CRYGD gene analysis in a family with autosomal dominant congenital cataract: evidence for molecular homogeneity and intrafamilial clinical heterogeneity in aculeiform cataract

Juan Carlos Zenteno,1,2 Maria Elena Morales,3 Veronica Moran-Barroso,4 Alejandra Sanchez-Navarro5

Departments of 1Genetics, 3Anterior Segment, 4Cornea, and the 2Research Unit, Instituto de Oftalmología Conde de Valenciana, Mexico City, Mexico; 3Department of Genetics, Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico

Purpose: To present a previously unreported four generation affected Mexican pedigree with congenital hereditary aculeiform cataract caused by a mutation in the γD-crystallin (CRYGD) gene.

Methods: A four generation family with 14 available members of whom 8 were affected was analyzed. Interventions included complete ophthalmological examination, cataract phenotype characterization, PCR amplification, and automated DNA sequencing of the 2 exons and exon/intron junctions of the CRYGD gene.

Results: A heterozygous missense mutation consisting of a G to A transition at nucleotide position 411 in exon 2 that predicts an Arg to His replacement in residue 58 (R58H) of the CRYGD protein was demonstrated. Intrafamilial clinical heterogeneity was observed as one affected member exhibited a coral-like cataract.

Conclusions: The R58H mutation described in this Mexican family is identical to that demonstrated previously in three unrelated families with aculeiform cataract, suggesting that this type of cataract has a specific molecular basis represented by the Arg to His change at residue 58 of CRYGD. However, intrafamilial clinical heterogeneity associated with this mutation can occur as evidenced by the identification of a subject in this family exhibiting a coral-like cataract. To our knowledge, this is the first example of phenotypic heterogeneity associated with the Arg 58 His CRYGD mutation.

Cataract is the most common treatable cause of visual loss in humans [1] and it may be broadly divided into early onset (congenital or juvenile) and age related cataract. Congenital cataracts are lens opacities that are present at birth or soon after birth and include hereditary cataracts or cataracts caused by infectious agents. Congenital cataracts are a significant cause of vision loss worldwide causing approximately one tenth of childhood blindness [2]. Two recent studies have estimated the incidence of congenital cataracts to be between 2.2 and 2.49 per 10,000 live births [3,4]. Roughly 50% of congenital cataracts are hereditary and familial studies have determined that approximately 30% of children with bilateral isolated congenital cataract had a genetic basis compared with 2% of unilateral cases [5]. Isolated congenital cataracts tend to be inherited in a Mendelian fashion with autosomal dominant (with high penetrance) being the most common form of familial transmission [6].

Classification of human inherited cataracts has been difficult because of the wide phenotypic spectrum observed. Attempts at classifying the distinctive lenticular morphology seen in congenital cataracts have resulted in a variety of nomenclature systems and as a result, no agreed classification exists.

Correspondence to: Juan Carlos Zenteno, MD, PhD, Departamento de Genetica, Conde de Valenciana Instituto de Oftalmología, Chimalpopoca 14, Col. Obrera, CP 06800, Mexico DF, Mexico; Phone: +(5255) 55-88-46-00, ext. 212; email: jczenteno@salud.gob.mx
METHODS

A four generation Mexican Mestizo family was referred to the Instituto de Oftalmología Conde de Valenciana (Mexico City, Mexico). Fourteen individuals participated in the study of whom 8 were affected (Figure 1). The study was approved by the institutional review board and patients gave their written consent to participate. All participants underwent full ophthalmologic examination including visual acuity testing, slit lamp examination with dilated pupils, intraocular pressure measurement, and fundus examination.

Blood samples were obtained by venipuncture, and genomic DNA was extracted using the QIAmp blood kit (Qiagen, Hilden, Germany). PCR amplification of the 3 coding exons and the intron-exon junctions of the CRYGD gene was achieved using 2 pairs of primers derived from the CRYGD sequence (Table 1). Each 25 µl PCR amplification reaction contained 1X buffer, 150 ng of genomic DNA, 0.2 mM of each dNTP, 2 U Taq polymerase, 1 mM of forward and reverse primers, and 1.5 mM MgCl2. PCR products were analyzed in 1.5% agarose gels from which the bands with the amplified templates were excised and the DNA subsequently purified with the help of the Qiaex II kit (Qiagen, Hilden, Germany).

Direct automated sequencing of CRYGD was performed with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) adding about 10 ng of template DNA in each reaction and using a temperature program which included 25 cycles of denaturation at 97 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for 4 min. All samples were analyzed in an ABI Prism 310 Genetic Analyzer (Applied Biosystems) and both DNA strands were investigated. Sequence variations were confirmed in each case using a newly PCR amplified fragment.

RESULTS

All affected family members showed a bilateral and symmetrical crystal-like cataract characterized by a centrally located crystal-like opacification originating from the fetal nucleus of the lens and radially projecting needle-like endings in all directions with a frosted or fiberglass appearance (Figure 2), a opacity phenotype identical to that previously described in aculeiform cataract [12]. The penetrance of the disease appeared to be complete and although all four generations were affected, a preponderance of affected females was observed. The cataract was a congenital cataract and there was no progressive development of lens opacities after birth. Although the needle-like endings were more conspicuous in some cases, the degree of both cataract opacification and morphology showed no significant variability among all except one affected subjects. Individual II-2 presented a lens opacity resembling a coral-like cataract as no conspicuous needle-like structures were apparent in both lenses (Figure 3). There was no family history of other ocular or systemic abnormalities and no other eye or somatic anomalies were evident on physical examination in the affected subjects. No affected individual required cataract surgery to improve their visual function.

After sequencing the entire coding region and intron-exon junctions of CRYGD in DNA from five affected patients (II:2, III:2, III:4, IV:1, and IV:3, in Figure 1), we identified a heterozygous missense mutation consisting in a G-to-A transition at nucleotide position 411 in exon 2 that predicts an Arg to His replacement in residue 58 (R58H) of the CRYGD protein (Figure 4). This mutation was absent in DNA from two unaffected relatives (III:1 and IV:2) or from 50 control alleles. As this particular mutation does not create or abolish a restriction enzyme site, all samples were directly sequenced. In their report, Héon et al. [13] did not observe this mutation in a group of 210 control individuals of various ethnic backgrounds.

![Figure 1](http://www.molvis.org/molvis/v11/a51/)

**Figure 1.** Simplified pedigree of the family with autosomal dominant aculeiform congenital cataract. Pedigree analysis of the family with congenital aculeiform cataract indicates autosomal dominant transmission of the disease. Squares and circles symbolized males and females, respectively. The open and filled symbols denote unaffected and affected members, respectively. The ages (in years) of affected subjects are shown to the left of their symbol. The diamond shaped symbol indicates a sibship of 3 subjects of unknown gender.
DISCUSSION

The genetic approach toward a better understanding of cataract formation has led to the identification of at least 17 independent autosomal dominant cataract gene loci and the characterization of 13 genes in this type of hereditary cataract [6,14]. Genes that have been previously reported to be associated with autosomal dominant congenital cataract are crystallins, connexins, cytoskeletal structure protein, and a homeobox encoding gene (for recent reviews see [6,15]). Mutations in the γ-crystallin encoding CRYG genes have been demonstrated to be the most frequent reason for isolated congenital cataracts [16]. The crystallins are proteins that constitute more than 90% of the soluble proteins in the lens and are critical to lens function, fulfilling a structural role for transparency and refraction [17]. In man, there are three classes of crystallins, the γ (CRYG), β (CRYB), and α (CRYA) that are distinguished according to the order of their elution on gel exclusion chromatography [17]. Even though the hereditary aspects of congenital cataracts have been recognized for nearly a century, at present no agreed phenotypic classification exists as a result of the wide variation observed in opacity morphologies. Aculeiform (meaning needle shaped) cataract is an uncommon form of congenital hereditary crystalline cataract originally described by Vogt in 1922 [18]. According to its appearance on slit-lamp examination and to its localization,
aculeiform cataract can be classified as a crystalline lens opacity affecting the nucleus and cortex [6]. Complete penetrance and minimal variable expressivity has been documented in most affected European and North American affected families [18,19]. This autosomal dominant type of lens opacification has been classically considered to be different from the coral-like cataract, which does not exhibit the needle-like projections. However, in the family described here with a clearly distinguishable aculeiform cataract phenotype, individual II:2 presented a opacity lacking the conspicuous needle-like projections and more compatible with a coral-like cataract [20]. Although it is possible that a second type of lens opacity could modify the aculeiform phenotype in patient II:2, no evidence of age related cataract was observed in this patient. In addition, the phenotype in patient I:1 is undoubtedly aculeiform cataract. Our data indicate that aculeiform and coral-like cataracts can coexist in a same family and that they can arise from a same genetic defect, suggesting that clinically differentiating both types of congenital opacifications is probably no longer justified. Recently, coral-like cataract has been shown to be caused by the P23T mutation in CRYGD [21,22].

In three previously described unrelated families with aculeiform cataract, Héon et al. [13] found the same G to A mutation at nucleotide 411 of CRYGD exon 2. This change, which changes arginine to histidine at residue 58 (R58H) of the protein, is identical to that encountered in the Mexican family with aculeiform cataract reported here. In conjunction, the available data strongly suggest that the CRYGD R58H mutation is exclusive of this type of lens opacification as no R58H mutation has been described to date in another type of hereditary cataract. However, as illustrated by the coral-like appearance of cataract in patient II-2, also harboring the R58H mutation, exceptions to this genotype-phenotype correlation can occur. To our knowledge, this is the first example of phenotypic heterogeneity associated with the R58H CRYGD mutation and this intrafamilial clinical variability can be attributed to the action of modifier genes and/or environmental factors. In this context, it would be interesting to analyze the probable contribution of recently identified CRYGD gene polymorphisms in modifying the final cataract phenotype. Different cataract phenotypes originated by the same molecular defect has been recently documented, for example Coppack-like cataract and cerulean cataract associated to a Q155X nonsense mutation in CRYBB2 [23] or lamellar and cerulean cataracts caused by the P23T change in CRYGD [24,25].

Recent functional studies on mutant CRYGD polypeptides carrying the R58H change have shown that they are less soluble and more prone to crystallization than the wild type protein [26]. In this context, aculeiform cataract is but one member of a group of protein condensation diseases in which the pathology is caused by a loss of solubility [26]. In summary, we present molecular data demonstrating that aculeiform cataract has a specific molecular basis represented by the R58H CRYGD mutation, the only genetic defect described so far in this type of cataract in unrelated pedigrees. However, intrafamilial clinical heterogeneity associated to this mutation can occur as evidenced by a subject in this family exhibiting a coral-like cataract, a phenotype classically considered distinct from aculeiform cataract.

ACKNOWLEDGEMENTS

The authors are grateful for the patronage of the Fundacion Conde de Valenciana for financial support.

REFERENCES