Chapter 2
Genetic Aspects of Gender Identity Development and Gender Dysphoria

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Abstract The development of gender identity, its variance, and gender dysphoria is thought to be a complex process involving biological and psychosocial factors. Heritability studies have demonstrated a genetic factor for the development of gender dysphoria. The brain is regarded as the anatomical substrate of gender identity, and sex differences of the brain are studied to elucidate the process of gender identity development. Many sex differences have been attributed to hormonal action, and the first genetic studies in transsexuals were focused on sex-steroid-related genes. To this day, a convincing candidate gene has not been identified, and it is now known that sex chromosomes have a direct effect on sex differentiation and that they may play a role in gender identity development. For future studies of the genetic base of gender dysphoria, new techniques, such as genome-wide studies, have become available. In addition, epigenetic studies may provide for a different association perspective of the genetics of gender dysphoria.

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2.1 Introduction

Gender identity development is a complex developmental process that is thought to be influenced by biological factors (such as hormonal and genetic factors) and psychosocial factors which also interact with each other. Gender identity is one of the most remarkable sex differences in humans. The brain is regarded as the anatomical substrate of gender identity, and numerous studies investigating gender identity development and variations in development, i.e., gender dysphoria, use sex differences of the brain as a proxy for gender identity. In Chap. 1 the role of (gonadal) hormones is addressed in mostly animal models, using behavioral outcome measures to evaluate sex differentiation in either feminine or masculine directions. In Chap. 3 the psychological factors that contribute to gender identity development are presented. In this chapter the contribution of genetics to gender identity and gender dysphoria is explored with a focus on the brain and its sex differences.

Sex differences in the human brain are prominent and have important implications for each sex, as they uniquely affect biochemical processes, may contribute to the susceptibility to specific diseases, and may influence specific behavior. These differences manifest themselves in a variety of ways, both structural and functional. In human brains there are region-specific dimorphisms with some structures larger in females (caudate nucleus, hippocampus, Broca's area, anterior commissure, and right parietal lobe) and some larger in males (hypothalamus, stria terminalis, and amygdala) (Goldstein et al., 2001). In addition to neuroanatomical differences, males and females exhibit different patterns in neurochemistry, the transmission, regulation, and processing of biomolecules involved in neurosignaling (Ngun, Ghahramani, Sanchez, Bocklandt, & Vilain, 2011). For example, men are more sensitive to cholinergic stimulation of the hypothalamic–pituitary–adrenal cortisol axis than women (Rubin, Sekula, O'Toole, Rhodes, & Czambel, 1999). Besides biological differences, men and women differ in many psychological and behavioral aspects; men perform better on specific visuospatial tasks (e.g., mental rotation) (Voyer, Voyer, & Bryden, 1995) and mathematical problem solving (Benbow, Lubinski, Shea, & Eftekhari-Sanjani, 2000). Women do better on verbal fluency, articulation, and verbal memory tests (Becker, 2008).

Of interest with respect to gender identity and gender dysphoria are the findings that male-to-female (MtF) transsexuals have some sex-specific brain characteristics more similar to females than males. Two sex-dimorphic nuclei, the central portion of the bed nucleus of the stria terminalis (BSTc) and the interstitial nucleus of the anterior hypothalamus 3 (INAH3), located in the hypothalamus, appear to be female sized and thus smaller when compared to men (Garcia-Falgueras & Swaab, 2008; Zhou, Hofman, Gooren, & Swaab, 1995). However, it is still unclear what their role is in the development of gender identity. The MtF subjects in which the BSTc was studied received estrogens prior to their demise, and thus a hormonally mediated effect could not be excluded (Zhou et al., 1995). On the other hand, in terms of INAH3, it was postulated that since the INAH3 did not differ between premenopausal and postmenopausal women, the feminization of the INAH3 in MtF

transsexuals was not due to estrogen treatment. It was implied that the INAH3 is at least partly a marker of an early atypical sexual differentiation of the brain and that the changes in INAH3 and the BSTc may belong to a complex network that may structurally and functionally be related to gender identity (Garcia-Falgueras & Swaab, 2008; Zhou et al., 1995).

These two reports illustrate the complexity of sex differentiation investigation and its potential pitfalls. Sex differences are well described, but the underlying mechanisms of development are not explained. As reviewed in Chap. 1, the role of (gonadal) hormones has been extensively researched in (mostly) animal models, paving a path for further exploration of hormonal influences on sex differentiation in humans. In this respect, the candidate genes pursued in past genetic studies of transsexuals are involved in sex steroid synthesis or action. In addition to hormonally mediated effects, more data nowadays have emerged of effects on sex differentiation that are not due to hormonal action but arise from expression of X and Y genes within non-gonadal cells and result in sex differences in the functioning of those cells (Ngun et al., 2011).

In this chapter we will first review the studies with transsexual populations, in search of genetic factors. The first approach is to establish whether heritable factors play a role in gender dysphoria development in heritability studies. The next step is to identify those factors found in studies exploring the association of gender dysphoria and sex-steroid-hormone-related genes. Subsequently, we will address direct (i.e., not hormonally mediated) genetic effects on sex differentiation and so present a novel concept of sex differentiation, as proposed by Arnold (2009b). Finally, we will discuss future directions of genetic research of gender dysphoria. New techniques have become available such as genome-wide association studies, which may have a place in the quest for the genetic base of gender identity development and gender dysphoria. In addition, the emerging field of epigenetics—the study of stable alterations in gene expression potential that arise during development, differentiation, and under the influence of the environment—may offer new insights as epigenetic mechanisms may be mediators between sex and its differentiation and genetic base.

2.2 Heritability Studies of Gender Dysphoria

The first step in studying genetic factors in gender dysphoria is to investigate the extent to which gender dysphoria can be attributed to genetic factors or to environmental factors (including hormonal levels during early development and psychosocial factors). Heritability studies provide answers to the question of whether a certain biological or psychological trait is heritable. Twin studies are a good example of this kind of design. If a certain trait is more concordant in monozygotic twins compared to dizygotic twins, this strongly indicates that such a trait is heritable. The measure of heritability is expressed as the part of the variance that is explained by shared genes relative to the total variation and varies from 0 to 1, where 0 means that there is no genetic explanation for a trait and 1 that it is completely determined by

genetic factors. Although this heritability index could also be assessed from family studies, most of the studies regarding gender dysphoria are done in twins.

The first heritability study is the one by Lippa and Hershberger (1999). They analyzed data from Loehlin and Nichols' (1976) classic twin study that recruited 839 same-sex pairs of twins who were high school juniors (ages 16–17) from the USA. They used several scales of masculinity–femininity including "gender diagnosticity," which refers to the Bayesian probability that an individual is predicted to be masculine or feminine based on gender-related interests. The estimated within-sex heritability of gender diagnosticity was 0.53, which indicates a moderate to high measure of heritability. Later, Loehlin and colleagues applied the same method to a larger cohort of five studies and found that gender diagnosticity is moderately heritable (somewhere between 25 and 47 % of the total variance) (Loehlin et al., 2005).

In 2000, Bailey and colleagues reported a heritability pattern for childhood gender nonconformity in 1891 adult twins. They found heritability estimates of 0.50–0.57 in men and 0.37–0.40 in women. This study focused on childhood gender nonconformity as a heritable component of adult sexual orientation and was performed in an adult population (median age 29 years) which can involve a considerable recall bias (Bailey, Dunne, & Martin, 2000).

In 2002, Coolidge and colleagues published a study of 314 twins (96 monozygotic and 61 dizygotic twins; mean ages 4–17 years) on the heritability of gender identity disorder. Gender identity disorder (GID) was assessed by a 6-item GID scale, based on DSM-IV criteria for GID and ranging from 4 to 24. A score of 15 or higher indicates a high likelihood for a clinical diagnosis of GID. The prevalence of clinically significant GID symptomatology in the twin sample was estimated to be 2.3 %. The model that best described the data included a significant additive genetic component accounting for 62 % of the variance, suggesting that there is a strong heritable component to GID. The effects were stronger in the older (11–17) than in the younger group (4–10). In this study the scoring of GID symptoms was done by the mother and thus might have some bias based on the type of twins (Coolidge, Thede, & Young, 2002).

In 2005, Knafo and colleagues reported a study in 5,799 twin pairs, between ages 3 and 4, for which parents rated their twin children's masculinity and femininity. Boys were selected as gender atypical if they were highly feminine relative to other boys, and girls were selected if they were highly masculine relative to other girls. Among fully gender atypical children (e.g., feminine boys also low on masculinity), heritability estimates were 0.21 in boys and 0.74 in girls (Knafo, Iervolino, & Plomin, 2005).

Van Beijsterveldt, Hudziak, and Boomsma (2006) studied the genetic and environmental influences on cross-gender behavior in twins at the age of 7 years ($n \sim 14{,}000$ twins) and 10 years old ($n \sim 8{,}500$ twins). The behavior was assessed by maternal report that the child behaves like or wishes to be the opposite sex. The prevalence was 3.2 % and 5.2 % for 7-year-old boys and girls, respectively, and decreased to 2.4 % and 3.3 % for 10-year-old boys and girls. Heritability estimates were 0.77 at age 7 and 0.71 at age 10 (Van Beijsterveldt et al., 2006).

Burri, Cherkas, Spector, and Rahman (2011) published a study in 4,426 British female twins focusing on sexual orientation, childhood gender typicality, and adult gender identity. The scale for adult gender identity was based on items such as "I don't feel very masculine" and "I pride myself on being feminine." Heritability for this scale was only 0.11 indicating a low influence of genes, which the authors attributed to a substantial measurement error (Burri et al., 2011).

Most studies have reported a considerable heritability of gender-related traits within sexes. However, a key question is whether these estimates provide an accurate impression of the heritability of gender dysphoria. Most of the studies focused on masculinity and femininity traits and do not include criteria for dysphoria. Only Coolidge's study used a questionnaire based on DSM-IV criteria for gender identity disorder (Coolidge et al., 2002). Van Beijsterveldt's study was based on two items of which one refers to "behaves like opposite sex" and the other "wishes to be of opposite sex" (Van Beijsterveldt et al., 2006). Another point of concern is that some studies were conducted with very young children, and it is not clear whether gender nonconforming behavior at very young ages corresponds to gender dysphoria during adolescence or adulthood.

Recently one paper reported data on twins of which one was diagnosed with gender identity disorder (Heylens et al., 2012). Of the 23 monozygotic female and male twins, 9 (39.1 %) were concordant for GID; in contrast, none of the 21 same-sex dizygotic female and male twins were concordant for GID. Although the sample size was rather low, this indicates a role for genetic factors in the development of GID.

2.3 Candidate Genes Involved in Gender Identity Development and Gender Dysphoria

A small number of candidate genes have been studied for gender dysphoria. Since conceptual frameworks determine experimental designs, the predominance of the classical theory of brain and behavioral sexual differentiation (see Chap. 1) presumably has led to investigations of genes that are involved in the biosynthesis of sex steroids or mechanisms of action. Assuming a pivotal role for sex hormones on sex differentiation of the brain and thus gender identity development and its variations, alterations in hormone levels or activity may contribute to the development of gender dysphoria. Therefore, the reported studies were aimed to identify differences in genes between transsexuals and the reference population. In this section we review these investigations. First, the production of steroid hormones and their physiological mode of action are briefly reviewed. Second, we discuss the genetic base of enzymes in these processes and how individuals are affected by either faults (i.e., mutations) or variants (i.e., polymorphisms). Finally, the outcome of association studies of sex-steroid-related genes in transsexuals is discussed.

2.3.1 Sex Steroid Biosynthesis and Mechanisms of Action

Steroidogenesis, the production of steroid hormones, mainly takes place in the gonad and adrenal gland. Cholesterol is the common precursor and is stepwise converted into biologically active steroid hormones. Several enzymes are needed to catalyze each step of the steroidogenesis. First, cholesterol is converted into pregnenolone (Miller & Auchus, 2011). Pregnenolone subsequently can be further used by $P450c_{17}$ (steroid 17-alpha-hydroxylase). This enzyme mediates both 17-alpha-hydroxylase and 17,20-lyase activity and allows the adrenal glands and gonads to synthesize both 17-alpha-hydroxylated glucocorticoids (via 17-alpha-hydroxylase activity) and sex steroids (via 17,20-lyase activity) (Chung et al., 1987; Kagimoto, Winter, Kagimoto, Simpson, & Waterman, 1988; Van Den Akker et al., 2002). In the latter, DHEA is produced. Like many other steroidogenic enzymes, steroid 17-alpha-hydroxylase is a form of cytochrome P450 (Miller, 2005). DHEA can be converted into androgens, namely, androstenedione and—via androstenediol—testosterone. The actions of androgens are mediated by the androgen receptor. This ligand-dependent transcription factor belongs to the superfamily of nuclear receptors. All members of this family share similar characteristics; these include a separate hormone-binding domain, a high-affinity DNA-binding domain, a tendency to form dimers (a structure built out of two equal molecules), and an enhanced affinity for the cell nucleus in the presence of bound hormones. Only one androgen receptor has been identified and cloned, despite the two different ligands, testosterone and dihydrotestosterone (Miller, 2005; Trapman et al., 1988). The irreversible conversion of testosterone into the more potent androgen, dihydrotestosterone, is catalyzed by 5-alpha-reductase type II (SRD5a2). The receptor–ligand complex translocates into the nucleus, where it transactivates genes with androgen-responsive elements resulting in the development of the male external genitalia, urethra, and prostate during embryogenesis. In addition, it is responsible for most androgen-mediated events of male sexual maturation at puberty (Wilson, Griffin, & Russell, 1993).

On the other hand, cP450arom (aromatase) is the enzyme that catalyzes the synthesis of estrogens from androgens. Therefore, the activity of this enzyme complex affects both androgen metabolism and estrogen synthesis. The biological importance of the aromatase complex is related not only to its role in the synthesis of estrogens but also to its potential influence on the balance of the androgen–estrogen ratio in different tissues (Belgorosky, Guercio, Pepe, Saraco, & Rivarola, 2009). Estrogen effects are exerted by the estrogen receptors (ERs), members of the steroid receptor family. To date, two ER subtypes have been cloned, called ERα and ERβ (Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996). Progesterone can be directly derived from pregnenolone and is produced by the corpus luteum, a temporary endocrine structure in women involved in the menstrual cycle and pregnancy. It plays a central role in the reproductive events associated with the establishment and maintenance of pregnancy. The progesterone receptor (PGR), a member of the steroid receptor superfamily, mediates the physiologic effects of progesterone (Akison & Robker, 2012).

2.3.2 Disorders and Polymorphisms of Steroidogenic Enzymes and Steroid Receptors

The information to synthesize steroidogenic enzymes and steroid receptors is stored in genes. Genes are made from a long molecule called DNA, which is copied and inherited across generations. The information is encoded in the DNA sequence (genetic code), which allows the genetic machinery to read the information in the genes in triplet sets of codons. The DNA sequence of a given gene consists of parts that are translated, i.e., used to construct the gene product, called exons. In addition, between exons are parts that are not translated, the so-called introns, which may have regulatory functions. Each unique form of a single gene is called an allele. Mutations are random changes in genes and can create new alleles. Mutations can produce new traits but also can cause disease when it leads to dysfunction of the encoded product. In addition, there are natural variations in a gene, DNA sequence, or chromosome, called polymorphisms, which have no adverse effects on the individual and occur with fairly high frequency in the general population. The most common type of polymorphism involves variation at a single base pair which leads to a single-nucleotide polymorphism, or SNP (pronounced "snip"). Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, an SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. SNPs occur normally throughout a person's DNA. They occur once in every 300 nucleotides on average, which means there are roughly ten million SNPs in the human genome. Most commonly, these variations are found in the DNA between genes. They can act as biological markers to locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function. Most SNPs have no effect on health or development. Some of these genetic differences, however, have proven to be very important in the study of human health. SNPs may help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing particular diseases, such as heart disease, diabetes, and cancer. Polymorphisms can also be much larger in size and involve long stretches of DNA (Cargill et al., 1999).

2.3.2.1 Steroidogenic Enzymes: Disorders and Polymorphisms Studied in the Transsexual Population

Faults, i.e., mutations in sex-steroid-related genes can lead to disorders of sex development (DSD). Herein, the sex differentiation of the genitals, both external and internal, may become ambiguous. Polymorphisms have a less profound effect. In this and the next paragraph, the genes which were studied in the transsexual population are reviewed. We use the term "transsexual" to refer to persons with extreme gender dysphoria who pursue sex reassignment, thus not to all people with gender dysphoria per se. The normal gene structure is discussed per gene first and then

followed by mutations causing DSD. Finally, we review the polymorphisms studied and how they may relate to transsexualism.

$Pc450_{17}$

The $Pc450_{17}$ (CYP17) gene was mapped to chromosome 10q24.3 (Fan et al., 1992). Mutations causing complete combined 17-alpha-hydroxylase and 17,20-lyase deficiency led to incapacity to produce androgens and thus result in 46XY individuals with female external genitalia (Auchus & Miller, 2012). An SNP has been identified (CYP17A2T>C SNP) (Carey et al., 1994) and is associated with elevated plasma levels of estradiol and progesterone (Feigelson et al., 1998). Estradiol and progesterone have been shown in rodents to play a role in sex differentiation of the brain (Menger, Bettscheider, Murgatroyd, & Spengler, 2010), and it is therefore of interest to study this gene in both FtM and MtF transsexuals.

5-Alpha Reductase (SRD5*a2)*

The SRD5a2 gene maps to chromosome 2p23 and spans over 40 kb of genomic DNA, containing 5 exons and 4 introns, and encodes a 254 amino acid protein (Labrie et al., 1992). Mutations in the gene causing deficiency will lead to hypovirilization in 46XY individuals (Andersson, Berman, Jenkins, & Russell, 1991). Several polymorphisms are known and have been associated with medical conditions such as polycystic ovaries (Graupp, Wehr, Schweighofer, Pieber, & Obermayer-Pietsch, 2011) and prostate cancer (Li, Coates, Gwinn, & Khoury, 2010). The SRD5A2 V89L SNP in codon 89 of exon 1 causes a replacement of the amino acid valine with leucine and has been shown to result in almost 30 % reduced enzyme activity and thus lower concentration of testosterone metabolites (Makridakis et al., 1997). It was found to be overrepresented when hypospadia cases were compared to normal controls (Thai et al., 2005). Therefore, this polymorphism may be associated with hypovirilization of other organs, i.e., the brain and thus with gender dysphoria.

Aromatase (cP450arom)

In humans, cP450arom is the product of a single gene—*CYP19*—located on chromosome 15q21.1. The protein-coding sequence is contained within nine exons (2–10), in which translation, initiation, and termination codons are present, and spans approximately 35 kb of DNA. There are multiple first exons that are not translated but are involved in tissue-specific expression. Several promoters are found within a 90-kb region upstream of the coding region (Means et al., 1989). cP450arom is expressed in a number of tissues, including parts of the placenta, gonads, and brain (including the hypothalamus, hippocampus, and amygdala), and is subjected to multifactor regulation by a diverse group of hormones and factors that differ markedly between tissues (Belgorosky et al., 2009).

Clinical presentation of aromatase deficiency is different in 46XX and 46XY individuals. Males (46XY) typically present after puberty with prolonged linear growth and tall stature, reduced bone mineralization, impaired fertility, insulin insensitivity, and dyslipidemia. Females (46XX) usually present at birth with ambiguous genitalia. In normal conditions aromatase protects the female fetus from adrenal-derived androgens. When aromatase is deficient, the fetus will be exposed to increased levels of androgens, resulting in virilization of the external genitalia. In one case, a 46XX individual was raised as a male and reported male gender identity, role, and orientation. The patient had spontaneous breast development that progressed to Tanner stage 4 at 14 years of age. A salpingo-oophorectomy and hysterectomy was performed (Lin et al., 2007). However, aromatase deficiency is rare, with fewer than 20 patients reported in the literature (Belgorosky et al., 2009).

A more common polymorphism was demonstrated in the fourth intron consisting of a (TTTA)n repeat (Polymeropoulos, Xiao, Rath, & Merril, 1991) and has been reported in association with variations in bone mineral density and fracture risk in both sexes (Masi et al., 2001; Van Pottelbergh, Goemaere, & Kaufman, 2003), breast (Dunning et al., 1999) and endometrial cancer (Berstein et al., 2001), and serum levels of sex steroids (Haiman et al., 2000). Also several SNPs were reported to be involved in hyperandrogenism in younger females (Petry et al., 2005). This suggests an important modulatory role for this enzyme in endocrine and metabolic function within the wider population. Aromatase is expressed in the brain of the human fetus (Montelli et al., 2012) and plays, as described in Chap. 1, a critical role in the differentiation of the developing brain in rodents (Roselli, Abdelgadir, & Resko, 1997). Therefore, CYP19 was an attractive candidate gene to study in the transsexual population.

2.3.2.2 Steroid Receptors: Disorders and Polymorphisms Studied in the Transsexual Population

Androgen Receptor (AR)

The AR gene is located on the X chromosome at Xq11. The gene consists of eight coding exons, and the structural organization is essentially identical to those of the genes coding for the other steroid hormone receptors (Brinkmann et al., 1989). A polyglutamine stretch, encoded by a polymorphic (CAG)*n*CAA repeat, is present in the NH_2-terminal domain (Sleddens, Oostra, Brinkmann, & Trapman, 1992). Variation in length (9–33 glutamine residues) is observed in the normal population. This polymorphism has functional significance, whereby the length of the CAG repeat is inversely proportional to the transcriptional activity of the androgen receptor (Palazzolo et al., 2008; Simanainen et al., 2011).

Androgen insensitivity syndrome is a disorder resulting from complete or partial resistance to the biological actions of androgens due to defective androgen receptor function. In complete androgen insensitivity syndrome, all tissues, including the brain, are unresponsive to testosterone, and the XY individual has a female phenotype and female gender identity. The clinical presentation of partial androgen

insensitivity syndrome however depends on the degree of responsiveness of the external genitalia to androgens. The number of CAG repeats can have modulatory effects on the phenotypic manifestation of some mutations of the androgen receptor gene noted in partial androgen insensitivity syndrome, particularly when the phenotype varies with the same mutation (Palazzolo et al., 2008; Werner et al., 2006). In addition, it has been associated with several androgen-related disorders such as polycystic ovary syndrome (Lin, Baracat, Maciel, Soares, & Baracat, 2013), male infertility (Davis-Dao, Tuazon, Sokol, & Cortessis, 2007), prostate cancer (Gu, Dong, Zhang, & Niu, 2012), and even with growth during childhood in boys (Voorhoeve, van Mechelen, Uitterlinden, Delemarre-van de Waal, & Lamberts, 2011). Increased androgen insensitivity of the brain may play a part in the development of gender dysphoria, and therefore variation in length of the (CAG)*n*CAA repeat was investigated in transsexuals.

Estrogen Receptor (ER)

The two subtypes of ER are highly homologous in DNA and ligand-binding domains, but overall homology is only 55 %. Both subtypes are widely distributed throughout the body, each with distinct tissue and cell patterns of expression. The ERα mRNA expression appears to dominate in the hypothalamus and amygdala, indicating that the alpha-subtype might modulate neuronal cell populations involved in autonomic and reproductive neuroendocrine functions, as well as emotional interpretation and processing. In contrast, the hippocampal formation, entorhinal cortex, and thalamus appear to be ERβ-dominant areas, suggesting a putative role for ERβ in cognition, non-emotional memory, and motor functions (Osterlund, Gustafsson, Keller, & Hurd, 2000). ERα plays a critical role in regulating reproductive neuroendocrine behavior and function. ERβ appears to play an important role in nonreproductive behaviors, such as learning and memory, anxiety, and mood (Ter Horst, 2010).

ESR1 is the gene that codes for the ERα protein and is located on chromosome 6 (6q25.1). Transcription is regulated by multiple promoters that give rise to mRNA variants with distinct 5′-untranslated regions. These promoters are utilized in a tissue-specific manner resulting in different levels of expression of mRNA variants in individual tissues (Kos, Reid, Denger, & Gannon, 2001). The polymorphic $-1174(TA)_n$ repeat in promoter region of the *ESR1* gene has been associated with somatic conditions such as coronary heart disease, bone mineral density, familial premature ovarian failure, and endometriosis (Ascenzi, Bocedi, & Marino, 2006). The functional importance of these repeat nucleotide sequences has not been clarified although reports have suggested that these sequences, even when situated in the non-translated regions, may influence the expression of a gene (Zhang & Yu, 2007). In addition, this polymorphism has been associated with anxiety, neuroticism, psychoticism, antisocial behavior, and conduct disorder in humans (reviews in Sundermann, Maki, & Bishop, 2010; Westberg & Eriksson, 2008). ESR2 is located on chromosome 14 (14q23) and codes for ERβ. The CA repeat in the 3′ noncoding

portion of the *ESR2* gene, c. 1092 + 3607(CA)$_n$, was suggested to be associated with bone mineral density in women (Ogawa et al., 2000) and with androgen and sex hormone-binding globulin (SHBG) levels. Women with relatively short repeat regions displayed higher hormone levels and lower SHBG levels than those with many CA repeats. In contrast, the TA repeat of the ER alpha gene did not have such an association (Westberg et al., 2001).

Progesterone Receptor (PGR)

The PGR gene is located on 11q21–q23 (Law et al., 1987) and uses separate promoters and translational start sites to produce two isoforms, PRA and PRB. Although PRA and PRB share several structural domains, they are distinct transcription factors that mediate their own response genes and physiologic effects with little overlap. PRB functions as a transcriptional activator in most cell and promoter contexts, while PRA is transcriptionally inactive and functions as a strong ligand-dependent transdominant repressor of steroid hormone receptor transcriptional activity. The first 140 amino acids of PRA contain an inhibitory domain (ID); deletion of the N-terminal 140 amino acids from PRA results in a receptor mutant that is functionally indistinguishable from PRB (Giangrande, Pollio, & McDonnell, 1997). The hPR gene has two promoters, regulating production of the hPR-B and -A protein isoforms. A +331G/A SNP was identified and is associated with an increased risk of endometrial cancer. Biochemical studies suggested that the +331G/A polymorphism increased transcription (De Vivo et al., 2002). A small (306-bp) insertion in intron G of the T2 allele of the human progesterone receptor was described and named "PROGINS" (Fuqua et al., 1991). Subsequently, two-point mutations were found to be linked to the PROGINS mutation. These two mutations included a G to T substitution in exon 4, causing a valine to leucine change (V660L), and a C to T substitution in exon 5 (H770H). These three variants of the progesterone receptor have been referred to in the literature as PROGINS complex (Agoulnik et al., 2004). Progesterone and PGR play an important role in the sexual differentiation of the rat brain. Numerous regions of the developing male rodent brain express PGR and show dramatic sex differences during critical windows of development, indicating a differential sensitivity of developing male and female brains to progesterone (Quadros & Wagner, 2008; Weisz & Ward, 1980).

2.3.3 Outcome of Association Studies of Sex-Steroid-Related Genes in Transsexualism

To date, only a few studies have investigated genes which may be related to the development of transsexualism. Two studies focused on one gene each (Bentz et al., 2007, 2008). Three studies comprised multiple genes, and therefore interactions between genes could be studied. Henningsson and colleagues (2005) were the first

to report on sex-steroid-related genes and (only) male-to-female (MtF) transsexualism. The study population were 29 Caucasian MtF transsexuals and 229 healthy male controls. The second multiple gene study was done in 112 Caucasian MtF transsexuals from Victoria, Australia (76 subjects), and from Los Angeles (36 subjects). Healthy Caucasian males from Australia served as controls ($n = 258$) (Hare et al., 2009). The last study was done in Japan, in which 74 MtF and 168 FtM transsexuals were compared with 275 healthy age- and origin-matched controls (106 males and 169 females). All subjects were Japanese (Ujike et al., 2009).

The androgen receptor is an attractive candidate gene to investigate because a complete loss of function results in a 46XY female phenotype with a female gender identity. In addition, as previously described, the (CAG)*n*CAA-repeat polymorphism is known to have modulatory effects. Henningsson et al. (2005) demonstrated that the prevalence of a long (CAG)*n*CAA repeat is higher in MtF transsexuals. This preliminary finding was later confirmed in a larger cohort consisting of 112 Caucasian MtF transsexuals (Hare et al., 2009). However, the Japanese case control association study did not find a significant difference in the distribution of long and short (CAG)*n*CAA repeats (Ujike et al., 2009).

The CYP 19 (aromatase) polymorphism and ERβ polymorphism were also studied in three transsexual populations. In the Swedish population, they demonstrated that the length of the (TTTA)*n* repeat in the fourth intron of the CYP19 gene did not differ from the control group, when dichotomized in short (<7 and long ≥7 repeats) (Henningsson et al., 2005). This finding was confirmed in the Australian/American (Hare et al., 2009) and the Japanese (Ujike et al., 2009) populations. The length of the dinucleotide CA repeat in intron 5 of the ERβ gene was also demonstrated not to differ from the control population (Hare et al., 2009; Henningsson et al., 2005; Ujike et al., 2009).

In one study, the TA repeat promoter region of ERα was investigated, and it was shown that the distribution of repeat numbers was not significantly different in FtM and MtF, compared with control females and males, respectively. In the Japanese study six polymorphisms of PGR were also investigated with identical results (Ujike et al., 2009). When interactions between genes and the odds for transsexualism were studied, only the first (relatively) small study population could demonstrate some interaction between AR and CYP19 (Henningsson et al., 2005). However, this was not confirmed in the two other studies (Hare et al., 2009; Ujike et al., 2009).

Bentz et al. (2007) studied the SRD5A2 V89L SNP in 100 MtF and 47 FtM transsexuals using 755 men and 915 women as controls and found that carriage of the mutant SRD5A2 Val89Leu L allele is not significantly associated with FtM and MtF transsexualism. The SRD5A2 was refuted as candidate gene of transsexualism. Subsequently, 102 MtF and 49 FtM transsexuals were investigated for the CYP17A2T>C SNP prevalence. There was no statistically significant difference between MtF and male controls in CYP17A2T>C SNP distribution. In contrast, FtM transsexuals did not follow the gender-specific allele distribution of their female pairs; rather, they had an allele distribution equivalent to MtF transsexuals and male controls. Therefore, the CYP17 was postulated to be a candidate gene for FtM transsexualism (Bentz et al., 2008). However, to date no further investigations have been reported.

2.3.4 *Evaluation of Past Genetic Studies in Transsexuals*

The heritability studies discussed in Sect. 2.2 provided a base to search for candidate genes involved in the development of gender identity and gender dysphoria. Given the fact that, as outlined in Chap. 1, sex steroids contribute largely to the sex differentiation of the brain, at least in animal models, it seemed only natural to investigate genes that are involved in either sex steroid biosynthesis or action. The results of these studies were rather disappointing. Of the genes involved in biosynthesis (CYP17, CYP19, and SRD5A2), only CYP17 appeared to be associated with FtM transsexualism, with an allele distribution equivalent to MtF transsexuals and male controls (Bentz et al., 2008). Of the sex steroid receptors investigated (AR, ERα, ERβ, and PGR), only the repeat length of the polyglutamine stretch of the AR was associated with MtF transsexualism (Hare et al., 2009; Henningsson et al., 2005). This finding, confirmed in two studies, does appear to support the theory of virilization of the brain through androgens since length of the CAG repeat is inversely proportional to the transcriptional activity of the androgen receptor, i.e., the longer the repeat, the higher the insensitivity for androgens thus leading to hypovirilization of the brain. However, the polymorphism of CYP17 is associated with higher estradiol and progesterone levels and yet is associated with FtM transsexualism. Given the fact that the allele distribution did not correspond to female controls, this paradox could be explained by a more male-typical expression pattern during embryonic and fetal development. As seen in rodents, levels of hormones are important but so are their timing and windows of susceptibility.

Hitherto, no strong candidate gene has emerged for the development of gender dysphoria. Because of the complexity of gender identity development, it does not seem likely that one single gene will be discovered. Gender identity is such a robust and prominent trait that polymorphisms of genes alone are unlikely to account for the development of gender dysphoria. In addition, the genes investigated involved hormonal pathways, and, nowadays, more data have emerged of effects on sex differentiation that are not attributable to hormonal action but that arise from expression of X and Y genes within non-gonadal cells and result in sex differences in the functioning of those cells (Ngun et al., 2011). These effects may provide a different angle to pursue the genetic basis of gender identity and gender dysphoria. In the Sect. 2.4, these so-called sex chromosome effects are further addressed.

2.4 The Classic Theory Revisited: The Unified Model

The classical theory of brain and sexual behavior and the research based on its conceptual framework of organizational and activational effects of sex hormones on cells are extensively addressed in Chaps. 1 and 3. In this section we will go beyond organization and activation by discussing sex chromosome effects in humans and animals, exploring phenomena that do not adhere to the classical theory, with its

focus on brain differentiation. Finally, we will present a new paradigm of sex differentiation that includes both the organizational–activational hypothesis and sex chromosome effects.

2.4.1 Sex Chromosome Effects

The male and female zygote, the cell formed by the union of a sperm cell and an egg cell, has an identical set of autosomes (nonsex chromosomes), which comprise about 95 % of the genome. Those common genetic factors make men and women very similar in their function. It is noteworthy that a recent meta-analysis of genome-wide-association studies comprising 114,863 individuals (61,094 women and 53,769 men) of European ancestry showed no autosomal differences between men and women (Boraska et al., 2012). The sex chromosomes, comprising the other 5 % of the genome, differ in three main ways in the zygote: (1) Only males have Y genes, (2) the two sexes differ in the copy number of X genes (although the sex-specific effect of this difference is largely eliminated by X inactivation), and (3) females receive a paternal X imprint that is lacking in the male. As the individual develops, however, other intrinsic genetic sex differences arise that are not caused by gonadal hormones. One X chromosome is randomly selected to be transcriptionally silenced in each female cell, and about half of the cells activate the maternal X chromosome and express the maternal X alleles or maternal imprint, and the other half express the paternal X alleles or imprint. Thus, female tissues are mosaic and might differ in phenotype from tissues that are not mosaic (male) (Arnold & Burgoyne, 2004; Migeon, 2007). In addition, X inactivation may make females more vulnerable than males to some genetic mutations or environmental perturbations at early stages of embryonic development, as was demonstrated in mouse models (Chen et al., 2008).

Patients with Turner syndrome have a basically female phenotype and were shown to have a 45X sex karyotype (Ford, Jones, Polani, De Almeida, & Briggs, 1959). In contrast, patients with Klinefelter syndrome have male genitalia, including testes, and are characterized by 47XXY (Jacobs & Strong, 1959). This appeared to confirm the concept of the male as the induced state, and the presence of a single Y seems to override the effects of even more than one X chromosome; even XXXXY individuals have testes (Hayek, Riccardi, Atkins, & Hendren, 1971). However, in Klinefelter syndrome (XXY) patients, as well as in XXXXY, the testes are very abnormal and the phenotype is partially feminized. The latter may include female bodily habitus and body-hair distribution, gynecomastia, and feminine pitch of the voice. The mechanism behind this feminization is not known, but it is presumed to be due to the presence of a significant level of estrogens, as the result of more than one active copy of an X chromosomal gene that directs the development of estrogen-producing cells. Likewise, in Turner syndrome (45X), the phenotype, though "basically" female, is in fact in fundamental ways different from 46XX females. For example, normal breast development may be lacking, and, most importantly, though

ovaries with oogonia (egg cell precursors) are present in fetal stages, in adults the ovaries are absent and replaced by "streak gonads" (underdeveloped and fibrous dysfunctional tissue). This suggests that two copies of a non-inactivated X chromosomal factor are required for normal postnatal development of the ovary. Thus, the "default" situation of absence of Y does not produce normal ovaries in the absence of a second X, and normal female phenotype is not a default state caused by absence of Y but rather a status that requires the presence of two functional copies of at least one X chromosomal gene (Blecher & Erickson, 2007).

In humans, sex chromosomal abnormalities can illustrate how sex chromosomes play a role in the phenotypic differences typically seen between 46XY men and 46XX women: 46XX girls score better than boys on tests of social cognitive skills (Skuse et al., 1997). Adolescent girls with Turner syndrome are more likely to have social difficulties compared to 46XX girls (McCauley, Feuillan, Kushner, & Ross, 2001). Since both 46XY boys and 45X girls experience more social adjustment problems compared to 46XX girls, there may be a genetic locus involved in social cognitive skills on the X chromosome, and this locus may be subject to imprinting (the process in which one specific allele of a gene comes to expression when it is inherited from one specific parent, either the father or the mother). Significant differences have been reported between 45Xp Turner syndrome girls (in which the X was of paternal origin) and 45Xm girls (in which the X was maternally derived) in terms of social skills: 45Xp girls had superior social competence and social skills compared to 45Xm girls, suggesting that the genes in this locus are expressed only from the paternal X (Skuse et al., 1997). This could potentially be one of the reasons why boys are more susceptible to disorders such as autism that affect social adjustment and social skills such as language. In boys, the X is only of maternal origin, and therefore this locus would be silenced (Skuse, 2000).

In animals, the mouse model mostly used to study sex chromosome effects is the "four core genotype" (FCG) model: The testis-determining gene (*Sry*) was moved from the Y chromosome to an autosome (by deletion of *Sry* from the Y and subsequent insertion of an *Sry* transgene onto an autosome), so that testis development occurred independently of the sex chromosome complement (i.e., XX or XY). Results were that XX mice developed testes, while XY mice that lacked *Sry* developed ovaries. This allowed the comparison of mice with the same-sex chromosome complement (XX versus XX(+Sry) or XY and XY(−Sry)) but with the opposite gonadal phenotype (ovaries versus testes). Thus, if a trait varies by gonadal phenotype (XX(+Sry) and XY differ from XY(−Sry) and XX), it is considered hormonal in origin, and if it varies by chromosome complement [XX and XX(+Sry) differ from XY and XY(−Sry)], it is considered genetic in origin (De Vries et al., 2002). To date, the model demonstrated that for most of the investigated traits, there were no differences between XX and XY mice that had the same gonadal sex. Thus, the organizational–activational framework accounted completely for the majority of the classic sex differences first studied in the FCG model (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012). However, the model could also demonstrate a sex chromosomal basis for sex differences in both brain structures and behavior. The vasopressin fiber density in the lateral septum, part of the limbic system, was

greater in XY mice with either testes or ovaries compared to XX mice with the same gonadal sex (De Vries et al., 2002; Gatewood et al., 2006). During embryogenesis, the increased susceptibility to neural tube defects in females is caused by XX versus XY differences in the genome and not by gonadal hormones (Chen et al., 2008). In addition, XY gonadal females were more aggressive than XX females. When tested for response to nociceptive stimuli (thermal and chemical), the XX mice showed greater or faster response than XY mice, irrespective of their gonadal sex (Arnold, 2009a).

The FCG model may have certain limitations. One is that the gonads may not be equal in XX and XY mice of the same gonadal sex and therefore secrete hormones differently during critical stages of development. However, it was found that XX and XY mice of the same gonadal sex do not differ in many traits that are responsive to the organizational effects of gonadal hormones, suggesting that these mice have similar hormonal levels. Also, the Sry transgene placed on an autosome (nonsex chromosome) may behave differently compared to the "regular" Sry gene. To address these limitations, mice were gonadectomized followed by an administration of equivalent doses of gonadal steroid hormones. In addition, it remains unclear when a sex difference is attributed to the complement of sex chromosomes within cells and what the underlying cause is. It may be caused by (a) gene or genes encoded on the Y chromosome; (b) higher dosage of X genes, particularly the ones that escape X inactivation in XX animals; or (c) the paternal imprint of the genes encoded on the X chromosome in XX animals, which changes the expression of these genes to exhibit a female-specific pattern (Ngun et al., 2011).

2.4.2 Genetic Aspects of Brain Sex Differentiation

Some sexual dimorphisms in the brain and other somatic tissues cannot be solely attributable to the effects of gonadal steroid hormones. For instance, the zebra finch male can sing a courtship song, whereas the female bird does not sing. This male-specific ability has been attributed to several brain regions that are much larger in the male. Based on the classical view, two pathways of development were conceptualized. Either the steroid hormones produced in the testes cause a masculine pattern of neural development or, alternatively, the steroid hormones secreted by the ovary cause female brain development and block masculinization, as was shown in other bird species for several male characteristics (Arnold, 1997). The initial studies indeed demonstrated that administration of estrogens or androgens to hatchling females caused masculine neural development (Gurney & Konishi, 1980). Androgens that cannot be converted to estrogens (dihydrotestosterone) caused less masculinization, and androgens that can be converted to estrogens (testosterone) were less potent than estradiol. Therefore, it was postulated that estradiol is the masculinizing agent in females. However, the masculinizing dosage of estrogen is very high and results in supraphysiological levels for both females and males. Therefore, it may be that the masculinizing actions of estrogen are pharmacologic

and do not normally occur in males. Also, none of the steroid treatment regimens have led to the development of a completely masculine neural circuit. To test the second theory—a feminizing and demasculinizing effect of estrogens—hatchling males were treated with estrogen but not much effect on anatomical differentiation of the song circuit was observed (Schlinger & Arnold, 1991). Also, androgen deprivation by either castration (Arnold, 1975) or treatment with the androgen receptor blocker, flutamide (Schlinger & Arnold, 1991), could not prevent masculine differentiation. To test the putative role of estrogen as the main mediator, males were treated with an inhibitor of estrogen synthesis, fadrozole. This treatment also failed to cause any consistent demasculinization of neural development in genetic males. Likewise, when (genetic) female embryos with viable and secreting testicular tissue and absent ovarian tissue were created, their neural song system showed little or no masculinization. In conclusion, in the zebra finch, testicular steroid secretion is not solely responsible for the masculinization of the brain (Arnold, 2009b).

In mammals, mice without both adrenals and gonads were studied. These steroidogenic factor 1 knockout (SF-1KO) mice do not express steroidogenic factor 1 (SF-1, NR5A1), a member of a nuclear receptor superfamily of transcription factors which is normally expressed in steroidogenic as well as non-steroidogenic tissues. They are born without gonads and adrenal glands and have nonfunctional gonadotropic cells in the pituitary and an altered organization of the ventromedial hypothalamus (VMH). In SF-1 KO mice, gonadal ridges form normally on embryonic day 10.5 (E10.5) but undergo apoptosis immediately thereafter, and gonadal ridges disappear by day E12.5 (Ikeda, Shen, Ingraham, & Parker, 1994). Consequently, SF-1 KO mice develop female internal and external genitalia without gonads irrespective of genetic sex. Because expression of steroidogenic enzymes in mouse fetal gonads is present around day E13 in testes (fetal ovaries are hormonally inactive) coinciding with the start of testosterone production (Budefeld, Jezek, Rozman, & Majdic, 2009), SF-1 KO mice are not exposed to endogenous gonadal hormones. Therefore, they represent an excellent model to study gonad-dependent versus gonad-independent brain sexual differentiation in the absence of *SF-1* gene. SF-1 KO mice normally die shortly after birth due to adrenal insufficiency but can be rescued by corticosteroid treatment and adrenal transplantation. It was found that the expression of neuronal nitric oxide synthetase in the caudal preoptic area/anterior hypothalamus was enhanced in wild-type and SF-1 KO males and considered the result of gonad-independent sex differentiation. This was also found in the anteroventral periventricular preoptic area (AVPV). Therefore, some sex differences present in agonadal SF-1 KO mice likely arise due to different complements of sex chromosomes or disruption of a functional *SF-1* gene and not due to differential exposure to sex steroids during prenatal/early postnatal development (Budefeld, Grgurevic, Tobet, & Majdic, 2008).

One other approach to investigate sex differentiation of the brain is to interfere directly with sex chromosome gene expression. Early transcription of the Y chromosomal gene SRY can be detected in the human zygote in the one-cell stage (Ao, Erickson, Winston, & Handyside, 1994; Fiddler, Abdel-Rahman, Rappolee, & Pergament, 1995), and a faster rate of cell division has been reported in male

embryos (Pergament, Fiddler, Cho, Johnson, & Holmgren, 1994). In mice, transcripts of Sry can be detected in the blastocyte stage (Zwingman, Erickson, Boyer, & Ao, 1993). In addition to the gonad, Sry is expressed in the embryonic murine brain, including the mesencephalon (midbrain) and the hypothalamus (Mayer, Mosler, Just, Pilgrim, & Reisert, 2000). Indeed, in the FCG mouse model, mesencephalic brain cells (neurons) were grown in cultures, and it was shown that cells of XY mice, irrespective of their gonadal sex, developed more dopaminergic neurons (Carruth, Reisert, & Arnold, 2002). This confirmed earlier studies in which rat embryonic mesencephalic cells in culture demonstrated sex differences in a developmental stage prior to sex steroid production (Reisert & Pilgrim, 1991). To explain this kind of differentiation, it was postulated that the expression of a gene on the Y chromosome in brain development would bias the development of neural circuits. Indeed, the expression of Sry was detected in both the human and mouse brain (Arnold, 1996). In the mouse brain, Sry messenger RNA is present through all developmental stages and in adulthood (Mayer et al., 2000). The translation of mRNA into the actual protein product can be inhibited with the so-called antisense oligodeoxynucleotides (ODN). Using this technique by infusing Sry antisense ODN in male rats, it was demonstrated that downregulation of Sry, specifically expressed in tyrosine hydroxylase-expressing neurons in the substantia nigra (SN), causes a significant decrease in tyrosine hydroxylase expression with no overall effect on neuronal numbers and that this decrease leads to motor deficits in male rats. This study was the first to report that Sry directly affects the biochemical properties of the dopaminergic neurons of the nigrostriatal system (Dewing et al., 2006). In addition, the effect of Sry antisense ODN in the SN on specific motor functions was assessed by the akinesia and limb-use asymmetry tests (Schallert, Fleming, Leasure, Tillerson, & Bland, 2000). In the akinesia test, animals with unilateral (one-sided) dopamine depletions take fewer steps with their forelimb opposite to the depletion side than they do with their forelimb at the same side of depletion. These results demonstrate a direct male-specific effect on the brain by a gene encoded only in the male genome, without any mediation by gonadal hormone (Dewing et al., 2006).

2.4.3 A New Paradigm on the Origins of Sex Differences in All Tissues

The classical paradigm of human and mammalian sexual development states that the genetic factors that determine sex only influence the fate of the gonad. A maleness factor produces testes (primary sex determination). These organs secrete hormones which cause male secondary sexual differentiation. In the absence of the maleness factor, by default the gonad becomes an ovary, and the absence of testicular hormones leads to female secondary differentiation. Building on this foundation, a new model which addresses both the classical organizational and activational effects and the more recently discovered sex chromosomes effects was proposed by Arnold in 2009 (Arnold, 2009b):

> All ontogenetic sex differences in phenotype derive from the differences in the effects of sex chromosome genes, which are the only factors that differ, on average, in the male and female zygote. A subset of X and Y genes represent the primary sex-specific factors causing sex differences in development and adult phenotype. Primary among these is Sry because it controls sexual differentiation of the gonads, and therefore sets up life-long sex differences in secretion of gonadal hormones. These hormones, especially testosterone and estradiol, act throughout the body in an organizational (long-lasting or permanent) and an activational (reversible) fashion at different times of life, to cause most known sex differences in phenotype, including sex differences in susceptibility to and progression of diseases. In addition to Sry, however, various X and Y genes have differential effects on male and female cells because of the constitutive sex differences in the copy number and/or parental imprint on these genes. Various sex-specific factors interact, acting synergistically or counteracting each other or otherwise conditioning the effects of each other. Thus, XX and XY cells are different prior to the secretion of gonadal hormones, and gonadal hormones affect XX and XY cells unequally.

The key in this model is that the previous emphasis on the action of gonadal hormones on non-gonadal tissue as the sole agent to cause sex differences is now shifted away towards primary sex-specific effects of X and Y genes. However, gonadal hormonal action does remain the most important among a variety of secondary factors downstream of the sex chromosomes. And thus doing so, the X and Y genes are given primacy, based on the fact that these are the only factors in the zygote that ultimately result in sex difference in phenotype. On the genetic level, three factors are considered to be determinates of sex differentiation: (1) Sry, (2) other putative Y and X genes, and (3) the constitutive difference in XX and XY genomes (as discussed in Sect. 2.2). The gonadal steroidal secretion still remains a major factor among the secondary proximate factors (those not sexually dimorphic in the zygote) because of its widespread and dominant effect on sexual phenotype. This proposed model is unified because it applies equally to all tissues and sex differences in phenotype. Previously, the sex differentiation of the gonads was seen as genetic and therefore classified as sex determination, and the sex differentiation of other non-gonadal tissues was considered to be caused by hormonal action. This implied a separation of gonads and other tissue, which has now disappeared. The reunification of gonads and other tissues provides an attractive concept but raises the question of how to integrate future investigations of direct genetic and hormonal factors and their interaction (Arnold, 2009b).

2.5 Conclusions and Future Perspectives

The results thus far show that heritability studies demonstrate that genetic components play a role in the causation of gender dysphoria. Second, it is unclear whether genetic variations (polymorphisms and mutations) that involved in sex hormone production or activity (including receptors) and that predispose for disorders of sex development also predispose for gender dysphoria. Most of the genetic studies measuring one or more of these variants show no clear difference between subjects with

gender dysphoria (i.e., most often patients referred for sex reassignment treatment) and control subjects. On the other hand, several studies show an increased prevalence of gender dysphoria in subjects with disorders of sex development (De Vries, Doreleijers, & Cohen-Kettenis, 2007). However, it is not clear whether this is the result of the underlying genetic disorder or associated with a more complicated psychosocial development.

Future studies on the genetic causes of gender dysphoria could develop along two lines of research. One way is to study the genetic causes of sex differences in the brain, supposing that gender dysphoria may be a result of deviations in the sexual differentiation of the brain. Under this assumption, future studies will require incorporating classic endocrine methods to manipulate hormone synthesis and action, modern molecular genetic methods to alter hormone action in a cell-type-specific manner, as well as methods to manipulate the copy number and expression of X and Y genes that underlie constitutive genetic differences in XX and XY cells. When investigating sexual differentiation, one should expand one's conceptual horizons beyond an appreciation for how gonadal hormones cause sex differences to include an understanding of the sex chromosomes and the direct effects of genes encoded on these chromosomes and the interaction of these genes with the rest of the genome (Arnold, 2009b). In this respect, the emerging field of epigenetics may provide promising opportunities.

Cells of a multicellular organism are genetically homogeneous but structurally and functionally heterogeneous, owing to the differential expression of genes. Many of these differences in gene expression arise during development, including sex differentiation, and are subsequently retained through cell division. Epigenetics refers to the study of stable alterations in gene expression potential that arise during (1) development, (2) differentiation, and (3) under the influence of the environment. In addition, epigenetic regulation of gene expression allows the integration of intrinsic and environmental signals in the genome, thus facilitating the adaptation of an organism to its changing environment through alterations in gene activity (Jaenisch & Bird, 2003). The epigenome refers to the ensemble of coordinated epigenetic marks that control the accessibility of the DNA to the machinery-driving gene expression. The best understood epigenetic marks are modifications of core histones, proteins that package DNA, and methylation of DNA itself (addition of a methyl group to the cytosine side chain in cytosine–guanine (CpG) dinucleotides) (Reik, 2007). In the context of sex differentiation, epigenetic changes offer a plausible mechanism by which early hormonal effects could be hardwired into the genome to channel adult hormonal and behavioral responses. In rodent models, steroid receptors are subjected to epigenetic programming. DNA methylation of ERα increases across development of the cortex in both sexes, concomitant with reduced gene expression. Furthermore, males exhibit more ERα promoter DNA methylation and reduced expression compared with females in certain regions. For the AR it was shown that methylation of its core promoter was increased by testosterone and decreased by estradiol. Epigenetic mechanisms mediate between the sex and the genetic blueprint. The expression potential of estrogen receptors, androgen

receptors, and progesterone receptors can be sex- and tissue-specific fine-tuned by epigenetic mechanisms. The transcriptional activities of these genes depend on the recruitment of the epigenetic machinery and modification of epigenetic marks governing gene expression. This process is dynamically regulated during brain development and shows a sex-dimorphic expression in distinct tissues and is under influence of sex steroid action. Moreover, the epigenetic machinery itself can show sex-specific differences in the expression and activity of single components in distinct tissues. Together, the mutual interplay between these regulatory layers critically determines how hormonal signals are sex-specifically translated into sustained changes of neuronal substrates during sexual development and differentiation (Menger et al., 2010). Studying the epigenome in transsexuals may help in identifying genes involved in gender identity development and also gain more insight in sex-steroid-mediated events in brain sex differentiation. Since the epigenome is also responsive to the environment, epigenetics may also provide a biological substrate for identifying psychological and social factors in gender identity development. It was shown in rats that differences in maternal grooming of male pups resulted in a sex-specific DNA methylation pattern of the ERα promoter. Simulation of this type of grooming in female pups resulted in a male-specific DNA methylation pattern of the ERα promoter (Kurian, Olesen, & Auger, 2010). These studies illustrate how early social experiences implement sex differences via epigenetic programming. Next to sex-steroid-related genes, sex chromosomes are also subjected to epigenetic programming. The X inactivation and possible imprinting of genes on the X chromosome that escape inactivation, as discussed in Sect. 2.4.1, are due to epigenetic mechanisms. In this perspective, the finding that MtF transsexuals have a significant excess of maternal aunts versus uncles prompted the hypothesis that MtF transsexuals may inherit feminizing X chromosomal imprinted genes. The underlying mechanism was postulated to be a failure to erase the imprint on the paternal X chromosome in the egg cell of the grandmother, followed by a second passage through the mother (Green & Keverne, 2000). Although the biological evidence, i.e., epigenome, is lacking, it does offer a new perspective to investigate sex chromosome effects in brain sex differentiation.

A second line is to apply genome-wide studies (including single-nucleotide polymorphisms, copy number variation, and also degrees of methylation) on subjects diagnosed with gender dysphoria. Such genome-wide studies have the advantage to have no prior hypothesis about which genes are involved, but in general these studies require a large number of participants. Important questions include whether genes predisposing for gender dysphoria are the same for male-to-female as for female-to-male transgendered persons. Second, in order to solve the power problem in this approach, the question arises as to which part of the genome is most likely to be associated with gender dysphoria. Given the above-mentioned literature, it is recommended to start with the study of the sex chromosomes.

Finally, the combination of magnetic resonance imaging (MRI) data and genome-wide arrays provides another option to study the genetic variation that underlies the phenotypic variation in those areas of the brain that are different in men and women.

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