Association between a functional dopamine D4 receptor promoter region polymorphism (-C521T) and pre-eclampsia: a family-based study

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Although many candidate genes have been studied in pre-eclampsia (PE), the important class of catecholamine receptors that contribute to sympathetic tone and blood pressure regulation has yet to be investigated. We therefore examined the dopamine D4 receptor (DRD4) gene. We performed a prospective family-based study in 50 families (patient and both her parents) who were genotyped for three DRD4 promoter regions. These single-nucleotide polymorphisms (SNPs) were tested for association using family-based association test (FBAT) that also included two quantitative measures, aspartate aminotransferase [serum glutamic oxalacetic transaminase (SGOT)] and systolic blood pressure. SNPs were assayed using a commercially available SNAPSHOT kit and PCR products were analysed in an ABI 310 DNA analyser. A significant association (preferential transmission of the T allele from a heterozygous parent to affected mother) was observed between the -C521T SNP and PE (P = 0.019). Significant association was also observed between the -521T allele and two-dimensional measures of PE : GOT (P = 0.039) and systolic blood pressure (P = 0.036). The DRD4 promoter region -C521T SNP that reduces transcriptional efficiency of this gene is suggested to contribute to developing PE. Additionally, DRD4 -521 TT homozygosity may be a marker for severe PE.

Key words: dopamine D4 receptor/family study/polymorphism/pre-eclampsia

Introduction

There is abundant evidence suggesting that not only pre-eclampsia (PE) but also gestational hypertension are partly genetic in origin (Morgan and Ward, 1999; Nilsson et al., 2004). Several models of inheritance have been proposed, and specific candidate genes that may account for maternal susceptibility have been suggested (Lachmeijer et al., 2002). However, similar to other complex or non-Mendelian disorders many genes appear to be involved, and there is no simple mode of inheritance. Estimates of heritability range as high as 50% (Ros et al., 2000; Nilsson et al., 2004). Increased activity in the sympathetic nervous system as well as elevated concentrations of circulating vaso-active substances are important factors in the pathophysiology of hypertension. In fact, sympathetic over-activity is a significant finding in PE (Schobel and Grassi, 1998; Greenwood et al., 2001). Norepinephrine is the principal neurotransmitter in the sympathetic nervous system and also a hormone released by the adrenal medulla during stress. Increased blood levels of monoamines such as norepinephrine (Kaaja et al., 1999) and serotonin (Laskowska et al., 2001) have been shown in PE as well as in intrauterine growth retardation. Several investigations also link dopaminergic mechanisms directly to PE (Murthy and Prema, 1983; De Almeida et al., 1994; Vaillancourt et al., 1997, 1998). Dopamine D2-like receptor binding sites are present in the human placenta, and dopamine via these D2-like receptors inhibits both basal- and hormone-stimulated secretion of human placental lactogen from trophoblastic cells. Differential expression of placental D2-like receptors is observed during normal pregnancy. When the relative levels of receptor proteins were analysed throughout pregnancy, there was a significant but transient decrease of approximately 23 percent of D2-like protein content at 9–16 weeks of gestation with a return to baseline levels at 17–18 weeks. The variations of placental D2 expression during normal and abnormal pregnancies argue for an important role of dopamine in human placental function, although this remains to be investigated further. Manyonda et al. (1998) reported that venous blood concentrations of noradrenaline were significantly higher in pre-eclamptic women compared with normotensive women, and they hypothesized that trophoblasts have the capacity to secrete catecholamines. Moreover, the higher levels of catecholamines found in the plasma of women with PE might be of placental origin, serving as a physiological signal to increase maternal blood flow to the fetoplacental unit (Manyonda et al., 1998). An interesting study (De Almeida et al., 1994) suggests that the dopaminergic vasodilatory tone in the umbilical artery is impaired in pre-eclampsia. Another investigation showed elevated plasma levels of dopamine β-hydroxylase, the rate limiting enzyme in norepinephrine synthesis, in PE (Murthy and Prema, 1983).

Despite considerable evidence suggesting a role for D2-like receptors in the aetiology of PE, to our knowledge no genetic studies have tested specific dopamine receptor polymorphisms for conferring risk for this disorder. We were therefore prompted to examine three dopamine D4 receptor (DRD4) promoter region polymorphism SNPs, one of which (-C521T) has been shown to partially regulate transcription of this gene (Okuyama et al., 2000). In particular, the DRD4 makes biological sense for contributing to PE because a mouse DRD4...
knockout exhibits elevated blood pressure (Bek et al., 2001; Li et al., 2001) and secondly, the DRD4 receptor is expressed in the human placenta (Matsumoto et al., 1995).

Materials and methods

Subjects
Fifty female patients following delivery with diagnosis of pre-eclampsia (and both their parents) were recruited from the Department of Obstetrics and Gynecology, Brno Zlon Medical Center and gave informed consent to participate in this protocol. Ten milliliters of peripheral blood was drawn and was frozen before transfer to the laboratory for DNA extraction. Diagnostic criteria for PE were elevated blood pressure >140/90 in the second half of pregnancy and proteinuria defined as at least 0.3 g of protein in a 24 h urine specimen. Because a 24 h urine specimen was not a criterion in our trial, we used a semiquantitative dipstick measure of 1+ or greater to indicate proteinuria at a level of at least 0.3 g per day. Pre-eclampsia was considered if one or more of the following criteria were present: systolic blood pressure of 160 mm Hg or higher or diastolic blood pressure of 110 mm Hg or higher in association with proteinuria (as defined above), proteinuria of more than 5 g per 24 h (or a dipstick finding of 3+ or 4+ in association with hypertension as defined above), or elevated blood pressure and proteinuria as defined above in association with any of the following: elevated serum creatinine level, pulmonary edema, oliguria (urinary output of less than 500 ml per 24 h or of less than 30 ml per hour for 3 or more hours), microangiopathic hemolysis, thrombocytopenia (a platelet count of less than 150,000 per cubic millimeter), hepatocellular dysfunction (as defined by an alanine aminotransferase level of more than 35 U per litre or an aspartate aminotransferase level of more than 35 U per litre), in utero growth restriction of less than 10% or oligohydramnios, or symptoms suggesting clinically significant end-organ involvement (headache, visual disturbances, or epigastric pain or right-upper-quadrant pain, according to diagnostic criteria of National High Blood Pressure Education Program Working Group Report on High Blood Pressure In Pregnancy (2000) (Zamorski and Green, 2001) We included only women with PE in their first delivery, regardless of the mode of delivery or gestational age at delivery. Exclusion criteria were chronic hypertension or other states that could have a confounding effect on pre-eclampsia (such as autoimmune diseases, diabetes etc.) We also assessed additional quantitative criteria—aspartate aminotransferase [serum glutamic oxaloacetic transaminase (SGOT)] level in patient’s blood, to define the severity of the PE. SGOT was measured with automated system ‘Hitachi 917’ using the Hico AST IFCC without pyridoxal phosphate activation (Bergmeyer et al., 1986).

The Hospital IRB board gave its approval for this project.

Genotyping
DNA was obtained from all family members including parents. DNA was extracted by Master Pure kit (Epigenex, Madison, WI).

DRD4 promoter region SNPs were assayed using an ABI SNAPSHOT kit and the products analysed in an ABI 310 DNA analyser. The primers for the first and second PCR reactions are shown in Table I. A ReddyMix master mix was used (Abgene, Surrey, UK) at a magnesium concentration of 1.5–2.5 mM MgCl₂. The first PCR reaction was carried out as follows. The sample was initially heated at 95°C for 5 min followed by 35 cycles of 95°C (30 s), 55°C (30 s), 72°C (90 s) and a final extension step of 72°C for 10 min.

Statistics
We tested for the presence of association between various polymorphisms and PE, blood pressure, and GOT levels using the family-based association test (FBAT) http://www.biostat.harvard.edu/~fbat/fbat.htm. Overall, FBAT tests for preferential transmission of an allele from a heterozygote parent to the affected offspring and examines whether transmission deviates from that expected by the null hypothesis (50% transmission). FBAT (Laird et al., 2000) employs a broad class of family-based association tests that are adjusted for admixture; use either dichotomous or measured phenotypes; accommodate phenotype-unknown subjects; use nuclear families, sibships or a combination of the two; permit multiple nuclear families from a single pedigree; incorporate di- or multi-allelic marker data; allow additive, dominant or recessive models and permit adjustment for covariates and gene-by-environment interactions. The test statistic is basically the covariance between a user-specified function of the genotype and a user-specified function of the trait. The distribution of the statistic is computed using the appropriate conditional distribution of offspring genotypes that adjusts for admixture. Haplotype analysis was carried out using the ‘hbat’ sub-routine available in the FBAT program. All the three SNPs markers were in Hardy–Weinberg equilibrium (using MERLIN) (Abecasis et al., 2002).

Results
As summarized in Table II, a significant association (preferential transmission of the T allele from a heterozygous parent to affected mother) is observed between the DRD4 -521 SNP and PE (P = 0.019). No association was observed for the other two DRD4 promoter region SNPs examined nor did haplotype analysis generate more significant association (data not shown). Using the genotype model, only the TT genotype showed significant association. Additionally, significant association was observed between DRD4 -521 and two dimensional measures of pre-eclampsia: SGOT (P = 0.039) and systolic blood pressure (P = 0.036). Interestingly, when SGOT values were grouped by the DRD4 -521 SNP, the three patients showing the highest enzyme levels (Figure 1A; patients: 40, 47, 34) all are homozygous for the TT genotype. The non-parametric Mann-Whitney test (z = –1.761, P = 0.078) showed a trend for a difference between the CC and TT genotypes vis-à-vis SGOT enzyme levels (Figure 1B).

Conclusion
This is the first report providing provisional evidence for an association between any of the five known dopamine receptors and PE. The T variant of the DRD4 -521T polymorphism reduces transcriptional efficiency by approximately 40% (Okayama et al., 2000) and consequently DRD4 receptor number. Indeed, these expression system results are consistent with the current findings. TT subjects would be expected to have reduced DRD4 receptor number, and similar to the DRD4 knockout mouse exhibit elevated blood pressure levels apparently exaggerated under particular physiological circumstances such as presenting during pregnancy.

This SNP is also associated with systolic blood pressure and GOT. Especially interesting is that the three patients with highly elevated GOT enzyme levels are TT homozygotes suggesting that this polymorphism contributes not only to risk but also to severity of illness. Finally, based on the current results, we hypothesize that homozygosity for the TT genotype is potentially a marker for severity of PE in a

Table I.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Polymorphism</th>
<th>First PCR primers (forward)</th>
<th>First PCR primers (reverse)</th>
<th>SNAPSHOT probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD4 -521</td>
<td>T/C</td>
<td>GCTTACTCTCTTGGCCCTCTTC</td>
<td>GACTCCTCACTCCATCCGT</td>
<td>CCGCTCGACACTCGYGGC (complimentary)</td>
</tr>
<tr>
<td>DRD4 -616</td>
<td>G/C</td>
<td>GACTCCTCACTCCATCCGT</td>
<td>CCGCTCGACACTCGYGGC (complimentary)</td>
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</tr>
<tr>
<td>DRD4 -809</td>
<td>A/G</td>
<td>GACTCCTCACTCCATCCGT</td>
<td>CCGCTCGACACTCGYGGC (complimentary)</td>
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</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism.
subset of patients; moreover, such patients may benefit from timely pharmacological treatment.

The current study focused attention on a gene not previously examined in conferring risk for PE. Towards testing association between the DRD4 promoter region polymorphisms, we genotyped 50 families including the proband and both parents allowing us to employ a robust family-based test that avoids the conundrum of population stratification that is thought to sometimes generate false–positive results in genetic association studies (Hamer and Sirota, 2000). The association was strongest for the DRD4 promoter region -C521T SNP, because

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Afreq</th>
<th>fam#</th>
<th>S</th>
<th>E(S)</th>
<th>Var(S)</th>
<th>Z</th>
<th>P</th>
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<tbody>
<tr>
<td>-521</td>
<td>C</td>
<td>0.528</td>
<td>20</td>
<td>8.000</td>
<td>8.250</td>
<td>4.562</td>
<td>-0.117</td>
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<td>-521</td>
<td>T</td>
<td>0.472</td>
<td>26</td>
<td>17.000</td>
<td>11.250</td>
<td>6.062</td>
<td>2.335</td>
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<td>18</td>
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<td>1.638</td>
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<tr>
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<td>10.000</td>
<td>5.750</td>
<td>0.834</td>
<td>0.404</td>
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</table>

Table II. Association between dopamine D4 receptor (DRD4) promoter region SNPs and pre-eclampsia model recessive; test bi-allelic

afreq, allele frequency; E(s), expected value of S under the null hypothesis of no association in the presence of linkage; fam#, number of informative families that is families with at least one heterozygote parent; S, test statistic for the observed number of transmitted alleles.

Figure 1. GOT values stratified by DRD4 promoter region polymorphism –521. (A) Patient identification number is on the left; numbers in parentheses represent >1 patient at that GOT value. (B) Error flag represents SEM.
association was observed not only with categorical definitions of PE but also with SGOT and systolic blood pressure measurements. We suggest that enzymes and receptors mediating catecholamine responses offer a potentially new and fruitful area of exploration for identifying common polymorphisms contributing risk to pre-eclampsia.

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References
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