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SHORT REPORT

TP63 gene mutation in ADULT syndrome

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***TP63* gene mutations have recently been shown to be disease causing in EEC and SHFM. Two other overlapping syndromes with ectrodactyly as a major feature, have been mapped to chromosome 3q27 close by the TP63 locus, namely the LMS and ADULT syndromes. Here, we report on a missense *TP63* gene mutation in an isolated ADULT syndrome case. This finding widens the spectrum of abnormalities to be ascribed to *TP63* gene in human and emphasise on the variable roles of the different Tp63 isotypes.** *European Journal of Human Genetics* (2001) **9**, 642–645.

Keywords: ADULT syndrome; EEC syndrome; SHFM; TP63; limb abnormality

Introduction

The acronym ADULT (acro-dermato-ungual-lacrimal-tooth, MIM 103285) defines a rare autosomal dominant syndrome characterised by ectrodactyly, onychodysplasia, hypodontia, obstruction of lacrimal ducts and excessive freckling.[1] The ADULT syndrome gene has recently been localised to chromosome 3q27 where two others syndromic forms of ectrodactyly have been mapped, namely the ectodermal ectrodactyly clefting (EEC3, MIM 604292)[2,3] and limb mammary syndromes (LMS, MIM 603543).[4] Because $p63^{-/-}$ knock out mice show the association of major limb defect with absence of mammary, lacrimal, salivary glands and eyelids,[5] the human *TP63* gene, mapping to 3q27, was considered as a strong candidate gene in those conditions. Eventually, *TP63* mutations were identified in both unrelated EEC cases and non syndromic split-hand-foot malformation (SHFM, MIM 183600) but not in the LMS syndrome.[3,6,7] Here we report a *TP63* missense mutation in an isolated case of ADULT syndrome.

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Material and methods

The patient, a female, is the elder child of healthy, unrelated parents. She was term born with low birth parameters (BW: 2500 g, BL: 46.5 cm, OFC: 34 cm). Growth and psychomotor development were in the normal range. She was first referred at 10.5 years of age for absent nipples. On examination, additional findings were observed, namely: (i) feet abnormalities with hypoplastic, over-riding toes, cutaneous 2–3 syndactyly and hypoplastic, discoloured toe nails; (ii) hands abnormalities with bilateral single palmar crease and Vth finger clinodactyly; (iii) freckling of the face, neck and chest, and; (iv) mild dysmorphic features with fine hair, malar hypoplasia, small mouth and multiple oral frenula (Figure 1a–d). Sweating and tears were normal. X-rays of hands and feet confirmed Vth fingers clinodactyly and hypoplastic toes (Figure 1e and f). Blood karyotype showed normal chromosomes, 46,XX.

Genomic DNA of the patient was extracted from leucocytes according to standard protocols. The coding region of the *TP63* gene, exons 2–15, was PCR-amplified using primers described elsewhere.[8] PCR was performed in a 50 μL volume containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM of each primer and 1 unit of Taq DNA polymerase (Eurobio^R). After a denaturation of 3 min at 95°C, the PCR consisted of 10 cycles of 10 s at 94°C, 10 s at 60°C (with a decrease of 1°C per cycle), and 10 s at 72°C, then 35 cycles of 10 s at 94°C, 10 s at 50°C, and 10 s at 72°C and was followed by a final extension of 7 min at 72°C. After

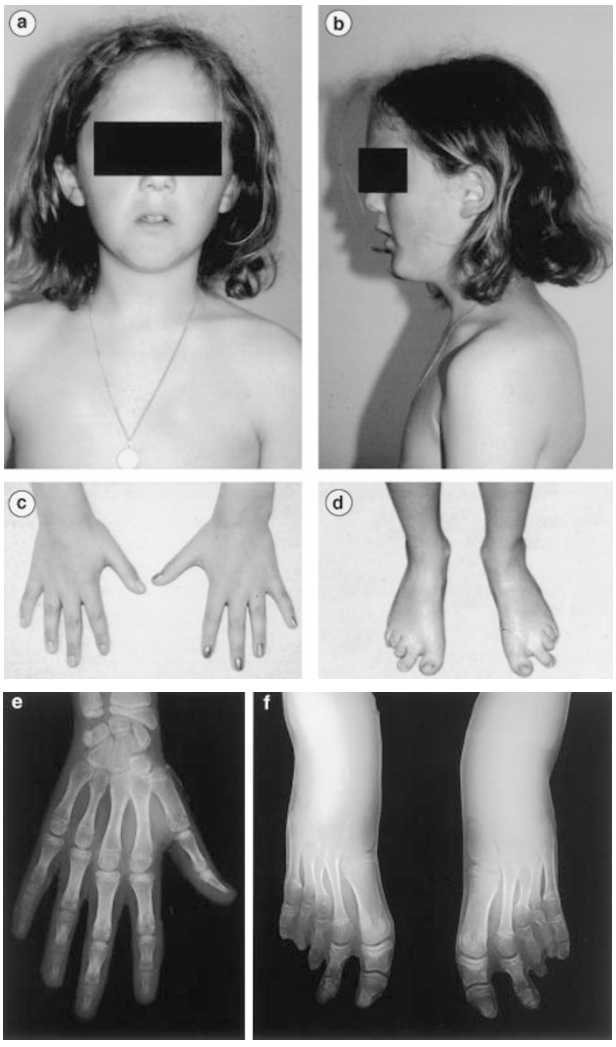

Figure 1 Patient with ADULT syndrome. (**a**) Note absent nipples, fine hair and small mouth. (**b**) Note malar hypoplasia. (**c**) Note bilateral clinodactyly of the Vth finger. (**d**) Note hypoplastic, over-riding toes, cutaneous 2–3 syndactyly and hypoplastic, discoloured toe nails. (**e**) X-ray of the left hand at the age of 11 years. Note clinodactyly of the Vth finger. (**f**) X-ray of the feet at the age of 11 years. Note bilateral hypoplasia of toes III, IV and V.

purification of the PCR products using QIAquick Gel Extraction Kit (Qiagen), sequencing analysis was performed using the PRISM Ampli Taq FS Ready Reaction Dye Terminators sequencing kit (PE Applied Biosystems) and a PE Applied Biosystems 377 automated DNA sequencer.

Results

TP63 sequence analysis revealed an heterozygous nucleotidic transversion at position 16 in exon 3′ (A>C), resulting in the substitution of a conserved asparagin by an histidine at codon 6 (N6H). The nucleotidic variation was inherited from the healthy father and was absent from a panel of 250 control chromosomes (Figure 2A).

Discussion

The *TP63* gene encodes six main isotypes with variable abilities to transactivate *TP53* target genes involved in apoptosis and/or cell cycle arrest. In mice, p63 is expressed in the apical ectodermal ridge, branchial arches and epideral appendage (Yang *et al*., 1999). All six Tp63 isotypes contain a DNA binding domain while only three isotypes contain a transactivation domain (TA, Figure 2B).[3,9] The mutation

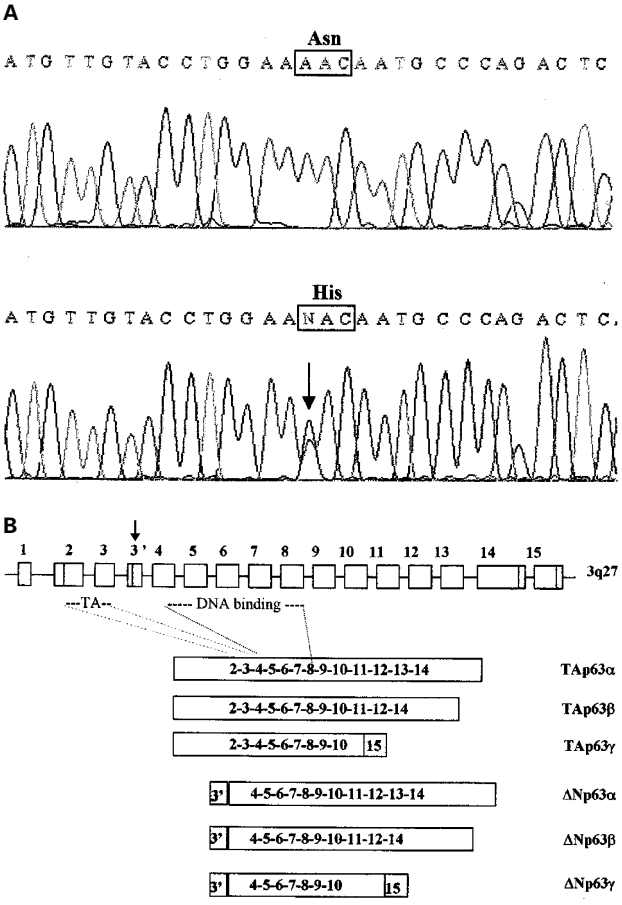

Figure 2 (**A**) N6H mutation of the *TP63* gene (exon 3′). The *TP63* gene was PCR-amplified from genomic DNA and directly sequenced. Sequences observed in the proband (bottom) and in a control (top) are shown. Heterozygous mutation in the ADULT patient is indicated by an arrow (N). The predicted amino-acid change is indicated above the nucleotide sequence. (**B**) The TP63 isotypes. The N6H mutation reported is indicated by an arrow. TA: trans activation domain, DNA binding: DNA binding domain.

Table 1 Clinical features in four overlapping syndromes

	SHFM	*EEC (TP63)*	*LMS*	*ADULT*	*LADD*
Limbs abnormalities	+	+	+ (75%)	+ (incomplete penetrance)	+ (radial ray)
Ectrodactyly	+	+	+	+	−
Syndactyly	+	+	+	+	+
Ectodermal dysplasia	−	+	+ (30%, hypohydrosis)	± (freckling)	−
CL/P	−	+	+ (20%, CP/bifid uvula)	−	occasional
Teeth abnormalities	−	+	+ (15%, hypodontia)	+ (hypodontia)	+ (hypodontia, abnormal shape)
Lacrimal ducts atresia	−	±	+ (50%)	+	+
Nipple/breast hypoplasia	−	−	+(100%)/+	+/+	−
Hair abnormalities	−	+ (sparse)	−	+ (fine)	−
Nails abnormalities	−	?	+ (30%, dysplasia)	+ (dysplasia)	+ (dysplasia)
Ears abnormalities	−	−	−	−	+ (cup shape)

Trait penetrance is given in brackets.

reported here affects exon 3′ present only in the isotypes lacking the transactivation domain of the Tp63 protein (ΔN-p63α, β and γ). Conversely, so far, all but one mutation detected in EEC3 patients are within the DNA binding domain of the protein.[3,6,7] ΔN-p63α, the major TP63 isotype in basal cells of epithelial tissues, has been shown *in vitro* to have a dominant-negative effect towards transactivation activities mediated by TP53 and TA-p63.[3,9] Therefore, although we cannot exclude that the N6H mutation is a rare variant, one may hypothesise that ADULT syndrome results in a release of the dominant-negative control of ΔN isotypes.

The *TP63* N6H mutation that we identified was inherited from the healthy father in whom frecking of the back and shoulders was the only feature of ADULT syndrome that could be noticed. DNA of the proband's paternal grand parents was not available for DNA analysis. Thus, while we cannot exclude mosaic in the proband's father, incomplete penetrance is the most likely hypothesis. Non penetrance in obligate carriers has been documented in families with EEC3,[6] as well as in the four generations family with ADULT syndrome reported by Propping and Zerres.[1] In this family, watch glass nails was the only feature in an affected male, and four out of eight affected individuals had normal hands and mild abnormalities of the feet (cutaneous syndactyly or short second toe). Van Bokhoven and coworkers mention a *TP63* gene mutation in an ADULT syndrome case. Whether this is a sporadic or familial case is not specified.[10]

Finally, there is a considerable overlap between EEC, LMS, ADULT and lacrimo-auriculo-dento-digital syndromes ((LADD, MIM 149730, Table 1). Both linkage analysis in a three generations family described elsewhere,[11] and direct DNA sequencing in an isolated case excluded *TP63* as the disease causing gene in LADD (personal data not shown). LMS and ADULT syndromes share features in accordance with the pattern of expression of TP63 in mice that are only occasionally found in EEC3, namely lacrimal duct atresia and breast/nipple hypoplasia. However, no nucleotidic variation in the *TP63* binding domain could be identified in LMS. It is therefore tempting to speculate that LMS, as ADULT syndrome, might result from an as yet unidentified defect of the Tp63 isotypes lacking the transcriptional domain, inducing an alteration of the regulation of the TP63 function.

In conclusion, we showed that ADULT syndrome could be ascribed to *TP63* gene mutation. This finding allows the lumping of two clinical entities and widens the spectrum of malformations resulting from *TP63* mutations in human.

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