Gene 523 (2013) 88–91

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Novel deletion mutation of *TRPS1* gene in a Chinese patient of trichorhinophalangeal syndrome type I

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ARTICLE INFO

Article history:
Accepted 7 March 2013
Available online 17 March 2013

Keywords:
Trichorhinophalangeal syndrome
TRPS1 gene
Deletion mutation

ABSTRACT

Tricho–rhino–phalangeal syndrome (TRPS) is a rare autosomal dominant disorder. Deletion or mutation of the *TRPS1* gene leads to the tricho–rhino–phalangeal syndromes type I or type III. In this article, we describe a Chinese patient affected with type I TRPS and showing prominent pilar, rhinal and phalangeal abnormalities. Mutational screening and sequence analysis of *TRPS1* gene revealed a previously unidentified four-base-pair deletion of nucleotides 1783–1786 (c.1783_1786delACTT). The mutation causes a frame shift after codon 593, introducing a premature stop codon after 637 residues in the gene sequence. This deletion is an unquestionable loss-of-function mutation, deleting all the functionally important parts of the protein. Our novel discovery indicates that sparse hair and metacarpal defects of tricho–rhino–phalangeal syndromes in this patient are due to this *TRPS1* mutation. And this data further supports the critical role of *TRPS1* gene in hair and partial skeleton morphogenesis.

1. Introduction

The trichorhinophalangeal syndrome (TRPS) is a rare autosomal dominant disorder and was first described by Giedion (1966) who characterized it by craniofacial dysmorphism and bone deformities. It is a genetic disorder caused by either mutation or deletion of the human gene. Depending on the severity of the phenotype, three subtypes have been described: TRPS I (MIM 190350), TRPS II (MIM 150230) and TRPS III (MIM 190351). TRPS I is caused by mutation in the *TRPS1* gene (OMIM 604386), which is also the mutational site in type III TRPS (Giedion et al., 1973; Hall et al., 1974).

Type I trichorhinophalangeal syndrome is caused by haploinsufficiency of a specific zinc finger protein TRPS1. TRPS I patients have sparse scalp hair, a distinctively bulbous nose with dorsally tented nares, long flat philtrum, thin upper vermilion border and protruding ears. Skeletal abnormalities include cone-shaped epiphyses at the phalanges, hip malformations, short stature, and shortening of the metacarpals and metatarsals (Hatamura et al., 2001). Trichorhinophalangeal syndrome type II (TRPS2), also known as Langer–Giedion syndrome (150230), is a similar disorder with the additional features of skin abnormalities, multiple exostoses and mental retardation (Hilton et al., 2002). TRPS2 is a contiguous gene syndrome on 8q24.1, involving loss of functional copies of the *TRPS1* and *EXT1* (608177) genes, which has autosomal recessive inheritance and appears to be extremely rare.

Type III TRPS shares the same disease gene *TRPS1* and traits with Type I TRPS, but distinguished by the presence of severe brachydactyly, short metacarpals and severe short stature. Despite the phenotypic similarity, the allelic relationship of TRPS III and TRPS I has been undefined (Hiromasa et al., 2002). Investigations have demonstrated that most patients with nonsense mutations in the *TRPS1* gene have the less severe TRPS type I phenotype (Hiromasa et al., 2002; Howell and Wynnedavies, 1986), while patients with missense mutations in the GATA-type zinc-finger region of the *TRPS1* gene have the more severe TRPS type III phenotype (Hall et al., 1974). No mutation in the parents of sporadic patients or in apparently healthy relatives of familial patients has been found, which indicates complete penetrance of *TRPS1* mutations.

Abbreviations: TRPS, tricho–rhino–phalangeal syndrome; NLS, nuclear localization signals.

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http://dx.doi.org/10.1016/j.gene.2013.03.035

TRPS I was first described by Giedion in 1966. Giedion's patient, a girl, had 2 supernumerary incisors. He found 2 previous reports, each describing 2 affected sibs. Furthermore, the parents were consanguineous in 1 case. We report an additional observation in a patient of a Chinese family, who presented with distinct TRPS I characteristics such as sparse and slow-growing scalp hair, a bulbous tip of the nose, protruding ears, elongated and flat philtrum, thin upper vermillion border and skeletal disorders. We analyzed the exons in *TRPS1* gene in this Chinese patient with type I TRPS. Our mutation analysis revealed a four-base-pair deletion in the *TRPS1* gene while no abnormal mutation was detected in his sister's gene.

2. Materials and methods

2.1. Patient

We examined a 22-year-old Chinese man who was referred to the dermatology clinic in our hospital for evaluation of the diffuse sparse hair noted for years. His normal sister and a healthy person were the controls. The Declaration of Helsinki protocols were followed. The patient and two controls gave their written, informed consent. The present study was approved by the Ethics Committee of the Peking University Health Science Center.

2.2. Isolation of genomic DNA

Peripheral blood samples of the patient, his normal sister and the control individual were collected from Peking University Third Hospital. Genomic DNA was extracted using a Blood DNA Mini Kit (Biomed Biotechnologies, Inc., Beijing, PRC) according to the manufacturer's instructions. DNA integrity and quantity were verified by agarose gel electrophoresis.

2.3. Identification of TRPS1 mutation

DNA samples from the type I TRPS patient, his sister and the control were subjected to PCR amplification, and we screened for *TRPS1* mutation by direct sequencing of PCR products from genomic DNA. Nine pairs of primers were used to amplify all the seven exons of *TRPS1*. Exon1 and part of exon7 are 5 untranslated regions, so they were not detected here. Exon3, exon4, and exon7 were designed to be divided into two fragments because they are too long and difficult in sequencing. They are listed in Table 1.

After heating at 94 °C for 5 min, polymerase chain reaction (PCR) amplification was performed with 35 cycles: 94 °C for 30 s, annealing for 30 s (annealing temperatures are listed in Table 1), 72 °C for 30 s and followed by a final extension step at 72 °C for 7 min. Then fragments were obtained for accurate sequencing (by AuGCT Biotechnologies, Inc., Beijing, PRC).

Table 1
Primers of each exon.

Exons	Primer sequences		Product size (bp)	Anneal temp.°C
Exon2	Forward	5′-GCTCCAGATTTGCTCATGCT-3′	446	53
	Reverse	5′-AACTCCCCTAGCGAGTCGTA-3′		
Exon3—part 1	Forward	5′-ACATCCCGGTTACATAGGGT-3′	1043	55
	Reverse	5′-CTCTGTGCTTGCCCTGTT-3′		
Exon3 —part 2	Forward	5′-CTGAGTGAGAAGGCTGGCTT-3′	786	58
	Reverse	5′-TCTGGTACTGGGACCTTGGT-3′		
Exon4 —part 1	Forward	5′-CTGCAGAGGGCCCATTGAAT-3′	678	58
	Reverse	5′-TCCCTTGCTGGAGAAGTCCT-3′		
Exon4—part 2	Forward	5′-CCAAGACAGACAAGAGCTCG-3′	658	57
	Reverse	5′-AACAATTCCCGGTTCAGCC-3′		
Exon5	Forward	5′-CAACCCTTCAGAACGCTGTC-3′	811	57
	Reverse	5′-TACTTGCTGCCACTTTGGCC-3′		
Exon6	Forward	5′-AGCTCAGGTAGCATGTGCTC-3′	607	57
	Reverse	5′-CAAGCCAGGGAATGGGACTT-3′		
Exon7—part 1	Forward	5′-GCTTAGGGCAAAAGGAGGAG-3′	888	57
	Reverse	5′-GGTCTGGAATGCTTGATCGC-3′		
Exon7—part 2	Forward	5′-GCGATCAAGCATTCCAGACC-3′	748	58
	Reverse	5′-CCAATGGCCAGTCCAGTACT-3′		

2.4. T–A cloning to confirm the mutation

To determine the exact status of mutation, patient's genomic DNA was subjected to a second and independent amplification, using the same procedure described above. PCR amplification produced a 658-base-pair DNA fragment which was cloned into the pGEM®-T Easy Vectors (Tiangen Biotech [Beijing] CO., Ltd.) according to the manufacturer's instructions. PCR products were inserted into vectors and sequenced by the AuGCT Biotechnologies (Inc., Beijing, PRC) after 24 h of amplification in XL1-Blue chemically competent *Escherichia coli* cells (Inc., Beijing, PRC) by heat shock at 42 °C, according to manufacturer's indications.

3. Results

The patient was a 22-year-old man. He was short (150 cm), and had a distinctive face, with thin scalp hair and receding frontotemporal hairline, laterally sparse eyebrows, a “pear-shaped” nose, protruding ears, elongated philtrum and hypoplastic mandible. The general hair was sparse and thin, including the pubic hair, the beard and the axillary hair. X-ray skeletal investigations showed various abnormalities including short metacarpals and metatarsals with cone-shape epiphyses and cervical kyphosis, but absence of exostoses. There was no evidence of systemic involvement. His intelligence seemed to be normal. His parents were not consanguineous and his elderly sister was also normal. The diagnosis of TRPS I was based on clinical examination and X-ray skeletal investigations.

To determine the molecular basis of the patient's disorder, we analyzed the *TRPSI* gene. After direct sequencing of PCR products in two controls and one TRPS I patient, a heterozygous mutation in the fourth exon of *TRPS1* was identified (Fig. 1). Sequence analysis of the aberrantly fragments showed overlapping peaks after nucleotide 1783 of the cDNA (in exon4), indicating the presence of a frameshift mutation. The mutation of extron4 of *TRPS1* may play a major role in the pathogenic mechanism of this pedigree with TRPS I. As shown, the sequencing results of the patient (Fig. 1b) have double peaks after the red arrow, while the control individual (Fig. 1a) and patient's sister (Fig. 1c) are normal. We also used T–A cloning method to confirm the mutation. PCR amplification products of patient's genomic DNA were separated into two types by cloning into the T vectors. Compared with wild type (Fig. 2a), the sequence of mutation chain (Fig. 2b) misses four bases, ACTT (c. 1783_1786delACTT).

4. Discussion

Here we report the identification of a four-base-pair deletion mutation responsible for a Chinese patient suffered from tricho–rhino–phalangeal syndrome type I. Through mutation analysis, after subcloning the PCR products from the patient and analyzing the sequence of individual clones, the mutated allele was found to have a deletion of 4 bp, ACTT (nucleotides 1783–1786), which alters the *TRPS1* reading frame and results in the introduction of a stop codon after 637 residues (Fig. 3b). The novel deletion mutation (c. 1783_1786delACTT) in *TRPS1* gene we identified is predicted to result in a truncated GATA zinc finger domains, including two potential nuclear localization signals (NLS).

Tricho–rhino–phalangeal syndrome type I is one of autosomal dominant inheritance, a form with sparse scalp hair, bulbous tip of the nose, long flat philtrum, thin upper vermilion border, and protruding ears (Giedion et al., 1973). Skeletal abnormalities include cone-shaped epiphyses at the phalanges, hip malformations, and short