[MUSIC PLAYING]

**SELMA WITCHELL:** Thank you for inviting me to talk today about the biology of gender. And we'll start here.

We live in a very binary gender society. During pregnancy, everybody wants to know whether you're having a boy or a girl.

Decide now looking at ultrasounds in utero to wanting to know what the gender of the child is, the newborn gender is assigned by the appearance of the external genitalia. But what happens when the external genitalia are ambiguous? That involves consulting a pediatric endocrinologist to evaluate for a disorder of sex development.

This means we have disorders of sex development, which are patients with ambiguous genitalia, a disorder called congenital adrenal hyperplasia, gonadal dysgenesis, complete androgen insensitivity.

But there are also patients in whom the chromosomal sex and external genitalia are congruent. They have issues in terms of how they feel about themselves in terms of gender identity or concerns. These are the transgender youth.

Here at the Children's Hospital of Pittsburgh, we have a team approach with multiple subspecialties working together as a team.

As a background, humans have been fascinated by sex development for centuries. Both [INAUDIBLE] and Aristotle gave their opinions about how gender came about in sex.

Greek medicine included the concept of right and left and hot and cold polarities and numerical beliefs about the number 7 to justify multiple births and sex determination. They described the womb as being cold and dry, and testicles were hot and dry.

The womb was divided into seven cells, as one can see in this picture here. The three on the right were for boys, where it was close to the liver and warm. The three on the left were by the spleen for girls, where it was cold. And the one in the middle was for what they called the hermaphrodites, which were children who had ambiguous genitalia.

Hermaphroditism was invoked to explain the existence of children with ambiguous genitalia. Affected individuals were considered to have double genitals, and Hermaphrodite was the son of Hermes and Aphrodite. He was considered to represent in intermediate sex, and he was the Greek god who was the patron of sexual union.

This is an a sculpture that was a copy of a bronze sculpture from the second century BC to look at the sleeping Hermaphroditus, where one can see breast development, as well as male genital structures. Again, pointing out that people have been interested in sex for centuries.

This concept of transgendered individual is not new either. This is a painting entitled Dance to the Berdache. The berdache are two spirit people-- it's a term used by some Native American tribes to describe gender variant individuals in their communities.

Male berdaches combine the social roles assigned to both genders. They can dress like women, they could alternate, they could dress like men, and they could do careers related to both genders.

Yet in the 19th century, along the same time as this painting was made, there was an increased focus on categorization and cataloging. What was one to do with a person who seemed to be neither or both male and female?

And most importantly, how could one distinguish between normal and homosexual relations if one could not clearly divide all parties into male or female? Remember at this point in history, homosexuality was considered to be a crime.

Everyone, everyone, including every doubtful person had to have one true permanent sex. But what happens when assigned gender does not match gender identity?

Gender dysphoria is defined as the distress caused by incongruence between one's expressed experienced or firm gender and the gender assigned at birth based on external general structures.

This slide shows a cartoon of the gender bred person, which points out several of the concepts to gender identity. There is gender identity in terms of being woman or male or somewhere along the spectrum, recognizing that there is some fluidity in terms of gender identity. There's gender expression-- how does one dress, wear their hair, what occupations does one choose in terms of gender expression.

Biologic sex refers more to the external and internal general structures for maleness or femaleness. There's also the gender in terms of attraction-- who is one interested in in terms of being close and intimate with. Are you interested in the opposite sex, the same sex, or is there bisexuality or pan-sexuality?

Here is another example of a dual gender person We'wha, who was a Zuni American Indian who lived in the mid to late 19th century. So this concept of transgender is not new, yet it's important to recognize that today, transgender youth are a hidden and vulnerable population.

The data suggests that transgender youth have a higher risk than their non-transgendered peers for negative health outcomes and health risk behaviors, including substance use, depression, suicidality, anxiety, sexual and physical abuse, sexually transmitted diseases, social isolation, and homelessness.

This table shows the baseline data for hopefully a longitudinal study to look at outcome for adolescents with gender dysphoria in Los Angeles. One sees that over half were living with their parents, but 6% were in homeless shelters because their parents rejected them.

There was a broad range of body mass index, but over 20% were considered to be obese. At least 10% had severe depression, and very scary, over 25% had had at least one suicide attempt.

The goal of this study is to look at trajectory and interventions over time.

This slide shows our data from the GEM study here in Pittsburgh, looking at trans youth and non-trans youth, mean age about 17. Under the trans youth, one sees the differences, statistically significant for victimization, depression score, anxiety score, self-esteem score, cigarette smoking, alcohol use, and sexual activity.

So what, if any, are the hormonal, environmental, genetic, epigenetic features that are associated with gender dysphoria?

We know that there are multiple factors that influence gonadal development, starting from the undifferentiated or bipotential gonad in the early human embryo. There are a number of steps, genes, and other factors that influence differentiation to testis or ovary, that the testis is producing testosterone.

The brain is also a sexual organ. So is there during early fetal life exposure to androgens during these critical stages that influence development and organization for the biology and behavior?

We have some data looking at animal studies. This is an old study, looking at prenatal and postnatal androgen treatment in the behavior of female guinea pigs.

In these guinea pigs, testosterone promoted male typical or male typical mounting behavior. The lack of testosterone supported female typical behavior. This led to what was called the organizational activation hypothesis, which we'll continue to explore.

Organizational effects are permanent changes in brain that have long lasting effects. Activational effects are temporary or transient behaviors or consequences that require an exposure later in life.

So what is the molecular basis of gender dysphoria? To try and parse this out, people have looked at sex chromosome and y chromosome effects on human behavior, development, gender identity, and sexual orientation using individuals with disorders of sex development as experiments of nature and models, particularly females with congenital adrenal hyperplasia. The interest in females congenital adrenal hyperplasia comes from the fact that they have been exposed to high levels of androgens throughout the pregnancy.

Outcomes that have been assessed-- sexual behavior, aggression, male behavior particularly towards infant care, spatial abilities, play and toy preferences, gender identity, brain volumes, and functional MRI.

Back in the 50s and 60s, there was questions regarding nature versus nurture, with the hypothesis being that nurturing could influence gender identity. So girls with CAH were looked at, girls with CAH are more likely to play with boy typical toys and engage in male typical leisure activities and occupations.

Over half the study showed the girls with CAH have increased masculine identity, where the remainder of the studies found no difference. In general, the more severely virilized girls, presuming that meant greater androgen exposure in utero, showed the greatest degree of masculinization of their external genitalia as well as their behavior.

This is a more recent study, trying to better address the issue of gender identity in prenatal exposure to androgen, again using girls with congenital adrenal hyperplasia.

In the study, there were four groups of children ages 4 to 12 years of age. There were girls with congenital adrenal hyperplasia, control girls, boys with congenital adrenal hyperplasia, and control boys.

Three measures were used-- there was a gender identity questionnaire for the children, parent report gender identity questionnaire, and a parent interview for cross-gender identification.

The results showed that the younger children gave more ambiguous responses regarding their gender identity. However, the girls with CAH gave the greatest number of ambiguous responses.

More girls with CAH greater preferences for cross-gender roles, games, and peers. This slide is a graphic representation of these data, with higher values indicating more cross-gender identification.

And so there's blue indicates the gender identity composite, red gender role behavior composite. And one sees that girls with CAH had higher gender identity and gender role behavior than all the other groups.

However, most women with CAH are heterosexual. For women with CAH, physical change from female to male is relatively uncommon, but occurs more frequently than among the general population.

Looking at other forms of disorders of sex development, 46 xy individuals with minimal androgen exposure, such as women with complete androgen and sensitivity, are raised as females and generally identify as females and stay in the female gender.

In contrast, individuals with another endocrine disorder 5F reductase deficiency undergo ongoing androgen exposure and even if raised as females initially often undergo transgender and become males as they become teenagers and developed signs of virilization.

This androgen exposure seems to play a decisive role in the development of gender typical behavior in children with disorders of sex development.

There's an emerging theme here that there are organizational effects of prenatal androgen exposure, but it's hard to explore these in humans, so what about other animal species?

So rodents, guinea pigs, rats, mice have been used, and these are the results of a study using guinea pigs where they were male and female guinea pigs are looking at prenatal and neonatal testosterone on behavior.

The males who were not treated exhibited typical mounting behavior. The females who were not treated fitted typical female lordosis behavior.

The males that were castrated immediately after birth became non-masculinized and adopted female behavior and were subject to lordosis, whereas the females who are treated with testosterone or estradiol were masculinized.

And you might ask, well, how did estrogen mascunilize the fetus of these animals? Well, it turns out that in rodents, testosterone is aromatized in the brain to estradiol and actually estradiol that causes the masculine effects.

In another experiment, a single injection of testosterone given to female mice immediately neo-natally in the period of time where there's a surge of testosterone in male mice, caused them to lose her estrous cycle, become acyclic. And their body parameters in terms of weight gain and body mass were more typical of males than females, so this is only a single shot of testosterone lead to changes in behavior.

As I mentioned, in the rodent, estradiol is the masculinizing hormone and acts through multiple mechanisms as illustrated in the picture below. There are effects on cell proliferation, cell death, synapses, and histone acetylation.

An endpoint that can be measured is lordosis behavior. Again, testosterone effects on male rodent brains are mediated by aromatization of androgens to estrogen.

Females are protected from this because of a protein called alpha fetoprotein that circulates in the fetus. Androgen receptor knockout female mice, never manifest lordosis response. This would be expected-- they don't see androgen, so they would have no lordosis response.

However, if these mice are treated with estrogen in a susceptible period of time, they have male-like behavior. So that the estrogen administered post-natally was effective in causing them to change their behavior.

Switching to non-human-- to our own species, [AUDIO OUT] were done giving flutamide to males was associated with early puberty, a female pattern. Now what flutamide does is it prevents androgen actions in the male fetus. So in other words, these fetal primates were not allowed to see testosterone and therefore had a more female pattern of puberty.

Yet manipulating prenatal testosterone exposure did not alter the timing of females. So this seems to be testosterone effect on males.

Blocking pubertal hormones in the neonatal period in males also lead to a problem with their mini puberty-- so they didn't have the typical testosterone surge-- and that too changes parameters for their hypothalamic pituitary gonadal axis.

So in conclusion, prenatal testosterone exposure in males appeared to organize the hypothalamic pituitary gonadal axis.

Male monkeys, like human boys, showed an overwhelming preference for wheeled toys-- they like trains and airplanes and boy kind of toys. Female monkeys would play with those, but they like girl toys as well-- plush toys-- but didn't really show one preference for one kind of toy over another.

Male monkeys, like little boys, like rough and tumble play and wrestle with each other. Typically female monkeys don't engage in that kind of behavior, but female monkeys exposed to testosterone prenatally on day 40 of gestation showed more rough and tumble play than control females.

So again, we have this emerging theme of organizing effects of prenatal androgen exposure on the brain. The effects can be programmed early in life and persist throughout the lifespan. They can be programmed early in life in response to hormones that require an activation later in life, such as puberty.

They can be transient due to context or hormonal exposure, they can be direct or indirect, they can be related to differences in how a male or female offspring is reared, and there may be physical constraints due to somatic sex differences.

Some potential mechanisms responsible for sex difference include the sex chromosomes, both x and y. It can harbor multiple genes, just not-- in addition to SRY that affects sexual differentiation. There could be other genetic imprinted or epigenetic factors that influence sex differences.

Hormones and other factors act directly or indirectly on the developing brain and other cells to influence sex development. Distinct brain regions have different responses or patterns to sex-specific signals, which likely involve cell-specific responses, cell to cell communication, membrane and nuclear hormone receptors, and local steroid synthesis.

In other words, one size does not fit all. There may be very local effects.

There can be antagonistic factors-- the classic example in the ovary is SRY versus [INAUDIBLE]. Whichever one is predominant affects gonadal differentiation. So there may be other factors influencing brain development and brain identity.

Environmental factors such as endocrine disruptors, stress, differences in maternal behavior, social expectations, and learned behavior may also influence sex differences.

And this is just a brief synopsis of pathways involved in sexual differentiation of the brain. Gene dosage, epigenetics, other factors, influencing cell death, survival, local cell to cell communication, et cetera.

But back to humans. There can be children who preset at very young ages saying that they're in the wrong body.

We've had patients who've come in asking their parents why God gave them a penis because they didn't want one. There are also teenagers who present with gender dysphoria, who appear that the changes at puberty brought forth their concerns about their gender.

Dysphoria in adolescence is generally much more severe and exacerbated by puberty. The natal females who identify as male are very much stressed and distraught when they undergo menstrual periods because that reminds them every month that they are born a female when they identify as a male.

Many of the younger children no longer fulfill requirements for gender dysphoria as they become older. Many turn out to be homosexual, many desist. However, there are no long term data to know what happens when these particular population of children reach their 20s. So there seems to be a group of children who have gender dysphoria that seem to refer to their natal gender during early adolescence but we don't know the natural history of those children.

Most of the current experience is related to the Dutch, who've had the greatest experience of physicians throughout the world and in managing adolescents and children and adults with gender dysphoria, they have a protocol where they suppress puberty at 12 years of age with medications that are designed to stop pubertal progression. They offer cross-sex hormone treatment at 16 years of age and gender affirming surgery at 18 years of age.

These data reports and outcome study on 22 natal males and 33 natal females. They reported that following cross-sex hormone treatment and gender reassignment surgery, the gender dysphoria was largely resolved in these patients. Their well-being and functioning in society were fairly comparable to their peers, and none regretted the treatment. These individuals sought jobs and furthered their education.

However, there are several limitations to the Dutch outcome study. It was a relatively small sample size, they did not provide information on physical side effects, there is no information on bone health, no information on satisfaction with sexual functions, and no long term outcome data are available. However, I trust that the Dutch will report that in the future.

To get a further handle and understand the biology, people have looked at functional MRI in patients with gender concerns or disorders of sex development. They have looked at [INAUDIBLE] specific brain regions, activation of specific brain regions, and task-driven activation of specific brain regions, because men and women differ in some of their abilities with tasks, particularly spatial ability.

This is a study looking at functional neuroimaging to assess sexual arousal in three groups of patients-- women with complete androgen insensitivity-- these are xy individuals, so they have a y chromosome they have the SRY gene. They make androgens, their bodies just do not respond to it.

So they see themselves as women, and their brains, although testosterone is made in their bodies, their brains do not perceive it or recognize testosterone.

Control women and control men-- men showed greater amygdala activation to sexual images than did either typical women or the women with complete androgen insensitivity. Control women and the women both complete androgen insensitivity had highly similar patterns of brain activation.

Thus the y chromosome itself is insufficient for male typical brain responses. However, we cannot exclude from the study the effects of social experience on the brain responses of the women with congenital and complete androgen insensitivity, as all were raised as girls.

Further examining this population to look at a functional study. Again, the same three groups of patients-- women with complete androgen insensitivity, control women, and control men, and they did have a mental rotation task using functional MRI.

The control men showed a greater activation in the inferior parietal lobe than control women. Individuals with complete androgen insensitivity, again, not as a surprise, showed a female-like pattern.

The conclusion was that sex differences in regional brain function during mental rotation appear to be more related to gonadal hormone exposure than genetic sex. And again, this is said because even though women with complete androgen insensitivity make testosterone, their bodies don't recognize it. So their abilities with the functional MRI appear to be those comparable to normal women.

This is another study looking at teenagers with gender dysphoria. And again, it's looking at-- visio-spatial functioning and a functional MRI and using, again, the mental rotation task.

There were three groups-- there were control girls, control boys, and girls with gender dysphoria, but these were a specific group. These were natal females who identified as males, but they were interested in females as their sexual partner.

So the cartoon up on the top right indicates the study course. So they did functional MRIs and the mental rotation task.

Early, they treated the patients with [INAUDIBLE] with testosterone and then did these same studies at the end. The control girl showed greater activation in the frontal brain regions compared to control boys for the mental rotation task.

The girls with gender dysphoria who were interested sexually in girls showed decreased right frontal activation compared to control girls prior to testosterone treatment. And after testosterone treatment, the girls with gender dysphoria and boys show increases in the brain regions implicated in mental rotation.

And during this time period, the boys had their own endogenous testosterone because they were going through puberty. So that boys have undergoing normal puberty as well as the natal females with gender dysphoria receiving testosterone had fairly similar outcomes.

This is the brain activation pattern during the first mental rotation comparing control boys on the top, the girls with gender dysphoria and the control girls, and the girls with gender dysphoria had a pattern that was more similar to that of the boys.

Limitations for the groups different because girls with gender dysphoria were on [INAUDIBLE] to prevent pubertal progression. Effects of menstrual cycle were not controlled for in either group, and so that may have influenced the outcome.

And another is the girls with gender dysphoria shared interests and hobbies with the boys. And so their identification just because of feeling like they were a boy may have influenced the results of the testing.

Nevertheless, these studies suggest that atypical sexual differentiation to bring in the natal girls with gender dysphoria and provide new evidence in organizational and inactivational effects of testosterone on visio-spatial cognitive function, suggesting that there may be something different in the brains of the adolescents that we see with gender dysphoria.

For summarizing activational and organizational effects, there are potential factors, again, listed influencing the neurobiology of gender identity beginning with testosterone and estrogen in utero during critical perinatal period with critical peri-pubital and adult periods that may influence the expression.

Back to a little bit more practical information in terms of management for children and adolescents. We use pubertal suppression with medications called [INAUDIBLE] or histerelin which are reversible treatments to enable additional time to explore gender identity.

This prevents the pubetal progression that does not match the individual's self identity or gender identity. Particularly, it stops menses for the natal females who identify as males who are distraught and occasionally suicidal every month when they have their menstrual flow.

And it allows the individual to present better in their affirmed gender, particularly if the medication is started before the signs of puberty begin. In other words, prevent breast development in the natal females who identifies as male and prevents development of facial hair in the natal males who identify as female.

One important topic that needs to be mentioned is fertility preservation that needs to be discussed. WPATH and the Endocrine Society clearly state that individuals should consider fertility issues before initiation of cross-sex hormone treatment and surgical procedures.

The long term data suggests that the cross-sex hormone treatment may alter fertility and these individuals may lose their fertility. There are options for trans women, in other words, natal males who identify as females would require preservation of sperm. For the natal females who identify as males, there can be cryro-preservation of their osites, bagging of ovarian tissue, and in a situation where a partner has already been identified, there can be embryo banking.

Cross-sex steroid hormones are used. For the male to female would be estrogen, and there are several different ways to give estrogen-- transdermal, oral, or intramuscular. And for the females to male, testosterone can be given is intramuscular injections or transdermal or using a gel.

Monitoring is important during puberty suppression. We monitor gonadotropins to make sure the medications are effective, measure calcium, bone density, and bone age during cross-sex hormone treatment. These are monitored as well as blood pressure, blood count, basal metabolic panel, liver function studies, lipids, glucose, and hemoglobin A1C.

There are risks associated with use of cross-sex steroids. For the feminizing hormone treatment, there's a high risk for venous thromboembolic events. Liver functions may be elevated. There may be infertility and dyslipedema. There's a possible increased risk for breast cancer, hyperprolactinemia, hypertension, and cardiovascular disease.

For masculizing hormone treatments, would be polycythemia, male pattern baldness, and I tell all the boys-- or the females wishing to be boys, so I call them boys-- that once they start testosterone, there's a chance they may lose their hair. And once it's lost, it won't come back, just as in typical for male pattern baldness.

There may be hair growth in androgen dependent areas, sleep apnea, acne, and infertility. There's a possible risk for elevated liver function studies, dyslipedema, breast, cervical, uterine, ovarian cancer for the natal females who become males. They still have their internal female organs and are at risk for typical adult female disorders, including hypertension and cardiovascular disease.

Most importantly, we believe in a gender affirmative model. Gender variations are not disorders. Gender identity may be fluid, and is not binary.

Presentations of gender identity are diverse and can vary over time, even in the same individual. Gender reflects inter-weaving of biology, development, socialization, culture, and contexts. Gender is primarily informed by individuals' cognitions and emotions, not their genital structures.

Certain ethical considerations need to be addressed. One needs to be respectful of autonomy, to do no harm, to think about the patient's best interests. The patient who is making decisions and sees our lifelong decisions need to be confident, making good decisions, and understand the consequences of their decisions.

And one important piece is that the patient is consistent and persistent in their expressions of gender identity to some extent. Again, there's some fluidity, but overall, there should be general consistence and persistence to how they feel about themselves.

And we believe in a team approach, encircling the patient with the parents, the primary care physician. We have parent support groups, pediatric endocrinologist, adolescent physicians, and health care providers. We have support from behavioral health and social work and ultimately surgeons.

This complicated slide shows the multiple origins, because I think we're just at the beginning of understanding sex differences in the brain, and one can see that there's lots of factors listed on this slide, from hormones and genes and gonads and sex chromosomes and the environment.

And so with that, I'd like to thank you for attention.