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Genetic variants of estrogen beta and leptin receptors may cause gynecomastia in adolescent

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ABSTRACT

Objective: Gynecomastia is a benign breast enlargement in males that affects approximately one-third of adolescents. The exact mechanism is not fully understood; however, it has been proposed that estrogen receptors and aromatase enzyme activity may play important roles in the pathogenesis of gynecomastia. While many studies have reported that aromatase enzyme (CYP19) gene polymorphism is associated with gynecomastia, only one study has shown a relationship between estrogen receptor (ER) alpha and beta gene polymorphism and gynecomastia. Thus, the aim of this study was to evaluate the relationships between CYP19 (rs2414096), ER alpha (rs2234693), ER beta (rs4986938), leptin (rs7799039), and leptin receptor (rs1137101) gene polymorphisms and gynecomastia.

Methods: This study included 107 male adolescents with gynecomastia and 97 controls. Total serum testosterone (T) and estradiol (E2) levels were measured, and DNA was extracted from whole blood using the PCR–RFLP technique. The polymorphic distributions of CYP19, ER alpha, ER beta, leptin and leptin receptor genes were compared.

Results: The median E2 level was 12.41 (5.00–65.40) pg/ml in the control group and 16.86 (2.58–78.47) pg/ml in the study group ($p < 0.001$). The median T level was 2.19 (0.04–7.04) ng/ml in the control group and 1.46 (0.13–12.02) ng/ml in the study group ($p = 0.714$). There was a significant relationship between gynecomastia and leptin receptor rs1137101 ($p = 0.002$) and ER beta receptor rs4986938 gene polymorphisms ($p = 0.002$).

Conclusions: According to our results, increased E2 level and ER beta gene rs4986938 polymorphism might explain why some adolescents have gynecomastia. Leptin receptor gene rs1137101 polymorphism might affect susceptibility to gynecomastia.

1. Introduction

Gynecomastia is defined as benign enlargement of the breast tissue in males. It is generally encountered in infants, adolescents, and elderly men. This condition is observed in approximately 30% of adults, but it may be encountered at higher rates in adolescents (Braunstein, 1993; Kumanov et al., 2007). The highest incidence of pubertal gynecomastia was determined as 65% at 14 years of age (Niewoehner and Nuttal, 1984). While the exact mechanism is not fully understood, it has been proposed that estrogen receptors and aromatase enzyme (CYP19) activity may play important roles in the pathogenesis of gynecomastia (Lee et al., 1990). Gynecomastia develops mainly due to disequilibrium between estrogen and androgen in the breast tissue (Braunstein, 1999). CYP19 activity, which converts androstenedione and testosterone to estrone and estradiol, respectively, is the most important factor in the equilibrium. Activating mutation can cause familial gynecomastia by increasing the aromatase activity in this gene (Shozu et al., 2003). It has been reported that overexpression of CYP19 enlarged breast tissue in transgenic male mice (Li et al., 2002).

Abbreviations: CYP19, aromatase enzyme; ER, estrogen receptor; T, testosterone; E2, estradiol; STAT3, signal transducer and activator of transcription 3; SDS, standard deviation score; BMI, body mass index; FSH, Follicle stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosterone sulfate; PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval.

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Studies on the expression of estrogen receptors in patients with gynecomastia have been reported only in histological analyses (Andersen et al., 1987; Poulsen et al., 1985). While many studies have reported that CYP19 gene polymorphisms are associated with gynecomastia, only one study showed a relationship between estrogen receptor (ER) alpha 454-351A/G polymorphism and gynecomastia (Czajka-Oraniec et al., 2007, 2008).

Leptin synthesized in adipose tissue has effects on food intake, body fattening, energy equilibrium, and gonadal functions. It has been reported that there is a relationship between leptin and sex hormones, and that leptin might increase estrogen secretion by increasing CYP19 activity in adipose and breast tissues (Dundar et al., 2005; Garofalo et al., 2006; Williams, 2012). It has also been reported that estrogen increased leptin mRNA and protein synthesis in adipose tissue (Machinal-Quélin et al., 2002). Moreover, it was determined that estrogen and leptin affected the same receptors and pathways in the arcuate nucleus in the hypothalamus. Molecular studies showed that both hormones had effects on "signal transducer and activator of transcription 3" (STAT3), and that estrogen increased leptin-induced STAT3 phosphorylation (Binai et al., 2013). However, no study showing a correlation between leptin and leptin receptor polymorphisms and gynecomastia has been published in the literature. The aim of our study was to determine whether there are relationships between CYP19 (rs2414096), ER alpha (rs2234693), ER beta (rs4986938), leptin (rs7799039), and leptin receptor (rs1137101) gene polymorphisms and gynecomastia.

2. Material and methods

2.1. Study group

The present study included patients who presented at pediatric endocrinology outpatient clinics with a complaint of breast enlargement. Cases with testicular volume above 4 ml in the physical examination were accepted as pubertal gynecomastia patients. Auxologic parameters of the enrolled patients, such as height, weight, and body mass index (BMI) were measured, and standard deviation score (SDS) of the data was calculated (Kuczmarski et al., 2002). We evaluated 107 consecutive adolescent patients with gynecomastia from the databases of the pediatric endocrinology units of Harran University Medical Faculty, Izmir Behcet Uz Children's Hospital, Gaziantep Children's Hospital, and Mersin Children's Hospital. Ninety-seven unrelated, age- and sex-matched, healthy controls were selected from the same geographic area ($p = 0.436$). Patients with systemic diseases, chronic drug intake history, or syndromic diseases were excluded from the study. The investigations conformed to the principles outlined in the Declaration of Helsinki.

2.2. Ethical standard

Written informed consent was obtained from all of the patients in accordance with the guidelines of the various internal review boards. The protocol was also approved.

2.3. Hormonal evaluation

Follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol (E2), dehydroepiandrosterone sulfate (DHEAS), and prolactin levels were analyzed using the electrochemiluminescence immunometric assay (ECLIA) method with a Roche Elecsys E170 immuno-analyzer (Roche Diagnostics, Burgess Hill, UK).

2.4. Genotyping

Venous blood samples were collected into vacutainer plastic tubes containing sodium/potassium EDTA. DNA was extracted with a Genejet Genomic DNA purification kit (Thermo Scientific K0772). For all genotyping, PCR was performed in a 25-μl volume with 100 ng DNA, 100 μm dNTPs, 20 pmol of each primer, 1.5 mM $MgCl_2$, 1× PCR buffer with $(NH_4)_2SO_4$ and 2 U Taq DNA polymerase (Thermo Scientific EP0401). Amplification was performed on an automated thermal cycler (Techne Flexigene, Cambridge, UK). Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) conditions of polymorphisms of CYP19 (rs2414096), ER alpha (rs2234693), ER beta (rs4986938), leptin (rs7799039), and leptin receptor (rs1137101) genes are shown in Table 1 (Jin et al., 2009; Mammès et al., 2000; Matsuoka et al., 1997; Safarinejad et al., 2012). These gene polymorphisms were determined by fragment separation at 120 V for 40–50 min on 3.5% agarose gel containing 0.5 mg/ml ethidium bromide. A 100-bp DNA ladder (Fermentas, Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat E-BOX VX5).

2.5. Statistical analysis

Statistical analysis was performed with SPSS 18.0 software (SPSS Inc., Chicago, IL). Distribution of data was determined using the Shapiro–Wilks test. Continuous variables were expressed as mean ± standard error of the mean, and categorical variables as frequency and percentage. Categorical variables were compared using Pearson's Chi-square test. Continuous variables were compared by an independent sample *t* test or the Mann–Whitney *U* test for two groups. The Kruskal–Wallis test was used to determine differences among three groups. The Bonferroni-adjusted Mann–Whitney *U* test was used as a post hoc test after the Kruskal–Wallis test. p values less than 0.05 were considered statistically significant for all tests.

3. Results

When the healthy controls ($n = 97$, median age 14.16 years) and the gynecomastia patients ($n = 107$, median age 13.7) were compared, no differences were detected in age, height, height SDS, or weight ($p > 0.05$); however, weight SDS, BMI, and BMI SDS were markedly higher in the gynecomastia group ($p = 0.008$, $p = 0.046$, and $p = 0.004$, respectively). When hormonal levels were evaluated, there were no differences in FSH, prolactin, testosterone, or DHEAS between the groups. The median serum LH level was higher in the control group (2.4 vs. 1.53 IU/l, $p = 0.004$), and the median E2 (12.41 vs. 16.86 IU/l, $p < 0.001$) and DHEAS (112.3 vs. 1.46, $p = 0.037$) levels were higher in the gynecomastia group. The auxologic and hormonal data of all of the subjects are shown in Table 2.

The control group was at the Hardy–Weinberg equilibrium for all analyzed genes ($p > 0.05$). According to our results, leptin receptor gene rs1137101 polymorphism variants might be associated with gynecomastia. It was determined that GA and AA genotypes showed protective effects (χ^2 p value 0.002; OR (95% CI) 0.423 (0.225–0.797) and 0.298 (0.137–0.649), respectively) (Table 3). In addition, it was found that the A allele showed a protective effect ($p < 0.01$; OR (95% CI) 0.449 (0.297–0.678)) and that the G allele might increase gynecomastia risk (Table 4).

We did not find any association between leptin (rs7799039), CYP19 (rs2414096), and ER alpha (rs2234693) gene polymorphisms and gynecomastia (χ^2 $p = 0.330$, χ^2 $p = 0.501$, χ^2 $p = 0.775$, respectively). However, the GA and AA genotypes were determined as risk factors in the ER beta gene rs4986938 polymorphism for gynecomastia (χ^2 $p = 0.002$). It was also determined that the A allele increased gynecomastia risk (χ^2 $p < 0.01$; OR (95% CI) 2.189 (1.454–3.295).

First, we combined the leptin and leptin receptor polymorphisms (rs7799039, rs1137101) for haplotype analysis (Table 5). The results of this analysis suggested that GA and AA haplotypes might be protective factors for gynecomastia (OR (95% CI) 0.476 (0.266–0.8549) and 0.505 (0.290–0.879, respectively).