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Favism, the commonest form of severe hemolytic anemia in Palestinian children, varies in severity with three different variants of G6PD deficiency within the same community

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic abnormality known to predispose to acute hemolytic anemia (AHA), which can be triggered by certain drugs or infection. However, the commonest trigger is fava beans *(Vicia faba)* ingestion, causing AHA (favism), which may be life-threatening especially in children. G6PD deficiency is genetically highly heterogeneous, as nearly 200 different mutations have been observed. We have investigated the hematological features of acute favism in the Palestinian Gaza community that is characterized by the polymorphic coexistence of three different G6PD deficiency genes (*G6PD A−*, *G6PD Cairo*, *G6PD Med*). We have found by comparison to the general population (485 adults and 466 newborns) that children with favism, in terms of relative frequency, G6PD A− was under-represented, whereas G6PD Med was over-represented. We also found that the severity of anemia was significantly greater with G6PD Med and G6PD Cairo than with G6PD A−; and with G6PD Cairo, compared to the other two variants, there was greater hyperbilirubinemia, as well as persistence of mild anemia and reticulocytosis for as long as 4 months after recovery from favism. This is the first report determining a differential impact of different G6PD mutations on the clinical features of favism in the same population and the same environment.

1. Introduction

Favism (OMIM 134700) was recognized for over a century as a form of acute hemolytic anemia (AHA) that is life-threatening especially in children [1]. Once glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) deficiency was discovered [2], it became clear that G6PD deficiency was an essential inherited predisposition to develop favism [3]. Initially favism was characterized particularly in Greece [4], Italy [5], and in the Middle East [6]; but subsequently also in North Africa, Thailand, China, and in some 30 other countries (listed in [7]). With appropriate management, which often must include blood transfusion, full recovery from favism without sequelae is the rule [8]; however, if not promptly diagnosed and managed it is still a life-threatening condition.

The *G6PD* gene (OMIM 305900) maps to the sub-telomeric region of the long arm of the X chromosome (band Xq28). G6PD deficiency, which is therefore X-linked, is well known to be highly heterogeneous at the genetic level, since different mutations underlie different variants, many of which have polymorphic frequencies in areas where malaria is or has been endemic [7]. G6PD deficiency is never complete: nearly all G6PD mutations are missense mutations or in-frame deletions [9], and all have some residual G6PD enzyme activity; indeed, a classification

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of G6PD variants has been based on residual enzyme activity and on clinical expression [10]. Most large clinical studies of favism have been carried out in countries where G6PD Mediterranean (G6PD Med, having the amino acid replacement S188F) is by far the commonest G6PD deficiency variant. Since G6PD A− (the G6PD deficiency variant most common in people from Africa or of recent African descent) has a residual enzyme activity higher than G6PD Med, it was thought for some time that it would not be associated with the risk of favism; but subsequently favism was also documented with G6PD A− [11,12]. Favism can occur in males hemizygous and in females homozygous for G6PD deficiency; it can also occur in heterozygous females, but its severity will depend on the pattern of X-chromosome inactivation in the individual heterozygote. Favism can occur probably with any type of G6PD deficiency: whether its severity varies depending on which variant is involved cannot be assessed from the literature, because generally reports from different countries reflect not only different genetic variants, but also different contexts in terms of environment and of health facilities.

We have previously reported that favism is the single commonest cause of transfusion-requiring AHA in Palestinian children in Gaza [13,14]. In this population at least 3 different G6PD variants are present at polymorphic frequencies: G6PD Med, G6PD A−, and G6PD Cairo. Since the large majority of children with favism from the entire Gaza region are admitted and treated in the same hospital (the Al Nasser Pediatric Hospital), this enabled us to compare the clinical presentation, hematological features and the clinical course of favism, including follow-up, in children with three different genetic abnormalities of G6PD. Here we report that favism is more common with G6PD Med and with G6PD Cairo; although some children with G6PD A− had favism as severe as that seen with the other two variants. We also found that post-hemolytic recovery may be delayed with G6PD Cairo.

2. Materials and methods

2.1. Study subjects

From March 2009 to August 2015, 223 Palestinian children were admitted to Al Nasser Pediatric Hospital in Gaza for AHA due to G6PD deficiency. Of these, we successfully contacted the parents or guardians of 131 unrelated children, aged 2 to 8 years, and therefore were enrolled in the study: 120 were boys and 11 were girls. Blood samples for additional tests, including molecular typing of G6PD, were obtained during initial hospitalization from all children; however, due to restrictive conditions in Gaza we were only able to obtain complete records and follow-up samples for 79 children (71 boys and 8 girls). An additional 22 unrelated, healthy children provided normal hematological index ranges for the Gaza children community.

2.2. Population studies

To estimate the prevalence of G6PD variants in Gaza we genotyped 466 self-reported healthy, non-anemic, unrelated Gaza adults and 485 unrelated, consecutively born neonates at the maternity hospital-Al Shifa medical compound in Gaza.

All studies were approved by the Palestinian Review Board adhering to the Declaration of Helsinki (approval number PHRC/HC/48/13). Informed consent was obtained from all adults and from the parents or guardians of the children enrolled in the study.

2.3. Sample collection and processing

For the samples from children with AHA, the methodology has been described previously [13,14]. For adult controls, whole blood samples were collected in EDTA-anticoagulated tubes and 400 μL was spotted and dried on Ahlstrom 226 grade new-born screening filter paper (ID Biological Systems, Greenville, SC, USA). DNA was extracted and purified from the dried blood spots following a superparamagnetic-bead based DNA extraction procedure [15]. Neonate control samples (twins were excluded) were collected from the umbilical vein after cord clamping at delivery and stored in K3-EDTA collection tubes. About 400 μL of cord blood was spotted and dried onto Ahlstrom 226 grade new-born screening filter paper and DNA isolated by an automated, rapid extraction protocol [16].

2.4. Laboratory studies

Hematological and biochemical analyses were performed at Al Nasser Pediatric Hospital laboratories, Gaza, Palestine. The G6PD enzyme activity was measured spectrophotometrically at 340 nm using the commercially available G6PDH screening test (Randox Laboratories, Ltd., Antrim, UK), with normal activity of G6PD enzyme of 6.97–20.5 IU/g Hb, and according to manufacturer instructions. G6PD activity was expressed as IU/g Hb at 37 °C [17].

2.5. G6PD genotyping

All molecular analyses were conducted at Associated Regional and University Pathologists (ARUP) Laboratories, Salt Lake City, Utah, USA. Genotyping G6PD variants in the hospitalized cohort were conducted as described previously [14]. The adult control samples were screened for G6PD mutations using a single nucleotide extension (SNE) protocol (ABI Prism SNaPshot Multiplex System, Life Technologies Corporation, Carlsbad, CA, USA) designed at ARUP Laboratories. This protocol incorporates a multiplex analysis of 14 mutations commonly reported in G6PD deficiency in European-Arab-African populations. These mutations are: Orissa$^{c.131G}$, Aures$^{c.143C}$, A−$^{c.202A}$, Namoru$^{c.208C}$, A+$^{c.376G}$, Cairo$^{c.404C}$, Santa Maria$^{c.542T}$, Mediterranean$^{c.563T}$, Mexico City$^{c.680A}$, Seattle$^{c.844C}$, Kerala$^{c.949A}$, Betica$^{c.968C}$, Chatham$^{c.1003A}$, and Iowa$^{c.1156G}$ [9] Reactions were performed according to manufacturer's instructions using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, USA) and 3100 Genetic Analyzer (Life Technologies Corporation). Fragment analysis was conducted using GeneMarker software (SoftGenetics, LLC, State College, PA, USA). The neonate control samples were screened only for A−$^{c.202A}$, A+$^{c.376G}$, Cairo$^{c.404C}$, and Mediterranean$^{c.563T}$ variants by restriction enzyme digestion of *G6PD* exon 4, 5 and 6 polymerase chain reaction products, followed by Sanger sequencing to confirm the presence of the detected G6PD variant as described previously [14]. All restriction enzymes were obtained from New England Biolabs (Ipswich, MA, USA) and used according to manufacturer's directions. Exon 4 was interrogated with *Nla*III for A−$^{c.202A}$ variant, exon 5 was interrogated with BstCI for A+$^{c.376G}$ variant and *Fau*I for Cairo$^{c.404C}$ variant, and exon 6 was interrogated with *Mbo*II for Mediterranean$^{c.563T}$ variant. Restriction products were analyzed using the Qiaxcel DNA analyzer using the DNA screening kit and BioCalculator software (Qiagen Sciences, Germantown, MD, USA). Primers and probes used for the SNE protocol are outlined in Tables 1 and 2.

Table 1
Primers for amplification of G6PD gene. Divided into three regions: exons 1–2, exons 3–7, exons 8–13.

Primer ID	Size	Sequence (with M13 sequencing tails)
Exon 1 Forward	35	TGT AAA ACG ACG GCC AGT CAG CGG CAG CGG GTA TG
Exon 2 Reverse	39	CAG GAA ACA GCT ATG ACC GGC CCT GCA ACA ATT AGT TGG
Exon 3 Forward	39	TGT AAA ACG ACG GCC AGT CAC CAA GGG TGG AGG ATG ATG
Exon 7 Reverse	35	CAG GAA ACA GCT ATG ACC GCT CTG CCA CCC TGT GC
Exon 8 Forward	38	TGT AAA ACG ACG GCC AGT GCC CTT GAA CCA GGT GAA CA
Exon 13 Reverse	34	CAG GAA ACA GCT ATG ACC GGA AGG AGG GTG GCC G