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REFERENCES

Chen ChJ, Sierra H, Cordova M et al. (2014) Confocal microscopy guided laser ablation for superficial and early nodular basal cell carcinoma. JAMA Dermatol 150:994–8

Chren MM, Torres JS, Stuart SE et al. (2011) Recurrence after treatment of nonmelanoma skin cancer: a prospective cohort study. Arch Dermatol 147:540–6

Chren MM, Linos E, Torres JS et al. (2013) Tumor recurrence 5 years after treatment of cutaneous basal cell carcinoma and squamous cell carcinoma. J Invest Dermatol 133:1188–96

Guitera P, Menzies SW, Longo C et al. (2012) In vivo confocal microscopy for diagnosis of melanoma and basal cell carcinoma using a two-step method: analysis of 710 consecutive clinically equivocal cases. J Invest Dermatol 132:2386–94

Hohenleutner U, Hohenleutner S, Bäumler W et al. (1997) Fast and effective skin ablation with an Er:YAG laser: determination of ablation rates and thermal damage zones. Lasers Surg Med 20:242–7

Nori S, Rius-Díaz F, Cuevas J et al. (2004) Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. J Am Acad Dermatol 51:923–30

Pan ZY, Lin JR, Cheng TT et al. (2012) In vivo reflectance confocal microscopy of Basal cell carcinoma: feasibility of preoperative mapping of cancer margins. Dermatol Surg 38:1945–50

Patel YG, Nehal KS, Aranda YI et al. (2007) Confocal reflectance mosaicing of basal cell carcinomas in Mohs surgical skin excisions. J Biomed Opt 12:034027

Scope A, Mahmood U, Gareau DS et al. (2010) In vivo reflectance confocal microscopy of shave biopsy wounds: feasibility of intra-operative mapping of cancer margins. Br J Dermatol 163:1218–28

Sierra H, Larson BA, Chen C et al. (2013) Confocal microscopy to guide erbium:yttrium aluminum garnet laser ablation of basal cell carcinoma: an ex vivo feasibility study. J Biomed Opt 18:095001

Smucler R, Vlk M (2008) Combination of Er:YAG laser and photodynamic therapy in the treatment of nodular basal cell carcinoma. Lasers Surg Med 40:153–8

Smucler R, Kriz M, Lippert J et al. (2012) Ultrasound guided ablative-laser assisted photodynamic therapy of basal cell carcinoma (US-aL-PDT). Photomed Laser Surg 30:200–5

Tannous Z, Torres A, González S (2003) In vivo real-time confocal reflectance microscopy: a noninvasive guide for Mohs micrographic surgery facilitated by aluminum chloride, an excellent contrast enhancer. Dermatol Surg 29:839–46

Walsh JT, Flotte TJ, Anderson R et al. (1989) Er:YAG laser ablation of tissue: effect of pulse duration and tissue type on thermal damage. Lasers Surg Med 9:314–26

Pathogenicity of *POFUT1* in Dowling-Degos Disease: Additional Mutations and Clinical Overlap with Reticulate Acropigmentation of Kitamura

TO THE EDITOR

Dowling-Degos disease (DDD (MIM 179850, MIM 615327)) is an autosomal dominant form of a reticulate pigmentary disorder. Affected individuals develop a progressive and disfiguring post-pubertal reticulate hyperpigmentation and small hyperkeratotic dark-brown papules, which mainly affect the flexures, great skin folds, trunk, face, and extremities. We previously identified loss-of-function mutations in keratin 5 (*KRT5*) (Betz et al., 2006) in fewer than half of our DDD patients, and just recently we described mutations in *POGLUT1*,

which explain about one-third of our DDD cases (Basmanav et al., 2014). *POGLUT1* encodes protein *O*-glucosyltransferase 1 and is part of the Notch signaling pathway. Li et al. (2013) recently reported mutations in *POFUT1* (MIM 607491), encoding *O*-fucosyltransferase 1, also involved in the Notch pathway, in two Chinese families with DDD. Here, we report on the clinical and molecular findings in eight patients/families with DDD of different ethnicities.

After excluding *KRT5* and *POGLUT1* mutations, we screened a total of 24

DDD patients for mutations in *POFUT1* by Sanger sequencing. Ethical approval was obtained from the ethics committee of the Medical Faculty of the University of Düsseldorf; the participants provided written informed consent prior to blood sampling. Patient consent was received for publishing identifying information and photographs. The study was conducted in concordance with the Declaration of Helsinki Principles.

In sporadic cases from Germany (n = 1), Poland (n = 1), and India (n = 1) and familial cases from Denmark (n = 1) and Germany (n = 1), we identified five different mutations, designated c.86G > A (p.Trp29*), c.718C > T (p.Arg240Cys), c.785T > C (p.Met262Thr), c.1067C > T (p.Ser356Phe), and c.1096C > T (p.Arg366Trp) (Figure 1a,
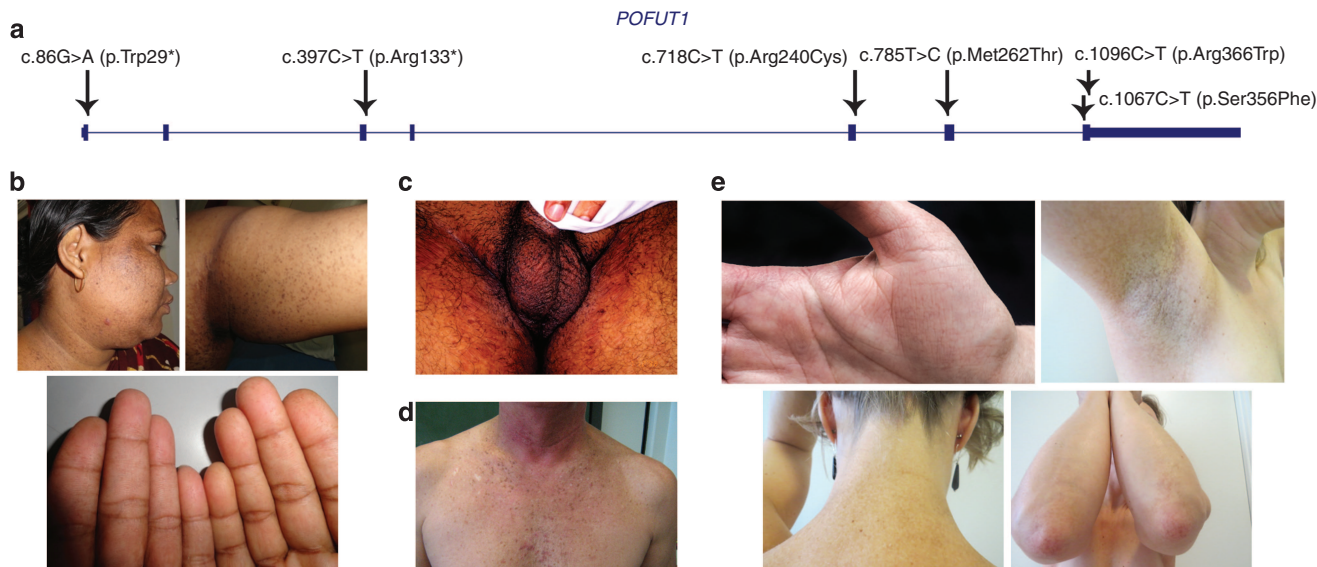
Figure 1. *POFUT1 mutations and clinical appearance of the patients.* (**a**) All mutations are marked by arrowheads on the exon structure of *POFUT1*. (**b**) Indian patient with hyperpigmented lesions on the face, axillae, and palmar aspect of fingers with pits. (**c**) Hyperpigmented lesions on the genitals of the Yemeni patient. (**d**) Hyperpigmented papules on the trunk of the Polish patient. (**e**) Multiple pits and interrupted dermatoglyphics in the palms; and hyperpigmentation on the axillae, neck, and elbows of the Danish patient.

Supplementary Table S1, S2 online). We also had performed exome sequencing in two affected individuals of a Yemeni family with seven members suspected to be affected by DDD (pipeline described in Basmanav *et al.*, 2014). Analysis of the data identified a stop mutation (c.397C>T; p.Arg133*) in *POFUT1* in both individuals (Figure 1a, Supplementary Table S1, S2 online). By Sanger sequencing of DNA from other family members, we confirmed that the mutation segregates with the disease phenotype. Subsequently, we identified the same mutation in two further, apparently unrelated, Yemeni families, also segregating in an autosomal dominant manner. Upon careful analysis of the phenotypes associated with each mutation (summarized in Supplementary Table S1 online), we noted a particular overlap in clinical symptoms. The mutation p.Trp29* was identified in the Indian sporadic patient who was clinically evaluated for hyperpigmented skin lesions, which were first noticed around 26 years of age. The skin lesions gradually spread from the face to the trunk and extremities with comedones in the inframammary folds and axillae (Figure 1b). Of interest, there were some hyperpigmented macules and pits on the palm and palmar aspect of fingers (pinpoint-like depressions)

(Figure 1b). Histopathological examination was consistent with DDD.

Another nonsense mutation p.Arg133* was found in patients of the three Yemeni families with all together 18 affected individuals, ranging from 18 to 50 years of age (Lestringant *et al.*, 1997). A female patient examined in more detail had brownish, slightly depressed macules and papules on her face, which appeared at about 12–15 years of age. Brown macules were also present on the dorsa of her hands and fingers, feet and toes, on the flexor aspect of her wrists, in her axillae, as well as in her palmar creases starting with skin colored pinpoint-like depressions. Other lesions included breaks in dermatoglyphics, facial and palmar pits, and numerous leukodermic macules on the outer aspects of both forearms. Her older brother presented with similar lesions but macules and papules were more profuse and darker, also involving the great folds, genitals, and waist (Figure 1c). Leukoderma was also present on his shins. Examination of 16 further Yemeni patients revealed identical findings. We hypothesize that there is an age-related development of cutaneous lesions. Initially, brown macules occur around puberty, followed by papules, in sun-exposed acral skin regions and/or in those

exposed to physical stimuli like rubbing and later developed proximally. Leukoderma manifests at about 22–25 years of age.

The mutation p.Arg240Cys was observed in a German female patient who presented with hyperkeratotic reddish-brown papules on the upper and lower legs, as well as the flexural sites of the lower arms, which appeared at the age of 51 years. The patient reported that her father had similar symptoms, which manifested at the age of 31 years. Another missense mutation, p.Met262Thr, was observed in a female Danish patient who had developed skin lesions since early puberty. In adulthood, she presented with reticulate dusky hyperpigmentation on her dorsal hands and feet, elbows, neck, and intertrigines, including, axillae, submammary region, and inguinal folds (Figure 1e). She also had melasma-like hyperpigmentation of her face and subtle pits around her mouth. Of particular note were multiple pits and interrupted dermatoglyphics in her palms (Figure 1e). A skin biopsy (neck) showed changes compatible with reticulate acropigmentation of Kitamura (RAK) as a continuum of DDD. The patient's affected mother showed similar mottled hyperpigmentation as well as perioral and palmar pits, but the skin lesions had fainted substantially over the

years and were hardly visible, particularly in winter time. Her 22-year-old daughter had multiple ephelides but no reticular or mottled dyspigmentation. The daughter and a younger brother of the patient carried the same mutation. As disease onset is late, they are expected to develop DDD too. One unaffected brother of the index patient did not carry the mutation.

In a male sporadic Polish patient carrying mutation p.Ser356Phe we observed reddish-brown papules and leukoderma on the trunk (Figure 1d), armpits, inguinal/genital region, as well as pits and interrupted dermatoglyphics in the palms. Age of onset was at 48 years, accompanied by itching and pain. In a sporadic male German patient we detected the mutation p.Arg366Trp. Clinically, he showed hyperpigmented papules on the trunk, which manifested at the age of 40 years.

The pathogenicity of the two nonsense mutations p.Trp29* and p.Arg133* identified here is beyond question as they lead to premature termination codons, which are either associated with nonsense-mediated mRNA degradation or formation of a largely non-functional truncated protein. All of the four missense mutations affect highly conserved amino-acid residues, and each one is predicted to be damaging by SIFT (http://sift.jcvi.org/) and Poly-Phen-2 (http://genetics.bwh.harvard.edu/pph2/) tools with maximal damage scores (Supplementary Table S2). We further assessed the effects of the missense mutations by homology protein modeling (Basmanav *et al.*, 2014). POFUT1 consists of 388 amino acids (Wang *et al.*, 2001). After cleavage of the signal peptide, the mature protein comprises amino-acid residues 27–388 and folds into two major domains. At the interface, residues from the N-terminal and the C-terminal domains form a deep substrate-binding pocket, in which the substrate, guanidine 5′-diphosphate-β-L-fucose (GDP-fucose), is mainly bound via the GDP moiety (Supplementary Figure S1a). The diphosphoester group of GDP forms hydrogen bonds to the side chains of residues Asn46, Arg240, Ser356, and Ser357. As a consequence, mutation Arg240Cys presumably results in a largely reduced

affinity toward GDP-fucose and a concomitant decrease in enzymatic activity of POFUT1, whereas mutation of Ser356Phe abolishes the formation of an important hydrogen bond of GDP-fucose, most likely resulting in destabilization of the protein (Supplementary Figure S1c). Even more drastic is the effect of the bulky side chain of Phe that blocks the binding cavity and hampers GDP-fucose binding (Supplementary Figure S1c online).

The fucose moiety is oriented by hydrogen bonds formed between two hydroxyl groups of the fucose with the side chain of Asn43 in POFUT1. Furthermore, there are van der Waals interactions of the fucose with side chains of hydrophobic residues like phenylalanine in *Caenorhabditis elegans* POFUT1 that is replaced by a methionine residue (Met262) in human POFUT1. Mutation Met262Thr presumably results in destabilization of fucose binding and reduces the catalytic activity of POFUT1 (Supplementary Figure S1b). The central β-sheet of the C-terminal domain of POFUT1 comprises five strands. The last strand, formed by residues 373–376, directly follows helix 18 of the C-terminal domain. A narrow turn is required to place the strand five correctly and complete the β-sheet. This turn is stabilized by the side chain of R366. The guanidinium group forms several hydrogen bonds to residues of strand β12 and β13, stabilizing the turn, as well as the β-sheet. Mutation of Arg366Trp most likely leads to destabilization of the C-terminal domain. Especially, strands β12 and β13 are affected and might destabilize the entire central β-sheet of the C-terminal domain (Supplementary Figure S1d online).

In summary, we identified six pathogenic *POFUT1* mutations in DDD patients of different ethnic origin. Two of these are protein truncating mutations, similar to those reported by Li *et al.* (2013), and four lead to amino-acid substitutions, thereby extending the mutation spectrum of *POFUT1*. Of interest, we observed the involvement of the acral regions with hyperpigmentation, palmar pits, and interrupted dermatoglyphics as distinct clinical features in patients with *POFUT1*

mutations, which differentiates them from DDD patients with mutations in *KRT5* and *POGLUT1* and illustrates a phenotypic overlap with RAK (Griffiths, 1976). The previous observation of cases with a certain clinical overlap between DDD and RAK already caused a controversy as to DDD and RAK either reflecting a single disease entity with variable phenotypic expression or two distinct diseases (Cox and Long, 1991; Lestringant *et al.*, 1997; Thami *et al.*, 1998; Shen *et al.*, 2011; Tang *et al.*, 2012; Kono *et al.*, 2013). Our current and previous findings (Basmanav *et al.*, 2014) suggest that a gene–phenotype correlation exists in the diverse reticulate hyperpigmentation disease spectrum, which should be further delineated on the basis of clinical examination and genetic analysis of more affected individuals.

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REFERENCES

Basmanav FB, Oprisoreanu AM, Pasternack SM et al. (2014) Mutations in POGLUT1, encoding protein O-glucosyltransferase 1, cause autosomal-dominant Dowling-Degos Disease. *Am J Hum Genet* 94:135–43

Betz RC, Planko L, Eigelshoven S et al. (2006) Loss-of-function mutations in the keratin 5 gene lead to Dowling-Degos disease. *Am J Hum Genet* 78:510–9

Cox NH, Long E (1991) Dowling-Degos disease and Kitamura's reticulate acropigmentation: support for the concept of a single disease. *Br J Dermatol* 125:169–71

Griffiths WA (1976) Reticulate acropigmentation of Kitamura. *Br J Dermatol* 95:437–43

Kono M, Sugiura K, Suganuma M et al. (2013) Whole-exome sequencing identifies ADAM10 mutations as a cause of reticulate acropigmentation of Kitamura, a clinical entity distinct from Dowling-Degos disease. *Hum Mol Genet* 22:3524–33

Lestringant GG, Masouye I, Frossard PM et al. (1997) Co-existence of leukoderma with features of Dowling-Degos disease: reticulate acropigmentation of Kitamura spectrum in five unrelated patients. *Dermatology* 195:337–43

Li M, Cheng R, Liang J et al. (2013) Mutations in POFUT1, encoding protein O-fucosyl-transferase 1, cause generalized Dowling-Degos disease. *Am J Hum Genet* 92:895–903

Shen Z, Chen L, Ye Q et al. (2011) Coexistent Dowling-Degos disease and reticulate acropigmentation of kitamura with progressive seborrheic keratosis. *Cutis* 87:73–5

Tang JC, Escandon J, Shiman M et al. (2012) Presentation of reticulate acropigmentation of Kitamura and Dowling-Degos disease overlap. *J Clin Aesthet Dermatol* 5:41–3

Thami GP, Jaswal R, Kanwar AJ et al. (1998) Overlap of reticulate acropigmentation of Kitamura, acropigmentation of Dohi and Dowling-Degos disease in four generations. *Dermatology* 196:350–1

Wang Y, Shao L, Shi S et al. (2001) Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase. *J Biol Chem* 276:40338–45

Acne Inversa Caused by Missense Mutations in *NCSTN* Is Not Fully Compatible with Impairments in Notch Signaling

TO THE EDITOR

Acne inversa (AI; also known as hidradenitis suppurativa; OMIM 142690) is a chronic recurrent follicular occlusion disorder. About 30–40% of patients with AI exhibit a highly penetrant, autosomal dominant mode of inheritance (Alikhan *et al.*, 2009). In many cases, AI patients harbor heterozygous mutations in genes encoding components of the γ-secretase complex, composed of presenilin (PS1 and 2), nicastrin (NCT), anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2) (Wang *et al.*, 2010; Jurisch-Yaksi *et al.*, 2013). PS is the catalytic center of γ-secretase that promotes intramembranous proteolysis of a number of membrane proteins, including the amyloid precursor protein (APP) and Notch 1–4, signaling receptors essential for cell lineage determination, cell proliferation and survival. Activation of Notch signaling occurs upon binding to the Delta/Serrate/Lag-2 family of ligands on the cell surface, leading to exposure of a sequence near the transmembrane domain that is a substrate of a metalloprotease, ADAM 10. This ''shedding'' event generates the membrane-tethered Notch extracellular truncation (NEXT) that is then subject to intramembranous processing by γ-secretase, to generate the Notch intracellular domain (NICD). NICD translocates to the nucleus to form a complex with a transcription factor, C-promoter binding factor-1 (CBF-1), that binds to CBF1-specific cognate DNA sequences to regulate gene expression (Fortini, 2009).

To date, 24 AI-specific mutations have been identified in genes encoding γ-secretase components, and 19 of these are in *NCSTN*, encoding nicastrin (Supplementary Table S1 online). Most mutations in *NCSTN* cause frameshift and premature translation termination as well as nonsense-mediated mRNA decay, leading to significantly reduced levels of NCT, findings which have led to the proposal that AI is caused by genetic haploinsufficiency because of reduced γ-secretase-mediated processing of Notch and signaling in the skin (Pink *et al.*, 2013). Interestingly, four missense mutations, V75I, D185N, P211R, and Q216P, have been identified in the large ectodomain of NCT (Li *et al.*, 2011; Pink *et al.*, 2012; Zhang *et al.*, 2013). These missense mutations could potentially disrupt the structure of this region and result in failed assembly of the γ-secretase complex, leading to impaired activity. To test this notion, we examined the activity of these NCT variants in mediating Notch processing and signaling.

We first coexpressed cDNAs encoding the NCT missense variants together with a constitutively activated membrane-bound Notch 1 derivative (mNΔE; Schroeter *et al.*, 1998) that is similar to NEXT, in *NCSTN*-deficient (*NCSTN*$^{-/-}$) fibroblasts. mNΔE is not subject to intramembranous processing in the absence of NCT (Figure 1a, lane 1), but coexpression of wild-type NCT rescues the generation of NICD