Neurobiology of Pediatric Gender Identity

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Introduction

Sex assigned at birth is usually based on the new born's physical sex characteristics, whereas gender identity refers to the thoughts and feelings someone has of belonging to a certain gender (male, female, among other non-binary possibilities). There are two main reasons for studying the neurobiology of gender diversity in youth. First, identifying the neurobiological correlates of gender identity in relation to sex assigned at birth will inform our understanding of the development of gender diversity. Second, because youth diagnosed with gender incongruence (GI) [1], a marked incongruence between one's experienced gender and sex assigned at birth**,** may be treated with puberty-suppression and cross-sex hormones, it is important to know the effects of these interventions on the development of the brain.

In this chapter, we review the literature investigating mechanisms that may underlie gender diversity from a neurobiological perspective. We specifically focus on the genetic aspects that may contribute to gender diversity and summarize the brain imaging research that included children and adolescents with GI.

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Genetic Effects

One area of interest in examining the biological mechanisms behind gender diversity and gender incongruence is to study if genetic factors influence the development of gender role (behavior interests and traits) and gender identity (the feeling of being a boy, girl or some alternative gender).

The contribution of genetic, shared and non-shared environmental factors can be studied in monozygotic (MZ) and dizygotic (DZ) twins. These studies can give an estimate of the heritability of a certain trait by examining the concordance and discordance of this trait in monozygotic (MZ) twins (100% genetically identical) compared to dizygotic (DZ) twins (share 50% of their genes). Discordance of a certain trait in MZ twins suggests the influence of environmental factors, whereas concordance would suggest genetic influence. One study summarized case studies of twins with GI and added their own clinical series, resulting in a total of 23 MZ and 21 DZ twin pairs [2]. None of the DZ twins were concordant for GI, whereas 39% of the MZ twins were, which was deemed to show that genetic factors play a considerable role in the development of GI.

Several studies in transgender and gender diverse (TGD) individuals have investigated polymorphisms in genes, mainly genes that are involved in the biosynthesis or action of sex steroids. The findings of these studies are fairly inconsistent. In TGD males (female assigned at birth, male gender identity), polymorphisms were found in the oestrogen receptor β (ERβ) [3] and CYP-17 gene in a Spanish cohort, but Ujike et al. [4] did not observe polymorphisms in these genes in a Japanese cohort. In TGD females (male assigned at birth, female gender identity), one study observed a polymorphism of CAG repeat length [5] which was not replicated by another study [6]. Another finding in TGD females was an increased prevalence of a long CA repeat in ERβ compared to control men [7]. These findings may be inconsistent due to small and heterogeneous samples. Interestingly, a recent study examined associations and interactions of the androgen receptor (AR), oestrogen receptor α (ERα), ERβ and aromatase in a large and more homogeneous population with regard to the onset of gender dysphoria and sexual orientation [8]. In androphilic (attracted to men) TGD females with an early onset of GI, an inverse allele interaction was found between ERβ and AR. In TGD males, ERα and ERβ were involved, but no interaction between the polymorphisms was observed. They thus seem to affect the development of gender dysphoria independently. Because all TGD females in this cohort were attracted to men and all TGD males were attracted to women, findings may also be explained by sexual orientation, which warrants further study.

Gender role, gender identity and related concepts have been examined in heritability studies in twins during childhood and adolescence (for a recent overview, see [9]). Masculinity and femininity were measured by the Pre-School Activities Inventory (PSAI) in 3–4-year-old twins in two related studies [10, 11]. The PSAI measures the gender role behavior in young children. For birth-assigned boys, they found that heritability accounted for 34% of the variation in PSAI scores, for birth-assigned girls this was 57%. For the femininity score in birth-assigned boys, the

heritability was 17% and for the masculinity score in birth-assigned girls 40% [11]. Heritability was thus observed to be higher for birth-assigned girls.

A study in somewhat older (7- and 10-year-old) twins examined the heritability of gender diversity in role and identity by two items of the Child Behavior Checklist (i.e. 'behaves like opposite sex' and 'wishes to be of opposite sex') [12]. Using a composite score of both items, heritability was estimated to be 77% at age 7 and 71% at age 10. No differences were observed between the sexes. In twins with a wider age range (4–17 years old), Coolidge and colleagues [13] presented items derived from the DSM-IV criteria for then-termed gender identity disorder (GID) to their parents. The study lacked statistical power to distinguish between genetic and environmental effects [9].

A study in a large sample of Japanese twins with an even wider age range (3–27 years old) examined items that were also based on the DSM-IV criteria for GID [14, 15]. For birth-assigned males, the majority of the variance was explained by environmental factors among children, adolescents and adults. For birth-assigned females, however, the estimate for heritability was considerable for children and adolescents. It is important to be aware that the DSM-IV criteria were more focused on gender role as compared to the current DSM-5 criteria for gender dysphoria [16].

It is also of interest to mention that studies in adult twins show that gender-related measures can predominantly be explained by genetic and unique environmental effects, whereas shared environmental effects (cultural factors) are negligible [9]. Shared environmental effects refer to aspects of the home environment for which siblings are perfectly correlated. In children and adolescents, shared environmental effects seem to be more important than in adults.

Based on the data of these heritability studies, the hypothesis is formulated that gender identity is a complex trait that results from a combination of multiple genetic and environmental factors with a heritable polygenic component [9]. This means, there is not one single gene that accounts for the development of gender identity, but many genes are asserted to contribute to the trait, in addition to other factors that are non-genetic.

Early Sexual Differentiation

Another area of interest in the study of biological contributions to the development of gender identity is to examine the role of sex hormones. Animal studies have shown that sex hormones drive the sexual differentiation of the brain by affecting the organization of the brain from prenatal development onwards (organizing effects) and by the influence of circulating hormones on brain and behavior later during life (activating effects) [17, 18]. For example, exposure to high levels of testosterone during prenatal development in female animals results in more 'masculine' animal behaviour [19]. In humans, similar processes are thought to occur, but are obviously difficult to study by experimental manipulation. However, studies in people with differences of sexual development (DSD, also referred to as intersex

conditions) have increased our understanding of the effects of sex hormones on behaviour. In one study, birth-assigned females with congenital adrenal hyperplasia, a condition in which they are exposed to elevated androgen levels prenatally, prefer masculine toys and display more male-typical play behaviour [20]. Individuals with Complete Androgen Insensitivity Syndrome (CAIS), who have an XY karyotype but are insensitive to the effects of androgens, are phenotypically female with regard to behavioural and external physical characteristics.

In addition, studies in individuals with various DSDs have mainly found evidence for effects of sex hormones on gender-typical behaviour and interests (gender role), but the effects on the development of gender identity are less clear. Gender dysphoria appears to be more prevalent in these groups [21], but other factors, such as medical interventions and social context, have been suggested to play a role in this respect as well [22]. Recent studies have observed that women with CAIS predominantly show female-typical brain structure and function, but also some male-typical neural characteristics [23]. In such studies, it is difficult to disentangle the effects of exposure to hormones, chromosomes and social factors.

A series of post-mortem studies observed a sex reversal in volume and neuron number of the central portion of the bed nucleus of the stria terminalis [24, 25], the interstitial nuclei 3 and 4 of the anterior hypothalamus [26] and kisspeptin expression [27] in TGD females. These findings were critical for the formulation of a sexual differentiation hypothesis for development of a transgender identity [28, 29]. Time windows for prenatal development of genitals (first trimester) and sexual differentiation of the brain (2nd and 3rd trimester) are different. Therefore, the findings of these post-mortem studies, which were sex-atypical for their sex assigned at birth but in line with their experienced gender identity, were hypothesized to be a result of exposure to atypical levels of prenatal sex hormones during a certain gestational period [30]. This has been the key hypothesis in many of the neuroimaging studies that evaluated if brain measures in TGD individuals showed more resemblance to those of people with whom they share their gender identity or with whom they share the sex assigned at birth [18, 31]. However, the findings of the post-mortem studies were all coming from one laboratory and have not been replicated thus far, and further work is needed to determine the strength of this hypothesis.

Adolescent Brain Development

In addition to the prenatal developmental period, adolescence has been recognized as sensitive period for brain development in terms of further neural sexual differentiation [32–38], and in terms of cognitive and social–emotional maturation [39–42], which includes the process of (gender) identity formation [43, 44].

Interestingly, in the case of gender incongruence, it has been suggested that early adolescence (ages 10–13 years) forms a critical period in the development of gender dysphoric feelings. Three factors in this period may be particularly important: (1) the physical changes due to puberty, (2) the changing social environment (in which one is treated according to one's gender more explicitly) and (3) the discovery of

sexuality [45, 46]. In those adolescents in whom GI continues into adolescence, feelings of discomfort and distress around their bodies usually worsen after the onset of puberty, which often triggers symptoms of social anxiety, depression, suicidal ideation and self-harming behaviours [47–49]. For a discussion with regard to developmental trajectories of gender dysphoria, see [50–52].

Pubertal suppression with gonadotropin-releasing hormone analogues (GnRHa) can be a great relief for those with persistent feelings of GI and provides extra time to make a balanced decision on further treatment steps without the distress of puberty [53]. The effects of this treatment are reversible in the sense that if treatment is discontinued, endogenous puberty will proceed. Subsequently, adolescents often benefit from gender-affirming hormonal treatment and later gender-affirming surgery in terms of psychological well-being [54–56]. However, these interventions induce changes that are only partly reversible. Moreover, longitudinal studies describing the impact of these hormonal interventions on adolescent brain development are currently lacking.

Two large-scale functional magnetic resonance imaging (fMRI) studies including gender-incongruent youth have been conducted at the Centre of Expertise on Gender Dysphoria, Amsterdam, the Netherlands. The first fMRI study initiated in 2007 (referred to as Cohort 1) includes a total of 93 TGD and 96 age-matched cisgender (i.e. not trans, when gender identity and sex assigned at birth align) participants between 11 and 23 years of age. This study looks at brain activations during verbal-fluency and executive functions. A second 2009 (f)MRI study with prospective longitudinal design also included pre-pubertal youth. This younger, ages 7–12 years, subsample compares 43 TGD youth with 41 cisgender controls. We will discuss the findings from these cohorts in subsequent sections.

Whereas a fair amount of magnetic resonance imaging (MRI) research comparing trans- and cisgender adults has focused on structural brain differences in terms of white matter microstructure [57–59], cortical thickness [60, 61], and white and grey matter volume [62–66], only one study (of Cohort 1) thus far has described morphological brain characteristics in TGD youth [67]. TGD participants (54 trans boys, 37 trans girls), as a combined sample of different treatment conditions, on a whole-brain level, showed no signs of any sex-atypical grey matter volumes and were thus comparable with the cisgender groups of the same sex assigned at birth. However, more focused analyses in only the sexually dimorphic regions (hypothalamus, cerebellum, superior mPFC) revealed subtle sex-atypical deviations in volume.

Interestingly, more recent studies in adults suggested differences between trans- and cisgender participants in the superior medial prefrontal cortex (mPFC) region [65]. Manzouri et al. [68] found smaller mPFC cortical thickness in trans men compared to *both* cisgender males and females, and Spizziri et al. [65] reported on *sex-typical* volumes of this region in oestradiol-treated TGD women. Thus, future studies comparing larger samples of treatment-naïve individuals with GI should investigate how regional grey matter volumes differ as a function of sex and gender identity, and how structural alterations relate to differences in brain function.

One of the first studies investigating differences in *functional* brain sexual differentiation in TGD youth was conducted by Soleman et al. in Cohort 1 [69]. A verbal-fluency fMRI paradigm, for which sex differences in performance favouring birth-assigned females had been reported [70], was used to compare 8 TGD girls (male sex assigned at birth, female gender identity) and 14 TGD boys (female sex assigned at birth, male gender identity), all treatment-naïve, to 25 male and 26 female cisgender controls. Group comparisons revealed significantly stronger brain activation in cis boys than in cis girls in the right rolandic operculum, suggesting increased use of verbalization in cisgender males [71]. Interestingly, in the TGD groups, differences in activation only reached trend level (TGD girls > TGD boys), suggesting less pronounced sexual differentiation of verbal-fluency processes.

Using an innovative olfactory fMRI paradigm, including the participants of Cohort 2, sex differences in neural responses of the hypothalamus were triggered by exposure to the putative human pheromone odour *androstadienone* [72]. The older, adolescent group (receiving GnRHa) was found to respond remarkably like their experienced gender, thus sex-*a*typical. A similar finding was previously reported in adult TGD women [73], suggesting a different, even sex-reversed sexual differentiation of this specific brain function among TGD individuals. However, whereas significant sex differences in activation were observed in the cisgender pre-pubertal children, their TGD peers showed responses that were either sex-typical (TGD girls) or not distinguishable from either of the two control groups (TGD boys).

In the most recent fMRI study, also including the participants of Cohort 2, Nota et al. [74] investigated sex assigned at birth (male versus female) and gender identity (cis- versus transgender) differences in resting state functional brain connectivity, that is, temporal correlations of fluctuations of the blood-oxygen-level-dependent (BOLD) signal among spatially distributed brain regions [71, 72]. Such intrinsic connectivity networks at rest have been found to be associated with behaviour and to represent distinct functional systems [75]. For example, the widely studied *Default Mode Network* (DMN) has been found to be implicated in mind-wandering and self-referential thinking [76–78]. A different processing of self has only recently been suggested as an alternative neurobiological explanation of GI [68, 79–81], and sex differences in functional connectivity networks have been reported [82, 83].

A comparison of the adolescent sub-samples revealed cisgender sex differences in functional connectivity within the sensory motor network (SMN) and the DMN, with sex-atypical (i.e. in line with their gender identity) functional connectivity patterns in these networks in both TGD girls (DMN and SMN) and boys (SMN). Of note, the adolescent TGD girls also showed a singular pattern of activation with stronger intrinsic connectivity within the visual network, compared to the three other groups (TGD boys, cisgender girls, cisgender boys). This finding was interpreted as a first hint towards transgender-specific alterations of brain functions, thus beyond the hypothesized difference from the cisgender sexual dimorphism. Furthermore, in the young sub-sample no significant group differences were found.

These age-specific findings in two different fMRI paradigms (olfactory and resting state) allow for alternative possible explanations. First, in line with the notion that the period of early adolescence seems decisive with regard to gender identity

development [45, 46], it may be that (neuro-)developmental processes (including further neural sexual differentiation) during early adolescence are needed for the sex-*a*typical brain activation pattern to be expressed or activated. Second, it is possible that the sex-*a*typical responses in TGD adolescents might be related to their puberty-suppression treatment [84]. However, given that in another study (involving participants of Cohort 1), those receiving GnRHa showed even exaggerated sex-typical brain activations [85], this possibility does not seem very likely. A third explanation may be that the younger, pre-pubertal sample was more pluripotent with regard to future developmental trajectories of their gender identity. The few longitudinal studies that allow estimations on the development of GI found that the majority of children with GI do not continue to experience GI and start puberty suppression in adolescence [86–88]. Future (follow-up) studies should therefore aim to address the unanswered question of what neurobiological mechanisms characterize and explain childhood GI.

Effects of Hormone Treatment

It has been suggested that puberty suppression could interfere with significant developmental brain changes, particularly within the prefrontal cortex, which underlie adolescence-specific changes in behaviour [89]. Examining participants from Cohort 1, investigators assessed the effects of GnRHa on executive function. This study compared TGD youth, receiving GnRHa treatment, with treatment-naïve TGD adolescents [85], and cisgender groups. Participants performed an fMRI version of the Tower of London task, indexing executive functioning. No differences in performance were found between treated and treatment-naïve TGD groups. Whereas the treatment-naïve TGD adolescents showed *sex-atypical* activations, the GnRHa-treated groups showed *sex-typical* patterns of task-related brain activation (in precuneus, dorso-lateral PFC). Though surprising, this finding of, even exaggerated, sex-typical brain functions is in line with a previous study in GnRHa-treated sheep [90].

In a recent case study of an 11-year-old trans girl (male birth-assigned sex), cognitive functions and changes in diffusion measures of white matter microstructure (indexing myelination, axonal diameter and white matter integrity, which all contribute to efficient information transfer) were assessed before initiation of GnRHa treatment and during two follow-up measurements at 13 and 14 years of age, respectively [91]. Performance intelligence quotient and memory deteriorated with treatment, and normative, testosterone-dependent white matter maturation (increase in diffusion parameters) was not observed. This study, although only for a single case, thus suggested long-term adverse effects of GnRHa on neurodevelopment and cognitive capacities. However, these findings require replication in larger samples and warrant special attention in upcoming studies.

Even though neuroimaging studies in TGD individuals have started to accumulate evidence on the effects of cross-sex hormonal treatments on brain functions and structure, knowledge on the effects of these treatments remains limited, especially in

TGD youth. In their study on regional brain volume differences between trans- and cisgender youth (Cohort 1), Hoekzema et al. [67] found that hypothalamus volumes in a mixed sample of treatment-naïve and hormone-treated TGD girls (N = 12 received cross-sex hormones) tended to be sex-*a*typical, thus smaller than in cisgender boys. This finding was in line with a prior study in TGD women, which showed a significant decrease in hypothalamus volume after having received oestradiol and anti-androgen treatment for 3 months [92]. Similarly, another study in TGD women reported on a general decrease of cortical thickness and subcortical volumes with oestradiol plus anti-androgen treatment [60]. Of note, given that the TGD female participants in all three studies had received both anti-androgen *and* oestradiol treatment, it is impossible to discern whether the observed effects on brain structure can be ascribed to the suppression of endogenous hormone production or to the addition of oestradiol. These studies, however, have major limitations of small sample sizes and cross-sectional study design. Furthermore, the clinical significance of these volumetric differences is unclear. Future research is needed in this regard.

Thus far, only one *longitudinal* study investigated cross-sex hormone treatment effects on spatial cognition, measured with an fMRI mental rotation task, in gender-incongruent youth (adolescent sub-sample of Cohort 2) [93]. Previously reported robust sex differences in performance (accuracy, reaction times) favouring birth-assigned males [94, 95] and sex differences in brain activation [96, 97] suggested that birth-assigned males and females use different strategies during mental rotation. Accordingly, cisgender girls were found to show stronger activations in the precuneus and precentral gyrus compared to cisgender boys. Interestingly, TGD boys differed significantly from the cisgender girls, but showed activation patterns comparable to those of the cisgender boys, thus suggesting sex-*a*typical spatial functioning. During a follow-up, 10 months after the TGD boys had initiated testosterone treatment, they showed significantly increased activation during mental rotation (in superior parietal, superior frontal cortex) relative to the previous session (under GnRHa), similar to the cisgender boys, and significantly different from the cisgender girls (who showed no changes between sessions). These findings in youth are generally in line with two fMRI studies in adult TGD men. Sommer et al. [98] found a trend for testosterone-related increases in brain activations during mental rotation, and Burke et al. [81] reported on significant changes in cortical thickness and functional connectivity that were associated with treatment-related changes in self- and own-body perception.

Gaps of Knowledge and Future Directions

First, with regard to the influence of genetic factors in the development of gender incongruence, the hypothesis that gender identity is a complex trait that results from a combination of multiple genetic and environmental factors with a heritable polygenic component [9] deserves further study. An interesting observation was that twin studies on gender role behaviour [11] and GI [15] suggest higher heritability estimates for birth-assigned girls than for birth-assigned boys. On the other hand, a study investigating click-evoked otoacoustic emissions as a retrospective marker of

perinatal androgen exposure found sex-typical emission strengths in birth-assigned girls with GI (7–16 years old), but sex-*a*typical in birth-assigned boys with GI (6–14 years old) [99]. These seemingly opposing findings may suggest that genetic factors and early hormonal influences weigh in differently for the two sexes in their contribution of the development of gender identity, gender role behaviour and also GI. Further work is needed to better understand whether and how the developmental trajectories of gender identity are different between TGD boys and girls.

We have seen that the studies in TGD adolescents show indications of sex-*a*typical (with regard to sex assigned at birth) patterns in several neuroimaging measures and demonstrate neurobiological patterns that are more in line with their identified TGD identity. However, for pre-pubertal TGD children this picture is less clear.

Neuroimaging studies in TGD adults point to a difference in neurobiological correlates as a function of sexual orientation (e.g. gynephilic versus androphilic trans women) [18] and for sexual orientation as a contributor to the sex-atypical findings in TGD individuals [100]. In the two large MRI projects in youth with GI thus far, the TGD groups differed from the cisgender comparison group (of the same at birth-assigned sex) not only in terms of gender identity but also in terms of sexual orientation. Given this limitation of the study design, it is possible that some of the differences in MRI findings were related to sexual orientation rather than gender identity. Future studies should therefore include control groups with various sexual orientations to clarify this aspect.

Recent neuroimaging findings provide tentative evidence for different own-body perception and altered underlying neural networks as alternative explanation for the body dysphoria experienced by TGD individuals [68, 79–81, 100, 101]. However, all these studies thus far included adult participants, and no study has examined the 'own-body perception hypothesis' in pre-pubertal and/or post-pubertal TGD youth. Given that the distress resulting from physical changes due to puberty was indicated as one important factor for future continuation of GI [46], it may be worthwhile for future studies to investigate the underlying mechanisms of a different body image development *in interaction* with less pronounced brain sexual differentiation in TGD adolescents.

Although the studies in TGD children and adolescents thus far have revealed some interesting findings, they all originate from one research centre and replication studies from other centres are urgently needed. Also, knowledge on the long-term effects of GnRHa treatment and cross-sex hormones on the brain is very limited, and systematic studies in larger, representative samples are currently lacking, partly due to the relatively recent introduction of these interventions in TGD adolescents.

Despite evidence of improvement in anxiety, depression and self-harm among TGD adolescents who receive these treatments [102, 103], the long-term effects of, in particular, GnRHa treatment in physically healthy young adolescents are unclear. Longitudinal evidence does exist, but only from studies in girls with idiopathic central precocious puberty, studies in healthy adult cisgender women and studies in animal models (sheep). Some of these studies have suggested adverse effects of GnRHa treatment on general intelligence [104, 105], information processing speed [106], emotion regulation [90, 107], reward-processing and associated amygdala

reactivity [108], spatial memory performance in sheep [109, 110], sex-specific (stronger in female sheep) effects on structural brain development [111] and regulation of gene expression in the sheep amygdala [112]. However, others found no differences between GnRHa-treated and treatment-naïve comparison groups for measures of spatial orientation [90], cognition and psychosocial functioning [107], but see [105] for a critical commentary.

Importantly, it should be acknowledged that withholding GnRHa treatment would be harmful to the psychological developmental of TGD youth, for example, due to the risk of stigmatization and living with prolonged distress [53]. Because such studies in TGD youth are still lacking, it is therefore critical to continue the study of the effects of puberty suppression in TGD youth on brain development, and to examine the effects on TGD adolescents' cognitive and socio-emotional functioning. Follow-up studies in the two cohorts that have been studied thus far during adulthood are highly recommended.

One should be aware that neuroimaging findings may reflect mechanisms that causally relate to gender incongruence, but may also be a result of the experience of living with an identity that is not typically congruent with their assigned sex at birth. In addition, studies thus far have investigated neurobiological correlates and descriptions of TGD anatomy and brain function on a group level. Therefore, MRI measures are not an appropriate tool to use diagnostically to, for example, confirm or exclude gender incongruence in a specific individual. Nevertheless, MRI has been proven to be informative to clinical and academic communities alike with regard to the existence and the aetiological factors underlying gender identity and -diversity. We are far from revealing a single neurobiological pattern that fully describes TGD neurobiology. Based on the current state of knowledge, it is more conceivable that there is no single distinctive neurobiological signature for gender incongruence at all. It remains an empirical question to what degree gender identity development is 'preprogrammed' (genetic, early hormonal influences) and what the relative contribution of environmental factors is. For understanding how to better serve our TGD youth, we should continue the search for neurobiological correlates of gender identity and gender diversity, as this will ultimately help to better characterize the cognitive and emotional needs of TGD youth and provide them with the best individualized-care approach.

Editorial Disclaimer This chapter describes a summary of the literature in this area by experts in the field, and conclusions drawn reflect the views of the authors, not the editors.

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