


RESEARCH ARTICLE

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# 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.1118T > 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 variants 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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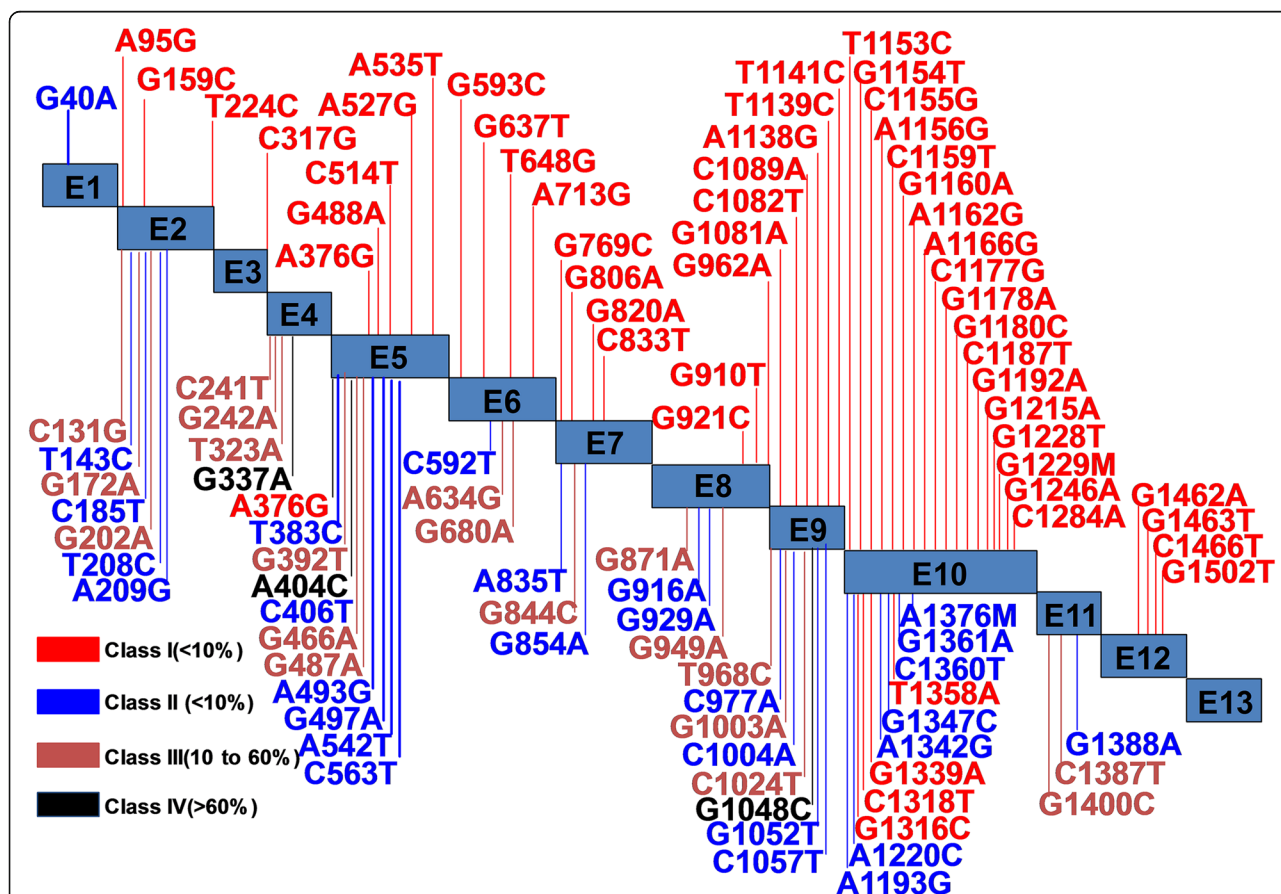
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### Background

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



**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  $\leq 1.0$  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/nucore/NG\\_009015.2/](https://www.ncbi.nlm.nih.gov/nucore/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/nucore/NM\\_000402](https://www.ncbi.nlm.nih.gov/nucore/NM_000402)), and its splice variant 2, which lacks exon 1 (NM\_001042351.2) ([https://www.ncbi.nlm.nih.gov/nucore/NM\\_001042351.2](https://www.ncbi.nlm.nih.gov/nucore/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 reaction (PCR) using *h*-Taq DNA polymerase (bio-WORLD, Ohio, USA). Then, shrimp alkaline phosphatase (USB, Cleveland, OH, USA) and exonuclease I (USB, Cleveland, OH, USA) were used 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 ChromasPro version 1.5 (Technelysium PtyLtd., 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](http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html)), Neural Network Splice site prediction (NNSPLICE) ([http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)), GeneSplicer (<http://www.cbcb.umd.edu/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 splice 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.1192 G > 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 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 G > 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 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 2 II2) and 5.1% (family 3 (II1), 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 II1, 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 II2, TBIL 57.2 unol/L, DBIL 10.9 unol/L; Family 3 II1, TBIL 154.27 unol/L, DBIL 17.5 unol/L).

### Pathogenicity analysis of the novel *G6PD* mutant

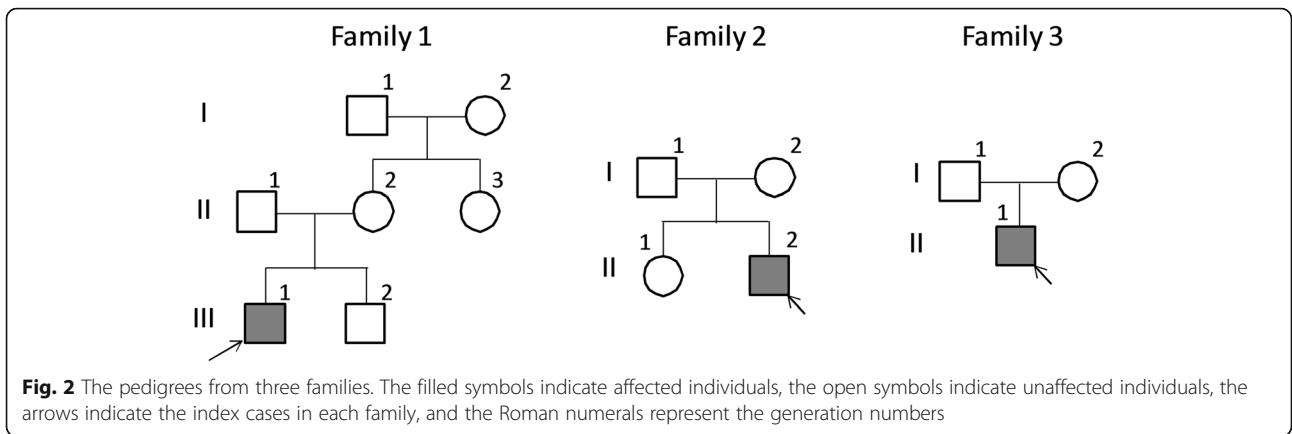
With bioinformatics tools to predict the effect of amino acid substitutions on protein function, the missense variant c.1118 T T > C, p.Phe373Ser was classified as pathogenic (Table 3). MutPred prediction score for this mutant was 0.902, revealing that this variant was a deleterious variant and has the 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 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 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

**Table 1** The detection rate of G6PD variants in infants with pathologic jaundice

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_001035810.1:p.His32Arg	50	11.39%	3.19%	10,403	CM910158
NM_000402.4:c.185A > G	NP_000393.4:p.His62Arg					
NM_001042351.2:c.466G > A	NM_000402.4:c.556G > A	16	3.64%	1.02%	10,364	CM880031
	NP_001035810.1:p.Glu156Lys					
	NP_000393.4:p.Glu186Lys					
NM_001042351.2:c.871G > A	NP_001035810.1:p.Val291Met	32	7.29%	2.04%	10,386	CM930275
	NP_000393.4:p.Val321Met					
NM_001042351.2:c.1004 C > A	NM_000402.4:c.1094C > A	12	2.73%	0.77%	NA	CM950506
	NP_001035810.1:p.Ala335Asp					
	NP_000393.4:p.Ala365Asp					
NM_001042351.2:c.1024C > T	NP_001035810.1:p.Leu342Phe	28	6.38%	1.79%	10,405	CM930276
NM_000402.4:c.1114C > T	NP_000393.4:p.Leu372Phe					
NM_001042351.2:c.1118T > C	NP_001035810.1:p.Phe373Ser	3	0.68%	0.19%	NA	NA
NM_000402.4: c.1208 T > C	NP_000393.4:p.Phe403Ser					
NM_001042351.2:c.1192 G > A	NP_001035810.1:p.Glu398 Lys	12	2.73%	0.77%	NA	CM910162
NM_000402.4:c.1282G > A	NP_000393.4:p.Glu428Lys					
NM_001042351.2:c.1376G > T	NP_001035810.1:p.Arg459Leu	140	31.89%	8.95%	100,058	CM910163
NM_000402.4:c.1466G > T	NP_000393.4:p.Arg489Leu					
NM_001042351.2:c.1388G > A	NP_001035810.1:p.Arg463His	146	33.26%	9.33%	100,059	CM910164
NM_000402.4:c.1478G > A	NP_000393.4:p.Arg493His					

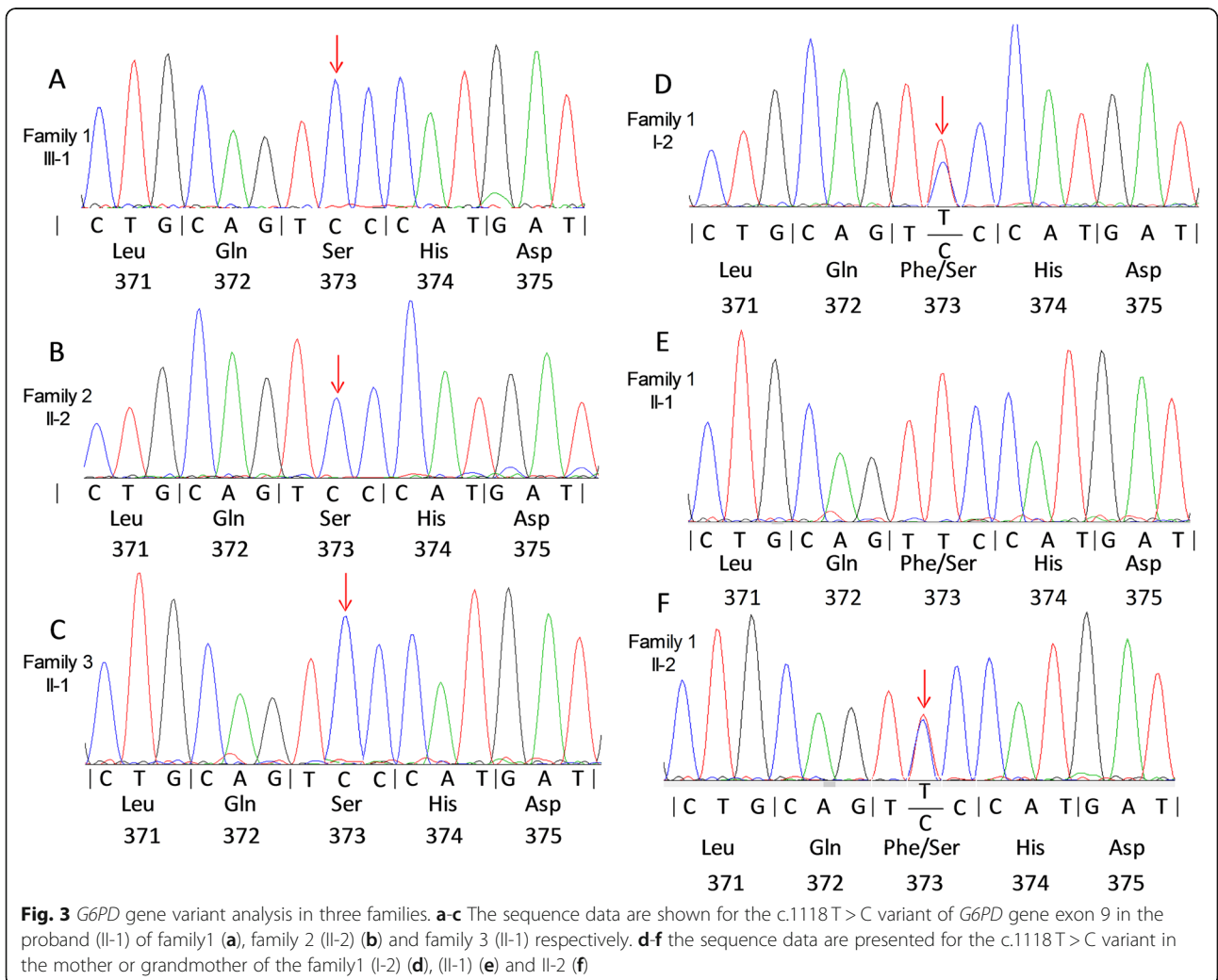
Note: In the database of Exome Aggregation Consortium (ExAC), ClinVar and HGMD, some G6PD variants are only expressed in G6PD splice variant 1 (NM\_000402.4) ([https://www.ncbi.nlm.nih.gov/nuccore/NM\\_000402](https://www.ncbi.nlm.nih.gov/nuccore/NM_000402)), some in splice variant 2, which lacks exon 1 (NM\_001042351.2) ([https://www.ncbi.nlm.nih.gov/nuccore/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 detect 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



**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

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. 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 family1 (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 family1 (I-2) (d), (II-1) (e) and II-2 (f)

**Table 2** The clinical data of three families

Items	Family 1						Family 2				Family 3			
	I1	I2	II1	II2	II3	III1	III2	I1	I2	II1	II2	I1	I2	II1
<b>patient</b>														
<b>age</b>	61	59	39	36	27	2.5	0.5	36	37	8	1	32	29	0.5
<b>Clinical diagnosis</b>	N	CHM	N	CHM	N	D-G6PD	N	N	CHM	CHM	D-G6PD	N	CHM	D-G6PD
<b>G6PD activity (%)</b>	97.5	63.8	98.3	65.8	96.4	3.5	98.1	96.2	67.2	66.1	4.6	95.8	61.8	5.1
<b>RBC (10<sup>12</sup>/L)</b>	4.78	3.28	3.98	3.24	4.54	2.72	4.59	4.98	3.78	3.04	1.73	4.51	3.46	2.61
<b>Hb(g/L)</b>	155	95	129	93	148	89	134	133	109	97	54	146	95	99
<b>HCT (%)</b>	0.44	0.29	0.36	0.28	0.42	0.26	0.40	0.41	0.33	0.28	0.15	0.43	0.29	0.29
<b>TBIL (unol/L)</b>	17.39	10.1	14.7	11.62	8.70	158.1	13.1	5.9	21.9	17.0	57.2	18.65	6.76	154.27
<b>DBIL (unol/L)</b>	5.4	2.3	4.1	2.8	2.0	19.5	2.8	2.0	6.1	5.2	10.9	6.8	3.0	17.5

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 10<sup>12</sup>/L, child 3.1–5.3 10<sup>12</sup>/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

### Conservation and stability analysis of the novel G6PD mutant

According to Alamut, both nucleotide c.1118T 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  $\Delta\Delta G$ : -1.54). I-Mutant2.0 predicts this variant to have decreased stability ( $\Delta\Delta G$ : -2.32). AUTO-MUTE predicts this variant to have decreased stability ( $\Delta\Delta G$ : -1.97). ProMaya (Protein Mutant stAbilitY Analyzer) predicts this variant to have decreased stability ( $\Delta\Delta G$ : -1.97). MutPred2 predicts this variant to have altered stability ( $P = 0.02$ ), and CUPSAT predicts this

variant to be destabilizing ( $\Delta\Delta G$ : -1.75). ProSMS predicts this variant to be destabilizing (probability 0.84).

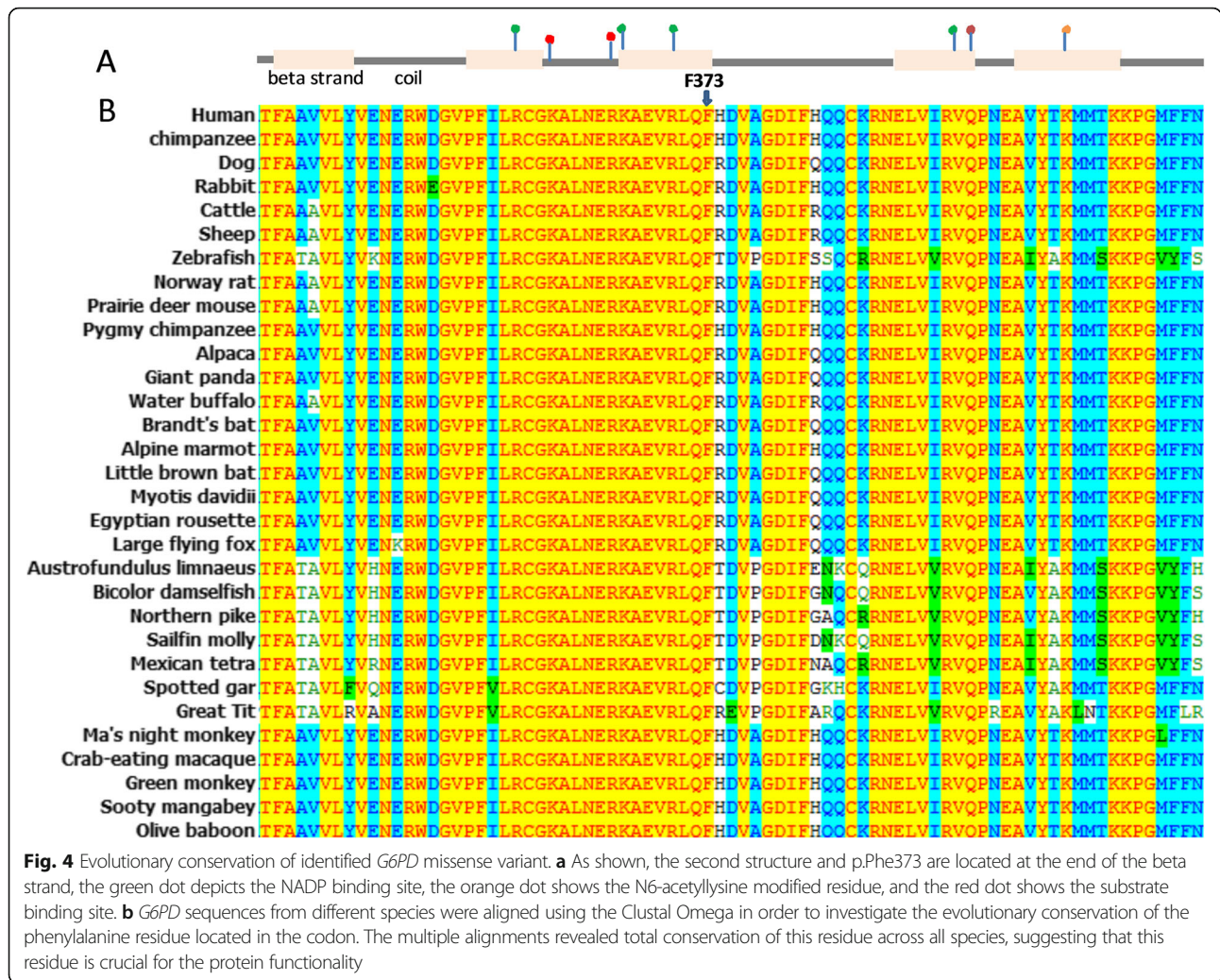
### Discussion

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

**Table 3** Disease causing variant and phenotype in Chinese patients with D-G6PD and hemizygote missense variant in G6PD

Genome (h19)	DNA	Protein	known variant	SIFT		PROVEAN		Variant taster		PolyPhen-2	
				score	prediction	score	prediction	prediction	probability	Prediction	Confidence
Chr X: 153760951 T>C	c.1118 T>C	p.Phe373Ser	No reports	0.000	Damaging	-6.58	Deleterious	disease-causing	1.0	Probably damaging	0.996



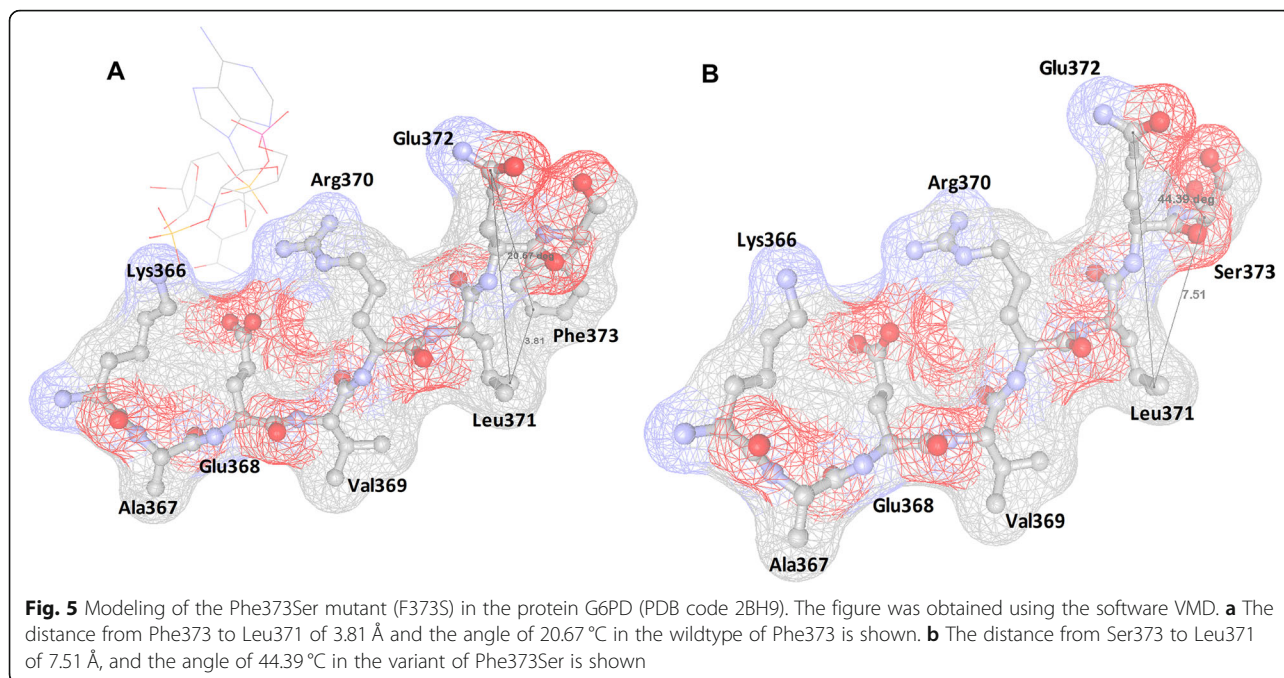
erythrocyte transfusion. The *G6PD* variant test is the gold standard for the diagnosis of D-G6PD. Almost all newborns with a positive result by *G6PD* serology screening will have a *G6PD* variant detected. Therefore, prenatal diagnosis should be performed when parents have clinical symptoms of D-G6PD, 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





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 taken 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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#### References

- Notaro R, Afolayan A, Luzzatto L. Human mutations in glucose 6-phosphate dehydrogenase reflect evolutionary history. *FASEB J*. 2000;14(3):485–94.
- Gomez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, Serrano-Posada H, Ortega-Cuellar D, Gonzalez-Valdez A, et al. Glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. *Int J Mol Sci*. 2016;17(12):2069.
- Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood*. 2008;111(1):16–24.
- Takizawa T, Huang IY, Ikuta T, Yoshida A. Human glucose-6-phosphate dehydrogenase: primary structure and cDNA cloning. *Proc Natl Acad Sci U S A*. 1986;83(12):4157–61.
- Hsia YE, Miyakawa F, Baltazar J, Ching NS, Yuen J, Westwood B, et al. Frequency of glucose-6-phosphate dehydrogenase (G6PD) mutations in Chinese, Filipinos, and Laotians from Hawaii. *Hum Genet*. 1993;92(5):470–6.
- Au WY, Lam V, Pang A, Lee WM, Chan JL, Song YQ, et al. Glucose-6-phosphate dehydrogenase deficiency in female octogenarians, nanogenarians, and centenarians. *J Gerontol A Biol Sci Med Sci*. 2006;61(10):1086–9.
- Belfield KD, Tichy EM. Review and drug therapy implications of glucose-6-phosphate dehydrogenase deficiency. *Am J Health Syst Pharm*. 2018;75(3):97–104.
- Ong KIC, Kosugi H, Thoeun S, Araki H, Thandar MM, Iwagami M, et al. Systematic review of the clinical manifestations of glucose-6-phosphate dehydrogenase deficiency in the greater Mekong subregion: implications for malaria elimination and beyond. *BMJ Glob Health*. 2017;2(3):e000415.
- Steiner M, Ludemann J, Krammer-Steiner B. Favism and Glucose-6-phosphate dehydrogenase deficiency. *N Engl J Med*. 2018;378(11):1068.
- Luzzatto L, Arese P. Favism and Glucose-6-phosphate dehydrogenase deficiency. *N Engl J Med*. 2018;378(11):1068–9.
- Peters AL, Van Noorden CJ. Glucose-6-phosphate dehydrogenase deficiency and malaria: cytochemical detection of heterozygous G6PD deficiency in women. *J Histochem Cytochem*. 2009;57(11):1003–11.
- Clarke GM, Rockett K, Kivinen K, Hubbard C, Jeffreys AE, Rowlands K, et al. Characterisation of the opposing effects of G6PD deficiency on cerebral malaria and severe malarial anaemia. *eLife*. 2017;6:e15085.
- Kim Y, Park J, Kim M. Diagnostic approaches for inherited hemolytic anemia in the genetic era. *Blood Res*. 2017;52(2):84–94.
- Vulliamy TJ, D'Urso M, Battistuzzi G, Estrada M, Foulkes NS, Martini G, et al. Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia. *Proc Natl Acad Sci U S A*. 1988;85(14):5171–5.
- Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Williams TN, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nat Commun*. 2010;1:104.
- Gomez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, Serrano-Posada H, Gonzalez-Valdez A, Martinez-Rosas V, et al. Functional and biochemical characterization of three recombinant human glucose-6-phosphate dehydrogenase mutants: Zacatecas, Vanua-Lava and Viangchan. *Int J Mol Sci*. 2016;17(5):787.
- Hwang S, Mruk K, Rahighi S, Raub AG, Chen CH, Dorn LE, et al. Correcting glucose-6-phosphate dehydrogenase deficiency with a small-molecule activator. *Nat Commun*. 2018;9(1):4045.
- Ramirez-Nava EJ, Ortega-Cuellar D, Serrano-Posada H, Gonzalez-Valdez A, Vanoye-Carlo A, Hernandez-Ochoa B, et al. Biochemical analysis of two single mutants that give rise to a polymorphic G6PD A-double mutant. *Int J Mol Sci*. 2017;18(11):2244.
- Gomez-Manzo S, Terron-Hernandez J, De la Mora-De la Mora I, Gonzalez-Valdez A, Marcial-Quino J, Garcia-Torres I, et al. The stability of G6PD is affected by mutations with different clinical phenotypes. *Int J Mol Sci*. 2014;15(11):21179–201.
- Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. *Blood Rev*. 2007;21(5):267–83.
- Scopes DA, Bautista JM, Naylor CE, Adams MJ, Mason PJ. Amino acid substitutions at the dimer interface of human glucose-6-phosphate dehydrogenase that increase thermostability and reduce the stabilising effect of NADP. *Eur J Biochem*. 1998;251(1–2):382–8.
- Mejia SA, Gutman LAB, Camarillo CO, Navarro RM, Becerra MCS, Santana LD, et al. Nicotinamide prevents sweet beverage-induced hepatic steatosis in rats by regulating the G6PD, NADPH/NADP(+) and GSH/GSSG ratios and reducing oxidative and inflammatory stress. *Eur J Pharmacol*. 2018;818:499–507.
- Verma A, Chandra S, Suthar MK, Doharey PK, Siddiqi MI, Saxena JK. NADP(+) binding effects tryptophan accessibility, folding and stability of recombinant B. malayi G6PD. *Int J Biol Macromol*. 2016;85:645–54.
- Cheng ML, Ho HY, Lin HY, Lai YC, Chiu DT. Effective NET formation in neutrophils from individuals with G6PD Taiwan-Hakka is associated with enhanced NADP(+) biosynthesis. *Free Radic Res*. 2013;47(9):699–709.
- Minucci A, Giardina B, Capoluongo E. Could G6PD-Buenos-Aires confirm the existence of the "structural NADP+ binding site" and its strategic role for the stability and/or activity enzyme? *Clin Biochem*. 2009;42(1–2):132–3.
- Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischARGE hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103(1):6–14.
- Stevenson DK, Fanaroff AA, Maisels MJ, Young BW, Wong RJ, Vreman HJ, et al. Prediction of hyperbilirubinemia in near-term and term infants. *J Perinatol*. 2001;21(Suppl 1):S63–72 discussion S83–7.
- Pahlavanzadeh M, Hekmatimoghaddam S, Teremahi Ardestani M, Ghafoorzadeh M, Aminorraaya M. G6PD enzyme deficiency in neonatal pathologic hyperbilirubinemia in Yazd. *Iran J Pediatr Hematol Oncol*. 2013;3(2):69–72.
- Moiz B, Arshad HM, Raheem A, Hayat H, Karim Ghanchi N, Beg MA. Frequency of G6PD Mediterranean in individuals with and without malaria in southern Pakistan. *Malar J*. 2017;16(1):426.
- Kawamoto F, Matsuoka H, Pham NM, Hayashi T, Kasahara Y, Dung NT, et al. Further molecular analysis of G6PD deficiency variants in southern Vietnam and a novel variant designated as G6PD Ho Chi Minh (173 A>G; 58 Asp>Gly): frequency distributions of variants compared with those in other southeast Asian countries. *Acta Med Okayama*. 2017;71(4):325–32.
- Huang S, Xu Y, Liu X, Zhou M, Wu X, Jia Y. Molecular newborn screening of four genetic diseases in Guizhou Province of South China. *Gene*. 2016;591(1):119–22.
- Yan T, Cai R, Mo O, Zhu D, Ouyang H, Huang L, et al. Incidence and complete molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Guangxi Zhuang autonomous region of southern China: description of four novel mutations. *Haematologica*. 2006;91(10):1321–8.
- Fu C, Luo S, Li Q, Xie B, Yang Q, Geng G, et al. Newborn screening of glucose-6-phosphate dehydrogenase deficiency in Guangxi, China:

determination of optimal cutoff value to identify heterozygous female neonates. *Sci Rep.* 2018;8(1):833.

34. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015; 17(5):405–24.
35. Smitherman H, Stark AR, Bhutani VK. Early recognition of neonatal hyperbilirubinemia and its emergent management. *Semin Fetal Neonatal Med.* 2006;11(3):214–24.
36. Lee J, Park J, Choi H, Kim J, Kwon A, Jang W, et al. Genetic profiles of Korean patients with glucose-6-phosphate dehydrogenase deficiency. *Ann Lab Med.* 2017;37(2):108–16.
37. Yang H, Wang Q, Zheng L, Zhan XF, Lin M, Lin F, et al. Incidence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency among neonates for newborn screening in Chaozhou, China. *Int J Lab Hematol.* 2015;37(3):410–9.
38. Peng Q, Li S, Ma K, Li W, Ma Q, He X, et al. Large cohort screening of G6PD deficiency and the mutational spectrum in the Dongguan District in southern China. *PLoS One.* 2015;10(3):e0120683.

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