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Increased serum androstenedione in adults with autism spectrum conditions

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Summary Molecular and behavioural evidence points to an association between sex-steroid hormones and autism spectrum conditions (ASC) and/or autistic traits. Prenatal androgen levels are associated with autistic traits, and several genes involved in steroidogenesis are associated with autism, Asperger Syndrome and/or autistic traits. Furthermore, higher rates of androgen-related conditions (such as Polycystic Ovary Syndrome, hirsutism, acne and hormone-related cancers) are reported in women with autism spectrum conditions. A key question therefore is if serum levels of gonadal and adrenal sex-steroids (particularly testosterone, estradiol, dehydro-epiandrosterone sulfate and androstenedione) are elevated in individuals with ASC. This was tested in a total sample of $n = 166$ participants. The final eligible sample for hormone analysis comprised $n = 128$ participants, $n = 58$ of whom had a diagnosis of Asperger Syndrome or high functioning autism (33 males and 25 females) and $n = 70$ of whom were age- and IQ-matched typical controls (39 males and 31 females). ASC diagnosis (without any interaction with sex) strongly predicted androstenedione levels ($p < 0.01$), and serum androstenedione levels were significantly elevated in the ASC group (Mann—Whitney $W = 2677$, $p = 0.002$), a result confirmed by permutation testing in females (permutation-corrected $p = 0.02$). This result is discussed in terms of androstenedione being the immediate precursor of, and being converted into, testosterone, dihydrotestosterone, or estrogens in hormone-sensitive tissues and organs.

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1. Introduction

Autism spectrum conditions (ASC) are neurodevelopmental and are characterized by difficulties in social interaction and communication skills, alongside restricted interests and stereotyped behaviours (APA, 1994). The foetal androgen theory of ASC proposes that foetal testosterone (fT) is one influence in the development of psychological and neural sex differences in the general population, and in the development of autistic traits (Baron-Cohen et al., 2004). Evidence in support of this theory comes from the Cambridge Foetal Androgen Study, in which typically developing children whose amniotic fT levels were measured in utero have been followed up at different points in development. Since amniotic testosterone levels measured are the product of renal clearance of hormones produced by the foetus (Finegan et al., 1991), fT can be regarded as a proxy measure of circulating testosterone in the foetus. Results from that longitudinal study reveal that fT levels are *inversely* correlated with social and language development, including measures of eye contact at 12 months, vocabulary size at 18 and 24 months, social skills at 48 months and empathy at 6—9 years (Lutchmaya et al., 2002a,b; Knickmeyer et al., 2005, 2006a; Chapman et al., 2006). In contrast, fT levels are *positively* correlated with autism-related behaviours such as autistic traits at 18—24 months and at 6—9 years (Auyeung et al., 2009, 2010), restricted interests at 48 months (Knickmeyer et al., 2005), and systemizing at 6—9 years (Auyeung et al., 2006). Consistent with these results, females with Congenital Adrenal Hyperplasia (CAH) (whose androgens are elevated prenatally due to a reduced efficiency of cortisol synthesis in the adrenal gland) have a higher than average numbers of autistic traits (Knickmeyer et al., 2006b).

To our knowledge, *postnatal* sex-hormone profiles at different stages of development and in adult life have not yet been systematically studied in relation to ASC and/or autistic traits in the general population. Schmidtova et al. (2010) found that children with autism (both prepubertal and pubertal) and (pubertal) children with Asperger Syndrome (AS) had significantly increased levels of salivary testosterone compared with control children. A further study from Schwarz et al. (2010) found elevated levels of free-testosterone and LH in females with AS compared with control females in a multi-analyte profiling of blood serum. In contrast, Croonenberghs et al. (2010) found lower concentration of serum testosterone in male adolescents with ASC, compared to matched controls. Some indirect evidence that circulating androgens may be dysregulated in ASC also comes from the atypical timing of puberty reported in ASC (Tordjman et al., 1997; Yoshimura et al., 2005; Knickmeyer et al., 2006c). In addition, it has been found that, in adulthood, a number of androgen-related medical conditions (such as Polycystic Ovary Syndrome (PCOS), hirsutism, acne, breast and ovarian cancers) and androgen-related characteristics (such as tomboyism, bisexualism and asexualism) are more common in women with ASC, and in their mothers (Ingudomnukul et al., 2007). This suggests that genetic factors might account for higher levels of androgen synthesis and/or increased local tissue sensitivity to circulating androgens in ASC.

Related to this, two sex-steroid hormone related genes (*SRD5A1* and *AR*) have been found to be associated with autism (Henningsson et al., 2009; Hu et al., 2009) and polymorphisms in genes involved in testosterone metabolism (*AR* and *SRD5A2*) have also shown association with ASC (Schmidtova et al., 2010). In another study, 10 other genes related to synthesis, metabolism, or transport of sex steroids (*HSD11B1*, *LHCGR*, *CYP17A1*, *CYP19A1*, *SCP2*, *CYP11B1*, *ESR1*, *ESR2*, *HSD17B4*, *HSD17B2*) showed nominal association with Asperger Syndrome and/or autistic traits in the general population. Of these, *CYP11B1*, *CYP17A1*, and *ESR2* survived familywise error rate correction by permutation testing (Chakrabarti et al., 2009). Given these converging lines of evidence, we tested if serum levels of gonadal and adrenal sex-steroids (specifically, testosterone, estradiol, dehydroepiandrosterone sulfate (DHEA-S) and androstenedione) are elevated in adults with ASC.

While testosterone and estradiol are the main sex hormones produced in the male and female gonads respectively, androstenedione is synthesized in both gonads and the adrenal cortex, and DHEA-S is the main androgen synthesized in the adrenal gland. Androstenedione and DHEA-S are both released in the peripheral blood circulation and are converted into testosterone, dihydrotestosterone or estrogens in hormone-sensitive tissues and organs (such as the skin, the pilosebaceous unit, adipose tissue, and most relevant, the brain) via intracrine mechanisms. Consequently, they contribute to the final pool of active androgens in peripheral target tissues and to the development of androgen-related conditions such as PCOS, hirsutism, and acne (Georgopoulos et al., 2009).

The objective of this study was to investigate if androgen biosynthesis is dysregulated in adult males and females with ASC, leading to increases in testosterone, testosterone to estradiol ratio, DHEA-S, or androstenedione in peripheral blood circulation. Since androgen related medical conditions and characteristics have been reported in females with ASC, we predicted that an increase in serum androgens, if present, would be seen particularly clearly in females.

2. Materials and methods

2.1. Participants and procedure

The study was approved by the Cambridge NHS NRES Research Ethics Committee and all participants signed a consent form to participate. The sample comprised n = 166 participants, 62 of whom had a diagnosis of ASC (33 males and 29 females) and n = 104 of whom were controls (49 males and 55 females). Participants with ASC were recruited via the Cambridge Autism Research Centre database of volunteers (www.autismresearchcentre.com), the National Autistic Society (UK), and local autism support groups in the UK. Controls were recruited from the general population via advertising. ASC diagnosis (Asperger Syndrome or high functioning autism) was made by psychiatrists or clinical psychologists based on Diagnostic and Statistical Manual-IV-Text Review Disorders criteria (DSM-IV-TR, 2000). As a check on diagnosis, a subgroup of 14 participants with ASC with available parental informants were also assessed using the Autism Diagnostic Interview — Revised (Lord et al., 1994), all of whom scored above the diagnostic cut off for autism. The 62 individuals with ASC were also given the Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) as a further check on diagnosis,