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G6P-DH SANTA CLARA AND G6P-DH VILLA CLARA: TWO NEW CUBAN VARIANTS

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Two new glucose 6-phosphate dehydrogenase G6P-DH variants designated G6P-DH Santa Clara and G6P-DH Villa Clara were identified in a Cuban family. These variants belong to class III. Their electrophoretic and kinetic properties allow the conclusion that G6P-DH Santa Clara and G6P-DH Villa Clara are probably two new variants.

INTRODUCTION

The X-chromosome linked glucose-6-phosphate-dehydrogenase (E.C 1.1.1.49) has its locus in position Xq28 is a polymorphic enzyme with more than 300 variants described up to now /8/. Some of these variants present characteristic clinical manifestations and others are asymptomatic /10/. In this report we describe a Cuban family with two new variants of G6P-DH.

FAMILY REPORT

The propositus was a 6 year old female with slight anaemia attended by the Haematology Service of the J.L. Miranda Teaching Pediatric Hospital of Villa Clara, (clinical history = 173398) sent to our laboratory for enzymatic studies.

A catalytic activity in haemolysate of 62 % was found in the propositus, while the electrophoresis in buffered phosphate starch gel at pH 7.0 developed a slow band and another similar to electrophoretic phenotype B. The father and the brother were asymptomatic, and their study revealed a catalytic activity of 46 and 46,2 % respectively. The father showed a slow band and the brother showed a similar band such as electrophoretic phenotype B. The mother showed an electrophoretic mobility B and 38 % of catalytic activity.

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MATERIALS AND METHODS

The enzymatic determinations were done from haemolysate according to the method described by Beutler /1/. G6P-DH was partially purified and characterized by conventional methods /10/. The electrophoretic mobility was studied in two buffered systems at different pH and Michael-Menten constants (Km) for glucose-6-phosphate, 2-d-glucose-6-phosphate and for NADP such as the Ki for NADPH. Moreover, the thermosensibility at 42 C^{o} and the optimum pH for each semipurified enzymes were studied.

Simultaneously a paired sample considered as control according to its kinetic property was studied.

RESULTS

The biochemical characteristics of the Gd(x)B variant are reported in literature, the control used in our laboratory and the variant of the father, and the brother of the propositus appear in Table I. According to the classification proposed by the group of experts of the WHO /10/ and McKusick /7/ both studied variants belong to class III.

In Annex I appear the variants of class III with similar electrophoretic mobility to G6P-DH Santa Clara reported by Yoshida and Beutler /9/, and the Annex II shows the rare variants of the same class, in which its electrophoretic mobility is similar to G6P-DH Villa Clara taken from the same report. Fig.1 corresponds to the effect of pH on G6P-DH (B)B, G6P-DH (B) Santa Clara and G6P-DH(B) Villa Clara. In Fig. 2 appear the thermostability curves of the control and the two variants. Fig. 3 shows the pedigree of the propositus family.

DISCUSSION

G6P-DH Santa Clara is a variant of G6P-DH(B), characterized by presenting no associated clinical manifestations. The characteristics of this enzyme demonstrate that it has not been described previously, because G6P-DH Tenganan and Cohe appearing with similar electrophoretic mobility in class III /9/, differ from it in the Km for G6P and NADP, such as the

TABLE I

Enzymatic properties of G6P-DH Santa Clara and G6P-DH Villa Clara compared with normal - B - enzyme

Variant	Pop. origin	Red cell enzyme activity (% of normal)	Electro-phoretic mobility (% of normal)	Km G6P (µmol/l)	Km NADP (µmol/l)	2d G6P utili-zation (% of G6P)	Ki NADPH (µmol/l)	Heat stability	pH optimum
- B - Reported range of normal	Various	100	100	50 - 70 *	2.9-4.4 *	< 4	21 - 37 **	normal	normal
Normal control - B -	Cuban	100	100	59	3.1	< 4	20.54	normal	normal
G6P-DH Santa Clara	Cuban	46	pH - 66.6 TRIS- 60.0	34.0	20.5	44.1	9.06	labile	peak AT 8.0
G6P-DH Villa Clara	Cuban	46.2	pH -100 TRIS-100	49	7.8	3	267.64	moderately increased	biphasic 7.6y9.0

* Mc Kusick 1983
** Luzzatto and Afolayan 1968

ANNEX I

Enzymatic properties of variants with similar electrophoretic mobility at G6P-DH Santa Clara (Class III)

Variant	Population origin	Electrophoretic mobility (% of normal)	Km G6P (µmol/l)	Km NADP (µmol/l)	2d G6P Utilization (% of G6P)	pH optimum	Heat stability
G6P-DH Tenganan *	Indonesia	TRIS - 64 TEB - 99 PHOS - 75	11.4	6.9	18.3	normal	low
G6P-DH Kobe *	Japanese	TRIS - 82 TEB - 89 PHOS - 61	143	4.7	4.6	acidic shift	very labile
G6P-DH Santa Clara	Cuban	TRIS - 60 PHOS - 66.6	34-34.8	20.5	44.1	peak at 8.0	labile

* Yoshida and Beutler, 1983

ANNEX II

Enzymatic properties of variants with
similar electrophoretic mobility at G6P-DH Villa Clara
(Class III)

Variant	Population origin	Electro-phoretic mobility (% of normal)	Km G6P (µmol/l)	Km NADP (µmol/l)	2d G6P Utilization (% of G6P)	pH optimum	Heat stability
G6P-DH Mahidol	Cambodia	TRIS-100 PHOS-100	40	-	4	normal	normal
G6P-DH Kamiube	Japanese	TRIS-100 TEB -100 PHOS-100	31 - 43	2.6 - 5.1	3 - 4	normal	normal
G6P-DH Villa Clara	Cuban	TRIS-100 PHOS-100	49	7.8	3	biphasic 7.6 y 9.0	moderately increased

* Yoshida and Beutler, 1983

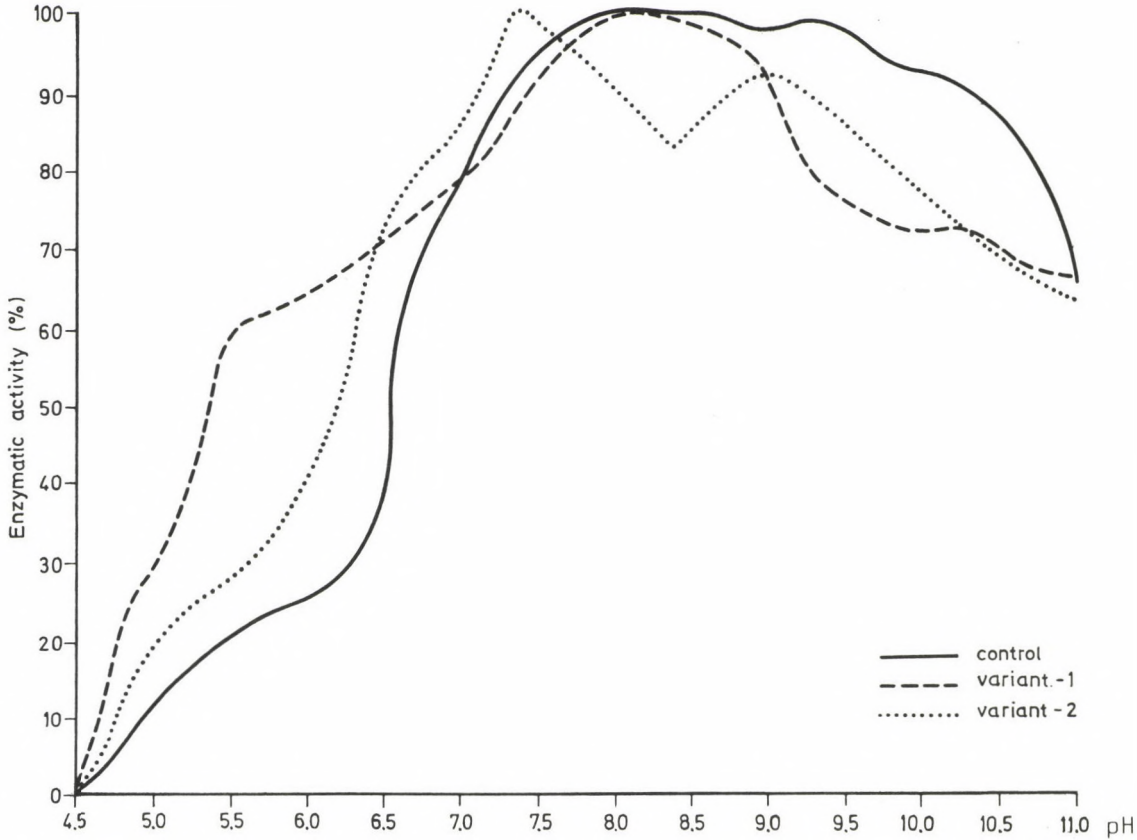

Fig. 1. pH curves of red cell G6P-DH Santa Clara, G6P-DH Villa Clara and G6P-DH (B)

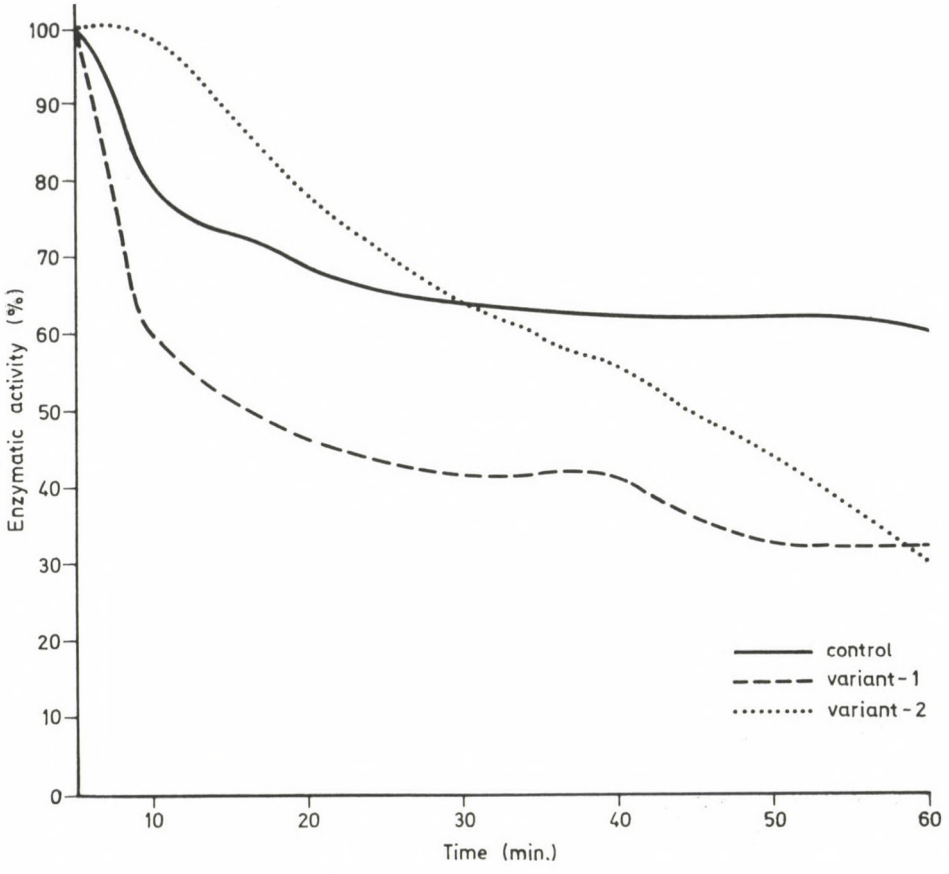

Fig. 2. Thermostability at 42°C of red cell G6P-DH Villa Clara, G6P-DH Santa Clara and G6P-DH (B)

using percentage of 2-d-G6P and behaviour in front of DH.

G6P-DH Villa Clara is also a variant of G6P-DH(B), having no associated clinical manifestations. The biochemical properties of this enzyme demonstrate that this variant has not been described previously, because the G6P-DH Mahidul and Kamiube variants belong to class III and having a similar electrophoretic mobility to our variant, but differ in behaviour in front of pH and in thermostability. The G6P-DH Villa Clara and Kamiube also differ in the Km for NADP. Although the 3 variants have Km values for G6P in an interval proposed as a normal range, our variant value is the highest.

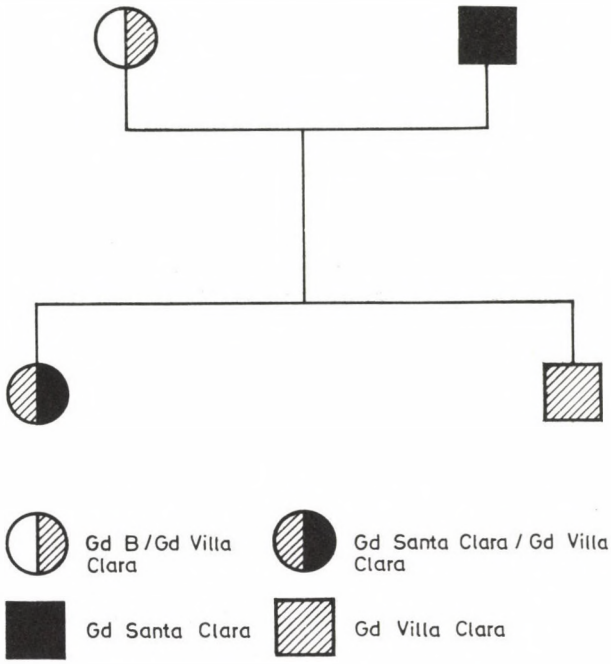

Fig. 3. Pedigree of family with the variants G6P-DH Santa Clara and G6P-DH Villa Clara

Of the described rare variants in the Cuban population compared with G6P-DH Varadero, Ciudad Habana, Matanzas, Giron, Caujeri Guantanamo, Pinar del Rio and Baire /2,3,4,5/, all are slow or rapid so that it can be sure that G6P-DH Villa Clara has not been described in our country so far. Although the G6P-DH Ciudad Habana, Baire and Pinar del Rio variants are slower than our variant, we proceeded to compare the 3 slow Cuban variants described before-hand and only G6P-DH Ciudad Habana presented enzymatic deficiency so that we only proceeded to compare it with G6P-DH Santa Clara. G6P-DH Ciudad Habana presents an enzymatic activity in haemolysate between 8-10 %, and its kinetic characteristics are: Km for G6P of 25 micromoles/l a 22 % utilization of 2-dG6P and the behaviour in front of pH is biphasic which demonstrates that both differ among them.

Keeping in account that the rare variant of G6P-DH in our population is of 0.004 /6/, the probability of finding a family with a double heterozygotic daughter is of 1 in 50000 families.

The presence of both variants in a double heterozygotic carrier has determined the sub-clinical manifestation of the deficiency in G6P-DH which demonstrates the effect of the doses in this enzyme linked to X-chromosome which can be given by a limitation in the half of the erythrocytic population.

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