Published in final edited form as:
Menopause. 2015 January ; 22(1): 108–113. doi:10.1097/GME.0000000000000291.

A potentially functional variant in the serotonin transporter gene is associated with pre and peri-menopausal hot flashes

May E Montasser, MS, PhD[1], **Ayelet Ziv-Gal, PhD**[2], **Jessica P Brown, PhD**[3], **Jodi A Flaws, PhD**[2], and **Istvan Merchenthaler, MD, PhD, DSc**[3,4]

[1]Department of Medicine, University of Maryland, School of Medicine, Baltimore, MD

[2]Department of Comparative Biosciences, University of Illinois, Urbana, IL

[3]Department of Epidemiology & Public Health, University of Maryland, School of Medicine, Baltimore, MD

[4]Department of Anatomy & Neurobiology, University of Maryland, School of Medicine, Baltimore, MD

Abstract

Objective—There is a recent increase in using selective serotonin reuptake inhibitors and/or serotonin-norepinephrine reuptake inhibitors (SSRIs/SNRIs) to relieve menopausal hot flashes (HF). However the response to them is heterogeneous. We hypothesized that this heterogeneity might be partially due to genetic variations in the genes encoding the serotonin and/or norepinephrine transporters (SLC6A4, SLC6A2). As a first step in testing the role of genetics in response to SSRIs/SNRIs, we examined the association between HF and genetic variants within these two genes.

Methods—We tested 29 haplotype tag SNPs within SLC6A4 and SLC6A2 for their association with HF separately in European American (EA; 396 cases, 392 controls) and African American (AA; 125 cases, 81 controls) pre/peri-menopausal women.

Results—We found that the minor allele of SLC6A4_rs11080121 was associated with protection against HF (OR=0.75, 95CI=0.60-0.94) only in EA. Bioinformatics analyses indicated that rs11080121 is fully correlated with rs1042173 in the 3'UTR of SLC6A4. The minor allele of rs1042173 seems to disrupt a conserved binding site for hsa-miR-590-3p microRNA.

Conclusions—Disrupting a microRNA binding site should lead to higher expression of SLC6A4, higher SLC6A4 will lead to depleted serotonin from the synaptic cleft, and that will trigger the presynaptic autoreceptor feedback mechanism to produce more serotonin, which is protective against HF.

Corresponding author: Istvan Merchenthaler, MD, PhD, DSc, Professor, Department of Epidemiology & Public Health, Professor, Department of Anatomy & Neurobiology, University of Maryland at Baltimore, School of Medicine, 10 S. Pine Str. MSTF Room 936, Baltimore, MD 21201, Ph: 410-706-1350; Fax: -4581, imerchen@epi.umaryland.edu.

Conflict of interest: none

This is the first study to test the association between HF in both EA and AA pre/peri-menopausal women and genetic variants in two neurotransmitter transporter genes: SLC6A2 and SLC6A4. This information can be used in tailoring pharmaceutical use of SSRIs/SNRIs for HF relief.

Keywords

Hot Flashes; Menopause; SLC6A4; SLC6A2; SSRIs/SNRIs

Introduction

Hot flashes (HF) are the most common symptoms of menopause. They are of concern because they significantly reduce the quality of life of millions of women and are the primary reason for seeking medical help during the menopause transition. Further, hot flushes are of concern because they might also be associated with other serious conditions, including cardiovascular disease and osteoporosis.[1-3]

Hot flushes are due to ovarian senescence and the falling levels or the lack of estrogens and progesterone. In addition, the irregular release of these hormones from the ovaries also contributes to the generation of hot flushes. In addition to estrogens and progesterone, environmental and genetic factors also play a role in the etiology of hot flushes. Confirmed environmental risk factors for HF include smoking, late menopausal stages, low levels of estrogen and inhibin A and B, high levels of follicle-stimulating hormone (FSH), African American race, and high body mass index (BMI).[4-5] Inconsistent or weak evidence also exists for other risk factors such as: alcohol use, physical inactivity, mood disorders such as depression and anxiety, life events such as childhood abuse and mental workload, and past hormone therapy.[4,5]

Since hormonal levels play a major role in the etiology of HF, several investigators hypothesized that genetic determinants of hormone levels might be linked to HF and thus, tested whether genetic polymorphisms in the genes encoding estrogen receptors (ESR) and the estrogen biosynthesis and metabolic pathway (CYP450) were associated with HFs. Indeed, it has been demonstrated that Chinese women who are carriers of the CYP1A1_rs2606345 variant report diminished vasomotor symptoms compared with non-carriers.[6] An association between CYP1B1 variants in African-American women and symptom reporting has been described by several groups.[6-8] Also, in addition to providing evidence that smoking and genetic variation in sex steroid metabolism impact menopausal end points separately,[9-11] a recent study has shown interactions between variants in sex steroid-metabolizing genes (CYP450 and Catechol O-methyl-transferase (COMT)) and smoking behavior on the risk of menopausal symptoms.[12] Estrogen receptor (ER) single nucleotide polymorphisms (SNPs) are also associated with the frequency of tamoxifen-induced HF,[13] suggesting that the ER genotype may be used to predict who may suffer HF during tamoxifen treatment. SNPs of the CYP19 gene may predict the risk of breast cancer in healthy patients,[14,15] are associated with lower estrogen levels and subsequent HF and arthralgia,[16] and may change the response of breast cancer progression to letrosole and tamoxifen treatment.[17,18]

Before 2002, estrogen therapy (ET) or the combined estrogen-progestin therapy (hormone therapy, HT) were widely used among pre, peri, and menopausal women to alleviate their HF and other menopausal symptoms such as depression, sleeplessness, diminished sexual desire, etc. However, the data from the Women's Health Initiative (WHI) studies published in 2002,[19] raised some serious concerns about the use of HT including the potential elevation of the incidence of breast cancer, deep venous thrombosis, and stroke. Although ET had fewer side effects than HT, it still was reported to increase the incidence of cerebrovascular stroke. Although re-analysis of the data by age group clearly showed that if ET or HT is taken within ten years of the cessation of the menstrual cycle, these side effects are minimal and the beneficial effects are dominant,[20] a large number of women stopped taking ET or HT. As a consequence, many women turned to other treatment options to relieve their menopausal symptoms. Among these, the use of selective serotonin reuptake inhibitors (SSRIs) and/or serotonin-norepinephrine reuptake inhibitors (SSRIs/SNRIs) become popular. However, it has become clear that SSRIs/SNRIs are less efficient than ET or HT and the response to them is heterogeneous.[21-23] This heterogeneous response might be due to single SNPs on the serotonin transporter (SERT) gene or the norepinephrine transporter (NET) gene, but no studies have examined whether genetic polymorphisms in SSRIs/SNRIs were associated with HF although a large number of publications reported association with psychiatric disorders.[24] Such an association with HF, however, would be important to explore in order to prescribe SSRIs/SNRIs only for those who lack such polymorphism(s) and consequently, will most likely benefit from the therapy.

As a first step in testing the genetic variants for the drug response, we first examined the association between HF and genetic variants within a norepinephrine transporter gene (SLC6A2), and a serotonin transporter gene (SLC6A4), in both European American and African American pre and peri-menopausal women.

Materials and Methods

Study population

Participants were recruited for a cross-sectional study of risk factors for menopausal hot flashes in midlife women. Detailed methods of participant recruitment are described elsewhere.[8,25] Briefly, between 2000 and 2004, generally healthy women residing in Baltimore city and the surrounding counties were sent recruitment letters via mail. Potential participants had to meet the following eligibility criteria: aged 45 to 54 years, not taking hormone therapy, not pregnant, and no history of cancer. Further, because the study was designed to examine the health of women undergoing the menopausal transition, women were eligible only if they reported having at least three menstrual periods in the last 12 months. Therefore, all women in the study were either pre- or peri-menopausal. Pre-menopausal women were those who experienced their last menstrual period within the past 3 months and reported 11 or more periods within the past year. Peri-menopausal women were those who experienced: 1) their last menstrual period within the past year but not within the past 3 months or 2) their last menstrual period within the past 3 months and experienced 10 or fewer periods within the past year. Eligible women were scheduled to come to the clinic during the morning hours and were instructed to fast overnight prior to the visit. During the

clinic visit, each participant had their weight and height measured, and a blood sample was drawn for genetic analyses. The participants also completed the detailed study survey, which included questions regarding demographics; medical, family, and reproductive history; past exogenous hormone use, menopausal symptoms; and lifestyle habits (e.g. smoking, diet). All participants in this study gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards.

Age and race/ethnicity were self-reported. Body mass index (BMI) was calculated based on height and weight measurements of the participant at the clinic visit and categorized either as normal BMI (≤24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), or obese (≥30.0 kg/m^2). Smoking status at the time of enrollment (current/former/never) was determined using the questions “Have you ever smoked cigarettes?” and “Do you still smoke cigarettes?” A woman’s history of ever experiencing midlife hot flashes was determined using the question “Have you ever experienced hot flashes?” Women experiencing hot flashes were further queried regarding hot flash severity (mild, moderate, or severe), frequency (daily, weekly, or monthly), and duration (number of months/years). Hot flash outcomes examined in the analyses were: ever experienced hot flashes, moderate or severe hot flashes, daily hot flashes, and hot flashes experienced for one year of greater (long duration). For all hot flash outcomes, the comparison group was never experienced hot flashes.

SNP selection, genotyping, and analysis

Thirty six haplotype tagging SNPs from the SLC6A2 gene and 7 haplotype tagging SNPs from the SLC6A4 gene were genotyped in 1125 individuals from several races (European American, African American, Hispanic, Asian, and other) using Taqman genotyping assays (Life Technologies, Foster City, CA) according to the manufacture’s recommendation.

After calling and cleaning the genotype data using Sequence Detection Systems version 2.4.1, 29 SNPs and 1021 persons were available for the association test. Haploview was used for SNP descriptive statistics.[26] The association test was performed separately for European Americans (EA; 396 cases, 392 controls) and African Americans (AA; 125 cases, 81 controls). Other ethnicities were not included in the analysis due to small sample size.

The Proc casecontrol was used to test the association between the HF status and each SNP using an additive genetic model with 10000 permutations to calculate the empirical p values. Proc logistic was used to calculate the odds ratio of the significant SNPs, adjusted for age, menopausal status (pre, peri), smoking status (current, former, never), and BMI. All statistical analyses were performed using SAS statistical software version 9.2 (SAS Institute, Cary, NC). False Discovery Rate (FDR) was used to correct for the multiple testing.[27]

Bioinformatics analyses were performed using several online utilities (including SNAP,[28] SNPinfo,[29] Regulome DB,[30] and DIANA-microT 3.0.[31]) in order to examine the predicted functionality of the significant SNPs and those that are highly correlated with them.

Results

Table 1 shows the basic characteristics of the study participants by HF status separately for EA and AA. Women who experienced HFs were more likely to be older and to be current or former smokers. EA women who experienced HF had a significantly higher BMI than EA women who did not experience HF. While there were a comparable number of women with and without HF among EA, the number of AA women who experienced HF was significantly higher than AA women who did not experience HFs.

Table 2 shows the basic statistics for the 29 SNPs that were used in the analysis. All SNPs were in Hardy Weinberg equilibrium ($p>0.001$) and had an average call rate of 98%. Minor allele frequency (MAF) for most of the SNPs was different between EA and AA, and in several cases the minor allele was switched between the two races (consistent with publically available data).

Table 3 shows the empirical p values for the association between each SNP and HF status separately for EA and AA women. Only two SNPs in the SLC6A4 gene were significantly associated with the HF status in EA after accounting for multiple testing, rs2066713 ($p= 0.0009$, FDR= 0.02) and rs11080121 ($p= 0.0038$, FDR=0.05). The odds ratios for the minor alleles of these two SNPs adjusted for age, menopausal status (pre, peri), smoking status (current, former, never), and BMI, showed that the minor allele (A) of rs2066713 was a risk factor for HF (OR=1.29, 95CI=1.03-1.60), while the minor allele (C) of rs11080121 was protective against HF (OR=0.75, 95CI=0.60-0.94).

Bioinformatics analyses identified 21 SNPs that are highly correlated with rs11080121 ($r^2>0.8$). One of these 21 SNPs (rs1042173, $r^2=1$) is in the 3'UTR of SLC6A4, and located in the micro RNA (miRNA) recognition element of two miRNAs (hsa-miR-142-5p, and hsa-miR-590-3p).

The minor allele C of rs1042173 seems to disrupt the first nucleotide of a conserved binding site for hsa-miR-590-3p. Three of these 21 SNPs (rs1906451, rs7224199, and rs4583306) had Regulome score of 1f (Likely to affect binding and linked to gene expression). Another one of these 21 SNPs (rs9303628) had a Regulome score of 2b (Likely to affect binding). No evidence of functionality was found for rs2066713, or any of its highly correlated SNPs.

Discussion

Although the precise mechanism of HF is not known, one explanation is that in asymptomatic women, there is a thermo-neutral zone (about 0.4C) within which fluctuations of the core body temperature do not trigger compensatory mechanisms such a HF or sweating.[32] In symptomatic women, the thermo-neutral zone may be reduced so that even minor fluctuations in core body temperature will reach the limits of the zone and initiate a thermoregulatory response. The narrowing of the zone may be due to noradrenaline (also called norepinephrine) activation due to the lack of sufficient estrogen and/or serotonin action. Estrogens enhance the synthesis of serotonin and inhibit the production of noradrenaline.[33-35] Therefore, the interventions which increase estrogen and serotonin levels and decrease noradrenaline levels may widen the thermo-neutral zone and therefore be

expected to reduce HF. Indeed, ET and HT are the most efficacious therapies in alleviating HF, but SSRIs and/or SSRIs/SNRIs also show some efficacies in preclinical,[36] and clinical studies.[6-8] However, the response to SSRIs/SNRIs therapies is heterogeneous, i.e., in some women SSRIs or SNRIs reduce the frequency and severity of HF, but in others they do not have beneficial effects. We hypothesized that this heterogeneous response may be due to SNPs in the serotonin and norepinephrine transporter genes.

To the best of our knowledge, this is the first study to test the association between HF in both EA and AA pre and peri-menopausal women and genetic variants in two neurotransmitter transporter genes: SLC6A2 and SLC6A4. SLC6A2 (norepinephrine transporter) and SLC6A4 (serotonin transporter) are both members of the sodium: neurotransmitter symporter family, which transports the neurotransmitters from synaptic clefts into presynaptic neurons. Several polymorphisms in these genes were repeatedly found to be associated with several psychiatric disorders as well as their response to different drugs.[24]

Our study identified two SNPs within the SLC6A4 gene that are significantly associated with HF status only in EA women. The minor allele C of one of these two SNPs (rs11080121) was found to be protective against HF (OR=0.75, 95CI=0.61-0.93). SLC6A4_rs11080121 was fully correlated with another SNP (rs1042173) in the 3'UTR of SLC6A4 which was found by bioinformatics analyses to have good evidences for functionality. The minor allele C of rs1042173 seems to disrupt the first nucleotide of a conserved binding site for hsa-miR-590-3p. Disrupting a binding site for miRNA which usually down-regulates gene expression,[37] could lead to higher expression of SLC6A4, which might look like a contradiction since SSRIs/SNRIs work by inhibiting the production of SLC6A4. However, the higher levels of SLC6A4 will increase the uptake of serotonin from the synaptic cleft back into presynaptic neurons, leading to lower level of serotonin available for transmission. This shortage in serotonin will activate the feedback mechanism that is orchestrated by the presynaptic autoreceptors and triggers the production of more serotonin in the presynaptic neurons.[38] The shortage in serotonin will also trigger the postsynaptic neurons to compensate by increasing the numbers of postsynaptic serotonin receptors.[39] Therefore, for a woman who has been born with a variant that increases the production of SLC6A4, the autoreceptor feedback mechanism will cause her neurons to release more serotonin, which will protect her from HF in adulthood, and that is consistent with our result. The autoreceptor feedback mechanism is the explanation for the contradiction between the protective effect of SSRIs that work by inhibiting the production of SLC6A4, and the higher risk of mood and depression behavior that have been observed in animals and humans harboring the short allele of 5-HTTLPR that reduces SLC6A4 expression.[38-40]

Interestingly, hsa-miR-590-3p was previously associated with 2 different neurological disorders: Alzheimer disease (AD) and Autism spectrum disorder (ASD). In AD patients, hsa-miR-590-3p was identified as a possible down-regulator for the heterogeneous nuclear ribonucleoprotein A1(hnRNP-A1) gene, that plays a role in neurodegenerative disorders.[41] In ASD, hsa-miR-590-3p was identified as the most important miRNA that is potentially regulating a long list of ASD-associated genes.[42] On the other hand hsa-miR-142-5p was

found to be associated with gastric cancer recurrence in combination with another miRNA,[43] and was recently strongly correlated with gene expression and methylation level in several cancer types.[44]

SLC6A4_rs11080121 was also highly correlated with several other SNPs that were found by bioinformatics analyses to have a high probability of affecting the binding of transcription factors and dysregulating gene expression. All the identified association warrant replication in another study and their predicted functionality need to be tested and confirmed experimentally. The associated SNPs also need to be examined for their association with differential response to SSRIs as a HF treatment especially among EA women to identify those who will better benefit from this medication, and use different type of medication with those who might not benefit from it.

Our study is limited by the small sample size in the AA population which might explain the lack of significance among them, however, the lack of association might also be a result of actual genetic heterogeneity between EA and AA. A larger study will be needed to clarify that.

Conclusion

In light of the current trend for personalized clinical medicine, our ultimate goal is to assist in the design of non-hormonal, tailored pharmaceutical treatments based on SNP status. The study described above is the first step toward this direction.

Acknowledgments

Financial support: This project received support from (R21AG033864-02, and R01 AG18400) from the NIH/NIA, and from The Mid-Atlantic Nutrition Obesity Research Center (P30 DK072488) from the NIH/NIDDK.

References

1. Whiteley J, Wagner JS, Bushmakin A, Kopenhafer L, Dibonaventura M, Racketa J. Impact of the severity of vasomotor symptoms on health status, resource use, and productivity. Menopause. 2013; 20(5):518–524. [PubMed: 23403500]
2. Dibonaventura MD, Chandran A, Hsu MA, Bushmakin A. Burden of vasomotor symptoms in france, germany, italy, spain, and the united kingdom. Int J Womens Health. 2013; 5:261–269. [PubMed: 23737678]
3. Archer DF, Sturdee DW, Baber R, et al. Menopausal hot flushes and night sweats: Where are we now? Climacteric. 2011; 14(5):515–528. [PubMed: 21848495]
4. Ziv-Gal A, Flaws JA. Factors that may influence the experience of hot flushes by healthy middle-aged women. J Womens Health (Larchmt). 2010; 19(10):1905–1914. [PubMed: 20831431]
5. Hunter MS, Gentry-Maharaj A, Ryan A, et al. Prevalence, frequency and problem rating of hot flushes persist in older postmenopausal women: Impact of age, body mass index, hysterectomy, hormone therapy use, lifestyle and mood in a cross-sectional cohort study of 10,418 british women aged 54-65. BJOG. 2012; 119(1):40–50. [PubMed: 22008610]
6. Crandall CJ, Crawford SL, Gold EB. Vasomotor symptom prevalence is associated with polymorphisms in sex steroid-metabolizing enzymes and receptors. Am J Med. 2006; 119(9 Suppl 1):S52–60. [PubMed: 16949389]
7. Rebbeck TR, Su HI, Sammel MD, et al. Effect of hormone metabolism genotypes on steroid hormone levels and menopausal symptoms in a prospective population-based cohort of women experiencing the menopausal transition. Menopause. 2010; 17(5):1026–1034. [PubMed: 20505544]

8. Schilling C, Gallicchio L, Miller SR, Langenberg P, Zacur H, Flaws JA. Genetic polymorphisms, hormone levels, and hot flashes in midlife women. Maturitas. 2007; 57(2):120–131. [PubMed: 17187946]

9. Cochran CJ, Gallicchio L, Miller SR, Zacur H, Flaws JA. Cigarette smoking, androgen levels, and hot flushes in midlife women. Obstet Gynecol. 2008; 112(5):1037–1044. [PubMed: 18978103]

10. Gallicchio L, Miller SR, Visvanathan K, et al. Cigarette smoking, estrogen levels, and hot flashes in midlife women. Maturitas. 2006; 53(2):133–143. [PubMed: 16368467]

11. Visvanathan K, Gallicchio L, Schilling C, et al. Cytochrome gene polymorphisms, serum estrogens, and hot flushes in midlife women. Obstet Gynecol. 2005; 106(6):1372–1381. [PubMed: 16319265]

12. Butts SF, Freeman EW, Sammel MD, Queen K, Lin H, Rebbeck TR. Joint effects of smoking and gene variants involved in sex steroid metabolism on hot flashes in late reproductive-age women. J Clin Endocrinol Metab. 2012; 97(6):E1032–42. [PubMed: 22466345]

13. Henry NL, Rae JM, Li L, et al. Association between CYP2D6 genotype and tamoxifen-induced hot flashes in a prospective cohort. Breast Cancer Res Treat. 2009; 117(3):571–575. [PubMed: 19153830]

14. Fasching PA, Loehberg CR, Strissel PL, et al. Single nucleotide polymorphisms of the aromatase gene (CYP19A1), HER2/neu status, and prognosis in breast cancer patients. Breast Cancer Res Treat. 2008; 112(1):89–98. [PubMed: 18049890]

15. Long JR, Kataoka N, Shu XO, et al. Genetic polymorphisms of the CYP19A1 gene and breast cancer survival. Cancer Epidemiol Biomarkers Prev. 2006; 15(11):2115–2122. [PubMed: 17119036]

16. Park IH, Lee YS, Lee KS, et al. Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer. Cancer Chemother Pharmacol. 2011; 68(5):1263–1271. [PubMed: 21442439]

17. Ma CX, Adjei AA, Salavaggione OE, et al. Human aromatase: Gene resequencing and functional genomics. Cancer Res. 2005; 65(23):11071–11082. [PubMed: 16322257]

18. Colomer R, Monzo M, Tusquets I, et al. A single-nucleotide polymorphism in the aromatase gene is associated with the efficacy of the aromatase inhibitor letrozole in advanced breast carcinoma. Clin Cancer Res. 2008; 14(3):811–816. [PubMed: 18245543]

19. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. JAMA. 2002; 288(3):321–333. [PubMed: 12117397]

20. MacLennan AH. HRT: A reappraisal of the risks and benefits. Med J Aust. 2007; 186(12):643–646. [PubMed: 17576182]

21. Loprinzi CL, Stearns V, Barton D. Centrally active nonhormonal hot flash therapies. Am J Med. 2005; 118(Suppl 12B):118–123. [PubMed: 16414336]

22. Loprinzi CL, Barton D, Rummans T. Newer antidepressants inhibit hot flashes. Menopause. 2006; 13(4):546–548. [PubMed: 16837874]

23. Nelson HD, Vesco KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: Systematic review and meta-analysis. JAMA. 2006; 295(17):2057–2071. [PubMed: 16670414]

24. Froehlich TE, McGough JJ, Stein MA. Progress and promise of attention-deficit hyperactivity disorder pharmacogenetics. CNS Drugs. 2010; 24(2):99–117. [PubMed: 20088618]

25. Ziv-Gal A, Gallicchio L, Miller SR, Zacur HA, Flaws JA. Genetic polymorphisms in the aryl hydrocarbon receptor signaling pathway as potential risk factors of menopausal hot flashes. Am J Obstet Gynecol. 2012; 207(3):202.e9–202.e18. [PubMed: 22840970]

26. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]

27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological). 1995; 57(1):289–300.

28. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: A web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008; 24(24):2938–2939. [PubMed: 18974171]

29. Xu Z, Taylor JA. SNPinfo: Integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res. 2009; 37(Web Server issue):W600–5. [PubMed: 19417063]
30. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 2012; 22(9):1790–1797. [PubMed: 22955989]
31. Maragkakis M, Reczko M, Simossis VA, et al. DIANA-microT web server: Elucidating microRNA functions through target prediction. Nucleic Acids Res. 2009; 37(Web Server issue):W273–6. [PubMed: 19406924]
32. Freedman RR. Pathophysiology and treatment of menopausal hot flashes. Semin Reprod Med. 2005; 23(2):117–125. [PubMed: 15852197]
33. Bethea CL, Lu NZ, Gundlah C, Streicher JM. Diverse actions of ovarian steroids in the serotonin neural system. Front Neuroendocrinol. 2002; 23(1):41–100. [PubMed: 11906203]
34. Freedman RR, Krell W. Reduced thermoregulatory null zone in postmenopausal women with hot flashes. Am J Obstet Gynecol. 1999; 181(1):66–70. [PubMed: 10411797]
35. Shanafelt TD, Barton DL, Adjei AA, Loprinzi CL. Pathophysiology and treatment of hot flashes. Mayo Clin Proc. 2002; 77(11):1207–1218. [PubMed: 12440557]
36. Deecher DC, Alfinito PD, Leventhal L, et al. Alleviation of thermoregulatory dysfunction with the new serotonin and norepinephrine reuptake inhibitor desvenlafaxine succinate in ovariectomized rodent models. Endocrinology. 2007; 148(3):1376–1383. [PubMed: 17122073]
37. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet. 2007; 8(2):93–103. [PubMed: 17230196]
38. Gobbi M, Crespi D, Foddi MC, et al. Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT1B autoreceptors, 5-HT3 and 5-HT4 receptors in rats. Naunyn Schmiedebergs Arch Pharmacol. 1997; 356(1):22–28. [PubMed: 9228186]
39. Eison AS, Mullins UL. Regulation of central 5-HT2A receptors: A review of in vivo studies. Behav Brain Res. 1996; 73(1-2):177–181. [PubMed: 8788498]
40. Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science. 2004; 306(5697):879–881. [PubMed: 15514160]
41. Villa C, Fenoglio C, De Riz M, et al. Role of hnRNP-A1 and miR-590-3p in neuronal death: Genetics and expression analysis in patients with alzheimer disease and frontotemporal lobar degeneration. Rejuvenation Res. 2011; 14(3):275–281. [PubMed: 21548758]
42. Vaishnavi V, Manikandan M, Tiwary BK, Munirajan AK. Insights on the functional impact of microRNAs present in autism-associated copy number variants. PLoS One. 2013; 8(2):e56781. [PubMed: 23451085]
43. Zhang X, Yan Z, Zhang J, et al. Combination of hsa-miR-375 and hsa-miR-142-5p as a predictor for recurrence risk in gastric cancer patients following surgical resection. Ann Oncol. 2011; 22(10):2257–2266. [PubMed: 21343377]
44. Andreopoulos B, Anastassiou D. Integrated analysis reveals hsa-miR-142 as a representative of a lymphocyte-specific gene expression and methylation signature. Cancer Inform. 2012; 11:61–75. [PubMed: 22570537]

Table 1

The basic characteristics of the study participants separately for European American (EA) and African American (AA) pre and peri-menopausal.

Variable	Cases	Controls	p
EA			
N (count)	396	392	0.8
Age (years)	48.96	48.06	<0.0001
BMI (Kg/m^2)	27.5	26.2	0.005
Smoking (count)			0.001
current	42	22	
former	168	141	
never	182	225	
Menopausal status (count)			<0.0001
pre	107	254	
peri	289	138	
AA			
N (count)	125	81	0.002
Age (years)	49.04	47.78	<0.0001
BMI (Kg/m^2)	32.7	32.2	0.6
Smoking (count)			0.005
current	23	4	
former	48	26	
never	53	49	
Menopausal status (count)			<0.0001
pre	50	60	
peri	75	21	

Table 2

Basic statistics of the 29 analyzed SNPs separately for European American (EA) and African American (AA) pre and peri-menopausal women.

		EA				AA			
SNP	**Position**	**%Geno**[a]	**MAF**[b]	**Alleles**[c]	**HWE**[d]	**%Geno**	**MAF**	**Alleles**	**HWE**
rs11080121	28528842	99.5	0.476	T/C	0.4372	100	0.226	T/C	0.3512
rs 140700	28543389	98.5	0.074	C/T	0.9813	98.5	0.042	C/T	0.6824
rs 8076005	28547210	95.9	0.163	A/G	0.6216	94.2	0.356	**G/A**	0.3002
rs 2066713	28551665	100	0.39	G/A	0.432	99.5	0.273	G/A	0.7937
rs 4251417	28551858	99.5	0.099	C/T	0.4476	99.5	0.017	C/T	0.8652
rs 16965628	28555425	97.6	0.066	G/C	0.3623	97.6	0.294	G/C	0.6535
rs 4404067	55683682	97.7	0.321	G/A	0.8974	96.6	0.497	**A/G**	0.1325
rs 4238784	55687065	98	0.222	A/G	0.7291	97.6	0.129	A/G	0.3879
rs 747107	55695721	98.5	0.155	C/T	0.2652	99	0.157	C/T	0.1801
rs 13333066	55703048	98.5	0.295	C/T	0.8932	95.1	0.362	C/T	0.4792
rs 187715	55704042	95.1	0.047	T/C	0.7303	94.2	0.015	T/C	0.954
rs 36026	55704795	99.6	0.103	C/A	0.1028	100	0.027	C/A	0.9554
rs 36024	55706391	93.4	0.416	G/A	0.5753	92.2	0.463	**A/G**	0.1496
rs 36021	55711950	95.8	0.431	A/T	0.6286	96.6	0.352	A/T	0.4489
rs 3785151	55712519	99.6	0.173	G/C	0.6334	98.5	0.429	**C/G**	0.7798
rs 36020	55713088	97.8	0.132	C/T	0.4086	97.6	0.052	C/T	0.7684
rs 16955591	55713786	99.4	0.132	C/T	0.7487	99	0.221	C/T	0.173
rs 3785152	55716550	99.7	0.109	C/T	0.5574	100	0.206	C/T	0.0173
rs 40147	55716840	98.7	0.306	G/A	0.959	99.5	0.21	G/A	0.0947
rs 1814270	55717077	99.9	0.433	T/C	0.1401	99	0.181	T/C	0.4755
rs 5564	55725975	99.5	0.078	A/G	0.0448	100	0.017	A/G	0.9554
rs 5568	55730124	99	0.34	A/C	0.1158	99.5	0.11	A/C	0.4319
rs 1566652	55731575	96.8	0.356	G/T	0.0119	98.5	0.131	G/T	0.5551
rs 5569	55731835	95.2	0.257	G/A	0.0616	95.1	0.094	G/A	0.2418
rs 2242447	55735912	98	0.346	T/C	0.7755	97.1	0.248	**C/T**	0.7783

		EA				AA			
SNP	**Position**	**%Geno**[a]	**MAF**[b]	**Alleles**[c]	**HWE**[d]	**%Geno**	**MAF**	**Alleles**	**HWE**
rs 42460	55737656	99.5	0.085	A/G	0.1988	99.5	0.1	A/G	0.3548
rs 16955708	55741456	99.2	0.152	T/C	0.8881	100	0.184	T/C	0.8415
rs 12596924	55744647	99.9	0.211	T/G	0.9591	98.1	0.374	**G/T**	0.4403
rs 258099	55747177	98	0.319	T/C	0.3883	97.6	0.249	**C/T**	0.6904

[a] %geno: the percentage of successful genotyping.

[b] MAF: minor allele frequency.

[c] Alleles are shown as major/minor allele, differential major/minor alleles between EA and AA are in bold.

[d] HWE: Hardy-Weinberg equilibrium calculated using controls only.

Table 3

Empirical p-values for the association between each SNP and the HF status in European American (EA) and African American (AA) pre and peri-menopausal women.

SNP	Position	p-value EA	p-value AA
rs11080121	28528842	0.0038	0.4211
rs 140700	28543389	0.7809	1
rs 8076005	28547210	0.8372	0.2793
rs 2066713	28551665	**0.0009**	0.2711
rs 4251417	28551858	0.3508	1
rs 16965628	28555425	0.6209	0.3597
rs 4404067	55683682	0.0971	0.3652
rs 4238784	55687065	0.2821	0.1732
rs 747107	55695721	0.8922	0.2721
rs 13333066	55703048	1	0.6882
rs 187715	55704042	0.6172	0.4079
rs 36026	55704795	0.2021	0.0505
rs 36024	55706391	0.4756	1
rs 36021	55711950	0.2207	0.0439
rs 3785151	55712519	1	0.2831
rs 36020	55713088	0.2951	0.1685
rs 16955591	55713786	1	0.4254
rs 3785152	55716550	0.7992	0.4202
rs 40147	55716840	0.4044	0.008
rs 1814270	55717077	0.4111	0.7029
rs 5564	55725975	0.3284	0.2506
rs 5568	55730124	0.577	0.5261
rs 1566652	55731575	0.3211	1
rs 5569	55731835	0.1599	0.2947
rs 2242447	55735912	0.7509	0.6529
rs 42460	55737656	0.7061	0.8616
rs 16955708	55741456	0.8353	0.5052
rs 12596924	55744647	0.4969	0.8423
rs 258099	55747177	0.7944	0.7375

Significant results in bold