**A gene-to-patient approach uplifts novel disease gene discovery and identifies 18 putative novel disease genes**

Eleanor G. Seaby1,2,3,4, Damian Smedley5, Ana Lisa Taylor Tavares5, Helen Brittain5, Genomics England Research Consortium5, Richard H. van Jaarsveld6, Diana Baralle1, Heidi L. Rehm2,3, Anne O’Donnell-Luria2,3,4, Sarah Ennis1

1. Faculty of Medicine, University of Southampton, Southampton, UK
2. Program in Medical and Population Genetics, Broad institute of MIT and Harvard, Boston, MA, USA
3. Center for Genomic Medicine, Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, USA
4. Division of Genetics and Genomics, Boston Children’s Hospital, Boston, MA, USA
5. Genomics England, London, UK
6. Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

Corresponding Author:

Dr Eleanor G. Seaby, Genomic Informatics Group, University Hospital Southampton, SO16 6YD, UK

[E.Seaby@soton.ac.uk](mailto:E.Seaby@soton.ac.uk)

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**List of abbreviations:**

**AF** – Allele frequency

**AnVIL** - Analysis, Visualisation, and Informatics Lab

**DECIPHER** - Database of genomic variation and phenotype in humans using Ensembl resources

**HPO** – Human Phenotype Ontology

**GECIP** - Genomics England Clinical Interpretation Partnership

**GEL** – Genomics England

**IMPC** – International Mouse Phenotyping Consortium

**LOEUF** – Loss-of-function Observed/Expected Upper-bound Fraction

**LoF** – Loss of function

**MGI** – Mouse Genome Informatics

**MME** – Matchmaker Exchange

**OMIM** - Online Mendelian Inheritance in Man

**pLoF** – Predicted loss of function

**RE** - Research environment

**VUS** - Variants of uncertain significance

**Abstract**

**Purpose:**

Exome and genome sequencing have drastically accelerated novel disease-gene discoveries. However, discovery is still hindered by myriad variants of uncertain significance found in genes of undetermined biological function. This necessitates intensive functional experiments on genes of equal-predicted causality leading to a major bottleneck.

**Methods:**

We apply the Loss-of-function Observed/Expected Upper-bound Fraction (LOEUF) metric of intolerance to gene inactivation to curate a list of predicted haploinsufficient disease genes. Using data from the 100,000 Genomes Project, we adopt a gene-to-patient approach that matches *de novo* loss-of-function variants in constrained genes to rare disease patients. Through large scale aggregation of data, we reduce excess analytical noise currently hindering novel discoveries.

**Results:**

Results from 13,949 trios revealed 643 rare, *de novo* predicted loss-of-function events filtered from 1,044 LOEUF-constrained genes. 168 variants occurred within 126 genes without a known disease-gene relationship. Of these, 27 genes had >1 kindred affected, and for 18 of these genes, multiple kindreds had overlapping phenotypes. Two years following initial analysis, 11/18 (61%) of these genes have been independently published as novel disease-gene discoveries.

**Conclusion:**

Utilising large cohorts and adopting gene-based approaches can rapidly and objectively accelerate dominantly-inherited novel gene discovery by targeting the most appropriate genes for functional validation.

**Introduction**

Next generation sequencing has revolutionised rare disease diagnostics; more patients than ever are receiving a molecular diagnosis for their rare genetic disorders. This has been driven by the ever-increasing rise in novel disease-gene discoveries, which is expanding the number of genes tested for in clinic.1 Making molecular genetic diagnoses is hugely important to patients and their families and can pave the way for therapeutic options, cascade testing, and family planning.2

However, most rare disease patients (up to 70% depending on clinical specialty) lack a definitive, molecular diagnosis.3 Clinical genetic testing often involves application of a gene panel either as the ordered test or by the analysis strategy applied to exome and genome sequencing.4 In the UK, the national genome sequencing programme only reports on variants in a pre-specified gene panel. Accredited clinical laboratories have no obligation to report on variants, including *de novo* variants outside of the panel applied.5 Yet many patients harbour pathogenic variants not captured by a gene panel, or in genes yet to be associated with disease. Indeed, approximately 50% of genes thought to cause disease through haploinsufficiency are yet to be associated with a clinical phenotype.6,7 Ergo, there is an unmet need for holistic and experimental approaches to identify novel disease genes and their associated phenotypes. These discoveries are critical for new genes to be added to diagnostic panels and for analytical approaches to uplift diagnostic rates.

Current barriers to novel gene discovery

Novel disease gene discovery is a protracted process that requires identifying multiple, unrelated patients with variants in the same gene affected with similar phenotypes. These ‘discoveries’ are then followed up with functional studies to provide evidence for gene causality.

Assessment of exome and genome data typically involves analysis of a small number of related individuals on a family-by-family basis. However, these analyses are time-consuming and resource intensive, often requiring commercial software and cross-checking public databases. Each family member has 3-4 million variants in their genome and ~30,000 variants in their genes. Assessing every potential pathogenic variant is simply impossible.3 Whilst filtering techniques can restrict variant lists considerably, tens to thousands of variants of uncertain significance (VUS) typically remain with little to distinguish pathogenicity between them, particularly for genes of unknown function.8 It is not possible to investigate all potential candidate variants since this necessitates intensive functional experiments on variants of ostensibly equal predicted causality, which is proving a major bottleneck. Researchers are reluctant to invest in expensive studies without persuasive evidence that a given candidate warrants pursuing; however, identifying which variants should be prioritized is challenged by the paucity of knowledge into the function of most human genes. Therefore, these VUSs end up as long lists of unreported variants present in a patient’s sequencing results that no one has time to resolve or investigate further. In many cases, these lists will contain the causal variant and thus represent missed opportunities for molecular diagnosis.

The Matchmaker Exchange

One popular route to pursue candidate variants is through the Matchmaker Exchange (MME)9. MME has proven successful in building case series of patients with shared phenotypes involving the same gene, which are later taken to publication.10 However, this relies on knowing which gene candidates, of many, are best to submit to MME. Due to institutional restrictions on data sharing, it is not possible to query MME and return a list of genotypes and phenotypes for all submissions. Each match with another submitter requires electronic correspondence whereby both parties may choose to share variant and genotype specific data. Furthermore, there may be multiple matches per patient, making this method cumbersome and difficult to manage for large cohorts. Therefore, there are clear advantages to reduce the number of candidate variants for ongoing investigation.

Gene constraint

Mutation is random, giving rise to new variants of which most do not have biological impact; however, some variants have greater consequences and may help us adapt and evolve, yet others may be harmful and cause disease. Natural selection purges deleterious variation from human populations as fewer individuals with damaging variants survive and reproduce. However, in large population databases, such as gnomAD, we still observe loss-of-function (LoF) variants because some genes are more tolerant than others to inactivation of one or even both gene alleles.11 We can exploit this principle to identify genes with fewer LoF variants observed in population datasets compared with random and expected variant rates, signifying genes most intolerant to LoF.11,12

Karczewski *et al.11* developed the Loss-of-function Observed/Expected Upper-bound Fraction (LOEUF) score, which compared for each gene in gnomAD, the number of observed LoF variants in 125,748 individuals compared with the number expected. LOEUF places >19,000 genes along a continuous spectrum of intolerance to gene inactivation, whereby low scores i.e., the fewest pLoF variants observed compared to expectation, are the most intolerant to LoF. Indeed, genes in the first LOEUF decile (equivalent to a score <0.2) have been validated as the most enriched for Online Mendelian Inheritance in (OMIM) haploinsufficient disease genes and show the greatest biological essentiality.11 Yet, as of January 2021, 65% of genes in the lowest LOEUF decile are yet to have an OMIM disease association2, leaving hundreds of undiscovered potential disease genes causing unrecognized phenotypes in patients.

Whilst statistical methods exist to identify potential novel disease genes using excess *de novo* mutation analysis, such as DeNovoWEST14 and DeNovolyzeR15, these methods require huge cohorts of similar phenotypes, such as autism spectrum disorder. This study takes a non-statistical approach across a more heterogenous cohort and aims to uplift novel disease gene discovery by targeting pLoF variants (with the greatest pathogenic potential) in genes whereby inactivation of a single copy of the gene is highly probable to cause dominant disease. We apply this method to the 100,000 Genomes Project, that has brought genome sequencing directly to patients with rare diseases in the UK.15 We move from a “patient-to-gene” approach to a “gene-to-patient” approach, whereby we are powered to identify and assign rare putative pathogenic variation in predicted disease genes to patients, cohort-wide.

**Material and Methods**

General methodological principle

We propose an objective filtering strategy that can be applied at scale. We apply the LOEUF metric of intolerance to gene inactivation to define a list of predicted haploinsufficient disease genes. We select genes with a LOEUF score <0.2 (first decile), which demonstrates the highest probability of representing autosomal dominant disease.11 By leveraging genomic and phenotypic data from rare disease trios in the 100,000 Genomes Project, we adopt an objective gene-to-patient approach that filters for rare, *de novo* pLoF variants in LoF constrained genes and matches these to rare disease patients. For this study, we exclude any variants in known OMIM disease genes (autosomal dominant or recessive) and focus only on novel disease genes. Where more than one patient with a *de novo* pLoF variant is found in the same gene, we call this a *novel disease gene contender* and then assess for phenotype overlap. This approach reduces analytical noise to focus on the most likely novel disease genes (**Figure 1**) and identifies suitable candidates for functional validation.

Data access

Access to the secure GEL research environment (RE) and high-performance cluster was obtained following information governance training and as a member of the Genomics England Clinical Interpretation Partnership (GeCIP): *Quantitative methods, machine learning, and functional genomics* and with approved project ID: RR359 - *Translational genomics: Optimising novel gene discovery for 100,000 rare disease patients*. This provided access, originally in 2019, to an aggregate *vcf* file of 20,050 rare disease families called using the Illumina Starling pipeline and passing quality control parameters as previously described.5 The majority of patients were children with neurodevelopmental disorders.5

Phenotype data for each patient were recorded by the referring clinician as a discrete list of human phenotype ontology (HPO) terms.16 The number of HPO terms varied considerably between patients with some individuals only having a single HPO term recorded. These data were stored within the RE in a LabKey data management system. The R LabKey package was used to extract HPO terms for each patient and merge these with genotype data.

Code availability

Code generated for this project is specific to data securely held within the GEL RE. Only users with the necessary permission and governance training can access these data. Scripts are available to GEL users within the RE machine learning directory.

Data filtering

Initial analysis was undertaken in October 2019. We selected full parent/offspring trios for *de novo* analysis, reducing the number of available families from 20,050 to 13,949. Bespoke scripts utilising bcftools17, VEP18 and Exomiser19 were developed to filter data. LOEUF scores were downloaded from gnomAD (<http://gnomad.broadinstitute.org/downloads>) and imported into the RE. We filtered out variants with an allele frequency (AF) >0.001 across all gnomAD populations and retained only *de novo* pLoF variants (canonical splice site, frameshift, stop gain/nonsense, start loss, stop loss) on RefSeq transcripts called by VEP in genes with a LOEUF score <0.2 to reflect genes with the greatest LoF constraint. To account for potential false positive pLoF calls, we applied LOFTEE v1.0 (<https://github.com/konradjk/loftee>), which removed low confidence variants such as those in the last exon. Variants remaining after LOFTEE filtering were deemed high confidence variants.

Merging genotype data with additional datasets

High confidence variants in putative disease genes that remained following the filtering approach in 2019 (AF < 0.001, *de novo*, pLoF, LOEUF <0.2) were classified as either found in a *known OMIM disease gene* (already associated with disease), or in a *non-OMIM disease gene* (not yet associated with disease); achieved by querying the OMIM application programme interface in October 2019. All novel disease gene contenders were compared with two mouse databases, the International Mouse Phenotyping Consortium (IMPC) database and Mouse Genome Informatics (MGI) database.20,21

Selecting high priority novel disease gene candidates

High confidence pLoF variants in novel disease gene contenders were selected as *candidate pathogenic variants*.

*Phenotype overlap*

We assessed for phenotype overlap between unrelated patients who shared a candidate pLoF variant in the same gene. To do this, we computationally compared HPO terms (using their coded identification number) between individuals and considered a phenotype overlap to be when any single HPO matched exactly. Genes were prioritized as Class 1 candidates if more than one unrelated patient harboured a candidate pathogenic variant in the same gene and there was a phenotype overlap (**Table 1**). These novel disease gene contenders were further curated against the literature to ascertain if there were existing publications implicating any of the genes as disease-causing, prior to being indexed in OMIM.

For novel disease gene contenders with only one pLoF variant in the cohort (i.e., unique to one individual), we curated high-level phenotypes for each patient by manually upscaling their HPO terms to align with the terminology used in the publicly accessible DECIPHER (DatabasE of genomiC variation and Phenotype in Humans using Ensembl Resources) database (<http://deciphergenomics.org>). For example, hydrocephalus was upscaled to ‘disorder of the nervous system’ and atrial septal defect was coded as ‘disorder of the cardiovascular system’.22 We then compared high-level phenotypes of GEL patients with DECIPHER patients harbouring *de novo* variants (pLoF or missense) in the same gene. We included *do novo* missense variants in DECIPHER to increase the number of genes with an associated phenotype for comparison. When high-level phenotypes matched, we classified these genes as Class 2 candidates. In Class 3 candidate genes, phenotypes did not match, or no comparison was available (**Table 1**).

Taking candidates forward

We requested permission to submit genes to GeneMatcher23 for Class 1 genes by filling in request forms within the RE. We completed Clinician Contact Request forms for all Class 1 candidates to obtain more detailed and current phenotype information from the patients’ referring clinician, in addition to obtaining consent to share genotypes, phenotypes, and consent for publication with any matches made using the GeneMatcher node of MME. Where we successfully matched with international colleagues through MME and a case series was already underway, we worked with the patients’ clinician to include their patient in the existing case series. Where no case series were established, we initiated a new interest group to lead on collecting phenotype data from collaborators and started functional experiments in *Xenopus* onnovel disease gene contenders.

Validation of method

To validate whether we could correctly predict novel disease genes, we compared our novel disease gene contenders in 2019 against an updated list of dominant OMIM disease genes from 2021, in addition to literature published between 2019 and 2021. If one of our predicted novel disease gene contenders from 2019 was added to OMIM or was published as a disease gene between 2019 and 2021, we manually compared the HPO terms of GEL patients with the clinical phenotypes reported in the literature and/or OMIM to assess concordance (**Figure 2**). We considered our method as having correctly predicted a disease gene when any of the patients in GEL had significant overlapping features with the clinical presentation published for variants in the same gene and the GEL variant would meet, at minimum, likely pathogenic status by ACMG-AMP guidelines.24,25 We further assessed whether any alternative diagnoses were made by NHS accredited genetics laboratories between 2019 and 2021.

**Results**

Data from the 100,000 Genomes Project (13,949 trios, involving 41,847 individuals) revealed 643 rare (AF <0.001), *de novo* pLoF events filtered in 1,044 pLoF-constrained genes (**Figure 3**). 475 variants were in 148 known OMIM genes (as of October 2019) and 168 were in novel disease gene contenders (involving 126 unique genes). Of these, 27 genes had more than one GEL kindred affected and 18 had overlapping phenotypes, meeting Class 1 criteria (**Table 1**). Of these Class 1 genes, five were absent from OMIM but had been published in the literature (**Supplementary Table 1**). Six more of these genes have since been published as disease-causing genes with matching phenotypes to our GEL probands (**Table 2**).

Nine genes had more than one GEL kindred affected but the phenotypes between patients were non-overlapping meaning that there were no exact matches of HPO terms between patients; 4 genes met Class 2 criteria with high-level phenotypes overlapping with DECIPHER entries, and 5 genes met Class 3 criteria (**Supplementary Table 1**).

Ninety-nine variants in 99 unique genes were identified in 98 individuals (**Supplementary Table 2**). Of these, 50 genes were classified as Class 2 candidates meaning their high-level phenotypes overlapped with individuals in DECIPHER harbouring *de novo* pLoF or missense variants in the same gene. Forty-nine genes were Class 3 meaning no patients within GEL or DECIPHER had matching phenotypes involving the same gene.

Investigating and validating putative disease genes

Between 2019 and 2021, 23/126 (18%) of our novel disease gene contenders have been published by independent groups. Class 1 candidates were the highest predictors of disease genes with 11/18 (61%) having been functionally validated and published confirming their status as new disease genes.

Of the Class 2 and Class 3 genes occurring in unique individuals, 2/50 (4%) and 10/49 (20%) have been published with evidence of causality, respectively. Of the remaining seven Class 1 genes yet to be validated, case series/and or functional experiments are underway. By 2021, 15 patients had likely pathogenic or pathogenic variants independently identified in alternative known disease genes by GEL diagnostic laboratories. In total, we identified 126 novel disease gene contenders.

**Discussion**

We rapidly applied an objective filtering strategy across a large cohort and identified 18 high-confidence putative novel disease genes of which 11/18 (61%) have since been validated through functional experiments and confirmed as disease-causing. Additionally, we identified a further 108 novel disease gene contenders.

In total, 23/126 (18%) of the genes identified in our study have been validated as disease-causing and diagnoses are being returned to patients who would otherwise have a negative genome report. This was achieved by a targeted gene-to-patient approach applied to the 100,000 Genomes Project with the power to detect very rare, pLoF variation in genes most intolerant to LoF. However, only in time will we determine the full specificity as well as sensitivity of this approach.

Class 1 genes and internal matches in GEL

Since initial analysis, 11/18 (61%) Class 1 genes (**Table 1**) have undergone functional validation and been published by independent groups confirming their status as novel disease-gene discoveries and we anticipate this number to increase over time. Class 1 genes outperformed Classes 2 and 3 (Fisher’s exact test <0.0001) likely due to the greater specificity and granularity of phenotypes available for internal matching within GEL.

There were nine genes whereby unrelated patients in GEL had pLoF variants in the same gene, yet no patient shared the same HPO term. However, three of these genes have since been published and the published phenotypes overlap with the GEL patients (**Supplementary Table 1**). This may be explained by variability in HPO terms reported in GEL; some patients had many HPO terms recorded, yet others had only one or two. In **Table 2**, patients with pLoF variants in *SPEN* and *TANC2* only overlapped by one HPO term (intellectual disability). Yet, when the disease phenotype was further delineated in published case series for both genes, many more features observed in the GEL patients were consistent with the reported phenotypic spectrum. This highlights the need for longitudinal and deep phenotyping data in automated gene discovery studies.

Due to the automated process of exact HPO term matching between GEL patients, we potentially missed overlapping phenotypes recorded with subtly different nomenclature, e.g. one patient with intellectual disability (HP:0001249) would not match another patient with mild intellectual disability (HP:0001256).

Class 2 and 3 genes

More Class 3 genes 10/50 (20%), were published as novel causal genes by 2021 than Class 2 genes 2/49 (4%); Fisher’s exact test 0.027. This may be due to small sample sizes but could reflect a weakness in Class 2 and 3 classification (**Table 1**). Comparing high-level phenotypes is potentially problematic as it lacks the granularity required to assess clinical overlap. Furthermore, we compared high-level phenotypes of GEL patients with patients in DECIPHER harbouring *de novo* missense variants, which are considerably more common and less likely to be pathogenic, increasing the possibility of false disease-phenotype associations. Additionally, it is possible that some patients in our cohort were also in DECIPHER, however due to data anonymity this could not be verified.

Lessons learnt

Class 2 and Class 3 genes may be better assessed through MME. Sharing more detailed phenotype data would provide the granularity to assess true clinical overlap. In GEL, this step involved contacting the patient’s clinician for permission to share data with matches through MME; and this process was not always successful. As MME involves manual correspondence between peers, this cannot be easily automated, highlighting the advantages of internal phenotype matching within the same cohort. We are fully utilising MME for novel gene candidates, however presenting these results is outside of the scope of this manuscript.

Novel gene discovery remains time-consuming

Whilst our method is rapid at identifying highly promising novel candidate genes, there remains persistent time requirements to validate any results through case series and functional experiments; however, the strength of the method is in rapidly identifying which VUSs to pursue and therefore shortening the process of discovery. We have identified seven Class 1 genes for which we are accruing case series on six of these and have started functional studies in four (**Table 2**). We believe our method provides the opportunity to identify the most salient candidates for follow-on studies, meaning that many patients will have their “most damaging” VUS investigated, when typically, no candidates would have been pursued.

Considerations and limitations

*De novo analysis applied to predominantly neurodevelopmental phenotypes:*

We utilised trios for *de novo* analysis, meaning that families without trio data were excluded. However, with plans to sequence 5 million more genomes in the UK, we believe our method will prove increasing more effective. Furthermore, we plan to refine our analysis to query all affected individuals in GEL with pLoF variants in our novel disease gene contenders, even if segregation data is unavailable.

The 100,000 Genomes Project is enriched for patients with rare neurodevelopmental disorders and therefore we risk comparing patient phenotypes within a cohort enriched for similar phenotypes. We were cautious of defining phenotype overlap as any two patients exactly matching on one HPO term, however due to the variability in number of HPO terms reported in GEL, this maximized sensitivity of Class 1 genes and enabled us to correctly predict *SPEN* and *TANC2* as novel disease genes.

*Statistical rigor:*

The large number of neurodevelopmental disorders in the dataset caused by many heterogenous genes precludes the reliability of statistical methods to confirm/refute novel disease gene contenders, although with ongoing genome sequencing in the UK this will likely be overcome. Further, we specifically focused on pLoF variants only, meaning that we are not powered even with 3 *de novo* variants per gene (the maximum we observed for class 1 candidates) to reach statistical significance using a case/control Fisher’s test and multiple test correction.14 Instead we rely on the established approach of identifying overlapping phenotypes to further prioritise the best candidates for functional validation.

*Minor allele frequency:*We used a liberal AF of <0.001, yet the highest variant frequency we observed was 0.0002. The presence of these rare, *de novo* variants within gnomAD could represent recurrent de novo variation.32 While a more restrictive AF would increase confidence of pathogenicity, pathogenic disease variants can be present in population databases due to incomplete penetrance, effects of cis-regulatory variation, and adult-onset disease.33 Nevertheless, our cohort is probably depleted for adult-onset diseases as early-onset conditions are more likely to have complete trios.

*Prioritising haploinsufficiency:*

Our method is enriched for haploinsufficient disease genes, and we did not prioritise biallelic observations in our analysis.11 Using a LOEUF score <0.2 (top decile), enabled us to select the genes most highly constrained for LoF, although the expectation was that these would be associated with dominant inheritance, meaning our approach is not enriched for autosomal recessive novel gene discovery.. Several genes in the top decile may be embryonically lethal, although we do not expect to observe these in our cohort. With higher LOEUF thresholds it is likely that further haploinsufficient disease genes and even more recessive disease genes will be found, but at the expense of increased noise.11

*Classification of pLoF variants*

We included start loss and stop loss within the category of LoF variants, however these variants often do not constitute true LoF and show selection signatures more similar to missense variants.34 We only observed 6 start/stop loss variants, and therefore potential misclassification of these variants is not expected to have substantively impacted our analysis. Our current analysis strategy also misses other LoF variants e.g. untranslated region variants, extended splice site, and structural variants (SV). Research into these potential LoF disrupting variants using tools such as UTRannotator35, spliceAI36 may further expand the disease gene candidate list.

*False positive pLoF variants*

Not all pLoF variants truly cause LoF; many are enriched for technical, rescue, and impact errors.11,33 Whilst *in silico* tools can identify some of these errors, manual curation is the most effective method to identify potential false positives.33 However, this process is extremely time-consuming and not yet standardised; therefore there is risk that we included false positive LoF variants in our analysis.33 We expect these false positives are more likely to be variants with higher allele frequencies in gnomAD or in Class 2 and 3 genes whereby detailed phenotype data cannot be assessed for overlap. Indeed, 15 of our pLoF variants in Class 2 and 3 genes were in individuals who had an alternative pathogenic variant (**Supplementary Table 2**). Whilst this does not rule out the potential for a second diagnosis, which occurs up to 5% of the time6, it does raise the possibility of a variant without functional impact.

**Conclusion**

Utilising a large cohort and adopting a highly efficient gene-based approach can accelerate novel gene discovery and target the most appropriate variants and genes for functional validation. This can uplift diagnostic rates and add new disease genes to clinical gene panels.

As rare disease cohorts continue to increase, there is increasing demand to automate analyses and reduce the burden of variants requiring analysis by clinical scientists. With increasing study sizes, our method should be better powered to detect rare pathogenic variation shared across individuals but necessitates real-time comparison to previously generated large datasets if the approach is to be used in routine diagnostics. Assessing phenotype overlap is an important step in our method and with drives towards data sharing, there is opportunity to securely access data and apply automated phenotype matching within and across cohorts using trusted research environments, such as the NHGRI’s Genomic Data Science Analysis, Visualisation, and Informatics Lab (AnVIL) space.37

We anticipate our method can be applied by other researchers to their own cohorts; however, we emphasize the importance of trio analyses, and encourage prudence when determining what constitutes LoF. We demonstrate that gene-based approaches can successfully identify novel disease genes, and with larger rare disease cohorts it is hoped that more discoveries will be identified for the benefit of patients, their families, and the wider scientific community.

**Declarations**

**Ethics approval and consent to participate**All patients included in this study consented to participate in the 100,000 Genomes Project - ethics approval by the Health Research Authority (NRES Committee East of England) REC: 14/EE/1112; IRAS: 166046. The ethical approval letter is available upon request.

**Consent for publication**  
Not applicable

**Availability of data and materials**  
The anonymised phenotype and genotype data that support the findings of this study are only available as a registered GeCIP member in the Genomics England Research Environment, but restrictions apply to the availability of these data due to data protection and are not publicly available. Information regarding how to apply for data access is available at the following url: <https://www.genomicsengland.co.uk/about-gecip/for-gecip-members/data-and-data-access/>. No individual’s variant or phenotype data can be shared or published due to data sharing restrictions. All data shared in this manuscript were approved for export by Genomics England.

**Competing interests**  
No competing interests or conflicts to declare.

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**Authors' contributions**

Conceptualization: E.G.S., S.E., D.B., A.oD.L., H.L.M.; Data curation: E.G.S, D.S., H.B; Formal analysis: E.G.S., D.S., A.L.T.T., H.B., R.v.J.; Funding acquisition: E.G.S., S.E., A.oD.L., H.L.M.; Investigation: E.G.S. D.S.; Methodology: E.G.S., D.S., S.E., A.oD.L., H.L.M.; Project Administration: E.G.S., S.E. G.E.R.C; Resources: E.G.S., D.S. G.E.R.S.; Software: E.G.S., D.S.; Supervision: S.E., D.B., A.oD.L., H.L.R.; Validation: E.G.S., H.B., A.L.T.T.; Visualization: E.G.S., D.S.; Writing-original draft: E.G.S.; Writing-reviewing & editing: E.G.S., D.S., A.L.T.T., H.B., R.V.J., D.B., A.oD.L., H.L.R., S.E.

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Figure titles and legends:

**Figure 1** | Method to uplift novel disease gene discovery

***(A)*** *A typical “patient-to-gene” approach, whereby patient A’s exome or genome is analysed and multiple candidates remain of similar predicted causality.* ***(B)*** *A proposed “gene-to-patient” approach to identify novel disease genes, that challenges the widely adopted diagnostic analytical paradigm of exome and genome sequencing. In this approach, a large-scale database is agnostically filtered for high confidence, rare, de novo, pLoF variants in genes with a LOEUF <0.2, and these variants are assigned to patients. Associated phenotypes are compared between patients with de novo pLoF variants in the same gene. Abbreviations: AF – Allele Frequency; GEL – Genomics England; MME – Matchmaker Exchange; OMIM – Online Mendelian Inheritance in Man; pLoF – predicted loss of function.*

**Figure 2** | Workflow applied for the validation of novel disease genes contenders

*Workflow applied to validate novel disease gene contenders as new disease genes. The red path shows the pathway for Gene 1, which was identified in 2019 and published in the literature between 2019 and 2021. Associated phenotypes of GEL patients with pLoF in Gene 1 were compared with the published phenotype and matched, validating Gene 1 as a correctly predicted new disease gene. The blue path shows the pathway for Gene 2; this gene was compared with OMIM and the literature and was not yet published/in OMIM and will be re-compared at a later stage. Gene 3 (thin grey line) was present in OMIM by 2021 and associated phenotypes in GEL patients with pLoF variants in Gene 3 overlapped with the published literature, validating this gene as a correctly predicted new disease gene.*

**Figure 3 |** Summary of Class 1, 2 and 3 results

*Summary of gene discovery results after filtering variants from GEL families.*

**Table 1** | Classification of novel disease gene contenders

**Table 2 |** 13 putative novel disease genes

*A table of 13 putative novel disease genes identified from analysis in 2019. Shared phenotypes between patients involving pLoF variants in the same gene are listed. Grey cells highlight candidates that have since been published in June 2021. For these, shared phenotypes between patients in GEL and patients included in publications by 2021 are recorded. Abbreviations: BMI – body mass index; GEL – Genomics England; HPO – Human phenotype ontology; MaxFreq – Maximum allele frequency in gnomAD v2.1.1 and 1000 Genomes phase 3 data; N/A – not available for comparison.*

**Genomics England Research Consortium**

Ambrose, J. C. 1 ; Arumugam, P.1 ; Bevers, R.1 ; Bleda, M. 1 ; Boardman-Pretty, F. 1,2 ; Boustred, C. R. 1 ; Brittain, H.1 ; Caulfield, M. J.1,2 ; Chan, G. C. 1 ; Fowler, T. 1 ; Giess A. 1; Hamblin, A.1; Henderson, S.1,2; Hubbard, T. J. P. 1 ; Jackson, R. 1 ; Jones, L. J. 1,2; Kasperaviciute, D. 1,2 ; Kayikci, M. 1 ; Kousathanas, A. 1; Lahnstein, L. 1 ; Leigh, S. E. A. 1 ; Leong, I. U. S. 1 ; Lopez, F. J. 1 ; Maleady-Crowe, F. 1 ; McEntagart, M.1; Minneci F. 1 ; Moutsianas, L. 1,2 ; Mueller, M. 1,2 ; Murugaesu, N. 1; Need, A. C. 1,2 ; O‘Donovan P. 1; Odhams, C. A. 1 ; Patch, C. 1,2 ; Perez-Gil, D. 1 ; Pereira, M. B.1 ; Pullinger, J. 1 ; Rahim, T. 1 ; Rendon, A. 1 ; Rogers, T. 1 ; Savage, K. 1 ; Sawant, K. 1; Scott, R. H. 1 ; Siddiq, A. 1 ; Sieghart, A. 1 ; Smith, S. C. 1; Sosinsky, A. 1,2 ; Stuckey, A. 1 ; Tanguy M. 1 ; Taylor Tavares, A. L.1; Thomas, E. R. A. 1,2 ; Thompson, S. R. 1 ; Tucci, A. 1,2 ; Welland, M. J. 1 ; Williams, E. 1 ; Witkowska, K. 1,2 ; Wood, S. M. 1,2.

1. Genomics England, London, UK

2. William Harvey Research Institute, Queen Mary University of London, London, EC1M 6BQ, UK.