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Sex steroid hormones and sex hormone binding globulin levels, CYP17 MSP AI (−34 T:C) and CYP19 codon 39 (Trp:Arg) variants in children with developmental stuttering

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ABSTRACT

Developmental stuttering is known to be a sexually dimorphic and male-biased speech motor control disorder. In the present case-control study, we investigated the relationship between developmental stuttering and steroid hormones. Serum levels of testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), oestradiol, progesterone, cortisol, and sex hormone binding globulin (SHBG), as well as the 2nd/4th digit ratio (2D:4D), an indicator of prenatal testosterone level, were compared between children who stutter (CWS) and children who do not stutter (CWNS). Moreover, two SNPs (CYP17 −34 T:C (MSP AI) and CYP19 T:C (Trp:Arg)) of cytochrome P450, which is involved in steroid metabolism pathways, were analysed between the groups. Our results showed significantly higher levels of testosterone, DHT, and oestradiol in CWS in comparison with CWNS. The severity of stuttering was positively correlated with the serum levels of testosterone, DHEA, and cortisol, whereas no association was seen between the stuttering and digit ratio, progesterone, or SHBG. The CYP17CC genotype was significantly associated with the disorder.

1. Introduction

Developmental stuttering is a common speech motor control disorder in which the flow of speech is disrupted by involuntary blocks, frequent repetitions, and prolonged speech sounds. The onset of this disorder is usually observed in children aged 2–5 years (Bloodstein & Ratner, 2008). Generally, 5% of children are affected by this disorder with a male to female ratio of 2:1 (Månsson, 2000; Yairi & Ambrose, 1999). Since the approximate rate of children's recovery is 80%, the overall prevalence of this disorder decreases to 1% in adult populations (Månsson, 2000). Because females are more likely to recover from this disorder than males, the male to female ratio for stuttering changes to 4:1 in adults (Bloodstein & Ratner, 2008; Yairi & Ambrose, 2005).

Although the role of sexual dimorphism in the onset of stuttering and the high rate of recovery in females have been addressed in many studies to date, the underlying physiological mechanisms of this gender-dependent disorder have not been well elucidated. This gender dependency of developmental stuttering suggests a role for androgens, the main male sex hormones, in the pathogenesis of the disorder. The role of testosterone in phenomena such as left-handedness (Hampson & Sankar, 2012) and some neurodevelopmental disorders such as attention deficit-hyperactivity disorder (ADHD) (Martel & Roberts, 2014; Roberts & Martel, 2013), autism spectrum disorder (ASD) (Baron-Cohen et al., 2015), and Tourette syndrome (TS) (Bortolato et al., 2013; James, 1995) has been reported. Controversies remain regarding the effects of testosterone on brain functions and disorders. A recent study by Papadatou-Pastou et al. did not reveal any association between salivary testosterone and handedness or cerebral lateralization for language (Papadatou-Pastou, Martin, & Mohr, 2017). Additionally, the role of prenatal testosterone exposure in the aetiology of ADHD has been challenged by some studies (Lemiere, Boets, & Danckaerts, 2010). Nevertheless, it has been suggested that some sex steroids, such as testosterone and oestrogen, contribute to the

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sex-biased distribution of stuttering (Geschwind & Galaburda, 1985). The comorbidity and association of stuttering with weak laterality (Kushner, 2012; Mohammadi, Khazaie, Rezaei, & Joghataei, 2016), ADHD (Donaher & Richels, 2012), and TS (Abwender et al., 1998; De Nil, Sasisekaran, Van Lieshout, & Sandor, 2005) may support the role of sex steroids in the pathogenesis of this disorder.

During speech and language processing, the left hemisphere of the human brain is more active in fluently speaking people; however, this left dominance is disrupted in people who stutter (Sato et al., 2011). The effects of prenatal testosterone on laterality have been explained by three hypotheses, and each hypothesis has proposed different directions and degrees of lateralization in relation to foetal testosterone exposure. According to the classic Geschwind-Behan-Galaburda hypothesis, foetal exposure to testosterone affects the development of the cerebral hemispheres. Therefore, more testosterone leads to greater dominance of the right hemisphere for language and handedness by either preventing the development of the left hemisphere (Galaburda, Corsiglia, Rosen, & Sherman, 1987; Geschwind & Galaburda, 1985) or enhancing the development of the right hemisphere (Rosen, Sherman, & Galaburda, 1991). The second hypothesis, named the corpus callosum hypothesis, proposed that the testosterone-mediated pruning of callosal neurons during pre- and post-natal development of the brain affects cerebral lateralization. According to this hypothesis, foetal testosterone enhances neural pruning in the corpus callosum, diminishes connectivity between the hemispheres and reinforces cerebral lateralization. In contrast with the Geschwind-Behan-Galaburda hypothesis, low but not high foetal testosterone would contribute to weak laterality by reducing neural pruning in the corpus callosum. This hypothesis proposed the effect of prenatal testosterone on the degree but not the direction of cerebral lateralization (Witelson, 1991; Witelson & Nowakowski, 1991). The final perspective, known as the sexual differentiation hypothesis, linked sexual dimorphism in laterality to testosterone-mediated sexual differentiation in the foetus. According to this hypothesis, higher exposure to testosterone in the foetus changes the degree of right-handedness or even leads to left-handedness but does not change the direction of cerebral language dominance and only influences the degree of language lateralization (Hines & Shipley, 1984).

Taking this information into consideration, a role for sex steroids in brain language processing and related disorders is suggested. According to Schaadt et al., brain exposure to testosterone and oestradiol in early infancy may delay language development (Schaadt, Hesse, & Friederici, 2015). Additionally, there is an association between testosterone and cerebral laterality for language processing in male subjects (Papadatou-Pastou & Martin, 2017). An association between stuttering and brain language processing (Connally, Ward, Howell, & Watkins, 2014; Wymbs, Ingham, Ingham, Paolini, & Grafton, 2013), as well as brain laterality for language (Code, Lincoln, & Dredge, 2005; Foundas et al., 2003), and the effect of sex steroids on brain language processing and laterality reinforces the probable association between sex steroids and stuttering; nevertheless, data regarding this important relationship are rare.

Moreover, the dopaminergic dysfunction in stuttering (Wu et al., 1997) together with the influence of steroid-dopamine interactions on brain functions and disorders (Purves-Tyson et al., 2014; Sanchez, Bourque, Morissette, & Di Paolo, 2010) strengthen the possible role of sex steroids in the neuropathology of stuttering. Fluctuations in the level of sex hormones during the menstrual cycle affect the severity of tics in women with TS, a disorder of basal ganglia circuits (Bortolato et al., 2013). Previous studies revealed more dysfluencies during the premenstrual period in women both with and without stuttering; this may confirm the role of steroids in stuttering pathology (Silverman, 1975; Silverman, Zimmer, & Silverman, 1974). Similarly, Kartalci et al., reported that after testosterone was injected into a 14-year-old boy with hypogonadism, he started stuttering despite lacking any previous speech disorder (Kartalcı, Özcan, Yüksel, & Ünal, 2012). Additionally, Selcuk et al. reported high serum testosterone levels in CWS aged 7–12 years compared to CWNS. They also showed that there is a remarkable positive correlation between the severity of stuttering and the testosterone level (Selcuk, Erbay, Ozcan, Kartalci, & Batcioglu, 2015).

Beside the role of sex steroids in brain language processing in children, the effect of prenatal exposure to testosterone on brain development and functions should not be ignored. Notably, the foetal testosterone level has been directly investigated in autism spectrum disorders (ASD), and the effect of this parameter on autistic traits has been confirmed (Auyeung et al., 2009). However, due to methodological problems in foetal blood sampling, the postnatal 2nd to 4th digit ratio (2D:4D) is used as an indirect indicator of the prenatal testosterone exposure; it is lower in males than in females (Manning, Scutt, Wilson, & Lewis-Jones, 1998). This method has been used to evaluate the correlation between prenatal exposure to testosterone and some neurodevelopmental conditions such as autism (Al-Zaid, Alhader, & Al-Ayadhi, 2015; Guyatt, Heron, Knight Ble, Golding, & Rai, 2015). The only study of the relationship between 2D:4D and stuttering was that published by Montag et al.; no significant difference was reported in the digit ratio between adults who stutter and the controls. The authors concluded that prenatal testosterone level was not significantly different between the studied groups; however, they reported a negative correlation between the left digit ratio and negative experiences of people who stuttered, using the Overall Assessment of the Speaker's Experience of Stuttering (OASES), suggesting that higher prenatal testosterone is linked to more negative experiences due to stuttering (Montag et al., 2015). The potential link between prenatal testosterone and stuttering still remains unclear.

Moreover, the expression of sex steroids is controlled by specific enzymes involved in the androgen metabolism pathways that may indirectly control stuttering. The cytochrome P450 (CYP) family of enzymes is among the more important regulators of sex steroid synthesis. CYP17 and CYP19, as members of the cytochrome P450 supergene family, were the focus of this study. The enzyme CYP17 plays a key role in the synthesis of sex steroids by steroid 17α-hydroxylation and by 17, 20-lyase activity, which converts 17 α-hydroxypregnenolone to dehydroepiandrosterone (DHEA) and 17α-hydroxyprogesterone to androstenedione, precursors of testosterone and oestrogens, respectively (Weston et al., 1998). In addition, the cytochrome P450 family 19 gene (CYP19), located on chromosome 15, controls three consecutive hydroxylation reactions that convert C19 androgens to aromatic C18 oestrogenic steroids. The enzyme CYP19 converts androstenedione and testosterone to oestrone and oestradiol, respectively (Kitawaki et al., 1999). Any genetic variations at the locus of this gene may alter the aromatase activity of this enzyme and thereby the levels of subsequent hormones (Surekha et al., 2014). In the present study, we investigated the role of the CYP17 −34 T:C (MSP AI) and the CYP19 T:C (Trp:Arg) single nucleotide polymorphisms in susceptibility to stuttering. A substitution of C for T at −34 bp in the 5′ promoter region of the CYP17 gene leads to increased gene expression and increases the oestradiol level in pre-menopausal women (Small et al., 2005). A polymorphism in the 3′ UTR of CYP19 increases mRNA expression (Kravitz, Meyer, Seeman, Greendale, & Sowers, 2006) resulting in an increased level of oestradiol and a decreased level of testosterone (Olson, Bandera, & Orlow, 2007). The CYP17 −34 T:C (MSP AI) polymorphism has been well studied in the context of breast and reproductive system conditions such as endometrial (Olson et al., 2007; Xu, Lin, Zhu, Zhang, & Yang, 2013), breast (Ebrahimi, Sabokbar, Eskandarieh, Peyghambari, & Shirkoohi, 2017; Haiman et al., 1999; Ye & Parry, 2002), and prostate cancers (Madigan et al., 2003; Ntais, Polycarpou, & Ioannidis, 2003). Additionally, the CYP19 T:C (Trp:Arg) polymorphism has been associated with the risk of breast cancer (Tuzuner et al., 2010). Due to the important role of sex steroids in brain development, associations between these SNPs and brain functions such as personality traits and cognition have been investigated. CYP19 genotypes have been related to cognitive functions in women in midlife