Common and recurrent \textit{HPGD} mutations in Caucasian individuals with primary hypertrophic osteoarthropathy

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Abstract

\textbf{Objective.} Homozygous recessive germline mutations of the 15-hydroxyprostaglandin dehydrogenase (\textit{HPGD}) gene, encoding 15-hydroxyprostaglandin dehydrogenase, result in persistent elevation of circulating PGE\textsubscript{2} levels, causing the syndrome of primary hypertrophic osteoarthropathy (PHO). Homozygous \textit{HPGD} mutations have so far been reported in 10 families, all but one displaying parental consanguinity. Only two of these families were of European origin. We wished to determine the role of \textit{HPGD} in causing PHO in non-consanguineous European families.

\textbf{Methods.} Five previously unreported families of Caucasian European origin, with one or more individuals affected with typical PHO, were characterized clinically and by complete sequencing of the \textit{HPGD} coding exons.

\textbf{Results.} Biallelic \textit{HPGD} mutations were identified in affected individuals in all the five families, confirming a very specific association of this phenotype with \textit{HPGD} mutations. The previously described c.175\_176delCT frameshift mutation was observed in association with two different alleles of an adjacent single nucleotide polymorphism.

\textbf{Conclusions.} Biallelic \textit{HPGD} mutations are found in the majority of patients with typical PHO, and sequencing of the \textit{HPGD} gene is a highly specific first-line investigation for patients presenting in this way, particularly during childhood. The c.175\_176delCT frameshift mutation appears to be recurrent and to be the commonest \textit{HPGD} mutation in Caucasian families.

\textbf{Key words:} Clubbing, Primary hypertrophic osteoarthropathy, Prostaglandin, 15-hydroxyprostaglandin dehydrogenase.

Introduction

Primary hypertrophic osteoarthropathy (PHO) is a notable example of a rare Mendelian mimic, whose genetic basis illuminates a much more common acquired disease process. Its hallmark is progressive digital clubbing, typically beginning early in childhood. Accompanying abnormalities include subperiosteal bone deposition, leading to thickening and abnormal modelling of long bones, acro-osteolysis, and effusions and pain of the large joints. Apart from the age of onset and lack of evidence for causative pathology, these skeletal features are indistinguishable from those found in patients with clubbing...
secondary to other diseases, particularly the severe forms of ‘hypertrophic pulmonary osteoarthropathy’ seen in association with lung cancer [1].

Dermatological features of PHO include skin thickening, seborrhea and hyperhidrosis, and indeed various inheritance patterns have been suggested on the basis of observations on families with some or all features of the disease [6]. However, we recently demonstrated that in four families with the full-blown, early-onset PHO syndrome, the underlying cause was homozygous mutations in 15-hydroxyprostaglandin dehydrogenase (HPGD), the gene encoding HPGD [7].

HPGD is the major enzyme responsible for PG degradation [8]. Affected patients consequently have chronically elevated levels of PGE2, and fail to excrete its metabolite (PGE-M; 11-β-hydroxy-9,15-dioxo-2,3,4,5-tetranor-propane-1,2,10-dioic acid). These findings suggest a simple unifying explanation for the pathogenesis of secondary hypertrophic osteoarthropathy and of clubbing secondary to a wide range of disease processes. Such disorders include not only neoplastic and inflammatory conditions (in which PG overproduction occurs), but also cyanotic congenital heart disease. In the latter case, the mechanism is likely to relate to the fact that in health, a large proportion of circulating PGE2 is cleared by HPGD in the lungs [9]. Right-to-left shunts that bypass the pulmonary circulation would therefore allow PGE2 to escape this clearance mechanism.

Recognition of HPGD deficiency as the underlying aetiology in patients presenting with a PHO phenotype may avoid the need for extensive searches for occult aetiology in patients presenting with a PHO phenotype. Since the HPGD gene is small, mutation analysis is currently the simplest way of confirming germline HPGD deficiency. However, it remains to be determined (i) what proportion of patients with PHO or with isolated finger clubbing [10] will harbour HPGD mutations and (ii) whether heterozygous HPGD mutations will account for similar phenotypes displaying dominant inheritance. The clinical phenotype of HPGD deficiency and its mutational spectrum also need to be defined in order to deduce genotype–phenotype correlations that will determine the predictive value of HPGD mutation analysis. Here, we report the clinical characteristics and the finding of biallelic HPGD mutations in all of eight typical early-onset PHO patients, from five previously unreported families of Caucasian European origin.

Patients and methods

All subjects or their parents gave written consent in compliance with the principles of the Declaration of Helsinki. The study was approved by the Bradford Teaching Hospitals Research Ethics Committee (reference 02/04/115). All families were Caucasian, with no known parental consanguinity.

Family A

This family has ancestry in Croatia. A 34-year-old female presented striking features of bilateral finger clubbing with periangual hyperaemia (Fig. 1A). Radiographs revealed typical features of hypertrophic osteoarthropathy, with metaphyseal new bone formation (Fig. 1B) and marked acro-osteolysis (Fig. 1C). She had suffered from palmoplantar hyperhidrosis since early childhood, as well as recurrent arthralgias. Rheumatic immunoserology was negative and a thoracic CT scan revealed no abnormalities.

Family B

Two affected brothers were seen. The first had been born at full term after an uneventful pregnancy, to healthy unrelated parents belonging to an Italian genetic isolate. Since childhood, he noticed progressive enlargement of the terminal phalanges and palmoplantar hyperkeratosis. At the age of 43 years, a chronic intestinal inflammatory disease was diagnosed, and the following year he had surgical resection of a 7 × 6.5 cm abdominal mass, located in the mesentery, whose histopathological diagnosis was desmoid tumour. He was then placed under surveillance for prevention of colorectal cancer.

When evaluated at the age of 49 years, intelligence and biometric parameters were normal. His facial skin was oily, thickened and furrowed. He showed bilateral finger clubbing and palmoplantar hyperkeratosis (Fig. 1D). There was no cardiac, pulmonary or hepatic disease.

His brother, 6 years older than him, was referred with similar features. He too showed bilateral clubbing with periangual hyperaemia (Fig. 1E) and palmoplantar hyperkeratosis. He had complained of severe and disabling arthralgia from the age of 14 years, and manifested enlargement of the large joints of the limbs with limitation of mobility. Skeletal radiography showed soft tissue swelling and periosteal reaction with cortical thickening, which had previously led to a diagnosis of Camurati–Engelmann disease. There was no digital clubbing in these patients’ mother, and abnormalities of the extremities were also not reported in their deceased father.

Family C

Two siblings presented with similar clinical features. There was a history of delayed closure of the fontanelles, recurrent periods of fever of unknown origin and vomiting in the first 3 years. In the female index case, the start of unaided walking was hampered by flexion contractures of both knees. At first presentation at the age of 6 years, there was ‘drumstick’ clubbing of fingers and toes, and both knees were enlarged, without signs of inflammation. She complained of paroxysmal bone pains, mainly at night. She had had intermittent palmoplantar hyperhidrosis since the first year of life. There was no skin abnormality and general growth and cognitive development were normal. The X-rays revealed Wormian bones in the skull.
and thickened diaphyseal cortices of the long bones. Towards puberty, the painful episodes were fading and joint contractures were responding well to physiotherapy. Introduction of treatment with a PG synthesis inhibitor (ibuprofen) led to a further substantial reduction of bone pains, but was ineffective in relieving hyperhidrosis. The patient’s 3-year-old younger brother presented an essentially identical clinical picture (Fig. 1F–I).

**Family D**

The patient was a female twin conceived by *in vitro* fertilization and born at 35 weeks. A congenital heart murmur resolved spontaneously. Enlargement of hands and feet began at the age of 1 year. Chest X-ray and sweat chloride testing were normal. When assessed at the age of 7 years, she was non-dysmorphic, normocephalic and of normal stature. She had mild bilateral limitation of knee extension, mild joint laxity of the hands and elbows, and coarse skin on the hands and feet. She had larger than normal hands and long fingers with excess soft tissue. Digital clubbing was most prominent on the thumbs. There was also clubbing of the toes with broadening of the distal phalanx. Neurological examination was normal. Radiology revealed acro-osteolysis of the fingers (Fig. 1J–L) and toes, periosteal reaction and normal bone mineralization; no other abnormalities were apparent on skeletal

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**Fig. 1** Clinical features of subjects with biallelic *HPGD* mutations. (A–C) Patient A1 (34 years old). Note the marked periungual hyperaemia in addition to finger clubbing (A). There is subperiosteal bone deposition in the tibial metaphysis (B). Acro-osteolysis is advanced, with loss of tufts of the terminal phalanges (C). (D, E) Family B: palmar hyperkeratosis in Patient B1 (D) and finger clubbing and periungual hyperaemia in Patient B2 (E). (F–I) Family C: index case at 6 (H) and 18 years of age (I) and younger affected brother at 3 (F) and 15 (G) years of age. Note the marked, non-inflammatory expansion of knees and ankles. (J–L) Patient D1 (7 years old). Dorsoventral (J) oblique (K) and lateral (L) views of the left hand. The arrows indicate the acro-osteolysis of the index finger (J, K), which is seen to be the end product of marked dorsoventral thinning of the terminal phalanx (L).
surveys. Full blood count and CRP were normal. When reviewed 7 months later, there was no evidence of disease progression. The patient later complained of pain in her extremities and is treated with NSAIDs. For the treatment of her mild knee contractures, she performs physical therapy exercises twice daily and wears orthotics. The maternal family is of British, German and Welsh ancestry and the paternal family ancestry is German. The patient’s father reported hyperhidrosis and her mother reported thickened skin on the hands around the cuticles.

Family E

Both parents were of Dutch ancestry. The male index case was born at 26 weeks’ gestation, requiring 3 months in intensive care, including a patent ductus arteriosus ligature. At the age of 3 years, juvenile arthritis was diagnosed elsewhere and treated unsuccessfully with NSAIDs and MTX; complete blood count, ESR, ANA and RF were reportedly normal. At the same time, his mother was diagnosed with palindromic rheumatism.

Although clubbing had been previously noted, the diagnosis of PHO was first entertained when referred to us at the age of 9 years. Pulmonary and cardiac evaluations were normal. Initial skeletal survey radiographs were read as normal; an MRI of his knee showed a minimal amount of SF and normal cartilage. Swelling and limited movement of both the knees persisted over the next 6 years. Despite treatment with NSAIDs, MTX, etanercept and steroid injection of the knees, his arthritis was unrelenting and subsequently extended to involve limited motion in the wrist, multiple phalangeal joints, both hips, and swelling and limitation of both knees and ankles without tenderness or morning stiffness. Clubbing of fingers and toes was pronounced and there was palmar hyperhidrosis. Most recent imaging revealed subtle irregularity of the tufts of his fingers, but no erosions or skeletal dysplasia. Intellectual and general physical development was normal at the age of 15 years.

The affected brother of the above patient was born at 31 weeks’ gestation and required 1 month of hospitalization. He too had a patent ductus arteriosus that closed after treatment with NSAIDs. Clubbing was noted at birth. He was diagnosed with eczema; the skin of his feet reportedly becomes dry and peels. All laboratory investigations had been normal. He reported knee pain intermittently with intercurrent infections or with strenuous activity, and was recently noted to have bony hypertrophy of both knees (without swelling or limitation) as well as clubbing of fingers and toes. He is otherwise intellectually and physically normal at the age of 5 years.

Genetic analysis

Mutation analysis of affected individuals was performed by direct sequencing of PCR products amplified from genomic DNA (supplementary table 1, available as supplementary data at Rheumatology Online), using BigDye terminator chemistry and a 3130 capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequence electropherograms were analysed using a new base-calling and mutation analysis software package, GeneScreen (http://dna.leeds.ac.uk/genescreen/), which we developed specifically for this purpose. GeneScreen will be described fully elsewhere; briefly, it determines the nature of sequence variants directly from electropherograms, in comparison with a reference sequence. A particular strength is its ability to resolve the superimposed allelic sequences in the presence of heterozygous insertions or deletions, determining the nature of the indel without the need for cloning of PCR products (Fig. 2B–D). The GeneScreen program and its detailed documentation are freely available for download from the above URL.

Results

Biallelic HPGD mutations were identified in affected individuals in all five families. Parental material was available in all except Family B, and was used to confirm the recessive inheritance of PHO in these families. The results of the genetic analyses are summarized in Fig. 2A.

In Family A, the affected woman was compound heterozygous for two different frameshifting HPGD mutations, both located in exon 2. The first of these, c.175_176delCT, is identical to that previously found in the homozygous state in affected members of a Polish family [7]. The second, c.120delA (Fig. 2B), has recently also been reported in a patient of Dutch origin [11]. It alters the encoded HPGD sequence after amino acid 40 and introduces a new stop codon after 30 novel amino acids. The patient’s mother was heterozygous for c.175_176delCT, whereas her father and brother were both homozygous for c.120delA. All these heterozygous individuals were asymptomatic, with no signs of clubbing.

In Family B, both affected brothers were homozygous for the c.175_176delCT mutation (Fig. 2C). Parental samples were not available for examination.

In Family C, both affected siblings were again compound heterozygous for the mutations c.120delA and c.175_176delCT. These were confirmed to be on opposite alleles on examining their parents, the father carrying c.120delA and the mother c.175_176delCT.

In Family D, the affected child was compound heterozygous for c.175_176delCT (inherited from her father) and a splice junction mutation c.325-1G > C (IVS3-1G > C) from her mother. Both these parents were healthy, although the father reported excessive sweating from the palms of his hands and was said to have mild clubbing (not examined).

In Family E, both affected brothers were again compound heterozygous for the mutations c.120delA and c.175_176delCT. These were again confirmed to be on opposite alleles on examining their parents, the father carrying c.120delA and the mother c.175_176delCT.

A silent polymorphic variant, c.156G > A (rs1050145), is situated between the c.120delA and c.175_176delCT mutations in exon 2. Both alleles (G/A) are of approximately equal frequency in HapMap data from individuals of European origin. In all three families (A, C and E) segregating c.120delA, this mutation was linked to the G allele of rs1050145. In contrast, the c.175_176delCT mutation
Fig. 2 Genetic analysis of the HPGD locus in PHO families. (A) Summary of results. Grey shading in Family B indicates deduced (rather than experimentally determined) genotypes. Alleles at the polymorphic position c.156 are indicated by G or A; delA refers to c.120delA and delCT refers to c.175_176delCT. (B–E) GeneScreen display of pathogenic HPGD mutations. In each case, both forward (F) and reverse (R) sequences are shown. (B) Heterozygous c.120delA. (C) Homozygous c.175_176delCT. (D) Heterozygous c.175_176delCT. (E) Heterozygous splice junction mutation c.325-1G>C.
was linked to an A allele of rs1050145 in Family B, but to a G allele in Families A, C and E (Fig. 2A), possibly indicating that it is a recurrent mutation (see ‘Discussion’ section).

**Discussion**

To date, eight different recessive HPGD mutations have been identified, in patients with either typical PHO [7, 11, 12] or isolated congenital digital clubbing [10] (Table 1). On the basis of this combined experience, the most common mutation appears to be c.175_176delCT, that accounts for 8 of 12 alleles among Caucasian families we have examined. We originally reported this variant in a non-consanguineous Polish family [7, 13] in which the affected individuals were homozygous for the change. The c.120delA mutation accounts for 3 of 12 of the disease alleles in the same group, and like c.175_176delCT, it is a frameshift mutation. Similarly, the novel c.325-1G>C splice acceptor mutation would be expected to result in incorrect exon splicing and a grossly aberrant protein structure. All three of these mutations are therefore expected to be null alleles, which is consistent with the reproducible and distinctive clinical phenotype of all subjects. Variability in age of presentation and in severity of symptoms may perhaps therefore be attributable to other factors, including those that affect the rate of synthesis of PGE2. In this respect, it may be relevant that we have previously noted that male individuals with null HPGD mutations (both homozygous and heterozygous) tend to have higher PGE2 levels and to develop clubbing at a younger age than their female counterparts [7].

In contrast, the family described by Tariq et al. [10] displayed a more limited clinical phenotype of severe congenital nail clubbing. Affected individuals were homozygous for a missense mutation, p.S193P; it is likely that this represents a hypomorphic rather than a null allele, since homozygous affected individuals showed a more modest (<2-fold) elevation of urinary PGE2 levels than we previously observed in individuals with full-blown PHO (<6-fold) [7].

Interestingly, we found the c.175_176delCT mutation in linkage with both variants of a common synonymous SNP located nearby (c.156G>A; rs1050145; Fig. 2A). This suggests that the high frequency of c.175_176delCT most likely does not reflect a common founder mutation, but rather this mutation has arisen independently on two or more occasions; it may therefore represent a mutation hotspot, where replication ‘slippage’ occurs within the duplicated CTCT sequence.

The desmoid tumour in Patient B1 is noteworthy; this association of two rare disorders has not, to our knowledge, been reported previously. The response of some desmoid tumours to anti-PG therapy was first noted nearly 30 years ago [14] and is still therapeutically useful [15]. A possible causative role for the chronically elevated PG therefore deserves consideration in this case.

Experience to date indicates that biallelic mutations of HPGD, resulting in chronic exposure to elevated levels of
PGE$_2$, underlie the majority of cases of typical PHO (including cases classified as pachydermoperiostosis or cranio-osteoarthropathy). Mutation analysis of $HPGD$ is simple and cheap, and should be considered as an early investigation in children presenting with features of these disorders.

### Rheumatology key messages

- Genetic deficiency of $HPGD$ commonly underlies PHO.
- Patients with idiopathic clubbing and other features of PHO may benefit from genetic testing for $HPGD$ mutations.

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### Supplementary data

Supplementary data are available at *Rheumatology* Online.

### References