

Association Study in Taiwanese Girls with Precocious Puberty

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Abstract

Background: The timing of puberty has a genetic component. Recently, genome-wide association studies have identified that rs314280 on 6q21 (near the *LIN28B* gene) and rs2090409 on 9q31.2 (in an intergenic region) are associated with age at menarche. In this study, we aimed to determine whether the two loci were associated with the timing of puberty in Taiwanese girls.

Results: A total of 117 girls were divided into 2 groups: 1) precocious puberty (n=50); 2) normal control subjects (n=45). The genotype proportions and allele frequencies in both groups were not significantly different.

Conclusion: These data suggest that rs314280 and rs2090409 polymorphisms are not a useful marker for prediction of the susceptibility of precocious puberty.

Introduction:

Precocious puberty refers to the appearance of physical and hormonal signs of pubertal development at an earlier age than is considered normal. The timing of puberty has a genetic component. A history of early puberty in a parent or sibling is relevant and decreases the likelihood that early puberty has an organic cause. One study from Israel estimated that precocious puberty was familial in one fourth of cases and that the predominant mode of inheritance was autosomal dominant.¹ Twins studies estimate that 44-95% of the variance in age at menarche may be heritable.²

Recently, four genome-wide association studies analyses establish that common genetic markers near a height-related gene, *LIN28B* (6q21), are related to menarche.³⁻⁶ In addition, combined analysis with replication sets from Iceland, Denmark and the Netherlands found a significant association between rs314280 [T] on 6q21, near the *LIN28B* gene, and age at menarche. *LIN28B* stands out as a trigger for menarche. Furthermore, the strongest signal was also found at 9q31.2 with rs2090409.^{3,5} However, the recombination region containing rs2090409 includes only a hypothetical gene (BC039487).

In our previous study, we have used single nucleotide polymorphisms as a tool in genetic studies of polygenic disorders.⁷ In this study, we tested the hypothesis that genetic variation in the rs314280 on 6q21 (near the *LIN28B* gene) and rs2090409 on

9q31.2 (in an intergenic region) confer susceptibility to precocious puberty.

METHODS AND RESULTS

The study included Taiwanese children with central precocious puberty (n=50) and normal control subjects (n=45). This study was approved by the Ethics Committee of China Medical University Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity and geographic location of origin. Central precocious puberty subjects and the controls were both recruited from the midland regions of Taiwan. Diagnosis of central precocious puberty followed the criteria of the Lawson Wilkins Pediatric Endocrine Society. Patients with peripheral precocious puberty were excluded. All cases were from unrelated kindred.

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood using a DNA extractor kit (Magna Pure LC DNA Isolation Kit, Roche, Mannheim, German). Genotypes of two SNPs (rs314280 and rs2090409) at chromosome 6p21 and 9q31.2 were performed using the Taqman SNP genotyping assay (ABI: Applied Biosystems Inc. Foster City, CA, USA). The primers and probes of SNPs were from the ABI assay on demand (AOD) kit. Reactions were carried out according to the manufacturer's protocol. Briefly, polymerase chain reaction (PCR) was performed in the presence of 2× TaqMan

Universal PCR Master Mix (ABI, Foster City, CA, USA), assay mix (Assay ID C_1361832_10 and C_16094353_10, Applied Biosystems, USA) and genomic DNA (15 ng). After initial denaturation for 10 min at 95°C, 40 cycles were run, each consisting of denaturation (95°C for 15 s), and annealing (60°C for 60 s). The probe fluorescence signal detection was performed using the ABI Prism 7900 Real Time PCR System.

The results showed that genotype proportions and allele frequencies for rs314280 on 6q21 and rs2090409 on 9q31.2 in both groups were not significantly different (Table 1). For rs314280, proportions of C homozygote, C/T heterozygote and T homozygote were respectively as follows: in patients, 58%, 30% and 12%; in controls, 42%, 51% and 7%. The frequencies of allele C and T in patients were 73% and 27% and in controls were 68% and 32%. For rs2090409, proportions of G homozygote, G/T heterozygote, and T homozygote were respectively as follows: in patients, 32%, 42%, and 26%; in controls, 36%, 38%, and 27%. The frequencies of allele G and T in patients were 53% and 47% and in controls were 54% and 46%.

Discussion

Precocious puberty is related age at menarche and age. Several candidate genes, encoding regulators of sex steroid secretion and action, have been proposed to regulate the timing of puberty, however, no common variants have yet been

established. In this issue, four separate papers report genome-wide association studies for age at menarche and/or age at natural menopause (3-6). Within this set of papers, genome-wide association analyses establish that common genetic markers near a height-related gene, *LIN28B* (6q21), are related to menarche (7-10). The collective findings may open promising new avenues for research into the mechanisms underlying ovarian aging, however, the extent to which this result can be extended to other populations remains to be assessed.

Our data suggest that 6q21 (rs314280) and 9q31.2 (rs2090409) polymorphisms are not a useful marker for prediction of the susceptibility of precocious puberty. This result is not consistent with the previous study (3-6). The discrepancy may be due to different illness classifications, and racial and disease variations. However, owing to the case number limitations, the power of this study might be too low to emphatically deny the association. Further studies using larger samples are needed.

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Table 1. Genotypic and allelic frequencies for rs314280 on 6q21 and rs2090409 on 9q31.2 polymorphisms

	Patient with precocious puberty	Control	<i>P</i> value
	N=50 (%)	N=45 (%)	
6q21 (rs314280)			
Genotype			
CC	29 (58)	19 (42)	0.1045
CT	15 (30)	23 (51)	
TT	6 (12)	3 (7)	
Allele frequency			
C	73 (73)	61 (68)	0.4305
T	27 (27)	29 (32)	
9q31.2 (rs2090409)			
Genotype			
GG	16 (32)	16 (36)	0.9055
GT	21 (42)	17 (38)	
TT	13 (26)	12 (27)	
Allele frequency			
G	53 (53)	49 (54)	0.8420
A	47 (47)	41 (46)	

p-values were calculated by χ^2 test.