Psychiatry Research 220 (2014) 987–990

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Self-injury in autism spectrum disorder: An effect of serotonin transporter gene promoter variants

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ARTICLE INFO

Article history:
Received 29 January 2013
Received in revised form
23 September 2014
Accepted 28 September 2014
Available online 6 October 2014

Keywords:
Autism
Serotonin
Serotonin transporter gene
Self-injury
Aggression

ABSTRACT

Self-injurious behavior in autism spectrum disorder (ASD) has been associated with lower whole blood serotonin levels and the role of serotonin transporter gene promoter region (5HTTLPR) polymorphisms is of interest because of their effects on transporter functioning. This study examined the association between self-injurious behavior in ASD and allelic frequencies of 5HTTLPR. Sixty-four children and adolescents with ASD who were not taking serotonergic medication at the time of the assessment were included in the analysis. Self-injury was assessed using the Autism Diagnostic Interview-Revised (ADI-R) and whole blood serotonin levels were measured using high-pressure liquid chromatography (HPLC) with fluorometic detection. DNA was extracted from saliva and PCR amplified with fluorescent primers. Self-injury significantly increased with the number of La alleles of the 5HTTLPR and decreased with the number of Lg alleles. Self-injury in ASD may be associated with a specific genotype of the serotonin transporter gene promoter region. Future studies should continue to explore subgroups to clarify the underlying clinical and genetic heterogeneity in ASD.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with significant phenotypic variability characterized by social and communication deficits in addition to restricted and repetitive behaviors. While environmental risk factors play a role, the etiology of ASD is primarily due to complex genetics; both common genetic polymorphisms and rare causal variants are now understood to play causative roles. Genetic heterogeneity contributes to the challenges in identifying susceptibility genes, so previous studies have attempted to identify subgroups of patients with homogeneous phenotypes. Focusing genetic analyses on such phenotypic differences may facilitate identification of the genetic factors underlying the variability within the disorder.

Most investigators have found that serotonin levels measured in whole blood are significantly higher on average in subjects with ASD than normal controls, and approximately one-third of autistic individuals have hyperserotonemia (Schain and Freedman, 1961; Gabriele et al., 2014). Whole blood serotonin levels in these studies were measured from the platelet content of serotonin since greater than 99 percent of whole blood serotonin is found in the platelet fraction (Anderson et al., 1987). In previous work, our group examined the relationship between whole blood serotonin and repetitive behaviors in ASD in order to clarify whether levels of this neurochemical were associated with specific behavioral symptoms of autism. An inverse relationship was found between whole blood serotonin level and aggression to self (Kolevzon et al., 2010) using the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). Of interest, much literature to date supports the presence of an inverse relationship between serotonin or its metabolite (5HIAA) and aggression (Moore et al., 2002; Siever, 2008; Seo et al., 2008; Lesch and Merschdorf, 2000). The relationship between serotonin and aggressive or violent behavior is evident in aggression to either self or others, and studies have

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http://dx.doi.org/10.1016/j.psychres.2014.09.018
0165-1781/

implicated the role of serotonin transporter gene (5HTT) polymorphisms to self-directed aggression, such as suicidal behavior.

Over 20 polymorphisms of 5HTT have been identified, some of particular interest because of their functional effects on gene expression and serotonin transporter functioning. One such polymorphism is a 44 base-pair insertion/deletion in the 5′-flanking regulatory region of the serotonin transporter gene promoter region (5HTTLPR) (Goveas et al., 2004), corresponding to two common allelic variants—long (L) allele and short (S) allele respectively. The short variant has lower mRNA transcriptional efficiency, leading to lower gene expression and subsequent lower serotonin reuptake activity (Goveas et al., 2004; Lesch et al., 1996). An association between self-directed injury, such as suicidal behavior, and 5-HTTLPR allele frequency has been demonstrated but with conflicting results. Some studies find higher frequency of the L allele in depressed suicide victims compared to non-suicidal controls (Du et al., 1999; Russ et al., 2000), while others show an association between the S allele and lethality of suicidal behavior (Bondy et al., 2000; Bellivier et al., 2000; Courtet et al., 2001). In addition, Hankin et al. (2011) found that youth carrying one or two copies of the S allele of 5-HTTLPR exhibited more traits of Borderline Personality Disorder, a condition that is often associated with non-suicidal self-injury. These data suggest self-injurious behavior as a possible phenotype associated with serotonin regulation.

The role of 5-HTT gene polymorphisms in ASD has been extensively explored, but with inconsistent results. Attempts to determine whether there is a transmission bias of either L or S allelic variants of 5HTTLPR in autism have yielded conflicting results, including over-transmission of S (Devlin et al., 2005; Sutcliffe et al., 2005) over-transmission of L (Klauck et al., 1997; Yirmiya et al., 2001), or no significant association of either allele (Huang and Santangelo, 2008). Studies of association between allelic frequency and phenotypic subgroups have likewise yielded disparate results. Over-transmission of the S allele has been demonstrated in patients with ASD compared with their unaffected siblings, and especially patients with severe social or communication deficits (Tordjman et al., 2001). Brune et al. (2006) also found that patients with SL or SS genotypes had more severe impairments in social and communication domains but patients with the LL genotype had more severe stereotyped, repetitive behavior, and aggression. However, Ramoz et al. (2006) found no association between variants at the 5HTTLPR locus in ASD or more specifically in relatively rigid-compulsive and obsessive–compulsive patients, a finding supported by McCauley et al. (2004). However, interpreting results from these studies may be confounded by the possibility of ethnicity effects; a meta-analysis by Huang and Santangelo (2008) found that in family based studies of ASD, United States samples with various ethnicities had preferential transmission of the S allele while European and Asian samples had no allelic association (Huang and Santangelo, 2008).

Adding further to this complexity, 5-HTTLPR has recently been shown to be more complex—with L subdivided into La and Lg variants and S into Sa and Sg variants. The Lg variant results from a single-base substitution (A > G) and has a binding site for a transcription factor that suppresses 5-HTT transcription (Hu et al., 2005, 2006; Kraft et al., 2005). The Lg variant therefore functions similar to the S variant and thus can be grouped with it. To extend our previous findings that found an inverse relationship between whole blood serotonin and self-injury as assessed by the ADI-R, this study examined the association between self-injurious behavior and allelic frequencies of La vs. S/Lg polymorphisms of 5HTTLPR. It was hypothesized that self-injury in children with ASD would be associated with an increased frequency of S or Lg (vs. La) alleles because S and Lg alleles are associated with decreased uptake of serotonin into platelets where serotonin is mainly stored.

2. Materials and methods

Subjects were part of an ongoing recruitment for multiplex families at the Seaver Autism Center for Research and Treatment. Affected families were required to have at least one case of autism and another case with autism or a sub-threshold autism-related disorder based on Diagnostic and Statistical Manual Fourth Edition (DSM-IV) criteria. Individuals known to have medical conditions associated with autism (e.g. tuberous sclerosis, fragile X syndrome, phenylketonuria) were excluded. Cases with suspected autism were evaluated using the ADI-R to examine the three core symptom domains of autism (communication, reciprocal interaction, and repetitive, restricted behaviors) used to make a diagnosis of autism based on the DSM-IV at the time.

Blood to analyze serotonin was collected from affected individuals. Affected cases 16 years old and younger were then screened to include only those with an ADI-R diagnosis of autism who were not taking serotonergic medication at the time of the assessment. Of 154 affected individuals, 78 (50%) were eligible for inclusion based on these criteria, but 14 did not have DNA available for 5HTT genotyping and thus were not included in this study. The ADI-R was used to assess the presence of self-injury. Whole blood serotonin levels were measured using high-pressure liquid chromatography (HPLC) with fluorometic detection using methods described elsewhere (Cook et al., 1993). DNA was extracted from saliva and PCR amplified with fluorescent primers. Different sized alleles were determined after separation and detection by an ABI 3730 Genetic Analyzer. Genotypes were scored and collapsed according to the number of La alleles (0, 1, or 2), and similarly for Lg and S (no Sg alleles were observed in this sample). Binary logistic regression, controlling age and sex, was used to examine the association between the frequency of La alleles and the presence of self-injury. Parallel analyses were performed for the number of Lg and S alleles. The associations of serotonin with the presence of self-injury using the ADI and the numbers of the respective alleles were also evaluated.

3. Results

The 64 subjects came from 44 families. The range of ages was from 2 to 16 years-old (mean 6.85, S.D.+2.75). Forty-five cases were male (70.3%). Whole blood serotonin levels ranged from 78 to 471 ng/ml and approximately one-third of subjects had levels greater than 252 ng/ml (skewness=0.902, SE=0.299). The ethnic composition of the sample was 78.1% white, 14.0% “other” and 7.9% was classified as unknown or ethnicity data was missing. For the S/La/Lg alleles of the 5HTTLPR gene, the frequency of genotypes among subjects was as follows: low group: S/S=6; S/Lg=6; Lg/Lg=1; intermediate group: S/La=30; Lg/La=5; high group: La/La=16. The presence of self-injury was associated with a lower serotonin level ($\chi^2=5.99$, d.f.=1, $p=0.014$) as previously reported (Kolevzon et al., 2010) despite the current sample being restricted only to patients with available DNA. Table 1 shows the sample broken down by serotonin expression groups and the presence or absence of self-injury. Logistic regression analysis, controlling for age and sex, demonstrates that the presence of self-injury increased with an increase in the number of La alleles ($\chi^2=6.17$, d.f.=1, $p=0.01$). For Lg, the association was in the opposite direction, self-injury decreased

Table 1
Self-injury by serotonin expression.

Genotype	*N*	Subjects with self-injury (%)
No La alleles—low expression		
Lg/Lg	1	0 (0)
Lg/S	6	1 (16.7)
S/S	6	1 (16.7)
Total	13	2 (15.4)
One La allele—medium expression		
La/Lg	5	2 (40)
La/S	30	18 (60)
Total	35	20 (57.1)
Two La alleles—high expression		
La/La	16	10 (62.5)
Total		
	64	32 (50)