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Melatonin Pathway Genes and Breast Cancer risk among Chinese women

Sandra L.Deming[1], **Wei Lu**[2], **Alicia Beeghly-Fadiel**[1], **Ying Zheng**[2], **Qiuyin Cai**[1], **Jirong Long**[1], **Xiao-Ou Shu**[1], **Yu-Tang Gao**[3], and **Wei Zheng**[1]

[1]Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN

[2]Shanghai Center for Disease Control and Prevention, Shanghai, China

[3]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Abstract

Previous studies suggest that melatonin may act on cancer growth through a variety of mechanisms, most notably by direct anti-proliferative effects on breast cancer cells and via interactions with the estrogen pathway. Three genes are largely responsible for mediating the downstream effects of melatonin: melatonin receptors 1a and 1b (*MTNR1a* and *MTNR1b*), and Arylalkylamine N-acetyltransferase (*AANAT*). It is hypothesized that genetic variation in these genes may lead to altered protein production or function. To address this question, we conducted a comprehensive evaluation of the association between common single nucleotide polymorphisms (SNPs) in the *MTNR1a, MTNR1b*, and *AANAT* genes and breast cancer risk among 2,073 cases and 2,083 controls, using a two-staged analysis of genome-wide association (GWAS) data among women of the Shanghai Breast Cancer Study. Results demonstrate two SNPS were consistently associated with breast cancer risk across both study stages. Compared with *MTNR1b rs10765576* major allele carriers (GG or GA), a decreased risk of breast cancer was associated with the *AA* genotype (OR=0.78, 95% CI=0.62–0.97, p=0.0281). Although no overall association was seen in the combined analysis, the effect of *MTNR1a rs7665392* was found to vary by menopausal status (p-value for interaction=0.001). Premenopausal women with the *GG* genotype were at increased risk for breast cancer as compared to major allele carriers (*TT* or *TG*) (OR=1.57, 95% CI=1.07–2.31, p=0.020), while post-menopausal women were at decreased risk (OR=0.58, 95% 0.36–0.95, p=0.030). No significant breast cancer associations were found for variants in *AANAT*. These results suggest that common genetic variation in the *MTNR1a* and *1b* genes may contribute to breast cancer susceptibility, and that associations may vary by menopausal status. Given that multiple variants in high linkage disequibrium with *MTNR1b* rs76653292 have been associated with altered function or expression of insulin and glucose family members, further research may focus on clarifying this relationship.

Keywords

Melatonin; gene; polymorphism; breast cancer; risk

Correspondence to: Dr. Sandra Deming, Vanderbilt Epidemiology Center, Institute for Medicine and Public Health, Vanderbilt University School of Medicine, Eighth floor, Suite 800. 2525 West End Ave, Nashville, TN 3720-1738.

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Introduction

Melatonin is a hormone produced by the pineal gland and is released in response to photic information from the retina. In humans, melatonin secretion increases soon after exposure to darkness, peaks during the middle of the night, and then decreases over the second half of the night [1]. Both laboratory and population-based studies have implicated melatonin in cancer etiology and progression through a number of mechanisms, including direct anti-proliferative effects on breast cancer cells, interaction with the estrogen pathway, stimulation of the immune system, and interactions with estrogen and insulin pathway members. The melatonin receptors 1a and 1b (*MTNR1a and MTNR1b*, respectively) are largely responsible for mediating the downstream effects of melatonin, while arylalkylamine N-acetyltransferase (*AANAT)* is the major enzyme in melatonin synthesis, and controls the day/night rhythm in melatonin production in the pineal gland [2,3]. All three have been identified as potentially important players in mediating breast cancer risk [4–7]. Studies have demonstrated that polymorphisms in the melatonin receptors are associated with blood glucose levels, insulin secretion, rheumatoid arthritis and idiopathic scoliosis, suggesting a functional role for these variants [8–12]. It is suggested as possible due to altered protein production or function. To date, however, no studies of genetic variation in melatonin pathway genes and breast cancer risk have been conducted. Therefore, this study was undertaken to comprehensively characterize the role of common genetic variation in relation in the *MTNR1a, MTNR1b* and *AANAT* genes to breast cancer risk among participants of the Shanghai Breast Cancer Study (SBCS).

Materials and Methods

Study participants were from the SBCS, a large, two-stage, population-based case-control study of incident breast cancer among women of urban Shanghai which has previously been described in detail [13,14]. Cases were identified via the use of a rapid-case ascertain system (Stage I), and the Shanghai Cancer Registry (Stages I and II). Controls were randomly selected from the Shanghai Resident Registry. Stage I participants included women aged 25–65 who were recruited from August 1996 to March 1998. Stage II participants were recruited from April 2002 to February 2005 and included women aged 25–70. In-person interviews were completed for 1,459 (91.1%) cases and 1,556 (90.3%) controls from Stage 1, and 1,989 cases (83.7%) and 1,989 controls (70.4%) from Stage 2. Blood samples were donated by 1,193 cases (81.8%) and 1,310 controls (84.2%) from Stage 1, and blood or buccal cell samples were donated by 1,932 (97.1%) cases and 1,857 (93.4%) controls from Stage 2. Institutional Review Board approval was garnered from relevant institutions in both China and the United States.

The Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix) was utilized to characterize a total of 8 SNPs in the *MTNR1a* gene, 9 SNPS in the *MTNR1b* gene, and 6 SNPs in the *AANAT* gene. These haplotype tagging SNPs were selected based on Han Chinese HapMap Project data using the Tagger program to capture SNPs with a minor allele frequency (MAF) of at least 0.05 with an $r^2 \geq 90\%$. Blinded duplicated samples and quality controls were included in the genotyping. All SNPs included in this analysis had concordance rates of at least 95% among duplicates within each platform, and an average call rate of 99.8% Hardy-Weinberg equilibrium (HWE) was assessed among controls by comparing the observed-to-expected genotype frequencies using χ^2 test. Logistic regression was utilized to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the association between genotype and breast cancer risk, while adjusting for age, education, and study stage when appropriate. Additive, dominant, recessive, and allelic models were considered. Multiplicative interactions between genetic variants and menopausal status were evaluated by comparing estimates of effect across strata for

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heterogeneity, as well as the statistical significance of interactions terms included in logistic regression models. The Adaptive Rank Truncated Product (ARTP) Method was utilized to produce an adjusted P-value for minimum P-value (*MinP*) to limit the effect of multiple comparisons when testing the null hypothesis that a collection of SNPs within a given gene is not associated with breast cancer risk [15]. Ten truncation points and 3,000 permuted datasets were used in the calculations. All statistical tests were 2-tailed and $p \leq 0.05$ values were considered statistically significant. Analyses were conducted using SAS v9.2 (SAS Institute, Cary, NC).

Results

This study included a total of 4,156 women, of which 2,123 were enrolled in Stage I and 1,943 were enrolled in stage II. Women in both study stages were comparable with respect to most demographic characteristics and breast cancer risk factors (Table 1). As expected, cases reported a significantly earlier age at menarche and older age at menopause. Additionally, cases were more like to have breast cancer among a first-degree family member, a higher body mass index (BMI), a higher waist-to-hip ratio (WHR), and were less likely to engage in regular physical activity as compared to controls.

A total of 23 haplotype-tagging SNPs were genotyped in this study; eight were excluded from the analysis due to minor allele frequencies (MAF) of less than 5% (rs16968964, rs11077821, rs9895222, rs11077823, rs4601728, rs12804291, rs12272268, rs12275765), and one (*rs1080964)* was excluded due to deviation from HWE among controls. Estimates of effect derived under additive, dominant, and recessive models are presented in Table 2 for all women combined. Although a number of SNPS were significantly associated with breast cancer risk in the stage-specific analysis (data not shown), only two SNPS, *rs7665392* and *rs10765576*, were found to have associations consistent within both study stages. However, p-values generated via the ARTP method to adjust for multiple comparisons suggest that among all women, there does not appear to be a significant association between the collection of SNPs in any single melatonin pathway gene and breast cancer risk (p-value = 0.30, 0.65, and 0.35 for *MTNR1a, MTNR1b*, and *AANAT* respectively). Given that previous studies have suggested that melatonin pathway effects may be mediated via interaction with estrogen pathway members, we conducted a stratified analysis to determine if the association between genetic variants and breast cancer risk differed by menopausal status (Table 3). With the exception of 1 variant (*rs7665392)*, we found no difference in effect by menopausal status under additive, dominant, or recessive models (data not shown).

Lastly, given evidence for an interaction between menopausal status and *MTNR1a rs7665392*, we examined the association between this SNP and breast cancer risk among other measures of estrogen exposure (Table 4). Although there were no significant interactions between age at menarche, years of menstruation, and total months of breast feeding and the *rs7665392* SNP in relation to breast cancer risk, the direction of the associations were not inconsistent with those found for menopausal status.

Discussion

We systematically evaluated common genetic variation among melatonin pathway genes in relation to breast cancer risk in a large, two-staged, population-based study of Chinese women. A total of 23 SNPS were genotyped in the *MTNR1a, MTNR1b*, and *AANAT* genes. We found that *MTNR1a* polymorphism *rs10765576* was significantly associated with altered breast cancer risk under a recessive model. While there was no evidence for an overall effect of the *MTNR1a rs7665392* with breast cancer risk, we found evidence of a significant interaction (p=0.001) between menopausal status and the *rs7665392*

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polymorphism. No association with breast cancer risk was found for SNPs in the *AANAT* gene.

To our knowledge, this investigation is the first to evaluate the association between common genetic variants in the aforementioned genes and cancer risk. Previous studies of genetic variation in the *MTNR1a, MTNR1b*, and *AANAT* genes have assessed their association with outcomes such as scoliosis, major depression, diabetes, blood glucose levels, polycystic ovary syndrome, and schizophrenia. There is a single published investigation of *AANAT* polymorphisms in which Wang and colleagues found no association with idiopathic scoliosis in Han Chinese [16]. Similarly, epidemiologic evidence is limited for the role of genetic variants in the *MTNR1a* gene and disease outcome. Haplotypes in the *MTNR1a* gene were found to be associated with non-tardive dyskinesia among schizophrenic inpatients receiving long-term antipsychotic treatment [17]. Another research group found no evidence for an association between *MTNR1a* polymorphisms and adolescent idiopathic scoliosis [18].

Our findings of a significant interaction between *MTNR1a* and menopausal status are consistent with *in vitro* data. Girgert and colleagues reported that membrane-bound melatonin receptor MT1 (*MTNR1a*) down-regulates estrogen responsive genes in breast cancer cells and that the more melatonin receptor the cell line expressed, the stronger the reduction in estradiol-induced genes, including p53, $p21^{WAF}$, and *c-myc* [19]. We might hypothesize that the *rs7665392* variant, or a yet unidentified functional variant in high linkage disequilibrium with this polymorphism, leads to decreased expression or function of the *MTNR1a* receptor and subsequently increased levels of estradiol-induced genes among pre-menopausal women with higher levels of estrogen. This interaction with the estrogen pathway and mechanism for increase in estradiol-induced genes is a plausible explanation for how the *rs7665392* variant might alter breast cancer risk. Furthermore, there have been six non-synonymous low frequency (<5% MAF) variants identified within the *MTNR1a* receptor which have been shown to dramatically alter function of the receptor, although their linkage with other known SNPs is not yet well characterized [20]. Further investigation to elucidate the function of the *rs7665392* or its linkage to other potentially function variants is warranted.

In contrast to the *AANAT* and *MTNR1a* genes, there is more extensive characterization of *MTNR1b* variants. That majority of the reports have focused on the relationship with insulin-related outcomes, sleep disturbances, and scoliosis, with no reports of cancer risk as an outcome. An investigation among Han Chinese women found an association between both the *rs10830963* minor allele genotypes and minor alleles and increased predisposition for polycystic ovary syndrome (PCOS), and was also associated with higher fasting plasma glucose concentrations [21]. The *rs10830963* polymorphism, as well as the *rs10830962* polymorphism that is frequently reported in high linkage with it in other studies, has also been found to be significantly associated with impaired fasting plasma glucose, reduced β-cell function, and increased diabetes risk [22–29]. However, neither the *rs10830963* nor *rs10830962* appeared to play a role in breast cancer risk in our population. There have been a number of additional SNPs in the *MTNR1b* gene that have also been identified to play a role in pre-diabetes. Although there is no functional information in the literature regarding the SNP we found to be associated in this study (*rs10765576*), this variant is in high linkage (r^2=0.97) with the *rs4753426* variant, which was significantly associated with impaired fasting glucose and b-cell function in another study [22].

Given that a number of polymorphisms in the *MTNR1b* gene have been associated with altered function of insulin pathway member, it is not necessarily a concern that we did not observe associations with breast cancer risk across all the variants we evaluated in this

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study. It is possible that some polymorphisms have a greater effect on function than others. Additionally, the SNPs that have been found to be associated with altered function of insulin pathway members are generally only in moderate linkage disequilibrium, and thus do not all reflect the same underlying association signal [30]. Given more recent evidence of the role of insulin and related pathway members in breast cancer etiology, it is a plausible mechanism by which variants in the *MTNR1b* pathway may alter breast cancer risk.

Study strengths included a large, two-stage, population-based study design. The large sample size also allowed for subgroup analysis by menopausal status. In terms of limitations, infrequent variation (<5% MAF) among our study population with respect to the SNPs included in the Affy Chip 6.0 for the *AANAT* gene precluded full characterization of its disease associations. Fine mapping to further assess low frequency SNPs in relationship to breast cancer risk in this gene is warranted, as we cannot rule out that one of these low frequency variants may be associated with breast cancer risk. Furthermore, given evidence of significant differences in circadian and melatonin genetic variations among worldwide populations [31], these results should be confirmed in other ethnic groups.

In summary, results from these analyses suggest that common genetic variation in the *MTNR1a* and *1b* genes may contribute to breast cancer susceptibility, and that the effect associated with the *rs7665392* polymorphism may vary by menopausal status. Given that previous studies have demonstrated that a number of variants in the *MTNR1b* genes are associated with altered function or expression of insulin and glucose family members, and this pathway has been implicated in breast cancer risk, further research may focus on clarifying the potential relationship between melatonin pathway genes, insulin pathway, and breast cancer risk.

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Table 1

Shanghai Breast Cancer Study participants genotyped for melatonin pathway genes.

Characteristics	Stage I (n=2,213)			Stage II (n=1,943)		
	Cases (n=1,104)	Controls (n=1,109)	P value	Cases (n=969)	Controls (n=974)	P value
Demographic Factors						
Age (years)	47.9 ± 7.9	48.2 ± 8.3	0.380	51.4 ± 8.3	51.4 ± 8.2	0.904
Education (less than middle school)	133 (12.1%)	114 (10.3%)	0.173	119 (12.3%)	91 (9.3%)	0.002
Postmenopausal	365 (33.1%)	408 (36.8)	0.066	441 (45.5%)	459(47.1%)	0.476
Ever used oral contraceptives	243 (22.0%)	240 (21.6%)	0.823	175 (18.1%)	180 (18.5%)	0.819
Reproductive risk factors						
Age at menarche (years)	14.5 ± 1.6	14.7 ± 1.7	<0.001	14.5 ± 1.7	14.7 ± 1.8	0.004
Age at menopause (years)[a]	48.1 ± 4.7	47.3 ± 5.0	0.031	48.7 ± 4.4	48.0 ± 4.5	0.027
Parity						
Nulliparious	54 (4.9%)	42 (3.8%)	0.21	47 (4.9%)	38 (3.9%)	0.03
1 birth	704 (63.8%)	688 (62.0%)		705 (72.8%)	669 (68.7%)	
2 or more births	346 (31.3%)	379 (34.2%)		217 (22.3%)	267 (27.4%)	
Other risk factors						
First degree relative with breast cancer	36 (3.3%)	29 (2.6%)	0.290	55 (5.7%)	33 (3.4%)	0.015
Body mass index (kg/m^2)[a]	24.2 ± 3.7	24.7 ± 3.5	0.706	24.7 ± 3.2	24.0 ± 3.4	0.257
Waist-Hip ratio	0.81 ± 0.06	0.80 ± 0.06	0.007	0.84 ± 0.05	0.82 ± 0.06	<0.0001
Regular physical activity	206 (18.7%)	291 (26.2%)	<0.0001	308 (31.8%)	338 (34.7%)	0.173

Continuous variables: mean values ± standard deviation, *P* value from *t*-tests;

Categorical variables: numbers and percentages, *P* values from χ^2 test

[a]Among postmenopausal women

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Table 2

MTNR1a, MTNR1b, and *AANAT* SNPs and breast cancer risk among all women, the Shanghai Breast Cancer Study

Gene	SNP	MAF[a]	HWE[b]	OR-het[c]	OR-hom[c]	P-trend	OR-dominant[c]	P-dominant	OR-recessive[c]	P-recessive
MTNR1a	rs6838290	0.0708	0.25	1.10(0.91– 1.32)	0.64(0.26– 1.54)	0.6119	1.07(0.89– 1.29)	0.4485	0.63(0.26– 1.52)	0.3034
	rs2165667	0.3168	0.29	1.04(0.91– 1.18)	1.08(0.88– 1.32)	0.4125	1.05(0.93– 1.18)	0.4513	1.06(0.87– 1.29)	0.5805
	rs6847693	0.3890	0.57	1.00(0.87– 1.15)	1.15(0.95– 1.39)	0.2130	1.04(0.91– 1.19)	0.5516	1.15(0.97– 1.36)	0.1128
	rs2165666	0.3176	0.23	1.03(0.91– 1.17)	1.07(0.88– 1.32)	0.4604	1.04(0.92– 1.18)	0.5253	1.06(0.87– 1.29)	0.5698
	rs7665392	0.2260	0.45	0.94(0.82– 1.08)	1.05(0.78– 1.41)	0.6593	0.95(0.84– 1.08)	0.4619	1.07(0.80– 1.43)	0.6424
	rs12642043	0.6512	0.88	0.91(0.75– 1.10)	0.86(0.71– 1.04)	0.1204	0.88(0.74– 1.06)	0.1742	0.93(0.82– 1.05)	0.2229
	rs4861722	0.2111	0.14	1.11(0.97– 1.27)	1.17(0.88– 1.54)	0.0792	1.12(0.99– 1.27)	0.0798	1.13(0.86– 1.48)	0.3981
	rs10030173	0.4819	0.31	0.98(0.84– 1.13)	0.89(0.74– 1.06)	0.2007	0.95(0.82– 1.09)	0.4505	0.90(0.78– 1.05)	0.1741
MTNR1b	rs10830962	0.4287	0.95	1.08(0.94– 1.24)	1.04(0.87– 1.24)	0.5484	1.07(0.94– 1.22)	0.3133	0.99(0.84– 1.16)	0.8924
	rs10765576	0.2948	0.09	1.04(0.91– 1.19)	0.79(0.63– 0.99)	0.2823	0.99(0.87– 1.12)	0.8774	0.78(0.62– 0.97)	0.0281
	rs10830963	0.4224	0.61	1.14(0.99– 1.32)	1.04(0.86– 1.25)	0.4252	1.12(0.97– 1.28)	0.1133	0.96(0.81– 1.13)	0.6251
	rs4611171	0.3023	0.54	0.97(0.85– 1.10)	0.85(0.68– 1.06)	0.2020	0.95(0.84– 1.07)	0.3818	0.86(0.69– 1.07)	0.1715
AANAT	rs8150	0.3547	0.46	1.07(0.93– 1.22)	1.16(083 1.39)	0.5699	1.09(0.96– 1.24)	0.2038	1.12(0.92– 1.35)	0.2525
	rs3826287	0.3474	0.96	1.10(0.96– 1.26)	1.19(0.97– 1.46)	0.3164	1.12(0.98– 1.27)	0.0869	1.13(0.93– 1.36)	0.2274

[a]Minor allele frequency among genotyped controls

[b]Hardy-Weinberg equibrium among genotyped controls

[c]ORs adjusted for age, education, and study stage.

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Table 3

Selected *MTNR1a* and *MTNR1b* SNPS and breast cancer risk, by stage and menopausal status, the Shanghai Breast Cancer Study

	cases	control	MAF[a]	OR-additive (AB)[b]	OR-additive (BB)[b]	p-trend	OR-recessive[b]	P-recessive
MTNR1a rs7665392								
Stage I	958	962	0.2443	0.77(0.64– 0.94)	0.80(0.54– 1.18)	0.0150	0.88(0.60– 1.29)	0.5074
Stage II	966	974	0.2079	1.15(0.95– 1.38)	1.47(0.93– 2.33)	0.0476	1.40(0.89– 2.20)	0.1514
Combined	1924	1936	0.2260	0.94(0.82– 1.08)	1.05(0.78– 1.41)	0.6593	1.07(0.80– 1.43)	0.6424
Premenopausal (combined)	1191	1141	0.2217	0.91(0.77– 1.08)	1.52(1.03– 2.24)	0.5720	1.57(1.07– 2.31)	0.0198
Premenopausal stage I	665	626	0.2188	0.78(0.62– 0.99)	1.18(0.73– 1.90)	0.3541	1.29(0.80– 2.06)	0.2954
Premenopausal stage II	526	515	0.2068	1.10(0.85– 1.42)	2.42(1.23– 4.76)	0.0446	2.33(1.20– 4.56)	0.0130
Postmenopausal (combined)	733	795	0.2321	1.00(0.81– 1.24)	0.58(0.35– 0.96)	0.1864	0.58(0.36– 0.95)	0.0303
Postmenopausal stage I	293	336	0.2634	0.76(0.55– 1.07)	0.33(0.15– 0.73)	0.0043	0.37(0.17– 0.80)	0.0113
Postmenopausal stage II	440	459	0.2092	1.21(0.92– 1.61)	0.87(0.45– 1.70)	0.4514	0.81(0.42– 1.57)	0.5402
MTNR1b rs10765576								
Stage I	959	963	0.3063	0.94(0.78– 1.13)	0.78(0.57– 1.08)	0.1489	0.80(0.59– 1.10)	0.1672
Stage II	967	974	0.2834	1.16(0.96– 1.40)	0.79(0.56– 1.11)	0.9937	0.74(0.53– 1.03)	0.0757
Combined	1926	1937	0.2948	1.04(0.91– 1.19)	0.79(0.63– 0.99)	0.2823	0.78(0.62– 0.97)	0.0281
Premenopausal (combined)	1193	1141	0.2958	0.99(0.83– 1.17)	0.73(0.54– 0.98)	0.1203	0.73(0.55– 0.98)	0.0339
Postmenopausal (combined)	733	796	0.2933	1.13(0.91– 1.39)	0.90(0.62– 1.32)	0.7961	0.85(0.59– 1.23)	0.3903

[a]Minor allele frequency among genotyped controls

[b]Model of effect: additive: AB or BB versus AA, or recessive: BB versus AA and AB; *AA* major allele homozygotes, *AB* heterozygotes, *BB* minor allele homozygotes. Major/minor alleles as determined by allele frequency among genotyped controls. ORs adjusted for age, education, and study stage.

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Table 4

Association between *MTNR1a rs7665392* and breast cancer risk by various estrogen-related factors in the combined dataset: The Shanghai Breast Cancer Study

	OR-recessive[a]	$P_{interaction}$
Characteristic or Risk Factor		
Menopausal status		0.001
Premenopausal (combined)	1.57(1.07– 2.31)	
Postmenopausal (combined)	0.58(0.36– 0.95)	
Age at menarche		0.58
Age 13 or less	1.15 (0.71–1.86)	
Greater than 13	0.96 (0.70–1.32)	
Years of menstruation		0.90
Greater than 30 years	1.05 (0.74–1.48)	
30 years or less	0.99 (0.66–1.48)	
Total Breastfeeding (months)		0.13
12 months or less	1.17 (0.85–1.62)	
Greater than 12 months	0.77 (0.48–1.23)	

[a]Model of effect: recessive: BB versus AA and AB; *AA* major allele homozygotes, *AB* heterozygotes, *BB* minor allele homozygotes. ORs adjusted for age, education, and study stage.