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

L. Sinibaldi, G. Harifi, I. Bottillo, M. Iannicelli, S. El Hassani, F. Brancati, B. Dallapiccola

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.
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-os teoarthropathy syndrome, or Curra rino idio pathic 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 E₂ (PGE₂) catabolising enzyme, highly expressed in the lung. In patients with HPGD mutations, the loss of enzymatic function causes a chronic elevation of circulating PGE₂; the consequent prolonged peripheral vasodilatation and the stimulating effect of PGE₂ 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 ar ticular 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 inter phalangeal art iculations 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-
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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.

Materials 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, preceded 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 3130x1, 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).

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.

<table>
<thead>
<tr>
<th>Exons</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GCTGGCTTGACAGTTTCCTC</td>
<td>AGTCTCGGAGTGTGGGC</td>
</tr>
<tr>
<td>2</td>
<td>GTGTGTTATTGTTGTTCCGTC</td>
<td>ACGTTCCCAGTGACAGATGTG</td>
</tr>
<tr>
<td>3</td>
<td>CCTCTCATGGCATAGGACATG</td>
<td>GTTCCATGACTCAAGAACCC</td>
</tr>
<tr>
<td>4</td>
<td>GATATCTTTTTTCACCTATGCG</td>
<td>TGAAGATTTTTTTTTGTTGCTCC</td>
</tr>
<tr>
<td>5</td>
<td>GAGTTTCACAAGCTACCTGG</td>
<td>TGAGATATGACGGTTGTTTAG</td>
</tr>
<tr>
<td>6</td>
<td>GAAACTGCTGAAAACCTACAAC</td>
<td>CTGTATAAGCTTTTTCTCCTCCC</td>
</tr>
<tr>
<td>7</td>
<td>CACATTCCCTTAAACATGTC</td>
<td>AGCTATGGCTAACACATAAGC</td>
</tr>
</tbody>
</table>
## Table II. Phenotypic and molecular findings in HPGD-mutated families.

<table>
<thead>
<tr>
<th>Family ID</th>
<th>ICNC PHO</th>
<th>PHO</th>
<th>% PHO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tariq et al., 2008</td>
<td>Uppal et al., 2008</td>
<td>Seifert et al., 2009</td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td>Pakistan</td>
<td>Pakistan</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Affected members</strong></td>
<td>11</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td><strong>Sex (F/M)</strong></td>
<td>7/4</td>
<td>4/2</td>
<td>1/1</td>
</tr>
<tr>
<td><strong>Age or range</strong></td>
<td>2-50 years</td>
<td>14-39 years</td>
<td>14, 35 years</td>
</tr>
</tbody>
</table>

### Skeletal
- **Digital clubbing**
  - Tariq et al.: 11/11
  - Uppal et al.: 6/6
  - Seifert et al.: 2/2
  - Yüksel-Konuk et al.: 1/1
  - this report: 100%
- **Periostosis**
  - Tariq et al.: 0/2
  - Uppal et al.: 3/3
  - Seifert et al.: 1/1
  - Yüksel-Konuk et al.: 3/3
  - this report: 100%
- **Acro-osteolysis**
  - Tariq et al.: 0/2
  - Uppal et al.: 3/3
  - Seifert et al.: 1/1
  - Yüksel-Konuk et al.: 3/3
  - this report: 100%
- **Arthralgia**
  - Tariq et al.: 0/11
  - Uppal et al.: 4/6
  - Seifert et al.: 2/2
  - Yüksel-Konuk et al.: 2/3
  - this report: 63%
- **Swollen joints**
  - Tariq et al.: 0/11
  - Uppal et al.: 2/6
  - Seifert et al.: 2/2
  - Yüksel-Konuk et al.: 0/3
  - this report: 33%

### Skin
- **Hyperhidrosis**
  - Tariq et al.: 0/11
  - Uppal et al.: 5/6
  - Seifert et al.: 0/2
  - Yüksel-Konuk et al.: 2/3
  - this report: 92%
- **Pachydermia**
  - Tariq et al.: 0/11
  - Uppal et al.: 3/6
  - Seifert et al.: 1/2
  - Yüksel-Konuk et al.: 2/3
  - this report: 54%
- **Seborrhoea**
  - Tariq et al.: 0/11
  - Uppal et al.: 5/6
  - Seifert et al.: 0/2
  - Yüksel-Konuk et al.: 0/3
  - this report: 67%
- **Acne**
  - Tariq et al.: 0/11
  - Uppal et al.: 3/6
  - Seifert et al.: 0/2
  - Yüksel-Konuk et al.: 0/3
  - this report: 28%
- **Eczema/flushing**
  - Tariq et al.: 0/11
  - Uppal et al.: 0/11
  - Seifert et al.: 0/2
  - Yüksel-Konuk et al.: 1/1
  - this report: 33%

### Developmental
- **Patent ductus arteriosus**
  - Tariq et al.: 0/11
  - Uppal et al.: 2/6
  - Seifert et al.: 1/2
  - Yüksel-Konuk et al.: 0/3
  - this report: 32%
- **Delayed cranial suture closure**
  - Tariq et al.: 0/11
  - Uppal et al.: 1/6
  - Seifert et al.: 2/2
  - Yüksel-Konuk et al.: 0/3
  - this report: 55%
- **Other**
  - Tariq et al.: -
  - Uppal et al.: -
  - Seifert et al.: -
  - Yüksel-Konuk et al.: -
  - this report: -

### DNA change
- Tariq et al.: Ex6_c.511T>C
- Uppal et al.: Ex4_c.18G>C
- Seifert et al.: Ex3_c.232_241delinsCA
- Yüksel-Konuk et al.: Ex1_c.52G>T
- this report: Ex1_c.1A>T

### Protein change
- Tariq et al.: p.S193P
- Uppal et al.: p.A140P
- Seifert et al.: p.A140P
- Yüksel-Konuk et al.: p.M1L
- this report: -

**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 bronchiectasis.
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.217+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 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.

References