in anticipation that the side effects will prove to be minimal, we propose to evaluate the safety and efficacy of NTBC for alkaptonuria patients. With the cooperation of Dr. C. Ronald Scott of the University of Washington, we now are attempting to secure NTBC for use in the treatment of alkaptonuria.

Whereas gene therapy generally involves specific tissue localization, pharmacotherapy routinely employs a wide range of targets. For many metabolic disorders, this provides a distinct advantage. For example, in the treatment of cystinosis, cysteamine has beneficial effects upon a variety of organs and tissues (Gahl et al. 1995), including the kidney, muscle, cornea, and thyroid (Kimonis et al. 1995). NTBC could have multisystemic salutary effects as well, meaning that we really *are* ready to try to cure alkaptonuria.

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (alkaptonuria [MIM 203500])

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0002-9297/98/6303-0042$02.00

Am. J. Hum. Genet. 63:921–926, 1998

Gene Localization for Aculeiform Cataract, on Chromosome 2q33-35

To the Editor:

Aculeiform cataract (MIM 115700) is a form of congenital crystalline cataract that originally was described by Vogt in 1922 and was referred to as "Spiesskatarakt" (Vogt 1922). Since its original description, this entity also has been referred to as "frosted cataract," "needle-shaped cataract," or "fasciculiform cataract" (Parker 1956). This phenotype is characterized by fiberglasslike or needlelike crystals projecting in different directions, through or close to the axial region of the lens (fig. 1). Some crystals may be >1 mm in length, and their biochemical composition is not known. This type of cataract is considered to be different from the corraliform cataract, which does not show the needlelike projections. This opacity does not appear to respect the sutures or the direction of the lens fibers (François 1963) and appears to originate from the fetal and postnatal nuclei, suggesting a congenital origin with some postnatal progression, if any. The opacity causes a variable degree of

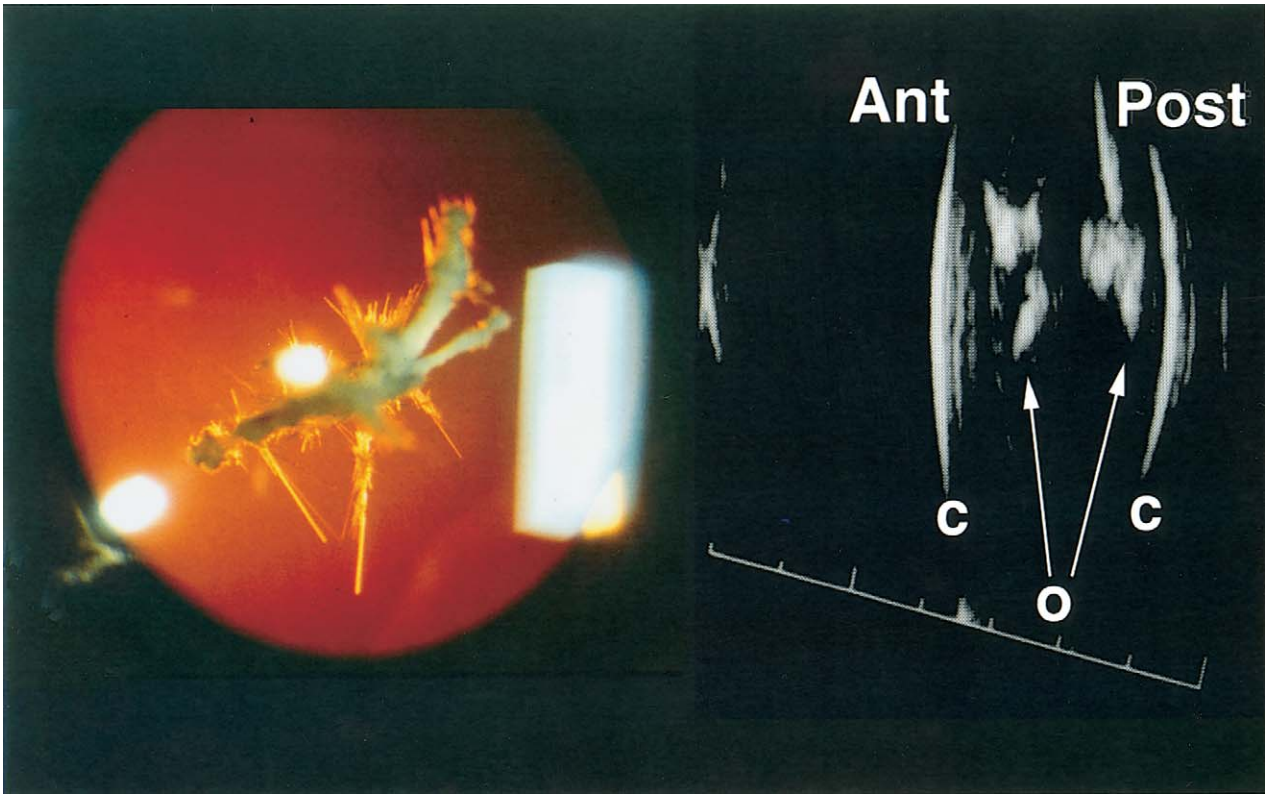

Figure 1 *Left,* Slit-lamp photography (retroillumination) of an individual affected with aculeiform cataract (A:IV-10; age 8 years) The central opacity projects in all directions, in needlelike endings, into the anterior and posterior cortex. *Right,* Ultrasound biomicroscopic evaluation of the opacity, showing involvement of the peripheral embryonal nucleus, extending into the cortical area of the lens. ant = anterior, post = posterior, c = capsule, and o = opacity.

vision loss, and surgery may be required to restore visual function.

Although usually bilateral, unilateral cases of aculeiform cataract have been described (Gifford and Punthenney 1937; Parker 1956; Rosselet 1961; Collier 1965). Dominant inheritance with complete penetrance and minimal variable expressivity has been reported in most affected European and North American pedigrees, with no sex predilection documented (Vogt 1922; Cords 1926).

A mapping study was performed with three unrelated families affected with the classic aculeiform-cataract phenotype, in an attempt to identify the disease-gene location. The families originated from Macedonia (family A) and the Neuchâtel area of Switzerland (families B and C) (fig. 2), and all affected individuals had the typical crystalline lens opacity.

A total of 19 affected family members, 17 unaffected family members, and 7 spouses were genotyped and studied by linkage analysis. The initial strategy consisted of screening 13 candidate loci related to congenital cataract and the crystallin genes (Cartier et al. 1994; Armitage et al. 1995; Eiberg et al. 1995; Berry et al. 1996; Ionides et al. 1997; Litt et al. 1997, 1998). Linkage was identified with short tandem-repeat–polymorphism markers in the 2q33-q35 region, around the γ-crystallin locus. Two-point maximum-likelihood data for markers in this region are summarized in table 1. When the data from all three families were combined, the maximum LOD score (Z_{max}) was 6.27 (recombination fraction [θ] 0), with marker D2S2208. The LOD-score results for family A alone remained >3 for at least six neighboring markers (data not shown).

The order of the markers used at the 2q33-35 locus, proximal to distal, and the intermarker distances were determined from published maps (Buetow et al. 1994; Gyapay et al. 1994; Murray et al. 1994; Dib et al. 1996) and genome databases (Cooperative Human Linkage Center and Genome Database) and are as follows (parentheses denote that intermarker distance is unknown): (D2S1391)–D2S2273–4 cM–D2S118, D2S389–8 cM–D2S116–6 cM–D2S155–2 cM–D2S2242, D2S2208–2 cM–D2S2321, D2S157–(CRYGA)–5 cM–D2S143–3 cM–D2S2382–1 cM–D2S164–(D2S434)–1 cM–D2S2249, D2S173–(Villin)–3 cM–D2S163–3 cM–D2S126–1 cM–PAX3, D2S2197–(D2S1363)–8 cM–

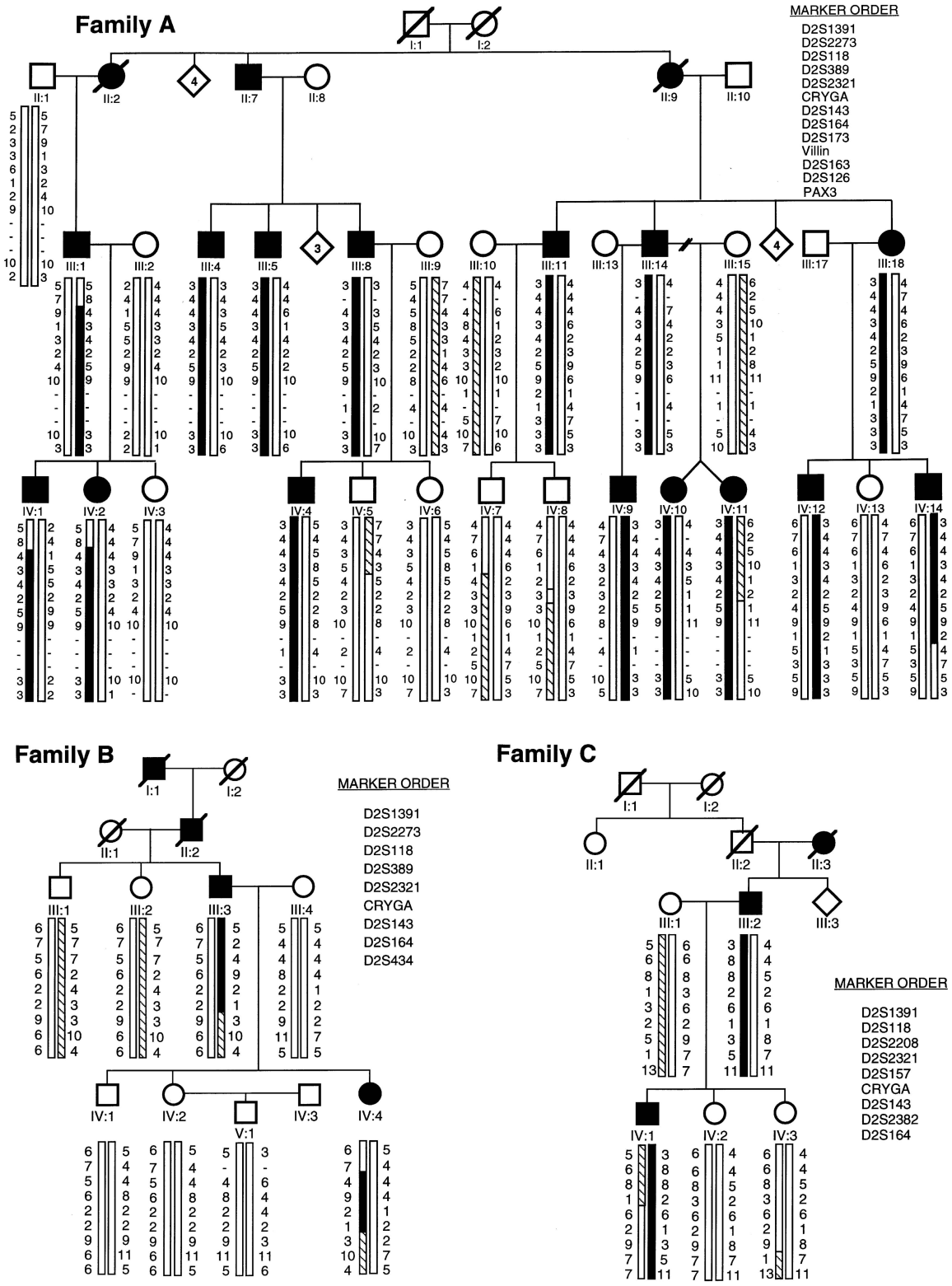

Figure 2 Pedigrees of families studied, with haplotypes for selected markers relevant to recombinant breakpoints on chromosome 2q33-35. Blackened squares and circles denote affected individuals, and diamonds denote nonparticipating relatives. A dash within a marker order denotes an untyped marker (deemed not critical to the identification of recombination events), which is not considered to be within the disease-gene interval. A haplotype cosegregating with the affected status is indicated by a blackened bar; the critical crossovers defining the proximal and distal boundaries of the aculeiform candidate region are shown in family B, individuals III:3 and IV:4, placing the disease locus between the markers D2S2273 and D2S143. Unblackened and patterned bars denote the non–disease-associated haplotypes.

Table 1

Two-Point Linkage Data for Aculeiform-Cataract Phenotype and Markers of 2q33-35 Region

	LOD Score at θ =						Maximum	
Marker	.00	.05	.10	.20	.30	.40	θ	Z_{max}
D2S1391	−1.96	2.18	2.17	1.79	1.22	1.08	.05	2.18
D2S118	5.25	4.77	4.28	3.22	2.09	.97	.00	5.25
D2S389	6.12	5.53	4.91	3.60	2.28	1.59	.00	6.12
D2S116	5.39	4.89	4.39	3.34	2.25	1.10	.00	5.39
D2S155	6.08	5.51	4.93	3.69	2.39	1.14	.00	6.08
D2S2208	6.27	5.64	5.04	3.79	2.48	1.15	.00	6.27
D2S2321	3.07	2.77	2.47	1.86	1.24	.61	.00	3.07
D2S157	3.36	3.08	2.77	2.15	1.47	.75	.00	3.36
CRYGA	1.36	1.26	1.14	.83	.53	.22	.00	1.36
D2S143	5.83	5.34	4.82	4.29	3.71	3.13	.00	5.83
D2S2382	3.33	3.13	2.87	2.22	1.49	.71	.00	3.33
D2S164	4.18	3.90	3.51	2.75	1.85	.88	.00	4.18
D2S126	−1.33	2.81	2.79	2.36	1.89	.89	.07	2.88
PAX3	2.81	2.49	2.18	1.61	1.01	.45	.00	2.81
D2S1363	3.39	3.15	2.84	2.16	1.41	.64	.00	3.39
D2S159	−10.04	−.60	−.13	.17	.12	.05	.20	.17

Note.—Linkage analysis was performed with the LINKAGE program package (version 5.1), and MLINK was used for pairwise analysis. A full-penetrance, equal allele frequency and a disease-gene frequency of .0001 were assumed for the disease locus.

D2S159. The marker CRYGA was an intragenic polymorphism of the γ-crystallin–A gene.

Critical recombination events observed in affected individuals defined an initial disease-gene interval of 27 cM between markers D2S2273 and D2S143 (fig. 2). Furthermore, observation of recombination events in the unaffected allele of individual C:IV-1 allowed ordering of markers D2S2321 and D2S157 (cen-D2S2321-D2S157-tel), which were nonrecombinant on the Généthon map (Dib et al. 1996).

Haplotype analysis showed a common affected haplotype for seven markers (D2S2242, D2S2208, D2S2321, D2S157, CRYGA, D2S143, and D2S2382) over a 10-cM interval in families B and C (see alleles within the box in table 2). Although no common ancestor could be identified through genealogical studies, both families are from the relatively small Neuchâtel area of Switzerland (population ~170,000). The shared haplotype, together with the recombination events observed between markers D2S2242 and D2S143, define a disease-gene interval of 7 cM (see the underlined alleles in table 2).

Several candidate genes are of interest in this interval, the most relevant being the γ-crystallin–gene cluster, CRYG (2q33-35). Although the precise position of CRYGA is unclear, haplotype analysis and observation of recombination events in families A and B suggest that CRYGA is distal to D2S155 and centromeric to D2S143. Another crystallin gene, CRYBA2, has been mapped to the 2q34-36 region (Hulsebos et al. 1995). However, physical mapping using radiation-hybrid cell lines placed CRYGA separate from and centromeric to CRYBA2 (Hulsebos et al. 1995). The gene order in the human 2q33-36 segment appears to be syntenic with that of genes on mouse chromosome 1, and, in the mouse, *Cryba2* is nonrecombinant with *Villin* (*Vil*) (10.6 cM telomeric to *Cryg*) (Hulsebos et al. 1995). Genotyping

Table 2

Haplotype Analysis of Aculeiform Cataract

	Intermarker Distance(cM)	Affected Haplotype in Family[a]		
Marker		A	B	C
D2S116		3	4	7
D2S155	6	1	3	6
D2S2242	2	8	1	1
D2S2208	0	6	8	8
D2S2321	2	4	2	2
D2S157	0	5	6	6
CRYGA		2	1	1
D2S143	5	5	3	3
D2S2382	3	6	5	5
D2S164	1	9	10	11
D2S434		5	4	3

[a] The region of allele sharing is circumscribed by the box, and the alleles that define the disease-gene interval when the recombination events shown in figure 2 are taken into account are underlined.

the three families using a dinucleotide repeat close to Villin confirms its location as being telomeric to CRYGA, since it is mapped below the recombination breakpoint in individual A:IV-14 (fig. 2). If synteny between the mouse genome and the human genome is assumed for this region, CRYBA2 would be located outside the disease-gene interval of interest. A developmental gene, PAX-3, was documented at the telomeric end of the interval. However, observation of recombination events centromeric to this gene, in the families studied, excluded the potential role of PAX-3 in this cataract phenotype (fig. 2).

The human γ-crystallin genes constitute a multigene family whose members are expressed only in the eye lens. The γ-crystallin–gene cluster contains six highly conserved genes (A–F), all mapped to chromosome 2q33-q35 (den Dunnen et al. 1985; Meakin et al. 1985) and specific to mammals (Cveki and Piatgorsky 1996) . The relative position of the γ-crystallins B–E have been established on a 40-kb DNA segment, but the exact locations for γ-crystallins A and F in the gene cluster are yet to be determined (Meakin et al. 1985). The γ-crystallin cluster is of great interest in the study of congenital cataract, since it is expressed early in development and is presumed to play a role in both fiber differentiation and maintenance of lens-fiber transparency (Papaconstantinou 1967). Furthermore, this locus has been associated with hereditary cataract in mouse and human (Oda et al. 1980; Lubsen et al. 1987; Cartier et al. 1992; Santhiya et al. 1995).

Although γ-crystallins E and F are considered to be pseudogenes, by virtue of an in-frame stop codon (Meakin et al. 1985), a low level of γ-crystallin–E transcript has been detected (Brakenhoff et al. 1994). Lubsen et al. (1987) reported a tight linkage between the γ-crystallin–gene cluster on chromosome 2 and a phenotype referred to as "Coppock-like cataract," confined to the embryonic nucleus (clearly distinct from the aculeiform cataract) (Lubsen et al. 1987). Recent work has demonstrated that sequence changes upstream from the γ-crystallin–E pseudogene result in a 10-fold increase in the activity of the γ-crystallin–E promoter. These data suggest a potential role for the γ-crystallin–E peptide in the Coppock-like cataract of human (Brakenhoff et al. 1994).

Of interest in the *Elo* and the *Cat2* mutant-mouse models, the γ-crystallin–E gene is the target of mutations and also is responsible for cataract phenotypes (Oda et al. 1980; Cartier et al. 1992; Santhiya et al. 1995). In both these mutants, the opacity involves the embryonic nucleus.

Recently, Rogaev et al. (1996) studied a large family, from the isolated Nokhurli population of Turkmenia, that is affected with polymorphic congenital cataract (PCC). This phenotype also mapped to the 2q33 locus, and it was characterized by a progressive, mostly peripheral, and highly variable opacity (Ginter et al. 1983, 1991). Whether PCC, Coppock-like cataract, and aculeiform cataract are allelic variants remains to be elucidated, but they clearly are three distinct clinical entities.

In summary, the localization of a gene for aculeiform cataract has been identified on chromosome 2q33-35, within a 7-cM interval. This condition appears to be genetically homogeneous. Refinement of the disease-gene interval and analysis of the γ-crystallin–gene cluster are currently underway, in an attempt to identify the disease-causing mutation(s). The molecular characterization of this phenotype may shed light on the complex cascade of events modulating lens differentiation.

Acknowledgments

The authors are grateful to the families for their enthusiastic participation and to Ms. Megan Priston for her excellent technical work. This work has been supported by Swiss National Science Foundation grant 32-43619.95 (to F.L.M. and D.F.S.) and by the Glaucoma Research Society of Ontario, the Weston Foundation, and the Imperial Oil Research Fund (all provided support to E.H.).

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Electronic-Database Information

URLs for data in this article are as follows:

Cooperative Human Linkage Center (CHLC), http://www.chlc.org
Généthon, http://www.genethon.fr
Genome Database, http://gdbwww.gdb.org
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0002-9297/98/6303-0043$02.00