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ORIGINAL PAPER

No evidence of the human chorionic gonadotropin-beta gene 5 βV79M polymorphism in Mexican women

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Abstract Human chorionic gonadotropin (hCG) is a placental hormone essential for the maintenance of pregnancy. Previous studies have shown a G to A transition in exon 3 of the hCGβ gene 5, which changes the naturally occurring valine to methionine in codon 79. The frequency of this transition varies among different ethnic groups, being high in USA women, and less common, or absent, in various European populations. The purpose of the present study was to determine the frequency of the βV79M allelic variant of the β-subunit of hCG in a Mexican population, and to compare this frequency with those found in other ethnic groups. Placental DNA from 161 pregnant Mexican women was genotyped for the βV79M by polymerase chain reaction (PCR)-restriction fragments length polymorphism analysis. No polymorphic βV79M alleles were identified in the population studied. The allele and genotypic frequencies of βV79M polymorphism in Mexican Mestizo women were significantly different from those reported for the US population, but not from five different European populations. In contrast to what has been found in women from the USA, it seems that the hCGβ V79M polymorphism is absent or extremely rare in Mexican Mestizo women.

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Introduction

Human chorionic gonadotropin (hCG) has an essential role in early pregnancy as it maintains steroid hormone production (mainly progesterone) by the corpus luteum until the placenta is functionally competent and assumes this steroidogenic function later in pregnancy [1, 2]. Additionally, hCG has several paracrine effects during the process of implantation [3, 4], angiogenesis, and placentation [5–7], as well as in the development of maternal immunotolerance [8]. Measurement of hCG in blood is widely used for early pregnancy testing and monitoring, biochemical prenatal screening, and assessment of gestational trophoblastic disease [9, 10], and it is also employed in assisted reproduction to induce final oocyte maturation prior to oocyte retrieval for IVF [11].

Human chorionic gonadotropin is a member of the glycoprotein hormone family, to which lutropin (LH), follitropin, and thyrotropin also belong [12]. All these hormones are heterodimers consisting of non-covalently bound α- and β-subunits. The β-subunit of hCG (hCGβ) is encoded by six non-allelic genes clustered in chromosome 19q13.3 [13]. Genes *β1* and *β2* are thought to be pseudogenes that are not expressed; β4 encodes LH, whereas *β7* and *β9* are alleles to *β6* and *β3*, respectively. Type I genes (*β6*/*β7*) encode a protein bearing an alanine residue at position 117, whereas hCGβ encoded by type II genes (*β3*/*β9*, *β5*, and *β8*) presents aspartic acid at this position. This heterogeneity does not apparently affect function or immunoreactivity of the heterodimer [14]. Type I genes (*β6*/*β7*) are mainly expressed in benign non-trophoblastic

tissues, whereas type II genes are expressed by trophoblastic and malignant tissues [15]. Of the six homologous genes, the *hCGβ* gene 5 is usually the most highly expressed [16]. Several polymorphisms had been described within the *hCGβ* gene, including a G to A transition that changes the naturally occurring valine amino acid residue to methionine in codon 79 (βV79M). This genetic variation generates atypical folding intermediates that impair the ability of hCGβ to assemble with the α-subnit to yield the functional α/β heterodimer [17].

The frequency of the βV79M allelic variant varies significantly among women from the USA and those from five different European populations. In North American women, the allele and genotype frequencies for this polymorphism are 4.2%, whereas in women from Finland, Denmark, Greece, Germany, and UK this alteration is virtually absent [17, 18]. Thus, the molecular characterization of the βV79M allelic variant in populations with different ethnic backgrounds seems important to establish the actual contribution of the βV79M allelic variant to hCGβ activity and the genetic susceptibility for miscarriage. The present study describes the allele and genotype βV79M frequencies in Mexican women.

Results

A total of 161 DNA samples were screened by the polymerase chain reaction (PCR)-restriction fragments length polymorphism (RFLP) method described below, including the analysis of positive controls. The study population was composed by Hispanic Mestizo women from Mexico. Except for the positive control samples, no mutant βV79M allele was detected among the samples genotyped. There were statistically significant differences in allelic and genotype frequencies between the Hispanic and USA populations (Table 1), but not with the five European populations [17, 18].

Table 1 Genotype and allele frequencies between Mexican and US population

Polymorphism		Mexican population	US population[a]	P[b]
βV79M	Genotypes			
	GG	161 (1.0)	320 (0.958)	
	GA	0 (0.0)	14 (0.042)	
	AA	0 (0.0)	0 (0)	
	GA + AA	0 (0.0)	14 (0.042)	0.008
	Alleles			
	G	322 (1.0)	654 (0.98)	
	A	0 (0.0)	14 (0.02)	0.008

Data are numbers of patients (frequency)

[a] Data obtained from Miller-Lindholm and colleagues [17]

[b] Calculated by χ^2

Discussion

Functional studies of the hCGβ subunit containing the βV79M polymorphism have shown that this amino acid replacement impairs the ability of the subunit to assemble with the hCG α-subunit to yield functionally competent α/β heterodimers. The inefficient assembly of βV79M hCG appears to be due to a decreased ability of the βV79M subunit to fold properly [17]. It has been proposed that defective folding of the βV79M subunit could result from non-native disulfide bridge formation involving cysteine 110, or to an altered hydrophobic collapse during early folding steps [19, 20]; in the first case, premature formation of the native disulfide bridge between cysteines 26 and 110 may hinder the ability of the βV79M subunit to assemble with the α-subunit, particularly because the 26–110 bridge functions as a seat belt that stabilizes the hCG heterodimer [19]. On the other hand, valine 79 is located in loop 3 (H3-β) of the hCGβ subunit [20]; it is thought that H3-β contributes to the secondary structure of the protein by causing a hydrophobic collapse during the initial stages of folding. Some of the key hydrophobic amino acids that contribute to formation of the H3-β structure via formation of hydrogen bonds surround valine at position 79 [20]. In this scenario, the substitution of valine with methionine may destabilize the initial steps in the hydrophobic collapse of the protein and result in an inability of the protein to form a stable folding intermediate. As correct folding and subunit assembly are crucial for hCG heterodimer activity, the presence of this genetic variant may impair the net amount of hCG produced, and thus lead, theoretically, to spontaneous miscarriage or infertility in women bearing this variant. Nevertheless, it is worth mentioning that the βV79M polymorphism may become clinically significant only when present in the homozygote state; since homozygocity for this polymorphism has not been detected even in North American women, it is unlikely that this alteration may play an etiologic role in idiopathic infertility or spontaneous miscarriage.

In the present study, no subjects bearing the polymorphic βV79M allele were identified. This finding is consistent with previous studies on the βV79M genotype in European women from Finland, Denmark, Greece, Germany, and the UK [18], strongly suggesting that the βV79M genotype is either virtually absent or extremely rare in the Hispanic Mestizo population. In contrast, a significantly high frequency of the βV79M variant has been found in a random population from the Midwest of the

USA [17]. Although differences in allele and genotype frequencies among the various populations studied may be attributed to a founder effect in that specific region of North America, additional studies including different ethnic groups within the United States are still necessary to confirm this possibility.

Using genetic markers from several chromosomes to define genetic admixture in Hispanic Mestizos from Mexico, Lisker and colleagues [21–23] have detected a mixture of 56% of Amerindian's genes, 40% of Caucasian genes, and 4% of black genes in this population. Likewise, genetic distances calculated from low molecular weight polypeptide allele frequencies have shown that Hispanic Mestizos from Mexico are related to Spaniards and Mexican Amerindians [24]. Although the presence of the βV79M genetic variant has not been determined in Hispanics from Spain, it is possible that individuals from this population are not carriers of the βV79M polymorphism.

In summary, the present study demonstrates that the βV79M genetic variant is absent or extremely rare among the Hispanic Mestizo population of women from Mexico and corroborates so far that the only population bearing this particular polymorphism is from the Midwest of the United States of North America. Since apparently this polymorphism is not present in Hispanic Mestizo women, a routine genotyping for this mutant allele seems unnecessary.

Materials and methods

Subjects

The study was approved by the institutional review board and the research ethics committee of our hospital. A total of 161 placenta samples were collected from term, uncomplicated pregnancies attended in the UMAE Hospital de Ginecología y Obstetricia "Luis Castelazo Ayala", IMSS, in Mexico City. Placental cotyledons containing the chorionic villus were dissected into small pieces to avoid non-parenchymal tissue contamination. The dissected cotyledons were then thoroughly washed with phosphate-buffered saline at 4°C and stored at −70°C until use.

Analysis of βV79M alleles

Genomic DNA was isolated from tissue samples using the QIAamp DNA Mini Kit (Qiagen, Inc., Valencia, CA, USA), following the manufacturer's instructions. The βV79M polymorphism was identified by PCR-RFLP analysis, as previously described [18]. Briefly, a 444 bp PCR product containing the V79M polymorphism was amplified using the forward primer 5′-TCTGAGACCTGTGGGGGCAA-3′ and the reverse primer 5′-GAGCCCACAGAAAGACTC

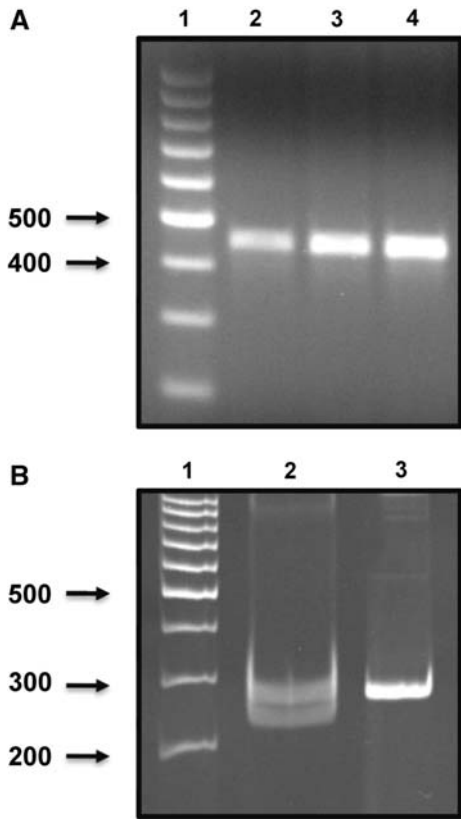

Fig. 1 Detection of the βV79M polymorphism by restriction enzyme digestion of polymerase chain reaction (PCR) products. **a** Polymorphism of the NcoI site in the 444-bp PCR fragment of the human chorionic gonadotropin. Lane 1, 100-bp DNA ladder; lanes 2–4, WT hCGβ samples (no NcoI cleavage site). **b** Polyacrilamide gel electrophoresis of the positive control for NcoI restriction endonuclease. An heterozygous sample for the TLR4 A896G polymorphism (which contains a NcoI site) was used as a positive control for restriction digestion. Lane 1, 100-pb DNA ladder; lane 2, heterozygous sample; lane 3, undigested PCR product

CT-3′. Primers were designed with specific nucleotide mismatches to all other highly homologous hCGβ and hLHβ genes to selectively amplify gene 5. PCR was performed using Platinum PCR SuperMix (Invitrogen Corp., Carlsbad, CA, USA) containing 200 ng genomic DNA, and 200 nM of each primer in a Mastercycler gradient PCR thermal cycler (Eppendorf, Hamburg, Germany). After initial denaturation at 94°C for 5 min, PCR was performed for 35 cycles with denaturation at 94°C for 60 s, annealing at 56°C for 90 s, and extension at 72°C for 90 s, followed by a final 7 min elongation at 72°C. After amplification, 10 μl of the PCR product were digested with 5 units of restriction endonuclease NcoI (New England Biolabs, Ipswich, MA, USA). The digested PCR products were separated in a 2% agarose gel, and visualized with ethidium bromide (Fig. 1a).

Since a positive genomic (βV79M) DNA was not available, the TLR4 A896G polymorphism (which contains

a NcoI site) was used as a positive control for restriction endonuclease [25]. Briefly, a PCR amplification strategy [26] that employs mismatch primers designed to detect the wild type and variant TLR 4 alleles based on the presence of a restriction site in the variant allele was used. The primers employed were 5′-GATTAGCAT ACTTAGAC TACTACC TCCATG-3′ (forward) and 5′-GATCAACT TCTGAAAAAGCATTCC CAC-3′ (reverse), with the underlined base in the forward primer indicating the nucleotide altered to create a Nco I restriction site in the presence of the polymorphism. PCR was carried out using Platinum PCR SuperMix (Invitrogen Corp.). After initial denaturation at 94°C for 5 min, 30 cycles of PCR were performed with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension for 30 s at 72°C, after which a 10 µl sample was digested with *Nco* I (New England Biolabs) and fractionated on a 12% polyacrilamide gel (Fig. 1b).

Statistical analysis

Statistical analysis was performed with SPSS for Mac, Rel. 16.0. (SPSS Inc., Chicago, IL, USA). The Chi-square test was used to evaluate differences among ethnic groups. A P-value <0.05 was considered as the limit for statistical significance.

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