The following full text is a publisher’s version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/154175

Please be advised that this information was generated on 2019-02-18 and may be subject to change.
The efficacy of microarray screening for autosomal recessive retinitis pigmentosa in routine clinical practice

Ramon A. C. van Huet,1 Laurence H.M. Pierrache,2,3 Magda A. Meester-Smoor,2,3 Caroline C.W. Klaver,3,4 L. Ingeborgh van den Born,2 Carel B. Hoyng,1 Ilse J. de Wijs,5 Rob W. J. Collin,6,8 Lies H. Hoefsloot,7 B. Jeroen Klevering1

1Department of Ophthalmology, Radboud University Medical Center, Nijmegen, The Netherlands; 2The Rotterdam Eye Hospital, Rotterdam, The Netherlands; 3Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands; 4Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 5Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands; 6Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands; 7Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

Purpose: To determine the efficacy of multiple versions of a commercially available arrayed primer extension (APEX) microarray chip for autosomal recessive retinitis pigmentosa (arRP).

Methods: We included 250 probands suspected of arRP who were genetically analyzed with the APEX microarray between January 2008 and November 2013. The mode of inheritance had to be autosomal recessive according to the pedigree (including isolated cases). If the microarray identified a heterozygous mutation, we performed Sanger sequencing of exons and exon–intron boundaries of that specific gene. The efficacy of this microarray chip with the additional Sanger sequencing approach was determined by the percentage of patients that received a molecular diagnosis. We also collected data from genetic tests other than the APEX analysis for arRP to provide a detailed description of the molecular diagnoses in our study cohort.

Results: The APEX microarray chip for arRP identified the molecular diagnosis in 21 (8.5%) of the patients in our cohort. Additional Sanger sequencing yielded a second mutation in 17 patients (6.8%), thereby establishing the molecular diagnosis. In total, 38 patients (15.2%) received a molecular diagnosis after analysis using the microarray and additional Sanger sequencing approach. Further genetic analyses after a negative result of the arRP microarray (n = 107) resulted in a molecular diagnosis of arRP (n = 23), autosomal dominant RP (n = 5), X-linked RP (n = 2), and choroideremia (n = 1).

Conclusions: The efficacy of the commercially available APEX microarray chips for arRP appears to be low, most likely caused by the limitations of this technique and the genetic and allelic heterogeneity of RP. Diagnostic yields up to 40% have been reported for next-generation sequencing (NGS) techniques that, as expected, thereby outperform targeted APEX analysis.

Retinitis pigmentosa (RP) is a group of hereditary diseases with an incidence of approximately 1:4,000 [1-4]. Although the clinical variation is high, RP is generally characterized by complaints of night blindness and peripheral visual field loss caused by progressive rod photoreceptor degeneration. In later stages of the disease, cones may also degenerate, which results in a decrease of central and color vision. The disease can be transmitted in all Mendelian patterns, including autosomal recessive in 50–60% of RP patients, autosomal dominant in 30–40%, and X-linked in 5–15% [1]. In addition, mitochondrial inheritance has been described in <1% of RP patients [5], and a few digenic cases have been reported [6,7]. To date, over 2,300 mutations in 45 genes have been associated with autosomal recessive RP (arRP; RetNet) [8]. This allelic and genetic heterogeneity complicates mutation detection in RP patients, since the phenotype is often not specific enough to link the disease to a particular gene. Furthermore, only just over 50% of the arRP cases can be linked to mutations in these genes [9,10].

Over time, multiple genotyping techniques have been developed to identify causative mutations in genes associated with RP, such as single-strand conformation analysis [11], denaturing high-performance liquid chromatography (HPLC) [12], resequencing microarrays [13], and arrayed primer extension (APEX) analysis [14-16]. Recently, next-generation sequencing (NGS) has exhibited potential in identifying causative mutations in a selected gene set (targeted NGS) [17] and in the whole exome [18].

Diagnostic genetic testing in nonsyndromic RP patients using the APEX microarray technology is popular, since it is a relatively low cost technique that enables screening of numerous mutations in multiple genes simultaneously. In the last decade, APEX chips have been developed for mutation
analysis of the ABCA4 gene (GenID: 24; OMIM 601691) in autosomal recessive Stargardt disease or cone–rod dystrophy [16,19], as well as for multiple gene microarrays for Leber congenital amaurosis (LCA) [15,20], Bardet–Biedl syndrome (BBS) [21], Usher syndrome [22], and autosomal dominant and recessive RP [23]. The efficacy with which these APEX chips lead to a molecular diagnosis is variable for the different disorders.

Identification of the genetic cause in these patients has become more important over time. This not only allows for a more accurate prognosis and appropriate genetic counseling for patients and their families, but also provides crucial information with regard to upcoming genetic therapies. The aim of this study was to evaluate the efficiency of the microarray chip for arRP in a cohort of recessive and isolated RP probands.

METHODS

Patients: For this study, we selected unrelated patients from the departments of ophthalmology of the Radboud University Medical Center (Nijmegen, Netherlands), Erasmus Medical Center (Rotterdam, Netherlands), and Rotterdam Eye Hospital (Rotterdam, Netherlands) that were clinically suspected of RP and were analyzed with an arRP microarray between January 2008 and November 2013. The microarray screenings were requested by the ophthalmologist who examined the patient when RP was suspected based on the simultaneous occurrence of at least two of the following criteria: (1) a history of night blindness or peripheral visual field loss, (2) a positive family history for RP, (3) perimetric results compatible with RP, and (4) reduced responses on electroretinography (ERG). We included both the probands of families that were suspected of RP with an autosomal recessive inheritance pattern and isolated cases; meanwhile, families with presumed dominant or X-linked inheritance patterns were excluded. Only probands were included; other patients within the same family were excluded, as well as patients with insufficient clinical data. For this retrospective study, the local ethics committee ruled that approval was not required, and according to the tenets of the Declaration of Helsinki, all participants gave informed consent for the use of their data.

For the selection procedure described above, we collected data from the medical records, including history and age of onset, best-corrected visual acuity (BCVA), fundus appearance, and full-field ERG results. Full-field ERG was performed according the International Society for Clinical Electrophysiology of Vision (ISCEV) standards [24].

Genetic microarray chip analyses: DNA was extracted from leukocytes acquired from peripheral venous blood samples according to automated nucleic acid isolation based on magnetic bead technology (Chemagic M1, Perkin Elmer chemagen Technologie GmbH, Baesweiler, Germany). We performed mutational screening using a commercially available genotyping microarray chip based on APEX technology (Asper Biotech, Tartu, Estonia) according to a protocol including polymerase chain reaction (PCR) DNA amplification, fragmentation of the amplification products and hybridization with the microarray slide as described previously [15]. An APEX reaction is based on a single base extension principle, which provides highly specific discrimination without allele-specific hybridization. In a single multiplex reaction, hundreds to thousands of variants can be analyzed simultaneously. The microarray chips used in this study included known pathogenic mutations in the coding regions and adjacent intronic sequences of genes associated with arRP.

The microarray chip initially included 501 mutations in 16 genes in 2006 [25], but was regularly updated as new mutations were discovered. The latest version (version 6.0) included 710 mutations in 28 genes (Table 1). During the inclusion period of this study, five versions of this array have been used, as follows: versions 4.0 (between January and April 2008), 4.1 (between April 2008 and February 2009), 5.0 (between February 2009 and September 2010), 5.3 (between September 2010 and July 2012), and 6.0 (between July 2012 and November 2013). Sanger sequencing was performed to confirm each mutation that was identified by the microarray chip. If only a single heterozygous mutation in a certain gene was found, all exons and intron–exon boundaries of this gene were analyzed with Sanger sequencing to search for the mutation on the second allele. The pathogenicity of a mutation was determined by our in-house protocol based on the criteria described by Cotton et al. [26], which evaluates pathogenicity according to evolutionary conservation of the altered nucleotide (phylogenetic profiling [PhyloP] score), the nature of the change at the amino acid level (Grantham score), and information from online in silico prediction tools SIFT and Polyphen-2. The effects of mutations on splice sites, if applicable, were determined by five predictor programs (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, and Human Splicing Finder) as provided in Alamut Visual (various versions, Interactive Biosoftware, Rouen, France). Reference sequences as provided by Alamut Visual (Interactive Biosoftware) have been used. Genes and mutations were annotated according to the HUGO Gene Nomenclature Committee (HGNC) and Human Genome Variation Society (HGVS) nomenclatures, respectively. The efficiency of each version of the microarray chip was determined by the
number of patients that had a molecular diagnosis after the analysis with the microarray chip. Patients were considered to have a molecular diagnosis when it was plausible that both alleles had been identified by a variant that was predicted to be pathogenic, meaning that the variants were predicted to significantly reduce or nullify the function of the protein. Identification of two pathogenic mutations (in combination with the presence of the RP phenotype) was considered pathogenic; segregation analysis—to evaluate whether the identified mutations are situated on separate alleles—was performed in some but not all families.

**Further genetic analyses:** To further evaluate the molecular diagnoses found in our study cohort, we also collected data from the genetic tests that had been performed after a negative result of the arRP microarray chip in these patients. The tests included targeted NGS (n = 16), microarray analyses for autosomal dominant RP, LCA, BBS, Usher syndrome, and ABCA4 mutation analysis (n = 28), or Sanger sequencing of selected genes (n = 88). The microanalyses were performed using the microarray chips available from Asper (Asper Biotech). Targeted NGS was performed by sequencing the exome with a 5500×l Genetic Analyzer (Life Technologies.

### Table 1. Overview of the genes analyzed by the latest APEX microarray chip for autosomal recessive retinitis pigmentosa (version 6.0).

| Gene symbol | Full gene name                                                                 | Number of mutations included in chip |
|-------------|-------------------------------------------------------------------------------|-------------------------------------|
| ABCA4       | ATP-binding cassette, sub-family A (ABC1), member 4                            | 1                                   |
| AIPL1       | Aryl hydrocarbon receptor interacting protein-like 1                           | 1                                   |
| CERKL       | Ceramide kinase-like                                                          | 5                                   |
| CNGA1       | Cyclic nucleotide gated channel alpha 1                                       | 5                                   |
| CNGA3       | Cyclic nucleotide gated channel alpha 3                                       | 1                                   |
| CNGB1       | Cyclic nucleotide gated channel beta 1                                        | 3                                   |
| CNGB3       | Cyclic nucleotide gated channel beta 3                                        | 1                                   |
| CRB1        | Crumbs homolog 1                                                              | 114                                 |
| EYS         | Eyes shut homolog                                                             | 68                                  |
| GRK1        | G protein-coupled receptor kinase 1                                           | 1                                   |
| IMPG2       | Interphotoreceptor matrix proteoglycan 2                                      | 6                                   |
| LRA7        | Lecithin retinol acyltransferase (phosphatidyl-choline-retinol O-acyltransferase) | 3                                   |
| MERTK       | C-mer proto-oncogene tyrosine kinase                                          | 14                                  |
| PDE6A       | Phosphodiesterase 6A, cGMP-specific, rod, alpha                              | 22                                  |
| PDE6B       | Phosphodiesterase 6B, cGMP-specific, rod, beta                               | 28                                  |
| NR2E3       | Nuclear receptor subfamily 2, group E, member 3                               | 31                                  |
| PROM1       | Prominin 1                                                                    | 2                                   |
| RBP3        | Retinol binding protein 3, interstitial                                        | 1                                   |
| RDH12       | Retinol dehydrogenase 12 (all-trans/9-cis/11-cis)                             | 45                                  |
| RGR         | Retinal G protein coupled receptor                                             | 7                                   |
| RHO         | Rhodopsin                                                                     | 2                                   |
| RLB1        | Retinaldehyde binding protein 1                                               | 13                                  |
| RP1         | Retinitis pigmentosa 1                                                         | 3                                   |
| RPE65       | Retinal pigment epithelium-specific protein 65 kDa                            | 100                                 |
| SAG         | S-antigen; retina and pineal gland (arrestin)                                 | 4                                   |
| TULP1       | Tubby like protein 1                                                           | 25                                  |
| CLRN1       | Clarin 1                                                                      | 12                                  |
| USH2A       | Usher syndrome 2A                                                             | 192                                 |

Total: 710

ATP, Adenosine triphosphate; cGMP, cyclic guanosine monophosphate; kDa, kiloDalton.
Carlsbad, CA) after DNA enrichment with the Agilent Sure-SelectXT Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, CA). Data were analyzed using LifeScope software (Life Technologies). Following this, the variants of 160 genes known to be involved in retinal disease were selected and ordered according to predicted pathogenicity. All identified mutations were confirmed by Sanger sequencing.

RESULTS

We included 250 probands (136 males, 54%) with the clinical diagnosis of autosomal recessive or isolated RP. Seven patients were analyzed with microarray version 4.0, 12 with version 4.1, 86 with version 5.0, 98 with version 5.3, and 47 with version 6.0. All mutations identified by the microarray chip were subsequently confirmed with Sanger sequencing.

Combining the results of all versions of the microarray chip, we identified mutations in 68 patients (27.7%). A total of 21 arRP patients (8.5%) received a confirmed molecular diagnosis by means of identification of a homozygous or two heterozygous pathogenic mutations. In 47 patients (18.8%), a single heterozygous pathogenic mutation was detected. In most patients (182, 72.8%), however, the microarray analysis did not reveal any causative mutation (Table 2).

Table 2 summarizes the numbers of patients with two, one, or no mutations after microarray screening for each microarray version, as well as the numbers of solved cases after additional Sanger sequencing.

In this study, we identified 65 different mutations in 12 genes (Table 3). Most mutations were identified in USH2A (48.5%; Gene ID: 7399; OMIM 608400), PDE6A (17.6%; Gene ID: 5145; OMIM 180071), and CRB1 (10.3%; Gene ID: 23418; OMIM 604210). Of the 65 variants identified in this study, 39 (60%) were missense mutations, 10 (15.4%) had effects on splicing, 9 (13.8%) caused a premature stop (nonsense mutations), and 7 (10.8%) resulted in a shift of the open reading frame. Fifty-nine mutations are (likely to be) pathogenic, whereas 6 mutations appear to have no significant effects on protein function (Table 3). The other eight mutations were identified by Sanger sequencing.

Further genetic analyses: Additional genetic tests were performed in 107 patients (43.6%) subsequent to the microarray analysis for arRP. An overview is provided in Table 4. The tests were selected based on the lack of family history or the acquisition of new history and ocular examination details after running the arRP APEX. These genetic tests resulted in a molecular diagnosis in 31 patients (30%), including arRP in 23 patients (21.5%), autosomal dominant RP in five patients (4.7%), X-linked RP in two patients (1.9%), and choroideremia in one patient (0.9%). The targeted NGS approach that covered 160 genes associated with hereditary blindness resulted in a molecular diagnosis in 12 patients (75%, Table 4).
| cDNA mutation (reference sequence) | Effect (RNA/protein) | EVS minor allele frequency in %† | Predicted pathogenicity‡ | Frequency of variant in this cohort (%) | Reference |
|-----------------------------------|---------------------|---------------------------------|--------------------------|------------------------------------------|-----------|
| **CERKL (NM_001030311.1)**       |                     |                                 |                          |                                           |           |
| c.487C>T                         | p.Arg283*           | 0.048                           | Pathogenic               | 1 (0.8)                                  | [44,45]   |
| **CLRN1 (NM_174878.2)**          |                     |                                 |                          |                                           |           |
| c.149_152delins8                 | p.Ser50fs           | 0.008                           | Pathogenic               | 1 (0.8)                                  | [46,47]   |
| **CNGA1 (NM_000087.3)**          |                     |                                 |                          |                                           |           |
| c.94C>T                          | p.Arg32*            | NA                              | Pathogenic               | 2 (1.7)                                  | [48]      |
| c.959C>T                         | p.Ser320Phe         | NA                              | Probably pathogenic      | 1 (0.8)                                  | [49]      |
| **CRB1 (NM_201253.1)**           |                     |                                 |                          |                                           |           |
| c.613_619del                      | p.Ile205fs          | NA                              | Pathogenic               | 1 (0.8)                                  | [50,51]   |
| c.614T>A                         | p.Ile205Lys         | NA                              | Possibly pathogenic      | 1 (0.8)                                  | This study|
| c.614T>C                         | p.Ile205Thr         | 0.038                           | Possibly pathogenic      | 1 (0.8)                                  | [52,53]   |
| c.1602G>T                        | p.Tyr3135*          | NA                              | Pathogenic               | 1 (0.8)                                  | [17]      |
| c.1892A>G                        | p.Tyr831Cys         | NA                              | Possibly pathogenic      | 2 (1.7)                                  | This study|
| c.2234C>T                        | p.Arg76Gln          | 0.032                           | Probably pathogenic      | 1 (0.8)                                  | [54]      |
| c.2681A>G                        | p.Asn894Ser         | 0.008                           | Possibly pathogenic      | 1 (0.8)                                  | [25,55]   |
| c.2842+5G>A                      | splicing            | NA                              | Possibly pathogenic      | 1 (0.8)                                  | [54]      |
| c.2945C>A                        | p.Arg311Gln         | 0.024                           | Possibly pathogenic      | 1 (0.8)                                  | [31]      |
| **EYS (NM_001142800.1)**         |                     |                                 |                          |                                           |           |
| c.9405T>A                        | p.Tyr3135*          | NA                              | Pathogenic               | 2 (1.7)                                  | [56]      |
| **NR2E3 (NM_014249.2)**          |                     |                                 |                          |                                           |           |
| c.119–2A>C                       | p.Arg257*           | 0.015                           | Pathogenic               | 1 (0.8)                                  | [46]      |
| c.227G>A                         | p.Arg76Gln          | 0.032                           | Probably pathogenic      | 1 (0.8)                                  | [46,47]   |
| c.932G>A                         | p.Arg311Gln         | 0.024                           | Possibly pathogenic      | 1 (0.8)                                  | [46]      |
| **PDE6A (NM_000440.2)**          |                     |                                 |                          |                                           |           |
| c.304C>A                         | p.Arg102Ser         | 0.015                           | Possibly pathogenic      | 10 (8.3)                                 | [25,57,58]|
| c.769C>T                         | p.Arg257*           | 0.015                           | Pathogenic               | 1 (0.8)                                  | [59]      |
| c.878C>T                         | p.Pro293Leu         | 0.361                           | Possibly benign          | 1 (0.8)                                  | [57]      |
| c.97del                          | p.Ile313fs          | NA                              | Pathogenic               | 1 (0.8)                                  | This study|
| c.1032C>T                        | p.Gln569Lys         | 0.015                           | Possibly pathogenic      | 1 (0.8)                                  | This study|
| c.1171G>A                        | p.Val391Met         | 1.699                           | Possibly pathogenic      | 4 (3.3)                                  | [57]      |
| c.1705C>A                        | p.His655Tyr         | 2.091                           | Possibly pathogenic      | 2 (1.7)                                  | [60]      |
| c.1963C>T                        |                     |                                 |                          |                                           |           |
| cDNA mutation (reference sequence) | Effect (RNA/protein) | EVS minor allele frequency in %† | Predicted pathogenicity‡ | Frequency of variant in this cohort (%) | Reference |
|-----------------------------------|---------------------|--------------------------------|--------------------------|----------------------------------------|-----------|
| **PDE6B** *(NM_000283.3)*        |                     |                                |                          |                                        |           |
| c.220C>T                          | p.Arg74Cys          | 0.038                          | Pathogenic               | 9 (7.5)                                | [61]      |
| c.655T>C                          | p.Tyr219His         | 0.538                          | Probably pathogenic      | 2 (1.7)                                | [17]      |
| c.1107+3A>G                       | splicing            | 0.015                          | Probably pathogenic      | 1 (0.8)                                | [17]      |
| c.1401+4_1401+48del               | splicing            | NA                             | Possibly pathogenic      | 1 (0.8)                                | This study |
| c.1798G>A                         | p.Asp600Asn         | NA                             | Possibly pathogenic      | 2 (1.7)                                | [58]      |
| c.2503+5G>C                       | splicing            | NA                             | Possibly pathogenic      | 1 (0.8)                                | [17]      |
| c.2503+2T>C                       | splicing            | NA                             | Probably pathogenic      | 1 (0.8)                                | This study |
| **PROM1** *(NM_006017.2)*         |                     |                                |                          |                                        |           |
| c.1354dup                         | p.Tyr452fs          | 0.049                          | Pathogenic               | 1 (0.8)                                | [62]      |
| **RDH12** *(NM_152443.2)*         |                     |                                |                          |                                        |           |
| c.379G>T                          | p.Gly127*           | NA                             | Pathogenic               | 4 (3.3)                                | [63]      |
| **RPE65** *(NM_000329.2)*         |                     |                                |                          |                                        |           |
| c.271C>T                          | p.Arg91Trp          | 0.015                          | Probably pathogenic      | 1 (0.8)                                | [64,65]   |
| c.963T>G                          | p.Asn321Lys         | 0.077                          | Possibly pathogenic      | 1 (0.8)                                | [66,67]   |
| c.1069dup                         | p.Asn356fs          | NA                             | Pathogenic               | 1 (0.8)                                | [68]      |
| **USH2A** *(NM_206933.2)*         |                     |                                |                          |                                        |           |
| c.486–14G>A                       | Splicing            | 0.008                          | Probably pathogenic      | 1 (0.8)                                | [69]      |
| c.949C>A                          | p.Arg317Arg (Splicing) | NA                           | Possibly pathogenic      | 1 (0.8)                                | [70-74]   |
| c.1256G>T                         | p.Cys419Phe         | 0.008                          | Pathogenic               | 3 (2.5)                                | [71,73,75]|
| c.1876C>T                         | p.Arg626*           | NA                             | Pathogenic               | 1 (0.8)                                | [71]      |
| c.2276G>T                         | p.Cys759Phe         | 0.154                          | Pathogenic               | 16 (13.3)                              | [52,76-80]|
| c.2299delG                        | p.Glu7678*21        | 0.176                          | Pathogenic               | 3 (2.5)                                | [81]      |
| c.2522C>A                         | p.Ser841Tyr         | 0.531                          | Possibly pathogenic      | 3 (2.5)                                | [73,82]   |
| c.3368A>G                         | p.Tyr1123Cys        | NA                             | Probably pathogenic      | 1 (0.8)                                | [83]      |
| c.5728C>T                         | p.Gln1910*          | NA                             | Pathogenic               | 1 (0.8)                                | This study|
| c.5975A>G                         | p.Tyr1992Cys        | 0.361                          | Possibly pathogenic      | 2 (1.7)                                | [80]      |
| c.6049+1G>A                       | Splicing            | NA                             | Pathogenic               | 1 (0.8)                                | This study|
| c.7054C>T                         | p.Pro2352Ser        | NA                             | Probably pathogenic      | 1 (0.8)                                | This study|
| c.8723_8724del                    | p.Val2908fs         | NA                             | Pathogenic               | 2 (1.7)                                | [70]      |
| c.9262G>A                         | p.Glu3088Lys        | 0.450                          | Probably benign          | 2 (1.7)                                | [80]      |
| c.9413G>A                         | p.Gly3138Asp        | NA                             | Probably pathogenic      | 1 (0.8)                                | This study|
| c.9433C>T                         | p.Leu3145Phe        | 0.008                          | Probably benign          | 1 (0.8)                                | EVS (rs26759873) |
| **c.2503+5G>C**                   | splicing            | NA                             | Possibly pathogenic      | 1 (0.8)                                | This study|

EVS (rs26759873)
| cDNA mutation (reference sequence) | Effect (RNA/protein) | EVS minor allele frequency in %† | Predicted pathogenicity‡ | Frequency of variant in this cohort (%) | Reference |
|-----------------------------------|----------------------|---------------------------------|-------------------------|----------------------------------------|-----------|
| c.9815C>T                        | p.Pro3272Leu         | NA                              | Possibly pathogenic     | 1 (0.8)                               | [84,85]   |
| c.10073G>A                       | p.Cys3358Tyr         | 0.054                           | Probably pathogenic     | 1 (0.8)                               | [25,80]   |
| c.10525A>T                       | p.Lys3509*           | NA                              | Pathogenic              | 1 (0.8)                               | [17]      |
| c.10561T>C                       | p.Trp3521Arg         | NA                              | Probably pathogenic     | 1 (0.8)                               | [74,80]   |
| c.11677C>A                       | p.Pro3893Thr         | 1.653                           | Probably benign         | 2 (1.7)                               | [74,86]   |
| c.12328T>G                       | p.Tyr4110Asp         | NA                              | Probably pathogenic     | 1 (0.8)                               | This study|
| c.12343C>T                       | p.Arg4115Cys         | 0.077                           | Probably pathogenic     | 5 (1.7)                               | [70,74,86]|
| c.13274C>T                       | p.Thr4425Met         | NA                              | Possibly pathogenic     | 3 (2.5)                               | [17,70,74,86]|
| c.14803C>T                       | p.Arg4935*           | 0.015                           | Pathogenic              | 1 (0.8)                               | [69,80,87]|
| c.15091C>T                       | p.Arg5031Trp         | 1.284                           | Probably benign         | 1 (0.8)                               | [74]      |
| c.15377T>C                       | p.Ile5126Thr         | 2.422                           | Probably pathogenic     | 1 (0.8)                               | [11,80,88]|
| c.15433G>A                       | p.Val5145Ile         | 0.408                           | Pathogenic              | 1 (0.8)                               | [52,78-80]|

†The overall allele frequency as provided in the Exome Variant Server in both European and African Americans. ‡The pathogenicity of the mutations was determined by our in-house protocol based on the criteria described by Cotton et al. [26], which evaluates pathogenicity by evolutionary conservation of the amino acid (phylogenetic profiling [PhyloP] score), the nature of the change (Grantham score), and information from online in silico prediction tools SIFT and Polyphen-2. * indicates a premature stop. Exome Variant Server (EVS); NA, not available.
Table 4. Results of genetic analyses other than the microarray chip for autosomal recessive RP in this study cohort.

| Gene name                  | Method                                      | N   | Results                                                                 | Molecular diagnosis                      |
|-----------------------------|---------------------------------------------|-----|-------------------------------------------------------------------------|------------------------------------------|
| Multiple                    | Targeted NGS on 160 blindness genes          | 2   | Heterozygous mutation in dominant gene                                   |                                          |
|                            | *PRPF31*                                    |     | c.18G>C; p.Glu6Asp                                                     | *PRPF31*-associated dominant RP         |
|                            | *BEST*                                      |     | c.682G>C; p.                                                      | *BEST*-associated dominant RP           |
|                            |                                             | 9   | Homozygous or compound heterozygous mutations                          |                                          |
|                            | *CNGB1*                                     |     | c.413–1G>A; splicing                                                  | *CNGB1*-associated recessive RP         |
|                            | *CRX*                                       |     | c.205C>T; p.Arg69Cys                                                   | *CRX*-associated recessive RP           |
|                            | *EYS*                                       |     | c.7919G>A; p.Trp2640*                                                  | *EYS*-associated recessive RP           |
|                            | *PDE6B*                                     |     | c.2193+1G>A; splicing c.1923_1971delinsTCTGGGA TA; p.Asn643fs          | *PDE6B*-associated recessive RP         |
|                            | *PDE6B*                                     |     | c.1189G>A; p.Gly397Arg c.1859A>G; p.His620Arg                          | *PDE6B*-associated recessive RP         |
|                            | *IMPG2*                                     |     | c.513T>G; p.Tyr171*                                                   | *IMPG2*-associated recessive RP         |
|                            | *TTC8*                                      |     | c.1363C>A; p.Gln455Lys                                                 | *TTC8*-associated recessive RP          |
|                            | *PRCD*                                      |     | c.2T>C; p.Met1? c.64C>T; p.Arg22*                                      | *PRCD*-associated recessive RP          |
|                            | *USH2A*                                     |     | c.6722C>T; p.Pro2241Leu c.13316C>T; p.Thr4439Ile                      | *USH2A*-associated recessive RP         |
|                            |                                             | 1   | Hemizygous mutation in *RPGR*                                          |                                          |
|                            | *RPGR*                                      |     | c.485_486del; p.Phe62fs                                                | *RPGR*-associated X-linked RP           |
|                            |                                             | 1   | Heterozygous mutation in recessive gene                                |                                          |
|                            | *USH2A*                                     |     | c.10510C>G; p.Pro3504Ala                                              | N/A                                      |
|                            |                                             | 3   | No mutations identified                                                |                                          |
| Autosomal dominant RP      |                                             | 2   | Heterozygous mutation in dominant gene                                  |                                          |
| microarray (APEX)          | *PRPF31*                                    |     | c.553G>T; p.Glu185*                                                   | *PRPF31*-associated dominant RP         |
|                            | *GUCY2D*                                    |     | c.2512C>T; p.Arg838Cys                                                 | *GUCY2D*-associated autosomal dominant cone-rod dystrophy |
|                            |                                             | 11  | No mutations identified                                                |                                          |
| LCA microarray (APEX)      |                                             | 4   | No mutations identified                                                |                                          |
| BBS microarray (APEX)      |                                             | 3   | No mutations identified                                                |                                          |
| Usher syndrome microarray  |                                             | 4   | No mutations identified                                                |                                          |
| (APEX)                     |                                             |     |                                                          |                                          |
| Gene name | Method | N  | Results                                      | Molecular diagnosis                      |
|-----------|--------|----|---------------------------------------------|------------------------------------------|
| ABCA4     | Sanger sequencing | 7  | Homozygous or compound heterozygous mutations |                                          |
| ABCA4     |        |    | c.5882G>A; p.Gly1961Glu                     | ABCA4-associated recessive retinal dystrophy |
| ABCA4     |        |    | c.3602T>G; p.Leu201Arg c.6320G>A; p.Arg2107His |                                          |
| ABCA4     |        |    | c.5461–10T>C; splicing c.6155del; p.Asn2052fs |                                          |
| ABCA4     |        |    | c.4469G>A; p.Cys1490Tyr                     |                                          |
| ABCA4     |        |    | c.5056G>A; p.Val1686Met c.6730–19G>A; splicing |                                          |
| ABCA4     |        |    | c.6658C>T; p.Gln2220*                      |                                          |
| ABCA4     |        |    | c.1622T>C; p.Leu541Pro c.3113C>T; p.Ala1030Val (both homozygously present) |                                          |
|           |        |    | 6 Heterozygous mutations                   |                                          |
| ABCA4     |        |    | c.1411G>A; p.Glu471Lys (2x)                | Carrier of ABCA4 mutation                |
| ABCA4     |        |    | c.3899G>A; p.Arg1300Gln                    |                                          |
| ABCA4     |        |    | c.4283C>T; p.Thr1428Met                     |                                          |
| ABCA4     |        |    | c.5882G>A; p.Gly1961Glu                    |                                          |
| ABCA4     |        |    | c.5908C>T; p.Leu1970Phe                    |                                          |
|           | Microarray (APEX) | 4  | No mutation identified                      | N/A                                      |
| BBS1      | Sanger sequencing | 1  | Homozygous mutation                        | BBS1-associated recessive RP             |
| BBS1      |        |    | c.1169T>G; p.Met390Arg                     |                                          |
| CHM       | Sanger sequencing | 1  | Hemizygous mutation                        | Choroideremia                            |
| CHM       |        |    | c.50–?_116+?del; deletion of exon 2       |                                          |
|           |        |    | 2 No mutations identified                  | N/A                                      |
| CNGA3     | Sanger sequencing | 1  | No mutations identified                     | N/A                                      |
| CNGB3     | Sanger sequencing | 3  | No mutations identified                     | N/A                                      |
| CRB1      | Sanger sequencing | 3  | No mutations identified                     | N/A                                      |
| EYS       | Sanger sequencing | 1  | Homozygous mutation                        | EYS-associated recessive RP              |
| EYS       |        |    | c.6714del; p.Ile2239fs                     |                                          |
| FAMIL61A  | Sanger sequencing | 1  | Compound heterozygous mutations            | FAMIL61A-associated recessive RP         |
| FAMIL61A  |        |    | c.1309A>T; p.Arg437* c.1501del; p.Cys501fs |                                          |
| KCNV2     | Sanger sequencing | 1  | No mutations identified                     | N/A                                      |
| MERTK     | Sanger sequencing | 1  | Homozygous mutation                        | MERKT-associated recessive RP           |
| MERTK     |        |    | c.1179dup; p.Leu394fs                      |                                          |
| Gene name | Method          | N   | Results                              | Molecular diagnosis                          |
|-----------|-----------------|-----|--------------------------------------|---------------------------------------------|
| NR2E3     | Sanger sequencing | 1   | Compound heterozygous mutations      | NR2E3-associated recessive RP                |
|           |                 |     | NR2E3 c.119–57_166del; frameshift c.1095C>G; splicing |                                             |
| PDE6A     | Sanger sequencing | 2   | No mutations identified              | N/A                                         |
| PDE6C     | Sanger sequencing | 1   | No mutations identified              | N/A                                         |
| PRPH2     | Sanger sequencing | 1   | Heterozygous mutations               | PRPH2-associated dominant RP                |
|           |                 |     | PRPH2 c.424C>T; p.Arg142Trp          |                                             |
| RHO       | Sanger sequencing | 1   | Homozygous mutation                  | RHO-associated recessive RP                 |
|           |                 |     | RHO c.759G>T; p.Met253Ile            |                                             |
| RP1       | Sanger sequencing | 1   | Homozygous mutation                  | RP1-associated recessive RP                 |
|           |                 |     | RP1 c.686del; p.Pro229fs             |                                             |
| RPE65     | Sanger sequencing | 1   | Heterozygous mutation                | Carrier of RPE65 mutation                   |
|           |                 |     | RPE65 c.11+5G>A; splicing            |                                             |
| RPGR      | Sanger sequencing | 1   | Hemizygous mutation                  | RPGR-associated X-linked RP                 |
|           |                 |     | RPGR c.2993_2996del; p.Glu998fs      |                                             |
| TRPM1     | Sanger sequencing | 1   | Compound heterozygous mutations      | Congenital stationary night blindness type IC|
|           |                 |     | TRPM1 c.1–27C>T; UTR 5'expressing defect c.2998C>T; p.Arg1000* |                                             |

* indicates a premature stop; fs=frameshift; UTR=untranslated region
DISCUSSION

Only a decade ago, microarray screening boosted diagnostic genetic analysis in genetic heterogeneous disorders such as RP by facilitating reliable fast analysis of multiple genes simultaneously with much lower costs than Sanger sequencing of the same genes. Nowadays, high-throughput NGS techniques like exome sequencing have become available and are selectively used in a diagnostic setting. The microarray technique, however, still has a prominent position in the diagnostic genetic analysis of RP, since NGS is currently only available for a small number of patients and has long lead times (>6 months). Therefore, we evaluated the efficiency of microarray screening in arRP and isolated RP cases to determine its place in the array of diagnostic genetic tests currently available.

The low efficacy of 15.2% solved cases after microarray screening and additional Sanger sequencing found in this study can be attributed to the method’s limitations in covering the genetic and clinical characteristics of autosomal recessive and simplex RP. First, the chip only analyzes a fixed set of mutations. The latest version of the chip includes 710 mutations in 28 genes, whereas over 2,300 mutations in 45 genes are associated with arRP nowadays [8] (and RetNet). Therefore, more frequent updates and inclusion of less frequent genes and mutations are necessary to increase the chip’s efficacy, although this will be costly and laborious to implement. Second, the APEX microarray approach does not identify variants other than the set of mutations present on the array. This rigid approach lowers the chance of mutation identification for arRP patients, since the frequency of private mutations is generally relatively high because of the immense mutational heterogeneity in arRP.

In addition to the disadvantages of the test itself, the heterogeneity of genetic and clinical characteristics of autosomal recessive and simplex RP complicates genetic analysis, since the correlation between a phenotype and specific mutations in a specific gene may be weak. Moreover, isolated RP cases, which are generally considered autosomal recessive, may also have autosomal dominant or X-linked modes of inheritance. For instance, X-linked RP caused by mutations in RPGR (Gene ID: 7399; OMIM 608400) or RP2 (Gene ID: 6102; OMIM 300757) account for 15% of male isolated cases with retinal degenerative disease [27], and de novo mutations in genes known to follow a dominant inheritance pattern account for 1–2% of isolated RP [17,28]. This is exemplified by the discovery of mutations in dominant and X-linked RP genes in seven isolated patients in the current study (Table 4). An approach that enables genetic analysis of autosomal recessive, dominant, and X-linked cases simultaneously, such as NGS, would therefore be preferable.

The microarray chip analyzes defects in the genes that are relatively frequently mutated in arRP. Yet, this contributes little to the chip’s efficacy, since mutations in the majority of genes account only for 1–2% or less of arRP cases [1,8,29]. Furthermore, the older versions of the chip included mutations that are considered benign (c.9262G>A in USH2A and c.878C>T in PDE6A, Table 3). These variants were probably detected in arRP cases previously, and have subsequently been added to the array, without a functional assessment of their pathogenicity, especially in the case of missense mutations. Recently, it has become clear that using in silico prediction tools, and especially databases with allele frequencies in large normal cohorts, like the Exome Variant Server (EVS), provides insight into the pathogenicity of a missense mutation, and should be used if functional assessment is missing. These benign mutations lower the microarray’s efficiency, and should ideally be removed from the chip. The two benign mutations identified with the microarray in this study were not on later versions of the chip.

In contrast to the microarray approach, NGS techniques such as whole-exome sequencing can handle the heterogeneity of arRP and provide a thorough genetic analysis. NGS has been reported to identify the genetic cause in 19% to 40% of arRP cases (and 50% to 82% of RP cases in general), which is significantly higher than the 15.2% solved cases after microarray screening and additional Sanger sequencing found in this study [10,17,30-36]. In whole-exome sequencing, all coding sequences (the exons) of all genes in the genome are sequenced, which enables the identification of known and novel mutations in known arRP genes. Mutations in genes that have not yet been associated with arRP can also be identified by this approach. In whole-genome sequencing, all genetic material is sequenced, including the exons as well as the introns, the noncoding sequences. This approach can theoretically solve even more arRP patients genetically, for instance through the identification of intronic pathologic mutations, which have been described in retinal degeneration [37-40]. Yet, the increasing number of DNA variants that will become available when employing these techniques poses a significant challenge to data interpretation.

Future perspectives of genetic testing in RP: The genetic and allelic heterogeneity and often nonspecific clinical appearance of RP complicates diagnostic genetic testing. Although APEX microarray analysis has been the most efficient diagnostic tool for RP for years, the introduction of NGS techniques in diagnostics have shown their superiority by identifying causative mutations in up to 40% of arRP cases [10,17,30-33].
However, NGS comes with its own difficulties, such as data management and analysis of the large datasets, and confirmation of the pathogenicity of identified variants [10,17,33]. The latter is crucial, since the large number of genes involved in arRP increases the risk of finding a pathogenic variant that is not causative, especially when considering that each person may be carrying ~1,500 variants in their coding sequence affecting protein function [41], and when considering retinal degeneration genes, clear-cut heterozygous pathogenic null mutations were reported in 1 out of 4 to 5 healthy controls that were analyzed with whole-genome sequencing [42]. Furthermore, the costs of data management and storage may rise with the use of whole-genome sequencing and the development of “third generation” technologies due to massive datasets [43]. The sequencing costs of NGS have been high initially, but the expenses have diminished over the years, especially since this technique became commercially available. Currently, the costs of diagnostic NGS have decreased to levels just above those of the APEX microarray analysis. Therefore, we conclude that NGS is by far more cost-effective and efficient than the microarray analysis in patients with arRP, and should be the diagnostic genetic analysis of preference.

ACKNOWLEDGMENTS

Publication of this article was supported by the Stichting A.F. Deutman Researchfonds Oogheelkunde, Nijmegen, The Netherlands; and Stichting Combined Ophthalmic Research Rotterdam (CORR), Rotterdam, The Netherlands. The funding organizations had no role in the design or conduct of this research.

REFERENCES

1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006; 368:1795-809. [PMID: 17113430].
2. Berson EL. Retinitis pigmentosa. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 1993; 34:1659-76. [PMID: 8473105].
3. Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 1984; 97:357-65. [PMID: 6702974].
4. Rosenberg T. Epidemiology of hereditary ocular disorders. Dev Ophthalmol 2003; 37:16-33. [PMID: 12876827].
5. Schrier SA, Falk MJ. Mitochondrial disorders and the eye. Curr Opin Ophthalmol 2011; 22:325-31. [PMID: 21730846].
6. Kajiwara K, Berson EL, Dryja TP. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 1994; 264:1604–8.
7. Dryja TP, Hahn LB, Kajiwara K, Berson EL. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa. Invest Ophthalmol Vis Sci 1997; 38:1972-82. [PMID: 9331261].
8. Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genet 2013; 84:132-41. [PMID: 23701314].
9. Wright AF, Chakarova CF, Abd El-Aziz MM, Bhattacharya SS. Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. Nat Rev Genet 2010; 11:273-84. [PMID: 20212494].
10. Glöckle N, Kohl S, Mohr J, Scheurenbrand T, Sprecher A, Weisschu h N, Bernd A, Rudolph G, Schubach M, Poloschek C, Zrenner E, Biskup S, Berger W, Wissinger B, Neidhardt J. 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; 22:99-104. [PMID: 23591405].
11. Yan D, Ouyang X, Patterson DM, Du LL, Jacobson SG, Liu XZ. Mutation analysis in the long isoform of USH2A in American patients with Usher Syndrome type II. J Hum Genet 2009; 54:732-8. [PMID: 19881469].
12. Hjortshøj TD, Gronskov K, Philip AR, Nishimura DY, Riise R, Sheffield VC, Rosenberg T, Brondum-Nielsen K. Bardet-Biedl syndrome in Denmark–report of 13 novel sequence variations in six genes. Hum Mutat 2010; 31:429-36. [PMID: 20210035].
13. Mandal MN, Heckenlively JR, Burch T, Chen L, Vasireddy V, Koenekoop RK, Sieving PA, Ayyagari R. Sequencing arrays for screening multiple genes associated with early-onset human retinal degenerations on a high-throughput platform. Invest Ophthalmol Vis Sci 2005; 46:3355-62. [PMID: 16123440].
14. Jaijo T, Aller E, Garcia-Garcia G, Aparisi MJ, Bernal S, Avila-Fernandez A, Barragan I, Baiget M, Ayuso C, Antinolo G, Diaz-Llopis M, Kulm M, Beneyto M, Najera C, Millan JM. Microarray-based mutation analysis of 183 Spanish families with Usher syndrome. Invest Ophthalmol Vis Sci 2010; 51:1311-7. [PMID: 19683999].
15. Zernant J, Kulm M, Dharmaraj S, den Hollander AI, Perrault I, Preisig MN, Lorenz B, Kaplan J, Cremers FP, Maumenee I, Koenekoop RK, Allikmets R. Genotyping microarray (disease chip) for Leber congenital amaurosis: detection of modifier alleles. Invest Ophthalmol Vis Sci 2005; 46:3052-9. [PMID: 16123401].
16. Klevering BJ, Yzer S, Rohrschneider K, Zonneveld M, Allikmets R, van den Born LI, Maugeri A, Hoyng CB, Cremers FP. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. Eur J Hum Genet 2004; 12:1024-32. [PMID: 15494742].
17. Neveling K, Collin RW, Gilissen C, van Huet RA, Visser L, Kwint MP, Gijssen SJ, Zonneveld MN, Wieskamp N, de Ligt J, Siemiatkowska AM, Hoefsloot LH, Buckley MF, Kellner U, Branh KME, 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; 33:963-72. [PMID: 22334370].

18. 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, Buijssen W, Derks RC, Wiekamp N, van den Heuvel B, Ligtenberg MJ, Kremers H, Koolen DA, van de Warrenburg BP, Cremers FP, Marcelis CL, Smitink 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; 34:1721-6. [PMID: 24123792].

19. Jaakson K, Zernant J, Kulm M, Hutchinson A, Tonisson N, Glavac D, Ravnkjav-Glavac M, Hawlina M, Meltzer MR, Caruso RC, Testa F, Maugeri A, Hoyng CB, Gouras P, Simonelli F, Lewis RA, Lupsik JR, Cremers FP, Allikmets R. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. Hum Mutat 2003; 22:395-403. [PMID: 14517951].

20. Yzer S, Leroy BP, de Baere E, de Ravel T, Zonneveld MN, Voesenek K, Kellner U, Ciriano JP, de Faber JT, Rohrschneider K, Roepman R, de Hollander AI, Cruijsberg JR, Meire F, Caesteels I, van Moll-Ramirez NG, Allikmets R, van den Born LI, Cremers FP. Microarray-based mutation detection and phenotypic characterization of patients with Leber congenital amaurosis. Invest Ophthalmol Vis Sci 2006; 47:1167-76. [PMID: 16505055].

21. Pereiro I, Hoskins BE, Marshall JD, Collin GB, Naggett JK, Pineiro-Gallego T, Ottmaa E, Katsanis N, Valverde D, Beales PL. Arrayed primer extension technology simplifies mutation detection in Bardet-Biedl and Alstrom syndrome. Eur J Hum Genet 2011; 19:485-8. [PMID: 21157946].

22. Cremers FP, Kimberling WJ, Kulm M, de Brouwer AP, van Wijk E, te Brinke H, Cremers CW, Hoefsloot LH, Banfi S, Simonelli F, Fleischhauer JC, Berger W, Kelley PM, Haralambous E, Bitner-Glindzicz M, Webster AR, Saithan Z, De Baere E, Leroy BP, Silvestri G, McKay GJ, Koenekoop RK, Millan JM, Rosenberg T, Joensuu T, Sankila EM, Weil D, Weston MD, Wissinger B, Kremer H. Development of a genotyping microarray for Usher syndrome. J Med Genet 2007; 44:153-60. [PMID: 16963483].

23. Koenekoop RK, Lopez I, den Hollander AI, Allikmets R, Cremers FP. Genetic testing for retinal dystrophies and dysfunctions: benefits, dilemmas and solutions. Clin Experiment Ophthalmol 2007; 35:473-85. [PMID: 17651254].

24. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 2009; 118:69-77. [PMID: 19030905].

25. Ávila-Fernández A, Cantalapiedra D, Aller E, Vallespin E, Aguirre-Lamban J, Blanco-Kelly F, Corton M, Riveiro-Alvarez R, Allikmets R, Trujillo-Tiebas MJ, Millan JM, Cremers FP, Ayuso C. Mutation analysis of 272 Spanish families affected by autosomal recessive retinitis pigmentosa using a genotyping microarray. Mol Vis 2010; 16:2550-8. [PMID: 21151602].

26. Cotton RG, Scriver CR. Proof of “disease causing” mutation. Hum Mutat 1998; 12:1-3. [PMID: 9633813].

27. Brannham K, Othman M, Brumm M, Karoukis AJ, Atmaca-Sonmez P, Yashar BM, Schwartz SB, Stover NB, Trzupke K, Wheaton D, Jennings B, Ciccarelli ML, Jayasundera KT, Lewis RA, Birch D, Bennett J, Sieving PA, Andreasson S, Duncan JL, Fishman GA, Iannaccone A, Weleber RG, Jacobson SG, Heckenlively JR, Swaroop A. Mutations in RPGR and RP2 account for 15% of males with simplex retinal degenerative disease. Invest Ophthalmol Vis Sci 2012; 53:8232-7. [PMID: 23150612].

28. Sohocki MM, Daiger SP, Bowne SJ, Rodriguez JA, Northrup H, Heckenlively JR, Birch DG, Mintz-Hittner H, Ruiz RS, Lewis RA, Saperstein DA, Sullivan LS. Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. Hum Mutat 2001; 17:42-51. [PMID: 11139241].

29. Berger W, Kloeckener-Gruissem B, Neidhardt J. The molecular basis of human retinal and vitreoretinal diseases. Prog Retin Eye Res 2010; 29:335-75. [PMID: 20362068].

30. 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; 54:2186-97. [PMID: 23462753].

31. Wang X, Wang H, Sun V, Tuan HF, Keser V, Wang K, Ren H, Lopez I, Zaneveld JE, Siddiqui S, Bowles S, Khan A, Salvo J, Jacobson SG, Iannaccone A, Wang F, Birch D, Heckenlively JR, Fishman GA, Traboulsi EL, Li Y, Wheaton D, Koenekoop RK, Chen R. Comprehensive molecular diagnosis of 179 Leber congenital amaurosis and juvenile retinitis pigmentosa patients by targeted next generation sequencing. J Med Genet 2013; 50:674-88. [PMID: 23847139].

32. Fu Q, Wang F, Wang H, Xu F, Zaneveld JE, Ren H, Keser V, Lopez I, Tuan HF, Salvo JS, Wang X, Zhao L, Wang K, Li Y, Koenekoop RK, Chen R, Sui R. Next-generation sequencing-based molecular diagnosis of a Chinese patient cohort with autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 2013; 54:4158-66. [PMID: 23661369].

33. Shanks ME, Downes SM, Copey RR, Lise S, Broxholme J, Hudspith KA, Kwasnieswka A, Davies WI, Hankins MW, Packham ER, Clouston P, Seller A, Wilkie AO, Taylor JC, Ragoussis J, Nemeth AH. 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; 21:274-80. [PMID: 22968150].

34. Xu Y, Guan L, Shen T, Zhang J, Xiao X, Jiang H, Li S, Yang J, Jia X, Yin Y, Guo X, Wang J, Zhang Q. Mutations of 60 known causative genes in 157 families with retinitis pigmentosa based on exome sequencing. Hum Genet 2014; 133:1255-71. [PMID: 24938718].

35. Zhao L, Wang F, Wang H, Li Y, Alexander S, Wang K, Willoughby CE, Zaneveld JE, Jiang L, Soens ZT, Earle P, Simpson D, Silvestri G, Chen R. Next-generation
sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Ireland. Hum Genet 2015; 134:217-30. [PMID: 25472526].

36. 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 Ophthalmo1 Vis Sci 2014; 55:6213-23. [PMID: 25097241].

37. Steele-Stallard HB, Le Quesne Stabej P, Lenassi E, Luxon LM, Claustres M, Roux AF, Webster AR, Bittner-Glindzicz M. Screening for duplications, deletions and a common intronic mutation detects 35% of second mutations in patients withUSH2A monoallelic mutations on Sanger sequencing. Orphanet J Rare Dis 2013; 8:122. [PMID: 23924366].

38. Braun TA, Mullins RF, Wagner AH, Andorf JL, Johnston RM, Bakall BB, Deluca AP, Fishman GA, Lam BL, Weleber RG, Cideciyan AV, Jacobson SG, Shepherd VC, Tucker BA, Stone EM. Non-exomic and synonymous variants in ABCA4 are an important cause of Stargardt disease. Hum Mol Genet 2013; 22:5136-45. [PMID: 23981662].

39. Webb TR, Parfitt DA, Gardner JC, Martinez A, Bevilacqua D, Davidson AE, Zito I, Thiselton DL, Ressa JH, Apergi M, Schwarz N, Kanuga N, Michaelides M, Cheatham ME, Gorin MB, Hardcastle AJ. Deep intronic mutation in OFD1, identified by targeted genomic next-generation sequencing, causes a severe form of X-linked retinitis pigmentosa (RP23). Hum Mol Genet 2012; 21:3647-54. [PMID: 22619378].

40. Pomares E, Riera M, Castro-Navarro J, Andres-Gutierrez A, Gonzalez-Duarte R, Marfany G. Identification of an intronic single-point mutation in RP2 as the cause of semidominant X-linked retinitis pigmentosa. Invest Ophthalmo1 Vis Sci 2009; 50:5107-14. [PMID: 19516003].

41. Ng PC, Levy S, Huang J, Stockwell TB, Walenz BP, Li K, Axelrod N, Busam DA, Strausberg RL, Venter JC. Genetic variation in an individual human exome. PLoS Genet 2008; 4:e1000160. [PMID: 18704161].

42. Nishiguchi KM, Rivolta C. Genes associated with retinitis pigmentosa and allied diseases are frequently mutated in the general population. PLoS ONE 2012; 7:e19092. [PMID: 22848652].

43. Schadt EE, Turner S, Kasarskis A. A window into third-generation sequencing. Hum Mol Genet 2010; 19:R2287-40. [PMID: 20858600].

44. Tuson M, Marfany G, Gonzalez-Duarte R. Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). Am J Hum Genet 2004; 74:128-38. [PMID: 14681825].

45. Nishiguchi KM, Tearle RG, Liu YP, Oh EC, Miyake N, Benaglio P, Harper S, Koskiniemi-Kuendig H, Venturini G, Sharon D, Koenenwoord 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; 110:16139-44. [PMID: 24043777].

46. Haider NB, Jacobson SG, Cideciyan AV, Swiderski R, Streb LM, Searby C, Beck G, Hockey R, Hanna DB, Gorman S, Duhl D, Carmi R, Bennett J, Weleber RG, Fishman GA, Wright AF, Stone EM, Sheffield VC. Mutation of a nuclear receptor gene, NR2E3, causes enhanced S cone syndrome, a disorder of retinal cell fate. Nat Genet 2000; 24:127-31. [PMID: 10655056].

47. Fields RR, Zhou G, Huang D, Davis JR, Moller C, Jacobson SG, Kimberling WJ, Sumegi J. Usher syndrome type III: revised genomic structure of the USH3 gene and identification of novel mutations. Am J Hum Genet 2002; 71:607-17. [PMID: 12145752].

48. Paloma E, Martinez-Mir A, Garcia-Sandoval B, Ayuso C, Vilagelieu L, Gonzalez-Duarte R, Balcels S. Novel homozygous mutation in the alpha subunit of the rod cGMP gated channel (CNGA1) in two Spanish sibs affected with autosomal recessive retinitis pigmentosa. J Med Genet 2002; 39:E66. [PMID: 12362048].

49. Dryja TP, Finn JT, Peng YW, McGee TL, Berson EL, Yau KW. Mutations in the gene encoding the alpha subunit of the rod cGMP-gated channel in autosomal recessive retinitis pigmentosa. Proc Natl Acad Sci USA 1995; 92:10177-81. [PMID: 7479749].

50. Corton M, Tatu SD, Avila-Fernandez A, Vallespin E, Tapias I, Cantalapiaedro D, Blanco-Kelly F, Riveiro-Alvarez R, Bernal S, Garcia-Sandoval B, Baiget M, Ayuso C. High frequency of CRB1 mutations as cause of Early-Onset Retinal Dystrophies in the Spanish population. Orphanet J Rare Dis 2013; 8:20. [PMID: 23379534].

51. Lottery AJ, Malik A, Shami SA, Sindhi M, Chohan B, Maqbool C, Moore PA, Denton MJ, Stone EM. CRB1 mutations may result in retinitis pigmentosa without para-arteriolar RPE preservation. Ophthalmic Genet 2001; 22:163-9. [PMID: 11559858].

52. Bernal S, Ayuso C, Antinolo G, Gimenez A, Borrego S, Tijuillo MJ, Marcos I, Calaf M, Del Rio E, Baiget M. Mutations in USH2A in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. J Med Genet 2003; 40:e8. [PMID: 12525556].

53. den Hollander AI, Davis J, van der Velde-Visser SD, Zonneveld MN, Pierrottet CO, Koenekekoop RK, Kellner U, van den Born LI, Heckenlively JR, Hoyng CB, Handford PA, Roepman R, Cremers FP. CRB1 mutation spectrum in inherited retinal dystrophies. Hum Mutat 2004; 24:355-69. [PMID: 15430723].

54. den Hollander AI, ten Brink JB, de Kok YJ, van Soest S, van den Born LI, van Driel MA, van de Pol DJ, Payne AM, Bhattacharya SS, Kellner U, Hoyng CB, Westerveld A, Brunner HG, Bleeker-Wagemakers EM, Deutman AF, Heckenlively JR, Cremers FP, Bergen AA. Mutations in a human homologue of Drosophila crumbs cause retinitis pigmentosa (RP12). Nat Genet 1999; 23:217-21. [PMID: 10508521].
55. Vallespin E, Cantalapiedra D, Riveiro-Alvarez R, Wilke R, Aguirre-Lamban J, Avila-Fernandez A, Lopez-Martinez MA, Gimenez A, Trujillo-Tiebas MJ, Ramos C, Ayuso C. Mutation screening of 299 Spanish families with retinal dystrophies by Leber congenital amaurosis genotyping microarray. Invest Ophthalmol Vis Sci 2007; 48:5653-61. [PMID: 18055816].

56. Collin RW, Littink KW, Klevering BJ, van den Born LI, Koenekoop RK, Zonneveld MN, Blokland EA, Strom TM, Hoying CB, den Hollander AI, Cremer FP. Identification of a 2 Mb human ortholog of Drosophila eyes shut/spacemaker that is mutated in patients with retinitis pigmentosa. Am J Hum Genet 2008; 83:594-603. [PMID: 18976725].

57. Dryja TP, Rucinski DE, Chen SH, Berson EL. Frequency of mutations in the gene encoding the alpha subunit of rod cyclic GMP-phosphodiesterase in autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 1999; 40:1859-65. [PMID: 10393062].

58. Tsang SH, Tsui I, Chou CL, Zernant J, Haamer E, Iranmanesh R, Tosi J, Allikmets R. A novel mutation and phenotypes in photoreceptor cGMP-phosphodiesterase 6 deficiency. Am J Ophthalmol 2008; 146:780-8. [PMID: 18723146].

59. Bocquet B, Marzouka NA, Hebrard M, Manes G, Senechal A, Aguirre-Lamban J, Avila-Fernandez A, Lopez-Martinez MA, Gimenez A, Trujillo-Tiebas MJ, Ramos C, Ayuso C. Mutations in the gene encoding the alpha subunit of rod cGMP-phosphodiesterase in autosomal recessive retinitis pigmentosa families detects novel mutations. Mol Vis 2013; 19:2048-82. [PMID: 24339724].

60. Simpson DA, Clark GR, Alexander S, Silvestri G, Willoughby CE. Molecular diagnosis for heterogeneous genetic diseases with targeted high-throughput DNA sequencing applied to retinitis pigmentosa. J Med Genet 2011; 48:145-51. [PMID: 21147909].

61. Veske A, Orth U, Rüther K, Zrenner E, Rosenberg T, Baehr W, Gal A. Mutations in the Gene for the B-Subunit of Rod Photoreceptor Cgmp-Specific Phosphodiesterase (PDEB) gene probe. In: Anderson R, LaVail M, Hollyfield J, editors. Degenerative Diseases of the Retina: Springer US; 1995. p. 313–22.

62. Pras E, Abu A, Rotenstreich Y, Avni I, Reish O, Morad Y, Reznik-Wolf H, Pras E. Cone-rod dystrophy and a frameshift mutation in the PROM1 gene. Mol Vis 2009; 15:1709–16. [PMID: 19718270].

63. Perrault I, Hanein S, Gerber S, Barbet F, Ducroq D, Dollfus H, Hamel C, Dufier JL, Mannich A, Kaplan J, Rozet JM. Retinal dehydrogenase 12 (RDH12) mutations in leber congenital amaurosis. Am J Hum Genet 2004; 75:639-46. [PMID: 15322982].

64. El Matri L, Ambresin A, Schorderet DF, Kawasaki A, Seeliger MW, Wenzel A, Arsenijevic Y, Borruat FX, Munier FL. Phenotype of three consanguineous Tunisian families with early-onset retinal degeneration caused by an R91W homozygous mutation in the RPE65 gene. Graefes Arch Clin Exp Ophthalmol 2006; 244:1104-12. [PMID: 16518657].

65. Samardzija M, von Lintig J, Tanimoto N, Oberhauser V, Thiersch M, Reme CE, Seeliger M, Grimm C, Wenzel A. R91W mutation in Rpe65 leads to milder early-onset retinal dystrophy due to the generation of low levels of 11-cis-retinal. Hum Mol Genet 2008; 17:281-92. [PMID: 17933883].

66. Lotusy AJ, Namerumalsamy P, Jacobson SG, Weleber RG, Fishman GA, Musarella MA, Hoyt CS, Heon E, Levin A, Jan J, Lamb M, Carr RE, Franklin A, Radha S, Andorf JL, Sheffield VC, Stone EM. Mutation analysis of 3 genes in patients with Leber congenital amaurosis. Arch Ophthalmol 2000; 118:538-43. [PMID: 10766140].

67. Simovich MJ, Miller B, Ezzeldin H, Kirkland BT, McLeod G, Fulmer C, Cremers FP, Cremers CW, Kremmer H. Identification of 51 novel exons of the USH2A gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Hum Mol Genet 2004; 13:281-92. [PMID: 15325563].

68. Singh HP, Lalali S, Narayanan R, Kannabiran C. Genetic analysis of Indian families with autosomal recessive retinitis pigmentosa by homozygosity screening. Invest Ophthalmol Vis Sci 2009; 50:4065-71. [PMID: 19339744].

69. Le Guédard-Méreuze S, Vache C, Baux D, Faugere V, Larrieu L, Abadie C, Jancakea C, Claustres M, Roux AF, Tuffery-Giraud S. Ex vivo splicing assays of mutations at non-canonical positions of splice sites in USHER genes. Hum Mutat 2010; 31:347-55. [PMID: 20052763].

70. van Wijk E, Pennings RJ, te Brinke H, Claassen A, Yntema HG, Hoeftsloot LH, Cremers FP, Cremers CW, Kremmer H. Identiﬁcation of 51 novel exons of the USH2A gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004; 74:738-44. [PMID: 15015129].

71. Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, Dryja TP. Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. Exp Eye Res 2004; 79:167-73. [PMID: 15325563].

72. Pennings RJ, Huygen PL, Orten DJ, Wagens A, van Aarem A, Kremmer H, Kimberling WJ, Cremers CW, Deutman AF. Evaluation of visual impairment in Usher syndrome 1b and Usher syndrome 2a. Acta Ophthalmol Scand 2004; 82:131-9. [PMID: 15043528].

73. Pennings RJ, Te Brinke H, Weston MD, Claassen A, Orten DJ, Weeckamp H, Van Aarem A, Huygen PL, Deutman AF, Hoeftsloot LH, Cremers FP, Cremers CW, Kimberling WJ, Kremmer H. USH2A mutation analysis in 70 Dutch families with Usher syndrome type II. Hum Mutat 2004; 24:185-97. [PMID: 15241801].

74. Dreyer B, Brox V, Tranebjærg L, Rosenberg T, Sadeghi AM, Moller C, Nilssen O. Spectrum of USH2A mutations in Scandinavian patients with Usher syndrome type II. Hum Mutat 2008; 29:451. [PMID: 18273898].

75. Weston MD, Eudy JD, Fujita S, Yao S, Usami S, Cremers C, Greenberg J, Ramesar R, Martini A, Moller C, Smith RJ, Sunnegi J, Kimberling WJ. Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type Ila. Am J Hum Genet 2000; 66:1199-210. [PMID: 10729113].
76. Rivolta C, Sweklo EA, Berson EL, Dryja TP. Missense mutation in the USH2A gene: association with recessive retinitis pigmentosa without hearing loss. Am J Hum Genet 2000; 66:1975-8. [PMID: 10775529].

77. Rivolta C, Berson EL, Dryja TP. Paternal uniparental heterodisomy with partial isodisomy of chromosome 1 in a patient with retinitis pigmentosa without hearing loss and a missense mutation in the Usher syndrome type II gene USH2A. Arch Ophthal 2002; 120:1566-71. [PMID: 12427073].

78. Dreyer B, Tranebjærg L, Rosenberg T, Weston MD, Kimberling WJ, Nilssen O. Identification of novel USH2A mutations: implications for the structure of USH2A protein. Eur J Hum Genet 2000; 8:500-6. [PMID: 10909849].

79. Clark GR, Crowe P, Muszynska D, O’Prey D, O’Neill J, Alexander S, Willoughby CE, McKay GJ, Silvestri G, Simpson DA. Development of a diagnostic genetic test for simplex and autosomal recessive retinitis pigmentosa. Ophthalmology 2010; 117:2169-77. [PMID: 20591486].

80. McGee TL, Seyedahmadi BJ, Sweeney MO, Dryja TP, Berson EL. 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; 47:499-506. [PMID: 20507924].

81. Aller E, Larrieu L, Jaijo T, Baux D, Espinos C, Gonzalez-Candelas F, Najera C, Palau F, Claustres M, Roux AF, Millan JM. The USH2A c.2299delG mutation: dating its common origin in a Southern European population. Eur J Hum Genet 2010; 18:788-93. [PMID: 20145675].

82. Bernal S, Meda C, Solans T, Ayuso C, Garcia-Sandoval B, Valverde D, Del Rio E, Baiget M. Clinical and genetic studies in Spanish patients with Usher syndrome type II: description of new mutations and evidence for a lack of genotype–phenotype correlation. Clin Genet 2005; 68:204-14. [PMID: 16098008].

83. Voizzi D, Aaspollu A, Athanasakis E, Berto A, Fabretto A, Licastro D, Kulm M, Testa F, Trevisi P, Vahter M, Ziviello C, Martini A, Simonelli F, Banfi S, Gasparini P. Molecular epidemiology of Usher syndrome in Italy. Mol Vis 2011; 17:1662-8. [PMID: 21738395].

84. Leijendeckers JM, Pennings RJ, Snik AF, Bosman AJ, Cremers CW. Audiometric characteristics of USH2a patients. Audiol Neurootol 2009; 14:223-31. [PMID: 19129697].

85. Herrera W, Aleman TS, Cideciyan AV, Roman AJ, Banin E, Ben-Yosef T, Gardner LM, Sumaroka A, Windsor EA, Schwartz SB, Stone EM, Liu XZ, Kimberling WJ, Jacobson SG. Retinal disease in Usher syndrome III caused by mutations in the clarin-1 gene. Invest Ophthalmol Vis Sci 2008; 49:2651-60. [PMID: 18281613].

86. Baux D, Larrieu L, Blanchet C, Hamel C, Ben Salah S, Vielle A, Gilbert-Dussardier B, Holder M, Calvas P, Philip N, Edery P, Bonneau D, Claustres M, Malcolm S, Roux AF. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. Hum Mutat 2007; 28:781-9. [PMID: 17405132].

87. Besnard T, Garcia-Garcia G, Baux D, Vache C, Faugere V, Larrieu L, Leonard S, Millan JM, Malcolm S, Claustres M, Roux AF. Experience of targeted Usher exome sequencing as a clinical test. Molecular genetics and genomic medicine. 2014; 2:30–43.

88. Le Quesne Stabej P, Saihan Z, Ranges N, Steele-Stallard HB, Ambrose J, Coffey A, Emmerson J, Haralambous E, Hughes Y, Steel KP, Luxon LM, Webster AR, Bitner-Glindzicz M. Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. J Med Genet 2012; 49:27-36. [PMID: 22135276].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 28 April 2015. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

© 2015 Molecular Vision