Exploring the mutational landscape of genes associated with inherited retinal disease using large genomic datasets: identifying loss of function intolerance and outlying propensities for missense changes

Alexander Tanner, Hwei Wuen Chan, Elena Schiff, Omar A Mahroo, Jose S Pulido

ABSTRACT

Background Large databases permit quantitative description of genes in terms of intolerance to loss of function (‘haploinsufficiency’) and prevalence of missense variants. We explored these parameters in inherited retinal disease (IRD) genes.

Methods IRD genes (from the ‘RetNet’ resource) were classified by probability of loss of function intolerance (pLI) using online Genome Aggregation Database (gnomAD) and Database of genomics variation and Phenotype in Humans using Ensembl Resources (DECIPHER) databases. Genes were identified having pLI ≥0.9 together with one or both of the following: upper bound of CI <0.35 for observed to expected (o/e) ratio of loss of function variants in the gnomAD resource; haploinsufficiency score <10 in the DECIPHER resource.

Results Of 280 analysed genes, 39 (13.9%) were predicted loss of function intolerant. A greater proportion of X-linked than autosomal IRD genes fulfilled these criteria, as expected. Most autosomal genes were associated with dominant disease. PANTHER analysis showed >100 fold enrichment of spliceosome tri-snRNP complex assembly. Most encoded proteins were longer than the median length in the UniProt database. Fourteen genes (11 of which were in the ‘haploinsufficient’ group) showed under-representation of missense variants. Six genes (SAMD11, ALMS1, WFS1, RP1L1, KCNV2, ADAMTS11) showed over-representation of missense variants.

Conclusion A minority of IRD-associated genes appear to be ‘haploinsufficient’. Over-representation of spliceosome pathways was observed. When interpreting genetic tests, variants found in genes with over-representation of missense variants should be interpreted with caution.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Large genomic datasets provide metrics for individual genes relating to under-representation of predicted loss of function variants and over-representation or under-representation of missense variants.

WHAT THIS STUDY ADDS

⇒ This study explores the above metrics for genes associated with inherited retinal disease: 39 were predicted ‘loss of function intolerant’ according to such metrics; 14 showed under-representation and 6 showed over-representation, of missense variants.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings can be compared with future studies of genes associated with other disorders. Also, when interpreting genetic tests, variants found in genes with over-representation of missense variants should be interpreted with caution.

INTRODUCTION

Inherited retinal diseases (IRDs) are a leading cause of blindness in children and the working age in many countries.1–4 Variants in over 250 genes are implicated. There are a number of unresolved questions relating to the spectrum of variants and mechanisms of disease.5 Some associated genes are ubiquitously expressed, yet pathogenic variants appear to give rise only to IRD.5 A number of genes show mutational hotspots, while other regions exist that rarely harbour disease-causing variants, either because the regions are highly conserved or because polymorphisms rarely cause disease. Identifying genes, or genetic regions, with particular characteristics might shed light on particular selection pressures, and also help in future interpretation of novel variants.6–7 The
range of genes and variants involved in IRDs has been recently reviewed comprehensively by Schneider et al., who discussed, among other things, the prevalence of different types of variant, as well as their amenability to various gene-based therapeutic approaches.

Metrics are available from large genomic datasets which can identify those genes in which loss of function variants appear to be under-represented (conventionally termed ‘haploinsufficient’ genes). Both databases were used to our study focused in particular on IRD genes. We also this group. Similar investigations have been per genes classified as having an under-

of these were, in the general population, found to have associated with retinal disease, and interrogated which converse approach: we took genes already known to be under-

landscape of IRD-

and could improve our understanding of the mutational potential highlighting particularly conserved pathways, still yield insights into aspects of those genes in particular, and so these metrics might not be affected. However, exploring these metrics for IRD genes might yield insights into aspects of those genes in particular, potentially highlighting particularly conserved pathways, and could improve our understanding of the mutational landscape of IRD-associated genes more generally.

For this study, we curated a list of IRD genes (from the Retinal Information Network online resource, https://sph.uth.edu/retnet/), and investigated the above metrics in two large genomic databases, namely Genome Aggregation Database (gnomAD) and DatabasE of genomic Variation and Phenotype in Humans using Ensembl Resources (DECIPHER). Both databases were used to identify genes with predicted ‘loss of function intolerance’, and the gnomAD resource was used additionally to identify those in which missense mutations were over-represented or under-represented. Genes of interest were evaluated in terms of associated pathways using the online gene ontology resource Protein Analysis Through Evolutionary Relationships (PANTHER).

The parameters investigated have been computed for each gene as a whole (based on the range of variants observed in the large datasets), rather than for any specific variants within the genes. Such parameters have been used, with some success, to identify candidate genes in whole genome data from patients with no molecular cause yet identified. In the present study, we took a converse approach: we took genes already known to be associated with retinal disease, and interrogated which of these were, in the general population, found to have an under-representation of loss of function variants, and also which had an under-representation or over-representation of missense variants.

We were interested to observe any particular patterns that emerged, estimating the proportion of IRD-associated genes classified as having an under-representation of loss of function variants and whether particular modes of inheritance were more commonly seen in this group. Similar investigations have been performed for loss of function intolerant genes in general, but our study focused in particular on IRD genes. We also explored whether such genes were more associated with syndromic disease, and whether certain pathways were over-represented. Identifying those genes with outlying propensities for missense variants could also be potentially useful: those IRD genes in which missense variants are over-represented may constitute ‘noisy genes’ such that missense variants in these genes, when found in patients, should be interpreted with caution.

## MATERIALS AND METHODS

### Databases and metrics

The gnomAD (https://gnomad.broadinstitute.org/) has over 141,456 individuals sequenced with 125,748 exomes and 15,708 genomes aligned against the Genome Reference Consortium Human genome build 37. Constraint variables are computed for most genes. Probability of loss of function intolerance (pLI) refers to the probability that loss of function mutations are selected against. The ratio of observed variants to the number expected (by random chance) (o/e) is also computed along with a CI. A pLI of 0.9 or greater suggests a high level of intolerance to loss of function, and this is confirmed when the CI of o/e for loss of function variants is 0.35 or lower. The o/e for missense variants can also be explored: for this study Z scores of 2.99 or greater, or −2.99 or less, were taken to indicate a significant over-representation or under-representation of missense variants (a Z value of −2.99 means that the chance of variants occurring randomly with such low frequency in the population is only 0.14% (0.0014)).

DECIPHER (https://decipher.sanger.ac.uk/) comprises genomic data from 36,000 children with rare diseases from over 270 specialist centres. Previously, a pLI separate to that from gnomAD was computed, but the pLI currently used is the gnomAD pLI. A haploinsufficiency score (HI) is also given where an index of less than 10% is taken to indicate that loss of function is significantly selected against.

### Gene classification

Genes listed in the Retinal Information Network online resource (https://sph.uth.edu/retnet/) were included in this study. Those which met both of the following criteria were identified: (1) a pLI in gnomAD of ≥0.9, and (2) an upper CI o/e limit for loss of function variants in gnomAD of <0.35 and/or an HI in DECIPHER of <10. These genes were taken as likely to be intolerant to loss of function. These genes were then evaluated in the PANTHER resource (http://pantherdb.org/) to identify common pathways in which the encoded proteins were involved, exploring which biological processes might be particularly over-represented in these gene groups (using the over-representation analysis). Also, genes with gnomAD missense (non-synonymous variants) o/e Z scores of −2.99 or less, or of 2.99 or greater, were identified and analysed similarly. The gene list curation from RetNet and investigation of metrics in gnomAD and DECIPHER were performed in September 2021;
the evaluation in PANTHER was performed in February 2022.

RESULTS
Intolerance of loss of function analysis
Of 309 genes and loci listed in the Retinal Information Network online resource, 29 were excluded (owing to one of the following: a mitochondrial location, no specific gene yet identified for the locus or lack of relevant data available on gnomAD and DECIPHER), leaving 280 available for inclusion (including 262 autosomal and 18 X-linked genes; online supplemental table 1). Of these, 39 genes (13.9%) met the specified criteria for loss of function intolerance. Of the IRD genes with pLI ≥0.9, there were no additional genes identified in the DECIPHER resource with an HI <10 that did not also have a gnomAD upper CI o/e limit for loss of function variants of <0.35. The 39 genes are listed in table 1. Of note, 8 of these genes are X-linked. Thus, the proportion of X-linked IRD genes fulfilling criteria for intolerance to loss of function (8/18) was 44.4% while the corresponding proportion of autosomal IRD genes (31/262) was significantly lower at 11.8% (p<0.0001 for difference in proportions). The majority of the 31 autosomal genes listed in table 1 are associated with dominantly inherited disorders. Only three genes are associated exclusively with recessively inherited disease; of note, implication of each of these genes with retinal disease has been established only by a single report, suggesting the association may not be secure. The final column of table 1 contains comments on the strength of evidence for association with monogenic retinal disease; these will be mentioned in the Discussion section.

The 39 genes identified as showing intolerance to loss of function were then evaluated for over-representation in biological processes using the PANTHER database. The analysis showed that the following process was enriched more than 100-fold: spliceosome tri-snRNP complex assembly (p=3.44×10^-6, false discovery rate (FDR) of 0.0049). Other processes showing significant over-representation included visual perception, eye morphogenesis, cellular protein localisation and nervous system development (online supplemental table 2) give these results in detail).

Exploration of size of encoded proteins
It has been noted that haploinsufficient genes are significantly longer than haplosufficient genes. From 20 394 reviewed genes on the Uniprot database (https://www.uniprot.org/), we found the median protein length was 415 amino acids. Only five of the 39 genes in table 1 encode a protein with fewer than 415 amino acids, and four of these (RSI, PRPS1, RP2, OPN1LW) are X-linked. Only one gene (OTX2) from table 1 was both autosomal and associated with a protein with fewer than 415 amino acids.

Analysis by frequencies of missense mutations
The 280 genes were also classified by observed/expected frequencies of non-synonymous missense variants. Fourteen genes (5.0%) were identified in which such variants appeared to be negatively selected (under-represented). These are given in table 2. Eleven of the 14 genes had already been classified as intolerant to loss of function (table 1); only KLHL7, PNPLA6 and PRPF6 are not present in table 1. As in table 1, the majority of genes in table 2 are associated with dominantly inherited disorders. PANTHER analysis revealed >100 fold enrichment of spliceosome tri-snRNP complex assembly process (p=3.00×10^-10, FDR of 4.76×10^-6).

Finally, those genes showing significantly more missense variants than expected were identified. Six genes (2.1%) met this criterion, listed in table 3. Most have long exons, and SAMD11 has relatively poor coverage on exome sequencing analyses. All of these genes were associated with recessively inherited diseases (exclusively autosomal recessive inheritance for 5 of the 6 genes). The PANTHER pathway analysis did not find particular biological processes enriched in this small number of genes.

DISCUSSION
In this study, we explored a novel classification of retinal disease-associated genes according to metrics relating to predicted tolerance to loss of function (as defined in two large genomic databases) and to under-representation or over-representation of missense variants (as computed in the gnomAD resource). We also sought to identify any broad biological pathways that were enriched in any of these groups.

We found that approximately 14% of IRD genes (as listed in the RETnet resource) overall were predicted to be intolerant to loss of function. The proportion for X-linked genes was significantly higher than that for autosomal genes. This might be expected as the mechanism of disease for many X-linked conditions, including X-linked retinal disease is frequently loss of function. For the autosomal genes, almost all were associated with dominantly inherited conditions (and those where exclusively recessive inheritance has been described have less strong evidence for association with retinal disease). Our finding of higher proportions of autosomal dominant and X-linked (and low proportion of autosomal recessive) Mendelian diseases associated with genes with high pLI is consistent with a previous study (not focusing on retinal disease) where these genes were compared with a random sample of other genes.

A number of genes encoding proteins involved in splicing complexes (PRPF3, SNRNP200, PRPF4, PRPF8, PRPF31) were found to fulfil criteria for intolerance to loss of function and are all associated with dominantly inherited disease. Of these, PRPF31 is known to be associated with disease resulting from haploinsufficiency. Why pathogenic variants in such ubiquitously expressed genes give only a rod-cone dystrophy is still not clear: rod photoreceptors appear uniquely vulnerable to loss of function in one PRPF31 allele. In contrast to haploinsufficiency, gain of function, often from specific missense variants, is
| Gene | Location | Mode of inheritance of disorders | Reported phenotypes | Comments on strength of association with retinal disease |
|------|----------|----------------------------------|--------------------|--------------------------------------------------------|
| COL11A1 | 1p21.1 | Dominant | Dominant Stickler syndrome type II; dominant Marshall syndrome | |
| MFN2 | 1p36.22 | Dominant | Dominant optic atrophy with neuropathy and myopathy; dominant Charcot-Marie-Tooth disease | |
| PRPF3 | 1q21.2 | Dominant | Dominant retinitis pigmentosa | |
| EFEMP1 | 2p16.1 | Dominant | Dominant drusen | |
| SNRNP200 | 2q11.2 | Dominant | Dominant retinitis pigmentosa | |
| ATXN7 | 3p14.1 | Dominant | Dominant spinocerebellar atrophy with macular dystrophy or retinal degeneration | |
| OPA1 | 3q29 | Dominant | Dominant optic atrophy; dominant optic atrophy with sensorineural hearing loss | |
| VCAN | 5q14.3 | Dominant | Dominant Wagner disease and erosive vitreoretinopathy | |
| NR2F1 | 5q15 | Dominant | Dominant optic atrophy with intellectual disability and developmental delay (Bosch-Boonstra optic atrophy) | |
| CTNNA1 | 5q31.2 | Dominant | Dominant macular pattern dystrophy (butterfly-shaped pigment dystrophy) | |
| RIMS1 | 6q13 | Dominant | Dominant cone-rod dystrophy | One family reported in detail and a single patient in a second report with a different phenotype. (Other variants reported, but lacking detailed information) |
| AHR | 7p21.1 | Recessive | Recessive retinitis pigmentosa | One family reported |
| KIAA1549 | 7q34 | Recessive | Recessive retinitis pigmentosa | One report of two families |
| GDF6 | 8q22.1 | Dominant and recessive | Recessive Leber congenital amaurosis; dominant Klippel-Feil syndrome; dominant microphthalmia | Single report of Leber congenital amaurosis |
| TOPORS | 9q21.1 | Dominant | Dominant retinitis pigmentosa | |
| PRPF4 | 9q32 | Dominant | Dominant retinitis pigmentosa | |
| HK1 | 10q22.1 | Dominant and recessive | Dominant retinitis pigmentosa; recessive nonspherocytic haemolytic anaemia; recessive hereditary neuropathy | |
| KIF11 | 10q23.33 | Dominant | Dominant microcephaly, lymphedema and chorioretinopathy | |
| TEAD1 | 11p15.3 | Dominant | Dominant atrophy areata/Sveinsson peripapillary degeneration | |
| FZD4 | 11q14.2 | Dominant | Dominant FEVR | |
| COL2A1 | 12q13.11 | Dominant | Dominant Stickler syndrome, type I; dominant bone dysplasias, developmental disorders, osteoarthritic diseases, syndromic disorders | |
| CCT2 | 12q15 | Recessive | Recessive Leber congenital amaurosis | One report |
| RB1 | 13q14.2 | Dominant | Dominant (or somatic) retinoblastoma; pinealoma; osteogenic sarcoma | |
| OTX2 | 14q22.3 | Dominant | Dominant syndromic microphthalmia; combined pituitary deficiency 6; early onset retinal dystrophy and pattern dystrophy | |

Continued
an important mechanism in much of dominant disease. The appearance of genes in which gain of function causes retinal disease in table 1 might suggest that loss of function adversely affects survival in a way other than by affecting the retina; heterozygosity for loss of function might have severe consequences for other systems. Many, but not all, of the genes in table 1 are also associated with syndromic or non-retinal disease. We also found that the majority of IRD genes identified as intolerant to loss of function encoded proteins above the median length in terms of amino acids.

Eight of the 31 autosomal genes have associations with monogenic retinal disease that arise from reports of relatively few families, suggesting less strong evidence for causative association. The first implication of RIMS1 in retinal disease came from a report of a single large family. A later report described a patient with the same RIMS1 variant, but a different phenotype (retinitis pigmentosa), and there have further variants reported, but without detailed evidence to secure a causative relationship. Implication of AHR in retinal disease comes from a report of a single family. For KIAA1549, there is a single report of two families. For GDF6, there is a single report of a patient with Leber Congenital Amaurosis, and in this case, both parents showed electroretinography (ERG) abnormalities. Similarly, for CCT2, there is a single report of Leber Congenital Amaurosis. With respect to ZNF423, one study reported a patient with recessive nephronophthisis (no ocular data) and two families with Joubert syndrome. For PITPNM3, one study reported two families; a subsequent study showed the carrier frequency of the variant in the general population was high for a dominant disease. Other reports of disease associated with this gene are either isolated cases or report variants that also have high carrier frequencies. A critical analysis of reported variants in autosomal dominant retinal dystrophies has questioned the evidence for disease association with PITPNM3 (and also with RIMS1), among other genes. Finally, while the C3 variants are associated with AMD, evidence of specific relationship is not proven to cause monogenic retinal disease.

Table 1 Continued

| Gene   | Location     | Mode of inheritance of disorders | Reported phenotypes                                                                 | Comments on strength of association with retinal disease |
|--------|--------------|----------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------|
| FBLN5  | 14q32.12     | Dominant                         | Dominant familial age-related macular degeneration; hereditary neuropathy with or without age-related macular degeneration |
| ZNF423 | 16q12.1      | Dominant and Recessive           | Recessive nephronophthisis; dominant Joubert syndrome                               | One report including two families with Joubert syndrome |
| PITPNM3| 17p13.2      | Dominant                         | Dominant cone-rod dystrophy                                                        | One report of two families                               |
| PRPF8  | 17p13.3      | Dominant                         | Dominant retinitis pigmentosa                                                      | Association with AMD, but not proven to cause monogenic retinal disease |
| C3     | 19p13.3      | Dominant and recessive           | Dominant susceptibility to atypical haemolytic-uraemic syndrome 5; recessive CS deficiency; polymorphisms confer risk for AMD |
| PRPF31 | 19q13.42     | Dominant                         | Dominant retinitis pigmentosa                                                      |
| JAG1   | 20p12.2      | Dominant                         | Dominant Alagille syndrome                                                         |
| RP2    | Xp11.23      | X-linked                         | Retinitis pigmentosa                                                               |
| RPGR   | Xp11.4       | X-linked                         | Retinitis pigmentosa; cone-rod dystrophy; macular dystrophy                        |
| DMD    | Xp21.2-p21.1 | X-linked                         | Duchenne muscular dystrophy                                                        | Electroretinogram may be abnormal                        |
| RS1    | Xp22.13      | X-linked                         | X-linked retinoschisis                                                             |
| OFD1   | Xp22.2       | X-linked                         | Joubert syndrome; orofaciodigital syndrome 1, Simpson-Golabi-Beahmel syndrome 2     |
| CHM    | Xq21.2       | X-linked                         | Choroideremia                                                                      |
| PRPS1  | Xq22.3       | X-linked                         | Retinitis pigmentosa, neuropathy, optic atrophy, deafness                           |
| OPN1LW | Xq28         | X-linked                         | Deuteranopia; blue-cone monochromacy                                               |

A number are associated also with syndromic or non-retinal disorders. The rightmost column contains comments, for some of the genes, on the strength of association with retinal disease (including highlighting those genes where there have been only single reports). These will be considered further in the Discussion section.
association with monogenic retinal disease is lacking. If these eight genes are excluded from the IRD list, the proportion of autosomal IRD genes meeting loss of function intolerance criteria is then 9.1%.

Interestingly, the only genes in table 1 associated with exclusively recessive inheritance are among those with evidence only from single reports. This might suggest that in cases where a novel genetic cause of recessive IRD is reported, with bi-allelic loss of function proposed as the disease mechanism, if that gene shows loss of function intolerance according to the criteria of the present study, such a report should be interpreted with caution.

We found that 5% of IRD genes showed significant under-representation of non-synonymous missense variants. The majority of these were also intolerant to loss of function, highlighting their importance in both terms. They were again mostly associated with dominantly inherited disorders, and a further gene encoding a splicing factor (PRPF6) emerged. Given that missense variants are relatively rare in these genes, any such novel variants found in patients with a consistent phenotype might be regarded as more likely to be pathogenic rather than incidental.

Only 2% of IRD genes met the criterion of significant over-representation of missense variants. These were all associated with autosomal recessive inheritance. This can make identifying the pathogenicity of novel missense variants in these genes more challenging. Fortunately, in KCNV2-associated retinopathy, electroretinography is pathognomonic for disease associated with this gene, facilitating judgements of pathogenicity of rare variants.39 On the other hand, variants in RP1L1 give rise to a dominantly inherited occult maculopathy or a recessively inherited rod-cone dystrophy. The phenotypic

| Table 2 | Genes which fulfilled criteria of negative selection for missense variants |
|---------|-------------------------------------------------|
| **Gene** | **Location** | **Mode of inheritance of disorders** | **Phenotypes** |
| PRPF3   | 1q21.2    | Dominant | Dominant retinitis pigmentosa |
| SNRNP200 | 2q11.2    | Dominant | Dominant retinitis pigmentosa |
| NR2F1   | 5q15      | Dominant | Dominant optic atrophy with intellectual disability and developmental delay (Bosch-Boonstra optic atrophy) |
| CTNNA1  | 5q31.2    | Dominant | Dominant macular pattern dystrophy (butterfly-shaped pigment dystrophy) |
| KLHL7   | 7p15.3    | Dominant | Dominant retinitis pigmentosa |
| HK1     | 10q22.1   | Dominant and recessive | Dominant retinitis pigmentosa; recessive nonspherocytic haemolytic anaemia; recessive hereditary neuropathy |
| KIF11   | 10q23.33  | Dominant | Dominant microcephaly, lymphedema and chorioretinopathy |
| COL2A1  | 12q13.11  | Dominant | Dominant Stickler syndrome, type I; dominant bone dysplasias, developmental disorders, osteoarthritic diseases, syndromic disorders |
| PRPF8   | 17p13.3   | Dominant | Dominant retinitis pigmentosa |
| PNPLA6  | 19p13.2   | Recessive | Boucher-Neuhauser syndrome with chorioretinal dystrophy |
| PRPF31  | 19q13.42  | Dominant | Dominant retinitis pigmentosa |
| JAG1    | 20p12.2   | Dominant | Dominant Alagille syndrome |
| PRPF6   | 20q13.33  | Dominant | Dominant retinitis pigmentosa |
| PRPS1   | Xq22.3    | X-linked | Retinitis pigmentosa, neuropathy, optic atrophy, deafness |

| Table 3 | Genes which fulfilled criteria of over-representation of missense variants |
|---------|-------------------------------------------------|
| **Gene** | **Location** | **Mode of inheritance of disorders** | **Phenotypes** |
| SAMD11  | 1p36.33   | Recessive | Recessive retinitis pigmentosa |
| ALMS1   | 2p13.1    | Recessive | Alstrom syndrome |
| WFS1    | 4p16.1    | Recessive | Recessive Wolfram syndrome (also autosomal dominant low frequency sensorineural hearing loss) |
| RP1L1   | 8p23.1    | Dominant and recessive | Dominant occult macular dystrophy; recessive retinitis pigmentosa |
| KCNV2   | 9p24.2    | Recessive | Cone dystrophy with supernormal rod response |
| ADAMTS18| 16q23.1   | Recessive | Knobloch syndrome; recessive early onset retinal dystrophy |
features are not unique to this gene; the identification of this gene as one of those in which missense variants are over-represented is consistent with the reported highly polymorphic nature of RP11, and further supports the notion that novel variants, particularly those outside the two known hotspots for pathogenic variants, should be interpreted with great caution in terms of potential pathogenicity.

We found that biological pathways relating to spliceosome complex assembly were enriched (>100 fold) in the group of retinal disease genes predicted to be loss of function intolerant. The spliceosome complex assembly pathway was also enriched in the group of genes with significant under-representation of missense variants. The importance of the splicing pathway is thus emphasised, together with the above-mentioned questions as to why only rod photoreceptors appear affected by heterogeneous pathogenic variants.

A number of limitations of our study deserve mention. There can be pitfalls of relying on pLI indicators as described by other authors; genes that do not meet the pLI criteria frequently may encode essential proteins where loss of function in one allele may cause dominant disease. The thresholds (criteria) used are somewhat arbitrary, but there was significant overlap between predictions from the gnomAD and DECIPHER databases. The reliance on only the PANTHER resource to investigate which pathways were over-represented might also represent a limitation. We therefore checked that similar results would be obtained from other resources. Entering the list of ‘haploinsufficient’ genes (from table 1) into the Reactome resource (https://reactome.org/) similarly showed that mRNA splicing was most significantly over-represented (p=0.0002). We also entered these genes into the Molecular Signatures Database (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp accessed 30 July 2022) to compute overlaps with other gene sets (selecting the Gene Ontology sets): the most significant overlap (after sensory perception gene sets, not unexpected for a group of IRD genes) was the ‘SPICIOSOMAL_TRI_SNRNP_COMPLEX’ gene set (p=5.74x10^-10). These findings support the validity of our results from the PANTHER analysis.

Our study is mainly exploratory, and many of the conclusions tentative. These metrics have been proposed to help identify candidate genes for unsolved diseases, whereas we have, conversely, applied these metrics to known disease-associated genes, with a view to further exploring the variant landscape of these genes.

Author affiliations
1 Ophthalmology, University College London Institute of Ophthalmology, London, UK
2 Ophthalmology, Moorfields Eye Hospital City Road Campus, London, UK
3 University College London Institute of Ophthalmology, London, UK
4 Department of Ophthalmology, University of Singapore, Singapore
5 Moorfields Eye Hospital City Road Campus, London, UK
6 Medical Retina Service, Moorfields Eye Hospital NHS Foundation Trust, London, UK
7 Wills Eye Hospital, Philadelphia, Pennsylvania, USA

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ORCID iDs
Alexander Tanner http://orcid.org/0000-0002-7194-0978
Hwee Wuen Chan http://orcid.org/0000-0001-8079-8398
Omar A Mahroo http://orcid.org/0000-0003-1254-0832

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