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Integrated in-depth bioinformatic analysis suggests *RELCH/KIAA1468*, *LINC02341*, and *AKAP11* as candidate genes for ages at menarche and menopause

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Abstract

Background: Polymorphisms of the *TNFRSF11A* and *TNFSF11* genes were reported for their association with age at menarche (AAM) and age at natural menopause (ANM). However, the biological mechanisms underlying this association remain largely unclear. **The aim of the study:** This study was to determine biological processes backing the observed genetic associations. **Materials and methods:** Forty-four SNPs were analyzed using *in silico* approach and ten publicly available online databases and tools. **Results:** *TNFRSF11A* and *TNFSF11* are highly pleiotropic genes that play a role in many metabolic processes. However, among that variety, lipid metabolism and cell survival and apoptosis seem the most biologically plausible mechanisms, through which these genes contribute to AAM and ANM. The analysis identified several mechanisms underlying the previously determined association of the *TNFRSF11A* and *TNFSF11* genes with AAM and ANM and suggested *RELCH*/*KIAA1468*, *LINC02341*, and *AKAP11* as new candidate genes for the traits. **Conclusion:** The *in silico* analysis is a powerful approach making it possible to uncover possible metabolic pathways underlying observed genetic associations.

Keywords: bioinformatics; in silico analysis; age at menarche; age at menopause; *TNFRSF11A*; *TNFSF11*

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Introduction. Tumor necrosis factor receptor superfamily, member 11a (*TNFRSF11A*), also known as receptor activator of nuclear factor-κB (NF-κB; *RANK*), and its ligand (*TNFSF11* or *RANKL*) have been implicated in various cellular processes related to proliferation and death, immunity, and tissue development. The *TNFRSF11A*/*TNFSF11* system is widely acknowledged as one of the key players in some primary postmenopausal disorders, such as osteoporosis [1] and cardiovascular diseases [2]. Also, these genes are expressed in mammary gland cells and were shown to con-

trol the development of a lactating mammary gland during pregnancy [3]; that is, they play a role in the reproductive system. Several candidate gene association studies suggested that *TNFRSF11A* and *TNFSF11* were associated with ages at menarche and menopause in different ethnic populations [4-7]. However, biological mechanisms, which underlie these associations, remain largely unclear. The exponential growth of biomolecular data and its mining into databases have provided not only a possibility of more accurate and substantiated choice of genetic markers for a study but also tools for comprehensive analysis to get deeper insights into probable functional assignments of the candidate genetic variants and mechanisms of their contribution to traits [8-10]. I took advantage of the recent advances in bioinformatics and used several online genomic databases to conduct a comprehensive *in silico* analysis of the *TNFRSF11A* and *TNFSF11* polymorphisms, which were reported as associated with age at menarche and menopause. This bioinformatic analysis aimed to get insights into possible mechanisms of these associations.

Materials and Methods

Selection of polymorphisms

Polymorphisms for the analysis were selected based on the published results of their association with ages at menarche and/or menopause. For this purpose, PubMed was screened using terms "TNFRSF11A", "TNFSF11", "RANK", "RANKL", "menarche", and "menopause" in various combinations. The search returned four articles with relevant results. These articles reported in total 44 SNPs (reference polymorphisms hereafter) associated with ages at menarche and/or menopause in three ethnic samples: Caucasians, Chinese, and Mexicans. The list of the selected polymorphisms and the map of the genomic regions, in which they are located, are given in Table 1 and Figure 1.

Beginning of Table 1

Information about the analyzed SNPs

Gene	SNP ID	Location in/around the gene	Association		Ethnicity	Reference
			AAM	ANM		
***TNFRSF11A* (*RANK*)**	rs3826620	Intron	+	+	Caucasian	[7]
			+		Chinese	[6]
	rs8086340	Intron		+	Caucasian	[7]
			+		Chinese	[6]
	rs11665260	Intron	+	+	Caucasian	[7]
	rs7239261	Intron	+		Chinese	[6]
	rs8094884	Intron	+		Chinese	[6]
	rs8089829	Intron	+		Chinese	[6]
	rs9956850	Intron	+		Chinese	[6]
	rs1805034	Exon Missense Ala/Val	+		Chinese	[6]
			+		Chinese	[5]
	rs4524034	Intron	+		Chinese	[6]
	rs4524035	Intron	+		Chinese	[6]
	rs12455775	Intron	+		Chinese	[6]
	rs17069904	Intron	+		Chinese	[6]
	rs12959396	Intron	+		Chinese	[6]
	rs2981003	5'-region, 5.8kb 3' of *KIAA1468*	+		Chinese	[6]
	rs2981004	5'-region, 6.2kb 3' of *KIAA1468*	+		Chinese	[6]
	rs6567263	5'-Region	+		Chinese	[6]
	rs7233197	Intron	+		Chinese	[6]
	rs4941125	Intron	+		Chinese	[6]
	rs4500848	Intron	+		Chinese	[5]

End of Table 1

Information about the analyzed SNPs

Gene	SNP ID	Location in/around the gene	Association		Ethnicity	Reference
			AAM	ANM		
	rs6567270	Intron	+		Chinese	[5]
	rs9962159	Intron		+	Chinese	[5]
***TNFSF11* (*RANKL*)**	rs12585014	5'-region	+		Mexican	[4]
			+	+	Caucasian	[7]
	rs9525641	Intron	+	+	Caucasian	[7]
	rs2200287	Intron	+		Caucasian	[7]
	rs1054016	3'-UTR	+		Caucasian	[7]
	rs346578	3'-UTR		+	Caucasian	[7]
	rs3742257	Intron	+	+	Caucasian	[7]
	rs922996	Intron	+	+	Caucasian	[7]
	rs7988338	Intron	+	+	Caucasian	[7]
	rs2277438	Intron	+	+	Caucasian	[7]
	rs9525645	Intron	+	+	Caucasian	[7]
	rs2148073	Intron	+	+	Caucasian	[7]
LINC02341	rs12874142	5'-region	+		Chinese	[6]
	rs7326472	5'-region	+		Chinese	[6]
	rs11147871	5'-region	+		Chinese	[6]
	rs9590697	5'-region	+		Chinese	[6]
	rs727243	5'-region	+		Chinese	[6]
	rs12864265	Intron	+		Chinese	[6]
	rs7316953	Intron	+		Chinese	[6]
	rs1324005	Intron	+		Chinese	[6]
	rs9525625	Intron	+		Chinese	[6]
	rs720824	Intron	+		Chinese	[6]
AKAP11	rs9525610	3'-UTR	+		Chinese	[6]
	rs238281	3'-UTR	+		Chinese	[6]
	rs9525613	3'-UTR	+		Chinese	[6]
	rs430586	3'-UTR	+		Chinese	[6]
	rs417768	3'-UTR	+		Chinese	[6]
	rs912100	3'-UTR	+		Chinese	[6]
	rs17063218	3'-UTR	+		Chinese	[6]
	rs17522044	3'-UTR	+		Chinese	[6]
	rs238270	3'-UTR	+		Chinese	[6]

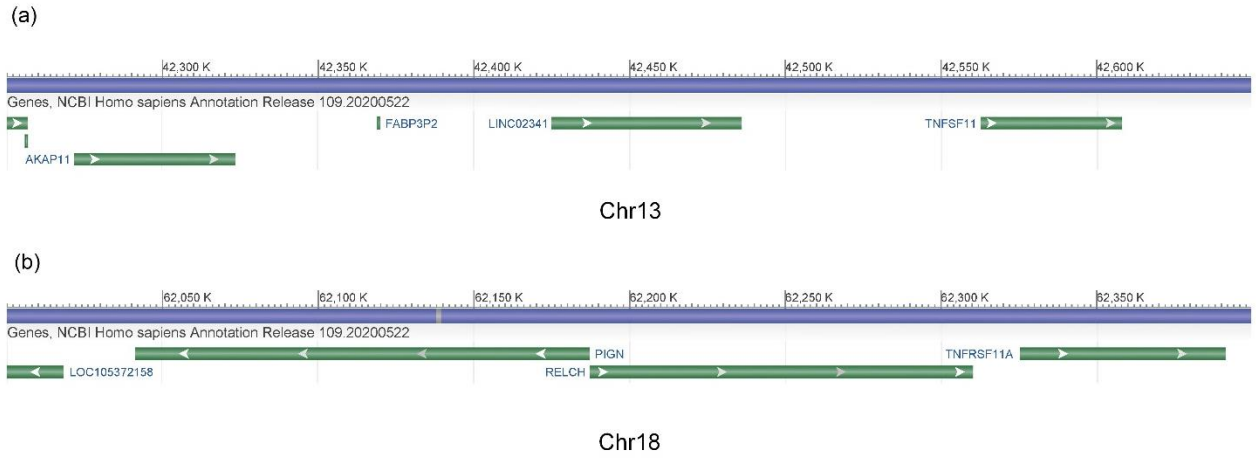

Fig. 1. Maps of the genomic regions where the analyzed SNPs and genes are located.

Bioinformatic analysis

In total ten bioinformatics tools were employed for the analyses.

The effect of non-synonymous SNPs on the protein function was analyzed using SIFT (https://sift.bii.a-star.edu.sg/) [11].

The integrated online tool, HaploReg v4.1 [12] was used to identify polymorphisms in strong linkage disequilibrium (LD) ($r^2 \geq 0.8$) with the AAM- and/or ANM-associated ones and to analyze them for their functional significance (chromatin states, motifs changes, protein interactions, regulatory potential, and eQTLs). The analysis was conducted separately for Caucasian and Chinese ethnicities using the data of the European and Asian populations from the 1000 Genomes Project Phase.

In addition to HaploReg (v4.1), three other databases were used to analyze regulatory effects of the polymorphisms: RegulomeDB (Version 1.1) (http://regulome.stanford.edu/) [13], rSNPBase (http://rsnp.psych.ac.cn/index.do) [14], and SNP Function Prediction (FuncPred) (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) [15], and GeneCards (https://www.genecards.org/) [16].

The effect of the 44 candidate SNPs for AAM and ANM on gene expression level (*cis*- and *trans*-eQTL) was estimated in peripheral blood using the Blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/) [17], and in other organs and tissues using the GTExportal data (http://www.gtexportal.org/) as of 07/27/2020. The false discovery rate (FDR) ≤0.05 was applied as the significance level.

The functional significance of the candidate genes for AAM in the various biological pathways was studied using the Gene Ontology Resource tools available at http://geneontology.org [18]. The results of multiple comparisons were adjusted with the FDR<0.05. The gene interaction networks were constructed using GeneMANIA (version 3.5.0) [19] available at http://genemania.org.

Results

Genomic location of the SNPs

First of all, 21 out of the 44 reference SNPs previously annotated to the regions of the *TNFRSF11A* and *TNFSF11* genes could also be mapped to the regions of the other genes (Table 1). Ten reference SNPs were located in the region of the *LINC02341* gene, five of them in the introns. Nine variants were located in the 3'-UTR of the *AKAP11* gene. Two SNPs, rs2981003 and rs2981004, were located in the 3'-UTR of the *RELCH*/*KIAA1468* gene.

Non-synonymous SNPs

Only one of all analyzed SNPs, rs1805034 in the *TNFRSF11A* gene, was missense. It results in an Ala/Val replacement in the respective protein. The replacement has SIFT Score = 1 and prediction value "tolerated".

SNPs in strong LD with the reference polymorphisms

The query against the HaploReg database returned in a total of 348 (224 unique) SNPs linked to the reference ones of the *TNFRSF11A* gene and 779 (322 unique) SNPs linked to the reference loci of the *TNFSF11* gene (Supplementary Table 1). The SNP association and linkage patterns were quite different between European and Asian populations. Specifically, two SNPs of the *TNFRSF11A* gene, rs3826620 and rs8086340, were associated with AAM and/or ANM in both Caucasians and Chinese [6, 7]. However, the HaploReg analysis returned no SNPs linked to rs3826620 in Europeans vs eight SNPs in Asians. In total, six loci were linked to the three reference SNPs in the European population and 218 were linked to the 19 reference SNPs in the Asian population. Out of these 224 unique SNPs, only three were shared between the European and Asian populations. Quite a few SNPs in the Asian population were located at/near the *RELCH*/*KIAA1468* and *PIGN* genes (Supplementary Table 1).

Even more striking ethnicity-related differences were observed for the *TNFSF11* gene polymorphisms: no shared SNPs in Europeans and Asians. In the Asian population, more

than half reference and linked to them polymorphisms were located at/near the *AKAP11* gene (Supplementary Table 1).

Regulatory effects

The results of the regulatory effect analysis are shown in Supplementary tables 1 and 2. They suggest that all reference SNPs can produce various regulatory effects, albeit to a different extent. For example, rs8086340 of the *TNFRSF11A* gene displays histone marks associated with promoters in six tissues and enhancers in 14 tissues, located in the DNase-1 hypersensitive region in 21 tissues, binding region for six proteins, and altered motif for the Foxm1 transcription factor (Supplementary Table 1).

Table 2

Effect of the reference AAM- and ANM-associated SNPs on the gene expression (*cis*-eQTL) in peripheral blood according to the Blood eQTL browser [17]

SNP	Gene/Region	Gene Expressed	*P*	FDR*
rs3826620	*TNFRSF11A*	*RELCH/KIAA1468*	$4.7*10^{-9}$	0.00
rs8086340	*TNFRSF11A*	*RELCH/KIAA1468*	$6.9*10^{-5}$	0.03
rs7239261	*TNFRSF11A*	*RELCH/KIAA1468*	$3.9*10^{-5}$	0.02
rs7233197	*TNFRSF11A*	*RELCH/KIAA1468*	$3.1*10^{-9}$	0.00
rs4941125	*TNFRSF11A*	*RELCH/KIAA1468*	$3.7*10^{-6}$	0.00
rs9962159	*TNFRSF11A*	*RELCH/KIAA1468*	$7.3*10^{-5}$	0.03
rs12874142	*80kb 3' of AKAP11*	*AKAP11*	$6.5*10^{-5}$	0.03
rs9525625	*117kb 3' of AKAP11*	*AKAP11*	$1.1*10^{-6}$	0.00
rs238281	*13kb 3' of AKAP11*	*AKAP11*	$1.9*10^{-32}$	0.00
rs9525613	*21kb 3' of AKAP11*	*AKAP11*	$2.6*10^{-8}$	0.00
rs430586	*23kb 3' of AKAP11*	*AKAP11*	$1.0*10^{-21}$	0.00
rs417768	*23kb 3' of AKAP11*	*AKAP11*	$1.4*10^{-21}$	0.00
rs912100	*24kb 3' of AKAP11*	*AKAP11*	$6.4*10^{-30}$	0.00
rs238270	*36kb 3' of AKAP11*	*AKAP11*	$1.4*10^{-20}$	0.00

Note: * FDR, False Discovery Rate

Expression QTLs

Several reference SNPs appeared to have a significant *cis*-eQTL effect on the expression of five genes, *RELCH/KIAA1468*, *PIGN*, *AKAP11*, *TNFRSF11A*, and *TNFSF11*, in various tissues and organs (Tables 2 and 3).

Pathway analysis

This analysis was conducted for *TNFRSF11A* and *TNFSF11* (because they were originally reported as associated with AAM and/or ANM), *LINC02341* (because several reference polymorphisms were mapped to this gene), and *RELCH/KIAA1468*, *PIGN*, *AKAP11* (because the expression of these genes might be affected by some reference SNPs according to the eQTL analysis).

According to the results of the PANTHER overrepresentation test, the *TNFRSF11A* and *TNFSF11* genes are involved in a broad range of biological processes, including regulation of ERK1 and ERK2 cascade, secretion of prostaglandins, bone remodeling, and mammary gland development (Supplementary Table 3). Apart from these two, *AKAP11* was suggested to contribute to the organism's homeostasis (Supplementary Table 3). No data was found for *RELCH/KIAA1468*, *PIGN*, and *LINC02341*.

The gene-gene interaction network inferred using GeneMANIA (Figure 2) suggested that the major contribution (64.32%) came from physical interactions between the proteins, followed by co-expression (25.88%), co-localization (5.61%), and common pathways (4.19%).

Beginning of Table 3

Effect of the reference AAM- and ANM-associated SNPs on the gene expression (*cis*-eQTL) in various tissues according to the GTEx browser

SNP	Gene/Region	Gene Expressed	Effect	Tissue
rs3826620	*TNFRSF11A*	*RELCH/KIAA1468*	Down	Nerve-tibial
rs11665260	*TNFRSF11A*	*RELCH/KIAA1468*	Down	Skin - sun exposed (lower leg)
rs8094884	*TNFRSF11A*	*RELCH/KIAA1468*	Up	Skin - sun exposed (lower leg)
rs8089829	*TNFRSF11A*	*RELCH/KIAA1468*	Down	Testis, nerve - tibial
rs9956850	*TNFRSF11A*	*RELCH/KIAA1468*	Up	Adipose-subcutaneous
		PIGN	Down	Aorta, coronary artery, adipose tissue, thyroid
rs17069904	*23kb 3' of AKAP11*	*TNFRSF11A*	Down	Esophagus - mucosa
		PIGN	Up	Adipose-subcutaneous, lung, muscle-skeletal
rs12959396	*24kb 3' of AKAP11*	*RELCH/KIAA1468*	Down	Testis
rs2981003	*36kb 3' of AKAP11*	*TNFRSF11A*	Up	Skin, esophagus-mucosa, brain, lung, mammary tissue, pancreas, pituitary, thyroid
		PIGN	Down	Adipose-subcutaneous, esophagus-mucosa, brain, lung, muscle-skeletal, thyroid, artery-tibial, nerve-tibial
rs2981004	*6.2kb 3' of KIAA1468*	*TNFRSF11A*	Up	Skin, esophagus-mucosa, brain, lung, mammary tissue, pancreas, pituitary, thyroid
rs6567263	*4.9kb 5' of TNFRSF11A*	*PIGN*	Down	Adipose-subcutaneous, esophagus-mucosa, brain, lung, muscle-skeletal, thyroid, artery-tibial, nerve-tibial
rs7233197	*TNFRSF11A*	*RELCH/KIAA1468*	Down	Skin, esophagus-mucosa, brain, lung, mammary tissue, pancreas, pituitary, thyroid, nerve-tibial
		PIGN	Down	Adipose-subcutaneous, esophagus-mucosa, brain, lung, muscle-skeletal, thyroid, artery-tibial, nerve-tibial
rs4941125	*TNFRSF11A*	*TNFRSF11A*	Up	Esophagus-mucosa, skin, thyroid
		PIGN	Down	Skin, thyroid, adipose-subcutaneous, small intestine-ileum
rs4500848	*TNFRSF11A*	*RELCH/KIAA1468*	Down	Nerve-tibial, adipose-subcutaneous, testis
		PIGN	Down	Skin, brain
rs9962159	*TNFRSF11A*	*TNFRSF11A*	Up	Skin, thyroid
		PIGN	Down	Skin, thyroid, adipose-subcutaneous
rs12874142	*80kb 3' of AKAP11*	*TNFSF11*	Up	Esophagus-mucosa
		AKAP11	Up	Esophagus-muscularis, brain, skin
rs9590697	*97kb 3' of AKAP11*	*TNFSF11*	Up	Esophagus-mucosa
		AKAP11	Up	Esophagus-muscularis, skin
rs727243	*98kb 3' of AKAP11*	*TNFSF11*	Up	Esophagus-mucosa
		AKAP11	Up	Esophagus-muscularis

End of Table 3

Effect of the reference AAM- and ANM-associated SNPs on the gene expression (*cis*-eQTL) in various tissues according to the GTEx browser

SNP	Gene/Region	Gene Expressed	Effect	Tissue
rs12864265	*117kb 3′ of AKAP11*	*TNFSF11*	Up	Esophagus-mucosa
		AKAP11	Up	Esophagus-muscularis, skin
rs7316953	*119kb 5′ of TNFSF11*	*TNFSF11*	Down	Esophagus-mucosa
		AKAP11	Down	Esophagus-muscularis
rs1324005	*119kb 5′ of TNFSF11*	*TNFSF11*	Up	Esophagus-mucosa
		AKAP11	Up	Esophagus-muscularis, skin
rs9525625	*119kb 5′ of TNFSF11*	*AKAP11*	Down	Esophagus-mucosa, esophagus-muscularis, brain
rs720824	*119kb 5′ of TNFSF11*	*TNFSF11*	Down	Esophagus-mucosa
		AKAP11	Down	Esophagus-muscularis
rs238281	*13kb 3′ of AKAP11*	*AKAP11*	Up	Artery, brain, colon, esophagus-mucosa, esophagus-muscularis, nerve-tibial, adipose-subcutaneous
rs9525613	*21kb 3′ of AKAP11*	*AKAP11*	Up	Artery-tibial
rs430586	*23kb 3′ of AKAP11*	*AKAP11*	Up/Down	Esophagus-mucosa, esophagus-muscularis, brain, adipose-subcutaneous, heart, muscle-skeletal
rs417768	*23kb 3′ of AKAP11*	*AKAP11*	Up/Down	Esophagus-mucosa, esophagus-muscularis, brain, adipose-subcutaneous, heart, muscle-skeletal
rs912100	*24kb 3′ of AKAP11*	*AKAP11*	Up/Down	Esophagus-mucosa, esophagus-muscularis, brain, adipose-subcutaneous, heart, muscle-skeletal, vagina, lung, colon, nerve-tibial
rs17063218	*25kb 3′ of AKAP11*	*AKAP11*	Up/Down	Esophagus-mucosa, esophagus-muscularis, brain, adipose-subcutaneous, heart, muscle-skeletal, vagina, lung, colon, nerve-tibial
rs17522044	*26kb 3′ of AKAP11*	*AKAP11*	Up	Esophagus-mucosa, esophagus-muscularis, heart, muscle-skeletal, nerve-tibial, artery-tibial
rs238270	*36kb 3′ of AKAP11*	*AKAP11*	Up/Down	Esophagus-mucosa, esophagus-muscularis, brain, adipose-subcutaneous, muscle-skeletal, nerve-tibial

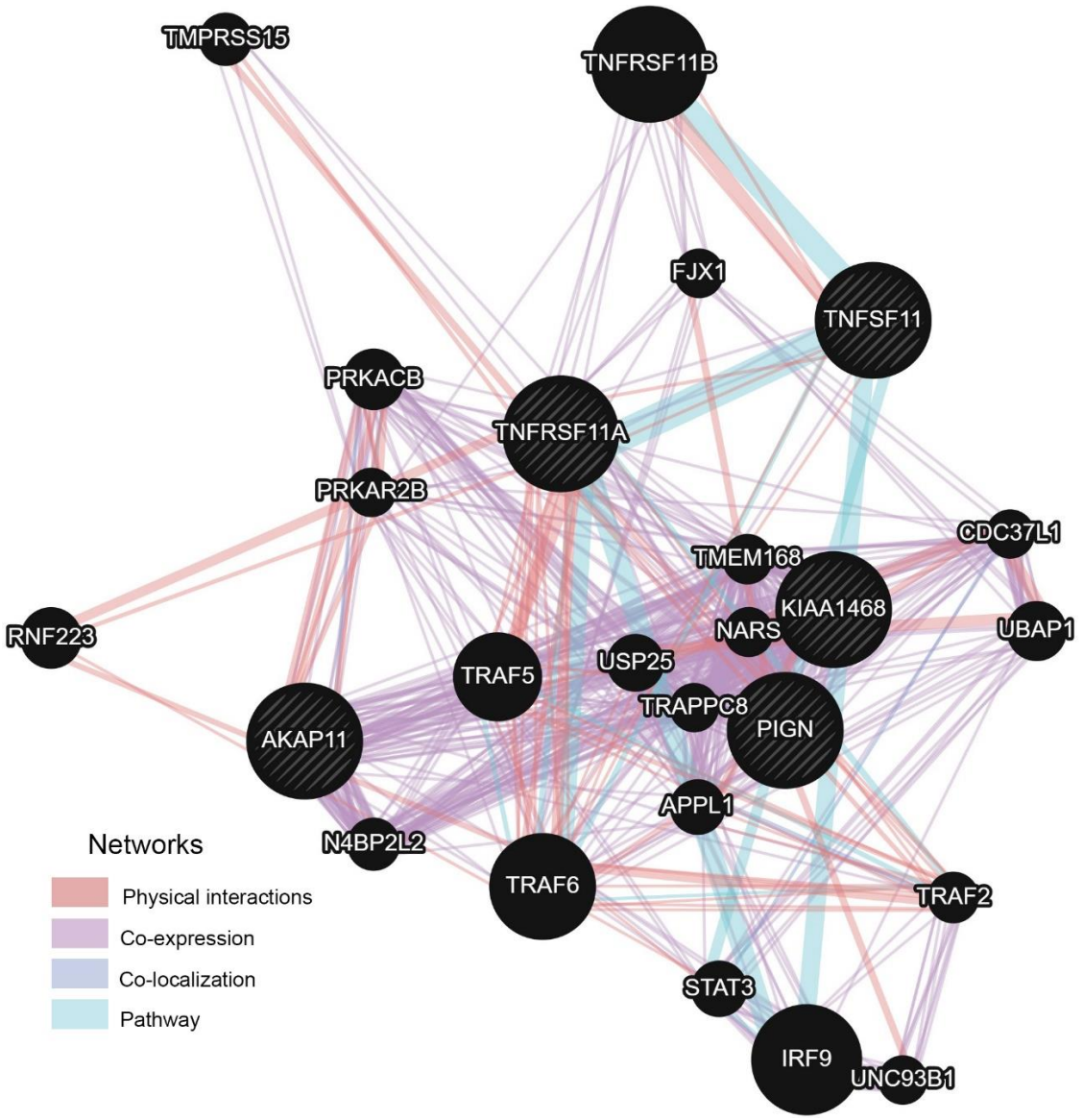

Fig. 2. The interaction networks of the candidate genes for age at menarche and natural menopause inferred using GeneMANIA. The candidate genes for the traits determined in the present study are cross-shaded

Discussion. This study provides evidence that, in addition to the *TNFRSF11A* and *TNFSF11* genes previously reported as associated with AAM and/or ANM, four other genes might be associated with these traits.

The *LINC02341* gene belongs to the class of long non-coding RNAs. There is not much information about *LINC02341* in public databases. Although long non-coding RNAs have not been studied well, there is a growing body of evidence that they are involved in transcriptional regulation [20]. Indeed, according to the GeneHancer database [21], *LINC02341* harbors enhancers for six genes, including *TNFSF11* and *AKAP11*, and binding sites for 76 transcription factors. The expression of the gene is relatively low and was documented in several tissues and organs, including lymph nodes, kidneys, placenta, and others [22]. The reference SNP, rs9525625, which is an intronic variant of the gene, was reported as a risk factor of inflammatory bowel disease [23].

The regions of two genes, *RELCH*/*KIAA1468* and *AKAP11*, also harbored several reference SNPs associated with AAM (Table 1). Besides, quite a few genetic variants in these genes were linked to the reference pol-

ymorphisms (Supplementary Table 1). These results suggested that the above genes might also contribute to the above trait.

RELCH (RAB11 binding and LisH domain, coiled-coil and HEAT repeat-containing, alias *KIAA1468*) encodes a protein playing a key role in intracellular cholesterol distribution [24]. The gene is ubiquitously expressed in human tissues and organs, including endocrine glands, endometrium, and ovaries [22]. The results of the GeneMANIA analysis suggested that this gene was co-expressed with *TNFRSF11A*, *AKAP11*, and *PIGN* (Figure 2).

A product of *AKAP11*, A-kinase anchoring protein 11, belongs to the protein family whose members, despite the diverse structure, have the same function of binding to the regulatory subunit of protein kinase A and targeting the enzyme to specific locations in the cell. It has similar to *RELCH* expression patterns [22] but is not co-expressed with *TNFRSF11A* and *PIGN* (Figure 2).

The *PIGN* gene encodes ethanolamine phosphate transferase, a key element of glycosylphosphatidylinositol-anchor biosynthesis. Mutations in the gene were associated with multiple congenital anomalies-hypotonia-seizures syndrome [25]. The gene is co-expressed with *TNFRSF11A* and *RELCH* (Figure 2).

RELCH and *AKAP11* are pleiotropic genes and were associated with multiple traits, including those related to menarche and menopause (e.g., bone phenotypes, obesity, development, etc.) [26, 27]. There is ample evidence that the above phenotypes have a shared genetic basis with AAM and ANM (see e.g., [6, 7, 28]. Together with the results of the *in silico* analysis of the present study, it suggests that *RELCH*/*KIAA1468*, *LINC02341*, and *AKAP11* may be candidate genes for AAM and/or ANM. This assumption is biologically plausible too.

A possible contribution of *PIGN* to AAM and/or ANM looks less obvious, largely due to the lack of data about the association of this gene with menarche- and menopause-related phenotypes. On the other hand, according to GeneHancer, this gene harbors binding sites of multiple transcription factors targeting the expression of *RELCH* and *TNFRSF11A*. Furthermore, given the involvement of this gene in the basic cellular and developmental processes [29] and tight linkage to the AAM-associated loci (Supplementary Table 1), the above possibility could not be ruled out.

The results of the Gene Ontology and GeneMANIA analyses (Supplementary tables 3, 4, Figure 2) suggested that the contribution of *TNFRSF11A* and *TNFSF11* to menarche and menopause timing is likely multifaceted. The *TNFRSF11A*/*TNFSF11*/*TNFRSF11B* (*RANK/RANKL/OPG*) signaling pathway has been widely acknowledged as a key player in bone remodeling [1]. Apart from this, the system plays an important role in the progesterone-driven proliferation of the mammary gland epithelium and the risk of breast cancer [30]. One of the possible ways through which *TNFRSF11A* can affect AAM and ANM is an interaction with *TRAF2*, a key element in the control of cell survival and apoptosis [31]. Involvement in the metabolism of lipids may be one more important biological mechanism of the AAM- and ANM-related role of *TNFRSF11A*. The relationship between obesity and AAM/ANM has been well documented [32, 33]. Arachidonic acid/prostaglandin E2 axis was implicated in uterine epithelium cell death induced by menopause [34]. The fatty acid composition was shown to be related to the menopausal status [35].

The lack of the GO Ontology data about *RELCH*/*KIAA1468*, *PIGN*, and *LINC02341* may suggest that their role in metabolic pathways is still poorly studied. On the other hand, there is extensive evidence about co-expression of *RELCH*/*KIAA1468* and *PIGN* with many genes, including those involved in the control of the basic cellular processes, e.g., cell proliferation [36] (Supplementary Table 4).

In general, a degree of gene pleiotropy seems to be inversely related to the relative contribution of the gene to the trait. Given that most genes in the human genome are pleiotropic [37], the expected contribution of each of them to a particular trait is quite mod-

est. Therefore, highly pleiotropic genes have a small effect size and often yield false negative results in GWAS unless their contribution to a particular trait is above the average for other traits (e.g., *TNFRSF11A*/*TNFSF11* contribution to bone remodeling).

The present study also sheds light on the frequently observed inconsistencies in associated polymorphisms and unsuccessful attempts to replicate candidate loci in different ethnic populations. Previous studies suggested that differences in population genetic structure might underlie the above disparities [38, 39]. The results of the present study suggest that, in addition to the allele frequencies, population-specific LD patterns are another important factor.

Conclusion. The *in silico* analysis of the *TNFRSF11A* and *TNFSF11* polymorphisms previously reported for association with AAM and/or ANM suggested *RELCH*/*KIAA1468*, *LINC02341*, and *AKAP11* genes as candidates for the traits. While this assumption is biologically plausible, candidate gene association studies are needed to verify it. In summary, the present study demonstrates that the in-depth analysis of rapidly expanding biological databases may provide new insights into possible factors and mechanisms underlying the observed association of genetic markers with a trait.

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Conflict of interests

The author has no conflict of interest to declare.

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