A novel G6PD deleterious variant identified in three families with severe glucose-6-phosphate dehydrogenase deficiency

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Abstract

Background: Glucose-6-phosphate dehydrogenase deficiency (D-G6PD) is an X-linked recessive disorder resulted from deleterious variants in the housekeeping gene Glucose-6-phosphate 1-dehydrogenase (G6PD), causing impaired response to oxidizing agents. Screening for new variations of the gene helps with early diagnosis of D-G6PD resulting in a reduction of disease related complications and ultimately increased life expectancy of the patients.

Methods: One thousand five hundred sixty-five infants with pathological jaundice were screened for G6PD variants by Sanger sequencing all of the 13 exons, and the junctions of exons and introns of the G6PD gene.

Results: We detected G6PD variants in 439 (28.1%) of the 1565 infants with pathological jaundice. In total, 9 types of G6PD variants were identified in our cohort; and a novel G6PD missense variant c.1118 T > C, p.Phe373Ser in exon 9 of the G6PD gene was detected in three families. Infants with this novel variant showed decreased activity of G6PD, severe anemia, and pathological jaundice, consistent with Class I G6PD deleterious variants. Analysis of the resulting protein’s structure revealed this novel variant affects G6PD protein stability, which could be responsible for the pathogenesis of D-G6PD in these patients.

Conclusions: High rates of G6PD variants were detected in infants with pathological jaundice, and a novel Class I G6PD deleterious variant was identified in our cohort. Our data reveal that variant analysis is helpful for the diagnosis of D-G6PD in patients, and also for the expansion of the spectrum of known G6PD variants used for carrier detection and prenatal diagnosis.

Keywords: D-G6PD, Variant, Neonatal jaundice, Infant

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Background
Glucose-6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme encoded by a housekeeping X-linked gene, the main function of this gene is to produce Nicotinamide Adenine Dinucleotide Phosphate (NADPH). NADPH is a key electron donor that protects against oxidizing agents and participate in reductive biosynthetic reactions [1, 2]. G6PD has extraordinary genetic diversity [1–3]. Many variants of G6PD, mostly generated from missense variants, have been reported to possess a various enzyme activity and associated clinical symptoms [2–6]. Glucose-6-phosphate dehydrogenase deficiency (D-G6PD), also named Favism, is the potential cause of clinical acute hemolysis, neonatal jaundice, or severe chronic non-spherocytic hemolytic anemia [2, 7–10].

More than 300 G6PD gene variants have been described in D-G6PD. These variants have been categorized into five classes, from Class I to Class V, by the World Health Organization (WHO) based on biochemical phenotype and clinical manifestations [11–13]. About 400 million people worldwide has been estimated to have D-G6PD [14]. This condition appears most frequently in certain parts of Africa, the Mediterranean, Asia, and the Middle East. Some genetic variants have reached a high incidence rate in people from certain parts of the world since they present with a selective advantage against malaria [15]. Mutations at different sites of the G6PD gene result in different effects on enzyme activity [16–19] (Fig. 1). The majority of variants of the G6PD gene result in red cell enzyme deficiency through decreasing enzyme stability [18, 19]. The polymorphic variants of the G6PD gene influence amino acid residues at multiple sites all over the enzyme and decrease the stability of the enzyme in the red cell, possibly by affecting protein folding [18–20]. These unfavorable variants of the G6PD gene mostly disturb residues at the dimer interface, or the residues responsible for association with a structural NADP

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Fig. 1 The common variants and classification in the G6PD gene. The G6PD gene variants are classified into Class I to Class IV based on the genotype and clinical manifestations (WHO classification). Red color indicates a Class I severe enzyme deficiency with chronic non-spherocytic hemolytic anemia (CNSHA). Blue color indicates a Class II severe enzyme deficiency with less than 10% of the normal activity. Orange color indicates a Class III mild to a moderate enzyme deficiency (10 to 60% of normal activity). Black color indicates a Class IV very mild or almost normal enzyme activity (> 60% normal activity and no clinical problem). Data from G6PD gene variant database (http://www.bioinf.org.uk)
molecule to stabilize the enzyme [21–25]. The de novo variants appear very rare, which causing the more severe condition of chronic non-spherocytic hemolytic anemia [20].

Pathological jaundice is an important condition and accounts for a large number of Neonatal Intensive Care Unit (NICU) admissions. Generally, jaundice in infants commonly presents in the first week of life. Pathological jaundice appears as early as the first day of life and can lead to adverse complications in the absence of timely intervention. A total serum bilirubin (TSB) level above the 95th percentile for an infant’s age (in hours) is defined as serum hyperbilirubinemia, which occurs in 8–9% of infants during the first week of life [26, 27]. The frequency of G6PD deficiency in infants with jaundice is well reported, however, the frequency of G6PD variants with G6PD deficiency in the infants with jaundice has not been studied to a great extent [28–30]. In this study, we screened for G6PD variants via DNA sequencing using blood samples from infants with pathological jaundice who were suspected to have D-G6P. We identified a new G6PD deleterious variant in three families with D-G6PD. Our data are indicative of the molecular mechanism underlying D-G6PD, and the importance of the molecular diagnosis and genetic screening for this disease.

Methods

Patient data and family consent

One thousand five hundred sixty-five infants born with pathologic jaundice at Renmin Hospital of Wuhan University between September 2015 to September 2018 were screened for G6PD gene variants. The identified novel variants were verified in 350 infants without jaundice as unrelated controls, and 80 blood donors serving as healthy controls were also screened. The red cell count (RBC), hemoglobin (Hb), hematocrit (HCT), total bilirubin (TBIL) and direct bilirubin (DBIL) were tested in all the newborns using routine clinical laboratory methods as previously reported [31–33]. Ethical Committee of Renmin Hospital of Wuhan University approved this study. Written consents were obtained from the families for reporting their clinical details.

G6PD enzymatic activity detected

The improved G6PD Nitroblue tetrazolium (NBT) Quantification Ratio Kit (Micky, Guangzhou, China) was utilized to measure G6PD enzymatic activity on a microplate reader (BioTek ELx808, USA) according to the manufacturer’s instructions. Those with a G6PD /6PGD ratio of ≤1.0 were classified as G6PD deficient [31–33].

Variant analysis with Sanger sequencing

Primer3 web version 4.1.0 (http://primer3.ut.ee/) was utilized to identify, select, and design primers specific to target regions for all the 13 exons and intron-exon boundaries (100 bp upstream and downstream of each exon) of G6PD (GenBank ID: NG_009015.2; https://www.ncbi.nlm.nih.gov/nuccore/NG_009015.2/) (Supplemental Table 1). The variants were analyzed in both G6PD splice variant 1 (NM_000402.4) (https://www.ncbi.nlm.nih.gov/nuccore/NM_000402.4), and its splice variant 2, which lacks exon 1 (NM_001042351.2) (https://www.ncbi.nlm.nih.gov/nuccore/NM_001042351.2). The G6PD gene has 3 promoters based on the Eukaryotic promoter database (https://epd.epfl.ch//index.php); the promoter2 sequence is also covered in the intro-exon1 region. The extracted genomic DNA was amplified by polymerase chain re-action (PCR) using k-Taq DNA polymerase (bios- WORLD, Ohio, USA). Then, shrimp alkaline phosphatase (USB, Cleveland, OH, USA) and exonuclease I (USB, Cleveland, OH, USA) were sued for purification of PCR products. The BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) was used for the purified PCR products. The products were then sequenced with 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing Analysis version 5.2 (Applied Biosystems, Foster City, CA, USA) and Chroma-sPro version 1.5 (Technelysium Pty Ltd., Tewantin, QLD, Australia) software were utilized for collected sequencing data analysis.

Bioinformatics analysis

Effect prediction of amino acid substitutions on protein function was performed by the bioinformatic tools Sorting Intolerant From Tolerant (SIFT) (http://sift.jcvi.org/), Polymorphism Phenotyping v2 (PolyPhen-2) (http://genetics.bwh.harvard.edu/pph2/index.shtml), and Mutation Taster (http://www.mutationtaster.org/). Splice-site prediction was also performed using Maximum Entropy Modeling of Short Sequence Motifs (Max-EntScan) (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_ scoreseq.html), Neural Network Splice site prediction (NNSPLICE) (http://www.fruitfly.org/seq_tools/splice.html), GeneSplicer (http://www.ncbi.nlm.nih.gov/software/genesplicer/) and Human Splicing Finder (http://www.umd.be/HSF/). The alignment tool MSA (Multiple Sequence Alignment, https://www.ebi.ac.uk/Tools/msa/clustalo/) was utilized for multiple sequence alignments of G6PD protein sequences from different species (mammals, fish, and reptiles). The conservation of the variant across species was assessed by Genomic Evolutionary Rate Profiling (GERP) scores (https://www.ebi.ac.uk/training/online/glossary/gerp-score), and Combined
Annotation-dependent Depletion (CADD) scores (https://cadd.gs.washington.edu/score).

Protein stability analyses and structure predictions were carried out using the software Triple-Helical collage Building Script (The BuScr) 1.06 (http://structbio.biochem.dal.ca/jrainey/THeBuScr.html), SCWRL 4.0 (http://dunbrack.fccc.edu/Software.php) and UCSF Chimera 1.5 (http://www.cgl.ucsf.edu/chimera/).

Results

**G6PD variants analyzed in infants with pathological jaundice**

One thousand five hundred sixty-five infants with pathological jaundice had G6PD variant screening performed by PCR amplification of the exons of the G6PD genes following Sanger sequencing. As a control, more than 300 unrelated infants (without jaundice) were also screened. There was a 28.1% variant detection rate with 439 infants out of 1565 infants found to have 9 different G6PD genetic variants. Detailed characteristics of each G6PD gene variants in two spice form and the number of times detected are summarized in Table 1. We also calculated the proportion of each variants in the 439 positive infants (Table 1). The most detected G6PD variants are c.1388G > A, p.Arg463His (9.33%) and c.1376G > T, p.Arg459Leu (8.95%). These two variants were found in 33.26 and 31.89% of subjects respectively, and represent the majority of variants found in our cohort. The order of incidence for the other variants from most frequent to least frequent is: c.95A > G, p.His32Arg (3.19%) with a proportion of 11.39%, c.871G > A, p.Val291Met (2.04%) with a proportion of 7.29%, c.1024C > T, p.Leu342Phe (1.79%) with a proportion of 6.38%, c.466G > A, p.Glu156Lys (1.02%) with a proportion of 3.64%, and c.1192G > A, p.Glu398Lys and c.1004 C > A, p.Ala335Asp with a very low incidence of 0.77%, and a proportion of 2.73%.

**Mapping and sequencing for the novel G6PD gene variant**

A novel G6PD variant, c.1118 T > C, p.Phe373Ser had the lowest incidence (0.19%) in our cohort with a proportion of 0.68% in our detected variants (Table 1). This variants appeared in 3 families, with a total of 14 family members affected (Family 1: 7affected; Family 2: 4 affected; Family 3: 3 affected) (Fig. 2). Analysis of all of the 13 exons of G6PD in the three probands when compared with the unrelated cohort revealed a hemizygous missense variant c.1118 T > C in exon 9. This results in a putative amino acid change from phenylalanine (TTC) to serine (TCC) in codon 373 p.Phe373Ser (Fig. 3a–c). Exon 9 of the G6PD gene was detected in all the other members of these three families. It was noted that the missense variant c.1118 T > C, p.Phe373Ser came from the mothers in the family and not the fathers (Fig. 3d–f) (data only showed the family 1). Moreover, c.1118 T > C, p.Phe373Ser was not present in 350 unrelated infants or in 80 healthy controls. The CADD score showed the G6PD c.1118 T > C is a potentially pathogenic variant (CADD score 25.5). The GERP score showed that the G6PD c.1118 T is highly conserved (score is 5.82).

**Novel Class I G6PD gene variant identified in the infants from 3 families**

To examine if the novel variants impact G6PD function we also examined RBC, Hb, HCT, TBIL, DBIL, and the G6PD activity in all persons from the three families. Results are shown in Table 2. The G6PD activity in three probands was 3.5% (Family 1 III1), 4.6% (Family 1 II2) and 5.1% (family 3 (I11), which is below the 10% of normal activity (100%). The Hb values in the three probands was examined and severe anemia was indicated in the infants (Family 1 III1, 89 g/L; Family 2 II2, 54 g/L; Family 3 III1, 99 g/L), and the mothers of the infants in the three probands (Family 1 II2, 93 g/L; Family 2 I2, 109 g/L; Family 3 I2, 95 g/L). The TBIL and DBIL data indicated the presence of jaundice in the three probands (Family 1 III1, TBIL 158.1 unol/L, DBIL 19.5 unol/L; Family 2 I2, TBIL 57.2 unol/L, DBIL 10.9 unol/L; Family 3 III1, TBIL 154.27 unol/L, DBIL 17.5 unol/L).

**Pathogenicity analysis of the novel G6PD mutant**

With bioinformatics tools to predict the effect of amino acid substitutions on protein function, the missense variant c.1118 T > C, p.Phe373Ser was classified as pathogenic (Table 3). MutPred prediction score for this mutant was 0.902, revealing that this variant was a deleterious variant and has the probability for a gain of disorder (statistically significant p = 0.007). MutPred2 prediction analysis showed that the amino acid substitution of this variant is pathogenic (score 0.885), affecting amino acids 137, 173 and 233, and also the eukaryotic linear motifs.

We further analyzed if the c.1118 T > C, p.Phe373Ser variant meet the pathogenic criteria of American College of Medical Genetics (ACMG) for the classification of variants [34]. Our data showed that the c.1118 T > C, p.Phe373Ser variant meets the criteria of 2 pathogenic strong (PS): de novo variant confirmed in parents (PS2) and appeared in several members of 3 families with increased segregation (PS1), and 2 pathogenic moderate (PM): a novel missense change at an amino acid (PM5) localizing at the well-studied functional domain (PM1). In addition, this
| Nucleotide changed                                      | Protein changed                                      | No. of the detected variants | Variant proportion (n = 439) | Variant frequency (n = 1565) | ClinVar Variation ID | HGMD Accession Number |
|--------------------------------------------------------|------------------------------------------------------|------------------------------|-------------------------------|-------------------------------|----------------------|-----------------------|
| NM_001042351.2:c.95A > G                                | NP_000393:4:p.His32Arg                              | 50                           | 11.39%                        | 3.19%                         | 10,403               | CM910158              |
| NM_000402.4:c.186A > G                                 | NP_000393:4:p.His62Arg                              | 16                           | 3.64%                         | 1.02%                         | 10,364               | CM880031              |
| NM_001042351.2:c.466G > A                              | NP_000393:4:p.Glu156Lys                              | 16                           | 3.64%                         | 1.02%                         | 10,364               | CM880031              |
| NM_000402.4:c.556G > A                                 | NP_000393:4:p.Glu186Lys                              | 32                           | 7.29%                         | 2.04%                         | 10,386               | CM930275              |
| NM_001042351.2:c.95A > G                                | NP_000393:4:p.His62Arg                              | 16                           | 3.64%                         | 1.02%                         | 10,364               | CM880031              |
| NM_000402.4:c.186A > G                                 | NP_000393:4:p.His62Arg                              | 12                           | 2.73%                         | 0.77%                         | NA                   | CM950506              |
| NM_001042351.2:c.1192G > A                              | NP_000393:4:p.Glu398Lys                              | 12                           | 2.73%                         | 0.77%                         | NA                   | NA                   |
| NM_000402.4:c.1282G > A                                | NP_000393:4:p.Glu398Lys                              | 12                           | 2.73%                         | 0.77%                         | NA                   | CM910162              |
| NM_001042351.2:c.1367G > T                              | NP_000393:4:p.Arg459Leu                              | 140                          | 31.89%                        | 8.95%                         | 100,058              | CM910163              |
| NM_000402.4:c.1466G > T                                | NP_000393:4:p.Arg459Leu                              | 140                          | 31.89%                        | 8.95%                         | 100,058              | CM910163              |
| Note: In the database of Exome Aggregation Consortium (ExAC), ClinVar and HGMD, some G6PD variants are only expressed in G6PD splice variant 1 (NM_000402.4) (https://www.ncbi.nlm.nih.gov/nuccore/NM_000402.4), some in splice variant 2, which lacks exon 1 (NM_001042351.2) (https://www.ncbi.nlm.nih.gov/nuccore/NM_001042351.2), and some in both splice variants. The G6PD mutant in the two splice variants are listed in the table. We were not able to amplify the first exon of the G6PD in the 1565 infants. This means we could only detected the G6PD splice variant 1 in the cohort. All the G6PD variants we detected in our cohort from the Wuhan area are in G6PD splice variant 2, which is also the reason why each G6PD mutant in splice variant 2 (NM_001042351.2) is listed first in the table.
variant meets 2 pathogenic supporting (PP) criteria of ACMG: multiple lines of computational evidence supporting a deleterious effect of this variant on G6PD function (pp3), and the patients with this variant showing the D-G6PD (phenotypes) (pp4). Taken together, these analyses indicated that the missense variant c.1118 T > C, p.Phe373Ser meets the pathogenic criteria of ACMG: 2 strong (PS1 and PS2) or 1 strong (PS1 and PS2) and 2 moderate (PM1 and PM5).

**Fig. 2** The pedigrees from three families. The filled symbols indicate affected individuals, the open symbols indicate unaffected individuals, the arrows indicate the index cases in each family, and the Roman numerals represent the generation numbers.

**Fig. 3** G6PD gene variant analysis in three families. a-c The sequence data are shown for the c.1118 T > C variant of G6PD gene exon 9 in the proband (II-1) of family 1 (a), family 2 (II-2) (b) and family 3 (II-1) respectively. d-f the sequence data are presented for the c.1118 T > C variant in the mother or grandmother of the family 1 (II-2) (d), (II-1) (e) and II-2 (f).
Conservation and stability analysis of the novel G6PD mutant

According to Alamut, both nucleotide c.1118 T and amino acid phenylalanine 373 are highly conserved. MultAlin Multiple sequence alignments of G6PD protein sequences from different species (Fig. 4) also showed high evolutionary conservation with respect to phenylalanine-373, which is substituted by serine in the affected members of the Chinese family.

The angle of the amino acid and distance of atoms around the Phe373 and Phe373Ser residue were studied using the software Swiss-PdbViewer (DeepView). When the phenylalanine residue was substituted by a serine residue, there was marked enlargement in the distance of the atoms from 3.81 Å (wild type) to 7.51 Å (variant type), and the angle of the three amino acid significantly expanded from 20.67 °C (wild type) to 44.39 °C (variant type) (Fig. 5).

The stability prediction for the variant and the properties of the structural environment along with its values for wildtype and mutant residues were also studied. SDM2 predicts this variant to have reduced stability (predicted pseudo ΔΔG: -1.54). 1-Mutant2.0 predicts this variant to have decreased stability (ΔΔG: -2.32). AUTO–MUTE predicts this variant to have decreased stability (ΔΔG: -1.97). ProMaya (Protein Mutant stAbilitY Analyzer) predicts this variant to have decreased stability (ΔΔG: -1.97). MutPred2 predicts this variant to have altered stability (P = 0.02), and CUPSAT predicts this variant to be destabilizing (ΔΔG: -1.75). ProSMS predicts this variant to be destabilizing (probability 0.84).

Discussion

Several factors play a role in infants developing pathological jaundice including the imbalance between production, conjugation, and elimination of bilirubin, environmental factors, and ethnicity [35]. G6PD deficiency is one of the common etiological factors for infant pathological jaundice and the G6PD variants are the major reason for the D-G6PD in China. We screened for G6PD gene variants in infants with pathologic jaundice in the Wuhan area and found that gene variants were detected in 28.1% of infants, which is comparable to 31.5% detection rate reported by Yazd in Neonatal Pathologic Hyperbilirubinemia [28]. Our data is the first report of the incidence of G6PD gene variants in infants with G6PD deficiency and pathological jaundice in the southeastern area of China.

Screening for the G6PD variants and prevention of the clinical manifestations of D-G6PD is essential for the public health. Many factors such as chemotherapeutic drugs, household and environmental agents trigger hemolytic anemia in patients with G6PD deficiency. The newborn screenings are usually performed 1 week after birth. This screening is helpful to prevent hemolysis prompted by treatment of infections, and other triggers. In addition, screening will allow for immediate treatment of severe anemia and hemolysis with resuscitation and

| Items Family 1 | Family 2 | Family 3 |
|----------------|----------|----------|
| patient        | I1       | I2       | I1       | I2       | I1       | I1       |
| age            | 61       | 59       | 39       | 36       | 27       | 2.5      |
| Clinical diagnosis | N        | CHM      | N        | CHM      | N        | D-G6PD   |
| G6PD activity (%) | 97.5     | 63.8     | 98.3     | 65.8     | 96.4     | 3.5      |
| RBC (10¹²/L)   | 4.78     | 3.28     | 3.98     | 3.24     | 4.54     | 2.72     |
| Hb(g/L)        | 155      | 95       | 129      | 93       | 148      | 89       |
| HCT (%)        | 0.44     | 0.29     | 0.36     | 0.28     | 0.42     | 0.26     |
| TBIL (unol/L)  | 17.39    | 10.1     | 14.7     | 11.62    | 8.70     | 4.15     |
| DBIL (unol/L)  | 5.4      | 2.3      | 4.1      | 2.8      | 2.0      | 19.5     |

All the test results of the patients were from the clinical tests in the clinical laboratory. The clinical laboratory performs quality control every day to ensure the reliability and accuracy of the test results.

N normal, CHM chronic hemolytic anemia, RBC BRI adult 3.8–5.1 × 10¹²/L, child 3.1–5.3 × 10¹²/L, Hb BRI adult 115–150 g/L, child 110–149 g/L, HCT BRI adult 0.35–0.45, child 0.40–0.50, TBIL BRI 2–22 unol/L, DBIL BRI 0–7.0 unol/L, BRI biological reference interval, RBC red cell count, Hb hemoglobin, HCT hematocrit, TBIL total bilirubin, DBIL direct bilirubin.

| Genome (h19) | DNA | Protein | known variant | SIFT ExAC or 1000G score | PROVEAN score | Variant taster | PolyPhen-2 Pred | Confidence |
|--------------|-----|---------|---------------|--------------------------|---------------|---------------|----------------|------------|
| Chr X:       | c.1118 | p.Phe373Ser | No reports | 0.000 | Damaging | -6.58 | Deleterious disease-causing | 1.0 | Probably damaging | 0.996 |
erythrocyte transfusion. The G6PD variant test is the gold standard for the diagnosis of D-G6PD. Almost all newborns with a positive result by G6PD serology screening will have a G6PD variant detected. Therefore, prenatal diagnosis should be performed when parents have clinical symptoms of D-G6PD, suspected D-G6PD, or older children impacted by D-G6PD following birth. The screening results should be shared with the parents and necessary education on D-G6PD should be provided. All of these approaches might be excellent prophylactic measures in preventing hemolytic crises later in life for the infants. Our study identified a novel G6PD variant, which will increase the spectrum of G6PD variants for diagnosis of D6PD deficiency, carrier detection and prenatal diagnosis.

Several methods have been used for screening the G6PD variants, for example, G6PD variant detection array, multicolor melting curve analysis, etc. However, these methods are difficult to detect the novel G6PD variants. We used Sanger sequencing following the PCR-amplification of all G6PD exons and the exon-intron boundaries, which can detect both the known and unknown variants in the patients. Next-generation sequencing is also used for identifying novel G6PD variants, however it is not a time or cost effective method and is not suitable for large-scale screening. Our method is a simple, quick and economic screening method for the G6PD variants.

Different G6PD gene variants cause different levels of enzyme deficiencies and disease manifestations [36]. Our data showed that c.1388 G > A, c.1376 G > T and c.95 A > G were the three most common G6PD variants, accounting for 76.54% of the total disease alleles in this study cohort. The detection rate and proportion of each variant was similar to other regions in south China [31–33, 37, 38]. Two common variants (detection rate > 5%) and three low frequency variants (detection rate 1–5%) found in this study belong to one of the 5 class deletion variants, with 2 variants (c.95A > G and c. 1192G > A) in Class I, 2 variants (c.1466G > T and c. 1388G > A) in

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Fig. 4 Evolutionary conservation of identified G6PD missense variant. a As shown, the second structure and p.Phe373 are located at the end of the beta strand, the green dot depicts the NADP binding site, the orange dot shows the N6-acetyllysine modified residue, and the red dot shows the substrate binding site. b G6PD sequences from different species were aligned using the Clustal Omega in order to investigate the evolutionary conservation of the phenylalanine residue located in the codon. The multiple alignments revealed total conservation of this residue across all species, suggesting that this residue is crucial for the protein functionality.
Class II, and 2 variants (c.466G > A and c.871G > A) in Class III, all of which are reported to cause different degrees of enzyme deficiencies [33].

We also identified a novel G6PD variant, c.1118 T > C. Infants with this variant appear pathologically jaundice. Pathogenicity analysis showed this is a deleterious variant; and it is pathogenic. Conservation and stability analysis showed that this variant would reduce the stability of the G6PD protein. Infants with this variant had severe anemia, which showed the morphological characteristics of the nonspherocytic hemolytic anemia (data not shown). Therefore, the identified novel variants c.1118 T > C belongs to the Class I G6PD. This data provides evidence that this novel G6PD variant is a cause of nonspherocytic hemolytic anemia, and has significant clinical impact on the pathology of G6PD-D although the frequency of the variant is low in this cohort. In the future, the functional analysis of the novel variant will be performed, particularly evaluating the effect of the variant on G6PD protein stability and cellular activity. Combining the cellular effect of the novel variant with the clinical cohort study focusing on the novel variant will emphasize its role in pathogenesis of D-G6PD.

Conclusions

High rates of G6PD variants are detected in infants with pathological jaundice, and a novel Class I G6PD variant has been identified in our cohort. Our data reveal that variant analysis is helpful for the diagnosis of D-G6PD and also in expanding the spectrum of G6PD variants evaluated for in carrier detection and prenatal diagnosis.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s12881-020-01090-2.

Additional file 1: Table S1. The primers for amplification and sequencing the exons of G6PD gene.

Abbreviations

G6PD: Glucose-6-phosphate dehydrogenase; D-G6PD: Glucose-6-phosphate dehydrogenase deficiency; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; RBC: Red cell count; Hb: Hemoglobin; HCT: Hematocrit; TBIL: Total bilirubin; DBIL: Direct bilirubin; NBT: Nitroblue tetrazolium

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Not applicable.

Authors’ contributions

All authors agree that the research in this manuscript is the authors’ own work and all the study objectives have been appropriately investigated, and reported. They have all read and approved the final version of manuscript. The authors’ contribution in the study was as follow: YT collected clinical data, performed data analysis/sequencing and took overall guidance. BL performed clinical data collection, DNA sequencing and data analysis. HZ collected clinical data, performed sequencing and data analysis. AB, ZW and JG performed clinical data collection/analysis. CS, MM, SK and BT drafted manuscript, developed manuscript concepts and analyzed data. YL performed clinical data collection/analysis, taken overall instruction, participated manuscript concept development, and provided the grant support.

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Availability of data and materials

All data supporting the results reported in a published article can be found. The patient datasets for the current study are not publicly accessible in
accordance with local health research ethics protocols; however, it may be available from the corresponding author. The accession numbers or direct web links and the full names of the data banks/repositories corresponding to all of the datasets obtained from web-based sources are given in the text and tables.

Ethics approval and consent to participate
Written informed consent was provided before enrollment in the study by all patients and their children in accordance with the Declaration of Helsinki. This study has been approved by the Ethical Committee of Renmin Hospital of Wuhan University and written informed consent was obtained from all patients or legal guardians of any participant under the age of 16.

Consent for publication
The written informed consent for publication of the clinical details was obtained from all participants or the parents or legal guardians of any participant under the age of 18.

Competing interests
The authors have declared no conflict of interest.

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