Profs*77		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1		1									
	c.1205C > A	p.Ser402X		McLennan et al., 2019(McLennan et al. 2019)	1			1								
	c.1215delG	p.G405fs*7		Dong et al., 2013(Dong et al. 2013)	1		1								9	9,22,73
	c.1222C>T	p.Arg408Trp		Xiao et al. 2017(Xiao et al. 2017)	1				1							
	c.1224dupG	p.Gln409Alafs*66		Wu et al, 2018(Wu et al. 2018)	1		1									
	c.1226_1227insA	p.Thr410Aspfs*65		Xie et al., 2018(Xie et al. 2018)	1											
	c.1234del	p.Val412X		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1				1							
	c.1261_1262delTC	p.S421Qfs*53		Glockle et al., 2014(Glockle et al. 2014)	1		1									
	c.1273C>T	p.Gln425X		ClinVar (pathogenic)	1				1							

intron 12																		
exon 13 c.1276-1374	c.1291C>T	p.Gln431X		ClinVar (pathogenic)	1			1										
	c.1305T>A	p.Y435X		Huang et al., 2015(Huang et al. 2015)	1			1										
	c.1373A>T	p.Gln458Leu		Xiao et al. 2017(Xiao et al. 2017)	1				1									
intron 13	c.1374+654C>G		deep intronic	Rio Frio et al., 2009(Rio Frio et al. 2009)	1	1												
exon 14 c.1375-1492 (39aa)	c.1462_1472del	p.Lys488Argfs*75		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1		1											
Deletion	upstream			Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1										1			
Deletion	exons 1-14 (whole gene)			Ivings et al., 2008(Ivings et al. 2008); Bowne et al., 2011(Bowne et al. 2011); Eisenberger et al., 2013(Eisenberger et al. 2013); Almoguera et al., 2015(Almoguera et al. 2015) Hariri et al., 2018(Hariri et al. 2018); Martin-	6										6			18, severe at 65

				Merida et al., 2018(Martin-Merida et al. 2018)												
30 kb deletion including putative promoter region of a novel gene OSCAR, the entire genomic content of genes NDUFA3, TFPT and most of the PRPF31 gene except for its terminal exon 14.				Abu-Safieh et al. 2006(Abu-Safieh et al. 2006)	1								1	6-30		
112 kb deletion encompassing over 90% of PRPF31 and five upstream genes: TFPT, OSCAR, NDUFA3, TARM-1, and VSTM-1				Rose et al., 2011(Rose et al. 2011)	1								1			

58.7kb deletion including TFPT, NDUFA3, OSCAR genes and 11 exons of the PRPF31		2 families in Sweden	Kohn et al., 2009(Kohn et al. 2009); Golovleva et al., 2010(Golovleva et al. 2010)	2										2		50s
Deletion	exon 1		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1										1		
Deletion	exons 1-3		Birtel et al., 2018(Birtel et al. 2018a)	1										1		
12kb deletion including exons 1-3 of PRPF31			Dong et al., 2013(Dong et al. 2013)	1										1		
Deletion	exons 1-5		Eisenberger et al., 2013(Eisenberger et al. 2013); Birtel et al., 2018(Birtel et al. 2018a)	2										2		
Deletion	Intron 1		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1										1		
Deletion	exons 2-3		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1										1		
Duplication	exons 2-5		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1										1		

Deletion	exons 2-5		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1											1		
Deletion	exons 2-14		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1											1		
Duplication	exons 4-5		Jespersgaard et al., 2019(Jespersgaard et al. 2019)	1											1		
Deletion	exons 4-13		Weisschuh et al., 2016(Weisschuh et al. 2016)	1											1		
Deletion	exon 9		Martin-Merida et al., 2018(Martin-Merida et al. 2018)	1											1		
Promoter mutation			Rose et al., 2012(Rose et al. 2012)	1											1		
Insertion/deletion	149 bp deleted/640 bp inserted	hg17 Deletion of 59,310,880-59,311,028/insertion of 59,292,594-59,291,955 reverse comp.	Sullivan et al., 2006b(Sullivan et al. 2006b)	1											1	14/16/25/46	37/46/50/52/77
Deletion	4.8 kb	hg 17 59,315,842-59,320,684	Sullivan et al., 2006b(Sullivan et al. 2006b)	1											1		
Deletion	11.3 kb	hg17 59,314,340-59,325,633	Sullivan et al., 2006b(Sullivan et al. 2006b)	1											1		
Deletion	32-42 kb	hg17 5' breakpoint: 59,290,949-59,295,848; ' breakpoint: 59,328,550-59,333,288	Sullivan et al., 2006b(Sullivan et al. 2006b)	1											1		

Deletion	>44.8 kb	hg17 5' breakpoint: <59,283,753 ; 3' breakpoint: 59,328,550–59,333,288	Sullivan et al., 2006b(Sullivan et al. 2006b)	1										1		
Deletion	19:54622548-54633842del11295		Carss et al., 2017(Carss et al. 2017)	1										1		
Deletion	19:54632279-54632481del203		Carss et al., 2017(Carss et al. 2017)	1										1		

Table 1. All reported pathogenic variants in *PRPF31* associated with adRP, from peer-reviewed publications and clinical variant database ClinVar (variants classified as pathogenic only). The location in cDNA, nature of the variant and impact on protein (if known) is included, alongside age of onset and age at diagnosis, where reported.

Figure legends

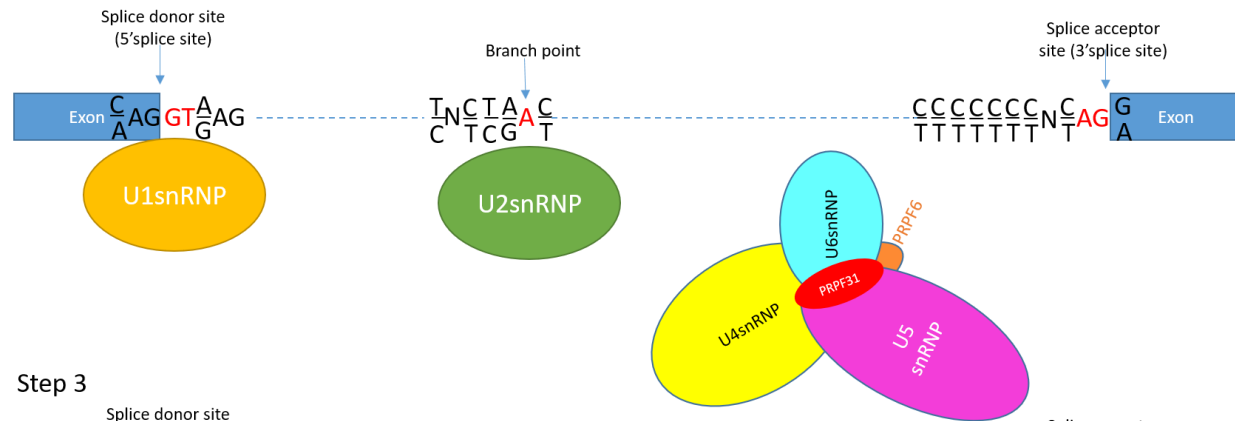
Figure 1. Schematic representation of the first four steps of pre-mRNA splicing by the major spliceosome, with *PRPF31* shown in red. In step 1, U1snRNP recognises and binds the splice donor site (the 5' splice site). In step 2, binding of U1snRNP to the splice donor site promotes the binding of U2snRNP to the branch site. Independently of this, the U4/U6.U5 tri-snRNP forms in the cell. In step 3, the U4/U6.U5 tri-snRNP is recruited to the pre-mRNA, where U6snRNP replaces U1snRNP. This forms the catalytically active spliceosome, which in step 4 cuts away the intron and joins the exons through two transesterification reactions.

Figure 2. Schematic representation of the protein and cDNA structure of *PRPF31*, showing major structural domains encoded by each exon.

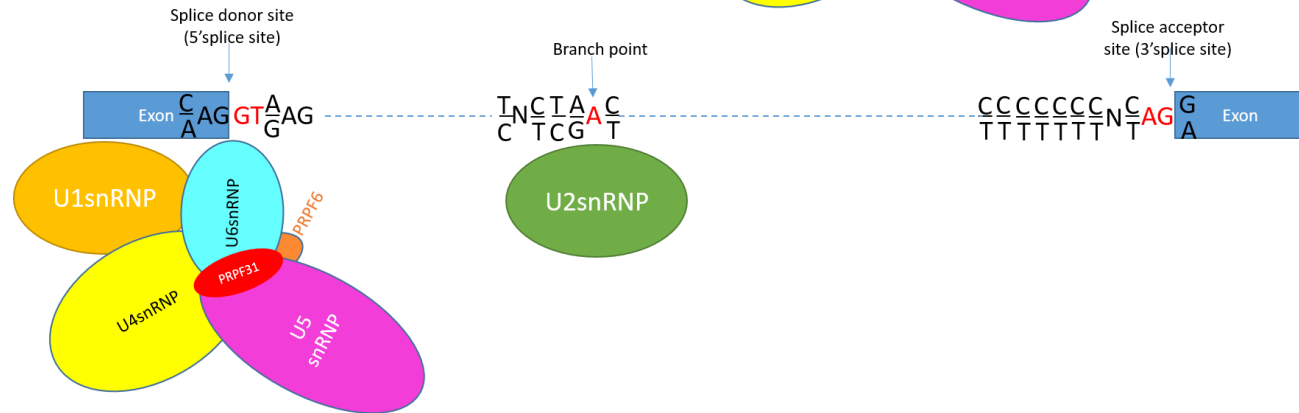
Figure 3. Schematic representation *PRPF31* gene, with all reported pathogenic variants labelled above, and total numbers of variants in each intron and exon displayed as a bar chart below. This shows that exons 7 and 8 are most enriched for pathogenic variants.

Figure 4. (a) Box and whisker plots showing upper and lower limits, median and interquartile range of reported age of onset of RP patients with different types of variant in *PRPF31* (b) Box and whisker plots showing upper and lower limits, median and interquartile range of reported age of diagnosis of RP patients with different types of variant in *PRPF31*.

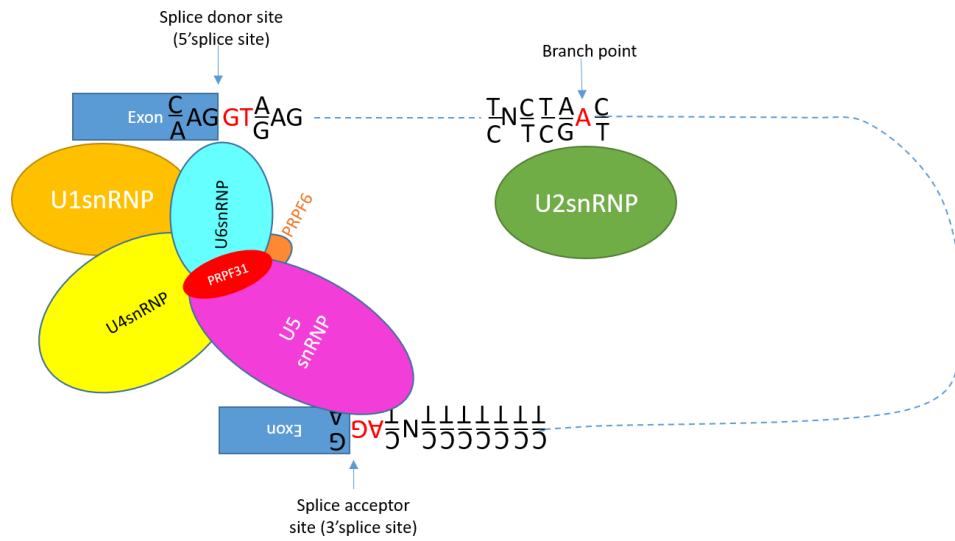
Step 1 and 2

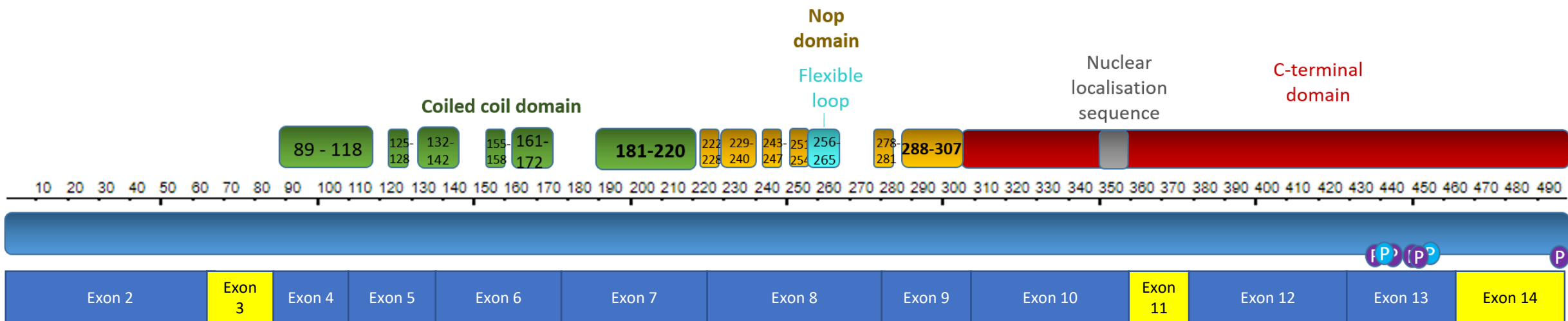


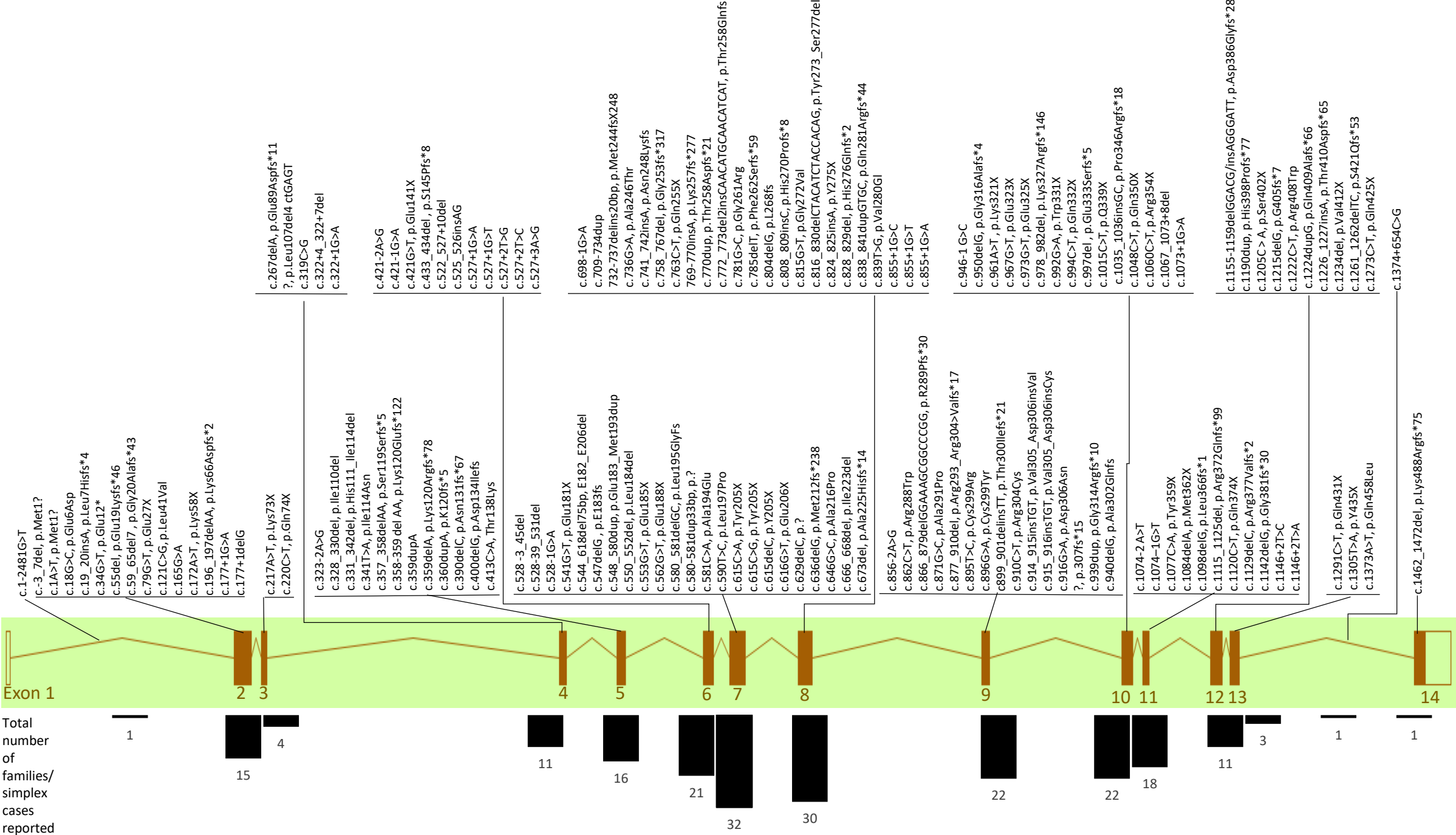
Step 3

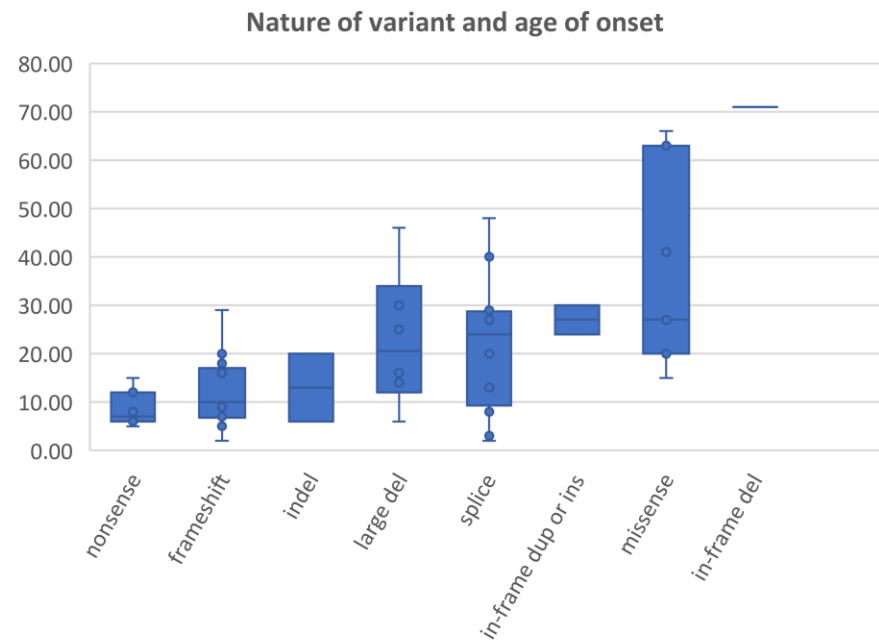


Step 4







a**b**