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ORIGINAL ARTICLE

FABP4: a novel candidate gene for polycystic ovary syndrome

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Abstract
Background Polycystic ovary syndrome (PCOS) is a complex multifactorial disorder involving a number of genetic and environmental factors. Adipocyte-fatty acid-binding protein (FABP4) is an adipokine regulating systemic insulin sensitivity, lipid and glucose metabolism. In humans serum FABP4 levels correlate significantly with features of PCOS. Previous researches showed strong evidences that *FABP4* impacted the developing of PCOS possibly through its protein alteration or transcription regulation. Thus, the present study is the first attempt to identify the possible genetic role of *FABP4* gene in the development of PCOS.

Methods About 1000 bp of the promoter region and four exons of *FABP4* gene of 178 PCOS patients and 171 healthy controls were directly sequenced.
Results Three polymorphisms, rs16909225, rs3834363, and rs16909220, were identified, of which rs16909225 and rs16909220 were completely linked ($r^2 = 1$) and not associated with the development of PCOS, while the −2-bp/−2-bp genotype of rs3834363 was significantly higher in PCOS than in the controls ($\chi^2 = 7.39$, df = 1, $P = 0.007$, OR = 1.80 95% CI: 1.18–2.75).
Conclusion The present study is the first to establish an association between FABP4 gene polymorphisms and the development of PCOS.

Keywords Polycystic ovary syndrome · Polymorphism · FABP4

Introduction

Polycystic ovary syndrome (PCOS) is one of the most prevalent female endocrinopathies, affecting up to 12% of premenopausal women [1]. Family and twin studies have suggested that PCOS is a complex, common genetic disorder, such that multiple susceptibility genes interact with lifestyle and environmental factors to result in disease [2, 3].

Previous studies have shown that the adipose fatty acid-binding protein gene, FABP4, might be a good candidate gene of PCOS developing [4–24].

First, the fatty acid-binding proteins (FABPs), with relative molecular mass of about 15,000 are cytoplasmic proteins and involved in the transport and metabolism of intracellular free fatty acid. Not only can they reversibly combine saturated and unsaturated long-chain fatty acids,

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eicosanoids and other lipids as well as varying their chain length and degree of saturation [4–6], but they can also facilitate the fatty acids and other lipid regulons to transfer through cells and get involved in the regulation of intracellular signaling pathways. In vitro studies have shown that FABPs have potential effects on the input, storage and output of fatty acids, and the metabolism of cholesterols and phospholipids as well [7–12]. Therefore, FABPs serve as a central hub in regulating metabolism, inflammation, immunization, and other areas. Nine members of this gene family have been identified, including one adipose tissue-specific form, FABP4 [13].

Second, FABP4, which is most often expressed in fat cells and monocytes/macrophages, is considered to be a marker of terminal differentiation of fat cells [4, 14–17]. It has also been shown to affect insulin sensitivity, lipid metabolism, and inflammatory responses, all of which are associated with atherosclerosis [18–20].

Third, the results obtained from recent years, various experimental models have demonstrated that FABPs are involved in the inflammatory and lipid-mediated pathways that are critical in metabolic syndrome. In particular, FABP4 has been linked to metabolic and immune responses [21–23]. Moreover, Aimin et al.'s [24] study suggested that the severity of the metabolic syndrome appears to parallel the concentration of FABP4; and these authors proposed that FABP4 should be considered the biological marker of metabolic syndrome.

According to the fact as mentioned above, these observations suggested to us that: nonsynonymous variations in the coding or promoter regions of the FABP4 gene may result in changes in protein or gene expression that impact the development of PCOS.

To examine this hypothesis, we performed a genetic analysis comparing a population of Chinese women with PCOS with normally cycling women from the same population.

Result

Clinical and laboratory variables

The clinical characteristics of women enrolled in the study are summarized in Table 1 (mean ± SD).

Gene sequencing

No variants were identified among the four exon regions. Through this study, we identified and confirmed three distinct single nucleotide polymorphisms (SNPs) (shown on Table 2), and all of them were previously reported in National Center for Biotechnology Information SNP database by rs16909225, rs3834363, and rs16909220, respectively. In addition, all of the SNPs were located in the promoter region.

Table 1 Demographic and clinical characteristics of the study population (mean ± SD)

Parameter	PCOS ($n = 178$)	Control ($n = 171$)	P
Age (year)	26.89 ± 3.91	31.10 ± 4.23	NS
BMI (kg/m^2)	22.94 ± 4.25	21.41 ± 2.48	<0.001
E2 levels (pg/ml)	231.3 ± 213.1	174.1 ± 123.1	0.002
FSH levels (mIU/ml)	6.92 ± 15.17	6.71 ± 2.15	NS
LH levels (mIU/ml)	13.46 ± 7.01	4.99 ± 3.13	<0.001
PRL levels (ng/ml)	17.57 ± 35.10	18.04 ± 19.69	NS
Total testosterone (ng/ml)	3.92 ± 9.70	2.09 ± 7.08	0.045
LH/FSH	2.53 ± 1.64	0.80 ± 0.64	0.001

Genotype and allele frequencies

The distribution of the different SNPs genotypes in the study population is shown in Table 2.

Among these three SNPs, rs16909225 and rs16909220 were completely linked ($r^2 = 1$). There were no statistical differences in rs16909220 and rs16909225 genotype and allele frequencies between PCOS case and controls ($\chi^2 = 4.478$, df = 2, $P = 0.107$ by genotype; $\chi^2 = 0.828$, df = 1, $P = 0.369$ by allele).

In contrast, there were significant differences in rs3834363 genotypic and allelic frequencies between PCOS cases and controls ($\chi^2 = 9.471$, df = 2, $P = 0.009$ by genotype; $\chi^2 = 10.53$, df = 1, $P = 0.001$ by allele).

Compared with controls, there was a higher −2/−2 genotype and −2 allele frequency of rs3834363 in the PCOS cases 59.0 vs. 44.4% by genotype; 75.8 vs. 64.6% by allele). And the association to PCOS reached significance ($\chi^2 = 7.39$, df = 1, $P = 0.007$, OR = 1.80 95% CI: 1.18–2.75 by genotype; $\chi^2 = 5.49$, df = 1, $P = 0.019$, OR = 2.28 95% CI: 2.13–4.59 by allele).

ANOVA test was made between clinical/biochemical parameters for the three different genotype groups (shown in Table 3). Comparisons also were made between clinical/biochemical parameters for the groups containing the −2/−2 and −2/+2 genotypes and the group containing the +2/+2 genotype and are presented in Table 3 in women with PCOS, but the statistical analyses failed to provide evidence of a distinction between these parameters and a specific genotype in these women (Table 3).

Table 2 The sequence variations, genotype distribution, and relative allele frequencies found in Fabp4 gene in Chinese with PCOS ($n = 178$) and controls ($n = 171$)

Group	No.	Genotype frequency (%)			Allele frequency (%)	
		T/T	T/C	C/C	T	C
rs no.	rs16909225					
Polymorphism	T–C					
Position (bp)	−911					
PCOS	178	160 (89.9)	16 (9.0)	2 (1.1)	336 (94.4)	20 (5.6)
Controls	171	146 (85.4)	25 (14.6)	0 (0)	317 (92.7)	25 (7.3)
		$\chi^2 = 4.478$, df = 2, $P = 0.107$			$\chi^2 = 0.828$, df = 1, $P = 0.369$ Permutation $P = 0.663$	
Group	No.	Genotype frequency (%)			Allele frequency (%)	
		−2-bp/−2-bp	−2-bp/+2-bp	+2-bp/+2-bp	−2-bp	+2-bp
rs no.	rs3834363					
Polymorphism	In/del CA					
Position (bp)	−798 to −799					
PCOS	178	105 (59.0)	60 (33.7)	13 (7.3)	270 (75.8)	86 (24.2)
Controls	171	76 (44.4)	69 (40.4)	26 (15.2)	221 (64.6)	121 (35.4)
		$\chi^2 = 9.471$, df = 2, $P = 0.009$			$\chi^2 = 10.53$, df = 1, $P = 0.001$ Permutation $P = 0.003$	
Group	No.	Genotype frequency (%)			Allele frequency (%)	
		G/G	G/C	C/C	G	C
rs no.	rs16909220					
Polymorphism	G–C					
Position (bp)	−737					
PCOS	178	160 (89.9)	16 (9.0)	2 (1.1)	336 (94.4)	20 (5.6)
Controls	171	146 (85.4)	25 (14.6)	0 (0)	317 (92.7)	25 (7.3)
		$\chi^2 = 4.478$, df = 2, $P = 0.107$			$\chi^2 = 0.828$, df = 1, $P = 0.369$ Permutation $P = 0.663$	

Table 3 Biochemical profile (mean ± SD) of Chinese PCOS women according to genotypes for rs3834363 variant of the Fabp4 gene

Parameter	−2-bp/−2-bp ($n = 105$)	−2-bp/+2-bp ($n = 60$)	−2-bp/−2-bp and −2-bp/+2-bp ($n = 165$)	+2-bp/+2-bp ($n = 13$)	P
Age (year)	26.81 ± 4.21	27.22 ± 3.51	26.96 ± 3.97	26 ± 3.14	NS
BMI (kg/m^2)	22.8 ± 3.92	22.92 ± 4.34	22.84 ± 4.06	24.12 ± 6.20	NS
E2 levels (pg/ml)	220.4 ± 182.1	231.2 ± 186.5	224.4 ± 183.2	319.2 ± 449.4	NS
FSH levels (mIU/ml)	5.67 ± 2.61	9.35 ± 25.86	7.01 ± 15.75	5.83 ± 1.77	NS
LH levels (mIU/ml)	13.37 ± 7.12	13.54 ± 6.57	13.43 ± 6.91	13.86 ± 8.56	NS
PRL levels (ng/ml)	20.05 ± 44.92	14.03 ± 8.43	17.86 ± 36.25	13.93 ± 14.15	NS
Total testosterone (ng/ml)	3.17 ± 6.16	4.73 ± 12.38	3.74 ± 8.93	6.22 ± 17.04	NS
LH/FSH	2.63 ± 1.87	2.36 ± 1.19	2.53 ± 1.65	2.55 ± 1.54	NS

Discussion

Multiple genetic pathways have been implicated in the pathogenesis of PCOS including steroid hormone metabolism, gonadotropin action, obesity and energy regulation, and insulin action through changes in protein function and/or regulation of gene transcription.

Unlike FABP4+/+ mice, the Fabp4 knock-out mouse does not develop insulin resistance in response to a high-fat diet, although the stress activated impact on fat metabolism does appear to be active, to some degree, in the Fabp4 knock-out mouse. Further, plasma concentrations of free fatty acids increase in the Fabp4 knock-out mouse whereas cholesterol and triglyceride levels decrease [25]. Therefore, these observations, and similarities in the regulation of expression of FABP4 for rodents and humans [26], suggested the possibility that genetic variations at this locus may bring about similar outcomes in humans.

Hu et al. [27] has shown that FABP4 serum levels in PCOS patients are greater than those of healthy controls. Our results indicate that there are no nonsynonymous variations in the promoter region and the four exon regions that we sequenced for the population of Chinese subjects used in this study; and, therefore, nonsynonymous protein variations in FABP4 do not appear to be a factor for the development of PCOS in this population. Together, these observations support the concept that changes in the transcription of the FABP4 gene may occur in at least some women with PCOS.

Although we discovered three common SNPs in the FABP4 promoter region, only one, rs3834363, displayed a statistically significant association with PCOS in our affected group of patients. A greater proportion of our subjects with PCOS carried the rs3834363 −2 bp allele compared to the +2 bp allele. While this was also true for control subjects, the relative ratios were 3:1 (PCOS) and 2:1 (controls), which suggests that the rs3834363 −2 bp allele may represent a genetic risk factor for PCOS in some populations of women. The reasons for this are not clear at this time; however, rs3834363 is positioned in the CAAT box/enhancer-binding protein (C/EBP) binding site, which has been shown to be critical for the transcription of the murine Fabp4 equivalent [28]. On the basis of these observations, we propose that the −2-bp allele may enhance the binding of the transcription factor in a manner that affects FABP4 production and contributes to the development of PCOS in affected women. Further research is required to determine the mechanisms that are involved in this process.

In conclusion, our data clearly demonstrate that changes in the FABP4 protein is not a factor in the etiology of PCOS in our population of women. Further, it appears that the −2-bp/−2-bp genotype and −2-bp allele of rs3834363 in the promoter region may represent a genetic risk factor for the development of PCOS in our population. To our knowledge, this study represents the first study to establish an association between FABP4 gene polymorphisms and the expression of PCOS.

Materials and methods

Subjects

Blood samples (349) were collected from Chinese women at The First Affiliated Hospital, Anhui Medical University, 178 of which were from patients with PCOS and the other 171 were from the healthy controls. The diagnosis of PCOS was based on the criteria of Rotterdam Revised 2003 (2 out of 3) diagnosis: oligomenorrhea or amenorrhea for at least 6 months; clinical and/or biochemical signs of hyperandrogenism; polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (10 ml). Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction were excluded. Control subjects of proven fertility, with normal menstrual cycles and ovary morphology, and without a history of subfertility treatment, were recruited from the Anhui Medical University. The study was approved by the Ethics Committee of the National Research Institute for Family Planning and informed consent was obtained from all participants.

Biochemical and hormonal measures

Blood samples were drawn on the 3rd day of menstrual onset in the early follicular phase of the cycle after an overnight fast. Because some of the patients were oligomenorrheic, we designed another approach to measure hormone levels if the patient were amenorrhea for more than 2 months. If the follicular diameter were lager than 10 mm, we used progesterone to induce withdrawal bleeding, and blood samples were drawn on the 3rd day after withdrawal bleeding; if the follicular diameter were smaller then 10 mm, blood samples were drawn on an empty stomach without other restrictive conditions. Plasma was harvested and stored at −20°C until total testosterone, prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) were determined.

We calculated the body mass index (BMI) to assess obesity and the specific values of LH and FSH.

Table 4 PCR and sequencing primers

PCR primer	Sequencing primer
I-f: 5′ acgcactaagacagagcgggtat 3′	I-I-f: 5′ ctcaaaagcatgaagggca 3′
I-r: 5′ cttggcaacaaatgctatgaaatg 3′	I-II-f: 5′ tgtcaccgcagcctgtttg 3′
	I-III-r: 5′ tttaaggcacctggaggg 3′
II-f: 5′ taagtaaaccaaggatacccaagt 3′	II-I-f: 5′ agacattgctataaacaca 3′
II-r: 5′ gtcacggaatcaacctaagtgt 3′	II-II-r: 5′ tctgggtatgttcgtggg 3′

DNA analysis

Blood samples from PCOS patients and controls were collected and stored at −20°C. Genomic DNA was extracted from peripheral blood leukocytes according to standard methods.

The FABP4 gene, which has been mapped to chromosome 8q21 (Mendelian Inheritance in Man no. 600434) and spanned about 7 kb of genomic DNA, consists of four exons, and encodes a 132-aa protein [29, 30].

Therefore, all four exon regions and the 1000 bp promoter region of FABP4 gene were amplified by polymerase chain reaction (PCR) with two pairs of FABP4 gene-specific primers. The promoter region and the first exon were amplified by using II-f and II-r primers, the other three exons were amplified by using I-f and I-r primers (shown in Table 4), all these primers were designed by GeneTool V 1.0.0.1 (Launcher Program for BioTools Inc Applications).

PCR products were denatured and annealed to form potential heteroduplexes. Forward products were sequenced using Big Dye Terminator 3.1 chemistry (Applied Biosystems Inc.) with the above primers and run on a 3730xl DNA analyzer (ABI).

Genetic and statistic analysis

In this study, statistical analyses were carried out using the Statistical Package for Social Sciences version 10.0 (SPSS 10.0). Differences between noncontiguous variables, genotype distribution, and allele frequency were tested by Chi-square analysis. ANOVA test and student's *t*-test was used to compare data of the clinical parameters (age, E2 levels, FSH levels, LH levels, PRL levels, and total testosterone levels) between different genotypes. Significant differences between or among groups was indicated by a P value <0.05.

Haploview was used to calculate linkage disequilibrium (LD, the D' statistic) between each pairwise combination of all the SNPs.

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