A novel mutation in the connexin 46 gene (GJA3) causes autosomal dominant zonular pulverulent cataract in a Hispanic family


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Purpose: A five-generation Hispanic pedigree with autosomal dominant zonular pulverulent cataract was studied to identify the causative mutation in connexin 46 (Cx46), a gap junction protein responsible for maintaining lens homeostasis.

Methods: Twenty-six individuals from the family were comprehensively clinically examined. DNA was extracted from their peripheral blood samples. The DNA was used for automated genotyping with fluorescently labeled microsatellite markers and for mutation detection by automated sequencing.

Results: A novel D3Y missense mutation in GJA3 segregated with autosomal dominant (AD) zonular pulverulent cataract throughout the family. The mutation was absent in the unaffected individuals in the family and in 230 control chromosomes.

Conclusions: A novel mutation causing AD zonular pulverulent cataract has been identified in a Hispanic Central American family. This is the first report of a mutation in GJA3 causing autosomal dominant congenital cataract (ADCC) in this ethnic group. It is also the first reported cataract-causing mutation in the NH2-terminal region of the Cx46 protein.

Cataract, opacification of the crystalline lens of the eye, is the most common cause of blindness in the world [1]. The World Health Organization estimates that 45 million people in the world are blind, 19 million of them as a result of cataract [1]. Cataracts may be broadly divided into adult onset and childhood onset (either congenital or infantile). Congenital cataract is defined as cataract which is present from birth and is responsible for approximately one-tenth of worldwide childhood blindness [2]. The incidence of congenital cataract is between 2.2 and 2.49 per 10,000 live births [3,4]. About one third of isolated congenital cataracts are familial [5], the most common mode of inheritance being autosomal dominant [4,5]. Fourteen genes and at least six additional loci have been implicated in autosomal dominant congenital cataract (ADCC) [6]. The genes that have been identified comprise seven crystallins [7-18], three transcription factors [19-21], one cytoskeletal protein [22], and three transmembrane proteins [23-34]. The transmembrane proteins consist of connexin 46 (Cx46) [23,26-30,32], connexin 50 (Cx50) [24,31,33,34], and major intrinsic protein of the lens (MIP) [25].

Cx46 is a member of the connexin family of proteins. The connexins comprise proteins important for the formation of gap junction channels. Connexin proteins form hexamers known as connexons in cell membranes. Connexons in neighboring cells dock to form gap junctions which allow the transport of small metabolites between cells [35]. In humans, at least 20 connexin genes have been associated with several different diseases including genetic deafness, skin disease, peripheral neuropathies, heart defects and cataracts [36]. The lens expresses three distinct connexins, connexin 43 (Cx43), Cx46, and Cx50, all of which appear to have different functions in maintaining lens homeostasis [36]. The lens is an avascular structure and lens fibers lose all intracellular organelles during development. The lens has therefore developed an extensive intercellular communication system using gap junctions to maintain tissue homeostasis and hence transparency [37]. Cx43 is expressed mainly in the lens epithelial cells, while Cx46 and Cx50 are expressed in lens fiber cells [38-40]. Hence, mutations in Cx46 and Cx50 may lead to congenital cataracts.

Pulverulent cataracts have a pulverized (powdery) appearance to opacification [41]. “Zonular pulverulent cataract” is a term which has been used to describe pulverulent cataracts which involve the nucleus minimally but markedly affect lamellar regions beyond it. AD zonular pulverulent cataracts have been described in association with mutations in GJA3 [30,32], GJA8 [24,31,33], and CRYGC [16] genes.

A linkage approach was used to investigate the known cataract genes and loci in a large Hispanic pedigree from Honduras with zonular pulverulent cataract with the aim of identifying the causative mutation. A novel causative mutation in the connexin 46 gene (GJA3) was identified.

METHODS

Patient ascertainment and collection of genetic material: A five-generation pedigree from Honduras with zonular pulverulent cataract and without gross chromosomal abnormalities was identified. Written informed consent for molecular studies, ethically approved by the Institutional Review Board of Self Regional Healthcare IRC, Greenwood, SC, was obtained from all individuals involved in the study. Both affected and unaffected individuals underwent full ophthalmic and clinical
examination. Peripheral blood samples were collected from which DNA was extracted for subsequent molecular genetic analysis.

**Linkage analysis:** Individuals were genotyped using fluorescently labeled microsatellite markers at known cataract loci. Alleles were assigned after the analysis of the PCR products on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK). Two point LOD scores were calculated using the FASTLINK package [42]. The pedigree was constructed using Cyrillic version 2.1.3 software (FamilyGenetix Ltd., Oxford, UK).

**Sequencing:** The entire coding region of GJA3 was sequenced using the same pairs of overlapping primers as used by Jiang et al. [28]. PCR products, amplified using ReddyMix PCR master mix (Abgene, Epsom, UK), were cycle sequenced with Big Dye Terminator Ready Reaction Mix (Applied Biosystems) and analyzed on an ABI PRISM 3100 DNA sequencer (Applied Biosystems).

**RESULTS**

The relevant part of the pedigree is shown in Figure 1. AD inheritance is supported by the presence of affected individuals in each of the five generations, equal numbers of affected males and females, and male-to-male transmission. Zonular pulverulent cataract (Figure 2) was fully penetrant and exhibited some variable expressivity. There were no other ocular or systemic abnormalities. Bilateral zonular pulverulent cataracts typically presented in the first few months of life and progressed to total opacity over time. Visual acuities of affected individuals at diagnosis ranged from 6/18 to counting fingers. In many cases, there was amblyopia. To date, almost all of the affected individuals have had cataract surgery.

Twenty-six members of the family, including 16 affected individuals and 10 unaffected individuals, were genotyped. The initial approach was to exclude all known cataract loci. Candidate loci implicated in ADCC (1p36, GJA8, CRYGC, CRYGD, BFSP2, and PITX3) were excluded by linkage analysis (LOD scores less than 1 at $\theta=0$). A LOD score of 2.53 was
obtained at θ=0 for D13S175, a marker genetically close to GJA3. The LOD score was not above 3 since this marker was relatively uninformative in the family. Haplotyping refined the region of possible linkage (Figure 1) as being between the tip of chromosome 13q above and the marker D13S1275 (the lower crossover) below. It was decided that the LOD score of 2.53 for marker D13S175 together with correlation between the family’s phenotype and the phenotype typical of cataracts associated with GJA3 mutations warranted screening of this gene.

GJA3 (GenBank NM_021954) was sequenced in both affected and unaffected individuals. The variant 7G→T, causing a novel heterozygous missense mutation D3Y, was identified in all 16 affected individuals but in neither 10 unaffected individuals nor 230 control chromosomes from an ethnically mixed panel with a high proportion of Hispanic individuals. The aspartate residue is conserved across species represented in GenBank (Figure 3). Its substitution by a tyrosine residue is therefore consistent with a significant change in the protein.

DISCUSSION

The investigation of this large Hispanic cataract pedigree has revealed a novel mutation, D3Y, in GJA3. The D3Y mutation is likely to be causative since it segregates with affected status throughout the pedigree and is absent both in unaffected individuals within the pedigree and in unaffected, unrelated controls. The mutation changes a negatively-charged amino acid aspartate (D) to an uncharged amino acid tyrosine (Y).

Nine different cataract-causing mutations in GJA3 [23,26-30,32] and four different cataract-causing mutations in the connexin 50 gene [23,26-30,32] have so far been reported [24,31,33,34] (Table 1). These are the only two connexin genes expressed in lens fibers. For both of them, there is a clear genotype-phenotype relationship. There is a strong association with prevalent congenital cataracts, either predominantly in the nuclear or lamellar regions of the lens. The phenotype in the Hispanic pedigree resembles those previously reported.

GJA3 mutations have previously been reported in pedigrees of Caucasian, Chinese, and Indian ancestry. Here, a novel GJA3 mutation is reported in a family of Hispanic Central American origin. This increases the diversity of ethnic groups in which these mutations cause cataract, adding to the evidence that mutations in GJA3 are an important cause of cataract in widely different ethnic groups on a worldwide scale.

All connexins have four transmembrane domains and two extracellular loops with cytoplasmic NH2- and COOH-termini. The previously reported mutations associated with congenital cataracts are summarized in Table 1.

GJA3 encodes a 435 amino acid protein in humans and is predominantly expressed in lens fiber cells [30]. The D3 residue of GJA3 is phylogenetically conserved from zebrafish to man (Figure 3), indicating that the aspartate is likely to be functionally important and that the mutation may therefore have a detrimental physiological effect. The 7G→T change results in an aspartate to tyrosine amino acid substitution within the NH2-terminal cytoplasmic tail. This is the first reported mutation within this region of GJA3 associated with congenital cataract. The mutation leads to replacement of a negatively charged amino acid with an uncharged amino acid at position 3 in the amino terminus. Substitutions in the amino acid residues of the NH2-terminus may interfere with the conformation and flexibility of the amino terminus and also with voltage gating [43,44]. Cx46 protein functions in gap junction communication between elongated fiber cells [38], which constitute the bulk of the lens mass and represent the target cells for cataract formation. Lens fiber cells are dependent on intercellular communication for their survival [37]. Given that the mutation affects the NH2-terminal domain, it is likely that it affects intercellular communication through the gap junction channel by affecting voltage gating. Functional work is re-

<table>
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<th>Gene</th>
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<td>[16]</td>
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Previously reported mutations in connexin genes associated with congenital cataract in widely different ethnic groups on a worldwide scale.

Figure 3. Cross species alignment of Connexin 46 and other connexins. Alignment of residues 1-60 of human Cx46 with mouse, rat, and zebrafish (Danio rerio) orthologues is shown, together with human Cx43 and Cx50. The D3 residue is marked in red.
quired to confirm that this is the mechanism by which the mutation affects the protein.

In summary, a novel mutation of the human GJA3 gene has been found to segregate with zonular pulverulent cataract. This expands the spectrum of GJA3 mutations causing AD pulverulent cataract both in terms of ethnicity and in terms of location of the mutation in the NH2-terminal region of the protein.

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