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De novo missense mutation, S541Y, in the *p63* gene underlying Rapp–Hodgkin ectodermal dysplasia syndrome

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Summary

Rapp–Hodgkin syndrome (RHS) is an autosomal dominant disorder characterized by ectodermal dysplasia and cleft lip/cleft palate. Very recently, mutations in *p63* have been identified as a cause of RHS; to date five such mutations have been identified. We describe a Thai girl with RHS. She had short stature, ectodermal dysplasia, epiphora, cleft lip, cleft palate, and normal development. Mutation analysis for the entire coding region of *p63* identified a novel and *de novo* mutation, 1622C → A (S541Y), in the SAM domain, predicting an abnormal α tail of the p63α protein isotypes. This observation supports that majority of patients with RHS are caused by mutations affecting the tail of p63α, a region that also contains most of the pathogenic mutations in ankyloblepharon-ectodermal dysplasia-clefting (AEC) syndrome.

Report

Rapp–Hodgkin syndrome (RHS, MIM 129400) is an autosomal dominant disorder first described in a mother and her two children with a combination of anhidrotic ectodermal dysplasia, cleft lip and cleft palate.[1] Its features include short stature, coarse and wiry hair, sparse eyelashes and eyebrows, epiphora due to obstructed lacrimal puncta, stenosis of external auditory canals, narrow nose, small mouth, cleft lip/cleft palate, cone-shaped incisors, hypodontia, hypospadias, dystrophic nails, and hypohidrosis.[2]

Very recently, mutations in the *p63* gene were demonstrated to cause RHS. The *p63* gene is a *p53* gene homologue with high amino acid identity; but unlike p53, p63 is a key regulator in limb, epithelial and craniofacial development, and has several isoforms. Two transcription initiation sites were initially described, one that would give rise to proteins containing the transactivating domain (the TA isotypes) and the other that lacks it (the ΔN isotypes). Alternative splicing at the 3′ end of the gene results in three different C termini, α, β and γ. The largest p63 isotype, TA-p63α.5, has a transactivating (TA), a DNA binding (DB), a polymerization, a sterile-α.5-motif (SAM), and a transactivation inhibitory (TI) domain.[3] So far, only five mutations in *p63* associated with RHS have been identified.[4–8]

Here we describe a Thai girl with RHS. She was born after uncomplicated pregnancy at term by spontaneous vaginal delivery to a 26-year-old, gravida 2, para 1 Thai mother and a 33-year-old unrelated Thai father. Birth weight was 3900 g. She was noted to have oral clefts from birth. Her development was appropriate for age. Physical examination at the age of 43 months revealed height of 86.5 cm (− 2.5 SD), weight 13 kg (− 1 SD) and head circumference 48 cm (− 1 SD). She had coarse hair, sparse eyebrows and eyelashes, epiphora, partly surgically corrected bilateral complete cleft lip and palate, rampant dental caries, and dystrophic finger and toe nails (Figs 1a–c). Her parents and elder brother were unaffected.

After informed consent had been obtained, peripheral blood (3 mL) was obtained from the girl and her parents and DNA was extracted by standard methods. The 16 exons of the *p63* gene, which contain its entire coding region, were PCR-amplified from genomic DNA using primers as previously described.[9] The PCR products

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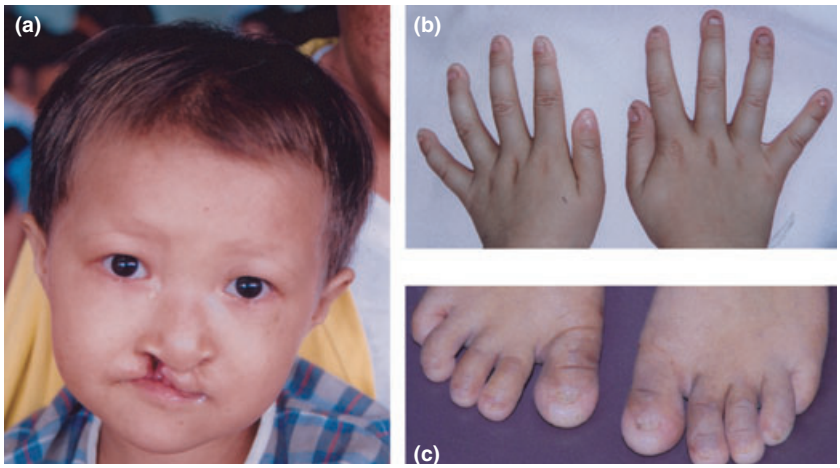

Figure 1 The proband has (a) coarse hair, sparse eyebrows and eyelashes, epiphora, partly surgically corrected bilateral complete cleft lip and palate, and dystrophic (b) finger and (c) toe nails.

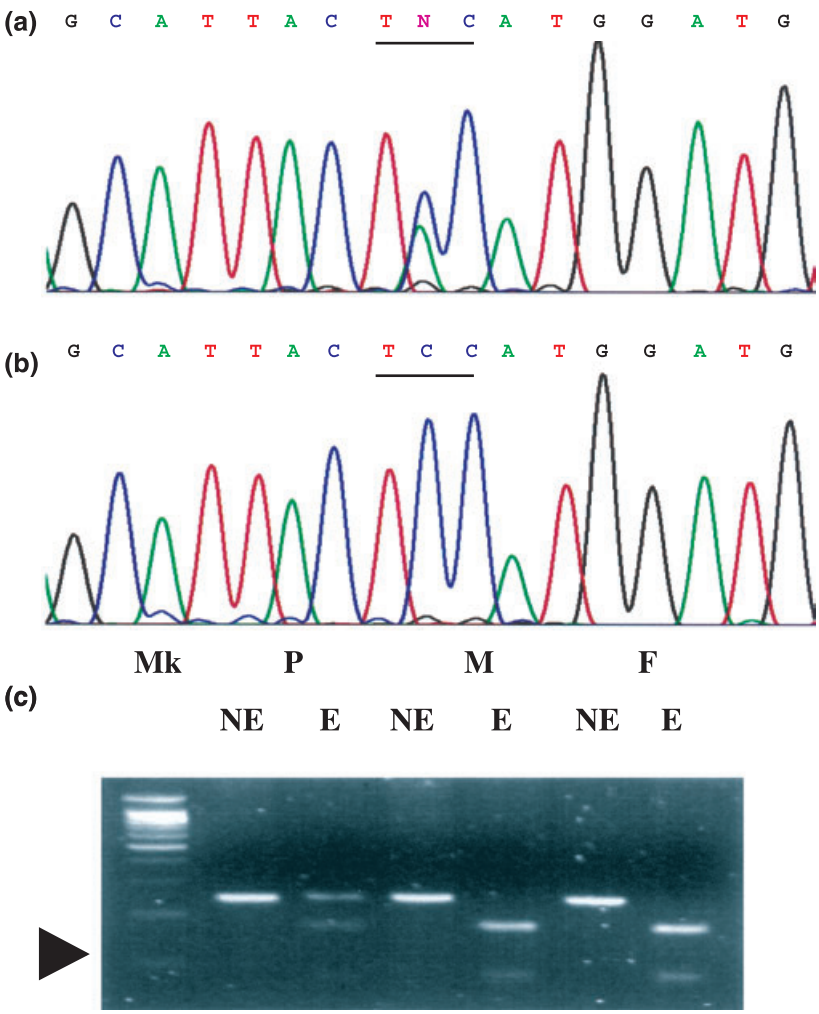

Figure 2 Mutation analysis. The sense sequence electropherogram of the *p63* exon 13 from (a) the proband and (b) control. The proband shows a heterozygous C → A mutation converting a serine residue (TCC) to tyrosine (TAC), designated as S541Y. The mutation is confirmed by restriction enzyme analysis (c). Mk in lane 1 represents a 100-bp marker with the band 100 bp indicated by an arrow; lanes 2 and 3 are the patient (P); lanes 4 and 5 from her mother (M); lanes 6 and 7 from her father (F). 'NE' denotes PCR products without adding restriction enzyme and therefore shows only the undigested 238-bp fragment. 'E' represents PCR products with the addition of a restriction enzyme, *Sty*I, in which the patient shows the 238-, 161- and 77-bp fragments while her parents have only the 161- and 77-bp fragments, indicating that the heterozygous S541Y in the patient is *de novo*.

were treated with ExoSAP-IT (USP Corporation; a mixture of exonuclease to eliminate residual oligonucleotide primers and shrimp alkali phosphatase to degrade dNTPs into deoxynucleotides) according to the company recommendations, and sent for direct sequencing at Macrogen Inc. (Seoul, Korea).

Direct sequencing analysis of the PCR products revealed that the girl was heterozygous for a C → A point mutation at nucleotide position 1622 (1622C → A) (numbered according to the TA-p63α isoform, GenBank accession AF075430) in exon 13 of *p63* (Fig. 2). The mutation was confirmed by digestion of the PCR products with the restriction enzyme *Sty*I, in which its recognition site is removed by the point mutation (Fig. 2c). The DNA change was expected to result in conversion of a serine (TCC) to tyrosine (TAC) (S541Y). This mutation has not been reported previously and was not detected in the patient's parents (Fig. 2c) or 100 control chromosomes. No other sequence variants for the remainder of the *p63* gene were found in the patient's DNA.

Our patient had short stature, ectodermal dysplasia, epiphora, cleft lip, cleft palate, and normal development, which are typical of RHS. However, the fact that RHS corresponds to a specific clinical entity is controversial. RHS displays some clinical overlap with other ectodermal dysplasia syndromes, notably Ectrodactyly Ectodermal Dysplasia-Clefting (EEC) syndrome (OMIM 129900) and Ankyloblepharon-Ectodermal Dysplasia-Clefting (AEC) syndrome (OMIM 106260). The EEC syndrome is characterized by the triad of ectrodactyly, ectodermal dysplasia, and oral clefts[3] while the AEC syndrome by ankyloblepharon, ectodermal dysplasia, and oral clefts.[10] In addition, there were reports of RHS and EEC[11] and RHS and AEC[5] within the same families. Yet, we believe that RHS was the most likely diagnosis in our patient since she did not present either ectrodactyly or ankyloblepharon, which argues against the diagnosis of EEC and AEC, respectively.

Recently, RHS, EEC syndrome, and AEC syndrome have been proved to be allelic disorders being caused by mutations in the *p63* gene.[4–6,10,12] The majority of the mutations in families with EEC syndrome gives rise to amino acid substitutions in the DB domain that is common to all known *p63* isoforms.[3] Most of mutations in patients with AEC syndrome are missense mutations within the SAM domain of *p63*.[3] These missense mutations affect only the α isotypes of p63, which behave as inhibitors of transactivation. All of the five mutations previously reported in patients with RHS were either in [1529T → C (I510T) in exon 12[8] and 1621T → C (S541P) in exon 13[4]] or downstream (1709delA[6] 1787delG[7] and 1859delA[5] in exon 14) the SAM domain, which predicting an abnormal α tail. There was a case of possible RHS associated with the mutation 836G → A (R279H) in exon 7 within the DB domain although there is a clinical impression that this could be a modified form of EEC syndrome.[6] The 1622C → A mutation found in our patient predicted the substitution of the codon 541. This is the third mutation in this particular serine residue: S541P[4] and S541Y (our patient) cause RHS, and S541F[8] causes AEC. This observation further supports that in the majority of patients RHS is caused by mutations affecting the α tail of the p63α protein isotypes.

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