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Letter

Association of *TAS2R38* Haplotypes and Menthol Cigarette Preference in an African American Cohort

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In a recent publication in *Nicotine & Tobacco Research*, Oncken *et al.*[1] examined 323 pregnant female Caucasian cigarette smokers, including menthol and nonmenthol users, and genotyped three *TAS2R38* bitter taste receptor gene polymorphisms (*rs713598*, *rs1726866*, and *rs10246939*). These polymorphisms specify whether an individual is a taster (associated with the PAV haplotype) or a nontaster (associated with the AVI haplotype) for a variety of bitter compounds, including the well-known phenylthiocarbamide and propylthiouracil.[2–3] The rationale behind this study was to test whether variations in the well-studied *TAS2R38* bitter taste receptor gene could contribute to the preference of smokers for menthol cigarettes, because menthol could mask the bitter taste of nicotine or other components of cigarette smoke. Oncken *et al.* reported the frequency of the PAV taster haplotype to be greater in menthol smokers than in nonmenthol smokers in both non-Hispanic (54% vs. 30%, respectively, $p < 0.001$) and Hispanic (53% vs. 25%, respectively, $p = 0.016$) women, confirming this initial hypothesis.

While this research reported an intriguing association between variations in a bitter taste receptor gene and preference for menthol cigarettes, the authors pointed out a number of weaknesses in their study, including the relatively small sample size and the fact that they only studied pregnant Caucasian women. The authors noted that it was not clear if these results generalize to other ethnicities, nonpregnant women or men. In addition, genotype–phenotype association studies typically require replication in an independent group in order to exclude any potential confounding population stratification and increase the confidence in the findings.[4] Moreover, focusing the attention on Caucasian individuals precludes the possibility of studying rarer alleles and haplotypes, since individuals of African descent carry greater levels of genetic diversity and more polymorphic sites when compared to non-African populations.[5] This is of particular importance when studying menthol cigarette smoking, since an average of 80% of adult African Americans are menthol smokers, while only 25–35% of Caucasian and Hispanic smokers use menthol.[6]

In order to overcome these limitations, we recruited 718 African Americans smokers (236 females and 482 males) in the Washington DC area with written informed consent under protocols reviewed and approved by the National Institutes of Health Combined Neurosciences IRB and the Western IRB (National Institutes of Health protocol 01-DC-0230). All procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. The average age of these individuals was 45.1 ($SD = 10.8$) and 406 (56.5%) of them were menthol smokers, with the remaining 312 (43.5%) being nonmenthol smokers. A higher percentage of menthol than nonmenthol smokers were female (39.6% vs. 24.2%, $p = 0.001$), in agreement with previously reported data.[6] No differences were found in the mean age of menthol smokers (45.4, $SD = 11.0$) and nonmenthol smokers (44.6, $SD = 10.6$; $p = 0.372$). We collected saliva samples from all participants using Oragene saliva collection kits (Genotek Inc, Kanata, ON, Canada) and we purified genomic DNA following the manufacturer's protocol. A dedicated set of primers was designed to completely sequence the single coding exon of the TAS2R38 gene using dideoxy Sanger sequencing, as previously published.[7] We constructed and calculated the frequencies of the taster PAV (45.47%), nontaster AVI (33.22%), and the rarer AAI (17.90%), AAV (2.37%), PVI (0.76%), and PAI (0.28%) haplotypes, and we examined associations between their frequency and menthol cigarette preference in the group. The nontaster AVI haplotype was inversely associated with menthol cigarette smoking, even after correction for sociodemographic factors (such as age, gender, marital status, and education level) and multiple testing correction, implemented in a logistic regression model.

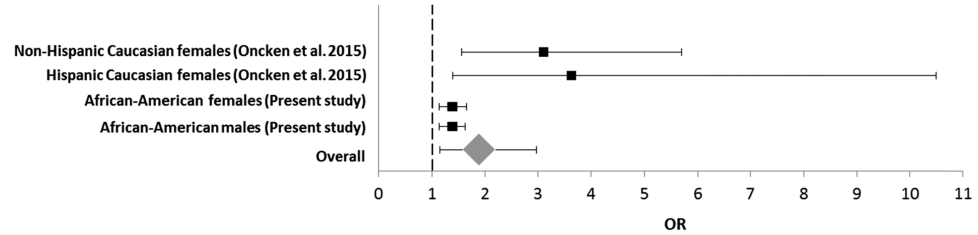

Figure 1. Forest plot illustrating the odds ratios and confidence intervals of the TAS2R38 PAV haplotype association with menthol cigarette smoking in both African American and Caucasian individuals.

The frequency of this haplotype was significantly lower in menthol smokers (29.8%) compared to nonmenthol smokers [37.7%; odds ratio (OR) = 0.70, p = 0.008]. This association was replicated in both female (OR = 0.89, p = 0.01) and male (OR = 0.72, p = 0.02) individuals and showed a gene dosage effect with 62.3%, 53.8%, and 44.0% of menthol users carrying zero, one, or two copies of this haplotype, respectively. Conversely, the taster PAV haplotype was higher in menthol smokers (48.2%), compared to nonmenthol smokers (42.1%; OR = 1.24, p = 0.04). The fact that the association between the PAV haplotype and menthol smoking was not as strong as previously found[1] could be due to the relatively greater number of intermediate sensitivity haplotypes (AAI, AAV, PVI, and PAI haplotypes) in our subject sample that did not show a significant difference in frequency between menthol and nonmenthol smokers (all p's > 0.1). In addition, since our population displayed a high prevalence of African-derived haplotypes, there was not a strong and complementary relation between the common nontaster AVI and taster PAV haplotypes (r = –0. 64), in comparison to the one previously shown in Caucasians[1] (r = –0.87). Lastly, when performing a meta-analysis including the results of both the present study and of Oncken *et al.*,[1] the association between *TAS2R38* PAV haplotype and menthol cigarette smoking was still significant (p = 0.025, using a random effects model) resulting in a common OR of 1.78 (95% confidence interval = 1.07 to 2.94; Fig.1), with some evidence (p = 0.019) of expected heterogeneity, considering the differences in subject characteristics.

Our results confirm the previous work by Oncken *et al.*,[1] stressing the validity and importance of their findings which we have now extended to men and to different ethnic groups. Together, these data suggest that genetic variations that modify the ability of tasting bitter compounds could explain the observed differences and preference toward mentholated tobacco use. This is of particular importance, considering the fact that the potential risks associated with adding menthol to cigarettes have been a subject of considerable recent study by the Food and Drug Administration (FDA)[8] and risks to specific racial and ethnic minorities, such as African Americans and Hispanics have been raised in this context.[9]

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Declaration of Interests

None declared.

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