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# Association of *G6PD*$^{202A,376G}$ with lower haemoglobin concentration but not increased haemolysis in patients with sickle cell anaemia

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## Summary

The genetic bases of the highly variable degrees of anaemia and haemolysis in persons with Hb SS are not fully known, but several studies have indicated that G6PD deficiency is not a factor. The *G6PD*$^{202A}$ and *G6PD*$^{376G}$ alleles and α-thalassaemia were determined by molecular genetic testing in 261 children and adolescents with Hb SS in a multicentre study. *G6PD*$^{202A,376G}$ (G6PD A−) was defined as hemizygosity for both alleles in males and homozygosity in females. Among the participants 41% were receiving hydroxycarbamide. The prevalence of *G6PD*$^{202A,376G}$ was 13·6% in males and 3·3% in females with an overall prevalence of 8·7%. *G6PD*$^{202A,376G}$ was associated with a 10 g/l decrease in haemoglobin concentration ($P = 0{\cdot}008$) but not with increased haemolysis as measured by lactate dehydrogenase, bilirubin, aspartate-aminotransferase, reticulocyte count or a haemolytic component derived from these markers ($P > 0{\cdot}09$). Similar results were found within a sub-group of children who were not receiving hydroxycarbamide. By comparison, single and double α-globin deletions were associated with progressively higher haemoglobin concentrations ($P = 0{\cdot}005$ for trend), progressively lower values for haemolytic component ($P = 0{\cdot}007$), and increased severe pain episodes ($P < 0{\cdot}001$). In conclusion, *G6PD*$^{202A,376G}$ may be associated with lower haemoglobin concentration in sickle cell anaemia by a mechanism other than increased haemolysis.

**Keywords:** sickle cell anaemia, G6PD, haemolysis, alpha-thalassaemia, haemoglobin concentration.

Individuals with sickle cell anaemia, i.e., homozygotes for the haemoglobin S mutation, have variability in the severity of anaemia and degree of haemolysis at steady state (Steinberg & Hebbel, 1983), and the reasons for this variability are not fully known (Christakis *et al*, 1990). α-Thalassaemia due to single or double *HBA1/2* deletions (Embury *et al*, 1982; Higgs *et al*, 1982; Chaar *et al*, 2006) and higher haemoglobin F (Hb F) percentages (Serjeant, 1975; Maude *et al*, 1987; Franco *et al*, 2006) are associated with higher haemoglobin concentrations and less haemolysis in this setting.

The glucose-6-phosphate dehydrogenase (G6PD) gene (*G6PD*) is located on the X chromosome. The normal allele is termed *G6PD* B. Mild G6PD deficiency (G6PD A−) has a prevalence of about 10% in predominantly African-American United States sickle cell disease patients based on reduced enzyme activity (Steinberg *et al*, 2003). The G6PD A− phenotype is usually caused by hemizygosity for both the *G6PD*$^{202A}$ and *G6PD*$^{376G}$ alleles in males and homozygosity for both of these alleles in females (Hirono & Beutler, 1988) and is referred to as *G6PD*$^{202A,376G}$ in this report. Hemizygosity for *G6PD*$^{376G}$ but not *G6PD*$^{202A}$ is termed G6PD A and is usually associated with normal enzymatic activity. *G6PD*$^{202A,376G}$ has a prevalence of about 12–13% in males and 2–3% in females in sub-Saharan Africa (Guindo *et al*, 2007; Johnson *et al*, 2009) and accounts for most of the G6PD deficiency in the population as determined by enzymatic assay (Johnson *et al*, 2009). Other genotypes, particularly inheritance of both the *G6PD*$^{376G}$ and *G6PD*$^{968C}$ alleles (*G6PD*$^{376G,968C}$), may contribute to the G6PD A− phenotype and even predominate over *G6PD*$^{202A,376G}$,

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especially in certain parts of West Africa (De Araujo *et al*, 2006; Clark *et al*, 2009).

G6PD A− may be associated with episodic haemolysis in individuals of African descent and has a frequency among sickle cell anaemia patients that is similar to the general background population (Bienzle *et al*, 1975; Las Heras Manso *et al*, 2008; Segeja *et al*, 2008). Little influence of G6PD A− on the degree of anaemia and of haemolysis was reported in several series of sickle cell anaemia patients (Gibbs *et al*, 1980; Steinberg *et al*, 1988; Bernaudin *et al*, 2008). Here we examined the effect of $G6PD^{202A,376G}$, as determined by molecular genetic testing, on the haemoglobin concentration and degree of haemolysis in a cohort of children and adolescents with haemoglobin SS (Hb SS) at steady state, those enrolled in the Pulmonary Hypertension and the Hypoxic Response in sickle cell disease (PUSH) Study (Minniti *et al*, 2009).

## Material and methods

### *Clinical study of participants*

Individuals 3–20 years of age with sickle cell anaemia (Hb SS) were recruited at three tertiary medical centres: Children's National Medical Center and Howard University Hospital in Washington, DC, and University of Michigan in Ann Arbor, Michigan. Children were recruited as outpatients when they presented for routine, ongoing care. To ensure that patients were enrolled at steady state, at least 3 weeks had to have elapsed since hospitalization, emergency department visit, or clinic visit for acute chest syndrome, pain crisis, infection or other sickle cell disease-related complication. Written informed consent was obtained for all participants. Parents or legal guardians provided consent for children aged 3–17 years. Participants aged 18–20 years provided consent for themselves. Participants aged 7–17 years provided additional written informed assent. Participants receiving regular blood transfusions or hydroxycarbamide therapy were not excluded. Detailed descriptions of experimental methods were published recently (Gordeuk *et al*, 2009; Minniti *et al*, 2009).

### *Molecular genetic testing*

*G6PD*. *G6PD* genotyping was conducted on a LightCycler480 (LC480) Real-Time PCR System (Roche Applied Science, Indianapolis, IN) using TaqMan custom probes (Applied Biosystems, Foster City, CA). In this report, $G6PD^{202A,376G}$ indicates boys who are hemizygous and girls who are homozygous for both the $G6PD^{202A}$ and the $G6PD^{376G}$ alleles (Table I), and this genotype is strongly associated with glucose-6-phosphate dehydrogenase deficiency (Hirono & Beutler, 1988). *HBB:* Haemoglobin S genotyping was conducted at ARUP Laboratories (Salt Lake City, UT) using loci-spanning probe – PCR as described (Pont-Kingdon *et al*, 2007). *HBA1/2:* α-thalassaemia genotyping was performed as described (Tan *et al*, 2001).

### *Echocardiography*

Transthoracic echocardiography was performed using the Philips Sono 5500/7500 or iE33, Acuson Sequoia, or General Electric VIVID 7 or VIVID I instruments. Systolic pulmonary artery pressure was estimated through measurement of tricuspid regurgitation velocity. Cardiac images were obtained, measurements performed, and studies interpreted centrally according to guidelines of the American Society of Echocardiography(Lai *et al*, 2006).

### *Statistical analysis*

Principal component analysis of age-adjusted values for lactate dehydrogenase (LDH), asparate aminotransferase (AST), reticulocyte count and total bilirubin was used to develop a haemolytic component that predicted 63% of variability among these factors and had a mean of 0 and standard deviation (SD) of 1·52. The arbitrary units of this variable were converted to positive SD units with a mean of 3·0 and SD of 1·00. Principal component analysis is used when the objective of the study is to investigate the role of an underlying mechanism (Genser *et al*, 2007), in this case the degree of haemolysis. The development of such a component resolves the problem of dealing with correlated predictors in multivariate analyses. Continuous and categorical variables were examined according to different genotypes by linear test for trend, student's *t* test or Chi-square. Multiple linear regression models were developed to identify the independent predictors of haemoglobin concentration and haemolytic component. These models were checked for model assumptions and outliers. An arbitrary level of 5% statistical significance was assumed to be significant. Statistical analyses were performed with STATA 10·0 (StataCorp, College Station, TX, USA).

**Table I.** Phenotypic characteristics of different $G6PD^{202,376}$ genotypes.

| Allele | Amino acids | Common designation | Enzymatic activity |
|---|---|---|---|
| 202G,376A | 68 Val,126 Asn | G6PD B | Normal |
| 202G,376G | 68 Val,126 Asp | G6PD A | Electrophoretic variant with normal activity |
| 202A,376G | 68 Met,126 Asp | G6PD A− | Deficient variant |

## Results

### *Demographics and genotypes*

Two hundred and sixty-one Hb SS patients were studied for $G6PD^{202A,376G}$ and for *HBA1/2* deletions. The median age was 12 years, 47% were females, and 98% were African Americans. The allele frequencies for $G6PD^{376G}$ (35·2%), $G6PD^{202A}$ (19·2%) and *HBA1/2* (α-thalassaemia; 17·0%) were similar to the frequencies in 57 controls from the background population (data not shown). Seventy-nine (30%) of the Hb SS patients had α thalassaemia, 72 with single *HBA1/2* deletion and seven with double *HBA1/2* deletions.

### *Haemoglobin concentration and haemolytic component according to G6PD alleles*

G6PD genotype and phenotype correlations are summarized in Table I. Nineteen (13·8%) of the 138 male Hb SS patients were hemizygous for both mutant alleles, i.e. had $G6PD^{202A,376G}$ (G6PD A−). Twenty-seven (19·6%) were hemizygous for $G6PD^{376G}$ but not $G6PD^{202A}$, i.e. had $G6PD^{202G,376G}$ (G6PD A). Ninety-two (66·7%) had neither the $G6PD^{376G}$ nor the $G6PD^{202A}$ mutations, i.e. had $G6PD^{202G,376A}$ (G6PD B). Table II shows that hemizygotes for both $G6PD^{376G}$ and $G6PD^{202A}$ had lower haemoglobin concentration but not higher haemolytic component than the G6PD B normal ($G6PD^{202G,376A}$) participants. In contrast, the males with G6PD A (hemizygotes for $G6PD^{376G}$ but not $G6PD^{202A}$) did not have either significantly lower haemoglobin concentration or higher haemolytic component compared to the G6PD B group. In further analyses, the males with $G6PD^{202A,376G}$ were compared to the combined group of males with the other two genotypes.

Four (3·3%) of the 123 female Hb SS patients were homozygotes for both the $G6PD^{376G}$ and $G6PD^{202A}$ alleles, i.e. had $G6PD^{202A,376G/202A,376G}$ (G6PD A−). Twelve (9·8%) had $G6PD^{376G}$ together with $G6PD^{202A}$ on one allele but not the other, i.e. had $G6PD^{202A,376G/202G,376A}$ or $G6PD^{202A,376G/202G,376G}$. Fifty-six (45·5%) had $G6PD^{376G}$ on at least one allele but not in combination with $G6PD^{202A}$, i.e. had $G6PD^{202G,376A/202G,376G}$ or $G6PD^{202G,376G/202G,376G}$. Fifty-one (41·5%) had $G6PD^{202G,376A/202G,376A}$ (G6PD B). Table III shows that the small number of females with $G6PD^{202A,376G/202A,376G}$ (G6PD A−) had a trend to lower haemoglobin concentrations but not higher haemolytic component compared to those who were homozygotes for both the $G6PD^{376A}$ and $G6PD^{202G}$ alleles (G6PD B). In contrast, the participants with other combinations of mutant alleles did not have lower haemoglobin concentrations compared to the G6PD B group. Therefore, in further analyses, females who were not homozygotes for both the $G6PD^{376G}$ and $G6PD^{202A}$ alleles were combined into one group for comparison.

### *Clinical and laboratory variables according to presence or absence of $G6PD^{202A,376G}$ (G6PD A−) in males and females combined*

Clinical characteristics according to $G6PD^{202A,376G}$ category are summarized in Table IV for all 261 participants. Neither α-thalassaemia genotypes nor Hb F levels varied significantly according to $G6PD^{202A,376G}$ status. Similar results were obtained in analyses restricted to 138 males (data not shown).

### *Associations of $G6PD^{202A,376G}$ (G6PD A−) with haemoglobin concentration and markers of haemolysis*

In unadjusted analysis, $G6PD^{202A,376G}$ was associated with a lower haemoglobin concentration of 10 g/l on average ($P = 0{\cdot}008$) but not with higher values for the haemolytic component ($P = 0{\cdot}6$) (Table IV). In multiple linear regression analysis that adjusted for degree of haemolysis, hydroxycarbamide treatment and recent blood transfusion, the association of $G6PD^{202A,376G}$ with lower haemoglobin concentration persisted (Fig 1). There was still no association of $G6PD^{202A,376G}$ with higher haemolytic component after adjusting for α-thalassaemia

**Table II.** Haemoglobin concentration and haemolytic component by different $G6PD^{202,376}$ genotypes in males. Results are given as mean (standard error).

| | $G6PD^{202G,376A}$ (G6PD B) $N = 92$ | $G6PD^{202G,376G}$ (G6PD A) $N = 27$ | $G6PD^{202A,376G}$ (G6PD A−) $N = 19$ |
|---|---|---|---|
| Haemoglobin concentration (g/l), unadjusted | 87 (1·5)[1] | 84 (2·5) | 79 (2·9)[2] |
| *P** | | 0·3 | 0·022 |
| Haemoglobin concentration (g/l), adjusted** | 88 (1·4)[1] | 84 (2·4) | 77 (3·0)[2] |
| *P** | | 0·1 | 0·001 |
| Haemolytic component (relative unit), unadjusted | 3·1 (0·1)[3] | 3·3 (0·2)[4] | 3·3 (0·2)[5] |
| *P** | | 0·5 | 0·4 |
| Haemolytic component (relative unit), adjusted*** | 3·1 (0·1)[3] | 3·3 (0·2)[4] | 3·4 (0·3)[5] |
| *P** | | 0·5 | 0·3 |

*Comparison with the $G6PD^{202G,376A}$ (G6PD B) group.

**Adjusted for recent blood transfusion and haemolytic component.

***Adjusted for hydroxycarbamide treatment and α-thalassaemia.

*N*: the number with the genotype in the data set. [1]*N*=86, [2]*N*=18, [3]*N*=80, [4]*N*=25, [5]*N* = 17.

**Table III.** Haemoglobin concentration and haemolytic component by different $G6PD^{202,376}$ genotypes in females. Results are given as mean (SE).

| | $G6PD^{202G,376A/202G,376A}$ (G6PD B) $N = 51$ | $G6PD^{202G,376A/202G,376G}$ or $G6PD^{202G,376G/202G,376G}$ $N = 56$ | $G6PD^{202A,376G/202G,376A}$ or $G6PD^{202A,376G/202G,376G}$ $N = 12$ | $G6PD^{202A,376G/202A,376G}$ (G6PD A−) $N = 4$ |
|---|---|---|---|---|
| Haemoglobin concentration (g/l), unadjusted | 86 (2·0)[1] | 87 (1·5)[2] | 89 (3·2) | 80 (11·3) |
| *P** | | 0·9 | 0·7 | 0·3 |
| Haemoglobin concentration (g/l), adjusted** | 87 (1·7)[1] | 88 (1·7)[2] | 89 (3·6) | 77 (5·4) |
| *P** | | 0·8 | 0·7 | 0·06 |
| Haemolytic component (relative unit), unadjusted | 2·9 (0·2)[3] | 2·9 (0·1)[4] | 2·8 (0·2) | 2·3 (0·6) |
| *P** | | 0·9 | 0·6 | 0·2 |
| Haemolytic component (relative unit), adjusted*** | 2·9 (0·1)[3] | 2·9 (0·1)[4] | 2·8 (0·3) | 2·1 (0·4) |
| *P** | | 0·9 | 0·8 | 0·1 |

*Comparison with the $G6PD^{202G,376A/202G,376A}$ (G6PD B) group.
**Adjusted for recent blood transfusion and haemolytic component.
***Adjusted for hydroxycarbamide treatment and α-thalassaemia.
[1]$N$=48, [2]$N$=53, [3]$N$=44, [4]$N$=51.

and hydroxycarbamide therapy ($P = 0·7$) (Fig 2). Similar analyses for the measurements from which the haemolytic component was derived revealed confirmatory findings: LDH ($P = 0·6$), AST ($P = 0·9$), total bilirubin ($P = 0·09$) and reticulocyte count ($P = 0·6$) were not significantly increased in individuals with $G6PD^{202A,376G}$ (Fig 2).

### *Associations of α-thalassaemia with haemoglobin concentration and the haemolytic component*

For comparison, we examined α-thalassaemia, a genetic variation that is known to influence haemoglobin concentration, haemolysis and clinical course in sickle cell anaemia (Embury *et al*, 1982; Higgs *et al*, 1982; Billett *et al*, 1986; Gill *et al*, 1995; Guasch *et al*, 1999; Mouele *et al*, 2000; Chaar *et al*, 2006; Taylor *et al*, 2008). In unadjusted analyses, single and double *HBA1/2* deletions were associated with progressively higher haemoglobin concentrations ($P = 0·005$), and these increases in haemoglobin concentration were paralleled by progressively decreasing values for the haemolytic component ($P = 0·007$). Single *HBA1/2* deletion was associated with a 4 g/l increase in haemoglobin concentration and double *HBA1/2* deletion with a 10 g/l increase. In multiple linear regression analysis that adjusted for the significant covariates of transfusion in past 2 months and white blood cell count, the association of *HBA1/2* deletions with higher haemoglobin concentrations persisted. After adjustment for these covariates, single *HBA1/2* deletion was associated with an estimated 4 g/l increase in haemoglobin concentration and double *HBA1/2* deletion with a 6 g/l increase ($P$ for trend = 0·012). Similarly, in multiple regression analysis that adjusted for hydroxycarbamide therapy, the association of *HBA1/2* deletions with lower values for the haemolytic component persisted ($P$ for trend = 0·005). Children with α-thalassaemia more commonly had a history of severe pain episodes in the past year compared to children without α-thalassaemia (46% vs. 23%, $P < 0·001$).

### *Sub-group of patients not receiving hydroxycarbamide*

Among 83 patients not receiving hydroxycarbamide treatment and with no recent blood transfusion, the haemoglobin concentration was lower in participants with $G6PD^{202A,376G}$ (G6PD A−) than the other children in unadjusted analysis (means of 64 vs. 81 g/l respectively; $P = 0·0005$) and after adjusting for α-thalassaemia and the haemolytic component ($P < 0·0001$). Neither the markers of haemolysis nor the haemolytic component were significantly higher in the $G6PD^{202A,376G}$ participants in this subgroup either in unadjusted or adjusted analysis. In this same subgroup, single and double deletion forms of α-thalassaemia were associated with higher haemoglobin concentrations and lower values for the haemolytic component ($P$ for trend <0·001).

## Discussion

The present findings suggest that $G6PD^{202A,376G}$ (G6PD A−), as determined by molecular genetic testing, contributes to the degree of anaemia in children and adolescents with sickle cell anaemia by a mechanism that may not include increased haemolysis. Our observation of a reduction in haemoglobin concentration with $G6PD^{202A,376G}$ applied to sickle cell disease children at steady state and was present either in unadjusted analysis or after adjustment for the degree of haemolysis and other important covariates. Likewise, the lack of an association

**Table IV.** Clinical and laboratory variables in children and adolescents with sickle cell anaemia based on G6PD status. Results are median (interquartile range) unless otherwise indicated.

| | All participants | | | | |
|---|---|---|---|---|---|
| Demographic variables | *N* | Other G6PD genotypes | *N* | *G6PD*$^{202A,376G}$ (G6PD A−) | *P* |
| Age (years) | 238 | 12 (7–16) | 23 | 12 (9–16) | 0·5 |
| Female, *N* (%) | 238 | 119 (50%) | 23 | 4* (17%) | 0·003 |
| Medical history | | | | | |
| Hydroxycarbamide treatment, *N* (%) | 234 | 94 (40%) | 23 | 11 (48%) | 0·5 |
| History of chronic transfusion program, *N* (%) | 231 | 40 (17%) | 23 | 4 (17%) | 0·9 |
| >10 units blood transfused life-time, *N* (%) | 228 | 53 (23%) | 23 | 10 (43%) | 0·033 |
| Blood transfusion | | | | | |
| Past 1 month | 58 | 29 (50%) | 6 | 3 (50%) | 0·3 |
| Past 2 months | | 16 (28%) | | 3 (50%) | |
| Past 3 months | | 13 (22%) | | 0 | |
| Two or more severe pain episodes in last 12 months, *N* (%) | 238 | 73 (31%) | 23 | 6 (26%) | 0·6 |
| Acute chest syndrome history | 234 | 73 (31%) | 23 | 11 (48%) | 0·11 |
| History of stroke, *N* (%) | 234 | 28 (12%) | 23 | 5 (22%) | 0·18 |
| Laboratory variables | | | | | |
| α-thalassaemia, *N* (%) | 232 | | 21 | | 0·4 |
| Single deletion *HBA1/2* | | 68 (29%) | | 4 (19%) | |
| Double deletion *HBA1/2* | | 7 (3%) | | 0 (0%) | |
| Haemoglobin F (%) | 123 | 12 (7–17) | 11 | 7 (3–20) | 0·3 |
| Haemoglobin (g/l) | 226 | 86 (77–96) | 22 | 76 (65–90) | 0·008 |
| Mean cell volume (fl) | 225 | 86 (81–93) | 22 | 88 (81–91) | 0·3 |
| White blood cells ($10^9$/l) | 225 | 11·5 (8·6–13·7) | 22 | 10·7 (7·7–13·0) | 0·6 |
| Erythropoietin (iu/l) | 232 | 70 (46–108) | 23 | 85 (39–140) | 0·5 |
| Reticulocyte count ($\times 10^9$/l) | 223 | 256 (188–351) | 21 | 238 (220–309) | 0·3 |
| Aspatate aminotransferase (u/l) | 227 | 45 (33–57) | 22 | 48 (34–60) | 0·8 |
| Total bilirubin (μmol/l) | 227 | 42·8 (30·8–63·3) | 22 | 54·7 (37·6–90·6) | 0·14 |
| Lactate dehydrogenase (u/l) | 218 | 458 (341–576) | 22 | 432 (315–571) | 0·6 |
| Haemolytic component (relative unit) | 212 | 3·1 (2·3–3·7) | 21 | 3·2 (2·7–3·6) | 0·6 |
| Tricuspid regurgitation velocity (m/s) | 219 | 2·4 (2·2–2·5) | 19 | 2·3 (2·2–2·5) | 0·7 |

*Four females were *G6PD*$^{202A,376G/202A,376G}$.

of *G6PD*$^{202A,376G}$ with haemolysis was observed either in unadjusted analysis or after adjustment for α-thalassaemia, hydroxycarbamide therapy and Hb F.

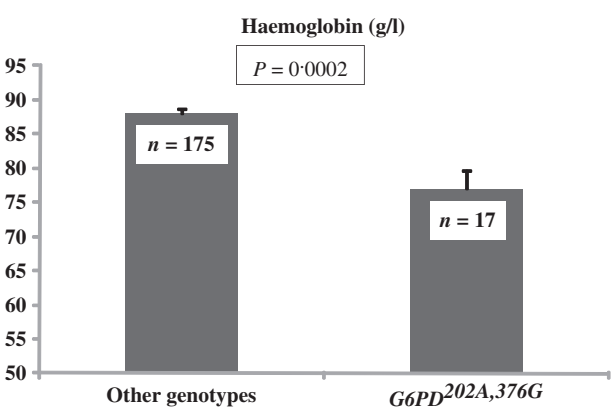


**Fig 1.** Estimated mean (standard error) haemoglobin concentration in sickle cell anaemia by *G6PD*$^{202A,376G}$ status. From ANOVA model with adjustment for degree of haemolysis, recent blood transfusion and hydroxycarbamide therapy.

Most previous studies reported that *G6PD* A− does not worsen anaemia or increase haemolysis in sickle cell disease (Gibbs *et al*, 1980; Steinberg *et al*, 1988; Bernaudin *et al*, 2008), although occasionally small series have reported a lower haemoglobin concentration with this phenotype (Bouanga *et al*, 1998). Our present finding that *G6PD*$^{202A,376G}$ is not associated with increased markers of haemolysis is thus consistent with previous large studies conducted in children (Bernaudin *et al*, 2008) or in children and adults (Gibbs *et al*, 1980; Steinberg *et al*, 1988) with sickle cell anaemia, but the association with lower haemoglobin levels differs from these studies. For instance, the study of Steinberg *et al* (1988) in children and adults reported that haemoglobin concentrations averaged 4–5 g/l lower with G6PD A− compared to G6PD A+ and G6PD B, but this difference was not statistically significant in multivariate analysis (Steinberg *et al*, 1988). Bernaudin *et al* (2008), a study in children, reported that the haemoglobin concentration averaged 4 g/l higher with G6PD deficiency, even though G6PD deficiency was significantly associated with increased trans-cranial Doppler velocity, which was linked to

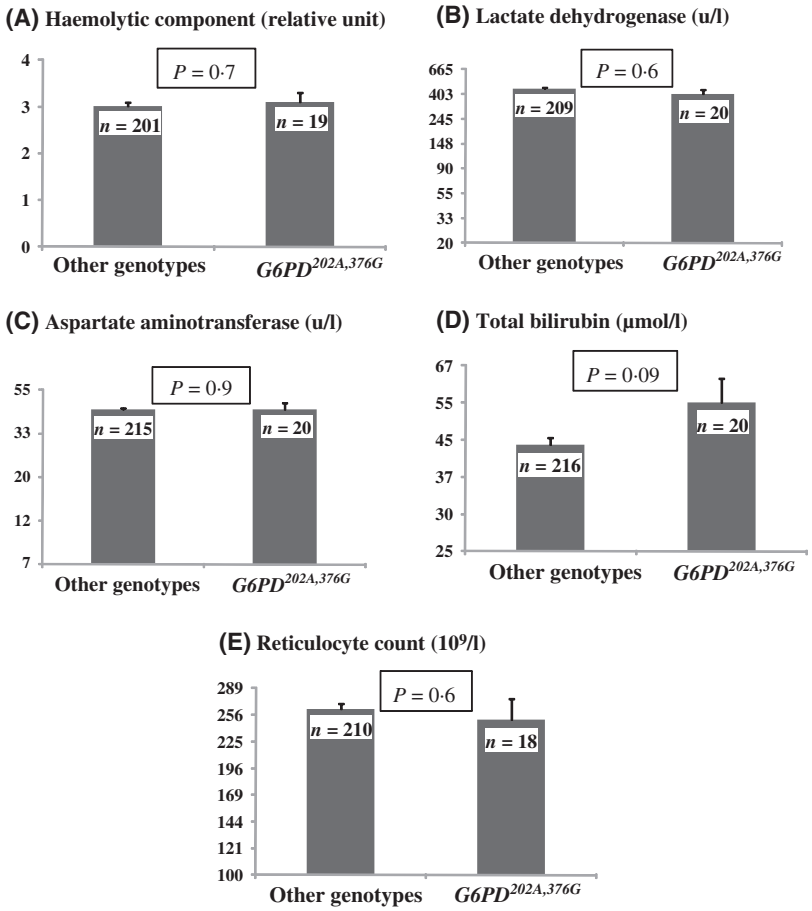


**Fig 2.** Estimated mean (standard error) haemolytic component, lactate dehydrogense, aspartate aminotransferase, total bilirubin, and reticulocyte count in sickle cell anaemia by G6PD status. From ANOVA model with adjustment for hydroxycarbamide treatment and α-thalasssaemia.

more severe anaemia (Bernaudin *et al*, 2008). An important difference from these previous studies is that we examined a single G6PD deficiency genotype, $G6PD^{202A,376G}$, rather than the G6PD A− phenotype determined enzymatically, which may include a variety of genotypes in addition to $G6PD^{202A,376G}$, such as $G6PD^{376G,968C}$. Also, enzymatic determination of G6PD may have decreased accuracy in hyperhaemolytic states because of higher enzyme concentrations in younger erythrocytes (Beutler *et al*, 1954; Marks *et al*, 1958). The facts that we studied the PUSH cohort in a prospective, cross-sectional manner with strictly defined steady state and that we performed *G6PD* genotyping by molecular genetic testing instead of electrophoresis or enzyme activity may help to explain the variance in the findings with previous studies (Steinberg *et al*, 1988; Bernaudin *et al*, 2008) and increase confidence in the present finding of lower haemoglobin concentration with $G6PD^{202A,376G}$. Furthermore, we performed molecular genetic testing for α-thalassmia in parallel to testing for $G6PD^{202A,376G}$ and observed higher haemoglobin concentrations, less haemolysis and more frequent pain crises with *HBA1/2* deletions. These findings with regard to α-thalassaemia are consistent with the observations of many other investigators (Embury *et al*, 1982; Higgs *et al*, 1982; Billett *et al*, 1986; Gill *et al*, 1995; Guasch *et al*, 1999; Mouele *et al*, 2000; Chaar *et al*, 2006; Taylor *et al*, 2008).

There are a number of limitations to our present study. The results are based on a single, cross-sectional analysis rather than on repeated determinations and there was small sample size in the $G6PD^{202A,376G}$ group. Furthermore, we did not perform molecular genetic testing for all of the mutations that could contribute to the G6PD A− phenotype, and we could not rule out $G6PD^{968C}$ in alleles classified as G6PD A in this study.

Given that the haemoglobin concentration was decreased without an apparent increase in haemolysis, our findings raise the possibility that $G6PD^{202A,376G}$ in sickle cell anaemia might be associated with decreased production of erythrocytes. Of note is a study indicating that G6PD plays an important role in erythroid maturation. With complete G6PD deficiency, erythroid precursors are unable to cope with high concentrations of oxygen radicals as haemoglobin synthesis is initiated, and they die by apoptosis (Paglialunga *et al*, 2004). These results may not be relevant for patients with relatively mild G6PD deficiency associated with $G6PD^{202A,376G}$. On the other hand, it is conceivable that any slight tendency to increased apoptosis of $G6PD^{202A,376G}$ erythroid precursors at a

minimally haemoglobinized stage might become more manifest in the setting of the markedly increased erythropoiesis and hypoxic conditions (low haemoglobin concentration and lower haemoglobin oxygen saturation) of sickle cell anaemia (Qian *et al*, 2001; Aispuru *et al*, 2008). Thus, based on our findings, studies of apoptotic markers in early G6PD A− erythroid progenitors exposed to varying degrees of oxidant stress and hypoxia may be of interest. Furthermore, elucidation of the precise pathogenesis of increased anaemia in G6PD-deficient sickle cell anaemia patients may help identify new disease pathways amenable to eventual treatment interventions.

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