INVITED REVIEW

Toward an elucidation of the molecular genetics of inherited retinal degenerations

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Abstract

While individually classed as rare diseases, hereditary retinal degenerations (IRDs) are the major cause of registered visual handicap in the developed world. Given their hereditary nature, some degree of intergenic heterogeneity was expected, with genes segregating in autosomal dominant, recessive, X-linked recessive, and more rarely in digenic or mitochondrial modes. Today, it is recognized that IRDs, as a group, represent one of the most genetically diverse of hereditary conditions - at least 260 genes having been implicated, with 70 genes identified in the most common IRD, retinitis pigmentosa (RP). However, targeted sequencing studies of exons from known IRD genes have resulted in the identification of candidate mutations in only approximately 60% of IRD cases. Given recent advances in the development of gene-based medicines, characterization of IRD patient cohorts for known IRD genes and elucidation of the molecular pathologies of disease in those remaining unresolved cases has become an endeavor of the highest priority. Here, we provide an outline of progress in this area.

Genetic Heterogeneity in IRDs

Inherited retinal degenerations (IRDs) represent the most frequent cause of visual dysfunction in those of working age, such conditions therefore having a highly significant impact on quality of life and health economics. The more common IRDs include Retinitis Pigmentosa (RP), Choroideremia, Leber congenital amaurosis (LCA), Usher syndrome, Congenital stationary night blindness (CSNB), Vitelliform macular dystrophy, Stargardt Macular Dystrophy, Best disease, Retinoschisis, cone-rod dystrophy and myocilin-based hereditary forms of open angle glaucoma (see www.sph.uth.tmc.edu/retnet/ for details of disorders, causative genes and mutations). Typically, IRDs result in gradual photoreceptor loss and compromised vision, often leading to registered blindness. Patterns of inheritance include autosomal recessive, autosomal dominant and X-linked, however, more rare mitochondrial and digenic forms of retinal dystrophies have been characterized.

Since the localization, through genetic linkage analysis, of the first IRD genes involved in X-linked recessive and autosomal dominant forms of RP (1–3), relentless progress has been made over three decades in defining an extensive portfolio of disease-causing genes, revealing an extreme level of genetic heterogeneity (4,5). Thus far, approximately 260 genes have been characterised, with more still to be identified (6–9; Tables 1–3). DNA
sequencing, and more recently, next-generation sequencing (NGS) technologies, in particular, now provide an opportunity to characterize the majority of the remaining genes (10–25).

IRD genes and encoded proteins are involved in a wide array of functions. It is of note that mutations in essentially all genes encoding components of the visual transduction and retinoid cycles have been implicated in disease etiology, as well as major structural components of photoreceptors (for extensive reviews of gene function see 26, 27; Tables 1–3). RP is the most common form of IRD, affecting approximately 1 in 3,000 people (4,5), the disease being characterized by progressive loss of rod photoreceptor function and their demise, followed by the death of the cone photoreceptors, initial symptoms of nyctalopia (night blindness) being followed usually by extensive loss of daytime vision as cones degenerate. Mutations within around 70 disease-causing genes have so far been implicated in RP (www.sph.uth.tmc.edu/retnet; date last accessed April 25, 2017), while other genes still remain to be identified, indicating that this condition may, perhaps, be better regarded as a cluster of related conditions rather than one unique disease. The situation for IRDs is further complicated by the diversity of clinical presentations that can be caused by mutations even within a single IRD gene, as well as overlapping clinical phenotypes which may be the result of mutations in entirely distinct genes, making it impossible for a diagnosis to be given in the majority of cases on the basis of disease phenotype alone (17,28–30). By way of example, in a recent NGS study of an Irish IRD patient cohort, new disease phenotypes were associated with the GNAT1 and SLC24A1 genes (28). In both cases a severe homozygous mutation in a known congenital stationary night blindness (CSNB) gene caused a late-onset form of RP involving photoreceptor loss (Fig. 1). Furthermore IRD phenotypes can be influenced by modifier loci (31–33). Given the limitations associated with the clinical diagnosis of specific forms of IRD, it is no surprise that the majority of patients with IRDs do not know the mutation(s) responsible for their condition. Recent advances in the development of gene-specific therapies for IRDs has made this issue all the more pressing, since this offers, for the first time, the prospect of modulating, halting or reversing the degeneration associated with some of these conditions (34); however, such therapies cannot, obviously, be administered without a precise knowledge of the underlying mutation to be treated.

High throughput NGS technologies offer an opportunity to rapidly characterize causative mutations in the growing number of IRD genes that have now been identified. These technologies have greatly accelerated DNA sequencing by providing a means of simultaneously sequencing many small fragments of DNA from different regions of the genome in a single reaction, and moreover by barcoding patient DNA to enable sequencing of samples from multiple patients in parallel. The short sequence reads from such fragments are then reconstructed by comparing the sequence to a reference genome; hg38 being currently the reference genome of choice for many such studies (hg38; GCA_000001405.15). NGS can be undertaken on the whole genome, or limited to expressed (coding) sequences (whole exome), or targeted to particular regions of the genome (employing target capture panel NGS); where genes of interest are, in essence, captured and sequenced. This form of sequencing has advanced at an exponential pace, with the cost per megabase of sequence halving regularly (35) and set to reduce further with the introduction of the new NovaSeq series from Illumina, among other innovations. IRDs have an ideal profile for NGS with emerging methods to define the function of candidate mutations, and more recently, next-generation sequencing (NGS) technologies, in particular, now provide an opportunity to characterize the majority of the remaining genes (10–25).

Table 1. Representative NGS studies of IRD patient populations

| IRD patient numbers | Patient origin (predominant) | NGS technology | Pathogenic/likely pathogenic mutations (%) | Reference |
|---------------------|-----------------------------|----------------|------------------------------------------|-----------|
| 100                 | Dutch                       | Target capture 111 genes | ~ 50% | Neveling el al. 2012 (10) |
| 20                  | France                      | Target capture 254 genes | 57% | Audo et al. 2012 (11) |
| 50                  | UK                          | Target capture 105 genes | 50-50% | O’Sullivan et al. 2012 (12) |
| 50                  | UK                          | Target capture 73 genes | 53% (early onset IRD;25% overall) | Shanks et al. 2013 (13) |
| 192                 | US                          | Target capture (GEDJ) 214 genes | 51% | Consugar et al. 2015 (14) |
| 105                 | eyeGENE® database           | Target capture 195 genes | 49.5% | Ge et al. 2015 (15) |
| 82                  | N Ireland                   | Target capture 186 genes | 60% | Zhao et al. 2015 (16) |
| 88                  | Italy                       | Target capture 72 genes | 59.1% | Bernardis et al. 2016 (17) |
| 309                 | Ireland                     | Target capture 216 genes | 57% | Carrigan et al. 2016 (18) |
| 537                 | Worldwide                   | Target capture 105 genes | 51% | Ellingford et al. 2016 (19) |
| 47                  | Spain                       | Target capture 75 genes | 57.4% | Perez-Carro et al. 2016 (20) |
| 88                  | European                    | Target capture & WES   | 61% | Weisschu et al. 2016 (21) |
| 58                  | Swiss                       | WES                  | 64% | Tiwari et al. 2016 (22) |
| 33                  | Mixed                       | WES                  | 55% | de Castro-Miro et al. 2016 (23) |
| 59                  | Spain                       | WES                  | 71.8% | Riera et al. 2017 (24) |
| 266                 | Dutch                       | Target panel & WES    | 52% | Haer-Wigman et al. 2017 (25) |

Next-Generation Sequencing of IRD Populations

Future exome and whole genome sequencing, in conjunction with emerging methods to define the function of candidate mutations in regulatory and intronic regions and to better characterize copy number variations (CNVs), all of which have been implicated as causative of some forms of IRD, will undoubtedly progress our understanding of the molecular genetics of IRDs to a new level. The situation for IRDs is further complicated by the diversity of clinical presentations that can be caused by mutations even within a single IRD gene, as well as overlapping clinical phenotypes which may be the result of mutations in entirely distinct genes, making it impossible for a diagnosis to be given in the majority of cases on the basis of disease phenotype alone (17,28–30). In both cases a severe homozygous mutation in a known congenital stationary night blindness (CSNB) gene caused a late-onset form of RP involving photoreceptor loss (Fig. 1). Furthermore IRD phenotypes can be influenced by modifier loci (31–33). Given the limitations associated with the clinical diagnosis of specific forms of IRD, it is no surprise that the majority of patients with IRDs do not know the mutation(s) responsible for their condition. Recent advances in the development of gene-specific therapies for IRDs has made this issue all the more pressing, since this offers, for the first time, the prospect of modulating, halting or reversing the degeneration associated with some of these conditions (34); however, such therapies cannot, obviously, be administered without a precise knowledge of the underlying mutation to be treated.
| Gene    | Location | Function                                                                                                                                                                                                 |
|---------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ABCA4   | 1p22.1   | ATP binding cassette subfamily A member 4; Photoreceptor-specific ATP-binding cassette (ABC) transporter with N-retinylidene-PE as a substrate.                                                              |
| AGBL5   | 2p23.3   | ATP/GTP binding protein like 5; Metallocarboxypeptidase that mediates protein deglutamylation; can remove glutamate residues from both carboxyl termini and side chains of protein substrates. |
| ARL6    | 3q11.2   | ADP ribosylation factor like GTPase 6; Involved in membrane protein trafficking at the base of the cilium.                                                                                                    |
| ARL2BP  | 16q13.3  | ADP ribosylation factor like GTPase 2 binding protein; Binds to ARL2.GTP with high affinity. Role in nuclear translocation, retention and transcriptional activity of STAT3. |
| BBS1    | 11q13.2  | Bardet-Biedl syndrome 1 protein; Required for BBSome complex assembly, its ciliary localization and ciligenesis.                                                                                               |
| BBS2    | 16q13    | Bardet-Biedl syndrome 2 protein; Required for BBSome complex assembly, its ciliary localization and ciligenesis.                                                                                               |
| BEST1   | 11q12.3  | Bestrophin 1; Forms calcium-sensitive chloride channels; highly permeable to bicarbonate and possibly in analogous synapes in retina.                                                                   |
| C2orf71 | 2p23.2   | Chromosome 2 open reading frame 71; Photoreceptor cilia protein connecting inner and outer segments.                                                                                                       |
| C8orf37 | 8q22.1   | Chromosome 8 Open Reading Frame 37; Co-localizes with polyglutamylated tubulin at the base of the primary cilium in RPE cells.                                                                                  |
| CERKL   | 2q31.3   | Ceramide kinase-like protein; has no detectable ceramide-kinase activity; protects cells from apoptosis in oxidative stress.                                                                                |
| CLRN1   | 3q25.1   | Clarin 1; A role in excitatory ribbon synapse junctions between hair cells and cochlear ganglion cells and possibly in analogous synapses in retina.                                                            |
| CNGA1   | 4p12     | Cyclic nucleotide gated channel alpha 1; Rod cGMP-gated channel alpha subunit; Forms cGMP-gated cation channel for depolarization of rod photoreceptors.                                                  |
| CNGB1   | 16q21    | Cyclic nucleotide gated channel beta 1; Rod cGMP-gated channel beta subunit; Forms cGMP-gated cation channel for depolarization of rod photoreceptors.                                                          |
| CRB1    | 1q31.3   | Crumbs 1; Plays a role in photoreceptor morphogenesis in the retina. May maintain cell polarization and adhesion.                                                                                             |
| CYP4V2  | 4q35.1   | Cytochrome P450 family 4 subfamily V member 2; Involved in fatty acid and steroid metabolism in the eye.                                                                                                   |
| DHDDS   | 1p36.11  | Dehydrodolichyl Diphosphate Synthase Subunit; forms the dehydrodolichyl diphosphate synthase (DSS) complex, an essential component of the dolichol monophosphate (Dol-P) biosynthetic machinery |
| DHX38   | 16q22.2  | DEAH-Box Helicase 38; Unknown function; probable ATP-binding RNA helicase involved in pre-mRNA splicing.                                                                                                 |
| EMC1    | 1p36.13  | ER membrane protein complex subunit 1; Unknown function.                                                                                                                                                    |
| EYS     | 6q12     | Eyes Shut Homolog (Drosophila); An extracellular matrix protein, one of the largest human genes at >2mb. Possibly involved in stability of photoreceptor ciliary axonemes.                                            |
| FAM161A | 2p15     | Family with sequence similarity 161 member A; Ciliary protein and a member of the Golgi-centrosomal interactome; involved in development of retinal progenitors.                                                |
| GPR125/ADGRA3 | 4p15.2 | Adhesion G protein-coupled receptor A3/G protein-coupled receptor 125; Involved in signal transduction.                                                                                         |
| HGSNAT  | 8p11.21  | Heparan-alpha-glucoaminide N-acetyltransferase; Lyosomal acetyltransferase that catalyzes transmembrane acetylation of the terminal glucosamine residues of heparan sulfate, prior to hydrolysis by N-acetylgalcosaminidase. |
| IDH3B   | 20p13    | Isocitrate Dehydrogenase 3 (NAD(+)) Beta; catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the citric acid cycle.                                                                               |
| IFT140  | 16p13.3  | Intraflagellar transport 140; Involved in ciliogenesis, cilia maintenance and retrograde ciliary transport.                                                                                               |
| IFT172  | 2p23.3   | Intraflagellar transport 172; Involved in formation and maintenance of cilia.                                                                                                                              |
| IMPG2   | 3q12.3   | Interphotoreceptor matrix proteoglycan 2; Involved in organization of interphotoreceptor matrix.                                                                                                             |
| KIAA1549| 7q34     | KIAA1549 protein; Unknown function.                                                                                                                                                                         |
| KIZ     | 20p11.23 | Kizuna centrosomal protein; Localizes to centrosomes, strengthening and stabilizing the pericentriolar region prior to spindle formation.                                                              |
| LRA1    | 4q32.1   | Lecithin retinol acyltransferase; Transfers the acyl group from phosphatidylcholine to all-trans retinol, producing all-trans retinyl esters.                                                              |
| MAK     | 6p24.2   | Male germ-cell associated kinase; Unknown function.                                                                                                                                                        |
| MERTK   | 2q13     | C-mer proto-oncogene receptor tyrosine kinase; A regulator of rod outer segment fragment phagocytosis.                                                                                                      |
| MVK     | 12q24.11 | Mevalonate kinase; Involved in the synthesis of isopentenyl diphosphate from (R)-mevalonate.                                                                                                                |
| NEK2    | 1q32.3   | NIMA (never in mitosis gene A)-related kinase 2; Involved in regulation of centrosome disjunction.                                                                                                         |

Continued
those whose genetic pathogenesis has been resolved, and those for whom new genes, or variants in regulatory sequences, splice variants or CNVs associated with disease genes, may be causative of disease and therefore additional analyses deploying whole exome or whole genome sequencing would be appropriate.

A variety of NGS studies, many employing a target panel-based NGS approach focused on sequencing the exons of known IRD genes, have been undertaken (10–25; Table 1). These studies support the view that IRDs represent an exemplar group of disorders for the application of panel-based NGS or WES as effective tools for detection of causative mutations. From such studies, some common patterns in findings have been observed, as have some unique findings, which at present, remain specific to individual studies. Of note, using target-capture based NGS, approximately 50–60% of IRD patients were found to carry disease causing or likely disease causing mutations in many studies. Given additional family studies indicating segregation of the disease, and replication of the findings in a certified diagnostic laboratory, these patients would be deemed to be categorised as ‘resolved’ in terms of the genetic etiology of the retinal pathology. Among recent examples of capture-panel NGS for IRDs, is a study of 537 IRD patients in which the variants identified were deemed to account, or likely account for the disease in 51% of cases and a recent study of Italian IRD patients, in which 59% of cases were resolved, similar to the identification levels obtained in many other NGS studies (17,19; Tables 1–3). Detection rates were significantly affected by the range of conditions under study, the number of genes included in the capture panel, as well as whether the rate was corrected based on previous screening of the same population, making precise comparisons of detection rates between studies challenging. Nonetheless, there is a consistent finding that between 25-50% of cases were not solved by targeted sequencing or WES. It is of interest that studies employing WES, rather than capture-panel NGS, have resulted in the identification of the underlying

| Gene       | Location | Function                                                                 |
|------------|----------|--------------------------------------------------------------------------|
| NEUROD1    | 2q31.3   | Neuronal differentiation protein 1; Acts as a transcriptional activator.  |
| NR2E3      | 15q23    | Nuclear receptor subfamily 2 group E3; Transcription factor, that is an activator of rod development and repressor of cone development. |
| NRL        | 14q11.2  | Neural retina leucine zipper; Transcription factor which regulates expression of rod-specific genes, involved in photoreceptor cell development and function |
| OFD1       | Xp22.2   | Oral-facial-digital syndrome 1 protein; Involved in biogenesis of the cilia and controlling centriole length during cell division. |
| PDE6A      | 5q32     | Alpha subunit of cGMP phosphodiesterase; involved in visual transduction cycle. |
| PDE6B      | 4p16.3   | Beta subunit of cGMP phosphodiesterase; involved in visual transduction cycle. |
| PDE6G      | 17q25.3  | Gamma subunit of cGMP phosphodiesterase; involved in visual transduction cycle. |
| POMGNT1    | 1p34.1   | Protein O-linked acetylglucosaminyltransferase 1 (beta 1,2-); Participates in O-mannosyl glycosylation. |
| PRCD       | 17q25.1  | Progressive rod-cone degeneration protein; Unknown function. |
| PRIM1      | 12q13.3  | DNA primase small subunit; Polymerase that synthesizes small RNA primers for Okazaki fragments made during discontinuous DNA replication. |
| RBP3       | 10q11.22 | Retinol-binding protein 3 (RBP); Shuttles 11-cis and all trans retinoids into RPE and visual pigments in photoreceptor cells. |
| RGR        | 10q23.1  | RPE-retinal G protein-coupled receptor; may be involved in regulating recycling of retinal. |
| RHO        | 3q22.1   | Rhodopsin; Light-induced isomerization of 11-cis to all-trans retinal leading to G-protein activation and release of all-trans retinal. |
| RLP1       | 15q26.1  | Retinaldehyde-binding protein 1; Soluble retinoid carrier essential for function of photoreceptors. |
| RP1        | 8q11.23-q12.1 | Oxygen-regulated protein 1; Involved in organization of photoreceptor outer segments. |
| RP1L1      | 8p23.1   | Retinitis pigmentosa 1-like 1 protein; Required for differentiation of photoreceptors and organization of photoreceptor outer segments. |
| RP2        | Xp11.3   | Retinitis pigmentosa 2 (X-linked); Involved in trafficking between Golgi and the ciliary membrane. |
| RPE65      | 1p31.2   | Retinoid isomerohydrolase; Involved in production of 11-cis retinal and visual pigment regeneration. |
| RPGR       | Xp11.4   | X-linked retinitis pigmentosa GTPase regulator; Involved in ciliogenesis. |
| SAG        | 2q37.1   | Rod photoreceptor-arrestin; Binds to photoactivated-phosphorylated rhodopsin, thereby apparently preventing the transducin-mediated activation of phosphodiesterase. |
| SLC7A14    | 3q26.2   | Solute carrier family 7 member 14; Unknown function. |
| SLC24A1    | 15q22.31 | Solute carrier family 24 (sodium/potassium/calcium exchanger) member 1; Critical component of visual transduction, controlling the calcium concentration of outer segments. |
| SPATA7     | 14q31.3  | Spermatogenesis associated protein 7; Required for recruitment and localization of RPGRIP1 to the photoreceptor cilium. |
| TRNT1      | 2p26.2   | CCA adding tRNA nucleotidyl transferase 1; Adds and repairs CCA sequences necessary for attachment of amino acids to the 3' terminus of tRNA molecules. |
| TTC8       | 14q31.3  | Tetratricopeptide repeat protein 8; Subunit of the BBSome complex required for proper cilia functions. |
| TULP1      | 6p21.3   | Tubby-like protein 1; Required for normal development of photoreceptor synapses. |
| USH2A      | 1q41     | Usherin; Involved in photoreceptor cell maintenance. |
| ZNF408     | 11p11.2  | Zinc finger protein 408; Unknown function. |
| ZNF513     | 2p23.3   | Zinc finger protein 513; Transcriptional regulator involved in retinal development and maintenance. |
genetic defect in similar levels of IRD patients ∼60% (22), although in a recent study of Spanish IRD patients, WES resulted in successful identification of pathogenic variants in approximately 70% of patients (24). Levels of mutation detection achieved for IRDs that are less genetically heterogeneous, such as choroideremia, were identified, about a third of these (n = 48) represented new Stargardt associated disease alleles (37). IRD mutation detection may remain elusive for a variety of reasons, possibly due to inadequate sequence coverage, inappropriate filtering of data (and loss of the pathogenic variant), variants in intronic sequences which affect splicing (such sequences do not form part of most capture panels), structural variants such as inversions, duplications and so on which can be difficult to resolve with NGS, regulatory variants or new, as yet uncharacterized, IRD genes and which therefore can be difficult to resolve with NGS, regulatory variants or new, as yet uncharacterized, IRD genes and which therefore were not included in capture panels, among many other causes. However from some NGS studies there are indicators that support the hypothesis that, within the known IRD genes included in many target capture panels, additional significant levels of pathological genetic variants are present but as yet remain undetected. For example, in a recent study in which 148 pathogenic or likely pathogenic mutations in the ABCA4 gene were identified, about a third of these (n = 48) represented new Stargardt associated disease alleles (37).

**Deciphering the Genetic Pathogenesis of Unresolved IRDS**

It is evident from this body of research that currently the genetic pathogenesis of IRDs remains unresolved in ∼40% of cases (10–25; Tables 1–3). IRD mutation detection may remain elusive for a variety of reasons, possibly due to inadequate sequence coverage, inappropriate filtering of data (and loss of the pathogenic variant), variants in intronic sequences which affect splicing (such sequences do not form part of most capture panels), structural variants such as inversions, duplications and so on which can be difficult to resolve with NGS, regulatory variants or new, as yet uncharacterized, IRD genes and which therefore were not included in capture panels, among many other causes. However from some NGS studies there are indicators that support the hypothesis that, within the known IRD genes included in many target capture panels, additional significant levels of pathological genetic variants are present but as yet remain undetected. For example, it has been found that there can be a preponderance of patients with one causative mutation in a recessive IRD gene; such over-representation of heterozygotes compared to a control population has been observed in a number of NGS studies, including our own study of the Irish IRD population, for example, for the ABCA4 gene causative of Stargardt disease.
The majority of clinically defined Irish Stargardt cases who did not have two ABCA4 mutations in the coding sequence, had a single ABCA4 mutation and this was highly significantly enriched compared to the frequency of ABCA4 mutant heterozygotes in a control Irish population (Carrigan M, unpublished data). Recent studies have focused on revealing the genetic variants underlying such observations. Various tools have been developed to detect copy number variations (CNVs) in NGS datasets and have been employed to successfully detect CNVs in IRD patient cohorts (38–40). Indeed, CNVs were identified in a recent WES study in approx. 10% of the 60 IRD patients assessed by exon coverage data analysis and confirmed by PCR (39). Alternative methods for CNV detection, that previously have been employed extensively in diagnostic laboratories for disorders other than IRDs, include quantitative PCR (qPCR), multiplex ligation-dependent probe amplification (MLPA) and comparative genomic hybridization. Using the latter, Van Cauwenbergh and colleagues developed a custom microarray (arrEYE) with coding and noncoding sequence from 166 known and candidate IRD genes and 196 noncoding RNAs for CNV detection in IRD patients (40); the CNV detection rate obtained with arrEYE from a first study of 57 IRD patients was 3.5%. In a recent study, 18% of unresolved cases (n = 28) were resolved by CNV mapping (38). It is clear that CNVs may represent a significant contributor to the unresolved cases of IRDs and that methods of CNV analysis, both computational and experimental, should, where possible be included in future IRD studies, which has not always been the case thus far.

In addition to CNVs, a proportion of the remaining unresolved IRD cases may be caused by variants that affect RNA splicing and thereby contribute to disease. Mutations that may have an impact on pre-mRNA splicing can be predicted using various in silico tools such as Human Splicing Finder (www.umd.be/HSF/; date last accessed April 25, 2017) among others, and can be confirmed by transcript analysis. RNA sequencing technologies are greatly enabling high throughput transcriptomics and undoubtedly will be applied to a greater extent to explore splice variants in IRDs in the future. A transcriptomic approach to assess the effects of IRD mutations on splicing may in principle be readily adopted for ubiquitously expressed IRD genes, and for tissue specific IRD genes, using iPS cells differentiated into appropriate cell lineages as per the example above. One approach to identify intronic variants with effects on RNA splicing in IRD patients involves deep intronic sequencing. For example, in a recent study of the USH2A gene, the most frequent cause of Usher syndrome type II (USH2) involving RP and sensorineural hearing loss, an analysis of the whole 800kb of the USH2A gene was prompted by the identification of patients with an USH2 phenotype and a single exonic mutation in the USH2A gene. Deep sequencing of intronic sequences enabled the identification of candidate splice mutations and subsequent functional validation of these mutations using reporter minigene assays leading to the resolution of 3 out of 5 USH2 patients with a single exonic mutation (41). In another study, the functional effect of a commonly observed mutation in the ABCA4 gene (the c.5461-10T→C variant) causative of Stargardt disease was analysed using patient-derived fibroblasts reprogrammed into induced pluripotent stem (iPS) cells and then differentiated into photoreceptor progenitor cells. This ABCA4 variant was found to induce skipping of exon 39 or exon 39 and 40 in the mature transcript again using a minigene assay (42). Recently homozygosity mapping and whole genome sequencing revealed a variant deep in intron 18 of the PROM1 gene causative of a recessive cone-rod dystrophy, resulting in the inclusion of a pseudo-exon in the mutant transcript, which was functionally validated again using a minigene assay (43). Detection of pathogenic IRD intronic variants will elude many of the current capture panel NGS strategies for IRDs which are focused solely on exons and hence the extension of screening studies in the future to include intronic sequences will aid in elucidating what proportion of IRD cases involve aberrant splicing.
been found to segregate in a family with autosomal dominantly IRD. Indeed a mutation in the seed region of microRNA-204 has associated target sites, may also be implicated in some forms of sequences, such as, promoter sequences or miRNAs, and their ‘unresolved’ pathogenic IRD mutations, variants in regulatory regions of the CHM gene has been implicated as causative of choroideremia (46), all highlighting the potential role of regulatory sequences in the NMNAT1 gene has been implicated in a form of rodentiremia (46), among others, will influence vector choice and route of administration. Strategies such as directed evolution are also expediting the generation of AAV serotypes with a predilection for specific retinal cell types (59,60). Additionally, the severity of disease and whether retention of a relatively intact target cell population is maintained over years, thereby providing a significant timeframe for therapeutic intervention, will also greatly influence therapeutic efficacy. For severe degenerative IRDs, where few or no photoreceptors remain, optogenetic therapies may provide an alternative option by enabling remaining cells to become photosensitive (61,62). While AAV has shown substantial promise for a number of ocular indications, preclinical and clinical studies have also been undertaken with a variety of other non-viral and viral vectors, for example, nanoparticles and lentiviral vectors amongst others (63,64). Moreover, methodologies for systemic delivery of potentially therapeutic compounds into the retina by modulation of permeability at the inner blood-retina barrier have barrier have been extensively tested in animal systems and have employed AAV vectors (65).

In conclusion, while significant advances in high throughput genomic and transcriptomic NGS has greatly facilitated an elucidation of much of the genetic architecture of IRD patient cohorts, substantial levels of unresolved IRD cases still remain. Recently it has become evident that a significant proportion of these will be accounted for by CNVs, splicing defects and regulatory variants. It still remains to be established within these unresolved cases, what proportion will be caused by new, as yet uncharacterised, retinal disease genes.

A Diagnostic Imperative Driven by Developments in Gene Therapy

There is an obvious rationale for pursuing such studies to their conclusion, in that gene therapies for a growing number of ocular disorders are now in clinical trial (47–51). To date, approximately 300 IRD patients have been treated with ocular gene therapies (www.clinicaltrial.gov). In some of these trials, a continued retinal degeneration has been observed (52) and the parameters determining this still need to be elucidated. It is of note however, that an adeno-associated virus (AAV)-RPE65 therapy (voretigene neparvovec) has successfully progressed through to Phase III clinical trial (CHOP/Spark Therapeutics; www.sparktx.com), as has an AAV-ND4 therapy for Leber hereditary optic neuropathy (LHON) (51). Data from many ocular gene therapy trials support the view that intracellular delivery of AAV is well tolerated in the human eye and hence represents a safe platform for gene delivery (48–51). A large number of gene therapies employing AAV vectors are also at the stage of preclinical evaluation in animal IRD models, in which benefit has been demonstrated (51,53–57). Thus far many gene therapies have been directed towards recessive forms of IRD, although strategies such as suppression and replacement or genome editing are being considered to address the 30% of IRDs that are dominantly inherited (53,58). The efficacy of the therapeutic approaches will be determined in part by features specific to individual IRDs. For example, the target cell type within the retina, whether photoreceptors, RPE cells or retinal ganglion cells (51,53–57), among others, will influence vector choice and route of administration. Strategies such as directed evolution are also expediting the generation of AAV serotypes with a predilection for specific retinal cell types (59,60). Additionally, the severity of disease and whether retention of a relatively intact target cell population is maintained over years, thereby providing a significant timeframe for therapeutic intervention, will also greatly influence therapeutic efficacy. For severe degenerative IRDs, where few or no photoreceptors remain, optogenetic therapies may provide an alternative option by enabling remaining cells to become photosensitive (61,62). While AAV has shown substantial promise for a number of ocular indications, preclinical and clinical studies have also been undertaken with a variety of other non-viral and viral vectors, for example, nanoparticles and lentiviral vectors amongst others (63,64). Moreover, methodologies for systemic delivery of potentially therapeutic compounds into the retina by modulation of permeability at the inner blood-retina barrier have barrier have been extensively tested in animal systems and have employed AAV vectors (65).

Given the rapid pace at which therapies are being developed, it is imperative to establish the genetic architecture of IRDs in different national and ethnic groups, in principle thereby facilitating participation of patients in future human clinical trials or access in the future to marketed gene therapies. In parallel with genotyping of IRDs, it is vital that patients are clinically profiled over time, as this will greatly augment our understanding of the natural histories of these ocular disorders and genotype-phenotype correlations. Knowledge regarding the natural history of a disorder is of direct value to patients, and moreover, will in principle facilitate future patient participation in clinical trials and will aid in informing the choice of appropriate primary and secondary endpoints for clinical trial design. Therefore as such, identification of the disease-causing mutations is of immediate clinical relevance to the entire IRD patient population.

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References

1. Bhattacharya, S.S., Wright, A.F., Clayton, J.F., Price, W.H., Phillips, C.I., McKeown, C.M., Jay, M., Bird, A.C., Pearson, P.L., Southern, E.M. et al. (1984) Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. Nature, 309, 253–255.

2. McWilliam, P., Farrar, G.J., Kenna, P.F., Bradley, D.G., Humphries, M.M., Sharp, E.M., McConnell, D.J., Lawler, M., Sheils, D., Ryan, C. and Humphries, P. (1989) Genomics, 3, 619–622.

3. Farrar, G.J., Kenna, P.F., Jordan, S.A., Kumar-Singh, R., Humphries, M.M., Sharp, E.M., Sheils, D.M. and Humphries, P. (1991) A three-base-pair deletion in the peripherin-RDS gene in one form of retinitis pigmentosa. Nature, 354, 478–480.

4. Ferrari, S., Di Iorio, E., Barbaro, V., Ponzin, D., Sorrentino, F.S. and Parmeggiani, F. (2011) Retinitis pigmentosa: genes and disease mechanisms. Curr. Genomics, 12, 238–249.

5. Chiang, J.P. and Truszpek, K. (2015) The current status of molecular diagnosis of inherited retinal dystrophies. Curr. Opin. Ophthalmol., 26, 346–351.

6. Arno, G., Agraval, S.A., Eblimit, A., Bellingham, J., Xu, M., Wang, F., Chakarova, C., Parfitt, D.A., Lane, A., Burgoyne, T. et al. (2016) Mutations in REEP6 Cause Autosomal-Recessive Retinitis Pigmentosa. Am. J. Hum. Genet., 99, 1305–1315.

7. Corton, M., Avila-Fernández, A., Campello, L., Sánchez, M., Benavides, B., López-Molina, M.I., Fernández-Sánchez, L., Sánchez-Alcudia, R., da Silva, L.R., Reyes, N. et al. (2016) Identification of the Photoreceptor Transcriptional Co-Repressor SAMD11 as Novel Cause of Autosomal Recessive Retinitis Pigmentosa. Sci. Rep., 6, 35370.

8. Pierrache, L.H.M., Kimchi, A., Ratnapriya, R., Roberts, L., Astuti, G.D.N., Obolensky, A., Beryozkin, A., Tjon-Fo-Sang, R., Lehman, A., Osman, I.S., Dharmat, R., Curry, K., C.P. et al. (2017) Whole-exome sequencing identifies biallelic IDH3A variants as a cause of retinitis pigmentosa accompanied by pseudocoloboma. Ophthalmology, doi: 10.1016/j.ophtha.2017.03.010. [Epub ahead of print]

9. Xu, M., Xie, Y.A., Abouzeid, H., Gordon, C.T., Fiorentino, A., Sun, Z., Lehman, A., Osman, I.S., Dharmat, R., Rivero-Alvarez, R. et al. (2017) Mutations in the spliceosome component CWC27 cause retinal degeneration with or without additional developmental anomalies. Am. J. Hum. Genet., 100, 592–604.

10. Neveling, K., Collin, R.W., Gilissen, C., van Huet, R.A., Visser, L., Kwint, M.P., Gijzen, S.J., Zonneveld, M.N., Wieskamp, N., de Ligt, J. et al. (2012) Next-generation genetic testing for retinitis pigmentosa. Hum. Mutat., 33, 963–972.

11. Audo, I., Bujakowska, K.M., Léveillé, T., Mohand-Saïd, S., Lancelot, M.E., Germain, A., Antonio, A., Michiels, C., Saraiva, J.P., Letexier, M. et al. (2012) Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. Orphanet J. Rare Dis., 7, 8.

12. O’Sullivan, J., Mullaney, B.G., Bhaskar, S.S., Dickerson, J.E., Hall, G., O’Grady, A., Webster, A., Ramsden, S.C. and Black, G.C. (2012) A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. J. Med. Genet., 49, 322–326.

13. Shanks, M.E., Downes, S.M., Copley, R.R., Lise, S., Broxholme, J., Hudspith, K.A., Kwasniewska, A., Davies, W.I., Hanksin, M.W., Packham, E.R. et al. (2013) Next-generation sequencing (NGS) as a diagnostic tool for retinal degeneration reveals a much higher detection rate in early-onset disease. Eur. J. Hum. Genet., 21, 274–280.

14. Consugar, M.B., Navarro-Gomez, D., Place, E.M., Bujakowska, M.K., Sousa, M.E., Fonseca-Kelly, Z.D., Taub, D.G., Janessian, M., Wang, D.Y., Au, E.D. et al. (2015) Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. Genet. Med., 17, 253–261.

15. Ge, Z., Bowles, K., Goetz, K., Scholl, H.P., Wang, F., Wang, X., Xu, S., Wang, K., Wang, H. and Chen, R. (2015) NGS-based Molecular diagnosis of 105 eyeGENE® probands with Retinitis Pigmentosa. Sci. Rep., 5, 18287.

16. Zhao, L., Wang, F., Wang, H., Li, Y., Alexander, S., Wang, K., Willoughby, C.E., Zaneveld, J.E., Jiang, L., Soens, Z.T. et al. (2015) Next-generation sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Irish. Hum. Genet., 134, 217–230.

17. Bernardis, I., Chiesi, L., Tenedini, E., Artuso, L., Ferret, L., Artusi, V., Simone, M.L., Manfredini, R., Camparini, M., Rinaldi, C. et al. (2016) Unravelling the complexity of inherited retinal dystrophies molecular testing: added value of targeted next-generation sequencing. Biomed. Res. Int., 2016, 6341870.

18. Carrigan, M., Diugnan, E., Malone, C., Stephenson, K., Saad, T., McDermott, C., Green, A., Keegan, D., Humphries, P., Kenna, P.F. and Farrar, G.J. (2016) Panel-based population next-generation sequencing for inherited retinal degenerations. Sci. Rep., 6, 33248.

19. Ellingford, J.M., Barton, S., Bhaskar, S., O’Sullivan, J., Williams, S.G., Lamb, J.A., Panda, B., Sergouniotis, P.I., Gillespie, R.L., Daiger, S.P. et al. (2016) Molecular findings from 537 individuals with inherited retinal disease. J. Med. Genet., doi: 10.1136/jmedgenet-2016-103837. [Epub ahead of print]

20. Perez-Carro, R., Corton, M., Sánchez-Navarro, I., Zurita, O., Sanchez-Bolivar, N., Sánchez-Alcudia, R., Leielveld, S.H., Aller, E., Lopez-Martínez, M.A., López-Molina, M.I. et al. (2016) Panel-based NGS reveals novel pathogenic mutations in autosomal recessive retinitis pigmentosa. Sci. Rep., 6, 19531.

21. Weisschnuh, N., Mayer, A.K., Strom, T.M., Kohl, S., Glück, N., Schubach, M., Andreasson, S., Bernd, A., Birch, D.G., Hamel, C.P. et al. (2016) Mutation detection in patients with retinal dystrophies using targeted next generation sequencing. PLoS One, 11, e0145951.

22. Tiwari, A., Bahr, A., Bahr, L., Fleischhauer, J., Zinkernagel, M.S., Winkler, N., Barthelmes, D., Berger, L., Gerth-Kahler, C., Neidhardt, J. et al. (2016) Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. Sci. Rep., 6, 28755.

23. de Castro-Miño, M., Tonda, R., Escudero-Ferruz, P., Andrés, R., Mayor-Lorenzo, A., Castro, J., Ciccioni, M., Hidalgo, D.A., Rodríguez-Ezcurra, J.J., Farrando, J. et al. (2016) Novel candidate genes and a wide spectrum of structural and point mutations responsible for inherited retinal dystrophies revealed by exome sequencing. PLoS One, 11, e0168966.
24. Riera, M., Navarro, R., Ruiz-Nogales, S., Méndez, P., Burés-Jelstrup, A., Corcóstegui, B. and Pomares, E. (2017) Whole exome sequencing using Ion Proton system enables reliable genetic diagnosis of inherited retinal dystrophies. Sci. Rep., 7, 42078.

25. Haer-Wigman, L., van Zeist-Stams, W.A., Pfundt, R., van den Born, L.I., Klaver, C.C., Verheij, J.B., Huygen, C.B., Breuning, M.H., Boon, C.J., Kievit, A.J. et al. (2017) Diagnostic exome sequencing in 266 Dutch patients with visual impairment. Eur, J. Hum. Genet, 25, 591–599.

26. Humphries, P., Humphries, M.M., Tam, L.C.S., Farrar, G.J., Kenna, P.F., Campbell, M. and Kiang, A.-S. (2012) Hereditary Retinopathies - Progress in Development of Genetic and Molecular Therapies. Springer-Verlag New York DOI 10.1007/978-1-4614-4499-2

27. Daiger, S.P., Sullivan, L.S. and Bowne, S.J. (2013) Genes and genetic diagnosis of inherited retinal dystrophies. Invest. Ophthalmol. Vis. Sci, 54, 100-102.

28. Carrigan, M., Duignan, E., Humphries, P., Palfi, A., Kenna, P.F. and Farrar, G.J. (2016) A novel homozygous truncating GNAT1 mutation implicated in retinal degeneration. Brt. J. Ophthalmol., 100, 495–500.

29. Shankar, S.P., Humphries, P., Palfi, A., Kenna, P.F., Sullivan, L.S., Connelly, K.N., Bowne, S.J., Stone, E.M. and Daiger, S.P. (2016) Autosomal dominant retinal dystrophies caused by a founder splice site mutation, c.828+3A>T, in PRPH2 and protein haplotypes in trans as modifiers. Invest. Ophthalmol. Vis. Sci., 57, 349–359.

30. Tee, J.J., Smith, A.J., Hardcastle, A.J. and Michaelides, M. (2016) RPGR-associated retinopathy: clinical features, molecular genetics, animal models and therapeutic options. Brt. J. Ophthalmol., 00, 1022–1027.

31. Vollrath, D., Yasumura, D., Benchorin, G., Matthes, M.T., Feng, W., Nguyen, N.M., Sedano, C.D., Calton, M.A. and LaVail, M.M. (2015) Tyro3 modulates Merk-associated retinal degeneration. PLoS Genet., 11, e1005723.

32. Rose, A.M., Shah, A.Z., Venturini, G., Krishna, A., Chakravarti, A., Rivolta, C. and Bhattacharya, S.S. (2016) Transcriptional regulation of PRPF31 gene expression by MSR1 repeat elements causes incomplete penetrance in retinitis pigmentosa. Sci. Rep., 6, 19450.

33. Conley, S.M., Stuck, M.W., Watson, J.N. and Naish, M.J. (2017) Rom1 converts Y141C-Prph2-associated pattern dystrophy to retinitis pigmentosa. Hum. Mol. Genet., 26, 509–518.

34. Pierce, E.A. and Bennett, J. (2015) The status of RPE65 gene therapy trials: safety and efficacy. Cold Spring Harb. Perspect. Med., 5, a017285.

35. Lecuit, M. and Eloit, M. (2015) The potential of whole genome sequencing using Ion Proton system enables reliable genetic diagnosis of inherited retinal dystrophies. Sci. Rep., 7, 42078.

36. Siemiatkowska, A.M., Collin, R.W., den Hollander, A.I. and Farrar, G.J. (2015) The potential of whole genome sequencing using Ion Proton system enables reliable genetic diagnosis of inherited retinal dystrophies. Sci. Rep., 7, 42078.

37. Schulz, H.L., Grassmann, F., Kellner, U., Spital, G., Rüther, K., Jagle, H., Hufendiek, K., Rating, P., Huchzermeyer, C., Baier, M.J. et al. (2017) Mutation spectrum of the ABCA4 Gene in 335 Stargardt Disease patients from a multicenter German cohort-impact of selected deep intronic variants and common SNPs. Invest. Ophthalmol. Vis. Sci., 58, 394–403.

38. Bujakowska, K.M., Fernandez-Godino, R., Place, E., Consugar, M., Navarro-Gomez, D., White, J., Bedoukian, E.C., Zhu, X., Xie, H.M., Gai, X. et al. (2016) Copy-number variation is an important contributor to the genetic causality of inherited retinal degenerations. Genet. Med., 2016 [Epub ahead of print]

39. Khatib, S., Hanany, M., Khalaeih, A., Beryozkin, A., Meyer, S., Abu- Diab, A., Abu Turky, F., Mizrahi-Meissonnier, L., Lieberman, S. et al. (2016) Identification of genomic deletions causing inherited retinal degenerations by coverage analysis of whole exome sequencing data. J. Med. Genet., 53, 600–607.

40. Van Cauwenbergh, C., Van Schil, K., Cannoodt, R., Bawens, M., Van Laethem, T., De Jaegere, S., Steyaert, W., Sante, T., Menten, B., Leroy, B.P. et al. (2017) arrEYE: a customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs. Genet. Med., 19, 457–466.

41. Liquori, A., Vaché, C., Baux, D., Blanchet, C., Hamel, C., Malcolm, S., Koenig, M., Claustres, M. and Roux, A.F. (2016) Whole USH2A gene sequencing identifies several new deep intronic mutations. Hum. Mutat., 37, 184–193.

42. Sangermano, R., Bax, N.M., Bawens, M., van den Born, L.I., Da Baere, E., Garanto, A., Collin, R.W., Goerich-Ramal, A.S., den Engelsman-van Dijk, A.H., Rohrschneider, K. et al. (2016) Photoreceptor progenitor mRNA analysis reveals exon skipping resulting from the ABCA4 c.5461+10T→C mutation in Stargardt Disease. Ophthalmology, 123, 1375–1385.

43. Mayer, A.K., Rohrschneider, K., Strom, T.M., Glöckel, N., Kohl, S., Wissinger, B. and Weisschu, N. (2016) Homozygosity mapping and whole-genome sequencing reveals a deep intronic PROM1 mutation causing cone-rod dystrophy by pseudexon activation. Eur. J. Hum. Genet., 24, 459–462.

44. Conte, I., Hadfeld, K.D., Barbato, S., Carrella, S., Pizzo, M., Bhat, R.S., Carissimo, A., Karali, M., Porter, L.F., Urquhart, J. et al. (2015) MIR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma. Proc. Natl. Acad. Sci. USA, 112, E3236–E3245.

45. Coppieters, F., Todeschini, A.L., Fujitaki, T., Baert, A., De Bruyne, M., Van Cauwenbergh, C., Verdin, H., Bawens, M., Ongenaeert, M., Kondo, M. et al. (2015) Hidden Genetic Variation in LCA9-Associated Congenital Blindness Explained by 5'UTR Mutations and Copy-Number Variations of NMNAT1. Hum. Mutat., 36, 1188–1196.

46. Radziwon, A., Arno, G.K., Wheaton, D., McDonagh, E.M., Baple, E.L., Webb-Jones, K.G., Birch, D., Webster, A.R. and MacDonald, I.M. (2017) Single-base substitutions in the CHM promoter as a cause of choroideremia. Hum. Mutat., [Epub ahead of print]

47. Farrar, G.J., Millington-Ward, S., Chadderton, N., Mansergh, F.C. and Palfi, A. (2014) Gene therapies for inherited retinal disorders. Vis. Neurosci., 31, 289–307.

48. Dalkara, D., Goureau, O., Marazova, K. and Sahel, J.A. (2016) Let there be light: gene and cell therapy for blindness. Hum. Gene. Ther., 27, 134–147.

49. Scholl, H.P., Strauss, R.W., Singh, M.S., Dalkara, D., Roska, B., Picaud, S. and Sahel, J.A. (2016) Emerging therapies for inherited retinal degeneration. Sci, Transl, Med., 8, 368rv6.

50. MacLaren, R.E., Bennett, J. and Schwartz, S.D. (2016) Gene therapy and stem cell transplantation in retinal disease: The New Frontier. Ophthalmology, 123, S98–S106.

51. Bennett, J. (2017) Taking Stock of Retinal Gene Therapy: Looking Back and Moving Forward. Mol. Ther., [Epub ahead of print]
52. Cideciyan, A.V., Jacobson, S.G., Beltran, W.A., Sumaroka, A., Swider, M., Iwabe, S., Roman, A.J., Olivas, M.B., Schwartz, S.B., Komáromy, A.M. et al. (2013) Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. Proc. Natl. Acad. Sci. U S A, 110, 517–525.

53. Millington-Ward, S., Chadderton, N., O'Reilly, M., Palfi, A., Millington-Ward, S., O'Reilly, M., Campbell, M., Ehrhardt, C., Wolfrum, U. et al. (2013) Intravitreal delivery of AAV-NDI1 provides functional benefit in a murine model of Leber hereditary optic neuropathy. Eur. J. Hum. Genet., 21, 62–68.

54. Mookherjee, S., Hiriyanna, S., Kaneshiro, K., Li, L., Li, Y., Li, W., Qian, H., Li, T., Khanna, H., Colosi, P. et al. (2015) Long-term rescue of cone photoreceptor degeneration in retinitis pigmentosa 2 (RP2)-knockout mice by gene replacement therapy. Hum. Mol. Genet., 24, 6446–6458.

55. LaVail, M.M., Yasumura, D., Matthes, M.T., Yang, H., Hauswirth, W.W., Deng, W.T. and Vollrath, D. (2016) Gene therapy for MERTK-associated retinal degenerations. Sci. Transl. Med., 5, 189ra76.

56. Castle, M.J., Turunen, H.T., Vandenberghe, L.H. and Wolfe, J.H. (2016) Controlling AAV tropism in the nervous system with natural and engineered capsids. Methods Mol. Biol., 1382, 133–149.

57. Zulliger, R., Conley, S.M. and Naash, M.I. (2015) Non-viral therapeutic approaches to ocular diseases: An overview and future directions. J. Control Release, 219, 471–487.

58. Lopes, V.S. and Williams, D.S. (2015) Gene therapy for the retinal degeneration of Usher syndrome caused by mutations in MYO7A. Cold Spring Harb. Perspect. Med., 5, pii: a017319.