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FOUR ADULT HEMOGLOBIN TYPES IN ONE MULATTO FAMILY

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SUMMARY

The studied family showed the presence of four different types of hemoglobin.

The family member who gave rise to this study (= propositus) presented Hb C and the hybrid Hb CG-phila.

The propositus has three children, all of which have Hb AC; none of the family members showed any clinical symptoms.

The investigation of the hemoglobin arose from the finding of target red cells in a blood test done during the pre-operatory examination for lower limb varicose vein stripping.

The hybrid Hb CG-phila is due to two gene pairs, each of which with individual expression, determining the synthesis and the particular type subunits.

The hybrid Hb CG-phila is formed by the combination velocity of the subunits $\alpha_2^{\text{G-phila}}\ \beta_2$; therefore the proportion of the hybrid Hb CG-phila is lower than Hb G-phila and Hb C.

The identification and molecular characterization of Hb G-phila showed the position $\alpha_2^{68\ \text{Asn}\rightarrow\text{Lys}}\ \beta_2$ and Hb C showed $\alpha_2\ \beta_2^{6\ \text{Glu}\rightarrow\text{Lys}}$.

KEYWORDS: Hb Gphiladelphia.

INTRODUCTION

This report presents the results of a study done in a family carrying Hb G-philadelphia (Hb G-phila).

Hb G-phila ($\alpha_2^{68\ \text{Asn}\rightarrow\text{Lys}}\ \beta_2$) has a frequency of 1:5,000 in Afro-Americans, suggesting that the gene responsible for it has origins in Africa[8].

The finding of Hb G-phila in a family of Brazilian mulattoes points towards a similar origin of North-American and Brazilian negroes, an important evidence for the tracing back of population migrations.

The reports of Hb G-phila cases in the literature describe its frequency as variable, ranging between 25%, 35%, 45% and 100%[4, 12]. It was found out that in the 25% heterozygotes Hb G-phila is determined by four genes of α globin[12].

Cases of Hb G-phila with 25% heterozygotes were also described in Caucasians, whereas cases with high percentages were described in negroes[12].

In the present family, it was possible to show that the case with 25% Hb G-phila, according to MOLCHANOVA et al.[12], correspond to a mutation AAC AAA in the gene of the α_2 globin, whereas the different mutation AAC AAG is present in the hybrid gene $\alpha_2\ \alpha_1$ with approximately 35% of Hb G-phila.

MATERIAL AND METHODS

A family study was made, starting with a female propositus, married to a healthy man (who was not tested) and with three children, all of which showed to have Hb AC, so none of them had inherited the hybrid Hb CG-phila.

The propositus mother and two maternal aunts were shown to be carriers of four different hemoglobin types: Hb

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A, Hb C, Hb G-phila and the hybrid Hb CG-phila. The propositus has four siblings, one of which carriers Hb AC, one Hb CC and the other two are normal.

The abnormal hemoglobins were detected by electrophoresis of hemoglobins on cellulose acetate with alkaline pH.

The determination of the hematologic parameters was performed by a Coulter counter model S, the hemoglobin electrophoresis in cellulose acetate with alkaline pH and in agar gel with acid pH, according to the International Committee for Standardization in Hematology (ICSH) recommendations[9].

Globin was first tested on cellulose acetate with alkaline pH and the hemolysate was precipitated in acetone/HCl.

The abnormal globin chain was studied by ion exchange chromatography on CM-cellulose in the presence of 8 M urea[6]. The structural analysis consisted of the separation of the peptides of the chain of an abnormal amino-ethylated globin by trypsin digestion, by means of high resolution liquid chromatography (HPLC), followed by the analysis of aminoacids and peptides with retention times different from normal[7]. Using this methodology, Hb G-phila and the component Hb C were identified.

RESULTS

The family study showed that the propositus' mother is the carrier of four types of hemoglobin (see Table 1, case # 03), as well as two maternal aunts (Table 1, cases # 01 and 02). The propositus (Table 1, case # 09) has Hb C and the hybrid Hb CG-phila, but, unlike her mother and two aunts, she does not have Hb A. She is married and has three children, all with Hb AC (see Table 1, cases # 10, 11 and 12).

None of the family members had any clinical symptoms.

The types of hemoglobin carried by the propositus' mother and two aunts are: Hb A, Hb C, Hb G-phila and the hybrid Hb CG-phila (see Table 1 and Figures 1 and 2).

Of the propositus' siblings, one had Hb CC one had Hb AC and the other two Hb AA. The propositus' father had Hb AC and the other two Hb AA. The propositus' father had Hb AC (see Figure 2).

On the structural analysis by HPLC, four peaks were identified, corresponding to chains β^A, β^C, α^A, $\alpha^{G\text{-phila}}$ (cases # 01, 02 and 03 on Table 1), in the propositus, only three peaks were seen, corresponding to chains β^C, β^A and $\alpha^{G\text{-phila}}$ (case # 09 on Table 1).

Fraction α^G underwent digestion and the resulting peptides were separated by HPLC, thus allowing the identification of $\alpha^{G\text{-phila}}$.

DISCUSSION

The reported family with Hb G-phila and Hb C illustrates a case of independent segregation. The presence of the hybrid Hb CG-phila suggests that this type of hemoglobin has both an abnormal α chain (Hb G-phila) and an abnormal β chain (Hb C).

TABLE 1
Hematological study of the family.

Nº	RBC M/mm³	Hb g/dl	Hct %	MCV fl	MCH pg	MCHC g/dl	HbA_2 %	HbA %	HbG %	HbC %	HbC/CG %	HbF %
01	5.46	13.9	43.3	79.3	25.4	32.1	–	44.4	23.9	21.9	10.2	0.3
02	6.00	14.6	47.2	78.8	24.4	31.0	–	41.4	24.2	24.7	9.7	0.1
03	5.24	13.4	40.6	77.3	25.6	33.0	–	42.0	23.2	21.9	12.6	0.3
04	5.28	14.5	44.9	85.0	27.4	32.3	–	67.0	–	32.5	–	0.5
05	4.01	9.9	31.7	79.0	24.7	31.2	2.4	97.5	–	–	–	0.1
06	5.53	15.0	47.7	86.3	27.1	31.4	3.4	96.0	–	–	–	0.6
07	4.60	12.6	38.8	84.3	27.4	32.4	–	–	–	95.0	–	0.5
08	5.16	14.0	44.5	86.2	27.1	31.5	–	47.6	–	51.6	–	0.8
09	4.41	11.1	36.7	83.2	25.2	30.3	–	–	–	70.1	27.9	2.0
10	4.72	10.7	33.5	70.8	22.6	31.9	–	67.0	–	32.5	–	0.5
11	5.08	13.0	39.6	77.8	25.6	31.9	–	67.0	–	33.3	–	0.9
12	4.73	11.6	35.3	74.5	24.4	32.8	–	62.7	–	36.1	–	1.2

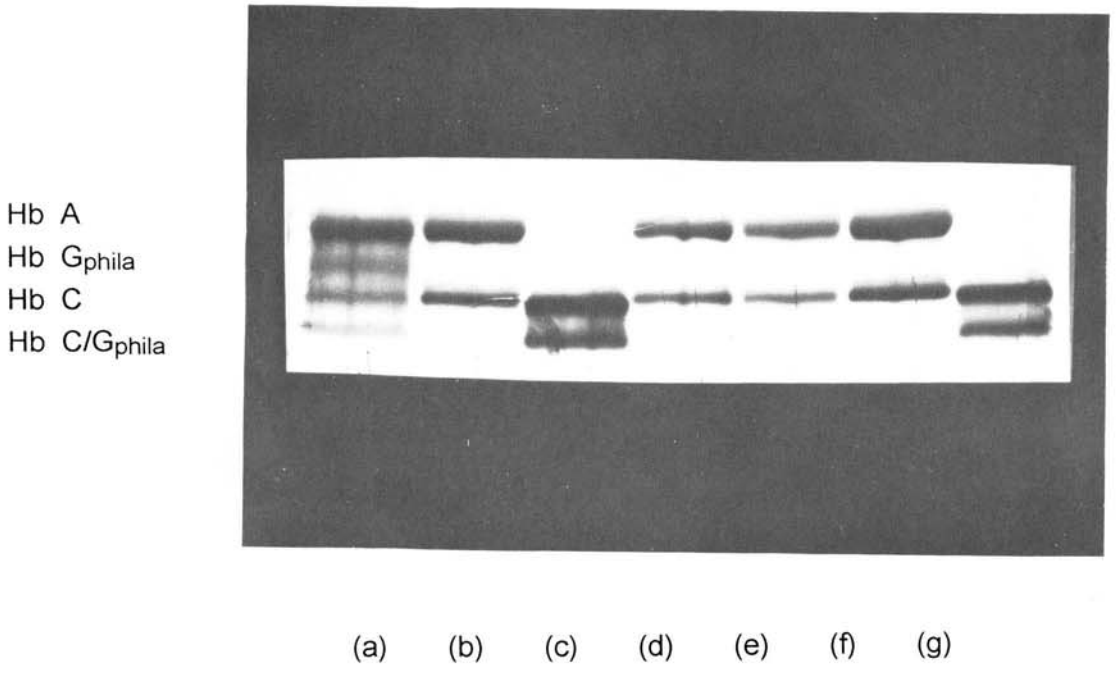

Fig. 1: Hemoglobin electrophoresis in alkaline pH. The mother with four hemoglobins; Hb A, Hb CG-phila, Hb C and hybrid Hb CG-phila, (a). The father with two hemoglobins types, Hb A and Hb C (b). The propositus with Hb C and hybrid Hb CG-phila (c, g). The brother with Hb A and Hb C (d, e, f).

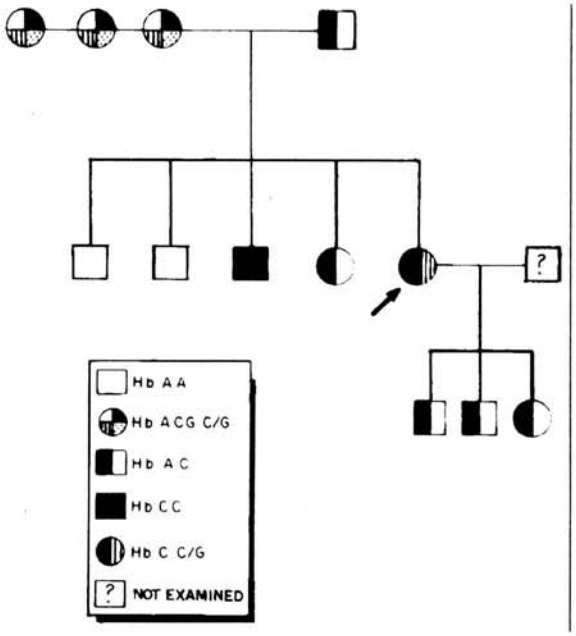

Fig. 2: Heredogram of the studied family.

A polypeptide chain, once synthesized, quickly combines with another one of the same kind, to form subunits $\alpha_2\beta_2$ or else these chains are synthesized as stable identical pairs. These hybrids can originate from the synthesis of two abnormal hemoglobins or from the intracellular exchange of subunits. ITANO[10] suggested that the proportion of hemoglobin that appears in the hemolysate is determined by the relative speed of hemoglobin synthesis. The normal subunits $\alpha_2^{A}\ \beta_2^{A}$ are more synthesized that the abnormal subunits $\alpha_2^{G\text{-phila}}\ \beta_2^{C}$.

Therefore there is a greater association of $\alpha_2^{A}\ \beta_2^{A}$ (Hb A) than of $\alpha_2^{G\text{-phila}}\ \beta_2^{A}$ (Hb G-phila) or of $\alpha_2^{A}\ \beta_2^{C}$ (Hb C).

The hemoglobin C/G-phila formed can be determined by the synthesis speed of subunits $\alpha_2^{G\text{-phila}}\ \beta_2^{C}$ and the amount of Hb CG-phila is significantly smaller than Hb G-phila and Hb C.

It seems likely that the synthesis of the α and β chains of the hemoglobin molecule is controlled by two pairs of genes, but each one of them has its own individual expression in determining synthesis.

Dissociation and recombination of Hb G-phila and Hb C are shown on Figure 3 and in the equation following it.

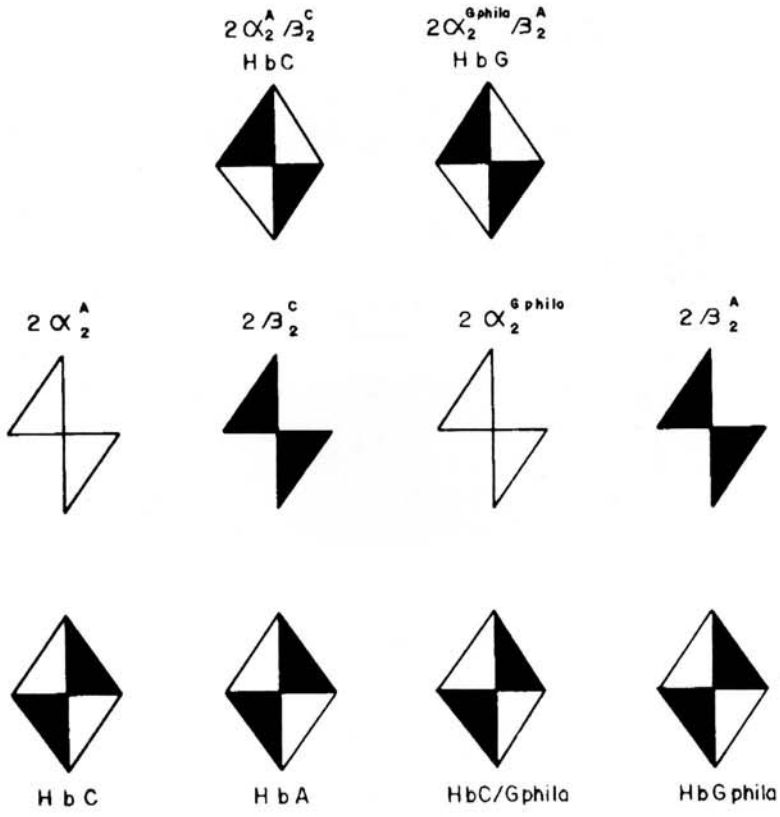

Fig. 3: The picture should show equation like:

$2\,\alpha_2{}^A\,\beta_2{}^C + \alpha_2{}^{G\text{-phila}}\,\beta_2{}^A \underline{\text{dissociation}} 2\,\alpha_2{}^A + \beta_2{}^C + \alpha_2{}^{G\text{-phila}} +$

Hb C Hb G-phila

$\beta_2{}^A \underline{\text{recombination}} \alpha_2{}^A\,\beta_2{}^C + \alpha_2{}^A\,\beta_2{}^A + \alpha_2{}^{G\text{-phila}}\,\beta_2{}^C + \alpha_2{}^{G\text{-phila}}\,\beta_2{}^A.$

Hb C Hb A Hb C/G-phila Hb G-phila

According to RAPER[13], if one of the genes undergoes a mutation, the resulting genotype will be α^A/α^A, β^A/β^C leading to the formation of subunits $\alpha_2{}^A\,\beta_2{}^A$ and $\beta_2{}^C$ and the production of Hb A and Hb C.

However, if two mutations occur, three different situations can arise:

1. The same mutation occurring twice:
   genotype α^A/α^A, β^C/β^C
   only subunits $\alpha_2{}^A$, $\beta_2{}^C$
   result Hb C

2. Two different mutations occurring at the same locus:
   genotype α^A/α^A, β^S/β^C
   subunits $\alpha_2{}^A$, $\beta_2{}^S$, $\beta_2{}^C$
   results Hb S and Hb C

3. Two different mutations occurring at two different loci:
   genotype $\alpha^A/\alpha^{G\text{-phila}}$, β^A/β^C
   subunits $\alpha_2{}^A$, $\alpha_2{}^{G\text{-phila}}$, $\beta_2{}^A$, $\beta_2{}^C$
   results Hb A, Hb G-phila, Hb C, hybrid Hb CG-phila

It can therefore be concluded that, in the family studied, two mutations occurred, affecting two different loci in part of the family members (The propositus' mother and two aunts) and leading to the genotypes of the propositus and her siblings and offspring by differential segregation.

The propositus has always led a normal life, keeping her home and her children and has not required any special medical care.

RESUMO

Quatro tipos de hemoglobina em uma família adulta de mulatos.

A família em estudo mostrou a presença de quatro diferentes tipos de hemoglobinas.

O membro da família que deu origem ao estudo (propositus) foi identificado como Hb C e o híbrido com Hb CG-phila.

O propositus tem três filhos todos portadores de Hb AC; nenhum membro da família apresentou sintomas clínicos.

A pesquisa da hemoglobina resultou da existência de hemácias em alvo, no pré-operatório de varizes de membros inferiores. O híbrido Hb CG-phila é constituído por dois pares de genes, mas cada um com expressão inidividual, determinando a síntese e o tipo particular das subunidades.

O híbrido Hb CG-phila é formado pela velocidade de combinação das subunidades $\alpha_2^{G\text{-phila}}$ β_2^{C} assim sendo a proporção do híbrido Hb CG-phila é menor do que a Hb G-phila e Hb C.

A identificação e caracterização molecular da Hb G-phila mostrou a posição $\alpha_2^{68\ Asn\rightarrow Lys}$ β_2 a Hb C α_2 $\beta 6\ Glu\rightarrow Lys$.

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