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Exome sequencing identifies *RDH12* compound heterozygous mutations in a family with severe retinitis pigmentosa

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ABSTRACT

Objective: Retinitis pigmentosa (RP) is the most prevalent type of inherited retinal degeneration and one of the commonest causes of genetically determined visual dysfunction worldwide. To date, approximately 35 genes have been associated with nonsyndromic autosomal recessive RP (arRP), however the small contribution of each gene to the total prevalence of arRP and the lack of a clear genotype–phenotype correlation complicate the genetic analysis in affected patients. Next generation sequencing technologies are powerful and cost-effective methods for detecting causative mutations in both sporadic and familial RP cases.
Methods: A Mexican family with 5 members affected from arRP was studied. All patients underwent a complete ophthalmologic examination. Molecular methods included genome-wide SNP homozygosity mapping, exome sequencing analysis, and Sanger-sequencing confirmation of causal mutations.
Results: No regions of shared homozygosity among affected subjects were identified. Exome sequencing in a single patient allowed the detection of two missense mutations in the *RDH12* gene: a c.446T>C transition predicting a novel p.L149P substitution, and a c.295C>A transversion predicting a previously reported p.L99I replacement. Sanger sequencing confirmed that all affected subjects carried both *RDH12* mutations.
Conclusions: This study adds to the molecular spectrum of *RDH12*-related retinopathy and offers an additional example of the power of exome sequencing in the diagnosis of recessively inherited retinal degenerations.

1. Introduction

Retinitis pigmentosa (RP) is an inherited retinal disorder in which progressive loss of photoreceptors, predominantly rods, leads to night blindness, peripheral visual field loss, and, at later stages, to irreversible blindness. Fundus examination findings in affected individuals include bone-spicule pigmentation, retinal blood vessel attenuation, and waxy pallor of the optic disk (Berson, 1993). The disease has an estimated prevalence of 1 in 3500–4000 (Bunker et al., 1984) and is a prototypic example of a genetically heterogeneous disorder as autosomal dominant, autosomal recessive, X-linked, or mitochondrial modes of inheritance can be observed in affected families (Hartong et al., 2006). To date, about 60 genes involved in human non-syndromic RP have been recognized (RetNet, at https://sph.uth.edu/retnet).

The most common and genetically heterogeneous RP subtype is the autosomal recessive form (arRP), with more than 35 genes implicated. Nonetheless, this group of identified genes is estimated to account for only 30 to 60% of cases with this mode of inheritance (Daiger et al., 2007; den Hollander et al., 2010). Due to this extraordinary locus heterogeneity, complete sequence analysis of all known arRP genes is time consuming and expensive and therefore, several approaches have been developed to uncover the causal mutation in familial and sporadic cases of the disease. For example, about one third of arRP genes were discovered by homozygosity mapping combined with a candidate gene selection approach (Bandah-Rozenfeld et al., 2010; Collin et al., 2011).

In arRP pedigrees that are not suitable for linkage mapping or in sporadic cases of unknown mode of transmission, next generation sequencing methodologies offer efficient strategies to identify the genetic basis of the disease (Tucker et al., 2011; Wang et al., 2012; Zuchner et al., 2011).

Abbreviations: A, adenine; arRP, autosomal recessive retinitis pigmentosa; Bp, base pairs; BRLMM, Bayesian robust linear model with Mahalanobis distance classifier; C, cytosine; DNA, desoxirribonucleic acid; ERG, electroretinogram; FAG, fluorescein retinal angiography; GCOS, GeneChip Operating Software; I, isoleucine; L, leucine; LCA, Leber congenital amaurosis; LM-PCR, ligation mediated-PCR; Logmar, logarithm of the minimum angle of resolution; MgCl2, magnesium chloride; NAD(P), nicotinamide adenine dinucleotide phosphate; Ng, nanogram; OCT, optical coherence tomography; P, proline; PCR, polymerase chain reaction; PolyPhen2, polymorphism phenotyping 2; RDH12, retinol dehydrogenase 12; RP, retinitis pigmentosa; SIFT, sorting intolerant from tolerant; SNP, single nucleotide polymorphism; T, thymine; μl, microliter.

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Here, we employed whole-exome sequencing to uncover the genetic cause of arRP in an inbred Mexican family in which homozygosity mapping failed to identify the disease locus. Compound heterozygosity for *RDH12* gene mutations was recognized as the origin of the disease. Our results show that exome sequencing is a powerful approach for molecular diagnostics of arRP families where autozygosity fails to identify the disease locus due to allelic heterogeneity.

2. Materials and methods

2.1. Subjects

The study was approved by the Institutional Review Board of the Institute of Ophthalmology "Conde de Valenciana", at Mexico City. All patient samples were collected with written informed consent and clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. A three generation family was studied and although the parents of affected subjects in the third generation were not aware of a common ancestry, they originate from the same small isolated village (currently ~8000 inhabitants), making a distant relationship possible. In addition, the absence of cases in previous generations, the consanguinity (first cousins) of the asymptomatic parents of an affected subject in the fourth generation IV-7 (Fig. 1), and the fact that males and females were diseased, strongly suggested autosomal recessive inheritance of RP.

Ophthalmologic examination of four affected siblings (III-1, III-2, III-3, and III-6, Fig. 1) and their parents (II-5 and II-6) and of the one affected nephew in the fourth generation (IV-7) included best-corrected visual acuity, slit-lamp biomicroscopy, fundus examination and photography, fundus fluorescein angiography, optical coherence tomography (OCT) for assessment of retinal thickness, and full-field flash electroretinograms (ERGs). ERGs were recorded following the standards of the International Society for Clinical Electrophysiology of Vision. Scotopic rod-driven responses and cone-driven photopic single flash and 30 Hz flicker stimuli were recorded sequentially.

2.2. Whole-genome SNP homozygosity mapping

A genome-wide linkage scan using an Affymetrix 250K NspI single nucleotide polymorphism (SNP) mapping array (Affymetrix, Inc., Santa Clara, CA) was undertaken to identify shared regions of homozygosity, as previously described (Zenteno et al., 2011). Briefly, 250 ng of pooled genomic DNA (83 ng from each of patients III-3, III-6, and IV-7, in Fig. 1) were first digested with the NspI restriction enzyme (New England Biolabs, Boston, MA) and then ligated to adaptors. Each NspI adaptor-ligated DNA was amplified in three 100 μl PCR reactions using AmpliTaq Platinum (Clontech Laboratories, Inc., Palo Alto, CA). Fragmented PCR products were then labeled, denatured and hybridized to the array following washing and staining steps on the Affymetrix GeneChip fluidics station 450. Fluorescence intensities were quantified with an Affymetrix array scanner 3000-7G and the data were collected by the Affymetrix GeneChip Operating Software (GCOS) v 1.4. Genotypes were generated using the GTYPE software for BRLMM analysis using default settings. The HomozygosityMapper software (www.homozygositymapper.org) was used to analyze the genotypes and for the identification of potential region(s) harboring the disease-associated gene (Seelow et al., 2009). Candidate regions within intervals >2 Mb were identified using GeneDistiller software (Seelow et al., 2008), available at www.genedistiller.org.

2.3. Exome capture and sequencing

Exome sequencing was performed on a single RP patient from this family (IV-7) by Ambry Genetics, Aliso Viejo, CA, USA. Samples were prepared using Illumina's protocol TruSeq DNA Sample Preparation Guide. Briefly, samples were sheared to an average size of 300–400 bp using sonication. DNA fragment ends were repaired and phosphorylated using Klenow, T4 DNA Polymerase and T4 Polynucleotide Kinase. Next, an 'A' base was added to the 3′ end of the blunted fragments, followed by ligation of Illumina Paired-End adapters via T-A mediated ligation. From here, samples were prepared using the NimbleGen protocol outlined in "NimbleGen SeqCap EZ Exome Library SR User's Guide" (Version 3.0). The libraries were amplified using LM-PCR and 1ug of amplified sample libraries were hybridized with Nimblegen's Exome Library baits for 64 h at 47 °C. Captured DNA was then washed and recovered using Streptavidin Dynabeads. The captured DNA was LM-PCR amplified for a total of 17 cycles. The amplified capture DNA library size and concentration were determined using an Agilent bioanalyzer. The captured library was then loaded on a HiSeq 2000 platform for sequencing with a mean exome coverage of 30×. Raw image files were processed by Illumina Pipeline v1.7 for base calling. SNPs and indels were called using an in-house developed software (Ambry Genetics). Identified variants were filtered against the Single Nucleotide Polymorphism database (dbSNP 129, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 genomes project (www.1000genomes.org), and Exome Variant Server (http://evs.gs.washington.edu/EVS/) databases.

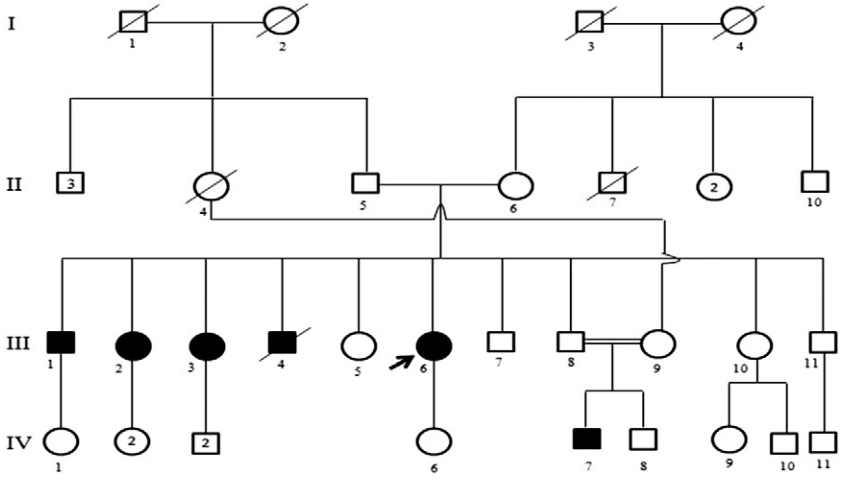

Fig. 1. Genealogy of the RP family showing the segregation of the p.L99I and p.L149P RDH12 mutations. Solid symbols designate affected subjects. Arrow indicates the propositus and slash indicates deceased individuals. Note that III-8 and III-9 are first cousins.