New Phenotype Associated With an Arg116Cys Mutation in the CRYAA Gene

Nuclear Cataract, Iris Coloboma, and Microphthalmia

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Objective: To describe a new phenotype with an arginine-to-cysteine mutation at position 116 (Arg116Cys) in the CRYAA gene.

Methods: We investigated a 4-generation French family with autosomal dominant cataract and performed a genetic linkage analysis using microsatellite DNA markers encompassing 15 known cataract loci. Exons 1, 2, and 3 and flanking intronic sequences of the CRYAA gene were amplified and analyzed using direct sequencing.

Results: All of the affected individuals had nuclear cataract and iris coloboma. Genetic analysis revealed the previously described Arg116Cys mutation in the CRYAA gene in the heterozygous state in all of the affected members of the family but not in unaffected individuals.

Conclusion: To our knowledge, this is the first case to date in which an Arg116Cys mutation in the CRYAA gene was associated with nuclear cataract and iris coloboma.

Clinical Relevance: This study indicates that an Arg116Cys mutation in the CRYAA gene could be associated with an unusual phenotype in affected individuals. In this family, the clinical observation of iris coloboma allows for the possibility of identifying individuals carrying the mutation. Iris coloboma is particularly important in terms of perinatal diagnosis because its detection in the newborn requires a careful and regular examination of the lens.
Logarithm of odds (LOD) scores were calculated using the LINKAGE version 5.2 package (Université Paris 7, Paris, France) and the parameters described for the SLINK program. Exons 1, 2, and 3 and flanking intronic sequences of the CRYAA gene were amplified as previously described and analyzed using direct sequencing.

**RESULTS**

All of the affected individuals had an autosomal dominant bilateral nuclear cataract with bilateral iris coloboma. A microphthalmia also appeared in 2 of 14 affected members. A SLINK simulation calculation yielded expected LOD scores of 3.5 at $\theta=0$, 3.2 at $\theta=0.05$, and 2.9 at $\theta=0.01$, indicating a high probability of finding a linkage in this family. Although we were able to exclude 14 of 15 candidate loci, we detected positive LOD scores with markers $D21S1912$ (LOD score = 2.6 at $\theta=0$) and $D21S1890$ (LOD score = 1.9 at $\theta=0$). Haplotype analysis indicated that a specific haplotype segregates with the disease (Figure 2). The CRYAA gene, which lies within the $D21S1260$-$D21S1890$ interval and encodes a crystallin protein, was considered to be a good candidate. Sequence analysis of the 3 CRYAA exons revealed the previously described Arg116Cys mutation in the heterozygous state in all of the affected members of this family but not in unaffected individuals. In addition, a synonymous change at the third base of codon 2 (GAC → GAT) (single nucleotide polymorphism rs872331, http://www.ncbi.nih.gov/SNP/) was observed on the CRYAA mutated allele. This polymorphism was found in the homozygous state in the 2 affected individuals (II:7 and III:5) who have both cataract and microphthalmia and in 5 of 12 other affected patients who have normal eye size. This frequent polymorphism was also observed in the heterozygous state in 5 of 12 patients with cataract and in 3 unaffected members of the family. Thus, its occurrence in the homozygous state did not correlate with microphthalmia and iris coloboma.

**COMMENT**

An Arg116Cys mutation of the CRYAA gene was identified in all of the affected members from the family with autosomal dominant cataract described here. This mutation was previously reported in 1 family with isolated autosomal dominant cataract but no iris coloboma. The preservation of a positive charge at position 116 was found to be critical for the structural and functional integrity of the $\alpha$A-crystallin. Indeed, Bera et al have demon-
strated that the Arg116 residue can be replaced by another positively charged amino acid, Lys, without any effect on protein structure and function. Moreover, mutation of Arg116 to Cys or to another neutral amino acid, Gly, showed very similar changes in structure, oligomerization, and chaperone function. This suggests that an extra Cys residue per se is not the cause of the changes. In contrast, the replacement of the Arg116 residue by a negatively charged amino acid, Asp, has a devastating effect on the secondary and tertiary protein structures. The αA-crystallin Arg116Cys mutant forms larger oligomerized heteroaggregates with αB-crystallin wherein its interaction with the βB2- and γC-crystallins is decreased. In addition, the mutant Arg116Cys protein has reduced chaperone activity.

In this study, an iris coloboma was observed in all of the patients with cataract, and 2 individuals have microphthalmia in addition to cataract and iris coloboma. Two families with different CRYAA mutations have been described. An Arg49Cys mutation was previously reported in a family with isolated autosomal dominant cataract whereas a Trp9Xaa mutation was identified in a family with autosomal recessive cataract. No iris coloboma was described in these 2 families. Litt et al noted that 5 of 13 individuals with the Arg116Cys mutation had microphthalmia in addition to their cataract. In contrast, no ocular abnormality aside from cataract was found either in the family with autosomal dominant cataract with a nonsense Trp9Xaa mutation of the human CRYAA gene or in the family with autosomal recessive cataract with a nonsense Trp9Xaa mutation of the CRYAA gene.

Coloboma may be caused by a defect in the closure of the optic fissure or by an abnormal development of the iris stroma and epithelium. Although some CRYAA transcripts have been detected in an iris complementary DNA library (National Eye Institute NEIBank library Nblib0016, http://neibank.nei.nih.gov), a direct implication of CRYAA in iris development remains to be demonstrated. Nevertheless, investigators have previously demonstrated that the lens produces growth factors and influences the development of the ciliary body and iris. Based on the features found, our results also indicate that CRYAA protein is probably involved in choroidal development. In the family we have described, the occurrence of an iris coloboma could be related to a higher expression level of the Arg116Cys αA-crystallin mutant allele compared with the previously described family.

Indeed, polymorphisms in the CRYAA promoter region appear to influence transcriptional activity and may be responsible for a different expression level of the mutant protein. In a family with autosomal dominant cataract with a mutation in MAF, a lens developmental gene that is expressed in early eye development, 1 of 5 affected patients had iris coloboma and 2 of 5 had microcornea. Ogino and Yasuda suggested that MAF may have a role in the embryogenesis of the eye and in the maintenance of lens clarity through its known role in crystallin gene regulation. Recently, a CYRBB1 mutation was found to lead to cataract and microcornea in 8 of 10 affected individuals. Willoughby et al identified a novel mutation in the CYRBB1 gene and provided the first molecular basis for cataract with microcornea in the absence of microphthalmia or coloboma. Thus, CYRBB1 also plays a role in early ocular development. Altogether, these results provide evidence for an early expression of MAF, CYRBB1, and CRYAA in the embryogenesis of the eye and confirm that mutations in each of these genes can result not only in a phenotype restricted to the lens but also in a complex variety of ocular phenotypes combining cataract, microphthalmia, and anterior segment dysgenesis such as microcornea or iris coloboma.

Mouse mutants with the Val142Glu CRYAA mutation or with a CRYAA gene homozygous invalidation have both microphthalmia and cataract. In the family described here, we suggest the possibility that the Arg116Cys mutation acting in combination with a trans genetic modifier such as a polymorphic allele of a gene involved in eye development may be responsible for the occurrence of microphthalmia in 2 individuals.

Autosomal dominant cataract is phenotypically and genetically highly heterogeneous, making it a major obstacle to the phenotype-genotype relationship and direct linkage of congenital cataract genes. We describe in this study a new phenotype associated with an Arg116Cys mutation of the CRYAA gene in a French family. In this family, all of the affected individuals had nuclear cataract and iris coloboma.

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