Genome and Hormones: Gender Differences in Physiology
Invited Review: Sex-based differences in gene expression

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Ostrer, Harry Invited Review: Sex-based differences in gene expression. J Appl Physiol 91: 2384–2388, 2001.—Certain diseases are more prevalent among women than men. The reasons for this increased prevalence are unknown, but there could be a genetic basis. Increased expression of X-linked genes in females, protective effects of Y-linked genes in males, or sex-limited gene expression that is developmentally or hormonally regulated could all account for these differences. Analysis of individuals with and without genetic sex reversal provides a means for distinguishing between genetic and hormonal causes. This can be complemented by genetic linkage and gene expression profiling to aid in the identification of candidate genes.

Y-linked genes; X-linked genes; sex determination; sex-limited gene expression

WHEN MENDEL’S LAWS OF HEREDITY were rediscovered, they were applied rapidly to the study of human traits (8, 9, 35, 56). Within a short time, it became apparent that a number of traits, although seemingly genetic, represented deviations from Mendel’s rules. The first deviation from Mendelism, sex-linked inheritance, sought to explain why men and women were phenotypically different and why some traits were manifested more commonly in one sex. E. B. Wilson and Nettie Stevens observed differences in the chromosomal constitution of males and females in certain species. This led to the chromosomal theory of sex determination and sex-based differences (63). Sex-linked inheritance was found to be applicable in humans for hemophilia and color blindness and subsequently for other traits (4). With the advent of molecular genetics in the latter 20th century and more recently the sequencing of the whole human genome, many X-linked and Y-linked genes have been identified that can account for some of the phenotypic variability.

Genetic studies of sex-based differences were complemented by work in developmental biology and endocrinology. The pioneer in this field was Alfred Jost, who conducted a series of experiments that provided the framework for how the reproductive apparatus of males develop in response to hormonal signals transmitted by the testes and how the absence of such signals leads to the development of the reproductive tract of females (23). In the 1950s, the fields of genetics and developmental biology coalesced and led to the identification of individuals with genetic sex reversal, that is, people with a phenotype of one sex and a chromosomal constitution of the other. The study of such individuals, whether as sporadic or familial cases, was invaluable for gaining insight into the genetic pathways involved in sex determination (41). The study of such individuals could also prove to be invaluable for understanding how hormones, sex-linked genes, and sex-specific developmental pathways could account for other phenotypic differences between males and females. Here, I review the molecular genetics of sex chromosomes and differences in gene expression between males and females, some of which are known to affect sexual differentiation, with the goal of creating a conceptual framework for how the genetic basis of phenotypic differences between males and females might be approached.

MOLECULAR GENETICS OF SEX CHROMOSOMES

Several features emerged from the original work with sex-linked transmission (36). Males have an XY chromosomal constitution, whereas females have an XX chromosomal constitution. As a result, the sex of the individual demonstrates a paternal origin effect.
X-linked traits do not show a pattern of male-to-male transmission. Rather, in sons, X-linked traits demonstrate a maternal origin effect. To compensate for differences in gene dosage between males and females, a mechanism of X chromosome inactivation developed (see Molecular genetics of the human X chromosome below).

Sex chromosomes differ from autosomes in their organization. The sex chromosomes have two regions, a pseudoautosomal segment shared between X and Y chromosomes and a sex-limited region. The pseudoautosomal regions of the X and Y chromosomes pair at the tips of their short and long arms and undergo recombination during meiosis in spermatocyte precursors (22). The term pseudoautosomal is used because alleles that are inherited within the region are not transmitted exclusively to males or females and thus behave as if they were inherited on autosomes (52). Sex-based differences in gene expression may occur from the sex-limited regions of the X or Y chromosomes. Genes within the sex-limited regions of the X and Y chromosomes are linked to the sexual phenotype of the individual. Genes in the sex-limited region of the Y chromosome have a male-only pattern of transmission.

Molecular genetics of the human Y chromosome. Some of the genes in the sex-limited region of the Y chromosome have functions that could occur only in males, such as testis determination or spermatogenesis (27). Testis determination is mediated by the SRY gene (for “sex-determining region Y”) (16, 53). This gene is conserved on the Y chromosomes of most mammals and functions as a developmental switch. Once expressed in the gonadal ridge of humans, it remains on through the period of testicular differentiation and leads the enhanced expression of SF1 and other sex-determining genes in humans (18). Sry has been shown to function as a transcriptional activator in mice (10). Its mechanism of action in humans is unknown, although it has been proposed to function as a repressor (33). The SRY gene has been shown to be expressed in early male embryos and in the central nervous system of humans and mice (32). This observation has led to the suggestion that expression of SRY could account for hormone-independent, somatic differences between males and females.

Genes that promote spermatogenesis have been shown to exist in three different regions of the Y chromosome because deletion of one or more of these regions is found in individuals with oligo/azospermia (20, 25, 31, 60). Deletions of these regions remove one or more of the candidate genes (DAZ, RBMY, USP9Y, and DBY). Of these, DAZ is a favored candidate because DAZ protein appears to be expressed in the right place at the right time. This protein is present in both the nuclei and cytoplasm of fetal gonocytes and in spermatogonial nuclei; during male meiosis, it relocates to the cytoplasm (46).

A number of other genes have been identified on the Y chromosome. Some of these are present as single genes on the Y, whereas others have multiple copies on the Y or homologous copies on the X and Y chromosomes (54). With the use of deletion mapping techniques, functional significance has been ascribed to some of these genes, including those for stature, suppression of gonadoblastoma, and prevention of the Turner syndrome phenotype (39, 40, 57). Differences in histocompatibility can also be ascribed to Y-linked genes. The SMCY gene encodes the HY antigen, which is ubiquitously expressed in male tissues, as early as two-cell embryos (1). HY is a minor histocompatibility antigen that can promote immunologically mediated rejection of male tissues transplanted into female mice (14). SMY has an X-linked ortholog from which it differs by the presence of an HY epitope (defined by the octamer peptide TENSQKDI) that presumably accounts for the antigenic difference between males and females (51). The UTY gene also encodes a male-specific histocompatibility antigen that is recognized by female T cells in a major histocompatibility complex-restricted manner (61).

Other genes with X-Y homologs may serve similar functions but may differ from each other in subtle ways. The RPS4Y and RPS4X genes encode ribosomal binding proteins that differ at 19 of 263 amino acids. Both genes are widely transcribed in human tissues, which suggests that the ribosomes of men and women are structurally, if not functionally, distinct (12).

Molecular genetics of the human X chromosome. X-linked traits are characterized by absence of male-to-male transmission; they may be transmitted from fathers to daughters or from mothers to daughters and sons. If one copy of the mutant gene is required for expression in females, then the trait is dominant. If two copies are required, then the trait is recessive. The presence of the hemizygous state in males makes it easier to map the genes for X-linked recessive conditions than the genes for autosomal disorders. To date, over 1,200 X-linked conditions have been described (34). Some conditions are distinctive in their phenotypic features, whereas others, such as mental retardation, are nonspecific (35). The pattern of inheritance can infer the X-linked nature of these conditions. Precise mapping to specific regions of the X chromosome can be performed by linkage to specific markers. X-linked dominant conditions, such as incontinentia pigmenti, Rett syndrome, microphthalmia with linear skin defects, and Aicardi syndrome, may not show Mendelian transmission because they are expressed only in females and usually as sporadic cases. The paucity of male cases for these conditions is presumed to result from lethality during gestation (2, 17, 28, 29, 42).

Most X-linked genes have dosage compensation between males and females by X chromosome inactivation, a process also known as “lyonization.” This process of random inactivation of one of the two X chromosomes starts in early embryos (30). The inactivation originates at a site on the long arm of the X chromosome (termed the “X chromosome inactivation center”) and spreads over the chromosome (49). The inactivation is stable and is transmitted to the progeny cells. X chromosome inactivation skips over some regions of the X chromosome affecting some genes but not others;
thus the degree of inactivation at a given locus is variable, ranging from complete to none at all (50). Some of the loci that escape X chromosome inactivation have homologous genes in either the pseudoautosomal or sex-limited regions of the Y chromosome (27).

Skewing may occur in which one X chromosome may be preferentially inactivated in a majority of cells. This skewing of X chromosome inactivation may be a heritable trait, may occur by chance alone, or may be the result of selection (7, 37, 43, 55). The stochastic nature of X chromosome inactivation has been highlighted by differences in the patterns of X chromosome inactivation among the tissues of an individual and by discordance in the patterns of X chromosome inactivation between monozygotic twins (13, 47). When this occurs for an X chromosome that contains a mutant gene, only one twin in the pair may have a population of cells in which inactivation of the X chromosome bearing a mutant gene predominated and thus is affected with the condition. This appears as discordance of the X-linked phenotype between the twins. Variability in X chromosome inactivation may likewise account for discordance in X-linked phenotypes among singleton sisters who harbor the same mutant allele.

On the other hand, heritable, skewed X chromosome inactivation may account for concordance of X-linked phenotypes among female relatives. Skewed inactivation in some cell types occurs because the expression of a mutant allele (or failure to express the normal allele) prevents cells from maturing along a developmental pathway. The B cells of females heterozygous for the X-linked agammaglobulinemia express only the X chromosome that encodes the normal allele (3, 7). Those precursor cells in which the chromosome with the normal gene is inactivated fail to mature along the B cell pathway. Individuals with balanced X-autosomal translocations tend to have selective inactivation of the normal (or untranslocated) X chromosome (64). Inactivation of the translocated X chromosome is lethal at a cellular level because the inactivation spreads to the autosomal segment and monosomy for the autosomal regions is selected against by the suboptimal dosage of autosomal genes. Skewed inactivation may also arise from mutations in the minimal promoter of the XIST gene; normally, X chromosome inactivation correlates with expression of the XIST gene on the chromosome being inactivated. Transmission of this mutant causes extreme skewing of X chromosome inactivation to be a heritable trait (43).

SEXUALLY DIMORPHIC GENE EXPRESSION FROM AUTOSOMES

In addition to SRY, the autosomal genes WT1, SOX9, SF1, WNT4, DMRT1 and FGFP play a role in gonadal development. Temporal and spatial differences have been observed for all of these genes in developing human testes and ovaries, thereby demonstrating a dimorphic genetic pathway for gonadal determination and development (4, 18, 19, 44, 59). Several bits of evidence suggest that the effects of SRY in causing male sexual differentiation may channel through SOX9. The expression of SOX9 in the gonads of 46,XY human embryos follows a pattern similar to that of SRY (18). The expression commences with testicular induction and increases over the next several days with maximal detection observed over the sex cords, most likely in Sertoli cells. A 46,XX male patient was observed with a chromosomal duplication encompassing the SOX9 gene. This finding suggested that enhanced expression of SOX9, even in the absence of SRY, is sufficient to cause testis determination (21). In XX Odsex mice, derepression of Sox9 expression in XX gonads leads to male development (5). Ordinarily, this derepression might be mediated by Sry.

Dimorphic gene expression may occur for other developmental pathways that could influence disease susceptibility. In addition to control from a regulatory sex chromosome-linked gene, such as SRY, genes under the control of sex steroids demonstrate dimorphic gene expression because of differences in the production of these hormones by men and women (48). Such differences have been observed for CYP11A1 in the brain, steroid sulfatase in trophoblasts, and estrogen receptor in the liver, among others. CYP11A1, the gene for the first enzyme in steroid biosynthesis (P450SCC, cholesterol side-chain cleavage enzyme), is expressed in the temporal and frontal lobe cortex of the brain and is significantly higher in women than in men (62). Steroid sulfatase is an enzyme that catalyzes the hydrolysis of the sulfate ester bonds of sulfated steroids, such as cholesterol, dehydroepiandrosterone, and estrone sulfate. Human cytotrophoblasts in primary culture show a gender-specific regulation of steroid sulfatase activity, with an increase of twofold to threefold in female, but not in male, cells (58). The estrogen receptor is a complex genomic unit in mice that exhibits alternative splicing that is regulated in a tissue-specific manner. Five variants have been described that are generated by alternative splicing that differ in their 5' untranslated regions. All encode a 66-kDa estrogen receptor protein, which the previously identified mRNA C variant generates. The expression of the H isoform mRNA is restricted to liver, although female mice produce around a fivefold higher level of this transcript than male mice (26). Most likely, many other sex-specific differences will be found.

Many different factors have been described that affect genes whose expression is influenced by steroid hormones (reviewed in Ref. 45). Some of these factors operate in trans, whereas others operate in cis. The trans elements include the availability of the hormones, their receptors, the receptor transcriptional coregulatory proteins, and basal transcription factors in the responsive tissue. The cis elements are the binding sites for the hormone receptors. For the estrogen receptor, specificity is conferred by the sequence of the DNA response element (24). By contrast, for the androgen receptor, specificity is conveyed by binding cooperatively to multiple androgen responsive elements in native promoters (15).
CONCLUSION

Studies to date have highlighted how differences in the genetic constitution of individuals, including sex chromosomes, sex-specific expression of genes, and hormonal concentrations, can influence not only their sexual phenotype but also other physical phenotypes, including susceptibility to disease. In addition, the genetic makeup of individuals (whether men or women) can influence the expression of certain autosomal genes in the next generation, a phenomenon that is known as “genomic imprinting.”

The process of teasing out sex-based differences in phenotypes should become more efficient. New technology for assaying for quantitative differences in the expression of thousands of genes simultaneously has emerged. These techniques, which commonly fall under the rubric of chip technology, could be applied to different classes of lymphocytes or different target tissues between affected and unaffected males and females (11). For such studies to be meaningful, a rigorous case-control design should be applied. To sort out between primary genetic and hormonal effects, individuals with genetic sex reversal who have the expected genetic constitution, but not the hormonal constitution, for the group most commonly affected might be included in these studies.

Candidate genes identified from these approaches can then be rigorously tested for their roles in disease susceptibility or disease protection using linkage or association. The identification of a new gene should meet several criteria to confirm the role of those mechanisms in the development of a specific phenotype. First, the gene should demonstrate linkage or a non-random association with a given phenotype that can be rigorously tested using a statistical method, such as LOD scores (for linkage) or $X^2$ analysis (for association). Second, the strength of the association should increase as the markers analyzed approach the gene(s) that causes the condition. Finally, there should be a biological assay that demonstrates that the expression of a causative gene has been altered as the result of a mutation that is thought to influence disease susceptibility.

REFERENCES


