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LETTER TO THE EDITOR

No association between *ADCYAP1R1* and post-traumatic stress disorder in two independent samples

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Ressler *et al.*[1] recently examined the pituitary adenylate cyclase-activating peptide (PACAP) ligand/type 1 receptor (PAC1) pathway in post-traumatic stress disorder (PTSD) in a predominantly African American (AA) sample. Association analyses examining 44 single nucleotide polymorphisms (SNPs) spanning the PACAP (encoded by *ADCYAP1*) and PAC1 (encoded by *ADCYAP1R1*) genes further identified rs2267735, a SNP, in a putative estrogen response element (ERE) within the *ADCYAP1R1* gene, which predicted PTSD diagnosis and symptoms in females. We sought to replicate the genetic association between rs2267735 and PTSD in two independent samples.

The first sample consisted of women from the Nurses' Health Study II (NHSII), a population-based prospective cohort of US female nurses predominantly of European descent. The protocol for the NHSII PTSD study has been published.[2] We assessed lifetime trauma exposure and PTSD in 2690 women using a clinically validated structured diagnostic telephone interview. rs2267735 was genotyped using a TaqMan assay at the Channing Laboratory of Brigham and Women's Hospital. Statistical analyses were performed using *PLINK*.[3]

The genotyping success rate for rs2267735 was 98.2%. Genotype distribution did not depart from Hardy–Weinberg equilibrium (HWE). To reduce potential bias by population stratification, we restricted analyses to 2528 women who self-reported as being European American (EA; mean age at interview = 55 years, s.d. = 4.4). A total of 584 subjects (23.1%) had a lifetime PTSD diagnosis. In comparison, 33.0% of the 763 women in Ressler's study had current PTSD.[1] The minor allele in the study by Ressler *et al.*[1] was C (63.6%). In the NHSII sample, the frequency of the C allele was 46.9%.

The putative risk allele 'C' reported by Ressler *et al.*[1] was not significantly associated with lifetime PTSD risk in either unadjusted or adjusted logistic regression models, whether tested under additive, recessive or dominant modes of inheritance. In the unadjusted model, 'CC' genotype carriers showed a small but non-statistically significant increased risk of PTSD compared with 'CG/GG' carriers (OR = 1.12, 95% CI = 0.90–1.40), whereas Ressler *et al.*[1] reported a significantly increased risk (OR = 1.66, 95% CI = 1.32–2.09). Our results were similar after adjusting for age at interview, age at worst trauma, trauma type, number of traumatic events and lifetime depression before PTSD (adjusted OR (aOR) = 1.18, 95% CI = 0.94–1.49). In contrast with the previous findings,[1] rs2267735 was not associated with total number of PTSD symptoms, PTSD symptom severity or symptom subscale scores (unadjusted $P = 0.48$–0.98). We also found no significant association for current PTSD (aOR for 'CC' carriers = 0.94, 95% CI = 0.64–1.39), defined as symptoms occurring within the past month, or chronic PTSD (aOR = 1.14, 95% CI = 0.84–1.55), defined as symptoms lasting more than 1 year. The NHSII PTSD sample had nearly perfect power (> 99%) to detect the effect size reported by Ressler *et al.*[1] an OR of 1.66 under a dominant model with regards to the G allele.

As rs2267735 was located within a predicted ERE,[1] we further stratified the sample by menopausal status, based on the hypothesis that the rs2267735–PTSD association may be stronger in pre-menopausal women. Menopausal status and type of menopause were assessed by biennial NHSII questionnaires. 'CC' genotype did not significantly increase risk of PTSD in the pre-menopausal (aOR = 1.29, 95% CI = 0.96–1.74, $n = 1471$) or natural menopause group (aOR = 0.86, 95% CI = 0.43–1.73, $n = 440$).

The second sample contained 6074 individuals recruited for genetic studies of substance dependence at five US sites.[4,5] All subjects were interviewed by trained interviewers using the Semi-Structured Assessment for Drug Dependence and Alcoholism,[6,7] which assessed PTSD based on *DSM-IV* criteria. Based on STRUCTURE[8] analysis using 41 ancestry-informative markers, 3353 participants were classified as AA (51.8% male, mean age = 41.1 years, s.d. = 9.1) and 2721 participants were classified as EA (57.7% male, mean age = 37.7 years, s.d. = 11.0). A total of 13.1% of the AAs and 15.0% of the EAs had a lifetime diagnosis of PTSD. rs2267735 was genotyped using a TaqMan assay at the Yale University School of Medicine. The genotype distributions were consistent with HapMap data and with HWE expectations in both populations. Age-adjusted logistic regression analyses were used to examine the association between rs2267735 genotype and PTSD risk in AA females, AA males, EA females and EA males, separately. No significant association was observed in any of these four subgroups under either additive or dominant genetic models with respect to the G allele (dominant model: AA female: OR = 1.13, 95% CI = 0.86–1.50; AA male: OR = 0.99, 95% CI = 0.73–1.32; EA female: OR = 1.07,

95% CI = 0.76–1.50; EA male: OR = 0.93, 95% CI = 0.65–1.32). Both AA and EA female samples had good power (93 and 77%, respectively) to detect an effect of the size reported by Ressler *et al.*[1] In addition, there was no evidence of association when restricting the analysis to AA females younger than 45 years ($n = 1198$, of which 186 had PTSD) who are likely to be pre-menopausal using an additive or dominant model (dominant model: OR = 1.29, 95% CI = 0.93–1.78).

In summary, we were unable to replicate the association between rs2267735 and PTSD in either AA or EA females in these two large independent samples. Our findings do not, however, exclude the possibility that the PACAP-PAC1 receptor pathway has a role in the production of PTSD. Our replication samples differ principally from Ressler's sample on three grounds: sample selection, demographic characteristics and participant's social context. Ressler's sample was recruited from patients in medical clinics, and comprised of relatively young (mean age = 39.6 years), low-income, largely unemployed, AAs, highly trauma exposed, inner city residents. In contrast, the NHSII sample was population-based, older EAs with much higher income and education. The Yale samples comprised of both AA and EA young substance dependence cases and unaffected controls, related or unrelated, who participated in the study focused on the genetics of substance dependence (mean age = 39 years).

Some might argue that a 'true' genetic effect would be observed across samples regardless of these differences. However, as we note elsewhere, animal models and human correlational studies suggest both individual-level and social environmental factors modify genetic effects.[9,10] Further research is needed to understand how selection factors, demographic characteristics and social context influence the role of the PACAP–PAC1 receptor pathway in PTSD.

Conflict of interest

The authors declare no conflict of interest.

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