Advanced Diagnostic Genetic Testing In Inherited Retinal Disease:Experience from a Single Tertiary Referral Centre in the UK National Health Service

Khan K[1,2,3], Ali N[1], Ravinder Chana[1,2], Genevieve Wright[1,2], Webster AR[1,2], Moore AT[1,2] Michaelides M[1,2],

1. Moorfields Eye Hospital, 162 City Road, London, EC1V 2PD, UK

2. UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK

3. St. James's University Hospital, Beckett Street, Leeds, LS9 7TF, UK

Author for Correspondence: Prof Michel Michaelides

UCL Institute of Ophthalmology

11-43 Bath Street, London, EC1V 9EL, UK

Tel: +44 207 608 6864

Michel.michaelides@ucl.ac.uk

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by grants from the National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology, Fight For Sight (UK), Moorfields Eye Hospital Special Trustees, Moorfields Eye Charity, the Foundation Fighting Blindness (USA), and Retinitis Pigmentosa Fighting Blindness. Michel Michaelides is a recipient of an FFB Career Development Award. This research has been funded/supported by the National Institute for Health Research Rare Diseases Translational Research Collaboration (NIHR RD-TRC). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

ABSTRACT

Background/Aims

In 2013, as part of our genetic investigation of patients with Inherited Retinal Disease, we utilised multigene panel testing of 105 genes known to cause retinal disease in our patient cohorts. This test was performed in a UK National Health Service (NHS) accredited laboratory.

Method

The results of all multigene panel tests requested between 1.4.13 and 31.8.14 were retrospectively reviewed. All patients had been previously seen at Moorfields Eye Hospital, London, UK and diagnosed with an inherited retinal dystrophy after clinical examination and detailed retinal imaging.

Result

The results were categorised into three groups: 1) Testing helped establish a certain molecular diagnosis in 45/115 (39%). Variants in USH2A (n=6) and RP1 (n=4) were most common.
2) Definitive conclusions could not be drawn from molecular testing alone in 13/115 (11%) as either insufficient pathogenic variants were discovered or those identified that were not consistent with the phenotype.
3) Testing did not identify any pathogenic variants responsible for the phenotype in 57/115 (50%).

Conclusion

Multigene panel testing performed in an NHS setting has enabled a molecular diagnosis to be confidently made in 40% of cases. Novel variants accounted for 38% of all identified variants. Detailed retinal phenotyping helped the interpretation of specific variants. Additional care needs to be taken when assessing polymorphisms in genes that have been infrequently associated with disease, as historical techniques were not as rigorous as contemporary ones. Future iterations of sequencing are likely to offer higher sensitivity, testing a broader range of genes, more rapidly and at a reduced cost.

Key words: genetics, NGS, diagnostics, retinal-dystrophy

INTRODUCTION

Inherited retinal disorders (IRD) are a genetically heterogeneous group of conditions, almost all of which are currently untreatable. They are associated with significant ocular morbidity and so, despite their low overall prevalence, they are a common cause of severe visual loss. In England and Wales they now represent the commonest cause of sight impairment registration in adults of working age [I], and the second commonest in childhood [II]. Managing patients with rare diseases requires specific expertise and for IRD this includes access to diagnostic genetic testing, a field that has rapidly evolved over the past decade.

The initial ophthalmic genetic discoveries were made in a research setting, using linkage analysis followed by Sanger sequencing [III, IV]. As a result, sequencing of single genes was subsequently offered as a clinical test. This technique still retains great utility for a few IRD where the phenotype is readily recognisable and where there is no or limited genetic heterogeneity (e.g. *BEST1* and *RS1*) and for regions not amenable to novel sequencing methods (e.g. ORF15 of *RPGR* and *OPN1LW*/*OPN1MW*). Sanger sequencing is however time consuming and costly, making it impractical for testing multiple genes in a large number of patients; there are over 300 genes currently associated with IRD. Subsequently genotyping technologies were developed which were capable of detecting hundreds of known sequence variants simultaneously (the Affymetrix array; Affymetrix, Santa, Clara, CA; Illumina platform; Illumina, San Diego, CA; or the Arrayed Primer Extension (Apex) chip; Asper Ophthalmics, Ltd., Tartu, Estonia) [V]. Whilst this approach offers a rapid and cost-effective form of analysis, it is limited to identifying only known disease-associated variants.

More recently it has become possible to rapidly sequence whole genes simultaneously, using massively parallel sequencing techniques (Next Generation Sequencing, NGS). In order to maximise effectiveness, both in the quality of information derived and cost, limiting sequencing to the coding regions of genes known to cause IRD has been performed. This “targeted capture” approach, also known as focussed exome sequencing or multigene panel testing, has been utilised in a clinical diagnostic setting in preference to whole exome sequencing. Whilst the former technique has key advantages, the main drawback is that the number of genes captured is limited by current knowledge, and may miss variants in novel genes, thus requiring regular updating. Despite these limitations, recent studies have reported diagnostic rates of approximately 50%, significantly higher than had been obtained with previous technologies [VI-X]. These reports have however generally involved only small numbers of patients.

In 2013, as part of our genetic investigation of patients with IRD, we utilised focused exome sequencing of 105 genes known to cause retinal disease in our patient cohorts. This test was performed in a National Health Service (NHS) accredited laboratory (National Genetics Reference Laboratory (NGRL), Manchester University Teaching Hospitals NHS Trust) as part of

routine clinical care and was not part of a research study. The present work describes the results of multigene panel testing in this cohort, and how it has influenced our clinical practice.

MATERIALS AND METHODS

Clinical cohort investigated

The results of all genetic tests (NGS, 105 genes) requested between 1.4.13 and 31.8.14 were retrospectively reviewed. All patients had been examined in the IRD clinics by one of three experienced clinicians (ATM, ARW, MM) at Moorfields Eye Hospital, London, UK and diagnosed with IRD. In order to refine the diagnosis, all patients consented to further genetic testing which was then requested in accordance with the retinal phenotype. Where a specific gene (e.g. *BEST1*, *RS1*, *NR2E3*, *RDH12*, *CRB1*), or small group of genes were suspected (e.g. *ABCA4*, *PRPH2*), patients were not subjected to the full 105 gene screen, as Sanger sequencing was requested. Patients with albinism, isolated foveal hypoplasia and inherited optic neuropathies were similarly not investigated by this route. Retinal dystrophies occurring as part of a syndromic diagnosis were included.

Genetic testing

A custom designed Sure Select Target Enrichment Kit (Agilent Technologies, Santa Clara, CA, USA) was used for targeted enrichment of 105 genes known to be mutated in patients with isolated and syndromic retinal disease. Samples were run on a SOLiD 4 sequencer (Life Technologies, Grand Island, NY, USA). The sequencing and variant calling methodologies have previously been reported [VIII]. The testing laboratory issued a clinical report detailing variants thought to account for the disease. If a novel variant was identified, a further comment as to their predicted pathogenicity and evolutionary conservation was also offered and segregation studies performed.

RESULTS

In 17 months, 115 multigene panel tests were requested. The results were available in all cases. Our findings have been grouped into three major categories:

1). *Testing helped to establish a certain molecular diagnosis*.

A specific molecular genetic diagnosis was identified in 45/115 (39%). This allowed the clinician to more confidently counsel the patient regarding the cause of their disease, inheritance pattern and therefore recurrence risk. In the majority of cases (28/45) variants already known to cause retinal disease were discovered. However, in over a third of cases, either one or both alleles harboured novel genetic variants that were predicted to be damaging (Table I). Variants in *USH2A* (n=6) and *RP1* (n=4) were most commonly identified, and together represented 22% of all cases. The remainder (78%) resulted

from variants in a total of 24 genes, with no one gene responsible for more than 4% of cases, underlining the genetically heterogeneous nature of IRD.

Pathogenic variants in *ABCA4*, *PRPH2* and *PROM1* were also identified, perhaps unexpected, as a separate testing pathway existed for macular dystrophies. Similarly one may have predicted that *RPGR* related RP may have been suspected from the pedigree structure or clinical features (e.g. early onset, severe disease, association with myopia, carrier changes in females). However, in all of these cases patients presented with typical, simplex retinitis pigmentosa, with no overt signs directing the investigating clinician towards a specific molecular diagnosis (Figure I).

2). *Definitive conclusions could not be drawn from molecular testing alone*.
In 13/115 (11%) patients genetic testing identified potentially pathogenic variants, but overall the results were thought to be inconclusive, as a definitive molecular diagnosis (from this test alone) could not be established. In the majority of cases (10/13 = 77%) only one of two recessive alleles was identified in patients thought to have a recessively inherited retinal dystrophy. In two of these (Patients 31953 and 32703), single alleles, known to be pathogenic from prior reports were identified in *CRB1* [XI-XIII]. For patient 31953 careful clinical phenotyping was helpful in supporting the hypothesis that *CRB1* was indeed the causative gene as this case demonstrated typical optical coherence tomography (OCT) characteristics quite specific to this gene (Figure II). Patient 32703 however did not exhibit any of these classical features and consequently was considered unsolved. In a minority of cases (3/13), a sufficient number of variants were identified however their pathogenicity was questionable (*PITPNM3*, *RDH12*, *PROM1*) (Table II).

3). *Testing did not identify any pathogenic variants responsible for the phenotype*.
In 57/115 (50%) cases testing did not identify any genetic variants that were thought to result in retinal disease. In one case a negative test was considered sufficient investigation to rule out a genetic aetiology, offering greater confidence to the diagnosis of acute zonal occult outer retinopathy (AZOOR). In all other cases the causative variants were considered elusive and a more comprehensive genetic study was undertaken with patients invited into whole exome (BRIDGE SPEED Project) or whole genome sequencing studies (100,000 Genomes Pilot Project, Genomics England, UK).

DISCUSSION

The present study has investigated the sensitivity of a specific focused exome-sequencing pipeline in a cohort of preselected patients with inherited retinal disease. This work represents the largest examination of such a technique, where patients have been recruited from a single clinical centre. 115 patients were tested, in whom the clinical phenotype had not suggested a candidate gene to screen. Pathogenic variants were identified in 45 cases. A

single pathogenic allele was identified in one patient which, when combined with additional phenotypic information established a definitive diagnosis. Overall therefore genetic testing enabled a diagnosis to be made in 46/115 (40%) cases where experienced clinical examiners could not establish a specific genetic diagnosis on phenotypic features alone.

Of the 46 confidently solved cases, 28 had recessive disease, 17 autosomal dominant disease and a single case of X-linked disease (patient 31664). In the majority of cases these inheritance patterns were not predicted from the family pedigree, as simplex cases are uninformative and dominant inheritance may be underestimated due to non-penetrance, particularly prevalent with variants of the splicing factors *PRPF8* and *PRPF31*. Similarly, autosomal recessive inheritance may be confused with autosomal dominant disease when two successive generations are affected, not an uncommon occurrence for genes where the carrier frequency for mutant alleles is high e.g. *ABCA4*. Herein pseudodominance was also observed in pedigree 20929 harbouring *USH2A* variants (Figure IIIa). The identification of X-linked disease in Patient 31664 was also surprising as this pedigree was predicted to segregate a dominant variant (Figure IIIb). The mother and son were both affected, however the maternal phenotype was particularly severe, even at a relatively young age, not thereby suggestive of typical *RPGR*-related retinopathy. Testing therefore provided the opportunity for more accurate genetic counselling.

Retinitis pigmentosa (RP) can occur either in isolation or as part of a syndrome, although this may not always be clear at the time of ophthalmic assessment. NGS has particular utility in the diagnosis of syndromic disease, especially where the extraocular phenotype may appear subclinical at first presentation [XIV]. Here we have identified two cases of syndromic disease – both patients with Usher Syndrome (USH). As patient 31899 and two of her affected siblings display the classical USH type 1 (USH1) phenotype, the identification of bi-allelic *MYO7A* variants, one of the five genes known to cause USH1, was not unexpected [XV-XX]. The gene responsible for the commonest cause of USH type 2 (USH2) is also the commonest cause of autosomal recessive isolated RP – *USH2A* (Daiger 207 [XXI]). Recently it has additionally been associated with RP and late onset hearing loss, a combination not originally described by von Gräfe and Usher (case D23 [XXII-XXIV]). The clinical interpretation of *USH2A* variants is therefore not straightforward, as variant may be associated with a wide range of hearing problems, and for those with isolated RP it may be uncertain if auditory problems will develop later. Here Patient 3381, with a clinical phenotype of RP and adult-onset hearing loss was found to carry alleles associated with both isolated RP (p.Cys934Trp) and USH2 (p.Arg4192His). As larger cohorts of well-characterised patients undergo NGS our understanding of allelic hierarchy in *USH2A* will improve, facilitating accurate genetic counseling. Unexpectedly, the same lady was also found to be a carrier for *PCDH15*, an USH1 gene, and a null allele in *RPGR* (p.Arg449*), highlighting that even with focused exome sequencing ‘incidental’ findings will be discovered and pose diagnostic challenge. A further cause of USH2 is variant of *GPR98* and we identified a patient with RP and a single heterozygous variant predicted to

introduce a cryptic splice site (Patient 3109; p.Asn157Ser). Although not formally tested with audiometry this patient did not report clinically significant hearing loss, an important feature to note, as to date all pathogenic variants in *GPR98* have been associated with dual sensory impairment [XXV]. The clinical information in this case therefore suggests that the cause of this patient's retinal dystrophy may be due to variants within of another gene. The alternative hypothesis, that mutation of *GPR98* is a hitherto unreported cause of isolated RP and a second allele is yet to be found, seems less likely.

As NGS offers an unbiased testing approach, identifying pathogenic variants in uncommon retinal disease genes is made easier, where previous candidate gene sequencing strategies would have delayed their discovery. This was particularly relevant for several genes included in the panel. Mutation of *PDE6G* has thus far been reported to cause RP in only a single Middle Eastern pedigree [XXVI]. Whilst *PDE6G* is an excellent candidate gene for retinal disease, the paucity of subsequent reports strengthening the association with RP leave the original conclusions open to scrutiny. We now record the second case of *PDE6G* related RP (Patient 30120). This family was also noteworthy as they were thought to segregate a dominant disease causing allele, as the proband's late mother, three of eight siblings and a niece were reported to be similarly affected, although none had been examined. NGS testing in this family not only facilitated the detection of bi-allelic variants in *PDE6G*, but also allowed the recurrence risk of disease to be downgraded from 50% to less than 1% for future generations.

Another rare cause of RP is heterozygous mutation of *KLHL7* (OMIM 612943) and both reports to date suggest that this is characterised by late onset disease [XXVII, XXVIII]. Here we have identified two patients with variants in *KLHL7*. The previously observed variant (p.Ala153Val) is again seen with onset of symptoms at an older age (Patient 28569), whilst the novel variant (p.Val141Ala) is associated with a much earlier onset (Patient 28819), resulting in significant visual field constriction (to approximately 5-10 degrees) and severe symptoms prior to the age of 30. These data highlight the cautious approach necessary when attempting to predict phenotypic outcomes from patients' genotype, especially with small cohorts of patients.

Mutation of *PITPNM3* (c.1878G>C, p.Gln626His) has also been identified to cause cone and cone-rod dystrophy, however since the original report in 2007 no further groups have replicated these findings [XXIX, XXX]. Here NGS identified the same sequence variant in 1/115 patients tested (Patient 32296), but this did not segregate with disease and despite acknowledgement of variable penetrance in the original report we could not confidently assign pathogenicity to this polymorphism. This same nucleotide change has also been observed in patients with IRD who are known to carry variants in other RP associated genes, and has also been identified in unaffected control samples (in-house databases, inherited retinal disease consortium, UK). Furthermore, this variant is observed at higher than expected frequencies in publically held databases (exome variant sever G/C: 36, C/C: 6467; 1000 genomes file shows global frequencies G/C: 5: C/C: 1087 accessed 1.7.15). It therefore remains likely that the genetic basis for retinal disease in Patient

32296 remains unexplained and that *PITPNM3* is yet to be confirmed as a disease-associated gene. Similarly the pathogenicity of the heterozygous variant in *RDH12* (Patient 32458) remains outstanding, as again only a single report exists [XXXI].

It is important to note that in 60% of our cases a clear molecular diagnosis was not obtained. In part this relates to the techniques involved. Whilst accurate at the time of design, the exponentially increasing rate of novel genetic discoveries means that the capture reagent, designed here to report on 105 genes, soon needs updating. Accordingly the latest version has been designed to cover 176 disease-associated genes. Even since then a further 62 genes have been discovered [XXXII]. The disadvantages of multigene panel testing are however offset by the accuracy, cost-effectiveness and speed that this technique offers, advantages that have allowed its use in a clinical rather than solely a research setting. Even if whole exome sequencing is performed, we still would not have a test with 100% sensitivity. Intronic and intergenic nucleotides, areas that are known to contain regulatory elements, would still not be sequenced and many structural variants would also be missed, even within the regions captured. As expected therefore, if these regions are interrogated, variant detection is improved. This was highlighted by Steele-Stallard and colleagues, who have reported finding 35% of missed second alleles in *USH2A* after screening for duplications, deletions and a common intronic variant [XXIII]. Whole genome sequencing will offer improved sensitivity but currently the costs involved limit its wider use. Irrespective of the capture reagent (multigene panel, whole exome or whole genome), one additional benefit of NGS platforms is the great “depth” of coverage that it offers, as each nucleotide is sequenced many times in parallel. This provides a bioinformatic opportunity to assess the relative number of allele counts in a more quantitative way than is possible with Sanger sequencing. Here the aforementioned *RPGR* variant load in Patient 3381 varied from that expected in the heterozygous state (approximately 50:50, wt:mt), findings that were confirmed by capillary sequencing, strongly suggesting that this patient is a mosaic for this variant (often associated with *de novo* somatic variant).

Although the advantages of NGS are many, we feel that there is still a role for Sanger sequencing, especially when one polymerase chain reaction may suffice (eg. *PROM1* p.Arg373Cys, *C1QTNF5* p.Arg163Ser, *EFEMP1* p.Arg345Trp). In a further subset of patients ancillary clinical tests may help direct genetic testing (eg. electroretinography for *NR2E3* and *KCNV2*, electrooculography for BEST1, fundus autofluorescence for *ABCA4*), however the costs associated with sequencing even a small gene may now not compare favourably to those for NGS. It is important to remember that as this cohort of patients were excluded from the present study the mutation detection rate may have artificially been lowered, as from a genetic point of view the “low hanging fruit” will have already been harvested. This hypothesis may be tested by comparing our results with those from two large, recently reported studies, where patients with recognisable genotypes were included (XXXIV, XXXV). The first tested 562 patients, recruited from multiple international clinics, finding a sufficient number of pathogenic alleles in 45% of

cases. In a further 5% a missed second allele was thought to have evaded detection (XXXIV). The second study tested 292 patients, again as part of an international collaboration but this time enriched for consanguineous pedigrees. A molecular genetic result was obtained in 60% (XXXV). These findings also highlight how both the ethnic background and pedigree structure of the study population can influence mutation detection rate, in addition to the underlying gene of interest.

In summary, the use of multigene panel testing performed in an NHS setting has enabled a molecular diagnosis to be confidently made in 40% (46/114) of cases. Novel variants accounted for 38% of all identified variants, although as testing becomes commonplace this figure is likely to reduce, aiding bioinformatics analysis. Obtaining a positive test result facilitated accurate counselling, often changing the estimated recurrence risk for future generations. Despite our current inability to initiate proven therapeutic interventions in the majority of cases many patients still place a high value on establishing a biological cause for their symptoms. Furthermore, as there are multiple planned and on-going clinical trials for IRD, obtaining a genetic diagnosis is likely to be necessary for enrolment [XXXVI]. Future iterations of this technique are likely to offer higher sensitivity, testing a broader range of genes, more rapidly and at a reduced cost.

REFERENCES

I. Liew G, Michaelides M, Bunce C. A comparison of the causes of blindness certifications in England and Wales in working age adults (16–64 years), 1999–2000 with 2009–2010. BMJ Open 2014;4:e004015 doi:10.1136/bmjopen-2013-004015

II. Rahi JS1, Cable N; British Childhood Visual Impairment Study Group. Severe visual impairment and blindness in children in the UK. Lancet. 2003 Oct 25;362(9393):1359-65.

III. Dryja TP, McGee TL, Reichel E et al. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature. 1990 Jan 25;343(6256):364-6.

IV. Cremers FP, Sankila EM, Brunsmann F et al. Deletions in patients with classical choroideremia vary in size from 45 kb to several megabases. Am J Hum Genet. 1990 Oct;47(4):622-8.

V. Kurg A, Tõnisson N, Georgiou I et al. Arrayed primer extension: solid-phase four-color DNA resequencing and mutation detection technology. Genet Test. 2000;4(1):1-7.

VI. JT Simpson, R Durbin. Efficient de novo assembly of large genomes using compressed data structures. Genome research 2012

VII. Neveling K, Collin RW, Gilissen C et al. Next-generation genetic testing for retinitis pigmentosa. Hum Mutat. 2012 Jun;33(6):963-72.

VIII. O'Sullivan J, Mullaney BG, Bhaskar SS et al. A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. J Med Genet. 2012 May;49(5):322-6.

IX. Shanks ME, Downes SM, Copley RR et al. 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. 2013 Mar;21(3):274-80. doi: 10.1038/ejhg.2012.172. Epub 2012 Sep 12. Erratum in: Eur J Hum Genet. 2013 Sep;21(9):1031.

X. Glöckle N, Kohl S2, Mohr J1 et al. Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet. 2014 Jan;22(1):99-104.

XI. Den Hollander AI, Davis J, van der Velde-Visser SD et al. CRB1 mutation spectrum in inherited retinal dystrophies. Hum Mutat. 2004 Nov;24(5):355-69.

XII. Henderson RH, Mackay DS, Li Z et al. Phenotypic variability in patients with retinal dystrophies due to mutations in CRB1. British Journal of Ophthalmology 2011, 95(6), 811-817.

XIII. Siemiatkowska AM, Arimadyo K, Moruz LM et al. Molecular genetic analysis of retinitis pigmentosa in Indonesia using genome-wide homozygosity mapping. Mol Vis. 2011;17:3013-24. Epub 2011 Nov 18.

XIV. Ellingford JM, Sergouniotis PI, Lennon R et al. Pinpointing clinical diagnosis through whole exome sequencing to direct patient care: a case of Senior-Loken syndrome. Lancet. 2015 May. Volume 385, No. 9980:1916

XV. Weil D, Blanchard S, Kaplan J et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 199537460–61.61

XVI. Jaijo T, Aller E, Oltra S et al. Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. Hum Mutat 200627290–291.291

XVII. Roux A F, Faugere V, Le Guedard S et al. Survey of the frequency of USH1 gene mutations in a cohort of Usher patients shows the importance of cadherin 23 and protocadherin 15 genes and establishes a detection rate of above 90%. J Med Genet 2006. 43763–768.768

XVIII. Liu X Z, Walsh J, Mburu P et al. Mutations in the myosin VIIA gene cause non- syndromic recessive deafness. Nat Genet 199716188–190.190

XIX. Weil D, Kussel P, Blanchard S et al. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin- VIIA gene. Nat Genet 199716191–193.193

XX. Liu X Z, Hope C, Walsh J et al. Mutations in the myosin VIIA gene cause a wide phenotypic spectrum, including atypical Usher syndrome. Am J Hum Genet 199863909–912.912

XXI. McGee TL, Seyedahmadi BJ, Sweeney MO et al. Novel mutations in the long isoform of the USH2A gene in patients with Usher syndrome type II or non-syndromic retinitis pigmentosa. J Med Genet. 2010 Jul;47(7):499-506.

XXII. Lenassi E, Vincent A, Li Z et al. A detailed clinical and molecular survey of subjects with nonsyndromic USH2A retinopathy reveals an allelic hierarchy of disease-causing variants. Eur J Hum Genet. 2015 Oct;23(10):1318-27.

XXIII. Usher C. On the inheritance of Retinitis pigmentosa with notes of cases. Roy. Lond. Ophthalmol. Hosp. Rep. 1914. 19: 130–236.

XXIV. von Gräfe A. Exceptionelles Verhalten des Gesichtsfeldes bei Pigmententartung der Netzhaut. Archiv für Ophthalmologie 1948. 4: 250–253.

XXV. Ouyang X M, Yan D, Du L L et al. Characterization of Usher syndrome type I gene mutations in an Usher syndrome patient population. Hum Genet 2005116292–299.299

XXVI. Dvir L, Srour G, Abu-Ras R et al. Autosomal-recessive early-onset retinitis pigmentosa caused by a mutation in PDE6G, the gene encoding the gamma subunit of rod cGMP phosphodiesterase. Am J Hum Genet. 2010 Aug 13;87(2):258-64.

XXVII. Friedman JS, Ray JW, Waseem N et al. Mutations in a BTB-Kelch protein, KLHL7, cause autosomal-dominant retinitis pigmentosa. Am J Hum Genet. 2009 Jun; 84(6):792-800.

XXVIII. Wen Y, Locke KG, Klein M et al. Phenotypic Characterization of 3 Families With Autosomal Dominant Retinitis Pigmentosa Due to Mutations in KLHL7. Arch Ophthalmol. 2011 Nov; 129(11): 1475–1482.

XXIX. Köhn L, Kadzhaev K, Burstedt MS et al. Mutation in the PYK2-binding domain of PITPNM3 causes autosomal dominant cone dystrophy (CORD5) in two Swedish families. Eur J Hum Genet. 2007 Jun;15(6):664-71.

XXX. Köhn L, Kohl S, Bowne SJ et al. PITPNM3 is an uncommon cause of cone and cone-rod dystrophies. Ophthalmic Genet. 2010 Sep;31(3):139-40.

XXXI. Fingert JH, Oh K, Chung M et al. Association of a novel mutation in the retinol dehydrogenase 12 (RDH12) gene with autosomal dominant retinitis pigmentosa. Arch Ophthalmol. 2008 Sep;126(9):1301-7. doi: 10.1001/archopht.126.9.1301.

XXXII. https://sph.uth.edu/Retnet/sum-dis.htm accessed 8.7.15

XXXIII. Steele-Stallard HB, Le Quesne Stabej P, Lenassi E et al: Screening for duplications, deletions and a common intronic mutation detects 35% of second mutations in patients with USH2A monoallelic mutations on Sanger sequencing. Orphanet J Rare Dis 2013; 8: 122

XXXIV. Ellingford JM, Barton S, Bhaskar S, Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. Ophthalmology. 2016 Feb 9. pii: S0161-6420(16)00030-0. doi: 10.1016/j.ophtha.2016.01.009. [Epub ahead of print]

XXXV. Patel N, Aldahmesh MA, Alkuraya H Expanding the clinical, allelic, and locus heterogeneity of retinal dystrophies. Genet Med. 2015 Sep 10. doi: 10.1038/gim.2015.127. [Epub ahead of print]

XXXVI. Smith J, Ward D, Michaelides M et al. New and emerging technologies for the treatment of inherited retinal diseases: a horizon scanning review. Eye (2015) 29, 1131–1140

LEGENDS

Table I: Novel Mutations identified in this study. Where compound heterozygous variants are identified, and only one is novel, the previously reported variant is shown in parentheses.

Table II: Genetic variants of questionable pathogenicity

Figure I: Colour fundus images from patients with pathogenic mutations in ABCA4, PROM1, PRPH2 and RPGR demonstrating signs typical of Retinitis Pigmentosa – pigment migration into the retina, retinal arteriolar attenuation and optic disc pallor.

Figure II: OCT phenotype of two patients with heterozygous CRB1 mutations and missed second allele. Macular thickening without significant oedema and relative loss of normal retinal lamination is highly indicative of CRB1-related retinopathy (2a,b) - whilst in the absence of these, or other stereotypical features, other genetic causes of RP cannot be excluded (2c,d).

Figure IIIA: Pedigree for Patient 20929. As the presence of two affected family members in different generations is unusual for rare, recessively inherited disorders this family were thought to harbour a dominant allele transmitted with variable penetrance. Genetic testing proved this hypothesis incorrect, identifying bi-allelic mutation of USH2A as the cause.

Figure IIIB: Pedigree for Patient 31664. As more females than males are affected, and some at a young age, autosomal dominant rather than X-linked disease was suspected. This family are now known to segregate a pathogenic variant in *RPGR*.

FIGURES/TABLES

Table I

Patient ID	Gene	Novel mutation
28819	*KLHL7*	c.422T>C, p.Val141Ala
31899	*MYO7A*	c.3728C>T, pProl1243Leu; (c.3476G>T, p.Gly1159Val)
30120	*PDE6G*	c.109C>T, p.Gln37*
3083	*PRPF8*	c.5804G>A, p.Arg1935His
27967	*RP1*	c.4576G>T, p.Glu1526* homozygous
31602	*RP1*	c.5446T>C, p.Cys1816Arg heterozygous
31735	*RP1*	c.2749C>T, p.Gln917*
31169	*RPGRIP1*	c.2608_2609insA homozygous
5041	*TULP1*	c.1313G>C, p.Arg438Pro heterozygous
3501	*USH2A*	c.8079G>A, p.Trp2693*; (c.12575, p.Arg4192His)
27978	*USH2A*	c.10342G>A, p.Glu3348Lys and c.14803C>T, p.Arg4935*; (c.6670G>T, p.Gly2224Cys)
30787	*C2orf71*	c.2315_2321delinsAAG homozygous
22588	*CEP290*	c.148C>T, p.His50Tyr; (c.4393C>T, p.Arg1465*)
32452	*EYS*	c.5928-2A>G homozygous
3259	*MERTK*	c.345C>G, p.Cys115Trp; c.634A>C, p.Thr212Pro
32408	*IMPG2*	c.3056G>A, p.Cys1019Tyr; (c.2716C>T, pArg906*)
29709	*IMPG2*	c.3113G>T, p.Cys1038Phe; (c.3423-7_3423-4delCTTT)

Table II

Gene	**ID**	**Variant**	**Reason to question pathogenicity**
PITPNM3	32296	c.1878G>C, p.Gln626His heterozygous	Identified in many control samples
RDH12	32458	c.763delG heterozygous	Doesn't fully segregate with disease
PROM1	1796	c.1414C>T, p.Arg472*	A dominant pedigree with a single recessive allele

Figure I

Figure Ia ABCA4

Figure Ib PROM1

Figure Ic PRPH2

Figure Id PRPH2

Figure Ie RPGR

Figure II

Figure II OCT RE, LE patient 31953

Figure II OCT RE, LE patient 32703

Figure IIIa

Figure IIIb