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Targeted exome sequencing identified a novel mutation hotspot and a deletion in Chinese primary hypertrophic osteoarthropathy patients

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ABSTRACT

Background: Primary hypertrophic osteoarthropathy (PHO) is a genetically and clinically heterogeneous systematic disorder caused by mutations in genes *HPGD* and *SLCO2A1*. The purpose of the present study is to provide useful information for the early and precise diagnosis of PHO and identify causative mutations in Chinese PHO children.

Methods and results: The clinical manifestations, radiographic features of seven Chinese pediatric patients were systematically analyzed. Targeted exome sequencing identified a previously reported c.310_311delCT mutation and a novel common splicing site mutation c.324 + 5G > A in the *HPGD* gene. Relative quantitative real time PCR validated a novel deletion of the exon 4 in the same gene. Neither mutations nor structural variations in the gene *SLCO2A1* were detected.

Conclusions: In the present study, homozygous or compound heterozygous *HPGD* mutations were identified in seven Chinese pediatric patients, suggesting an autosomal recessive inheritance. The c.310_311delCT mutation and the splicing site mutation c.324 + 5G > A were likely to be mutational hotspots in Chinese PHO patients. For the first time, a structural variation of the *HPGD* gene was reported. Homozygous, compound heterozygous mutations or structural variation identified in the *HPGD* gene proposed that targeted exome sequencing may be a preferable method for pediatric PHO diagnosis and mutation analysis.

1. Introduction

Primary hypertrophic osteoarthropathy (PHO; MIM 167100) is a genetically and clinically heterogeneous systematic disorder characterized by pathological changes affecting soft connective tissue, skin and bones, with unclear incidence and prevalence [1–7]. Key clinical presentations include digital clubbing, periostosis, and pachydermia [6,8]. Particular abnormal developmental manifestations, such as patent ductus arteriosus, have been found in PHO patients [9]. Digital clubbing typically presents early in childhood, followed with progressive skin thickening, swelling of periarticular tissue and new bone formation of the long bones, which usually progress for 5–20 years [6,10]. Therefore, the diagnosis of PHO could be hindered for the pediatricians for the incomplete clinical presentations [11–13].

Previously reports showed that the inheritance pattern of PHO was controversial [6]. In 2008, Uppal et al. utilized SNP array genotyping and identified for the first time that homozygous mutation in hydroxyprostaglandin dehydrogenase (*HPGD*) encoding 15-hydroxyprostaglandin dehydrogenase (15-PGDH) could be the cause of PHO, suggesting the likelihood of an autosome recessive inherited pattern of PHO [14]. Subsequently, several groups used Sanger sequencing of the *HPGD* gene and determined both homozygous and heterozygous mutations underling PHO, hinting both dominant and recessive inheritance of PHO [15–21]. The prostaglandin transporter encoding gene *SLCO2A1* was recently reported to be responsible for PHO [22]. However, it is universally known that besides point mutations, genomic aberrations such as copy number variations (CNV), microdeletion/-duplication, which cannot be readily detected by the Sanger method of

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DNA sequencing, can also be the causative factors of human diseases [23].

Therefore in the present study, we carefully described the clinical and genetic analyses of seven Chinese pediatric patients. Targeted exome sequencing, accompanied with Sanger sequencing and relative quantitative real-time PCR (RT-PCR), identified a previously reported recurrent c.310_311delCT mutation, a novel splicing site mutation c.324 + 5G > A and a structural variation in the *HPGD* gene. Our findings expanded the mutational spectrum of the *HPGD* gene and demonstrated an autosome recessive inheritance pattern of PHO.

2. Methods

2.1. Study subjects

Seven children (six boys and one girl) with digital clubbing were transferred to Shanghai Children's Medical Center by local hospitals during the period of March 2013 to June 2017. Informed consents to go through genetic analysis and research publications were signed and collected, according to the principles of the declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Shanghai Children's Medical Center. The medical history, physical examination and radiological data were obtained and systematically analyzed.

2.2. Targeted exome sequencing

Exome sequencing of 2747 genes, which are known to be closely associated with human inherited diseases, was performed in the seven pediatric patients according to experimental procedures described previously [24]. Peripheral blood was collected from patients and respective healthy parents. Genomic DNA was extracted using a QIAamp Blood DNA Mini Kit® (Qiagen, Germany), and concentration and quality were determined by Nanodrop Lite spectrophotometer (Thermo Fisher Scientific, USA). Approximately 3 μg of genomic DNA from each sample was randomly fragmented by shearing, and an adaptor-ligated DNA library was constructed, followed by capture and enrichment of exons and flanking intronic regions. The products were sequenced on an Illumina HiSeq 2000 System (Illumina, USA). Additionally, a group of 105 Chinese without PHO were recruited as controls to test whether the discovered mutations were polymorphisms.

2.3. Analysis of sequencing data

Base calling and quality assessment of sequencing reads were conducted by Illumina Sequence Control Software v4.0.4 with real-time analysis (RTA) (Illumina, USA). The NextGENe® (SoftGenetics LLC, USA) was utilized for the alignment of sequencing reads to the reference human genome (GRCh37.3, SNP135). All the single nucleotide variants (SNVs) and insertion-deletions (indels) were saved as VCF format files and uploaded to the online variation annotation tool Ingenuity® Variant Analysis™ for mutation annotation.

2.4. Validation of mutations in the HPGD gene

Sanger sequencing was utilized for the validation of mutations filtered by Ingenuity® Variant Analysis™. The primers for the amplification of targeteded regions of the *HPGD* gene (NM_000860) were designed by the Oligo 6.0 software. The specificity and reliability of primers were determined by online UCSC In-Silico PCR. The primers were listed as follow, forward 5′– CCTCTCATGGCATAGG ACATG–3′ and reverse 5′- GTTTCCATGACTCCAA GAACC–3′. The polymerase chain reactions (PCR) analysis was performed (Takara Biotechnology, Dalian, China) and the products were sequenced on an ABI3730XL (Applied Biosystems; Thermo Fisher Scientific, USA). The sequencing data was analyzed and the results were exported by Chromas 2.5.1.

2.5. RT PCR for validation of the deletion in the HPGD gene

RT-PCR primers for the candidate deleted region in the *HPGD* gene suggested by exome-sequencing data were designed by Primer 3 (http://primer3.ut.ee/) and detailed as below, forward, 5′–TGCCCTT ACCTGCTAAAGATGA–3′ and reverse, 5′–TCACTTATGCCTCTGGATT AGG–3′. The *GAPDH* gene was amplified as the internal control and the primers were forward, 5′–AAGAAGGTGGTGAAGCAGG C–3′ and reverse, 5′–GAGTGGGTGTCGCTGTTGAA–3′. The Agilent female and male genomic DNA, which are reference controls for the Agilent oligonucleotide compared genomic hybridization (CGH) microarray assays, were used as healthy controls. RT-PCR was performed in all the patients except for patient 1 in triplicate using a SYBR Green PCR Master Mix (Ambion, Thermo Fisher Scientific, USA) in a Fast 7500 sequence-detection system (Applied Biosystems, Thermo Fisher Scientific, USA). Relative copy number changes were calculated using the DeltaDeltaCt method [25].

2.6. Pathogenicity predictions for the validated mutations

The potential pathogenicity of single nucleotide substitutions was analyzed with two prediction tools: Human Splicing Finder (HSF v.2.4, http://www.umd.be/HSF/) and NNSPLICING 0.9 (http://www.fruitfly.org/seq _tools/splicing.html).

3. Results

3.1. Patient description

The clinical manifestations of seven HPO Chinese patients were detailed in Table 1 and Fig. 1. All of them developed digital clubbing and pachydermia, without obvious periostosis. Patient 5 showed joint swelling. Patient 3 and her little brother patient 4 presented with left pulmonary artery stenosis, patent ductus arteriosus (PDA) and patent foramen ovale. After pulmonary artery plaque enlargement formation operation and PDA closure operation, the postoperative recovery was very well. None of the patients presented with delayed closure of cranial suture, anemia, or hypoalbuminemia. The magnetic resonance imaging (MRI) of the brain, chest computed tomography (CT) and X-ray of thoracic vertebra and lumbar vertebra were normal, respectively.

Table 1
Clinical and molecular features of PHO patients.

Patient	Sex	Age (Years)	Phenotypes	Mutations
1	M	7	Digital clubbing; Pachydermia	Homozygous c.310-311del CT
2	M	6	Digital clubbing; Pachydermia	Homozygous c.310-311del CT
3	F	7	Digital clubbing; Pachydermia; Pulmonary stenosis; Patent ductus arteriosus	Heterozygous c.310-311del CT and c.324 + 5G > A
4	M	5	Digital clubbing; Pachydermia; Pulmonary stenosis; Patent ductus arteriosus	Heterozygous c.310-311del CT and c.324 + 5G > A
5	M	5	Digital clubbing; Pachydermia; joint swelling	Heterozygous c.310-311del CT and a Deletion of exon 4
6	M	7	Digital clubbing; Pachydermia	Heterozygous c.310-311del CT and c.324 + 5G > A
7	M	7	Digital clubbing; Pachydermia	Heterozygous c.310-311del CT and c.324 + 5G > A