RESEARCH ARTICLE

# Glucose-6-phosphate dehydrogenase deficiency in northern Mexico and description of a novel mutation

N. GARCÍA-MAGALLANES[1,2], F. LUQUE-ORTEGA[1], E. M. AGUILAR-MEDINA[1], R. RAMOS-PAYÁN[1], C. GALAVIZ-HERNÁNDEZ[3], J. G. ROMERO-QUINTANA[1], L. DEL POZO-YAUNER[4], H. RANGEL-VILLALOBOS[5] and E. ARÁMBULA-MERAZ[1]*

[1]*Doctorado Regional en Biotecnología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Universitarios s/n Ciudad Universitaria, 80010 Culiacán, México*
[2]*Ingeniería en Biotecnología, Universidad Politécnica de Sinaloa, Carretera municipal libre Mazatlán Higueras Km 3 col. Genaro Estrado, 82125 Mazatlán, México*
[3]*Laboratorio de Biología Molecular, CIIDIR-IPN, Unidad Durango. Sigma #119 Fracc. 20 de Noviembre II, 34220 Durango, México*
[4]*Instituto Nacional de Medicina Genómica, Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan México, D.F. C.P. 14610, México*
[5]*Instituto de Investigación en Genética Molecular, Centro Universitario de la Ciénega, Universidad de Guadalajara, Av. Universidad #1115, Col Paso Blanco, CP 47810 Ocotlán, México*

## Abstract

Glucose-6-phosphate dehydrogenase deficiency (G6PD) is the most common enzyme pathology in humans; it is X-linked inherited and causes neonatal hyperbilirubinaemia, chronic nonspherocytic haemolytic anaemia and drug-induced acute haemolytic anaemia. G6PD deficiency has scarcely been studied in the northern region of Mexico, which is important because of the genetic heterogeneity described in Mexican population. Therefore, samples from the northern Mexico were biochemically screened for G6PD deficiency, and PCR-RFLPs, and DNA sequencing used to identify mutations in positive samples. The frequency of G6PD deficiency in the population was 0.95% ($n = 1993$); the mutations in 86% of these samples were G6PD $A^{-202A/376G}$, G6PD $A^{-376G/968C}$ and G6PD Santamaria$^{376G/542T}$. Contrary to previous reports, we demonstrated that G6PD deficiency distribution is relatively homogenous throughout the country ($P = 0.48336$), and the unique exception with high frequency of G6PD deficiency does not involve a coastal population (Chihuahua: 2.4%). Analysis of eight polymorphic sites showed only 10 haplotypes. In one individual we identified a new G6PD mutation named Mexico $DF^{193A>G}$ (rs199474830), which probably results in a damaging functional effect, according to PolyPhen analysis. Proteomic impact of the mutation is also described.

[García-Magallanes N., Luque-Ortega F., Aguilar-Medina E. M., Ramos-Payán R., Galaviz-Hernández C., Romero-Quintana J. G., Del Pozo-Yauner L., Rangel-Villalobos H. and Arámbula-Meraz E. 2014 Glucose-6-phosphate dehydrogenase deficiency in northern Mexico and description of a novel mutation. *J. Genet.* **93**, 325–330]

## Introduction

G6PD deficiency is the most common of inherited and clinically significant enzyme defects. This disease has a broad biochemical and genetic heterogeneity and more than 330 million people present this deficiency (Nkhoma *et al*. 2009). Individuals with G6PD deficiency are usually asymptomatic; however, massive intravascular haemolysis is observed after intake of some drugs or chemicals. This disease is inherited in a recessive X-linked manner, and clinical manifestations include: drug or food induced acute haemolytic anaemia (Cappellini and Fiorelli 2008), nonspherocytic chronic haemolytic anaemia, and neonatal hyperbilirubinaemia that could lead to kernicterus and death (De Gurrola *et al*. 2008).

The frequencies of G6PD deficiency in 10 states of Mexico have been reported previously, with an average prevalence of 0.71% and a total of 18 variants of G6PD identified (Vaca *et al*. 1982, 2002; Medina *et al*. 1997). However, these studies have focussed on the central and southern

*For correspondence. E-mail: eliakymarambula@hotmail.com.

**Keywords.** G6PD deficiency; variants; northwest Mexico; haplotypes.

regions, including some states located in the Gulf of Mexico and Pacific Ocean. Conversely, scarce information of the G6PD deficiency has been obtained from the northern region, which potentially is different considering the heterogeneous ancestry described throughout the Mexican territory (Rubi-Castellanos *et al*. 2009): the European ancestry increases in the northern region, and in the same way the Amerindian counterpart increases in the centre and southeast regions. Therefore, the aims of this study were the following: (i) analyse the prevalence of G6PD deficiency in Mexico, including previously unstudied northern populations; and (ii) increase the knowledge concerning the genetic and molecular background of this disease in Mexico. Contrary to expectations, results show a relatively homogeneous G6PD deficiency frequency in the total Mexican population; in addition, a novel mutation named G6PD Mexico DF is described.

## Materials and methods

### *Samples*

Three sets of male samples were analysed (groups A, A2 and B). Group A corresponds to 1993 samples from volunteer blood donors whose mother and grandmother were from the following northern states: Sonora ($n = 587$), Baja California ($n = 670$), Baja California Sur ($n = 236$) and Chihuahua ($n = 500$). Blood samples were taken from major hospital(s) located in the main city of each Mexican state aforementioned, which receive cases from different smaller cities. Based on the current average prevalence of G6PD deficiency described in Mexico (0.71%), the population sample size allows reliable estimation of G6PD variant frequency in the northern region. Subgroup A2 comprised 100 individuals of group A with positive activity during the G6PD deficiency screening, in which the haplotype analysis was performed. Group B included nine G6PD deficient samples received in our laboratory from the Hospital General of Mexico (Mexico city) for molecular analyses.

### *Biochemical analyses*

In the Mexican population sample from the North (group A), detection of the enzymatic deficiency was performed by the fluorescent spot test (Beutler *et al*. 1979).

### *Molecular analyses*

In individuals from all groups (A, A2 and B), genomic DNA was extracted from peripheral blood leucocytes using the Gustincich method (Gustincich *et al*. 1991). Based on the previous detection of G6PD mutations in Mexican populations (Vaca *et al*. 1982, 2002; Medina *et al*. 1997), G6PD-deficient individuals from groups A and B were tested for the following variants: G6PD $A^{-202A/376G}$, G6PD $A^{-376G/968C}$, G6PD $A^{-376G/680T}$, G6PD Seattle$^{844C}$, G6PD Mediterranean$^{563T}$, G6PD Santamaría$^{376G/542T}$ and G6PD Canton$^{1376T}$. Exons were amplified by PCR with previously reported primers (Beutler *et al*. 1991), followed by restriction analyses with the *Nla*III, *Fok*I, *BspE*I, *Mbo*II, *BstN*I, *Nla*III, *Nci*I and *Afl*III enzymes to detect nucleotide (nt) substitutions 202G>A, 376A>G, 542A>T, 563C>T, 680T>A, 844G>C, 968T>C and 1376G>T, respectively.

The G6PD-deficient individuals with negative results for these mutations were evaluated through PCR amplification of exons 2–11; PCR products were screened by means of the single strand conformational polymorphism (SSCP) technique, according to Arámbula and Vaca (2002). Exons displaying mobility shift were sequenced to identify the mutation causing G6PD deficiency.

Haplotype analyses in all samples included four silent polymorphisms and the following four disease-causing mutations on *G6PD* gene: intron 5 (*Pvu*II), nt 1116 (*Pst*I), intron 11 (*Nla*III), and nt 1311 (*Bc*II), using proper restriction enzymes, following Vaca *et al*. (2002) and Vulliamy *et al*. (1991). PCR amplifications were subjected to electrophoresis in 7.5% polyacrylamide gels followed by silver staining.

### *Data analyses*

Allele and haplotype frequencies were estimated by the gene counting method. Exact tests were carried out to evaluate population differentiation by pairwise comparisons and to check homogeneity among all populations based on allele or haplotype distributions. *P* values were empirically determined by 20,000 simulations. The software TFPGA was employed for these purposes (Miller 1997). In addition, we employed the software PolyPhen-2 to predict the functional effects of this mutation in humans.

## Results

We identified a total of 19 G6PD-deficient individuals (0.95%) in the studied northern population sample of Mexico (table 1). A total of 28 G6PD-deficient samples were found, including 19 individuals from group A (68%), and nine from group B (32%). Among these samples, mutation was identified in 24 deficient patients, 22 of which presented the variant G6PD $A^{-202A/376G}$ (15 from group A and seven from B), one G6PD Santamaría$^{542T}$ (group A), and one G6PD $A^{-376G/968C}$ (group B).

In four individuals (three from group A and one from group B), the mutations were not found by means of the PCR-RFLP technique. These samples were submitted to SSCP identifying an electrophoretic mobility shift in the exon 4 of only one sample, leading to its sequencing. Results revealed the mutation X55448:c.193A>G that changes the amino acid threonine for alanine at position 65 (p.Thr65Ala). This mutation has not been previously reported in literature, hence, we called it G6PD Mexico DF$^{193A>G}$ (rs199474830). The PolyPhen analysis gave two main predictions regarding the functional effect of this

**Table 1.** Results of biochemical screening and molecular analysis of G6PD deficiency in population samples from four northern Mexican populations and Mexico City.

| State (sample size) | Biochemical screening | | Molecular analysis | | | | |
|---|---|---|---|---|---|---|---|
| | Deficient (*n*) | Per cent | A | B | C | D | E |
| Baja California (670) | 3 | 0.45 | 3 | – | – | – | – |
| Baja California Sur (236) | 1 | 0.42 | 1 | – | – | – | – |
| Chihuahua (500) | 12 | 2.4 | 8 | 1 | – | – | 3 |
| Sonora (587) | 3 | 0.51 | 3 | – | – | – | – |
| Mexico City (9) | 9 | – | 7 | – | 1 | 1 | – |
| Total (1993) | 28 | 0.95 | 22 | 1 | 1 | 1 | 3 |

A, G6PD $A^{-202A/376G}$; B, G6PD Santamaría$^{542T}$; C, G6PD $A^{-376G/968C}$; D, G6PD Mexico $DF^{193G}$; E, unknown.

mutation: (i) probably damaging with a score of 0.979 (sensitivity, 0.76; specificity, 0.96); and (ii) possibly damaging with a score of 0.903 (sensitivity, 0.69; specificity, 0.90).

The proteomic analysis of the mutation G6PD Mexico $DF^{193A>G}$starts with the location of the amino acid Thr65 that is situated in the second β-strand of the β–α–β motif of the coenzyme domain. This side chain contacts with other residues as well as with the peptide backbone that might contribute importantly to the conformational stability of β–α–β motif, where the conserved catalytic NADP-binding site is located (figure 1). In the crystallographic structure of the human G6PD enzyme, variant Canton (Au *et al*. 2000), the hydroxyl group of Thr65 forms a H-bond with the main chain carbonyl group of Pro62, stabilizing the turn connecting the α-helix with the second β strand of the β–α–β motif

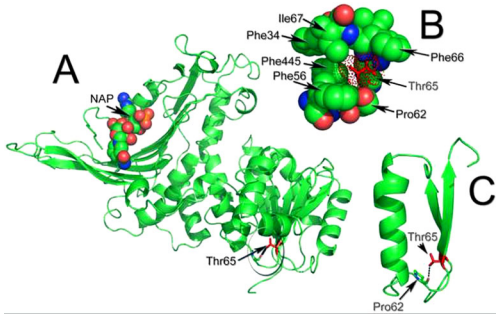


**Figure 1.** Structural environment of Thr65 in human G6PD. (A) Cartoon representation of the crystallographic structure of human glucose-6-phosphate dehydrogenase (variant Canton R459L), complexed with structural $NADP^+$ (PDB ID 1QKI). Residue Thr65 is represented in stick format and coloured in red. Structural $NADP^+$ is represented in space-filled format. (B) Structural environment of Thr65 in the native structure of human G6PD, the same protein of panel A. Residue Thr65 is represented in stick format and the Van der Waals radio of its atoms is represented in dotted format and coloured in red. The residues forming the walls of the nonpolar pocket in which Thr65 is placed in the native folding are identified and are represented in space-filled format. (C) Cartoon representation of the segment of G6PD, the same protein represented in A, spanning from His27 to Leu75. Residues Pro62 and Thr65 are represented in stick format and the H-bond formed between the hydroxyl group of Thr65 and the main chain carbonyl group of Pro62 is represented as a dotted line in black. The molecular graphics figures were prepared with PyMOL.

(figure 1 C). Since the side chain of Ala lacks a H-bond donor group, the Thr65Ala mutation eliminates the aforementioned H-bond and by this way it might destabilize the β−α−β folding. Thr65Ala mutation might also be destabilizing by causing a packing defect. In the native state of the G6PD human protein, Thr65 is buried in a pocket formed by several nonpolar residues, as Phe34, Phe56, Pro62, Phe66 and Ile67, among others (figure 1 B). The shorter side chain of Ala might not fill optimally the pocket, loosening the domain packing by disrupting the interaction network that stabilizes it. Mutations leading to a packing defect in globular proteins are generally associated with decreased thermodynamic stability, although the magnitude of the destabilizing effect is context dependent (Eriksson *et al*. 1992). Thus, we postulate that Thr65Ala mutation might lead to an unstable coenzyme union site region of the enzyme, due to the combined effect of the suppression of a peptide backbone H-bond and by disrupting the domain packing.

Finally, the haplotype analysis with eight polymorphic sites was performed in 99 individuals of subgroup A2 with the G6PD B variant, one with G6PD $A^{+376G}$, 22 with G6PD $A^{-202A/376G}$, one with G6PD $A^{-376G/968C}$ , one with G6PD Santamaria$^{376G/542T}$ and one with G6PD Mexico $DF^{193A>G}$. In the present study, only 11 among the 256 possible haplotypes were found (table 2); this is consistent with the strong linkage disequilibrium between silent and other G6PD coding sequence polymorphisms.

## Discussion

### *Biochemical analyses*

The biochemical result (0.95%) is slightly higher than the report of Vaca *et al*. (2002), who reported a frequency of 0.71% for 10 states from different regions of the country. The frequency of G6PD-deficient individuals in the northern states studied here (Sonora, Baja California and Baja California Sur) was 0.47% in 1493 blood donors. A previous study in northwestern Mexico to detect inborn errors of erythrocyte metabolism in blood donors revealed a similar frequency (0.37%) (Vaca *et al*. 1982). On the other side, Chihuahua reached the highest frequency (2.4%) with

**Table 2.** G6PD haplotypes in population samples from four northern Mexican populations and Mexico City.

| Variant | Haplotype | E-4 202A *Nla*III | E-5 376G *Fok*I | E-6 542T *Bsp*EI | E-9 968C *Nci*I | I-5 611G *Pvu*II | E-10 1116G *Pst*I | E-11 1311T "*Bcl*I" | I-11 93C *Nla*II | *n* | Per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G6PD B | I | − | − | − | − | − | + | − | − | 71 | 56.8 |
| G6PD B | II | − | − | − | − | − | + | + | + | 15 | 12 |
| G6PD B | III | − | − | − | − | − | + | − | + | 5 | 4 |
| G6PD B | IV | − | − | − | − | − | − | − | + | 1 | 0.8 |
| G6PD B | V | − | − | − | − | − | + | + | − | 7 | 5.6 |
| G6PD $A^{376G}$ | VI | − | + | − | − | + | + | − | + | 1 | 0.8 |
| G6PD $A^{-202A/376G}$ | VII | + | + | − | − | + | + | − | + | 21 | 16.8 |
| G6PD $A^{-202A/376G}$ | VIII | + | + | − | − | − | + | − | + | 1 | 0.8 |
| G6PD $A^{-376G/968C}$ | IX | − | + | − | + | − | + | − | + | 1 | 0.8 |
| G6PD Santamaria$^{542T}$ | X | − | + | + | − | − | + | − | + | 1 | 0.8 |
| G6PD Mexico DF$^{193G}$ | XI | − | − | − | − | + | + | + | + | 1 | 0.8 |

*n*, number of chromosomes.

respect to the rest of the states analysed in this study and previous reports, including the states of Nayarit (1.26%), Veracruz (1.17%) and Guerrero (1.03%) (Vaca *et al*. 2002).

A high frequency of G6PD deficiency has been claimed for Mexican states with historically known influence of African ancestry, like those on the Pacific shore and the Gulf of Mexico (Medina *et al*. 1997; Vaca *et al*. 2002). Although G6PD-deficiency prevalence in Mexico seems slightly heterogeneous ($P = 0.02748$), the distribution is homogeneous when the high frequency G6PD deficiency in the northern state of Chihuahua is excluded (2.4%; $P = 0.48336$). The elevated frequency of G6PD deficiency observed in Chihuahua probably is explained by the African slaves recruited to work in mines after the Spanish conquest. In fact, during the XVII and XVIII centuries, reduction of local native population by epidemics and wars forced conquerors to import manpower, followed by documented interracial marriages (Indians and free Mulatto, Blacks and Indians, Spanish and Blacks, among others) (Guevara 2011). In addition, African ancestry may have come to Mexico via the Spaniards (Rubi-Castellanos *et al*. 2009), who also received African lineages from Middle East and North Africa.

### *Molecular analyses of G6PD-deficient samples*

Previous studies regarding G6PD deficiency in Mexico agree with our findings in general (Vaca *et al*. 2002) and selected populations (Medina *et al*. 1997), reporting 58% and 79% for the G6PD $A^{-202A/376G}$mutation, respectively. Conversely, in a previous study, Vaca *et al*. (2002) found 42 individuals with G6PD $A^-$ variants, 16 of them with G6PD $A^{-376G/968C}$, meanwhile Medina *et al*. (1997) reported 15 G6PD $A^-$ individuals and five of them as G6PD $A^{-376G/968C}$. These frequency differences may be due to heterogeneous African admixture, because most of the deficient persons in our study come from the Pacific and North shore states, which could have a different African ancestry in which the G6PD $A^{-202A/376G}$ variant is more common. We particularly note the smaller influence of the European variants such as the G6PD $A^{-376G/968C}$variant that reaches polymorphic frequencies in Spain, and G6PD Santamaria$^{376G/542T}$ that is more frequent in Spain and Canary Islands (Vaca *et al*. 2002). Table 1 shows sample distribution of the G6PD $A^{-202A/376G}$ and G6PD Santamaria$^{376G/542T}$ variants observed in this study. G6PD $A^{-202A/376G}$ presented a 1.6% frequency in the Chihuahua state. This is a class III variant with a 10 to 60% residual enzymatic activity regarding the normal reference; thus, patients do not require strict treatment like the class I and II variants, only some drug restriction is needed to prevent haemolysis. In Sonora, Baja California and Baja California Sur states, this is the unique G6PD variant observed with frequencies of 0.45, 0.42 and 0.51%, respectively.

### *PCR-SSCP and DNA sequencing*

In four individuals (three from group A and one of group B), mutations were not found by means of the PCR-RFLP technique. These samples were submitted to SSCP identifying an electrophoretic mobility shift in the exon 4 of only one sample, leading to its sequencing. Results revealed the mutation X55448:c.193A>G that changes the amino acid threonine for alanine at position 65 (p.Thr65Ala). This mutation has not been previously reported in literature, thus we named it G6PD Mexico DF$^{193A>G}$ (rs199474830). Another pathogenic variant (near of G6PD Mexico DF$^{193A>G}$), G6PD Amazonia$^{185C>A}$ described by Hamel *et al*. (2002), causes a change Pro62His (class III); G6PD Musashino$^{185C>T}$described by Hirono *et al*. (1997), that changes Pro62Phe (class III); and finally G6PD Songklanagarind$^{196T>A}$described by Laosombat *et al*. (2005) that causes change of Phe66Ile (class II). Based on the biochemical analyses result, using the program Provean 1.0 and comparing with another G6PD mutations, we were able to infer its pathogenic effect. In the remaining three samples, the mutation responsible for the G6PD deficiency remains to be identified.

***Proteomic analysis of the new mutation***

In one individual we identified a new G6PD mutation named Mexico DF$^{193A>G}$ (rs199474830), which according to PolyPhen analysis results in a probably damaging functional effect, which is in agreement with the deeper proteomic analysis carried out of the mutation (figure 1). Although probably this mutation has a very low frequency in the Mexican population, we can predict a significant health impact in the males of the family receiving this mutation. The geographic extent of this mutation in our country deserves further research.

***Intrapopulation comparison of G6PD haplotypes***

In those individuals who presented the G6PD B variant, five different haplotypes were found (I through V), the most frequent haplotypes were I and II present in 56.8 and 12% of the samples, respectively. In another study, two more haplotypes were found for this variant (Vaca *et al*. 2002), besides the five haplotypes here described. In our study, the sample with the G6PD$^{376G}$ nondeficient variant had the haplotype VI. The haplotypes VII and VIII were found in samples with the variant G6PD A$^{-20A/376G}$ and haplotype VII was the most frequent with 16.8%. Conversely, Vaca *et al*. (2002) and Medina *et al*. (1997) found haplotype VII on samples with G6PD A$^{-202A/376G}$ variant with frequencies of 9.21% and 75%, respectively.

The sole African origin of G6PD A$^{-202A/376G}$ has been associated with the *Pvu*II/*Pst*I/1311/*Nla*III haplotype (+/+/−/+ haplotype VII of this research), independently of the individual ethnic origin. This statement is confirmed by our results because unrelated samples presented the same haplotype and came from different states of the country.

Variant G6PD A$^{-376G/968C}$ has been identified in Mexico and South of Europe, and has been associated with two haplotypes: −/+/−/+ or −/+/−/− (Medina *et al*. 1997; Hamel *et al*. 2002; Vaca *et al*. 2002). In this study, the sample presented the haplotype IX (−/+/−/+), reported on the majority of analyses in Mexicans, and in agreement with the Spanish influence that has contributed to the present day genetic pool of the Mexican population (Rubi-Castellanos *et al*. 2009).

When the variant G6PD Santamaria$^{376G/542T}$ was analysed, the haplotype X was found, similar to that observed in the Spanish population (Pinto *et al*. 1996). The sample with the novel mutation Mexico DF$^{193A>G}$, presented in this article, had the haplotype +/+/+/+, previously not reported or associated with any other variant, reinforcing the view that it constitutes a new mutation.

In agreement with historical records (Rubi-Castellanos *et al*. 2009), haplotypes found in the northern region of Mexico reveal that the G6PD deficiency was imported from Europe and Africa, probably during and after the Spanish conquest involving the African slave trading, because the G6PD variants and haplotypes identified here correlate with mutations previously described in these populations (G6PD A$^{-202A/376G}$and G6PD Santamaria$^{376G/542T}$, respectively).

***Interpopulation comparison of G6PD variants***

The G6PD variants found in the North region of Mexico have been observed in Portuguese, African, Brazilian and Spanish populations, but with different frequencies. Manco *et al*. (2007) analysed 70 unrelated G6PD-deficient patients, and found G6PD A$^{-202A/376G}$as the most common mutation (63.4%); all these samples present the *Fok*I/*Pvu*II/*BspH*I/*Pst*I/1311/*Nla*III (+/+/−/+/−/+) haploype. Our results are similar for majority of A$^{-}$ alleles reported in sub-Saharan Africa. This is consistent with the Africa origin hypothesis of the G6PD A$^{-202A/376G}$variant by malaria-selective pressure (Ruwende *et al*. 1995; Tishkoff *et al*. 2001). The G6PD Santamaria$^{376G/542T}$ is another variant described in this study; Manco *et al*. (2007) and Hamel *et al*. (2002) have found this mutation in Portuguese and Brazilian populations, respectively. The same haplotype has been found in all G6PD Santamaria$^{376G/542T}$ cases, suggesting a common origin for this variant in all countries. Comparison of G6PD variants found in Mexico with respect to Asian populations with elevated frequencies of G6PD mutations such as those found in India (Chalvam *et al*. 2011), did not offer significant matches given the low gene flow between Asia and America after the European contact.

## Conclusion

The overall prevalence of G6PD deficiency in northern Mexico was 0.95%. Contrary to previous reports, we demonstrated that G6PD deficiency distribution is relatively homogeneous throughout the country; the sole exception being elevated frequency of G6PD deficiency that does not involve a coastal population (Chihuahua: 2.4%). As previously reported, specific deficient G6PD variants are commonly found in the Mexican population, such as G6PD A$^{-}$. A new G6PD variant named Mexico DF$^{193A>G}$ is described, including the structural and functional impact in the protein. Our findings are in agreement with historical records that involve both European and 'principally' African origins for the presence of G6PD variants in Mexico.

**Acknowledgements**

This work was supported by PROFAPI (grant 123) and COECYT approved to E. A-M. We thank the QFB's Aurelio Hernández, Sandra Ochoa, Aaron Dávila, Nurse Rosario García and Dr José Guillermo Romero for providing blood samples.

## References

Arámbula E. and Vaca G. 2002 Genotyping by 'cold single-strand conformation polymorphism' of the UGT1A1 promoter polymorphism in Mexican mestizos. *Blood Cells Mol. Dis.* **28**, 86–90.

Au S. W., Gover S., Lam V. M. and Adams M. J. 2000 Human glucose-6-phosphate dehydrogenase: the crystal structure reveals a structural NADP(+) molecule and provides insights into enzyme deficiency. *Structure* **15**, 293–303.

Beutler E., Blume K. G., Kaplan J. C., Löhr G. W., Ramot B. and Valentine W. N. 1979 International Committee for Standardization in Haematology: recommended screening test for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. *Br. J. Haematol.* **43**, 465–467.

Beutler E., Kuhl W., Ramirez E. and Lisker R. 1991 Some Mexican glucose-6-phosphate dehydrogenase variants revisited. *Hum. Genet.* **86**, 371–374.

Cappellini M. D. and Fiorelli G. 2008 Glucose-6-phosphate dehydrogenase deficiency. *Lancet* **371**, 64–74.

Chalvam R., Colah R. B., Mohanty D., Ghosh K. and Mukherjee M. B. 2011 Restriction fragment length polymorphism (RFLP) of the X chromosome linked glucose-6-phosphate dehydrogenase (G6PD) locus in India. *Ann. Hum. Biol.* **38**, 106–109.

De Gurrola G. C., Araúz J. J., Durán E., Aguilar Medina M., Ramos Payán R., GarcíaMagallanes N. *et al.* 2008 Kernicterus by glucose-6-phosphate dehydrogenase deficiency: a case report and review of the literature. *J. Med. Case Rep.* **2**, 146.

Eriksson A. E., Baase W. A., Zhang X. J., Heinz D. W., Blaber M., Baldwin E. P. and Matthews B. W. 1992 Response of a protein structure to cavity-creating mutations and its relation to the hydrophobic effect. *Science* **255**, 178–183.

Guevara S. A. 2011 Presidio y población indígena en la nueva Vizcaya. Siglo XVII y XVIII Universidad Michoacana de San Nicolás de Hidalgo.

Gustincich S., Manfioletti G., Del Sal G., Schneider C. and Carninci P. 1991 A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques* **11**, 298–300, 302.

Hamel A. R., Cabral I. R., Sales T. S., Costa F. F. and Olalla Saad S. T. 2002 Molecular heterogeneity of G6PD deficiency in an Amazonian population and description of four new variants. *Blood Cells Mol. Dis.* **28**, 399–406.

Hirono A., Fujii H., Takano T., Chiba Y., Azuno Y. and Miwa S. 1997 Molecular analysis of eight biochemically unique glucose-6-phosphate dehydrogenase variants found in Japan. *Blood.* **89**, 4624–4627.

Laosombat V., Sattayasevana B., Janejindamai W., Viprakasit V., Shirakawa T., Nishiyama K. and Matsuo M. 2005 Molecular heterogeneity of glucose-6-phosphate dehydrogenase (G6PD) variants in the south of Thailand and identification of a novel variant (G6PD Songklanagarind). *Blood Cells Mol. Dis.* **34**, 191–196.

Manco L., Gonçalves P., Antunes P., Maduro F., Abade A. and Ribeiro M. L. 2007 Mutations and haplotype diversity in 70 Portuguese G6PD-deficient individuals: an overview on the origin and evolution of mutated alleles. *Haematologica* **92**, 1713–1714.

Medina M. D., Vaca G., Lopez Guido B., Westwood B. and Beutler E. 1997 Molecular genetics of glucose-6-phosphate dehydrogenase deficiency in Mexico. *Blood Cells Mol. Dis.* **23**, 88–94.

Miller M. P. 1997 Tools for population genetic analysis (TFPGA) version 1.3. Department of Biological Sciences-Box 5640, Northern Arizona University, http://herb.bio.nau.ed/~miller.

Nkhoma E. T., Poole C., Vannappagari V., Hall S. A. and Beutler E. 2009 The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol. Dis.* **42**, 267–278.

Pinto F. M., González A. M., Hernández M., Larruga J. M. and Cabrera V. M. 1996 Sub-Saharan influence on the Canary Islands population deduced from G6PD gene sequence analysis. *Hum. Biol.* **68**, 517–522.

Rubi-Castellanos R., Martínez Cortés G., Muñoz Valle J. F., González Martín A., Cerda Flores R. M., Anaya Palafox M. and Rangel Villalobos H. 2009 Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. *Am. J. Phys. Anthropol.* **139**, 284–294.

Ruwende C., Khoo S. C., Snow R. W., Yates S. N. R., Kwiatkowski D., Gupta S. *et al.* 1995 Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. *Nature* **376**, 246–249.

Tishkoff S. A., Varkonyi R., Cahinhinan N., Abbes S., Argyropoulos G., Destro Bisol G. *et al.* 2001 Haplotype divestity and linkage disequilibrium at human G6PD; recent origin of alleles that confer malarial resistence. *Science* **293**, 455–461.

Vaca G., Ibarra B., Hernandez A., Velazquez A. L., Gonzalez Quiroga G., Romero F. *et al.* 1982 Screening for inborn errors of the erythrocyte metabolism in Northwestern Mexico. *Acta Anthropogenet.* **6**, 255–264.

Vaca G., Arambula E. and Esparza A. 2002 Molecular heterogeneity of glucose-6-phosphate dehydrogenase deficiency in Mexico: overall results of a 7-year project. *Blood Cells Mol. Dis.* **28**, 436–444.

Vulliamy T. J., Othman A., Town M., Nathwani A., Falusi A. G., Mason P. J. and Luzzatto L. 1991 Polymorphic sites in the African population detected by sequence analysis of the glucose-6-phosphate dehydrogenase gene outline the evolution of the variants A and $A^{-}$. *Proc. Natl. Acad. Sci. USA* **88**, 8568–8571.

Received 2 October 2013, in revised form 29 November 2013; accepted 3 December 2013
Published on the Web: 23 June 2014