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RESEARCH PAPER

Polymorphisms of the *TNF*, *LTA*, and *TNFRSF1B* genes are associated with onsets of menarche and menopause in US women of European ancestry

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ABSTRACT

Background: The *TNF*, *LTA* and *TNFRSF1B* genes have been implicated in various traits related to menarche and menopause.

Aim: To analyse the *TNF*, *LTA* and *TNFRSF1B* genes for their association with ages at menarche (AM) and natural menopause (ANM).

Subjects and methods: The study sample consisted of 314 unrelated females of European ancestry. Twenty SNPs located in and near the genes were analysed using various statistical methods. In addition, the functional significance of the loci associated with AM and ANM was analysed *in silico*.

Results: Locus rs2229094 of the *LTA* gene was associated with AM according to the additive ($\beta = -0.295$, $p_{perm} = 0.016$) and recessive ($\beta = -0.940$, $p_{perm} = 0.016$) genetic models. Haplotype GG rs1148459-rs590368 of the *TNFRSF1B* gene was associated with AM ($\beta = 0.307$, $p_{perm} = 0.023$). Haplotype GCA rs2844484-rs2229094-rs1799964 was associated with ANM after adjustment for covariates ($\beta = -1.020$, $p_{perm} = 0.035$). All studied loci were associated with ANM after adjustment for breastfeeding (raw $p < 0.05$). In addition, eight of the most significant models of interlocus interactions were associated with AM and five with ANM.

Conclusion: The results of the present study suggest that the *TNF*, *LTA* and *TNFRSF1B* genes are associated with AM and ANM.

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Age-at-onset; association; genes; menopause; menarche; polymorphisms

Introduction

Menarche and menopause are the key physiological events in the female life delimiting the reproductive period and the timing of their onsets has been associated with many adverse health consequences in later life. In particular, age at menarche (AM) has been implicated in the risk of various cancers of the reproductive system (Hsieh et al. 1990; Petridou et al. 1996; Chiaffarino et al. 2001; Xu et al. 2004; Jordan et al. 2005), psychological discomfort (Harlow et al. 2004), cardiovascular disease (Lakshman et al. 2009), obesity (Laitinen et al. 2001; Wang et al. 2006), preeclampsia (Rudra and Williams 2005) and osteoporosis (Ito et al. 1995; Gerdhem and Obrant 2004). Age at natural menopause (ANM) is a risk factor for common postmenopausal health problems, such as cardiovascular disease (Jacobsen et al. 1999), osteoporosis (Kritz-Silverstein and Barrett-Connor 1993; Harlow and Signorello 2000), various cancers (Cramer 1990; Purdie and Green 2001; Schildkraut et al. 2001; Velie et al. 2005), etc.

The tumour necrosis factor (*TNF*), lymphotoxin alpha (*LTA*) and TNF receptor superfamily member 1B (*TNFRSF1B*) genes are members of the tumour necrosis factor and its receptor superfamilies, which have been implicated in multiple cellular processes, including cell–cell signalling, cell proliferation and apoptosis, in many tissues. There is ample evidence suggesting the contribution of these genes to various health problems associated with AM and ANM, such as osteoporosis (Khosla 2013), obesity and breast cancer (To et al. 2013; Rose and Vona-Davis 2014), and cardiovascular diseases (Pawluk et al. 2020), to name a few. Given the above data, we tested the hypothesis that the *TNF*, *LTA* and *TNFRSF1B* genes may be associated with AM and ANM.

Subjects and methods

Study participants

The participants of the study have been recruited following the protocol approved by the Creighton University Institutional Review Board, including signed informed consent. As the study sample came from our previous studies (Liu et al. 2010b; Lu et al. 2010), the recruitment process adopted the same exclusion criteria (Deng et al. 2002), i.e. presence of chronic diseases of vital organs (brain, heart, lung, kidney, liver), systemic metabolic disorders (diabetes

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mellitus, diseases of the thyroid gland, etc.), various malnutrition conditions (e.g. chronic ulcerative colitis, diarrhoea, etc.) and menopause age below 40 years (indication of possible premature ovarian insufficiency). The information about lifestyle habits of the participants (e.g. alcohol and tobacco consumption) and clinical conditions (e.g. use of oral contraceptives, parity, lactation, etc.) was collected through a questionnaire and from personal medical records. The total study sample included 314 otherwise healthy females of self-reported European ancestry. A subsample of 197 women who had natural menopause was used for the association analysis of ANM.

Genotyping

DNA was isolated from peripheral blood buffy coat using a commercial kit (Gentra Systems, Inc. Minneapolis, MN). The SNPs for the analysis were chosen based on the following criteria: (1) the regulatory effect (regSNP); (2) the effect on gene expression (eSNP); and (3) MAF $>$ 0.05. In total, 20 SNPs located in or around the *TNFRSF1B*, *LTA* and *TNF* genes were genotyped off-site using the Integrated BeadArray System (Illumina, Inc., San Diego, USA).

Statistical analyses

All selected polymorphisms were checked for correspondence to the Hardy–Weinberg equilibrium (HWE) by the χ^2-test.

The association of the SNPs with AM and ANM was analysed using multivariate linear and logistic regression approaches. In the first case, both phenotypes were treated as quantitative traits. In the second case, AM and ANM were converted into qualitative variables as follows: the values below the sample mean were classified as 'earlier age' (affected) and above the mean as 'later age' (unaffected).

Haplotype blocks were determined using the algorithm implemented in HaploView (Barrett et al. 2005). The haplotype association analysis was conducted using PLINK v. 1.07 (Purcell et al. 2007). As the participants came from our previous studies (Liu et al. 2010a, 2010b), the same set of covariates (i.e. alcohol consumption, smoking, and breastfeeding) were used in the ANM association analysis. None of the above covariates were used for the analyses of AM, as they were all assumed to occur after menarche. The analyses were conducted using PLINK v.1.9 (Chang et al. 2015) available at http://www.cog-genomics.org/plink/1.9/. The adjustment for multiple testing was done using the adaptive permutation test (Che et al. 2014).

The most significant models of intergenic interactions for two-, three-, and four-locus models were determined by MB-MDR (Model-Based Multifactor Dimensionality Reduction) (Calle et al. 2008; 2010; Ponomarenko 2019) and respective software (v. 2.6) implemented in the R programming environment.

The in silico analysis of functional SNPs, regulatory effects, and pathways

The predictive effect of non-synonymous SNPs was analysed using the SIFT online tool (https://sift.bii.a-star.edu.sg/) (Vaser et al. 2016). The networks of intergenic interactions were determined using GeneMANIA (http://genemania.org) (Warde-Farley et al. 2010), with the maximum number of genes limited to 15 to infer the most significant interactions.

The SNPs associated with the studied traits and their proxies were analysed for their functional significance. HaploReg (v4.1) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) (Ward and Kellis 2012) and the data of the European population from the 1000 Genomes Project Phase 1 were used to identify the SNPs in strong linkage disequilibrium (LD) ($r^2 \geq 0.8$).

The eQTL significance of the AM/ANM candidate SNPs was estimated using the data of GTExportal (http://www.gtexportal.org/) as of 10.12. 2017 (Release V7 updated on 09/05/2017 (dbGaP Accession phs000424. v7.p2) and the Blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/) (Westra et al. 2013). The False Discovery Rate (FDR) $\leq$ 0.05 was applied to determine significant eQTLs.

Results

Characteristics of the study participants

The phenotypic characteristics of the participants are provided in Table 1. The mean AM of the sample was 13.0 ± 0.1 years and the mean ANM in the respective subsample was 49.0 ± 0.3 years. This is about the mean ANM in US Caucasians (McKnight et al. 2011). The categorised subsamples for AM and ANM had significantly different mean values of the respective traits ($p < 0.001$, Table 1).

SNP and haplotype associations

The summary information about the analysed SNPs is given in Table 2. All studied SNPs but three (rs945439, rs1061622 and rs235214) showed no deviation from the Hardy-Weinberg equilibrium.

Table 1. Characteristics of the study participants.

	Population-based analysis	
Participant characteristics	Age at menarche	Age at natural menopause
No.	314	197
Age, years	61.0 ± 0.5*	62.2 ± 3.6
Age at menarche, years	13.0 ± 0.1	13.0 ± 0.1
≤13.0 ($n = 223$)	12.3 ± 0.1	NA
>13.0 ($n = 91$)	14.7 ± 0.1	NA
Age at menopause, years	NA	49.0 ± 0.3
≤49.0 ($n = 89$)	NA	46.1 ± 0.3
>49.0 ($n = 108$)	NA	52.1 ± 0.2
Height, cm	162.7 ± 0.4	162.3 ± 0.5
Weight, kg	74.0 ± 0.9	72.2 ± 1.1
Smoking, % of sample	15.9	15.7
Alcohol consumption, % of sample	64.6	67.5
Breastfeeding, % of sample	55.7	55.8

*Mean ± SE.

Table 2. Summary information about the studied SNPs in the total sample.

Gene	SNP ID	Allele variants†	Position in the gene region	MAF	*p*, HWE
TNFRSF1B	rs1148459	G/**A**	5′-UTR	0.465	0.365
	rs590368	G/**A**	5′-Transcribed region	0.375	0.469
	rs652625	T/**A**	5′-Transcribed region	0.054	0.611
	rs625847	G/**A**	5′-Transcribed region	0.043	1
	rs496888	T/**C**	Intron 1	0.315	0.360
	rs499646	G/**A**	Intron 1	0.045	0.467
	rs17037696	C/**T**	Intron 1	0.207	0.059
	rs945439	A/**G**	Exon 2	0.210	0.017
	rs1061622 Met196Arg	T/**G**	Exon 6	0.210	0.017
	rs2275416	G/**A**	Intron 7	0.177	0.242
	rs5746059	A/**G**	Intron 10	0.202	0.222
	rs1061628	C/**T**	3′-UTR	0.392	0.480
	rs235214	C/**T**	3′-UTR	0.142	0.010
	rs4846100	C/**G**	LOC107984917	0.470	0.258
LTA	rs2844484	G/**A**	Intron 2	0.465	0.429
	rs2229094 Cys13Arg	T/**C**	Exon 6	0.224	1
	rs2229092 His51Pro	A/**C**	Exon 7	0.048	1
TNF	rs1799964	G/**A**	5′-Transcribed region	0.174	0.844
	rs3093664	A/**G**	Intron 3	0.079	0.419
	rs769178	G/**T**	3′-UTR	0.093	0.319

†The minor allele is in bold.

Locus rs2229094 of the *LTA* gene was individually associated with AM according to the additive ($\beta = -0.295$, $p = 0.029$) and recessive ($\beta = -0.940$, $p = 0.011$) models. The association remained significant after the correction by the permutation test ($p_{perm} = 0.016$ in both cases). Women with genotype CC had the onset of menarche at the age of 12.07 ± 1.16 years, which is 0.85 years earlier than in women with genotype CT (12.92 ± 1.31 years) and nearly 1 year earlier than in the TT homozygotes (13.06 ± 1.45 years). Haplotype GG rs1148459-rs590368 of the *TNFRSF1B* gene was associated with AM ($\beta = 0.307$, $p = 0.026$, $p_{perm} = 0.023$). Haplotype GCA rs2844484-rs2229094-rs1799964 was associated with ANM after adjustment for covariates ($\beta = -1.020$, $p = 0.043$, $p_{perm} = 0.035$).

Thirteen studied polymorphisms manifested an association with ANM according to the results of the linear regression analysis and after adjustment for breastfeeding. The logistic regression analysis yielded a significant association for all the studied SNPs (Table 3). On the other hand, all these associations became non-significant after the multiple testing correction.

The MB-MDR analysis determined the eight most significant models of interlocus interactions associated with AM and five associated with ANM (Table 4). Eight studied SNPs contributed to the significant models for AM and 14 to those for ANM. Two loci, rs590368 and rs1148459, contribute to the most significant models for AM at all three levels (two-, three- and four-locus). These two loci and rs4846100 appear in the largest number of the models (Table 4). The most significant associations with later menarche were determined for the following genotype combinations: rs769178 GT × rs590368 GA × rs4846100 CG × rs1148459 GA ($B = 1.87$, $p = 0.00007$) and rs769178 GT × rs2229094 TT × rs4846100 CG × rs1148459 GA ($\beta = 1.69$, $p = 0.0001$).

Locus rs4846100 contributed to the largest number of the three- and four-locus models for ANM (three out of five). Genotype combinations rs769178 GT × rs3093664 AA × rs4846100 GG ($\beta = -4.60$, $p = 0.002$), rs1799964 GA × rs5746059 AA × rs235214 CC × rs1148459 GA ($\beta = -3.86$, $p = 0.001$), rs2844484 GA × rs496888 CC × rs17037696 CT × rs1148459 GA ($\beta = -10.03$, $p = 0.007$) seem to produce the most significant effect on early menopause, whereas the combination of genotypes rs590368 AA × rs496888 TC × rs4846100 CC × rs2275416 GG ($\beta = 7.12$, $p = 0.007$) affects later menopause.

In silico *analysis of functional significance*

Three analysed SNPs, rs1061622 (Met196Arg), rs2229094 (Cys13Arg), and rs2229092 (His51Pro), were missense. Locus rs1061622 is located in exon 6 of the *TNFRSF1B* gene. The other two are located in exons 6 and 7, respectively, of the *LTA* gene. According to the SIFT database, all three have a predictive value 'tolerated'.

According to the GTEx portal data, six of the studied loci have the eQTL value and affect expression of 50 genes in various tissues and organs (Supplementary Table S1a). Among those are psoriasis susceptibility candidates (*PSORS1C1*, *PSORS1C2* and *PSORS1C3*), members of the major histocompatibility complex (*HLA-B*, *-C*, *-S*, *-DRA*, *-DRB5*, *MICA*, *MICB*) and others. The above effects were reported ubiquitously for the adipose tissue, brain and organs of the cardiovascular system.

Eleven of the studied SNPs manifested the sQTL significance (Supplementary Table S1b). They affected splicing of 21 genes in various tissues and organs. Eighteen of these genes were affected by loci rs2844484, rs2229094, rs1799964, rs3093664 and rs769178, which demonstrated the most pronounced effect. Importantly, these variants also demonstrate the most significant eQTL effect.

The results of the HaploReg database analysis suggested that 12 SNPs associated with AM/ANM were in strong LD with 93 polymorphic variants. Most of the linked SNPs (51) were intronic, one was located in 3′-UTR, two in 5′-UTR and 39 in intergenic regions. All these variants were annotated to have functional significance, albeit to a different extent (Supplementary Table S2). One of the most common functional annotations of these linked loci is their location in histone marks of enhancers and promoters, modification of regulatory motifs, and eQTL value. Some of these SNPs are characterised by the remarkable functional potential. For example, rs2516479 (linked to rs2844484) is located in the evolutionary conserved region, overlaps with promoter histone marks in five tissues, enhancer histone marks in 22 tissues, DNAse hypersensitivity region in 36 tissues, binds 29 proteins, modifies four transcription factor-binding motifs and manifests eQTL significance (Supplementary Table S2).

According to the GeneMANIA (Warde-Farley et al. 2010) pathway and gene network analysis, the *TNF*, *LTA* and *TNFRSF1B* genes, apart from interacting with each other, also physically interact with another 18 genes (Supplementary

Table 3. Association of the studied polymorphisms with ANM according to the three models of inheritance.

			Additive				Dominant				Recessive			
			Linear		Logistic		Linear		Logistic		Linear		Logistic	
Gene	SNP	Allele	β	p	OR (95% CI)	p	β	p	OR (95% CI)	p	β	p	OR (95% CI)	p
TNFRSF1B	rs1148459	A	1.027	0.052	0.492 (0.277−0.874)	**0.016**	1.030	0.051	0.492 (0.277−0.874)	**0.015**	1.032	0.051	0.494 (0.278−0.878)	**0.016**
	rs590368	A	1.044	**0.049**	0.476 (0.267−0.849)	**0.012**	1.056	**0.047**	0.476 (0.267−0.848)	**0.012**	1.022	0.054	0.479 (0.269−0.855)	**0.013**
	rs652625	A	1.038	**0.049**	0.493 (0.278−0.875)	**0.016**	1.035	0.050	0.496 (0.279−0.879)	**0.016**	1.056	**0.046**	NA	NA
	rs625847	A	1.037	**0.049**	0.493 (0.278−0.875)	**0.016**	1.037	**0.049**	0.493 (0.278−0.875)	**0.016**	NA	NA	NA	NA
	rs496888	C	1.039	**0.049**	0.498 (0.281−0.884)	**0.017**	1.036	0.050	0.500 (0.281−0.887)	**0.018**	1.041	**0.049**	0.494 (0.278−0.876)	**0.016**
	rs499646	A	1.032	0.050	0.496 (0.279−0.881)	**0.017**	1.027	0.052	0.498 (0.281−0.885)	**0.017**	1.056	**0.046**	NA	NA
	rs17037696	T	1.032	0.051	0.499 (0.281−0.885)	**0.017**	1.034	0.050	0.297 (0.280−0.881)	**0.017**	1.032	0.051	0.498 (0.280−0.886)	**0.018**
	rs945439	G	1.034	0.050	0.499 (0.281−0.885)	**0.017**	1.040	**0.049**	0.297 (0.280−0.881)	**0.017**	1.035	0.050	0.491 (0.275−0.885)	**0.016**
	rs1061622	G	1.037	**0.049**	0.495 (0.279−0.879)	**0.016**	1.038	**0.049**	0.495 (0.279−0.877)	**0.016**	1.035	0.050	0.491 (0.275−0.875)	**0.016**
	rs2275416	A	1.037	**0.049**	0.495 (0.279−0.879)	**0.016**	1.036	0.050	0.495 (0.279−0.879)	**0.016**	1.025	0.052	0.498 (0.281−0.884)	**0.017**
	rs5746059	G	1.012	0.057	0.498 (0.280−0.888)	**0.018**	1.002	0.059	0.501 (0.281−0.892)	**0.019**	1.041	**0.049**	0.493 (0.278−0.875)	**0.016**
	rs1061628	T	1.039	**0.049**	0.495 (0.279−0.878)	**0.016**	1.038	0.050	0.496 (0.279−0.880)	**0.017**	1.037	**0.049**	0.495 (0.279−0.878)	**0.016**
	rs235214	T	1.037	**0.049**	0.495 (0.279−0.879)	**0.016**	1.037	**0.049**	0.495 (0.279−0.879)	**0.016**	NA	NA	NA	NA
	rs4846100	G	1.035	0.050	0.496 (0.280−0.879)	**0.016**	1.037	**0.049**	0.495 (0.279−0.877)	**0.016**	1.029	0.051	0.497 (0.280−0.882)	**0.017**
LTA	rs2844484	A	1.001	0.058	0.504 (0.284−0.896)	**0.020**	1.001	0.058	0.502 (0.283−0.891)	**0.019**	1.004	0.057	0.503 (0.283−0.895)	**0.020**
	rs2229094	C	1.009	0.054	0.503 (0.283−0.892)	**0.019**	1.011	0.054	0.502 (0.283−0.891)	**0.019**	1.004	0.057	0.503 (0.283−0.892)	**0.019**
	rs2229092	C	1.009	0.055	0.503 (0.283−0.893)	**0.019**	1.009	0.055	0.503 (0.283−0.893)	**0.019**	NA	NA	NA	NA
TNF	rs1799964	A	1.022	0.051	0.502 (0.283−0.891)	**0.019**	1.007	0.055	0.503 (0.283−0.892)	**0.019**	1.041	**0.048**	0.504 (0.284−0.895)	**0.019**
	rs3093664	G	1.039	0.050	0.484 (0.272−0.864)	**0.014**	1.036	0.051	0.482 (0.270−0.861)	**0.014**	1.028	0.052	0.494 (0.278−0.878)	**0.016**
	rs769178	T	1.003	0.058	0.504 (0.284−0.894)	**0.019**	1.003	0.058	0.503 (0.284−0.893)	**0.019**	1.031	0.051	NA	NA

All analyses were adjusted for breastfeeding. Values of $p < 0.05$ are in bold.

Table 4. The most significant models of the SNP-SNP interactions associated with AM and ANM.

N	Models of SNP × SNP interactions	NH	*Beta*H	WH	NL	*Beta*L	WL	P_{perm}
			AM					
Two-locus models								
1	rs2229094 × rs4846100	1	0.54	10.36	2	−0.52	5.82	0.032
2	rs3093664 × rs17037696	1	1.41	9.12	0	NA	NA	0.035
3	rs590368 × rs1148459	2	0.65	9.08	0	NA	NA	0.048
Three-locus models								
1	rs769178 × rs590368 × rs1148459	4	0.96	26.96	1	−0.46	26.96	<0.001
2	rs769178 × rs4846100 × rs1148459	3	1.26	25.87	1	−0.51	25.87	0.001
Four-locus models								
1	rs769178 × rs590368 × rs4846100 × rs1148459	5	1.63	46.04	4	−0.82	14.6	<0.001
2	rs769178 × rs2229094 × rs4846100 × rs1148459	6	1.35	43.73	1	−2.98	4.57	<0.001
3	rs769178 × rs2229094 × rs1148459 × rs1061628	8	1.13	41.77	4	−1.23	16.15	<0.001
			ANM†					
Three-locus models								
1	rs769178 × rs3093664 × rs4846100	0	NA	NA	2	−5.29	14.65	0.026
2	rs3093664 × rs4846100 × rs1061628	0	NA	NA	4	−3.63	15.04	0.032
Four-locus models								
1	rs1799964 × rs5746059 × rs235214 × rs1148459	0	NA	NA	4	−4.49	31.66	<0.001
2	rs590368 × rs496888 × rs4846100 × rs2275416	5	4.87	30.68	2	−2.99	9.37	0.016
3	rs2844484 × rs496888 × rs17037696 × rs1148459	1	6.09	5.44	6	−4.39	32.97	0.032

†Adjusted for breastfeeding.

NH, number of significant genotypic combinations associated with the late menarche/menopause; *Beta*H, coefficient of the linear regression for significant genotypic combinations associated with the late menarche/menopause (change of the transformed age at menopause); WH, the Wald test value for significant genotypic combinations associated with the late menarche/menopause; NL, number of significant genotypic combinations associated with the early menarche/menopause; *Beta*L, coefficient of the linear regression for significant genotypic combinations associated with the early menarche/menopause; WL, the Wald test value for significant genotypic combinations associated with the early menarche/menopause; p_{perm}, p values after the permutation test.

Table S3). Most of these genes are involved in the TNF signalling pathway and thus contribute to multiple cellular processes associated with it. Overall, the abovementioned networks were characterised predominantly by physical interactions (77.6%), co-expression (8.0%) and predicted interactions (5.4%).

Discussion

This study first reports that the *TNF*, *LTA* and *TNFRSF1B* genes may be associated with AM and ANM. The individual associations of the SNPs with ANM became significant only when breastfeeding was used as a covariate. Despite the relatively low significance level ($p = 0.013$–0.049, Table 3) that becomes non-significant after the multiple testing adjustment, the observed associations are unlikely random because the probability that all 20 studied loci are associated with the trait purely by chance is negligible.

TNF, *LTA* and *TNFRSF1B* are pleiotropic genes contributing to the wide variety of cellular processes. They are also important elements of the immune system and are involved in cytokine-mediated, TNF-mediated and apoptotic signalling pathways. The age-related immune system decline in women is thought to occur largely due to menopausal oestrogen

deprivation (Gameiro et al. 2010). Interestingly, lactation produces a similar effect by inducing chronic suppression of oestrogens and progestogens (Gust et al. 2020).

Despite there having been several GWAS and other large-scale studies of probable causative variants/genes for AM (e.g. Shi et al. 2016; Day et al. 2017; Wang et al. 2019), none of them reported the associations for the genes analysed in the present study. One of the reasons for this can be a relatively weak effect of the genes, which fades due to the commonly used adjustment for multiple testing, which is usually conservative. On the other hand, some data which support the observed relationships between the analysed genes and AM (or puberty in a broad sense) do exist. For example, a recent study reported that the level of circulating TNFRSF1B might affect breast development in adolescent girls (Michels et al. 2020).

The data about the association of the *TNF*, *LTA* and *TNFRSF1B* genes with ANM is literally absent. We have found only one study reporting the association of *TNF* with ANM (He et al. 2010). However, the reported variant, rs909253, which was referred in the above study to the 5′-region of the *TNF* gene, in fact maps to an intron of the *LTA* gene (according to the dbSNP, https://www.ncbi.nlm.nih.gov/snp/). On the other hand, there are studies reporting that the above three genes may be involved in the processes related to menopause and, more specifically, timing of this physiological event. For example, postmenopausal status was characterised by the elevated plasma level of TNF (Sites et al. 2002; Taleb-Belkadi et al. 2016) that might induce hot flashes in postmenopausal women (Huang et al. 2017). In the 5-year observational study of postmenopausal women, Razmjou et al. (2018) determined the higher level of soluble TNFRSF1B at the onset of menopause as compared to the late postmenopausal period. A more recent study suggested that higher levels of serum TNFRSF1B increased the risk for earlier menopause (Bertone-Johnson et al. 2019).

It is worth noting that, while the individual associations of the loci analysed in the present study were relatively weak, the effect of the interlocus interactions was much more significant and remained so after the multiple testing correction (Table 3). These results suggest that the effect of a particular gene on the trait is expressed mostly through the interactions with other genes of the pathway. In such a way, an effect of the observed weak association can be, in fact, much stronger because of the amplification through the pathway.

It should be acknowledged that some analysed loci manifested a departure from the Hardy-Weinberg equilibrium. This might be due to several factors, e.g. population admixture (Deng et al. 2001), segmental duplication or location of the SNP in a copy number variation (Lee et al. 2008). This might introduce some bias to the observed associations for the respective loci but did not substantially impact the overall results.

Although this article provides evidence for association of the *TNF*, *LTA* and *TNFRSF1B* genes with AM and ANM in females of European ancestry, the exact mechanism of this association remains unclear and warrants further studies. In addition, due to the well-known inconsistencies of the association results in different ethnicities (Dvornyk et al. 2003, 2005), the study of these genes in other ethnic populations are needed.

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Data availability statement

The data that supports the finding of this study are available from the corresponding author upon request.

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