Hayashi S, Imoto I, Aizu Y, Okamoto N, Mizuno S, Kurosawa K, Okamoto N, Honda S, Araki S, Mizutani S, Numabe H, Saitoh S, Kosho T, Fukushima Y, Mitsubuchi H, Endo F, Chinen Y, Kosaki R, Okuyama T, Ohki H, Yoshihashi H, Ono M, Takada F, Ono H, Yagi M, Matsumoto H, Makita Y, Hata A, Inazawa J	Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies.	J Hum Genet	56(2)	110-24	2011
Naiki M, Mizuno S, Yamada K, Yamada Y, Kimura R, Oshiro M, Okamoto N, Makita Y, Seishima M, Wakamatsu N	MBTPS2 mutation causes BRESEK/BRESHECK syndrome.	Am J Med Genet A			In press
Niihori T, Aoki Y, Okamoto N, Kurosawa K, Ohashi H, Mizuno S, Kawame H, Inazawa J, Ohura T, Arai H, Nabatame S, Kikuchi K, Kuroki Y, Miura M, Tanaka T, Ohtake A, Omori I, Ihara K, Mabe H, Watanabe K, Niijima S, Okano E, Numabe H, Matsubara Y. Matsubara Y. Inazawa J, Ohura T, Arai H, Nabatame S, Kikuchi K, Kuroki Y, Miura M, Tanaka T, Ohtake A, Omori I, Ihara K, Mabe H, Watanabe K, Niijima S, Okano E, Numabe H, Matsubara Y	HRAS mutants identified in Costello syndrome patients can induce cellular senescence: possible implications for the pathogenesis of Costello syndrome.	J Hum Genet	56(10)	707-15	2011

Seiji Mizuno, Daisuke Fukushi, Reiko Kimura, Kenichiro Yamada, Yasukazu Yamada, Toshiyuki Kumagai, Nobuaki Wakamatsu	Clinical and genomic characterization of siblings with a distal duplication of chromosome 9q (9q34.1-qter)	Am J Med Genet A	155 (9)	2274-80	2011
Liang JS, Shimojima K, Takayama R, Natsume J, Shichiji M, Hirasawa K, Imai K, Okanishi T, Mizuno S, Okumura A, Sugawara M, Ito T, Ikeda H, Takahashi Y, Oguni H, Imai K, Osawa M, Yamamoto T	CDKL5 alterations lead to early epileptic encephalopathy in both genders.	Epilepsia	52(10)	1835-42	2011
Miyajima Y, Kitase Y, Mizuno S, Sakai H, Matsumoto N, Ogawa A	Acute lymphoblastic leukemia in a pediatric patient with Marfan's syndrome	Rinsho Ketsueki	52(1)	28-31	2011
Waga C, Okamoto N, Ondo Y, Fukumura-Kato R, Goto YI, Kohsaka S, Uchino S	Novel variants of the SHANK3 gene in Japanese autistic patients with severe delayed speech development.	Psychiatr Genet	21	208-11	2011
Sasaki K, Okamoto N, Kosaki K, Yorifuji T, Shimokawa O, Mishima H, Yoshiura KI, Harada N	Maternal uniparental isodisomy and heterodisomy on chromosome 6 encompassing a CUL7 gene mutation causing 3M syndrome.	Clin Genet	80(5)	478-83	2011
Hiraki Y, Nishimura A, Hayashidani M, Terada Y, Nishimura G, Okamoto N, Nishina S, Tsurusaki Y, Doi H, Saitsu H, Miyake N, Matsumot	A de novo deletion of 20q11.2-q12 in a boy presenting with abnormal hands and feet, retinal dysplasia, and intractable feeding difficulty.	Am J Med Genet A	155	409-14	2011
Okamoto N, Hatsukawa Y, Shimojima K, Yamamoto T	Submicroscopic deletion in 7q31 encompassing CADPS2 and TSPAN12 in a child with autism spectrum disorder and PHPV.	Am J Med Genet A	155	1568-73	2011

Shimizu K, Okamoto N, Miyake N, Taira K, Sato Y, Matsuda K, Akimaru N, Ohashi H, Wakui K, Fukushima Y, Matsumoto N, Kosho T	Delineation of dermatan 4-O-sulfotransferase 1 deficient Ehlers-Danlos syndrome: Observation of two additional patients and comprehensive review of 20 reported patients.	Am J Med Genet A	155A	1949-58	2011
Hayashi S, Okamoto N, Chinen Y, Takanashi JI, Makita Y, Hata A, Imoto I, Inazawa J	Novel intragenic duplications and mutations of CASK in patients with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH).	Hum Genet	131(1)	99-110	2012
Yukiko Kawazu, Noboru Inamura, Futoshi Kayatani, Nobuhiko Okamoto, Hiroko Morisaki	Prenatal complex congenital heart disease with Loeys–Dietz syndrome.	Cardiology in the Young			In press
Tsurusaki Y, Okamoto N, Suzuki Y, Doi H, Saitsu H, Miyake N, Matsumoto N	Exome sequencing of two patients in a family with atypical X-linked leukodystrophy.	Clin Genet	80	161-6	2011
Misako Naiki,,Seiji Mizuno,Kenichiro Yamada, Yasukazu Yamada, Reiko Kimura, Makoto Oshiro, Nobuhiko Okamoto, Yoshio Makita, Mariko Seishima, and Nobuaki Wakamats	MBTPS2 mutation causes BRESEK/BRESHECK syndrome	Am J Med Genet			In press
Okamoto N, Tamura D, Nishimura G, Shimojima K, Yamamoto T	Submicroscopic deletion of 12q13 including HOXC gene cluster with skeletal anomalies and global developmental delay.	Am J Med Genet A	155	2997-3001	2011
Soneda A, Teruya H, Furuya N, Yoshihashi H, Enomoto K, Ishikawa A, Matsui K, Kurosawa K	Proportion of malformations and genetic disorders among cases encountered at a high-care unit in a children's hospital.	Eur J Pediatr	171	301-5	2012

Kurosawa K, Masuno M, Kuroki Y	Trens of occurrence of twin births in Japan.	Am J Med Genet Part A	158A	75-77	2012
Yagihashi T, Kosaki K ,Okamoto N ,Mizuno S, Kurosawa K,Takahashi T, Sato Y, Kosaki R	Age-dependent change in behavioral phenotype in Rubinstein-Taybisyndrome.	Congenit Anomalies			In press

〔V〕

資　　料

RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

EEC Syndrome-Like Phenotype in a Patient With an *IRF6* Mutation

Rika Kosaki,[1]* Tsuyoshi Kaneko,[2] Chiharu Torii,[3] and Kenjiro Kosaki[3]

[1]Division of Medical Genetics, National Center for Child Health and Development, Tokyo, Japan

[2]Division of Plastic and Reconstructive Surgery, National Center for Child Health and Development, Tokyo, Japan

[3]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

Received 9 September 2011; Accepted 27 December 2011

TO THE EDITOR:

Mutations in the *IRF6* gene lead to van der Woude syndrome (VWS) and popliteal pterygium syndrome (PPS) [Kondo et al., 2002]. VWS is characterized by lower lip pits and cleft lip and palate, whereas PPS is characterized by multiple pterygiums (wing-like triangular membranes located behind major joints). Mutations in the *TP63* gene lead to ectrodactyly-ectodermal dysplasia-clefting syndrome (EEC) [Celli et al., 1999], which is characterized by a middle-ray deficiency of the hands or feet and cleft lip and palate. Hence, the phenotypic spectra of *IRF6* and *TP63* mutations share a unique malformation: cleft lip and palate. Here, we report on a patient with EEC-like features and an *IRF6* mutation. This clinical observation lends further support to the concept that a protein–protein interaction occurs between IRF6 and TP63, as recently demonstrated in vitro [Moretti et al., 2010], and that the genetic interaction occurring between these two genes in mice [Thomason et al., 2010] is indeed relevant to humans.

The proposita was a Japanese female born after 39 weeks of gestation to a 26-year-old gravida 1 para 1 woman who had syndactyly of the left hand and cleft lip and palate. The 26-year-old father had a normal phenotype. Consanguinity was specifically denied. The patient was delivered vaginally and had a birth weight of 3,120 g (+0.3 SD). Her length was 50.5 cm (+1SD), and her head circumference was 33.5 cm (+0.2 SD). She had middle ray defects of the feet with syndactyly (Fig. 1), nail dysplasia of the index finger as an indication of middle ray defect of the right hand, bilateral cleft lip and cleft palate with lower-lip pits (Fig. 1), and accessory nipples. Skin over the hallux was not present. She was clinically diagnosed as having EEC syndrome at the age of 3 months. Syndromes that include lip pits, particularly PPS, were considered but were thought to be unlikely given the limb findings. However, PPS was not completely ruled out even with the absence of popliteal pterygia, because popliteal pterygia were present in only 70% of patients with PPS. At the age of 2 years and 8 months, the patient's height was 84.7 cm (−1.3 SD) and her weight was 12.5 kg (0 SD). Development was normal. She sat without support at 6 months, walked alone at 1 year and 3 months, and spoke two-word sentences at 2 years.

PCR-sequencing of the exons of the *IRF6* gene and the *TP63* gene revealed that the proposita and her mother had a heterozygous *IRF6* mutation, 250 C>T (R84C), within a highly conserved helix–loop–helix DNA binding domain. The *TP63* mutation analysis was non-contributory. Since the R84C mutation in *IRF6* has been shown to be the most common recurrent mutation among patients with PPS [Desmyter et al., 2010], we concluded that the R84C mutation represented a pathogenic change and that the *IRF6* mutation led to an EEC-like phenotype in the proposita. The mother of the proposita, who had cleft lip and palate and syndactyly of one hand, also had the R84C mutation in the *IRF6* gene.

Here, we documented a patient with an *IRF6* mutation who had ectrodactyly, cleft lip and palate, and paramedian lower-lip pits. The combination of ectrodactyly and cleft lip and palate was highly suggestive of a diagnosis of EEC syndrome, with the paramedian lower-lip pits being an atypical and additional feature. Alternatively, the combination of cleft lip and palate and paramedian lower-lip pits was compatible with a diagnosis of VWS, with ectrodactyly being an atypical and additional feature. Furthermore, ectrodactyly has been described in some patients who were clinically diagnosed as having PPS [Aron et al., 1988].

The diagnostic dilemma was eventually resolved using molecular testing, which demonstrated that the phenotypic effects of the *IRF6* mutation can mimic EEC syndrome. This clinical observation may be associated with recently demonstrated in vitro data indicating

How to Cite this Article:
Kosaki R, Kaneko T, Torii C, Kosaki K. 2012. EEC syndrome-like phenotype in a patient with an *IRF6* mutation.
Am J Med Genet Part A.

Grant sponsor: The Ministry of Health, Labour, and Welfare of Japan.
*Correspondence to:
Rika Kosaki, M.D., Division of Medical Genetics, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan. E-mail: kosaki-r@ncchd.go.jp
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.35273

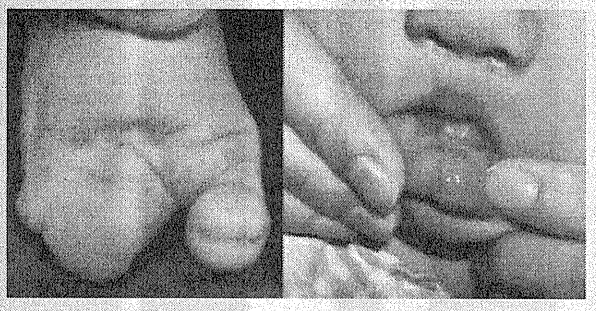

FIG. 1. Clinical features of the proposita at the age of 2 years and 8 months: Middle ray defects of the feet (left) with syndactyly and repaired cleft lip with paramedian lower lip pits (right).

that *TP63*, the causative gene for EEC, and *IRF6* operate within the same regulatory loop [Moretti et al., 2010] and also with in vivo data showing a genetic interaction between a null mutation of *Tp63* and an *Irf6* knock-in mutation of R84C [Thomason et al., 2010], with the specific R84C mutation being that identified in the family documented herein.

As mentioned above, *IRF6* mutations are known to be associated with both the VWS and the PPS phenotype [Kondo et al., 2002]. The specific R84C mutation of *IRF6* was originally thought to be strongly associated with the PPS phenotype, but occasional patients with R84C and the VWS phenotype have been previously described [Little et al., 2009]. Here, we further added an EEC-like phenotype to the list of possible phenotypic consequences of the R84C mutation, expanding the pleiotropic spectrum of the R84C mutation.

Interestingly, the mother of the proposita also harbored the R84C mutation and had cleft lip and palate and syndactyly of one hand but did not exhibit paramedian lower-lip pits (the cardinal feature of VWS) or ectrodactyly (the cardinal feature of EEC). This case shows the variable expressivity associated with the R84C mutation. How such variations in expressivity arise remain unclear. However, the recent documentation of genetic interactions between *TP63* and *IRF6* using mice heterozygous for both *Tp63* and the *Irf6* knock-in mutation R84C provides a clue [Little et al., 2009; Thomason et al., 2010]. As *TP63* transactivates *IRF6* by binding to an upstream enhancer element [Thomason et al., 2010], sequence variations outside the coding sequence of *TP63*, including enhancer elements, may be responsible for the variable expressivity. Further studies are awaited.

In summary, we documented a patient with EEC syndrome-like features including ectrodactyly and cleft lip who had a mutation in *IRF6* but not in *TP63*. Close inspection of the lower lip is recommended when evaluating patients with an EEC syndrome phenotype so that the molecular basis of the condition may be determined correctly.

REFERENCES

Aron N, Tajoori S, Gang R. 1988. The popliteal web syndrome. Eur J Plastic Surg 11:93–94.

Celli J, Duijf P, Hamel BC, Bamshad M, Kramer B, Smits AP, Newbury-Ecob R, Hennekam RC, Van Buggenhout G, van Haeringen A, Woods CG, van Essen AJ, de Waal R, Vriend G, Haber DA, Yang A, McKeon F, Brunner HG, van Bokhoven H. 1999. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 99:143–153.

Desmyter L, Ghassibe M, Revencu N, Boute O, Lees M, Francois G, Verellen-Dumoulin C, Sznajer Y, Moncla A, Benateau H, Claes K, Devriendt K, Mathieu M, Van Maldergem L, Addor MC, Drouin-Garraud V, Mortier G, Bouma M, Dieux-Coeslier A, Genevieve D, Goldenberg A, Gozu A, Makrythanasis P, McEntagart U, Sanchez A, Vilain C, Vermeer S, Connell F, Verheij J, Manouvrier S, Pierquin G, Odent S, Holder-Espinasse M, Vincent-Delorme C, Gillerot Y, Vanwijck R, Bayet B, Vikkula M. 2010. IRF6 screening of syndromic and a priori non-syndromic cleft lip and palate patients: Identification of a new type of minor VWS sign. Mol Syndromol 1:67–74.Epub.

Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. 2002. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet 32:285–289.

Little HJ, Rorick NK, Su LI, Baldock C, Malhotra S, Jowitt T, Gakhar L, Subramanian R, Schutte BC, Dixon MJ, Shore P. 2009. Missense mutations that cause Van der Woude syndrome and popliteal pterygium syndrome affect the DNA-binding and transcriptional activation functions of IRF6. Hum Mol Genet 18:535–545.

Moretti F, Marinari B, Lo Iacono N, Botti E, Giunta A, Spallone G, Garaffo G, Vernersson-Lindahl E, Merlo G, Mills AA, Ballaro C, Alema S, Chimenti S, Guerrini L, Costanzo A. 2010. A regulatory feedback loop involving p63 and IRF6 links the pathogenesis of 2 genetically different human ectodermal dysplasias. J Clin Invest 120:1570–1577.

Thomason HA, Zhou H, Kouwenhoven EN, Dotto GP, Restivo G, Nguyen BC, Little H, Dixon MJ, van Bokhoven H, Dixon J. 2010. Cooperation between the transcription factors p63 and IRF6 is essential to prevent cleft palate in mice. J Clin Invest 120:1561–1569.