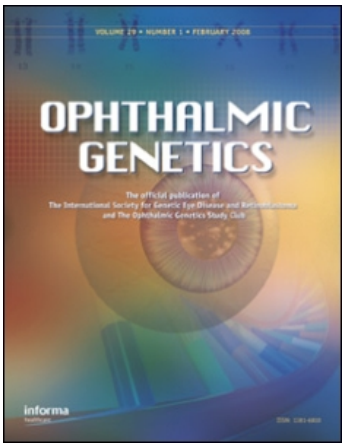

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CASE REPORT

Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome in a Girl with Chromosome Translocation t(2;3)(q33;q23)

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We report on a young female patient with the clinical features of blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, OMIM 110100) and a balanced chromosome translocation 46,XX,t(2;3)(q33;q23)dn.BPES is a rare autosomal dominant congenital disorder characterized by the eponymous oculo-facial features that are, in female patients, associated either with (type 1 BPES) or without (type 2 BPES) premature ovarian failure. Both types of BPES are caused by heterozygous mutations in the *FOXL2* gene, which is located in chromosome band 3q23. Chromosome aberrations such as balanced rearrangements have only rarely been observed in BPES patients but can provide valuable information about regulatory regions of *FOXL2*. The translocation in this patient broadens our knowledge of pathogenic mechanisms in BPES and highlights the importance of conventional cytogenetic investigations in patients with negative results of *FOXL2* mutation screening as a prerequisite for optimal management and genetic counseling.

Keywords *FOXL2*; balanced chromosome translocation; blepharophimosis-ptosis-epicanthus inversus syndrome; BPES; premature ovarian failure

INTRODUCTION

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, OMIM 110100) is a rare autosomal dominant congenital disorder characterized by short palpebral fissures, epicanthus inversus, ptosis of the eyelids and additional ocular and non-ocular features.[1] In female patients, BPES can be associated with premature ovarian failure (POF), menstrual irregularities or infertility (type 1 BPES); or it is not associated with fertility problems (type 2 BPES).[2,3]

In the majority of patients, BPES is caused by heterozygous mutations of the *FOXL2* gene which encodes a forkhead transcription factor that is expressed in the developing eyelid and ovaries[4,5] BPES is rarely associated with chromosome aberrations such as deletions or rearrangements,[6,7] and an autosomal

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recessive form of BPES associated with a homozygous *FOXL2* mutation has recently been reported in a consanguineous Indian family.[8]

Here, we report a young BPES patient in whom *FOXL2* mutation analysis failed to identify a mutation, but subsequent chromosome analysis revealed a *de novo* balanced chromosome translocation involving the *FOXL2* locus.

CLINICAL REPORT

The patient was born to healthy and non-consanguineous parents after an uneventful pregnancy in the 37th gestational week. Birth parameters were within the normal range [length 47 cm (50th centile); weight 3365 g (50th centile); head circumference 32 cm (10th centile)]. Psychomotor development was normal.

On examination at the age of 14 months, her height was 75 cm (25th centile) and head circumference 46 cm (25–50th centile). Facial dysmorphic features included short palpebral fissures (blepharophimosis) and lateral displacement of inner canthi (telecanthus), bilateral ptosis of eyelids, bilateral epicanthus inversus, sparse eyebrows, long philtrum, flat nasal bridge and anteverted nares (Figure 1A). Ophthalmologic investigations revealed no abnormalities of the eye or the retina. The clinical features were strongly suggestive of blepharophimosis-ptosis-epicanthus inversus syndrome.

MATERIAL AND METHODS

FOXL2 Mutation Analysis

For *FOXL2* mutation analysis we used the primers as reported in Crisponi et al. (2001).[4] Details of the PCR and sequencing conditions are available upon request.

Karyotyping, Array CGH and FISH

Cytogenetic investigations (GTG banding) on 25 metaphases obtained from PHA-stimulated peripheral lymphocytes were performed according to standard protocols.

Array comparative genomic hybridisation (array CGH) analysis was performed on a "32k" BAC array as described previously.[9] Aberrations were only considered if at least three adjacent clones were involved unless they coincided with published DNA copy number variants as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/). Detailed step-by-step protocols are provided on our website (http://www.molgen.mpg.de/~abt_rop/ molecular_cytogenetics/).

For fluorescence *in situ* hybridization (FISH) experiments, a permanent lymphoblastoid cell line of the patient was established by EBV transformation according to standard protocols after informed consent. FISH was performed using three BAC clones (RP11-186B11; RP11-809A16; RP11-34M23) at and around the *FOXL2* locus in 3q32. The BAC clones were selected from the Human "32k" BAC Re-Array set (http://bacpac.chori.org/pHumanMinSet.htm; kindly provided by Pieter de Jong, Children's Hospital Oakland Research Institute). DNA samples were prepared according to standard protocols and were labelled by nick translation with either biotin-16-dUTP or digoxigenin-11-dUTP. Immunocytochemical detection of probes was performed as described elsewhere.[10] Chromosomes were counterstained with 46-diamino-2-phenyl-indole (DAPI). Metaphases were analysed with a Zeiss epifluorescence microscope.

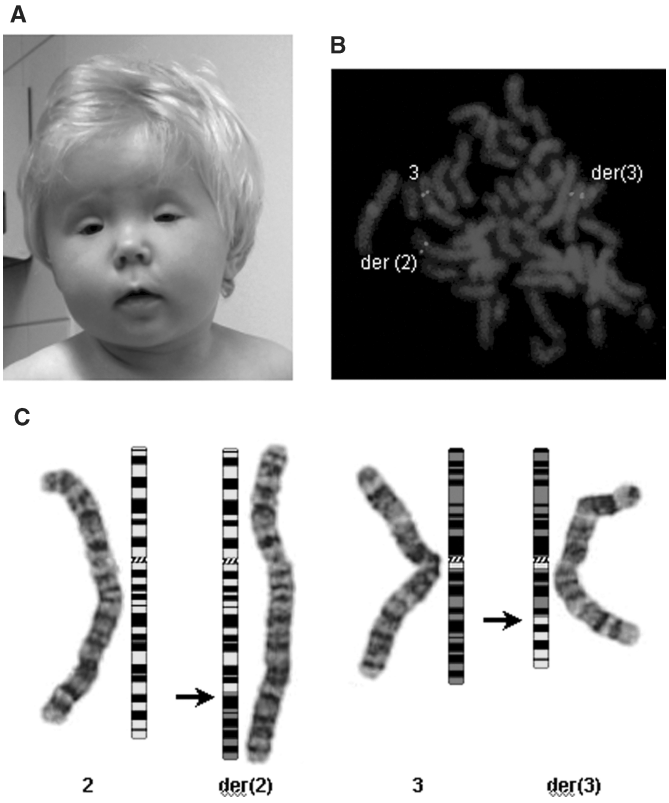

FIG. 1A. (A) The patient at age 14 months. Note short palpebral fissures, low nasal bridge, ptosis and epicanthus inversus. (B) Hybridization signals (red) of the breakpoint-spanning BAC RP11-809A16 on the normal chromosome 3, derivative chromosome 2 [der(2)], and derivative chromosome 3 [der(3)]. (C) Partial karyogram showing G-banded homologues and ideograms of the normal chromosomes 2 (yellow) and 3 (red), and of the translocation chromosomes. The breakpoints are in chromosome bands 2q33 and 3q23 (arrows).

RESULTS

No Mutation in FOXL2

Sequencing of FOXL2 failed to detect pathogenic mutations.

A Chromosome Breakpoint Affects the FOXL2 Locus

Chromosome analysis in the patient revealed a balanced reciprocal translocation between one homologue of chromosome 2 and one homologue of chromosome 3 in all metaphases

(Figure 1C). The chromosomes of the parents were normal. The patient's karyotype was determined as 46,XX,t(2;3)(q33;q23)dn. Analysis by whole genome array CGH confirmed the balanced character of the translocation and revealed no copy number changes elsewhere in the genome that were no known polymorphisms.

Molecular cytogenetic analysis using FISH revealed a breakpoint-spanning BAC clone (RP11-809A16) with split signals on both derivative chromosomes, and one signal on the unaffected chromosome 3 (Figure 1B). The neighboring BAC clones yielded signals proximal to the breakpoint (RP11-186B11) and distal to the breakpoint (RP11-34M23) (data not shown).

The breakpoint-spanning BAC RP11-809A16 has a size of about 170 kb and harbors the *FOXL2* gene. Thus, the precise position of the breakpoint is either within *FOXL2* or, given the small genomic size of 2.7 kb of this single-exon gene, upstream or downstream of *FOXL2*.

DISCUSSION

Most BPES patients have mutations within *FOXL2*, and the *FOXL2* mutation database lists more than 100 pathogenic alleles.[5] In contrast, genomic aberrations such as microdeletions or chromosome rearrangements involving the *FOXL2* locus are rare,[6,7] and very few balanced chromosome translocations have been reported to be associated with BPES.[11–13]

The unambiguous clinical features of our patient led us to analyze *FOXL2,* which, however, failed to identify a causative mutation. Subsequent chromosome analysis revealed a *de novo* balanced translocation t(2;3)(q33;q23), and by molecular cytogenetic analysis we could show that the breakpoint in 3q23 affects *FOXL2* either by direct disruption or a position effect. Position effects on the expression of *FOXL2* by translocation breakpoints or extragenic deletions more than 200 kb upstream of the transcription start site have been reported recently.[7,11] Similar position effects spanning up to 1 Mb have also been observed in other disorders and provide valuable information about long-range *cis*-acting regulatory elements of the respective genes.[14]

The presence of a chromosome rearrangement in a patient with a monogenic disorder illustrates the importance of conventional cytogenetic analyses in situations where molecular genetic tests of the respective genes are available, but re-sequencing of these genes fails to identify causative mutations. In BPES patients, molecular and/or cytogenetic confirmation of the clinical diagnosis is important not only to exclude other disorders associated with blepharophimosis [e.g. Michels syndrome (OMIM 257920), Ohdo syndrome (OMIM 249620), Marden-Walker syndrome (OMIM 248700), Dubowitz syndrome (OMIM 223370) or the syndrome reported by Khan et al.,[15] but also because female patients can be at risk to develop premature ovarian failure or other fertility problems (BPES type 1). Firm establishment of the diagnosis in childhood provides the rationale for pre-symptomatic tests of ovarian function, early therapeutic intervention (e.g. hormone replacement therapy, ovarian tissue cryopreservation) and/or counseling for reproductive choices (e.g. to plan pregnancies earlier rather than later in life).[16–20] Knowledge of a balanced chromosome rearrangement is even more important for those patients who are able to procreate, since the formation of germ cells with unbalanced chromosome complements can lead to reduced fertility rates, higher risks for abortions and increased risks for the birth of disabled children with unbalanced chromosome aberrations.

In summary, the unusual chromosome disorder of this patient adds to our understanding of the pathogenesis of BPES and highlights the importance of conventional cytogenetic investigations in patients with negative results in *FOXL2* mutation screening as a prerequisite for optimal management and genetic counseling.

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