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Biological aspects of gender disorders

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The scientific community is very interested in the biological aspects of gender disorders and sexual orientation. There are different levels to define an individual's sex: chromosomal, gonadic, and phenotypic sex. Concerning the psychological sex, men and women are different by virtue of their own gender identity, which means they recognize themselves as belonging to a determinate sex. They are different also as a result of their own role identity, a set of behaviors, tendencies, and cognitive and emotional attitudes, commonly defined as "male" and "female". Transsexuality is a disorder characterized by the development of a gender identity opposed to phenotypic sex, whereas homosexuality is not a disturbance of gender identity but only of sexual attraction, expressing sexual orientation towards people of the same sex. We started from a critical review of literature on genetic and hormonal mechanisms involved in sexual differentiation. We re-examined the neuro-anatomic and functional differences between men and women, with special reference to their role in psychosexual differentiation and to their possible implication in the genesis of homosexuality and identity gender disorders. Homosexuality and transsexuality are conditions without a well defined etiology. Although the influence of educational and environmental factors in humans is undeniable, it seems that organic neurohormonal prenatal and postnatal factors might contribute in a determinant way in the development of these two conditions. This "organicistic neurohormal theory" might find support in the study of particular situations in which the human fetus is exposed to an abnormal hormonal environment *in utero*.

Key words: **Gender identity - Homosexuality - Sexual and gender disorders - Sex differentiation - Disorders of sex development - Adrenal hyperplasia, congenital.**

Sex determination in humans occurs at the time of fertilization when two haploid gametes (oocyte and sperm) fuse to generate a diploid zygote. Normal oocyte have a single X chromosome, while normal sperm contains either a single Y chromosome or a single X chromosome, resulting in 46 XY or 46 XX zygotes, respectively, following fertilization. Therefore, chromosomal or genetic sex describes the complement of sex chromosomes present in an individual: 46 XY, which is "male" and 46 XX, which is "female".

There are, however, different aspects to take into consideration when defining the "sex" of an individual. Testes and ovaries, for example, are structurally and functionally different, and on the basis of these differences individuals can be defined as male

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or female. This sex definition is referred to as gonadic sex.

On the other hand, the developing gonad produces several steroid and peptide hormones that mediate sexual differentiation and result in the morphological or phenotypic sex seen at birth with internal (better defined as somatic sex) and external genitalia.

The sex of an individual can also be defined on the basis of minor biological parameters. Regarding psychological sex, for example, males and females are distinguished in terms of appropriate gender identity, *i.e.*, for recognizing and feeling they belong to a particular sex. This complex area of psychosexuality also includes all behaviors, tendencies, cognitive and emotional attitudes, commonly defined as "male" and "female", constituting the gender identity ("role of gender" or "gender identity" of the individual).

Finally, we have to remember that the social and legal attribution (birth certificate) of a determined sex indicates, respectively, the social and "official" sex of a subject.

It appears clear that the terms "sex" and "gender" are often inappropriately considered synonyms, but in reality, they cannot be used as such.

Sexual differentiation

The determination of chromosomal sex happens at the moment of fecundation. Conversely, complete sexual differentiation includes a series of complex sequential hormone-related processes, able to define gonadic and phenotypical sex. In fact, human embryos chromosomally either male or feminine, develop in the same manner in about the first two months of gestation. Only after this period they anatomically and physiologically differentiate according to their own chromosomal sex.

The structures of the genital tract in this "indifferent" stage are represented by the gonads. These originate in the urogenital crest at the 4th-5th week, by two systems of genital ducts (Wolffian and Müllerian) and by a common outlet for the genital ducts and urinary tract. In this phase, the gonads are particularly undifferentiated and bipotential, which is to say male and female gonads cannot be distinguished morphologically and they can differentiate either as testicles or ovaries.

It is the chromosomal sex that then conditions the differentiation of the gonads of the embryo. In particular, it is the presence of the male Y chromosome that influences the sexual differentiation into testicles and it determines the male gonadic sex. In fact, the undifferentiated embryo gonad has a spontaneous tendency to develop as female gonad, except that there is a molecule that actively induces the differentiation towards testes. This substance, defined traditionally as TDF (testis determining factor) is codified on the Y chromosome in the SRY (sex-determining region), is constituted by 204 AA, and is expressed when it forms the primordial structures of the testicle and persists up to the 18th week of gestation.

It generally follows that in the presence of the Y chromosome, the gonad differentiates into a testis, independent of the rest of the chromosomal constitution and, in particular, of the number of X chromosomes, while in the absence of the Y chromosome, the bipotential embryo gonad develops into an ovary. This also occurs, although in an incomplete fashion, in the presence of only one X chromosome. The determination of gonadic sex depends on embryonic chromosomal sex and develops through multiple molecular events. These orientate the development of germ cells, their migration towards the urogenital crest and the formation of the testes (in the presence of the Y chromosome) or the ovaries (in the absence of the Y chromosome and, in a complete manner, in the presence of the second X chromosome).

If the chromosomal sex conditions the gonadic sex, the latter then conditions the morphological sex. The embryos of both sexes show two systems of genital ducts: Wolffian and Müllerian. In the female, the Fallopian tubes and the uterus originate from the Müllerian ducts, whereas in the

male the epididimus, deferent ducts, seminal vescicles and ejaculation ducts originate from the Wolffian ducts. Simultaneous with the differentiation of the structures of Wolffian (male) or Müllerian (female) origin, each sex has the involution of the opposite structures. The mechanism of differentiation of the genital ducts has been clarified by Jost's classic experiments, which show that the differentiation into male is due to the presence of the testicles and their secretions. For the internal female differentiation, on the other hand, there is no need for hormonal stimulation and, consequently, the presence of the ovary is not indispensible. In other words, it is the presence or absence of the testes (and not of the ovaries) that determines the somatic sex.[1, 2]

This process is shown through the secretion of two different testicular substances: testosterone, which stimulates the development of the Wolffian ducts acting by local diffusion, and the inhibiting factor of the Müllerian ducts which, in a similar manner, determines the regression of the Müllerian ducts. External genital too, as well as genital ducts, differentiate "spontaneously" in the direction of the female phenotype in the absence of the secretions of the fetal testis. In this way, in female subjects, the external genitals spontaneously lead to a modest differentiation with the development of the clitoris, labia minora and labia majora.

Vice versa, in the male the differentiation of the external genitals (penis, scrotum, prostate) is due to the action of dihydrotestosterone (DHT), derived from testosterone by the action of the enzyme 5-alfa reductase. Finally, it is important to remember that the so-called target tissues, *i.e.*, those whose differentiation is induced by hormonal substances, play an important role in the process of differentiation of morphological sex. The target tissues have to be able to "respond" to this induction. This occurs thanks to the presence in their cells of receptors for inducer substances, the production of which is under genetic control.

The testosterone-receptor complex determines the evolution of Wolffian ducts in the male internal genitals, while the DHT–receptor complex determines the differentiation of the male external genitals. In the same way, the anti-Müllerian factor-receptor complex permits the involution of the precursors of the female genitals tracts.

The androgen receptor (which is unique for testosterone and DHT) is present in the target cells of both males and females, and, consequently, both sexes are sensitive to the action of the androgens, and the limiting factor is the quantity of the androgens present.[3, 4]

Definitely, both the male and female embryos possess common primordial undifferentiated structures, which occur "spontaneously" in the direction of the female phenotype, unless there is interference of recognizable masculinizing signals.

In conclusion, the process of sexual differentiation is substantially complete at birth. After the long latent period of childhood during which the hormonal secretions are minimal, the process of sexual maturation leads to subjects capable of reproduction.

This process is seen in the puberal phase, and the typical body and behavioral changes for each sex are due to a net increase in the concentrations of sexual steroids in this period, essentially testosterone in males and estradiol in females. The causes of this increase are complex, but it can be simplified by saying that at the moment of puberty there is an activation, or rather a disinhibition, of the secretion of a hypothalamic hormone (GnRH) which, through the secretion of pituitary hormones (the gonadotropins FSH and LH), stimulates the gonads to produce sexual hormones.[5]

Importance of genetic factors

In addition to the SRY, many genes have been discovered that contribute in early or late phases to the process of sex determination and differentiation. It is probable that many others will be discovered in the future, attesting to the biological complexity underlying these processes.[6]

Table I lists principal genes involved in the physiological process of sexual differ-

TABLE I.—*Genes involved in sexual differentiation.*

Principal genes	Other genes
SRY	LIM1, EMX2, LHX9,
GATA 4	WNT4, M33, DHH, FGF9,
WT1 e SF1	DMRT1/2, ATRX, ARX,
DAX1	SOX8, FATE, Vanin-1,
SOX9	Tescalcin, RSPO, Beta-cat,
AMH	FST, CBX2, PAX 2, TSPYL1, MAMLD1, FOXL2

entiation. In particular, mutations of various genes can lead, due to a defect or an excess of the codified proteins, to a variety of dysgenic syndromes concerning the gonads and/or the Wolffian and Müllerian ducts with possible associations of renal pathologies.

Thereafter, Table II indicates the principal alterations of the sexual differentiation caused by genetic defects.

GATA4, WT1 and SF1 appear in the first stages of development and, together with other genes, increase the SRY expression,[7, 8] which, as stated, is fundamental for all the successive phases of sexual differentiation. If the SRY gene is transposed on the X chromosome, in fact, there is a condition known as "sex reversal" and this can happen either naturally [9] or experimentally by using XY female mice and XX male mice.[10] Again, if the SRY gene is blocked during sex differentiation (as happens in the case of exposure to atrazine pesticides) the differentiation process in both sexes is halted, with the presence of ambiguous genitals at birth as well as ambiguous sexual behavior.[11]

The SOX 9 gene is also indispensible for normal development,[12] as it can still induce complete male differentiation in experimental models, also in the absence of SRY.

Another gene activated by SRY is FGF 9, which is fundamental in the processes of proliferation and differentiation of Sertoli cells, the formation of the spermatic cords, the increase of Leydig cells and the number of peritubular myoid cells.[13] Finally, DAX 1 is essential for the early development of the testes, for the beginning of spermatogenesis and the proliferation and differentiation of Leydig cells. Its overexpression, moreover, interferes with the development because it induces the expression of the repressors of SRY, SFI e SOX 9.[9, 14]

Psychosexual differentiation

Psychosexual differentiation is influenced by chromosomal, hormonal, social and familiar components. In particular, sexual dimorphism of the central nervous system (CNS) regarding both the hypothalamus (or, more

TABLE II.—*Genes and alterations of sexual differentiation.*

Gene	Alterations
SRY	Male: testicular dysgenesis, persistence of Müllerian ducts, female or ambiguous external genitalia
WT1	Male: female or ambiguous external genitalia, gonadal dysgenesis
SF1	Male: testicular absence or dysgenesis, female or ambiguous external genitalia, persistence of Müllerian ducts female: premature ovarian failure
SOX 9	Male: testicular dysgenesis, sometimes persistence of Müllerian ducts
AMH	Male: persistence of Fallopian tubes and utero
WNT4	Male: testicular dysgenesis, ambiguous external genitalia or persistence of Müllerian ducts Female: Müllerian aplasia, ambiguous external genitalia, ovotestis, testicular dysgenesis, ovarian hyperandrogenism
DHH	Male: testicular dysgenesis Both: ambiguous external genitalia
DMRT1	Male: testicular dysgenesis, female or ambiguous external genitalia, persistence of Müllerian ducts
ATRX	Male: testicular dysgenesis, ambiguous external genitalia
CBX2	Both: ambiguous external genitalia, presence of ovaries with oocytes, presence of Müllerian structures
RSPO1	Female: presence of testes, ambiguous external genitalia, absence of Müllerian structures
TSPYL1	Male: testicular dysgenesis, ambiguous external genitalia
MAMLD1	Male: hypospadias and cryptorchidism
FOXL2	Female: primary ovarian failure and sterility

precisely, the hypothalamus-pituitary-gonadal axis) and the neocortex is a complicated and important aspect. This corresponds, finally, to the development of masculine or feminine psychosexuality. The problem of sexual dimorphism of the hypothalamus-pituitary-gonadal system and of other regions in CNS has been the topic of many experimental studies, all above on animals. The role of the gonadic hormones in such delicate processes is still being debated.

Hypothalamus-pituitary axis

The hypothalamus, through the secretion of GnRH, stimulates the gonadotropins pituitary secretion, which, in turn, stimulates the gonadic production of testosterone in the male and estrogens in the female. Gonadic hormones then "reverse check" the gonadotropins secretion in two different ways: through negative feedback and positive feedback.

Negative feedback refers to the tonic inhibition exercised by both estrogen and testosterone on pituitary gonadotropins.

Positive feedback, on the other hand, refers to the ability of estrogen to determine, under certain conditions, a phasic release of gonadotropins, which in females characteristically occurs at the moment of the preovulatory peak during the menstrual cycle.[15, 16]

Positive feedback has always been considered an exclusive prerogative of the female, not evocable in the male. For this reason a substantial anatomo-functional difference in the hypothalamus of the two sexes was long hypothesized, and this difference appeared definitive and unchangeable starting from the perinatal period. Testosterone has been considered responsible for this irreversible sexual "imprinting" of the hypothalamus. Its presence in the perinatal period would definitively masculinize the hypothalamus (with the loss of the ability to respond positively to estrogens), while the absence of testosterone could allow it to evolve into a female direction (with the consequent evocability of the positive estrogenic feedback).

In reality, the phenomenon of perinatal imprinting of the hypothalamus can actually be proposed only in inferior mammals and in particular in rats. In male rats, in fact, a positive feedback to the sexual steroids cannot be evoked either in the prenatal or postnatal phase, a tonic release of gonadotropins being observed.[17, 18]

Vice versa, in primates, and, particularly, in humans, the hypothalamus is not definitively marked by testosterone produced in the perinatal period; the imprinting phenomenon seems, therefore, nonexistent. In fact, in particular experimental conditions, positive feedback can be evoked not only in human females, but also in human males.[19-21] Studies conducted on primates show that castrated males can respond to estrogenic stimulation with the LH surge and can exhibit a 28-day ovarian cycle if they undergo an ovary transplant. Moreover, the male primates and the androgenized females seem to respond to the positive feedback effect of estradiol with a larger LH surge than do the females.[22, 23]

There are no apparent substantial differences at the level of the hypothalamus-pituitary-gonadal axis between normal subjects and subjects with alterations of sexual differentiation (Klinefelter's syndrome, 46, XY disorders of the sexual differentiation) or psychosexuality (transsexuals).

Finally, it can be maintained that the human hypothalamus is not "marked" as male or female, but rather takes a male or female direction according to the sexual hormonal environment.

Cerebral cortex

The sexual differentiation of brain structures includes a series of a morphofunctional changes thanks to interactions between developing neurons and the pericellular space including nervous cells, hormones, nutrients and chemical substances (such as drugs) coming from the maternal circulation. All these elements could have a long-term effect on the process of sexual neurodifferentiation.

Regarding the anatomic aspects, there is

agreement that the male brain shows greater asymmetry than the female brain.[24] Raisman e Field [25] demonstrated a higher number of dendritic spines in the dorsal preoptic area of females than in males.

Other differences include: differences of size of specific brain regions, extent of dendrites, density and pattern of synaptic connections, and morphology of astrocytes.

Table III lists a synthesis of the main anatomic brain regions investigated in the study of brain sexual dimorphism (adapted by Wilson and Davies [6]). These dimorphisms could justify functional differences. For example, the gonadotropin release patterns in females could be related to a larger anteroventral periventricular nucleus, as well as to more dendritic arborization and synapse density in the arcuate nucleus than in males.[26] Different studies showed differences in different cognitive abilities between males and females: on average, females have a higher performance in verbal tasks, such as speech and language,[27, 28] whereas males are better at visual spatial tasks and orientation tasks.[29]

The brain lateralization and the anatomical differences of CNS between males and females are probably related to testosterone secretion. In particular, Witelson and Nowakowski [30] hypothesized that the asymmetry of the male brain could be caused by a reduction of commissural fibers in the corpus callosum induced by testosterone. Conversely, in females, in the absence of testosterone more interhemispheric connections might allow a higher number of information exchanges between hemispheres and a lower functional asymmetry. Not all the authors, however, agree on the role of testosterone in the genesis of these differences.[31]

Furthermore, some studies on animals, examined the role of estrogens in brain differentiation. Until the 80s, it was thought that the female brain occurred "by default" in the absence of testosterone, but Dörner [32] showed that antiestrogens prevent a nor-

TABLE III.—*Principal anatomical regions involved in the brain sexual dimorphism.*[6]

Region	Volume larger in:	Notes
VNO	Male	
AOB	Male	
meAmg	Male	
PMv	Male	
BNST:		
Posterior medial	Male	
Anterior medial	Female	
Anterior lateral	Female	
Central nucleus	Male	Seen only in adulthood in humans
SDN-POA	Male	
AVPN	Female	
SON	Male	
SCN	Male	
VMN	Male	More calbindin, vasopressin and VIP cells in females
ARC	No sex difference	More axodendritic spine and shaft synapses in male vlVMN More axodendritic shaft synapses in females more axosomatic synapses in males; no difference in spine synapses
LC	Female	
SNB	Male	
AC	Male (rat) Female (human) No sex difference	

VNO: vomeronasal organ; AOB: accessory olfactory bulb; mAmg: medial amygdaloid nucleus; PMv: ventral premamillary nucleus; BNST: bed nucleus of the stria terminalis; SDN-POA: sexual dimorphic nucleus of the POA; POA: preoptic area; AVPN: anteroventral periventricular nucleus; SON: supra-optic nucleus; SNC: suprachiasmatic nucleus; VMN: ventromedial nucleus; ARC: arcuate nucleus; LC: locus coeruleus; SNB: spinal nucleus of the bulbocavernosus; AC: anterior commissure; vl: ventrolateral.

mal female sexual receptivity and an appropriate release of LH. Additionally, estrogens might play a role in the modulation of the development of the hypothalamus preoptic area,[33, 34] probably through mechanisms controlled by binding proteins such as the alfa-fetoprotein.[35]

The ontogenesis of brain sexual dimorphism can occur through different mechanisms, including neurogenesis, differentiation, cellular death, axon guidance, and synaptogenesis by the action of nuclear transcription factors and/or molecule intercellular signaling. Additionally, some peptides with functions yet to be determined might represent biological markers of sexual differentiation, since a potential relationship between sex hormones and biological molecules such as GABA, NO, BDNF and CREB exists, recently described as probable molecular mediators. In future experiments it will be important to directly test the interaction between molecules and the developing hypothalamus.[34]

This topic appears more complex if one considers that recent experiments question the dogma that sexual brain differentiation is related exclusively to the activation of the steroid hormone receptors belonging to the superfamily of nuclear receptors.

In fact, still today, the activation of the steroid hormones receptors appears to be the prevailing factor, and in any case it seems that other different molecular mechanisms could contribute to the hormonal action on sexual differentiation. For example, different proteins belonging to the steroid hormone receptor superfamily could bind to extranuclear steroid receptor pools.[36] Furthermore, there are some effects modulated by sexual steroids that occur too quickly to be regulated by nuclear transcription, and others that cannot be blocked by transcription inhibitors.[37, 38]

Some authors maintain that sexual dimorphism of CNS is not related only to sex hormones: for example, the transcription of SRY causes both the production of androgens and the biosynthesis of dopamine in the substantia nigra of adult male rats, essential in the physiology of the motor system.[39]

Hormones and psychosexuality

The relationship between gonadal hormones and differentiation of psychosexuality into male or female is still controversial, in particular regarding gender identity and sexual orientation. Sexual orientation includes partner sex, erotic interest, behavior, fantasies, and attraction, and is usually directed to the sex opposite the psychological sex.

It is considered that in inferior mammals sexual behavior is strictly controlled by sexual hormones and is determined by the prenatal hormonal environment. It appears, however, that already in inferior primates this influence is limited and in human beings, predictably, even less so. In fact, it appears undeniable that organic factors are determinant in humans as well; prenatal neuro-hormonal factors, educational, environmental, and social factors, and postnatal experiences make an important or perhaps even prevalent contribution in determining the sexual behavior of an individual. Organicistic theories are based on evidence of clear anatomic and functional differences between the male and female brain, and their influence on psychosexuality and cognitive functions.

According to the neuro-hormonal or androgenetic theory, the psychological sex of a person depends on the hormonal environmental surrounding the CNS in the critical phase of fetal development which can be defined as the period of neuro-organization of sexuality. In particular, from the second to the fifth month of gestation, the hormonal environment involving limbic-hypothalamic structures might influence the future development of gender identity and sexual orientation. However, the more complex aspects of sexual behavior typical of each sex could depend on hormonal environment effects on the specialized areas of CNS throughout the entire gestation period and in the perinatal period.[10, 40, 41] Also in this case the presence of an adequate quantity of testosterone (or other androgens) could determine masculinization or defeminization of CNS structures controlling sexuality.[42, 43]

These areas, in the absence of said masculinizing signals, seem to be orientated towards a feminine development, in analogy with the whole biological process of sexual differentiation.

Additionally, testosterone and other androgens are subjected to important metabolic transformations in the CNS both in the fetal and the neonatal period. Two important enzymatic systems related to these reactions are described: 5 alpha reductase, responsible for conversion of testosterone to 5α-dihydrotestosterone, and aromatase, which converts androgens into estrogens. It seems that the products of these enzymatic reactions can mediate the testosterone action on sexual differentiation of the brain and, consequently, on sexual behavior.

Estradiol is derived from aromatisation of testosterone by cytochrome P450. It can bind to the estrogenic alpha receptor which induces masculinization. Conversely, the beta receptor induces defeminization of sexual behavior in some inferior mammalian species,[44-46] but not in humans, as demonstrated by humans with defective estrogenic receptors or aromatase deficiency.[47] Yet in rats, some typical male behaviors (*e.g.*, aggressiveness) require the presence of both testosterone and estradiol for their expression.[11, 48]

Instead, there is no clear evidence concerning the role of testosterone secretion in the neonatal period on the masculinization of the primate brain, where the androgenic postnatal suppression blocks the correct development of external genitalia and of male sexual behavior, which, however, can occur again after the administration of testosterone in adults, showing the predominant influence of prenatal androgenic exposure.[49]

Also puberty is probably involved in the definition of psychosexual differentiation.[50, 51] In this phase, the brain should already be differentiated and sexual maturity should consequently be achieved. There are, instead, other modifications both steroid dependent and not, such as, for example: reduction of bulbous cavernosum dendrites (testosterone-dependent), reduction of amigdala medial nucleus (testosterone-independent), higher response to female pheromones with an increase of dopaminergic activity in the preoptical medial area. It could be hypothesized that alterations of these processes may be involved in atypical sexual behavior.

In conclusion, the neuro-hormonal theory of prenatal psychosexual organization is based essentially on animal experiments conducted on different mammalians, including primates. In these experiments, it was possible to obtain a reversal of sexual orientation, modifying with different methods the hormonal environment and, in particular, androgen levels in the prenatal or perinatal period.

Further interesting experimental evidence supporting this theory is the following: high levels of stress in pregnant rats caused a higher frequency of partial reversal of sexual orientation in male offspring. It is believed that maternal stress may decrease the secretion of testosterone in the fetus. This reduction of hormone secretion could explain the abnormal psychosexual orientation observed. Obviously, it is very difficult to apply these results obtained by animal models to humans.

The higher complexity of the structure of the human brain can predict a higher influence of postnatal experiences on psychosexual development. Moreover it is impossible, for ethical reasons, to conduct similar experiments on humans. However, there are some diseases or accidents which expose the fetus *in utero* to an abnormal hormonal environment, which could be considered an experimental human model.

Biological aspects of homosexuality and of disorders of gender identity

First of all, it is important to clarify certain terms and classifications.

We define "disorders of sexual differentiation" (DSD) as congenital conditions in which the development of chromosomal, gonadic and phenotypical sex is atypical, different from the normal evolution of a male or a female, and leads to abnormal development of the sex organs.

A recent classification removes the definitions of "hermaphroditism" and "pseudohermaphroditism", preferring to speak about:

— disorders of chromosomal sex (Klinefelter's syndrome and variants, Turner's syndrome and variants, mixed gonadal dysgenesis, chimeras/mosaics);

— 46, XY DSD (disorders of testicular development, disorders of androgen synthesis or action, other);

— 46, XX DSD (disorders of ovarian development, excess of androgens, other).

In humans, there is no evidence that chromosomal sex (XX, XY, XXY, X0, XYY, etc.) has a direct influence on gender identity and sexual orientation. Conversely, the influence could be indirect and depends on the embryonic gonads and their hormonal production.

The diagnostic criteria of "gender identity disorder", also called "gender dysphoria", are classified in the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision, American Psychiatric Association) as follows:

a) strong and persistent cross-gender identification (not merely a desire for any perceived cultural advantages of being the other sex);

b) persistent discomfort with his or her sex or sense of inappropriateness in the gender role of that sex;

c) the disturbance is not concurrent with a physical intersex condition;

d) the disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

In particular, transsexualism is the development in an individual of a gender identity different from the phenotypical sex. We can also consider it as an extreme "gender dysphoria", with a mismatch between gender identity and role and primary in respect of secondary sexual characteristics.[57]

Homosexuality, referring exclusively to the sexual attraction towards members of the same sex, is not included in gender identity disorders.

Morphological differences and neuroendocrine functions

Over the years, different studies have compared the brain morphology and hormonal and functional aspects between homosexual, heterosexual and transsexual individuals. Some studies have shown differences between homosexuals and heterosexuals regarding the volume of the Interstitial Nuclei of the Anterior Hypothalamus,[58] the anterior commissure [42] and the suprachiasmatic nucleus (SCN);[59] however, these data have not been confirmed.[60, 61]

Similarly, autoptic findings have stressed some differences in transsexuals concerning the bed nucleus of the stria terminalis (BSTc),[62] but limited to only a few patients.

In general, these studies are controversial in terms of their trustworthiness and they remain difficult to interpret.

Regarding the different neuroendocrine functions, in the 1970s the hypothesis of an atypical positive feedback of estrogens in homosexuals was considered. Dorner described that homosexuals showed higher positive feedback to estrogens than did heterosexuals and bisexuals, showing a feminine differentiation in the central nervous system. This research was also conducted on transsexuals with similar results, while in transsexual women a suppression of positive feedback to estrogens was revealed. These results, criticized because of the low number of subjects studied, have not been confirmed by other authors, and the theory of positive feedback of estrogens as neurondocrine marker of sexual orientation has, for the moment, been abandoned.

As regards the hormonal pattern of adult homosexuals and transsexuals compared with heterosexuals, no substantial differences have been shown with sufficient evidence [63, 64, 54]. This regards both plasmatic values of gonadal hormones and gonadotropins, and the secretion pattern of gonadotropins. In particular, hypoandrogenism and hyperestrogenism in humans causes a reduction of libido and sexual potency, but usually do not cause homosexuality or bisexuality.

The presence of a normal hormonal pattern in transsexuals and homosexuals could reveal that the postpuberal testosterone production is not related to the quantity of testosterone, which reaches the CNS in the prenatal period, the phase in which the psychological sex is determined.

Prenatal organization of the psychosexuality

One hypothesis for the basis of homosexuality and transsexuality is the existence of an alteration of the sexual differentiation of the brain *in utero* for hormonal modifications in a critical phase of fetal growth. An example is an unfit androgenization *in utero* in the case of transsexuality or male homosexuality. This hypothesis finds some support in two particular situations.

In these circumstances, the human fetus is exposed to an abnormal hormonal ambient *in utero*. The first condition is the adrenogenital syndrome, an enzymatic anomaly with genetic pathogenesis. In this syndrome, there is an overproduction of androgen from the adrenergic glands, which starts at the early age.

The second condition is the exposure *in utero* to sexual hormones having an estrogenic or progestinic action, which were given to the mother for the treatment of pregnancy problems.

These drugs could provoke the phenomena of masculinization or feminization based on the type, dose and administration period of the drugs. In particular, these phenomena influence the typical behavior of each sex and they prescind from any anomalies associated with the morphological sex, as in adrenogenital syndromes.[54, 65, 66]

It seems that the effects of estrogens and anti-androgens in males could modify the gender identity and the lateralization of cerebral functions.[67]

In the first clinical situation, the adrenogenital syndrome can present with different degrees of gravity, based on the enzymatic deficiency at the origin. In the classical form (salt loss or simple virilizing), the female fetus is exposed to high androgen levels during the development, with possible virilitazion of genitals and development of male behavior. In the non-classic form, however, manifestations of androgen excess occur after birth, during childhood or adolescence, in the form of hirsutism, oligoamenorrhea and acne.

Yet in 1960, Erhardt and Money [68] showed how young girls affected by adrenogenital syndrome tend to adopt male behavior, preferring boys' toys, competitive sports and unfeminine clothing. They also demonstrated that these people have more masculine features, including various spatial skills in three dimensions.[69, 70] It seems also that the more masculine behavior in these young girls during childhood is strictly related to the degree of virilization of the genitals, which is an indicator of prenatal exposure to androgens.[71] The majority of patients affected by the adrenogenital syndrome present with a female gender identity, despite some typical male features, proving the direct effect of prenatal androgen exposure on the human brain.[72] Several studies showed an incidence of homosexuality and bisexuality in 1/3 of girls affected by this syndrome.[73, 74]

As far as the classic form is concerned, there are authors who have also documented a higher incidence of sex reversal.[75, 76] In a 2007 review, De Vries *et al*.[77] observed that 5% of adolescents affected by DSD and women affected by adrenogenital syndrome present some form of gender dysphoria, which can result in a sex reversal also.

Instead, in the non-classical form, the fetal hyperandrogenism could be sufficient to provoke a slight male differentiation of the brain, even without virilization, with a tendency to bisexuality and homosexuality with respect to the general population. It is believed that sexual orientation in these cases can be partially predicted both from the degree of prenatal exposure to androgens and from postnatal psychosocial influences.[78]

Other evidence of the pre and postnatal hormonal influence on psychosexuality is the androgen insensitivity syndrome. In this syndrome, patients with XY karyotype have testicles producing normal testosterone levels, but they are ineffective due to

receptorial resistance. The degree of virilization depends on the degree of receptorial resistance: complete androgen insensitivity syndrome (CAIS) or partial androgen insensitivity syndrome (PAIS). Some authors [79] compared women affected by CAIS with normal female subjects, finding that the former have a greater preference for typical male toys and activities during childhood, even though they maintain a feminine gender identity.

Women affected by PAIS showed a greater incidence of gender dysphoria, with sex reversal in some cases.[80]

Maternal prenatal stress

Maternal stress has been considered a possible cause of sex reversal in men. A high percent of male homosexuals in Germany during the Second World War (1941-1946) allows for the hypothesis that severe stress the women were under could have had some role in their sons' homosexuality.[81]

The following studies confirmed that 2/3 of male homosexuals' mothers referred stressful events during pregnancy (bereavement, divorce, financial problems and similar), against 10% of mothers of heterosexual males.[81, 82]

It is possible that a fetal testosterone reduction caused by maternal stress plays a role in the determination of sex reversal, as hypothesized in animal models.

Neurohormonal "organicistic" theory: indirect evidence

Neurohormonal organicistic theory could find indirect support from the following observations:

— homosexuality and transsexuality are prevalent in males at a rate of M:F 3/1 for transsexuality, in line with the hypothesis that also psychological sex has a basal feminine orientation in absence of virilizing signals;[83, 84]

— homosexuality and, in particular, transsexuality, which express themselves clearly in puberty, are associated already in childhood with behaviors, attitudes and mannerisms typical of the opposite sex. This is to be expected if a prenatal neuro-organization of psychological sex as a whole is supported;[85, 86]

— different studies exist which evaluate gene polymorphisms possibly involved in homosexuality, supposing that homosexuality is a common finding in humans and in animals, as well as in transsexuals. Moreover, cases of male homosexual identical twins and cases of familiarity for homosexual orientation have been reported.[85, 87-90] There are, however, no conclusive studies on this topic:[19]

— attempts to interfere with homosexual and transsexual psychological sex through psychotherapy and/or social pressure are rarely effective, in line with the hypothesis of prenatal programming of psychological sex at the hypothalamic limbic level.[84]

At present, some authors who do not accept an absolute prenatal hormonal determinism maintain that the hormonal prenatal milieu simply changes the threshold for the development of an appropriate psychosexuality, interacting in any case with a series of postnatal variables.

So, in females androgenized *in utero*, the anomalous hormonal fetal environment could simply reduce the threshold for the appearance of the phenomenon of sexual ambivalence, and could raise, vice versa, the threshold for the complete emergence of femininity.

A similar approach could be used for explaining a syndrome that is usually presented as proof of pre and postnatal hormonal conditioning of psychosexuality, *i.e.*, the 46 XY DSD syndrome (male pseudohermafroditism) due to congenital deficit of 5 alpha reductase, an enzyme responsible for the conversion of testosterone into DHT. This disease was studied by the American endocrinologist Imperato-Mc Ginley *et al.*[91, 92] in a large group of inhabitants of an isolated mountainous region of the Dominican Republic. These individuals, who are genetically and gonadically male, are born with a considerable external genitalia ambiguity due to a deficiency in DHT, and they are

recognized to be, and are raised as, females. At puberty, in relation to the increase in testosterone and despite the enzymatic deficiency, they demonstrate a high degree of virilization.

Surprisingly, it has been observed that in the same period the majority of subjects examined changed gender identity from female to male, and showed sexual preferences for women. The authors' conclusion is that "androgens play a role as inductors (*in utero* and in neonatal period) and as activators (in puberty) in the gender identity evolution in the male". They also deduced that when the sex in which someone is raised is opposite from the biological, hormone-mediated sex, the biological sex prevails.

This means that "the degree of androgen exposure on the human brain *in utero*, in the immediate postnatal period and then at puberty, has a higher effect in determining male gender identity than sex imposed by the environment". An analogous phenomenon is described by other authors.[93, 94]

However these conclusions do not take into consideration the effective importance that socio-cultural determiners have on their psychosexual evolution, and for this reason they were the subject of criticism. In fact, it is difficult to admit that these individuals have female social status during their childhood and adolescence. In their village they are ridiculed by being called "machehembra" (half woman half man) and probably for this reason up to puberty they have an ambivalent or imprecise gender identity. At puberty, neither feminized nor virilized, they find themselves having to "choose" the sex they belong to, and it is probable that they sense from their family and society that choosing the male sex will allow them to have a better life. Also in this case, the hormonal determiners and the environmental influences concur in the development of psychosexuality.

A confirmation of the role of environmental postnatal factors comes from the study of comparative biographies of subjects affected by sexual ambiguity due to anomalous sexual differentiation (for example, the adrenogenital syndrome).

If individuals which are identical from an organic hormonal point of view but with a different official status are studied, it can be observed that these individuals develop a gender identity and sexual preference in agreement with the sex attributed to them at birth and according to the sex in which they are raised. In this same sense, Gooren's studies [19] indicate that prenatal androgenization may predict the development of male gender identity in a non determinant way. In fact, 40-50% of 46 XY DSD individuals with apparently normal prenatal exposure to androgens do not develop a male gender identity. Similarly, there are male to female transsexuals who develop a female gender identity despite seemingly normal prenatal androgenization and female to male transsexuals without evidence of prenatal androgen exposure. Again, 46 XX individuals with normal prenatal exposure to androgens show in adulthood a marked masculinization of gender role, but they have no type of gender dysphoria.

It is not clear yet what the importance of hormonal imprinting on gender identity development is. As of today, no biological or psychological studies have given a satisfactory explanation of transsexualism.

While it is probable that a prenatal program based on neurohormonal influence on CNS might determine the subsequent development of gender identity and sexual orientation, it should be considered that in humans an equally or probably even more important role is represented by the social environmental influence in the post natal period.

Apart from every determinism, it seems reasonable to suppose that the interaction between constitutional prenatal factors with educational-social conditioning in childhood leads to the development of psychological sex in its various components.

Riassunto

Aspetti biologici dei disturbi di genere

La comunità scientifica è molto interessata dagli aspetti biologici dei disturbi di genere e dall'orienta-

mento sessuale. Esistono vari livelli per definire il sesso di un individuo: sesso cromosomico, gonadico e fenotipico. Per quanto concerne il sesso psicologico, uomini e donne sono diversi in virtù della loro identità di genere, il che significa che riconoscono se stessi come appartenenti a un determinato sesso. Essi sono diversi anche come conseguenza della loro identità di ruolo, una serie di comportamenti, tendenze e attitudini cognitive ed emotive, comunemente definite come "maschili" e "femminili". La transessualità è un disturbo caratterizzato dallo sviluppo di un'identità di genere opposta al sesso fenotipico, mentre l'omosessualità non è un disturbo di identità di genere ma solo di attrazione sessuale, poiché esprime un orientamento sessuale verso persone dello stesso sesso. Abbiamo cominciato con una rassegna critica della letteratura sui meccanismi genetici e ormonali implicati nel differenziamento sessuale. Abbiamo riesaminato le differenze neuro-anatomiche e funzionali tra uomini e donne, con particolare riferimento al ruolo che esse rivestono nel differenziamento psicosessuale e alla loro possibile implicazione nella genesi dell'omosessualità e dei disordini dell'identità di genere. L'omosessualità e la transessualità sono condizioni prive di un'eziologia ben definita. Sebbene l'influenza dei fattori ambientali ed educativi negli esseri umani sia innegabile, fattori neuro-ormonali organici, prenatali e postnatali, potrebbero contribuire in un determinato modo allo sviluppo di queste due condizioni. Tale teoria "neurormonale organicistica" potrebbe trovare supporto nello studio di particolari situazioni nelle quali il feto umano è esposto a un ambiente ormonale anormale nell'utero.

Parole chiave: Sesso, identità - Omosessualità - Disturbi sessuali e di genere - Differenziazione sessuale - Disordini della differenziazione sessuale - Iperplasia surrenalica, congenita.

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