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Full Length Research Paper

Gene mapping in a highly inbred consanguineous foveal hypoplasia family to cytogenetic region 16q24.1

Venkata Ramana Anandula[1], Rohit Shetty[2], AjoyVincent[2], Ramprasad VL[3] and N Ramesh[1]*

[1]Department of Biotechnology, JJ College of Arts and Science, Pudukottai, Tamil Nadu, 622422, India.
[2]Department of Cornea and Refractive Services, Narayana Nethralaya Super Specialty Eye Hospital and Post Graduate Institute of Ophthalmology, 121/C, Chord Road, Rajajinagar 1st 'R' Block, Bangalore – 560 010, India.
[3]Spinco Biotech, Chennai, India.

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A highly inbred uncle-niece, second degree consanguineous Foveal hypoplasia affected family with four members was subjected to gene mapping. Members underwent detailed ophthalmic evaluation including best corrected distance and near vision measurements, color vision assessment, fundus evaluation and flourescence angiography, horizontal corneal diameter measurement, total axial length measurement, optical coherence tomography and full-field electroretinogram. Peripheral blood was drawn from the members and DNA was extracted using Macherey-Nagel maxi kit (Germany). Gene mapping was performed by the Affymetrix SNP 6.0 Genechip through homozygosity mapping technique. Two point, multipoint analyses and haplotyping for all the 400 odd markers on chromosome 16 were performed. LOD score of 2.3 was obtained for the marker rs254347 and the disease segregated with the haplotypes. No other region showed similar significant association. The gene for foveal hypoplasia may be located on chromosome 16, near the SNP marker rs254347 at cytogenetic region 16q24.1.

Key words: Haplotyping, homozygosity mapping, chromosomes, consanguinity.

INTRODUCTION

Foveal hypoplasia is characterized by absence or abnormal foveomacular reflex, unclear definition of the foveomacular area, and presence of capillaries running abnormally close to the macula. Patients have decreased visual acuity and congenital nystagmus. It occurs in isolation as well as in association with other anatomical eye disorders such as aniridia, albinism, microphthalmos, presenile cataracts, peripheral retinal rosettes, and achromatopsia (Francois, 1961; Duke-Elder, 1963; Waardenburg, 1963; Curran and Robb, 1976; O'Donnell and Pappas, 1982). Foveal hypoplasia is a recognised cause of poor vision in early childhood (McGuire and Weinreb, 2003). Mutations with PAX 6 gene are documented with Isolated foveal hypoplasia (Azuma and Yamada, 1998) and anterior segment dysgenesis (Hanson and Churchill, 1999).

Occurrence of isolated and sporadic foveal hypoplasia are rare (Curran and Robb, 1976; Oliver and Dotan, 1987) and under diagnosed because of congenital nystagmus (Oliver and Dotan, 1987). Foveal hypoplasia with or without ocular associations has been described (Azuma and Nishina, 1996; Azuma and Yamaguchi, 1999; Pal and Mohamed, 2004). Foveal hypoplasia, microphthalmos, and retinochoroidal coloboma occur because of defects in embryogenesis at various stages of development of the fetus (Taylor, 2005). Southern India has many highly consanguineous recessive pedigrees with very rare inherited Mendelian disorders. We identified a consanguineous foveal hypoplasia affected family for gene mapping.

MATERIALS AND METHODS

Clinical

Members of the family underwent detailed ophthalmic evaluation including fundus evaluations, flourescence angiography, horizontal corneal diameter measurement, total axial length measurement, optical coherence tomography and full-field electroretinogram.

*Corresponding author. E-mail: ramanagene@gmail.com. Tel: 04322 – 260103. Fax: 04322 – 260103.

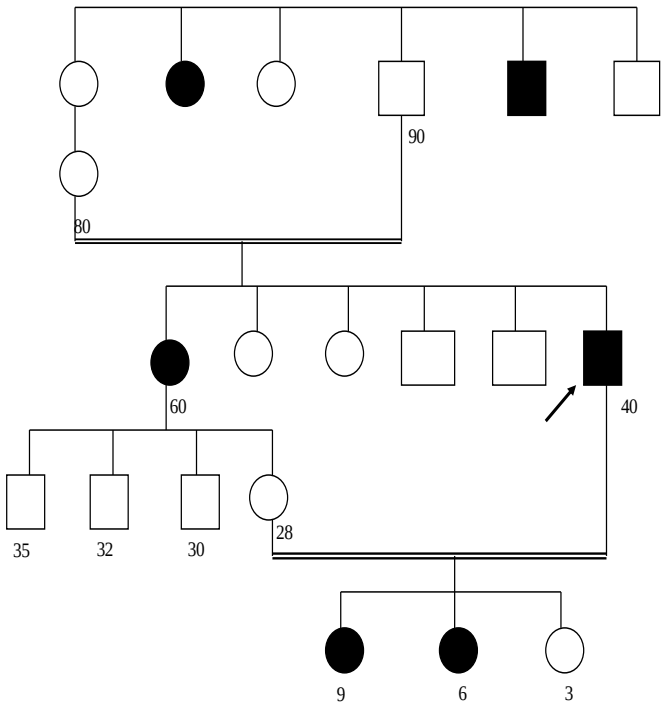

Figure 1. Pedigree of a five generation family associated with foveal hypoplasia showing autosomal recessive (pseudo dominant) inheritance. The fully shaded symbol indicates affected members. The open symbol indicates unaffected members.

Table 1. Protocol for PCR mix concentration.

Reagents	Volume (µl)	Concentration
PCR buffer (10X)	10	-
dNTP (2.5 mM each)	10	250 µM
MgCl2 (25 mM)	10	2.5 mM
PCR Primer Xba (10 µM)	7.5	0.75 µM
AmpliTaq Gold® (5 U/µl)	2	0.1 U/µl
H2O	50.5	-

Pedigree

Detailed pedigree was taken from the proband and peripheral blood was collected from the affected as well as unaffected members (Figure 1).

Laboratory

Ten milliliters of heparinized blood was drawn from affected patients and their parents after obtaining written informed consent from both parents. DNA was extracted using Machery & Nagel (M&N) Kit according to manufacturer's instructions. High throughput genotyping was done using Affymetrix SNP 6.0 Gene Chip. The protocol is as follows, 250 ng of total genomic DNA was incubated with 10 units of restriction endonuclease. The digested DNA was incubated with 0.25 µM adaptors and DNA ligase. All fragments resulting from restriction enzyme digestion, regardless of size, are substrates for adaptor ligation. A generic primer, which recognizes the adaptor sequence, was used to amplify ligated DNA fragments. The PCR mix was prepared (Table 1) and PCR reaction conditions were optimized as follows: Initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at 68°C for 15 s. This was followed by 30 cycles and final extension at 68°C for 7 min to selectively amplify fragments in the 250 to 1100 bp size range. The amplification products were concentrated with a Qiagen PCR purification column (Qiagen) and 80 µg of DNA was digested with fragmentation reagent (Affymetrix SNP 6.0 genotyping assay kit). Samples were labeled with 25 to 30 units of terminal deoxytransferase (Affymetrix SNP 6.0 genotyping assay kit) 30 biotinylated ddATP (Affymetrix SNP 6.0 genotyping assay kit).

Following heat inactivation at 95°C for 10 min, samples are injected into microarray cartridges and hybridized overnight. Microarrays were washed in a fluidics station (Affymetrix), followed by a staining protocol with streptavidin avidin phycoerythrin (molecular probes), and biotinylated anti-streptavidin (Vector Lab), and a final wash of SSPE buffer. Microarrays are scanned according to manufacturer's directions (Affymetrix). The scanned data was analyzed using Gene Chip® genotyping analysis software (GTYPE v.4.1). This software was specifically designed to give highly automated SNP allele calls for the Gene Chip mapping arrays and provides an integrated analysis workflow to enable high content, high throughput, and accurate mapping analysis.

The primary results were produced as a simple excel spreadsheet. The results were then sorted by chromosome and genetic distance, using a simple ''data sort'' excel command. The column of sorted SNP allele calls was copied. The sorted data were processed and analysed using ExcludeAR (Woods, 2004) program, a freeware spreadsheet. The sorted primary SNP allele data were pasted into Exclude AR cell. ExcludeAR3 was used for the interpretation of the data obtained from the three affected individuals and unaffected mother and child (Figure 2).

RESULTS

The regions of significant homozygosity in this family were on chromosome 16. Two point, multipoint analyses and haplotyping for all the 400 odd markers on chromosome 16 were performed. LOD score of 2.3 was obtained for the marker rs254347, and the disease was segregating with the haplotypes. No other region showed similar significant association. The likelihood of the foveal hypoplasia gene in this south Indian consanguineous family to be on chromosome 16 is quite high and was strongly associated with the SNP marker rs254347 at the cytogenetic region 16q24.1. Further work needs to be done to identify the disease causing gene (Figure 3).

DISCUSSION

Foveal hypoplasia is used clinically to describe maculae where the foveal pit is poorly demarcated with absence of the foveal avascular zone, implying the lack of centrally specialized structures needed for good visual acquity. Foveal hypoplasia is suggested to have autosomal recessive inheritance pattern (Curran and Robb, 1976). The dominant Gly64Val mutation in PAX6 causes foveal hypoplasia, cataracts, and corneal epithelial changes comparable to those seen in aniridia (Glaser and Jepeal,

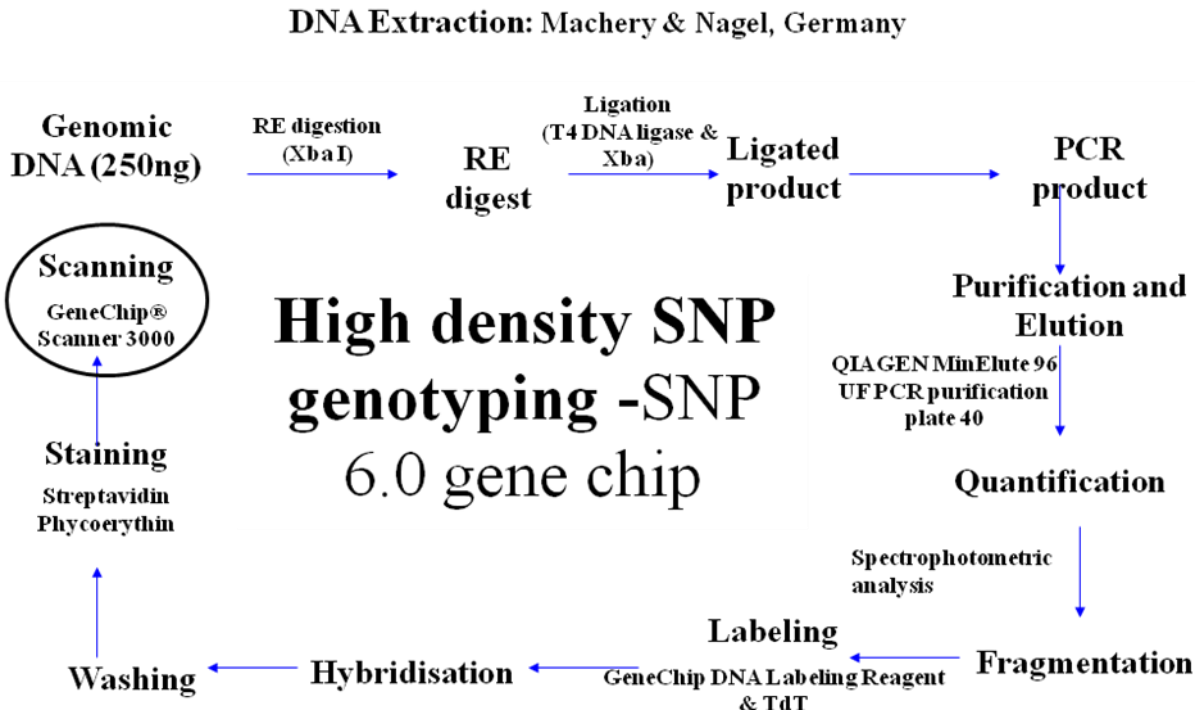

Figure 2. Schematic representation of High density SNP genotyping-SNP 6.0 gene chip.

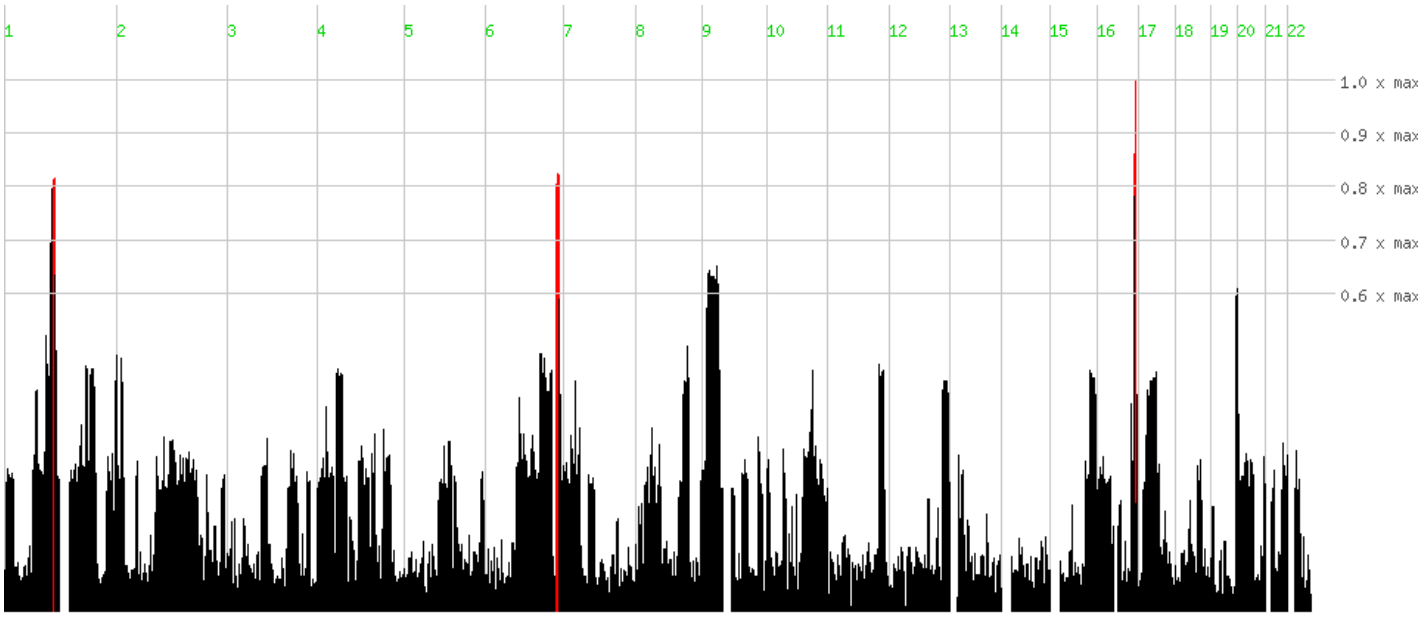

Figure 3. Whole genome linkage data, LOD score of 2.3 was obtained for the marker rs254347 and the disease was segregating with the haplotypes.

1994). By inference, it therefore seems likely that the defective protein in this family, like PAX6, will have a significant role in the developmental processes that control the formation of the eye. The PAX proteins are transcriptional regulators that recognize the target genes via the DNA binding function of the paired domain (Bopp and Burri, 1986; Walther, 1991; Walther and Guenet, 1991). We did gene mapping in the autosomal recessive family to find gene loci and our data clearly suggests that the consanguineous family we studied was strongly

associated with SNP marker rs254347 on chromosome 16 at 16q24.1 region. The identification of the gene involved may contribute to a better understanding of eye embryogenesis and development.

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