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

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 expression[1] launched innumerable association studies. In part, this flurry of activity arose from the common nature of 5-HTTLPR variants. White individuals exhibit approximately 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 neuroticism[1] 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.

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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 expression.[2] 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 al[3] 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 × 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 × environment interaction at the 5-HTTLPR for depression following stressful life events.[4] Karg et al[5] 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 stressors.[5]

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 stress.[6] 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 stress.[6] 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 stress.[7]

But aren't we really after brain mechanisms, not just behavioral par-

allels? 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 stimuli.[8] 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 that 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.

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