Association of 5-HTTLPR genotypes with antisocial behavior in response to childhood environment: a study in young adults of Portuguese origin

To the Editors:

The *SLC6A4* gene (chr: 17q11.2) encodes the serotonin transporter protein (5-HTT) involved in the reuptake of serotonin at brain synapses. In humans, the functional polymorphism 5-HTTLPR (serotonin transporter gene-linked polymorphic region) located in the *SLC6A4* promoter region consists of different lengths of a repetitive 20- to 23-bp sequence, the most common alleles are a L (long, 16-repeats) and a S (short, 14-repeats) (Heils et al., 1996). It was shown that the short variant is associated with lower transcriptional efficiency, resulting in a 30-40 % reduction of the serotonin transporter and a two-fold reduction in serotonin transport from the brain synapses (Lesch et al., 1996). SNP rs25531 A>G, located near upstream 5-HTTLPR (Bonvicini et al., 2010), results in four haplotypic forms denoted L-A, L-G, S-A and S-G, though the S-G is infrequently observed. Furthermore, it is described that the L-G haplotype has a transcriptional activity similar to that of the S allele (Hu et al., 2006).

Several studies have reported significant associations between the 5-HTTLPR S-allele and antisocial behavior (ASB) including impulsivity, aggression and conduct disorders in both children and young adults; however, other studies reported no such associations (Ficks and Waldman, 2014; Iofrida et al., 2014). In addition, several studies showed an association between the S-allele and ASB only if adversity during childhood was experienced (Reif et al., 2007; Li and Lee, 2010; Ciccheti et al., 2012). Moreover, a considerable number of studies have highlighted a differential susceptibility to the

environment, in that the S-allele carriers are more vulnerable to negative environments, but profited significantly more from positive environmental inputs (Iofrida et al., 2014). Thus, the 5-HTTLPR polymorphism has been suggested as a typical genetic marker of differential susceptibility (Beaver and Belsky, 2012).

This study aims to replicate previous associations between the 5-HTTLPR genotypes and self-reported ASB in relation to childhood environment, in a general population sample of young adults.

The study sample included 205 healthy young adults of European Portuguese descent, recruited in the general population, being 103 males and 102 females, aged between 18-37 years (mean age 22.17 years). Written informed consent was obtained from all participants. ASB was measured by self-reports based on Guo et al. (2008) questionnaire. The items included 10 modified questions asking for events occurring in the last 12 months about stealing, breaking and entering, drug selling and graffiti, involvement in physical fighting, deliberately damaging property, and pulling a knife on someone. An ASB score based on the scaling weights used by Guo et al. (2008) was calculated for each individual. Childhood maltreatment was assessed by retrospective self-reports based on three questions regarding verbal, physical or sexual abuses. Buccal swabs were submitted to DNA extraction and genotyping for the 5-HTTLPR polymorphism was performed by PCR followed agarose gels electrophoresis using primers described in Gelerneter et al. (1997). The same PCR products were digested with the MspI enzyme to genotype rs25531 A- and G-alleles and to infer 5-HTTLPR/rs25531 haplotypes according to Bonvicini et al. (2010). The haplotype classification was transformed into a biallelic model according to *SLC6A4* allele expression levels: L-A as L1 for the high activity allele, and L-G, S-A and S-G as S1 for

the low activity allele. Then, the six observed phased haplotypes were clustered in three genotypes denoted as L1L1 (LALA), L1S1 (LASA, LALG and LASG) and S1S1 (SASA and LGSA). Genotype and allele frequencies, Hardy-Weinberg equilibrium probability values, linear regression models and the interaction of childhood maltreatment with genotypes (GxE effects) on ASB, were done using the PLINK software v.1.07 (Purcell et al., 2007).

Genotypes and allele frequencies estimated for the two studied polymorphisms and derived 5-HTTLPR/rs25531 haplotypes are detailed in Supplementary Table 1.

The chi-square test between individuals that had been exposed or not exposed to adverse childhood environment and the clustered 5-HTTLPR/rs25531 genotypes showed no significant results for the overall sample or stratified by sex ($p>0.05$), suggesting that retrospective self-reports of childhood adversity are not associated with a particular genotype.

Testing the association between clustered 5-HTTLPR/rs25531 genotypes and ASB outcomes, for the whole study sample, without adjusting for environmental factors, linear regression analysis showed no statistical significant values ($p>0.05$), (Table 1) indicating that 5-HTTLPR genotype alone did not predict ASB patterns.

When the population was split per childhood environment, the analysis revealed in individuals that had been exposed to adverse childhood experiences a clear grading tendency towards higher ASB levels in carriers of S1 alleles, when compared to homozygous L1L1 (or LALA) (Table 1; Supplementary Figure 1). Homozygous individuals for the S1 allele present the higher mean score values of ASB and in males regression analysis yielded near significant association ($\beta=0.152$; $p=0.05$) between the S1 allele and higher ASB score levels. The work by Reif et al. (2007) in adult male

Caucasians referred for forensic assessment report similar interaction effect between childhood environment and 5-HTTLPR genotypes showing that high adversity during childhood impacted only the later-life violence if the short promoter alleles were present.

Most interestingly, in those individuals that were not exposed to childhood adversity, homozygous individuals L1L1 were more prone to commit ASB as opposed to heterozygous L1S1 and homozygous S1S1 (Table 1; Supplementary Figure 1). The S1-allele was found negatively related with ASB score levels (β=-0.071 in the overall population and β=-0.079 in males), reaching a significant value when testing for the overall population (p=0.019). Thus, the present findings suggest that those individuals homozygous S1S1 are most antisocial in the presence of adversity during childhood but least antisocial in the absence of early adversity (i.e. a cross-over interaction).

In concordance, investigating differences in ASB for each genotype group, L1L1, L1S1, and S1S1, in relation to the presence or absence of childhood adversity, higher scores of ASB were found in participants that come from childhood adverse environments both for S1S1 and L1S1 genotypes, but for the L1L1 subjects the higher ASB scores were found in absence of childhood adversity (Table 1).

Accordingly, a formal test for GxE effects showed a significant interaction between the clustered 5-HTTLPR/rs25531 genotypes and childhood environment that affects the ASB score ($p_{interaction}$=0.012).

Despite the interesting results, there are some limitations of this study: i) the use of two retrospective self-reports susceptible to recollection and reporting bias; ii) childhood adversity was assessed with three simple questions rather than a standardised questionnaire; iii) low statistical power.

In conclusion, the present results provide support for possible differential susceptibility properties of the 5-HTTLPR for ASB outcomes in response to childhood environments in a general population sample of young adults, in line with previous reports showing that S-allele carriers are both more vulnerable to negative environments and show more positive outcomes from positive environmental conditions. However, further studies should be carried out in order to overcome the limitations observed in the present investigation.

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Legends:

Figure 1. Graphical analyses of mean score levels of antisocial behavior (ASB) among genotypes in total population, males and females, stratified by childhood environment. Genotypes of 5-HTTLPR and rs25531 polymorphisms were transformed into a biallelic model according to *SLC6A4* allele expression levels (L1L1 includes LALA phased haplotypes; L1S1 includes LASA, LALG and LASG phased haplotypes, and S1S1 includes SASA and LGSA phased haplotypes). Graphical analyses were performed with software R version 3.1.3.

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