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Associations between polymorphisms in sex steroid related genes and autistic-like traits

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Summary Sex differences in psychiatric disorders are common, which is particularly striking in autism spectrum disorders (ASDs) that are four times more prevalent in boys. High levels of testosterone during early development have been hypothesized to be a risk factor for ASDs, supported by several studies showing fetal testosterone levels, as well as indirect measures of prenatal androgenization, to be associated with ASDs and autistic-like traits (ALTs). Further, the importance of sex steroid related genes in ASDs is supported by studies reporting associations between polymorphisms in genes involved in sex steroid synthesis/metabolism and ASDs and ALTs. The aim of the present study was to investigate possible associations between 29 single nucleotide polymorphisms (SNPs) in eight genes related to sex steroids and autistic features. Individuals included in the study belong to a subset (n = 1771) from The Child and Adolescent Twin Study in Sweden (CATSS), which are all assessed for ALTs. For two SNPs, rs2747648 located in the 3′-UTR of *ESR1* encoding the estrogen receptor alpha and rs523349 (Leu89Val) located in *SRD5A2* encoding 5-alpha-reductase, type 2, highly significant associations with ALTs were found in boys and girls, respectively. The results of the present study suggest that SNPs in sex steroid related genes, known to affect gene expression (rs2747648 in *ESR1*) and enzymatic activity (Leu89Val in *SRD5A2*), seem to be associated with ALTs in a general population. In conclusion, the current findings provide further support for a role of sex steroids in the pathophysiology of ASDs.

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1. Introduction

The importance of genetic factors in the development of autism spectrum disorders (ASDs), including autistic disorder, Asperger syndrome and pervasive developmental disorder-not otherwise specified (PDD-NOS), has been clearly demonstrated in twin and family studies, generating concordance rates of 70–90% in monozygotic twins compared to 3–5% in siblings (Rosenberg et al., 2009; Lichtenstein et al., 2010). Recent genetic studies have concluded that both rare (Devlin and Scherer, 2012) and common (Klei et al., 2012) genetic variants impact the liability for ASDs. ASDs represent a spectrum of impairments, theoretically divided into three dimensions: language impairments, social interaction impairments and restricted and repetitive behavior (Happe and Ronald, 2008). This division has been confirmed in several studies and it has been indicated that the dimensions have partly separate genetic influences when investigated in the general population (Ronald et al., 2011). ASDs represent the upper extreme of autistic-like traits (ALTs) that are continuously distributed in the general population (Constantino and Todd, 2003). It was recently shown that ALTs below the threshold for a diagnosis and ASDs share common genetic influences (Lundstrom et al., 2012).

ASDs are more prevalent in boys than in girls, with ratios of 3:1 for classic autism and 10:1 for Asperger syndrome (Gillberg et al., 2006). During development, males are exposed to testosterone in the second trimester in utero, which induces masculine patterns of neural and behavioral development (McCarthy, 2010). It has been hypothesized that high levels of testosterone during early development may be a risk factor for ASDs and ALTs, producing phenotypic changes associated with ''extreme maleness'' (Baron-Cohen et al., 2011; Pfaff et al., 2011). This theory has been supported by several studies showing fetal testosterone levels (for refs. see Baron-Cohen et al., 2011), as well as indirect measures of prenatal androgenization (De Bruin et al., 2009; Von Horn et al., 2010; for refs. see Baron-Cohen et al., 2011), to be associated with autistic features. The relationship between prenatal and postnatal androgen exposure is unclear, but several studies show an association between elevated androgen levels in adults and ASDs (Tordjman et al., 1997; Ruta et al., 2011; Bejerot et al., 2012). Furthermore, some studies have indicated that genetic polymorphisms in the androgen receptor (AR) gene (Henningsson et al., 2009) as well as in other sex steroid-related genes (Chakrabarti et al., 2009) may be associated with the risk of developing ASDs or ALTs.

Comparisons of brain size development between children with ASDs and typically developing children have lent further support to the theory of an ''extreme maleness'' involved in the disorder. Infant males have, on average, larger brains than females and the brains of children with autism are even larger (Courchesne et al., 2011). In addition, the amygdala in autistic children has been shown to be abnormally large. This enlargement seems to persist through early childhood (Nordahl et al., 2012), exactly during the period of sex-differential amygdala growth in typically developing boys. Lombardo et al. (2012) have shown that gray matter volume in brain areas of importance for autism, such as right temporoparietal junction/posterior superior temporal sulcus (RTPJ/pSTS), and amygdala, was greater in males compared to females and influenced by fetal testosterone.

The sex steroid testosterone is synthesized in several steps from the precursor cholesterol. During this process the enzyme cytochrome P450, family 17 (CYP17) catalyzes the conversion of pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and the final precursor androstenedione. DHEA can either be transformed to androstenedione, or to the other final precursor androstenediol. When metabolized, testosterone can be converted to the more potent androgen dihydrotestosterone by 5 alpha-reductase enzymes (type 1 and type 2). Both testosterone and dihydrotestosterone exert their effect by binding to ARs. In addition, testosterone can be transformed to the most potent estrogen, estradiol, by the enzyme aromatase, also named cytochrome P450, family 19 (CYP19), and act through binding to estrogen receptors (ER-alpha and ER-beta). Circulating estradiol, testosterone and dihydrotestosterone are transported in the blood stream bound to the proteins sex hormone binding globulin (SHBG) and albumin, which thereby affect the bioavailable fraction of the sex steroids.

In the present study candidate genes were chosen based on their relation to sex steroids, either to synthesis (*CYP17*, *CYP19*), metabolism (*SRD5A1*, *SRD5A2*), transport (*SHBG*), or as a receptor (*AR*, *ESR1* (estrogen receptor 1), *ESR2* (estrogen receptor 2)). The aim of this study was to investigate whether 29 single nucleotide polymorphisms (SNPs) in these genes are associated with ALTs in the general population. Some of the SNPs have been associated with ASD in previous studies, while the majority of the SNPs were selected either based on functionality, shown in vivo or in vitro, or based on previous associations with diseases or traits related to sex steroids and/or brain function (see Table 1).

2. Materials and methods

2.1. Subjects and measurements

Subjects included in this study are a subset (N = 1771) from The Child and Adolescent Twin Study in Sweden (CATSS) (Anckarsater et al., 2011). The subjects, born in Sweden in 1992 or 1995, were contacted and their parents participated in a telephone interview containing, among other instruments, the Autism-Tics, ADHD, and other Comorbidities inventory (A-TAC) (Hansson et al., 2005; Larson et al., 2010). The A-TAC is a sensitive and validated tool for screening general populations on childhood ASDs and associated conditions and can also be used as a dimensional measure. Each of the 17 items on ALTs (six corresponding to language impairment, six to social interaction impairment and five to restricted and repetitive behavior) has three response categories: ''no'' (coded 0), ''yes, to some extent'' (coded 0.5), and ''yes'' (coded 1.0). The measure of total ASD score is the sum of these 17 items, while the measures of the modules (language impairment, social interaction impairment, and restricted and repetitive behavior) are the sum of the specific items corresponding to respective module (Anckarsater et al., 2011). In the investigated subset of CATSS, the mean (sd) of the total ASD score is 1.24 (2.18) in boys and 0.66 (1.39) in girls. A cut-off score of 8.5, used as a validated proxy for clinical diagnoses of ASDs, shows a sensitivity of 0.71 and a specificity of 0.95, while a cut-off score of 4.5, which is a screening-diagnosis for ASDs, which has a sensitivity of 0.96