Mutational analysis of the inhibin alpha gene in preeclamptic women

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ABSTRACT. Background: Preeclampsia (PE) is a disorder that occurs in at least 5–10% of pregnancies and affects both the mother and the unborn baby. A dramatic increase of maternal serum inhibin A concentration in the second and third trimester of pregnancy is a common feature of PE and inhibin A measurement may add significant prognostic information for predicting PE in pregnant women. Design: We evaluated the presence and prevalence of gene polymorphisms for inhibin α subunit (INHα) in patients affected by PE (no.=50; study group), and in the general population (control group composed of 103 women and 42 men). Methods: DNA extraction, single strand conformation polymorphism analysis, DNA sequencing, restriction fragment length polymorphism analysis, and Fisher’s exact test were used. Results: A 769G→A transition was found in INHα1, but not in INHα2 or INHα3 fragment. This variant was found in 10/145 normal controls (7.6%), and in 1/50 preeclamptic patients (2%), without significant difference between the two groups (p=0.29). Conclusions: The prevalence of INHα gene variants is not increased in PE. Due to its frequency, the 769G→A transition may be considered a polymorphism present in the general Italian population. (J. Endocrinol. Invest. 28: 30–33, 2005) © 2005, Editrice Kurtis

INTRODUCTION

Preeclampsia (PE) is a disorder that occurs in at least 5–10% of pregnancies and affects both the mother and the fetus. It is a complex disorder with a wide clinical spectrum but its initial hallmark is new onset hypertension with significant proteinuria in the latter half of pregnancy, that usually resolves after delivery (1). The aetiology and pathogenesis of PE are unclear but general consensus exists on the involvement of an abnormal placentation, probably due to an altered interaction between the placenta and the maternal vascular function. Finally, a genetic predisposing for PE has been also suggested (1, 2). The prevention of PE would mean a big step forward in prenatal medicine, in order to avoid maternal and fetal complications associated with this gestational disease, and a plethora of biophysical and biochemical parameters have been investigated (3). Among them, the measurement of inhibin A, a dimeric growth factor made up of two subunits (α and βA) and belonging to the transforming growth factor-β (TGF-β) superfamily (4), is the most promising. During pregnancy, human placenta secretes high amounts of inhibin A from the first trimester of gestation, with the highest values at term (5), higher than in non-pregnant state (3, 5). Nevertheless, a further dramatic increase of maternal serum inhibin A concentration in the second and third trimester of pregnancy is a common feature of PE, and its measurement may add significant prognostic information for predicting PE and its complications in pregnant women (3). Therefore, in the present study we investigated whether mutations in the inhibin α gene exist in preeclamptic patients, and if a difference in their prevalence compared to the general population may be observed.

MATERIALS AND METHODS

Patients information
Gene polymorphism for inhibin α subunit was researched in: 1) woman (no.=50, age range: from 31 to 39 yr) suffering from PE
DNA extraction and polymerase chain reaction

Genomic DNA from all consenting subjects was extracted from 3 ml samples of blood using the Lahiri and Nurngerger protocol (7). Three regions of the α-inhibin (INHα) gene were analysed by PCR. The primers overlapping fragment of 200-300 bp of the functional region were used as previously described (8) for PCR analysis (Table 1).

PCR was carried out using Taq DNA Polymerase (Invitrogen, Milan, Italy) and PCR buffer. Genomic DNA was amplified in 50 μl volume reaction containing 5 μl of PCR buffer (1X), 25 nmol of each dNTP, 5 nmol of forward and reverse primer, 0.2 μl of Taq DNA Polymerase. A nil DNA reaction was used as a negative control for all PCR reactions. Standard PCR conditions comprised 94 C denaturation for 1 min, 58 C annealing for 1 min, 72 C extension for 1 min for 30 cycles. To ensure that a single band of expected size was present after amplification, electrophoresis of 10 μl of each product was carried out in a 2% agarose gel and visualized under UV light using an ethidium bromide stain.

Single strand conformation polymorphism

For rapid detection of mutations all samples of 50 patients with PE and controls were analysed by non-radioactive single stranded conformation polymorphism (SSCP) analysis. Amplified samples were denatured 1:1 in formamide denaturing solution at 95 C for 5 min and thereafter directly placed on ice to prevent re-annealing of the single stranded product. 6 μl of the sample were applied to the gel (GeneGel Excel 12.5/24, Amersham Biosciences, Milan, Italy) and run on GenePhor Electrophoresis Unit (Amersham Biosciences, Milan, Italy). Electrophoresis was performed at 4 C for 2 and a half h (INHα2) or for 3 h (INHα1 and INHα3) at 400 V. The DNA fragments were visualized by silver staining (DNA Silver Staining Kit, Amersham Biosciences, Milan, Italy).

Table 1 - Primers for mutation detection flanking the three inhibin gene fragments. The size of the PCR products generated by each set of primers is indicated. The location of the primers is shown with reference to corresponding nucleotides in the corresponding genes. PCR primers were designed spanning the mature peptides of each gene for INHα (nucleotides 841-1242) (8).

<table>
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<tr>
<th>Fragment</th>
<th>Size (bp)</th>
<th>Primers (5’ to 3’)</th>
<th>Location</th>
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| INHα1    | 243      | Forward: GGCCCCACACTCGGACCAGAG  
Reverse: AGCCCCACACACGACAGTAG   | 792-811  
1034-1011 |
| INHα2    | 139      | Forward: GCTGGGCTGAGAGCGGTGGAT  
Reverse: GGATGAGGGCTGGGGCTGGGTAGG   | 963-983  
1101-1078 |
| INHα3    | 254      | Forward: CTACCCCAAGCAGGGCTACTCTCT  
Reverse: TATTTTCCAACTCGCTTCTTCTTTC   | 1079-1102  
1332-1309 |

INHα: inhibin α.

DNA sequencing

Each sample showing an anomalous SSCP electrophoretic pattern was analysed by DNA sequencing. PCR samples were purified with Jetquick PCR product purification spin kit (Genomed, Bad Oeynhausen, Germany), and sequencing in both directions was performed by an automated sequencer (Applied Biosystem ABI PRISM Model 3700).

Restriction fragment length polymorphism analysis

The INHα1 PCR products were analysed for the Bst711 restriction enzyme polymorphism (RFLP), since the occurrence of the 769G→A transition abolishes the Bst711 restriction site. The digestion was performed as previously described (8, 9) and the product analysed by 3% NuSieve agarose gel electrophoresis. Wild-type DNA yields three fragments of 85, 25 and 134 bp, whereas a homozygous sample for the mutation gives only two fragments of 85 and 159 bp. A heterozygous sample will display all four fragments.

Statistical analysis

Fisher’s exact test was used to compute the statistical difference between groups, and it was assumed significant when p<0.05. Power calculations indicated that a sample of 50 preeclamptic women and 145 controls would detect a proportion difference of at least 20% in the prevalence of INHα gene polymorphism, with a significance (α)=0.05 and a power of 80% (10).

RESULTS

In all PE samples evaluated, the only variant detected by SSCP was in the INHα1 fragment, but not INHα2 and INHα3 fragments of the INHα gene. In fact, only a INHα1 PCR product from one sample showed a different electrophoretic pattern (Fig. 1) based on the conformation of the fragment and thus the base sequence. This sample was analysed by DNA sequencing, confirming the presence of a G→A missense substitution at nucleotide 769. The mutation alters the codon 257 from GCT to ACT, resulting in an alanine to threonine amino acid substitution in the INHα gene subunit. To confirm our findings, RFLP analysis was performed in all the preeclamptic samples showing that the incidence of the 769G→A variant was 2%.