Advanced onset of menarche and higher bone mineral density depending on vitamin D receptor gene polymorphism

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Abstract

Bone mineral density (BMD) at distal forearm, and weight and height of healthy Japanese girls aged 18–19 years were measured and their age at menarche was obtained through a questionnaire. A statistically significant association was found between BMD at distal radius and vitamin D receptor (VDR) gene polymorphism at the ApaI site. The age at menarche in the population with Aa genotype was significantly earlier than that in the aa population. In addition, BMD was significantly dependent on the earlier onset of menarche in the population with genotype Aa but not in the population with genotype aa. BMD was also positively associated with the body mass index (BMI) in the population with genotype Aa. Statistical analysis suggested a stronger effect of VDR genotype on age at menarche than on BMI. Thus, we show that VDR gene polymorphism advances the age at menarche and increases BMD in cooperation with age at menarche.

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Introduction

Vitamin D receptor (VDR) is a member of the ligand-dependent receptor superfamily mediating the signal of classical steroid hormones (1). VDR acts as a trans-acting factor that interacts with specific cis-acting elements termed VDREs at the upstream of many target genes involved in calcium metabolism and bone formation. The VDR gene contains nine exons extending over 43.5 kbp in chromosome 12 (2) and restriction fragment length polymorphism (RFLP) analysis indicated the presence of polymorphism alleles (3). The polymorphisms defined by the restriction enzymes BsmI (alternatively TaqI) and ApaI were reported to be strong genetic components of bone mineral density (BMD) accounting for as much as 80% of the adult BMD (4). As corrected by the authors themselves (5), however, the association between BMD and VDR genotype is now a controversial issue in the etiology of osteoporosis. A recent meta-analysis has concluded that there is a lack of significant differences in BMD between VDR gene polymorphisms (6). Nevertheless, it is clear that BMD is heritable since BMD in monozygotic (MZ) and dizygotic (DZ) twins is interclassically related (7).

There may be several explanations for the discrepancies in the studies of the relationship between BMD and the VDR genotype. First, the sample size and consequent statistical power of the studies were different. Secondly, there may be a bias in the association due to the population admixture. For example, a recent study on prepubertal American girls of Mexican descent showed an affirmative relation of ApaI and BsmI polymorphisms to BMD (8) while the opposite association of BsmI polymorphism had been reported for American premenopausal women (9). A twin study in Britain on postmenopausal women showed an affirmative influence of the TaqI polymorphism on BMD (10), whereas a study on a cohort of pre- and postmenopausal women in Scotland reported a significant negative association between BsmI polymorphism and BMD (11). These results suggest that the relationship depends on the age of the study population. Thirdly, linkage disequilibrium may be a possible explanation for the discrepancy. We can assume that the VDR gene locus itself may not be causally related to BMD, but is linked to a neighboring bone metabolism-related gene, and the low frequency of the cross-over between the VDR gene locus and this putative gene during meiosis may give the misleading interpretation that VDR gene polymorphism is related to BMD. Since such a linkage disequilibrium may be found in some populations but not in others, we need to accumulate information from various populations with different genetic backgrounds. Fourthly, we need to assume a complex interaction between the genetic background and environmental factors such as calcium intake and the experience of physical activity.

To clarify the controversial association between BMD and VDR genotype mainly from the second and third...
aspects described above, we selected a population of Japanese girls within a narrow range of the postpubertal period (aged 18–19 years). This study population showed a statistically significant association of BMD with the VDR gene polymorphism at the ApaI site but in the opposite direction to that originally reported by Morrison et al. (4). Their age at menarche was also statistically significantly advanced by the polymorphism. Probably due to the age of our study population (18–19 years), the BMD was clearly associated with the early onset of menarche. Interestingly, this association was clear in the Aa genotype but not in the aa genotype, suggesting that the VDR genotype affects BMD in cooperation with the age at menarche in addition to accelerating the onset of menarche.

**Subjects and methods**

One hundred and twenty 18–19-year-old girls participated in the study. All were healthy and had no history of diseases affecting bone metabolism. This study was approved by the college where they were students, and procedures for the study were explained to their parents and their informed consent was obtained.

BMD was measured at the ulnar and radius of the nondominant arm by dual energy X-ray absorptiometry (DXA, DTX200, Rødovre, Osteometer, Denmark). At the same time, weight and height were measured and the body mass index (BMI) was calculated. A self-administered questionnaire was used to assess the age at menarche and menstrual status.

DNA was extracted from white blood cells and a 740 base pair fragment of the vitamin D receptor gene in chromosome 12 (2) was amplified by the polymerase chain reaction (PCR). Detection of ApaI and TaqI sites was facilitated by amplification with one primer in intron 8 (5'-CAGAGCATGGACAGGGAGCAAG-3') (12) and the other in exon 9 (5'-GCAACTCCTCATGGCT-GAGGTCTCA-3') (12) producing a 740 bp fragment. Each sample was subjected to 30 amplification cycles in fast capillary using a Takara thermocycler (Takara, Kyoto, Japan). The RFLPs were coded as Aa (ApaI) or Tt (TaqI), where the upper case letter signifies absence of the site and the lower case signifies presence of the site.

The data were analyzed by the SPSS software package. Analysis of variance was used to examine the mean difference of BMD, age at menarche and BMI between VDR genotypes using multiple comparison procedures. Linear regression analysis was used to examine the relationships between BMD and age at menarche and between BMD and BMI. The associations of BMD with age at menarche and with BMI in each VDR genotype were examined by ANCOVA.

**Results**

The characteristics of the study population are summarized in Table 1.

![Table 1](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.4 (0.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.5 (5.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.2 (6.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.0 (2.3)</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.3 (1.0)</td>
</tr>
<tr>
<td>BMD of distal radius (g/cm²)</td>
<td>0.503 (0.049)</td>
</tr>
<tr>
<td>BMD of distal ulna (g/cm²)</td>
<td>0.418 (0.044)</td>
</tr>
<tr>
<td>BMD of distal forearm (g/cm²)</td>
<td>0.468 (0.044)</td>
</tr>
</tbody>
</table>

Apparently healthy Japanese girls aged 18–19 years participated in this study and their age was within a narrow range of the postpubertal period when the peak bone mass is expected to be attained. Age at menarche was obtained through a questionnaire and the participant’s memory of the fact was checked through an interview.

The RFLPs at the ApaI and TaqI sites in the VDR gene were determined as in Table 2. Since the frequency of haplotype t was only 0.09, the statistical analysis of the TaqI site polymorphism was not effective due to the uneven sample number of the TT, Tt and tt genotypes. On the other hand, an adequate frequency of haplotypes A and a of 0.28 and 0.78 respectively, revealed a statistically comparable population of the Aa and aa genotypes.

BMD at distal radius was associated with the ApaI site polymorphism and the value BMD in genotype Aa was statistically significantly higher than that in genotype aa (Table 3). The same trend was also observed for BMD at distal ulna but the difference was not significant. Whereas this result showed the association of BMD with ApaI site genotypes, it was in the opposite direction to that originally reported by Morrison et al. (4). A similar trend in the association of BMD to the VDR genotype was observed for the TaqI site polymorphism. The age at menarche in the population with the aa genotype was statistically higher than that of the population with Aa genotype (Table 3). The difference in the mean value was only 0.4 year but the Aa and aa genotypes showed discrete distribution of age at menarche (see Fig. 1). The
trend towards later age at menarche in the TT genotype compared with that in the Tt genotype was also observed but the uneven sample number failed to show any statistical significance. It is important to notice in Table 3 that BMI was not affected significantly by the genotype.

Linear regression analysis in Fig. 1 showed that higher BMD was significantly associated with earlier onset of menarche. An interesting observation is that the correlation was found only in the Aa genotype (slope $-0.023$, $P < 0.05$) but not in the aa genotype (slope $-0.0034$, not significant (NS)). The same analysis of the Tt and TT genotypes indicated a similar trend (not shown). These results indicate that the effect of the early onset of menarche on BMD is dependent on the VDR genotype. In other words, VDR genotype supports the effect of early onset of menarche on BMD via an unknown factor, in addition to accelerating the onset of menarche itself. The menstrual status (menstrual cycle) of the individuals interrogated through a questionnaire had no effect on this association.

As reported in other studies, BMD was also associated with BMI in our study population. We found that the effect of BMI on BMD was also dependent on the VDR genotype (Fig. 2). This suggested again that the VDR genotype supports the effect of BMI on BMD via unknown factor(s). In this case, however, the difference in the slope between genotype Aa and aa was not clear.

No significant correlation was found between BMI and age at menarche (data not shown). Nevertheless, both a higher BMI and earlier onset of menarche increased BMD depending on the VDR genotype. In Table 4, we examined the difference in BMD between genotypes Aa and aa by means of ANCOVA, using the age at menarche and/or the BMI as the adjusting variables. The difference was significant after adjusting for either the age at menarche or for the BMI, whereas it was not significant after adjusting for both age at menarche and BMI. This shows that both age at menarche and BMI are needed for the genotype-dependent increase in BMD.

Table 3 BMD, age at menarche and BMI according to vitamin D receptor genotype. Values are means with s.d. in parentheses.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>BMD (g/cm²)</th>
<th>Age at menarche (years)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Distal radius</td>
<td>Distal ulnar</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>7</td>
<td>0.502 (0.061)</td>
<td>0.405 (0.044)</td>
<td>12.0 (1.1)</td>
</tr>
<tr>
<td>Aa</td>
<td>53</td>
<td>0.515 (0.054)*</td>
<td>0.422 (0.050)</td>
<td>12.1 (1.0)*</td>
</tr>
<tr>
<td>aa</td>
<td>60</td>
<td>0.492 (0.039)</td>
<td>0.416 (0.039)</td>
<td>12.5 (1.0)</td>
</tr>
<tr>
<td>TT</td>
<td>99</td>
<td>0.499 (0.048)</td>
<td>0.416 (0.043)</td>
<td>12.4 (1.1)</td>
</tr>
<tr>
<td>Tt</td>
<td>20</td>
<td>0.518 (0.051)</td>
<td>0.426 (0.050)</td>
<td>12.0 (0.9)</td>
</tr>
<tr>
<td>tt</td>
<td>1</td>
<td>0.564</td>
<td>0.468</td>
<td>13.0</td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared with aa (ANOVA using multiple comparison procedure (Tukey’s method)).
Discussion

Having a population of healthy Japanese girls aged 18–19 years, we studied the relationship between VDR gene polymorphisms and BMD through a narrow window, viewing a specified period of postpubertal development, and found an opposite association of ApaI polymorphism. Considering that most previous studies had permitted a population admixture in terms of age distribution, this study is included among limited examples enrolling study populations with a specified age range. Sainz et al. studied a prepubertal population of American girls and showed an affirmative association of ApaI and BsmI polymorphisms with BMD (8). Ferrari et al. confirmed the affirmative association of BsmI polymorphism in prepubertal and adolescent girls but they could not find any association in older populations (13). A hasty interpretation of our results may conclude that the association switches from affirmative to opposite during growth in the prepubertal through postpubertal period. Before reaching such a conclusion, however, studies with a larger sample size are needed to cover those developmental stages when rapid bone formation occurs before the accumulation of environmental effects.

Linkage disequilibrium has been assumed to explain the controversial relationship between VDR gene polymorphisms and BMD (14). This situation is thought to arise because the mutation in the VDR gene first occurred in an individual with haplotype having a strong phenotype in BMD but not due to the VDR gene, and the mutation and the haplotype have remained in association due to their close proximity in the population derived from that individual. Because such a situation can be found in a limited population but not in others, the original observation by Morrison et al. in Australia may not be repeated in other populations. Nevertheless, this hypothesis hardly explains the discrepancy among Japanese populations since Tokita et al. (12) and Yamagata et al. (15) reported the affirmative association of the BsmI polymorphisms.

We found that the VDR gene polymorphism at the ApaI site is statistically significantly associated with the age at menarche. Since the correlation for the onset of menarche between MZ twins was significantly higher than between DZ twins (16), it is clear that menarche is influenced by genetic variations. However, the gene responsible for the age at menarche is not well understood except for the recent report on the CYP17 gene. Age at menarche among women with CYP17

Table 4 Mean (±S.D.) BMD of radius adjusted by age at menarche and/or BMI.

<table>
<thead>
<tr>
<th>Adjusted variable</th>
<th>Aa (g/cm²)</th>
<th>aa (g/cm²)</th>
<th>Difference in mean BMD (g/cm²) between Aa and aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.515 (0.054)</td>
<td>0.492 (0.039)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Menarche</td>
<td>0.512 (0.044)</td>
<td>0.494 (0.046)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>0.512 (0.044)</td>
<td>0.495 (0.046)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Menarche and BMI</td>
<td>0.510 (0.044)</td>
<td>0.497 (0.046)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.
Statistical analysis was performed by analysis of covariance (ANCOVA).
genotype Al/Al was shown to be statistically significantly later than in women with genotype A2/A2 (17). CYP17 gene codes for cytochrome P450/17α having both steroid 17α-hydroxylase and 17,20-lyase activities. Conversion from A1 to A2 genotypes creates an additional SP-1 type promoter 34 bp upstream from the initiation site of the translation, and may up-regulate the transcription of the CYP17 gene. Consequent up-regulation may affect the function of reproductive organs by altering steroid hormone metabolism. The VDR is distributed ubiquitously in many tissues including not only the principal sites of calcium metabolism but also reproductive organs such as ovary, oviduct and uterus (18). An interesting phenotype recently reported for the VDR gene-disrupted mice was the impaired development of the ovary (19). It is possible that VDR expressed in the reproductive organs affects their development and alters the age at menarche. A later age at menarche is known to be a determinant of osteoporosis in the postmenopausal period, and an earlier menarche decreases the risk probably by increasing the peak bone mass obtained in earlier life (20, 21). The age of 18–19 years of our study population is about the time of peak bone mass attained at individually different stages of post-pubertal maturation. Probably reflecting such a situation, increased BMD depended on early age of menarche in a significant fashion (Fig. 1). Our finding was that this dependency was observed mainly in the Aa but not in the aa genotypes. This shows that the VDR genotype not only accelerates the onset of menarche but also supports the effect of the early onset of menarche on BMD. An important question to be addressed here is what factor(s) is mediating this effect of the VDR genotype.

As reported by other investigators (22), BMD was positively correlated with BMI and we found that the correlation is also dependent on the VDR genotype (Fig. 2). BMI was distinct from age at menarche in that the association of the age at menarche with BMD depended on early age of menarche in a significant fashion (Fig. 1). Our finding was that this dependency was observed mainly in the Aa but not in the aa genotypes. We suggest that BMI may mediate, at least partially, the supporting effect of the VDR genotype on the association of the age at menarche with BMD. Conversely, the age at menarche may mediate the supporting effect of the VDR genotype on the association of BMI with BMD.

References


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