Association of SHBG gene polymorphism with menarche

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The age of menarche may be subject to hereditary influences but the specific determinants are unknown. Our aim was to investigate the possible association of a functional (TAAAA)n polymorphism in the promoter of the sex hormone-binding globulin (SHBG) gene with the timing of menarche. This polymorphism has been associated with polycystic ovary syndrome (PCOS) and is considered to contribute to SHBG levels. We studied 130 healthy normal-weight adolescent females from a closed community in North–Western Greece. Information on menarche was obtained through interviews. The BMI was recorded. Genomic DNA was isolated from peripheral blood leukocytes for genotyping the TAAAA repeat region. We subdivided our subjects into two groups based on median age of menarche: those with menarche <13 years and those with menarche ≥ 13 years. Genotype analysis revealed six (TAAAA)n alleles containing 5–10 TAAAA repeats. The distribution of alleles was different in the two groups. Girls with late menarche had more frequently longer TAAAA alleles (>8 repeats), while girls with early menarche had shorter alleles at a greater frequency (P = 0.048). The major contribution to early menarche was by the 6 TAAAA repeat allele. Furthermore, carriers of the longer allele genotypes had later menarche (13.24 ± 1.15 years) than those with shorter allele genotypes (12.67 ± 1.15, P = 0.018). These findings provide evidence for a genetic contribution of SHBG gene to the age of menarche.

Key words: gene/menarche/polymorphisms/SHBG

Introduction

The timing of menarche is regulated by both environmental and genetic factors. While there is a variety of environmental stressors that influence the development of the reproductive system, genetic analysis in twins has produced strong evidence that genetic factors contribute greatly to the age of menarche (Treloar and Martin, 1990; Kaprio et al., 1995). These studies have shown that there is a significant relation between age at menarche in mothers and daughters and the relation for the onset of menarche between monozygotic twins is significantly higher than between dizygotic twins (Treloar and Martin, 1990). However, the genes responsible for the age of menarche are not well defined.

Estrogens play a crucial role in regulating the pre-ovulatory gonadotrophin surge and they are important determinants of menarche (Mahesh and Brann, 1998). In this regard, sex hormone-binding globulin (SHBG), through binding testosterone and estradiol, regulates the access of sex steroids to their target tissues and might therefore play a role in menarche.

Recently, a (TAAAA)n pentanucleotide repeat polymorphism at the 5’ boundary of the human SHBG promoter has been described, and has been reported to influence its transcriptional activity in vitro (Hogeveen et al., 2001). This functional polymorphism has been found to be associated with polycystic ovary syndrome (PCOS) and may contribute to individual differences in plasma SHBG levels (Xita et al., 2003, Cousin et al., 2004).

The aim of this study was to investigate the possible association of the (TAAAA)n polymorphism of the SHBG gene with the age of menarche in a group of healthy normal-weight adolescents.

Materials and methods

Subjects and methods

The study population consisted of 130 healthy normal-weight adolescent girls from a closed rural community in North–Western Greece. Information on menarche was obtained through interviews with the adolescents and their mothers and through diaries. The BMI of each subject, calculated as weight (kg)/height² (m²), was recorded. Blood samples were obtained from all girls for the genetic analysis. The details of the protocol were explained to the girls and their parents, who gave their consent, and the protocol was approved by the Hospital Ethics Committee.

Genotype analysis

Genomic DNA was isolated from peripheral blood leukocytes. Genotyping of the TAAAA repeat region within the Alu sequence in the SHBG promoter was accomplished as previously described (Xita et al., 2003).

Statistical analysis

Statistical analysis of differences in allele and genotype frequencies between girls with earlier menarche and those with later menarche was performed using the χ²-test with the Yates correction. Differences in menarche were assessed with the non-parametric Mann–Whitney U-test. Analysis of covariance was performed including BMI as a covariate. P-value of <0.05 was set as statistically significant. All results are reported as the mean ± SD. All analyses used the Statistica Software Package (version 5.1, Statsoft Inc, USA).

Results

The general characteristics of the study population are presented in Table I. The mean age of menarche was 12.9 ± 1.2 years and the
The distribution of SHBG (TAAAA) 8 repeats between the two subgroups (Figure 2). There was no difference in the frequency of the TAAAA allele with present at a greater frequency (39.9% versus 29.6%, \( P = 0.04 \)). Furthermore, a cut-point sensitivity analysis revealed that girls with late menarche more frequently had long TAAAA alleles (>8 repeats) than girls with early menarche (30.2% versus 20.3%) in whom shorter TAAAA alleles (<8 repeats) were present at a greater frequency (39.9% versus 29.6%, \( P = 0.048 \)). There was no difference in the frequency of the TAAAA allele with 8 repeats between the two subgroups (Figure 2).

With regard to genotypes of the (TAAAA)\(_h\) polymorphism of the SHBG gene, we compared differences in age of menarche between carriers (heterozygotes and homozygotes) and non-carriers of each allele. Carriers of (TAAAA)\(_h\) allele tended to have earlier menarche than non-carriers of this allele (12.7 ± 1.2 versus 13.0 ± 1.2 years, \( P = 0.018 \)). On the other hand, carriers of (TAAAA)\(_{10}\) allele tended to have later menarche compared to non-carriers of these alleles, although the differences failed to reach statistical significance (13.0 ± 1.3 versus 12.8 ± 1.2 years, \( P = 0.1 \) and 13.4 ± 1.0 versus 12.8 ± 1.2 years, \( P = 0.1 \)). There was no difference in age of menarche among carriers of (TAAAA)\(_8\) allele, while the analysis of carriers of (TAAAA)\(_3\) and (TAAAA)\(_7\) was limited due to the small number of carriers of these alleles. However, when the frequencies of the various genotypes were compared between the two groups of girls (girls with early versus late menarche), then carriers of the longer allele genotypes (genotypes 8/9, 8/10, 9/9, 9/10) were more frequent in group 2 than in group 1 (34.8% versus 17.2%). In contrast, carriers of shorter allele genotypes (5/8, 6/6, 6/7, 7/7, 7/8, 8/8) were more frequent in group 1 than in group 2 (67.2% versus 47%, \( P = 0.02 \)). Thus, carriers of the longer allele genotypes had late menarche (13.24 ± 1.15 years) than those with shorter allele genotypes (12.67 ± 1.15, \( P = 0.018 \)) and this association was independent of BMI (Figure 3). From this comparison, girls (\( n = 23 \)) with extreme genotype combinations, having one shorter and one longer than 8 repeat alleles, were excluded (genotypes 5/9, 6/9, 6/10, 7/9 and 7/10).

**Discussion**

In the present study we analysed a polymorphic (TAAAA)\(_h\) repeat of the human SHBG gene in a group of Greek adolescent females, in order to evaluate whether variations in this pentanucleotide repeat are associated with age at menarche. The study population was a homogeneous population that was selected with the anticipation that cultural and environmental heterogeneity would be minimized, since regional and environmental factors may create some variability in the age of menarche. Furthermore, the subjects of this study were adolescents and bias due to incorrectness in self-report of ages at menarche was diminished, since they participated in the study one or two years after menarche and they or their mothers were able to remember the exact year and month of menarche. In addition they were normal-weight girls and therefore bias due to effects of body weight on menarche was avoided.

The (TAAAA)\(_h\) pentanucleotide repeat is located upstream of the SHBG promoter region within an Alu sequence and appears to have functional significance. Hogeveen et al. (2001), using transient transfection experiments in human HepG2 hepatoblastoma cells, have shown that the transcriptional activity of the SHBG promoter may be related to the number of TAAAA repeats. In particular, the allele with six repeats was found to have the lower transcriptional activity. In addition, Haiman et al. (2005) recently studied this polymorphism in healthy post-menopausal women and found that carriers of the six repeat allele had significantly lower SHBG levels than non-carriers.

In this study we have shown that early menarche in adolescent Greek girls is associated with shorter (TAAAA)\(_h\) alleles, whereas late menarche (±13 years) is associated with longer repeat alleles. However, the major contribution to the significance of this association was the 6 TAAAA repeat allele that was found more frequently among girls with early menarche than in girls with late menarche. Taking into account that this particular allele has been associated with decreased in vitro transcriptional activity (Hogeveen et al., 2001) and lower plasma SHBG levels in healthy post-menopausal women (Haiman et al., 2005), we may infer that the early menarche in the carriers of this allele may reflect exposure of target

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche, median (IQR), years</td>
<td>13.0 (12.0–13.7)</td>
<td>13.0 (12.0–13.7)</td>
</tr>
<tr>
<td>Age at evaluation median (IQR), years</td>
<td>16.9 (15.2–18.5)</td>
<td>16.9 (15.2–18.5)</td>
</tr>
<tr>
<td>Height, mean (SD), cm</td>
<td>161.6 (5.0)</td>
<td>161.6 (5.0)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>51.8 (6.8)</td>
<td>51.8 (6.8)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>19.8 (2.3)</td>
<td>19.8 (2.3)</td>
</tr>
<tr>
<td>(TAAAA)(_8) alleles n(%)</td>
<td>2 (0.8)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>(TAAAA)(_9) alleles</td>
<td>76 (29.2)</td>
<td>76 (29.2)</td>
</tr>
<tr>
<td>(TAAAA)(_{10}) alleles</td>
<td>12 (4.6)</td>
<td>12 (4.6)</td>
</tr>
<tr>
<td>(TAAAA)(_{11}) alleles</td>
<td>104 (40.0)</td>
<td>104 (40.0)</td>
</tr>
<tr>
<td>(TAAAA)(_{12}) alleles</td>
<td>54 (20.8)</td>
<td>54 (20.8)</td>
</tr>
<tr>
<td>(TAAAA)(_{13}) alleles</td>
<td>12 (4.6)</td>
<td>12 (4.6)</td>
</tr>
</tbody>
</table>

IQR = interquartile range.

Figure 1. Distribution of SHBG gene alleles in girls with early menarche (group 1) and girls with late menarche (group 2).
its earliest phase starts in an androgen-dominated state, but during before and during early puberty compared with mid- and late puberty, it has been shown that there is a relatively transient hyperandrogenism that might have provided more insight into the underlying mechanism of the association that we observed. However, to be meaningful, the measurement of SHBG should have been done at the time.

One limitation of the study is absence of measurement of SHBG that might have provided more insight into the underlying mechanism for this is not yet identified (Treloar and Martin, 1990; Kaprio et al., 1995). We have previously reported an association of polymorphisms of the estrogen receptor (ERa) gene with the age of menarche (Stavrou et al., 2002). This was not confirmed by Gorai et al. (2003) who reported that estrogen-metabolizing gene polymorphisms and especially polymorphisms of CYP17 gene influence the onset of menarche. Other studies of CYP17 gene polymorphisms for possible association with menarche produced inconsistent results (Feigelson et al., 1997; Dunning et al., 1998; Weston et al., 1998; Haiman et al., 1999; Goodman et al., 2001; Lai et al., 2001; Ambrosone et al., 2003; Wu et al., 2003). Clearly, further studies are required to identify the genetic factors influencing the timing of menarche.

As a conclusion, this study provides evidence for a possible association of the pentanucleotide TAAAA repeat polymorphism in the promoter region of the SHBG gene with age at menarche. Differences at the age of menarche, determined by genetic factors, may also affect endocrine manifestations and influence the reproductive fitness during the female reproductive life.

References


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