Insights and barriers to clinical use of serotonin transporter pharmacogenetics in antidepressant therapy

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“It is a distinct possibility that combinatorial approaches utilizing *SLC6A4* as ‘a piece to the puzzle’ may reach widespread clinical adoption before our understanding of how best to predict 5-HTT phenotype is fully refined.”

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Antidepressants are the first-line pharmacotherapy for a variety of affective and anxiety disorders and are among the most widely used medications in the USA, where one in eight adults are currently treated with a drug from the class [1]. Unfortunately, a large percentage of patients do not respond to initial therapy and a similarly large fraction experience adverse drug reactions (ADRs) including drowsiness, nausea and sexual dysfunction. The high failure rate and trial-and-error medication selection strategy results in ongoing morbidity and substantial healthcare costs. Because of the considerable variability in treatment outcomes, depression has become a popular target for precision medicine approaches. Yet, to date, there is no reliable biomarker routinely used in clinical practice to guide antidepressant selection.

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly used antidepressants in the USA. SSRIs reduce the reuptake of serotonin (5-HT) from the synaptic cleft by antagonizing the serotonin transporter (5-HTT). The gene *SLC6A4* encodes 5-HTT, and not surprisingly, this gene has been among the most commonly studied candidate genes for psychiatric disease risk and response to psychiatric medications.

There is known functional variation in *SLC6A4*; the most studied of which is the serotonin transporter linked polymorphic region (5-HTTLPR), located in the gene's promoter region. This variable number of tandem repeat (VNTR) polymorphism can result in short (S) or long (L) alleles depending on the number of repeats. Consistent *in vitro* data indicate that the S allele is associated with reduced expression [2]. Theoretically, this polymorphism could impact 5-HTT saturation and 5-HT availability during SSRI treatment, potentially altering drug efficacy and ADR risk.

Resultantly, the potential for association of 5-HTTLPR and SSRI outcomes has been examined repeatedly and in some of the largest antidepressant clinical trials available. Separate analyses of nearly 2000 patients treated with citalopram in the STAR*D study found a positive association with the L allele and remission [3] or no association [4,5], depending on phenotype definitions and sample stratification. A study of approximately 800 patients treated with escitalopram in the Genome Based Therapeutic Drugs for Depression (GENDEP) project found better treatment response for L allele carriers, an effect driven primarily by males [6]. The association between 5-HTTLPR and efficacy was recently examined in a meta-analysis of more than 30 studies, resulting in odds ratios of 1.5–2 favoring the L allele [7].

Moreover, 5-HTTLPR may affect the ADR burden of SSRIs. The single largest study of 5-HTTLPR and SSRI ADR burden used data from citalopram-treated white non-Hispanic patients in the STAR*D study and found decreased ADR burden associated with the L allele [4]. On the other hand, analyses of the escitalopram-treated patients in the large GENDEP project revealed no association between ADR rate and 5-HTTLPR [6]. The

body of literature concerning 5-HTTLPR and SSRI tolerability ($n = 40$ studies) was recently reviewed, noting fairly consistent evidence linking the L allele to decreased ADR burden [8]. The strongest evidence was related to gastrointestinal side effects and antidepressant-induced mania.

Given that the same 5-HTTLPR allele (L) is associated with better response and fewer ADRs, it is appealing to imagine the use of 5-HTTLPR to identify ideal and poor SSRI candidates in clinical practice. Numerous unanswered questions and technical challenges have prevented widespread adoption as of yet. One essential but inadequately answered question is whether 'poor SSRI candidates' as identified by 5-HTTLPR (those with S/S genotypes) respond as expected, better or worse to non-SSRI therapies. Of note, some alternative antidepressants, including serotonin and norepinephrine reuptake inhibitors and tertiary amine tricyclic antidepressants, have pronounced effects at 5-HTT and theoretically may not be ideal treatment alternatives in individuals with S/S genotypes. Alternative medications with mechanisms independent of 5-HTT (e.g., bupropion) may work as expected in 'poor SSRI candidate' genotypes, but studies of 5-HTTLPR and treatment outcomes for these drugs are more sparse.

Furthermore, the literature supporting a relationship between 5-HTTLPR and SSRI outcomes include discrepant findings and potential confounders. For one, a SNP (rs25531) in the same region as 5-HTTLPR appears to have functional consequence [2]. It is most commonly thought that guanine at rs25531 negates the increased expression from the 5-HTTLPR L allele, while the combination of the 5-HTTLPR L allele and adenine at rs25531 results in increased expression. This results in a triallelic polymorphic site, with the possibility of low (S, L_G) or high expression (L_A) alleles. Older literature does not consider rs25531, which may result in misclassification of L_G alleles as high expression, thus potentially diluting genotype effects. Analyses of large datasets have demonstrated associations using the triallelic system which are not significant while using the older L versus S classification [4]. What is more, technical errors in 5-HTTLPR assays have been described, leading to false associations and manuscript retractions [9].

Some researchers have suggested differential 5-HTTLPR effects by ethnicity, and the aforementioned efficacy meta-analysis examined studies of Caucasian and Asian populations separately [7]. Whether genotype effects differ by ethnicity is not clear, but it is known that 5-HTTLPR is more polymorphic (a more balanced distribution of L and S alleles) in Caucasian populations than Asian populations. The L allele frequency is roughly 60% in Caucasians, 75% in African-Americans and 25% in east Asian populations [10]. This difference can affect statistical power and may help explain some of the null findings in small studies of east Asian populations. On the other hand, if ethnicity does indeed interact with 5-HTTLPR, it is important to note that available studies are almost exclusively comprised of non-Hispanic Caucasian and east Asian populations [7,8]. In addition to ethnicity, some researchers have hypothesized about differential effects of genotype by sex [6,11].

Pharmacogenetic studies of *SLC6A4* are further complicated by other polymorphisms that reportedly have functional effects. An additional SNP near 5-HTTLPR (rs25532), has been recently described to modulate gene expression but is not typically genotyped in pharmacogenetic studies of SSRIs [2]. Outside the promoter region, a VNTR polymorphism in intron 2 [12], polymorphisms in the 3'-untranslated region and epigenetics may all ultimately contribute to *SLC6A4* expression [13]. While some existing pharmacogenetic studies consider the effects of the intron 2 VNTR, these other polymorphisms and epigenetic factors are generally not accounted for and likely contribute to unexplained variation in the existing literature. Unfortunately, there is no consensus in how best to form functional categorizations for association studies utilizing these common polymorphisms together with or without epigenetic information.

Despite these limitations and unanswered questions, there are numerous ongoing clinical trials examining pharmacogenomics-guided antidepressant therapy. These studies largely use combinatorial genetic approaches, in other words, utilizing *SLC6A4* genotype in combination with other pharmacogenes such as *CYP2C19* and *CYP2D6* to form a treatment recommendation. This combinatorial approach has proven useful for germline pharmacogenomic studies outside of psychiatry, for example in formulating warfarin dosing recommendations [14]. But in contrast to the warfarin literature, in-progress antidepressant pharmacogenetic clinical trials often use proprietary algorithms to generate treatment recommendations.

Clinical trials using proprietary combinatorial strategies are valuable in answering whether current combinatorial pharmacogenomics approaches can improve patient outcomes. Positive results from these studies would be an important milestone for psychiatric pharmacogenomics, but results will likely be generalizable only to the specific testing/reporting product used in the trial. Because the clinical trial funders are incentivized to protect their intellectual property, these studies are unlikely to provide gene-level data to help settle unanswered questions about how *SLC6A4* contributes to antidepressant outcomes.

Unfortunately, we find ourselves in a place where systematic review and meta-analyses of existing literature is complicated by a handful of potentially major confounders, including but not limited to ethnicity, systematic genotyping errors and ungenotyped functional polymorphisms. Researchers performing genetic association studies in *SLC6A4* should be encouraged to re-evaluate older findings in light of newer developments, such as the functional effect of rs25531 (and perhaps rs25532) in conjunction with 5-HTTLPR. Biobanking of samples should be encouraged, so that these analyses can evolve as our understanding of how to appropriately account for other *SLC6A4* polymorphisms and epigenetics grows. Going forward, clinical trials testing the utility of open source algorithms would be an ideal complement to the current wave of trials using branded psychiatric pharmacogenomics tools.

Although twenty years have lapsed since the publication of the first antidepressant pharmacogenetic study of 5-HTTLPR [15], surprising knowledge gaps and barriers to clinical use remain. It is a distinct possibility that combinatorial approaches utilizing *SLC6A4* as ‘a piece to the puzzle’ may reach widespread clinical adoption before our understanding of how best to predict 5-HTT phenotype is fully refined. And while this outcome may be frustrating to the academician, if it helps the clinician to find the right medication at the right dose for the patient in a safe and cost-efficient manner, it is a future we should all look forward to.

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References

- 1 Pratt LA, Brody DJ, Gu Q. Antidepressant use among persons aged 12 and over: United States, 2011–2014. NCHS data brief, no 283 (2017). www.cdc.gov/nchs/products/databriefs/db283.htm
- 2 Iurescia S, Seripa D, Rinaldi M. Role of the 5-HTTLPR and SNP promoter polymorphisms on serotonin transporter gene expression: a closer look at genetic architecture and *in vitro* functional studies of common and uncommon allelic variants. *Mol. Neurobiol.* 53(8), 5510–5526 (2016).
- 3 Mrazek DA, Rush AJ, Biernacka JM *et al.* SLC6A4 variation and citalopram response. *Am. J. Med. Genet. B Neuropsych. Genet.* 150B(3), 341–351 (2009).
- 4 Hu XZ, Rush AJ, Charney D *et al.* Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Arch. General Psychiatr.* 64(7), 783–792 (2007).
- 5 Kraft JB, Peters EJ, Slager SL *et al.* Analysis of association between the serotonin transporter and antidepressant response in a large clinical sample. *Biol. Psychiatr.* 61(6), 734–742 (2007).
- 6 Huez-Diaz P, Uher R, Smith R *et al.* Moderation of antidepressant response by the serotonin transporter gene. *Br. J. Psychiatr.* 195(1), 30–38 (2009).
- 7 Porcelli S, Fabbri C, Serretti A. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with antidepressant efficacy. *Eur. Neuropsychopharmacol.* 22(4), 239–258 (2012).
- 8 Zhu J, Klein-Fedyshin M, Stevenson JM. Serotonin transporter gene polymorphisms and selective serotonin reuptake inhibitor tolerability: review of pharmacogenetic evidence. *Pharmacotherapy* 37(9), 1089–1104 (2017).
- 9 Janssen PK, Olivier B, Zwinderman AH, Waldinger MD. Measurement errors in polymerase chain reaction are a confounding factor for a correct interpretation of 5-HTTLPR polymorphism effects on lifelong premature ejaculation: a critical analysis of a previously published meta-analysis of six studies. *PLoS ONE* 9(3), e88031 (2014).
- 10 Goldman N, Glei DA, Lin YH, Weinstein M. The serotonin transporter polymorphism (5-HTTLPR): allelic variation and links with depressive symptoms. *Depress. Anxiety* 27(3), 260–269 (2010).
- 11 Bishop JR, Ellingrod VL, Akroush M, Moline J. The association of serotonin transporter genotypes and selective serotonin reuptake inhibitor (SSRI)-associated sexual side effects: possible relationship to oral contraceptives. *Hum. Psychopharmacol.* 24(3), 207–215 (2009).
- 12 Mackenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc. Natl Acad. Sci. USA* 96(26), 15251–15255 (1999).
- 13 Iurescia S, Seripa D, Rinaldi M. Looking beyond the 5-HTTLPR polymorphism: genetic and epigenetic layers of regulation affecting the serotonin transporter gene expression. *Mol. Neurobiol.* 54(10), 8386–8403 (2016).
- 14 Gage BF, Bass AR, Lin H *et al.* Effect of genotype-guided warfarin dosing on clinical events and anticoagulation control among patients undergoing hip or knee arthroplasty: the GIFT randomized clinical trial. *JAMA* 318(12), 1115–1124 (2017).

- 15 Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol. Psychiatr.* 3(6), 508–511 (1998).