mutant function more precisely, and additional patient recruitment would be very helpful.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to the patient who consented to his image being used.

Smail Hadj-Rabia[1,2,8], Jacob Mashiah[1,2,8], Patrice Roll[3,4], Amandine Boyer[5], Patrice Bourgeois[5], Philippe Khau Van Kien[6,7], Nicolas Lévy[3,5], Annachiara De Sandre-Giovannoli[3,5], Christine Bodemer[1,2] and Claire Navarro[3]

[1]Department of Dermatology, Reference Center for Genodermatoses and Rare Skin Diseases (MAGEC), INSERM U781, Université Paris Descartes—Sorbonne Paris Cité, Paris, France; [2]Institut Imagine, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; [3]Aix Marseille Université, GMGF, INSERM, UMR_S 910, Marseille, France; [4]Laboratoire de Biologie Cellulaire, Assistance Publique-Hôpitaux de Marseille, Hôpital d'Enfants La Timone, Marseille, France; [5]Laboratoire de Génétique Moléculaire, Assistance Publique-Hôpitaux de Marseille, Hôpital d'Enfants La Timone, Marseille, France; [6]Laboratoire de Génétique Moléculaire, Centre de Compétences Maladies Vasculaires Rares, CHU de Montpellier, Montpellier, France and [7]Laboratoire de Cytologie Clinique et Cytogénétique, UF Génétique Médicale, CHU de Nîmes, Nîmes, France
E-mail: claire.navarro@univ-amu.fr or smail.hadj@inserm.fr
[8]These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Ahmad Z, Zackai E, Medne L *et al.* (2010) Early onset mandibuloacral dysplasia due to compound heterozygous mutations in ZMPSTE24. *Am J Med Genet A* 152A:2703–10

Araújo-Vilar D, Lado-Abeal J, Palos-Paz F *et al.* (2008) A novel phenotypic expression associated with a new mutation in LMNA gene, characterized by partial lipodystrophy, insulin resistance, aortic stenosis and hypertrophic cardiomyopathy. *Clin Endocrinol (Oxf)* 69:61–8

Bonne G, Di Barletta MR, Varnous S *et al.* (1999) Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 21:285–8

De Sandre-Giovannoli A, Bernard R, Cau P *et al.* (2003) Lamin a truncation in Hutchinson-Gilford progeria. *Science* 300:2055

Doubaj Y, De Sandre-Giovannoli A, Vera EV *et al.* (2012) An inherited LMNA gene mutation in atypical progeria syndrome. *Am J Med Genet A* 158A:2881s–7s

Eriksson M, Brown WT, Gordon LB *et al.* (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423:293–8

Gottron H (1940) Familiäre Akrogerie. *Arch Dermatol Syph* 181:571

Hashimoto C, Abe M, Onozawa N *et al.* (2004) Acrogeria (Gottron type): a vascular disorder? *Br J Dermatol* 151:497–501

Jansen T, de Paepe A, Luytinck N *et al.* (2000) COL3A1 mutation leading to acrogeria (Gottron Type). *Br J Dermatol* 142:178–80

Kane MS, Lindsay ME, Judge DP *et al.* (2013) LMNA-associated cardiocutaneous progeria: an inherited autosomal dominant premature aging syndrome with late onset. *Am J Med Genet A* 161A:1599–611

Novelli G, Muchir A, Sangiuolo F *et al.* (2002) Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet* 71:426–31

Pope FM, Narcisi P, Nicholls AC *et al.* (1996) COL3A1 mutations cause variable clinical phenotypes including acrogeria and vascular rupture. *Br J Dermatol* 135:163–81

Prokocimer M, Davidovich M, Nissim-Rafinia M *et al.* (2009) Nuclear lamins: key regulators of nuclear structure and activities. *J Cell Mol Med* 13:1059–85

Schreiber KH, Kennedy BK (2013) When lamins go bad: nuclear structure and disease. *Cell* 152:1365–75

A Mutation in *TP63* Causing a Mild Ectodermal Dysplasia Phenotype

Journal of Investigative Dermatology (2014) **134,** 2277–2280; doi:10.1038/jid.2014.159; published online 24 April 2014

TO THE EDITOR

The transcription factor p63 has been shown to have a pivotal role during ectodermal, orofacial, and limb development (Mills *et al.*, 1999; Yang *et al.*, 1999; Rinne *et al.*, 2007). So far, mutations in *TP63* encoding p63 have been linked to three major phenotypes: ectodermal defects, split hand/foot malformation, or orofacial clefting (Celli *et al.*, 1999; van Bokhoven *et al.*, 1999; Ianakiev *et al.*, 2000; McGrath *et al.*, 2001; Rinne *et al.*, 2007). Ectodermal dysplasia syndromes associated with *TP63* mutation are always inherited in an autosomal dominant manner (Koster, 2010) and include the ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome (EEC), the ankyloble-pharon–ectodermal defects–cleft lip/palate syndrome (AEC), the limb–mammary syndrome, the acro–dermato–ungual–lacrimal–tooth syndrome, and the Rapp–Hodgkin syndrome (RHS; Rinne *et al.*, 2007). Through large-scale mutation screening, several phenotype–genotype correlations have emerged: although mutations altering the p63 DNA–binding domain have been shown to cause EEC, mutations affecting the sterile alpha-motif domain of the protein cause mostly AEC (Mills *et al.*, 1999; Yang *et al.*, 1999; McGrath *et al.*, 2001; Lo Iacono *et al.*, 2008; Koster, 2010). In the present study, we identified a mutation affecting a conserved residue of the p63 transcription inhibitory (TI) domain causing a phenotype previously unreported to the best of our knowledge.

Abbreviations: AEC, ankyloblepharon–ectodermal defects–cleft lip/palate syndrome; EEC, ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome; RHS, Rapp–Hodgkin syndrome; TI, transcription inhibitory

Accepted article preview online 27 March 2014; published online 24 April 2014

We assessed a family of Jewish Ashkenazi origin. Patients presented with a variable phenotype (Figure 1). Most affected individuals demonstrated hypodontia, diastema, dystrophic nails, and thin and fragile skin on their feet. In addition, some of the patients had coarse and dry hair, and lower-lip pits. Hypohydrosis was absent and psychomotor development was normal.

After having obtained informed written consent according to a protocol approved by our institutional review board and by the Israel National Committee for Human Genetic Studies in adherence with the declaration of Helsinki Principles, genomic DNA was extracted from peripheral blood lymphocytes obtained from affected and healthy family members. Given the clinical features displayed by the patients, we searched for a causative mutation in a panel of candidate genes previously found to be associated with similar clinical manifestations (Visinoni *et al.*, 2009). No mutations were identified in the coding sequences of *WNT10A*, *PVRL1*, *FZD6*, or *IRF6* (for list of primers, see Supplementary Table S1 online). In contrast, mutation analysis identified a heterozygous missense mutation in *TP63*, c.G1904T, predicted to result in a single amino acid substitution, p.G635V (p.G596V, according to previous nomenclature (Yang *et al.*, 1998); Figure 2a). Using direct sequencing, we confirmed cosegregation of the mutation with the disease phenotype throughout the entire family. The mutation was not found in any major public databases including in a total of more than 8000 individual sequences deposited in the 1000 Genomes Project (http://www.1000genomes.org/) and in the NHLBI Grand Opportunity Exome Sequencing Project (http://evs.gs.washington.edu/EVS/). Mutation p.G635V affects a moderately well-conserved amino acid residue (ConSurf score = 5; range = 1–9; http://consurf.tau.ac.il; Ashkenazy *et al.*, 2010). The mutant amino acid residue resides within the TI domain. This domain is specific for the p63 alpha isoforms, which are required for ectodermal and limb development and for the maintenance of mature skin (Wolff *et al.*, 2009). Therefore, we set out to examine the effect of the mutation on the transactivating activity of p63.

We cloned wild-type p63 delta N alpha (ΔNp63α) isoform into the pcDNA-HA expression vector. We then introduced c.G1904T into the p63 construct using the Quick Change Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The wild-type p63 construct, the mutant p63 construct, or an equal mixture of both constructs were co-transfected into primary keratinocytes using Lipofectamine 2000 (Life Technologies, Grand Island, NY)

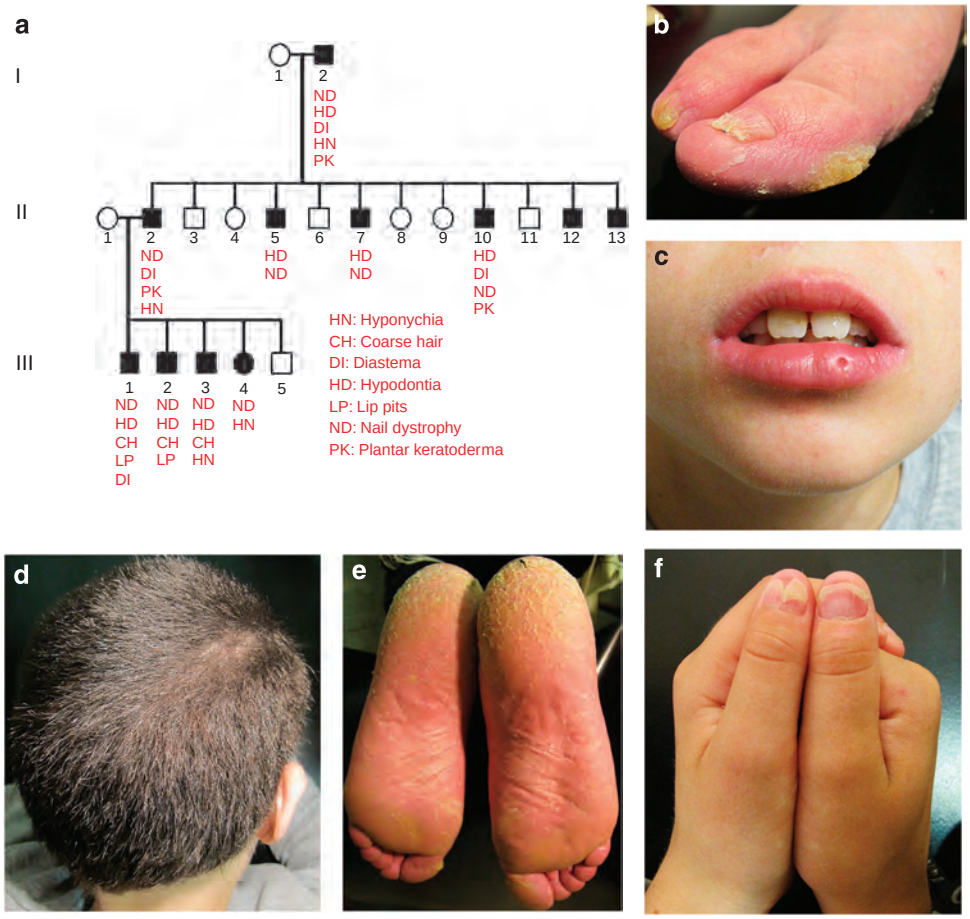

Figure 1. Clinical features. (**a**) Pedigree of the family. (**b**) Hyponychia and keratoderma in individual II-2. (**c**) Diastema and lower-lip pit in individual III-1. (**d**) Coarse hair in individual III-1. (**e**) Palmoplantar keratoderma in individual II-2. (**f**) Nail dystrophy in individual III-3.

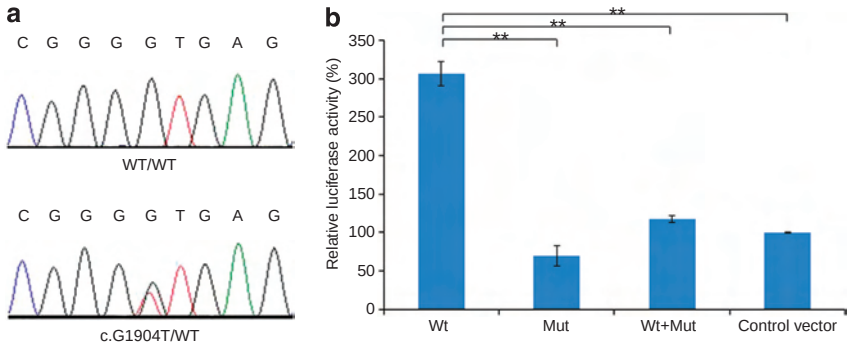

Figure 2. Molecular analysis. (**a**) Direct sequencing of *TP63* revealed a heterozygous transversion at cDNA position 1,904 (c.G1904T) (lower panel). The wild-type (WT) sequence is given for comparison (upper panel). (**b**) Primary human keratinocytes were transfected with a luciferase reporter gene under the regulation of the p63-responsive C40 enhancer element alone (control vector) or together with either a wild-type *TP63* expression vector (Wt), a c.G1904T-containing *TP63* expression vector (Mut), or both constructs (Wt + Mut). Forty-eight hours after transfection, luciferase activity was measured. Measurement data are expressed as the percentage of relative luciferase activity corrected for transfection efficiency as determined by *Renilla* activity, compared with maximal wild-type p63 activity. Data shown represent mean values ± s.d. of two independent experiments (**$P<0.01$).

together with a plasmid carrying the C40 enhancer sequence (which contains a highly conserved binding site for the p63 protein (Antonini *et al.*, 2006)) located upstream to the luciferase gene and with pRL-TK renilla luciferase. Forty-eight hours after transfection, luciferase activity was found to be significantly suppressed in the presence of the mutant p63 compared with wild-type p63 (Figure 2b), suggesting that p.G635V alters the transcriptional regulation of p63 targets.

Of interest, p.G635V, which was found in the present study to result in a loss-of-function effect (Figure 2b), causes a constellation of signs overlapping with, but not identical to, RHS (Supplementary Table S2 online) while affecting a region of the gene in which nonsense/truncating mutations have been shown to cause split hand/foot malformation (Rinne *et al.*, 2007) and missense mutations have been associated with the AEC phenotype (Rinne *et al.*, 2009). Phenotypic heterogeneity is typical of p63-associated syndromes and is thought to result from the different biological effects of alterations of the molecule's various domains, as well as from both inherited and environmental modifying traits (Vanbokhoven *et al.*, 2011). In this regard, as *IRF6* can act as a modifier gene (Gritli-Linde, 2010), increasing the risk for cleft lip in individuals carrying *TP63* mutations, and given the fact that some of our patients displayed lower-lip pits (never previously reported to our knowledge in p63 syndromes), we excluded by direct sequencing any pathogenic mutation in the coding region of this gene in our patients, as well as in its enhancer (not shown). The fact that p63 regulates IRF6 (Moretti *et al.*, 2010) and that abnormal IRF6 activity leads to lip pit formation (Kondo *et al.*, 2002) may explain how *TP63* mutations affect lip pit formation.

In summary, the present observations expand the spectrum of clinical manifestations associated with *TP63* mutations and re-emphasize the need for a better understanding of the molecular basis of phenotypic heterogeneity in p63 syndromes.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank the patients and their family members for their participation in the study. We are grateful to Caterina Missero for the C40 enhancer plasmid. This work has been supported, in part, by the European ERA-Net Research Program on Rare Diseases (E-Rare-2; Skindev) to ES and DA.

Tomer Goldsmith[1], Ori Eytan[1,2], Lee Magal[1], Michal Solomon[3], Shirli Israeli[1], Emily Warshauer[1], Meital Grafi-Cohen[1], Daniel Aberdam[4,5], Hans van Bokhoven[6], Huiqing Zhou[6,7], Ofer Sarig[1], Eli Sprecher[1,2] and Janna Nousbeck[1]

[1]Department of Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [2]Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]Department of Dermatology, Chaim Sheba Medical Center, Tel Hashomer, Israel; [4]INSERM U976, Paris, France; [5]University of Paris Diderot, Paris, France; [6]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands and [7]Department of Molecular Developmental Biology, Radboud University, Radboud, The Netherlands
E-mail: elisp@tlvmc.gov.il

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Antonini D, Rossi B, Han R *et al.* (2006) An autoregulatory loop directs the tissue-specific expression of p63 through a long-range evolutionarily conserved enhancer. *Mol Cell Biol* 26:3308–18

Ashkenazy H, Erez E, Martz E *et al.* (2010) ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res* 38:W529–33

Celli J, Duijf P, Hamel BC *et al.* (1999) Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. *Cell* 99:143–53

Gritli-Linde A (2010) p63 and IRF6: brothers in arms against cleft palate. *J Clin Invest* 120:1386–9

Ianakiev P, Kilpatrick MW, Toudjarska I *et al.* (2000) Split-hand/split-foot malformation is caused by mutations in the p63 gene on 3q27. *Am J Hum Genet* 67:59–66

Kondo S, Schutte BC, Richardson RJ *et al.* (2002) Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nat Genet* 32:285–9

Koster MI (2010) p63 in skin development and ectodermal dysplasias. *J Invest Dermatol* 130:2352–8

Lo Iacono N, Mantero S, Chiarelli A *et al.* (2008) Regulation of Dlx5 and Dlx6 gene expression by p63 is involved in EEC and SHFM congenital limb defects. *Development* 135:1377–88

McGrath JA, Duijf PH, Doetsch V *et al.* (2001) Hay-Wells syndrome is caused by hetero-

zygous missense mutations in the SAM domain of p63. *Hum Mol Genet* 10:221–9

Mills AA, Zheng B, Wang XJ *et al.* (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* 398: 708–13

Moretti F, Marinari B, Lo Iacono N *et al.* (2010) A regulatory feedback loop involving p63 and IRF6 links the pathogenesis of 2 genetically different human ectodermal dysplasias. *J Clin Invest* 120:1570–7

Rinne T, Bolat E, Meijer R *et al.* (2009) Spectrum of p63 mutations in a selected patient cohort affected with ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC). *Am J Med Genet A* 149A:1948–51

Rinne T, Brunner HG, van Bokhoven H (2007) p63-associated disorders. *Cell Cycle* 6: 262–8

van Bokhoven H, Jung M, Smits AP *et al.* (1999) Limb mammary syndrome: a new genetic disorder with mammary hypoplasia, ectrodactyly, and other Hand/Foot anomalies maps to human chromosome 3q27. *Am J Hum Genet* 64:538–46

Vanbokhoven H, Melino G, Candi E *et al.* (2011) p63, a story of mice and men. *J Invest Dermatol* 131:1196–207

Visinoni AF, Lisboa-Costa T, Pagnan NA *et al.* (2009) Ectodermal dysplasias: clinical and molecular review. *Am J Med Genet A* 149A: 1980–2002

Wolff S, Talos F, Palacios G *et al.* (2009) The alpha/beta carboxy-terminal domains of p63 are required for skin and limb development. New insights from the Brdm2 mouse which is not a complete p63 knockout but expresses p63 gamma-like proteins. *Cell Death Differ* 16:1108–17

Yang A, Kaghad M, Wang Y *et al.* (1998) p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol Cell* 2:305–16

Yang A, Schweitzer R, Sun D *et al.* (1999) p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* 398:714–8

JIDOpen

Proinflammatory Cytokines and Chemokines at the Skin Interface during Powassan Virus Transmission

Journal of Investigative Dermatology (2014) **134,** 2280–2283; doi:10.1038/jid.2014.150; published online 17 April 2014

TO THE EDITOR

Powassan virus (POWV) is an emerging North American tick-borne flavivirus transmitted to humans by infected tick bites. Ticks transmit pathogens during the complex feeding process of penetrating the skin and stay attached for several days to acquire blood. This process is facilitated by a repertoire of pharmacologically active proteins/factors in tick saliva (Ribeiro *et al.*, 2006; Kazimírová and Štibrániová, 2013). Thus, skin acts as the interface of the host–pathogen–vector interactions (Wikel, 2013). Skin provides the first line of defense against mechanical and environmental damage and infectious agents (Nestle *et al.*, 2009). In a previous study, which examined cutaneous bite-site lesions from uninfected *Ixodes scapularis* nymphs, a rapid, proinflammatory progression of the early host response was identified, culminating in the infiltration of innate immune cells by 12 hours after tick infestation (Heinze *et al.*, 2012). Successful transmission of tick-borne POWV has been shown to occur within 15 minutes of *I. scapularis* attachment (Ebel and Kramer, 2004). In addition, it was demonstrated that during early feeding time points the viral load in the tick salivary glands increases (Alekseev and Chunikhin, 1990). Therefore, the early cutaneous interactions between host immunity and initial tick-mediated immunomodulation are central to successful disease-causing agent transmission. In this study, we sought to characterize tick-induced changes in cutaneous gene expression at the early stages of attachment and feeding by POWV-infected *I. scapularis* nymphs. This will allow us to demonstrate the effect of a tick-borne virus on immune response at the tick–host interface.

In this study, we generated POWV-infected *I. scapularis* nymphs by synchronous infection (McNally *et al.*, 2012) and allowed them to feed on 6-week-old female Balb/C mice. Uninfected ticks were used as control. Each treatment group consisted of four mice, each with a capsule containing one tick. At least three out of four mice had successful tick attachment at each time point, providing us with sufficient sample sizes to perform statistical analyses. Three and six hours after tick attachment (hours post infection, h.p.i.), 4 mm mouse skin biopsies were harvested along with the feeding ticks. Ticks and skin were checked for POWV infection, and all the infected ticks/skin biopsies used in this experiment contained POWV RNA. Total RNA was extracted from each skin biopsy and cutaneous immune responses were analyzed by pathway-specific PCR arrays (Supplementary Table S1 online). In total, 456 genes were analyzed with these arrays. Relative fold differences of the immune genes were calculated as previously described (Heinze *et al.*, 2012). These data were then uploaded to ingenuity pathway analysis software for further analysis. Comparative analysis between POWV-infected and uninfected tick attachment sites at 3 and 6 h.p.i. was performed (Supplementary Table S2 online). When all significantly modulated ($P \leqslant 0.05$) host genes in the uninfected versus POWV-infected 3 h.p.i. tick-feeding sites were taken into account, there were 40 upregulated genes and 11 downregulated genes (Figure 1a). Of all significantly modulated host genes in the 6 h.p.i. uninfected versus POWV-

Abbreviations: POWV, Powassan virus; TNF, tumor necrosis factor

Accepted article preview online 21 March 2014; published online 17 April 2014