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Viewpoint

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# 5HTTLPR: White Knight or Dark Blight?

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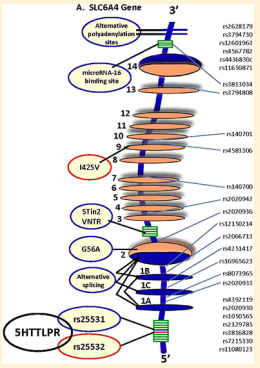
**ABSTRACT:** In over 100 neuroscience genetics reports on *SLC6A4* published in the first part of 2012, >40% reported data from genotyping only the serotonin transporter-linked promoter region [5HTTLPR] indel, omitting genotyping of two nearby SNPs that substantially alter 5HTTLPR allele frequencies and functionality. And 25% of these papers did not report ethnicity of the subjects genotyped, another factor that alters allele frequencies. This field thus seems stultified. Improved science for the present and future will be better served by attention to more complete methods for genotyping and subject sample reporting.




**KEYWORDS:** *serotonin, 5HTTLPR, serotonin transporter gene, SLC6A4*

The purpose of this Viewpoint is to alert neuroscience authors, readers, reviewers, and editors to the fact that, in scientific publications that include genotyping of the serotonin transporter gene [*SLC6A4*], substantial oversights and omissions have been present since 2006, and are continuing to be published.

Based on a recent search of publications from 2012 catalogued in PubMed that included *SLC6A4* genotyping (see below), 44% reported only the promoter polymorphism 5HTTLPR (simple long "L" versus short "S" allele classifications), neglecting other promoter region variants that alter the functional effects of the "L" versus "S" variants. This lack of detailed analysis leads to errors in interactive variant consequences and thus statistical inaccuracy in overstating or understating the conclusions presented.

The history of this story is as follows:

1. In our 1996 publication in *Science*, we reported differences in anxiety-related personality traits in normal individuals that seemed relatable to the *SLC6A4* 5HTTLPR.[1] Despite calculations in this publication that the results from 505 individuals only contributed "a modest but replicable 3 to 4% of total variance and 7 to 9% of the genetic variance," today's PubMed lists >2100 publications (since 2000) on the serotonin transporter gene and its variants.
2. In 2006, an important advance in *SLC6A4* variant consequences was reported: the discovery of the rs25531 variant (Figure 1).[2] Now consistently confirmed, this variant is located in close proximity to the 5HTTLPR. It comprises a single base substitution (A > G) that yields a "$L_G$" allele, which is functionally equivalent to the 5HTTLPR "S" allele.[2,3] Importantly, up to 15% of individuals previously considered in all studies of 5HTTLPR to be "L" carriers should instead be functionally classified with "S" carriers. This error rate of ±15% might have influenced study results, leading to false positives or false negatives.

   Of note, there are large ethnic differences in the presence of different variants: Caucasians have ∼22% "S"/"$L_G$" alleles while Asians have ∼60% "S"/"$L_G$" alleles, with other populations having intermediate values (Figure 2).[2] These differences have been found to be of particular importance in association studies of drug treatment responses and drug side effects.[4,5]
3. Most recently in 2008, a promoter region *SLC6A4* variant, rs25532, was reported (Figure 1).[6] Located close to the much-studied 5HTTLPR, this variant showed an allele-dependent effect on serotonin transporter expression of up to 7-fold when tested in a serotonergic, raphe-derived cell line.[6] Since this report 4 years ago, not a single publication has included genotyping of this variant or evaluations of its relationship to disease, drug response, or to other phenotypes.
4. We thought we might have reached a watershed in 2006, with the discovery of rs25531 regulating the 5HTTLPR and thus comprising a triallele.[2] However, further reports by other groups have identified additional SNPs, some involving amino acid changes across the gene that affect transporter function and regulation such as G56A and I425 V.[7,8] In addition, the STin2 VNTR [9] and 3′ untranslated region gene variants[10,11] have been

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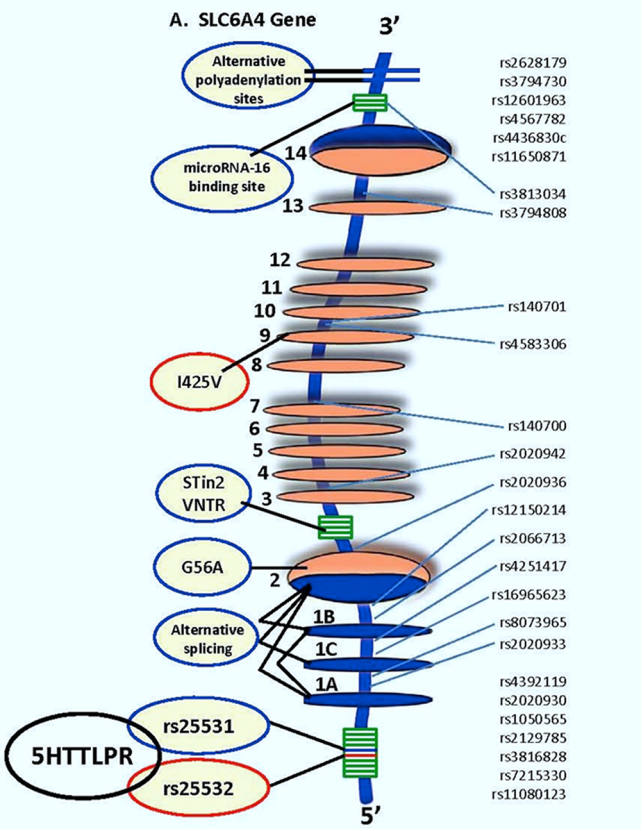


**Figure 1.** Human *SERT* gene (*SLC6A4*) organization showing multiple functional variants. [Adapted from ref 4.]

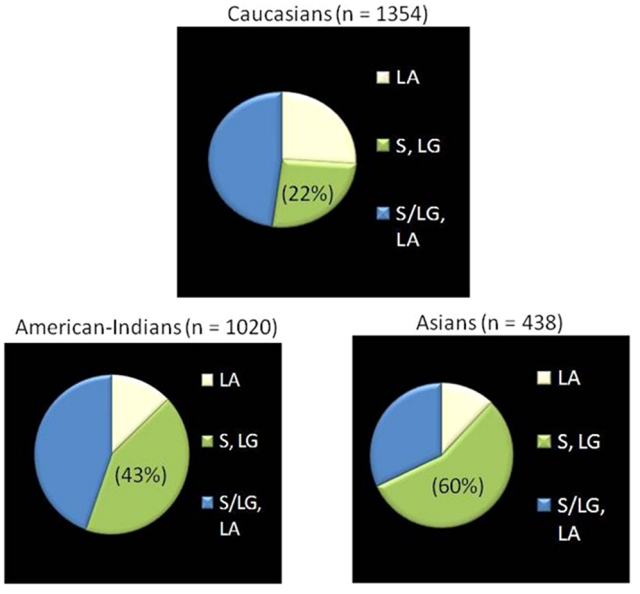


**Figure 2.** Distributions (%) of *SLC6A4* alleles in different global populations. [Adapted from ref 2.]

identified as functional *SLC6A4* elements. The latter are of increasing interest since they affect microRNA-mediated translational regulation; therefore, it is likely that variants located within or near such microRNA sites will have a marked impact on *SLC6A4* expression.[10,11]

However, despite concerns expressed to editors and reviewers, papers continue to be published that not only neglect all but the 5HTTLPR, but also ignore the use of now accessible methods to more fully evaluate the multiple known functional elements in this gene.[3,4,12,13] For example, when we surveyed the papers catalogued in PubMed for January 2012 to November 2012 using the search terms "serotonin transporter gene", "SLC6A4", "serotonin transporter genotype", "5HTTLPR", and "5-HTTLPR" (conducted separately, omitting duplicates), we identified 102 relevant papers. In 27/102 of these, we could find no mention of "ethnicity" (or "white" or "Caucasian" or "Asian"). In 45/102 papers, we could find no mention of any other *SLC6A4* variant other than 5HTTLPR. Perhaps, in these postmedieval times, 72/102 papers missing one or both of these elements might be considered analogous to sending Don Quixote into battle with an arm tied behind his back (due to inattention to genotyping other now-known functional variants) and a hobbled horse (due to inattention to ethnicity).

Basing research on finely tuned and available methods of genotyping *SLC6A4* only stands to have a positive impact in the field. By including more detailed analyses, positive and negative findings alike will be more accurate and will serve to move the field forward.

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A database containing all references identified from PubMed searches used for the analyses herein are available by request to the authors.


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