High-Resolution Homozygosity Mapping Is a Powerful Tool to Detect Novel Mutations Causative of Autosomal Recessive RP in the Dutch Population

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Purpose. To determine the genetic defects underlying autosomal recessive retinitis pigmentosa (arRP) in the Dutch population and in a subset of patients originating from other countries. The hypothesis was that, because there has been little migration over the past centuries in certain areas of The Netherlands, a significant fraction of Dutch arRP patients carry their genetic defect in the homozygous state.

Methods. High-resolution genome-wide SNP genotyping on SNP arrays and subsequent homozygosity mapping were performed in a large cohort of 186 mainly nonconsanguineous arRP families living in The Netherlands. Candidate genes residing in homozygous regions were sequenced.

Results. In ~94% of the affected individuals, large homozygous sequences were identified in their genome. In 42 probands, at least one of these homozygous regions contained one of the 26 known arRP genes. Sequence analysis of the corresponding genes in each of these patients revealed 21 mutations and two possible pathogenic changes, 14 of which were novel. All mutations were identified in only a single family, illustrating the genetic diversity within the Dutch population.

Conclusions. This report demonstrates that homozygosity mapping is a powerful tool for identifying the genetic defect underlying genetically heterogeneous recessive disorders like RP, even in populations with little consanguinity. (*Invest Ophthalmol Vis Sci.* 2011;52:2227–2239) DOI:10.1167/iovs.10-6185

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Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of progressive retinal disorders with a worldwide prevalence of approximately 1 in every 4000 individuals.[1] Patients with RP typically present with night blindness in the first or second decade of life, followed by visual field loss and deterioration of visual acuity that leads to legal blindness. Abnormalities on ophthalmoscopy include a waxy pallor of the optic disc, attenuated vessels, and atrophy of the (mid)peripheral retinal pigment epithelium (RPE) with bone spicule pigmentation.[2,3]

To date, 26 genes have been identified in which mutations are associated with autosomal recessive RP (arRP) (http://www.sph.uth.tmc.edu/retnet/). The proteins encoded by these genes exert different roles within the retina—for instance, in the regulation of transcription, in transport processes via the photoreceptor connecting cilium, in the phototransduction cascade, or in vitamin A metabolism.[4] About one third of these genes were discovered by homozygosity mapping combined with a candidate gene selection approach, often in large consanguineous pedigrees with multiple affected individuals. In many Western countries, including The Netherlands, arRP families are small and generally have no more than one or two affected individuals. Conventional linkage analysis is therefore not suitable for detecting the genomic region harboring the genetic defect. Because of the extreme heterogeneity of arRP, complete sequence analysis of all known arRP genes is time consuming and expensive. Detection of mutations using the Asper Biotech arRP microarray (Tartu, Estonia)[5] seems cost effective and efficient, but allows only the identification of previously reported mutations, some of which may be specific for certain ethnic groups.

There has been little migration over the past centuries in some parts of The Netherlands, which led us to believe that The Netherlands consists of partially overlapping subpopulations (Fig. 1). As a result, many people share a common ancestor, between 5 and 20 generations removed, and patients with a recessive disorder inherit the same mutant allele from both parents. Not only is the mutation homozygous, but also stretches of DNA surrounding the mutation, although the sizes of the homozygous segments are smaller than those observed in patients born in consanguineous marriages. The use of high-resolution SNP genotyping to detect homozygous mutations has been shown to be effective in a German patient with Leber congenital amaurosis and in Dutch families with arRP and autosomal recessive cone dysfunction.[6–8]

In this study, 186 families, mainly living in The Netherlands, were genotyped on high-resolution SNP arrays. In 42 families that showed significant homozygous stretches encompassing one of the known arRP genes, the respective genes were sequenced, which resulted in the identification of 21 disease-causing mutations and two variants that are potentially pathogenic. We demonstrated that high-resolution homozygosity mapping is a powerful tool for detecting causative mutations in patients with arRP, also from nonconsanguineous populations.

Materials and Methods

Subjects and Clinical Evaluation

Two hundred thirty RP patients from 186 families, mainly living in The Netherlands, were included in the study. The diagnosis of RP was based on an ophthalmic examination that included best corrected visual acuity, slit lamp biomicroscopy, ophthalmoscopy, and fundus photography. Electroretinograms (ERG), recorded according to the protocol of the International Society for Clinical Electrophysiology of Vision (ISCEV),[9] and Goldmann visual field measurements were available from most of the patients. Some of the patients were clinically re-examined after the identification of the genetic defect.

After an explanation of the nature of this phenotype–genotype study, an informed consent adhering to the tenets of the Declaration of Helsinki was obtained from all patients and their unaffected relatives. Blood samples from these individuals were collected for molecular genetic testing. DNA samples of 180 unrelated Dutch or 90 unrelated Turkish control individuals were used.

Homozygosity Mapping

Genomic DNA was isolated from lymphocytes by standard salting-out procedures.[10] DNA samples of all affected individuals were genotyped on a SNP microarray (GeneChip Genome-Wide Human SNP Array 5.0, Affymetrix, Santa Clara, CA) that contains 500,000 polymorphic SNPs in addition to 420,000 nonpolymorphic probes for the detection of germline copy number variations. Array experiments were performed according to protocols provided by the manufacturer. The array data were genotyped with a genotyping data analysis program (Genotype Console, ver. 2.1; Affymetrix) and regions of homozygosity were identified (Genomics Solution ver. 6.1; Partek, Inc., St. Louis, MO). Regions containing more than 250 consecutive homozygous SNPs were considered to be large homozygous regions, on average corresponding to a genomic size of 1 Mb.

Mutation Analysis

In patients with known arRP genes residing within the large homozygous regions, all exons and intron–exon boundaries of these genes were amplified by PCR. PCR conditions and primer sequences are available on request. PCR products were purified (Nucleospin Plasmid Quick Pure columns; Machery Nagel, Düren, Germany) and sequenced

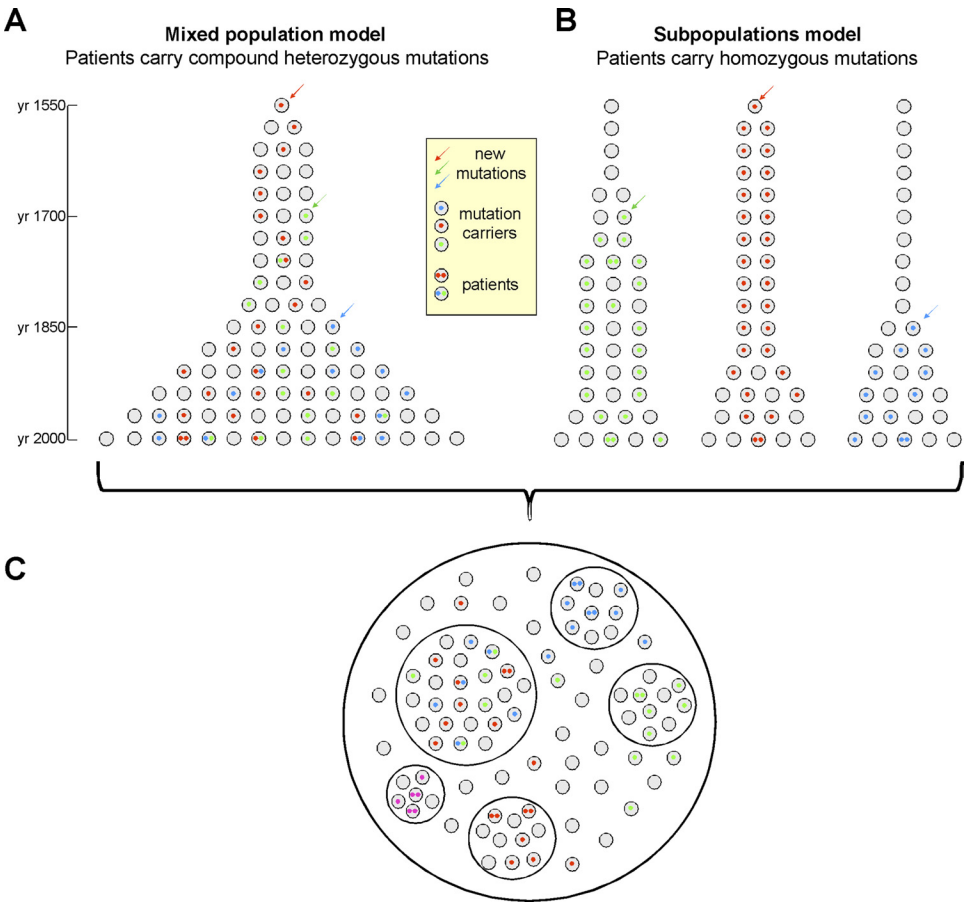

Figure 1. Hypothetical population models for The Netherlands. **(A)** Mixed model in which all individuals of The Netherlands in the last centuries have mixed without any geographic, religious, or social restrictions. **(B)** Subpopulations have lived side-by-side for several generations with no or limited mixture. **(C)** Based on our findings in arRP patients, we assume that The Netherlands consists of partially overlapping subpopulations. New mutation denotes either de novo mutations or the introduction of a new mutation through immigration of mutation carriers.

in sense and antisense directions with dye termination chemistry on a DNA analyzer (model 3730 or 2100; Applied Biosystems, Inc., Foster City, CA). The prevalence of novel missense mutations was analyzed in ethnically matched control individuals, either by amplification–refractory mutation system (ARMS) analysis or by restriction enzyme digestion. If ethnically matched control individuals were not available, Dutch control individuals were used.

Bioinformatic Analysis and Evolutionary Comparison Missense Mutations

For each of the missense changes identified in this study, the pathogenicity was analyzed by calculating Grantham scores (that compare the differences in physical properties of the amino acids side chains)[11] and PhyloP scores, that are a measure for evolutionary conservation of the mutated nucleotide. In addition, for the novel missense mutations detected in this study, the corresponding human protein sequences and those of their orthologues were derived from the UniProt and NCBI databases, and sequence alignments were made with commercial software (Align progam, in VectorNTI Advance 11.0 software; Invitrogen, Carlsbad, CA). Accession numbers of these protein sequences are presented in the legend to Figure 2.

Online Web Resources

National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/ National Institutes of Health, Bethesda, MD.

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/omim/ National Institutes of Health.

Retinal Network RetNet: http://www.sph.uth.tmc.edu/retnet/ University of Texas Houston Health Science Center, Houston, TX.

SMART protein domain database: http://smart.embl-heidelberg.de/ European Molecular Biology Laboratory, Heidelberg, Germany.

The ConSeq server: http://conseq.tau.ac.il/ Tel Aviv University, Tel Aviv, Israel.[12]

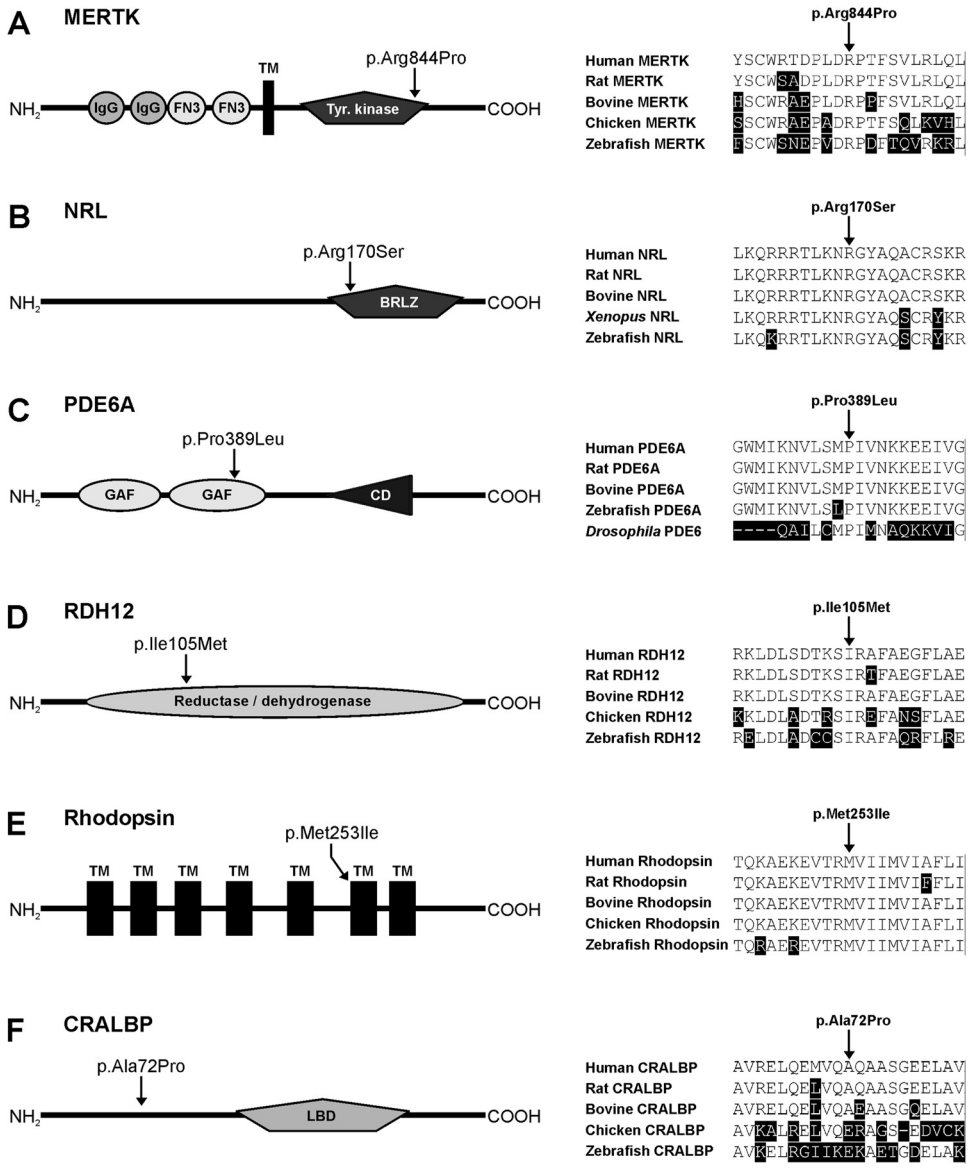

FIGURE 2. Domain structure and evolutionary conservation of proteins with missense mutations. Graphic overview of the proteins encoded by genes in which novel missense mutations were identified: (**A**) *MERTK*, (**B**) *NRL*, (**C**) *PDE6A*, (**D**) *RDH12*, (**E**) rhodopsin, and (**F**) *CRALBP*. Important structural or functional domains are depicted, as well as the position of the amino acid substitution. For each of the amino acids that is replaced, plus a series of surrounding amino acids, the evolutionary conservation is presented in human, rat, and bovine, as well as two of the following nonmammalian species: chicken, *Xenopus tropicalis*, zebrafish, and *Drosophila melanogaster*. Amino acids that are present in at least three of the five species are depicted in *black* on a *white* background, whereas other, nonconserved, amino acids are indicated in *white* on a *black* background. *Arrows*: position of the substituted amino acids. IgG, Immunoglobulin G-like domain; FN3, fibronectin type 3-like domain; TM, transmembrane of membrane-spanning region; BRLZ, basic region leucine zipper motif; GAF, cyclic-GMP-binding domain; CD; catalytic domain; LBD, ligand-binding domain, e.g., domain responsible for retinaldehyde binding. Accession numbers of the protein sequences are as follows: human MERTK (Q12866), rat MERTK (P57097), bovine MERTK (XM_580552), chicken MERTK (Q90777), and zebrafish MERTK (XP_001919423); human NRL (P54845), rat NRL (NP_001099506), bovine NRL (XP_599808), *Xenopus tropicalis* NRL (A4IHY9), and zebrafish NRL (Q4U1T8); human PDE6A (P16499), rat PDE6A (NP_001100856), bovine PDE6A (P11541), zebrafish PDE6A (Q800E7), and *Drosophila melanogaster* PDE6 (Q9VFI9); human RDH12 (Q96NR8), rat RDH12 (NP_001101507), bovine RDH12 (P59837), chicken RDH12 (XM_421193), and zebrafish RDH12 (Q6DG78); human rhodopsin (P08100), rat rhodopsin (P51489), bovine rhodopsin (P02699), chicken rhodopsin (P22328), and zebrafish rhodopsin (P35359); and human CRALBP (P12271), rat CRALBP (NP_001099744), bovine CRALBP (P10123), chicken CRALBP (NP_001019865), and zebrafish CRALBP (AAH65863).

The Human Gene Mutation database: http://www.hgmd.cf.ac.uk/ac/index.php/ Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, Wales, UK.

UCSC Human genome Browser working draft hg18, March 2006: http://genome.ucsc.edu/ UCSC Genome Bioinformatics, University of California at Santa Cruz, Santa Cruz, CA.

UniProt: http://www.uniprot.org/ The Uniprot Consortium.[13]

RESULTS

Homozygosity Mapping

To determine the genetic causes underlying arRP in the Dutch population, we analyzed 230 patients with a diagnosis of RP from 186 families on high-resolution SNP arrays (Affymetrix). Of these, one family had five affected individuals, three families had four affected siblings, three families had three affected siblings, 25 families had two affected individuals, and 154 families had only one affected individual. To find genomic regions harboring the underlying causative genetic defect, we mapped continuous homozygous stretches, regarding genomic regions containing 250 or more consecutive homozygous SNPs as actual homozygous regions.

According to these criteria, several families showed a relatively high number (>20) of very large homozygous regions, up to even 90 Mb of genomic DNA. Detailed analysis of their family history revealed that 21 families in our cohort were born of consanguineous marriage (e.g., between first or second cousins). In the remaining 165 families, 158 probands carried one or more large homozygous regions, with an average of five segments per patient. The size of these regions ranged from 0.6 Mb up to 38.5 Mb, with an average size of 3.8 Mb per region.

In 42 RP probands (including those of consanguineous marriages), one or more of their homozygous regions contained one of the known arRP genes. In those patients, all exons and intron–exon boundaries of the relevant gene were sequenced. A complete overview of the patients and their homozygous regions encompassing the known arRP genes is presented in Table 1. In 23 cases, a mutation was identified, most often in the patient's largest or second largest homozygous region. In only two patients, both born of consanguineous marriage, were the mutations detected in the 3rd and 13th homozygous largest regions, respectively, although the size of these regions still exceeded 5 Mb of genomic DNA (Table 1). Of the 23 mutations that were identified, 14 have not been reported before (Table 2). Twelve mutations are predicted to result in premature termination of the protein and as such are considered to be true loss-of-function mutations. The remaining 11 mutations are missense changes, for which the pathogenicity is not always certain. For all missense changes, Grantham and PhyloP scores were calculated, which consider biophysical properties of amino acid side chains and evolutionary conservation of nucleotide residues, respectively, to predict the pathogenicity of the mutations (Table 3). In addition, for those mutations that have not been reported previously, the conservation and the position of the mutated amino acid within predicted functional domains of the corresponding protein were analyzed (Fig. 2). All mutations that were identified in this study are discussed in more detail in the following sections. Clinical characteristics of the probands with these mutations are presented in Table 4.

ABCA4. Mutations in *ABCA4* have been described to cause autosomal recessive Stargardt disease (STGD1), cone-rod dystrophy (CRD), or RP, depending on the severity of the combinations of mutations.[22–24] In patient 20922, a splice site mutation in *ABCA4* was detected (c.768G>T) that has been reported to be an allele with a severe effect occurring in Dutch patients with STGD1 or RP.[14] On clinical re-examination and re-evaluation of retrospective data, CRD rather than RP was diagnosed in individual 20922.

CRB1. In patient 18389, only a single homozygous region was detected, that harbored the *CRB1* gene, in which various mutations cause either Leber congenital amaurosis (LCA) or a specific type of arRP, called RP with preserved para-arteriolar retinal pigment epithelium (PPRPE, RP12).[15,25,26] Mutation analysis revealed a missense mutation (c.482C>T; p.Ala161Val) that has been described in an RP patient with PPRPE. Our patient initially also showed signs of PPRPE that were less pronounced at recent examination, because of progressive degeneration.

EYS. About 2 years ago, we and others simultaneously identified the *EYS* gene, the human orthologue of the *Drosophila* eyes shut/spacemaker.[6,27] Recent studies have implicated *EYS* as one of the most frequently mutated genes in patients with arRP from different ethnic groups.[28–31] In three Dutch patients who had homozygous regions encompassing *EYS*, protein-truncating mutations were identified, of which two are novel and one has been described previously (Table 1). Detailed clinical characteristics of the patients with *EYS* mutations are described elsewhere.[31]

LRAT. In patient 43329, who was born of a consanguineous marriage, one of her largest homozygous regions contained the *LRAT* gene. Mutations in *LRAT* are causative of early-onset RP.[32] In patient 43329, a novel 1-bp deletion was detected that is predicted to result in premature termination of the encoded protein (c.519del; p.Ile174SerfsX12). On identification of the mutation, two affected siblings were also genotyped and found to carry the same mutation homozygously. All three patients displayed an early-onset form of RP. Interestingly, the proband had undergone surgery for a bilateral cataract at the age of 1 month, whereas her two affected siblings did not have cataracts.

MERTK. In two patients, homozygous mutations in *MERTK* were identified. Mutations in *MERTK* cause typical RP.[33] Patient 18864 is part of a larger family segregating RP, LCA, and early-onset severe retinal dystrophy (EOSRD). Genome-wide SNP analysis combined with linkage analysis revealed that three distant relatives carried compound heterozygous mutations in *CEP290*, causing either LCA or EOSRD.[34] Patient 18864, whose parents are consanguineous, had multiple large homozygous regions, one of which contained *MERTK*. Mutation analysis revealed a previously unreported 1-bp duplication that is predicted to result in premature termination of the protein (c.1179dup; p.Leu394SerfsX3). Patient 25688 had homozygous SNP calls for the complete chromosome 2, strongly suggesting uniparental isodisomy, although her parents were unfortunately not available to confirm this. Sequence analysis of *MERTK*, which is located on chromosome 2, revealed a novel missense mutation, substituting a proline residue for an arginine residue (c.2531G>C; p.Arg844Pro). This change was not detected in 360 Dutch control alleles. The MERTK protein is essential for correct phagocytosis of the photoreceptor outer segments by the RPE[35] and is a type I transmembrane protein composed of two IgG-like and two fibronectin type 3-like domains in the extracellular part of the protein and a tyrosine kinase domain in the cytoplasmic tail (Fig. 2A). The proline residue that is substituted for an arginine is part of this kinase domain and is highly conserved during evolution. Not only in other vertebrate MERTK proteins (Fig. 2A) but even in several related tyrosine kinases, like the insulin receptor, the insulin-like growth factor 1 receptor, the hepatocyte growth factor receptor and the tyrosine-protein kinase transforming protein Abl, an arginine residue is present at this position (data not shown). These data indicate that this arginine residue plays a crucial role within the tyrosine kinase domain of MERTK and further support the causality of the mutation identified in this study.

TABLE 1. Homozygous Regions Harboring Known arRP Genes

Proband	Country of Origin	Consanguinity	Homozygous Regions (*n*)	arRP Gene in Region	Size Hom. Region (Mb)	Individual Ranking Region	Mutation Identified
8640	The Netherlands	No	4	*RLBP1*	3.2	2	Yes
				NR2E3	16.6	1	No
9458	The Netherlands	No	7	*RP1*	26.8	1	No
9860	The Netherlands	No	15	*PDE6A*	14.8	2	No
11319	The Netherlands	No	8	*EYS*	37.0	2	No
15374	The Netherlands	No	4	*ABCA4*	10.6	1	No
16544	The Netherlands	No	6	*EYS*	11.2	1	Yes
17959	Morocco	No	10	*NR2E3*	11.2	2	Yes
18336	The Netherlands	No	4	*RGR*	1.7	1	No
				CRB1	2.5	2	No
18389	India	No	1	*CRB1*	31.3	1	Yes
18777	Turkey	Yes	48	*MERTK*	91.6	1	No
				PDE6A	34.8	3	No
				EYS	34.6	6	No
18864	The Netherlands	Yes	16	*MERTK*	67.1	1	Yes
19081	The Netherlands	No	14	*EYS*	33.5	1	No
20463	Unknown	No	6	*RDH12*	4.9	1	Yes
20922	The Netherlands	No	11	*ABCA4*	11.0	1	Yes
20984	The Netherlands	Yes	18	*CNGB1*	68.8	1	No
				CERKL	22.9	4	No
				RHO	15.8	5	No
				PDE6B	6.2	12	No
21211	The Netherlands	No	6	*CNGA1*	5.9	3	No
22218	Somalia	Yes	24	*RDH12*	5.0	13	Yes
22315	The Netherlands	No	3	*CRB1*	2.8	1	No
22565*	The Netherlands	No	4	*NR2E3*	3.8	2	Yes
22891	The Netherlands	No	5	*EYS*	9.7	1	Yes
23718	The Netherlands	No	5	*NR2E3*	20.7	1	Yes
25402	Turkey	Suspected	36	*PDE6A*	10.3	6	No
25688	The Netherlands	No	4	*MERTK*	Chr2 (UPD)	1	Yes
25846	The Netherlands	No	8	*RP1*	8.3	1	Yes
26722	The Netherlands	No	5	*EYS*	13.9	1	Yes
27775	The Netherlands	Yes	17	*EYS*	29.0	1	No
27790	The Netherlands	No	3	*PDE6A*	7.5	1	No
29998*	Turkey	Yes	1	*PDE6B*	4.2	1	Yes
30228	The Netherlands	No	14	*RDH12*	7.9	2	No
31035	The Netherlands	No	4	*EYS*	1.8	4	No
32111	Morocco	No	6	*TULP1*	13.8	1	No
32666	Morocco	No	9	*NRL*	19.1	1	Yes
33672	Turkey	Yes	12	*RDH12*	32.6	1	Yes
33685	Turkey	Yes	10	*NR2E3*	33.3	2	No
				RLBP1	33.3	2	No
				TULP1	12.8	3	No
33747	The Netherlands	No	7	*PDE6A*	12.4	1	Yes
34219	Turkey	No	4	*PDE6A*	1.5	2	Yes
37370	Turkey	Suspected	19	*EYS*	49.9	1	No
37799	Serbia	No	4	*RGR*	3.3	2	Yes
40845	The Netherlands	No	5	*CNGB1*	6.6	1	No
41611	The Netherlands	No	16	*PDE6A*	3.0	2	No
42981	Turkey	Yes	30	*RHO*	82.3	1	Yes
				RGR	46.9	2	No
43329	Turkey	Yes	14	*LRAT*	23.1	3	Yes
44014*	Morocco	Yes	5	*RLBP1*	4.0	2	Yes

Overview of homozygous regions harboring known arRP genes in RP probands. Listed are the country of origin, whether the probands were born of consanguineous marriage, the total number of homozygous regions, the size of the homozygous region that contains the corresponding arRP gene, the individual ranking of that region for each of the individual patients, and whether a mutation in the corresponding gene was identified. Data of patients in whom the causative mutation has been identified are shaded.

* For three probands, affected relatives were also genotyped on the SNP arrays, and for these, only homozygous regions shared by all affected individuals are listed. UPD, uniparental disomy.

NR2E3. Mutations in the *NR2E3* gene cause clumped pigmentary retinal degeneration (CPRD) or enhanced S-cone syndrome (ESCS), a type of retinal dystrophy characterized by an increased number of S-cones.[16,36,37] In three families that had initially been diagnosed with atypical arRP, homozygous mutations in *NR2E3* were detected. On retrospective data analysis and clinical re-evaluation, features of ESCS were observed in all patients. Individual 17959 carried a missense mutation (c.310C>T; p.Arg104Trp) that had been described in an ESCS patient, whereas individual 23718 was homozygous for the frequent mutation in *NR2E3* that abolishes the splice acceptor site of exon 2 (c.119-2A>C).[16] Finally, in patient 22565 as well as in his affected brother, a novel 2-bp deletion was identified that is predicted to result

TABLE 2. Homozygous Mutations in Known arRP Genes Identified in the Study

Proband	arRP Gene	Mutation (cDNA)	Mutation (Protein)	Phenotype	Affected Relatives with the Mutation (*n*)	Reference
20922	*ABCA4*	c.768G>T	p.Val256Val/aberrant splicing	CRD	—	14
18389	*CRB1*	c.482C>T	p.Ala161Val	RP	1	15
16544	*EYS*	c.6799_6800del	p.Gln2267GlufsX15	RP	—	This study
22891	*EYS*	c.4350_4356del	p.Lys1450LysfsX3	RP	—	This study
26722	*EYS*	c.6714del	p.Pro2238ProfsX16	RP	—	6
43329	*LRAT*	c.519del	p.Ile174SerfsX12	EOSRD	2	This study
18864	*MERTK*	c.1179dup	p.Leu394SerfsX3	RP	—	This study
25688	*MERTK*	c.2531G>C	p.Arg844Pro	RP	—	This study
17959	*NR2E3*	c.310C>T	p.Arg104Trp	ESCS	—	16
22565	*NR2E3*	c.724_725del	p.Ser242GlnfsX17	ESCS/CPRD	1	This study
23718	*NR2E3*	c.119-2A>C	aberrant splicing	ESCS	—	16
32666	*NRL*	c.508C>A	p.Arg170Ser	CPRD	—	This study
33747	*PDE6A*	c.305G>A	p.Arg102His	RP	1	17
34219	*PDE6A*	c.1166C>T	p.Pro389Leu	RP	—	This study
29998	*PDE6B*	c.2399del	p.Leu800ArgfsX17	RP	4	This study
20463	*RDH12*	c.164C>T	p.Thr55Met	EOSRD	—	18
22218	*RDH12*	c.315C>G	p.Ile105Met	RP	—	This study
33672	*RDH12*	c.658+591_*603+669delins CT	deletion C-terminus	RP	—	This study
37799	*RGR*	c.196A>C	p.Ser66Arg	RP	—	19
42981	*RHO*	c.759G>T	p.Met253Ile	RP	—	This study
8640	*RLBP1*	c.214G>C	p.Ala72Pro	RP	—	This study
44014	*RLBP1*	c.525_954del	deletion C-terminus	RPA	1	20
25846	*RP1*	c.686del	p.Pro229GlnfsX35	RP	—	This study

For each mutation, the predicted effect on the protein level is presented. For multiplex families, the number of affected individuals who also carry the mutation homozygously is presented.

in premature termination of the protein (c.724_725del; p.Ser242GlnfsX17).

NRL. Patient 32666, originating from Morocco, displayed several homozygous regions, of which the largest contained the *NRL* gene. Mutations in *NRL* have been described in patients with autosomal dominant RP and autosomal recessive CPRD.[38,39] In patient 32666, a homozygous missense mutation was identified that substitutes a serine for an arginine residue

TABLE 3. Missense Variants and Evaluation of Pathogenicity

Gene	Mutation (Protein)	Frequency in Controls	Segregation In Family	Location Amino Acid in Predicted Functional Domain	PhyloP Score	Grantham Score	Reported Functional Proof of Evidence	Reference
Pathogenic Variants								
NR2E3	p.Arg104Trp	NA	NA	DNA binding domain	2.39	101	Reduced DNA binding and transcriptional activation	16, 21
RDH12	p.Thr55Met	NA	NA	Dehydrogenase domain	5.64	81	Reduced reductase and dehydrogenase activity	18
Probably Pathogenic Variants								
CRB1	p.Ala161Val	0/360	NA	Calcium binding EGF-like domain	5.43	64	—	15
MERTK	p.Arg844Pro	0/360	Yes	Tyrosine kinase domain	6.15	103	—	—
NRL	p.Arg170Ser	0/360*	NA	DNA binding domain	2.53	110	—	—
PDE6A	p.Arg102His	NA	Yes	cGMP binding domain	5.10	29	—	17
PDE6A	p.Pro389Leu	0/360	NA	cGMP binding domain	6.22	98	—	—
RGR	p.Ser66Arg	NA	NA	Transmembrane domain	1.70	110	—	19
RHO	p.Met253Ile	0/180	Yes	Transmembrane domain	6.67	10	—	—
Potentially Pathogenic Variants								
RDH12	p.Ile105Met	0/360*	NA	Dehydrogenase domain	0.91	10	—	—
RLBP1	p.Ala72Pro	0/360	NA	—	1.96	27	—	—

Assessment of pathogenicity for the missense variants identified in the study. The variant in *PDE6A* was homozygously present in an affected brother, whereas the *MERTK* and *RHO* variants were heterozygously present in the two parents as well as heterozygously present or absent in other unaffected relatives.

* For these patients, ethnically matched control individuals were not available, and hence 360 Dutch control alleles were screened. NA, not analyzed.

TABLE 4. Clinical Characteristics of Probands with Mutations in Known arRP Genes

Proband	Defective Gene	Mutation (cDNA)	Mutation (protein)	Sex	Diagnosis	Age at Diagnosis (y)	Age at Last Exam (y)	Visual Acuity		Funduscopy	ERG	Goldmann Perimetry
								RE	LE			
20922	*ABCA4*	c.768G>T	p.Val256Val/ aberrant splicing	M	CRD	7	17	CF	20/200	Optic disc pallor, attenuated vessels, atrophic macula with pigmentary clumping, peripheral degeneration	NA	Central scotoma, peripheral field relatively intact
18389	*CRB1*	c.482C>T	p.Ala161Val	M	RP	8	40	LP	LP	Severe pigmentary retinopathy, colobomatous macular appearance	NR	NP
16544	*EYS*	c.6799_6800del	p.Gln2267GlufsX15	M	RP	15	39	20/50	20/40	Waxy optic disc, moderately attenuated vessels, peripheral bone spicules	NR	Marked decrease, small temporal islands
22891	*EYS*	c.4350_4356del	p.Lys1450LysfsX3	M	RP	17	25	20/20	20/25	Pink optic disc, attenuated vessels, cystoid maculopathy, mild RPE atrophy in the midperiphery, with scarce bone spicules	SR	Intact periphery, midperipheral sensitivity loss, paracentral scotoma
26722	*EYS*	c.6714del	p.Pro2238ProfsX16	M	RP	22	42	20/25	20/25	Waxy optic disc, attenuated vessels, mild peripheral bone spicules	NR	Marked constriction
43329	*LRAT*	c.519del	p.Ile174SerfsX12	F	RP	5	7	CF	20/320	Pink optic disc, vessels normal, preserved RPE posterior pole, subtle RPE changes in the periphery	NR	NP
18864	*MERTK*	c.1179dup	p.Leu394SerfsX3	F	RP	13	26	20/300	20/160	Attenuated vessels, RPE changes in the macula, RPE atrophy in the periphery, with bone spicules	NR	Severely constricted
25688	*MERTK*	c.2531G>C	p.Arg844Pro	M	RP	16	46	CF	CF	Attenuated retinal vessels, peripheral pigmentations	NA	NA
17959	*NR2E3*	c.310C>T	p.Arg104Trp	M	ESCS	12	16	20/20	20/20	Pink optic disc, sheathing of peripheral vessels, RPE changes in the macula, round atrophic RPE spots in the midperiphery with nummular pigmentations	Typical†	Intact periphery, central sensitivity loss
22565	*NR2E3*	c.724_725del	p.Ser242GlnfsX17	M	ESCS/CPRD	7	61	20/80	20/63	Pink optic disc, vascular sheathing, RPE changes in the macula, pronounced atrophy of RPE in the periphery with subretinal pigmentations	NR‡	Severely constricted
23718	*NR2E3*	c.119-2A>C	Aberrant splicing	M	ESCS	19	25	20/63	20/100	Pink optic disc, mild attenuated vessels, schisis macula, round atrophic RPE spots midperiphery with nummular pigmentations	Typical†	Intact periphery, midperipheral and central sensitivity loss
32666	*NRL*	c.508C>A	p.Arg170Ser	M	CPRD	10	30	20/125	20/200	Pink optic disc, attenuated arterioles, cystoid maculopathy RE > LE, peripheral patchy RPE atrophy with small nummular pigmentations	NR	Moderately constricted, midperipheral and central sensitivity loss

(*continues*)

TABLE 4 (*continued*). Clinical Characteristics of Probands with Mutations in Known arRP Genes

Proband	Defective Gene	Mutation (cDNA)	Mutation (protein)	Sex	Diagnosis	Age at Diagnosis (y)	Age at Last Exam (y)	Visual Acuity RE	Visual Acuity LE	Funduscopy	ERG	Goldmann Perimetry
20966§	*PDE6A*	c.305G>A	p.Arg102His	F	RP	20	57	20/400	20/80	Attenuated retinal vessels, peripheral pigmentation	NR	Severely constricted
34219	*PDE6A*	c.1166C>T	p.Pro389Leu	F	RP	24	27	20/20	20/20	Optic disc pallor, attenuated vessels, preserved macula with inner limiting membrane wrinkling, RPE atrophy periphery with bone spicules	NR	Large, midperipheral annular scotoma
29998	*PDE6B*	c.2399del	p.Leu800ArgfsX17	M	RP	16	47	20/63	20/63	Optic disc pallor, attenuated vessels, atrophic maculopathy, perimacular remnant of normal RPE, RPE atrophy periphery with bone spicules	NA	NA
20463	*RDH12*	c.164C>T	p.Thr55Met	M	LCA/RP	5	5	20/160	20/60	Severe diffuse loss of RPE	NA	Severely constricted
22218	*RDH12*	c.315C>G	p.Ile105Met	F	RP	24	25	20/50	20/80	Optic disc pallor, attenuated vessels, with sheathing, pericentral RPE atrophy involving the macular region with bone spicules	NR	Moderately constricted, midperipheral and central sensitivity loss
33672	*RDH12*	c.658+591_*603 +669delinsCT	Deletion C-terminus	M	RP	26	29	20/400	20/400	Optic disc pallor, attenuated vessels with sheathing, atrophic maculopathy, RPE atrophy of posterior pole and periphery with bone spicules and white dot lesions	NR	Severely constricted
37799	*RGR*	c.196A>C	p.Ser66Arg	M	RP	6	36	LP	LP	Pink optic discs, attenuated vessels, RPE changes macula, paving-stone-like degeneration in the periphery, RPE atrophy and bone spicules	NP	NP
42981	*RHO*	c.759G>T	p.Met253Ile	M	RP	16	18	20/25	20/25	Pink optic disc, attenuated vessels, cystoid maculopathy, atrophy RPE periphery with bone spicules	NR	Intact periphery, midperipheral sensitivity loss
8640	*RLBP1*	c.214G>C	p.Ala72Pro	M	RP	41	53	20/25	20/20	Attenuated vessels, perifoveal RPE changes, RPE atrophy along inferior arcade, no dots	SR	Pericentral scotoma
44014	*RLBP1*	c.525_954del	Deletion C-terminus	F	RPA	24	32	20/63	20/40	Narrowed retinal vessels, small white dots characteristic of RPA	NR	Incomplete ring scotoma
25846	*RP1*	c.686del	p.Pro229GlnfsX35	M	RP	13	30	HM	LP	Optic disc pallor, attenuated vessels, subfoveal RPE atrophy, peripheral RPE atrophy with bone spicules	NR	NP

Detailed description of clinical findings in the probands with the mutations identified in the study. For multiplex families, clinical details of probands are generally representative of the phenotype in the complete family. If present, unusual clinical features in any of the siblings are presented in the main text. LE, left eye; RE, right eye; CF, counting fingers; HM, hand motion; LP, light perception; NR, non- recordable; SR, severely reduced; NA, not available; NP, not performed

†"Typical" absent rod-specific responses, waveform of maximum scotopic response is similar to those of the photopic responses with prolonged implicit times of the b-wave, high amplitudes of S-cone–specific testing.

‡ERG last performed in 1967, not repeated because of extensive retinal degeneration.

§Proband of the family with individual 33747.

(c.508C>A; p.Arg170Ser). The allele was not detected in 180 Dutch control individuals who were tested in the absence of proper ethnically matched control individuals. Clinical re-examination revealed that patient 32666 also showed features of CPRD (Table 4). The *NRL* gene encodes the neural retina leucine zipper (NRL) protein, which is a basic motif leucine zipper transcription factor that is preferentially expressed in rod photoreceptor cells.[40] The C-terminal part of the protein harbors a basic region leucine zipper (BRLZ) domain that is involved in the DNA-protein interaction with its transcriptional targets, among which is the gene encoding the rod photopigment rhodopsin.[41] The arginine residue that is mutated to a serine in the CPRD patient from this study is located in the DNA-binding domain of NRL (Fig. 2B) and is completely conserved throughout vertebrate evolution. Positively charged arginine and lysine residues are often enriched in DNA-binding domains and play a crucial role in the interaction with the DNA. These data therefore suggest that the replacement of a serine for an arginine at position 170 reduces DNA-binding and subsequent transcriptional activity of NRL.

***PDE6A* and *PDE6B*.** Mutations in the genes encoding the α- and β-subunits of the phosphodiesterase 6 enzyme (*PDE6A* and *PDE6B*, respectively) are both associated with arRP[42–44] and are considered to be a relatively frequent cause of the disease, each accounting for 4% to 5% of cases.[3] In two RP patients in our cohort, homozygous missense variants in *PDE6A* were identified. In patient 33747, a missense mutation was found (c.305G>A; p.R102H) that had been reported previously,[17] and segregated with RP in the family, being homozygously present in the proband 20966 that was not included in the genome-wide SNP analysis. In patient 34219, a novel missense mutation (c.1166C>T; p.Pro389Leu) was identified that was not detected in 360 ethnically matched control alleles. At its N terminus, the PDE6A enzyme contains two structural motifs termed GAF domains because of their presence in c*G*MP-regulated PDE, *a*denylyl cyclases and the *E. coli* protein *F*h1A.[45] These domains have the ability to bind cyclic GMP, and several missense mutations affecting residues in these domains have been described.[17] The catalytic domain of the enzyme is located in the C-terminal half of the protein, which is evolutionarily well conserved among cyclic nucleotide phosphodiesterases.[46] The p.Pro387Leu mutation identified in this study replaces a highly conserved proline residue in the second GAF domain of the protein (Fig. 2C) and as such is likely to impair the function of the phosphodiesterase enzyme in rod photoreceptor cells.

In a consanguineous family originating from Turkey, with four affected siblings and an additional affected cousin, only one genomic region was detected that was homozygous in all five affected individuals. Sequence analysis of *PDE6B*, which resides within this region, revealed a 1-bp deletion (c.2399del; p.Leu800ArgfsX17) that completely segregates with an early-onset form of RP in this family and was heterozygously present or absent in 11 nonaffected relatives.

***RDH12*.** Mutations in the *RDH12* gene, encoding the retinol hydrogenase 12 enzyme RDH12, are associated with LCA as well as early-onset progressive RP.[18,47,48] In our cohort, two patients carried missense mutations in *RDH12*. In patient 20463, classified as EOSRD, sequence analysis revealed a missense change (c.164C>T; p.Thr55Met) that has previously been reported heterozygously in a patient with EOSRD, in conjunction with an *RDH12* nonsense mutation on the counter allele.[18] In patient 22218, who originates from Somalia, a novel homozygous missense mutation in *RDH12* was identified, substituting a methionine residue for an isoleucine (c.315C>G; p.Ile105Met). This change was not detected in 360 Dutch control individuals, who were tested in the absence of control individuals from Somalia. RDH12 is a cytoplasmic enzyme that plays a crucial role in the visual cycle by converting all-*trans*-retinal to all-*trans*-retinol[49] and contains a large domain that is responsible for the actual dehydrogenase activity (Fig. 2D). The isoleucine residue that was mutated in patient 22218 is completely conserved throughout vertebrate evolution (Fig. 2D). Many missense mutations in the *RDH12* gene have been described to cause early-onset retinal degeneration and, for several of the mutant proteins, the biochemical properties have been analyzed in vitro.[18] In some of these cases, the mutant proteins were still able to convert all-*trans*-retinal to all-*trans*-retinol and vice versa. In a cellular transfection assay, many of the mutant proteins appeared to have reduced protein stability. These data suggest that the p.Ile105Met mutation, like other missense mutations in *RDH12*, may impair the function of the RDH12 enzyme, either directly, by affecting residues that are important in the enzymatic function, or indirectly, by reducing protein stability or altering protein confirmation.

Individual 33672 also had a large homozygous region encompassing *RDH12*. During the mutation analysis, the final two exons (8 and 9) of this gene could not be amplified, suggesting a genomic deletion. Further PCR analysis using primers in introns 8, 9, and 10 revealed a homozygous deletion (c.658+591_*603+669delinsCT) that is predicted to result in the absence of a large C-terminal part of the protein.

***RGR*.** A little more than a decade ago, the *RGR* gene was identified as causing arRP.[19] In one of the families described in that study, a homozygous missense mutation (p.Ser66Arg) was identified in five siblings with typical symptoms of RP. The same missense mutation was identified in individual 37999 from our cohort.

***RHO*.** Mutations in the *RHO* gene, encoding the rhodopsin pigment protein of rod photoreceptor cells are a major cause of autosomal dominantly inherited RP, but only rarely cause recessive RP.[3] In patient 42981, who originated from Turkey and was born of a consanguineous marriage, a novel homozygous missense mutation was identified (c.759G>T; p.Met253Ile) that was not detected in 180 ethnically matched control alleles. Clinically, this patient displayed typical RP with cystoid maculopathy. Both parents, who carried the mutation heterozygously, showed no symptoms of RP, excluding an autosomal dominant pattern of inheritance. The rhodopsin protein spans the membranes of the rod outer discs seven times (Fig. 2E). The homozygous missense mutation described in this study substitutes an isoleucine residue for a conserved methionine residue that is located in the sixth transmembrane region of rhodopsin (Fig. 2E). Besides several protein-truncating mutations, numerous missense mutations that are equally distributed over the gene have been described to cause autosomal dominantly inherited RP. Interestingly, only a few mutations have been reported to be associated with the recessively inherited form, among which are a protein-truncating mutation[50] and a substitution of a lysine for a glycine residue at position 150 of the protein.[51,52] The underlying mechanisms by which these mutations and the p.Met253Ile mutation described here cause the recessively inherited form remain unclear, although this missense change may be a mild mutation that is only pathogenic if present on both alleles.

***RLBP1*.** An affected sib pair originating from Morocco displayed two shared homozygous regions, the second largest of which encompassed the *RLBP1* gene. Mutations in *RLBP1* are causative of specific retinal phenotypes called fundus albipunctatus (FA), retinitis punctata albescens (RPA), or Bothnia dystrophy, all of which are characterized by the presence of white dots on the fundus.[53–56] The proband 44014 and her affected sister displayed these white dots and reported night blindness and progressive retinal degeneration, characteristic of RPA. Mutation analysis of *RLBP1* revealed a homozygous genomic

deletion that is predicted to result in the absence of the 143 most C-terminal amino acids of the protein (c.525_954del). This mutation has been reported in other RPA patients from Morocco,[20] suggesting a founder effect. In addition to the Moroccan sisters, an isolated Dutch RP patient was homozygous for a genomic region harboring *RLBP1*. Mutation analysis revealed a novel missense change substituting a proline for an alanine residue (c.214G>C; p.Ala72Pro) that was not detected in 360 ethnically matched control alleles. In this RP patient, no white dots were observed at the time of examination. The *RLBP1* gene encodes the cellular retinaldehyde-binding protein (CRALBP) that carries 11-*cis*-retinaldehyde or 11-*cis*-retinal as physiologic ligands[57] and plays a role in the visual cycle. The CRALBP protein is expressed in the RPE, and proteins with previously described missense mutations have been shown to have a reduced solubility.[56] The amino acid substitution described in this study (p.Ala72Pro) does not affect a residue located in the Sec14 domain that is important for retinaldehyde binding nor is the alanine residue that is mutated completely conserved throughout vertebrate evolution (Fig. 2F). Molecular modeling of CRALBP, however, has shown that residues 66 to 119, in which the mutated alanine residue resides, consists of four α-helices that are arranged antiparallel to each other.[58] Alanines are amino acids that are abundantly present in α-helices, whereas proline residues, because of their intrinsic property of inducing bends in three-dimensional protein structures are hardly present in these structures.[59] Therefore, the substitution of a proline for an alanine may disrupt the helical structure of the N-terminal part of CRALBP, and as such, like other missense mutations in *RLBP1*, may reduce protein stability and function.

RP1. Like mutations in *RHO*, mutations in *RP1* can cause both autosomal dominant and, occasionally, autosomal recessive RP.[60–62] In a Dutch patient who showed typical symptoms of RP with an onset in the first decade of life, a homozygous 1-bp deletion was identified that is predicted to cause a frameshift and premature termination of the RP1 protein (c.686delC; p.Pro229GlnfsX35). Both parents who carried the mutation heterozygously, did not show any symptoms of RP.

Causality of Mutations

In recessive disorders, mutations in the respective genes generally result in loss-of-function of the encoded proteins. Nonsense, frameshift and splice mutations are considered to be such loss-of-function alleles. In the case of missense mutations, however, when only a single amino acid is substituted, additional evidence is needed to prove causality of the mutation. One of the methods that provide evidence of the pathogenicity of a mutation is to generate a mutant protein and assess its function in a specific cellular or biochemical assay. Alternatively, bioinformatic software tools that mainly consider evolutionary conservation and biophysical properties of amino acid side chains are used to predict the pathogenicity of a missense change. In this study, 23 mutations have been identified, 12 of which are nonsense, frameshift, and splice mutations or genomic deletions that result in premature termination of the protein and as such are considered to be true loss-of-function mutations. The remaining 11 mutations are missense changes. For those missense mutations that are novel, a comparison of their evolutionary conservation and their location within functional protein domains has been discussed herein and presented in Figure 2. In addition, for all missense changes, including those reported previously, we evaluated PhyloP scores, which consider evolutionary conservation, and Grantham scores, which simply address the biophysical properties of the amino acid side chains (Table 3). Nucleotide changes with PhyloP scores $\geq$2 were considered to be potentially causative, whereas for Grantham scores $\geq$60, amino acid changes are considered to have potentially damaging effects.[63] Together with the absence of all alleles in control individuals, their prior identification in RP patients, the location of the amino acids that are substituted within predicted functional domains, and previously reported functional evidence for pathogenicity, most of the mutations are considered to be pathogenic and thus causative of RP in the corresponding patients. The pathogenicity remains unclear for only two variants: p.Ile105Met in *RDH12* and p.Ala72Pro in *RLBP1* (Table 3).

Discussion

In this study, we applied genome-wide, high-resolution homozygosity mapping to identify the genetic defect underlying arRP in 186 different families. Most of the families were of Dutch origin and showed no consanguinity. In 42 probands, significant homozygous regions harbored a known arRP gene, and subsequent sequence analysis of the corresponding genes revealed 21 mutations and two potentially pathogenic variants. Knowledge of a patient's genetic defect is beneficial mainly for three reasons. First of all, it helps in establishing genotype–phenotype correlations and therefore enables a more accurate disease diagnosis and prognosis. Second, it facilitates genetic counseling in families, and finally, it enables the selection of patients eligible for gene augmentation or other forms of therapy, as exemplified below.

The RP patient cohort used in this study was collected over the past two-and-a-half decades. Hence, for some of our patients, lack of specialized equipment and limited knowledge of the different subtypes of RP and allied diseases hampered establishment of the correct diagnosis. Identification of the genetic defect may lead to re-examination of the patient and, subsequently, a more accurate diagnosis. For instance the three families with mutations in *NR2E3* received an initial diagnosis of atypical RP, whereas on clinical re-evaluation and extended electroretinography, all patients showed symptoms of ESCS, a phenotype specifically associated with mutations in this gene.

Most of the cases in our cohort were sporadic, for which the mode of inheritance could be either autosomal recessive or X-linked recessive or even de novo autosomal dominant. Mutations in *RP2* and *RPGR*, causative of X-linked RP,[64,65] for instance, are thought to account for 5% to 15% of all RP cases.[3] Since half of the sporadic cases in our cohort involved males, X-linked inheritance cannot be ruled out in several cases. Identifying an autosomal recessive mutation in these isolated male cases excludes X-linked inheritance and as such is reassuring for relatives, although one could also opt to first screen isolated males for *RPGR* mutations prior to homozygosity mapping.

Also, the identification and knowledge of the genetic defect may be crucial for an RP patient in terms of future therapy. During the past few years, the development of gene augmentation therapies has received an enormous boost due to the successes of therapeutic trials in LCA and EOSRD patients with *RPE65* mutations.[66–69] These results clearly demonstrate the possibility of slowing down disease progression or even of restoring vision in patients with retinal dystrophies, although not all different genetic subtypes may be treated in a similar manner,[70] and as such stress the importance of identifying the individual genetic defects in patients with retinal dystrophies.

Many arRP genes have been identified in consanguineous pedigrees with multiple affected individuals, by classic gene linkage and homozygosity mapping studies. Seven families from our study in which the causative mutation was identified were consanguineous. Furthermore, 12 families originated from countries outside Europe, including India, Morocco, Ser-

bia, Somalia, and Turkey. Because of the migration of certain ethnic populations to other countries and the tendency to marry within the same ethnic group for socioeconomic or religious reasons, certain recessive mutations and the diseases that are associated with them remain present in these relatively genetically isolated communities. Although a percentage of our cohort represented patients born of consanguineous marriage, we showed in the present study the power of applying high-resolution homozygosity mapping in nonconsanguineous populations. We and others have applied this method, not only for the identification of mutations in known disease genes,[7,71,72] but also to identify novel disease genes.[6,8,73–75] The amount and size of homozygous segments in an individual's genome largely depend on the degree of relatedness between the parents. Children born of first-cousin marriage are predicted to have homozygous stretches covering 6.25% of their genome, but due to additional consanguineous loops in these families, homozygosity is often observed in 10% to 11% of their genome.[76] Although this percentage is much smaller in individuals whose parents are more distantly related, long stretches of homozygosity are also often present in individuals in nonconsanguineous populations.[77,78] In the 165 nonconsanguineous families, 158 probands showed at least one homozygous region in his or her genome. On average, each proband carried five significant homozygous regions with an average size of 3.8 Mb per region, corresponding to approximately 0.5% of the total genome. The homozygous regions ranged in size from 0.6 to 38.5 Mb. The number of Dutch individuals who have tracts of homozygosity in their genomes appears to be somewhat higher than in other Caucasians, whereas the size range is comparable, although the type of SNP arrays used as well as the thresholds for assigning a region as homozygous are different between our study and others.[77,79] The threshold that we used for labeling a genomic region homozygous was 250 consecutive SNPs (~1 Mb on average). Although the choice of this threshold is rather arbitrary, it appears to be a valuable cutoff for distinguishing truly homozygous regions that are identical by descent from apparent but false-positive homozygous regions caused by haplotype blocks. In only one patient, in whom an arRP gene was sequenced (*CNGA1* in patient 21211), was a heterozygous SNP identified, indicating that the region identified by homozygosity mapping was not truly homozygous. In general, the success rate of this approach depends mainly on the demographic properties of the population that is studied, and in populations with high genetic heterogeneity, this approach may not be very useful.

In total, 42 of the 186 probands harbored a known arRP gene in one of their homozygous regions harboring a known recessive RP gene. In half (21/42 probands) of those cases, the causative mutation indeed was identified within these genes, whereas for two variants, the pathogenicity was debatable. These results illustrate that once a homozygous region is identified that overlaps with a known arRP gene, there is a reasonable chance that the causative mutation will be identified in the corresponding gene. Since most of the mutations that were identified were novel, those would not have been identified using an array-based approach that analyzes only known arRP mutations (e.g., APEX-based analysis).[5,80]

Besides the 42 probands for which homozygous regions overlapped with known arRP genes, 137 probands (or multiplex families) carried homozygous regions not harboring any of the known genes. These data have already aided the identification of *EYS, C2ORF71*, and *IMPG2* as three new genes associated with arRP.[6,73,74] An interesting challenge that remains is how to solve the genetic puzzle of the remaining families. We have just entered an era in which next-generation sequencing (NGS) applications will become state of the art and have already proven their enormous potential for the identification of novel disease genes, using both unbiased sequencing of all exons in the genome[81,82] and targeted approaches in which linkage intervals or specific genomic regions were analyzed in a high-throughput manner.[83,84] NGS technology, by designing targeted arrays that will enrich DNA of all known retinal dystrophy genes or by performing genome-wide exome sequencing, will be instrumental in identifying the causative alleles in the remaining patients of our cohort, both with homozygous and compound heterozygous mutations. However, as long as the costs of NGS efforts are considerable, a rapid introduction of these methods in routine diagnostics is unlikely to occur. Therefore, homozygosity mapping has proven itself and will remain a cost- and time-effective way of identifying genetic defects in patients with arRP in The Netherlands and in many other populations with a comparable demographic structure.

In conclusion, this study revealed the power of high-resolution homozygosity mapping as an initial step in locating the position of the genetic defect in recessive RP patients from nonconsanguineous populations. Combined with other novel technologies, it will ensure a rapid identification of the causative mutations in many patients with RP. With such advances, these patients will benefit in the near future by becoming eligible for genetic therapies that are now rapidly being developed.

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