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Additional Functional Variation at the SLC6A4 Gene

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TO THE EDITOR

The serotonin transporter gene (*SLC6A4* or *HTT*) remains one of the most studied genes in psychiatric genetics. A large number of association studies have been published on a polymorphism in the 5' flanking region (*HTTLPR*), primarily on the role of this polymorphism in susceptibility to major depression and anxiety disorders, and treatment response to SSRI drugs [Serretti et al., 2006]. Although association to complex behavioral phenotypes has been inconsistent and meta-analyses reveal modest effects, imaging genetic studies have found stronger effects of *HTTLPR* on intermediate phenotypes related to emotion including fMRI responses to emotional stimuli, brain connectivity, and brain volume. It is widely accepted that in postmortem brain, lymphoblasts, and transfected cell lines, the L allele, which contains 16 copies of an imperfect repeat sequence, leads to higher levels of transcription than the S variant, which contains 14 copies. Furthermore, we described the influence on transcription of a relatively common A to G SNP (rs25531) within an L allele repeat (such that there are S, L_G, and L_A alleles) and noted that additional variation at the *SLC6A4* gene or elsewhere must exist to fully account for variation in *SLC6A4* mRNA expression [Hu et al., 2006]. The effect of the L_G allele was demonstrated in 62 lymphoblastoid cell lines selected for genotype and was verified and mechanistically explained by in vitro transfection experiments. The L_G allele creates a binding site for the transcription factor AP2, which can function as either an activator or repressor of transcription. In a transfected neuronal cell line, the L_G allele reduced reporter gene transcription relative to the L_A that could be rescued when the cells were simultaneously treated with a synthetic DNA incorporating the L_G allele. Taken together, the evidence supported the idea that the L_G allele exerted its effect by transcriptional repression.

Philibert et al. [*Am J Med Genet B Neuropsychiatr Genet*, published online November 6, 2007] described a combined effect of *HTTLPR* genotype and epigenetic modifications on *SLC6A4* mRNA expression in lymphoblast cell lines, emphasizing the effect of methylation of a CpG island near *SLC6A4* exon 1. This constitutes a potentially important advance in understanding *SLC6A4* expression, especially if the methylation pattern in lymphoblasts reflects that in brain.

However, while Philibert et al. did not replicate the functional effect of the L_G allele, evidence continues to accumulate that this relatively common allele (0.25 in Africans) alters function. Recently, Wendland et al. [2008] provided evidence that the L_A and L_G alleles and a previously uncharacterized SNP, rs25532 (C>T) in the antepenultimate repeat unit of the *HTTLPR* region, alter *SLC6A4* expression. The T allele of rs25532 decreased luciferase reporter gene expression levels by 15–30% in S constructs and 25–80% in L_A constructs, depending on the cell line transfected. The C allele of rs25532 is only observed on the L_G background but was not analyzed in the reporter gene assay. Additional sequence variants with putative function are also present. SNPs rs16965628 and rs2020933, previously associated with allelic expression

imbalance by Martin et al. [2007], were used to subdivide L_A alleles for haplotype analyses [Wendland et al., 2008]. Extensions of the function-guided approach have refined our understanding of *SLC6A4* expression and will undoubtedly aid in haplotype-based analyses for detecting associations.

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