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SHORT COMMUNICATION

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A locus for congenital preauricular fistula maps to chromosome 8q11.1–q13.3

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Abstract The incidence of congenital preauricular fistula (CPF) is >1.1% in both Chinese and Caucasians, but it is even higher in Blacks. We mapped the locus for CPF to chromosome 8q11.1–q13.3 by linkage analysis of a family composed of 7 affected and 11 nonaffected members. The two-point LOD score was 2.40, shown by markers D8S285 and D8S1113 at a recombination fraction (θ) of 0.00. Results from three other markers (D8S1110, D8S260, and D8S1136) in the same region further support the linkage. Haplotype analysis for this family confined the locus to within an interval of approximately 26.7 cM, flanked by markers D8S532 and D8S279. A LOD score of <3 is likely due to the limitation of family size.

Key words Congenital preauricular fistula · Linkage analysis · Gene mapping · Genotyping · Haplotyping · Mutation

Introduction

Congenital preauricular fistula (CPF, MIM 128700) is a common auricular abnormality, the incidence of which is 1.2% in Chinese (He and Jiang 1983), 1.1% in Caucasians (Gualandri 1969), and higher in Blacks (Simpkiss and Lowe 1961). Familial CPF associated with major congenital disorders is not observed, whereas serious congenital anomalies have been detected in one third of sporadic cases of CPF (Meggyessy and Mehes 1982). CPF occurs in the upper anterior end of the ear helix as a kind of narrow, winding fistula, sometimes with branches. The formation of CPF is closely associated with embryological development of the auricle, which is developed from the first and second branchial arches. It is hypothesized that this is either due to malfusion of the cumular nodules of the first and second branchial arches, or due to incomplete closure of the first branchial cleft (He and Jiang 1983). CPF is generally classified into the following three types: simple, infectious, and secretory. The majority of patients are seen by physicians because of infection, but the simple and secretory types of CPF are usually also found in patients with infectious CPF. Gualandri (1969) found 321 cases of CPF in 29309 school children in Milan, 93 of whom had a condition of autosomal dominant inheritance with high penetrance. Another report based on a large kindred also suggested this mode of inheritance (Bhalla et al. 1979). However, the gene underlying CPF has not been assigned.

Here we report the result of a genome-wide linkage analysis on a Chinese family with CPF.

Patients and methods

Family and patients

A Chinese family with three generations comprising 18 members (7 affected and 11 unaffected individuals) from Shanghai was recruited in this study. Informed consent was obtained from all the family members for participation in a linkage analysis, and they underwent general medical and detailed otorhinolaryngologic evaluations in Shanghai Chang Zheng Hospital. Except for preauricular fistulae, they did not express congenital defects such as hearing impairment, renal dysplasia, and branchial fistulae, which

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are all characteristic of branchio-oto-renal (BOR) syndrome. Results of clinical evaluations showed that the affected family members had simple preauricular fistulae but not any syndromes. Among the seven affected members, individuals II-1 and II-8 (Fig. 1) showed a preauricular fistula on the left ear, whereas individuals II-6, II-11, III-1, III-6, and III-7 had the fistulae bilaterally.

Genotyping and linkage analysis

Peripheral blood samples were taken from all available members in the family, and DNA was extracted by the standard method (Yang et al. 2000). A set of dinucleotide repeat microsatellite markers based on the Généthon genetic map (Dib et al. 1996), with a 10-cM resolution, was purchased from Perkin-Elmer Applied Biosystems (Foster City, CA, USA) and recruited for genome-wide genotyping. We also selected additional markers for denser mapping of candidate chromosomal regions. The forward primer of each primer pair was labeled with fluorescent dyes (FAM, HEX, or NED) to perform multiplex analysis on an ABI 377 DNA sequencer (Perkin-Elmer Applied Biosystems).

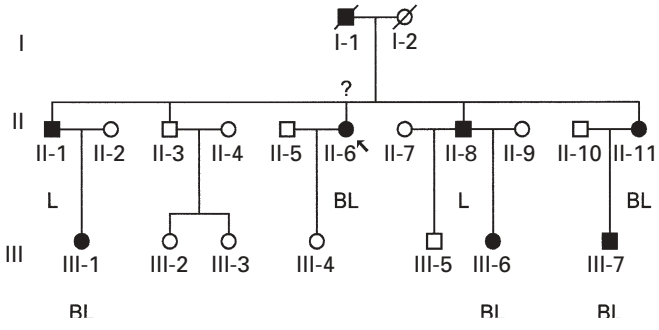

Fig. 1. Pedigree of congenital preauricular fistula. *Open*, *solid*, *square*, and *circle* symbols are unaffected, affected, men, and women, respectively. *BL*, Bilaterally affected; *L*, affected in the left ear only. The affected side of individual I-1 was unknown

Two-point LOD scores for the putative disease locus were calculated in each marker by the use of the MLINK program of FASTLINK version 5.1 (Lathrop et al. 1984). An autosomal dominant model with 99% penetrance was assumed, and the disease-gene frequency was set at 0.001 for the LOD score calculation. LOD scores were calculated at recombination fractions (θ) of 0.00, 0.01, 0.05, 0.10, 0.20, 0.30, and 0.40. Equal recombination frequencies were assumed between male and female family members. The maximum score (Zmax) was calculated by the ILINK program of the LINKAGE software package.

In a candidate gene, *EYA1*, we sequenced all 17 exons using an ABI 3100 DNA sequencer (Perkin-Elmer Applied Biosystems; Gao et al. 2001). Primers were synthesized according to the sequences published (Abdelhak et al. 1997; Vincent et al. 1997; Kumar et al. 1998; Azuma et al. 2000).

Results

By carrying out genome-wide scanning, two adjacent markers (D8S285 and D8S260) at chromosome 8q gave LOD scores of 2.40 and 2.10, respectively. Further analysis of four markers (D8S1110, D8S1113, D8S1136, and D8S2324) also strongly supported this region as a candidate. The highest LOD score (Zmax) of 2.40 at $\theta = 0.00$ was obtained at marker loci D8S285 and D8S1113. D8S260 and D8S1136 adjacent to D8S1113 also revealed a LOD score of >2. More markers (D8S505, D8S1722, D8S532, D8S543, D8S279, and D8S275) recruited to define the CPF locus in the denser map resulted in proportional positive scores according to their locations (Table 1).

Haplotypes of the family were constructed with all the markers listed earlier (Fig. 2). A disease-associated haplotype shared by all the affected individuals (II-1, II-6, II-8, II-11, III-1, III-6, and III-7) was observed. Recombination events were found in two affected individuals (III-6 and III-7) and in two unaffected individuals (III-4 and III-5). Based on these recombination events, we confined the CPF

Table 1. Two-point linkage data in the CPF family

	LOD score at recombination fraction (θ)								
Locus	.00	.01	.05	.10	.20	.30	.40	Zmax	θmax
D8S505	−3.19	−0.23	0.34	0.48	0.43	0.25	0.07	0.49	.126
D8S1722	−2.59	0.36	0.90	0.99	0.83	0.50	0.14	0.99	.10
D8S532	−2.54	0.36	0.90	0.99	0.83	0.51	0.15	0.99	.10
D8S1110	1.20	1.18	1.07	0.93	0.63	0.33	0.08	1.20	.00
D8S285	2.40	2.35	2.18	1.94	1.43	0.86	0.28	2.40	.00
D8S1113	2.40	2.35	2.18	1.94	1.43	0.86	0.28	2.40	.00
D8S260	2.10	2.06	1.90	1.69	1.23	0.72	0.22	2.10	.00
D8S1136	2.10	2.06	1.90	1.69	1.23	0.72	0.22	2.10	.00
D8S543	0.92	0.90	0.82	0.72	0.50	0.28	0.09	0.92	.00
D8S279	0.29	0.56	0.88	0.93	0.76	0.45	0.12	0.94	.092
D8S2324	−0.99	−0.67	−0.17	0.06	0.18	0.13	0.04	0.18	.204
D8S275	−4.42	−2.38	−1.33	−0.81	−0.31	−0.11	−0.02	−0.00	.501

CPF, Congenital preauricular fistula

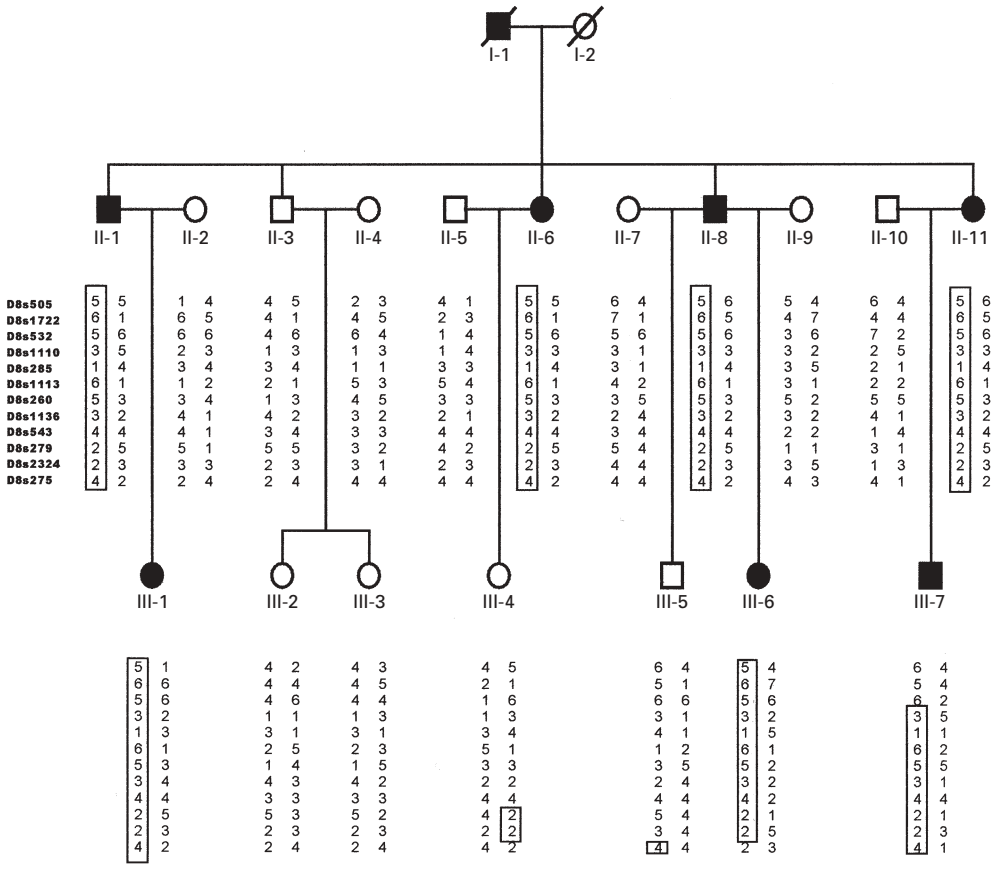

Fig. 2. The pedigree of congenital preauricular fistula showing haplotypes for polymorphic markers at 8q11.1–13.3. Marker orders were determined from the généthon sex-averaged genetic map and Genome Database. *Open*, *solid*, *square*, and *circle* symbols are unaffected, affected, men, and women, respectively. The haplotype shared by affected members is *boxed*

locus to an interval of <26.7 cM flanked by markers D8S532 and D8S279 at 8q11.1–q13.3.

No mutations in *EYA1* were found in any affected members in the family.

Discussion

We successfully assigned the CPF locus to 8q11.1–q13.3. Because of the limitation of the size of the family we analyzed, the Zmax obtained was 2.40, lower than a significant value of 3.00. Positive results at multiple markers within the region, as well as the shared haplotype by all the affected members, strongly supported the linkage between the disease locus and the marker loci. In addition, the critical recombinants narrowed the linked interval to approximately 26.7 cM near markers D8S532 and D8S279.

The putative CPF gene may have functions in the developmental process of the auricle. By searching the database (Dib et al. 1996) for candidate genes in the region between D8S532 and D8S279, we considered *EYA1*, the human homologue of the *Drosophila* eyes absent gene, as the most likely candidate gene for CPF. *EYA1* has been reported to be responsible for BOR syndrome or branchial-otic syndrome (Abdelhak et al. 1997; Vincent et al. 1997). BOR syndrome, first described by Melnick et al. (1976), is characterized by branchial anomalies affecting the outer middle and/or inner ear that frequently lead to sensorineural, con-

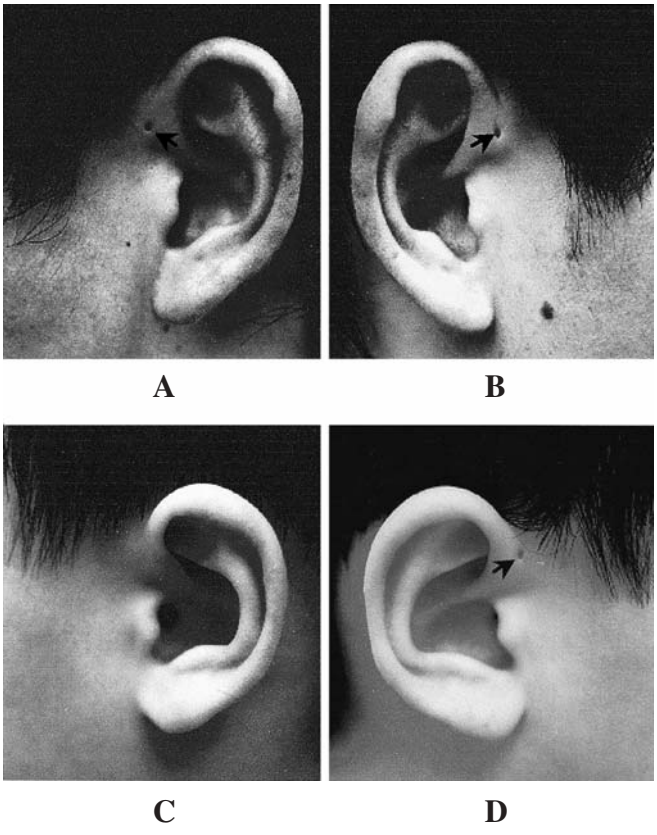

Fig. 3.A–D. Congenital preauricular fistula in a bilaterally affected member (**A** and **B**) and in a unilaterally affected member (**C** and **D**)

ductive, or mixed hearing loss (Fraser et al. 1980), and by a wide spectrum of renal anomalies ranging form mild hypoplasia to lethal bilateral renal aplasia. We found no mutation in *EYA1* in affected members in our family. However, because BOR syndrome and CPF are both heterogeneous, we cannot fully exclude *EYA1* from the candidacy for CPF in our family.

Interestingly, we have found phenotypes to be discordant between generations in this family (Figs. 1 and 3). For example, the fistula of II-1 appeared on his left ear, whereas those of his daughter (III-1) were bilateral. Bilateral fistulae appeared in both II-11 and his son (III-7). A similar phenomenon was also found in some other family members. We performed statistical analysis in 54 CPF families (no DNA samples available), and clinical information was collected by a physician at Chang Zheng Hospital in Shanghai. Of 186 affected individuals, 91 had CPF bilaterally and 95 had CPF unilaterally (left and right ear involvement in 48 and in 47 individuals, respectively). Because we were not able to find any distinct patterns in the study, a larger number of families may be required to further our understanding of CPF.

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