Clin Genet 2001: 60: 314–315
Printed in Ireland. All rights reserved

CLINICAL GENETICS
ISSN 0009-9163

Letter to the Editor

A *de novo* mutation (*R279C*) in the *P63* gene in a patient with EEC syndrome

To the Editor:

Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome is an autosomal dominant disorder characterized by ectrodactyly, ectodermal dysplasia and cleft lip with or without cleft palate (1). Celli et al. demonstrated that heterozygous mutations in the *P63* gene, a cell cycle regulator on chromosome 3q27, lead to the EEC syndrome (2). To date, a total of twelve heterozygous nucleotide changes in *P63* has been detected in EEC syndrome patients (2–4). We report here the first Asian EEC syndrome patient with a *P63* mutation.

The patient was delivered at 40 weeks gestation to a 22-year-old G1P0 woman. The birth weight of 2820 g was in the 25th percentile, and the patient's length of 49 cm was in the 50th percentile. The patient's head circumference of 33 cm was in the 25th percentile. At birth, the diagnosis of the EEC syndrome was made based on middle ray defects of both hands and feet (Fig. 1), sparse scalp, eyebrow, and eyelash hair, thin and dry skin, and cleft lip with cleft palate. After obtaining informed consent from the parents, the coding sequence of *P63* was screened for mutations by polymerase chain reaction (PCR) sequencing with primers designed by Celli et al. (2). Genomic DNA was extracted from whole blood with a QIAamp DNA Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Sequencing of the PCR product amplified from exon 7 and flanking introns of *P63* with primer 7F (5′-GGGAAG AACTGAGAAGGAACA AC-3′) and primer 7R (5′-CAGCCACGATTTCACTTTGCC-3′) revealed a heterozygous C to T transition at nucleotide 980 (with respect to the adenine of the start codon) (Fig. 2). This mutation predicts an arginine-to-cysteine substitution at amino acid 279 within the DNA binding domain (numbering based on the TA-P63 isotype). The C to T transition was not present in either parent or 200 ethnically matched control chromosomes. No other potentially pathogenic *P63* sequence variations were identified.

We conclude that the *R279C* mutation is pathogenic for the following reasons: 1) The *R279C* substitution occurred within the DNA-binding domain showing an extreme degree of evolutionary sequence conservation among vertebrates. Furthermore, protein sequences flanking the mutation are strictly conserved among three related proteins: P53, P63, and P73. 2) A crystallographic study of the P53 protein predicts the arginine residue at 248, which corresponds to the arginine 279 of P63, to extend into the minor groove of the DNA (5). 3) The R279C substitution was not identified in either parent or normal controls. The arginine residue at 279 was mutated to histidine in 2 patients with the EEC syndrome (2, 3). Hence, the arginine residue at 279 represents a mutational hotspot.

Amino acid substitutions in the P63 protein sequence involving the sterile alpha motif (SAM) domain, as opposed to the DNA-binding domain, lead to the Hay–Wells syndrome (OMIM 106260) characterized by ankyloblephalon (fused eyelids) and severe scalp dermatitis as distinguishing features (6). Twelve patients with the Hay–Wells

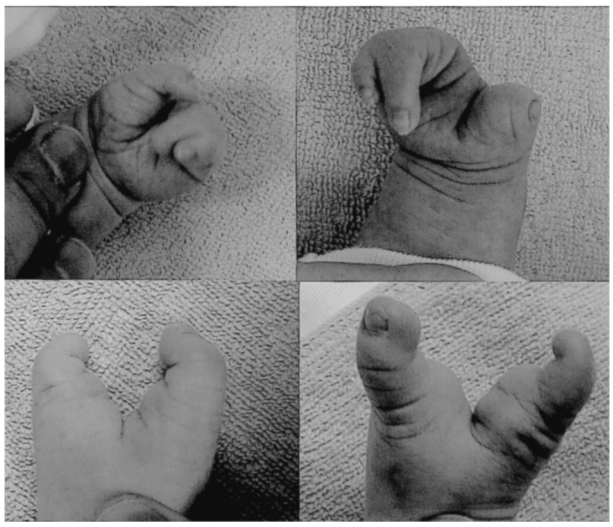

Fig. 1. Hands and feet. Note middle ray defect accompanied by syndactyly.

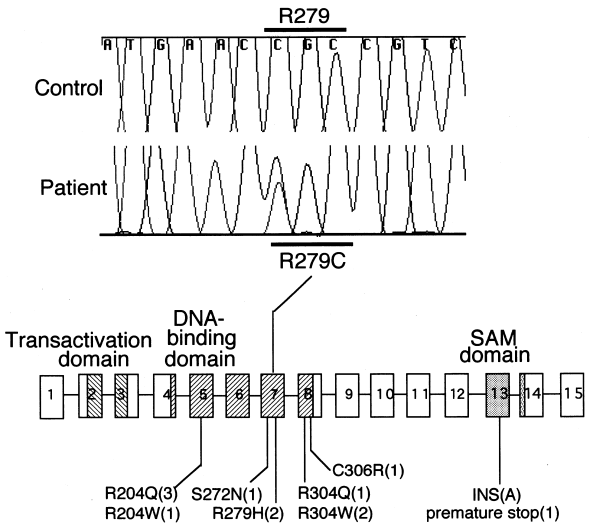

Fig. 2. R279C mutation in the EEC syndrome patient. (Top) A heterozygous C to T transition at nucleotide 980 within exon 7 leads to an arginine-to-cysteine substitution at amino acid 279 within the DNA-binding domain. (Bottom) Schematic representation of the human *P63* gene and position of mutations found in EEC syndrome patients to date. Numbers of patients with each mutation are indicated in parentheses.

syndrome have been reported to have mutations in the SAM domain. Documentation of an amino acid substitution within the DNA-binding domain in the present case, who had the EEC phenotype, further supports the notion that the location of the missense mutation within the P63 protein may be related to the phenotype.

R Kosaki
H Ohashi
H Yoshihashi
T Suzuki
K Kosaki

Acknowledgements

This work was supported in part by a grant from the CREST, Japan Science and Technology Corporation.

References

1. Penchaszadeh VB, de Negrotti TC. Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome: dominant inheritance and variable expression. J Med Genet 1976: 13 (4): 281–284.
2. Celli J, Duijf P, Hamel BC et al. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 1999: 99 (2): 143–153.
3. Ianakiev P, Kilpatrick MW, Toudjarska I, Basel D, Beighton P, Tsipouras P. Split hand/split-foot malformation is caused by mutations in the *p63* gene on 3q27. Am J Hum Genet 2000: 67 (1): 59–66.
4. Wessagowit V, Mellerio JE, Pembroke AC, McGrath JA. Heterozygous germline missense mutation in the *p63* gene underlying EEC syndrome. Clin Exp Dermatol 2000: 25 (5): 441–443.
5. Cho Y, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 1994: 265 (5170): 346–355.
6. McGrath JA, Duijf PH, Doetsch V et al. Hay–Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63. Hum Mol Genet 2001: 10 (3): 221–229.

Correspondence:
Kenjiro Kosaki
Division of Medical Genetics and Dysmorphology
Department of Pediatrics
Keio University School of Medicine
35 Shinanomachi
Shinjuku-ku
Tokyo 160-8582, Japan
Telephone: +81-3-3353-1211 ext 62368
Fax: +81-3-5379-1978
E-mail: kkosaki@sc.itc.keio.ac.jp