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Identification of mutations in the prostaglandin transporter gene *SLCO2A1* and its phenotype–genotype correlation in Japanese patients with pachydermoperiostosis

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ABSTRACT

Background: Pachydermoperiostosis (PDP) is a rare genetic disorder characterized by 3 major symptoms: pachydermia including cutis verticis gyrata (CVG), periostosis, and finger clubbing. Recently, a homozygous mutation in the gene *HPGD*, which encodes 15-hydroxyprostaglandin dehydrogenase (15-PGDH), was found to be associated with PDP. However, mutations in *HPGD* have not been identified in Japanese PDP patients.

Objective: We aimed to identify a novel responsible gene for PDP using whole exome sequencing by next-generation DNA sequencer (NGS).

Methods: Five patients, including 2 patient-parent trios were enrolled in this study. Entire coding regions were sequenced by NGS to identify candidate mutations associated with PDP. The candidate mutations were subsequently sequenced using the Sanger method. To determine clinical characteristics, we analyzed histological samples, as well as serum and urinary prostaglandin E2 (PGE2) levels for each of the 5 PDP patients, and 1 additional patient with idiopathic CVG.

Results: From initial analyses of whole exome sequencing data, we identified mutations in the solute carrier organic anion transporter family, member 2A1 (*SLCO2A1*) gene, encoding prostaglandin transporter, in 3 of the PDP patients. Follow-up Sanger sequencing showed 5 different *SLCO2A1* mutations (c.940+1G>A, p.E427_P430del, p.G104*, p.T347I, p.Q556H) in 4 unrelated PDP patients. In addition, the splice-site mutation c.940+1G>A identified in 3 of 4 PDP patients was determined to be a

Abbreviations: CVG, cutis verticisgyrata; NGS, next-generation DNA sequencer; PDP, pachydermoperiostosis; SLCO2A1, solute carrier organic anion transporter family member 2A1; PGT, prostaglandin transporter; SNP, single nucleotide polymorphism.

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founder mutation in the Japanese population. Furthermore, it is likely that the combination of these *SLCO2A1* mutations in PDP patients is also associated with disease severity.
Conclusion: We found that *SLCO2A1* is a novel gene responsible for PDP. Although the *SLCO2A1* gene is only the second gene discovered to be associated with PDP, it is likely to be a major cause of PDP in the Japanese population.

1. Introduction

Pachydermoperiostosis (PDP), also known as primary hypertrophic osteoarthropathy, is a rare autosomal recessive condition characterized by 3 major symptoms: cutis verticis gyrata (CVG), periostosis, and finger clubbing. In addition, several other symptoms, including sebaceous hyperplasia, hyperhidrosis, and arthropathy have also been reported [1,2]. The phenotypic spectrum of PDP is broad, and is generally categorized into 3 primary forms: the complete form, which involves all 3 major symptoms, including CVG; the incomplete form, which has all three symptoms but solely lacks CVG; and the "form fruste," characterized by the occurrence of pachydermia and minimal or absent skeletal changes [3].

To date, homozygous and compound heterozygous mutations in the *HPGD* gene, which encodes 15-hydroxyprostaglandin dehydrogenase (15-PGDH), have been identified as the main causative factor of PDP (MIM#259100) [4–10]. The primary function of 15-PGDH is an enzyme to catabolize for prostaglandin E2 (PGE2), prostaglandin F2 (PGF2), and prostaglandin B1 (PGB1). The identified *HPGD* mutation results in chronic elevation of PGE2 levels in serum, but it is unclear whether this elevation of PGE2 is associated with PDP phenotypes. Furthermore, several cases of PDP patients with congenital clubbed nails and *HPGD* mutations have also been reported [3–8]. We have also attempted to find *HPGD* mutations in Japanese PDP patients; however, no *HPGD* mutations have been identified so far, suggesting the existence of other causative gene(s) responsible for PDP in the Japanese population.

Recent advances in DNA sequencing techniques, such as the advent of next-generation sequencer (NGS), now allow for the analysis of all coding regions in exons (whole exome sequencing). In this study, we identified 5 different mutations in the solute carrier organic anion transporter family, member 2A1 (*SLCO2A1*) gene, which encodes prostaglandin transporter (PGT), in 4 unrelated PDP patients using whole exome sequencing and Sanger sequencing approaches. In addition, we assessed the potential impacts of the identified *SLCO2A1* mutations on disease severity and tested for associations between these variants and the clinical forms.

2. Patients and methods

2.1. Clinical report

PDP was diagnosed in the patients in our study, all of whom were of Japanese descent, on the basis of established clinical and radiological criteria [1]. All individuals participating in the study gave their written informed consent. This study was approved by the ethics committee of the National Center for Child Health and Development, and Keio University School of Medicine. *HPGD* mutation analyses had been performed previously [9], and no mutations were detected in any of the patients.

2.1.1. Patient1 (P1)

Clinical details for this patient have been reported in full elsewhere [11]. Briefly, at the age of 19, the patient was referred to evaluate his endocrinological status. He had a 6-year history of clubbing of fingers and toes. On physical examination, a coarse face, greasiness of facial skin (Fig. 1, P1), and hyperhidrosis were observed. Marked thickening of the scalp (CVG) was not evident. A skin biopsy specimen from the forehead skin showed thickening of the dermis. Interwoven collagen bundles, hypertrophic sebaceous glands, and increased density of sweat glands were subtle but evident in the dermis [11]. Elastic fibers and fibrosis were not observed only in the superficial dermis. Endocrinological examinations showed no notable findings. Radiological examination showed the presence of periostosis of the diaphysis of the radius, ulna, tibia, and fibula. On the basis of these observations, the patient was diagnosed with the incomplete type of PDP. At the age of 21, hydrarthros is developed in the knee joints. Swelling in knee joints was evident, but the patient did not complain of arthralgia or local joint heat. He was born with normal measurements following an uneventful pregnancy. None of the patient's immediate family members, including both parents and 2-year-old sister, had PDP or associated symptoms.

2.1.2. Patient 2 (P2)

This patient was 23 years old at the time of the study. At the age of 12, he noticed enlargement of fingers and toes, swelling of elbow and knee joints, as well as hyperhidrosis. At the age of 14, he presented with clubbing of fingers and toes, periostosis, and pachydermia. He was then diagnosed with PDP. At the age of 15, he was referred to one of the authors. Prominent swelling of the lower legs, paw-like fingers, and greasiness of the facial skin were observed. Radiological examination showed periostosis of the diaphysis of the radius and a cauliflower-like appearance of phalanx. Endocrinological examinations showed no notable findings. By the age of 23, the patient showed no clinical symptoms of CVG. He was diagnosed with the incomplete form of PDP. No skin biopsy specimen of this patient was available. The patient has no sibling, and his parents did not show any signs of the disease.

2.1.3. Patient 3 (P3)

The case of this patient has also been reported elsewhere [12]. At the time of the study, the patient was 41 years old. He first presented with thickening and furrowing of the scalp (CVG) and forehead (Fig. 1, P3), which the patient had noticed at the age of 17. His facial skin appeared greasy, and digital clubbing was apparent. Radiological examination showed periostosis of the diaphysis of the radius and ulna. Arthropathy was not evident. A skin biopsy specimen from the scalp and forehead (Supplementary Fig. 1) showed thickening of the dermis, which was filled with hypertrophic sebaceous glands and dense thickened collagen bundles. Abundant sweat glands and mucin deposition were also seen in the dermis. These findings met the diagnostic criteria of the complete form of PDP. His familial history was unavailable.

2.1.4. Patient 4 (P4)

The case of this patient has been reported elsewhere [13]. At the time of this study, the patient was 25 years old, and had a 7-year history of digital clubbing and acne on the scalp. He developed a peptic ulcer at the age of 14. Since the age of 22, the patient showed thickening and furrowing of the forehead skin and scalp. Physical examination showed digital clubbing, greasiness of facial skin, and