Hum Genet (2008) 122:589–593
DOI 10.1007/s00439-007-0435-y

ORIGINAL INVESTIGATION

Localization of a novel gene for congenital nonsyndromic simple microphthalmia to chromosome 2q11-14

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Received: 7 June 2007 / Accepted: 25 September 2007 / Published online: 9 October 2007

Abstract Microphthalmia is a clinically and genetically heterogeneous disorder of eye development. The genetic basis of nonsyndromic microphthalmia is not yet fully understood. Previous studies indicated that disease pedigrees from different genetic backgrounds could be attributed to completely different gene loci. To investigate the etiology in a large autosomal-dominant inherited simple microphthalmia (nanophthalmia) pedigree, which is the first genetically analyzed Chinese microphthalmia pedigree, we performed a whole-genome scan using 382 microsatellite DNA markers after the exclusion of reported candidates associated with microphthalmia. Strong evidence indicated that microphthalmia in this family was mapped to an unreported new locus on chromosome 2q. A significantly positive two-point LOD score was obtained with a maximum 3.290 at a recombination fraction of 0.00 for marker D2S2265. Subsequent haplotype analysis and recombination data further confined the disease-causing gene to a 15-cM interval between D2S1890 and D2S347 on 2q11-14. Our results further underlined the degree of heterogeneity in microphthalmia from Chinese background and localized a novel gene which regulates eye embryogenesis.

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Introduction

Microphthalmia (small eye [MIM 309700]) is an ocular developmental malformation characterized by a small eye (2/3 of the normal volume), short axial length (less than 20 mm), high hyperopia, narrow palpebral fissure and small fossa orbitalis. The reported prevalence of microphthalmia at birth is 0.009% (1/11,077) in China (Hu 1987) and 0.015% (~1.5/10,000) in white population (Bermejo and Martinez-Frias 1998). About 80% of microphthalmia cases occur as part of syndromes that include other systemic malformations, especially cardiac defects, facial clefts, microcephaly, and hydrocephnary (Kallen et al. 1996; Warburg 1993). Such syndromic disease pattern made it inconvenient to clarify the relationship between phenotype and genotype in microphthalmia.

Previous studies demonstrated that microphthalmia is a genetically heterogeneous disorder. Investigations in microphthalmia cases and pedigrees have mapped microphthalmia from different genetic background to different chromosomal regions (Bessant et al. 1998; Graham et al. 1991; Morle et al. 2000; Othman et al. 1998) and different gene mutations such as *OTX2* (Ragge et al. 2005), *CHX10* (Bar-Yosef et al. 2004; Ferda Percin et al. 2000), *PAX6* (Hanson 2003), *SOX2* (Hagstrom et al. 2005), *SHH* (Schimmenti et al. 2003) and *RX* (Voronina et al. 2004). Furthermore,

mutations in different genes could cause identical microphthalmia phenotype; and on the other hand, mutations in the same gene give rise to quite different microphthalmia phenotypes (Ferda Percin et al. 2000; Bar-Yosef et al. 2004; Ragge et al. 2005). The extreme heterogeneity has complicated the efforts to clarify the genetic basis of microphthalmia.

More studies in microphthalmia pedigrees from different genetic backgrounds, especially large and nonsyndromic pedigrees are helpful for revealing the genetic etiology of microphthalmia. Nonsyndromic pedigree can clearly map the phenotype to one member of the genes which direct eye development, and large pedigrees are most informative for gene mapping and linkage analysis of traits with genetic heterogeneity (Sanna-Cherchi et al. 2005).

In the current study, we performed a whole-genome scan in a large six-generation pedigree of autosomal-dominant congenital simple microphthalmia (nanophthalmia) of China origin, which is the first Chinese background microphthalmia pedigree analyzed at molecular level. The disease pattern of the pedigree is nonsyndromic. The karyotype in this pedigree was normal (46, XX.) (Yu et al. 2004), and our prophase work (Yin et al. 2006; Yu et al.2004) have excluded the linkage of microphthalmia in this pedigree with the reported eight candidate loci (*MITF*, *CHX10, NNO1, NNO2, PAX6, RX, MCOP* and *SOX2*) (Bessant et al. 1998; Ferda Percin et al. 2000; Hagstrom et al. 2005; Hanson 2003; Morle et al. 2000; Othman et al. 1998; Tassabehji et al. 1994; Voronina et al. 2004). In order to further identify the gene responsible for this Chinese microphthalmia family, we performed a whole-genome scan analysis by using 382 micro-satellite markers in the 22 pairs of autosomes. Linkage and haplotype analyses indicated that a novel locus was responsible for the microphthalmia in this family.

Subjects and methods

Subjects

A six-generation microphthalmia pedigree containing 101 individuals from Zhejiang Province in China was studied. Autosomal-dominant inheritance of the phenotype was ascertained by the presence of affected individuals in each of the six generations and with a male–male transmission. The penetrance in this pedigree is almost 100%, and affected members showed nonsyndromic microphthalmia without other ocular or systemic abnormalities. Blood samples from 28 individuals including 12 affected family members and 16 unaffected family members were collected and subjected to further analysis. All patients gave written informed consent before they were enrolled in this study.

Phenotypes of the patients

A detailed ophthalmologic examination showed that affected members had the phenotype of a small eye globe, enophthalmos, narrow palpebral fissure, light ptosis, superficial anterior chamber, lens thickness, small cornea and normal pupil. Affected members demonstrated high hyperopia, within the range +6.00 to +11.25 diopters (mean 8.25 dopters). The proband III-38, who came from a consanguineous marriage, showed the phenotype as following: bilateral small eye globe, 19.9 mm axial length, narrow palpebral fissure and light cernuous, 9.7 mm horizontal diameter and 9.4 mm vertical diameter of cornea, 4.5 mm axial length of lens, left eye with a refraction of 9.75 diopters and right eye with a refraction of 9.25 diopters, light anterior chamber and normal slit lamp biomicroscopy. Based on the clinical diagnosis, the type of microphthalmia in this pedigree was classified as congenital simple microphthalmia (nanophthalmia). Nanophthalmia (OMIM 600165), also referred to as "simple (or pure) microphthalmia" (Brockhurst 1974; Vingolo et al. 1994; Weiss et al. 1989), is a relatively rare condition characterized by a small eye (globe) in the absence of any systemic abnormalities.

Genotyping and statistical methods

Peripheral blood was collected from each individual with informed consent. Genomic DNA was extracted by using the standard phenol/chloroform method and stored at −20°C. The whole-genome scan was carried out by using 382 fluorescent microsatellite markers with an average spacing of 10-cM scattering on human genome (ABI prism linkage mapping set LMSV2.5). PCR was performed in a 15 μl volume with 10 ng genomic DNA as template, 1.5 μl PCR 10× buffer, 1.5 μl dNTP mix (2.5 mM), 1 μl primers, 1 μl $MgCl_2$ (15 mM), 0.12 U of Taq DNA polymerase (Takara) and 9 μl d.w. water. Thermal cycling in a Gene Amp 9700 (Perkin Elmer) thermal cycler was performed at 95°C for 12 min, followed by 10 cycles at 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s and 20 cycles at 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s, followed by a 10 min extension at 72°C and a hold at 10°C. PCR products were denatured and size fractionated on 6% denaturing polyacrylamide gel run on an ABI 3730 automated sequencer. GS500 size standard was used as internal standard run in the same lane with the markers. Alleles read and scored by use of GeneMapper V4.0 software (ABI Perkin Elmer) were confirmed by eye. Subsequently, additional 12 fluorescent markers from Genome database and Généthon database namely, D1S434, D1S2833, D1S227, D6S291, D8S552, D8S520, D11S912, D2S2187, D2S1890, D2S308, D2S283, D2S2265 were analyzed for fine mapping.

Two-point LOD scores were calculated by using MLINK program of Linkage software package (version 5.2). Microphthalmia of this family was assumed to be in an autosomal dominant model with a penetrance of 100%. The gene frequency of disease was set to 0.0001, and the mutation rate was set to zero. The recombination frequencies between male and female were assumed equal. Allele frequencies for all markers were calculated from ethnically matched population. Multipoint analysis was computed using FASTMAP (http://www.mds.qmw.ac.uk). Haplotypes were constructed with software Simwalk2 (http://www.genetics.ucla.edu).

Results

Linkage analysis

After initial scanning, markers located on six regions on chromosome 1p36, 1q41, 2q14, 6p21, 8p22 and 11q24 demonstrated two-point LOD scores >1.0 (Table 1). Further fine mapping excluded the linkage of microphthalmia with chromosome 1, 6, 8 and 11 because markers from these chromosomes gave LOD scores <-2. A maximum LOD score (Z_{max}) of 3.290 at recombination 0.00 was obtained at microsatellite marker D2S2265 on chromosome 2q14. Four other markers (D2S2264, D2S160, D2S308, and D2S283) around D2S2265 also gave positive LOD scores at recombination fraction 0.00, which showed a suggestive linkage to microphthalmia (Table 2). Multipoint linkage analysis of these markers did not increase the highest LOD score.

Haplotye analysis and recombination mapping

Eight microsatellite markers with the flanking region of D2S2216 and D2S347 were used to construct the haplotypes. Inspection of the haplotype transmission data revealed that a common affected haplotype expanding from D2S2264 to D2S2265 was inherited by all 12 affected members in this family (Fig. 1), which is consistent with the complete penetrance of the trait. The informative recombination events were present in affected individuals III16, III17 and IV20 between markers D2S1890 and D2S160, which localized the disease-causing gene centomeric to the marker D2S1890, but no crossovers were observed in telomeric marker D2S347. Thus, the disease gene responsible for microphthalmia in this family was assigned to a region of approximately 15 cM on chromosome 2q11-14 between D2S1890 and D2S347.

Discussion

It does not come as a surprise that a novel locus was linked to our Chinese congenital simple microphthalmia pedigree.

Table 1 Markers with two-point LOD scores values >1.0 in the first genome-scan

Marker	Location	Recombination fraction (θ)								
		0.00	0.01	0.05	0.10	0.20	0.30	0.39	Z_{max}	θ_{max}
D1S2267	1p36	1.768	1.739	1.622	1.466	1.128	0.766	0.428	1.766	0.00
D1S213	1q41	1.258	1.278	1.309	1.272	1.041	0.685	0.327	1.278	0.01
D2S160	2q14	1.848	1.814	1.676	1.500	1.138	0.770	0.433	1.848	0.00
D6S610	6p21	1.002	0.980	0.891	0.778	0.554	0.350	0.187	1.002	0.00
D8S550	8p22	1.635	1.587	1.398	1.162	0.701	0.295	0.058	1.635	0.00
D11S4151	11q24	1.113	1.092	1.005	0.894	0.667	0.442	0.244	1.113	0.00

Table 2 Two-point LOD scores between CMIC and markers on chromosome 2q

Marker	Recombination fraction						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D2S2187	$-\alpha$	−2.741	−1.883	−0.154	0.271	0.306	0.186
D2S2264	1.551	1.519	1.386	1.214	0.855	0.498	0.193
D2S1890	−3.416	−1.297	−0.635	−0.372	−0.140	−0.031	0.000
D2S160	1.804	1.771	1.637	1.465	1.113	0.754	0.386
D2S308	0.823	0.800	0.709	0.596	0.384	0.211	0.088
D2S283	1.346	1.319	1.209	1.068	0.782	0.509	0.256
D2S2265	3.290	3.228	2.978	2.656	1.980	1.268	0.560
D2S347	$-\alpha$	0.030	0.565	0.661	0.558	0.349	0.137

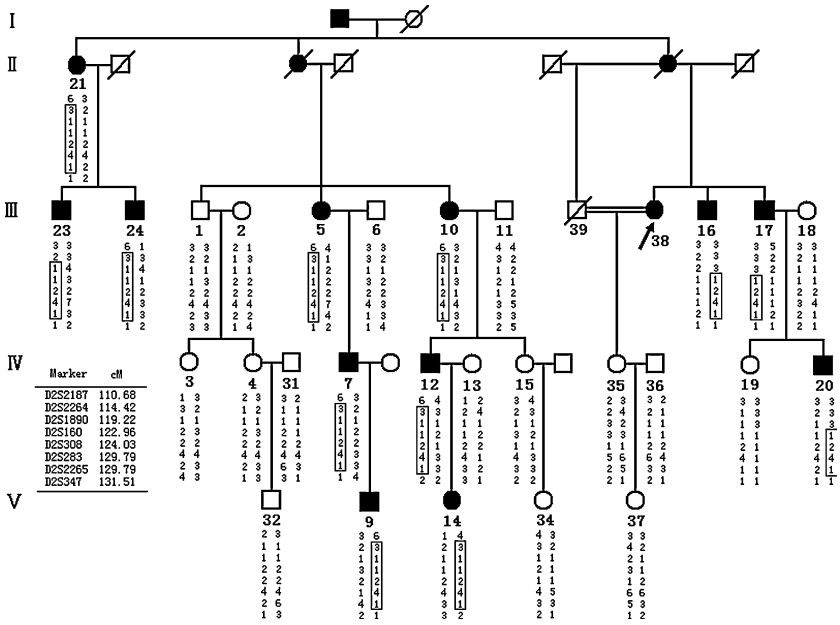

Fig. 1 Pedigree of the family studied and haplotypes obtained with eight microsatellite DNA markers on chromosome 2q. *Solid symbols* represent affected individuals; *open symbols* represent unaffected individuals. The sequence of markers is from centromere to telomere. The affected haplotype is shown in *rectangles*

More than 40 transcription factors and growth factors are found to be involved in mammalian eye development (Jean et al. 1998). Mutations in any of these genes could cause obstruction of eye morphogenesis and eventually result in eye morphogenesis abnormal. Previous studies also indicated that microphthalmia cases or pedigrees from different genetic background could be attributed to completely different disease-causing genes (Bessant et al. 1998; Morle et al. 2000; Othman et al. 1998). The pedigree studied here is the first Chinese background microphthalmia family analyzed at molecular level. Our result supplied new information to this disorder.

Within the 2q11-14 region defined by D2S1890 and D2S347, a total of 119 genes have been mapped in the NCBI Map Viewer (Build 35.1), and no genes have been reported to be associated with the microphthalmia phenotype. After analyzing gene function in this region, we selected *Gli2, Inhibin beta B* and *En-1* as the most probable genes that might be responsible for the microphthalmia phenotype in our pedigree because only these three genes have been reported to be related with eye development (Friedman and O'Leary 1996; Furimsky and Wallace 2006; Itasaki and Nakamura 1996; Ying et al. 1997). We performed mutation screen, by direct sequencing, to these three genes in our pedigree. Our results demonstrated that there is no pathogenic mutation in the coding region, 3′-UTR, 5′-UTR, and intron/extron splicing region of these three genes. Thus a novel gene in this interval, which has yet to be recognized, might be responsible for the microphthalmia phenotype of this pedigree.

It is expected that identification of new loci in such non-syndromic microphthalmia pedigree can help identify new members of eye development signaling cascades. Mapping genes in such a way has been an important tool for the detailed analysis of the complex process of eye development (Ferda Percin et al. 2000; Hodgkinson et al. 1993). More future studies on the expression pattern of disease-causing gene and its correlation with the eye phenotypes could help in understanding the molecular basis of inherited eye abnormalities in humans (Ragge et al. 2005).

In conclusion, we provided genome-wide evidence for the localization of a novel gene for autosomal-dominant simple microphthalmia on chromosome 2q11-14. Our results from Chinese background pedigree further underlined the degree of heterogeneity in microphthalmia and can facilitate in finding the new essential gene related to eye embryogenesis.

Acknowledgment We are grateful to the family members for participating in this research. We would like to acknowledge Dr. Gui-Sheng Wu and Dr. Qing-Peng Kong for their help in preparing the manuscript. We thank the anonymous reviewers for helpful comments on the earlier version of the manuscript. This work was supported by the Key State Research Program of China (2006CB943900), Science and Technology Committee of Yunnan Province and the National Natural Science Foundation of China.

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