Identification of a genetic locus for autosomal dominant infantile cataract on chromosome 20p12.1-p11.23 in a Chinese family

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Purpose: To map a gene responsible for infantile cataract in a large four-generation, non-consanguineous Chinese family.

Methods: Twenty-two family members including 17 cataract patients in the Chinese family were analyzed clinically. All family members were genotyped with 382 microsatellite markers that provide genome-wide coverage every 10 cM. Linkage analysis was performed to identify the chromosomal location of the infantile cataract gene in the family. Candidate genes were studied by direct DNA sequence analysis.

Results: Genome-wide linkage analysis provided evidence for a genetic locus for infantile cataract on chromosome 20p12.2-20p11.23. The maximum LOD score was 5.15 for marker D20S471 at a recombination fraction of 0. Fine mapping defined the cataract gene within a 7.4 Mb interval between markers D20S915 and D20S912. No mutation was detected in potential candidate genes, BFSP1 and CHMP4B.

Conclusions: Our results suggest that there is a new gene for infantile cataract on chromosome 20p12.2-p11.23. Our results suggest that new genes for infantile cataract could be found through further study of candidate genes at the 20q locus, which may provide insights into the pathogenic mechanisms of cataracts.

Congenital cataract is a significant cause of hereditary visual impairment in childhood. Its incidence is estimated to be 2.2-2.49 per 10,000 live births and may account for one-third of total blindness in infants [1-3]. Congenital cataract is genetically and clinically highly heterogeneous, and various inheritance patterns have been reported including autosomal dominant, autosomal recessive, and X-linked forms [4,5]. To date, 21 genetic loci have been identified, and 16 causative genes have been identified for autosomal dominant congenital cataract (ADCC), the most common familial form [6]. The phenotype of ADCC also varies markedly in morphology, affecting the nuclear, cortical, polar, and other sections of the lens [7]. Total cataract is characterized by opacity of all lens fibers. For autosomal dominant total cataract, one mutation in the heat shock transcription factor (HSF4) gene was reported by us [8], and another mutation was identified in a gene encoding the major intrinsic protein (MIP, MIP26) of the lens [9]. For autosomal recessive total cataract, a mutation was reported in the gene encoding the α-A component of α-crystallin (CRYAA) [10].

There are three ADCC families linked to chromosome 20 including a Japanese autosomal dominant posterior cataract family linked to chromosome 20p12–20q12 [11] and a Chinese family with autosomal dominant progressive congenital zonular nuclear cataract that is linked to chromosome 20p12.2-20p11.23 [12]. Very recently, a white (U.S.A.) family with progressive childhood posterior subcapsular cataracts has been reported to be linked to 20q, and CHMP4B was identified as the pathogenic gene for ADCC at this locus [13].

In this study, we analyzed a four-generation, non-consanguineous Chinese family diagnosed as having infantile total cataract. It is different from other congenital cataract because there is no amblyopia presented posteriorly with intraocular lens transplantation. Because the cataract was present at the age of 10–12 years and showed progressive development of lens opacities within one to two years and decreased visual acuity. Our genome-wide linkage screen mapped the new infantile cataract pathogenic gene on chromosome 20p12.2-20p11.23. The maximum LOD score reached 5.15 for marker D20S471 at a recombination fraction of 0. The infantile cataract gene was further defined within a 12.5 cM (7.5 Mb) interval between markers D20S915 and D20S912. In this region, the potential candidate genes include...
BFSP1 and the newly-reported pathogenic gene, CHMP4B. However, direct DNA sequence analysis did not identify any mutation in the two candidate genes. Our results suggest that a gene for infantile total cataract is located on chromosome 20p11.23.

**METHODS**

**Study participants:** We recruited a four-generation, non-consanguineous Chinese family from the Henan province with multiple family members diagnosed as having total cataract. The family displayed an autosomal dominant inheritance pattern (Figure 1). There are 17 patients. Blood samples were obtained from 14 living patients.

Complete medical history analyses and ophthalmic examinations were performed on all living family members. The examinations include assessment of visual acuity and a detailed examination of the ocular lens under a slit lamp to determine the disease status. The results are shown in Table 1. In this family, the phenotype of lens opacity showed total cataract in morphology (Figure 2). The initial clinical manifestation for all affected members was opacity of the lens and decreased visual acuity at the age of 10–12 years. Interestingly, the cataract phenotype starts to manifest as early as just after birth (Figure 2).

Informed consent was obtained from all participants, and the study was in accordance with the tenets of the Declaration of Helsinki on human subject research.

**Genotyping:** Genomic DNA was extracted from the peripheral blood of 22 family members with a DNA isolation kit for mammalian blood (Wizard Genomic DNA Purification kit; Promega, Madison, WI). The DNA samples were quantified by a spectrophotometer and diluted to 25 ng/μl for polymerase chain reaction (PCR) amplification.

The initial genome-wide screen was performed with 382 highly polymorphic fluorescent markers (PRISM Linkage Mapping Set MD-10, Applied Biosystems, Foster City, CA) that have an average spacing interval of 10 cM over the entire human genome. Other fluorescently labeled markers were selected and designed according to the Marshfield Clinic Medical Genetics database for fine mapping. Each PCR genotyping reaction was performed in a 5 μl volume containing 50 ng of genomic DNA, 10 μM dye-labeled primer pairs, 0.5 μl 10X PCR buffer (GeneAmp Buffer II, Applied Biosystems), 0.5 μl 10 mM dNTP mix, and 0.2 U of Taq DNA polymerase (AmpliTaq Gold Enzyme, Applied Biosystems). Amplification was performed in a GeneAmp 9700 PCR system (Applied Biosystems). PCR products (1 μl) from each DNA sample was pooled and mixed with 0.2 μl of Liz Size Standard-500 and 9 μl of Hi-Di formamide (both from Applied Biosystems, Foster City, CA). The mixture was then fractionated by electrophoresis and visualized on a 3100 Genetic Analyzer (Applied Biosystems). The GenScan 3.1 and GeneMapper 2.5 software (Applied Biosystems) were used to analyze the alleles.

**Linkage analysis:** Two-point linkage analysis was performed using the MLINK program from the LINKAGE program package (version 5.2). The linkage was performed assuming an autosomal dominant inheritance pattern, 99% penetrance, and a disease gene frequency of 0.0001.

Multipoint linkage analysis was computed using GENEHUNTER version 2.1 running on the Linux operating system, and the marker order and positions were based on the Marshfield Clinic Medical Genetics map (Table 2). Haplotype analysis was performed using the Cyrillic 2.1 (Cyrillic Software, Setauket, NY) program and manual prediction.
Mutational analysis: All exons and exon-intron boundaries of candidate genes, BFSP1 and CHMP4B, were amplified by PCR, purified, and sequenced using the BigDye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems).

RESULTS

We clinically characterized a large Chinese family with a diagnosis of total cataract, which manifests as early as at birth (infantile cataract, Figure 2). A genome-wide genotyping scan was performed for 22 members of the family including 14 living patients, five normal individuals, and three married spouses, using 382 microsatellite markers covering the entire human autosome every 10 cM. We obtained a positive two-point LOD score of 2.73 at θ=0 with marker D20S195 (Table 2). The two markers flanking D20S195 were uninformative. Fine mapping was then carried out with markers near D20S195, and multiple markers showed LOD scores greater than 3.0 (Table 2). Marker D20S471 showed the highest LOD score of 5.15. Multipoint linkage analysis showed a peak LOD score of nearly 5.0 for the chromosomal region from D20S910 to D20S471 (Figure 3). These results revealed that the gene for infantile total cataract in the Chinese family is linked to markers D20S910 to D20S471 on chromosome 20 with significant LOD scores.

Haplotype analysis was constructed for eight markers on chromosome 20p12.2-p11.23. Obligate recombination events...
were identified in patient IV-4 between D20S604 and D20S915 and in individual IV-7 between D20S915 and D20S910. Thus, D20S915 was defined as the left flanking marker for the locus. One non-obligate recombination event was detected in a normal individual, IV-8, between D20S471 and D20S912, which defines D20S912 as the right flanking marker for the locus.

Mutational analysis of two candidate genes on chromosome 20, BFSP1 and CHMP4B, did not reveal any disease-associated mutation.

**DISCUSSION**

Using genome-wide genetic linkage analysis, we have shown that a gene for an autosomal dominant infantile total cataract is located on the short arm of chromosome 20, within a 7.4 Mb interval between markers D20S915 and D20S912. Recently, Shiels et al. [13] reported linkage of autosomal dominant progressive childhood posterior sub-capsular cataracts to chromosome 20q in a Caucasian family and identified the pathogenic gene as CHMP4B in a refined disease interval of 3.5 cm. We used direct DNA sequence analysis for mutational analysis of CHMP4B, but no mutation was identified. Furthermore, CHMP4B is located outside of the refined disease locus. Thus, the infantile cataract gene on chromosome 20p12.2-p11.23 in the Chinese family is not CHMP4B.

One Japanese autosomal dominant posterior cataract family has been linked to chromosome 20p12–20q12 [11]. A Chinese autosomal dominant progressive congenital zonular nuclear cataract family has been linked to chromosome 20p12.2-p11.23 [12]. Interestingly, the Chinese family with infantile total cataract under this study is also linked to the same region. It is possible that the same gene is responsible for three different types of cataracts in the Japanese family, the Chinese family, and the family under this study. Our infantile total cataract locus is 2 cM smaller than the progressive congenital zonular nuclear cataract locus identified in the other Chinese family and much smaller than the posterior cataract locus identified in the Japanese family.

On the other hand, due to the highly clinical and genetic heterogeneity of congenital cataracts, it may be possible that the distinct genes contributed to the different cataract phenotype at this specific chromosomal region. Future identification of these specific genes should be able to distinguish the two hypotheses.

There are no other obvious candidate genes for autosomal dominant total cataract on chromosome 20p12.2-p11.23 except for BFSP1. BFSP1 encodes the beaded filament structural protein 1, a lens-specific intermediate filament-like protein, which functions as a major cytoskeletal element of the eye lens and is essential to the optical properties of eye lens. Mutations in BFSP2 have been reported to be associated with autosomal dominant congenital zonular cataract [14,15]. In a consanguineous family of Indian origin with autosomal recessive juvenile onset cortical cataract, linkage was detected with markers between D20S852 and D20S912 (peak LOD=5.4 with D20S860), and one homozygous deletion in BFSP1 was identified [16]. Heterozygous carriers did not develop cataracts. Direct DNA sequence analysis of the entire coding region and exon-intron boundaries of BFSP1 has been previously conducted in the Japanese and Chinese cataract families linked to chromosome 20, but no mutation was identified [11,12]. We also performed direct DNA sequence analysis of BFSP1 for the proband from the family under this study but did not detect any mutation. Although we cannot exclude the possibility that a BFSP1 mutation in the promoter or an intron may be associated with cataract in the family, this is unlikely because our results are consistent with the findings by Yamada et al. [11], Li et al. [12], and Ramachandran et al. [16] that heterozygous carriers for a BFSP1 deletion were phenotypically normal.

In summary, these results indicate that there is new gene on chromosome 20p12.2-p11.23 that is responsible for infantile total cataract. The disease gene interval has been defined between markers D20S915 and D20S912, a 7.4 Mb region. Future studies of the candidate genes within the locus should identify the specific gene, which will provide further important insights into the genetic basis of infantile cataracts.

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