Exploring Appropriate Reference Intervals and Clinical Decision Limits for Glucose-6-Phosphate Dehydrogenase Activity in Individuals From Guangzhou, China

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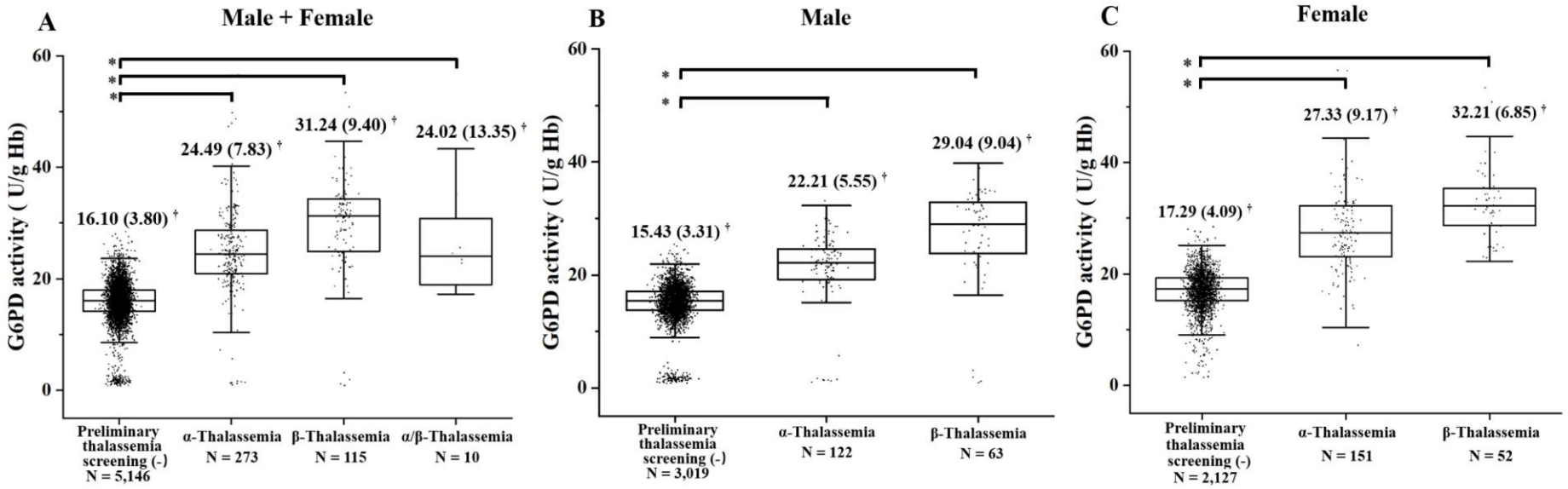
Supplemental Data Text S1. Description of the protocol shown in Fig. 1. We enrolled 5,852 individuals in this study, and 5,810 remained after applying the exclusion criteria. All individuals in the selected population (N=5,810) were quantitatively tested for G6PD activity, and samples with low G6PD activity (N=404) were tested for *G6PD* variants. The selected population also underwent preliminary screening for thalassemia according to MCH and MCV. The preliminary screening identified thalassemia-negative individuals who comprised Group A (N=5,146). Group A was subdivided into Group B (N=260) and Group C (N=4,886), where Group B had *G6PD* variants and Group C had a normal *G6PD* genotype. Individuals in Group A with low G6PD activity (N=377) were evaluated in terms of the *G6PD*-variant probability and follow-up analysis. According to the Hb-electrophoresis and thalassemia-genotyping results, the thalassemia-positive group identified during preliminary screening was subdivided into an α-thalassemia group, a β-thalassemia group, and an α/β complex thalassemia group.

Supplemental Data Text S2. Description of the results presented in Fig. 2. The distributions of G6PD activities in the enrolled population (N=5,810) are shown in Fig. 2A and B. The distributions of G6PD activities in Group A (thalassemia-negative group identified during preliminary screening, N=5,146) are shown in Fig. 2C and 2D. In both groups, the distributions of male and female were abnormal ($P<0.001$), with male showing a bimodal distribution and female showing a unimodal distribution. The distributions of G6PD activities in Group C (thalassemia-negative group with a normal *G6PD* genotype identified during preliminary screening, N=4,886) are shown in Fig. 2E and 2F. G6PD activities showed a normal distribution (NS) and unimodal distributions in both male and female.

Supplemental Data Text S3. Basis for the specific classifications of the hemolysis risk. For male, 10% of the NMM was used as the cut-off value between the high and medium hemolysis risk groups, according to the following data: (1) The G6PD activity of three male with severe hemolysis in this follow-up analysis were all less than 10% of the NMM. (2) This finding was consistent with the hemolytic risk prediction threshold of the WHO for primaquine use in 2016 [10]. In addition, 45% of the NMM was used as the cut-off value between the medium and low hemolysis risk groups, according to the following data: (1) The G6PD activity of four male with hemolysis in follow-up analysis was less than 45% of the NMM. (2) The G6PD activity of all male hemizygotes was less than 45% of the NMM. (3) The cut-off value of 45% of the NMM is consistent with the new classification scheme for G6PD variants released by the WHO in 2022 [5]. That classification scheme indicates that male hemizygotes or female homozygotes with a G6PD activity of less than 45% of the NMM are at risk for acute hemolysis. (4) The *G6PD* variant-positive rate was 100% when the G6PD activity in male and female was less than 45% of the NMM (6.98 U/g Hb). For female, 30% of the NMM was used as the cut-off value between the high and medium hemolysis risk groups, according to the following data: (1) The G6PD activity of one woman with severe hemolysis in the follow-up analysis was 27.46% of the NMM. (2) This finding was consistent with the hemolytic risk-prediction threshold of the WHO for primaquine use in 2016 [10]. Considering the wide distribution of G6PD activities observed in female heterozygotes, the cut-off values between the medium and low hemolysis risk groups in female were determined using the lower limit of the RI (79% of the NMM) for female G6PD activity, as established in this study, based on the following data: (1) The G6PD activity of three female with hemolysis in the follow-up analysis was less than 79% of the NMM. (2) This finding was consistent with the hemolytic risk-prediction threshold of the WHO for primaquine use in 2016 [10].

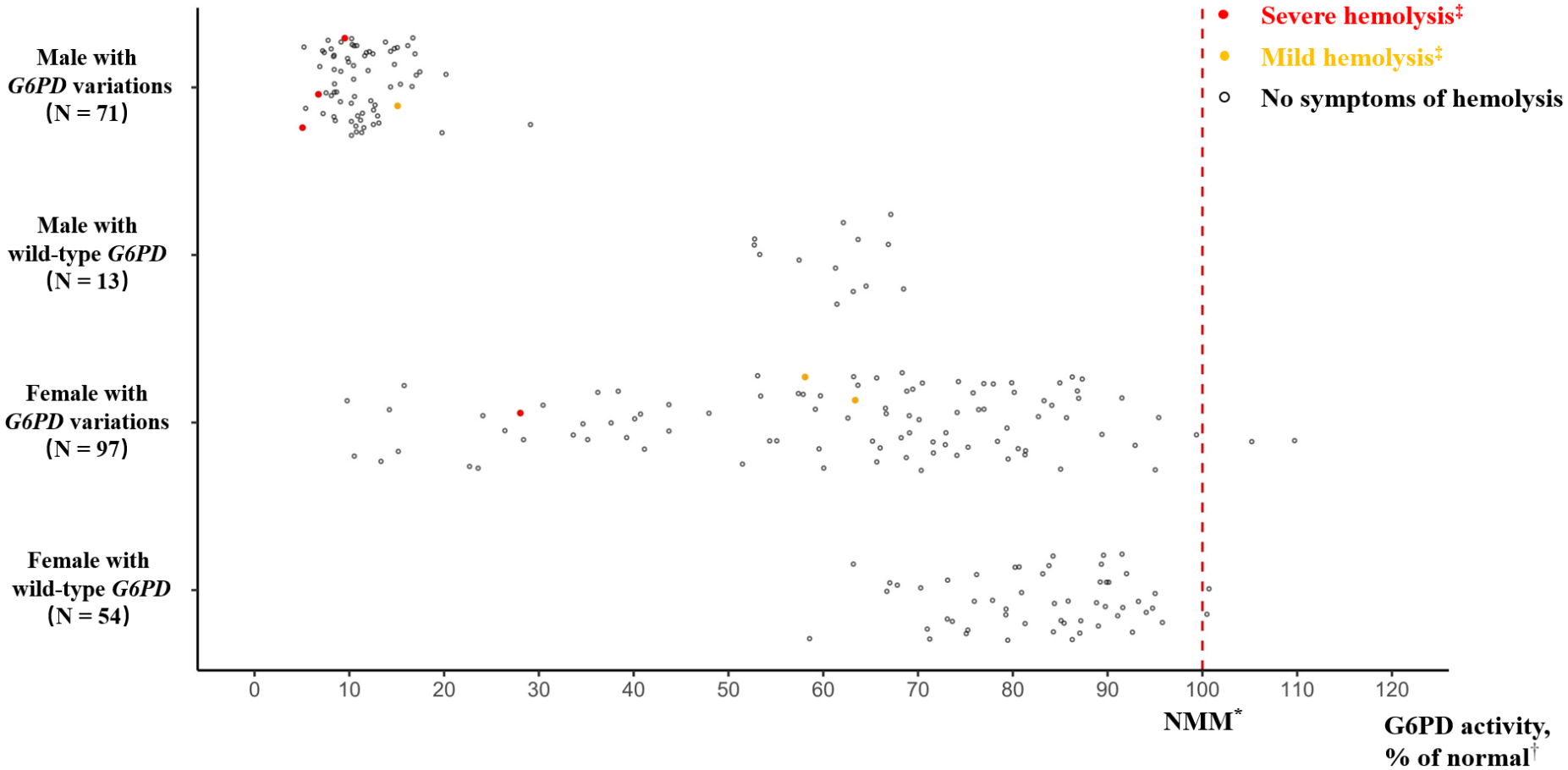


Supplemental Data Fig. S1. Effects of thalassemia on the measured G6PD activity.

*Asterisks represent statistical differences at $P<0.001$, as determined using the Mann–Whitney U-test.

†The G6PD-activity levels in each group are represented as the median (interquartile range).

Abbreviations: α-thalassemia, α-thalassemia group; β-thalassemia, β-thalassemia group; α/β-thalassemia, α/β-complex thalassemia group; G6PD, glucose-6-phosphate dehydrogenase.



Supplemental Data Fig. S2. G6PD-activity distribution in the follow-up population.

*Refers to NMM_2 (15.51 U/g Hb).

†Indicates the percentage of NMM_2 (15.51 U/g Hb).

‡Severe hemolysis refers to patients who were hospitalized due to sudden acute hemolysis. Mild hemolysis refers to patients who showed symptoms but were not hospitalized.

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; NMM_2, normal male median in group 2.

Supplemental Data Table S1. AMM and NMM calculations

Group	M_0 (U/g Hb)	AMM (U/g Hb)	NMM (U/g Hb)
Group 1* (N=3,284)	15.60	15.66	15.75
Group 2* (N=3,019)	15.01	15.47	15.51

*Group 1 indicates the male in the enrolled population, and Group 2 indicates the male in Group A who were identified as thalassemia-negative during preliminary screening.
Abbreviations: AMM, Adjusted male median; NMM, normal male median; M_0, initial median of the male population; U/g Hb, units per g of Hb.

Supplemental Data Table S2. Follow-up population characteristics

Age (yrs)	Total number of individuals where follow-up was attempted	Total number of individuals successfully followed up on (male/female)*	Total number of symptomatic people (male/female)	Total number of asymptomatic people
18–29	101	81 (21/60)	3 (2/1)	78
30–39	112	85 (36/49)	3 (1/2)	82
40–49	55	37 (13/24)	0	37
50–59	48	27 (14/13)	2 (1/1)	25
60–69	13	4 (0/4)	0	4
70–83	12	1 (0/1)	0	1
Total	341	235 (84/151)	8 (4/4)	227

*106 individuals were lost to follow-up (due to wrong contact information, refusal of follow-up, or three or more missed phone calls), and 235 individuals were successfully followed up.