



Published in final edited form as:

*Am J Med Genet B Neuropsychiatr Genet.* 2012 July ; 159B(5): 611–612. doi:10.1002/ajmg.b.32054.

## The Relationship of the Serotonin Transporter (SLC6A4) Extra Long Variant to Gene Expression in an African American Sample

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### TO THE EDITOR

The serotonin transporter (*SLC6A4*) is a key regulator of serotonergic neurotransmission. Like most genes, *SLC6A4* expression is partially regulated by genetic variation. The most heavily studied variant affecting this expression is the polymorphism known as the serotonin transporter linked polymorphic region (5HTTLPR). In those of northern European ancestry, this variation exclusively consists of two alleles; a long (l) variant that consists of 16 repeats and a short (s) variant that consists of 14 repeats of ~22 bp element. In a large number of studies, it has been repeatedly demonstrated that the short variant is associated with 60% of the transcriptional activity as long allele [Lesch et al., 1996]. Unfortunately, the mechanism through which this differential expression is conveyed is unclear but may be mediated via the diverse interactions of a number of transcription factors including CCCTC-binding factor (CTCF) [Ali et al., 2010].

In individuals of other ancestry, in particular the African-Americans, another 5HTTLPR variant exists and is referred to as the extra long (xl) allele which is ~81 bp longer than the l allele [Gelernter et al., 1997]. Although it has been speculated that this longer variant act as “super long” alleles with respect to transcriptional activity, to the best of our knowledge, this activity has not been directly examined, depriving the field of hard data on which to build a better understanding of the regulatory mechanisms through which variation at this locus effects gene expression.

It is also particularly useful to examine regulation of gene activity in an African American population because several race-by-genotype interaction effects have been reported [Widom and Brzustowicz, 2006; Propper et al., 2007], suggesting to some that the regulation of serotonin reuptake may be differentially related to 5-HTTLPR genotype in African American versus European-American individuals [Williams et al., 2003], with opposite effects expected in the two groups. Because we have previously examined the relationship of 5-HTTLPR genotype to mRNA production in a European-American sample [Philibert et

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The authors have no conflicts of interest with respect to this communication.

al., 2008], it is possible for us to directly compare the direction of association between genotype and gene activity in each using the same methods.

In order to examine transcriptional properties of these alleles, we genotyped DNA from female subjects participating in the Family and Community Health Care Takers Study (FACHS) [Cutrona et al., 2000]. The FACHS is a longitudinal examination of health and health-related behaviors in care taker/offspring dyads in rural African American families whose methods and procedures have been approved by the University of Iowa Institutional Review Board study.

To accomplish our objective, we genotyped DNA from FACHS female subjects for whom we had lymphoblast cell lines to identify individuals homozygous for short or long variant, as well as individuals who were either heterozygous or homozygous for the xl allele. We next measured *SLC6A4* gene expression in RNA from lymphoblast cell lines for each of these individuals and analyzed the relationship between genotype and gene expression.

The genotyping of the 5HTTLPR was conducted as previously described [Philibert et al., 2008]. The amount of *SLC6A4* transcript as well as the levels of five housekeeping gene transcripts (*CALR*, *UBC*, *RPL7A*, *RPS19*, and *RPS20*), were determined in triplicate as previously described using Taqman™ reagents (Applied Biosystems, Foster City, for *SLC6A4*, we used Hs00169010), and a BioMark Platform (Fluidigm, South San Francisco, CA) as previously described [Shields et al., in submission]. Next, the relative amount of *SLC6A4* transcript was then normalized to total RNA levels using the geometric mean of five housekeeping genes and the resulting C<sub>t</sub> scores converted to z-scores. Finally, the resulting genotype and gene expression data were analyzed using the regression subroutine contained within JMP version 9 (SAS Institute, Cary, SC).

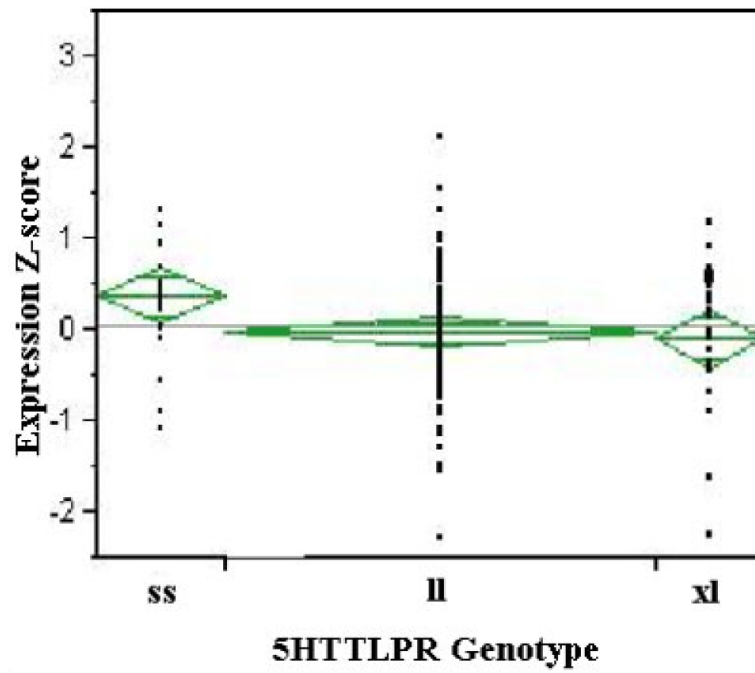
DNA from a total of 480 female FACHS subjects were genotyped with the overall frequencies of the s, l and xl alleles being 26%, 70%, and 4%, respectively. From the material derived from these subjects, we selected 86 cell lines that were homozygous for the long allele, 26 that were homozygous for the short allele, 21 that were heterozygous for the extra-long allele (8 s,xl and 13 l,xl) and one that was homozygous for the xl allele.

Figure 1 gives the relationship between genotype and expression level. Ordinal regression analysis using a simple 0 (ss), 1 (ll) and 2 (at least one xl allele) model demonstrated a significant relationship between genotype and gene expression ( $P < 0.05$ ) with the order of transcriptional efficiency being  $xl > l > s$ . This pattern is consistent with the hypothesis that the xl variant will be associated with increased transcriptional efficiency. It is also noteworthy that the relative transcriptional efficiency of the l and s alleles is in the same direction in this African American sample as was reported earlier by our lab and others for a European American sample [Philibert et al., 2008]. Accordingly, there is support for a simple model in which length of the polymorphic region is linearly associated with transcriptional activity, but no support for the hypothesis that regulation of the highly conserved serotonin transmitter system is differentially regulated by African Americans and European Americans. Conversely, because the xl allele is present, there is potential for the 5HTTLPR to provide an expanded range of potential mRNA responses and environmental interactions in gene–environment interaction studies in African American cohorts.

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**FIG. 1.**

The relationship between 5HTTLPR genotype and SLC6A4 transcript levels in lymphoblast RNA prepared from blood contributed by female FACHS subjects. Higher z-scores denote lower levels of expression with each unit of z-score being equal to approximately 1.6 Ct counts. Group sizes: ss (n = 26), ll (n = 86), or at least one xl allele (n = 24). [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmgb>]