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TNFRSF11A and *TNFSF11* are associated with age at menarche and natural menopause in white women

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Abstract

OBJECTIVE—Menarche and menopause mark lower and upper limits of the female reproductive period. Timing of these events influences female's health in later life. The onsets of menarche and menopause have a strong genetic basis. We tested two genes, *TNFRSF11A* (*RANK*) and *TNFSF11* (*RANKL*), for their association with age at menarche (AM) and age at natural menopause (ANM).

METHODS—Nineteen SNPs of *TNFRSF11A* and 12 SNPs of *TNFSF11* were genotyped in a random sample of 306 unrelated white women. This sample was analyzed for association of the SNPs and common haplotypes with AM. Then a subsample of 211 females with natural menopause was analyzed for association of both genes with ANM. Smoking, alcohol intake and duration of lactation were applied as covariates in the association analyses.

RESULTS—Three polymorphisms of *TNFSF11* were associated with AM: rs2200287 ($P = 0.005$), rs9525641 ($P = 0.039$), and rs1054016 ($P = 0.047$). Two SNPs of this gene, rs346578 and rs9525641, showed association with ANM ($P = 0.007$ and $P = 0.011$, respectively). Two SNPs of *TNFRSF11A*, were associated with AM (rs3826620, $P = 0.022$) and ANM (rs8086340, $P = 0.015$). Multiple SNP/SNP and SNP/environment interaction effects on AM and ANM were detected for both genes. One polymorphism of *TNFRSF11A*, rs4436867, was not directly associated with either trait, but indicated significant interactions with four *TNFSF11* polymorphisms on ANM. Two other *TNFRSF11A* polymorphisms, rs4941125 and rs7235803, showed interaction effects with several *TNFSF11* polymorphisms on AM. Both genes manifested significant interaction with the duration of breastfeeding in their effect on ANM.

CONCLUSIONS—The *TNFRSF11A* and *TNFSF11* genes are associated with the onset of AM and ANM in white women.

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Summary

The article reports for the first time possible association of two genes, *TNFRSF11A* (*RANK*) and its ligand, *TNFSF11* (*RANKL*), with age at menarche and natural menopause in white females.

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Keywords

age at natural menopause; age at menarche; association; *TNFRSF11A*; *TNFSF11*; polymorphisms; haplotypes

Introduction

The reproductive period of a woman's life is limited by two key physiological events, menarche and menopause. Although these events per se are experienced by virtually all women, their timing is quite variable. The onset of menarche and menopause was implicated in many consequences for female health in later life. Early age at menarche (AM) was shown to increase the risk for various cancers, including ovarian [1;2], endometrial 3, and breast 4[;5] ones, to promote obesity [6;7], and to cause psychological discomfort 8. Women with the later menarche are at higher risk of getting osteoporosis 9;10 and preeclampsia [11]. In turn, age at natural menopause (ANM) is also associated with many postmenopausal health complications, such as osteoporosis [12;13], cardiovascular disease 14, dementia 15, and various cancers of reproductive organs [16–19]. The postmenopausal health problems are thought to be related to the overall exposure of a female organism to estrogen, i.e., duration of the reproductive period. Therefore, knowing the factors underlying AM and ANM may help to forecast potential health problems and to improve overall female well-being.

Because both AM and ANM are complex traits, multiple environmental and genetic factors contribute to them [20–22]. A genetic component of AM variance is estimated to be about 45–74% [23–25], whereas that of ANM ranges between 63–74% [23;26].

During the last few years, several genomic regions and candidate genes, which may potentially contribute to AM and ANM, have been determined [27–33]. However, this list is probably incomplete. Tumor necrosis factor receptor superfamily, member 11a (*TNFRSF11A*), also known as receptor activator of nuclear factor-κB (*RANK*) and its ligand (*TNFSF11* or *RANKL*) participate in a wide variety of processes controlling cell death and proliferation, immunity, and development of the lymphoid tissue [34;35]. The *TNFRSF11A/TNFSF11* system is widely acknowledged as one of the key players in some primary postmenopausal disorders, such as osteoporosis [36;37] and atherosclerosis [38]. In addition, these genes are expressed in mammalian gland cells and were shown to control the development of a lactating mammary gland during pregnancy [39], i.e., play a role in the reproductive system. Collectively, the above data suggest that *TNFRSF11A* and *TNFSF11* may contribute to AM and ANM.

Methods

Study participants

The study participants were recruited from the metropolitan area of Omaha, NE as described previously 40. The study protocol was approved by the institutional review board of Creighton University. Each participant signed an informed consent agreement before the enrollment in the project. To minimize an effect of the potential non-genetic confounding factors, the following exclusion criteria, which were developed previously [41], were applied to the recruitment: chronic diseases of vital organs (brain, lung, heart, liver, kidney), systemic metabolic diseases (including diabetes, hypo- and hyperparathyroidism, hyperthyroidism, etc.), malnutrition conditions (chronic diarrhea, chronic ulcerative colitis, etc.), ANM below 40 years (as an indicator of probable premature ovarian failure). The participant's compliance to the exclusion criteria was evaluated through nurse-administered questionnaires and/or medical records. The recruited females provided the following information about their reproductive history and lifestyle factors: use of hormonal contraceptives, parity, duration of

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breastfeeding, smoking habits and use of alcohol. The total study sample consisted of 306 otherwise healthy Caucasian women of European descent. For the population association and haplotype analysis of AM and ANM, the total sample was partitioned into two subsamples of 306 and 211 participants, respectively. Women with surgical menopause were excluded from the analysis of ANM.

AM was defined as the age at the first menstrual period minus the date of birth; ANM was calculated as the age at the last menstrual period (years) followed by one year without menses. The summary of the data about the participants are given in Table 1.

Genotyping

Genomic DNA was isolated from leukocytes of peripheral blood using a commercially supplied kit (Gentra Systems, Inc. Minneapolis, MN, USA) and according to the manufacturer's protocol. SNPs genotyping was performed off-site using Integrated BeadArray System (Illumina, Inc., USA). In total 19 SNPs located within and in close proximity to *TNFRSF11A* and 12 SNPs for *TNFSF11* were genotyped.

Statistical analyses

The concordance of the SNPs to the Hardy–Weinberg equilibrium (HWE) was checked using the χ^2-test. Consistency of genotype data with Mendelian inheritance was verified using PedCheck [42]. Some SNPs had only a few minor allele homozygotes in the sample. In order to increase power of the analysis, those homozygotes were pooled with heterozygotes, and thus, two groups (with or without the minor allele) instead of the three (minor allele homozygote, heterozygote, and major allele homozygote) were analyzed. The participants of the ANM subsample were divided into categories according to the number of pregnancies and months of breastfeeding (Table 1).

The effects of the studied SNPs and environmental factors and their interactions on AM and ANM were estimated by stepwise multiple regression analysis and univariate ANOVA. In the latter case, each SNP was analyzed independently. Haplotype blocks were initially determined using the procedure implemented in Haploview 43 and then analyzed for their association with the traits under study. As the current population sample came from our previous studies, which showed the significant effect of smoking, alcohol consumption, and duration of breastfeeding on ANM 28[;44], these variables were used as covariates in the subsequent association analysis of ANM. No lifestyle factors were used as covariates for the AM association analyses, as they all occurred presumably after menarche. The analyses were conducted using SPSS (v. 16.0.1, SPSS, Inc., Chicago, IL) and PLINK [45], available at http://pngu.mgh.harvard.edu/~purcell/plink/.

Results

Study participants' characteristics

In total 306 women were recruited for the study. Their mean (±SE) AM was 13.0 ± 0.1 years and the mean ANM was 45.7 ± 0.4 years. The ANM of the total sample was relatively low due to the inclusion of participants who had experienced surgical menopause (hysterectomy of oophorectomy). The mean age at surgical menopause for this sample is about 40 years [46]. The ANM in the respective subsample was higher (48.9 ± 0.3 years, Table 1) but still lower than the average ANM for the US female population (about 51 years). This difference may be attributed to the interpopulation variation in ANM across the country.

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SNP association analyses

All studied SNPs were in agreement with the Hardy-Weinberg equilibrium (Table 2). Three *TNFSF11* polymorphisms showed association with AM: rs2200287 ($P = 0.005$), rs9525641 ($P = 0.039$), and rs1054016 ($P = 0.047$) (Table 3). After correction for multiple testing, only rs2200287 showed nearly significant ($P = 0.063$) association. Two SNPs of this gene, rs346578 and rs9525641, were significantly associated with ANM ($P = 0.007$ and $P = 0.011$, respectively). After correction for multiple testing, the rs346578 association became nearly significant ($P = 0.080$) and another became non-significant ($P = 0.130$). On the other hand, when being analyzed without the covariates, the association of rs346578 remained significant ($P = 0.032$) even after the multiple testing correction.

One SNP of *TNFRSF11A*, rs3826620, was significantly associated with AM ($P = 0.022$), and another, rs8086340, was associated with ANM ($P = 0.015$). Neither association remained significant after correction for multiple testing. The effects of the *TNFRSF11A* and *TNFSF11* polymorphisms on the respective traits are modest (Table 3). For example, homozygotes at the minor allele of rs3826620 have about 0.7 year later menarche (13.7 ± 0.3 yr) than the average for the sample (13.0 ± 0.1 yr). Likewise, a minor allele of rs8086340 confers about 1 yr later menopause (50.8 ± 0.6 yr) than the mean ANM for the studied sample (48.9 ± 0.3 yr).

Several haplotypes of both genes were found to be associated with the studied traits. Specifically, the AT haplotype of polymorphisms rs3826620 and rs12969194 of the *TNFRSF11A* gene was significantly associated with AM ($P = 0.022$) and TA haplotype of the same polymorphisms was significantly associated with ANM ($P = 0.046$). Two identified haplotype blocks of *TNFSF11* are associated with the studied traits (Table 4). Interestingly, the haplotypes, which are associate with both AM and ANM, have an opposite effect on the traits. For example, the ATTG haplotype confers earlier AM but later ANM, and so does the AATAAG haplotype (Table 4). Overall, the effect of the haplotypes is modest: each of them explains, on average, about 2% of the trait variance.

Several significant interaction effects between the *TNFRSF11A* and *TNFSF11* polymorphisms on the studied traits were also detected (Table 5). The rs4436867 polymorphism of *TNFRSF11A* interacts with the four *TNFSF11* polymorphisms in their effect on ANM. Two *TNFRSF11A* polymorphisms, rs4941125 and rs7235803, manifest an interaction effect with several *TNFSF11* polymorphisms on AM. Interestingly, none of the listed SNPs manifests the interaction effect on both AM and ANM (Table 5).

Both studied genes seem to interact with various lifestyle factors in their effect on ANM. In terms of the number of the interactions, duration of breastfeeding has the strongest effect on ANM: all *TNFSF11* polymorphisms and 17 out of 19 studied *TNFRSF11A* SNPs indicated significant ($P < 0.05$) interaction with this factor. The rs8086340 polymorphism of *TNFRSF11A* interacts with smoking ($P = 0.028$) and the rs9525641 polymorphism of *TNFSF11* showed to interact with alcohol consumption ($P = 0.037$). In addition, many polymorphisms of both genes showed nearly significant ($P \leq 0.07$) interactions with these two factors (data not shown).

Discussion

TNFRSF11A and *TNFSF11* are two functionally linked genes and are therefore frequently considered together as to their role in determination of various traits. However, virtually no data exist about their probable contribution to menarche and/or menopause. Some speculations may be made based on the known function of these genes. At the molecular level, their primary function is the activation of the transcription factor NF-κB [47]. In turn, the transcription factor

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NF-κB signaling pathway controls expression of a wide variety of genes involved in cell proliferation and survival [48]. Therefore, *TNFRSF11A* and *TNFSF11* are pleiotropic genes and thus were implicated for various complex processes, e.g., bone remodeling, vascular diseases, and immunity [34;35;37;38]. Furthermore, there is evidence that the transcription factor NF-κB signaling pathway is associated with cell aging and age-related diseases [49–52]. Recent data from the integrated microarray study of nine tissues reinforced the role of this pathway in senescence [53]. It seems probable that *TNFRSF11A* and *TNFSF11* contribute to AM and ANM by activating the transcription factor NF-κB signaling pathway and, respectively, triggering the mechanisms of aging. The effect of these genes on timing of menarche and menopause is likely estrogen-dependent. For example, there is ample evidence about estrogen modulation of *TNFRSF11A* and *TNFSF11* expression in osteogenic cell lineages that was implicated in postmenopausal bone loss due to the cessation of the ovarian function and, respectively, estrogen depletion [54–57]. Although no data about the estrogen effect on *TNFRSF11A* and *TNFSF11* during puberty are available, but we can assume the opposite mechanism due to the activation of the ovaries. This possibility should not be rejected, as the contribution of estrogen and estrogen metabolizing genes to AM is evidenced by several studies [29;58–61]. However, the details of the estrogen effect on *TNFRSF11A* and *TNFSF11* during menarche are yet to be determined. Despite the fact that most of the single SNP associations determined in this study became non-significant after correction for multiple testing, they should not be rejected. The Bonferroni correction is conservative and tends to reject the null hypothesis and mask a real association, especially when the effect of the given SNP is weak, which is common for pleiotropic genes like those in the present study,

In addition to estrogen, expression of *TNFRSF11A* and *TNFSF11* is controlled by other hormones [62–65]. Given that the hormonal status during menarche and menopause changes drastically [66–68], this may suggest respective alterations in the genes' activity. There are also data about the prolactin-controlled role of *TNFRSF11A* and *TNFSF11* in mammary gland development during pregnancy [69;70]. The strong interactions between these genes and the duration of lactation reported here are in a good agreement with these data.

The observed interactions between *TNFSF11* and smoking and alcohol consumption are supported by available literature data, which documented the stimulating effect of these factors on the *TNFSF11* expression [71–76]. We have not found any experimental evidence about effect of these factors on *TNFRSF11A* and, therefore, it is difficult to hypothesize the mechanism of this effect. One of the possible ways may be that they may influence *TNFRSF11A* through *TNFSF11*. However, the observed significant interactions should be treated with certain caution, as sample size is small and, respectively, statistical power to detect interactions is limited.

Another possible problem is a recalling bias, which may be introduced when obtaining the data through a questionnaire. According to the previous studies, the accuracy of long-term recall of AAM and self-reported ANM varied between 70% and 84% [77–80].

The question about shared genetic basis of AM and ANM is still disputed. These traits were reported either correlating [81;82] or not [23;83] phenotypically. However, recent genetic studies provide more and more evidence that some genes [27;60;84] and genomic regions [30;32] may contribute to both AM and ANM. Similar to our previous study of the methylenetetrahydrofolate reductase gene [44], the present work gives further support to the shared genetic basis for AM and ANM.

Despite the fairly good knowledge of the NF-κB signaling pathway per se [85], a probable contribution of its components (including *TNFRSF11A* and *TNFSF11*) to AM and ANM

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remains largely elusive. This study provides evidence for a possible role of this pathway in timing of menarche and menopause.

Conclusion

This study firstly reports *TNFRSF11A* and *TNFSF11*, two important genes for various complex traits, as probable contributors to the onset of menarche and natural menopause. This contribution incorporates direct association, SNP/SNP and SNP/environment interactions. However, the currently available data on these genes are limited and do not allow for making any definite conclusions about an exact mechanism of this genes' effect on AM and ANM. More studies, including the functional ones, on other cohorts of women are needed to determine this mechanism and verify the results of the present study.

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Table 1

Study participants' characteristics

Characteristics	Population-based analysis subsamples	
	Menarche	Natural menopause
No.	306	211
Age, y	61.1 ± 2.4	66.6 ± 3.4
Age at menarche, y	13.0 ± 0.1	13.1 ± 0.1
Age at menopause, y	45.7 ± 0.4	48.9 ± 0.3
Height, cm	163.2 ± 0.0	161.8 ± 0.5
Weight, kg	73.4 ± 0.9	73.1 ± 1.1
Use of oral contraceptive, % of sample	63.4	52.8
Smoking, % of sample	16.1	15.7
Alcohol consumption, % of sample	70.2	66.2
Breastfeeding, % of sample	57.1	56.5
Months of breastfeeding		
None	43.6	43.8
1–6	23.5	23.8
7–12	11.4	11.4
13–24	11.7	8.6
25 and more	9.7	12.4
No. of pregnancies	4.0 ± 0.1	4.3 ± 0.2
Pregnancies, % of sample		
None	3.6	1.4
1 or 2	30.4	25.0
3 or 4	39.9	37.5
5 and more	26.1	36.1

Values are in mean ± SE, unless otherwise indicated.

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Table 2

Summary information about the studied SNPs in the total sample

Gene	SNP ID	Allele variants	Location in the gene	MAF	*P*, HWE
TNFRSF11A (RANK)	rs12956925	C/**T**	Intron 1	0.181	0.238
	rs4436867	A/**G**	Intron 1	0.227	0.128
	rs4941125	C/**T**	Intron 1	0.343	0.138
	rs7235803	A/**G**	Intron 1	0.364	0.281
	rs8086340	A/**G**	Intron 1	0.438	0.534
	rs3826619	A/**G**	Intron 2	0.101	0.474
	rs11664594	A/**T**	Intron 3	0.348	0.202
	rs3826620	T/**A**	Intron 3	0.266	0.705
	rs11665260 (rs12969194)	T/**A**	Intron 4	0.283	0.682
	rs17069904	G/**A**	Intron 7	0.093	0.815
	rs4303637	T/**C**	Intron 7	0.332	0.344
	rs12959396	T/**G**	Intron 9	0.482	0.631
	rs17069906	A/**G**	Intron 9	0.025	0.660
	rs4426449	G/**A**	Intron 9	0.356	0.089
	rs6567274	A/**G**	Intron 9	0.351	0.090
	rs9646629	T/**G**	Intron 9	0.357	0.587
	rs884205	G/**A**	3′-UTR	0.253	1.000
	rs2957127	G/**A**	3′-UTR	0.439	0.562
	rs3017365	G/**A**	3′-UTR	0.496	0.647
TNFSF11 (RANKL)	rs12585014	G/**A**	5′-region	0.437	0.719
	rs7988338	G/**A**	5′-region	0.170	0.367
	rs9525641	C/**T**	Intron 1	0.454	0.802
	rs2277438	A/**G**	Intron 1	0.168	0.303
	rs9525645	A/**G**	Intron 2	0.170	0.367
	rs2148073	C/**G**	Intron 2	0.168	0.328
	rs2200287	G/**A**	Intron 2	0.382	0.659
	rs3742257	T/**C**	Intron 2	0.489	0.486
	rs922996	C/**T**	Intron 4	0.495	0.774

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Gene	SNP ID	Allele variants	Location in the gene	MAF	*P*, HWE
	rs1054016	G/**T**	3′-UTR	0.428	0.641
	rs346578	G/**A**	3′-UTR	0.066	1.000
	rs9567004	A/**G**	3′-UTR	0.014	1.000

TNFRSF11A, tumor necrosis factor receptor superfamily, member 11a; *TNFSF11*, tumor necrosis factor (ligand) superfamily, member 11; the minor allele is bold; HWE, Hardy-Weinberg equilibrium.

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Table 3

Significant associations for the single SNPs of the *TNFRSF11A* (underlined) and *TNFSF11* genes with AM and ANM (mean ± standard error) in white women

Gene/SNP	11*	12	22	*P*
		AM		
rs3826620	13.7 ± 0.3 (7.5)	13.0 ± 0.1 (38.2)	12.9 ± 0.1 (54.3)	0. 022
rs9525641	12.7 ± 0.2 (20.3)	13.0 ± 0.1 (50.3)	13.1 ± 0.1 (29.4)	0. 039
rs2200287	13.2 ± 0.2 (14.1)	13.1 ± 0.1 (48.4)	12.8 ± 0.1 (37.5)	0. 005
rs1054016	12.7 ± 0.2 (17.6)	13.0 ± 0.1 (50.3)	13.0 ± 0.1 (32.1)	0. 047
		ANM		
rs8086340	50.8 ± 0.6 (16.6)	49.1 ± 0.3 (54.5)	48.9 ± 0.6 (28.9)	0.015/0.034**
rs346578		48.4 ± 0.7 (12.4)	49.5 ± 0.3 (87.6)	0. 007/0.003
rs9525641	49.9 ± 0.7 (20.9)	49.5 ± 0.4 (52.6)	49.9 ± 0.7 (36.5)	0. 011/0.020

* 11, 12, 22 denote homozygote at minor allele, heterozygote and homozygote at major allele, respectively (the missed values for homozygotes at minor allele indicate that they were combined with heterozygotes into a single group); numbers in brackets indicate percentage of the studied sample;

** adjusted for covariates/crude

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Table 4

Association of the *TNFSF11* haplotypes with AM and ANM in white women

Trait	Haplotype block	Haplotype	Frequency	β	R^2	*P*
AM	rs2200287|rs3742257|rs922996|rs1054016	ATTG	0.436	−0.234	0.014	0.048
		GCCT	0.375	0.345	0.027	0.005
	rs12585014|rs7988338|rs9525641|rs2277438|rs9525645|rs2148073	AATAAG	0.458	−0.242	0.015	0.039
		AACAAG	0.375	0.303	0.021	0.015
ANM	rs2200287|rs3742257|rs922996|rs1054016	ATTG	0.436	0.926	0.026	0.020
		ATTT	0.325	−1.462	0.020	0.043
	rs12585014|rs7988338|rs9525641|rs2277438|rs9525645|rs2148073	AATAAG	0.458	0.919	0.026	0.020

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Table 5

Results of the SNP-SNP interaction effects between polymorphisms of the *TNFRSF11A* and *TNFSF11* genes on AM and ANM (*P* value) in white women

SNP		***TNFRSF11A***		
		rs4436867	**rs4941125**	**rs7235803**
TNFSF11	rs2148073		0.028	0.009
	rs2200287	**0.007**		
	rs2277438		0.032	0.011
	rs3742257	**0.047**		
	rs7988338		0.028	0.009
	rs9525641	**0.009**		
	rs9525645		0.028	0.009
	rs12585014		0.036	
	rs346578	**0.043**		

TNFRSF11A, tumor necrosis factor receptor superfamily, member 11a; *TNFSF11*, tumor necrosis factor (ligand) superfamily, member 11; the estimates of interaction effects on ANM are shown in bold.