Identification of a new *TWIST* mutation (7p21) with variable eyelid manifestations supports locus homogeneity of BPES at 3q22

Helene Dollfus, Govindasamy Kumaramanickavel, Partha Biswas, Corinne Stoetzel, Renaud Quillet, Michael Denton, Marion Maw, Fabienne Perrin-Schmitt

EDITOR—Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder of eyelid development defined by small palpebral fissures, epicanthus inversus, and ptosis.^{1,2} BPES type I (OMIM 110100) is characterised by female infertility, whereas BPES type II (OMIM 601649) is transmitted by both females and males. Most cases of BPES types I and II map to chromosome 3q22-q23 (*BPES1*).³⁻⁷ However, a second locus (*BPES2*) was reported in the chromosome 7p13-p21 region on the basis of patients presenting with eyelid anomalies carrying chromosomal abnormalities in the 7p21 region⁸⁻¹¹ and the further linkage data of a large Indian family diagnosed initially with BPES type II.⁷ The *TWIST* gene, mapped on chromosome 7p21, codes for a transcription

factor with a bHLH domain.¹² *TWIST* mutations¹³⁻¹⁸ have been reported in the heterozygous state in patients presenting with the Saethre-Chotzen syndrome (SCS, OMIM 101400). This disorder is a common autosomal dominant form of syndromic craniosynostosis defined by craniostenosis, minor limb and ear abnormalities, and frequent ptosis of the eyelids.¹⁹ In the present study, molecular genetic analysis at *TWIST* and subsequent clinical re-evaluation of the Indian family were used to investigate the possibility that prominent eyelid malformations may represent a clinical variant in the spectrum of phenotypes associated with SCS.

The four generation Indian family originates from the Bihar region in the western part of India. The members of the family were initially referred in 1995 because of palpebral anomalies.⁷ Clinical re-evaluation of the family took place at the Anandalok Eye Hospital in Calcutta in June 2000. Nineteen members of the family, including 17 affected persons, were examined in detail (photographs and x rays available on request for all affected members).

DNA samples, extracted from Guthrie cards, were available from 31 members of the Indian family.⁷ These samples were PCR amplified for *TWIST* and PCR products were subjected to single strand sequence conformation polymorphism (SSCP) and direct sequencing analyses. The primer pair that allowed detection of the mutations reported here was forward primer, VB56 (5' - GAG GCGCCCCGCTCTTCTCTCTG - 3') and reverse primer TQ 53 (5' - CGTCTGAAGAACGGCGCGAA - 3'). A specific migration pattern was observed after amplification of the DNA of all 16 members of the Indian family who were previously reported to have an abnormal clinical examination. In all cases, the abnormal SSCP pattern cosegregated with the previously reported haplotype of linked chromosome 7p markers⁷ (fig 1). Direct sequence analyses were performed on the PCR products of all 31 DNA samples. All 16 samples with an abnormal SSCP pattern showed the same heterozygous mutation, namely a C to T transition at position +82, changing a CAG codon to TAG, which is predicted to result in premature termination of the protein at codon 28 (28 C→T) positioned far upstream of the bHLH motif and probably within the recently reported histone acetyltransferase interaction domain.²⁰ This nonsense *TWIST* mutation probably results in the absence of stable protein synthesis from the

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LGME du CNRS,
INSERM U184, Faculté
de Médecine, 11 rue
Humann, 67085
Strasbourg, France
H Dollfus
C Stoetzel
R Quillet
F Perrin-Schmitt

Sankara Nethralaya,
Medical Research
Foundation, 18 College
Road, Chennai, India
G Kumaramanickavel

Anandalok, Asutosh
Mukherjee Road,
Calcutta, India
P Biswas

Biochemistry
Department,
University of Otago,
PO Box 56, Dunedin,
New Zealand
M Denton
M Maw

Correspondence to:
Dr Dollfus,
helene.dollfus@medecine.
u-strasbg.fr

Figure 1 Abnormal SSCP pattern and sequence analysis showing the *TWIST* mutation (identification of patients was done according to Maw et al). (A) Abnormal SSCP band (arrow) of affected patients (arrow heads) compared to unaffected members of the family. (B) Sequence analysis: antisense sequence of II.4, an unaffected member of the family, compared to IV.2, a patient carrying the mutation. All the patients with an abnormal SSCP band carried the 28 C→T mutation.

Figure 2 Photographs of two members of the family carrying the mutation showing variable phenotypes. (A) Full face of patient II.1 showing bilateral ptosis and reduced palpebral aperture. (B) Profile of patient II.1. (C) Full face of patient IV.6 showing asymmetrical orbits and narrow forehead. (D) Profile of patient IV.6 with obvious exophthalmos.

mutant allele and suggests that haploinsufficiency at the *TWIST* locus is responsible for the phenotype in this Indian family.

The phenotype of family members carrying the mutation showed very mild clinical features of SCS in some subjects without evidence of obvious craniostenosis but more pronounced eyelid malformation compared to other subjects with all the clinical and radiographic evidence of craniostenosis as well as brachydactyly and external ear anomalies (fig 2). Collectively, the phenotype of the affected members of this family was compatible with the diagnosis of SCS with variable expressivity and more pronounced eyelid manifestations in some of them.

Variable expressivity in SCS has been extensively underlined as a pitfall in the establishment of the diagnosis.^{19–21} In most cases, the details of the dysmorphic gestalt could not be correlated with the functional defects expected from the *TWIST* mutations detected. That the clinical features of some members of the Indian family were easily confused with BPES indicates that palpebral anomalies may be the main manifestation of haploinsufficiency at the *TWIST* locus in some SCS patients.

In conclusion, genetic and phenotypic assessment of the Indian family, previously reported as locus *BPES2*, now suggests that autosomal dominant BPES (types I and II) is probably a homogeneous disorder with a unique locus on chromosome 3q22–q23 (locus *BPES1*).

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Association between the defective Pro369Ser mutation and in vivo intrahepatic α 1-antitrypsin accumulation

Susana Seixas, Ana Isabel Lopes, Jorge Rocha, Lúcia Silva, Carlos Salgueiro, Jaime Salazar-de-Sousa, Amélia Batista

EDITOR— α 1-antitrypsin (PI), the major inhibitor of neutrophil elastase in the lower respiratory tract, is a highly polymorphic glycoprotein synthesised in the liver that has several rare gene products in which serum protein levels are reduced or even undetectable.¹ Early onset pulmonary emphysema, resulting from unopposed elastase activity, and neonatal cholestasis probably resulting from the retention of the defective protein in the liver,² are the two most common clinical manifestations of PI deficiency and are mainly associated with PI*Z, the most common deficient allele. In addition, other rare alleles occasionally associated with liver injury have been shown to share with PI*Z an increased tendency for intracellular accumulation. Recently, a complete intracellular transport block has been reported for a newly identified^{3,4} defective Pro369Ser allele (Mwürzburg) by in vitro expression studies in human cell cultures. Adenovirus mediated transfer of the mutant gene into the mouse reproduced the consequences of this block and no traceable amounts of the variant protein could be detected in the plasma after in vivo recombinant expression.³ However, no detectable intrahepatocytic PI inclusions were found in the mice expressing the Mwürzburg mutant³

and no liver biopsy material has yet been presented from patients with this defective allele.

Case report

We report a carrier of the Mwürzburg allele with evidence for in vivo intrahepatic accumulation of PI. The patient is a white Portuguese boy who presented at the age of 1.5 months with cholestasis associated with a recent cytomegalovirus (CMV) infection. A percutaneous liver biopsy performed at the age of 2.5 months showed significant portal fibrosis with porto-portal bridging, giant cell transformation, moderate cholestasis, and an intense portal-acinar inflammatory infiltrate. Periodic acid-Schiff staining after diastase treatment (PAS-D) additionally showed the presence of positive, diastase resistant, intracellular inclusions. Immunoperoxidase staining specific for PI was positive (fig 1). Serum PI concentration, determined by automated nephelometry (Behring), was found to be 92% of normal on admission and dropped to 45% of normal at the age of 24 months, after CMV serology (IgM) and antigens became negative and following progressive decline of transaminase levels to their normal upper limit. The PI

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Instituto de Patologia e Imunologia Molecular, Universidade do Porto (IPATIMUP), R Dr Roberto Frias s/n, 4200 Porto, Portugal
S Seixas
J Rocha

Faculdade de Ciências, Universidade do Porto, Portugal
S Seixas
J Rocha

Unidade de Gastroenterologia Pediátrica, Hospital de Santa Maria, Lisboa, Portugal
I A Lopes
J Salazar de Sousa

Serviço de Pediatria, Hospital do Barreiro, Barreiro, Portugal
L Silva
C Salgueiro

Serviço de Anatomia Patológica, Hospital de Santa Maria, Lisboa, Portugal
A Batista

Correspondence to:
Dr Rocha,
jrocha@ipatimup.pt

Figure 1 Tissue section from the patient's percutaneous liver biopsy. PAS positive diastase resistant inclusions were found in the cytoplasm of several hepatocytes (PAS-D). PI is identified by immunoperoxidase in inset (immunoperoxidase staining).