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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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Abstract

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 ($P=0.008$) but not with increased haemolysis as measured by lactate dehydrogenase, bilirubin, aspartate-aminotransferase, reticulocyte count or a haemolytic component derived from these markers ($P>0.09$). 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 ($P=0.005$ for trend), progressively lower values for haemolytic component ($P=0.007$), and increased severe pain episodes ($P<0.001$).

Conclusions—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; α -thalassaemia; haemoglobin concentration

Introduction

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 and 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 (Chaar, *et al* 2006, Embury, *et al* 1982, Higgs, *et al* 1982) and higher haemoglobin F (Hb F) percentages (Franco, *et al* 2006, Maude, *et al* 1987, Serjeant 1975) are associated with higher haemoglobin concentrations and less haemolysis in this setting.

The glucose-6-phosphate dehydrogenase (G6PD) gene (*GSPD*) 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 and Beutler 1988) and is referred to *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}, especially in certain parts of West Africa (Clark, *et al* 2009, De Araujo, *et al* 2006).

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 (Bernaudin, *et al* 2008, Gibbs, *et al* 1980, Steinberg, *et al* 1988). 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 new 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 to 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 three 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 to 17 years. Participants aged 18 to 20 years provided consent for themselves. Participants aged 7 to 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 1), and this genotype is strongly associated with glucose-6-phosphate dehydrogenase deficiency (Hirono and 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), aspartate 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 was used when the objective of the study was 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 resolved 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).

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 1. 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 2 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 3 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 4 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 ($P = 0.008$) but not with higher values for the haemolytic component ($P = 0.6$) (Table 4). 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 (Figure 1). There was still no association of *G6PD*^{202A,376G} with higher haemolytic component after adjusting for α -thalassaemia and hydroxycarbamide therapy ($P = 0.7$) (Figure 2). Similar analyses for the measurements from which the haemolytic component was derived revealed confirmatory findings: LDH ($P = 0.5$), 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} (Figure 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 (Billett, *et al* 1986, Chaar, *et al* 2006, Embury, *et al* 1982, Gill, *et al* 1995, Guasch, *et al* 1999, Higgs, *et al* 1982, Moule, *et al* 2000, 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 two 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 of *G6PD*^{202A,376G} with haemolysis was observed either in unadjusted analysis or after adjustment for α -thalassaemia, hydroxycarbamide therapy and Hb F.

Most previous studies reported that *G6PD A-* does not worsen anaemia or increase haemolysis in sickle cell disease (Bernaudin, *et al* 2008, Gibbs, *et al* 1980, Steinberg, *et al* 1988), 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* (1998) in children and adults reported that haemoglobin concentrations averaged 4 to 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 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 (Bernaudin, *et al* 2008, Steinberg, *et al* 1988) and increase confidence in the present finding of lower haemoglobin concentration with *G6PD*^{202A,376G}. Furthermore, we performed molecular genetic testing for α -thalassaemia 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 (Billett, *et al* 1986, Chaar, *et al* 2006, Embury, *et al* 1982, Gill, *et al* 1995, Guasch, *et al* 1999, Higgs, *et al* 1982, Mouele, *et al* 2000, 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 (Aispuru, *et al* 2008, Qian, *et al* 2001). 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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References

- Aispuru GR, Aguirre MV, Aquino-Esperanza JA, Lettieri CN, Juaristi JA, Brandan NC. Erythroid expansion and survival in response to acute anemia stress: the role of EPO receptor, GATA-1, Bcl-xL and caspase-3. *Cell Biol Int*. 2008; 32:966–978. [PubMed: 18485751]
- Bernaudin F, Verlhac S, Chevret S, Torres M, Coic L, Arnaud C, Kamdem A, Hau I, Grazia Neonato M, Delacourt C. *G6PD* deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. *Blood*. 2008; 112:4314–4317. [PubMed: 18772456]
- Beutler E, Dern RJ, Alving AS. The hemolytic effect of primaquine. IV. The relationship of cell age to hemolysis. *J Lab Clin Med*. 1954; 44:439–442. [PubMed: 13201853]

- Bienze U, Sodeinde O, Effiong CE, Luzzatto L. Glucose 6-phosphate dehydrogenase deficiency and sickle cell anemia: frequency and features of the association in an African community. *Blood*. 1975; 46:591–597. [PubMed: 1174693]
- Billett HH, Kim K, Fabry ME, Nagel RL. The percentage of dense red cells does not predict incidence of sickle cell painful crisis. *Blood*. 1986; 68:301–303. [PubMed: 3719102]
- Bouanga JC, Mouele R, Prehu C, Wajcman H, Feingold J, Galacteros F. Glucose-6-phosphate dehydrogenase deficiency and homozygous sickle cell disease in Congo. *Hum Hered*. 1998; 48:192–197. [PubMed: 9694250]
- Chaar V, Keclard L, Etienne-Julan M, Diara JP, Elion J, Krishnamoorthy R, Romana M. UGT1A1 polymorphism outweighs the modest effect of deletional (–3.7 kb) alpha-thalassemia on cholelithogenesis in sickle cell anemia. *Am J Hematol*. 2006; 81:377–379. [PubMed: 16628735]
- Christakis J, Vavatsi N, Hassapopoulou H, Papadopoulou M, Mandraveli K, Loukopoulos D, Morris JS, Serjeant BE, Serjeant GR. Comparison of homozygous sickle cell disease in northern Greece and Jamaica. *Lancet*. 1990; 335:637–640. [PubMed: 1690325]
- Clark TG, Fry AE, Auburn S, Campino S, Diakite M, Green A, Richardson A, Teo YY, Small K, Wilson J, Jallow M, Sisay-Joof F, Pinder M, Sabeti P, Kwiatkowski DP, Rockett KA. Allelic heterogeneity of G6PD deficiency in West Africa and severe malaria susceptibility. *Eur J Hum Genet*. 2009; 17:1080–1085. [PubMed: 19223928]
- De Araujo C, Migot-Nabias F, Guitard J, Pelleau S, Vulliamy T, Ducrocq R. The role of the G6PD Aeth376G/968C allele in glucose-6-phosphate dehydrogenase deficiency in the seer population of Senegal. *Haematologica*. 2006; 91:262–263. [PubMed: 16461316]
- Embury SH, Dozy AM, Miller J, Davis JR Jr, Kleman KM, Preisler H, Vichinsky E, Lande WN, Lubin BH, Kan YW, Mentzer WC. Concurrent sickle-cell anemia and alpha-thalassemia: effect on severity of anemia. *N Engl J Med*. 1982; 306:270–274. [PubMed: 6172710]
- Franco RS, Yasin Z, Palascak MB, Ciraolo P, Joiner CH, Rucknagel DL. The effect of fetal hemoglobin on the survival characteristics of sickle cells. *Blood*. 2006; 108:1073–1076. [PubMed: 16861353]
- Genser B, Cooper PJ, Yazdanbakhsh M, Barreto ML, Rodrigues LC. A guide to modern statistical analysis of immunological data. *BMC Immunol*. 2007; 8:27. [PubMed: 17963513]
- Gibbs WN, Wardle J, Serjeant GR. Glucose-6-phosphate dehydrogenase deficiency and homozygous sickle cell disease in Jamaica. *Br J Haematol*. 1980; 45:73–80. [PubMed: 7378331]
- Gill FM, Sleeper LA, Weiner SJ, Brown AK, Bellevue R, Grover R, Pegelow CH, Vichinsky E. Clinical events in the first decade in a cohort of infants with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Blood*. 1995; 86:776–783. [PubMed: 7606007]
- Gordeuk VR, Campbell A, Rana S, Nouraie M, Niu X, Minniti CP, Sable C, Darbari D, Dham N, Onyekwere O, Ammosova T, Nekhai S, Kato GJ, Gladwin MT, Castro OL. Relationship of erythropoietin, fetal hemoglobin, and hydroxyurea treatment to tricuspid regurgitation velocity in children with sickle cell disease. *Blood*. 2009; 114:4639–4644. [PubMed: 19724057]
- Guasch A, Zayas CF, Eckman JR, Muralidharan K, Zhang W, Elsas LJ. Evidence that microdeletions in the alpha globin gene protect against the development of sickle cell glomerulopathy in humans. *J Am Soc Nephrol*. 1999; 10:1014–1019. [PubMed: 10232687]
- Guindo A, Fairhurst RM, Doumbo OK, Wellems TE, Diallo DA. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. *PLoS Med*. 2007; 4:e66. [PubMed: 17355169]
- Higgs DR, Aldridge BE, Lamb J, Clegg JB, Weatherall DJ, Hayes RJ, Grandison Y, Lowrie Y, Mason KP, Serjeant BE, Serjeant GR. The interaction of alpha-thalassemia and homozygous sickle-cell disease. *N Engl J Med*. 1982; 306:1441–1446. [PubMed: 6176865]
- Hirono A, Beutler E. Molecular cloning and nucleotide sequence of cDNA for human glucose-6-phosphate dehydrogenase variant A(-). *Proc Natl Acad Sci U S A*. 1988; 85:3951–3954. [PubMed: 2836867]
- Johnson MK, Clark TD, Njama-Meya D, Rosenthal PJ, Parikh S. Impact of the method of G6PD deficiency assessment on genetic association studies of malaria susceptibility. *PLoS ONE*. 2009; 4:e7246. [PubMed: 19789650]

- Lai WW, Geva T, Shirali GS, Frommelt PC, Humes RA, Brook MM, Pignatelli RH, Rychik J. Guidelines and standards for performance of a pediatric echocardiogram: a report from the Task Force of the Pediatric Council of the American Society of Echocardiography. *J Am Soc Echocardiogr*. 2006; 19:1413–1430. [PubMed: 17138024]
- Las Heras Manso G, Junca Piera J, Feliu Frasnado E, Rovira Fernandez JM, Gil Garcia M. Hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in the sub-Saharan immigrant population of the Center and South Maresme region, Catalonia, Spain. *Med Clin (Barc)*. 2008; 131:5–9. [PubMed: 18582416]
- Marks PA, Johnson AB, Hirschberg E. Effect of Age on the Enzyme Activity in Erythrocytes. *Proc Natl Acad Sci U S A*. 1958; 44:529–536. [PubMed: 16590234]
- Maude GH, Hayes RJ, Serjeant GR. The haematology of steady state homozygous sickle cell disease: interrelationships between haematological indices. *Br J Haematol*. 1987; 66:549–558. [PubMed: 2444245]
- Minniti CP, Sable C, Campbell A, Rana S, Ensing G, Dham N, Onyekwere O, Nourai M, Kato GJ, Gladwin MT, Castro OL, Gordeuk VR. Elevated tricuspid regurgitant jet velocity in children and adolescents with sickle cell disease: association with hemolysis and hemoglobin oxygen desaturation. *Haematologica*. 2009; 94:340–347. [PubMed: 19211639]
- Mouele R, Pambou O, Feingold J, Galacteros F. alpha-thalassemia in Bantu population from Congo-Brazzaville: its interaction with sickle cell anemia. *Hum Hered*. 2000; 50:118–125. [PubMed: 10799970]
- Paglalunga F, Fico A, Iaccarino I, Notaro R, Luzzatto L, Martini G, Filosa S. G6PD is indispensable for erythropoiesis after the embryonic-adult hemoglobin switch. *Blood*. 2004; 104:3148–3152. [PubMed: 15271799]
- Pont-Kingdon G, Chou LS, Damjanovich K, Sumner K, Herrmann M, Erali M, Lyon E. Multiplex genotyping by melting analysis of loci-spanning probes: beta-globin as an example. *Biotechniques*. 2007; 42:193–197. [PubMed: 17373484]
- Qian J, Ramroop K, McLeod A, Bandari P, Livingston DH, Harrison JS, Rameshwar P. Induction of hypoxia-inducible factor-1alpha and activation of caspase-3 in hypoxia-reoxygenated bone marrow stroma is negatively regulated by the delayed production of substance P. *J Immunol*. 2001; 167:4600–4608. [PubMed: 11591789]
- Segeja MD, Mmbando BP, Kamugisha ML, Akida JA, Savaeli ZX, Minja DT, Msangeni HA, Lemnge MM. Prevalence of glucose-6-phosphate dehydrogenase deficiency and haemoglobin S in high and moderate malaria transmission areas of Muheza, north-eastern Tanzania. *Tanzan J Health Res*. 2008; 10:9–13. [PubMed: 18680959]
- Serjeant GR. Fetal haemoglobin in homozygous sickle cell disease. *Clin Haematol*. 1975; 4:109–122. [PubMed: 1102178]
- Steinberg MH, Hebbel RP. Clinical diversity of sickle cell anemia: genetic and cellular modulation of disease severity. *Am J Hematol*. 1983; 14:405–416. [PubMed: 6190397]
- Steinberg MH, West MS, Gallagher D, Mentzer W. Effects of glucose-6-phosphate dehydrogenase deficiency upon sickle cell anemia. *Blood*. 1988; 71:748–752. [PubMed: 3345344]
- Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, Orringer E, Bellevue R, Olivieri N, Eckman J, Varma M, Ramirez G, Adler B, Smith W, Carlos T, Ataga K, DeCastro L, Bigelow C, Sauntharajah Y, Telfer M, Vichinsky E, Claster S, Shurin S, Bridges K, Waclawiw M, Bonds D, Terrin M. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. *JAMA*. 2003; 289:1645–1651. [PubMed: 12672732]
- Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. *Blood*. 2001; 98:250–251. [PubMed: 11439976]
- Taylor, JG; Nolan, VG.; Mendelsohn, L.; Kato, GJ.; Gladwin, MT.; Steinberg, MH. Chronic hyper-hemolysis in sickle cell anemia: association of vascular complications and mortality with less frequent vasoocclusive pain. *PLoS ONE*. 2008; 3:e2095. [PubMed: 18461136]

Figure 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 and recent blood transfusion

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

Table 1Phenotypic 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

Table 2

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

	<i>G6PD</i> ^{202G,376A} (G6PD B) N=92	<i>G6PD</i> ^{202G,376G} (G6PD A) N=27	<i>G6PD</i> ^{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 3

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

	<i>G6PD</i> ^{202G,376A/202G,376A} (G6PD B) N=51	<i>G6PD</i> ^{202G,376A/202G,376G} or <i>G6PD</i> ^{202G,376G/202G,376G} N=56	<i>G6PD</i> ^{202A,376G/202G,376A} or <i>G6PD</i> ^{202A,376G/202G,376G} N=12	<i>G6PD</i> ^{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

Table 4

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			
	N	Other G6PD genotypes	N	G6PD ^{202A,376G} (G6PD A+)
Demographic variables				
Age (years)	238	12 (7–16)	23	12 (9–16)
Female, N (%)	238	119 (50%)	23	4* (17%)
Medical history				
Hydroxycarbamide treatment, N (%)	234	94 (40%)	23	11 (48%)
History of chronic transfusion program, N (%)	231	40 (17%)	23	4 (17%)
>10 units blood transfused life-time, N (%)	228	53 (23%)	23	10 (43%)
Blood transfusion	58		6	
past 1 month		29 (50%)		3 (50%)
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%)
Acute chest syndrome history	234	73 (31%)	23	11 (48%)
History of stroke, N (%)	234	28 (12%)	23	5 (22%)
Laboratory variables				
α -thalassaemia, N (%)	232		21	
single deletion <i>HBA1/2</i>		68 (29%)		4 (19%)
Double deletion <i>HBA1/2</i>		7 (3%)		0 (0%)
Haemoglobin F (%)	123	12 (7–17)	11	7 (3–20)
Haemoglobin (g/l)	226	86 (77–96)	22	76 (65–90)
Mean Cell Volume (fl)	225	86 (81–93)	22	88 (81–91)
White blood cells ($10^9/l$)	225	11.5 (8.6–13.7)	22	10.7 (7.7–13.0)
Erythropoietin (iu/l)	232	70 (46–108)	23	85 (39–140)
Reticulocyte count ($\times 10^9/l$)	223	256 (188–351)	21	238 (220–309)
Aspartate aminotransferase (u/l)	227	45 (33–57)	22	48 (34–60)
Total bilirubin ($\mu\text{mol/l}$)	227	42.8 (30.8–63.3)	22	54.7 (37.6–90.6)
				0.14

Demographic variables	All participants				P
	N	Other G6PD genotypes	N	G6PD ^{202A,376G} (G6PD A-)	
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}.