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ARTICLE

HPGD mutations cause cranioosteoarthropathy but not autosomal dominant digital clubbing

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Cranio-osteoarthropathy, clinically classified as a variant of primary hypertrophic osteoarthropathy, is a very rare autosomal-recessive condition characterized by delayed closure of the cranial sutures and fontanels, digital clubbing, arthropathy, and periostosis. Recently, mutations in the gene *HPGD*, which encodes the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase, were reported in four families affected with primary hypertrophic osteoarthropathy and one family with autosomal-recessive isolated nail clubbing. We report the clinical and molecular findings in four patients from two families affected with cranio-osteoarthropathy and one family with isolated, autosomal dominant digital clubbing. Genome-wide homozygosity mapping identified a locus for cranio-osteoarthropathy harboring the *HPGD* gene in one affected family. We detected two novel homozygous mutations in *HPGD* in these families: a missense mutation affecting the NAD^+ binding motif and a frameshift mutation. The clinical presentation in our patients was variable. Digital clubbing and hyperhidrosis were present in all cases. Delayed closure of the cranial sutures and fontanels, periostosis, and arthropathy were not consistent clinical features. No *HPGD* mutation was detected in a familial case of autosomal dominant isolated digital clubbing. The failure to identify any mutation in a family with an autosomal dominant type of isolated digital clubbing suggests that *HPGD* is not the major gene for this condition.
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Introduction

Primary hypertrophic osteoarthropathy (PHO, MIM 259100, 167100) is genetically and clinically heterogeneous and is characterized by digital clubbing, arthropathy, and periostosis, which consists of new bone formation around the periosteum of long bones. For PHO, both autosomal-recessive and dominant inheritance have previously been postulated.[1] In contrast, hypertrophic osteoarthropathy (HO) develops as a secondary consequence to different acquired diseases such as intrathoracic tumors and shows clinical overlap with PHO.

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Two variants of PHO have been distinguished clinically (Supplementary Table 1). In addition to features of PHO, pachydermoperiostosis (MIM 167100, 259100) includes clinical signs of pachydermia, such as coarsening of the facial features as early as in adolescence, seborrheic hyperplasia, skin thickening, and excessive sweating.[2] Cranio-osteoarthropathy (COA, Currarino idiopathic osteoarthropathy, MIM 259100) shows, in addition to the other signs of PHO, poor neurocranium ossification with delayed closure of the cranial sutures and fontanels and increased number of wormian bones.[3–5] Clinical signs of pachydermia are absent in patients with COA.[3] An autosomal-recessive mode of inheritance has been suggested in COA by analysis of the affected pedigrees. Emphasizing the clinical overlap between pachydermoperiostosis and COA, O'Connell *et al* suggested that these disorders may be allelic.[6]

Recently, homozygous mutations in the 15-hydroxyprostaglandin dehydrogenase (*HPGD*) gene located at 4q33-q34 have been described to cause PHO.[7] *HPGD* encodes the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH, EC 1.1.1.141), which is a prostaglandin E_2 (PGE_2) catabolizing enzyme. Mutations reported so far in PHO very likely result in loss of enzymatic function. Elevated urine PGE_2 levels were found in all homozygous and some of the heterozygous mutational carriers.[7] These findings support the idea that impaired metabolism of PGE_2 is critical in PHO as well as in HO pathogenesis. PGE_2 is a ubiquitous lipid mediator regulated by a number of enzymes, including different COX enzymes. The COX enzymes are the pharmacological targets of non-steroidal anti-inflammatory drugs (NSAIDs). Owing to this pathway, NSAIDs are able to block PGE_2 synthesis. Further studies should clarify whether progression of PHO could be influenced by NSAID treatment.

Hpgd−/− mice are affected by patent ductus arteriosus and die shortly after birth because of high-output heart failure, but do not show other manifestations of PHO.[8] These findings in mice indicate that PGE_2 metabolism by 15-PGDH is required for the remodeling of the ductus arteriosus. In humans, an increased frequency of 25% for a patent ductus arteriosus has been documented in patients with PHO, whereas the general risk is about 0.05 % in full-term neonates.[6,7]

Recently, a homozygous missense mutation in *HPGD* has been identified in a single family affected with isolated congenital nail clubbing as an autosomal-recessive trait.[9]

In this study, we found significant linkage of COA to a region harboring the *HPGD* gene in one affected family and report novel mutations in *HPGD* causing COA in two families. However, in a third family with dominant inheritance of isolated digital nail clubbing, we found no mutation in *HPGD* indicating another underlying gene defect.

Material and methods

Patients

Written informed consent was obtained from all participants or their legal guardians.

Genome-wide linkage scan

Genotyping was performed using the 250k Affymetrix SNP chip (GeneChip Mapping 250K Nsp Assay Kit, GeneChip Mapping 250K Sty Assay Kit Affymetrix, Inc. (US), Santa Clara, CA, USA) in family, 1. We analyzed the data using ALLEGRO v1.2c (http://www.decode.com/software/allegro/), GENEHUNTER v2.1r5 (http://www.broad.mit.edu/ftp/distribution/software/genehunter/) under the graphical user-interface easyLINKAGE v5.08 (http://compbio.charite.de/genetik/hoffmann/easyLINKAGE/).[10,11] We assumed a recessive model with complete penetrance, disease allele frequency of 0.001, and equally distributed marker alleles. To save analysis time and to avoid linkage disequilibrium-related artifacts, we analyzed informative single nucleotide polymorphisms at every 0.5 cM along the genome. We included all available family members of family 1 and reconstructed haplotypes by ALLEGRO and manually. Next, we sequenced functional candidate genes within the linkage interval by standard sequencing procedures.[12]

Mutational analysis

Genomic DNA was isolated from the peripheral blood using standard techniques. For mutation screening, we amplified the coding region of *HPGD* (NM_000860), including the flanking intronic sequences and the predicted promoter region. Primer sequences and PCR conditions are available on request. PCR products were purified using the enzymes exonuclease I and shrimp alkaline phosphatase treatment, and directly sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) and analyzed on an automated DNA Analyzer (3730 Applied Biosystems).

Molecular modeling of the 15-PGDH

The multiple sequence alignment was performed using CLUSTALW implemented in the BioEDIT V7.0.5.3 (Tom Hall, Ibis Biosciences, Carlsbad, CA, USA) program. For studying the missense mutation G18C, we calculated its putative effect on 15-PGDH (2GDZ.pdb) using the mutate tool followed by energy minimization and computation of H-bonds, both implemented in the DeepView software (Swiss Pdp Viewer).

Results

Clinical reports

Family 1 This Turkish family is multiply consanguineous and the affected patients were offspring of first-cousin

parents (Supplementary Figure 1b). The affected propositus (patient V-1) was first seen at the age of 13 months for mild developmental delay and skeletal anomalies, such as widening of the cranial sutures and clubbing of the distal phalanges. He was born at 34 weeks of gestation after an uneventful pregnancy with normal measurements (weight 2185 g, length 44 cm, occipitofrontal head circumference (OFC) 32.5 cm). After birth, echocardiogram showed an atrioseptal defect II and a persistent ductus arteriosus; subsequently, the patient was treated with indometacin for 5 days. Feeding difficulties and atopic eczema were noted since his fifth month of life.

At the age of 10 months, muscular hypotonia, patent cranial sutures, and fontanels as well as clubbing of the distal phalanges were documented. The patient sat at the age of 9 months and was able to stand with support at the age of 13 months. He used first words at the age of 13 months.

Physical examination at the age of 13 months showed a height of 75 cm (−0.5 SD), weight of 7.5 kg (−2.5 SD), and OFC of 45 cm (−1.6 SD); open anterior and posterior fontanels, clubbing of all the digits and toes, large nails, sweaty hands and feet, and atopic eczema were still observed (Table 1 and Figures 1a and b).

Hand radiographs at the age of 9 months and 18 months, respectively, showed some diaphyseal constriction and tufted ends of the terminal phalanges (Figure 1c). Skull radiograph at the age of 22 months showed multiple wormian bones (Figure 1d). Computed tomography (CT) of the skull at the age of 25 months showed widely open anterior (23×50 mm) and posterior (30×30 mm) fontanels, patent cranial sutures, especially the lambdoid suture with a maximal wide of 34 mm, and a bone island with a length of 50 mm within the open sagittal suture (Figures 1e and f). Periosteal new bone formation could be excluded in the X-rays of his long bones taken at the age of 13 months. Calcium metabolism parameters were all within normal limits. His karyotype was normal (46,XY) at the 550 band level.

A female cousin of the mother (patient IV-1) who had first-cousin parents was also affected. During the first year of life, she suffered from an atopic eczema that disappeared later on. CT of the skull at the age of 9 months showed widely open cranial sutures, and multiple wormian bones. Her cognitive development was normal. Examination at the age of 4 years showed clubbing of all the digits and toes, hyperhidrosis of the palms and soles, and closed cranial sutures and fontanels. The maternal aunt of the index patient (patient IV-5) was reported to have 'swelling' of her distal fingers but no other specific physical problems or complaints. Her parents were first cousins.

Family 2 Patient 2 is the second child of parents of Dutch origin. Her elder sister is healthy. The father was reported to have psoriatic arthropathy. Genealogical studies disclosed that they are third cousins. The proposita was born after 37 weeks of gestation and with a birth weight of 3000 g. At the age of 3 weeks, it was noticed that her anterior and posterior fontanels were large accompanied by widely open cranial sutures. At the age 6 months, cranial sutures were still wide, with a patent posterior fontanel. During infancy she had eczema.

First complaints in terms of painful joints appeared when she was almost 5 years old.

During an upper airway infection, her knees became very painful and subsequently she was hardly able to stand. Thereafter, the hand joints became painful, mostly pronounced in the distal interphalangeal joints. Later on, the ankles and feet were involved, too. During childhood, pain increased gradually. Physical exercise, upper airway infections, and high humidity turned out to be triggers for exacerbation of the arthralgias. At the age 10 years, she complained about constant pain in her hands while writing. Since the age of 5.5 years she has been treated with indometacin, a non-steroid anti-inflammatory drug, 30 mg/24 h in three doses, which gave a good response initially. After withdrawal of this drug, the joint problems reappeared and indometacin therapy was restarted.

Table 1 Summary of clinical data

Patient	V-1	IV-1	IV-5	2
Age/sex	13 months/male	4 years/female	11 years/female	9 years/female
Skeletal				
Delayed cranial suture closure	+	+	−	+
Clubbing of the fingers and toes	+	+	+	+
Periostosis	−	n.d.	n.d.	−
Arthralgia	−	−	−	+
Skin				
Hyperhidrosis	+	+	+	+
Eczema	+	+	−	+
Others				
Patent ductus arteriosus	+	−	−	−

n.d., not documented.

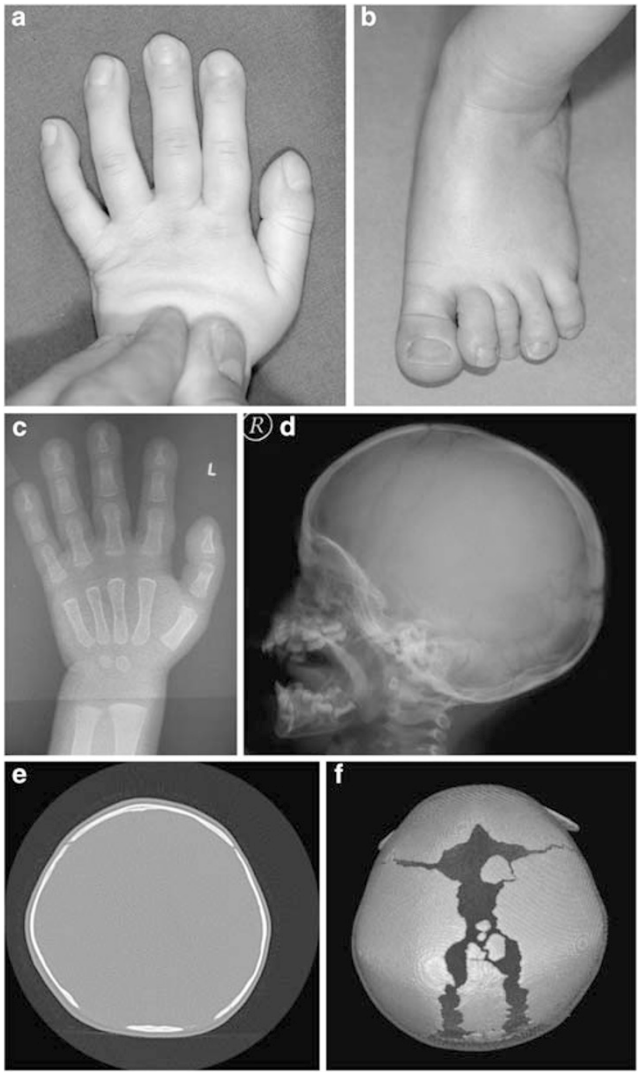

Figure 1 Patient V-1. (**a** and **b**) Club-shaped terminal phalanges of the fingers and toes at the age of 13 months. (**c**) Hand radiograph at the age of 9 months. Constricted diaphyses of the terminal phalanges and tufted ends. (**d**) Skull radiograph at the age of 22 months showing multiple wormian bones. (**e**) CT scan of the skull at the age of 25 months. Axial scan showing widely open lambdoid sutures. (**f**) 3D CT reconstruction showing widely open fontanels and patent cranial sutures.

Hyperhidrosis of the hands and feet was recalled by her parents, being present since her birth. Her psychomotor development was normal with an above average intelligence (IQ 132 at the age of 6 years).

At the age of 9 years and 9 months, height was 136 cm (−1 SD), weight 27.6 kg (−1 SD), and head circumference was 52 cm (−0.5 SD). Physical examination showed clubbing of the fingers and toes (Table 1 and Figures 2a and b). Radiographs of the skull at the age of 5 years showed an increased number of wormian bones. A hand radiograph at the age of 5 years and 10 months showed diaphyseal constriction and broad ends of the terminal phalanges (Figure 2c). No signs of periostosis were visible in the X-rays of her long bones at the age of 9 years. Dermatologic examination at the age of 8 years excluded cutaneous anomalies.

Family 3 The female index patient, her mother, and maternal grandfather have bilateral clubbing of all the fingers and toes. No associated medical problems were observed in the mother and grandfather.

Apart from probably isolated digital clubbing, the index patient developed mild hepatosplenomegaly and subsequently a common variable immunodeficiency at the age of 2 years. Psychomotor development was normal. Physical examination at the age of 4.5 years showed height of 99 cm (−1.9 SD), weight of 13.9 kg (−1.7 SD), and an OFC of 50 cm (−0.5 SD), and clubbing of all the fingers and toes. Laboratory tests for inborn errors of metabolism and karyotyping were normal.

Mapping of a locus for COA to chromosome 4

We carried out a genome-wide linkage analysis in family 1 and found significant linkage of the disease to a 7 cM genetic interval on chromosome 4q33 (Supplementary material). While sequencing candidate genes of this region, mutations in the *HPGD* gene were identified in four affected families with PHO.[7]

Mutational analysis

For mutational analysis, we analyzed the coding region of the *HPGD* gene as well as their flanking intronic sequences.

In family 1, sequencing showed a homozygous G>T transversion in exon 1 in all three affected individuals (Figure 3a). This mutation predicts the amino-acid substitution Gly18Cys. It was not found in any of 150 control individuals. This residue is highly conserved in all available 15-PGDH homologous sequences comprising mammals, *Xenopus tropicalis*, and bony fish (Figure 3b). For further analysis, we used a proposed 3D structure model of the binary 15-PGDH–NAD^+ complex (2GDZ.pdb) (Figure 3c). Molecular modeling of the Gly18 to Cys18 exchange predicts disruption of the coenzyme NAD^+ to 15-PGDH binding by introducing a new H-bond donor. The thiol group of Cys18 is predicted to form a novel H-bond with Asn91. In consequence, this novel H-bond causes a sterical change of the 15-PGDH NAD^+ binding pocket disturbing proper NAD^+ H-bonding to residues Asp36, Asp64, and Val186 (Figure 3c). This prediction suggests that the mutation Gly18Cys causes a loss of 15-PGDH function due to a failure of NAD^+ binding or enzymatic conversion.

In family 2, we identified a homozygous deletion of one nucleotide, c.120delA (p.Glu40fsX31), in exon 2 (Figure 3a). This alters the reading frame from residue 40

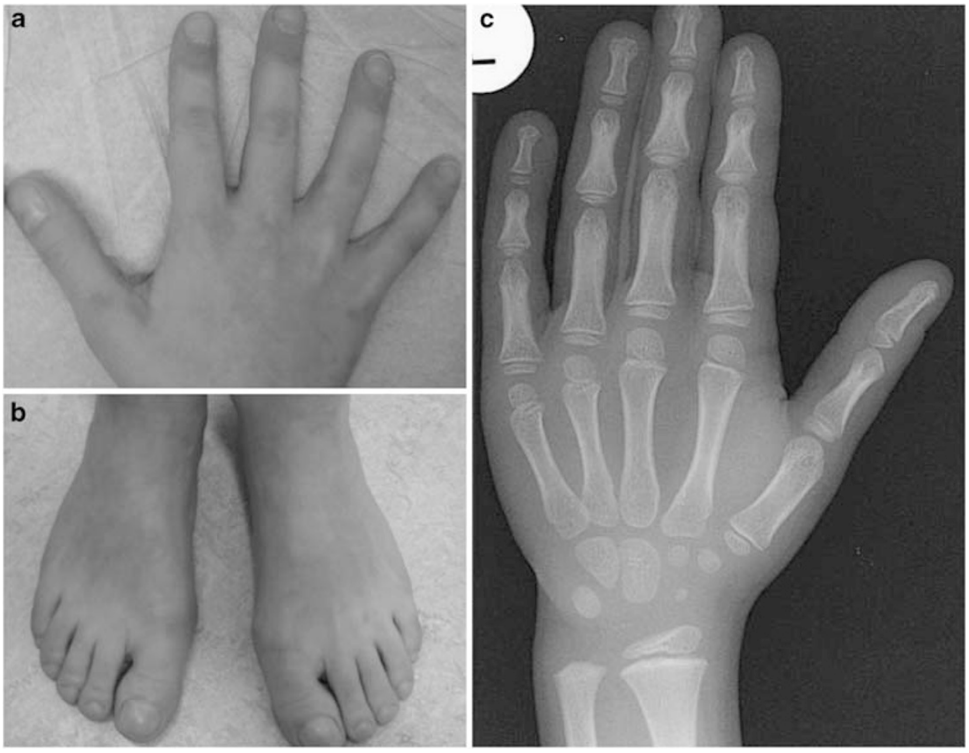

Figure 2 Patient 2. (**a** and **b**) Clubbing of all the digits and toes at the age of 9 years and 9 months. (**c**) Hand radiograph at the age of 5 years and 10 months showing diaphyseal constriction and broad ends of the terminal phalanges.

and truncates the HPGD protein at residue 71 after 31 altered amino acids. The predicted truncated protein lacks the entire PGE_2-binding domain. Each mutation co-segregated in the respective family.

In family 3 with autosomal dominant isolated digital clubbing, no mutation was detected.

Discussion

In this study, genome-wide homozygosity mapping identified a locus for COA harboring the *HPGD* gene in a consanguineous family. Novel homozygous *HPGD* mutations were found in this family as well as in a second family affected with COA. Furthermore, we excluded *HPGD* mutations in a family with digital clubbing as an autosomal dominant trait.

15-PGDH is a member of the short-chain dehydrogenase/reductase (SDR) family. The amino-acid substitution p.Gly18Cys found in family 1 alters the highly conserved glycine-rich motif Gly-X-X-X-Gly-X-Gly at its last position. This NAD^+ binding motif is located at the N-terminus in all members of the SDR family and is thought to be part of the putative coenzyme binding site [13]. In a 3D structural model of the 5-PDGH–NAD^+ complex, we predicted that the Gly18Cys exchange introduces a new H-bond donor within the NAD^+ binding pocket causing a sterical change, which probably reduces NAD^+ H-bonding. On the basis of this information, we consider that the altered NAD^+ binding to 15-PDGH and consequently the failure of the PGE_2 to NAD^+ proton transfer results in a loss of enzymatic catabolism of PGE_2 into the main metabolite PGE-M finally causing COA.

The frameshift mutation, c.120delA (p.Glu40fsX31), identified in family 2 is predicted to result in a premature stop of protein translation causing a truncated HPGD protein or lead to degradation of *HPGD* by nonsense-mediated mRNA decay.

Mutations in *HPGD* have been identified in four families affected with PHO and in a single family with isolated congenital nail clubbing as an autosomal-recessive trait.[7,9,14] Two of four mutations detected so far result in truncation of the *HPGD* transcript due to nucleotide deletions.[7,9,14] These two mutations alter the open reading frame and result in truncated proteins, which lack the PGD_2-binding domain.[7] By analyzing the crystal structure of HPGD, the reported homozygous amino-acid substitution Ala140Pro is predicted to disrupt binding of PGE_2.[7] Expression of recombinant human HPGD in *Escherichia coli* showed that the P140 mutant protein had no detectable activity, indicating that Ala140Pro also is a functionally null allele.[7] Another homozygous missense mutation p.S193P is predicted to disrupt the helical structure by reduced stability of the helix number eleven due to introduction of proline residue.[9] The current distribution of mutations shows no putative mutation hotspot region.

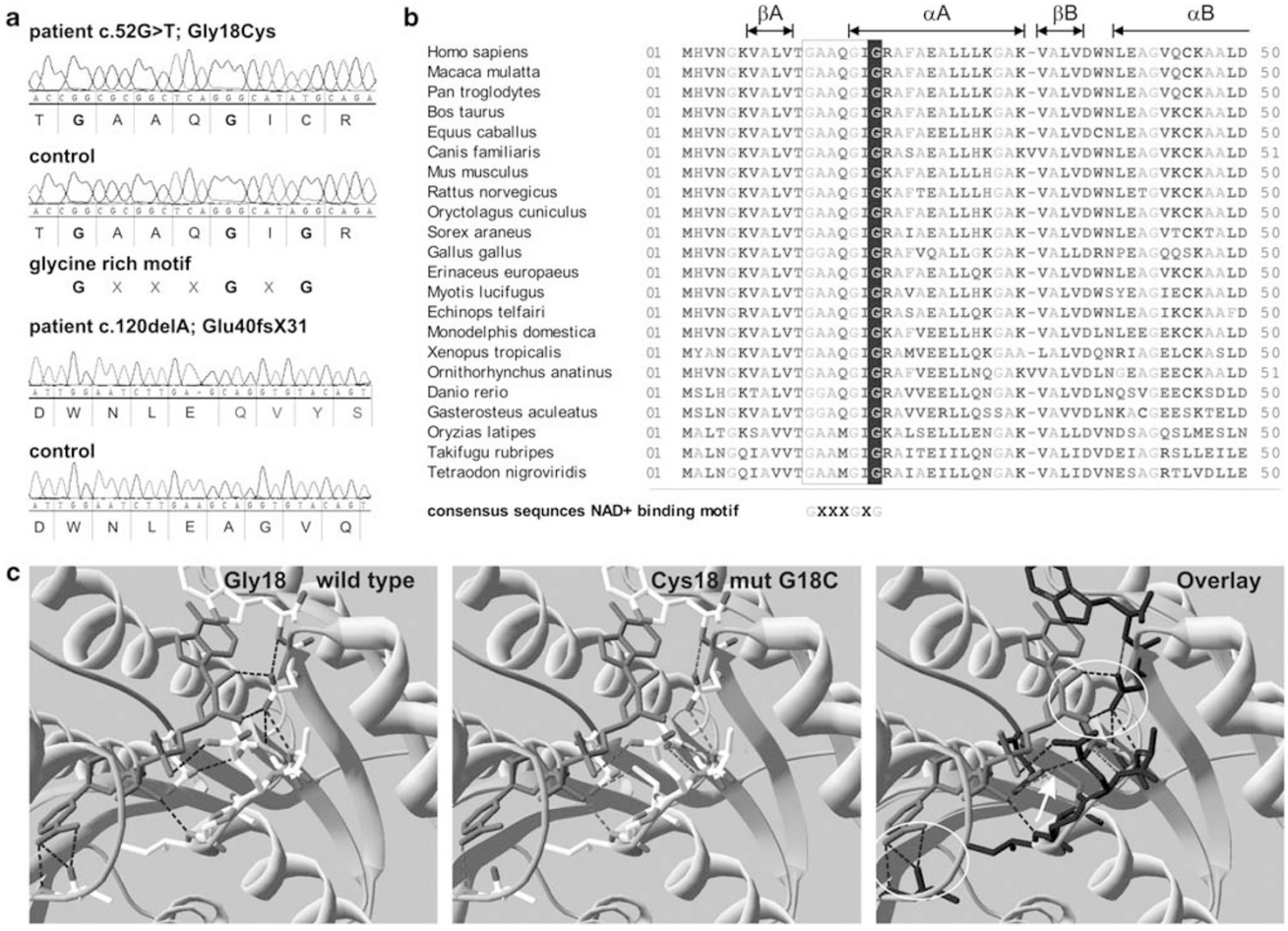

Figure 3 (**a**) Sequence analysis of families 1 and 2 showed a homozygous missense mutation c.52G>T and a homozygous deletion c.120delA, respectively. (**b**) Protein sequence conservation plot of the 15-PGDH NAD^+-binding site showed high conservation of the residue Gly18 within the glycine-rich motif. (**c**) Constructed 3D model of the 15-PGDH–NAD^+ complex: this model indicates an interaction of NAD^+ (stick) with residues Asn91 and Val186 in wild-type Gly18 (left) and the abolished interactions in mutated Gly18Cys 15-PGDH (middle). An overlay of the wild-type (black) and Gly18Cys (arrow) mutant (red) complexes shows the steric alteration and loss of H-bonds (right). The color reproduction of the figure is available on the html full text version of the paper.

Taken together the clinical data of the patients with COA reported here, there is marked intrafamilial and interfamilial variability. Some of the clinical signs and symptoms evolve with time. All patients showed clubbing of the fingers and toes and hyperhidrosis of palms and soles starting in infancy. Delayed closure of the cranial sutures and widely open fontanels were noted in 3/4 patients. Skull ossification normalized with time in all patients reported here. After a few years, severe periodic arthralgia and joint swelling were present in patient 2 harboring a homozygous frameshift mutation, whereas patients carrying the homozygous missense mutation c.52G>T did not have significant joint problems in childhood and early adolescence. Diaphyseal periostosis of long bones was not detected in the two patients (V-1, -2) whose radiographs were available. Solely in patient V-1, a patent ductus arteriosus was diagnosed and treated in the neonatal period. Pachydermia was not present in the patients reported here.

A variety of clinical and radiological manifestations in patients with PHO is mediated by the abnormal prostaglandin metabolism. Digital clubbing is suggested to be caused by prolonged local vasodilatation that can be induced by an enhanced peripheral vasodilatory effect due to chronic elevation of circulating PGE_2.[7,15] PGE_2 is also able to stimulate the activity of osteoblasts.[7] Increased levels of PGE_2 in PHO patients could explain the increased bone formation around the periosteum. The sensitivity of the ductus arteriosus to PGE_2 already has been proven and applied by treatment with NSAIDs to close the vessels in neonates with PDA as well as by treatment with PGE_2 to maintain the patency of vessels.

Hereditary digital clubbing has been reported occasionally as an isolated, painless anomaly presenting at birth, and without any underlying disease.[9] An autosomal-recessive variant has been found to be caused by a missense mutation in *HPGD* in a consanguineous family from

Pakistan.[9,14] Heterozygous individuals of this family have clinically normal fingers and toes. Autosomal dominant digital clubbing (MIM 119900) has been rarely reported and was thought to be distinct from PHO. We did not identify a mutation in *HPGD* in family 3 with this type. In conclusion, *HPGD* mutations seem not be the sole cause of hereditary isolated digital clubbing. Further studies are necessary to identify the major gene causative for this condition.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)