Polymorphisms in Genes Involved in Testosterone Metabolism in Slovak Autistic Boys

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Abstract: Autism spectrum disorders (ASDs) are neurodevelopment disorders which are characterized by impairments in the following core domains: social interaction, language development, verbal/nonverbal communication, repetitive and restricted behaviors. The androgen theory of autism proposes that autism spectrum disorders develop in part due to elevated fetal testosterone levels, which correlate with a number of autistic traits. The present study evaluates androgen and estrogen levels in saliva as well as polymorphisms in genes for androgen receptor (AR), 5-alpha reductase (SRD5A2), and estrogen receptor alpha (ESR1) in the Slovak population of prepubertal (under 10 years) and pubertal (over 10 years) children with autism spectrum disorders. The examined prepubertal patients with autism, pubertal patients with autism, and prepubertal patients with Asperger syndrome had significantly increased levels of salivary testosterone (P < 0.05, P < 0.01, and P < 0.05, respectively) in comparison with control subjects. We found a lower number of (CAG)n repeats in the AR gene in boys with Asperger syndrome (P < 0.001). Autistic boys had an increased frequency of the T allele in the SRD5A2 gene in comparison with the control group. The frequencies of T and C alleles in ESR1 gene were comparable in all assessed groups. The modulating influence of studying genotypes on the effect of testosterone could provide insight into the pathogenesis of autism spectrum disorders.

Key Words: autism, testosterone, androgen receptor

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Autism spectrum disorders (ASDs) are neurodevelopment disorders with a genetic origin and heritability of about 90%. ASDs are characterized by impairments in these core domains: social interaction, language development and verbal/nonverbal communication, repetitive and restricted behaviors, narrow interests, abnormal movement patterns, and sensory dysfunction.

ASDs are more common in males than in females (4:1 for classic autism and 9:1 for Asperger syndrome). The androgen theory of autism proposes that autism spectrum disorders develop, in part, due to elevated fetal testosterone levels, which are positively correlated with a number of autistic traits and inversely correlated with social development and empathy.1 Studies of amniotic testosterone suggest that fetal testosterone is related to specific sexually dimorphic aspects of cognition and behavior.2 The extreme manifestation of some male typical traits mainly in neuroanatomy and cognition represents one of many theories of autism. Several studies were conducted to clarify the effects of fetal testosterone on male and female neuroanatomical structures. Grimshaw et al3 reported that 8-year-old girls with higher levels of fetal testosterone perform a mental rotation task faster than girls with lower levels and 10-year-old girls with higher levels of fetal testosterone show a more masculine pattern of cerebral lateralization.4 Knickmeyer et al5 confirmed in 4-year-old children that fetal testosterone decreases social cognition or social interest in both boys and girls, resulting in poor quality of social relationships. Fetal testosterone levels were positively associated with higher scores on the Childhood Autism Spectrum Test.6 These findings are consistent with the androgen theory of autism that proposes an influence of prenatal androgen exposure on the development of autistic traits in children.6

Further studies have shown elevated blood androgen metabolites in patients with ASDs in comparison with controls.7,8 Tordjman et al7 observed precocious secondary sexual characteristics (growth of pubic hair, increase of testis volume) in prepubertal autistic children suggesting high androgenic activity in patients with ASDs. Geier and Geier9 found significantly increased levels of serum testosterone, free testosterone, dehydroepiandrosterone, and androstenedione in patients with ASDs. Genes participating in testosterone metabolism mediate and modulate the effects of androgens in tissues. Polymorphism in the number of (CAG)n repeats in the first exon of the androgen receptor gene is well-known. The normal number of (CAG)n repeats ranges from 9 to 37 with the average number of repeats 21 ± 2.10 The length of (CAG)n repeats is inversely related to the transcriptional activity of androgen-dependent genes.11,12 Thus, fewer (CAG)n repeats in the androgen receptor is associated with stronger androgen effect and increased prenatal androgen sensitivity as measured by the ratio between the second and fourth digit.13 Relatively long (CAG)n fragments are associated with decreased transactivation function of androgen receptor.14,15 The results of Henningsson et al16 support the hypothesis that (CAG)n repeats influence the susceptibility for autism, but argue against the possibility that mutations in the AR gene are common in subjects with this condition. The SRD5A2 gene codes for the enzyme 5-alpha-reductase that converts testosterone to the more efficient androgen—dihydrotestosterone. SRD5A2 gene polymorphisms influence the activity of the enzyme through modulation of the mRNA stability.17 A49T (alanine to threonine) substitution amplifies the enzymatic activity 5-fold in vitro18 and this could contribute to higher dihydrotestosterone levels. We know of no study that reveals the association of SRD5A2 gene polymorphisms and ASDs.

ESR1 encodes one of the receptors for estrogens. Polymorphisms related to impaired/improved cognition are located in the first intron. Here, a restriction site for PvuII was found. A T→C transition leads to its absence and subsequently to modulation of cognitive abilities via modulation of effects of estradiol—a metabolite of testosterone.

The present study evaluates androgen and estrogen levels as well as polymorphisms in AR, SRD5A2, and ESR1 in the Slovak population of prepubertal (<10 years) and pubertal (>10 years) children with autism spectrum disorders.
PATIENTS AND METHODS

Patients
After signing an informed consent, 54 autistic boys with classic autism, 47 boys with Asperger syndrome, and 107 age matched healthy controls were included in the study. Probands and control boys were divided into 2 groups according to age: 4 to 10 year-olds and 11 to 18 year-olds. We divided the patients into the same age groups. Patients with Asperger syndrome were selected by a trained psychologist according to the following criteria: absence of delayed speech development, no impairments in word and logic cognition, and good verbal skills. Autistic children were selected according to impairments in 3 behavioral domains: social interaction, communication, and restricted interests and activities. The diagnosis used standard protocols ICD-10 and DSM-IV. Healthy control boys were primary school and high school students. The study was approved by the Ethical Committee of Comenius University, Faculty of Medicine and was conducted in compliance with the Code of Ethics of the World Medical Association.

Methods
DNA samples were isolated from buccal cells in saliva using a commercial kit (Omega Biotek) and subsequently particular gene segments were amplified by PCR. Polymorphism in SRD5A2 gene (rs9282858; restriction endonuclease—MwoI) and polymorphism in ESR1 gene (rs2234693; restriction endonuclease—PvuII) were determined by RFLP analysis. AR (CAG)n polymorphism was determined by fragment analysis with fluorescently labeled primer on ABI 3100 Avant genetic analyser. Salivary testosterone and estradiol levels were measured by ELISA analysis (DRG Diagnostics, Germany). Differences in testosterone and estradiol levels and in selected genetic markers in boys with ASDs and control boys were statistically evaluated by 1 way ANOVA and the post hoc LSD test. Qualitative parameters were evaluated with χ² test. XLstatistics 5, SPSS 15, and Microsoft Excel 2007 were used. The level of significance was set to 0.05. Data are presented as mean ± SD.

RESULTS
Prepubertal autistic boys had significantly increased salivary testosterone (116.6 ± 106.9 pmol/L) in comparison with the control group (64.4 ± 52.5 pmol/L) (P = 0.01) (Fig. 1). Prepubertal Asperger boys had increased testosterone levels by 122.7% in comparison with control group (P = 0.05) (Fig. 1). We did not find any significant differences between Aspergers (143.4 ± 179.8 pmol/L) and the autistic group (116.6 ± 106.9 pmol/L) (P = 0.07) (Fig. 1). Differences in salivary estradiol levels in prepubertal boys in control group (10.0 ± 3.1 pmol/L), Asperger group (7.7 ± 4.0 pmol/L), and autistic group (10.0 ± 4.1 pmol/L) were not significant (P = 0.16) (Fig. 2).

In pubertal boys, differences were seen only between autistic and control boys. Autistic boys had significantly increased overall levels of salivary testosterone (Fig. 3) and no differences in salivary estradiol levels (Fig. 4).
We also investigated the genotype status in AR, SRD5A2, and ESR1 gene. Figure 5 shows differences in (CAG)n repeats in the first exon of AR gene between the groups. Differences between groups were statistically significant (P = 0.002). Boys with Asperger syndrome had lower number of (CAG)n repeats in comparison with control group. Lower number of repeats was found in Asperger compared with autistic boys. Differences between autistic boys and control group were not found.

We found differences in the length of restriction fragments in SRD5A2 gene between the groups (P < 0.001) (Fig. 6). The most frequent genotype was AT in autistic boys (77.05%), while AA genotype was shown as the most frequent in Asperger (73.3%) and control group (76.92%).

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DisCUSSION

The patients with autism (prepubertal and pubertal) and Asperger syndrome (prepubertal) had significantly higher levels of salivary testosterone in comparison with control subjects. This observation is consistent with previous studies. Geier and Geier found that the T allele is the more frequent in autistic boys. Increased genotype was the most frequent in Asperger syndrome. It suggests that the T allele has a higher frequency in autistic boys. The wide phenotypic variability of ASDs suggests interaction of multiple genes. Many linkage studies, candidate gene association studies and cytogenetic studies have been conducted to reveal chromosomal regions with autism susceptibility loci. Wang et al. have completed a genetic analysis in a large number of ASD cases (more than 10,000 subjects) and they have identified common genetic variants on 5p14.1 that are associated with susceptibility to ASDs. The International Molecular Genetic Study of Autism Consortium previously identified linkage loci on chromosomes 7 and 2, termed AUTS1 and AUTS3, respectively. The genome scan carried out by the Autism Genome Project using Affymetrix 10K single nucleotide polymorphism arrays and 1181 multiplex families, also provided some support for both the chromosome 2q and 7p loci within the families of inferred European ancestry.

All these previously mentioned studies were focused on identification of genetic loci with high frequency in ASDs. We concentrated on loci with potentially high effect but with low frequency. We selected genetic regions which are involved in testosterone metabolism because testosterone and its metabolites appear to participate in the pathophysiology of autism. Two previous studies have assessed microsatellites in the AR to address the issue of altered X-inactivation patterns in autism. Hemmingson et al. found that the length of CAG triplets is shorter in patients with ASD than in controls, but these differences were significant only in females. Our finding of fewer (CAG)n repeats (within the normal range of 9–37 repeats) in boys with Asperger syndrome suggests that Asperger boys might have higher transactivation activity of the androgen receptor and thus the final androgen effect of comparable testosterone concentrations might be stronger than in the control group. In both pubertal and prepubertal groups of autistic boys we found that the levels of salivary testosterone are higher in comparison with the control boys, but we did not find any differences in the number of (CAG)n repeats in androgen receptor gene.
The moderating influence of AR genotype on the effect of testosterone could provide an example of gene-environment interaction where hormonal environment influences the phenotype more or less depending on a specific variant gene. Comparing the length of (CAG)_n repeats in boys with autism spectrum disorder, we find a lower number of (CAG)_n repeats in the AR gene is consistent with the hypothesis that boys with more “efficient” (CAG)_n repeats have more masculinized androgen receptor and consequently stronger androgen effect. Excessive testosterone levels in autism and lower number of (CAG)_n repeats, compared with those with a less efficient AR (higher number of (CAG)_n repeats).

The SRD5A2 gene codes for 5-alpha-reductase that converts testosterone to dihydrotestosterone. An A49T (alanine to threonine) substitution polymorphism in SRD5A2 gene amplifies the enzyme activity 5-fold in vitro and consequently the T allele increases dihydrotestosterone levels in cells. We found differences in distribution of A/T alleles between groups. Autistic boys had an increased frequency of T allele in comparison with the control group. Because of amplified 5-alpha-reductase activity, the autistic boys are probably influenced by higher androgen levels. We did not find a higher frequency of T allele in autistic boys compared with the control group. Autistic patients probably do not have amplified 5-alpha-reductase’s activity and do not have elevated levels of dihydrotestosterone in comparison with autistic boys.

Testosterone may act via 2 pathways. The first represents conversion to dihydrotestosterone via the enzyme 5-alpha-reductase. The second is the conversion to estradiol by aromatase. Our results suggest that autistic boys may not have the amplified 5-alpha-reductase activity and might not have elevated levels of dihydrotestosterone. Consequently, testosterone is accessible for conversion to estradiol in the brain.

The effects of estradiol in brain are mediated by estrogen receptors. We analyzed single nucleotide polymorphism in ESR1 gene. T→C transition may participate on the modulation of spatial cognition by increasing estrogen receptor activity, by regulation of gene expression or by changing the estradiol serum levels. We did not find any differences in distribution of TT, TC, or CC genotypes. The frequencies of T and C alleles were comparable in all assessed groups.

Increased levels of testosterone (mainly in pubertal boys) as well as the AT genotype of SRD5A2 is observed only in autistic boys. CAG repeat differences are only observed in Asperger patients. Earlier studies show similar variability.

CONCLUSION

Boys with autism syndrome have significantly higher testosterone levels while estradiol levels were comparable with control group. We find a lower number of (CAG)_n repeats in boys with Asperger syndrome suggest a higher transactivation activity of the androgen receptor and consequently stronger androgen effect. Excessive testosterone levels in autism and lower number of (CAG)_n repeats in Asperger syndrome support the hypothesis that androgens are important in the pathogenesis of ASD. Our findings bolster the theory that the variation within the AR receptor gene may influence ASD susceptibility. It is clear that additional research should be directed toward understanding the role of steroid hormones and related genetic polymorphisms in ASD pathogenesis.

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