Published in final edited form as:

Arch Gen Psychiatry. 2011 May ; 68(5): 457–458. doi:10.1001/archgenpsychiatry.2011.34.

Genetic Indeterminism, the 5-HTTLPR, and the Paths Forward in Neuropsychiatric Genetics

Randy D. Blakely^{1,2,3} and Jeremy Veenstra-VanderWeele^{1,2,3,4}

¹Dept of Psychiatry, Vanderbilt University School of Medicine, Nashville, TN 37232-8548

²Dept of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232-8548

³Dept of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232-8548

⁴Center for Molecular Neuroscience, Vanderbilt University School of Medicine, Nashville, TN 37232-8548

The serotonin transporter gene (5-HTT, SERT, *SLC6A4*) is arguably both the most and least loved gene in psychiatric genetics. Fifteen years ago, the discovery of a common, functional promoter polymorphism (5-HTTLPR) that modulates SERT expression¹ launched innumerable association studies. In part, this flurry of activity arose from the common nature of 5-HTTLPR variants. White individuals exhibit a 40/60 split in allele frequency for “short” (s) vs “long” (l) alleles, respectively. Not surprisingly, initial findings from these studies caught the imagination of clinicians and patients alike: finally a gene that could explain our neuroticism¹, or maybe depression, or maybe...ah, well, complex brain disorders are, after all, complex. With inconsistent findings, feelings about the 5-HTTLPR among many psychiatric geneticists moved from excitement to consternation.

We shouldn’t be surprised at the emergence of challenges in studying the 5-HTTLPR in neuropsychiatric disorders. Most studies seeking to define its impact were small and only examined 5-HTTLPR, despite evidence of other common polymorphisms that can affect SERT expression². The lack of replication also likely reflects a limited relationship between our clinical labels, such as ‘depression’ and the underlying biological processes. Of course, if we understood the biology, we wouldn’t still be conducting association studies. But without having biologically determined phenotypes, how can we expect to find consistent relationships between genes and behavior? Imagine enrolling patients in a genetic study of type 2 diabetes based on how subjects feel after a meal!

The field has not been without signs of progress. In an elegant longitudinal study, Caspi et al³ described association of 5-HTTLPR variants with depression, but only in the context of childhood maltreatment or stressful life events. Such findings are typically discussed in the context of a gene X environment interaction. The stance that genetic influences on neuropsychiatric disorders are indeterminate was given its clearest example.

As might be expected, not all subsequent studies reached the same conclusion. Indeed, a recent well-publicized meta-analysis sided with a lack of consistent evidence of gene X environment interaction at the 5-HTTLPR for depression following stressful life events.⁴ Karg et al⁵ now present a more comprehensive meta-analysis that reports significant association of the 5-HTTLPR with depression in the face of childhood maltreatment or specific medical conditions, with weaker evidence following stressful life events. The studies included in this meta-analysis are limited to the *DSM* definition of “major depressive disorder” or corresponding quantitative scales. When coupled with variability in stressors, we are admittedly left with a strikingly vague phenotype. This doesn’t sound like a recipe for success, and yet, the meta-analysis is strongly supportive of the association. The Karg et

al findings also support the idea that improved phenotyping can yield stronger findings, as greater significance was observed among high quality studies and among studies that used more objective measures of stressors⁵.

Karg et al are unlikely to convince all readers. Genetic studies in human populations are by their very nature observational, looking for correlations between genotypes and behavioral phenotypes. Meta-analyses are also not without flaws given the heterogeneity of subjects and phenotypes within the compiled studies. Experiments are necessary to move from correlation to proof of causation, but such experiments will not (hopefully) come with humans. Here, the tools of molecular and behavioral neuroscientists must merge in the study of tractable model organisms. Indeed, we now have multiple models, ranging from worms to mice to monkeys, where the serotonin system is coming under ever-intensifying scrutiny. Ultimately, of course, these models have to stand the test of translation. Nonetheless, environmental perturbations achieved in the context of defined genetic manipulations present a considerable opportunity to reach beyond genetic correlations.

With respect to modeling the consequences of altered SERT expression, one example is transgenic mice where portions of the SERT gene are deleted (SERT KO). These animals show dramatic increases in anxiety-like behavior and susceptibility to stress⁶. Sounds promising. But are there people bearing deletions of both copies of their SERT gene? Not so far as we know. A more realistic model of the effects of the 5-HTTLPR *s* allele may be mice lacking just 1 copy of SERT, who also show increased anxiety-like behavior and susceptibility to stress⁶. Other more nuanced models are no doubt needed, as the 5-HTTLPR may also impact SERT gene regulation. Rhesus monkeys actually share the 5-HTTLPR with humans, and *s* allele carriers demonstrate increased susceptibility to both experimentally delivered adverse early experience and adult stress⁷.

But aren't we really after brain mechanisms, not just behavioral parallels? In mice, monkeys, and humans, SERT genotype affects volume and connectivity of critical brain regions. Furthermore, meta-analysis supports association of the 5-HTTLPR *s* allele with enhanced amygdala activation in response to fear-inducing stimuli⁸. Although much work remains to understand the mechanisms leading to these functional magnetic resonance imaging findings, they argue that genotype can be connected more readily to discrete measures of brain function than to the catchall of 'depression'. Such efforts are but one version of a "phenomic" approach to extract biologically meaningful connections that would unlikely arise from *DSM*-based scans. In this reverse genetics approach, the concept is to use a functional genetic variant, such as the 5-HTTLPR, as the tool to find biologically determined phenotypes that are under its influence. Indeed, if the initial studies with the 5-HTTLPR had used a neurocognitive battery rather than self-report of personality traits or *DSM* disorder definition, we would likely be discussing alterations in cognitive flexibility, a concept emerging across mouse, monkey, and human studies of 5-HTT.⁹

Although the study of Karg et al is notable, the effect size of the common 5-HTTLPR *s* allele on risk for mental illness remains quite small. Many studies of common gene variation, whether derived from candidate genes or genome-wide analyses, have been similarly disappointing, particularly when oriented toward rather vague disease categories. On the other hand, studies of rare gene variants may have a larger impact on risk in certain individuals offer a path forward and are often more easily transferred to animal models. Multiple SERT amino acid variants have been identified in obsessive compulsive disorder and autism that all show increased 5-HT transport.¹⁰ We recently generated a mouse model of the most common of these variants, Gly56Ala, and believe that this model can help define the impact of SERT gene variation on brain development, 5-HT signaling, and behavior.¹⁰ Even with such a model, progress is not likely to come by focusing on a single variant, no

matter how penetrant, but rather will derive from the elucidation of a broader network of genetic variation and its environmental influences that, together, drive risk for mental illness. It is time to move beyond observational studies of single variants, particularly in *DSM*-defined neuropsychiatric disorders, including everyone's favorite whipping boy, the 5-HTTLPR.

References

1. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996 Nov 29; 274(5292):1527–1531. [PubMed: 8929413]
2. Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR, Murphy DL. A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive-compulsive disorder. *Hum Mol Genet*. Mar 1; 2008 17(5):717–723. [PubMed: 18055562]
3. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. Jul 18; 2003 301(5631):386–389. [PubMed: 12869766]
4. Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA*. Jun 17; 2009 301(23):2462–2471. [PubMed: 19531786]
5. Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress and depression meta-analysis revisited: evidence of genetic moderation. *Arch Gen Psychiatry*. 2011
6. Murphy DL, Lesch KP. Targeting the murine serotonin transporter: insights into human neurobiology. *Nat Rev Neurosci*. Feb; 2008 9(2):85–96. [PubMed: 18209729]
7. Barr CS, Newman TK, Shannon C, Parker C, Dvoskin RL, Becker ML, Schwandt M, Champoux M, Lesch KP, Goldman D, Suomi SJ, Higley JD. Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biol Psychiatry*. Apr 1; 2004 55(7):733–738. [PubMed: 15039002]
8. Munafo MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biol Psychiatry*. May 1; 2008 63(9):852–857. [PubMed: 17949693]
9. Jedema HP, Gianaros PJ, Greer PJ, Kerr DD, Liu S, Higley JD, Suomi SJ, Olsen AS, Porter JN, Lopresti BJ, Hariri AR, Bradberry CW. Cognitive impact of genetic variation of the serotonin transporter in primates is associated with differences in brain morphology rather than serotonin neurotransmission. *Mol Psychiatry*. May.2010 (5):512–522. 446. [PubMed: 19721434]
10. Veenstra-Vanderweele J, Jessen TN, Thompson BJ, Carter M, Prasad HC, Steiner JA, Sutcliffe JS, Blakely RD. Modeling rare gene variation to gain insight into the oldest biomarker in autism: construction of the serotonin transporter Gly56Ala knock-in mouse. *J Neurodevelopmental Disorders*. 2009; 1:158–171.