

Further Genetic Heterogeneity for Autosomal Dominant Human Sutural Cataracts

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Key Words

Sutural cataract · γ -Crystallin · Linkage analysis ·
Humans · Mutation · Polymorphism

Abstract

A unique sutural cataract was observed in a 4-generation German family to be transmitted as an isolated autosomal, dominant trait. Since mutations in the γ -crystallin encoding *CRYG* genes have previously been demonstrated to be the most frequent reason for isolated congenital cataracts, all 4 active *CRYG* genes have been sequenced. A single base-pair change in the *CRYGA* gene has been shown, leading to a premature stop codon. This was not observed in 170 control individuals. However, it did not segregate with the disease phenotype. This is the first truncating mutation in an active *CRYG* gene without a dominant phenotype. As the *CRYGA* mutation did not explain the cataract, several other candidate loci (*CCV*, *GJA8*, *CRYBB2*, *BFSP2*, *MIP*, *GJA8*, *central pouch-like*, *CRYBA1*) were investigated by microsatellite markers and linkage analysis, but they were excluded based on the combination of haplotype analysis and two-point linkage analysis. The phenotype in this family is due to a mutation in another sutural cataract gene yet to be identified.

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Introduction

Cataract, an opacification of the eye lens, is a leading cause of visual impairment or blindness during infancy and early childhood [1]. An estimated 200,000 children are blind with bilateral cataract worldwide; 20,000–40,000 children affected with developmental cataract are born each year [2]. In the developed nations of Europe and Northern America, national surveillance or cross-sectional studies revealed a prevalence of 1–4 cases per 10,000 children [3–5].

Most frequently, congenital cataracts without other clinical symptoms have been found in the γ -crystallin encoding *CRYG* genes [6–10]. Moreover, other genes have also been reported to be involved in congenital dominant cataract formation like *CRYAA* [11, 12], *CRYBB2* [13–15], *GJA3* [16], *GJA8* [17–19] and *BFSB2* (also known as CP49 [20]). Similar clues are observed in the mouse as the most prominent model system for cataract [for an overview, see ref. 21].

Among those mutations listed, only very few have been characterized as sutural opacities. In man, this is a mutation in the *CRYBA1* gene [22] and in the mouse the *Cat2^{ns}* [23]. We tested for linkage of some of the known cataract loci with the unique phenotype observed in a 4-generation German family.

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0030-3747/03/0352-0071\$19.50/0

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Table 1. Haplotype analysis with two-point analysis

Marker	Family member												LOD ($\Theta = 0$)
	I-2 (+)	II-2 (+)	II-4 (+)	II-5 (-)	III-1 (-)	III-2 (+)	III-3 (+)	III-4 (-)	IV-1 (+)	IV-2 (+)			
<i>CCV</i>													
D1S468	2 1	3 1	1 1	1 2	3 1	2 1	2 1	2 1	3 2	1 2		$-\infty$	
D1S2845	1 2	6 2	2 2	2 1	5 4	7 2	3 2	3 2	5 7	4 7		$-\infty$	
D1S508	3 1	5 3	4 1	4 3	1 2	1 3	3 1	3 4	1 1	2 1		$-\infty$	
<i>GJA8</i>													
D1S2669	1 2	3 1	3 2	3 2	1 3	1 3	1 2	1 2	1 1	3 1		$-\infty$	
D1S514	1 2	2 1	4 2	4 2	2 2	3 2	2 2	2 2	2 3	2 3		$-\infty$	
D1S1595	1 4	nd	4 4	nd	nd	1 4	3 4	4 4	5 4	7 1		nd	
<i>CRYG</i>													
D2S2321	4 2	2 2	2 4	2 4	4 3	3 2	2 2	4 4	4 2	3 2		0.85	
CRYG	2 2	1 2	1 2	3 2	3 2	2 2	3 1	3 2	3 2	2 2		$-\infty$	
D2S1434	4 2	2 2	2 4	2 4	4 3	3 2	2 2	4 4	4 2	3 2		0.85	
<i>BFSP2</i>													
D3S606	5 3	6 3	6 5	3 3	6 2	4 6	1 5	7 6	2 6	6 4		$-\infty$	
D3S3637	1 2	3 2	3 1	3 2	1 5	4 3	3 1	2 3	5 3	5 3		$-\infty$	
<i>MIP26</i>													
D12S375	3 2	1 2	1 2	1 2	1 3	2 2	2 2	2 2	1 2	3 2		$-\infty$	
D12S1052	1 2	2 2	2 2	2 2	2 3	2 2	1 2	1 2	2 2	3 2		$-\infty$	
D12S1064	5 4	6 4	2 4	2 4	3 3	6 4	1 4	2 4	3 4	3 6		$-\infty$	
<i>Gja3</i>													
D13S1316	2 4	6 4	6 2	6 2	1 5	3 4	4 2	4 6	5 4	1 4		$-\infty$	
D13S175	2 2	3 2	3 2	3 2	2 3	1 2	4 2	5 3	3 2	2 2		1.51	
D13S1275	5 1	3 1	3 5	3 5	4 1	1 3	2 5	6 3	4 3	4 3		$-\infty$	
D13S2332	3 1	2 1	2 3	2 3	2 4	3 2	2 3	2 2	2 2	2 2		$-\infty$	
<i>CPC</i>													
D15S659	4 2	2 2	2 4	2 4	1 2	2 2	4 2	3 4	1 2	1 2		-0.1	
D15S643	3 1	2 1	3 3	2 3	2 1	3 2	3 3	4 3	2 2	2 2		$-\infty$	
D15S153	3 4	1 4	4 3	1 3	2 4	5 1	2 4	1 3	2 1	2 1		$-\infty$	
<i>CRYBA3</i>													
D17S122	2 2	3 2	3 2	3 2	1 3	4 2	3 2	3 3	3 4	3 2		$-\infty$	
D17S1294	4 2	1 2	1 4	1 2	3 2	2 2	2 4	2 1	3 2	2 2		$-\infty$	
<i>CRYBB2</i>													
D22S431	2 3	3 3	1 3	1 3	4 3	3 3	4 3	1 3	4 3	3 3		$-\infty$	
D22S258	3 2	2 2	2 2	2 2	1 2	2 2	1 2	1 2	1 3	2 3		$-\infty$	

'+' = Affected; '-' = unaffected; nd = not determined.

Materials and Methods

Clinical Documentation

The proband and the accompanying parents or relatives underwent clinical eye examination by a senior pediatric ophthalmologist at the Eye Clinic of the Ludwig-Maximilians University of Munich, Germany, by either Scheimpflug (Topcon-SL45) or slit lamp (Zeiss, Oberkochen, Germany) examination. Clinical details were recorded in a standard questionnaire including the age of onset and time of diagnosis of the cataract in the proband as well as the proband's and her family's health history. This study respected the tenets of the Declaration of Helsinki, as the family members were fully informed

of their role in the study and the outcome, prior to securing their informed consent, prepared according to standard norms.

Molecular Analysis

Blood samples (5–10 ml) were collected from the affected and unaffected members of the family and genomic DNA was isolated according to standard procedures. Candidate loci were analyzed by a combination of linkage analysis and haplotype analysis as well as by mutational analysis of the coding sequence available for selected genes. Specifically, the γ -crystallin gene cluster (*CRYG*) was analyzed by sequencing. PCR of the four active *CRYG* genes (*CRYGA*, *CRYGB*, *CRYGC*, *CRYGD*) was performed on genomic DNA sam-

ples in reactions of 20 μ l with a denaturation step at 95 °C for 2 min, for 1 cycle, followed by 40 cycles of 95 °C denaturation for 45 s, annealing at 60 °C for 45 s, extension at 72 °C for 45 s and with a final extension at 72 °C for 5 min with either a Stratagene Robocycler (Stratagene, Heidelberg, Germany) or a Perkin Elmer Thermocycler (Perkin Elmer, Weiterstadt, Germany), using primers according to Santhiya et al. [10]. Sequence analysis was performed commercially (SequiServe, Vaterstetten, Germany) after purification of PCR fragments through Nucleospin® extraction columns (Macherey-Nagel, Düren, Germany). The interesting sequence variation suggestive of a mutation was further investigated by restriction analysis (*AvaII*).

Mapping of Candidate Loci and Linkage Analysis

In addition to the *CRYG* gene cluster, a group of candidate genes/loci was selected from their previously documented association with sutural lens opacities (table 1). Polymorphic markers were selected from the publicly available databases to cover the genetic intervals of interest. The most informative markers used are listed in table 1.

Assuming an autosomal dominant mode of inheritance and complete penetrance, our family was not powerful enough to detect significant linkage (LOD score >3 , odds for linkage of at least 1,000:1; $p = 0.05$). The power to detect linkage was investigated using SLINK. Two hundred replicates of the pedigree were generated assuming a completely penetrant AD disease locus with disease allele frequency of 0.01 and no phenocopies. The predicted maximum LOD score is 2.10 (average 1.60); however, the structure of the family allowed us to obtain significant information by haplotype analysis.

Genotyping was done masked to the disease status. Genetic maps used for the selection of short tandem repeat polymorphisms were obtained from the literature, those characterized by the Cooperative Human Linkage Center (www.chcl.org/), Généthon (www.genethon.fr/) and others available through the Internet, such as Marshfield (www.marshmed.org/genetics/) and GDB. Primers were obtained from Research Genetics Inc. (screening set 6A), ACGT Inc., or Dalton Inc. (Toronto, Canada). A fluorescent dye label was incorporated on the 5'-end of one of the primers and the amplification products were electrophoresed using ALF automated sequencers (Pharmacia, Piscataway, USA) and analyzed using the fragment manager software. The amplification protocol has previously been described [14]. Their intermarker distances (cM) were determined from genome database genetic maps.

Cyrillic (V2.1.3) was used for data management and pedigree drawing. Two-point linkage analysis between the cataract phenotype and the genotype information used the MLINK component of LINKAGE (v5.1 [24]). A full penetrance and a disease-gene frequency of 0.0001 were assumed for the disease locus. The allele frequencies were assumed to be equal for each marker since the true population allele frequencies for each marker could not be reliably estimated.

Results and Discussion

Phenotype Description

A 4-generation family (fig. 1) with the clinical feature of progressive congenital sutural cataract (*cataract stellaris*) was examined at the Eye Hospital of the Ludwig-Maximilians University, Munich, Germany. The phenotype

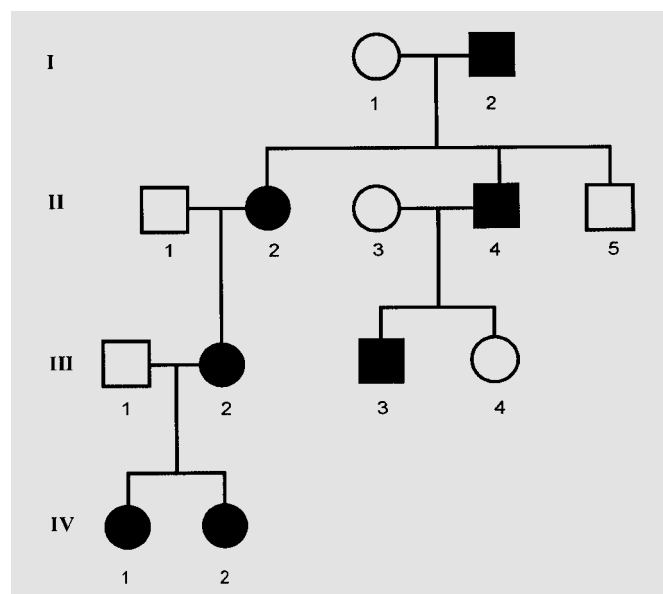


Fig. 1. Pedigree of the sutural cataract family. The pedigree of the 4-generation German family demonstrates the autosomal dominant mode of inheritance. The numbers next to the patient's symbol indicate the collected blood sample used for linkage or molecular analysis.

of two sisters (IV/1, IV/2) was analyzed by skiascopy, slit lamp examination, funduscopy and documented with a Scheimpflug camera (fig. 2) and with a slit lamp camera (fig. 3; Zeiss, Oberkochen, Germany).

In the older sister (IV/1; fig. 1), visual acuity for distance and near on the right eye was 20/50 (cc. $+3.0/-2.0/175^\circ = 0.4$) and on the left eye 20/50 (cc. $+3.0/-2.0/180^\circ = 0.4$). The cornea was clear and the anterior chamber without irregularities. The lens showed dense opacities of the anterior and posterior λ -suture and a diffuse moderate opacification of the whole lens. Vitreous and retina were normal. Binocular vision was reduced (Titmus fly +; Bagolini +; Titmus rings up to ring 2, normally ring 9), and the symbols on the Lang chart could not be recognized.

Cataract extraction with intraocular lens implantation was performed successfully. Postoperative visual acuity with best correction was 0.8 (20/25) on both eyes. Moderate reduction of postoperative visual acuity is explained by a relative amblyopia.

In the younger sister (IV-2), visual acuity was 20/40 with best correction of $+2.5/-0.5/180^\circ$ on the right eye (0.5) and on the left eye 0.6 (20/30) with a correction of $+2.0/-0.75/175^\circ$. The lens also showed dense opacities of

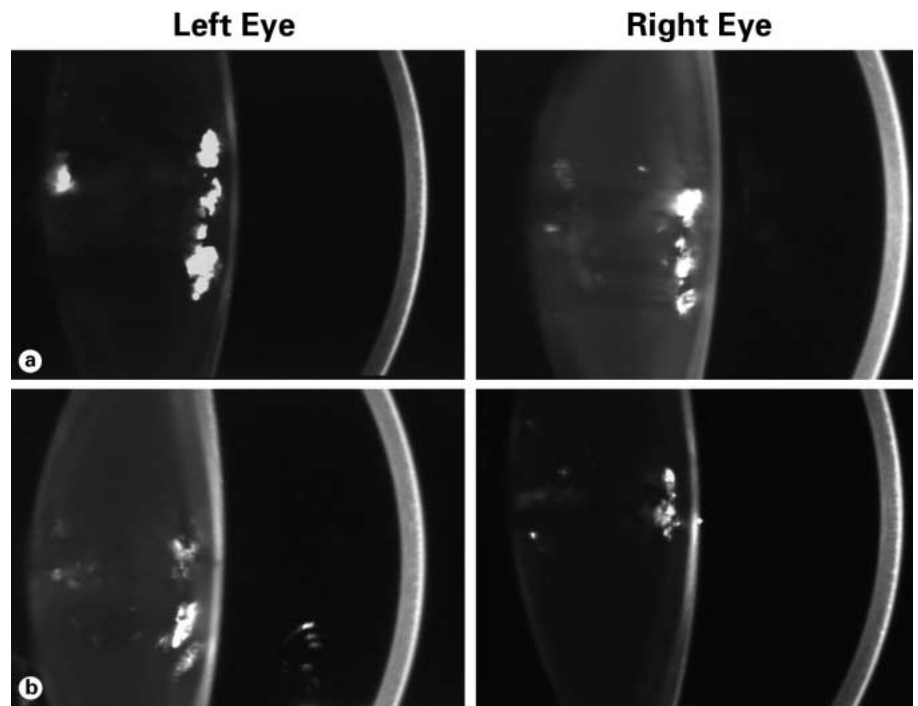


Fig. 2. Scheimpflug analysis. The Scheimpflug pictures show dense opacities of the lens more prominent at the anterior than the posterior λ -suture. The spots in the anterior chamber (b) are artifacts due to reflexions. The age of the patients were 16 (a; IV-1) and 11 (b; IV-2) years.

the λ -sutures and moderate diffuse opacification of the lens and the nucleus. Binocular vision was nearly identical to her sister. The Titmus rings were recognized up to ring 3 (Titmus fly +, Bagolini +), and cataract surgery is planned. In both patients, no strabismus was present.

The mother (III/2) of the two girls demonstrated at the age of 42 years less opacities in the λ -suture but snowflake-like subcapsular opacities in the central as well as in the more peripheral areas of the lens and diffuse opacification of the whole lens, but the overall severity was not significantly different from her daughters (fig. 3). Functional testing in the mother was not performed; she was operated in 1998 at another hospital. In all family members, no further clinical manifestations were observed cosegregating with the ocular phenotype, suggesting that the cataract in this family was an isolated feature.

Analysis of the CRYG Candidate Genes

Since it has been shown in mouse and man that *CRYG* genes are most frequently causative for congenital, isolated cataracts, we sequenced all 4 active human *CRYG* genes also in the proband and her father. In two of the four *CRYG* genes (*CRYGA*, *CRYGD*), we observed polymorphic changes. The T→C transversion at position 196 of the *CRYGA* gene (exon 3) leads to a Pro instead of a Lys. However, this Pro is very common in human (10 of 10

cases tested in a previous study [10]) and in all other *Cryg* genes of mouse, rat and bovine. Two further polymorphic sites have been observed in the *CRYGD* gene (AG92-93GA; QV→QM; T286C without effect on the amino acid composition); both have also been observed in a previous study [10] with a high frequency and without cosegregation with congenital cataracts. All three polymorphisms were also observed in the proband's father. In contrast, the described silent polymorphic site in the *CRYGB* gene at position 2437 (ccC→ccT; Pro→Pro; acc. M19364 [6]) could not be observed in the proband nor in her father.

However, an additional insertion of C at position 43 in the *CRYGA* was observed in the proband (IV-1, fig. 4) in a heterozygous situation. The mutation leads to a shift of the open reading frame; computer-assisted translation suggests that only a short peptide will be formed consisting of the 13 N-terminal amino acids of the γ A-crystallin and 7 novel amino acids (fig. 4). Since all other previously reported truncations of the *Cryg* genes in the mouse lead to dominant cataracts, this mutation was suggested to be causative for the phenotype in this family. Using a restriction enzyme digestion by *Ava*II, we could find this particular polymorphism neither in additional 170 randomly collected human DNA samples of Caucasian origin nor in 1 Indian family suffering from dominant cataracts (C132



Fig. 3. Slit-lamp analysis. Dense opacifications were presently more expressed at the anterior λ -suture. The anterior and posterior suture are demonstrated in their inverse position. There is also a diffuse opacification of the whole lens as compared with young individuals of the same age. The age of the patients were 16 (a; IV-1) and 11 (b); IV-2) years; the age of the mother (c; III-2) was 42 years.

[10]). However, the sequence change observed did not segregate with the disease phenotype and was observed only in the proband and her unaffected father, but in none of the other affected members of the family; we conclude that this particular polymorphism is very rare (<1%; fig. 5).

To our knowledge, it is the first truncating *CRYG* change without (dominant) consequences on the clinical phenotype. It might be speculated that this small peptide (18 amino acids) is either unstable and will be degraded or does not have a dominant negative effect as described

in the other truncated γ -crystallin, which have usually a molecular weight of 16–18 kD [25–27]. As in the ψ *CRYGE* and ψ *CRYGF*, the loss of function of a further *CRYG* gene also seems to be without pathological consequences. However, the mouse *Cryga* gene is expressed earlier (from E12.5 onward) and at a higher level than the other *Cryg* genes [28]. From this point of view, it would be expected that mutations in the *Cryga* gene should lead to severe cataracts. However, there has been no report on a *CRYGA* mutation in man so far, and also in the mouse, only one mutation has been reported [25]. In the human

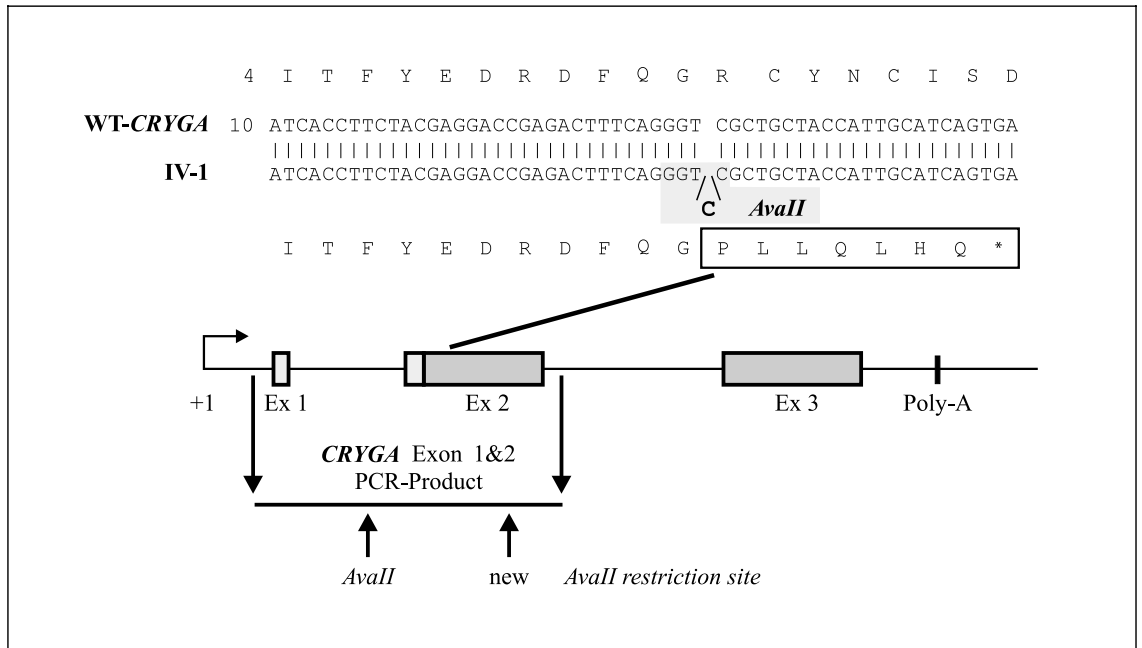


Fig. 4. Sequence analysis of *CRYGA*, exon 2. Sequence analysis of exon 2 of the *CRYGA* gene in the proband (IV-1) revealed an insertion of a C at position 43 (given in bold, counted according to its position in the cDNA). It leads to a short peptide of just 20 amino acids including 7 foreign amino acids (boxed) because of a stop codon in the new reading frame. The genomic *CRYG* gene structure is indicated below the DNA and deduced amino acid sequence. The new *AvaII* restriction site is shaded in grey; the place of the regular and new *AvaII* sites are indicated below the genomic structure.

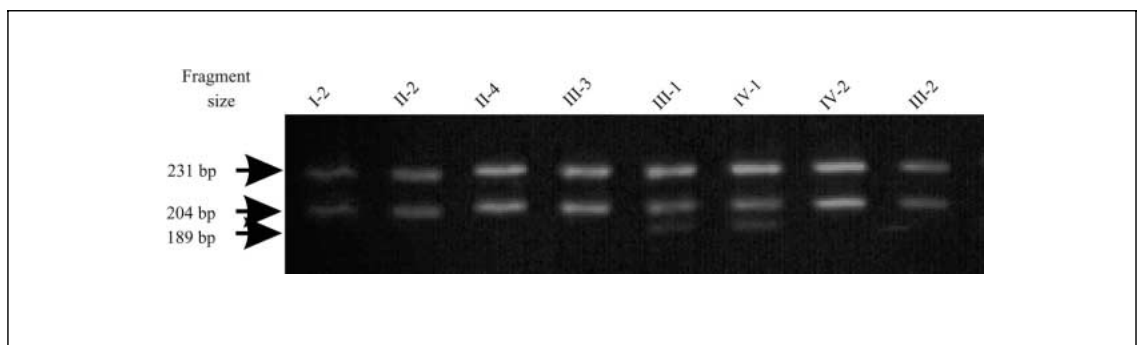


Fig. 5. Restriction digest of *CRYGA* by *AvaII*. The *AvaII* restriction analysis of the PCR fragment containing exons 1 and 2 are given. The carriers of the insertion (III-1 and IV-1) demonstrate 3 bands indicating the presence of two restriction sites, whereas all other affected family members exhibit only two bands indicating the presence of just one restriction site.

embryonic lens, *CRYGA* contributes 14% to the overall *CRYG* transcripts (for comparison: *CRYGC* and *CRYGD* together 81%, *CRYGB* only 5% [29]). Therefore, the contribution of *CRYGA* to the functional integrity of the lens is less compared with *CRYGC* and *CRYGD*.

Linkage of the Cataract Phenotype to Known Cataract Loci

Following the sequence analysis of the *CRYG* genes, we proceeded to the genetic analysis of the other sutural cataract candidate loci (table 1). Using linkage analysis

and haplotype analysis, the candidate loci considered were genetically excluded, suggesting another sutural cataract locus is yet to be identified.

Conclusion

A specific form of sutural opacities has been observed in a 4-generation German family. A newly found truncation of the γ A-crystallin after 20 amino acids is not involved in the formation of this type of cataract. Map-

ping experiments did not show linkage to known sutural cataract loci. This suggests that this unique form of sutural opacity represents a novel genetic subtype expanding the genetic heterogeneity of this condition.

Acknowledgment

The expert technical assistance of Margret Bahnweg and Erika Bürkle is gratefully acknowledged. Oligonucleotides have been manufactured by Utz Linzner (working group Bioinformatics at the GSF Institute of Experimental Genetics).

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