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Arguable Assumptions, Debatable Conclusions

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To the Editor:

Munafò *et al.* (1) conducted a meta-analysis of a subset of studies that examined the interaction between the serotonin transporter (5-HTTLPR) gene and stressful life events (SLEs) in conferring risk for depression. 5-HTTLPR has two common alleles of varying length, which are designated “s” for short and “l” for long. They concluded that the prior “positive results for the 5-HTTLPR $\times$ SLE interactions in logistic regression models are compatible with chance findings” and stated further that “the 5-HTTLPR and SLE interaction effect is negligible.” These conclusions, however, were based on assumptions in the simulation model that are inconsistent with prior research findings and a bias in sampling strategy that call into question the authors’ conclusions.

In the simulation reported in the Munafò article, the environmental effect was specified as having two levels—unexposed (E_1) and exposed (E_2)—corresponding to zero and one + SLE. As can be seen in Figure 1, in the original report by Caspi *et al.* (2) the presence of only one SLE conferred no risk for depression, regardless of genotype. Risk for depression in association with the “s” allele of 5-HTTLPR only began to increase significantly in the presence of three or more SLEs and was most pronounced in individuals with four or more SLEs. Collapsing subjects across the various levels of exposure to SLEs added significant noise in the model and compromised efforts to detect the 5-HTTLPR $\times$ SLE interaction in the meta-analysis.

The nonrepresentative nature of the sample of studies included in the meta-analysis is also a limitation of this report. Munafò *et al.* identified 14 studies of 33 published reports that met their criteria for inclusion in the meta-analysis but were unfortunately only able to obtain data from 5 of the 14 studies. The meta-analysis was conducted with data from less than one-half the studies that met the stated inclusion criteria, with a trend for negative studies to be over-represented in the meta-analysis. Of the 14 studies meeting the inclusion criteria for the meta-analysis, 75% (3/4) of the negative studies and only 20% (2/10) of the positive studies were included in the analysis (Fisher exact test: $p < .10$).

There has been a proliferation of studies examining this gene $\times$ environment interaction over the past 2 years and, to date, no comprehensive meta-analysis of all studies. Since the

publication of the article by Munafò *et al.*, a second meta-analysis was published that similarly concluded that there is no evidence that the serotonin transporter genotype alone or in interaction with SLEs is associated with an elevated risk for depression (3). Although a larger number of studies were included in the second meta-analysis, 12 studies were published after the dataset for this second meta-analysis was closed, with only 3 of the 12 studies including adequate data in the published reports to be examined in the second meta-analysis (K.R. Merikangas, written communication, July 2009). Seven of the nine studies excluded because they were published after the dataset was closed were full or partial replications (4–10), as were four other earlier studies that were not included in the second meta-analysis because the data were either not received or received in an incompatible format (11–14). Like the publication by Munafò *et al.*, the second meta-analysis also included an excess representation of nonreplications. It was conducted with data from one-half of the 26 studies that met the inclusion criteria for the meta-analysis (3), with a trend again for negative studies to be over-represented. Of the 26 studies meeting the inclusion criteria for the second meta-analysis, 78% (7/9) of the negative studies and only 35% (6/17) of the positive studies were included in the analysis (Fisher exact test: $p < .10$).

Most studies in the literature (17/26) have replicated in part or fully the finding that individuals with the “s” allele of 5-HTTLPR are at higher risk for depression after exposure to multiple SLEs or experiences of child maltreatment, but to date a comprehensive meta-analysis of published results has not been completed. The serotonin transporter is a protein critical to the regulation of serotonin levels in the brain, on the basis of its ability to remove serotonin from the synapse and terminate its action. Serotonin transporter protein (5-HTT) knockout mice show stress-induced anxious- and depressive-like behaviors and increased dendritic spine density in the amygdala (15). This latter finding is interesting in light of the fact that the “s” allele of 5-HTTLPR has been associated with increased amygdala activation to negative stimuli in multiple imaging genomic studies (16). In experimental stress-induction paradigms, the “s” allele of 5-HTTLPR has also been associated with increased hypothalamic pituitary adrenal axis activity in both humans (17) and nonhuman primates (18) and heightened brain activity in stress-responsive brain regions (19).

A comprehensive meta-analysis is required to determine the strength of the 5-HTTLPR $\times$ SLE interaction in conferring risk for depression. In addition, we believe more work is needed to unravel the mechanisms by which stress might confer vulnerability to depression in individuals with the “s” allele of 5-HTTLPR, to further characterize allelic variants of this gene, and identify additional genetic (e.g., *BDNF*) and environmental (e.g., social supports) factors that can modify the magnitude of the 5-HTTLPR $\times$ SLE interaction.

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Figure 1.

Results of multiple regression analyses estimating the association between number of stressful life events (between ages 21 years and 26 years) and the probability of major depressive disorder by age 26 as a function of serotonin transporter protein genotype. Among the 146 s/s homozygotes, 43 (29%), 37 (25%), 28 (19%), 15 (10%), and 23 (16%) study members experienced zero, one, two, three, and four or more stressful events, respectively. Among the 435 s/l heterozygotes, 141 (32%), 101 (23%), 76 (17%), 49 (11%), and 68 (16%) experienced zero, one, two, three, and four or more stressful events. Among the 264 l/l homozygotes, 79 (29%), 73 (28%), 57 (21%), 26 (10%), and 29 (11%) experienced zero, one, two, three, and four or more stressful events. The main effect of the serotonin transporter gene was not significant ($b = -.15$, $SE = .14$, $z = 1.07$, $p = .29$), the main effect of life events was significant ($b = .37$, $SE = .06$, $z = 5.99$, $p = .001$), and the gene $\times$ environment interaction was in the predicted direction ($b = -.19$, $SE = .10$, $z = 1.91$, $p = .056$). Life events predicted a diagnosis of major depression among s carriers ($b = .52$, $SE = .16$, $z = 3.28$, $p = .001$ among s/s homozygotes, and $b = .39$, $SE = .09$, $z = 4.24$, $p = .001$ among s/l heterozygotes) but not among l/l homozygotes ($b = .16$, $SE = .13$, $z = 1.18$, $p = .24$). From Caspi *et al.*, Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003;301:386–389. Reprinted with permission from AAAS.