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# Primary Hypertrophic Osteoarthropathy: an update on patient features and treatment

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**MANUSCRIPT WORD COUNT**: 1511

Hypertrophic osteoarthropathy (HO) is a disorder characterized by changes to the skin and bones, and occurs either in a rare familial primary form, also called pachydermoperiostosis (PHO), with a 9:1 male:female prevalence ratio, or more commonly secondary to an underlying pathology.(1) Key features include digital clubbing, periostosis with bone and joint enlargement and skin changes, such as pachydermia, abnormal furrowing, seborrhea and hyperhidrosis. Specific developmental abnormalities have been found in some PHO patients, such as wide cranial sutures, Wormian bones and patent *ductus arteriosus*.(2) In adults, when all major clinical features are present, PHO is relatively easy to diagnose. However, for the pediatrician, who often has to deal with an incomplete clinical presentation(3), diagnosis may be a challenge. The recent discovery of mutations in two prostaglandin pathway genes *HPGD* and *SLCO2A1* has clarified the autosomal recessive inheritance (MIM #259100, MIM #614441) of this genetically heterogeneous condition(4–7). Here we present four previously undescribed patients who exemplify the gene-dependent presentations of PHO and provide diagnostic and treatment advice.

The subjects' written consent was obtained in conformance to the Declaration of Helsinki.

Key clinical features are listed in Table 1. Secondary causes of hypertrophic osteoarthropathy were excluded(8) and DNA sequence analysis (supplementary data) confirmed the clinical diagnoses of PHO/PDP.

**Case details**

*Patient 1*

A 20 year old male patient had begun limping aged 14 months. At 3 years old chronic arthritis of both knees was investigated by arthroscopy, with non-specific chronic synovial

inflammation at biopsy. Oligoarticular juvenile idiopathic arthritis (JIA) was diagnosed and the patient was treated with non-steroidal anti-inflammatory drugs (NSAIDs), with clinical improvement. At age 5 coarse facies was noted. Tests for autoimmune diseases and mucopolysaccharidosis were normal as well. In the following years, the frequent relapses with arthritis, required synovectomy, intra-articular steroid injections, immunosuppressive therapies (sulfasalazine and then methotrexate) and TNFα-blocking agents (etanercept and adalimumab), with modest efficacy. From the age of 12 years, "watch-glass" nails and digital clubbing started to develop (supplementary Figure S1), associated with a worsening pachydermia and furrowing of the forehead, leading to the clinical suspicion of PHO. The diagnosis was supported by the elevated prostaglandin E2 (PGE2) levels (Supplementary Table) and by sequencing *HPGD,* which identified two previously described heterozygous mutations (2,4)(Table 1, supplementary FigureS2). High dose therapy with NSAIDs (ibuprofen 30 mg/kg/day) was started and is currently maintained, with clinical improvement even if not complete recovery.

*Patient 2*

A 21 year old male patient was first admitted at age 13 due to isolated swelling and pain in the knees. This was diagnosed as oligoarticular JIA and treated with local steroid injections, and afterwards with NSAIDs and salazopyrin because of persisting arthritis. Arthroscopy with biopsy and synovial fluid analysis confirmed non-specific chronic synovitis. In the following years the extension of the disease to wrists, hips, ankles and spine, led to the use of methotrexate (15 mg/m$^2$/week) and NSAIDs (diclofenac 2 mg/kg/day), with low efficacy. At age 18 the patient started to develop severe aplastic anemia requiring multiple

transfusions, and thrombocytopenia, progressively associated with mild leukopenia (Figure 1). Bone marrow examination showed hypocellularity (<5% to 40%), trilinear hematopoeisis with dyserythropoiesis and moderate reticular fibrosis. Maintenance therapy of prednisone 35 mg/day was started and then tapered gradually. Hyperhidrosis and thickening of the skin, with *cutis verticis gyrata* and seborrhea also became increasingly evident (Supplementary Figure S3). No digital clubbing was observed. PHO was suspected, and elevated PGE2 (Supplementary Table) and DNA sequencing confirmed this diagnosis. Compound heterozygosity for two nonsense mutations in *SLCO2A1* was found: c.754C>T (p.R252X), previously described (9), and c.794C>G (p.S265X), which was novel (Table 1, supplementary Figure S3). The patient continued on ibuprofen 400 mg 3 times per day, combined with low-dose steroid therapy (prednisone 10mg/day) for myelofibrosis. The haematological picture improved dramatically, achieving complete normalization. The patient had no further joint relapses, but follow-up X-rays of hands, knees and ankles showed metacarpal and fibular bilateral periostosis (Supplementary figure S4). Generalized hyperhidrosis required oxybutynin 2.5 mg three times per day, with great efficacy. Considering the stabilization of the rheumatological and haematological picture, and the risk of worsening thrombocytopenia, NSAIDs were stopped and used again only in the last year for episodes of back pain.

*Patient 3*

A 15 year-old girl was first evaluated aged 9 years for arthralgias in knees, back and ankles. She also had hyperhidrosis and a history of delayed suture closure. Over time, more pronounced coarse facial features developed. At 11 years old she had signs of active

arthritis in knees, hands, hips and feet, with normal lab tests and X-rays. Just MRI showed chronic arthritis in the left wrist. Therapy with NSAIDs (indomethacin 1 mg/kg/day) was started, with remarkable improvement of the joint involvement and pain relief. At 15 years old, the patient relapsed with arthritis of the knees, associated, for the first time, with digital clubbing, hyperhidrosis and mild anemia. A brief period of methotrexate (15 mg/m$^2$/week) was tried with no effect. PHO was diagnosed through genetic testing of *HPGD*, identifying the homozygous c.120delA mutation (Table 1). Indomethacin was doubled, with significant clinical improvement, both in the joint and skin involvement. The dysmorphic features did not progress.

*Patient 4*

This male child was evaluated at the age of 2.5 years after pre term birth (33+4 weeks; birth weight 1700 g) for swelling and stiffness of the knees, gradually followed by involvement of ankles, toes and hands. Throughout the course of his disease he had required surgery for a patent *ductus arteriosus* and physical therapy, including helmet remodeling, in the first months of life for brachyplagiocephaly. Delayed suture closure and late motor development and speech/language were observed. Physical examination also identified hyperhidrosis of the hands. Blood tests including ANA, RF and inflammatory markers, ultrasound scan of the skull and MRI of the knees were normal. Treatment with NSAIDs (ibuprofen 20 mg/kg/day) was started, with positive results. At 3 years, bony enlargement of the distal phalanges and a thickened skull were revealed by X-rays. At age 4, PHO was diagnosed by the identification of two compound heterozygous *HPGD* mutations: c.175_176delCT and the novel c.298T>C (p.W100R) (Table 1, supplementary data and Figure S5). The patient was treated with

ibuprofen, initially at 20 mg/kg/day and subsequently as necessary, with a stable clinical pattern requiring therapy around 2-3 times a week.

Here we have described four PHO/PDP patients with *HPGD* or *SLCO2A1* deficiency (OMIM#259100, PHOAR1 and #614441, PHOAR2, respectively).

Although mutations in either *HPGD* or *SLCO2A1* cause PHO/PDP, there are phenotypic differences associated with each gene exemplified in patients 1 and 2 who had mutations in *HPGD* and *SLCO2A1,* respectively. Patient 1 developed initial symptoms much earlier than patient 2. The onset of PHO is usually before the age of 20, but its bi-phasic incidence is now attributed to the genetic heterogeneity, with onset of symptoms in early childhood for *HPGD* patients and during puberty/early adulthood for *SLCO2A1* patients (5,6,9–13).

Patients with *SLCO2A1* mutations, such as patient 2, have been described as having more severe symptoms overall (6,9,11), the presence of skin folds (as cutis *verticis gyrata*) (5,12) and chronic anaemia and/or pancytopaenia, secondary to hypocellular myelofibrosis (5,14,15). On the other hand, patients 3 and 4 exhibited delayed cranial suture closure, which has led to the use of the term “cranio-osteoarthropathy”. This has only been seen in patients with *HPGD* mutations (2,4,12,16).

The histories of our patients suggest approaches for clinical practice. For example, young children with swollen and painful joints and a history of patent *ductus arteriosus*, delayed closure of the cranial sutures and fontanelles or Wormian bones, should be investigated immediately for PHO, as clubbing, periostosis and skin changes might be absent. When PHO/PDP is suspected, secondary causes have to be excluded. Blood and early morning urine can be considered for PG measurement, but their detection is not feasible in all

laboratories and implications in differential diagnosis, follow-up and treatment still need to be studied and standardized with age-related values.(13) In contrast, sequence analysis of both genes is simple and cheap, and therefore should be considered as an early investigation when PHO/PDP is suspected, as it can provide confirmation of diagnosis. The presence of chronic arthritis often leads to a diagnosis of JIA. Nevertheless, a JIA patient, non-responder to DMARDs and biological therapies, with a better response to NSAIDs, should be considered as suffering from another disease and investigated for further clinical signs. Indeed, *HPGD* and *SLCO2A1* patients usually experience some positive effects on joint pain and swelling using NSAIDS (5,12,16–18), but it is uncertain if either the use of indomethacin compared with ibuprofen could provide better results clinically, as for patient 3, or if the phenotype of this patient is milder as a consequence of being female.

Myelofibrosis is a life-threatening complication; consequently, when germline *SLCO2A1* mutations are detected, the patient should be followed up periodically for myelofibrosis. The use of steroids can produce haematological improvement, but no effects on skin or digital clubbing, and longer-term treatment may help in symptom stabilization (19). NSAIDs appear to be the best option for treatment, while other treatments can be evaluated individually, but support for their use is largely anecdotal (20–22).

**KEY MESSAGES**

- The histories of our patients and the study of their genotype suggest approaches for clinical practice.
- Sequencing of genes should be considered as an early investigation in patients suspected for PHO.
- The use of NSAIDs according to symptoms is the best and up to now the only therapeutic option in PHO patients.
- Steroid therapy for myelofibrosis is an important possibility.

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**REFERENCES**


1. Friederich N. Hyperostose des gesammtenskelettes. Arch Path Anat. 1868;43:83–7.

2. Seifert W, Beninde J, Hoffmann K, Lindner TH, Bassir C, Aksu F, et al. HPGD mutations cause cranioosteoarthropathy but not autosomal dominant digital clubbing. Eur J Hum Genet [Internet]. 2009 Dec [cited 2014 Sep 29];17(12):1570–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2987025&tool=pmcentrez&rendertype=abstract

3. Latos-Bielenska A, Marik I, Kuklik M, Materna-Kiryluk A, Povysil C, Kozlowski K. Pachydermoperiostosis-critical analysis with report of five unusual cases. Eur J Pediatr [Internet]. 2007 Dec [cited 2014 Sep 18];166(12):1237–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17285282

4. Uppal S, Diggle CP, Carr IM, Fishwick CWG, Ahmed M, Ibrahim GH, et al. Mutations in 15-hydroxyprostaglandin dehydrogenase cause primary hypertrophic osteoarthropathy. Nat Genet [Internet]. 2008 Jun [cited 2014 Sep 29];40(6):789–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18500342

5. Diggle CP, Parry D a, Logan C V, Laissue P, Rivera C, Restrepo CM, et al. Prostaglandin transporter mutations cause pachydermoperiostosis with myelofibrosis. Hum Mutat [Internet]. 2012 Aug [cited 2014 Sep 29];33(8):1175–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22553128

6. Seifert W, Kühnisch J, Tüysüz B, Specker C, Brouwers A, Horn D. Mutations in the prostaglandin transporter encoding gene SLCO2A1 cause primary hypertrophic osteoarthropathy and isolated digital clubbing. Hum Mutat [Internet]. 2012 Apr [cited 2014 Sep 29];33(4):660–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22331663

7. Zhang Z, Xia W, He J, Zhang Z, Ke Y, Yue H, et al. Exome sequencing identifies SLCO2A1 mutations as a cause of primary hypertrophic osteoarthropathy. Am J Hum Genet [Internet]. The American Society of Human Genetics; 2012 Jan 13 [cited 2014 Aug 27];90(1):125–32. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3257902&tool=pmcentrez&rendertype=abstract

8. Pineda C, Martínez-Lavín M. Hypertrophic osteoarthropathy: what a rheumatologist should know about this uncommon condition. Rheum Dis Clin North Am [Internet]. Elsevier Inc; 2013 May [cited 2014 Sep 29];39(2):383–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23597970

9. Cheng R, Li M, Guo Y, Yao Y, Gao C, Yao Z. Three novel mutations in the SLCO2A1 gene in two Chinese families with primary hypertrophic osteoarthropathy. Eur J Dermatol [Internet]. Jan [cited 2014 Aug 27];23(5):636–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24153155

10. Zhang Z, He J-W, Fu W-Z, Zhang C-Q, Zhang Z-L. A novel mutation in the SLCO2A1 gene in a Chinese family with primary hypertrophic osteoarthropathy. Gene [Internet]. Elsevier B.V.; 2013 May 25 [cited 2014 Sep 29];521(1):191–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23531451

11. Madruga Dias J a C, Rosa RS, Perpétuo I, Rodrigues AM, Janeiro A, Costa MM, et al. Pachydermoperiostosis in an African patient caused by a Chinese/Japanese SLCO2A1 mutation-case report and review of literature. Semin Arthritis Rheum [Internet]. Elsevier; 2014 Feb [cited 2014 Aug 27];43(4):566–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24012041

12. Tüysüz B, Yılmaz S, Kasapçopur O, Erener-Ercan T, Ceyhun E, Bilguvar K, et al. Primary hypertrophic osteoarthropathy caused by homozygous deletion in HPGD gene in a family: changing clinical and radiological findings with long-term follow-up. Rheumatol Int [Internet]. 2014 May 12 [cited 2014 Sep 29]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24816859

13. Zhang Z, He JW, Fu WZ, Zhang CQ, Zhang ZL. Mutations in the SLCO2A1 gene and primary hypertrophic osteoarthropathy: A clinical and biochemical characterization. J Clin Endocrinol Metab. 2013;98(5):923–33.

14. Castori M, Sinibaldi L, Mingarelli R, Lachman RS, Rimoin DL, Dallapiccola B. Pachydermoperiostosis: an update. Clin Genet [Internet]. 2005 Dec [cited 2014 Sep 29];68(6):477–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16283874

15. Fontenay-Roupie M, Dupuy E, Berrou E, Tobelem G, Bryckaert M. Increased proliferation of bone marrow-derived fibroblasts in primitive hypertrophic osteoarthropathy with severe myelofibrosis. Blood [Internet]. 1995 Jun 1;85(11):3229–38. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7756654

16. Bergmann C, Wobser M, Morbach H, Falkenbach A, Wittenhagen D, Lassay L, et al. Primary hypertrophic osteoarthropathy with digital clubbing and palmoplantar hyperhidrosis caused by 15-PGHD/HPGD loss-of-function mutations. Exp Dermatol [Internet]. 2011 Jun [cited 2014 Sep 29];20(6):531–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21426412

17. Erken E, Köroğlu Ç, Yıldız F, Özer HTE, Gülek B, Tolun A. A novel recessive 15-hydroxyprostaglandin dehydrogenase mutation in a family with primary hypertrophic osteoarthropathy. Mod Rheumatol [Internet]. 2013 Apr 25 [cited 2014 Sep 29]; Available from: http://link.springer.com/10.1007/s10165-013-0882-3

18. Diggle CP, Carr IM, Zitt E, Wusik K, Hopkin RJ, Prada CE, et al. Common and recurrent HPGD mutations in Caucasian individuals with primary hypertrophic osteoarthropathy. Rheumatology (Oxford) [Internet]. 2010 Jun [cited 2014 Sep 29];49(6):1056–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20299379

19. Ninomiya S, Hara T, Tsurumi H, Kanemura N, Kasahara S, Ogawa Y, et al. Myelofibrosis Successfully Treated with Prednisolone in a Patient with Pachydermoperiostosis.

Intern Med [Internet]. 2011 [cited 2014 Sep 29];50(19):2207–11. Available from: http://joi.jlc.jst.go.jp/JST.JSTAGE/internalmedicine/50.5717?from=CrossRef

20. Jojima H, Kinoshita K, Naito M. A case of pachydermoperiostosis treated by oral administration of a bisphosphonate and arthroscopic synovectomy. Mod Rheumatol [Internet]. 2007 Jan;17(4):330–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17694269

21. Warwas S, Specker C, Jager M. Arthroscopic synovectomy and radiosynoviorthesis: a treatment option for recurrent arthritis symptoms in patients with pachydermoperiostosis. Reumatismo. 2013;65(2):82–5.

22. Da Costa FV, de Magalhães Souza Fialho SC, Zimmermann AF, Neves FS, Werner de Castro GR, Pereira IA. Infliximab treatment in pachydermoperiostosis: a rare disease without an effective therapeutic option. J Clin Rheumatol [Internet]. 2010 Jun [cited 2014 Sep 29];16(4):183–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20414127

| | Patient No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| gender | | **male** | **male** | **female** | **male** |
| Age (years) | Current | 20 | 21 | 15 | 5 |
| | At disease onset | 1.3 | 13 | 1 | 2 |
| | At first observation | 2.2 | 13 | 9 | 2.5 |
| Clinical symptoms | Joint enlargement at onset | Knees, ankles and hands | Knees | Knees, back, ankles | Knees |
| | Developmental problems | Mild cognitive delay | | | Motor development and speech-language delay; ADHD patent *ductus arteriosus*, delayed suture closure |
| | Dysmorphic features | Broad nasal bridge, mild hypertelorism, coarse facial features | Coarse facial features | Delay in suture closure, broad nasal bridge, coarse facial features | Plagiocephaly, abnormal eye slits, slightly dysplastic ears, not palpable left testicle |
| | Skin abn | Pachydermia | Hyperhydrosis, seborrhea, *cutis verticis gyrate*, pachydermia | Hyperhydrosis, pachydermia | Hyperhydrosis |
| | Hematological abn | | Aplastic anemia, thrombocytopenia, splenomegaly, late-onset leukopenia | Anemia | |
| | Digital clubbing | Watch-glass nails and clubbing | | Watch-glass nails and clubbing | |
| Diagnosis | CRP, ESR | neg | neg | neg | neg |
| | ANA | neg | neg | neg | neg |
| | Urine crystals | neg | neg | neg | neg |
| | Test for MPS | neg | | | |
| | Bone marrow | | Aplastic marrow with focal hematopoiesis , mainly erythropoiesis | No abn | |

| | | | | | |
|---|---|---|---|---|---|
| | | | (less than 1%) | | |
| | PG* | x | x | | |
| Imaging | X ray | no abn | no abn | Acro-osteolysis | Enlargement of the bone of the distal phalanges, thickening of the skull |
| | MRI | Aspecific enhancement of the peri-articular soft tissue in the knees | Mild synovitis in the knees | Wrists and hands: no abn | Knees and skull: no abn |
| Genetic test PHO | HPGD | c.120delA + c.175_176delCT | | c.120delA | c.175_176delCT + c.298T > C |
| | SLCO2A1 | | c.754C > T + c.794C > G | | |
| Further negative genetic tests | | Karyotype, VCFS and CACP genetics | | | FMR1 gene and CGH- array test |
| Therapy** | Knee steroid injection | x | x | x | |
| | Synoviectomy | x | x | | |
| | NSAIDs | x | x | x | x |
| | Salazopyrin | x | x | | |
| | MTX | x | x | | |
| | Systemic steroid therapy | | x | | |
| | Etanercept | x | | | |
| | Adalimumab | x | | | |

Table 1. Patients' clinical features. No: number; abn: abnormalities; CRP: C-reactive protein; ESR: erythrosedimentation rate; ANA: antinuclear antibodies; MPS: mucopolisaccaridosis; PG: prostaglandin; PHO: primary hypertrophic hosteoarthropathy; VCFS: velo-cardio-facial syndrome; CACP: Camptodactyly-Arthropathy-Coxa vara-Pericarditis; MTX: Methotrexate. *For prostaglandin dosage see Table 2.**For dose and period of therapy, see the text.

**Supplementary Information**

**Additional clinical features of patients:**

Patient 1:

The watch-glass nails and digital clubbing are shown in supplementary figure 1. In addition this patient also presented at age 5 with additional dysmorphic features: broad nasal bridge and mild hypertelorism, without a specific diagnosis. Cytogenetic testing for velocardiofacial syndrome and *PRG4* analysis for camptodactyly-arthropathy-coxavara-pericarditis (CACP) syndrome were normal.

Patient 2:

The facial skin changes are shown in supplementary figure S3 and periostosis in supplementary figure S4.

Patient 4:

Psychological support was started because the child developed attention-deficit/hyperactivity disorder (ADHD); array CGH analysis and *FMR1* testing were normal.

**Role of *HPGD* or *SLCO2A1* in the prostaglandin metabolism**

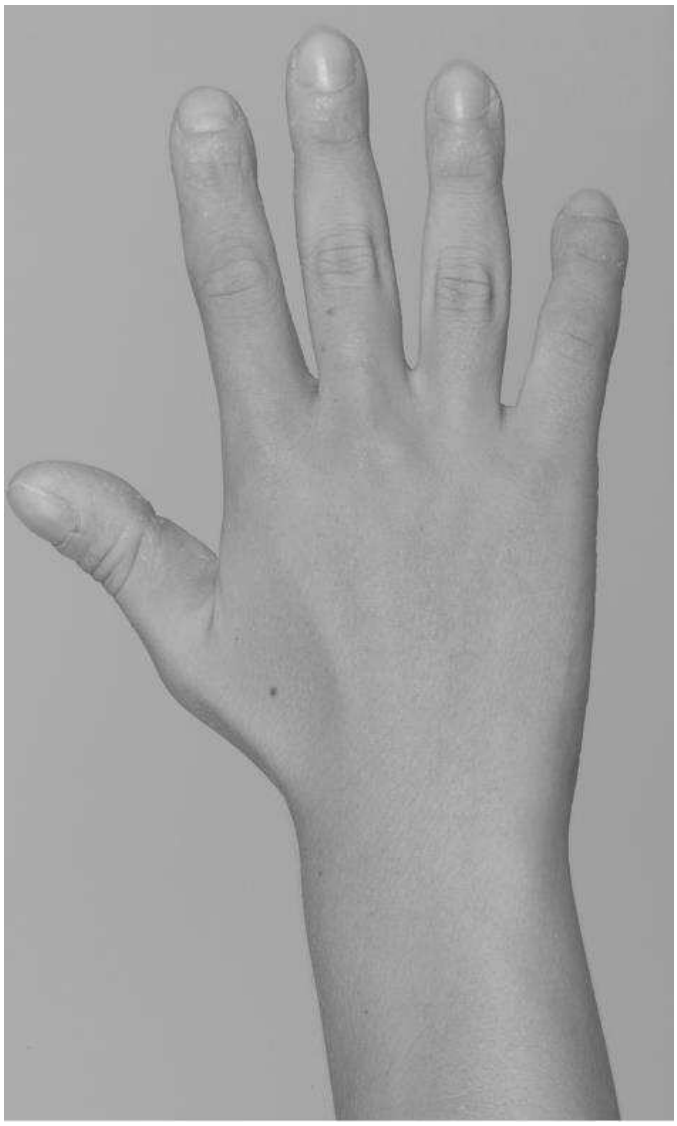
*HPGD* or *SLCO2A1* are genes both involved in $PGE_2$ degradation, through a 2-step process: firstly an active uptake into the cell by the transmembrane PG transporter encoded by *SLCO2A1*; then oxidation by HPGD. Normally $PGE_2$ in vivo has a short half-life, however aberrant HPGD or SLCO2A1 allow the levels to rise. When *HPGD* is mutated, $PGE_2$ is not readily catabolized and the levels of the PGE2 metabolites that are more stable than $PGE_2$, are very low (1). However, when *SLCO2A1* is mutated, some metabolites, can in addition be

elevated, suggesting that some access to intracellular HPGD must be achieved (2,3). The measurement of both $PGE_2$ and its metabolites can therefore be useful in detecting altered prostaglandin levels, and aid in discriminating between the *SLCO2A1* and *HPGD* genetic basis of PHO. The increased levels of $PGE_2$ are presumed to mediate not only osteoclast activation (and thereby acro-osteolysis), but also osteoblast activation, and hence new bone formation as in periostosis. The accompanying vasodilatatory effect may mediate digital clubbing (1).

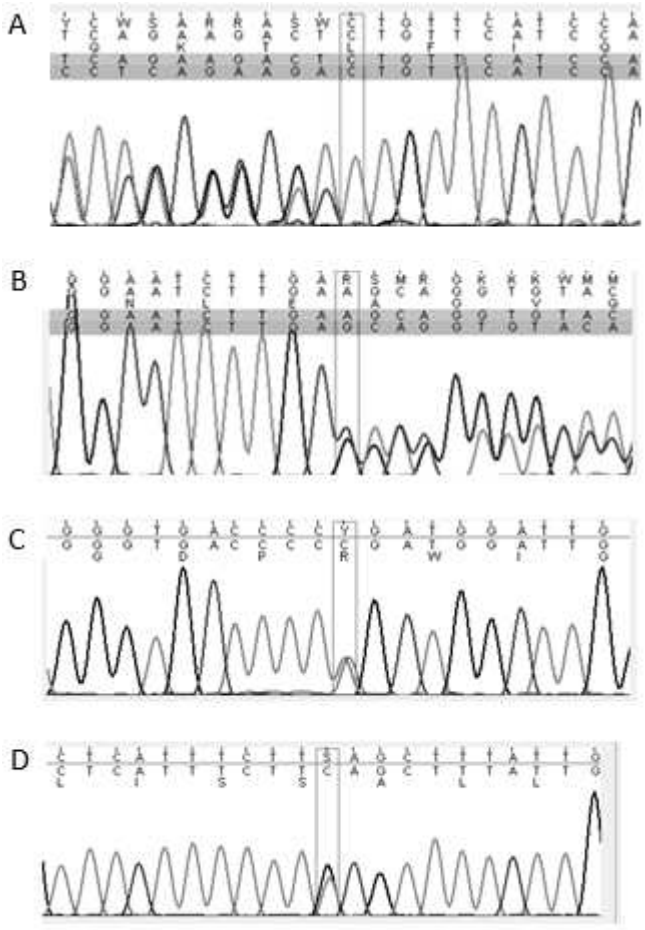
Measurement of systemic and urinary levels can be a challenge, and is not a standard test presently available for most clinicians. Blood samples can be collected in clinic for prostaglandin analysis. Early morning urine samples can also be collected and stored in a home freezer by the patient until collected on dry ice. These samples can be processed for mass spectrometry in specialized laboratories that have developed appropriate assays. This approach can identify sufficiently elevated levels of $PGE_2$ in PHO patients, though the levels in normal individuals can be below the level of detection. The levels of the metabolite can be more easily detected in normal individuals, as can elevated changes in PHO patients, though individuals with *HPGD* mutations are likely to not have detectable levels. Ideally both $PGE_2$ and PGE-M levels should be used to correctly identify individuals with altered levels. ELISA's are commercially available to measure PG levels, though there is data to indicate that there is intra-assay variation with this method (1-3). Prostaglandin levels can also be variable between different days when collected (1,3), and can vary with age of patient (4) and gender (1). This indicates the need for appropriate normal control samples and ideally multiple sampling. The value of monitoring $PGE_2$ levels during the course of the disease and any treatment, for example with NSAID therapy, remains to be determined, though it could be a means to help tailor treatment for PHO individuals.

*(An additional list of references is provided at the end of the supplementary section)*

**Supplementary Figure S1.** Patient 1 watch-glass nails and digital clubbing



**Supplementary Figure S2.** Sanger sequence data.



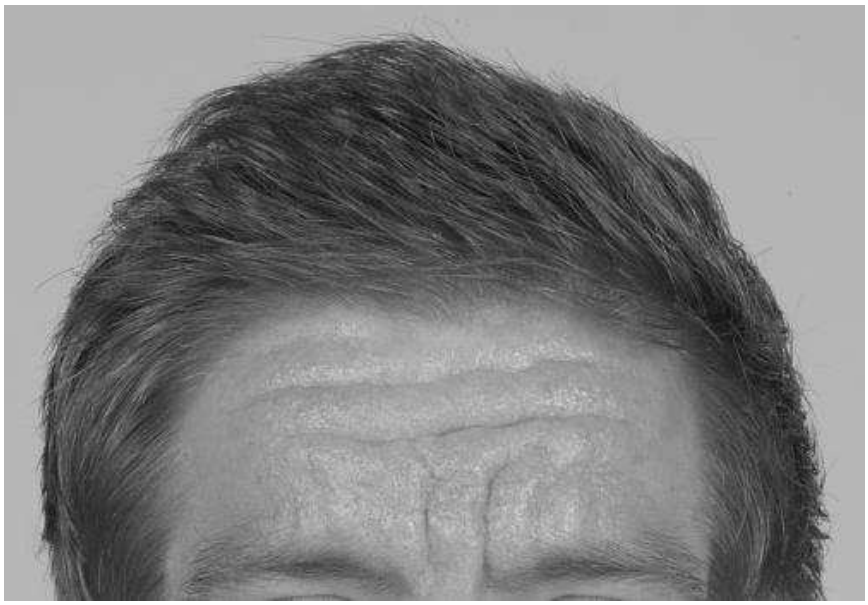

Sanger sequencing of PCR amplicons was used to detect sequence variants in *HPGD* and *SLCO2A1*. Analysis of Sanger sequence data was performed using the GeneScreen program(5).

Analysis of patient 1 data identified in *HPGD* (A) a heterozygous c.175-176delCT p.LysVfsX8 mutation using a reverse sequencing primer and (B) a heterozygous c.120delA p.Glu40fsX31 mutation using a forward sequencing primer. These mutations have been described previously in PHO patients(1,6).

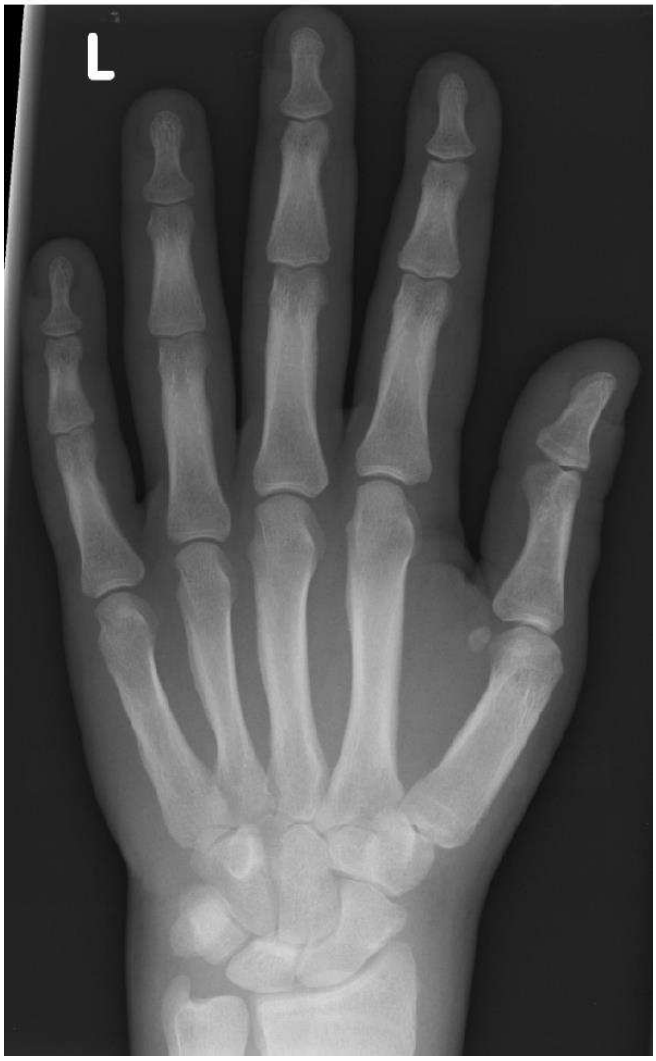
Analysis of patient 2 data identified in *SLCO2A1* (C) a heterozygous c.754C>T p.R252X mutation and (D) a heterozygous c.794C>G p.S265X mutation. c.754C>T

(p.R252X) had been previously described in a patient with isolated digital clubbing where a single heterozygous mutation was identified (6), while c.794C>G (p.S265X) was novel.

**Supplementary Figure S3:** Patient 2 pachydermia with furrowing of the forehead.



**Supplementary Figure S4**. Patient 2: X ray of the left hand showing periostosis.

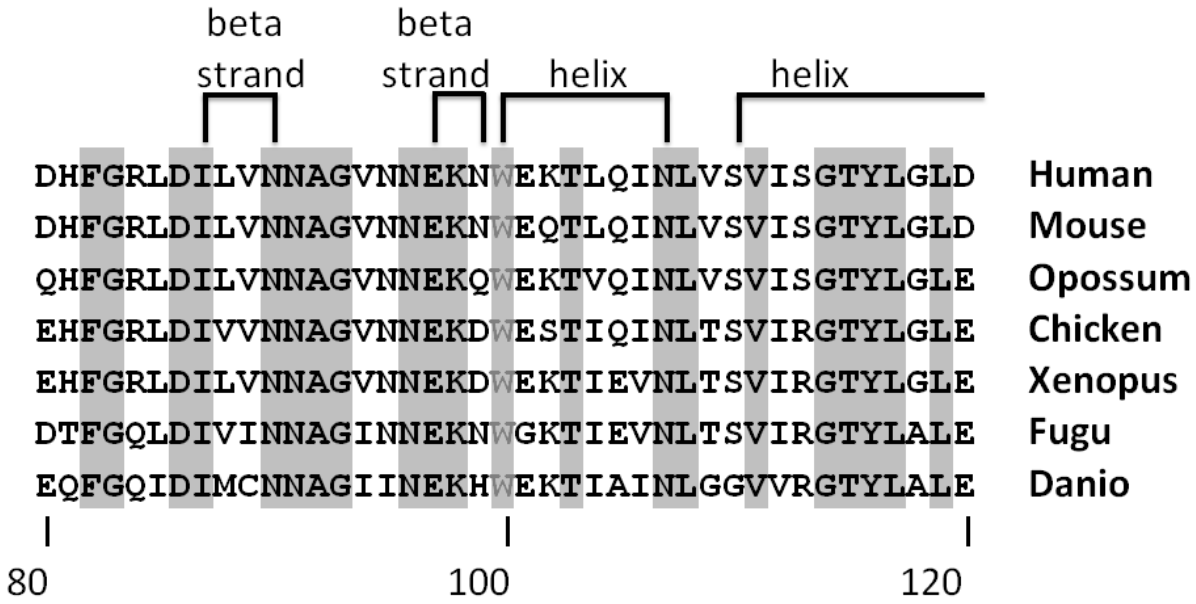


**Supplementary Figure S5.** Bioinformatic analysis of the novel HPGD missense variant c.298T>C.

Population frequencies of the c.298T>C were assessed using the 1000 Genomes dataset (www.1000genomes.org), where it was found to be a rare variant. Investigating the potential functional impact of the p.W100R using PolyPhen-2 and SIFT it was predicted it was likely to have a damaging effect (7,8).

The alignment of the HPGD protein is shown from amino acid 80 to 120 for seven species. The tryptophan at position 100 is in red text. Grey shading indicates sequence identity across all seven species. The sequences used for comparison were human NP_000851, mouse NP_032304, opossum ENSMODT00000004532, chicken NP_001264713, Xenopus tropicalis (frog) NP_001007992, Takifugu rubripes (Japanese puffer fish) ENSTRUT00000025332, and Danio rerio (zebra fish) LOC393297.

Protein data from UniProt (http://www.uniprot.org/) indicated that the p.W100R was at the start of an eight amino acid sized helix.


beta strand
beta strand
helix
helix
DHFGRLDILVNNAGVNNEKNWEKTLQINLVSVISGTYLGLD Human
DHFGRLDILVNNAGVNNEKNWEQTLQINLVSVISGTYLGLD Mouse
QHFGRLDILVNNAGVNNEKQWEKTVQINLVSVISGTYLGLE Opossum
EHFGRLDIVVNNAGVNNEKDWESTIQINLTSVIRGTYLGLE Chicken
EHFGRLDILVNNAGVNNEKDWEKTIEVNLTSVIRGTYLGLE Xenopus
DTFGQLDIVINNAGINNEKNWGKTIEVNLTSVIRGTYLALE Fugu
EQFGQIDIMCNNAGIINEKHWEKTIAINLGGVVRGTYLALE Danio
80
100
120

**Supplementary Table 1.** Prostaglandin analysis

An analytical platform with high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was used to detect prostaglandin (PG) levels in patients 1 and 2 (9).

| | **PGE2** (nM) | **11betaPGE2** (nM) | **PGE1** (nM) |
|---|---|---|---|
| **Pt1** | 3 | 2.15 | 3.3 |
| **Pt2** | 2.08 | 4.2 | 4.95 |
| **LODs** | 0.6793 | 0.7930 | 0.0201 |

Prostaglandin dosage in plasma during treatment with Ibuprofen. Pt: patient; PG: prostaglandin; LODs: limits of detections, intended for the lowest quantity of metabolites detectable with the assay in subjects not affected by PHO. PGE2 metabolites were undetectable in the serum from 150 normal controls using this method.

**Supplementary References**


1. Uppal S, Diggle CP, Carr IM, Fishwick CWG, Ahmed M, Ibrahim GH, et al. Mutations in 15-hydroxyprostaglandin dehydrogenase cause primary hypertrophic osteoarthropathy. Nat Genet 2008;40:789–93.
2. Zhang Z, Xia W, He J, Zhang Z, Ke Y, Yue H, et al. Exome sequencing identifies SLCO2A1 mutations as a cause of primary hypertrophic osteoarthropathy. Am J Hum Genet 2012;90:125–32.
3. Diggle CP, Parry D a, Logan C V, Laissue P, Rivera C, Restrepo CM, et al. Prostaglandin transporter mutations cause pachydermoperiostosis with myelofibrosis. Hum Mutat 2012;33:1175–81.
4. Zhang Z, He JW, Fu WZ, Zhang CQ, Zhang ZL. Mutations in the SLCO2A1 gene and primary hypertrophic osteoarthropathy: A clinical and biochemical characterization. J Clin Endocrinol Metab. 2013;98:923–33.
5. Carr IM, Camm N, Taylor GR et al. GeneScreen: a program for high-throughput mutation detection in DNA sequence electropherograms. J Med Genet 2011;48:123-30.
6. Seifert W, Beninde J, Hoffmann K, Lindner TH, Bassir C, Aksu F, et al. HPGD mutations cause cranioosteoarthropathy but not autosomal dominant digital clubbing. Eur J Hum Genet 2009;17:1570–6.
7. Adzhubei I A, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods; 2010;7:248–9.

8. Ng PC. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31:3812–4.
9. Strassburg K, Huijbrechts AML, Kortekaas KA, Lindeman JH, Pedersen TL, Dane A, et al. Quantitative profiling of oxylipins through comprehensive LC-MS/MS analysis: application in cardiac surgery. Anal Bioanal Chem. 2012;404:1413–26.