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Whole Exome Sequencing Reveals a Mutation in *CRYBB2* in a Large Mexican Family with Autosomal Dominant Pulverulent Cataract

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Key Words
Autosomal dominant pulverulent cataract · Clinical heterogeneity · *CRYBB2* · *CRYBB2P1* · Whole exome sequencing

Abstract
Congenital cataract, an important cause of reversible blindness, is due to several causes including Mendelian inheritance. Thirty percent of cataracts are hereditary with participation of the gamma crystallin genes. Clinical and genetic heterogeneity is observed in patients with gene mutations and congenital cataract; about 40 genetic loci have been associated with hereditary cataract. In this study, we identified the underlying genetic cause of an autosomal dominant pulverulent cataract (ADPC) in a large Mexican family. Twenty-one affected patients and 20 healthy members of a family with ADPC were included. Genomic DNA was analyzed by whole exome sequencing in the proband, a normal daughter, and in an affected son, whereas DNA Sanger sequencing was performed in all members of the family. After the bioinformatics analysis, all samples were genotyped using Sanger sequencing to eliminate variants that do not cosegregate with the cataract. We observed a perfect cosegregation of a nonsense mutation c.475C>T (p.Q155*) in exon 6 of the *CRYBB2* gene with ADPC. We calculated a logarithm of the odds score of 5.5. This mutation was not detected in healthy members of the family and in 100 normal controls. This is the first Mexican family with ADPC associated with a p.Q155* mutation. Interestingly, this specific mutation in the *CRYBB2* gene seems to be exclusively associated with pulverulent/cerulean cataract (with some clinical variability) independent of the population's genetic background.

A cataract is any opacity of the crystalline lens. There are many causes for cataracts, but they are normally associated with the breakdown of the lens architecture. It is generally thought that mutations in the crystalline or other lens proteins cause protein aggregation and, consequently, opacity of the crystalline lens [Shiels and Hejtmancik, 2007]. Three major crystalline lens components are α-, β-, and γ-crystallins. β-crystallin is subdivided into acidic (βA1, βA2, βA3, and βA4) and basic (βB1, βB2, and βB3) proteins. β-Crystallins form heterogeneous oligomers in the lens [Liu and Liang, 2005]. Hereditary congenital cataracts (HCCs) tend to be inherited in a Mendelian fashion, but they are clinically and genetically heterogeneous with high penetrance [Shafie et al., 2006; Bateman et al., 2007]. HCCs represent 10% of the treatable causes of childhood blindness [Lund et al., 1992; Gilbert et al.,

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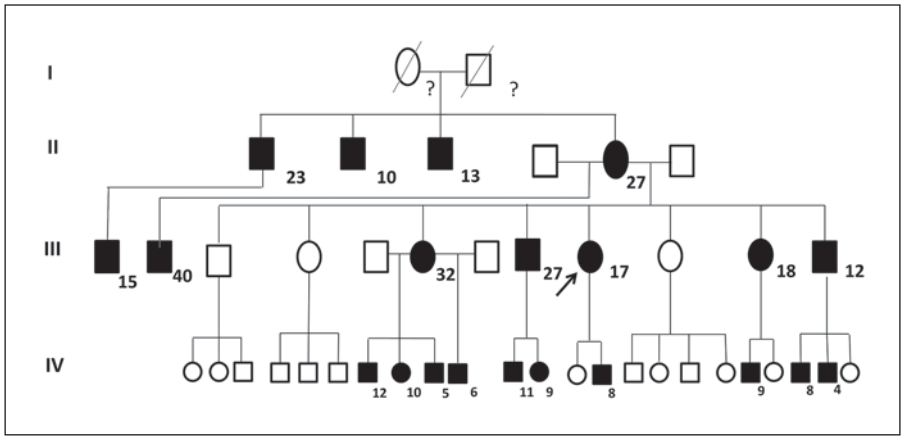

Fig. 1. Pedigree of the family showing affected patients with autosomal dominant pulverulent cataract (black symbols) and unaffected subjects (open symbols). The arrow indicates the proband. A diagonal line indicates that the person is deceased. Numbers indicate the age at diagnosis or at surgery.

1993]. In developed countries, the prevalence of HCCs ranges from 0.63 to 13.6 per 10,000 births [Stoll et al., 1992; Bermejo and Martinez-Frias, 1998; Muñoz and West, 2002; Bhatti et al., 2003]. Thirty percent of cataracts are hereditary with a predominance of the nonsyndromic autosomal dominant forms [Rahi and Dezateux, 2000]. Currently, there are over 40 genetic loci which have been associated with HCCs [Hejtmancik, 2008]. Mutations in the *CRYBB2* gene (OMIM 123620) have been associated with HCCs. Presently, there are 12 missense mutations [Santhiya et al., 2004, 2010; Pauli et al., 2007; Lou et al., 2009; Mothobi et al., 2009; Wang et al., 2011; Yao et al., 2011; Weisschuh et al., 2012; Chen et al., 2013; Faletra et al., 2013; Gillespie et al., 2014; Sun et al., 2014] and 2 nonsense mutations, p.Q155* [Litt et al., 1997; Gill et al., 2000; Vanita et al., 2001; Yao et al., 2005; Bateman et al., 2007; Devi et al., 2008; Li et al., 2008; Wang et al., 2009] and p.Y159* [Hansen et al., 2009] (table 1). Both types of mutation present clinical variability [Shiels et al., 2010]. The *CRYBB2* gene belongs to the β-crystallin family together with 7 other genes (4 *CRYBA* and 3 *CRYBB*) in different chromosomes. Four β-crystallin genes *(CRYBB1*, *BB2*, *BB3*, and *BA4)* are expressed in the eye. There is one pseudogene *(CRYBB2P1)* that is transcribed in several tissues, except in the eye, but there are no proteins associated with this pseudogene (UCSC Genome Bioinformatics Group). The *CRYBB2* gene encompasses 6 exons with the start of the translation in the second exon. This exon encodes the NH-2 terminal site and the other 4 exons encode one Greek key motif each [Inana et al., 1983]. The N- and C-terminal regions of the β-crystallin [Lubsen et al., 1988] are postulated to be essential for the maintenance of lens transparency [Bax et al., 1990; Norledge et al., 1997]. The *CRYBB2* gene has 97% whole homology with the pseudogene *CRYBB2P1* and 95% homology with exon 6. Whole exome sequencing (WES) has been successfully applied for molecular characterization of heterogeneous conditions and in the identification of novel genes [Bamshad et al., 2011; Goh and Choi, 2012]. Since the specific cataract phenotype is typically not sufficient to predict which gene is mutated in a family [Hejtmancik, 2008], WES represents an efficient method of screening to identify affected genes in congenital cataracts. In the present study, we analyzed an extensive Mexican pedigree in a family affected by autosomal dominant pulverulent cataract (ADPC).

Material and Methods

Patients

Twenty-one affected patients and 20 healthy members of a family with ADPCs (fig. 1) were recruited from the Ophthalmology Service of the General Hospital of Mexico. All patients presented with pulverulent cataracts and underwent surgery at different ages (figs. 1, 2). Genomic DNA was analyzed by WES in the proband, normal daughter, and an affected son, whereas DNA sequencing was performed in all members of the family.

Whole Exome Sequencing

Two μg of genomic DNA were submitted to Axeq Technologies (Rockville, Md., USA) for human exome capture using the SureSelect Human All Exon kit (Agilent Technologies, Santa Clara, Calif., USA). Axeq Technologies performed sample validation, library preparation, exon enrichment, clustering and sequencing using an Illumina HiSeq 2000 Sequencer (Illumina Inc., San Diego, Calif., USA). Approximately 63,000,000 reads of an average size of 107 bp per sample were returned as 2 FASTQ files (one file per orientation). Each pair of FASTQ files was aligned to the human genome (hg19) using the Novoalign mapping tool (http://www.novocraft.com). All parameters were kept at the default settings, as recommended by Novocraft. SAMtools (http://samtools.sourceforge.net/) [Li et al., 2009] was used to sort the SAM files, create BAM files and generate their index files. Picard (http://picard.sourceforge.net/) was used to remove all the PCR duplicates from the BAM files. The Genome Analysis Toolkit (GATK) [McKenna et al., 2010] was used for local

Fig. 2. Sample photographs of the pulverulent/cerulean cataract of the family.

realignments, base quality recalibration and variant calling. Parameters were set as described in GATK's Best Practices v3. GATK generated standard variant call format files (http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcf-variant-call-format-version-41). These files were annotated using snpEff (snpeff.sourceforge.net) [Cingolani et al., 2012] and ANNOVAR [Wang et al., 2010]. From this point on, we focused our analysis only on the putative coding of nonsynonymous variants. We removed false positive calls by using a post-calling filter that ensures that each variant has a mapping quality >30, a base quality >20, and a coverage ≥10, so that the variant in reads from both orientations is present. Common variants were removed using allele frequency information from the NHLBI ESP (http://evs.gs.washington.edu/EVS). A common variant was defined as a variant present in the ESP with a minor allele frequency of more than 1%. Every variant was annotated for potential functional effects in the protein using 3 variant effect predictors: SIFT [Kumar et al., 2009], PolyPhen-2 (Prediction of functional effects of human nsSNPs; http://genetics.bwh.harvard.edu/pph2) [Adzhubei et al., 2010], and MutationTaster (www.mutationtaster.org) [Schwarz et al., 2010].

DdeI Digestion, PCR Amplification, and DNA Sequencing of Exon 6 in the CRYBB2 Gene

CRYBB2 and *CRYBB2P1* genes share a high level of homology. To ensure specific amplification of exon 6 of the *CRYBB2* gene, we developed a test [Gill et al., 2000]. It consists of digesting the genomic DNA before amplification with the restriction enzyme *Dde*I. *Dde*I disrupts exon 6 of the *CRYBB2* pseudogene and interrupts its amplification. Specifically, we digested 400 ng genomic DNA with 3 units *Dde*I (New England Biolabs, Beverly, Mass., USA) in a total volume of 10 µl. After an overnight incubation at 37°C, we amplified 100 ng of the *Dde*I-digested genomic DNA using the following conditions: (a) forward primer 5′-CTATCTCTCTCCCCTCGCCTCT-3′, (b) reverse primer 3′-CTAGTTGGAGGGGTGGAAGGCACC-5′, (c) 1.5 mM MgCl2, (d) PCR buffer (GeneAmp II; Perkin-Elmer, Norwalk, Conn., USA), (e) 5% dimethyl sulfoxide (DMSO), (f) 1 unit DNA polymerase (AmpliTaq, Perkin-Elmer, Norwalk, Conn., USA), (g) 200 mM dNTPs, and (h) 50 ng exon 6 primer in a final PCR volume of 20 µl. The hot-start addition of DNA polymerase, after an initial 3 min denaturation (94°C), was followed by 35 cycles of 30 s at 94°C, 35 s at 58°C, and 40 s at 72°C, and it was completed with an 8-min extension at 72°C in a thermocycler (Robocycler Gradient 96; Stratagene; LaJolla, Calif., USA). Amplicons were analyzed with ABI BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems, Foster City, Calif., USA) on an ABI3100 Genetic Analyzer (Applied Biosystems). Detected variants were further sequenced in affected and nonaffected members of the family and in 100 normal controls.

Results

The initial raw variant call format contained 195,062 putative variants. We isolated 11,502 high-quality, nonsynonymous putative coding variants using our quality filter in conjunction with the annotation tags generated by snpEff [Cingolani et al., 2012]. A similar number of nonsynonymous putative coding variants were isolated by using our quality filters and ANNOVAR [Wang et al., 2010]. The results from WES quickly and efficiently narrowed down the number of variants to 3 possible damaging mutations in genes, *GJA3*, *CRYBA*, and *CRYBB2*. At this point, we validated the variants by Sanger sequencing in all members of the family. After performing the sequencing screening in the 21 affected patients and 20 healthy members of the family, we identified that only the mutation p.Q155* in the gene *CRYBB2* cosegregated perfectly with the disorder (fig. 3), unlike in the case of the *GJA3* and *CRYBA* gene variants. To obtain a statistical value for the segregation, we calculated the logarithm of the odds score (LOD). We used a multipoint parametric linkage analysis, Merlin [Abecasis et al., 2002]. We applied an autosomal dominant of inheritance, assuming a disease allele frequency of 0.0001. We obtained the significant LOD score of 5.5. In terms of significance, an LOD score of 5 means the odds are 100,000:1 that the genes and the disorder are linked. DNA sequencing analysis confirmed the WES finding (fig. 4).

Discussion

A congenital cataract presents genetic heterogeneity and can be inherited in one of the 3 Mendelian patterns (autosomal dominant, autosomal recessive, and X-linked) with at least 155 reported disease-causing gene mutations

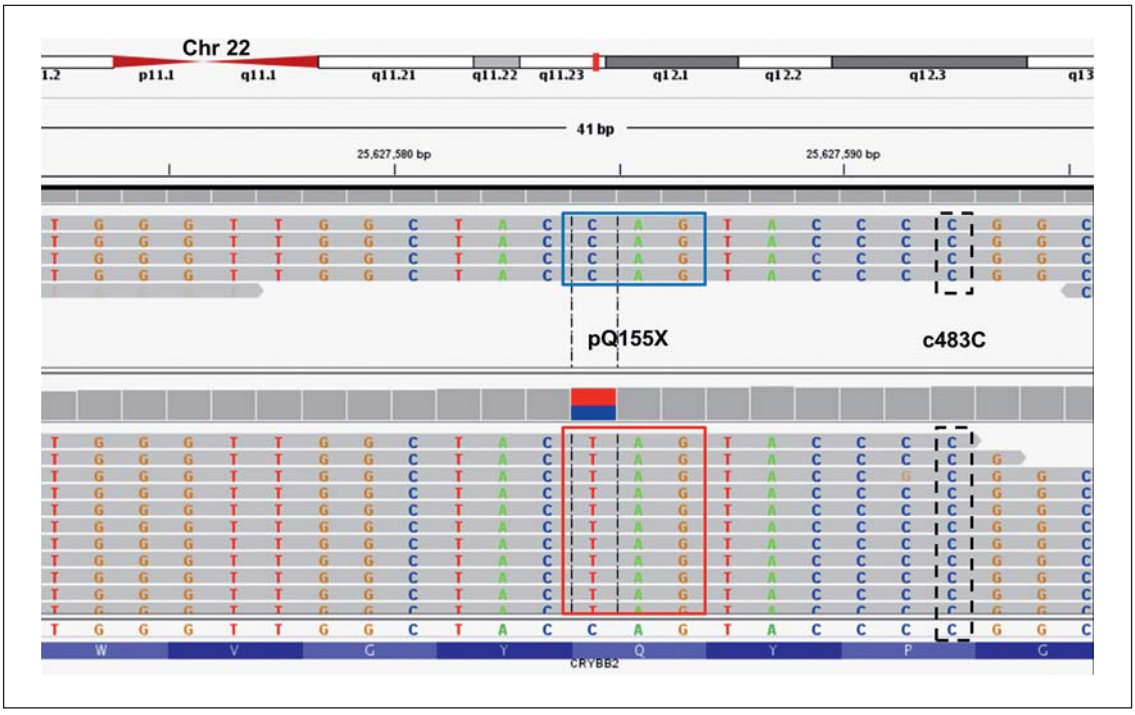

Fig. 3. WES with the heterogeneous change (C and T nucleotides).

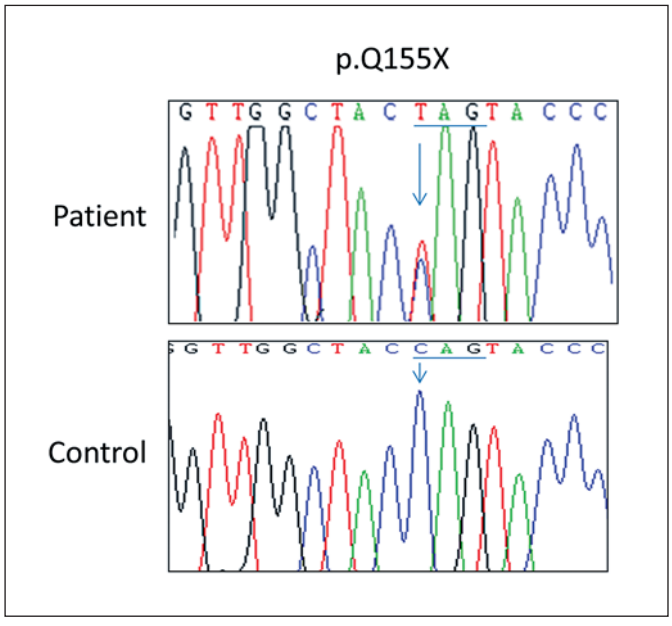

Fig. 4. DNA sequencing analysis confirming the heterozygous change of c.475C>T after digesting with restriction enzyme *Dde*I. Normal control shows the homozygous state due to elimination of the *CRYBB2P1* pseudogene.

[Huang and He, 2010]. For the ADPCs presented by the Mexican family in this study, WES identified the c.475C>T mutation (p.Q155*) in exon 6 in the *CRYBB2* gene. In addition, the variants c.895C>A (p.L299M) in *GJA3* and c.331A>C (p.I111L) in *CRYGB* were also detected. These variants were considered polymorphisms because they were detected in the SNP data base (rs968566 in the *GJA3* gene and rs796287 in the *CRYGB* gene; http://www.ncbi.nlm.nih.gov/snp) and were present in some nonaffected members and absent in some affected members of the family. The nonsense c.475C>T (p.Q155*) mutation has been reported previously in 5 different populations [Litt et al., 1997; Gill et al., 2000; Vanita et al., 2001; Yao et al., 2005; Bateman et al., 2007; Devi et al., 2008; Li et al., 2008], and it predicted a stop codon (p.Q155*) with a loss of 51 amino acids and the 4 Greek key domains in the C-terminal region of the CRYBB2 protein. It is very interesting to note, in spite of the great clinical and genetic heterogeneity in congenital cataracts, that the nonsense mutation p.Q155*, with a chain termination in the human β-crystallin gene *CRYBB2,* seems to be associated exclusively with ADPC (with some clinical variability), independent of the studied population. In addition, it has been demonstrated in a mouse model that the presence of

Table 1. Mutation p.Q155X in the *CRYBB2* gene

hg19 Location	Nuc_Acc	trans_change	Protein	prot_change	rsid	pmid	Country	Reference
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	9158139	USA	Litt et al., 1997
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	10634616	Switzerland	Gill et al., 2000
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	11424921	India	Vanita et al., 2001
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	17234267	Chile	Bateman et al., 2011
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	18587492	India	Devi et al., 2008
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	18449377	China	Li et al., 2008
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	19321936	China	Wang et al., 2009
chr22:25627584C>T	NM_000496.2	c.463C>T	NP_000487.1	p.Q155*	rs74315489	16179907	China	Yao et al., 2005

hereditary cataracts are due to a mutation consistent in the deletion of 4 amino acids near the C-terminus of β-B2-crystallin [Chambers and Russell, 1991]. Normal oligomerization of β-B2-crystallin is not possible due to the lack of these amino acids involved in the stabilization of the native tertiary structure of β-B2-crystallin of the bovine lens [Bax et al., 1990]. Protein-protein interaction, ordered structure and stability which reduced the cause of changes in biophysical properties were also observed with the p.Q155* mutation [Liu and Liang, 2005].

Previous reports indicated that gene conversion seemed to be the cause of cerulean cataract in 2 families due to a second variant (c.483C>T) in exon 6 of the *CRYBB2* gene [Bateman et al., 2007]. The rest of the families only describe the point mutation c.475C>T, similar to our family. We excluded the possibility of gene conversion because the second variant c.483C>T was not identified.

The families with cataracts and mutations in the *CRYBB2* gene have clinical variability with different ages of onset. In a previous report, the authors speculated on the cataracts with the c.475C>T mutation and proposed that they may be influenced by the modifiers' *cis* genetic factors among families and *trans* genetic and epistatic factors within each family [Vanita et al., 2001]. More studies are necessary to consider the influence or other factors apart from p.Q155* in patients with congenital cataracts. Moreover, due to the genetic heterogeneity and the great number of genes involved in HCCs, WES is an important tool because it provides high coverage to detect variants in genes associated with the disease. A successful model to identify disease-causing genes in HCCs implicates the use of trio sequencing (proband and parents) to be genotyped.

In conclusion, this is the first Mexican family with ADPCs associated with a p.Q155* mutation in the *CRYBB2* gene. Interestingly, this specific mutation in the *CRYBB2* gene seems to be associated exclusively with pulverulent/cerulean cataracts (with some clinical variability) independent of the population's genetic background. In addition, this study verifies that WES has become a more powerful tool for the detection of genetic origins of cataracts because of its high genetic and clinical heterogeneity.

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Statement of Ethics

Written informed consent was obtained from the participants or their parents prior to the study. The process followed the tenets of the Declaration of Helsinki and followed the Guidance of Sample Collection of Human Genetic Diseases of the Ethics Committee of the General Hospital of Mexico.

Disclosure Statement

The authors report no conflicts of interest.

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