A novel homozygous splice site mutation in the *HPGD* gene causes mild primary hypertrophic osteoarthropathy

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Abstract

Objectives

Homozygous mutations in HPGD gene, encoding 15-hydroxyprostaglandin dehydrogenase, have recently been associated with primary hypertrophic osteoarthropathy (PHO). So far, only 7 HPGD alterations are known. In order to expand this mutational spectrum and better delineate the HPGD-related phenotype, we report the clinical and molecular characterisation of a 13-year-old boy and compare his features to known mutated patients.

Methods

The HPGD gene exons 1-7 and exon-intron junctions were analysed by direct sequencing. Previously published HPGD-mutated patients were systematically reviewed based on the original clinical description.

Results

A novel homozygous c.217+1G>A mutation affecting the obligatory donor splice site of HPGD exon 2 was identified in our proband who showed a mild form of PHO. Review of HPGD-mutated patients outlined all patients manifested digital clubbing, periostosis and acro-osteolysis. Hyperhidrosis (92%), arthralgia (65%) and eczema (33%) were variably associated features. Pachydermia (54%) was mild and mostly limited to palms and sole; cutis vertigis gyrata, blepharoptosis and severe skin thickening were never observed. Besides digital clubbing, PHO infants often presented patent ductus arteriosus (PDA) (32%) and delayed cranial sutures closure (55%).

Conclusion

The present findings broaden the allelic spectrum of HPGD gene to include a novel c.217+1G>A mutation. Mutated patients display a homogeneous phenotype mainly consisting in digital clubbing, periostosis, acro-osteolysis, hyperhidrosis and mild pachydermia. Earliest manifestations include delayed closure of the cranial sutures and PDA. In conclusion, the information reported herein would facilitate the diagnosis of PHO due to HPGD mutations.

Key words

15-hydroxyprostaglandindehydrogenase, *HPGD*, primary hypertrophic osteoarthropathy, digital clubbing, pachydermoperiostosis.

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Introduction

Primary hypertrophic osteoarthropathy (PHO; OMIM no. 259100) is a hereditary systemic disorder characterised by digital clubbing, arthropathy, periostosis and acro-osteolysis of long bones and cutaneous manifestations including skin thickening (hence the term pachydermoperiostosis, PDP) mostly of palms and soles (1, 2). Additional skin manifestations include excessive sweating and acne. Three clinical presentations of PHO are generally recognised: a complete form characterised by periostosis and pachydermia; an incomplete form with periostosis without pachydermia; and a 'forme fruste' with pachydermia and minimal or absent skeletal anomalies (9, 11). Patent ductus arteriosus (PDA) may be present (6), as well as delayed cranial sutures closure prompting some author to consider cranio-osteoarthropathy syndrome, or Currarino idiopathic osteoarthropathy (4, 13), as a distinct disorder. Of note, these features are clinically indistinguishable from those found in PHO phenocopy, the so-called secondary hypertrophic osteoarthropathy (SHO), occurring after systemic conditions such as pulmonary and congenital heart diseases (3).

Both autosomal dominant and recessive inheritance have been suggested in PHO (OMIM %167100; no. 259100) (2). Recently, homozygous mutations in the *HPGD* gene have been identified in a subset of patients with PHO (12). *HPGD* encodes the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141), a prostaglandin E_2 (PGE_2) catabolising enzyme, highly expressed in the lung. In patients with *HPGD* mutations, the loss of enzymatic function causes a chronic elevation of circulating PGE_2; the consequent prolonged peripheral vasodilatation and the stimulating effect of PGE_2 on osteoblasts and osteoclasts are consistent with digital clubbing, acro-osteolysis and periosteal bone formation observed in PHO patients (12).

Four PHO families and five additional *HPGD* mutated kindred were reported so far (8, 12, 14). Moreover, Tariq *et al.* (2009) described a large Pakistani family with isolated congenital nail clubbing (ICNC; OMIM no. 119900) harbouring a distinct homozygous *HPGD* mutation. Indeed, digital clubbing was the unifying feature in the ten so far reported *HPGD* families, while phenotypic variability was observed with respect to cutaneous (mainly pachydermia) and arthropathic manifestations, delayed closure of cranial sutures and PDA. Since no large screening of *HPGD* gene is yet available, report of novel PHO mutated patients may help elucidating the *HPGD*-related phenotype.

We describe a 13-year-old boy with a mild form of PHO harbouring a novel homozygous splice-site mutation in *HPGD* and review the features of the 34 mutated individuals from families reported to date.

Clinical report

A 13-year-old boy, the second of three siblings born to first-cousin unaffected parents, was referred to our Institute for arthralgia and fingers and toes broadening. No cardiopulmonary or hepatic disease was reported nor was apparent at time of evaluation. Personal history revealed polyarthritis since the age of 5, which was managed as an acute articular rheumatism. Progressive fingers and toes deformities associated with curving of nails and swelling of ankles became also apparent from the age of 5. These features were accompanied by pain of legs and forearms with local cyanosis and hyperhidrosis.

On clinical examination at 13 years of age, clubbing of fingers and toes was apparent (Fig. 1 a-c) with bilateral reducible hyperextension of distal interphalangeal articulations of the 2nd and 3rd fingers. Maculopapular acne of the back was also evident. Skeletal radiographs of the long bones showed bilateral diaphyseal periostosis and acro-osteolysis (Fig. 2 a, b). Linear growth and psychomotor development were normal. Blood count showed hypochromic, microcytic, iron-deficient anaemia. Calcium and phosphorous serum levels, serum protein electrophoresis, liver and kidney function assessment were all within normal ranges. Echocardiography, abdominal ultrasound, chest x-ray and computed tomography scan were unremarkable. A diagnosis of PHO was then suggested. His arthralgia was re-

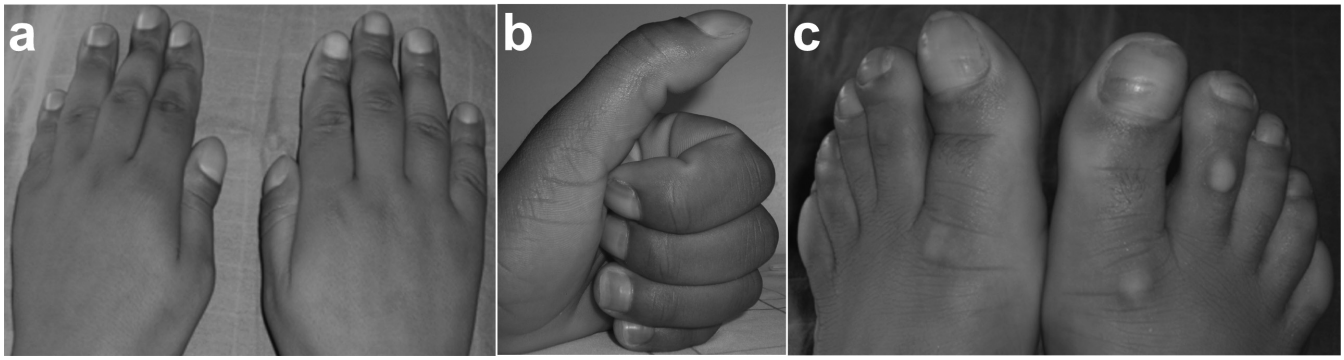

Fig. 1. Hands and feet aspect observed in the patient at 13 years of age with mild clubbing of fingers (a, b) and toes (c). Note "turtle carapace-like" nails particularly evident on the lateral view of the 1st digit (b).

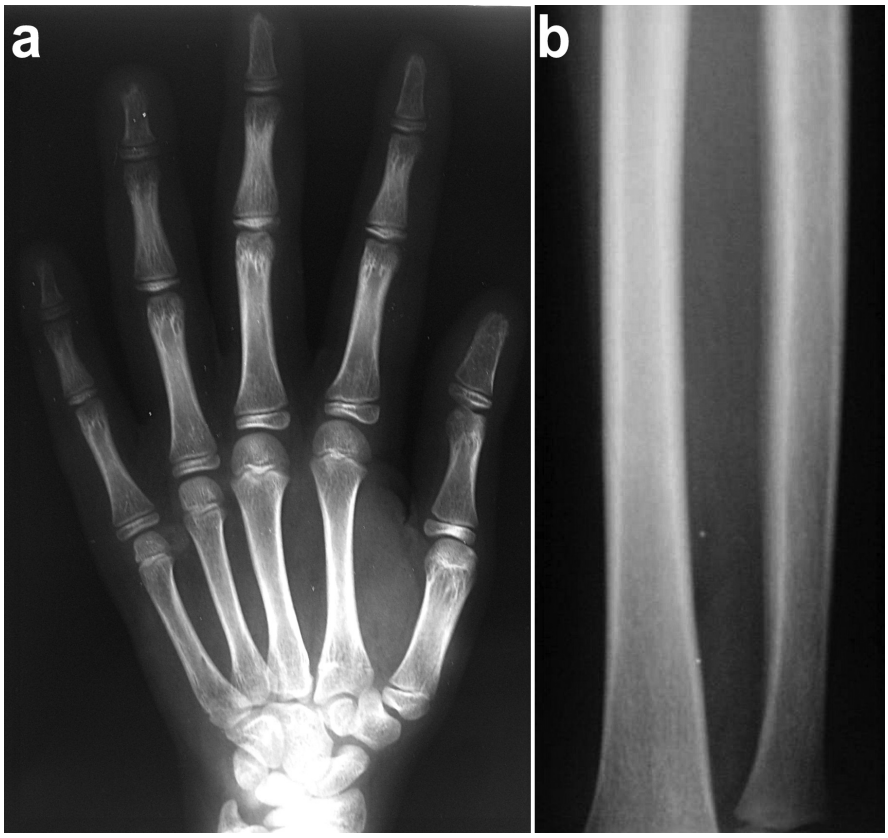

Fig. 2. Skeletal manifestations consisted of irregular terminal phalanges with acro-osteolysis more evident at the tips of the 2nd, 3rd and 4th terminal phalanges (a) and irregular mild periosteal reaction around the diaphyseal portions of the long bones of the forearm.

Table I. *HPGD* gene primers used for genomic amplification and sequencing.

Exons	Forward primer (5' to 3')	Reverse primer (5' to 3')
1	GCTGGCTTGACAGTTTCCTC	AGTCTCGGAGTGTGTGGGC
2	GTGTGTTTATTGTTTGTCCGTC	ACGTTCCCAGTTGACAGATTG
3	CCTCTCATGGCATAGGACATG	GTTTCCATGACTCCAAGAACC
4	GTATTCCTTTTCTCACTTATGC	TGAAGATTTGTTTTTGTGGTCC
5	GAGTTTCACAAAGCTATCTGG	TGAGATATGACGGTTGTTGTAG
6	GAAACTGCTGAAACCTACAAC	CTGTATAAGCTTATTTCTTCCC
7	CACATTTCCCTATAACATGTTC	AGCTATGGCTAACACATAAGC

lieved with diclofenac sodium (75 mg/day for 9 months). Anaemia recovered after oral iron supplementation therapy. Clinical evaluation of the parents excluded the presence of digital clubbing.

Matherials and methods

Peripheral blood samples of proband and parents were collected after obtaining an informed consent. Genomic DNA was extracted according to standard procedures and *HPGD* exons were amplified in seven PCR fragments (Table I). PCR amplifications were performed with 50 ng of genomic DNA in a 25 μL volume. Exon 1 was amplified in a reaction containing 1X reaction buffer B, 200 μM dNTPs, 0.5 μM of each primer, 3% DMSO and 0.5 U KAPA2G Fast Hot Start DNA polymerase (Kapa Biosystems, Boston, Massachusetts, United States). Thermal cycler conditions were 35 cycles of 95°C for 10 seconds, 59°C for 10 seconds, and 72°C for 2 seconds, preceeded by 1 minute at 95°C and followed by a final elongation step at 72°C for 30 seconds. PCRs of exons 2-7 were performed with Ampli Taq Gold Polymerase (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions at 56°C annealing temperature. Direct sequencing was performed using BigDye Terminator v1.1 Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA) on an automated capillary sequencer (ABI 3130xl, Applied Biosystems).

Identified single nucleotide substitution was analysed with two splicing prediction tools: Human Splicing Finder (HSF v.2.4, http://www.umd.be/HSF/) and NNSPLICE 0.9 (http://www.fruitfly.org/seq_tools/ splice.html).

Table II. Phenotypic and molecular findings in *HPGD*-mutated families.

	ICNC	PHO										% PHO
	Tariq *et al.*, 2008	Uppal *et al.*, 2008				Seifert *et al.*, 2009		Yüksel-Konuk *et al.*, 2009			this report	
Family ID		A	B	C	D	1	2	1	2	3		
Origin	Pakstan	Pakistan	Pakistan	Bangladesh	Poland	Turkey	Netherlands	Turkey	Turkey	Turkey	Morocco	
Consanguinity	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	
Affected members	11	6	2	2	3	3	1	2	3	1	1	
Sex (F/M)	7/4	4/2	1/1	2/0	1/2	2/1	F	2/0	3/0	M	M	
Age or range	2-50 years	14-39 years	10, 14 years	14, 35 years	13-21 years	13 months-11 years	9 years	21-24 years	23-34 years	9 years	13 years	
Skeletal												
Digital clubbing	11/11	6/6	2/2	2/2	3/3	3/3	1/1	2/2	3/3	1/1	1/1	100
Periostosis	0/2	3/3	1/1	1/1	3/3	1/1	1/1	1/1	2/2	1/1	1/1	100
Acro-osteolytis	0/2	3/3	1/1	1/1	3/3	1/1	1/1	1/1	2/2	1/1	1/1	100
Arthralgia	0/11	4/6	2/2	2/2	2/3	0/3	1/1	N.E.	N.E.	0/1	1/1	63
Swollen joints	0/11	2/6	1/2	2/2	0/3	0/3	0/1	0/2	3/3	0/1	0/1	33
Skin												
Hyperhidrosis	0/11	5/6	2/2	2/2	3/3	3/3	1/1	1/2	3/3	1/1	1/1	92
Pachydermia	0/11	3/6	1/2	2/2	3/3	0/3	0/1	2/2	1/3	1/1	0/1	54
Seborrhoea	0/11	5/6	1/2	2/2	2/3	0/3	0/1	N.E.	N.E.	N.E.	1/1	67
Acne	0/11	3/6	0/2	1/2	0/3	0/3	0/1	N.E.	N.E.	N.E.	1/1	28
Eczema/Flushing	0/11	0/6	1/2	2/2	0/3	2/3	1/1	N.E.	N.E.	N.E.	0/1	33
Developmental												
Patent ductus arteriosus	0/11	2/6	1/2	1/2	0/3	1/3	0/1	N.E.	N.E.	1/1	0/1	32
Delayed cranial suture closure	0/11	1/6	2/2	1/2	3/3	2/3	1/1	1/2	N.E.	N.E.	0/1	55
Other*	-	-	-	-	-	-	-	-	SB	ASD	0/1	
DNA change	Ex6_c.511T>C	Ex4_c.418G>C	Ex4_c.418G>C	Ex3_c.232_241 delinsCA	Ex2_c.175_176 delCT	Ex1_c.52G>T	Ex2_c.120delA	Ex4_c.418G>C	Ex1_c.1A>T	Ex4_c.418G>C	IVS2_c.G217+1G>A	
Protein change	p.S193P	p.A140P	p.A140P	p.V78QfsX11	p.L59VfsX8	Gly18Cys	p.Glu40fsX31	p.A140P	p.M1L	p.A140P	splice	

ICNC: Isolated congenital nail clubbing; PHO: primary hypertrophic osteoarthropathy; F: female; M: male; N.E.: not evaluated. Other associated features found in single patients; ASD: atrial septal defect; SB: saccular bronchiectasiasis.

Results

HPGD sequence analysis identified in the proband a novel G to A homozygous substitution affecting the obligatory donor splice site of exon 2 (c.217+1G>A). This change was absent in 150 unaffected individuals and segregated from heterozygous parents. Bioinformatics analysis predicted that the G nucleotide at position c.217+1 belonged to the donor splice site of exon 2. In detail, HSF software pointed out that the c.217+1G>A mutation significantly decreases the consensus value of this splice site (-27.98%) and NNSPLICE indicated the abolition of the donor splice motif. Both tools predicted the activation of alternative donor sites in intron 2. Based on these data, we infer the c.217+1G>A mutation decreases the strength of the consensus exon 2 donor site, putting into action an alternative downstream site. These results point to the possible retention of a share of intron 2. Results of the review of mutated *HPGD* patients are shown in Table II.

Discussion

We report on a 13-year-old boy affected by PHO, homozygous for the novel c.G217+1G>A mutation in the *HPGD* gene. So far, only seven distinct *HPGD* pathogenic alterations have been identified consisting in 4 missense and 3 non-sense/frameshift (8,10,12,14). Yet, we describe the first splice site mutation in the gene which is predicted to cause the formation of an abnormal transcript.

Among previously reported *HPGD* mutations, six were identified in single families and are distributed in exons 1, 2, 3 and 6, while the p.A140P alteration, recurring in 4 out of 11 families, is located in exon 4 (8, 10, 12, 14). Of note, all are homozygous mutations, consanguinity being reported in ten out of eleven families (Table II).

Comparison of clinical characteristics observed in the 35 *HPGD*-mutated patients delineates a homogeneous phenotype defined by digital clubbing, periostosis, acro-osteolysis, which are constant features, and mild skin involvement especially with respect to pachydermia. In fact, skin thickening was not clinically evident in the family reported by Tariq *et al*. (2009) nor in 11 out of 24 *HPGD*-mutated PHO patients. When present, pachydermia was mild and limited to palms and soles while puckering of face and forehead was reported only in one patient (7). The association of *HPGD* mutations to a rather mild PHO clinical appearance is also supported by the exclusion of mutations in a patient with severe pachydermia of hands and feet, facial furrowing with redundant skin on cheeks and forehead, blepharoptosis, cutis verticis gyrata, seborrhoea, folliculitis and hyperhidrosis (2). A mild form of pachydermoperiostosis was recently delineated in which pachydermia was absent or limited to palmar skin (5). Notably, in this family, a mutation in the *HPGD* gene is probable. However, additional skin manifestations including hyperhidrosis (92% of the cases) and seborrhoea (67%) are consistent features of PHO.

Osteoarticular manifestations are constant findings in *HPGD*-mutated patients. In particular, digital clubbing of fingers and toes is observed in all patients and can manifest since the first months of life. Periostosis and acro-osteolysis have been detected in every radiologically investigated patient. More than 2/3 of mutated individuals complained of joints pain which notably appeared much earlier than usually reported in PHO. Relief of arthralgia by nonsteroidal anti-inflammatory therapy was observed in treated patients (8, present patient).

Delayed closure of cranial sutures occurs in more than half of the *HPGD*-mutated patients while PDA was reported in about one third of the cases. However, these figures are likely to be underestimated as these manifestations may not be evident in adults. Of note, together with digital clubbing, these features highlight the juvenile PHO phenotype.

No obvious genotype-phenotype correlation emerges from comparison between *HPGD* mutation sites and clinical outcome, although the number of mutated patients is still limited (Table II). Notably, the p.S193P mutation causes isolated congenital nail clubbing. This alteration localises closer to the C-terminal domain of the protein compared to other *HPGD* mutations possibly affecting its function less severely (10).

In conclusion, a homogeneous phenotype results from *HPGD* mutations consisting in digital clubbing, periostosis, acro-osteolysis, hyperhidrosis and mild pachydermia. Large scale screening are needed to confirm these observations and to assess the role of *HPGD* gene among the whole PHO clinical spectrum.

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