Mutations in the Prostaglandin Transporter SLCO2A1 Cause Primary Hypertrophic Osteoarthropathy with Digital Clubbing

Journal of Investigative Dermatology (2012) **132,** 2473–2476; doi:10.1038/jid.2012.146; published online 14 June 2012

TO THE EDITOR

Digital clubbing with enlargement of the distal phalanges and overcurvature of the nails frequently occurs as a secondary feature of chronic hypoxia in bronchopulmonary or cardiovascular disease and systemic inflammatory or neoplastic processes. Genetic causes are much less common, but their decipherment provides a better understanding of those more common diseases. Mutations in the *15-hydroxyprostaglandin dehydrogenase* (*HPGD*) gene encoding the major prostaglandin (PG) PGE_2-catabolizing enzyme were the first reported cause of primary hypertrophic osteoarthropathy (PHO), whose most prominent feature is digital clubbing (Uppal *et al.*, 2008; Tariq *et al.*, 2009; Diggle *et al.*, 2010; Bergmann *et al.*, 2011). Very recently, Zhang *et al.* (2012) identified *SLCO2A1* mutations in patients with autosomal recessive PHO. Intriguingly, individuals with PHO share many characteristics with patients who display HO secondary to systemic pathologies. In addition to digital clubbing, these are glandular hypertrophy and osteoblast proliferation, which cause thickening of the skin, bothersome palmoplantar hyperhidrosis, and joint disease (pachydermoperiostosis). Excessive sebum production (seborrhea) may lead to severe facial acne. Acroosteolysis of the distal phalanges of the fingers and toes due to osteoclast stimulation is also commonly observed (Castori *et al.*, 2005).

Termination and clearance of PG signaling is regulated by a two-step process (Nomura *et al.*, 2004). First, extracellular PGs are taken up by a carrier-mediated process across the plasma membrane. For many prostanoids (e.g., PGE_2), this transport is mediated by the PG carrier SLCO2A1 (Kanai *et al.*, 1995). In a second step, oxidation of prostanoids takes place within the cell via HPGD. In this study, we hypothesized that PHO can also be caused by mutations in the gene encoding the PG transporter SLCO2A1. SLCO2A1 is located on 3q22, comprises 14 exons, and encodes a 643-amino-acid type IV transmembrane protein that belongs to the solute carrier organic anion transporter family (Lu and Schuster, 1998). SLCO2A1 is broadly expressed in fetal and adult tissues, and it contains 12 putative transmembrane domains, three known N-glycosylation sites, and one KAZAL-like domain. We performed direct sequencing of the coding region and adjacent exon–intron boundaries of *SLCO2A1* on an ABI 3500xL genetic analyzer (Applied Biosystems, Darmstadt, Germany) in seven PHO patients of five unrelated pedigrees who were previously found to be negative for mutations in *HPGD*. Primer sequences and conditions are available on request. Blood samples were collected from all patients and family members, and genomic DNA was extracted using standard procedures. Institutional approval for the experiments were obtained, and written patient consent and protocols were in accordance with the Declaration of Helsinki Principles. All patients were of normal intelligence and height, and did not suffer from pulmonary disease or any other interfering disease.

The 27-year-old male index patient of consanguineous family 1 from India (Figure 1) carries the homozygous 1-bp deletion c.1292delC (p.Pro431LeufsX49) in exon 9 (Figure 2). At age 25, he complained about swelling of fingers and toes, thickening and furrowing of facial skin, and increasing knee pain, which did not significantly improve despite administration of nonsteroidal anti-inflammatory drugs. Seborrhea was successfully treated with 20 mg per day isotretinoin. His renal PGE_2 clearance was considerably increased (731 ng per hour per 1.73 m^2, normal level 4–27), similar to PGE-M, which showed a high clearance of 2,960 ng per hour per 1.73 m^2 (normal 62–482).

In another consanguineous Indian pedigree (Figure 1), we identified in both affected brothers (26- and 28-year-old) the homozygous missense mutation c.763G>A (p.Gly255Arg) in exon 6. Digital clubbing with hyperhidrosis and pain and swelling in ankle and knee joints started at about age 17 years.

In a 53-year-old Japanese patient, we identified two heterozygous mutations: c.1668G>C (p.Gln556His) in exon 12 and c.940+1G>A, which affects the canonical donor splice site of intron 7. At the age of 20 years, he recognized enlarged wrists and ankles, with digital clubbing and arthralgia in both knees. X-rays showed periostosis with cortical thickening and ectopic ossification. PGE2 levels were significantly elevated (1,762 pg ml^{-1} in serum, normal range 25–200; and 7,063 ng $mmol^{-1}$ creatinine in urine, normal level <50; Cayman, Cayman Biochemical, Ann Arbor, MI).

In a consanguineous Japanese pedigree with two affected brothers, 21 and 19 years of age, we identified the above splicing mutation c.940+1G>A in the homozygous state (Figure 2). PGE2 levels measured with the same enzyme immunoassay kit were even more increased in these patients. Levels were as follows (elder brother first): 2,375 and 17,701 pg ml^{-1} in serum, and

Abbreviations: HPGD, 15-hydroxyprostaglandin dehydrogenase; *NMD, nonsense-mediated decay; PG, prostaglandin; PHO, hypertrophic osteoarthropathy*

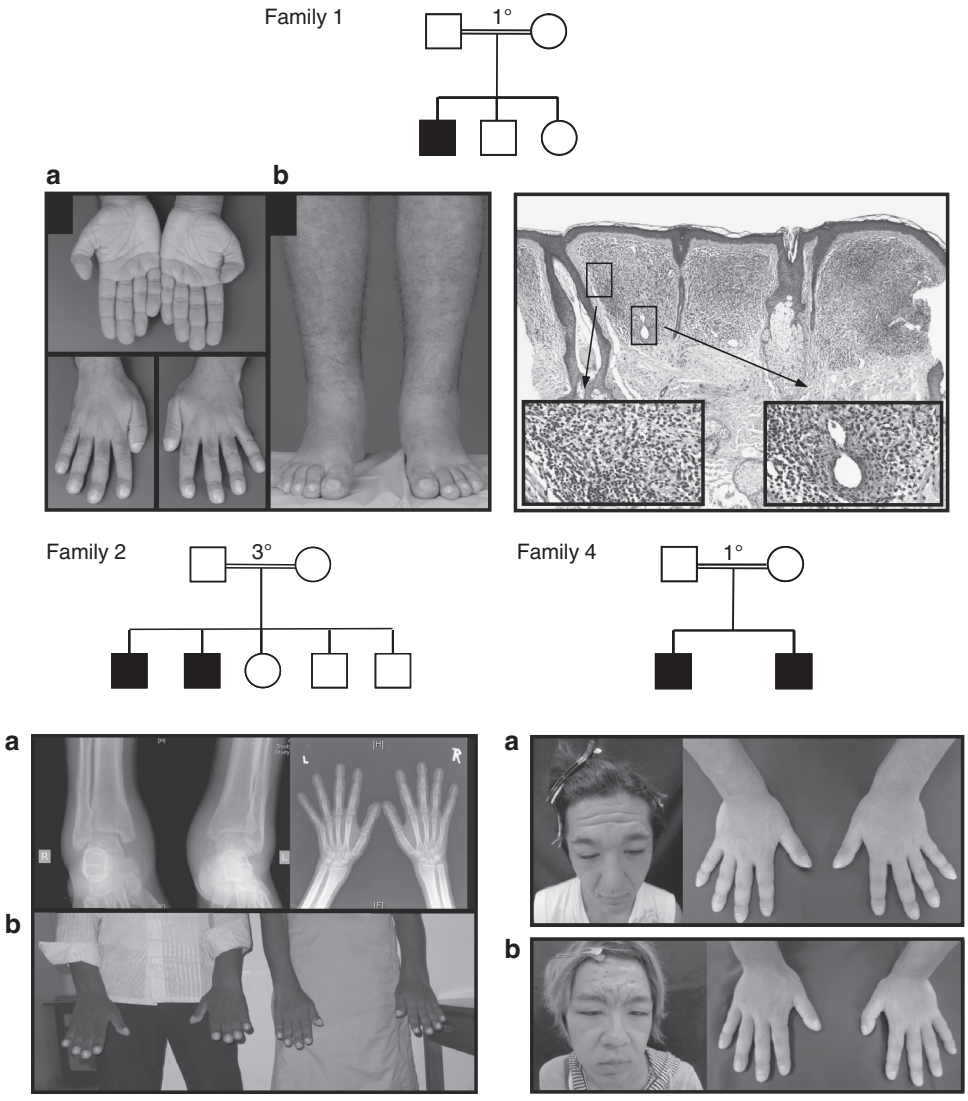

Figure 1. Pedigrees and phenotypes of affected individuals with *SLCO2A1* mutations. Top left: clinical features of the propositus of family 1. (**a**) Paw-like hands with moderate pachydermia, digital clubbing, and overcurvature of fingernails. (**b**) Clubbing of toes and moderate lymphoedema of lower legs. Top right: punch biopsy of the forehead. The epidermis shows normal differentiation, whereas the upper dermis demonstrates a dense diffuse inflammatory infiltrate composed of lymphocytes, plasma cells, and eosinophils. Some blood vessels show fibrinoid degeneration and neutrophils perivascularly with signs of cytoclasia, and some show extravasated erythrocytes. Lymph vessels appear dilated, and mild perifollicular fibrosis can be observed. Sebaceous glands are prominent and hair follicles contain demodex mites. Periodic acid Schiff (PAS) staining showed no other infectious agents. Mucin deposits are absent. Bottom left: (**a**) X-rays of ankle joints and hands of the elder affected brother of family 2 displaying extensive soft-tissue swelling with irregular periosteal proliferation, cortical thickening, and acroosteolysis. (**b**) Digital clubbing of the two affected brothers. Bottom right: (**a, b**) thickening and furrowing of the facial skin and digital clubbing of the hands of both affected brothers in pedigree 4 (**a**, elder brother, **b**, younger one). Subjects in the photographs have provided written, informed consent for the publication of their photographs.

9,260 and 17,701 ng mmol^{-1} creatinine in urine, respectively. Other PG metabolites were not measured.

The Japanese families, both carrying c.940+1G>A, are not known to be related. Interestingly, this splicing mutation was also present in the heterozygous state in one of the Han Chinese patients described recently (Zhang *et al.*, 2012). Although we cannot prove this, it might be hypothesized that this mutation represents an ancient founder allele rather than a recurrent mutation, as our patients harbor identical single-nucleotide polymorphisms, most probably *in cis* on the same allele (rs10935090, rs4634113, rs6767412, rs34550074, rs117837593, http://genome.ucsc.edu). c.940+1G>A destructs the canonical donor splice site of intron 7. If the splice resulted from exon skipping (the most common splicing mechanism), this mutation would result in an out-of-frame transcript and probably nonsense-mediated decay (NMD). NMD would be identical with loss-of-function, i.e., a ''most likely devastating chatacter'' of this mutation. NMD can also be postulated for c.1292delC (p.Pro431LeufsX49), identified homozygously in family 1.

Overall, it might be hypothesized that all or most SLCO2A1 mutations represent

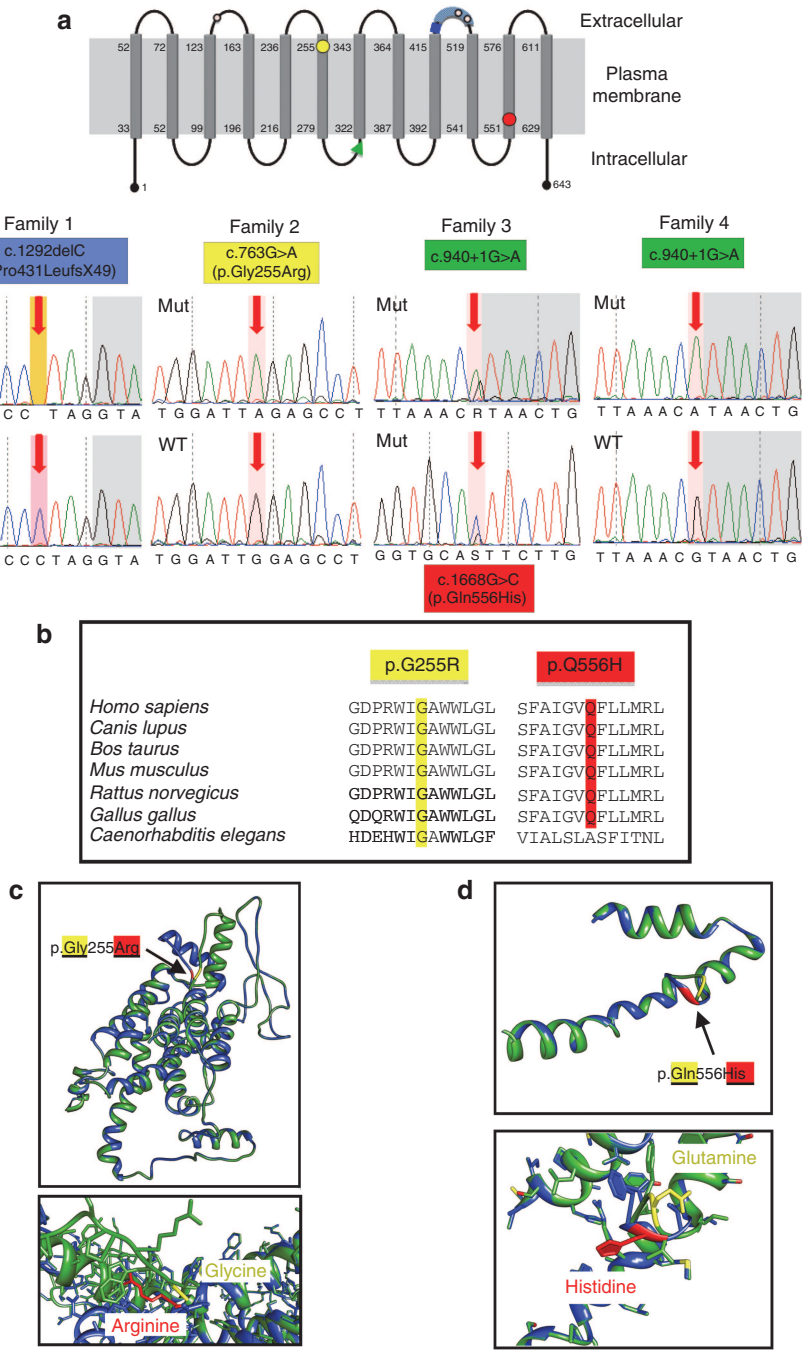

Figure 2. Electropherograms and localization and effect of identified *SLCO2A1* mutations in the prostaglandin transporter protein. Top: (**a**) scheme of the prostaglandin transporter SLCO2A1 (not drawn to scale). The SLCO2A1 protein contains 12 transmembrane domains with intracellularly located N and C terminus, 11 connecting loops in between, 3 glycosylation sites (highlighted as small circles), and a KAZAL-like domain (highlighted in bluish). The positions of the amino acids affected by mutations identified in this study are indicated by circles (in yellow and red for the two missense mutations G255R and Q556H), a green triangle (for the mutation c.940 + 1G > A affecting the donor splice site of intron 7), and a blue square (for the frameshift mutation p.Pro431LeufsX). Below are depicted the respective electropherograms of all *SLCO2A1* mutations identified in this study. The propositus of family 3 carried two heterozygous mutations, whereas the patients of the other families showed homozygosity for this locus in line with parental consanguinity in all three pedigrees. Middle: (**b**) conservation of the amino acids Gly (G) 255 and Gln (Q) 556 in SLCO2A1. The two missense mutations p.G255R and p.Q556H affect evolutionarily highly conserved amino acids in the SLCO2A1 protein (highlighted in yellow and red). Bottom left: (**c**) protein modeling of the missense mutation p.Gly255Arg in comparison with the wild-type (WT) sequence of SLCO2A1. Illustrated is the amino-acid region comprising amino acids 35–392 (only the protein ribbon is pictured). Both sequences (WT in green, mutated sequence in blue) put on top of each other. The corresponding position (Gly255) is marked in different colors (WT: yellow; mutated amino acid: red). The protein conformation has changed considerably; glycine (polar, neutral) present in the WT sequence is known to be much smaller than the basic amino acid arginine present in the mutant protein, whose side chain consists of a three-carbon aliphatic straight chain. Bottom right: (**d**) protein modeling of the missense mutation p.Gln556His in comparison with the WT sequence of SLCO2A1 according to the given illustration for p.Gly255Arg. Shown is the protein region comprising amino acids 529–579. WT glutamine is a polar and neutral amino acid that has been exchanged by the basic residue histidine with an imidazole ring, causing marked conformational changes.

truncating alleles or affect residues that are indispensable for PG transporter activity, such as the missense mutations c.763G>A (p.Gly255Arg) and c.1668G>C (p.Gln556His) identified. Both changes are nonconservative, affect evolutionarily highly conserved amino acids (Figure 2), and were predicted *in silico* by all bioinformatic tools of pathogenic relevance (Supplementary Table 1 online). The pathogenic character of c.763G>A (p.Gly255Arg) and the impact of the glycine residue at position 255 is further strengthened by the fact that the same codon was mutated (c.764G>A; p.Gly255Glu) in a patient described by Zhang *et al.* (2012). To corroborate these data, we constructed molecular models of wild-type and mutated SLCO2A1 proteins to visualize changes of protein folding and structure (Meng *et al.*, 2006; Bordoli *et al.*, 2009; Yang *et al.*, 2011). Both mutations are located in a transmembrane domain and considerably affect the protein structure (Figure 2), which was additionally confirmed by Ramachandran plots (Supplementary Figure S1 online).

In this report, we show that *SLCO2A1* is a frequent cause of autosomal recessive PHO, confirming the data recently published by Zhang *et al.*, 2012. Although most patients harbor mutations in *HPGD* and *SLCO2A1*, further genetic heterogeneity is likely, and candidate approaches targeting other PG-signaling components seem promising.

CONFLICT OF INTEREST

JB, VF, NB, HB, and CB are employees of Bioscientia, a member of Sonic Healthcare.

ACKNOWLEDGMENTS

We thank the families for their cooperation and interest in the study.

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SUPPLEMENTARY MATERIAL

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

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Modulation of Lymphocyte Function *In Vivo* via Inhibition of Calcineurin or Purine Synthesis in Patients with Atopic Dermatitis

Journal of Investigative Dermatology (2012) **132,** 2476–2479; doi:10.1038/jid.2012.150; published online 24 May 2012

TO THE EDITOR

In atopic dermatitis (AD), lymphocytes have an important role via regulation of the immune response, production of Igs, and sustaining cutaneous inflammation.

In AD, activated T cells can be found in blood, and most importantly in affected skin a marked increase of activated T cells is present (Akdis *et al.*, 2006). Serum levels of T-cell chemokines CCL17 (thymus and activation regulated chemokine, TARC) and

Abbreviations: AD, atopic dermatitis; CsA, cyclosporine A; EC-MPS, enteric-coated mycophenolate sodium; FUCT-VII, fucosyl transferase VII; MPA, mycophenolic acid; TARC, thymus and activation regulated chemokine; TNF–AIP3, tumor necrosis factor–alpha inducible protein-3