Heterozygous germline missense mutation in the *p63* gene underlying EEC syndrome

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Summary

Mutations in the *p63* gene have recently been delineated as the molecular basis for some cases of the ectrodactyly, ectodermal dysplasia and cleft lip/palate (EEC) syndrome, an autosomal dominant disorder (MIM 129900). In this report, we describe a 35-year-old woman with EEC syndrome and document a heterozygous germline missense mutation, R304W, in exon 8 of the *p63* gene. As with most other *p63* mutations in EEC syndrome, this mutation has arisen *de novo and* is located within the core DNA-binding domain of *p63*. Identification of this mutation has implications for genetic counselling and the feasibility of future DNA-based prenatal diagnosis in this individual.

Report

The ectrodactyly, ectodermal dysplasia and cleft lip/palate (EEC) syndrome is a rare autosomal dominant developmental disorder that shows highly variable expression and reduced penetrance (MIM 129900). Several other syndromes may show overlapping clinical features.[1,2] The main clinical features of EEC syndrome are the developmental anomalies reflecting perturbation of a molecular signalling pathway common to the formation of limbs, hair, teeth and apocrine glands.[3]

Clues to the pathogenesis of EEC syndrome emerged from studies of knock-out mice, specifically from mice with ablation of the *p63* gene.[4,5] *p63* is a homologue of the archetypal tumour suppressor gene, *p53* and is abundantly expressed in proliferating basal epithelial cells, including epidermis.[6,7] *p63*−/− mice show limb and palate abnormalities as well a lack of epidermis, hair, whiskers, teeth, lacrimal and salivary glands, thus demonstrating the relevance of *p63* in tissue development. Subsequently, genetic linkage and positional cloning studies in families with EEC syndrome identified *p63* as a candidate gene and a series of nine heterozygous mutations (eight missense and one frameshift) were reported.[8] We now report the clinical and molecular assessment of a further patient with EEC syndrome.

The patient is a 35-year-old woman of nonconsanguineous parents. She is the only affected member of her family and does not have any children. From birth, she has had ectrodactyly of both hands (Fig. 1) and the right foot, as well as syndactyly of the left third and fourth toes and shortening of the left second toe. Other congenital abnormalities have included bilateral cleft lip and palate, a broad flattened nose, and bilateral nasolacrimal duct stenosis. All teeth have been poorly formed and extensive caries has led to complete loss of all teeth. Her nails have always been dystrophic and her scalp and body hair have constantly been sparse and wiry. She has also had a low vaginal stenosis requiring surgical correction. Other operations have included cleft lip and palate repair, orthopaedic surgery to the right foot, dilatation and stenting of the nasolacrimal ducts and then dacryocystorhinostomies for epiphora, and two operations to correct her nasal deformity.

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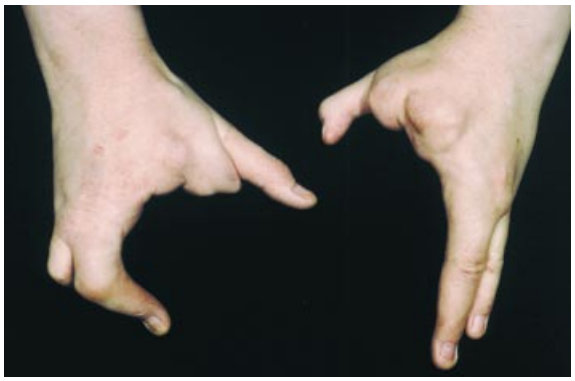

Figure 1 Clinical appearance of the split hand (lobster claw) malformation in the patient with EEC syndrome.

To investigate potential abnormalities of *p63*, DNA was first extracted from peripheral blood samples taken from the affected individual and her clinically normal parents using a standard cold water lysis method. Individual exons of the *p63* gene were then amplified by polymerase chain reaction (PCR) as described previously.[8] Specifically, to amplify exon 8 and flanking introns of *p63* the following primers were used: forward primer 5′-GTAGATCTTCAGGGGACTTTC-3′, reverse primer 5′-CCAACATCAGGAGAAGGATTC-3′. The expected PCR product size was 260 base pairs (bp). For PCR amplification, 250 ng genomic DNA was used as the template in an amplification buffer containing 6.25 pmol of the primers, 37.5 nmol $MgCl_2$, 5 mmol of each nucleotide triphosphate and 1.25 U *Taq* polymerase (Perkin-Elmer) in a total volume of 25 μL in an OmniGene thermal cycler (Hybaid). The amplification conditions were 94 °C for 5 min, followed by 38 cycles of 94 °C for 45 s, 53 °C for 45 s, 72 °C for 45 s. Aliquots (5 μL) of the PCR products were analysed by 2% agarose gel electrophoresis. PCR products were then sequenced directly using Big Dye labelling in an ABI 310 genetic analyser (Perkin-Elmer).

In the patient's DNA, we identified a heterozygous point mutation at nucleotide position 910 within exon 8 of the *p63* gene (based on nucleotide numbering of the p63α isotype with the A of the start codon as nucleotide 1).[8] The mutation comprised a C → T substitution at a CpG dinucleotide which converts an arginine residue (CGG) to tryptophan (TGG) (Fig. 2). This mutation, designated R304W, results in the loss of a restriction site for the endonuclease *Hpa*II, which was used to verify the mutation and to establish that it was not present in amplified DNA from either parent nor in 100 control chromosomes. No other potentially pathogenic *p63* sequence variations were identified.

Of the nine *p63* mutations previously documented in

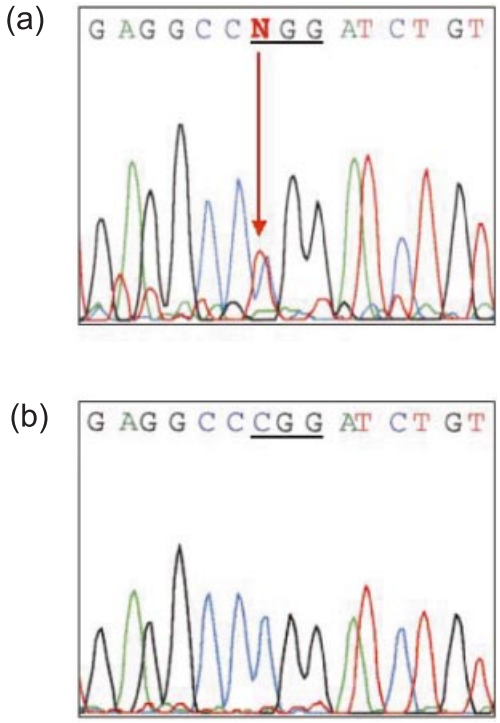

Figure 2 Nucleotide sequencing of exon 8 of the *p63* gene in the patient's DNA (a) reveals a heterozygous C → T substitution that converts an arginine residue (CGG) to tryptophan (TGG): R304W. Sequencing of control DNA (b) shows homozygous wild-type sequence (CGG).

EEC syndrome, R304W has been seen in one other patient.[8] Eight of the mutations, including R304W, have comprised missense mutations clustered within the core DNA-binding domain of *p63*. This particular arginine residue is conserved with respect to other similar genes, including *p53* and *p73*, and has been shown to bind directly to DNA.[7] Functional studies have shown that mutation of this arginine residue affects all six known *p63* isotypes and results in alterations in gene transcriptional activity.[8] Also noteworthy is the observation that five of the nine *p63* mutations have arisen *de novo*. Our findings provide further evidence for this genetic basis of the EEC syndrome. At present, the collective mutation findings cannot offer an adequate explanation for the phenotypic heterogeneity in EEC syndrome. The effects of environment and other genes acting as possible modifiers of phenotypic expression are currently unknown although a potentially relevant locus other than *p63* (on chromosome 19q) has been implicated in one EEC family.[9]

From a practical perspective, delineation of this mutation in *p63* has important implications for the

feasibility of prenatal testing. EEC is an autosomal dominant disorder, so offspring of the affected individual have a 50% possibility of being affected. We can now offer DNA-based testing using PCR amplification and restriction endonuclease digestion using fetal DNA from chorionic villi or amniocytes or by using a preimplantation genetic diagnosis approach.[10]

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