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Genetics

Polymorphism in the Tumor Necrosis Factor-α Gene in Women with Preeclampsia

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***Purpose*:** We determined whether genetic variability in the gene encoding for tumor necrosis factor-α (TNF-α) contributes to individual differences in susceptibility to the development of preeclampsia.

***Methods*:** The study involved 133 preeclamptic and 115 healthy control pregnant women who were genotyped for C-850T polymorphism in the *TNF-α* gene promoter. Chi-square analysis was used to assess genotype and allele frequency differences between preeclamptic women and controls.

***Results*:** A significantly different genotype distribution of C-850T polymorphism was observed between the two groups, with the frequency of the variant T allele being significantly reduced in the preeclamptic group (4.5%) when compared with the control group (9.6%) ($P = 0.03$; OR $= 0.45$, 95% CI $= 0.22$–0.92). Accordingly, the odds ratio for preeclampsia associated with the pooled TT and CT genotypes was 0.367 ($P = 0.02$; 95% CI $= 0.159$–0.847).

***Conclusions*:** The T allele of the *TNF-α* gene may modify individual preeclampsia risk, being protective against the development of the complication.

KEY WORDS: Polymorphism; preeclampsia; TNF-α.

INTRODUCTION

There is increasing epidemiological evidence suggesting a relationship between genetic factors and preeclampsia, although the mode of inheritance is not yet resolved (1). First-degree relatives are known to have a fivefold increased risk of developing the disease compared with women with no family history of preeclampsia (2). Clinical studies have also shown that impaired glucose tolerance and cardiovascular disorders are more frequent in women with a history of preeclampsia (3,4). A variety of candidate genes have been proposed as important contributors to preeclampsia, such as the genes encoding HLA-Dr beta, HLA-G, CuZn superoxide dismutase, angiotensin-converting enzyme, factor 5, microsomal epoxide hydrolase, and methylenetetrahydrofolate reductase (5–11). Furthermore, a promising, but as yet unidentified locus in *2p13* has been reported in linkage studies in Iceland and Australia (12,13). However, the significance of these genes or loci in the pathogenesis of preeclampsia remains uncertain, since overall the evidence of association or linkage has been ambiguous (14–16).

The *TNF-α* gene is one of the candidates, since evidence has accumulated to suggest a relationship between preeclampsia and TNF-α. First, increased serum TNF-α activity has been identified in preeclampsia (17) although Livingston *et al.* did

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not find a significant increase in TNF-α production in severe preeclampsia (18). Sources other than the placenta seem to contribute to the elevated concentrations of TNF-α (19). Second, Chen *et al.* reported that high expression of TNF-α may be associated with the TNF1 allele, whose frequency was found to be markedly increased in preeclamptic patients (20). These observations are consistent with the concept of a major role for TNF-α in mediating endothelial disturbances and suggest a key role for TNF-α in the development of preeclampsia. However, the results of Lachmeijer *et al.* did not show evidence for association or linkage with familial preeclampsia in the Dutch population (21). Third, recent data have also suggested a role for TNF-α in insulin resistance, obesity, and hyperlipidemia, which are also common features associated with preeclampsia (22). In addition, Reister *et al.* found that macrophages, residing in excess in the placental bed of preeclamptic women, are able to limit extravillous trophoblast invasion of spiral arterial segments through apoptosis mediated by the combination of TNF-α secretion and tryptophan deletion (23). Taken together, these reports suggest that the role of TNF-α in the development of preeclampsia is evident but not completely understood. The Finnish population is considered to be a genetic isolate, and thus ideal for genetic association studies (24). This study was undertaken to determine the relationship between *TNF-α* gene C-850T polymorphism and preeclampsia.

MATERIAL AND METHODS

Written approval for the study was obtained from the Ethics Committee of Kuopio University Hospital. Informed consent was obtained from all patients and the 115 controls and documented.

Information was collected retrospectively in connection with 133 preeclamptic pregnancies of primiparous women and 115 control women with no history of preeclampsia who delivered at Kuopio University Hospital between January 1994 and December 1998. To ensure homogeneity of the genetic background, the controls, originating from a regional population and with no clinical signs of the disorder, were enrolled by random selection in this case-control study.

Preeclampsia was defined as the development of hypertension and new-onset proteinuria (>300 mg of urinary protein in 24 h) in women with no proteinuria at baseline. Hypertension was defined according to current guidelines that accept 140 and/or 90 mmHg of systolic and diastolic pressure respectively, or higher, as hypertension, when measured on two consecutive occasions at least 24 h apart (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000) (25). Women with chronic hypertension were excluded from the study.

DNA was extracted from peripheral blood lymphocytes by using a standard phenol–chloroform extraction method. The C-850T polymorphism in the promoter of the *TNF-α* gene was genotyped by using a PCR-RFLP method as previously described (26). The PCR product (133 bp) was amplified with primers TP203FM (mismatch) and TP0306R, using 25 ng of genomic DNA. Subsequently, the 133 bp PCR product was digested with *Hin*cII (MBI Fermentas, Lithuania) restriction enzyme and subjected to 3% agarose gel electrophoresis. In the case of a C allele at position-850, *Hin*cII digestion produces 108 bp and 25 bp fragments, whereas the 133 bp fragment remains undigested when the T allele is located at this position. Statistical analyses for comparing individual allele and genotype frequencies as well as pooled genotype frequencies (CT + TT vs. CC) were carried out using Pearson's chi-square test (two-sided asymptotic P values) with SPSS 9.0 software and the level of statistical significance was defined as $P < 0.05$. Hardy–Weinberg distribution of genotypes in the preeclamptic and control groups was assessed by using the Associate program, Version 2.31.

RESULTS

The mean (SD) maternal ages in the study and control groups were 28.8 (6.4) and 28.7 (5.4) years, respectively. The mean (SD) gestational age at the development of preeclampsia was 31.7 (±3.5) weeks. When only the first-degree relatives of the index patients were taken into account, a positive family history was reported in 30 affected women, who had six affected sisters and 28 affected mothers.

Genotype and allele distributions of the *TNF-α* C-850T polymorphism differed significantly between the preeclamptic and control groups (Table I). The frequency of the T allele was 4.5% among preeclamptic women and it was 9.6% among control women ($P = 0.03$), giving a protective odds ratio of 0.45 (95% CI = 0.22–0.92) for the T allele. The odds ratio for preeclampsia associated with the pooled TT and CT genotypes was 0.367 ($P = 0.02$; 95% CI = 0.16–0.85).

Table I. Genotype and Allele Frequencies of the *TNF-α* Gene Promoter C-850T Polymorphism Among Women with Preeclampsia and Healthy Pregnant Controls

	Preeclamptic women ($N = 133$)		Controls ($N = 115$)	
	n	%	*n*	%
Genotype frequencies				
CC	124	93.2	96	83.5
CT	6	4.5	16	13.9
TT	3	2.3	3	2.6
Allele frequencies				
C	254	95.5	208	90.4
T	12	4.5	22	9.6

Note. Asymptotic *P*-values for the genotype and allele data: $P = 0.03$ and 0.03, respectively. Hardy–Weinberg equilibrium *P*-values for the preeclamptic and control genotypes: $P = 0.0005$ and 0.07, respectively.

DISCUSSION

Here we report an association between the *TNF-α* gene C-850T promoter polymorphism and preeclampsia. The frequency of the T allele in the control group was 9.6%, whereas in the preeclamptic group it was 4.5%, indicating that the T allele exerts a protective effect against preeclampsia. Interestingly, the genotype distribution in the preeclamptic group differed significantly from Hardy–Weinberg equilibrium ($P = 0.0005$), which is something that may be expected with a disease-associated gene. On the other hand, a similar trend in deviation from Hardy–Weinberg equilibrium, although not significant ($P = 0.07$), was observed in the control group. Deviations in both groups were considered to originate partly from the small number of CT and TT genotypes, which clearly affected the Hardy–Weinberg equilibrium calculations, and thus the deviations were not considered to be a result of genotyping errors.

Although the pattern of inheritance is not yet resolved, investigations into the genetic etiology of preeclampsia have yielded intriguing results implying that genes are responsible for the disease, rather than a common environment (27). Familial genetic predisposition can be investigated in association studies and the results of the present study suggest that there is an association between preeclampsia and *TNF-α* gene C-850T promoter polymorphism. TNF-α may act through the influence of cytokines on lipid metabolism which has been related to the etiology of preeclampsia. Accordingly, cells treated with TNF-α release arachidonic acid, the concentrations of which are increased in preeclampsia (28,29). However, any mechanism remains speculative at this point. On the other hand, the possibility of indirect mechanisms has to be taken into account, since the association between *TNF-α* polymorphism and preeclampsia may reflect linkage disequilibrium between *TNF-α* and a closely located functional variant of another gene that contributes to the risk of the disease (30). Clearly, relating genotype to phenotype will be one of the future challenges in diseases that result from varying susceptibility to a wide range of environmental factors, mediated by many different genes.

In summary, we have demonstrated an association between *TNF-α* polymorphism and preeclampsia susceptibility, with the T allele being protective against preeclampsia. However, it is not known whether C-850T polymorphism has a functional status in the *TNF-α* gene (26) and thus it is possible that the present association actually reflects linkage disequilibrium with a functional variant located in some other part of the *TNF-α* gene or with a closely located functional variant of another gene. How this polymorphism produces or contributes to the clinical syndrome remains unclear, but previous studies have shown that TNF-α levels are altered in pregnancies complicated by preeclampsia. Chen *et al*. have also reported a significant association between *TNF-α* polymorphism and preeclampsia (20) but Dizon-Townson *et al*. found no evidence for such association in another population (31). This discrepancy does not exclude the possibility that alternative mutations or polymorphisms of the *TNF-α* gene might segregate with preeclampsia in a population with a different genetic background. The conflicting results of these studies may reflect not only differences across populations but also the multifaceted nature of preeclampsia. However, TNF-α polymorphism may be of biological significance in the development of preeclampsia, as shown by its pathophysiological effects, and therefore the *TNF-α* gene has to be considered a strong candidate associated with the disease. Further studies are needed to address the issue whether TNF-α levels are altered in pregnant women having the T allele or whether the observed association between the *TNF-α* gene and preeclampsia reflects linkage disequilibrium with another closely located gene.

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