Interactions between Genetic Variants in AMH and AMHR2 May Modify Age at Natural Menopause

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Abstract

The onset of menopause has important implications on women’s fertility and health. We previously identified genetic variants in genes involved in initial follicle recruitment as potential modifiers of age at natural menopause. The objective of this study was to extend our previous study, by searching for pairwise interactions between tagging single nucleotide polymorphisms (tSNPs) in the 5 genes previously selected (AMH, AMHR2, BMP15, FOXL2, GDF9). We performed a cross-sectional study among 3445 women with a natural menopause participating in the Prospect-EPIC study, a population-based prospective cohort study, initiated between 1993 and 1997. Based on the model-based multifactor dimensionality reduction (MB-MDR) test with a permutation-based maxT correction for multiple testing, we found a statistically significant interaction between rs10407022 in AMH and rs11170547 in AMHR2 (p = 0.019) associated with age at natural menopause. Rs10407022 did not have a statistically significant main effect. However, rs10407022 is an eQTL SNP that has been shown to influence mRNA expression levels in lymphoblastoid cell lines. This study provides additional insights into the genetic background of age at natural menopause and suggests a role of the AMH signaling pathway in the onset of natural menopause. However, these results remain suggestive and replication by independent studies is necessary.

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Introduction

The timing of the end of a women’s reproductive life has important health implications. An early onset of menopause is associated with a higher risk of cardiovascular diseases, osteoporosis, and overall mortality, whereas a later menopausal age may increase the risk of breast, ovarian, and endometrial cancer [1–4]. The underlying biological mechanisms for these associations remain poorly understood and much effort has been devoted to explain the observed variation in age at natural menopause (ANM) in an attempt to comprehend the etiology of these complex traits. The age at which menopause occurs varies between 40 to 60 years, with an average of 50–51 years in women of Northern European descent [5,6]. Numerous studies focused on lifestyle and reproductive factors in association with ANM. Some evidence for an association with ANM has been observed for smoking, parity (i.e., rs2002555: $\beta = 0.38, p = 0.005$ for parous women; $\beta = -0.22, p = 0.58$ for nulliparous women and rs11170547: $\beta = 0.41, p = 0.001$ for parous women; $\beta = -0.38, p = 0.44$ for nulliparous women). In the present study, we aim to extend the previous study by exploring gene-gene interactions among genes DNA repair [9–11]. Despite the large efforts made in unraveling the genetic background of ANM, only a small part can be explained through genetic factors identified so far. Most studies investigated the effect of only one SNP at a time, while it is obvious from biological studies that biological processes are influenced by multiple genes in complex networks [12]. Investigating gene-gene interactions might be a first step towards complex interaction analysis.

In a previous study, we investigated genetic variants in genes involved in initial follicle recruitment in association with ANM among 3445 Dutch women participating in Prospect-EPIC [13]. In that study, we observed an association between ANM and two single nucleotide polymorphisms (SNPs) in AMHR2 (rs2002555 ($\beta = 0.30, p = 0.021$) and rs11170547 ($\beta = 0.31, p = 0.049$)), and one SNP in BMP15 (rs6521896 ($\beta = 0.41, p = 0.007$)) [13]. Moreover, we found that the two SNPs in the AMHR2 gene were associated with age at natural menopause in interaction with parity (i.e., rs2002555: $\beta = 0.38, p = 0.005$ for parous women; $\beta = -0.22, p = 0.58$ for nulliparous women and rs11170547: $\beta = 0.41, p = 0.001$ for parous women; $\beta = -0.38, p = 0.44$ for nulliparous women). In the present study, we aim to extend the previous study by exploring gene-gene interactions among genes.
involved in initial follicle recruitment in association with ANM. In addition, we aim to further explore gene-environment interactions between these genes and parity, smoking and BMI.

Materials and Methods

Ethics Statement
The study was approved by the Institutional Review Board of the University Medical Center Utrecht. All women signed informed consent.

Study Design
Prospect-EPIC is one of the two Dutch prospective cohort studies participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) which is a multi-center cohort study including 10 European countries [14]. Between 1993 and 1997, a total of 17,357 women aged 50–69 years, living in Utrecht and vicinity were recruited through the national breast cancer screening program. At recruitment, all women completed a questionnaire with detailed questions on reproductive factors, physical activity, smoking, education level, and other lifestyle-related factors. Moreover, all women underwent physical examination and donated a 30-ml nonfasting blood sample. A full description of the study design and cohort has been published elsewhere [15].

Study Population
We describe an extension of a previous study by Voorhuis et al. for which participant selection has been described in detail [13]. Briefly, women were excluded if they were pre- or perimenopausal at time of enrollment (n = 3,497), if they experienced a surgical menopause (n = 4,449), if they used hormone therapy (n = 2,161), if they were younger than 58 years at inclusion (n = 2248), when menopausal status or age was unknown (n = 1,194), or when buffy coat samples were missing or DNA extraction failed (n = 192). Eventually, a total of 3,616 postmenopausal women with a known ANM were eligible for the current study.

Outcome Measure
ANM was extracted from the baseline questionnaire. Natural menopause was defined according to the World Health Organization as amenorrhea for at least 12 consecutive months without other obvious reasons [16].

Laboratory Methods
Methods of blood collection, DNA extraction and genotyping have been described in detail [13]. Duplicate samples were included to assess the quality of the genotyping process. Women with a call rate smaller than 95% were excluded (n = 171). The average genotyping success rate in the remaining 3445 samples was 99.3%.

Gene and SNP Selection
The selection procedure for genes and SNPs has been described in detail previously [13]. A total of 23 tagging SNPs were selected among 5 genes involved in initial follicle recruitment: rs10407022, rs7249235, rs733846, rs886363, rs3746158, and rs4806834 in AMH; rs2002555 and rs11170547 in AMHR2; rs3810682, rs6521896, rs17249566, rs5961233, and rs3897937 in BMP15; rs7641989, rs13064974, rs11924939, and rs10804661 in FOXL2; and rs10491279, rs254286, rs803224, rs4705974, rs30177, and rs1174063 in GDF9. We excluded two SNPs with a minor allele frequency smaller than 0.05 (i.e., rs4806834 and rs17249566), leaving a total of 21 SNPs. For the investigation of gene-gene interactions it should be avoided to include SNPs that are in linkage disequilibrium (LD) with each other in order to prevent spurious interactions. Therefore, we used PLINK to generate a pruned subset of SNPs considering a window of 21 SNPs, a shift of 1 SNP forward, and an r² of 0.75. The r² of 0.75 was advised by the developers of MB-MDR (personal communication). None of the SNPs were removed based on these parameters.

Data Analysis
Deviation from HWE was tested in PLINK v1.07 using a X² test with 1 degree of freedom. We corrected for age at inclusion by using rank-transformed age-adjusted residuals for ANM (GenA-BEL v1.6–7). Missing genotypes were imputed using BEAGLE v3.3.2.

All possible pairwise interactions (n = 210) were investigated using model-based multifactor dimensionality reduction (MB-MDR) v2.7.5 [17,18]. MB-MDR is an extension of the multifactor dimensionality reduction (MDR) method, a nonparametric exhaustive data mining method that considers all possible interactions between SNPs and classifies individuals into high and low risk groups [19]. MB-MDR, in contrast to MDR, is capable of analyzing quantitative traits and is able to adjust for main effects. Moreover, it introduces an additional ‘no evidence’ group. A full description of MB-MDR is available in references [17] and [18]. As suggested by the authors, we adjusted for main effects by adjusting for the lower-order effects of the SNPs in the SNP-pair under investigation using a co-dominant coding scheme. This method provides the best balance between type I error and power [17]. Multiple testing was accounted for by adopting a permutation-based maxT correction with 999 replicates.

Gene-environment interactions were evaluated using MB-MDR by including the environmental factor of interest as a categorical variable in the MB-MDR analysis. We investigated interactions between all SNPs and parity (parous [yes/no]), smoking (never, current, former), and BMI (<20, 20–25, ≥25).

Results
Characteristics of the 3445 women in our study cohort have been described previously [13]. Briefly, the mean age at inclusion and at natural menopause were 63 (SD: 3.4) and 50 years (SD: 4.2), respectively. The majority of women delivered one or more children (84.6%). Only 34.5% used oral contraceptives. Half of women were ever smokers (50.7%).

No significant deviations from Hardy-Weinberg equilibrium were observed. Results from the single SNP analysis have been published previously [13]. Interaction results for the top 10 SNP-SNP interaction models are presented in Table 1. The interaction between rs1170547 in AMHR2 and rs10407022 in AMH was statistically significant after correction for multiple testing (p = 0.019).

We also tested the interaction between each SNP and parity (parous [yes/no]), smoking (never, current, former), and BMI (<20, 20–25, ≥25). After correction for multiple testing no significant gene-environment interaction was observed. Our MB-MDR analysis did thus not replicate the previously observed interaction between AMHR2 and parity based on linear regression.

Discussion
In this large cross-sectional study we investigated interactions between 21 SNPs in genes involved in initial follicle recruitment in association with ANM. We observed a statistically significant
pairwise interaction between rs10407022 in AMH and rs11170547 in AMHR2 after permutation-based maxT correction. No gene-environment interactions were observed between these SNPs and parity, smoking or BMI. The present study is the first study investigating interactions between these 5 genes involved in initial follicle recruitment in relation to ANM. We previously observed statistically significant associations between the two SNPs that tag the gene encoding the AMH receptor (rsAMHR2, rs2002555 and rs11170547) and ANM [13]. No associations for tSNPs in AMH with ANM were found. In the present study we extended this study and searched for pairwise interactions between the SNPs in the genes previously selected. We found an interaction between SNPs in AMH gene and its receptor gene AMHR2. This might imply that complex interactions between these genes play a role in ovarian aging and thus in onset of menopause. However, this is the first report of an interaction between AMH and AMHR2, therefore, replication by independent studies is necessary to confirm these findings.

One of the SNPs involved in this interaction, rs10407022 in the AMH gene, is an expression quantitative trait locus (eQTL) for AMHR2. This SNP is the only known eQTL SNP in these genes [20].

Anti-Müllerian hormone (AMH), produced solely by small, growing follicles in the ovary, is a validated biomarker of ovarian aging, as serum levels of this hormone are strongly correlated with growing follicles in the ovary, is a validated biomarker of ovarian aging. Moreover, this missense mutation is predicted by SIFT to have damaging protein function [22]. We have shown that this SNP by itself does not influence ANM, but that it may modify AMH signaling pathway in the onset of natural menopause. Interestingly, this SNP is the only known eQTL SNP in these genes [20].

Later, the previously observed interactions between parity and SNPs in AMHR2 using linear regression models [13]. This interaction was a replication of a finding by Kevenaar et al. [25]. The lack of replication in the present study might be attributed to either the different parameterization in linear regression (additive models) compared to MB-MDR (non-parametric) or to the very stringent correction for multiple testing used with MB-MDR. On the other hand, the previously observed interactions between parity and SNPs in AMHR2 might be false positive findings. In fact, a clear biological mechanism for these interactions has not been found.

In conclusion, we observed a pairwise interaction between 2 SNPs in AMH and AMHR2 in association with ANM. More studies are needed to provide additional evidence for a role of the AMH signaling pathway in the onset of natural menopause.

Author Contributions
Conceived and designed the experiments: MGMB MV YTvdS PHMP LJS MJCE FJB NCO-M. Analyzed the data: MGMB MV NCO-M. Wrote the paper: MGMB MV YTvdS PHMP LJS MJCE FJB NCO-M.

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