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SLC6A4 Polymorphism, Population Genetics, and Psychiatric Traits

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In 1997, my colleagues and I published an article about allele frequencies at two polymorphisms mapped to the serotonin transporter protein gene, *SLC6A4* (Gelernter et al. 1997). One variant, a 44 bp variable number of tandem repeats (VNTR) polymorphism, has acquired the trivial name "5HTTLPR," for 5-HTT (5-hydroxytryptophan, or serotonin) length promoter polymorphic region. This variant maps to the promoter region at the locus. The most common alleles are referred to as "short," or "s," and "long," or "l." Alleles with more repeats than the "long" allele – that are, in fact "longer" – are often binned by investigators with the "l" allele, a practice that in our view lacks biological justification. In our paper we also considered another *SLC6A4* VNTR variant, this one intronic, which has been given the trivial name Stin2. We reported allele frequencies in three different populations – European-Americans (EAs), African-Americans (AAs), and Japanese; and we also included a phenotype association study for alcohol dependence (AD) in the EA part of the sample. The association study was negative, but in the population genetics part of the study, we showed major differences by population, allelewise and haplotypewise. Now – why would such a paper have been cited 284 times (according to SCOPUS, as of December 2013)?

First, there is the critical function of the protein product of the gene. Serotonin is a key neurotransmitter, and the serotonin transporter protein modulates serotoninergic neurotransmission via the mechanism of synaptic reuptake (Rudnick 2006). This protein is the major target of the selective serotonin reuptake inhibitors, a set of medications including fluoxetine (trade name Prozac) widely used in psychiatry for depression, anxiety, and other disorders. Serotonergic dysfunction has been implicated in a host of psychiatric disorders and traits including affective disorders, schizophrenia, anxiety, and autism. In the era of candidate gene studies, *SLC6A4* was a prime target from the very day it was cloned.

Second, there was the functionality of the variants. This had been reported for 5HTTLPR in 1996 (Lesch et al. 1996; Heils et al 1996) and was a major impetus for the work that resulted in our paper but since then, evidence has continued to mount and now it is very strong. At the time of our report, the functional implications of the Stin2 variant had not yet been worked out, but it was subsequently reported that this variant is also functional (MacKenzie and Quinn 1999).

Third, there were the population differences. In principle this – i.e. the reporting of population allele frequencies with the idea that they might be consequential -- was not a

novel idea. But practice had not yet fully integrated this principle and association studies with mixed populations were common then. They are still seen now even without stratification control, albeit less frequently and in specialty journals. The range of allele and haplotype frequencies across populations was too wide for most investigators to ignore – it was very easier to explain "positive" findings on the basis of stratification – and although the genetically-informed had been making this argument for many years, it finally began to take hold in the field (even more so because of the development of analytic methods such as the structured association approach (Pritchard et al. 2000) that can control for stratification artifact). In our early phenotype-driven work involving this marker, we approached the issue by using the family-controlled TDT approach to show that the "l" allele is associated with obsessive-compulsive disorder (McDougle et al. 1998). (While that study used a sample that nowadays would be considered unsuitably small, the main finding was replicated by Hu et al (2006).)

One additional point is that we had worked out effective and reliable 5HTTLPR genotyping conditions and reported those as well; these methods are still in use in our lab. The article that originally described the variant (Heils et al 1996) contained an error at the 3' end at one of the reported PCR primers and, while this will be difficult for many readers to remember, there was a time when routine PCR genotyping often posed a challenge.

These factors were enough to generate considerable interest in the locus and in the polymorphism we studied, especially 5HTTLPR. But there was more to come: Fourth, there was a second wind for studies of this locus occasioned by one of the first strong gene-by-environment (GxE) studies of a psychiatric trait, a study by Caspi et al. (2003) published in *Science*. Interest in *SLC6A4* was waning slightly in the wake of the first set of candidate gene publications that followed the Lesch et al. (1996) article. In that wave the focus was on personality (as in the Lesch paper itself), and also anxiety traits, affective disorders, and alcohol dependence. The Caspi et al. article focused attention on GxE interactions involving this locus. It was a powerful finding, but beyond that, while it was not the first major GxE finding (nor, for that matter, not the first from Caspi's research group), it had great impact on the focus of the research community and also caught the imagination of the public. Thus started a further wave of studies involving this variant that addressed GxE issues.

To look a little more closely at the GxE finding – at its basis is an observation that subjects with one or more copies of the "s" allele are at increased risk for psychiatric consequences of stress. In Caspi et al. (2003), the consequence measured was depression-related symptoms. In other studies, e.g. several of ours (Kaufman et al. 2004; Kilpatrick et al. 2007; Xie et al. 2009, replicated in Xie et al. 2012) the consequence was posttraumatic stress disorder-related symptoms. These findings have been controversial in some quarters (Risch et al. 2009) but other investigators have found them to be robust (Karg et al. 2010).

Where does this leave us – what is the importance of this locus in the GWAS and sequencing era? Note that since 5HTTLPR is a VNTR, it cannot be genotyped via microarray – and that although a moderate-throughput Taqman assay has been created, this assay distinguishes only the most common "l" and "s" variants. The longer-than-"l" variants are left on the table – worse, they are not separated from "l," and this may be important from

a functional standpoint in those non-European populations were these other alleles are prevalent. The upshot is that even today it takes comparatively high effort to obtain accurate genotypes for this variant. Nevertheless the relationship of variation at this locus to phenotype is still of intense interest to the research community. If 5HTTLPR is not, strictly speaking, the last candidate gene standing, it is at least important, unusual, and interesting enough to be one of a very few. Interest in the gene determined the fate of the population genetics article, and resulted in a large number of citations to an unlikely candidate article.

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