Genetic Spectrum and Clinical Early Natural History of Glucose-6-Phosphate Dehydrogenase Deficiency in Mexican Children Detected Through Newborn Screening.

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TITLE:

Genetic spectrum and clinical early natural history of glucose-6-phosphate dehydrogenase deficiency in Mexican children detected through newborn screening

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase deficiency (G6PDd) newborn screening is still a matter of debate due to its highly heterogeneous birth prevalence and clinical expression as well as the lack of enough knowledge on its natural history. Herein, we describe the early natural clinical course and the underlying *GDPD* genotypes in infants with G6PDd

detected by newborn screening and later studied in a single follow-up center. G6PDd newborns were categorized into three groups: group 1: hospitalized with or without neonatal jaundice (NNJ); group 2: nonhospitalized with NNJ; and group 3: asymptomatic.

Results: A total of 81 newborns (80 males, one female) were included. Most individuals (46.9%) had NNJ without other symptoms, followed by asymptomatic (42.0%) and hospitalized (11.1%) patients, although the hospitalization of only 3 of these patients was related to G6PDd, including NNJ or acute hemolytic anemia (AHA). Nine different G6PDd genotypes were found; the G6PD A$-^{202A/376G}$ genotype was the most frequent (60.5%), followed by the G6PD$^{376G/542T}$ (Santamaria, 22.2%) and the Union-Maewo (rs398123546, 7.4%) genotypes. These genotypes produce a wide range of clinical and biochemical phenotypes with significant overlapping residual enzymatic activity values among class I, II or III variants. Some G6PD A$-^{202A/376G}$ individuals had enzymatic values that were close to the cutoff value (5.3 U/g Hb, 4.6 and 4.8 U/g Hb in the groups with and without NNJ, respectively), while others showed extremely low enzymatic values (1.1 U/g Hb and 1.4 U/g Hb in the groups with and without NNJ, respectively).

Conclusion: Wide variability in residual enzymatic activity was noted in G6PDd individuals with the same G6PD genotype. This feature, along with a documented heterogeneous mutational spectrum, makes it difficult to categorize G6PD variants according to current WHO classification and precludes the prediction of complications such as AHA, which can occur even with residual enzymatic activity (>10%) and/or be associated with the common and mild G6PD A$-^{376G/968C}$ and G6PD A$-^{202A/376G}$ haplotypes.

Key words: Glucose-6-phosphate dehydrogenase deficiency; G6PD deficiency; neonatal jaundice; genetic disorders; newborn screening; hemolytic anemia.

Abbreviations

ACMG NBS: American College of Medical Genetics newborn screening panel
ASx: asymptomatic
AHA: acute hemolytic anemia
CNSHA: chronic non spherocytic hemolytic anemia
DBil: direct bilirubin
DBS: dry blood sample
G6PD: glucose-6-phosphate dehydrogenase
G6PDd: glucose-6-phosphate dehydrogenase deficiency
Hb: hemoglobin
HTC: hematocrit
IBil: indirect bilirubin
NADPH: nicotinamide adenine dinucleotide phosphate
NNJ: neonatal jaundice
Ret: reticulocytes
TBil: total bilirubin
WHO: World Health Organization

BACKGROUND

Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) is a cytosolic enzyme that catalyzes the first step of the pentose phosphate pathway to provide reduced equivalents to biosynthesis processes and to neutralize cell oxidative stress [1]. G6PD deficiency (G6PDd) is considered the most common human enzymopathy, which is inherited as a polymorphic X-linked trait and attributed to nearly 230 hypomorphic variants in the *G6PD* gene (Xq28, MIM *305900) [2, 3, 4]. G6PDd affects more than 500 million people, although it has a worldwide distribution with very large variations in its prevalence ranging from zero in the original Amerindian populations to 20% in regions of Africa and Asia [4]. In addition, G6PDd has a great variety in its clinical expression, with most patients being asymptomatic, while others develop serious events of acute hemolytic anemia (AHA) that can be life-threatening or chronic [4, 5]. Neonatal jaundice (NNJ) is one of the clinical manifestations of G6PDd, and sometimes, its severity can lead to kernicterus [3, 6]. The WHO G6PDd classification from 1967 [7] establishes five classes of G6PDd based on the levels of enzyme residual activity determined in hemizygous males and according to associated clinical manifestations: class I: <10% with chronic nonspherocytic hemolytic anemia (CNSHA) and acute exacerbations; class II: <10% without clinical manifestations in the steady state; class III: 10-60% asymptomatic in the steady state; class IV: 100% asymptomatic; and class V: >100% no clinical manifestations. However, Luzzato 2016 proposed a revised classification based on adult screening as follows: class I: residual activity <10%; class II+III: <30%; and class IV>85% (with the elimination of class V, [6].

Particularly, population screening of G6PDd has been carried out in malaria endemic areas to prevent drug interactions that should trigger acute hemolytic crises in deficient individuals [8-10], but G6PDd mass newborn screening is still a matter of debate, and its implementation

has been limited to few countries, mainly countries in Asia and Latin America [10-14]. Although some G6PDd newborn screening experiences in high-income countries such as Sweden have been reported [15], most of them do not include G6PDd detection in their recommended uniform screening panel [16], based on the argument of its highly heterogeneous birth prevalence and clinical expression as well as the lack of enough knowledge on its natural history [17].

Despite several publications regarding the results obtained for some G6PDd newborn screening programs, most of them are focused on its prevalence, cutoff and enzyme value distributions, and mutational spectrum [12-14, 18], but few of them try to establish the phenotype-genotype correlation [19] reports about detailed clinical follow-up or medical interventions on G6PDd newborns are limited.

In Mexico, a country with nearly 2 million births annually (https://www.inegi.org.mx/sistemas/olap/consulta/general_ver4/MDXQueryDatos.asp?#Regreso&c=23699), the detection of G6PDd was added to the mandatory neonatal screening panel established by the Ministry of Health since 2015; this panel also includes congenital hypothyroidism, phenylketonuria, congenital adrenal hyperplasia, galactosemia and cystic fibrosis [20]. The first results of the G6PDd Mexican screening program confirmed its regional disparity in prevalence, ranging from 0.2 to 20%, as well as the identified difficulties in classifying affected patients [21].

The aim of this work is to present the G6PD activity levels, the underlying deficient *G6PD* genotypes, the phenotype-genotype correlation, and the clinical characteristics of a group of Mexican infants with G6PDd detected by newborn screening and later studied in a single follow-up center, aiming to produce a more comprehensive clinical classification system.

METHODS

1. Population study.

Eighty-four infants (81 males, 3 females) with a suspicious (positive) result in the newborn screening for G6PDd were evaluated at the National Institute of Pediatrics from February 2018 to March 2020. The study algorithm is shown in Figure 1. Confirmed patients were called for medical evaluation, including a record of risk factors and genetic counseling. False positive patients were informed and discharged to the first level attention medical units for healthy child control. Confirmed G6PDd patients were categorized into 3 groups according to their clinical antecedents: group 1: hospitalized patients in the neonatal period with or without NNJ; group 2: nonhospitalized patients with NNJ; and group 3: asymptomatic newborns. Enzymatic and molecular studies were also offered for the siblings of G6PDd children. Clinical description was performed under the criteria of the Phenomizer (Orphanet) database [22, 23, http://www.human-phenotype-ontology.org]. The study was approved by the Institutional Research, Ethics and Biosecurity Boards (protocol registry 039/2018), and written informed consent was obtained from the parents of each participant.

2. Blood samples for biochemical and genotype confirmation of G6PDd.

Six drops of blood were extracted from each subject by heel puncture, deposited on a Guthrie card and allowed to dry for 3 hours at room temperature. For older siblings, the sample was obtained by finger puncture.

3. Confirmatory studies (short follow-up).

3.1 Enzyme activity quantification. To prevent G6PD activity decay in dried blood spots (DBS, Maldonado 2018), all the samples were stored at -20°C and analyzed 48 hours after extraction. G6PD activity was determined by the fluorometric method using commercial kits (test kit 6199860, LabSystems Diagnostics Oy, Vantaa, Finland). Briefly, 3 mm DBS disks

were allocated into a 96-well microplate with calibrators and duplicate controls. A reaction mixture was reconstituted with a buffer solution. Then, 150 μL of the reaction mixture was added to each well. The plates were incubated for 30 minutes while being shaken. Then, 150 μL of cold copper reagent was added, and finally, the product of the enzymatic reaction was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The cutoff value was established after enzymatic activity quantification of 564 DBS using the fifth percentile value of 5.3 U/g Hb.

3.2 G6PD molecular analysis. By processing 3-4 DBS punches of 3.2 mm in diameter, genomic DNA was obtained by the salting-out precipitation method (Gentra Puregene Blood Kit, QIAGEN, Hilden, Germany). PCR amplification of exons 3-4, 5, 6-7 and 9-10 of the *G6PD* gene (exon numbering according to NM_001042351.2), including their exon-intron boundaries, and further direct automated Sanger sequencing (primer and PCR conditions are available upon request) were applied to characterize the main hypomorphic haplotypes that account for ~90% of G6PDd alleles in the Mexican population [24]: G6PD A$^{-202A/376G}$ (c.[202G>A; 376A>G] or p.[Val68Met; Asn126Asp]), G6PD A$^{-376G/968C}$ (c.[376A>G; 968T>C] or p.[Asn126Asp; Leu323Pro]) and G6PD Santamaria$^{376G/542T}$ (c.[376A>G; 542A>T] or p.[Asn126Asp; Asp181Val]). The employed sequencing strategy allows the identification of other variants allocated at these *G6PD* gene regions and described previously as rare G6PDd variants in the Mexican population (i.e., Viangchan or p.(Val291Met) variant, rs137852327, [24]. In the patients with biochemically confirmed G6PDd but an initial normal Sanger sequencing result, further sequencing of exons 2, 8 and 11-13 of *G6PD* was subsequently applied to exclude the presence of other rare *G6PD* deficient variants, which have also been described in Mexican G6PDd patients (i.e., Union-

Maewo or p.(Arg454Cys) variant, rs398123546). Whole *G6PD* sequencing was applied to confirm all suspected false-positive cases.

4. Continuous data are presented as medians with maximum and minimum values; categorical data are presented as counts and percentages. To establish differences between groups, one-way ANOVA was used. Data were analyzed using the R program (http://cran.r-project.org/).

RESULTS

A total of 81 patients (80 males and one female) showed enzymatic activity below the cutoff value (5.3 U/g Hb), so they were considered to have G6PDd and then subjected to molecular analysis. Three individuals (1 male and 2 females) had normal G6PD activity, and no hypomorphic variant was found after whole *G6PD* Sanger sequencing, so they were classified as false-positive patients. The number of patients in each clinical group is presented in Figure 1, showing that the majority of individuals (46.9%, 38/81) had NNJ without other symptoms, 42.0% (34/81) were asymptomatic at the moment of the study, and 11.1% (9/81) were symptomatic patients (including NNJ) who required hospitalization. None of the hospitalized patients had been exposed to medications prior to their admission, and all were breastfed or receiving mixed feeding with human milk and starter infant formula.

Across the studied population, we found 9 different *G6PD* variants, all of which were previously described (Table 1). The most frequent deficient haplotype was G6PD A$^{-202A/376G}$, which was found in 60.5% of the deficient patients, followed by G6PD Santamaria$^{376G/542T}$ which was found in 22.2% of the deficient patients, and Union-Maewo (p.(Arg454Cys) [rs398123546]), which was found in 7.4% of the deficient patients. The mean enzymatic activity of each variant and its geographical origin are shown in Table 1.

Of the 81 patients, 9/81 (11.1%) patients, in accordance with the Luzzato 2016 classification [6], were class II, while 2/81 (2.4%) and 70/81 (86.4%) were class II-III and class III, respectively (Table 2). However, there is an overlapping residual G6PD activity between classes II and III (Table 1).

The hematologic parameters and G6PD activity related to the studied groups are shown in Table 2, while a detailed description of the patients who required hospitalization in the newborn period (Group 3) and its categorization according to the WHO and Luzzatto classification systems [6, 7] are shown in Table 3. In four families, one or more siblings were G6PDd, and their results are presented in Table 4. In the studied newborns, 41/81 (50.61%) had NNJ, and 2/81 (2.47%) had hemolytic anemia (Figure 1, Table 3). The genotypes, enzymatic activity, and geographical origin of each of 81 patients are shown in Supplementary Table 1.

DISCUSSION

G6PDd is widely heterogeneous in terms of biochemical, clinical, and molecular manifestations [25]. In the present study, different G6PD mutations produced a wide range of clinical and biochemical phenotypes with significant overlapping residual enzymatic activity values between class I, II or III variants. WHO classification has been established according to enzymatic activity and severity of the patients, assuming that class I patients are more serious than class III patients. In class II newborns, the residual activity ranged from 0.56% to 12.36%, while in class III newborns, the residual activity ranged from 5.6 to 43.82%, and in a significant number of individuals, there was an overlap of the values (Table 1). However, our results revealed some discrepancies with the WHO classification, since the most severe patients with AHA were associated with class III genotypes (patients 2 and 11

bearing the G6PD A$^{-376G/968C}$ and G6PD A$^{-202A/376G}$ genotypes, respectively; Table 3). Usually, AHA is expected to be more common and severe in association with the Mediterranean variant than in the A− variant [5], but our results do not agree with this observation since AHA was found in A− patients. Moreover, we did not find a correlation between the clinical severity and the WHO *G6PD* variant categorization. Remarkably, only 7/9 patients could be categorized with the Luzzato classification, and the other two patients could not be classified as they did not meet the clinical or biochemical criteria (Table 3).

Several authors have found that the prevalence of G6PDd is higher in jaundiced newborns than in the control population, ranging from 8.9 to 28.1% [26-30]. Badejoko et al. found in a prospective observational study that 68.2% of G6PDd newborns presented with hyperbilirubinemia [31]. To our knowledge, the proportion of patients with NNJ in the reviewed publications on the results of G6PDd newborn screening is not stated. Instead, we found a high proportion of patients with NNJ (44/81, 54.32%), but only four of them (9%, 4/44) required hospitalization for jaundice management (Table 3). Even some individuals carrying the hypomorphic G6PD A$^{-202A/376G}$ haplotype had enzymatic values that were close to the cutoff value (5.3 U/g Hb, 4.6 and 4.8 U/g Hb in the groups with and without NNJ, respectively), while other individuals showed extremely low enzymatic values (1.1 U/g Hb and 1.4 U/g Hb in the NNJ and without NNJ groups, respectively). In fact, we identified four G6PDd families with more than one affected patient (Table 4), in which only two patients had antecedent NNJ and one of them had a hemyzygous G6PD A$^{-202A/376G}$ genotype (class III) experienced two episodes of AHA at the ages of 9 and 24 months old, respectively (Table 4). All these observations supported the idea that identical *G6PD* genotypes could lead to a wide range of phenotypes [4]; therefore, information that relies only on the *G6PD* genotype seems to be not useful for the prediction of clinical severity, as other causes, such as

enzymatic kinetics or residual catalytic function related to structural stability [4, 6, 32]. Recently, Mansour-Hendili et al described patients with unexplained congenital hemolytic anemia that carried genetic variations in more than one gene, where G6PD variants were detected in combination with heterozygous β-spectrin, α-spectrin, solute carrier family 4 (anion exchanger) member 1, piezo-type mechanosensitive ion channel component 1, or hemoglobin-β locus [33], so other genetic as well as other epigenetic factors that are currently unknown, could be involved and must be further assessed. Moreover, there were no significant differences among the enzymatic residual activity documented in the three groups of studies, although slightly lower enzymatic residual values were noted for newborns that required hospitalization (Table 2). Nevertheless, one limitation of this study design is that it does not allow us to know the cases of kernicterus that could have occurred; due to the severity of kernicterus, it is to be expected that such patients would be hospitalized or even deceased and not come to our newborn screening follow-up center.

Regarding the identified genotypic *G6PD* spectrum, the most frequently identified hypomorphic *G6PD* allele comes from Africa (G6PD $A^{-202A/376G}$), followed by the class II Union-Maewo or p.(Arg454Cys) variant (Table 1), whose origin was presumably traced to the Philippines [12, 34]. Hemizygous Union-Maewo genotypes were found in six of our patients, and all of them showed the lowest enzymatic activity (mean 0.7, interval 0.05-0.1 U/g Hb), but only three of them had NNJ, and none of them required hospitalization or showed AHA. The Union-Maewo variant comprised 66% of G6PDd-responsible genotypes in patients who came from the Mexican Pacific coast (Guerrero, Supplemental Table 1), which could be a feature historically related to the intense commercial exchange (which included slave trade) established between the Philippines (Manila Galleon) and the Mexican

Pacific coast during the 16th-17th centuries [35]. The full sequencing of the coding region of the *G6PD* gene allowed for the identification of very rare variants, including the Mahidol, Belem and Akrokorinthos variants, which have been described mainly in specific populations from Thailand, Brazil and Greece, respectively [36-38]. To the best of our knowledge, we describe for the first time their presence in the Mexican population. Remarkably, none of the two patients with the Akrokorinthos variant had any known Greek or Mediterranean ancestry, and both families were originally from the state of Guerrero in the Pacific Coast. The patients with Belem and Mahidol variants came from the metropolitan area of Mexico City and denied having a known ancestry from Brazil or Southeast Asia, respectively (Supplemental Table 1). The identified heterogeneous genotypic spectrum may reflect the well-known multiethnicity of the Mexican population [39].

In summary, the present work shows that the same *G6PD* variant can lead to highly variable enzymatic residual activity as well as a wide phenotypic spectrum in the first month of life, which is in accordance with previously reported results [15]. The absence of an absolute or predictive phenotype-genotype correlation (Supplementary Table 1) precludes the elaboration of guidelines on management, which agrees with statements of the ACMG NBS Expert Panel, who rejected the inclusion of G6PDd in the US newborn mandatory screening panel, due to the very limited data about the natural history of the disease, then encouraged the collection and publication of all the relevant clinical findings of the G6PDd screening programs [17]. Our work provides information on the early natural history of G6PDd newborns, and the present cohort will remain under surveillance.

Conclusion

There is wide variability in the enzymatic activity in G6PDd individuals, even in those with the same *G6PD* genotype. This feature, along with a documented heterogeneous mutational spectrum, hinders the categorization of *G6PD* variants according to the current WHO classification and, importantly, precludes the prediction of complications such as AHA, which can occur even with residual enzymatic activity >10% and/or is associated with the common and mild G6PD A$^{-376G/968C}$ and G6PD A$^{-202A/376G}$ variants.

Declarations

Ethics approval and consent to participate: This study was approved prior to data collection by the research, biosecurity, and ethics committees of the National Institute of Pediatrics (039/2018).

Consent for publication: All participants provided written consent to participate and have data published in an anonymized form.

Availability of data and materials: The datasets analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no potential conflicts of interest with respect to research, authorship or publication of this work.

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Authors' contributions:

MVA conducted the literature review, designed, and conceived the study, interpreted the data, drafted and critically revised the manuscript. MAAO designed the experimental procedure for the genotyping methods and their analyses, acquired funding for performing the genotyping experiments, and drafted and critically revised the manuscript. AGA

interpreted the genotyping data and critically revised the manuscript. LBM and CLC contributed to the recruitment and clinical evaluation of patients. IIG conceived and designed the study, designed the experimental procedures for biochemical analyses, interpreted the biochemical data, drafted, and revised the manuscript and approved the final version of the manuscript to be published.

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Tables and Figures.

Figure 1. Study algorithm. *Infants with a positive newborn screening test came from different primary care health centers. The clinical and biochemical approach of the 84 unrelated individuals initially referred to our center as G6PDd started with a new measurement of the G6PD activity and further *G6PD* genotyping experiments that unequivocally confirmed the G6PDd status in 81 patients, with or without neonatal jaundice (NNJ).

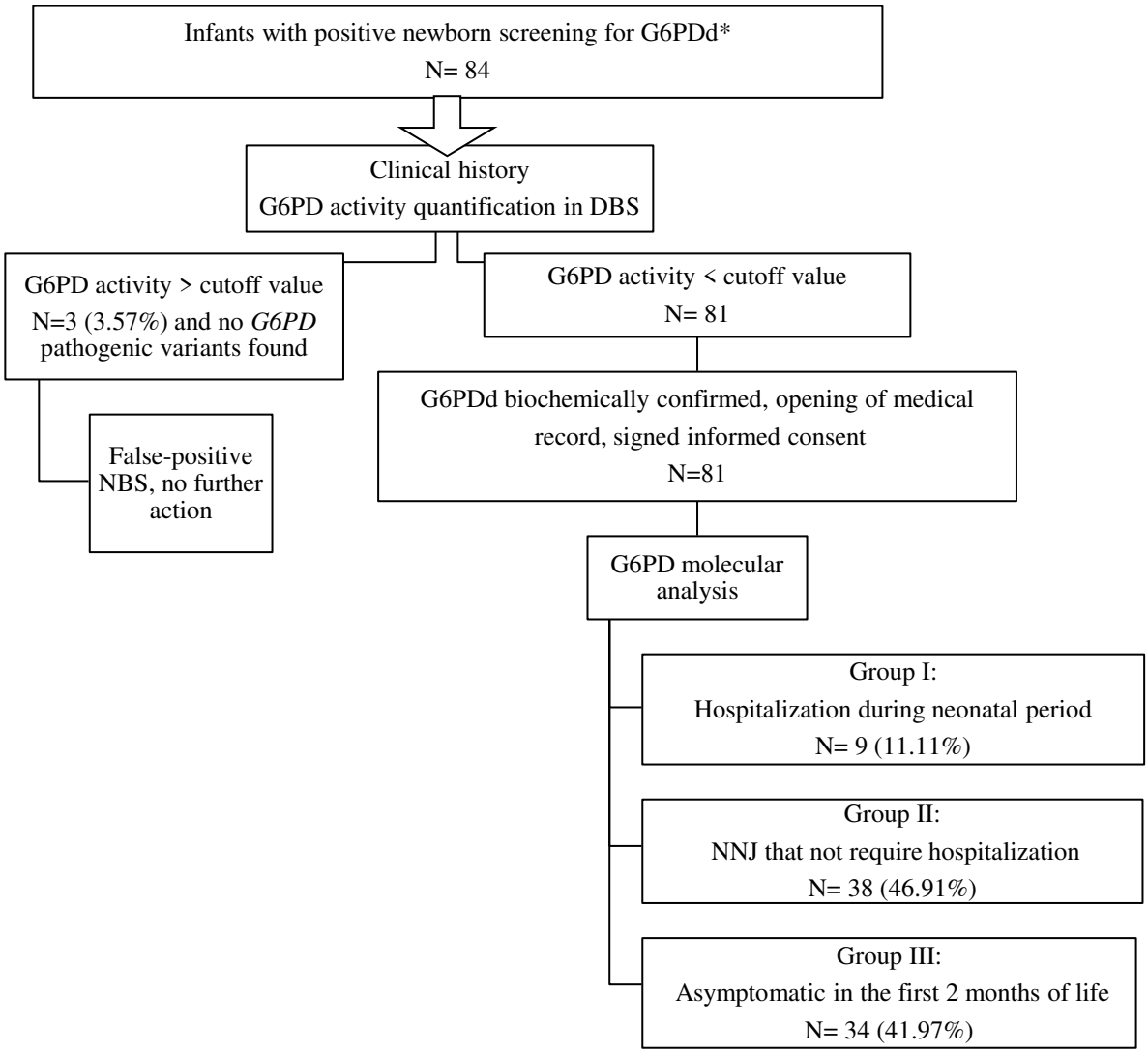
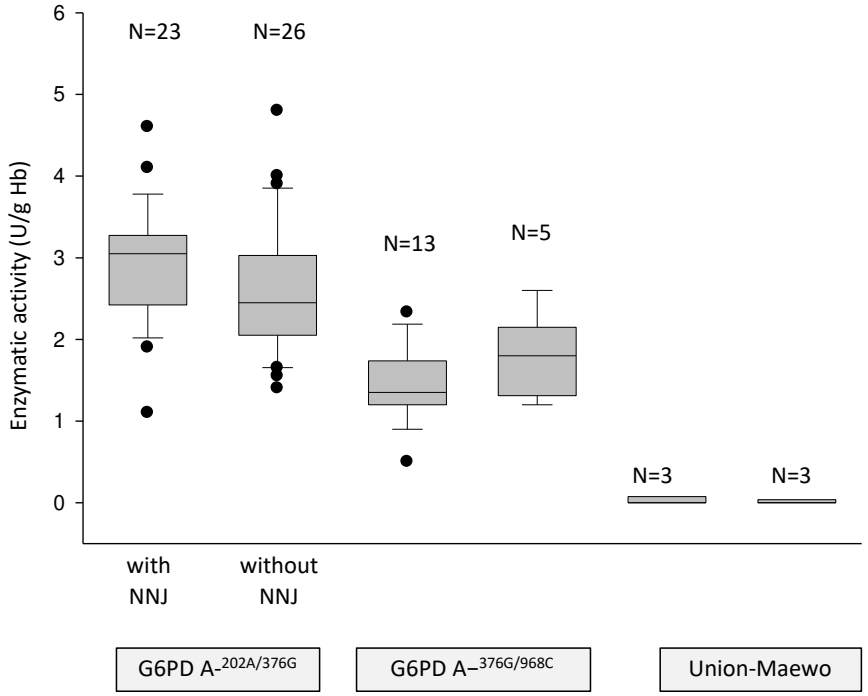


Figure 2. Documented G6PD deficient activity according to the identified *G6PD* patient genotypes (n=81), with or without (w/o) NNJ (neonatal jaundice). Panel A) Box and whisker plot genotypes with more than 3 affected individuals; Panel B) Enzymatic activity documented in less than 3 affected individuals with corroborated *G6PD* genotypes. Filled triangles represent patients with NNJ, and open triangles are patients without NNJ.

A)



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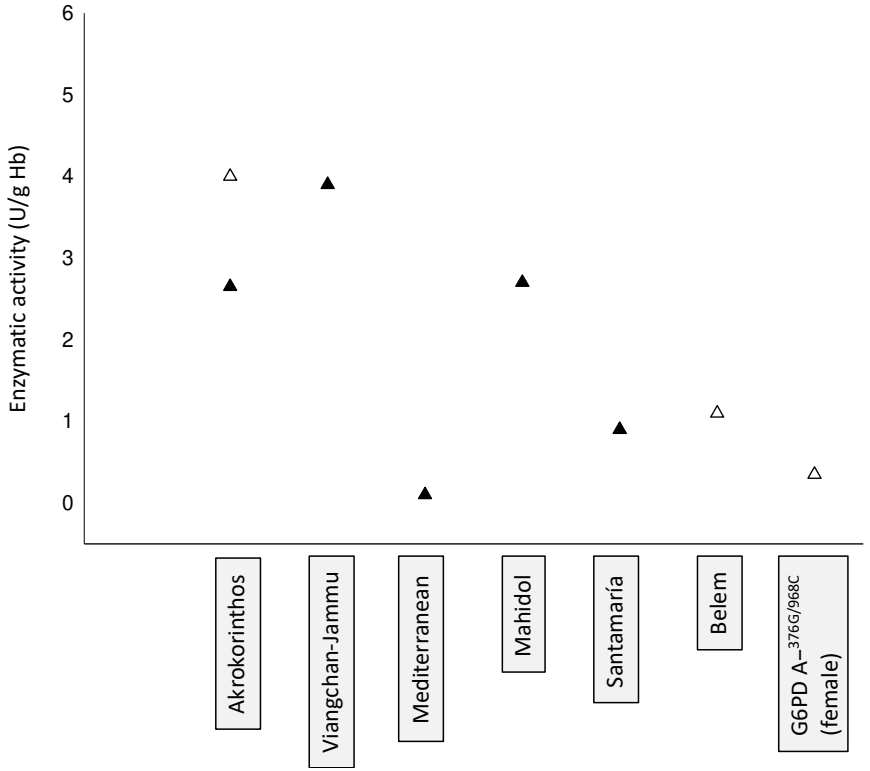

Table 1. Frequency of *G6PD* variants identified in the 81 Mexican G6PDd patients, according to WHO class and its mean enzymatic activity value.

					Present study		
Legacy name	**WHO class**	***G6PD* genotype (according to NM_001042351.2)**	**Protein Change (according to NP_001035810.1)**	**Geographical origin**	**Frequency (%)**	**G6PD activity U/g Hb (min-max)**	**G6PD residual activity %**
Males (80)							
G6PD A$^{-202A/376G}$	III	c.[202G>A; 376A>G]	p.[Val68Met; Asn126Asp]	African	49 (60.5)	2.76 (1.1-4.8)	31.04
G6PD A$^{-376G/968C}$	III	c.[376A>G; 968T>C]	p.[Asn126Asp; Leu323Pro]	African	18 (22.2)	1.55 (0.5-2.6)	17.37 (5.62 -29.21)
Union-Maewo	II	c.[1360C>T]	p.[Arg454Cys]	Asian (Philippines)	6 (7.4)	0.07 (0.05-0.1)	0.73 (0.56-1.12)
Akrokorinthos	II-III	c.[463C>G]	p.[His155Asp]	Greece	2 (2.5)	3.33 (2.6-4)	37.36 (29.78 – 44.94)
Belem	II	c.[409C>T]	p.[Leu137Phe]	Brazil	1 (1.2)	1.1	12.36
Mediterranean	II	c.[563C>T]	p.[Ser188Phe]	Mediterranean	1 (1.2)	0.1	1.12
Santamaria	II	c.[376A>G; 542A>T]	p.[Asn126Asp; Asp181Val]	Costa Rica, Italy	1 (1.2)	0.9	10.11
Mahidol	III	c.[487G>A]	p.[Gly163Ser]	Asian	1 (1.2)	2.7	30.34
Viangchan-Jammu	III	c.[871G>A]	p.[Val291Met]	Asian (China)	1 (1.2)	3.9	43.82
Female (1)							
Heterozygous G6PD A$^{-376G/968C}$	III	c.[376A>G; 968T>C];[=]	p.[Asn126Asp; Leu323Pro];[=]	African	1 (1.2)	0.35	3.93

Table 2. G6PD activity according to the different clinical groups.

Legacy name	Relative proportion of patients*	Mean G6PD-Activity U/g Hb	Mean G6PD residual activity %**
Group 1. Hospitalization during neonatal period n=9			
G6PD A$^{-202A/376G}$	4/49	3 (1.55 – 4.60)	34.1 (17.42-51.66)
G6PD A$^{-376G/968C}$	3/18	1.6 (1.2-2.0)	17.6 (13.48-22.47)
G6PD A$^{-202A/376G}$ (Heterozygous female)	1/1	0.35	3.93
Mediterranean	1/1	0.10	1.12
		1.92 (0.10-4.60)	**21.60 (1.12-51.69)**
Group 2. NNJ non-hospitalized, n=38			
G6PD A$^{-202A/376G}$	21/49	2.9 (1.10-4.10)	32 (12.36-46.07)
G6PD A$^{-376G/968C}$	10/18	1.4 (0.50-2.33)	15.9 (5.62-26.22)
Union-Maewo	3/6	0.1 (0.05-0.10)	0.8 (0.78-1.12)
Akrokorinthos	1/1	2.7	29.8
Santamaria	1/1	0.9	10.1
Mahidol	1/1	2.7	30.3
Viangchan-Jammu	1/1	3.9	43.8
		2.22 (0.10-4.10)	**24.97 (1.12-46.07)**
Group 3. Asymptomatic during the neonatal period, n=34			
G6PD A$^{-202A/376G}$	24/49	2.6 (1.40-4.80)	29.6 (15.73-53.93)
G6PD A$^{-376G/968C}$	5/18	1.8 (1.20-2.60)	20.1 (13.48-29.21)
Union-Maewo	3/6	0.1 (0.05-0.06)	0.6 (0.56-0.67)
Akrokorinthos	1/1	4.0	44.9
Belem	1/1	1.1	12.4
		2.28 (0.05-4.8)	**25.62 (0.56-53.93)**

*Denominator means the total number of patients of each variant. ** There were no significant differences between the groups.

Table 3. Clinical description and type of G6PD variants found in the patients belonging to Group I (hospitalization during neonatal period).

ID	***G6PD* variant**	**WHO class**	**Luzzato 2016 classification**	**G6PD activity U/g Hb**	**G6PD activity %**	**NNJ**	Hospitalization				
							Clinical data (HPO ID)*	Age (days)	Cause	Cause related to G6PDd	Length (days)
30	Mediterranean	II	I	0.10	1.12	Yes	Seizures (HP:0001250)	7	Treatment of seizures	No	7
71	G6PD A$^{-376G/968C}$ (heterozygous female)	III	I	0.35	3.93	No	Neonatal asphyxia (HP:0012768) Neonatal hypoglycemia (HP:0001998)	<1	Clinical surveillance	Unclear	3
14	G6PD A$^{-376G/968C}$	III	II+III	1.20	13.48	Yes	NNJ (HP:0000952)	<1	Phototherapy	Yes	15
11**	G6PD A$^{-376G/968C}$	III	II+III	1.50	16.85	Yes	NNJ (HP:0000952)	7	Phototherapy	Yes	3
							AHA (HP:0001878)	37	Anemia diagnostic approach requiring blood transfusion	Yes	4
2	G6PD A$^{-202A/376G}$	III	II+III	1.55	17.42	No	Cough (HP:0012735) Rhinitis (HP:0012384) Fever (HP:0001945) Neutropenia (HP:0001875) Lethagy (HP:0001254) AHA (HP:0001878) Melanocitic nevus (HP:0000995)	60	Upper respiratory tract infection, anemia diagnostic approach and transfusion	Yes	1
39	G6PD A$^{-376G/968C}$	III	II+III	2.00	22.47	Yes	NNJ (HP:0000952)	4	Phototherapy	Yes	1

15	G6PD A$^{-202A/376G}$	III	Does not meet criteria***	3.00	33.71	No	Fever (HP:0001945)	42	Diagnostic approach of sepsis	Not clearly	3
57	G6PD A$^{-202A/376G}$	III	II-III	3.00	33.71	Yes	NNJ ((HP:0000952) Tachypnea (HP:0002789) Neonatal sepsis (HP:0040187)	<1	Sepsis diagnostic approach and NNJ treatment	Yes	15
19	G6PD A$^{-202A/376G}$	III	Does not meet criteria	4.60	51.69	Yes	Transient apnea (HP:0002104) Neonatal sepsis (HP:00401879)	<1	Diagnostic approach and sepsis treatment	Unclear	7

*HPO ID= The Phenomizer (Orphanet) uses the latest Orphanet date and a different algorithm for ranking the differential diagnoses (Köhler, 2009; Köhler 2018), http://www.human-phenotype-ontology.org

** Patient with 2 hospitalizations

*** Does not meet criteria means that the observed residual activity is higher than that established by Luzzato et al. 2016.

Table 4. Enzymatic activity of the abnormal siblings of the G6PDd newborn index patients.

Patient	Relationship and age	Legacy name	Genotype (*G6PD* variant)	WHO class	% Residual enzymatic activity	Outcome until the time of this study
Family 1	Index newborn	G6PD A$^{-202A/376G}$	Hemizygous c.[202G>A; 376A>G]	III	1.4	Asymptomatic
	Half-brother, 19 years	G6PD A$^{-202A/376G}$	Hemizygous c.[202G>A; 376A>G]	III	1.85	NNJ, healthy at the time of this study
Family 2	Index newborn	Mediterranean	Hemizygous c.[563C>T]	II	0.1	NNJ, seizures
	Brother, 15 years	Mediterranean	Hemizygous c.[563C>T]	II	0.2	Healthy at the time of this study
Family 3	Index newborn	G6PD A$^{-202A/376G}$	Hemizygous c.[202G>A; 376A>G]	III	4	Asymptomatic
	Brother, 3 years	G6PD A$^{-202A/376G}$	Hemizygous c.[202G>A; 376A>G]	III	2.8	NNJ requiring hospital management, AHA at 9 months and 2 years of age requiring blood transfusions
Family 4	Index newborn	G6PD A$^{-376G/968C}$	Hemizygous c.[376A>G; 968T>C]	III	1.35	Asymptomatic
	Brother, 4 years	G6PD A$^{-376G/968C}$	Hemizygous c.[376A>G; 968T>C]	III	1.3	Asymptomatic

Supplemetary Table 1. Individual genotypes, geographic origin, residual enzymatic activity and concordance classification.

Patient ID	G6PD variant	Geographic origin	Enzyme activity in red cells (% of normal)	Clinical features		Present work classification*	Classification according WHO 1967	Genotype Phenotype concordance
				AHA	NNJ			
34	Union-Maewo	Edo. Mex	0.6	no	yes	2	I	no
50	Union-Maewo	Guerrero	0.6	none		3	I	no
8	Union-Maewo	Guerrero	0.7	none		3	I	no
56	Union-Maewo	Edo. Mex	0.7	none		3	I	no
17	Union-Maewo	Guerrero	0.8	no	yes	2	I	no
30	Mediterranean	CDMX	1.1	no	no	1	I	yes
10	Union-Maewo	Guerrero	1.1	no	yes	2	I	no
71	G6PD A + (female)	Guanajuato	3.9	no	no	1	I	yes
74	G6PD $A^{-376G/968C}$	Oaxaca	5.6	no	yes	2	I	no
21	Santamaria	Oaxaca	10.1	no	yes	2	III	no
80	G6PD $A^{-376G/968C}$	Guerrero	11.2	no	yes	2	III	no
81	G6PD $A^{-202A/376G}$	China	12.4	no	yes	2	III	no
41	Belem	CDMX	12.4	none		3	III	yes
14	G6PD $A^{-376G/968C}$	CDMX	13.5	no	yes	1	III	no
43	G6PD $A^{-376G/968C}$	CDMX	13.5	no	yes	2	III	no
55	G6PD $A^{-376G/968C}$	Edo. Mex	13.5	no	yes	2	III	no
13	G6PD $A^{-376G/968C}$	Edo. Mex	13.5	none		3	III	yes
58	G6PD $A^{-376G/968C}$	Guerrero	14.6	no	yes	2	III	no
65	G6PD $A^{-376G/968C}$	CDMX	15.2	no	yes	2	III	no
72	G6PD $A^{-376G/968C}$	CDMX	15.2	none		3	III	yes
26	G6PD $A^{-202A/376G}$	Edo. Mex	15.7	none		3	III	yes
11	G6PD $A^{-376G/968C}$	CDMX	16.9	yes	yes	1	III	no
53	G6PD $A^{-376G/968C}$	Guerrero	16.9	no	yes	2	III	no
2	G6PD $A^{-202A/376G}$	CDMX	17.4	yes	no	1	III	no

59	G6PD A$^{-376G/968C}$	Guerrero	18.5	no		yes	2	III	no
3	G6PD A$^{-202A/376G}$	Edo. Mex	18.5		none		3	III	yes
45	G6PD A$^{-202A/376G}$	CDMX	19.1		none		3	III	yes
33	G6PD A$^{-202A/376G}$	CDMX	20.2		none		3	III	yes
73	G6PD A$^{-376G/968C}$	Guerrero	20.2		none		3	III	yes
68	G6PD A$^{-202A/376G}$	Guerrero	21.3	no		yes	2	III	no
61	G6PD A$^{-202A/376G}$	CDMX	22.1		none		3	III	yes
39	G6PD A$^{-376G/968C}$	CDMX	22.5	no		yes	1	III	no
46	G6PD A$^{-376G/968C}$	CDMX	22.5		none		3	III	yes
63	G6PD A$^{-202A/376G}$	CDMX	23	no		yes	2	III	no
67	G6PD A$^{-202A/376G}$	Edo. Mex	23		none		3	III	yes
31	G6PD A$^{-202A/376G}$	Oaxaca	23.6	no		yes	2	III	no
9	G6PD A$^{-376G/968C}$	Guerrero	24.2	no		yes	2	III	no
37	G6PD A$^{-202A/376G}$	CDMX	24.2		none		3	III	yes
16	G6PD A$^{-202A/376G}$	CDMX	24.7	no		yes	2	III	no
60	G6PD A$^{-202A/376G}$	Edo. Mex	24.7		none		3	III	yes
25	G6PD A$^{-202A/376G}$	CDMX	25.3		none		3	III	yes
38	G6PD A$^{-202A/376G}$	Edo. Mex	25.8		none		3	III	yes
62	G6PD A$^{-202A/376G}$	CDMX	25.8		none		3	III	yes
6	G6PD A$^{-376G/968C}$	Oaxaca	26.2	no		yes	2	III	no
7	G6PD A$^{-202A/376G}$	Edo. Mex	27	no		yes	2	III	no
4	G6PD A$^{-202A/376G}$	Edo. Mex	27		none		3	III	yes
51	G6PD A$^{-202A/376G}$	CDMX	28.1	no		yes	2	III	no
5	G6PD A$^{-202A/376G}$	Guerrero	28.1		none		3	III	yes
69	G6PD A$^{-202A/376G}$	Edo. Mex	28.7		none		3	III	yes
18	G6PD A$^{-202A/376G}$	Guerrero	29.2	no		yes	2	III	no
64	G6PD A$^{-376G/968C}$	CDMX	29.2		none		3	III	yes
47	Akrokorinthos	Guerrero	29.8	no		yes	2	III	no

36	Mahidol	CDMX	30.3	no		yes	2	III	no
23	G6PD A$^{-202A/376G}$	Edo. Mex	30.3		none		3	III	yes
24	G6PD A$^{-202A/376G}$	CDMX	31.5		none		3	III	yes
15	G6PD A$^{-202A/376G}$	Guerrero	33.7	no		no	1	III	no
57	G6PD A$^{-202A/376G}$	Edo. Mex	33.7	no		yes	1	III	no
35	G6PD A$^{-202A/376G}$	CDMX	33.7	no		yes	2	III	no
48	G6PD A$^{-202A/376G}$	Hidalgo	33.7	no		yes	2	III	no
40	G6PD A$^{-202A/376G}$	Edo. Mex	33.7		none		3	III	yes
66	G6PD A$^{-202A/376G}$	CDMX	34.1		none		3	III	yes
22	G6PD A$^{-202A/376G}$	Guerrero	34.3	no		yes	2	III	no
27	G6PD A$^{-202A/376G}$	Veracruz	34.8	no		yes	2	III	no
32	G6PD A$^{-202A/376G}$	CDMX	34.8	no		yes	2	III	no
54	G6PD A$^{-202A/376G}$	CDMX	36	no		yes	2	III	no
76	G6PD A$^{-202A/376G}$	CDMX	36	no		yes	2	III	no
77	G6PD A$^{-202A/376G}$	Edo. Mex	36	no		yes	2	III	no
44	G6PD A$^{-202A/376G}$	Edo. Mex	36		none		3	III	yes
78	G6PD A$^{-202A/376G}$	Guerrero	36		none		3	III	yes
49	G6PD A$^{-202A/376G}$	Guerrero	37.1	no		yes	2	III	no
42	G6PD A$^{-202A/376G}$	Guerrero	38.8		none		3	III	yes
70	G6PD A$^{-202A/376G}$	CDMX	39.3	no		yes	2	III	no
28	G6PD A$^{-202A/376G}$	Guerrero	40.4	no		yes	2	III	no
12	G6PD A$^{-202A/376G}$	Edo. Mex	41.6	no		yes	2	III	no
79	Viangchan-Jammu	Edo. Mex	43.8	no		yes	2	III	no
1	G6PD A$^{-202A/376G}$	Guerrero	43.8		none		3	III	yes
29	Akrokorinthos	Guerrero	44.9		none		3	III	yes
52	G6PD A$^{-202A/376G}$	Tlaxcala	44.9		none		3	III	yes
75	G6PD A$^{-202A/376G}$	Guerrero	46.1	no		yes	2	III	no
19	G6PD A$^{-202A/376G}$	Michoacan	51.7	no		no	1	III	no

20	G6PD $A^{-202A/376G}$	Guerrero	53.9	none	3	III	yes

***** Group 1: Hospitalized G6PDd patients in the neonatal period with or without NNJ; Group 2: Non-hospitalized G6PDd patients with NNJ, and Group 3: asymptomatic G6PDd newborns

Figures

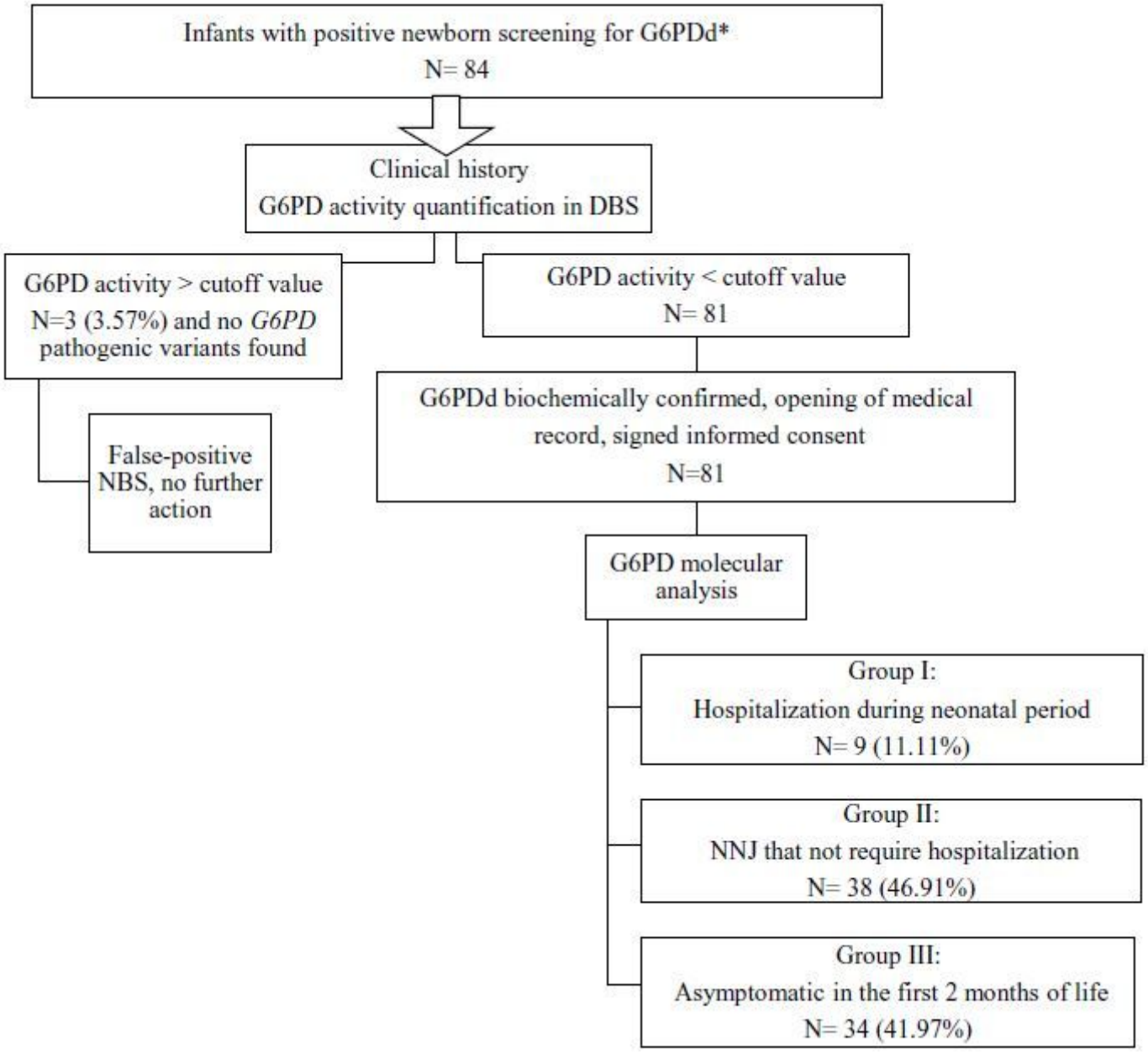

Figure 1

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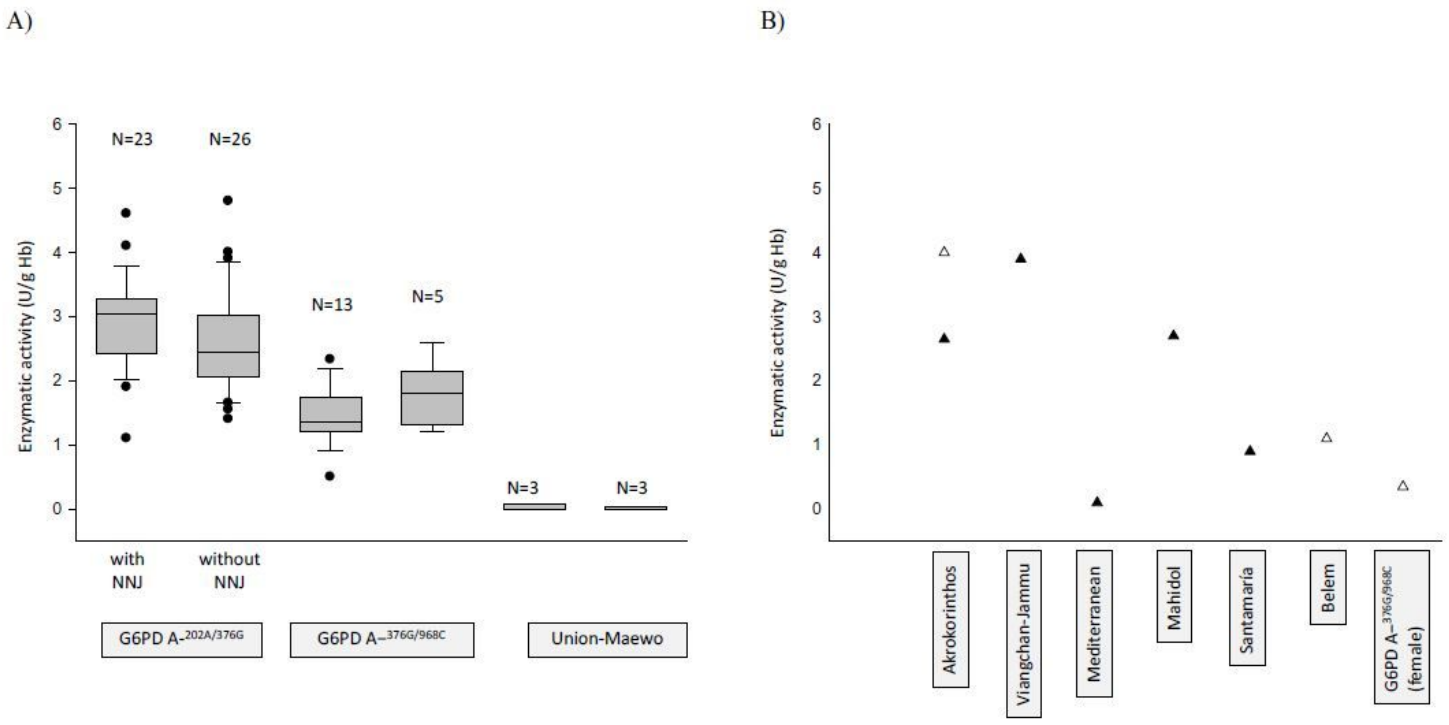

Figure 2

Documented G6PD deficient activity according to the identified G6PD patient genotypes (n=81), with or without (w/o) NNJ (neonatal jaundice). Panel A) Box and whisker plot genotypes with more than 3 affected individuals; Panel B) Enzymatic activity documented in less than 3 affected individuals with corroborated G6PD genotypes. Filled triangles represent patients with NNJ, and open triangles are patients without NNJ.