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Original Research

Effect of the FSH receptor polymorphism on the age at menarche

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Summary

Summary: Estrogen and estrogen receptor (ER) genes have an effect on the age at menarche. Follicle stimulating hormone (FSH) is an important hormone that plays a major role in the regulation of folliculogenesis and estrogen synthesis. The aim of this study was to investigate the influence of the FSH receptor (FSHR) polymorphisms Asn680Ser and Thr307Ala on age at menarche. *Materials and Methods:* FSH receptor gene polymorphisms were investigated in women who were admitted to Selcuk University between May 2013 and November 2014. Information about age at menarche was obtained through interviews. Polymorphic analysis of the FSH receptor gene was performed in 209 healthy female patients who had undergone an annual health examination. Genomic DNA was obtained from peripheral blood leukocytes, and polymorphisms were investigated using restriction fragment length polymorphism analysis. *Results:* The mean age at menarche was 12.95 ± 1.62 years. The overall frequencies for Asn680Ser variants NN, NS, and SS were 40.2%, 36.4%, and 23.4%, respectively, whereas Thr307Ala variants TT, TA, and AA were 34.4%, 41.1%, and 24.4%, respectively. Menarche occurred 3.6 months earlier in subjects with TN homozygote genotype than in AS homozygotes, and the mean age at menarche was 2.5 months earlier in TN homozygotes compared with that in all other study subjects, but the differences were not statistically significant ($p = 0.966$ and $p = 0.986$, respectively). *Conclusions:* The Ala307Thr and Ser680Asn polymorphisms of the FSHR gene are not associated with age at menarche.

Key words: Menarche age; FSH receptor; Polymorphism; Estrogen receptor.

Introduction

Menarche is the first menstrual cycle and indicates the onset of the reproductive period in the life of a woman. Age at menarche has long-term effects on the gynecological system as well as other systems of the body. Although the patients who experienced menarche at an early age were more likely to develop breast cancer, endometrial cancer, the risk for cardiovascular disease, blood pressure alterations, and glucose intolerance, these women had higher long-term results on bone mineral densitometry [1-5].

Menarche occurs as a result of the maturation of the hypothalamic–pituitary–ovarian axis due to the effect of environmental and genetic factors, among others. Environmental factors include, but are not limited to, physical activity, height, weight, ethnicity, and socioeconomic level. The effect of genetic factors on the age at menarche has been reported to be between 57-82%. Because menarche is associated with a significant change in hormone levels, especially estrogen, the genes involved in the metabolism of the hormones or those encoding the hormonal receptors have been the most commonly studied genetic factors for their effect on age at menarche [6]. The effect of the estrogen receptor (ER) gene, CYP17 gene, the progesterone receptor (PR) gene, and single nucleotide polymorphisms (SNPs) on the age at menarche has been studied in various populations and different results have been reported [6-11].

Estrogen and progesterone, which regulate the cyclic changes of the reproductive system, are released as a result of the effect of pituitary gonadotropins. Follicle stimulating hormone (FSH) is the most important hormone in follicle formation and estrogen synthesis. It acts via the FSH-receptor (FSHR) located on the granulosa cell membrane. In FSHR knockout mice, during folliculogenesis, a blockade occurs before the formation of antral follicle and a rapid loss of ovarian function is observed [12, 13]. The FSHR gene has two SNPs on exon 10 that change amino acids at the Threonin307Alanin (Thr307Ala) and Asparagin680Serin (Asn680Ser) positions [14, 15].

Menarche is a complex process that results from the effects of follicular steroid release promoted by the gonadotropins on the endometrium, but whether there is a correlation between FSHR polymorphisms and age at menarche has not been studied to date.

This study aimed to investigate the effect of

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Asn680Ser and Thr307Ala polymorphisms in the FSHR gene on the age at menarche in the Turkish population.

Materials and Methods

FSHR gene polymorphism was investigated in 209 healthy female subjects with regular menses who were admitted to the Gynecology and Obstetrics Outpatient Clinic at Selçuk University between May 2013 and November 2014. The subjects were sampled consecutively. Subjects with any systemic and gynecological disease, who had a history of precocious puberty or delayed puberty, and who were using hormonal therapies were excluded. This study was approved by the Clinical Trials Ethics Committee at Selçuk University and was conducted according to the principles of the Declaration of Helsinki. Each subject gave written informed consent.

Patients and controls provided a 3-cc venous whole blood sample. The blood samples were transferred into EDTA tubes and stored in a refrigerator at 4°C until use. As recommended by the manufacturer, genomic DNA was isolated from peripheral blood leukocytes using a genomic DNA extraction kit.

For the detection of Thr307Ala and Asn680Ser polymorphisms, polymerase chain reaction (PCR) and restriction enzyme cutting analyses (RFLP, restriction fragment length polymorphism) were performed as described by *Suda et al.* [14].

PCR was performed with 25 volume containing 25 ng DNA template, 2.5 µl PCR buffer, 1.25 mM MgCl2, 1.5 U Tag DNA polymerase, 200 nM dNTP, and 10 pM primer for each reaction. In PCR, all PCR fragments were amplified by an initial cycle at 95°C for two minutes, then a cycle at 95°C for one minute, a cycle at 58°C for 50 seconds, and another cycle at 72°C for 30 seconds and finally, a total of 40 cycles of denaturation at 72°C for ten minutes.

For RFLP analysis of the Asn680Ser polymorphism, PCR fragments were stained using ethidium bromide (EtBr) in the electrophoresis with Brsl enzyme and agarose gel 2.5%, and three different patterns were revealed. Based on this RFLP analysis, the patients were classified into three groups as follows: NN (680 Asn/Asn), NS (680 Asn/Ser), and SS (680 Ser/Ser).

For RFLP analysis of the Thr307Ala polymorphism, PCR fragments were stained using ethidium bromide (EtBr) in the electrophoresis with Brsl enzyme and agarose gel 2.5%, and three different patterns were revealed. Based on this RFLP analysis, the patients were classified into three groups as follows: TT (307 Thr/Thr), TA (307 Thr/Ala), and AA (307 Ala/Ala).

SGP Hardy-Weinberg equilibrium was evaluated using the χ^2 test. Distribution normality was assessed using the Kolmogorov-Smirnov test. Data are expressed as mean ± standard deviation or number and percentage. Intergroup differences were analyzed using the Kruskal-Wallis test. Adjustment for BMI was performed using multiple logistic regression analysis. For data analysis, SPSS 17 software was used. $P < 0.05$ was considered statistically significant.

Results

Demographics of the study participants are shown in Table 1. Mean age at menarche was 12.95 ± 1.62 years. The incidences of the Thr307Ala SNP genotype variants TT, TA, and AA were, respectively, 34.4%, 41.1%, and 24.4%, whereas the incidences of the Asn680Ser SNP genotype variants NN, NS, and SS were, respectively, 40.2%, 36.4%, and 23.4% (Table 2). Neither Thr307Ala nor Asn680Ser SNPs achieved the Hardy-Weinberg equilibrium (p = 0.0148 and p = 0.0003, respectively).

Thr307Ala SNP and Asn680Ser SNP genotypes were not different in terms of mean age at menarche. When haplotypes were evaluated, it was seen that the mean age at menarche was greater by approximately 3.6 months in AS homozygotes (n=36, 17.2%) compared with TN homozygotes (n=52, 24.9%), but the difference was not statistically significant (p = 0.966) (Table 3). When the mean age at

Table 1. — *Patient characteristics.*

Mean age at menarche, mean ± SD, years	12.95 ± 1.62
Height, mean ± SD, cm	162.85 ± 6.26
Weight, mean ± SD, kg	59.28 ± 9.80
Body mass index, mean ± SD, kg/m²	22.39 ±3.70

Table 2. — *Genotype frequency of FSHR polymorphism.*

Genotype	n. (%)
Thr307Ala	
TT	72 (34.4%)
TA	86 (41.1%)
AA	51 (24.4%)
Allelotype	
T	230 (55%)
A	188 (45%)
Asn680Ser	
NN	84 (40.2%)
NS	76 (36.4%)
SS	49 (23.4%)
Allelotype	
N	244 (58%)
S	174 (42%)

Table 3. — *Mean menarche age for different genotypes of the FSHR gene.*

	Mean age at menarche ± SD	*p*
Thr307Ala		
TT	12.888 ± 2.03	
TA	12.976 ± 1.33	0.904
AA	13.019 ± 1.44	
Asn680Ser		
NN	12.821 ± 1.90	
NS	13.131 ± 1.32	0.624
SS	12.918 ± 1.55	
Haplotype		
TTNN	12.784 ± 2.22	
AASS	13.083 ± 1.57	0.966
Others	12.991 ± 1.33	
Haplotype		
TTNN	12.784 ± 2.22	0.986
Others	13.012 ± 1.39	

menarche seen in TN homozygotes was compared with that observed in TN heterozygotes or in those without the TN allele, it was seen that the age at menarche was earlier by approximately 2.5 months, but this difference did not reach statistical significance ($p = 0.986$). When the assessments were repeated following adjustment for BMI, the difference remained statistically insignificant across the groups ($p > 0.05$).

Discussion

One of the best examples that demonstrates that SNPs may have an effect on clinical outcomes is the FSHR polymorphism. Different responses of patients with various FSH-receptor polymorphic variants to controlled ovarian hyperstimulation administered during in vitro fertilization (IVF) therapies guided the investigators to study FSH-receptor polymorphisms in several areas of the gynecological discipline. No correlation was found between polycystic ovarian syndrome and FSH-receptor polymorphism in Turkish and Chinese populations [15-20]. Although the study performed by Wang *et al.* in the Taiwanese population reported a decreased risk for endometriosis in subjects with SS and NS genotypes, the current authors previous study of this issue did not reveal a correlation between the risk for endometriosis and FSH-receptor SNPs [16, 21].

The studies that examined the correlation between FSH-receptor genotype and menstruation demonstrated that, among FSH-receptor inactivating mutations, Ala189Val, Val221Gly, Pro348Arg, Asp224Val, Pro587His, and Ala575Val were correlated with primary amenorrhea and Ile160Thr with secondary amenorrhea [14]. Greb *et al.* examined the effects of FSH-R Asn680Ser polymorphism on menstrual cycle characteristics and reported that the SS genotype had a higher ovarian threshold for FSH, decreased negative pituitary feedback, and longer menstrual cycles [22].

Menarche results from the effects of the steroids released that have a gonadotropin effect on the endometrium upon the maturation of the hypothalamus-pituitary-ovarian axis. Although some studies that investigated the effects of Msp1 SNP of the CYP17 gene and XbaI and Pvull SNPs of the ERα gene on age at menarche reported that these SNPs did not affect age at menarche, Stavrou *et al.* reported in 2002 that menarche occurred approximately six months later in the people with XbaI SNP XX genotype and in 2006 that the age at menarche was greater by seven months in the people with the ERβ gene A1730G SGP AA genotype [7-10]. Taylor *et al.* investigated the effect of the PR gene SNP on age at menarche and reported that menarche occurred one year later in the people who encode two copies of the Val660Leu variant for rs1042838 SNP [11] In the present study, menarche age seen in those with the TN homozygote FSHR polymorphism was earlier by approximately 2.5 months compared with TN heterozygotes and in those without the TN allele. It was also detected that the mean age at menarche was later by approximately 3.6 months in AS homozygotes compared with TN homozygotes, but the differences were not statistically significant.

The age at menarche had a secular decrease worldwide. When reviewing the results of the studies that analyzed age at menarche in this country, it can be seen that the mean menarche age result in this study (12.95 ± 1.62 years) was similar to the results obtained in other studies (13.04 years and 13.10 years) [23, 24].

Conclusion

This study demonstrated that the FSHR polymorphism has no effect on age at menarche. The subjects who led the authors to these study data represent a heterogeneous population. Although genetic factors and SNPs can affect age at menarche, combined effects of the genetic and environmental factors are probably much more significant.

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References

[1] Schatzkin. A, Palmer J.R., Rosenberg L., Helmrich S.P., Miller D.R., Kaufman D.W., *et al.*: "Risk factors for breast cancer in black women". *J. Nat. Cancer. Inst*., 1987, *78*, 213.
[2] Peeters P.H., Verbeek A.L., Krol A., Matthyssen M.M., de Waard F.: "Age at menarche and breast cancer risk in nulliparous women". *Breast Cancer Res. Treat*., 1995, *33*, 55.
[3] Dossus L., Allen N., Kaaks R., Bakken K., Lund E., Tionneland A., *et al.*: "Reproductive risk factors and endometrial cancer: the European prospective investigation into cancer and nutrition". *Int. J. Cancer,* 2010, *127*, 442.
[4] Fujita M., Tase T., Kakugawa Y., Hoshi S., Nishino Y., Nagase S., Ito K., *et al.*: "Smoking, earlier menarche and low parity as independent risk factors for gynecological cancers in Japanese: a case-control study". *Tohoku. J. Exp. Med.,* 2008, *196*, 590.
[5] Lakshman R., Forouhi N.G., Sharp S.J., Luben R., Bingham S.A., Khaw K.T.,*et al.*: "Early age at menarche associated with cardiovascular disease and mortality". *J. Clin. Endocrinol. Metab*., 2009, 94(12), 4953.
[6] Dvornyk V., Ul-Haq W.: "Genetics of age at menarche: a systematic review". *Human. Reprod. Update,* 2012, *18*, 198.
[7] Pei Y.F., Zhang L., Deng H.W., Dvornyk V.: "CYP17 MspA1 polymorphism and age at menarche: a meta-analysis". *Dis. Markers,* 2008, *25*, 87.
[8] Manuck S.B., Craig A.E., Flory J.D., Halder I., Ferrell R.E.: "Reported early family environment covaries with menarcheal age as a function of polymorphic variation in estrogen receptor-α". *Dev. Psychopathol*., 2011, *23*, 69.
[9] Stavrou I., Zois C, Ioannidis J.P., Tsatsoulis A.: "Association of polymorphisms of the oestrogen receptor alpha gene with the age of menarche". *Hum. Reprod.*, 2002, *17*, 1101
[10] Stavrou I., Zois C., Ioannidis J.P., Tsatsoulis A.: "Combined estrogen receptor alpha and estrogen receptor beta genotypes influence the age of menarche". *Hum. Reprod*. 2006, *21*, 554.
[11] Taylor K.C., Small C.M., Epstein M.P., Sherman S.L., Tang W., Wilson M.M., *et al.*: "Associations of progesterone receptor polymor-

phisms with age at menarche and menstrual cycle length". *Horm. Res. Paediat*., 2010, *74*, 421.
[12] Danilovich N., Roy I., Sairam M.R.: "Emergence of uterine pathology during accelerated biological aging in FSH receptor-haploinsufficient mice". *Endocrinology,* 2002, *143*, 3618.
[13] Kumar T.R.: "What have we learned about gonadotropin function from gonadotropin subunit and receptor knockout mice?" *Reproduction*, 2005, *130*, 293.
[14] Sudo S., Kudo M., Wada S., Sato O., Hsueh A.J., Fujimoto S.: "Genetic and functional analysis of polymorphisms in the human FSH receptor gene". *Mol. Hum. Reprod*., 2002, *8*, 893.
[15] Unsal T., Konac E., Yesilkaya E., Yilmaz A., Bideci A., Ilke O.H., *et al.*: "Genetic polymorphisms of FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome". *J. Assist. Reprod. Genet*., 2009, *26*, 205.
[16] Wang H.S., Cheng B.H., Wu H.M., Yen C.F., Liu C.T., Chao A., Wang T.H.: "A mutant single nucleotide polymorphism of follicle-stimulating hormone receptor is associated with a lower risk of endometriosis". *Fertil. Steril.,* 2011, *95*, 455.
[17] Gromoll J., Simoni M.: "Genetic complexity of FSH receptor function". *Trends.Endocrinol. Metab*., 2005, *16*, 368.
[18] Falconer H., Andersson E., Aanesen A., Fried G.: "Follicle-stimulating hormone receptor polymorphisms in a population of infertile women". *Acta. Obstet. Gynecol. Scand.,* 2005, *84*, 806.
[19] de Koning C.H., Benjamins T., Harms P., Homburg R., van Montfrans J.M., Gromoll J., et al: "The distribution of FSH receptor isoforms is related to basal FSH levels in subfertile women with normal menstrual cycles". *Hum. Reprod*., 2006, *21*, 443.
[20] Wu X.Q., Xu S.M., Liu J.F., Bi X.Y., Wu Y.X., Liu J.: "Association between FSHR polymorphisms and polycystic ovary syndrome among Chinese women in north China". *J. Assist. Reprod. Genet*., 2014, *31*, 371.
[21] Kerimoglu O.S., Yılmaz S.A., Pekin A., Nergiz S., İncesu F., Dogan N.U., *et al.*: "Follicle-stimulating hormone receptor gene polymorphisms in women with endometriosis". *Arch. Gynecol. Obstet.,* 2015, *291*, 1411.
[22] Greb R.R., Grieshaber K., Gromoll J., Sonntag B., Nieschlag E., Kiesel L., Simoni M.: "A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle". *J. Clin. Endocrinol. Metab*., 2005, *90*, 4866.
[23] Ekerbicer H.C., Celik M., Kiran H., Kiran G.: "Age at menarche in Turkish adolescents in Kahramanmaras, Eastern Mediterranean region of Turkey". *Eur. J. Contracept. Reprod*. *Health Care,* 2007, *12*, 289.
[24] Tekgül N., Saltık D., Vatansever K. "Secular trend of menarche age in an immigrant urban city in Turkey: Izmir". *Turk. J. Pediatr.,* 2014, *56*, 138.

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