

Lack of association between C-850T polymorphism of the gene encoding tumor necrosis factor- α and polycystic ovary syndrome

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ABSTRACT

In the present study, we determined whether genetic variability in the gene encoding tumor necrosis factor- α (TNF- α) contributes to individual differences in susceptibility to the development of polycystic ovary syndrome (PCOS). The study involved 87 Caucasian Finnish women with PCOS and 115 healthy control women who were genotyped for the C-850T polymorphism in the TNF- α gene promoter. Analysis by χ^2 was used to assess genotype and allele frequency differences between PCOS women and controls. A similar genotype distribution for the C-850T polymorphism was observed in the two groups, with the frequency of the variant T allele being 8.6% in the PCOS group and 9.6% in the control group ($p = 0.862$). Accordingly, the profile of genotype frequencies was similar in the groups. The observed profiles of allele and genotype frequencies confirm an equilibrium state between C-850T polymorphism and PCOS and suggest that polymorphism of the TNF- α gene is unlikely to contribute to the risk of PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS), characterized by anovulation and hyperandrogenism, is the most common endocrinopathy in women of reproductive age¹. Two essential features are accumulation of multiple small antral non-atretic follicles and arrested follicular maturation². The genetic defect causing PCOS is unknown, and the initiating event remains undefined.

Normal folliculogenesis is a complex process; a large number of areas could be subject to derangement and cause PCOS. Any interference of the finely balanced sequence of events could lead to this complex syndrome. Thus, there are many possible predisposing genes, and any selection of candidate genes in the field of PCOS research will be incomplete.

Tumor necrosis factor- α (TNF- α) immunoreactivity and mRNA have been detected in oocytes and granulosa cells in humans, and TNF- α is secreted locally by granulosa-luteal cells³⁻⁵.

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Serum TNF- α has been shown to be increased in normal-weight women with PCOS, suggesting that factors other than obesity are the cause of elevated serum TNF- α in normal-weight women with PCOS⁶. Recent published findings suggest that the TNF- α system might contribute to the pathogenesis of hyperandrogenism⁷. Although an understanding of the exact physiological function of TNF- α awaits additional studies, this pro-inflammatory cytokine has been proposed to be an organizing factor in follicular development.

In the present study, a candidate gene approach was applied on the basis of this *a priori* information linking the pathogenesis of PCOS to TNF- α . Prior studies have, however, failed to demonstrate an association between a functional variant of the TNF- α gene promoter and PCOS. These data do not exclude the possibility that variation in the poorly characterized regulatory areas of the TNF- α gene contains polymorphisms that would have associations with PCOS in other populations. Failures in using this approach may also have been partly due to the huge variation of predisposing genes in a population. We have carried out a genetic association study to clarify whether TNF- α polymorphism is associated with PCOS in a Finnish population considered to represent a genetic isolate and to be ideal for genetic association studies⁸.

MATERIALS AND METHODS

Information was collected retrospectively from 87 women with PCOS in the endocrinology/infertility clinic at Kuopio University Hospital, Finland, and from 115 non-hirsute, fertile control women with regular cycles and normal ovaries who delivered at Kuopio University Hospital between January 1999 and December 1999. In the study group, the indications for referral were menstrual cycle disturbances, infertility and symptoms of hyperandrogenism.

In this study the diagnosis of PCOS was based on the observation of anovulation and polycystic ovaries on ultrasound examination and exclusion of other reasons for anovulation and hyperandrogenism such as hypothyroidism, hyperprolactinemia, hypercortisolism and late-onset congenital adrenal hyperplasia. In addition, study groups had one or more of the following clinical or biochemical disturbances: hirsutism, infertility, laboratory

testing revealing androgen excess (serum total testosterone concentration > 2.5 nmol/l or free plasma testosterone > 40 pmol/l, evaluated by using sex hormone binding globulin) and an elevated luteinizing hormone/follicle stimulating hormone ratio (> 2). Hirsutism was defined by the presence of excessive body hair in an androgen-dependent pattern, with a modified Ferriman-Gallwey score of 8 or more⁹.

DNA was extracted from peripheral blood lymphocytes using a standard phenol-chloroform extraction method. The C-850T polymorphism in the promoter of the TNF- α gene was genotyped with a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method as previously described¹⁰. The PCR product (133 bp) was amplified with primers TP203FM (mismatch) and TPO306R, using 25 ng of genomic DNA. Subsequently, the 133 bp PCR product was digested with Hinc II (MBI Fermentas, Lithuania) restriction enzyme and subjected to 3% agarose gel electrophoresis. In the case of a C allele at position-850, Hinc II digestion produces 108-bp and 25-bp fragments. In contrast, 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 the Fisher exact test (two-sided Monte Carlo estimate with 99% confidence level) with SPSS 9.0 software, and the level of statistical significance was defined as $p < 0.05$.

Sample size and power determination were performed using nQuery Advisor 4.0 software (Statistical Solutions, Saugus, MA, USA). The Hardy-Weinberg distribution of genotypes in the PCOS and control groups was assessed with the Associate program, version 2.31, and were found to be in equilibrium in both patient and control groups.

Written approval for the study was obtained from the Ethics Committee of Kuopio University Hospital. The protocol was approved by the investigation review board. Written informed consent was obtained from all subjects.

RESULTS

The clinical characteristics of the patients are presented in Table 1. A total of 87 women with

PCOS and 115 healthy control women were genotyped for C-850T polymorphism in the TNF- α gene promoter, and genotype and allele frequencies are shown in Table 2. The distribution of C and T alleles was equal in the PCOS and unaffected cases ($p = 0.862$), as was the distribution of genotypes ($p = 0.945$, respectively). The odds ratio for PCOS associated with the pooled TT and CT genotypes was 0.88 (95% CI 0.41–1.91). The genotypes were found to be in Hardy–Weinberg equilibrium in both patient and control groups. Furthermore, all patients presenting with alopecia areata ($n = 4$) had the CC genotype.

Table 1 Clinical characteristics of the women with polycystic ovary syndrome (PCOS)

	<i>n</i> = 87	%
Ovulatory disorders	87	100
oligoamenorrhea*	61	70.1
irregular periods [†]	26	29.9
Ultrasound: PCOS	87	100
Hyperandrogenism	52	59.8
hirsutism [‡]	34	39.1
acne	15	17.2
alopecia areata	4	4.6
total testosterone > 2.5 nmol/l**	36	41.4
Infertility	65	74.7
Obesity (BMI > 27 kg/m ²)	52	59.8

BMI, body mass index; *period > 3 months; [†]low progesterone levels; [‡]Ferriman–Gallwey score of > 8; **or free plasma testosterone > 40 pmol/l, evaluated by using sex hormone binding globulin

Table 2 Tumour necrosis factor genotype and allele frequencies in women with polycystic ovary syndrome (PCOS) and in healthy, fertile controls

	PCOS women (<i>n</i> = 87)		Controls (<i>n</i> = 115)	
	<i>n</i>	%	<i>n</i>	%
<i>Genotype</i>				
CC	74	85.1	96	83.5
CT	11	12.6	16	13.9
TT	2	2.3	3	2.6
<i>Allele</i>				
C	159	91.4	208	90.4
T	15	8.6	22	9.6

DISCUSSION

Given the familial tendency of PCOS and the likely pathogenetic role of TNF- α in the disease we investigated the role of TNF- α polymorphism in patients with PCOS¹¹. In this study no association was demonstrated between alleles at the single polymorphism located in the promoter region of the TNF- α gene and PCOS. The results of the TNF- α promoter polymorphism are in accordance with those of a previous study by Milner and colleagues which failed to show an association between PCOS and the -308 polymorphism¹². On the other hand, -308A carriers have shown increased androgen secretion⁶, although the group studies did not represent PCOS patients. The comparability of findings of PCOS studies is partly hampered by the lack of a universally accepted definition of PCOS. In this study the polycystic morphology of ovaries in PCOS patients was confirmed by ultrasound.

Familial genetic predisposition can be investigated in association studies; the results of the present study suggest that there is an equilibrium state between TNF- α genotypes and alleles in PCOS women and in women in the control group. We did not find a significant relationship between TNF- α polymorphism and PCOS, but the limitation of our study is its relatively small sample size, which theoretically increases the likelihood of a type II error. Power analysis based on data from the current study showed that more than 5495 patients and controls would be necessary to have an 80% chance of showing a difference between TNF- α frequencies at a significance level of $p < 0.05$. These figures suggest that it is unlikely that our findings are false negatives.

Unlike many other diseases the genetic studies of PCOS have focused on finding and testing candidate genes¹³. There are two main reasons for this. First, localizing PCOS genes by classic systematic linkage study has been difficult because of the heterogeneity of the disease etiology, uncertainty of the type of inheritance and non-existent male phenotype. Second, it has been possible to name several attractive candidate genes, such as the genes affecting steroid synthesis, insulin resistance and follicle maturation. One of the strengths of the candidate gene approach in the current study is the Finnish closed heritage, which is genetically

relatively homogenous. Collectively, this report presents no evidence that indicates that the C-850T polymorphism in the TNF- α gene has an

association with PCOS. Other genes should therefore be targets for further studies in the field of PCOS research.

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