ADULT ectodermal dysplasia syndrome resulting from the missense mutation R298Q in the *p63* gene

I. Chan,* J. I. Harper,† J. E. Mellerio*† and J. A. McGrath*

**Genetic Skin Disease Group, St John's Institute of Dermatology, The Guy's, King's, and St Thomas' School of Medicine, St Thomas' Hospital, London, and †Department of Dermatology, The Great Ormond Street Hospital for Children, London, UK*

Summary

Several ectodermal dysplasia syndromes have been shown to result from mutations in the gene that encodes the transcription factor p63. We describe an 11-year-old boy, with clinically normal parents, who had a developmental disorder that resembled EEC (ectrodactyly ectodermal dysplasia-clefting) syndrome (OMIM 604292). He had ectrodactyly and missing middle fingers bilaterally, onychodysplasia, hypodontia with missing teeth, hypohidrosis and lacrimal duct obstruction. DNA sequencing disclosed a heterozygous G → A substitution at nucleotide 893, that converts an arginine residue (CGA) to glutamine (CAA), the mutation being designated R298Q. This mutation occurs within the DNA-binding domain of p63, and is close to many of the published EEC syndrome mutations. However, R298Q has been described once previously in a large German pedigree, not with EEC syndrome, but another ectodermal dysplasia disorder, ADULT (acro-dermato-ungual-lacrimal-tooth) syndrome (OMIM 103285). Further clinical assessment in our patient revealed that, apart from not having cleft lip and/or palate, he had an exfoliative dermatitis of his hands and feet, and some freckling on his face and shoulders. Collectively, these features support a diagnosis of ADULT syndrome. This study has identified a specific genotype–phenotype correlation in a rare ectodermal dysplasia syndrome and the findings are useful in improving genetic counselling in this family.

Over 170 different ectodermal dysplasias and related syndromes have been described, many of which have overlapping clinical features.[1,2] These conditions encompass a spectrum of developmental abnormalities of skin, hair, teeth, nails, and sweat glands. Recently, a number of these syndromes has been shown to result from mutations in the gene that encodes the transcription factor, p63.[3] For example, several cases of EEC (ectrodactyly ectodermal dysplasia-clefting) syndrome (OMIM 604292) have been reported with autosomal dominant mutations (mostly missense) in the DNA-binding domain of p63.[3–5] Other syndromes such as limb–mammary syndrome (LMS), ankyloblepharon-ectrodactyly ectodermal dysplasia (AEC), Rapp-Hodgkin Syndrome (RHS), and split-hand–split-foot malformation syndrome (SHSF) may also result from mutations in *p63*.[3–8]

We report an 11-year-old boy who was born to clinically normal and unrelated parents. He was of normal intelligence but had a form of ectodermal dysplasia that resembled EEC syndrome. Specifically, he had ectrodactyly with missing middle fingers bilaterally requiring seven reconstructive surgical procedures to improve function, onychodysplasia, hypodontia with missing teeth, mild hypohidrosis and lacrimal duct obstruction (Fig. 1). Although he did not have a cleft lip and/or palate, these clinical features gave an EEC syndrome Roelfsema and Cobben score of 8/18.[9]

Following informed consent, DNA was extracted from a peripheral blood sample taken from the affected individual using a standard cold water lysis method. Individual exons of the *p63* gene were amplified by PCR

Correspondence: J. A. McGrath, Genetic Skin Disease Group, St John's Institute of Dermatology, St Thomas' Hospital, Lambeth Palace Road, London, SE1 7EH, UK.
E-mail: john.mcgrath@kcl.ac.uk

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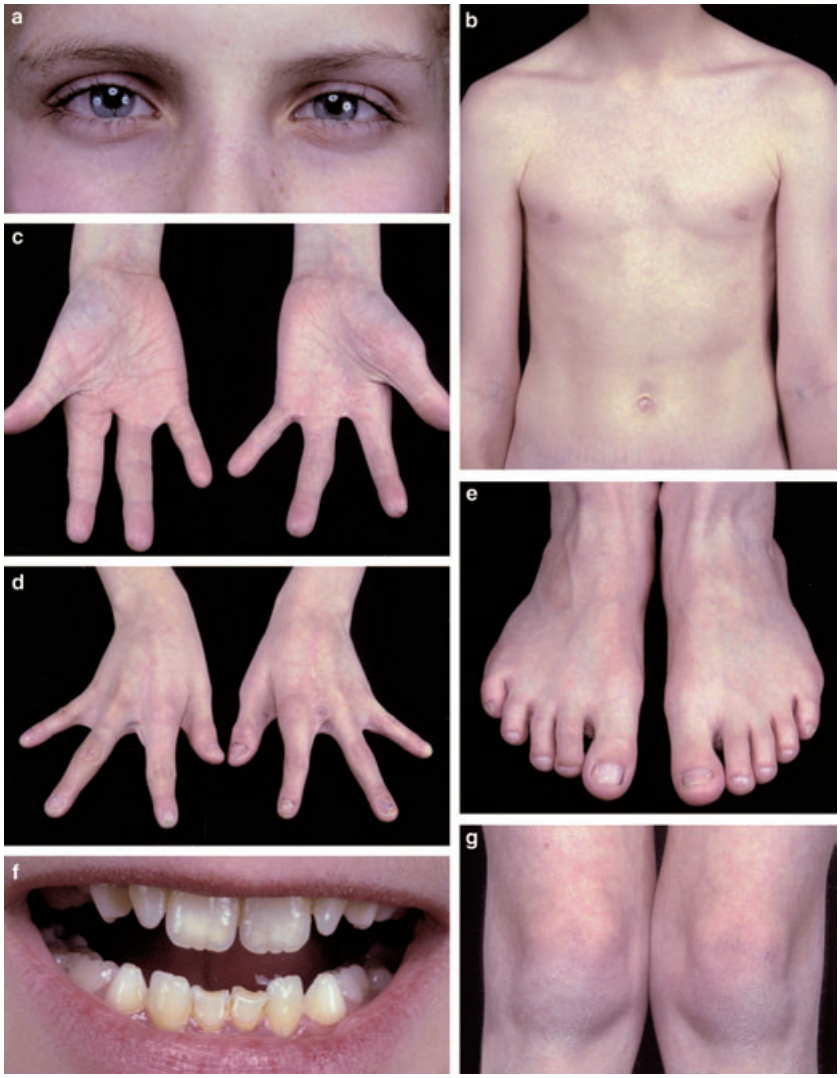

Figure 1 Clinical features of an 11-year-old boy with ADULT syndrome. (a) There is freckling on the cheeks. (b) The right nipple is hypoplastic. (c and d) Ectrodactyly with bilateral missing middle fingers and surgical scars from reconstructive operations. There is an exfoliative dermatitis of the fingers and palms as well as marked nail dystrophy. (e) Structurally normal feet apart from mild dysplasia of the toenails. (f) The teeth are hypoplastic with poorly formed enamel. (g) Dry skin on the knees.

and sequenced.[7] Specifically, to amplify exon 8 and flanking introns of *p63* the following primers were used: forward primer 5′-TGGCTAAGCTGGTAGTACGT-3′, reverse primer 5′-CACAGGTCTTATCATGCAGC-3′. The expected PCR product size was 352 base pairs (bp). For PCR amplification, 250 ng of 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 2.5 U *Taq* polymerase (Applied Biosystems) in a total volume of 50 μL in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). The amplification conditions were 94 °C for 5 min, followed by 38 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s. Aliquots (5 μL) of the PCR products were analysed by electrophoresis through 2.5% agarose gel. PCR products were then purified using QIAquick PCR Purification Kit (Qiagen) and sequenced directly in an ABI 310 genetic analyser (Applied Biosystems).

Amplified DNA from the affected individual disclosed a heterozygous nucleotide substitution (G → A) in nucleotide 893 within exon 8 of the *p63 gene* (Fig. 2). This transition converts an arginine residue (CGA) to glutamine (CAA). This sequence change was not present in his mother's DNA; genomic DNA from the father was not available for study. This missense mutation (presumed *de novo*) is designated R298Q. The site of the mutation is similar to several other pathogenic amino acid substitutions reported in EEC syndrome.[3–5]

However, a literature search revealed that R298Q had been reported previously in a German family, not with EEC syndrome, but with a seemingly unique form of ectodermal dysplasia termed ADULT (acro-dermato-

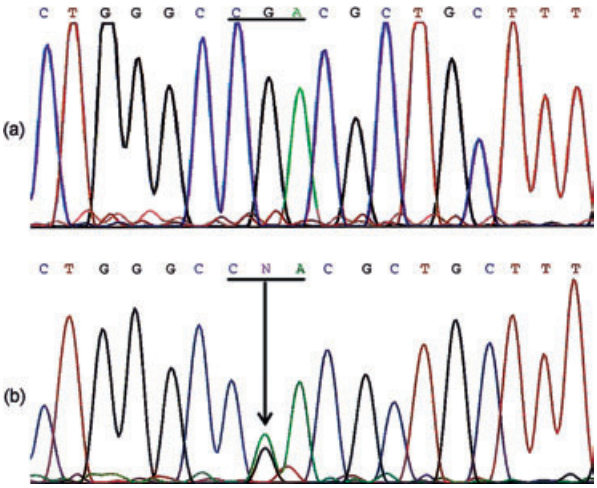

Figure 2 Automated sequencing of part of exon 8 of the *p63* gene in (a) normal control DNA and (b) the affected child. Compared to the wild-type sequence in (a), the affected individual has a heterozygous nucleotide substitution (G → A) at nucleotide 893. This transition converts an arginine residue (CGA) to glutamine (CAA) and this missense mutation is designated R298Q.

ungual-lacrimal-tooth) syndrome (OMIM 103285).[10,11] These reports suggested that ADULT syndrome had subtle but distinctive differences from EEC syndrome. We therefore reviewed the clinical features in our patient. In keeping with the affected German individuals, he did not have clefting of the lip or palate and had an exfoliative dermatitis of his hands and feet as well as very dry skin over the knees and shins. In addition, he had some freckling on his face and shoulders and his right nipple appeared hypoplastic (Fig. 1). X-rays confirmed some missing secondary dentition, but it remains to be seen if he will experience early loss of his permanent teeth. His hair growth is normal and not particularly thin. Overall, the clinical similarities support a diagnosis of ADULT syndrome in our patient. However, it should be noted that the phenotype of ADULT syndrome in the German family showed variable penetrance, making it difficult to diagnose clinically this specific syndrome.

Given the proximity of the ADULT syndrome-associated mutation R298Q to other EEC syndrome mutations, the question arises why should this result in a specific phenotype? The answer, albeit a partial one, may lie in functional studies. p63 is thought to have an important role in both the development and differentiation of epidermis. In embryogenesis, p63 is required for the initiation of epithelial stratification, whereas in the mature epidermis, it maintains the proliferative potential of the basal keratinocytes.[12] However, the six isoforms of p63, ΔN-p63α, β, γ and TA-p63α, β, γ, exist in a dynamic complex that counterbalances the effects of each other, thereby regulating growth, differentiation and apoptosis. Disturbances to this interactive balanced system may underscore the various ectodermal dysplasia syndromes associated with mutations in the *p63* gene.

Of note, the consequences of the mutation R298Q have been shown to differ from other missense mutations in the *p63* gene.[11] Specifically, R298Q has been found to induce significant transactivation capacity to ΔN-p63γ, one of the six isoforms of p63.[11] In contrast, EEC syndrome missense mutations result in loss-of-function for this isoform of p63. Thus, the molecular consequences of this particular mutation in the R298 residue of p63 may not only cause unique gene dysfunction but might also account for the specific ectodermal abnormalities that underlie ADULT syndrome. That said, there has been one other case of ADULT syndrome reported which resulted from a different heterozygous missense mutation, N6H, located upstream of the DNA-binding domain of p63.[13] This particular mutation is atypical for ectodermal dysplasia syndromes and its functional effects on the p63 isoforms are unclear.[13,14]

From a practical perspective, elucidation of this mutation in *p63* in our patient has enabled us to provide both an accurate diagnosis and more precise genetic counselling. In particular, clefting is a disfiguring craniofacial anomaly that requires complex multidisciplinary treatment and has lifelong implications for affected individuals. The lack of clefting of either lip or palate in ADULT syndrome can provide some reassurance to patients with this autosomal dominant disorder who are considering having their own children. This study highlights the sometimes subtle variation in phenotypes resulting from mutations in *p63* and helps define genotype–phenotype correlation in these syndromes.

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