REVIEW

Clinical applications of high-throughput genetic diagnosis in inherited retinal dystrophies: Present challenges and future directions

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Abstract

The advent of next generation sequencing (NGS) techniques has greatly simplified the molecular diagnosis and gene identification in very rare and highly heterogeneous Mendelian disorders. Over the last two years, these approaches, especially whole exome sequencing (WES), alone or combined with homozygosity mapping and linkage analysis, have proved to be successful in the identification of more than 25 new causative retinal dystrophy genes. NGS-approaches have also identified a wealth of new mutations in previously reported genes and have provided more comprehensive information concerning the landscape of genotype-phenotype correlations and the genetic complexity/diversity of human control populations. Although whole genome sequencing is far more informative than WES, the functional meaning of the genetic variants identified by the latter can be more easily interpreted, and final diagnosis of inherited retinal dystrophies is extremely successful, reaching 80%, particularly for recessive cases. Even considering the present limitations of WES, the reductions in costs and time, the continual technical improvements, the implementation of refined bioinformatic tools and the unbiased comprehensive genetic information it provides, make WES a very promising diagnostic tool for routine clinical and genetic diagnosis in the future.

Key words: Next generation sequencing; Identification of novel causative genes; Inherited retinal dystrophies; Genetic diagnosis; Whole exome sequencing

Core tip: This review focuses on the application of next generation sequencing (NGS)-based methods [whole genome sequencing, whole exome sequencing (WES), targeted exome sequencing] for genetic diagnosis and novel gene identification in hereditary retinal dystrophies. Advances over the last two years concerning NGS accuracy, reliability, development of bioinformatics tools, together with the drop in costs and time required for the

analysis have allowed thirty novel genes to be identified, plus a large number of new mutations in previously reported genes. NGS techniques (particularly WES) are revolutionizing genetic diagnosis and have clear applications in clinical practice, helping to pave the way for personalized medicine. Present challenges and future directions are also discussed.

INTRODUCTION

Inherited retinal dystrophies (IRDs) consist of a group of highly heterogeneous disorders at the genetic and clinical level. Until recently, the ever increasing number of causative genes (more than 200 so far) and mutations (more than 5000) (https://sph.uth.edu/retnet/) posed an enormous challenge for molecular diagnosis and limited the effectiveness of conventional mutational screening. However, the advent of next generation sequencing (NGS) technologies has completely revolutionized genetic diagnosis[1,2]. Since the first application of exome sequencing using NGS to identify the causative gene in a very rare autosomal recessive disorder[3], more than 150 new Mendelian disease genes have been reported using similar approaches[4]. Focusing on *IRD* genes, NGS approaches [whole exome sequencing (WES), or whole genome sequencing (WGS)] have rapidly identified new causative genes, increasing the success rate of molecular diagnosis from 40% to almost 80%, depending on the number of cases analysed and the informativity of the family[5-7]. It is foreseeable that NGS-based methods will be the technique of choice for future routine DNA diagnosis in IRDs and similar heterogeneous Mendelian disorders, since accuracy and efficiency increase while costs and time requirements drop continually[8,9].

NGS-BASED DIAGNOSIS

The challenge posed by the molecular diagnosis of heterogeneous disorders prompted researchers to devise novel conceptual and technical approaches to help clinicians classify diseases, inform patients and families, and offer genetic counselling and prenatal diagnosis. The approaches they devised also provide the basis for a more efficient molecular-based therapy. Since the draft of the human genome was published, several high-throughput techniques have been devised. In the field of IRDs, commercially available microarrays for direct mutational screening (http://www.asperbio.com/asper-ophthalmics), customized resequencing microarrays

(restricted to several large diagnostic centres/units)[10] and whole genome or targeted gene *SNP* genotyping arrays for linkage analysis (cosegregation and homozygosity studies) have paved the way either for mutation screening in reported known genes, or for the highlighting of new loci for candidate causal genes[11]. Diagnostic efficiency ranged from 15%-44% in direct mutation screening microarrays-depending on the pathogenic allelic frequencies in the population, to 30%-70% for resequencing microarrays-depending on the number of genes included and the sequence quality[12]. Indeed, direct analysis of known mutations and genes requires constant updating, and even so, many mutations remain undetected because they are private[13]. Moreover, SNP genotyping for homozygosity mapping and cosegregation analysis has become a very informative genetic tool in many cases[14,15].

WES EFFICIENCY IN THE DIAGNOSIS OF MENDELIAN DISORDERS

A survey of the IRD (syndromic and non-syndromic) genes identified in the last two years (up to 29) showed that all the approaches used to identify them involved NGS. The success of NGS as a diagnostic tool is due to: (1) the power of an unbiased genome scale analysis; (2) the increasing number of databases containing information on SNP allelic frequencies in different populations, which allows rare presumptive mutations to be discriminated from frequent genetic variants; (3) the relative simplicity of the currently standardized protocols; (4) powerful bioinformatics analysis; and (5) the fact that the data gathered is useful on its own.

Nonetheless, additional genetic information is still instrumental to increase the yield of molecular diagnosis since, despite the power of WES, gene identification in recessive IRDs (24) is far more successful than it is in dominant cases (5) (Table 1). The difference in this outcome is to be expected, since finding the relevant causative mutation in heterozygosis amidst the great number of genetic variants identified by WES (more than 20.000 on average) is not a straight-forward endeavour[16]. In contrast, the requirement of a double heterozygous mutation (or even homozygosis) in the same gene for recessive cases, greatly diminishes the noise associated with such massive collection of data, and thus the number of putative causative genes, after the data has been filtered. While WES alone has pinpointed the causative gene in around 30% of the recessive IRD cases (years 2013-2014), adRD causative genes have proved to be more difficult to identify and require a combination of genetic approaches, such as linkage analysis, deletion mapping and targeted capture of candidates, to eventually single out the pathogenic mutation in a novel gene (10%)[17]. The informativity of these genetic approaches has also greatly favoured gene identification in recessive cases (60%)[9] (Table 1).

Table 1 List of retinal dystrophy causative and candidate genes identified in 2013-2014 and the strategy of identification

Gene	Retinal phenotype	Methodological approach
ABCD5	Recessive CRD, spastic parapesis, white matter disease	Homozygosity mapping combined with WES[25]
ADAMTS18	arRD early onset	Homozygosity mapping combined with WES[43]
ARLBP2	arRP	Homozygosity mapping combined with WES[44]
BBIP1	arBBS	WES[45]
C12orf65	Recessive optic atrophy, spastic paraplegia and neuropathy	Linkage mapping WES[46,47]
C21orf2	Recessive CRD	Homozygosity mapping combined with WES[25]
CSPP1	Recessive JS	WES[48-50]
DHX38	arRP (early onset with macular coloboma)	Homozygosity mapping combined with candidate gene approach[51]
DTHD1	Recessive LCA, myopathy	Homozygosity mapping combined with WES[25]
EMC1	arRP	Homozygosity mapping combined with WES[25]
GDF6	arRD	Candidate gene sequencing[52]
GPR125	arRP	Homozygosity mapping combined with WES[25]
HK1	adRP, nonspherocytic hemolytic anemia, and neuropathy	Linkage mapping and WES[53]
IFT27	arBBS	Homozygosity mapping combined with candidate gene approach[42]
IMPG1	Dominant MD	Linkage mapping
	Recessive MD	WES and candidate gene sequencing[54-56]
ITM2B	Dominant RD, dementia	WES combined with linkage mapping[57]
KIAA1549	arRP	Homozygosity mapping combined with WES[25]
KIZ	arRP, arCRD	WES[58]
LRIT3	arCSNB	WES[59]
MVK	arRP, recessive mevalonic aciduria	WES[60]
NEK2	arRP	WGS[6]
NR2F1	Dominant optic atrophy, intellectual disability	Deletion mapping
		WES and deletion mapping[61,62]
PCYT1A	arCRD with skeletal disease	WES and targeted candidate gene sequencing[63,64]
POC1B	Recessive CRD	WES[65]
PRPF4	adRP	Targeted capture NGS[41]
RAB28	arCRD	Homozygosity mapping combined with WES[66]
RDH11	arRP	WES[67]
SLC7A14		WES[68]
TUB	arRD with obesity	Homozygosity mapping combined with WES[69,70]
TTLL5	Recessive cone and CRD	WES[71]

ar: Autosomal recessive; ad: Autosomal dominant; CRD: Cone-rod dystrophy; RD: Retinal dystrophy; RP: Retinitis pigmentosa; BBS: Bardet-Biedl syndrome; JS: Joubert syndrome; LCA: Leber congenital amaurosis; MD: Macular dystrophy; CSNB: Congenital stationary night blindness; WES: Whole exome sequencing; WGS: Whole genome sequencing; NGS: Next generation sequencing.

WGS *VS* WES *VS* TARGETED EXOME SEQUENCING IN ROUTINE DIAGNOSIS

At present, many groups rely on NGS-based techniques for genetic diagnosis of IRDs (and other Mendelian disorders)[18]; WES is the most common of these approaches (Table 1). Nonetheless, a few attempts using WGS or targeted exome sequencing have also been reported. In the latter, long polymerase chain reaction amplimers spanning the exons of reported *RP* genes[17] or lately, customized exome capture of the coding exons of a selected set of genes, have been developed with a wide range of diagnosis success (40%-80%)[19-22]. Customized approaches allow different degrees of refinement and are dependent on the optimization of the techniques and the prioritization of the type of mutations to be identified. For instance, if copy number variants (CNVs) are suspected, the coverage and high quality of the reads constitute one focus of improvements[23]. Nonetheless, the cost of customized capture arrays for a list of causative disease genes is still much higher than that of conventional capture arrays for WES, and the genetic information provided is limited to the candidates analysed. Mutations in non-selected or previously unreported genes will

remain undetected. WES is becoming the most popular choice, particularly since the reliability of the technique and the quality of the analysis software have greatly increased (though there is still room for improvement), and microRNAs and transcript untranslated regions are also included in some exon capture array versions[24]. Overall, the reported success rate for IRD diagnosis in randomly selected familiar and simplex cases account for 74%-80% of the mutation pool in some studies[25].

WGS for the molecular diagnosis of retinal dystrophy (RD) has been attempted with moderate success (56% of molecular diagnosis and the identification of a new causative gene)[6]. The main reason behind this massive genome sequencing approach was to analyse coding and noncoding regions in order to detect structural and copy number variants and to evaluate highly polymorphic SNPs. Although the WGS reported in this work facilitated the detection of two structural pathogenic variants (which would probably have escaped detection with WES), the fact that no pathogenic mutation in the large noncoding fraction of the genome was identified, and that 7 out of 16 patients remained undiagnosed after the considerable effort required to screen the whole genome, pose some questions about the suitability of WGS in routine RD genetic diagnosis.

Table 2 Possible genetic cause in undiagnosed patients after whole exome sequencing

Genetic variants	Technical restrains	Alternative approaches
MicroRNAs and lncRNAs	Not sequenced	Inclusion in the capture
Deep intronic	Not sequenced	RNASeq
		WGS
		Targeted re-sequencing
Variants in regulatory regions	Not sequenced	WGS
		Targeted re-sequencing
Large deletions	Mostly undetected	Detectable in homozygosis
		In heterozygosis can be detected in comparison with controls
		(if high coverage)
		WGS
		Targeted re-seq
CNVs	Mostly undetected	High coverage
		WGS
		Targeted re-seq
		CGH
Pathogenic trinucleotide repeats	Short reads not covering the whole expansion	Triple repeat based PCR
Structural chromosomal variants	Undetectable	FISH
		WGS
		Targeted Long PCR coupled to NGS
Aneuploidies	Undetectable	Conventional cytogenetics FISH
		WGS

lncRNAs: Long non-coding RNAs; CNVs: Copy number variants; RNASeq: RNA sequencing; WGS: Whole genome sequencing; CGH: Comparative genome hybrdization; PCR: Polymerase chain reaction; FISH: Fluorescent *in situ* hybridization; NGS: Next generation sequencing.

PRESENT LIMITATIONS OF WES IN GENETIC DIAGNOSIS

Although NGS-based methodologies allow comprehensive genomic analysis on an unprecedented scale, none of them is free from technical constrains. The conventional WES diagnostic strategy is based on exon capture by nucleic acid hybridization. Even though continuous improvements to the method have continually been implemented (capture optimization, and higher coverage and sequencing accuracy), not all the pathogenic mutations can be detected[26]. One main issue that needs to be addressed without delay is the implementation of unified bioinformatics tools for accurate mapping and reliable variant-calling software, particularly for small indels (insertions/deletions) and CNVs[8,27]. Other pending issues include the detection of mutations in genomic regions that escape the capture methods currently available, such as small exons, regulatory regions, deep intronic variants and chromosomal structural variations that do not affect exons (inversions and deletions) (Table 2).

When the main focus is basic research and the analysis is restricted to a small genomic region high-lighted by linkage or homozygosity, custom targeted genome re-sequencing is a viable alternative to WES[22,28]. However, for daily routine diagnosis, standard WES offers an appealing compromise between cost, time, comprehensiveness of data processing and efficiency.

UNEXPECTEDLY HIGH NUMBER OF IRD RECESSIVE PATHOGENIC VARIANTS IN THE CONTROL POPULATION

Knowledge of the underlying genetic structure of human populations provides very valuable clues to help successfully identify pathogenic genes[25,29], particularly in highly consanguineous cohorts where homozygosity by descent is suspected. Current data indicates that this assumption should be extended even in the absence of a positive family history, where both parents may be heterozygous for the same pathogenic allele. Not only may the unsuspected homozygosity of pathogenic alleles reveal a founder effect-which is informative in itself- but it is also one of the most useful genetic assumptions that can lead to the identification of novel causative alleles after WES[25,30].

Notably, the wealth of genome information gathered by WES suggests that control individuals carry 10-20 pathogenic recessive mutations causative of Mendelian disorders[3]. RD stands out as one of the most highly genetically heterogeneous monogenic disorders, and when we focus on the IRD causative genes-even when only null alleles are considered-22% of the control population (1 in 4-5 individuals) is heterozygous for at least one pathogenic mutation[31]. This high prevalence is still an underestimate because missense and splicing mutations have not been included, and neither have all the *IRD* genes been identified, which overall would probably account for 1 in 2 control individuals carrying a pathogenic recessive *RD* mutation. Such a high frequency of unaffected carriers has an important impact on genetic diagnosis since: (1) consanguinity would increase the risk of blindness in the offspring; (2) the comparison of a newly identified genetic variant with control individuals in databases to assess pathogenicity could be misleading; and (3) many patients would by chance bear an additional pathogenic allele besides the causative mutations, which would hamper the molecular diagnosis. This last point would lead to

Main candidate gene	Disease
CNGB3, CNGA3	Achromatopsia
RHO	adRP
VMD2	Best disease
CYP4V2	Bietti crystalline dystrophy
RDS/PRPH2	Central areolar choroidal dystrophy
CHM	Choroideremia
LRPO5, FZD4, TSAPN12	Familiar exudative vitreoretinopathy
RDH5, RLBP1	Fundus albipunctatus
NR2E3	Goldman-Favre-Enhanced S-cone syndrome
CEP290	LCA
MFRP	Nanophthalmia
NDP	Norrie disease
SAG	Oguchi disease
RS1	Retinoschisis
RECQL4	Rothmund-Thompson syndrome
ABCA4, RDS/PRPH2	Stargardt disease
USH2A	Usher syndrome
VCN	Wagner syndrome
RPGR	XLCD, XLCRD
RPGR, RP2	XLRP, RP simplex

adRP: Autosomal dominant retinitis pigmentosa; LCA: Leber congenital amaurosis; XLCD: X-linked cone dystrophy; XLCRD: X-linked cone-rod dystrophy; XLRP: X-linked retinitis pigmentosa.

false assumptions of dominant effects of recessive alleles, and explain compound heterozygosis in some consanguineous pedigrees, and open the can of worms of digenic inheritance[31]. In addition, reports of the synergic addition of pathogenic alleles in families with several phenotypes are now emerging, which would seem to call for a new conceptual molecular framework for genotype/phenotype correlations.

Another issue revealed by WES when trios (two parental samples in addition to the patient sample) are analysed is the unexpectedly high frequency of *de novo* mutations, which strengthens the case for reconsidering dominance along side recessivity in simplex cases[22].

PENETRANCE AND EXPRESSIVITY REVISITED: MODIFIER GENES AND WES

Incomplete penetrance and variable expressivity are two genetic phenomena frequently associated with human disease, mainly due to additional genetic factors influencing the final phenotype. From the molecular point of view, genes and proteins interacting and/or regulating the function of the causative gene exert a modifying effect, which could enhance or diminish the pathological outcome in patients bearing the same causative mutation. Identifying the modifier genes has been, and still is, one of the most important challenges in clinical and genetic diagnosis. WES is instrumental in unveiling modifier alleles by direct comparison of the DNA sequences of affected members of the same family, frequently displaying different phenotype severity[6,28,32].

As there is a continual increase in WES-generated data on genetic variants, the pool of modifier genes likewise grows and diagnostic inferences will become more accurate, thus providing the grounds for a more precise prognosis.

EMPOWERING GENETIC DIAGNOSIS OF IRDS BY WES: CANDIDATE PRIORITIZATION CRITERIA, GENETIC INFORMATION AND INTERACTION NETWORKS

So far, NGS-based approaches have mostly been considered for the identification of causative genes in very rare Mendelian disorders when the gene is unknown or mutation screening involves a large number of genes and exons, as is the case of highly heterogeneous diseases. However, after progressive and substantial methodological refining, WES and other NGS-based techniques have leapt from bench to bedside, and are now feasible and attractive alternatives for routine diagnosis. They allow for comprehensive genomic screening, are increasingly affordable and robust, and last but not least, the bioinformatics analysis is becoming more accurate and user-friendly (even though a common standard framework for downstream variant mapping and calling analysis is still lacking)[8,33].

Monogenic disorders caused by mutations in a major gene also will benefit from WES (NGS)-based diagnosis. The costs of Sanger sequencing of a large gene (*e.g.*, *ABCA4*, *CEP290*, *etc.*) are no less than those of full exome sequencing (WES), but the benefits from the comprehensive information gleaned *via* the latter technique are far superior. To mention just a few: minor causative genes are included in the analysis, additional disease causing alleles in modifier genes will be also detected-and so their impact in the population genetic reservoir can be assessed; the molecular basis of rare clinical entities with ambiguous diagnosis can be identified; genotype-phenotype correlations will be more precisely defined; and genetic data on the patient drug response (pharmacogenetics) will be included. Indeed, the analysis of NGS-based data should be prioritized for the genes and variants that are most prevalent for a particular IRD and pattern of inheritance (for instance, in X-linked disorders) (Table 3). If no pathogenic variants are identified, the list of candidates should be expanded following prioritization criteria that include less frequent causative candidates for the same (or similar) phenotype, and finally, all the variants detected by WES under all possible assumptions of Mendelian inheritance should be considered[22,34]. This is particularly relevant in simplex cases and pedigrees with a small number of patients, where dominant *de novo*, X-linked or very rare recessive mutations should be carefully considered. In this context, exhaustive human gene mutation repositories will be extremely informative tools to perform a rapid screening of reported mutations

and thus, simplify the genomic analysis[35].

Indeed, intersection with previous or parallel genetic analysis has been and still is instrumental in pinpointing pathogenic alleles. For instance, SNP genotyping microarrays (6K Illumina) for linkage or homozygosity studies (Table 1 and references therein), or SNP-based cosegregation chips[12] highlight the genetic loci where the gene/mutation identification efforts should be focused. This greatly simplifies matters and provides statistical support for the final molecular diagnosis. In fact, only one third of the novel *IRD* genes identified by NGS over the last two years (Table 1) were discovered without resorting to candidate prioritization using genetic data.

TAKING ON THE FUTURE: PARTS LIST, MAP, DIAGNOSIS, THERAPY

How many novel *IRD* causative genes remain to be identified? Based on the latest NGS results where all new genes explain either rare syndromic disorders with an accompanying IRD phenotype or cases with private mutations affecting very few patients, it seems very unlikely that any novel gene will account for a substantial fraction of unassigned cases[6]. As most technical approaches do not cover the whole panoply of causative mutations, a percentage of mutations in already reported genes might have been overlooked. In fact, transcriptome analysis of healthy human retinas revealed more than one hundred previously unannotated genes, almost 30000 unreported exons (around a 3% increase) and over 20000 3' and 5' alternative splicing sites[36]. This unprecedented transcript diversity is a serious challenge for mutation identification, as these regions are not yet included in commercial exome enrichment kits and RNASeq of patient neural tissues is not feasible. Thus, optimization of molecular diagnosis in IRD demands, on the one hand, technical improvements for easy implementation and accuracy, and on the other, the widening of the genomic regions to include novel genes, exons and other regions of interest.

The great wealth of data gathered by conventional as well as high-throughput approaches demands a framework based on systems biology[37]. To this end, unveiling the genetic networks underlying IRDs, although still fragmentary, is a valid approach. Ongoing efforts to integrate interactomes of photoreceptors[38-40] are beginning to show the first promising candidates[41,42]. Further work will allow the translation of this genetic information to the cellular and tissular contexts. Only a comprehensive view of the retinal pathways in health and disease can pave the way for effective therapies.

Finally, although not the main aim of this review, we should not overlook that any genetic laboratory working on WES and WGS data should abide to strict ethical guidelines that concern incidental findings relevant to the patient's health status but unrelated to the focus of the genetic testing.

CONCLUSION

To sum up, the generalized implementation of NGS-based analysis will foster more reliable genotype/phenotype correlations and provide a more holistic view of the genetic factors that cause and modify the severity of the phenotype. Even though 100% diagnosis will not be reached soon and there are new challenges and questions to address, the comprehensive genetic data gathered by NGS will definitely help the clinicians and patients in securing diagnosis, improving prognosis and recommending therapy. It is foreseeable that in the near future, clinical management of the patient will become more personalized and thus more effective.

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REFERENCES

1   **Shendure J**. Next-generation human genetics. *Genome Biol* 2011; **12**: 408 [PMID: 21920048 DOI: 10.1186/gb-2011-12-9-408]
2   **Bamshad MJ**, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, Shendure J. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet* 2011; **12**: 745-755 [PMID: 21946919]
3   **Ng SB**, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet* 2010; **42**: 30-35 [PMID: 19915526 DOI: 10.1038/ng.499]
4   **Zhang X**. Exome sequencing greatly expedites the progressive research of Mendelian diseases. *Front Med* 2014; **8**: 42-57 [PMID: 24384736 DOI: 10.1007/s11684-014-0303-9]
5   **Neveling K**, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, Rodenburg RJ, Yntema HG, Spruijt L, Vermeer S, Rinne T, van Gassen KL, Bodmer D, Lugtenberg D, de Reuver R, Buijsman W, Derks RC, Wieskamp N, van den Heuvel B, Ligtenberg MJ, Kremer H, Koolen DA, van de Warrenburg BP, Cremers FP, Marcelis CL, Smeitink JA, Wortmann SB, van Zelst-Stams WA, Veltman JA, Brunner HG, Scheffer H, Nelen MR. A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum Mutat* 2013; **34**: 1721-1726 [PMID: 24123792 DOI: 10.1002/humu.22450]
6   **Nishiguchi KM**, Tearle RG, Liu YP, Oh EC, Miyake N, Benaglio P, Harper S, Koskiniemi-Kuendig H, Venturini G, Sharon D, Koenekoop RK, Nakamura M, Kondo M, Ueno S, Yasuma TR, Beckmann JS, Ikegawa S, Matsumoto N, Terasaki H, Berson EL, Katsanis N, Rivolta C. Whole genome sequencing in patients with retinitis pigmentosa reveals pathogenic DNA structural changes and NEK2 as a new disease gene. *Proc Natl Acad Sci USA* 2013; **110**: 16139-16144 [PMID: 24043777 DOI: 10.1073/pnas.1308243110]
7   **Licastro D**, Mutarelli M, Peluso I, Neveling K, Wieskamp N, Rispoli R, Vozzi D, Athanasakis E, D'Eustacchio A, Pizzo M, D'Amico F, Ziviello C, Simonelli F, Fabretto A, Scheffer H, Gasparini P, Banfi S, Nigro V. Molecular diagnosis of Usher syndrome: application of two different next generation sequencing-based procedures. *PLoS One* 2012; **7**: e43799 [PMID: 22952768 DOI: 10.1371/journal.pone.0043799]
8   **Ratnapriya R**, Swaroop A. Genetic architecture of retinal and macular degenerative diseases: the promise and challenges of next-generation sequencing. *Genome Med* 2013; **5**: 84 [PMID:

24112618]

9   **Gilissen C**, Hoischen A, Brunner HG, Veltman JA. Disease gene identification strategies for exome sequencing. *Eur J Hum Genet* 2012; **20**: 490-497 [PMID: 22258526 DOI: 10.1038/ejhg.2011.258]

10 **Kim C**, Kim KJ, Bok J, Lee EJ, Kim DJ, Oh JH, Park SP, Shin JY, Lee JY, Yu HG. Microarray-based mutation detection and phenotypic characterization in Korean patients with retinitis pigmentosa. *Mol Vis* 2012; **18**: 2398-2410 [PMID: 23049240]

11 **Pomares E**, Riera M, Permanyer J, Méndez P, Castro-Navarro J, Andrés-Gutiérrez A, Marfany G, Gonzàlez-Duarte R. Comprehensive SNP-chip for retinitis pigmentosa-Leber congenital amaurosis diagnosis: new mutations and detection of mutational founder effects. *Eur J Hum Genet* 2010; **18**: 118-124 [PMID: 19584904 DOI: 10.1038/ejhg.2009.114]

12 **de Castro-Miró M**, Pomares E, Lorés-Motta L, Tonda R, Dopazo J, Marfany G, Gonzàlez-Duarte R. Combined genetic and high-throughput strategies for molecular diagnosis of inherited retinal dystrophies. *PLoS One* 2014; **9**: e88410 [PMID: 24516651 DOI: 10.1371/journal.pone.0088410]

13 **Corton M**, Nishiguchi KM, Avila-Fernández A, Nikopoulos K, Riveiro-Alvarez R, Tatu SD, Ayuso C, Rivolta C. Exome sequencing of index patients with retinal dystrophies as a tool for molecular diagnosis. *PLoS One* 2013; **8**: e65574 [PMID: 23940504 DOI: 10.1371/journal.pone.0065574]

14 **Avila-Fernandez A**, Corton M, Nishiguchi KM, Muñoz-Sanz N, Benavides-Mori B, Blanco-Kelly F, Riveiro-Alvarez R, Garcia-Sandoval B, Rivolta C, Ayuso C. Identification of an RP1 prevalent founder mutation and related phenotype in Spanish patients with early-onset autosomal recessive retinitis. *Ophthalmology* 2012; **119**: 2616-2621 [PMID: 22917891 DOI: 10.1016/j.ophtha.2012.06.033]

15 **Coussa RG**, Otto EA, Gee HY, Arthurs P, Ren H, Lopez I, Keser V, Fu Q, Faingold R, Khan A, Schwartzentruber J, Majewski J, Hildebrandt F, Koenekoop RK. WDR19: an ancient, retrograde, intraflagellar ciliary protein is mutated in autosomal recessive retinitis pigmentosa and in Senior-Loken syndrome. *Clin Genet* 2013; **84**: 150-159 [PMID: 23683095 DOI: 10.1111/cge.12196]

16 **Koboldt DC**, Larson DE, Sullivan LS, Bowne SJ, Steinberg KM, Churchill JD, Buhr AC, Nutter N, Pierce EA, Blanton SH, Weinstock GM, Wilson RK, Daiger SP. Exome-based mapping and variant prioritization for inherited Mendelian disorders. *Am J Hum Genet* 2014; **94**: 373-384 [PMID: 24560519 DOI: 10.1016/j.ajhg.2014.01.016]

17 **Daiger SP**, Bowne SJ, Sullivan LS, Blanton SH, Weinstock GM, Koboldt DC, Fulton RS, Larsen D, Humphries P, Humphries MM, Pierce EA, Chen R, Li Y. Application of next-generation sequencing to identify genes and mutations causing autosomal dominant retinitis pigmentosa (adRP). *Adv Exp Med Biol* 2014; **801**: 123-129 [PMID: 24664689 DOI: 10.1007/978-1-4614-3209-8_16]

18 **Foo JN**, Liu JJ, Tan EK. Whole-genome and whole-exome sequencing in neurological diseases. *Nat Rev Neurol* 2012; **8**: 508-517 [PMID: 22847385 DOI: 10.1038/nrneurol.2012.148]

19 **Audo I**, Bujakowska KM, Léveillard T, Mohand-Saïd S, Lancelot ME, Germain A, Antonio A, Michiels C, Saraiva JP, Letexier M, Sahel JA, Bhattacharya SS, Zeitz C. Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. *Orphanet J Rare Dis* 2012; **7**: 8 [PMID: 22277662 DOI: 10.1186/1750-1172-7-8]

20 **Chen X**, Zhao K, Sheng X, Li Y, Gao X, Zhang X, Kang X, Pan X, Liu Y, Jiang C, Shi H, Chen X, Rong W, Chen LJ, Lai TY, Liu Y, Wang X, Yuan S, Liu Q, Vollrath D, Pang CP, Zhao C. Targeted sequencing of 179 genes associated with hereditary retinal dystrophies and 10 candidate genes identifies novel and known mutations in patients with various retinal diseases. *Invest Ophthalmol Vis Sci* 2013; **54**: 2186-2197 [PMID: 23462753 DOI: 10.1167/iovs.12-10967]

21 **Wang J**, Zhang VW, Feng Y, Tian X, Li FY, Truong C, Wang G, Chiang PW, Lewis RA, Wong LJ. Dependable and efficient clinical utility of target capture-based deep sequencing in molecular diagnosis of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2014; **55**: 6213-6223 [PMID: 25097241 DOI: 10.1167/iovs.14-14936]

22 **Neveling K**, Collin RW, Gilissen C, van Huet RA, Visser L, Kwint MP, Gijsen SJ, Zonneveld MN, Wieskamp N, de Ligt J, Siemiatkowska AM, Hoefsloot LH, Buckley MF, Kellner U, Branham KE, den Hollander AI, Hoischen A, Hoyng C, Klevering BJ, van den Born LI, Veltman JA, Cremers FP, Scheffer H. Next-generation genetic testing for retinitis pigmentosa. *Hum Mutat* 2012; **33**: 963-972 [PMID: 22334370 DOI: 10.1002/humu.22045]

23 **Eisenberger T**, Neuhaus C, Khan AO, Decker C, Preising MN, Friedburg C, Bieg A, Gliem M, Charbel Issa P, Holz FG, Baig SM, Hellenbroich Y, Galvez A, Platzer K, Wollnik B, Laddach N, Ghaffari SR, Rafati M, Botzenhart E, Tinschert S, Börger D, Bohring A, Schreml J, Körtge-Jung S, Schell-Apacik C, Bakur K, Al-Aama JY, Neuhann T, Herkenrath P, Nürnberg G, Nürnberg P, Davis JS, Gal A, Bergmann C, Lorenz B, Bolz HJ. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One* 2013; **8**: e78496 [PMID: 24265693 DOI: 10.1371/journal.pone.0078496]

24 **Asan Y**, Jiang H, Tyler-Smith C, Xue Y, Jiang T, Wang J, Wu M, Liu X, Tian G, Wang J, Wang J, Yang H, Zhang X. Comprehensive comparison of three commercial human whole-exome capture platforms. *Genome Biol* 2011; **12**: R95 [PMID: 21955857 DOI: 10.1186/gb-2011-12-9-r95]

25 **Abu-Safieh L**, Alrashed M, Anazi S, Alkuraya H, Khan AO, Al-Owain M, Al-Zahrani J, Al-Abdi L, Hashem M, Al-Tarimi S, Sebai MA, Shamia A, Ray-Zack MD, Nassan M, Al-Hassnan ZN, Rahbeeni Z, Waheeb S, Alkharashi A, Abboud E, Al-Hazzaa SA, Alkuraya FS. Autozygome-guided exome sequencing in retinal dystrophy patients reveals pathogenetic mutations and novel candidate disease genes. *Genome Res* 2013; **23**: 236-247 [PMID: 23105016 DOI: 10.1101/gr.144105.112]

26 **Chilamakuri CS**, Lorenz S, Madoui MA, Vodák D, Sun J, Hovig E, Myklebost O, Meza-Zepeda LA. Performance comparison of four exome capture systems for deep sequencing. *BMC Genomics* 2014; **15**: 449 [PMID: 24912484 DOI: 10.1186/1471-2164-15-449]

27 **Maranhao B**, Biswas P, Duncan JL, Branham KE, Silva GA, Naeem MA, Khan SN, Riazuddin S, Hejtmancik JF, Heckenlively JR, Riazuddin SA, Lee PL, Ayyagari R. exomeSuite: Whole exome sequence variant filtering tool for rapid identification of putative disease causing SNVs/indels. *Genomics* 2014; **103**: 169-176 [PMID: 24603341 DOI: 10.1016/j.ygeno.2014.02.006]

28 **Estrada-Cuzcano A**, Neveling K, Kohl S, Banin E, Rotenstreich Y, Sharon D, Falik-Zaccai TC, Hipp S, Roepman R, Wissinger B, Letteboer SJ, Mans DA, Blokland EA, Kwint MP, Gijsen SJ, van Huet RA, Collin RW, Scheffer H, Veltman JA, Zrenner E, den Hollander AI, Klevering BJ, Cremers FP. Mutations in C8orf37, encoding a ciliary protein, are associated with autosomal-recessive retinal dystrophies with early macular involvement. *Am J Hum Genet* 2012; **90**: 102-109 [PMID: 22177090 DOI: 10.1016/j.ajhg.2011.11.015]

29 **Collin RW**, van den Born LI, Klevering BJ, de Castro-Miró M, Littink KW, Arimadyo K, Azam M, Yazar V, Zonneveld MN, Paun CC, Siemiatkowska AM, Strom TM, Hehir-Kwa JY, Kroes HY, de Faber JT, van Schooneveld MJ, Heckenlively JR, Hoyng CB, den Hollander AI, Cremers FP. High-resolution homozygosity mapping is a powerful tool to detect novel mutations causative of autosomal recessive RP in the Dutch population. *Invest Ophthalmol Vis Sci* 2011; **52**: 2227-2239 [PMID: 21217109 DOI: 10.1167/iovs.10-6185]

30 **Alazami AM**, Alshammari MJ, Salih MA, Alzahrani F, Hijazi H, Seidahmed MZ, Abu Safieh L, Aldosary M, Khan AO, Alkuraya FS. Molecular characterization of Joubert syndrome in Saudi Arabia. *Hum Mutat* 2012; **33**: 1423-1428 [PMID: 22693042 DOI: 10.1002/humu.22134]

31 **Nishiguchi KM**, Rivolta C. Genes associated with retinitis pigmentosa and allied diseases are frequently mutated in the general population. *PLoS One* 2012; **7**: e41902 [PMID: 22848652 DOI: 10.1371/journal.pone.0041902]

32 **Khateb S**, Zelinger L, Mizrahi-Meissonnier L, Ayuso C, Koenekoop

RK, Laxer U, Gross M, Banin E, Sharon D. A homozygous nonsense CEP250 mutation combined with a heterozygous nonsense C2orf71 mutation is associated with atypical Usher syndrome. *J Med Genet* 2014; **51**: 460-469 [PMID: 24780881 DOI: 10.1136/jmedgenet-2014-102287]

33   **Mefford HC**. Diagnostic exome sequencing--are we there yet? *N Engl J Med* 2012; **367**: 1951-1953 [PMID: 23033977 DOI: 10.1056/NEJMe1211659]

34   **Neveling K**, den Hollander AI, Cremers FP, Collin RW. Identification and analysis of inherited retinal disease genes. *Methods Mol Biol* 2013; **935**: 3-23 [PMID: 23150357 DOI: 10.1007/978-1-62703-080-9_1]

35   **Stenson PD**, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet* 2014; **133**: 1-9 [PMID: 24077912]

36   **Farkas MH**, Grant GR, White JA, Sousa ME, Consugar MB, Pierce EA. Transcriptome analyses of the human retina identify unprecedented transcript diversity and 3.5 Mb of novel transcribed sequence via significant alternative splicing and novel genes. *BMC Genomics* 2013; **14**: 486 [PMID: 23865674 DOI: 10.1186/1471-2164-14-486]

37   **Vidal M**, Cusick ME, Barabási AL. Interactome networks and human disease. *Cell* 2011; **144**: 986-998 [PMID: 21414488 DOI: 10.1016/j.cell.2011.02.016]

38   **Rachel RA**, Li T, Swaroop A. Photoreceptor sensory cilia and ciliopathies: focus on CEP290, RPGR and their interacting proteins. *Cilia* 2012; **1**: 22 [PMID: 23351659 DOI: 10.1186/2046-2530-1-22]

39   **Sorusch N**, Wunderlich K, Bauss K, Nagel-Wolfrum K, Wolfrum U. Usher syndrome protein network functions in the retina and their relation to other retinal ciliopathies. *Adv Exp Med Biol* 2014; **801**: 527-533 [PMID: 24664740 DOI: 10.1007/978-1-4614-3209-8_67]

40   **Estrada-Cuzcano A**, Roepman R, Cremers FP, den Hollander AI, Mans DA. Non-syndromic retinal ciliopathies: translating gene discovery into therapy. *Hum Mol Genet* 2012; **21**: R111-R124 [PMID: 22843501 DOI: 10.1093/hmg/dds298]

41   **Chen X**, Liu Y, Sheng X, Tam PO, Zhao K, Chen X, Rong W, Liu Y, Liu X, Pan X, Chen LJ, Zhao Q, Vollrath D, Pang CP, Zhao C. PRPF4 mutations cause autosomal dominant retinitis pigmentosa. *Hum Mol Genet* 2014; **23**: 2926-2939 [PMID: 24419317 DOI: 10.1093/hmg/ddu005]

42   **Aldahmesh MA**, Li Y, Alhashem A, Anazi S, Alkuraya H, Hashem M, Awaji AA, Sogaty S, Alkharashi A, Alzahrani S, Al Hazzaa SA, Xiong Y, Kong S, Sun Z, Alkuraya FS. IFT27, encoding a small GTPase component of IFT particles, is mutated in a consanguineous family with Bardet-Biedl syndrome. *Hum Mol Genet* 2014; **23**: 3307-3315 [PMID: 24488770 DOI: 10.1093/hmg/ddu044]

43   **Peluso I**, Conte I, Testa F, Dharmalingam G, Pizzo M, Collin RW, Meola N, Barbato S, Mutarelli M, Ziviello C, Barbarulo AM, Nigro V, Melone MA, Simonelli F, Banfi S. The ADAMTS18 gene is responsible for autosomal recessive early onset severe retinal dystrophy. *Orphanet J Rare Dis* 2013; **8**: 16 [PMID: 23356391 DOI: 10.1186/1750-1172-8-16]

44   **Davidson AE**, Schwarz N, Zelinger L, Stern-Schneider G, Shoemark A, Spitzbarth B, Gross M, Laxer U, Sosna J, Sergouniotis PI, Waseem NH, Wilson R, Kahn RA, Plagnol V, Wolfrum U, Banin E, Hardcastle AJ, Cheetham ME, Sharon D, Webster AR. Mutations in ARL2BP, encoding ADP-ribosylation-factor-like 2 binding protein, cause autosomal-recessive retinitis pigmentosa. *Am J Hum Genet* 2013; **93**: 321-329 [PMID: 23849777 DOI: 10.1016/j.ajhg.2013.06.003]

45   **Scheidecker S**, Etard C, Pierce NW, Geoffroy V, Schaefer E, Muller J, Chennen K, Flori E, Pelletier V, Poch O, Marion V, Stoetzel C, Strähle U, Nachury MV, Dollfus H. Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (BBS18). *J Med Genet* 2014; **51**: 132-136 [PMID: 24026985 DOI: 10.1136/jmedgenet-2013-101785]

46   **Shimazaki H**, Takiyama Y, Ishiura H, Sakai C, Matsushima Y, Hatakeyama H, Honda J, Sakoe K, Naoi T, Namekawa M, Fukuda Y, Takahashi Y, Goto J, Tsuji S, Goto Y, Nakano I. A homozygous mutation of C12orf65 causes spastic paraplegia with optic atrophy and neuropathy (SPG55). *J Med Genet* 2012; **49**: 777-784 [PMID: 23188110 DOI: 10.1136/jmedgenet-2012-101212]

47   **Tucci A**, Liu YT, Preza E, Pitceathly RD, Chalasani A, Plagnol V, Land JM, Trabzuni D, Ryten M, Jaunmuktane Z, Reilly MM, Brandner S, Hargreaves I, Hardy J, Singleton AB, Abramov AY, Houlden H. Novel C12orf65 mutations in patients with axonal neuropathy and optic atrophy. *J Neurol Neurosurg Psychiatry* 2014; **85**: 486-492 [PMID: 24198383 DOI: 10.1136/jnnp-2013-306387]

48   **Akizu N**, Silhavy JL, Rosti RO, Scott E, Fenstermaker AG, Schroth J, Zaki MS, Sanchez H, Gupta N, Kabra M, Kara M, Ben-Omran T, Rosti B, Guemez-Gamboa A, Spencer E, Pan R, Cai N, Abdellateef M, Gabriel S, Halbritter J, Hildebrandt F, van Bokhoven H, Gunel M, Gleeson JG. Mutations in CSPP1 lead to classical Joubert syndrome. *Am J Hum Genet* 2014; **94**: 80-86 [PMID: 24360807 DOI: 10.1016/j.ajhg.2013.11.015]

49   **Tuz K**, Bachmann-Gagescu R, O'Day DR, Hua K, Isabella CR, Phelps IG, Stolarski AE, O'Roak BJ, Dempsey JC, Lourenco C, Alswaid A, Bönnemann CG, Medne L, Nampoothiri S, Stark Z, Leventer RJ, Topçu M, Cansu A, Jagadeesh S, Done S, Ishak GE, Glass IA, Shendure J, Neuhauss SC, Haldeman-Englert CR, Doherty D, Ferland RJ. Mutations in CSPP1 cause primary cilia abnormalities and Joubert syndrome with or without Jeune asphyxiating thoracic dystrophy. *Am J Hum Genet* 2014; **94**: 62-72 [PMID: 24360808 DOI: 10.1016/j.ajhg.2013.11.019]

50   **Shaheen R**, Shamseldin HE, Loucks CM, Seidahmed MZ, Ansari S, Ibrahim Khalil M, Al-Yacoub N, Davis EE, Mola NA, Szymanska K, Herridge W, Chudley AE, Chodirker BN, Schwartzentruber J, Majewski J, Katsanis N, Poizat C, Johnson CA, Parboosingh J, Boycott KM, Innes AM, Alkuraya FS. Mutations in CSPP1, encoding a core centrosomal protein, cause a range of ciliopathy phenotypes in humans. *Am J Hum Genet* 2014; **94**: 73-79 [PMID: 24360803 DOI: 10.1016/j.ajhg.2013.11.010]

51   **Ajmal M**, Khan MI, Neveling K, Khan YM, Azam M, Waheed NK, Hamel CP, Ben-Yosef T, De Baere E, Koenekoop RK, Collin RW, Qamar R, Cremers FP. A missense mutation in the splicing factor gene DHX38 is associated with early-onset retinitis pigmentosa with macular coloboma. *J Med Genet* 2014; **51**: 444-448 [PMID: 24737827 DOI: 10.1136/jmedgenet-2014-102316]

52   **Asai-Coakwell M**, March L, Dai XH, Duval M, Lopez I, French CR, Famulski J, De Baere E, Francis PJ, Sundaresan P, Sauvé Y, Koenekoop RK, Berry FB, Allison WT, Waskiewicz AJ, Lehmann OJ. Contribution of growth differentiation factor 6-dependent cell survival to early-onset retinal dystrophies. *Hum Mol Genet* 2013; **22**: 1432-1442 [PMID: 23307924 DOI: 10.1093/hmg/dds560]

53   **Sullivan LS**, Koboldt DC, Bowne SJ, Lang S, Blanton SH, Cadena E, Avery CE, Lewis RA, Webb-Jones K, Wheaton DH, Birch DG, Coussa R, Ren H, Lopez I, Chakarova C, Koenekoop RK, Garcia CA, Fulton RS, Wilson RK, Weinstock GM, Daiger SP. A dominant mutation in hexokinase 1 (HK1) causes retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2014; **55**: 7147-7158 [PMID: 25190649 DOI: 10.1167/iovs.14-15419]

54   **Gehrig A**, Felbor U, Kelsell RE, Hunt DM, Maumenee IH, Weber BH. Assessment of the interphotoreceptor matrix proteoglycan-1 (IMPG1) gene localised to 6q13-q15 in autosomal dominant Stargardt-like disease (ADSTGD), progressive bifocal chorioretinal atrophy (PBCRA), and North Carolina macular dystrophy (MCDR1). *J Med Genet* 1998; **35**: 641-645 [PMID: 9719369 DOI: 10.1136/jmg.35.8.641]

55   **Manes G**, Meunier I, Avila-Fernández A, Banfi S, Le Meur G, Zanlonghi X, Corton M, Simonelli F, Brabet P, Labesse G, Audo I, Mohand-Said S, Zeitz C, Sahel JA, Weber M, Dollfus H, Dhaenens CM, Allorge D, De Baere E, Koenekoop RK, Kohl S, Cremers FP, Hollyfield JG, Sénéchal A, Hebrard M, Bocquet B, Ayuso García C, Hamel CP. Mutations in IMPG1 cause vitelliform macular dystrophies. *Am J Hum Genet* 2013; **93**: 571-578 [PMID: 23993198 DOI: 10.1016/j.ajhg.2013.07.018]

56   **van Lith-Verhoeven JJ**, Hoyng CB, van den Helm B, Deutman AF, Brink HM, Kemperman MH, de Jong WH, Kremer H, Cremers FP. The benign concentric annular macular dystrophy locus maps to 6p12.3-q16. *Invest Ophthalmol Vis Sci* 2004; **45**: 30-35 [PMID: 14691150 DOI: 10.1167/iovs.03-0392]

57   **Audo I**, Bujakowska K, Orhan E, El Shamieh S, Sennlaub F, Guillonneau X, Antonio A, Michiels C, Lancelot ME, Letexier M, Saraiva JP, Nguyen H, Luu TD, Léveillard T, Poch O, Dollfus H, Paques M, Goureau O, Mohand-Saïd S, Bhattacharya SS, Sahel JA, Zeitz C. The familial dementia gene revisited: a missense mutation revealed by whole-exome sequencing identifies ITM2B as a candidate gene underlying a novel autosomal dominant retinal dystrophy in a large family. *Hum Mol Genet* 2014; **23**: 491-501 [PMID: 24026677 DOI: 10.1093/hmg/ddt439]

58   **El Shamieh S**, Neuillé M, Terray A, Orhan E, Condroyer C, Démontant V, Michiels C, Antonio A, Boyard F, Lancelot ME, Letexier M, Saraiva JP, Léveillard T, Mohand-Saïd S, Goureau O, Sahel JA, Zeitz C, Audo I. Whole-exome sequencing identifies KIZ as a ciliary gene associated with autosomal-recessive rod-cone dystrophy. *Am J Hum Genet* 2014; **94**: 625-633 [PMID: 24680887 DOI: 10.1016/j.ajhg.2014.03.005]

59   **Zeitz C**, Jacobson SG, Hamel CP, Bujakowska K, Neuillé M, Orhan E, Zanlonghi X, Lancelot ME, Michiels C, Schwartz SB, Bocquet B, Antonio A, Audier C, Letexier M, Saraiva JP, Luu TD, Sennlaub F, Nguyen H, Poch O, Dollfus H, Lecompte O, Kohl S, Sahel JA, Bhattacharya SS, Audo I. Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet* 2013; **92**: 67-75 [PMID: 23246293 DOI: 10.1016/j.ajhg.2012.10.023]

60   **Siemiatkowska AM**, van den Born LI, van Hagen PM, Stoffels M, Neveling K, Henkes A, Kipping-Geertsema M, Hoefsloot LH, Hoyng CB, Simon A, den Hollander AI, Cremers FP, Collin RW. Mutations in the mevalonate kinase (MVK) gene cause nonsyndromic retinitis pigmentosa. *Ophthalmology* 2013; **120**: 2697-2705 [PMID: 24084495 DOI: 10.1016/j.ophtha.2013.07.052]

61   **Al-Kateb H**, Shimony JS, Vineyard M, Manwaring L, Kulkarni S, Shinawi M. NR2F1 haploinsufficiency is associated with optic atrophy, dysmorphism and global developmental delay. *Am J Med Genet A* 2013; **161A**: 377-381 [PMID: 23300014 DOI: 10.1002/ajmg.a.35650]

62   **Bosch DG**, Boonstra FN, Gonzaga-Jauregui C, Xu M, de Ligt J, Jhangiani S, Wiszniewski W, Muzny DM, Yntema HG, Pfundt R, Vissers LE, Spruijt L, Blokland EA, Chen CA, Lewis RA, Tsai SY, Gibbs RA, Tsai MJ, Lupski JR, Zoghbi HY, Cremers FP, de Vries BB, Schaaf CP. NR2F1 mutations cause optic atrophy with intellectual disability. *Am J Hum Genet* 2014; **94**: 303-309 [PMID: 24462372 DOI: 10.1016/j.ajhg.2014.01.002]

63   **Hoover-Fong J**, Sobreira N, Jurgens J, Modaff P, Blout C, Moser A, Kim OH, Cho TJ, Cho SY, Kim SJ, Jin DK, Kitoh H, Park WY, Ling H, Hetrick KN, Doheny KF, Valle D, Pauli RM. Mutations in PCYT1A, encoding a key regulator of phosphatidylcholine metabolism, cause spondylometaphyseal dysplasia with cone-rod dystrophy. *Am J Hum Genet* 2014; **94**: 105-112 [PMID: 24387990 DOI: 10.1016/j.ajhg.2013.11.018]

64   **Yamamoto GL**, Baratela WA, Almeida TF, Lazar M, Afonso CL, Oyamada MK, Suzuki L, Oliveira LA, Ramos ES, Kim CA, Passos-Bueno MR, Bertola DR. Mutations in PCYT1A cause spondylometaphyseal dysplasia with cone-rod dystrophy. *Am J Hum Genet* 2014; **94**: 113-119 [PMID: 24387991 DOI: 10.1016/j.ajhg.2013.11.022]

65   **Roosing S**, Lamers IJ, de Vrieze E, van den Born LI, Lambertus S, Arts HH, Peters TA, Hoyng CB, Kremer H, Hetterschijt L, Letteboer SJ, van Wijk E, Roepman R, den Hollander AI, Cremers FP. Disruption of the basal body protein POC1B results in autosomal-recessive cone-rod dystrophy. *Am J Hum Genet* 2014; **95**: 131-142 [PMID: 25018096 DOI: 10.1016/j.ajhg.2014.06.012]

66   **Roosing S**, Rohrschneider K, Beryozkin A, Sharon D, Weisschuh N, Staller J, Kohl S, Zelinger L, Peters TA, Neveling K, Strom TM, van den Born LI, Hoyng CB, Klaver CC, Roepman R, Wissinger B, Banin E, Cremers FP, den Hollander AI. Mutations in RAB28, encoding a farnesylated small GTPase, are associated with autosomal-recessive cone-rod dystrophy. *Am J Hum Genet* 2013; **93**: 110-117 [PMID: 23746546 DOI: 10.1016/j.ajhg.2013.05.005]

67   **Xie YA**, Lee W, Cai C, Gambin T, Nõupuu K, Sujirakul T, Ayuso C, Jhangiani S, Muzny D, Boerwinkle E, Gibbs R, Greenstein VC, Lupski JR, Tsang SH, Allikmets R. New syndrome with retinitis pigmentosa is caused by nonsense mutations in retinol dehydrogenase RDH11. *Hum Mol Genet* 2014; **23**: 5774-5780 [PMID: 24916380 DOI: 10.1093/hmg/ddu291]

68   **Jin ZB**, Huang XF, Lv JN, Xiang L, Li DQ, Chen J, Huang C, Wu J, Lu F, Qu J. SLC7A14 linked to autosomal recessive retinitis pigmentosa. *Nat Commun* 2014; **5**: 3517 [PMID: 24670872 DOI: 10.1038/ncomms4517]

69   **Chang B**, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR. Retinal degeneration mutants in the mouse. *Vision Res* 2002; **42**: 517-525 [PMID: 11853768 DOI: 10.1016/S0042-6989(01)00146-8]

70   **Borman AD**, Pearce LR, Mackay DS, Nagel-Wolfrum K, Davidson AE, Henderson R, Garg S, Waseem NH, Webster AR, Plagnol V, Wolfrum U, Farooqi IS, Moore AT. A homozygous mutation in the TUB gene associated with retinal dystrophy and obesity. *Hum Mutat* 2014; **35**: 289-293 [PMID: 24375934 DOI: 10.1002/humu.22482]

71   **Sergouniotis PI**, Chakarova C, Murphy C, Becker M, Lenassi E, Arno G, Lek M, MacArthur DG, Bhattacharya SS, Moore AT, Holder GE, Robson AG, Wolfrum U, Webster AR, Plagnol V. Biallelic variants in TTLL5, encoding a tubulin glutamylase, cause retinal dystrophy. *Am J Hum Genet* 2014; **94**: 760-769 [PMID: 24791901 DOI: 10.1016/j.ajhg.2014.04.003]