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Association of genetic variation in the tachykinin receptor 3 locus with hot flashes and night sweats in the Women's Health Initiative Study

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Abstract

Objective: Vasomotor symptoms (VMS, ie, hot flashes or night sweats) are reported by many, but not all, women. The extent to which VMS are genetically determined is unknown. We evaluated the relationship of genetic variation and VMS.

Methods: In this observational study, we accessed data from three genome-wide association studies (GWAS) (SNP Health Association Resource cohort [SHARe], WHI Memory Study cohort [WHIMS+], and Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials [GARNET] studies, total n = 17,695) of European American, African American, and Hispanic American postmenopausal women aged 50 to 79 years at baseline in the Women's Health Initiative Study. We examined genetic variation in relation to VMS (yes/no) in each study and using trans-ethnic inverse variance fixed-effects meta-analysis. A total of 11,078,977 single-nucleotide polymorphisms (SNPs) met the quality criteria.

Results: After adjustment for covariates and population structure, three SNPs (on chromosomes 3 and 11) were associated with VMS at the genome-wide threshold of 5×10^{-8} in the African American SHARe GWAS, but were not associated in the other cohorts. In the meta-analysis, 14 SNPs, all located on chromosome 4 in the tachykinin receptor 3 (*TACR3*) locus, however, had $P < 5 \times 10^{-8}$. These SNPs' effect sizes were similar across studies/participants' ancestry (odds ratio ~1.5).

Conclusions: Genetic variation in *TACR3* may contribute to the risk of VMS. To our knowledge, this is the first GWAS to examine SNPs associated with VMS. These results support the biological hypothesis of a role for *TACR3* in VMS, which was previously hypothesized from animal and human studies. Further study of these variants may lead to new insights into the biological pathways involved in VMS, which are poorly understood.

Key Words: Genome-wide association study – Hot flashes – Menopause – Vasomotor symptoms.

Menopausal vasomotor symptoms (VMS), defined as hot flashes or night sweats, are reported by the majority of US women regardless of ethnic

ancestry. More than 70% of late perimenopausal women, and more than 60% of postmenopausal women, experience VMS.¹⁻³ Moreover, VMS may last many years, lasting more

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than 7 years for more than half of women, and 10% of women report having VMS 12 years after the final menstrual period.^{4,5} Ethnicity (African American [AA] vs white), greater body mass index (BMI), lower education level, current smoking, anxiety, and depressive symptoms are associated with an increased risk for frequent VMS.¹

The extent to which VMS are genetically determined is unclear. Genome-wide association studies (GWAS), which are studies of common genetic variation across the entire human genome, identify genetic associations with observable traits.⁶ In GWAS, each person's complete chromosomal deoxyribonucleic acid is scanned by machines that quickly survey each person's genome for strategically selected markers of genetic variation called single-nucleotide polymorphisms (SNPs).⁷ GWAS can provide valuable clues about genomic function and pathophysiologic mechanisms.⁶

GWAS regarding VMS have never been performed among US women. Candidate gene studies suggest that genetic variants may be associated with VMS, including SNPs in estrogen receptor genes and the estrogen metabolism pathway (cytochrome P450 [*CYP*] *CYP1A1*, *CYP1A2*, *CYP1B1*, and *CYP19A1*, catechol-O-methyltransferase [*COMT*], 17-beta-hydroxysteroid dehydrogenase [*17HSD*], and sulfotransferase A1 [*SULT1A1*] genes).⁸⁻¹⁶ Other candidate gene studies have suggested associations of drug metabolism-related SNPs^{17,18} and serotonin transporter SNPs¹⁹ with VMS. Another candidate gene study suggested associations between genes that control angiogenesis (endothelial nitric oxide synthase [*eNOS*] and hypoxia inducible factor-1 alpha [*HIF1α*]) and VMS.²⁰ Because the pathophysiology of VMS is not well understood, and because GWAS does not require a priori hypotheses regarding associations, GWAS is an invaluable tool to help fill knowledge gaps regarding the pathophysiology of VMS.

The goal of this study was to evaluate the relationship of genetic variation (assessed by GWAS) and VMS in the Women's Health Initiative Study, which collected GWAS data and information regarding VMS in several independent cohorts of postmenopausal women.²¹ We hypothesized that we would find multiple genetic variants to be associated with VMS. Elucidation of these variants may lead to new insights into the biological pathways involved in VMS, which are poorly understood. By using a meta-analytic approach to combine GWAS that span multiple ethnic groups (a trans-ethnic meta-analysis),²² we found that genetic variation in the tachykinin receptor 3 gene (*TACR3*), which encodes the receptor for neurokinin B (NKB), was associated with VMS. NKB mRNA-expressing neurons are located predominantly in the infundibular nucleus and the anterior hypothalamic area.²³ In humans, NKB neurons co-localize with the gonadotropin-releasing hormone tract in the infundibular stalk, and the NKB pathway is implicated in pubertal development and hypogonadotropic hypogonadism.²³⁻²⁵

METHODS

The Women's Health Initiative Study and Women's Health Initiative Genetic Studies

The Women's Health Initiative Observational Study (WHI-OS) and Clinical Trials (WHI-CT) were carried out at 40 US clinical centers between 1993 and 1998.^{21,26} Participants were postmenopausal women aged 50 to 79 years at baseline, free from serious cardiac, pulmonary, renal, and hepatic conditions, with at least 3 years' life expectancy. The WHI-CTs performed randomized controlled trial evaluation of three distinct interventions: a low-fat eating pattern, menopausal hormone therapy (HT), and calcium plus vitamin D supplementation.²¹ The WHI-OS was designed to provide information about disease risk factors, including cancer, cardiovascular disease, and fractures.²¹ The combined studies enrolled 161,808 participants (93,676 in the WHI-OS and 68,132 in the WHI-CTs). The WHI Hormone Therapy trials did not exclude women on the basis of having severe VMS, but they excluded women who did not wish to forgo the use of personal menopausal HT.²¹

There were several independent GWAS studies conducted within the WHI study: (1) the SNP Health Association Resource cohort (SHARe) (n = 12,157 AA [SHARe-AA] and Hispanic American [SHARe-HA]), (2) the Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials (GARNET) cohort (n = 4,416 women of non-Hispanic, European ancestry), and (3) the WHI Memory Study cohort (WHIMS+) (n = 5,840 women of non-Hispanic, European ancestry).²⁷ We used all available GWAS data from all three studies, the characteristics of which are shown in Table 1.

Genotyping was performed using baseline blood samples collected at the time of enrollment. Of the 22,413 total women for whom genotyping data were available in all three GWAS studies combined, data from 21,753 passed quality control criteria, including minimal genotyping efficiencies and no near relatives (see Supplemental Digital Content 1, <http://links.lww.com/MENO/A193> for details). Of these women, information regarding baseline self-reported VMS was available for 20,482 women. Of these 20,482 women, information regarding primary analysis covariates was missing for 2,787 women. Therefore, our total analytic sample consisted of 17,695 participants from three ethnic groups (GARNET non-Hispanic European n = 3,282; SHARe AA n = 6,732 and SHARe Hispanic n = 2,778; and WHIMS+ non-Hispanic European n = 4,903) (Fig. 1).

Participants were asked to complete baseline self-assessment questionnaires. Weight and height were measured at baseline visit with standardized protocols. BMI was calculated as body weight in kilograms (kg) divided by the square of height in meters.

Each institution obtained human subjects committee approval. Each participant provided written informed consent.

Outcome: VMS

On baseline questionnaires, participants were asked whether they had ever experienced VMS (hot flashes and/

TABLE 1. Sample sizes and racial/ethnic composition of the WHI GWAS studies^a

Study name	Study population (synopsis)	GWAS method	Link to further information on dbGAP ^b
SHARe (SNP Health Association Resource cohort), AA & H GWAS	African American (AA) and Hispanic (H) participants in the WHI CT or OS; racial/ethnic distribution; 70% AA, 30% H	Affymetrix 6.0	http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000386.v6.p3
GARNET (Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials), HT GWAS	Cases and controls from the WHI HT trials; cases: all of the WHI participants with confirmed cases of CHD, stroke, VTE, or incident diabetes, or more than one of the case conditions in the WHI HT trials; controls: free of those conditions by the end of the WHI HT trials; racial/ethnic distribution: white (87%), black (5%), Hispanic (3%), Asian/Pacific Islander (1.8%), American Indian (0.7%), unknown (1.9%)	Illumina HumanOmni1-Quad v1-0 B	http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000315.v6.p3
WHIMS+ (WHI Memory Study cohort), EA GWAS	WHI HT Trial participants of non-Hispanic European ancestry from three subgroups: (1) WHIMS participants who were not in GARNET; (2) women at least 65 y old at enrollment who were neither in WHIMS nor GARNET; (3) women younger than age 65 at enrollment who were neither in WHIMS nor GARNET; racial/ethnic distribution: non-Hispanic European ancestry	Illumina HumanOmniExpress Exome-8v1_B	http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000675.v2.p3

CHD, coronary heart disease (myocardial infarction or coronary death); CT, Clinical Trials; EA, European Americans; HT, hormone therapy; OS, Observational Study; QC, quality control; SNP, single-nucleotide polymorphism; VTE, venous thromboembolism; WHI, Women's Health Initiative.

^aAll participants were postmenopausal women. Further information regarding the genome-wide association studies (GWAS) within the Women's Health Initiative (WHI) Clinical Trials and Observational Studies is available at http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000746.v1.p3 and at <https://www.whi.org/researchers/data/SitePages/GWAS%20Data.aspx>.

^bdbGAP denotes the Database of Genotype and Phenotype created by the National Center for Biotechnology Information (NCBI).

or night sweats). Hot flashes and night sweats were assessed as individual symptoms on the symptom checklist.^{3,28}

Primary exposure: genotyping

For SHARe, genome-wide SNP genotyping was performed using the Affymetrix Genome-wide Human SNP Array 6.0 (Affymetrix, Inc, Santa Clara, CA).²⁹⁻³¹ An ND-8000 spectrophotometer was used to quantitate genomic DNA. DNA quality was assessed using gel electrophoresis. Affymetrix Genotypic Console software was used to process data for genotype calling by the Birdseed calling algorithm version 2.0 (Affymetrix, Inc).²⁹ For GARNET, genotyping was performed with the Illumina Human Omni Quad 1.0M chip (Illumina, Inc., San Diego, CA).³¹ WHIMS+ used the Illumina Omni Express Array.

All three substudies were imputed to the same 1000 Genomes reference panel. Before imputation, we excluded data from samples with low genotyping efficiency (sample call rates <95% for SHARe, <98% for GARNET, and <97% for WHIMS+); SNP assays with locus call rates <90% for SHARe and <98% for GARNET and WHIMS+; and SNPs that deviated from Hardy-Weinberg equilibrium (P value threshold $<1 \times 10^{-6}$ for SHARe and $<1 \times 10^{-4}$ for GARNET

and WHIMS+). SNP assays with a minor allele frequency <0.01 were removed from SHARe and WHIMS+. Imputation was carried out with MINIMAC-OMP (version 1, Ann Arbor, MI^{32,33}) for WHIMS+ and GARNET, and MACH for SHARe

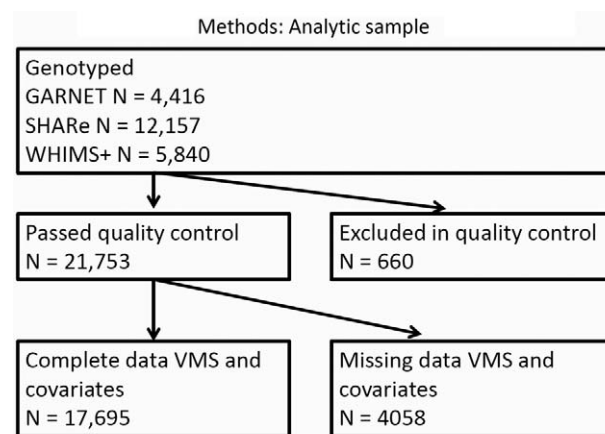


FIG. 1. Flow diagram of analytic cohort. GARNET, Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials; SHARe, SNP Health Association Resource cohort; VMS, vasomotor symptoms; WHIMS+, WHI Memory Study cohort.

(version 1.0, Ann Arbor, MI^{34,35}). All SNPs in the final analytic dataset were imputed, including those that had been directly genotyped. Any SNP whose postimputation minor allele frequency was <0.01 when averaged across the studies, or that had an imputation quality R^2 measure of <0.01 , was removed. Data from genetically related individuals were excluded. Allelic R^2 , the squared correlation between the imputed allele dosage with the highest posterior probability and true allele dosage, is a measure of imputation accuracy.³⁶ An SNP was required to meet the minimal thresholds in all three component studies to be included in the meta-analysis. In total, 19,335,715 SNPs met inclusion criteria in at least one of the studies; 11,078,977 SNPs were available for analysis across all of the studies. A detailed description of the imputation and harmonization effort of the WHI GWAS data is available.³⁷

Other covariates

Baseline self-report questionnaires were used to gather information regarding age, race/ethnicity, education, income, cigarette smoking, alcohol intake, physical activity,³⁸⁻⁴⁰ years since menopause, and oophorectomy. Clinic staff examined the labels of drug containers to collect information regarding medication use.

Statistical analysis

In each separate study, and subsequently in the meta-analysis of all the studies, we analyzed associations between genetic variants and VMS using additive models. In the primary analyses, ever experiencing VMS was categorized as a binary (yes vs no) variable. Principal components were calculated with *R* using a subset of 5,665 SNPs and all nonduplicate samples across WHI GWAS studies. We used the first 10 principal components as covariates in each of these analyses to adjust for ethnic differences between participants that remained after separating the samples based on self-reported ethnicity.⁴¹

We included the following covariates: bilateral oophorectomy, age at baseline, smoking (never/past/current), alcohol intake (drinks/d, continuous), physical activity (total metabolic equivalents [METs]/wk), population structure (principal components 1-10), BMI (continuous), education (0-8 y, some high school, high school diploma, some school after high school, college degree or higher), income (total annual household income $< \$20,000$, $\$20,000$ - $\$49,999$, $> \$50,000$), and menopausal estrogen therapy use (ever use, yes/no).

In a secondary analysis, we repeated the analyses in the subset of women who reporting never having used menopausal HT because HT could mask VMS.

For the meta-analysis, we used *R* version 3.2.2.⁴² The effect size for each SNP from each of the sample datasets was estimated using inverse variance fixed-effects meta-analysis using *rmeta* package for *R* version 2.16 (Vienna, Austria).^{42,43}

As part of our quality control, we plotted the quantile-quantile plot (q-q plot) of the test statistics as compared with their expected values under the null to determine whether our adjustments for study and ethnic differences were

effective.^{44,45} As we expected a small number of significant results (10-20), we removed the 14 most significant results before producing the q-q plot.

RESULTS

At baseline, the mean age of participants was 63.8 years, mean BMI was 29.7 kg/m², 9.6% were current smokers, and 39.3% of participants reported current use of menopausal HT (Table 2). Thirty-eight percent of participants were AA, 16% were Hispanic, and 46% were non-Hispanic European ancestry.

At baseline, 69% of participants reported ever experiencing VMS. Sixty-three percent of participants of non-Hispanic European ancestry experienced VMS, compared with 77% of AA participants ($P < 0.001$ vs participants of non-Hispanic European ancestry) and 68% of Hispanic participants ($P < 0.001$ vs participants of non-Hispanic European ancestry).

After removal of the 14 most significant results, q-q plot of the test statistics as compared with the expected distribution suggests that the likelihood of substantial hidden population substructure or differential genotype calling between cases and controls was unlikely ($\lambda = 0.9987071$) (Supplemental Fig. 1, Supplemental Digital Content 1, <http://links.lww.com/MENO/A193>).

The Manhattan signal plot is a genome-wide plot of $-\log_{10}$ of the P value for the SNP-phenotype association versus chromosomal position.⁴⁶ That is, the association statistics are shown as $-\log_{10}$ of the P value; a P value of 0.01 would be plotted as “2” on the y axis. The Manhattan signal plots show the statistical significance of SNP-VMS associations versus chromosomal position for all genotyped SNPs for each of the four separate GWAS (Fig. 2A-D). In the individual studies, three SNPs reached the P value threshold of 5×10^{-8} , all of them in the SHARe-AA study: rs75699757 and rs11518608 on chromosome 11, and rs148680409 on chromosome 3 (Fig. 2B). The odds ratios [ORs] for VMS were statistically significantly higher for rs75699757 (OR 1.98) and for rs11518608 (OR 1.07) in SHARe, indicating that the minor allele was positively associated with risk of VMS; in contrast, the minor alleles of these two SNPs were *inversely* associated with VMS in the SHARe-HA and WHIMS+ studies (Table 3). The opposite directions of the ORs across the individual studies account for the lack of statistically significant association of these two SNPs with VMS in the meta-analysis. Although rs148680409 passed the initial quality control and was associated with a 7.47-fold higher odds of VMS in the SHARe-AA study, this SNP did not pass the quality control criteria for the meta-analysis. SNPs on chromosome 4 were not associated with VMS in the individual studies, even in SHARe, which had the largest sample size.

In the Manhattan signal plot for the meta-analysis (Fig. 2E), only the locus on chromosome 4 exceeds genome-wide significance. After adjustment for bilateral oophorectomy, age, smoking, alcohol intake, physical activity, population structure, BMI, education, income, and menopausal HT use,

TABLE 2. Baseline characteristics of participants included in the analytic sample

	SHARe-AA		SHARe-HA		GARNET-EA		WHIMS+ EA		Total		P
	N	%	N	%	N	%	N	%	N	%	
Any vasomotor symptoms (eg, hot flashes, night sweats)											<0.001
No	1,527	22.7	902	32.5	1,144	34.9	1,846	37.7	5,419	30.6	
Yes	5,205	77.3	1,876	67.5	2,138	65.1	3,057	62.3	12,276	69.4	
Smoking status											<0.001
Never	3,217	47.8	1,741	62.7	1,625	49.5	2,520	51.4	9,103	51.4	
Past	2,731	40.6	848	30.5	1,276	38.9	2,038	41.6	6,893	39.0	
Current	784	11.6	189	6.8	381	11.6	345	7.0	1,699	9.6	
Alcohol intake daily											<0.001
Nondrinker	4,172	62.0	1,435	51.7	1,558	47.5	2,009	41.0	9,174	51.8	
≤1 drink	2,257	33.5	1,190	42.8	1,323	40.3	2,207	45.0	6,977	39.4	
>1 drink	303	4.5	153	5.5	401	12.2	687	14.0	1,544	8.7	
Education											<0.001
0-8 y	123	1.8	282	10.2	34	1.0	38	0.8	477	2.7	
Some high school	468	7.0	219	7.9	150	4.6	174	3.5	1,011	5.7	
High school diploma/GED	870	12.9	485	17.5	758	23.1	1,051	21.4	3,164	17.9	
School after high school	2,671	39.7	1,102	39.7	1,375	41.9	2,004	40.9	7,152	40.4	
College degree or higher	2,600	38.6	690	24.8	965	29.4	1,636	33.4	5,891	33.3	
Income, annual household											<0.001
<\$20,000	1,810	26.9	912	32.8	824	25.1	1,006	20.5	4,552	25.7	
\$20,000-\$49,999	2,909	43.2	1,141	41.1	1,649	50.2	2,585	52.7	8,284	46.8	
>\$50,000	2,013	29.9	725	26.1	809	24.6	1,312	26.8	4,859	27.5	
Bilateral oophorectomy											<0.001
No	5,133	76.2	2,280	82.1	2,583	78.7	4,271	87.1	14,267	80.6	
Yes	1,599	23.8	498	17.9	699	21.3	632	12.9	3,428	19.4	
Menopausal hormone therapy use ever											<0.001
No	3,952	58.7	1,344	48.4	2,090	63.7	3,348	68.3	10,734	60.7	
Yes	2,780	41.3	1,434	51.6	1,192	36.3	1,555	31.7	6,961	39.3	
Age (mean, SD)	61.3	6.9	60.2	6.6	65.7	6.9	68.2	5.8	17,695	63.8, 7.3	<0.001
Body mass index, kg/m ² (mean, SD)	31.0	6.6	28.8	5.8	29.7	6.2	28.4	5.7	17,695	29.7, 6.3	<0.001
Physical activity (mean, SD) ^a	9.7	12.7	10.8	13.5	10.2	12.7	11.7	13.2	17,695	10.5, 13.0	<0.001

AA, African American; EA, European Americans; GARNET, Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials; GED, General Educational Development; HA, Hispanic American; MET, metabolic equivalent; SHARe, SNP Health Association Resource cohort; SNP, single-nucleotide polymorphism; WHIMS+, WHI Memory Study cohort.

^aTotal energy expended from recreational physical activity (MET-h/wk).

14 SNPs were associated with increased odds of VMS at a *P* value threshold $<5 \times 10^{-8}$ (Table 3). The regional Manhattan plot provides a detailed view of the SNPs on chromosome 4 that were significantly associated with VMS (Supplemental Fig. 2, Supplemental Digital Content 1, <http://links.lww.com/MENO/A193>). These 14 SNPs are located within the same region of chromosome 4, the gene for *TACR3*. Per allele, the SNPs were associated with 1.20- to 1.83-fold higher odds of VMS.

The magnitudes of associations were consistent in each individual study. Although the GARNET substudy was enriched with women who had cardiovascular disease and/or diabetes, the magnitudes of associations among the GARNET substudy participants were similar to those of the other substudies.

The linkage disequilibrium (LD) plot (Fig. 3) shows the strength of the associations, the LD pattern (r^2 estimates), and the position of the associated SNPs relative to the most strongly associated SNP (rs74827981, represented by a purple circle) and the genes in the region (LocusZoom⁴⁷). The SNPs that we found to be associated with VMS are clustered tightly within the region of the *TACR3* gene, and are not in LD with other SNPs found in other genes within the 3Mb region.

In secondary analyses designated a priori, we repeated the analyses among participants who had never used HT. Among

the HT never users, the strongest signals and corresponding ORs were similar to those of the main analysis (Supplemental Table 3, Supplemental Digital Content 1, <http://links.lww.com/MENO/A193>).

To facilitate comparison of our results with previous and future studies, we provide the rs numbers and allele frequencies corresponding to the University of California Santa Cruz (UCSC) genome browser⁴⁸ chromosomal position of each of the 14 SNPs that met the *P* value threshold $<5 \times 10^{-8}$ (Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/MENO/A193>). Supplemental Table 2 (Supplemental Digital Content 1, <http://links.lww.com/MENO/A193>) displays the observed allele frequencies for the 14 SNPs that were associated with higher odds of VMS.

DISCUSSION

To our knowledge, this is the first published GWAS study to focus on genetic variation in relation to VMS. In this trans-ethnic meta-analysis of several studies, we identified several SNPs that were associated with experiencing hot flashes. The SNPs that were significantly associated with hot flashes at $P < 5 \times 10^{-8}$ were all located on chromosome 4 in the intronic regions of the *TACR3* gene. *TACR3* encodes the NK3R receptor (NK3R). NKB, a member of the tachykinin

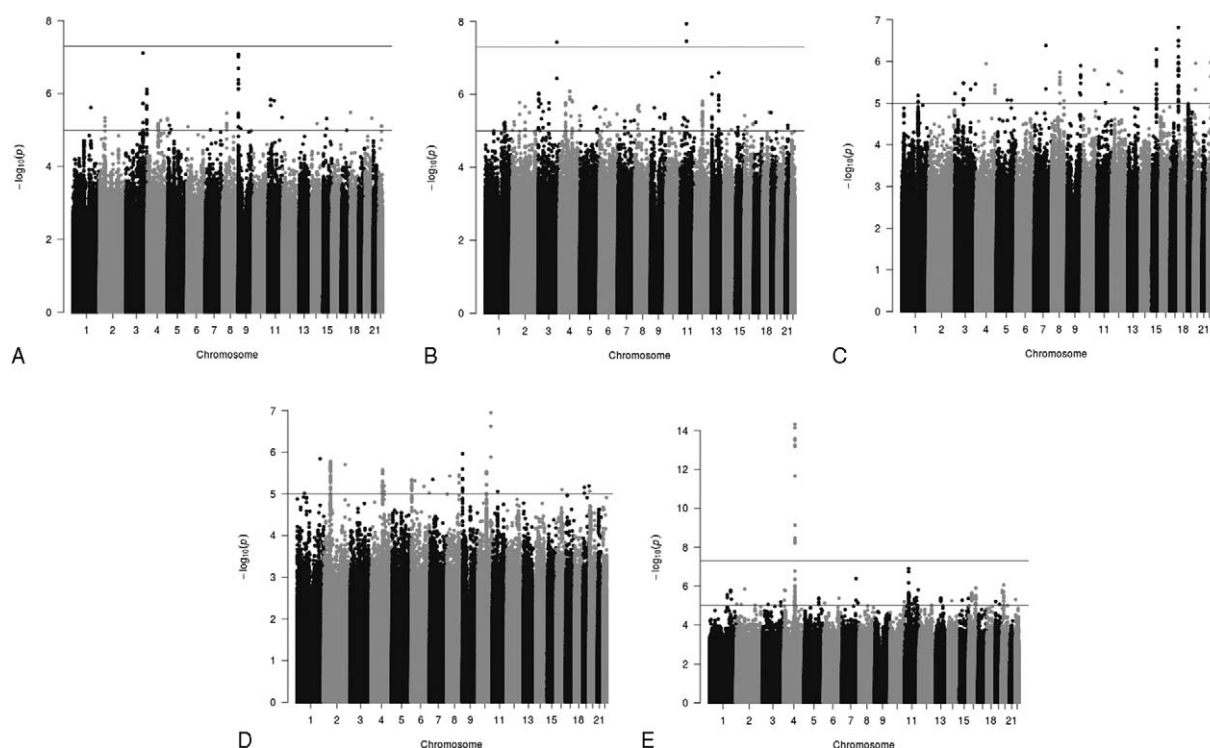


FIG. 2. Manhattan signal plot of meta-analysis of associations of all genotyped single-nucleotide polymorphisms (SNPs) with vasomotor symptoms. Manhattan plot for participants of (A) GARNET study, (B) SHARE-AA study, (C) SHARE-HA study, and (D) WHIMS+ study. (E) Manhattan plot of meta-analysis showing statistical significance of all genotyped SNPs. Blue line denotes $P < 5 \times 10^{-5}$; red line denotes $P < 5 \times 10^{-8}$. AA, African American; GARNET, Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials; HA, Hispanic American; SHARE, SNP Health Association Resource cohort; WHIMS+, WHI Memory Study cohort.

TABLE 3. GWAS results by study sample and combined in meta-analysis: vasomotor symptoms ever versus never^a

RefsnpID ^b	CHR:POS ^c	GARNET study		SHARE-AA study		SHARE-HA study		WHIMS+ study		Meta-analysis P value
		OR (SE)	P	OR (SE)	P	OR (SE)	P	OR (SE)	P	
rs74827081	4:104556732	1.65 (0.18)	7.88e-6	1.83 (0.34)	1.25e-3	1.64 (0.24)	7.46e-4	1.54 (0.15)	6.41e-6	4.77e-15
rs74589515	4:104584258	1.60 (0.18)	2.71e-5	1.80 (0.32)	1.11e-3	1.61 (0.23)	9.62e-4	1.57 (0.15)	2.92e-6	7.11e-15
rs79246187	4:104580809	1.61 (0.18)	2.1e-5	1.51 (0.23)	7.46e-3	1.60 (0.23)	1.02e-3	1.57 (0.15)	2.63e-6	2.64e-14
rs112390256	4:104575473	1.64 (0.18)	9.46e-6	1.51 (0.24)	8.92e-3	1.61 (0.23)	9.87e-4	1.54 (0.15)	4.97e-6	2.81e-14
rs75544266	4:104584997	1.60 (0.18)	2.84e-5	1.51 (0.23)	6.55e-3	1.60 (0.23)	9.42e-4	1.57 (0.15)	2.89e-6	3.15e-14
rs78154848	4:104562840	1.65 (0.18)	8.16e-6	1.46 (0.24)	1.94e-2	1.62 (0.24)	8.61e-4	1.54 (0.15)	6.05e-6	5.65e-14
rs76643670	4:104562842	1.65 (0.18)	8.16e-6	1.46 (0.24)	1.94e-2	1.62 (0.24)	8.59e-4	1.54 (0.15)	6.05e-6	5.65e-14
rs78289784	4:104580155	1.61 (0.18)	2.12e-5	1.46 (0.23)	1.51e-2	1.60 (0.23)	9.92e-4	1.56 (0.15)	3.28e-6	6.55e-14
rs77322567	4:104569676	1.64 (0.18)	8.62e-6	1.23 (0.13)	5.12e-2	1.55 (0.22)	2.24e-3	1.53 (0.15)	6.52e-6	2.17e-12
rs78141901	4:104593977	1.42 (0.16)	2.67e-3	1.80 (0.37)	3.88e-3	1.35 (0.23)	7.62e-2	1.55 (0.16)	1.31e-5	7.27e-10
rs78844131	4:104600029	1.41 (0.16)	2.85e-3	1.49 (0.26)	2.08e-2	1.30 (0.22)	1.17e-1	1.56 (0.16)	1.14e-5	3.34e-09
rs79852843	4:104628587	1.44 (0.17)	1.68e-3	1.49 (0.29)	4.45e-2	1.22 (0.20)	2.22e-1	1.58 (0.16)	5.08e-6	4.38e-09
rs80328778	4:104612447	1.42 (0.16)	2.15e-3	1.46 (0.27)	3.67e-2	1.24 (0.21)	1.95e-1	1.57 (0.16)	7.06e-6	4.97e-09
rs112623956	4:104623714	1.42 (0.16)	2.33e-3	1.49 (0.27)	2.83e-2	1.20 (0.20)	2.67e-1	1.57 (0.16)	6.19e-6	6.19e-09
rs75699757	11:56818268	1.08 (0.08)	3.01e-01	1.98 (0.24)	1.16e-08	0.84 (0.10)	1.16e-01	0.86 (0.05)	1.51e-02	7.07e-01
rs11518608	11:56767184	1.07 (0.08)	3.67e-01	1.91 (0.22)	3.47e-08	0.87 (0.10)	2.13e-01	0.91 (0.06)	1.09e-01	3.80e-01
rs148680409	3:173330544	Missing	Missing	7.47 (2.73)	3.69e-08	2.90 (3.33)	3.54e-01	Missing	Missing	Missing

AA, African American; GARNET, Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials; HA, Hispanic American; SHARE, SNP Health Association Resource cohort; SNP, single-nucleotide polymorphism; WHIMS+, WHI Memory Study cohort.

^aTable displays the results that were statistically significant (P value threshold $< 5 \times 10^{-8}$) in the meta-analysis of data from all three genome-wide association study (GWAS) studies and/or in the individual studies. Odds ratio (OR) is expressed as allelic OR (SE). Reference group is “never had vasomotor symptoms.” Adjusted for bilateral oophorectomy, age, smoking, alcohol intake, physical activity, population structure, body mass index, education, income, and menopausal estrogen therapy use. GARNET study denotes the Genome-Wide Association Studies of Treatment Response in Randomized Clinical Trials cohort of women of non-Hispanic European ancestry.

^bRefsnp identification numbers (IDs) were obtained from the SNP locations for Homo sapiens (dbSNP Build 144) Bioconductor package.

^cCHR:POS denotes chromosome assignment and position of SNP according to Build 37.

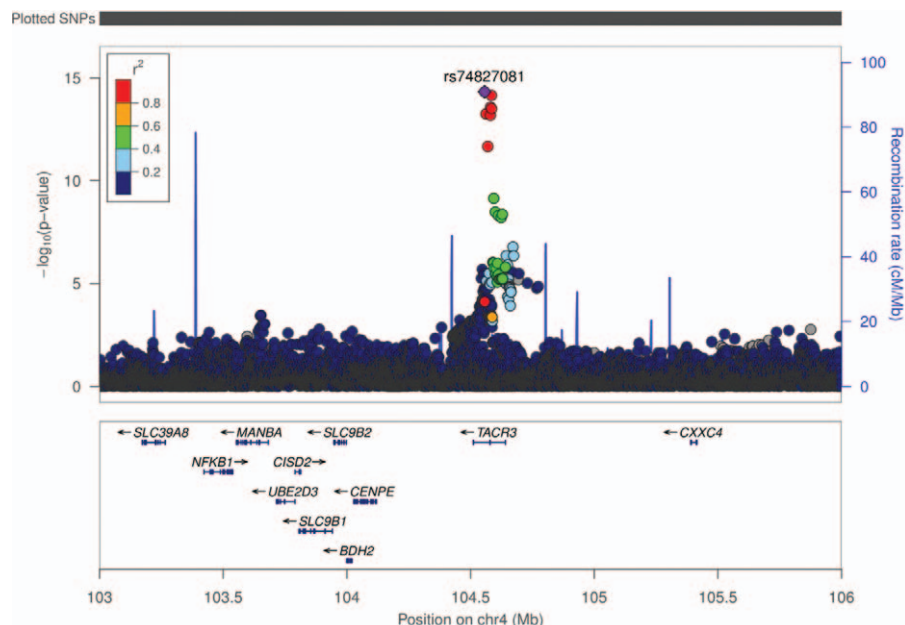


FIG. 3. Linkage disequilibrium plot showing the position of the single-nucleotide polymorphisms (SNPs) and their r^2 values relative to the mostly strongly associated SNP (rs74827981) and genes in the region.

family of peptides, preferentially binds to NK3R.²³ The meta-analysis combined studies of women with different ethnicities. Because regions of high LD (haplotype blocks) will vary by ethnicity, this trans-ethnic approach to meta-analysis can increase statistical power and narrow the region that should be considered for fine-mapping.²² The effect sizes (ORs) were very similar across racial/ethnic groups, suggesting a relatively ancient origin of the mutation. Statistical power was greatest in European Americans, which can be at least partially accounted for by the differences in minor allele frequencies among the racial/ethnic groups.

There are no previous GWAS studies of hot flashes or hot flushes posted in the GWAS Catalog (<https://www.ebi.ac.uk/gwas/>), although age at menarche has been associated with upstream gene variants of *TACR3* in a prior GWAS.⁴⁹ None of the SNPs that were associated with VMS in the current study produced any search results in the GWAS catalog, highlighting that our research question is novel.

The associations that we observed between genetic variation in NKB pathway genes and hot flashes are biologically plausible. The strongest evidence comes from a recent randomized double-blind placebo-controlled crossover study of healthy women, demonstrating that the infusion of NKB induces hot flashes.⁵⁰ Other lines of evidence also implicate a role for NKB in VMS. First, colocalization studies support physiologic interaction between the NKB pathway and gonadotropin pathways. For example, NKB and estrogen receptor alpha messenger RNA are co-expressed in the human infundibular nucleus²³ and the descending gonadotropin-releasing hormone tract that courses through the infundibular stalk to the neurohypophysis is exposed to dense NKB fiber plexuses.⁵¹ In rats, monkeys, and sheep, NKB fibers project from the arcuate nucleus (the area corresponding to the human

infundibular nucleus) to the median eminence, a site with dense gonadotropin-releasing hormone terminals.²³ Second, menopause and/or oophorectomy are associated with changes in NKB gene expression in human and animal studies. For example, NKB gene expression in the human infundibular nucleus increases after menopause, and in monkeys and rats, ovariectomy induces similar increases in NKB gene expression that are reversed by estradiol therapy.^{23,24} Compared with premenopausal women, postmenopausal women have hypertrophy of neurons expressing NKB.²³ Third, in humans, inactivating mutations of the NKB gene (*TAC3*) and *TACR3* induce hypogonadotropic hypogonadism. For example, in humans, *TAC3* and *TACR3* inactivating mutations are associated with absent pubertal development as well as low circulating serum luteinizing hormone and gonadal steroid levels.^{23,25} In rhesus monkeys, intervention to slow the frequency of gonadotropin-releasing hormone pulses can reproduce the pattern of low serum luteinizing hormone seen in humans with *TAC3* and *TACR3* mutations, suggesting that women with deficits in NKB/NK3R signaling have changes in the pattern of gonadotropin-releasing hormone pulses.²³ Finally, the infusion of a potent selective NK3R agonist (senktide) into the median preoptic nucleus of the rat markedly reduces core body temperature, suggesting that hypertrophy of NKB neurons characterizing postmenopausal women could be involved in the biology underlying menopausal hot flashes.^{23,52}

On the National Institutes of Health Common Fund's Genotype-Tissue Expression (GTEx) website (<https://commonfund.nih.gov/GTEx/index>), the highest gene expression (reads per kilobase of transcript per million mapped reads) for *TACR3* is located in the hypothalamus, followed by the amygdala (<http://www.gtexportal.org/home/gene/TACR3>).

Searches for the *TACR3* SNPs associated with VMS in the current study produced no results in GTEx.

The results of the current study do not confirm the results of the previous candidate gene studies, and previous candidate gene studies of genetic variation on chromosome 4 found no associations between SNPs in candidate genes (sulfotransferase family 1E, estrogen-preferring, member 1 [SULT1E1]; UDP glucuronosyltransferase 2 family, polypeptide B7 [UGT2B7]; UDP glucuronosyltransferase 2 family, polypeptide B15 [UGT2B15]; kinase insert domain receptor [VEGFR2]).^{10,13,20} According to the National Center for Biotechnology Information gene dbSNP webpage, the locations of these genes (SULT1E1 69841212..69860765; UGT2B7 69051249..69112987; UGT2B15; 68646597..68670776; VEGFR2 55078259..55125595) are, however, not located in close proximity to the *TACR3* gene (103589468..103719816).

This study has potential limitations. Although it would be interesting to investigate gene-gene or gene-environment interactions, we do not have sufficient power with the current sample size to carry forward these analyses. The variants identified are noncoding and have not been implicated as regulatory. As is the case with all GWAS studies, genetic variation in intronic regions could indicate that SNPs exert effects on splicing or regulation of transcription or methylation sites of other genes, or they could affect translation, induce posttranslational modifications, lead to changes in mRNA stability, or affect ligand binding.⁵³ Alternatively, the identified SNPs may not be causal, but may instead be tightly linked with the underlying true causal variants.

It is likely that there are still rare variants of moderate effect as well as common variants of small effect that we have not detected. By combining studies across ethnic groups using meta-analysis, we down-weight the importance of ethnic-specific association. In addition, each of the cohorts were genotyped using SNP platforms that include approximately 1 million SNPs, which means that many SNPs and single-nucleotide variations are not directly genotyped, that is we could fail to discover a rare variant (or even a common variant) that is not included in the SNP array. There are, however, two related points that ameliorate this concern. First, LD leads to high correlation between the variants at an SNP that was genotyped and the variants of loci that were not genotyped. Second, based on this principle of LD, our densely genotyped marker sets, and well-characterized reference panels, we imputed 10-fold more SNPs than were directly genotyped.

Although VMS were self-reported in this study, self-reporting of VMS reflects clinical practice. Also, because individuals are unaware of their genotypes, self-report should not be differentially biased between *TACR3* variant carriers and those individuals homozygous for the major allele. The numbers of women with severe VMS at baseline were too low to allow us to reliably examine genetic variation in relation to the severity of VMS. It is possible that women with severe VMS did not consent for inclusion in the study. Strengths of this study include the large sample size, ethnic

diversity of participants, and detailed information regarding potential confounders. The GWAS approach provides an unbiased source of information regarding previously unknown functional pathways.

We are encouraged by the GWAS results and our bioinformatics analyses, which lend support to the hypothesis of the NKB pathway as a target for the treatment of VMS symptoms, but confirmation of these results is necessary before clinical implications can be made. Necessary steps in testing this hypothesis include replication and fine-mapping of *TACR3*, perhaps by targeted sequencing followed by *in-silico* annotation, characterization of variant function using epigenetic and expression data, and ultimately functional studies using cell lines, human tissue, and isogenetic or mouse models.⁵⁴

CONCLUSIONS

In conclusion, several SNPs located in the area coding for *TACR3* were associated with increased odds of VMS at a $P < 5 \times 10^{-8}$. Per allele, each of these SNPs was associated with 1.4- to 1.6-fold higher odds of VMS. The ORs were consistent in several cohorts from diverse ethnic groups. To our knowledge, the present study is the first GWAS of VMS. Ultimately, elucidation of the mechanisms responsible for VMS could yield novel therapies for hot flashes. Our results should inform the study of biological pathways responsible for VMS. Interrelations between genetic markers and other known environmental risk factors for VMS require study.

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For a list of all the investigators who have contributed to WHI science, please visit: <https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf>.

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