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Two Tunisian patients with Peters plus syndrome harbouring a novel splice site mutation in the *B3GALTL* gene that modulates the mRNA secondary structure

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ABSTRACT

Peters plus syndrome is an autosomal recessive rare disorder comprising ocular anterior segment dysgenesis, short stature, hand abnormalities, distinctive facial features, and often other major/minor additional defects. Peters plus syndrome is related to mutations in the *B3GALTL* gene with only seven recently reported mutations, leading to the inactivation of the B1, 3-glucosyltransferase. In this study, we screened the *B3GALTL* gene in two unrelated patients with typical Peters plus syndrome. A novel homozygous c.597-2A>G mutation was identified in both patients. Bioinformatic analyses showed that this mutation modulates the pre mRNA secondary structure of the gene, and decreases the score value related to the formation of splicing loops. Moreover, the c.597-2A>G mutation is located in a CpG Island of the *B3GALTL* gene, suggesting a potential epigenetic role of this position including gene's methylation and regulation. These data confirm an important role of the *B3GALTL* gene test that provides diagnosis confirmation and improves genetic counseling for the families.

1. Introduction

Peters anomaly is one of the main causes of congenital corneal opacities. It is a rare form of disrupted anterior chamber development, either sporadic or inherited, that presents as corneal opacity from birth with the opaque cornea obstructing the pupil and thus causing visual loss (Ozeki et al., 2000). In addition, there is anterior chamber dysgenesis with connection between the cornea and the iris and/or the lens in some cases. Peters plus syndrome (PPS) was first coined in 1984 by the Dutch ophthalmologist Mary Van Schooneveld (Van Schooneveld et al, 1984). It is transmitted according to the autosomic recessive inheritance and it associates to the Peters anomaly other major/minor additional defects essentially typical facial dysmorphism including long philtrum, cleft lip/palate, short stature and limbs, broad hands and feet, brachydactyly, and variable mental delay (Maillette and Hennekam, 2002). Additional clinical features were also found to be associated to PPS including hearing loss and abnormal ears (Reis et al., 2008), central nervous system abnormalities with hydrocephalus (Aliferis et al, 2010), hypoplastic left heart (Shimizu et al, 2010) and vertebral defects (Faletra et al, 2011). Phenotypic heterogeneity was often shown in PPS with a severity ranging from death in the early childhood to general delay in growth and development (Heinonen and Maki, 2009).

The genetic defect responsible for PPS was recently identified in the 13q12.3 region by Lesnik Oberstein et al. after the detection of a microdeletion by array-based comparative genomic hybridization (Lesnik Oberstein et al, 2006). The responsible *B3GALTL* gene spans 132 kb of genomic DNA and contains 15 exons. It is expressed in a wide range of human tissues (dbEST Web site), and produces three transcripts resulting of three different alternative polyA sites, all in exon 15 (Heinonen et al, 2003). The B3GALTL protein contains 498 residues and contains a short cytoplasmic N-terminal tail (residues 1–4), a transmembrane region (residues 5–28), a stem region (residues 29–260), and a C-terminal catalytic domain (residues 261–498) (Heinonen et al, 2003). This protein was predicted to be a glycosyltransferase: the β 1,3-glycosyltransferase involved in the synthesis of the disaccharide Glc-β1, 3-Fuc-O- that occurs on thrombospondin type 1 repeats (TSRs) of many biologically important proteins (Hess et al, 2008).

Up to now, only seven mutations in the *B3GALTL* gene were identified in patients with PPS. The most frequent mutation occurring in 75% of

Abbreviations: A, adenosine; bp, base pair(s); *B3GALTL*, Beta 1,3-galactosyltransferase-like gene; B3GALTL, Beta-1,3-glucosyltransferase; C, cytidine; cDNA, DNA complementary to RNA; dNTP, deoxyribonucleoside triphosphate; EST, expressed sequence tag; EtdBr, ethidium bromide; G, guanosine; RFLP, restriction-fragment length polymorphism; T, thymidine; U, uridine; U2AF, U2 auxiliary factor; UTR, unstranslated region(s); UV, ultraviolet.

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cases is the c.660 + 1G > A located at the donor splice site (5'ss) of exon 8 and identified in several populations (Reis et al., 2008). Other mutations were also reported: the c.230insT mutation in exon 4, the c.459 + 1G > A in intron 6, the c.347 + 5G > A in intron 5, the c.1065-1G > A in intron 13 (Faletra et al, 2011) and the c.1178G > A in exon 13 (Dassie-Ajdid et al., 2009). More recently, a p.Tyr366X mutation in exon 13 was identified in a French patient (Aliferis et al, 2010).

This study represents the first screening of the *B3GALTL* gene in two Tunisian patients with typical PPS. We identified the first mutation in the acceptor splice site (3'ss) of the exon 8 of the *B3GALTL* gene. The mutation is located in a CpG Island of the gene and modulates the pre mRNA secondary structure and the score value related to the splicing. These finding confirm the association of *B3GALTL* mutations with typical PPS disease features in Tunisian patients.

2. Materials and methods

2.1. Patients

Two Tunisian patients from two unrelated families were suspected to be affected by PPS. Clinical investigations including paediatric and ophthalmologic exams were performed for the patients after informed consent.

2.2. Methods

2.2.1. DNA extraction

Appropriate informed consent was obtained from all participants and the study protocol adhered to the principles of the Declaration of Helsinki. Two families, including two patients with PPS from the same region were enrolled in the study. In addition, 60 healthy Tunisian unrelated controls from the same region of the families were tested for the mutation. Genomic DNA was isolated from whole blood and DNA extraction from blood leukocytes was performed according to a previously described protocol (Kawasaki, 1990).

2.2.2. Sequencing of the B3GALTL gene

Mutation analysis was performed by PCR amplification of each of the 15 encoding exons of *B3GALTL* gene and the intron–exon boundaries using appropriate primers chosen so that at least 30 to 50 bp of flanking intronic sequences are readable (Table 1). PCR amplification of each exon was performed in a thermal cycler (Gene Amp PCR system 9700, Applied Biosystem) and was carried out in 50 μl with 100 ng of genomic DNA, 10 pmol of each primer, 125 μM dNTPs, 1.5 mM MgCl2, 5 mM KCl, 10 mM Tris–HCl, pH 8.8 and 1 U of Taq DNA polymerase (Promega). The PCR reaction was performed for 35 rounds, each consisting of 30 s at 95 °C, 30 s at 57 °C and 30 s at 72 °C followed by a final elongation step of 10 min at 72 °C. The expected PCR products were electrophoresed on a 2% agarose gel in TAE buffer (40 mM tris acetate, 1 mM EDTA, pH 7.7), stained with 0.5 μg/ml ethidium bromide and then visualized under UV light.

PCR products were then sequenced using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and loaded on an "ABI 3100 Avant" automated sequencer. The blast homology searches were performed using software available at the National Center for Biotechnology Information Web site.

2.2.3. RFLP (restriction fragment length polymorphism) analysis

Screening of the 3'ss mutation of the exon 8 in both families' members as well as the 60 control individuals were examined by RFLP since the mutation abolishes a BfaI restriction site. After PCR amplification, 10 μl of exon 8 PCR product was digested with 10 U of the BfaI restriction endonuclease (Fermentas) at 37 °C overnight, and then separated on 2% agarose gel by electrophoresis.

2.2.4. Computational analyses

The effect of the c.597-2A > G mutation on pre mRNA splicing was first assessed using the consensus score calculation method to quantify the influence of the mutation on formation of splicing loops (Carmel et al, 2004). The corresponding software is available online at http://ast.bioinfo.tau.ac.il/splicesite.

Prediction of the c.597-2A > G mutation effect on mRNA secondary structure was performed by using the MFOLD program (Zuker, 2003).

In the aim to search if the c.597-2A > G is situated in a CpG region, we used the EMBOSS CpGReport available at: www.ebi.ac.uk/Tools/emboss/cpgplot/index/html web site.

3. Results

3.1. Clinical evaluation

3.1.1. Patient 1

The patient is a 7 year old son of non consanguineous parents, and without particular familial history. Pregnancy's follow up revealed short limbs in prenatal echography. The patient was underweight at birth (2500 g), he presented a length of 47 cm and an OFC of 33 cm. He also presented at birth disproportionate short stature and microcephaly related to the agenesis of corpus collusum (Fig. 1A). He had axial hypotonia and facial dysmorphism with dolichocephaly, round face, small nose with reverted nostrils and short columella. Flat and long philtrum, big mouth with thin lips and retrognatism were also shown (Fig. 1A).

Short limbs in the proximal segment and brachydactyly with bilateral clinodactyly of fifth finger were also found. At the age of one month, the weight was 2800 g (−2–3SD), the length 48 cm (−3SD) and OFC: 35 cm. Ophthalmologic examinations showed total corneal opaqueness

Table 1
Sequence of the primers required for the amplification of the *B3GALTL* gene.

Exon	Forward primer	Reverse primer	PCR size product
1	5′GCAGAGGAGAAAGGAAGAGGAG3′	5′GGACCCAAGACCGAAAGG3′	674 bp
2	5′GCAAAACTGTTGGATGTGAGAA3′	5′CAAATGAATCTTAGAAGGGAGGAA3′	176 bp
3	5′TCAGTGTACT′GCTGGCTTTGTTA3′	5′GGCCAAAGACAGAATATTTGAA3′	237 bp
4	5′TTTTGTAAAAAGAAATACCTGAAAACT3′	5′TGGAAAACATCAAGGTAATCAAGA3′	217 bp
5	5′TTTCGGAGTAGTCAATTCATACTTATC3	5′GGGCGTAAAAGTGTTCAGGTAG3′	187 bp
6	5′CCCTTCATTCACTTCCTACTGA3′	5′GAAACACTGTCCCCCAAAATAA3′	249 bp
7	5′TGAAATGATTGTTTTTAAAGTGACA3′	5′CAATGAAATTAAAGTTGATGCACAC3′	246 bp
8	5′TCTTGCTTGACACTTCTTTTGG3′	5′AAAATGCAAGATTAGGGAATGC3′	212 bp
9	5′AAAATGCAAGATTAGGGAATGC3′	5′GGATGACATTATGCACAGCATC3′	225 bp
10	5′GAGCGTGTGAGATCTAGTGTGG3′	5′GGTAGTCTGGATTTTAGCGTTCA3′	196 bp
11	5′TGTGTAACTGTGCCAGTGATTG3′	5′CAGAATGGCAATGTTTAATAAGGA3′	204 bp
12	5′CACTTCAAAACTAAAAAGAAATGAACA3′	5′AAAGCCATTTAGGATGAGAATGA3′	216 bp
13	5′CTGTTCCATAACCACGTTTGAA3′	5′TCGTGTGGAAAGGGAAGTTTAT3′	239 bp
14	5′GTGGAGCTTCTCTAACCCCTTT3′	5′AGAAGAATCTGTCCCTCACCTG3′	242 bp
15	5′TGAAATGATTGTTTTTAAAGTGACA3′	5′CAATGAAATTAAAGTTGATGCACAC3′	246 bp