SHORT COMMUNICATION

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Heterozygous 17-bp deletion in the forkhead transcription factor gene, *FOXL2*, in a Japanese family with blepharophimosis-ptosis-epicanthus inversus syndrome

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Abstract We examined mutations in the forkhead transcription factor gene, *FOXL2*, in three members of a Japanese family with autosomal dominant blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and in 100 healthy controls. The *FOXL2* was analyzed by direct genomic sequencing. A novel 17-bp deletion at nucleotides 1092–1108 in *FOXL2* was found in the three affected patients. No mutation was found in any of the 100 healthy controls. The 17-bp deletion in *FOXL2* may be involved in the pathogenesis of BPES in Japanese patients.

Key words Forkhead transcription factor gene · *FOXL2* · Blepharophimosis-ptosis-epicanthus inversus syndrome · BPES · Japanese patients

Introduction

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is a combination of congenital anomalies of the eyelid characterized by reduction of the horizontal fissure length, congenital ptosis, and epicanthus inversus (Amano et al. 1995; Matsuoka et al. 1997; Oley et al. 1988; Stromme et al. 1996). The syndrome may occur either sporadically or in an autosomal dominant mode. Two types of the syndrome have been proposed: type I with infertility in affected females and type II transmitted by females and males (Zlotogora et al. 1983). Genetic and chromosomal studies have indicated that one of the BPES loci exists at the 3q region (Amati et al. 1996; Messiaen et al. 1996; Toomes et al. 1998). Crisponi et al. (2001) cloned a forkhead transcription factor gene, *FOXL2*, from that region and reported on mutations in the gene in five families and two sporadic

patients with BPES. They suspected that the mutated *FOXL2* gene produced truncated proteins in type I families and larger proteins in type II families (Crisponi et al. 2001). De Baere et al. (2001) also reported on the other 17 mutations of the *FOXL2* gene in BPES patients. They demonstrated a genotype–phenotype correlation in which truncated *FOXL2* proteins caused type I BPES and extended proteins caused type II BPES. We examined *FOXL2* genotypes in three Japanese patients from a family with BPES.

Patients and methods

Case reports

The pedigree of the affected family is shown in Fig. 1. The proband's parents had a nonconsanguineous marriage. A girl (proband, patient III-7) was born after a full-term pregnancy. At the time of birth, her mother and father were 30 and 33 years old, respectively. Her birth weight was 2600 g. The patient had had abnormal eyelids bilaterally since birth. Her developmental milestones were within normal ranges. At the age of 5 years, she was referred to our clinic. On examination, her corrected visual acuity was 0.1 in the right eye and 0.6 in the left eye. Her horizontal palpebral fissures were 15 mm in both eyes (normal, 20–26 mm), indicating blepharophimosis. Her palpebral widths were 1 mm in the right eye and 3 mm in the left eye (normal, 4–6 mm), indicating ptosis. Her right pupil was occluded by the upper eyelid. Epicanthus inversus was seen bilaterally (Fig. 2a). Cornea, anterior chamber, iris, pupil, lens, vitreous, and fundus appeared normal bilaterally. Her height was 108 cm and her body weight was 16.8 kg. No other physical abnormality was noted. Her psychomotor development appeared normal. The 38-year-old father, patient II-8, had had BPES since birth (Fig. 2b). No other mental or physical abnormality was noted. The 71-year-old paternal grandfather, patient I-3, had had BPES since birth. He had undergone eyelid surgeries several times during childhood. However, the

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Fig. 1. Family tree. *Open squares*, normal man; *Solid squares*, affected man; *Open circles*, normal woman; *Solid circles*, affected woman; *arrow*, proband

Fig. 2a-c. Eyelid photograph of **a** patient III-7 (proband), **b** patient II-8 (proband's father), and **c** patient I-3 (proband's paternal grandfather). A bilateral reduction in horizontal fissure length, short palpebral width, and epicanthus inversus are seen in three patients

BPES remained (Fig. 2c). No other mental or physical abnormality was noted. The 74-year-old paternal grandaunt, patient I-2, was reported to have had BPES since birth. She had three sons. One hundred subjects with normal eyelids served as controls.

Informed consent was obtained from all individuals and/or their parents. Ten-millimeter samples of peripheral venous blood were collected from the three patients and healthy subjects. Genomic DNA was extracted from each sample. *FOXL2* was amplified by polymerase chain reaction (PCR), according to a slight modification of the method described by Crisponi et al. (2001). In brief, four

Fig. 3. Polyacrylamide gel electrophoresis. *Lane 1*, patient III-7 (proband); *lane 2*, patient II-8 (proband's father); *lane 3*, patient I-3 (proband's paternal grandfather); *lane 4*, normal control; *lane 5*, DNA markers. Two allele-fragments are found in *lanes 1, 2, and 3*, and one fragment in *lane 4*

primer pairs were used according to the original method, and another primer pair (1, sense: 5'-TACACACGCGT GCAGAGCAT-3', and 2, antisense: 5'-AGAGCAATG CATCATGGCGA-3') was used. A total of 100ng DNA in 50μl of a solution containing 10mM Tris-HCl buffer (pH 8.0), 2.5mM MgCl₂, 50mM KCl, 0.2mM dNTPs, and 2.5U Taq polymerase were used for each reaction. After initial denaturation at 95°C for 5min, 30 cycles of PCR were performed. Each cycle consisted of denaturation at 95°C for 45sec, primer annealing at 61°C or at 63°C for 45sec, and extension at 71°C for 90sec. All PCR products were sequenced with an automated DNA sequencer (ABI 310, Perkin Elmer Cetus, Foster, CA, USA). For the agarose gel study, 5μl of PCR product (primer pairs 1 and 2), 1μl of loading dye (40% [w/v] sucrose, 0.25% [w/v] bromophenol blue, and 1mM ethylenediaminetetraacetic acid) were combined, and were run on 3.5% agarose gels in 0.5 × Tris[hydroxymethyl]aminomethane borate ethylenediaminetetraacetic acid (TBE) at 100V for 120min. The agarose gels were then stained for 15min at room temperature with a 1μl/ml ethidium bromide, 0.5 × TBE solution. Bands were visualized on an ultraviolet-transilluminator and photodocumented.

Results

Agarose gel electrophoresis showed two allele-fragments in patients III-7, II-8, and I-3, but one fragment in healthy controls (Fig. 3), suggesting heterozygosity in this region in the three affected patients. Sequence analysis revealed heterozygous 17-bp deletion at nucleotides 1092–1108 in *FOXL2* in three affected patients of the family with BPES

Fig. 4a,b. The sequence analysis around nt 1100 of *FOXL2*. **a**, normal control; **b**, patient III-7. Heterozygous 17-bp deletion at nt 1092–1108 is seen in patient III-7

(Fig. 4). No mutation was found in any of the 100 control subjects.

Discussion

The pedigree showed an autosomal dominant inheritance of BPES in our present family. Type II BPES was suspected in this family because the affected woman (patient I-2 with BPES) had three sons. To our knowledge, the 17-bp deletion in *FOXL2* in the three affected members of this family has not been previously reported. It is likely that the deletion in this family may be involved in the pathogenesis of BPES, because no mutation was found in the 100 healthy controls.

FOXL2 is a possible winged, forkhead transcription factor gene (Crisponi et al. 2001). Forkhead, winged helix proteins are crucial in several signal transduction pathways of embryogenesis, tumorigenesis, and maintenance of differentiated cell states (Kaufmann et al. 1996). Crisponi et al. (2001) reported that *FOXL2*, the mouse homolog for *FOXL2*, was expressed strongly in developing eyelids in mouse embryo, and that the gene might control the devel-

opment of the eyelids. In eyelid development, fusion and subsequent reopening of the eyelids are a common feature in the mouse and the human embryo (Findlater et al. 1993; Pearson et al. 1980). Insufficient reopening of the eyelid is believed to cause BPES (Conlon et al. 1994).

Crisponi et al. (2001) and De Baere et al. (2001) reported that the mutations in *FOXL2* were found in families with BPES. They demonstrated a genotype–phenotype correlation in which truncated *FOXL2* proteins cause type I BPES and extended proteins cause type II. The heterozygous 17-bp deletion in *FOXL2* observed in three patients described here may cause a frame shift at codon 285 that exists downstream of the forkhead domain, leading to elongation of the coding region and to abnormal development of the eyelids.

Our patients with type II BPES had a 17-bp deletion at nucleotides 1092–1108 in the *FOXL2* gene. Crisponi et al. (2001) and De Baere et al. (2001) reported a family with BPES having 17-bp duplication at nucleotides 1092–1108. Although the exact mechanism remains unclear, it seems possible that a close correlation may exist between the roles of deletion or duplication in the pathogenesis of BPES. It remains unclear why mutations in the same gene cause two different types of BPES. Further research is needed to resolve this issue.

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