SUMMARY

Objective: The neurobiological basis of Gender Dysphoria (GD) is not yet fully known. In recent years, the role of prenatal exposure to testosterone has been emphasized in the development of the GD. The aim of this study was to compare the 2D:4D digit ratio, which is considered to be a morphological indicator of exposure to testosterone in the prenatal period, in individuals with GD.

Method: The study included 99 participants diagnosed with GD comprising 54 assigned the female gender at birth [AFB-GD], 45 assigned the male gender at birth [AMB-GD], and 58 female and 58 male participants making up the control group. The right and the left hands of the participants were photocopied and finger lengths were measured using digital calipers.

Results: The 2D:4D digit ratio on the right hand of the AFB-GD group was significantly lower (p=0.028) than that of the female controls, but it did not differ significantly as compared to male controls. The ratio on the left hand of the AFB-GD group did not significantly differ from that of the female controls, but it was higher than that of the male controls (p=0.045). The 2D:4D digit ratio on the right hand of the AMB-GD group did not differ significantly from that of the male controls, but they had a lower finger ratio as compared to the female controls (p<0.001). The ratio on the left hand of the AMB-GD group did not differ significantly from those of the male and female controls.

Conclusion: The results of this study present suggestive evidence that the AFB-GD individuals were exposed to testosterone in the prenatal period.

Keywords: Gender dysphoria, 2D:4D digit ratio, testosterone, prenatal exposure to testosterone

INTRODUCTION

Gender Dysphoria (GD) is the distress caused by the discrepancy between an individual’s gender identity and the gender assigned at birth (American Psychiatric Association 2013). Whereas the prevalence of GD was reported in the DSM-IV as 1/30000 and 1/100000, respectively, in individuals assigned male and female gender identities at birth (American Psychiatric Association 1994), this was reported in the DSM-5, respectively, as 0.005-0.014% and 0.002-0.003% (American Psychiatric Association 2013). Two meta-analyses reported in the recent years have shown the prevalence of GD as 4.6 per 100000 (Arcelus et al. 2015) and as 6.8 per 100000 (Collin et al. 2016). However, it should be kept in mind that these ratios are related to individuals consulting clinics for medical intervention and that the prevalence of GD is predicted to be higher in the general population (Ahs et al. 2018, Byne et al. 2018).

Since the determinants of gender identity and their development are not yet known exactly, it can be said that the underlying reasons for having a gender identity different from the sex assigned at birth have not be fully elucidated. Studies conducted in recent years have placed emphasis on the involvement of complex biological mechanisms in the development of gender identity (Smith et al. 2015, Zucker...
et al. 2016). One of the biological determinants of gender identity was proposed to be exposure to androgens in prenatal period (Cohen-Kettenis and Gooren 1999, Berenbaum and Beltrz 2011, Erickson-Schroth 2013). According to this assumption, the basic mechanism determining gender identity develops as a result of the direct effect of testosterone, an important androgen, on the developing human brain, and that the prenatal exposure levels the fetal brain to testosterone is the main determinant of gender identity (Swaab and García-Falgueras 2009, Savic et al. 2010, Bao and Swaab 2011). It was proposed that male gender identity develops as a result of the fetal brain’s exposure to high levels of testosterone in the prenatal period and that the female gender identity develops if the fetal brain is not exposed to the same effect (Bao and Swaab 2011, Smith et al. 2015).

In the early phases of prenatal development, testosterone is thought to play a role in the sexual differentiation of the brain with lasting effects on behaviour (Bao and Swaab 2011, Hines et al. 2015). Previous studies gave indications that exposure to high levels of testosterone in the prenatal period may affect sexual orientation (Lippa 2003, Peters et al. 2007, Grimbos et al. 2010, Hiraishi et al. 2012), gender identity (Manning 2017) and gender-specific behaviours (Hampson et al. 2008, Auyeung et al. 2009).

Studies investigating the role of prenatal exposure to testosterone in the development of gender identity and GD have focused on the complete androgen insensitivity syndrome (CAIS) (Kulshreshtha et al. 2009), and on twins with one of the pair with GD assigned the female gender at birth (AFB-GD) and the other assigned the male gender at birth (Turan and Demirel 2017). Another variable investigated in relation to the development of GD due to prenatal exposure to testosterone is the ratio of the length of the 2nd (index) finger to the length of the 4th (ring) finger (2D:4D) (Schneider et al. 2006, Wallien et al. 2008, Kraemer et al. 2009, Hisasue et al. 2012, Vujovic et al. 2014, Leinung and Wu 2017, Manning 2017, Voracek et al. 2018). Patients diagnosed with CAIS are born with normal female phenotype despite having the XY karyotype and nearly all develop heterosexual female gender identity, which was attributed to the absence of an effect of testosterone on the fetus (Kulshreshtha et al. 2009). Investigations on twins demonstrated that the fetus born with female gender whose twin had the male gender, developed ‘male’ morphological, physiological, cognitive, perceptual and behavioral characteristics (Miller 1994, van Anders et al. 2006, Voracek and Dressler 2007, Tapp et al. 2011). It was proposed that testosterone prenatally produced by the fetus born with male identity, diffusing from the fetal membranes or arriving via the mother had affected the fetus born with the female gender (Tapp et al. 2011). The reports on the role of prenatal exposure to testosterone on GD development were also supported by the identification of the 2D:4D ratio as a morphological indicant of the androgen effect (Manning et al. 1998). Low 2D:4D ratios were taken as indicative of prenatal exposure to high level of testosterone, whereas the high ratios meant that the fetus was not exposed to androgens (Manning et al. 2003, Hönkopp et al. 2007, Berenbaum et al. 2009, Hönkopp and Watson 2010, Xu and Zheng 2015).

Results of studies on rodents indicated that androgen and estrogen receptor levels in the 4th digit of the right hind paw are higher than those of the 2nd digit, and that the activity of these receptors influences the 2D:4D ratio by regulating gene expression and cell proliferation in the skeletal system (Zheng and Cohn 2011, Manning et al. 2014). It is argued that the human right hand is more sensitive to androgens in the early stages of embryological development and, in this context, the 2D:4D digit ratio in the right hand may appear more prominent (Manning et al. 1998, Hönkopp and Watson 2010, Xu and Zheng 2015).

There are in the literature a limited number of controlled studies with conflicting results on the 2D:4D ratio in individuals with GD (Leung and Wu 2017). It was found in the early studies that the right hand 2D:4D digit ratios of individuals with AMB-GD identity were higher than those of the males in the control group, similarly to individuals without GD and assigned the female gender at birth. There was not any difference between the 2D:4D digit ratios of AFB-GD individuals and the females in the control group (Schneider et al. 2006, Kraemer et al. 2009). In contrast, the results of all studies undertaken subsequently showed that the 2D:4D ratios of the AMB-GD groups did not differ from those of the males in the control group whereas the AFB-GD individuals had a lower 2D:4D ratio when compared with the females in the control group (Wallien et al. 2008, Hisasue et al. 2012, Vujovic et al. 2014, Leinung and Wu 2017).

Despite the accumulated evidence, it can be said that the role of prenatal exposure to testosterone in the development of gender identity and GD is not completely clear and that there is a need for further research on this subject. The aim of this study is to compare the data on the 2D:4D digit ratios of individuals with GD and control group participants assigned the same gender identity at birth, and to assess the differences in relation to prenatal exposure to testosterone. Given the uncertainty in the reports on the subject, it is believed that this investigation would make a contribution to the literature on the significance of prenatal exposure to testosterone and GD.

**METHOD**

**Participants**

This study initially enrolled 104 individuals consulting Cerrahpaşa Medical Faculty, Department of Mental Health and Diseases for sex reassignment, after psychiatric diagnosis with AFB-GD (n=56) and AMB-GD (n=48), on the basis of the DSM-5 diagnostic criteria, by two psychiatry
committees. The control group consisted of 116 individuals (58 AFB and 58 AMB) matched with the patients on age and gender identity at birth. The inclusion criteria of the study consisted of being ≥ 18 years of age, having been diagnosed according to the DSM-5 criteria on “GD in Adolescents and Adults”, and having signed a written informed consent form for participating in the study. The exclusion criteria included having any neurological, metabolic, endocrinological pathology and any disorder related to sexual development, having any congenital hand deformity or having had any hand injury, having a history of psychiatric disorder or cognitive inadequacy, and being illiterate. Given the arguments for a relationship between sexual orientation and the 2D:4D digit ratio (Xu and Zheng 2016), a total of five participants, two AFB-GD participants sexually attracted to males, and to both males and females, and three AMB-GD participants who were sexually attracted to females or both females and males were excluded from the study to eliminate the possible impact of sexual orientation on finger length. Hence, a total of 99 subjects with GD (54 AFB-GD, 45 AMB-GD) were included in the study. The control group consisted of medical students and hospital staff members who identified themselves as “heterosexual”, and gave the same response to the questions “What is your legal sex?” (written on the identity card, driver’s license or passport) and “Which gender do you feel you belong to?”

Measurements and Procedures

The right and left hands of the participants were photocopied with a standard photocopy machine. The participants were asked to remove their rings and gently press their palms on the glass surface of the photocopier while slightly opening their fingers. Measurements were made on the photocopied images, from the middle of the basal crease on the ventral surface of the 2nd and the 4th fingers to the tip of the fingers for evaluation. All independent measurements were made using digital calipers with 0.01 mm precision by two researchers (T.S. and Ş.T) one of whom (T.S.) was blind to the data on the sex assigned at birth and gender identity of the participants. The inter-rater reliability showed a high correlation for both hands (right hand: r=0.88, left hand: r=0.91). For further analysis, the measurements made by the two raters were averaged.

Sexual orientations of the participants were assessed by asking the following question: “How would you describe your sexual orientation?” with the response choices of, “only males are attractive to me,” “only females are attractive to me,” “both females and males are attractive to me,” “neither females nor males are attractive to me,” and “other” (Turan et al. 2018).

This study was evaluated and approved by the Ethics Committee of Cerrahpaşa Medical Faculty.

Statistical Analysis

Statistical analyses were performed using the SPSS version 22.0 statistical package program. Number, percentage, and median (min.-max.) values were used for descriptive statistics. Normal or non-normal distribution of the continual data was assessed by the Kruskal Wallis test. The Mann Whitney U test was used for pairwise comparisons of the data which were determined to have significant differences in the Kruskal Wallis test. The p value of ≤0.05 was accepted for statistical significance.

Results

The median age of the participants of the AFB-GD group, the female control group, the AMB-GD group and the male control group were determined to be, respectively, 25.0 (22.0-27.00), 23.5 (22.0-26.00), 24.0 (22.0-27.00) and 24.0 (22.0-26.00). There were not significant intergroup age differences (KW = 1.423, p = 0.700). The female controls had significantly higher 2D:4D digit ratios in both the right (p<0.001) and the left (p=0.003) hands when compared with the male controls. Significant differences were found in both the right hand (p<0.001) and the left hand (p=0.004) when the 2D:4D digit ratios of the four groups with and without GD and of male or female gender identity at birth were compared.

Comparison of Right Hand 2D:4D Ratios

The right hand 2D:4D digit ratios of the AFB-GD participants were significantly lower when compared with the female controls (p=0.028) but did not differ significantly when compared with the male controls (p=0.855). The right hand 2D:4D digit ratios of the AMB-GD participants did not differ significantly from those of the male controls (p=1.000), but when compared with the female controls, the 2D:4D digit ratios were significantly higher in the female control group (p<0.001).

Comparison of Left Hand 2D:4D Ratios

Significant difference was not found between the left hand 2D:4D digit ratios of the AFB-GD participants and the female controls (p=1.000). But, the AFB-GD participants had higher 2D:4D digit ratios on the left hand when compared with male controls (p=0.045). The left hand 2D:4D digit ratios did not differ significantly between the AMB-GD participants and the male controls (p=0.182). Also, the 2D:4D digit ratios did not differ significantly when compared between the AMB-GD participants and the female controls (p=1.000).

The distribution of 2D:4D digit ratios in the four groups is shown in Table 1 and Figure 1.
DISCUSSION

In this study, male and female individuals with and without GD and matched on the basis of age and gender identity at birth, were compared on the right and left hands with respect to the 2D:4D ratio which has been proposed to be an indicator of prenatal exposure to high level of testosterone. It was determined that the right hand 2D:4D digit ratios of the AFB-GD participants were significantly lower compared to female controls (as also seen with the participants without GD and assigned the male gender at birth), but were not significantly different when compared with male controls. On the other hand, the 2D:4D digit ratios of the AMB-GD participants did not differ significantly from the male controls on both the right and the left hand, but the 2D:4D digit ratios on the right hand, but not on the left hand, were lower when compared with the female controls.

There are disagreements in the results of a few controlled studies investigating the 2D:4D digit ratio presumed to be an indicator of prenatal exposure to testosterone (Leinung and Wu 2017). Although the pioneering investigations by Schneider et al. (2006) followed by Kraemer et al. (2009) demonstrated that the 2D:4D digit ratios in right hands of AMB-GD participants were higher than those of the male controls (as was also observed with GD participants without GD and assigned female gender identity at birth), a similar difference was not found between AFB-GD participants and female controls. However, the results of all the other subsequent investigations (Wallien et al. 2008, Hisasue et al. 2012, Vujovic et al. 2014, Leinung and Wu 2017), including the results of our study, contradicted these earlier results. The meta-analysis by Voracek et al. (2018) showed that the right hand 2D:4D digit ratios of AMB-GD individuals showed similarity to those with female gender identity at birth but without GD. The same observation was not made on the left hand. The AFB-GD individuals did not show any similarity of 2D:4D digit ratios on both the right and the left hands with those participants without GD and assigned the male gender at birth. It was argued that investigations on the 2D:4D ratio in relation to GD were too few to allow reaching a definite conclusion; and that the effect of prenatal exposure to testosterone on the 2D:4D ratio was small at best, emphasizing that the results of the analyses were affected by the large numbers of the control groups, pointing out the importance of the numbers in the control groups in the future investigations (Voracek et al. 2018).

Wallien et al. (2008) found a lower 2D:4D digit ratio in AFB-GD individuals as compared to female controls, but a significant difference was not determined between AMB-GD individuals and male controls. Similarly to our results, controlled study on AFB-GD showed that the right hand 2D:4D digit ratios were lower than in the female controls (Hisasue et al. 2012). Also, a recent study using the direct measurement method on AFB-GD individuals demonstrated that while the 2D:4D digit ratio in the dominant hand was found to be lower than in the female controls, there was not a similar difference between AMB-GD individuals and the male controls (Leinung and Wu 2017). In agreement with the results of these studies, we found that the right hand 2D:4D digit ratios of the AFB-GD participants were lower as compared to the female controls. This result suggests that the AFB-GD participants had been prenatally exposed to high levels of androgens and that they had developed morphological characteristics seen in finger length ratios matching those of individuals without GD and assigned the male gender at birth; and that, therefore, the development of male gender identity could be related prenatal exposure to testosterone. However, contrary to our expectation, it is unclear why AMB-GD participants did not show similar finger length ratios with those without GD and assigned the
female gender at birth. Breedlove (2010) explains the failure to find a difference in finger length ratios of homosexual men and control men by suggesting a “ceiling effect” of prenatal exposure of all males to androgen stimulation which should also hold for AMB-GD individuals. Another possibility is the different response or sensitivity of the brain and the peripheral skeletal system of AMB-GD individuals to androgens (Leinung and Wu 2017). In addition, it should be noted that prenatal exposure to testosterone may not be the only determinant of the development of gender identity in men, and that other factors, which have not yet been identified may also have a role.

Previous studies have shown that the right hand is more sensitive to androgens in the early stages of pregnancy in humans (Manning et al. 1998, Hönekopp and Watson 2010, Xu and Zheng 2015). Also, in the rodent model used by Zheng and Cohn (2011), the 2D:4D digit ratio in the right hind paw of mice was more sensitive to prenatal exposure to androgens than that of the left hind paw. It was determined in these experiments that although on day 12.5 of the embryonic development, when the articular cartilage tissue starts to be distinguished, the 2D:4D digit ratio did not differ between male and female mice, the development of a small but significant difference was detected especially on the hind right paw on the 17th day. These results suggest that the gender dependent difference in the 2D:4D digit ratio in mice develops over a limited interval of the embryonic period, and that the 2D:4D digit ratio of the right hind paw is at least initially more sensitive to prenatal androgens than that of the left hind paw (Zheng and Cohn 2011, Manning et al. 2014). On the other hand, measurement of human finger digit lengths by X-ray imaging in a controlled study, the AFB-GD group had the lowest left hand 2D:4D digit ratio among all compared groups (Vujovic et al. 2014). These findings suggest that both the right and left hands may be affected by androgens to some extent in the prenatal period, but this effect may be more potent on the right hand.

There are several limitations to this study. Firstly, the number of participants investigated was low. Secondly, an indirect method was used to measure finger lengths. It has been emphasized that indirect measurement is not the best method for research (Manning 2017). However, there are authors who defend the use of indirect measurement methods in research as long as different measurement methods are not used in the same study (Xu and Zheng 2015). Another limitation is that the data on gender assigned at birth, gender identity and sexual orientation were based on statements made by the participants. Individuals may experience difficulty in providing such information. Finally, gender binary was used in the study which points out that gender identities other than “male” and “female” have been ignored. In this context, it can be said that it is appropriate to investigate prenatal exposure to testosterone and other developmental factors by using methods that can evaluate gender identity dimensionally.

This study demonstrates evidence for prenatal exposure of AFB-GD individuals to testosterone. The results of measurements on the right hand point to a relationship between the development of male gender identity and the low 2D:4D ratio as the effect of prenatal exposure to testosterone. On the other hand, it can be said that there is not any evidence related to the development of female gender identity. In this context, it is necessary to investigate whether or not there is a difference in the effect of prenatal testosterone on the target organ in AMB-GD individuals.

In conclusion, these findings suggest that prenatal exposure to testosterone may not be the only determinant in the development of gender identity, but that prenatal exposure to testosterone may be associated with the development of male gender identity in AFB individuals. There is need for studies using more sensitive methods on larger experimental groups to investigate how gender identity develops and how GD occurs in relation to this.

REFERENCES


