A recurrent mutation in CRYGD is associated with autosomal dominant congenital coralliform cataract in two unrelated Chinese families

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Purpose: Congenital cataract is a clinically and genetically heterogeneous lens disorder. The purpose of this study was to identify the mutation responsible for autosomal dominant congenital coralliform cataracts in two Chinese families and to investigate the relationship between virulence genes and lens morphology.

Methods: Patients received a physical examination, and blood samples were collected for DNA extraction. Mutation analysis was performed by direct sequencing of the candidate genes: gammaC-crystallin (CRYGC), gammaD-crystallin (CRYGD), gammaS-crystallin (CRYGS), gap-junction protein, alpha 8 (GJA8), gap-junction protein, alpha 3 (GJA3), and alphaA-crystallin (CRYAA).

Results: The affected individuals in two families had congenital coralliform cataracts. Mutational analysis of the CRYGD identified a C→A transversion at nucleotide position c.70 in exon 2, which resulted in a threonine substitution for proline at amino-acid residue 24 (P24T). This mutation was identified in all affected individuals but was not found in healthy relatives or 100 control chromosomes from the same ethnic background.

Conclusions: Our results indicated that the P24T mutation of CRYGD was responsible for two Chinese pedigrees with congenital coralliform cataracts. CRYGD and coralliform cataracts are highly related, and P24T may be a hot-point mutation for this disorder.

Congenital cataract is a significant cause of vision loss, resulting in approximately one third of all cases of blindness in infants. Congenital cataracts occur in approximately 1–6/10,000 of live births, and one quarter of congenital cataracts are hereditary [1-4]. Hereditary (i.e., Mendelian) cataracts are mostly inherited as autosomal dominant forms, but X-linked and autosomal recessive forms are also observed. Congenital cataracts are a clinically and genetically heterogeneous lens disorder. Phenotypically identical cataracts can result from mutations at different genetic loci and can have different inheritance patterns. While in the same genetic locus or a single large family, phenotypically different cataract types can also be found. To date, about forty genetic loci have been linked to congenital cataracts, and 26 genes have been cloned [5], including crystallins, connexins, heat shock transcription factor-4, aquaporin-0, and beaded filament structural protein-2. The types of mutations and the morphology of the cataracts are considered related [5]. In this study, we report on two congenital coralliform cataract pedigrees caused by the P24T mutation of the gammaD-crystallin (CRYGD) gene.

Methods

Patients and clinical data: Family 1 enrolled in this study was from the Inner Mongolia Autonomous Region, China. The patients had completed a cataract operation, but their vision recovery was not optimal. Family 2 was from Liaoning province, China. These patients had, in part, received cataract operations of one eye; their vision recovery was less than satisfactory. Clinical examination, peripheral blood collection, and DNA extraction were performed at the Department of Ophthalmology, Peking Union Medical College Hospital. Informed consent in accordance with the Declaration of Helsinki and the Institutional Review Board and Ethics Committee of Peking City was obtained from all participants. Family 1 included four confirmed patients, and Family 2 included seven confirmed patients (Figure 1). Clinical data for these subjects was ascertained by detailed ocular examinations.

Mutation analysis and haplotype analysis: The reported genes, gammaC-crystallin (CRYGC), CRYGD, gammaS-crystallin (CRYGS), gap-junction protein, alpha 8 (GJA8), gap-junction protein, alpha 3 (GJA3), and alphaA-crystallin (CRYAA) were selected as candidate genes. All coding exons for these genes were amplified by polymerase chain reaction (PCR) using a set of previously described paired primers [6] and presented in Table 1. The PCR products were sequenced using an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA). Microsatellite markers close to CRYGD loci
| Exon      | primer (5′-3′)                  | Product length (bp) | Annealing temperature (°C) |
|-----------|---------------------------------|---------------------|---------------------------|
| CRYGD-1–2F| AGAACACGAAAATGCCTTG            | 579                 | 60                        |
| CRYGD-1–2R| TGCTTGAAACCATCCAGTA            |                     |                           |
| CRYGD-3F  | GCTGGACCTGCTAAACAATGC          |                     |                           |
| CRYGD-3R  | CATCTTTGCTGCCATTGG            | 498                 | 58                        |
| CRYGS-1F  | TGACTAGAAACCAGCCATA            |                     |                           |
| CRYGS-1R  | TTAAGTGAAAGCAGGTTAGG           | 166                 | 58                        |
| CRYGS-2F  | AATTAAGCCACCCAGCTCCT           |                     |                           |
| CRYGS-2R  | AGCCAAGAGAAAGGAGCATG           | 356                 | 58                        |
| CRYGS-3F  | CCTCCTGTGTATTTCCAATA           |                     |                           |
| CRYGS-3R  | GATGATGCCATATGGGACAC          | 399                 | 58                        |
| GJA8–1F   | CGGGGCTCTTCTTTTCTCTAGTCC       |                     |                           |
| GJA8–1R   | AGGCCCAAGGTGGCCCAACTCC         | 750                 | 67                        |
| GJA8–2F   | CAGCCGGTGCCCTGCC               |                     |                           |
| GJA8–2R   | GGTGCGTGGAGTGAGTCGTC          | 770                 | 66                        |
| GJA3–1F   | CGCTGTTACATGACATTTTC          | 496                 | 58                        |
| GJA3–1R   | GACGTAGTGCCTGGCAAGCAG         |                     |                           |
| GJA3–2F   | GCAGGAAATCTCCCTGC             | 532                 | 58                        |
| GJA3–2R   | GGTACAAGGCTAGCATTTGGA         |                     |                           |
| GJA3–3F   | TCCTGGTTACCCACCTACTAT         | 579                 | 58                        |
| GJA3–3R   | TGACATTTTGTTGGTGGTTT          |                     |                           |
| CRYAA-1 F | CACGCCTTTCCAGAGAAATC          | 466                 | 58                        |
| CRYAA-1 R | CTCTGCAAGGGGATAGTG            |                     |                           |
| CRYAA-2 F | CTTGAGTGTGGAGGAAGAGG          | 377                 | 58                        |
| CRYAA-2 R | TCCCTCTCCAGGGTTGAAG           |                     |                           |
| CRYAA-3 F | CCCCCCTCTTGCAGTCTG            | 989                 | 58                        |
| CRYAA-3 R | GCTTGGCTTCCAGAGAAAGGGA        |                     |                           |
| CRYGC-1–2F| GCAGTATGTTACAGGAGCAGG          |                     |                           |
| CRYGC-1–2R| CCTCCTGTAACCCACATTTG          | 644                 | 58                        |
| CRYGC-3F  | ATCCATGCCCACCTACCA            |                     |                           |
| CRYGC-3R  | CCCACCCCATTCCACTCTTTA         | 527                 | 58                        |
were selected. PCR products from each DNA sample were separated using an ABI 3730XL DNA Analyzer (PE Biosystems).

RESULTS

Clinical findings: All patients enrolled in this study were afflicted with coralliform congenital cataracts (Figure 2), and none of the participants had any other clinical related ophthalmic syndromes.

Mutation analysis and haplotype analysis: By directly sequencing the coding region of CRYGD, we identified a C→A transversion at nucleotide position c.70 in exon 2 of CRYGD. This mutation resulted in a threonine substitution for proline at amino-acid residue 24 (P24T; Figure 3). This mutation was only identified in the patients and was not found in healthy relatives or the 100 control chromosomes from the same ethnic background. The disease haplotype cosegregated in all affected members in each family. The haplotype indicated that the two families did not share the same microsatellite markers group. This confirmed that these two families were unrelated (Figure 1).

DISCUSSION

Coralliform cataract is a special form of congenital cataracts. Several studies have shown that mutations in the CRYGD gene, located at 2q33–35, can result in congenital coralliform cataracts, and the P24T mutation of CRYGD has been reported in multiple cases. In the two autosomal dominant congenital coralliform cataract pedigrees in this study, we identified a recurrent P24T mutation. This mutation has been found in ten pedigrees, including two cerulean, one lamellar, six coralliform (including our two pedigrees and an unreported pedigree from Tianjin Eye Hospital, China), and one fasciculiform phenotype. From the reported pedigrees, the congenital coralliform cataracts all resulted from CRYGD mutations. This information indicated that the coralliform phenotype and the CRYGD gene are closely related. Our results supported the idea that virulence genes and lens morphology are related [7-12].

Results of biophysical analysis have shown that the P24T mutant protein has a significantly lower solubility compared with wild-type human γD crystallin. With synchrotron radiation circular dichroism spectroscopy, Evans et al. [13] found that the P24T mutant has a slightly increased content of beta-sheets, due to the substitution of the Pro24 residue, which may be attributed to the extension of an edge beta-strand. This indicates that the insolubility of the P24T mutant protein, rather than the loss of stability, likely causes the occurrences of congenital cataracts. Based on nuclear magnetic resonance analysis, jung et al. found that the pivotal local conformation and dynamics of the threonine substitution in the P24T mutant
are different from that of wild-type γD crystallin [14]. The substitution alters motional behavior of the associated region of the protein, speculating that the P24T substitution may initiate aggregation or polymerization. Such aggregation could result in reduced solubility and formation of high-molecular weight complexes.

Up to now, fourteen mutations in CRYGD have been reported [15-19]. Several studies have verified that mutation of CRYGD can lead to a decrease in solubility of the mutant protein compared to wild type. However, the conformation and stability of the mutant protein undergoes little change.

In conclusion, mutations in CRYGD are responsible for coralliform cataracts, and the P24T mutation may be a hot-point mutation affecting the formation of congenital coralliform cataracts.

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