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An autosomal dominant posterior polar cataract locus maps to human chromosome 20p12–q12

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We assigned the locus for a previously reported new type of autosomal dominant posterior polar cataract (CPP3) to 20p12–q12 by a genome-wide two-point linkage analysis with microsatellite markers. CPP3 is characterized by progressive, disc-shaped, posterior subcapsular opacity. The disease was seen in 10 members of a Japanese family and transmitted in an autosomal dominant fashion through four generations. We obtained a maximum lod score (Z_{max}) of 3.61 with a recombination fraction (θ) of 0.00 for markers *D20S917*, *D20S885* and *D20S874*. Haplotype analysis gave the disease gene localization at a 15.7-cM interval between *D20S851* and *D20S96* loci on chromosome 20p12–q12. Since the *BFSP1* that encodes the lens-specific beaded filament structural protein 1 (filensin) has been mapped around the CPP3 region, we performed sequence analysis on its entire coding region. However, no base substitution or deletion was detected in the CPP3 patients. The mapping of the CPP3 locus to 20p12–q12 not only expands our understanding of the genetic heterogeneity in autosomal dominant posterior polar cataracts but also is a clue for the positional cloning of the disease gene. *European Journal of Human Genetics* (2000) **8**, 535–539.

Keywords: autosomal dominant congenital cataract; posterior polar cataract; mapping; linkage analysis; haplotype analysis; *BFSP1*; filensin; chromosome 20

Introduction

Congenital cataract is one of the causes of visual impairment during infancy, and the prevalence is estimated to be 1.2–6.0 in 10 000 infants.[1] Approximately a quarter of the diseases is hereditary.[2] Among the various forms of congenital cataracts (zonular, polar, total, and membranous), posterior polar cataract (CPP) (MIM 116600) is the genetic term given to subcapsular opacities in the posterior polar regions of the lens. Posterior subcapsular opacities of the lens may occur not only in congenital cataracts but also in senile, diabetic, and steroidal-treated patients, and sometimes in those with neurofibromatosis type II or a type of retinitis pigmentosa. Posterior lens opacities impair visual acuity because of their central or axial position; however, little is known about the underlying pathogenesis.

Previous clinical and genetic studies on autosomal dominant congenital cataracts have identified at least 10 different loci, although allelic heterogeneity may exist in some of them. An autosomal dominant posterior polar cataract, defined as CPP1 (or CTPP1), has been tentatively assigned to 16q,[3] and another form, CPP2 (or CTPP2), is linked to 1p36.[4] Chromosomal localizations or regions assigned for other cataract loci are 1p36,[5] 1q,[6] 2q,[7–9] 12q,[10] 13cen,[11] 16q,[12] 17p[13] 17q,[14,15] 21q[16] and 22q.[17] Autosomal dominant congenital cataracts including above loci have been reviewed recently.[18–21]

We previously identified a new form of CPP, defined as CPP3 (or CTPP3), in a Japanese family in which 10 members in four generations were affected, and a genetic study

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excluded all known autosomal dominant congenital cataract loci.[21] Here we report the results of a genome-wide two-point linkage analysis of this CPP3 family.

Materials and methods

Family study

The proband is a 45-year-old Japanese man (III-6, Figure 1) with bilateral congenital posterior polar cataract. He belongs to a family in which a total of 10 members suffer (or suffered) from the cataract. The family descended from a group of people who immigrated from another region of Japan to Moriyama district in Nagasaki prefecture after a big religious war (the Shimabara war) about 400 years ago. Clinical data of the family have been described in detail elsewhere, and the cataract seen in 10 of them is referred to as CPP3.[21] In brief, representative manifestations of the disease include bilateral, disc-shaped, posterior polar cataracts, which progress gradually with diffuse cortical opacities, although variable expressivity is present within the family. The time of surgery also varied significantly. After informed consent was obtained, blood samples were prepared from seven affected members and nine unaffected members and/or their spouses. Their lymphocytes were immortalized as Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines. High-molecular weight genomic DNA was extracted directly from the blood samples or from their lymphoblastoid cell lines (Figure 1).

Genotyping and linkage analysis

A genome-wide screening was performed on the CPP3 family members using microsatellite polymorphic markers chosen to be spaced at 5–10 cM. Sets of polymerase chain reaction (PCR) primers for 450 Généthon microsatellite repeat markers[22] were synthesized. In each set, a forward primer was labeled with fluorescence dye, Cy5 (Amersham Pharmacia Biotech, Uppsala, Sweden), and its reverse primer was unlabeled. Genotypes of the family members for each marker locus were determined according to the DNA sequencer-assisted method.[23] The resulting data were analyzed with a software (Fragment Manager™ version 1.2, Amersham Pharmacia Biotech) to determine genotypes.

Two-point lod scores were calculated by MLINK of the FASTLINK software version 4.0P[24] under the following assumptions: a gene frequency of 0.0001, full penetrance, and equal allele frequencies at all the loci examined. Recombination distances between the marker loci were based on the Généthon linkage map.[22]

Sequence analysis of *BFSP1* coding region

The human beaded filament structural protein 1 gene (*BFSP1*, GenBank accession number: AF039655) that encodes filensin has been mapped to chromosome 20p11.23–p12.1.[25] In order to confirm the gene assignment, the Stanford G3 radiation hybrid panel (Research Genetics, Huntsville, AL, USA) was screened with a primer set designed from the 3'-UTR sequence of *BFSP1*. Results from the PCR were submitted to the Stanford Human Genome Center. Since the gene structure for *BFSP1*, its eight coding exons,[26] 3'-ends intronic sequences of exon 3, and the intron–exon boundary sequences of exon 4–8 (GenBank accession Nos. Y16718, Y16719, Y16720, Y16721, Y16722, and Y16723, respectively) have been described, we determined intronic sequences flanking exon 1, exon 2 and exon 3 of the gene. A P1-derived artificial chromosome (PAC) library[27] was screened with the same primers for the radiation hybrid mapping, and an isolated PAC clone was used as a sequencing template.

In order to analyze the entire *BFSP1* exon sequence and splice signals in the CPP3 patients, PCR products from two patients (III-2 and III-6) were directly sequenced for the open reading frame (ORF) region, with primers designed from the exon-flanking intronic sequences (Table 1). The sequences in the patients were compared to those of normal control individuals as well as to published gene sequences.

Results

The genome-wide two-point linkage analysis gave a high lod score ($Z_{max} = 3.61$, $\theta = 0.00$) at *D20S917*, *D20S885* and *D20S874* marker regions on chromosome 20. For further fine mapping of the disease locus, another series of 30 polymorphic markers[22] derived from the regions was tested. An overview of two-point lod scores between the CPP3 locus and relevant 12 markers is shown in Table 2.

Haplotype analysis of the affected family members revealed three recombination events that narrowed the disease gene localization (Figure 1). One recombination occurred in individual III-6 between *D20S851* and *D20S917*, and the recombinant chromosome was inherited by his two children; another recombination was observed in III-2 between *D20S96* and *D20S888*, and the other recombination was found in his daughter (IV-2) between *D20S874* and *D20S96*. Thus, a haplotype segment common to all the affected members ranges from *D20S917* to *D20S899*, and a critical region for CPP3 lies between the *D20S851* and *D20S96* loci.

The radiation hybrid panel mapping revealed that *BFSP1* is linked to an STS, *D20S114*, with a lod score of >6.0. This indicated that *BFSP1* is localized 10.89 cR from the STS and between *D20S1015* and *D20S182* GDB loci. The resulting intronic sequences flanking exon 1, exon 2 and exon 3 of *BFSP1* have been deposited at GenBank (GenBank accession numbers, AF191045, AF191046, and AF191047). Sequence analysis on the *BFSP1* ORF region revealed no evidence of mutation in the two CPP3 patients, but two silent polymorphisms were detected in exons 7 and 8 in normal control individuals (data not shown).

Discussion

We assigned an autosomal dominant posterior polar cataract (CPP3) to chromosome 20p12–q12, to which no cataract loci

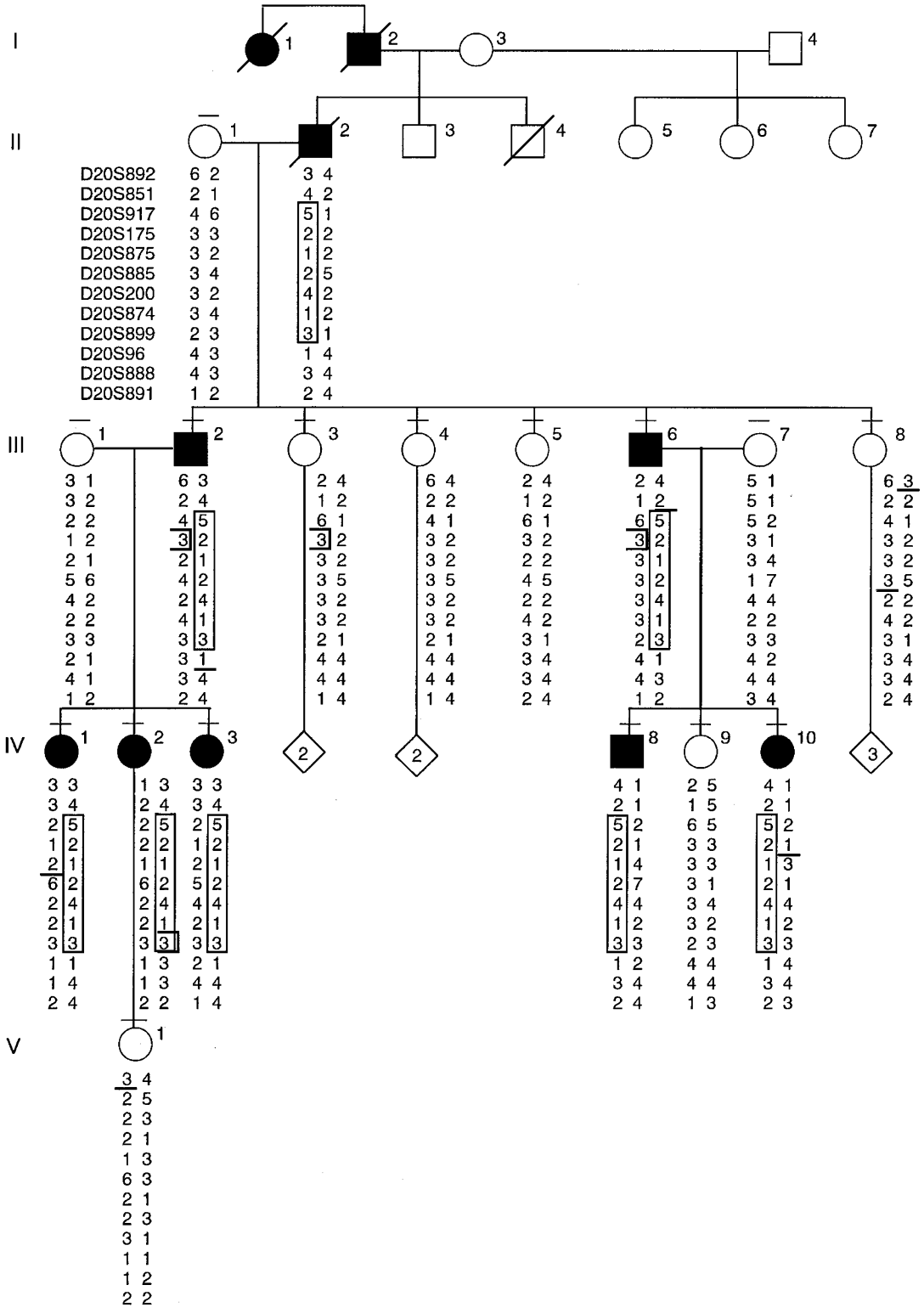

Figure 1 Pedigree of a posterior polar cataract (CPP3) family and haplotypes of markers on chromosome 20. Solid and open squares/circles depict affected and unaffected individuals, respectively. Bars on the individual symbols indicate those examined ophthalmologically. Common haplotypes segregating with the disease phenotype are boxed. Heavy short lines, and heavy double short lines depict definite recombination sites and recombination sites that could have occurred on either side of the corresponding marker(s), respectively.

Table 1 PCR primers used in the present study

Exon	*Primer*	*Direction*	*Sequence (5′–3′)*
1a	B1F	Forward	CCTCATTGCCCAGCGTACCTTCC
	B1R	Reverse	CATCCAGCTGCCTCCGGAGCC
1b	B2F2	Forward	AAGGAGCAGTACGAGCACGC
	B2R2	Reverse	GAGGTCATCGATCGACAGGG
2	PF2F	Forward	TGCCCCTGGTTACCCCAC
	PF2R	Reverse	ACCTGCACTTCCACCATTCC
3	PF3F	Forward	CTCCCAGGTGGTCTGTGTG
	PF3R	Reverse	TCATGCAGTCTGTTTCAGCC
4	SF4.1F	Forward	GAAGGAAAACCAGCGCAG
	SF4.1R	Reverse	CAGGTACAGCTTCCCTCCAG
5	SF5F	Forward	CCTCTCTGCATGTCCCATGAG
	PF5R	Reverse	TAGGATATGTTCAGCGTGTG
6	PF6F	Forward	TGGTGAGGTCTGTCTCTTAG
	PF6R	Reverse	ACCTGCTGGGACACTTATG
7	PF7F	Forward	GTGAGCAATTTGGTCTAGAG
	PF7R	Reverse	TTTCCAGATGGATCTGAAGC
8a	PF8F	Forward	TTCTGCTCCAGATGAAGG
	Fex9R	Reverse	TGCCCATTCTCTAAAGGGG
8b	SF8.1F	Forward	AAGACTCTGTGCTTTATGACG
	PF8R	Reverse	CAGATGGGTCAACTATGGTC

had yet been assigned. Although, the size of the critical region is 33.9 cM, based on the sex average map,[22] most of the meioses scored in this family occurred in males, it is more appropriate to estimate the size from the male linkage map than from the sex average map.[22] In this case, the critical region decreases to 15.7 cM. CPP3 is a new clinical form of autosomal dominant cataract characterized by bilateral, disc-shaped posterior polar opacities which grow larger and denser, progress with cortical opacities, and finally lead to total cataract.[21] Two autosomal dominant forms of CPP, CPP1 and CPP2, have been identified. Maumenee[3] reported a loose linkage (Z_{max} = 1.8, θ = 0) of CPP1 to the haptoglobin gene at 16q, and Ionides *et al*[4] mapped CPP2 to 1p36 (Z_{map} = 3.14, θ = 0). Therefore, CPP3 is genetically distinct from these two cataracts and is a third locus for autosomal dominant CPP. Recently, Ionides *et al*[20] reported a family with a progressive posterior polar cataract that looks similar clinically to CPP3, although the cataract locus has not yet been assigned. It remains to be seen whether the cataract reported by Ionides *et al*[20] and CPP3 are genetically distinct.

Several genes and ESTs that may be related to eye diseases have been assigned to the region to which we assigned the CPP3 locus. They include *PLCB4* (β-4 phospholipase C gene, MIM 600810), *BFSP1* (filensin gene, MIM 603307), *PLCG1* (γ-1 phospholipase C gene, MIM 172420), *MYBL2* (locus for V-myb avian myeloblastosis viral oncogene homolog-like 2, MIM 601415), *SDC4* (gene for amphiglycan ryudocan, MIM 600017), and *KRLM* (Kreisler mouse maf-related leucine zipper homolog, GenBank accession number, NM 005461). Among them, *BFSP1* would have been a potential candidate for the *CPP3* gene, because its encoding protein, filensin, is a lens-specific intermediate filament (IF) referred to as the beaded filament.[28] Although the function of IF is not yet fully understood, it has been suggested that IF plays an important role in supporting cellular architecture and providing mechanical strength. Indeed, truncated keratin intermediate filament heterodimer can impair the mechanical stability of epithelial cells, and may be related causally to epidermolysis bullosa simplex.[29] Filensin has been believed to be functionally important in lens fiber cell differentiation and in maintaining lens fiber cell conformation and transparency.[30]

BFSP1 contains 8 exons with an ORF of 1995 bp, the entire gene size is approximately 35 Kb, and some intron–exon junctions have been sequenced.[26] This allowed us to analyze patients in the CPP3 family as to whether they have a *BFSP1* mutation. However, there was no evidence of base substitutions or deletions in the protein coding region of the gene in our patients. Thus, it is unlikely that *BFSP1* mutations cause CPP3, though it remains to be determined whether there is a mutation in the non-coding regions of *BFSP1*, including the promoter region and introns.

Understanding of the *CPP3* gene will give insight into specific features of lens function and dysfunction.

Table 2 Two-point lod scores at 11 markers on chromosome 20

		lod score at θ =							
Markers	*Distance (cM)*[a]	*0.00*	*0.001*	*0.05*	*0.10*	*0.15*	*0.20*	*0.30*	*0.40*
D20S892	6.2	–∞	–5.39	–0.51	0.16	0.45	0.57	0.57	0.36
D20S851	1.4	–∞	0.61	2.04	2.06	1.94	1.75	1.25	0.63
D20S917	1.1	3.61	3.61	3.32	3.02	2.69	2.35	1.60	0.76
D20S175	7.7	1.81	1.80	1.67	1.53	1.38	1.22	0.88	0.48
D20S875	0.0	2.71	2.70	2.49	2.25	2.00	1.74	1.16	0.53
D20S885	2.1	3.61	3.61	3.32	3.02	2.69	2.35	1.60	0.76
D20S200	0.0	3.01	3.01	2.74	2.47	2.18	1.87	1.23	0.54
D20S874	2.3	3.61	3.61	3.32	3.02	2.69	2.35	1.60	0.76
D20S899	1.1	2.41	2.40	2.21	2.00	1.77	1.54	1.02	0.45
D20S96	0.1	–∞	0.61	2.04	2.06	1.94	1.75	1.23	0.59
D20S888	1.8	–∞	–3.79	–0.59	–0.19	–0.05	–0.01	–0.03	–0.04
D20S891	0.0	–∞	–2.69	0.49	0.85	0.96	0.95	0.73	0.38

[a]Recombination distances based on previously published data for the male linkage map[22] are shown in centimorgans (cM).

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