

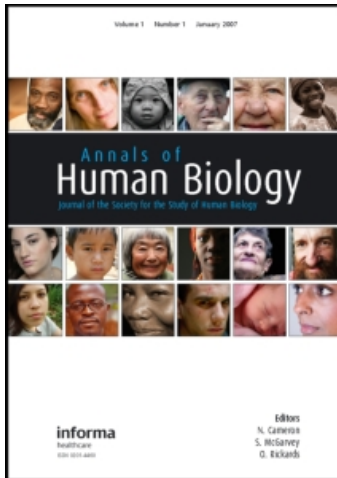
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SHORT REPORT

Hemoglobin variants, hematological parameters and β -globin gene cluster haplotypes in an isolated Amerindian group from the Orinoco River Delta

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Abstract

Background: Several previous studies reported that the Venezuelan Warao Indians presented unusual genetic characteristics.

Aim: The present study checked previous reports of a high frequency of hereditary persistence of fetal hemoglobin (HPFH) and examined other hematological traits.

Subjects and methods: Standard hematology, electrophoresis on cellulose acetate, fetal hemoglobin alkali denaturation, γ -globin chain, DNA amplification and sequencing, and denaturing gradient gel electrophoresis determinations were performed in 269 individuals living in two localities of the Orinoco River Delta.

Results: Two β^s genes, in apparently non-related individuals, were found. HPFH, detected in this same population of Warao Indians 25 years ago, was present in heterozygous form in five individuals from a large kindred, with hemoglobin F levels ranging from 3.7% to 8%, and with a pancellular distribution. The HPFH mutation was of the deletional type. β -globin gene haplotypes were determined by direct counting (through family studies) in 150 chromosomes; 26% of the 150 examined cluster presented haplotype 2, 22% haplotype 6, and 13% a new, Warao haplotype. Haplotype 3, of probable African origin, was also found with a frequency of 5%.

Conclusions: The presence of the HPFH mutation was confirmed, and the new β -globin gene haplotype together with the presence of other rare variants indicates that the Warao are very distinctive in relation to other Native Americans. Evidence was also found of a slight admixture from Africa-derived subjects (Layrisse et al. 1988).

Keywords: β -globin gene haplotypes, Amerindians, HPFH

Introduction

When the Spaniards arrived in Venezuela they found a large native population which had probably migrated around 20 000 years earlier from Northeast Asia (Arends 1992). The Warao (Warrau, Guarauno, Guarao) are an Indian tribe of 20 981 subjects, according to the Venezuelan Indian Census (OCEI 1995), who live in the Orinoco River Delta, between 8° 20' and 10° N and 59° 30' and 63° W. They are grouped into several independent subtribes that subsist through fishing and swamp scavenging. Early studies of genetic markers – hemoglobin variants, haptoglobin, albumin, transferrin, lipoprotein, blood groups, histocompatibility antigens and group-specific component (Gc) – in these Indians showed no signs of admixture with European- or African-derived persons (Arends 1975, 1992; Arends and Gallango 1964; Arends et al. 1969; Layrisse et al. 1976).

One of the first reports of genetic markers presented for this Amerindian group was done by Arends (1963). In subsequent studies Arends (1975) found that 6.7% of Warao individuals had high persistence of fetal hemoglobin (HPFH). The aim of the present study was to ascertain if there had been any changes, 25 years later, studying the hemoglobin variants, β -thalassemia mutations, HPFH, and haplotypes of β -globin in the same villages.

Subjects and methods

Blood samples from 269 subjects from the Warao etnia, 143 (57 children, 42 females and 44 males) living in Cangrejito and 126 (53 children, 55 females and 18 males) living in Winikina, were collected. Both villages are located in the Delta region of the Orinoco River in the northeastern part of Venezuela, very close to the Atlantic coast. These villages consisted of 421 individuals, 206 of them females and 215 males living in 77 households, according to the Venezuelan Indian 1992 census (OCEI 1995). Twenty-five per cent of the families are polygamous. They live at the margins of the river and are not a readily accessible group. Approval by the Indian leader of each community was obtained before sample collections and clinical evaluations were performed on each individual.

The hemoglobin analyses were carried out on all 269 individuals, by electrophoresis on cellulose acetate at pH 8.0 and citrate agar at pH 6.0; hemoglobin A₂ was measured using a cation exchange column (Huisman et al. 1975) (Helena Laboratories, Beamont, TX, USA) and fetal hemoglobin by alkali denaturalization (Betke et al. 1959) and by ion exchange HPLC-CE using the VARIANT™ system (BioRad Laboratories, Hercules, CA, USA). The blood counts and erythrocyte indices were determined using an electronic cell counter. The intracellular distribution of fetal hemoglobin was determined by the acid elution test of Kleihauer et al. (1957). The A γ - and G γ -globin chain determinations were performed using the globin chain electrophoresis technique (Alter et al. 1980).

DNA from peripheral blood leukocytes was prepared as previously described (Poncz et al. 1982). The β -globin haplotypes were determined in 150 chromosomes, from 75 unrelated individuals living in Cangrejito and Winiquina, by the analysis of five polymorphic sites of the β -globin gene cluster, amplified by the polymerase chain reaction (PCR) using the primers described by Sutton et al. (1989) and Guerreiro et al. (1992); subsequently they were digested with HincII 5'- ϵ ; HindIII G γ ; HindIII A γ ; HincII 3 $\phi\beta$ and HincII $\phi\beta$ endonuclease restriction enzymes to identify the different polymorphisms. Each chromosome arrangement was then classified according to the Long et al. (1990) nomenclature and the diploid genotype was established through family studies. To study the HPFH mutation, the A γ and G γ promoter gene was amplified independently and the presence of the mutation in these genes was searched for by

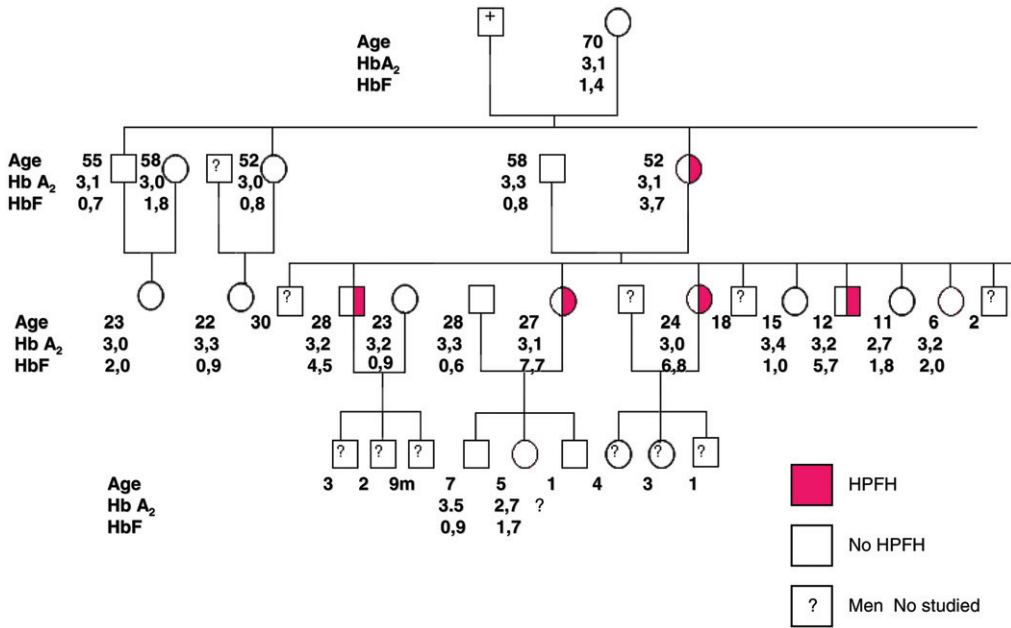


Figure 1. Pedigree of Warao family with HPFH. Values for Hb A and Hb F are frequencies (%); ages are given in years, except where marked 'm' (months).

denaturing gradient gel electrophoresis (DGGE), as previously described by Gottardi et al. (1992). The γ^A and γ^G promoter globin gene was sequenced using the sequenase PCR product sequencing Kit (United States Biochemicals, USB, Cleveland, OH, USA).

Results

The hemoglobin analyses were carried out in all 269 individuals. In both villages, the degree of anemia was higher in children than adults. The Hb A₂ levels were 3.05% ± 0.69. The levels of Hb F were 0.72% ± 0.07. Two of the individuals studied from Cangrejito were carriers of the β^s allele; they were not related and morphologically did not show any sign of non-Indian admixture. Three members of a Winiquina family were carriers of the β^C allele. No hemoglobin S homozygotes were found. Both β^s alleles occurred within the CAR haplotype, and the β^A haplotypes which they were associated with were the 2 and 6 haplotypes, respectively.

The HPFH trait, found in this same Warao tribe 25 years ago, was present in heterozygous form in five individuals from a large kindred (Figure 1), with Hb F between 3.7% and 8.0% and with a pancellular distribution. The analysis of the γ^A - and γ^G -globin chains showed that the HPFH detected was of the deletional type, HPFH-4. This mutation was associated with haplotype 2.

The frequency of the haplotype defined for 150 chromosomes examined are given in Table I. We found 21 of the 32 possible haplotypes. The most common was haplotype 2 (+----) with a frequency of 26.0%, followed by haplotype 6 (-++-+)

Table I. Frequencies (%) of the β -globin gene haplotypes in the Warao.

Haplotypes*	Restriction sites	No. of chromosomes	Frequency (%)
1	-----	7	4.66
2	+-----	39	26.0
3	-----+	8	5.33
4	-+----+	1	0.66
5	-+--++	8	5.33
6	-++-++	33	22.0
7	-++--	2	1.33
8	+--++	1	0.66
9	-++++	3	2.00
10	+++++	1	0.66
11	---++	5	3.33
12	++---	1	0.66
14	+++--	3	2.00
15	+++++	2	1.33
16	-+---	3	2.00
Haplotype Warao	+++--	20	13.3
Atypical 1	---+-	4	2.66
Atypical 2	+----	4	2.66
Atypical 3	+----	3	2.00
Atypical 4	+++++	1	0.66
Atypical 5	---+-	1	0.66
Total		150	100.00

*1-16: According to Long et al. (1990).

with a frequency of 22.0%. A new, Warao haplotype (+ + + - -) was found with a frequency of 13.3%. The remainder haplotypes showed frequencies $\leq 5.3\%$.

Eight individuals were homozygous for haplotype 2, six for haplotype 6, two for the Warao haplotype and one for haplotype 7 and Atypical 3, respectively. All the other individuals (76%) were heterozygotes. Haplotype 3 (- - - - +), of possible African origin, was found in eight chromosomes, with a frequency of 5.3%.

Discussion

To our knowledge this is the first report of β -globin haplotypes in a Venezuelan Indian tribe. The 2 and 6 haplotypes were the most frequent in this study. The Warao haplotype, described for the first time in the present study, has not been found either in any Mestizo or African-derived Venezuelan population (Castro de Guerra et al. 1997; Arends et al. 2000) or any other Amerindian populations (Guerreiro et al. 1992) and its high frequency in the present sample is notable. Further studies, in other Warao subtribes, are needed to establish the pattern of distribution.

Nevertheless, Pante-De-Sousa et al. (1999) reported the presence of a rare haplotype (+ + + - -) in an individual from a population with a high African component in the Brazilian Amazon and they report that it was earlier described by Antonarakis et al. (1984), suggesting that its presence possibly was due to the mixture with non-African populations. Hence, in individuals with the Atypical 'Warao' haplotype, two additional polymorphic sites were studied, and we found that the Warao genotype differed from the haplotypes

reported by Antonarakis et al. for at least two restriction sites – the 5' β Hinf I and the 3' β Hpa I. Therefore it was confirmed that the Atypical 'Warao' haplotype was found for the first time, and its presence could be explained by genetic drift (possibly a founder effect), being a very isolated and small population.

Admixture with African-derived persons has occurred, as indicated by the finding of two β^s genes, in apparently non-related individuals, and a frequency of 5.3% of the African haplotype 3 (---+). This is probably due to the fact that the Warao occasionally venture out of the delta to trade overseas in Trinidad and upstream towards more populated and mixed Venezuelan villages.

The Warao have provided a rich source of unique findings: HPFH at a frequency of 6.7% (Arends 1975), which was confirmed in this study, the slow-moving Warao albumin found in 2% of individuals (Arends et al. 1969), the high frequency of HLA-Bw62, a possibly new allele at the HLA-D1 locus, and a unique HLA-DR/DQ association (Layrisse et al. 1988).

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