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MINIREVIEW

Genetics of Sex Determination: Exceptions That Prove the Rule

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The dogma that male and female embryos develop in identical fashion until *SRY* initiates Sertoli cell differentiation in the hitherto bipotential gonad is in need of reevaluation in the light of data that do not fit into this scheme. One of the exceptions that proves the rule of sex determination is true hermaphroditism, the existence of individuals with both testicular and ovarian tissue. Furthermore, the two types of tissues are asymmetrically distributed, ovaries being more common on the left side and testes and ovotestes on the right. Hermaphrodite mice also exhibit bilateral asymmetry of gonad differentiation, but in the opposite direction: ovaries on the right, testes and ovotestes on the left. To explain these asymmetries, it is necessary to consider the relationship between growth and gonadal differentiation. The idea that accelerated growth precedes histological differentiation of the testis has recently been confirmed by the finding that *Sry* induces cell proliferation in fetal mouse gonads, suggesting that the differentiation of Sertoli cells may be dependent on a critical cell number. Recent evidence has also shown that XY embryos develop faster than their XX counterparts at very early stages of development, and it has been reported that *SRY* and *ZFY* are expressed in early human and murine embryos. The relationship between growth and sex differentiation links the mammalian system with those of nonmammalian vertebrates with temperature-dependent sex determination. Early growth differences between male and female human embryos question the belief that all sex differences in later life are due to gonadal hormones.

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NORMAL SEX DETERMINATION

In humans and other mammals, sex is determined at fertilization by the sex chromosome of the sperm. Those containing a Y chromosome give rise to XY males, while X-bearing sperm form XX females. It has been widely assumed that the early development of genetically male and female embryos proceeds in identical fashion, until the formation of the genital ridge or "bipotential" gonad. Then their paths diverge. The Y chromosome carries the "testis-determining" gene *SRY* (*Sry* in the mouse). Therefore, if a Y chromosome is present, it will trigger the indifferent gonad into the path of testicular development.

On the basis of electron-microscopical studies on the gonads of rat embryos, Jost *et al.* (1) concluded that the appearance of Sertoli cells is the first indication of testicular differentiation, and it was assumed that *SRY* functions by causing the differentiation of supporting cells into Sertoli cells (2). Once formed, the Sertoli cells were believed to direct the formation of the other cells of the testis. Sertoli cells secrete anti-Müllerian hormone, while fetal Leydig cells secrete testosterone (3). In the absence of *SRY*, the supporting cells develop into follicle cells and the gonad becomes an ovary. Whereas the differentiation of the male reproductive tract depends on hormones secreted by the fetal testis, the fetal ovary

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seems not to be required for the development of the female tract, which is believed to occur simply in the absence of testicular secretions (4). It follows that the differentiation of the genital ridge into a testis is a pivotal event in the development of the male phenotype. For this reason, a "testis-determining factor," *TDF* (*Tdy* in the mouse), was mapped onto the Y chromosome before any candidate gene had been proposed.

SRY has many properties expected of *TDF*. Two of the most important are the existence of mutations in *SRY* in patients with XY gonadal dysgenesis (XY females) (5), albeit in only a minority (6), while the role of *Sry* in testis development was confirmed in transgenic experiments, in which a proportion of XX mice bearing *Sry* developed as males (5). It is believed that *Sry* is particularly involved in the development of Sertoli cells, since it was found that in XX↔XY chimeric mice the proportion of XY cells in Sertoli cells was much higher than in other cell types (7).

It has become evident, however, that sex determination cannot be explained by a simple switch; indeed, a considerable number of non-Y-chromosomal genes are now known whose normal function is required in the process (2), including *SOX9, WT1,* and *DAX-1*. Haploinsufficiency of *SOX9* causes campomelic dysplasia as well as female development in XY individuals (5), while heterozygous deletions of *WT1* predispose to childhood tumors and are also associated with genitourinary abnormalities, which can include incomplete masculinization. By contrast, *DAX-1* causes XY sex reversal when the gene is duplicated (8). As yet, it has not been possible to elucidate the mode of action of the sex-determining genes in a molecular pathway.

It may be instructive, therefore, to look at some anomalies.

HERMAPHRODITISM AND BILATERAL ASYMMETRY

One of the exceptions that proves the rule of the genetics of sex determination is the existence of individuals with both testicular and ovarian tissue. Apart from a small minority of patients with more than one cell line, true hermaphroditism requires an explanation of either the presence of ovarian tissue in the presence of *SRY* or the presence of testicular tissue in the absence of the gene. An even greater difficulty, which is rarely considered in molecular models of sex determination, is that the two types of tissue are not symmetrically distributed within the body. When large numbers of patients are examined, it is found that ovaries occur about twice as often on the left as on the right side, whereas testes and ovotestes are more frequent on the right (9,10) (Fig. 1).

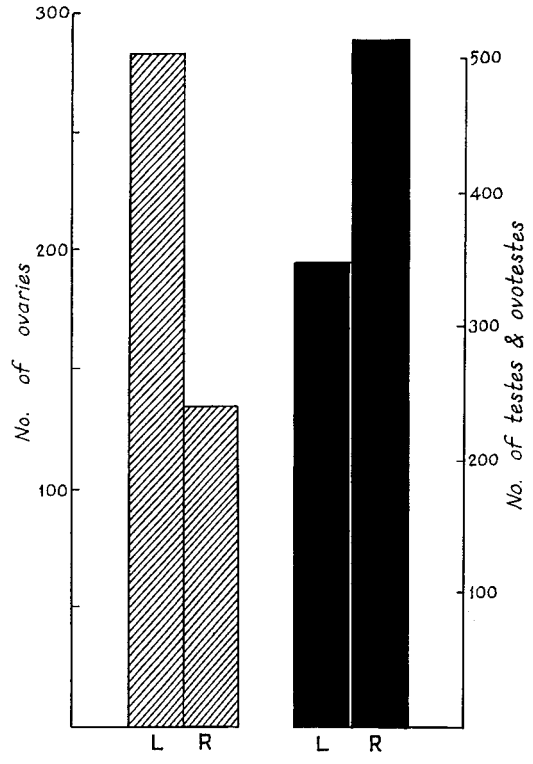

FIG. 1. Distribution of 414 ovaries and 860 testes and ovotestes in 637 patients with true hermaphroditism. Combined data by van Niekirk and Retief (9) and Krob *et al.* (10). L, left; R, right. Illustration from Mittwoch (14).

Hermaphroditism is not the only abnormality that exhibits bilateral asymmetry. In one of the most common congenital anomalies, cleft lip with or without cleft palate (CL/P), unilateral clefts are far more frequent on the left side than the right, as was clearly shown in the pioneering investigation by Fogh-Anderson (11) and confirmed in two later studies (12,13). While transforming growth factor alpha has been shown to play a major role in the expression of the CL/P phenotype, epigenetic factors resulting in minor growth differences between the left and right side of the developing face seem to be equally important (14).

Hermaphrodite mice also exhibit bilateral asymmetry, but in the opposite direction to that in humans: ovaries are preferentially situated on the

TABLE 1
Distribution of Gonads in Lateral and Unilateral Hermaphrodite Mice

	Left	Right	Total
Ovary	30	61	91
Testis	212	36	248
Ovotestis	80	225	305
Total	322	322	644

Note. Combined data from Eicher *et al.* (15), Ward *et al.* (16), and Biddle *et al.* (17, 18). Mice with bilateral ovotestes have been excluded.

right side and testes and ovotestes on the left side. The data shown in Table 1 give the combined results of the distribution of gonads in unilateral and lateral hermaphrodite mice (but excluding bilateral hermaphrodites) from four investigations (15–18).

To explain these asymmetries, it will be necessary to give a brief review of the relationship between gonadal differentiation and growth.

GONADAL DIFFERENTIATION: HISTOLOGICAL VERSUS QUANTITATIVE CRITERIA

The tendency to equate the beginning of gonadal differentiation with the time when histological differentiation can first be detected has persisted, as illustrated in the statement by Swain and Lovell-Badge (2) that "The gonad" (of the mouse) "initially develops in a non-sex specific manner, being morphologically identical in XX and XY embryos until ~12.0 dpc." By limiting the process of differentiation to the appearance of morphological differences, however, the authors have overlooked a substantial body of evidence that differences in size between male and female gonads precede overt differences in histology.

Following the demonstration that in embryos of the rat, the volumes of XY gonads had exceeded those of XX gonads before any histological difference could be detected (19,20), a similar quantitative difference was observed in the gonads of other mammalian species (21), including the marsupial *Monodelphis domestica* (22,23). In human fetuses, it was found that, on average, right gonads exceeded those on the left in wet weight, DNA, and protein content (24), while testes exceeded ovaries in the same criteria when related to crown-rump length (25).

This relationship could provide an explanation for the bilateral asymmetry of the gonads in human true hermaphroditism: if testes need to grow faster than ovaries, and right gonads grow faster than left gonads, we may conclude that the predisposition for testicular differentiation is inherently greater in right than in left gonads. This is of no consequence in normal sexual differentiation, which is firmly controlled by the sex chromosomes. In the development of hermaphroditism, however, we witness the breakdown of the sex chromosome mechanism, and we also see the bilaterally asymmetrical development of testes and ovaries in accordance with the basic asymmetry of normal gonadal development (21).

The difference in the direction of asymmetry between humans and mice could be connected with the difference in size between human and murine fetal gonads. An analysis of kidney weights in wild mice showed that right gonads tended to be heavier than those on the left, but the data suggested that this difference decreased with increasing kidney weight and that the direction of asymmetry may eventually be reversed (26). In contrast to mice, Henry Gray (27) wrote of human kidneys that "The left is nearly always heavier than the right, by about two drachms."

A relationship between bilateral asymmetry and organ size is further substantiated by an analysis of left and right gonadal volumes in embryos of the gray short-tailed opossum *M. domestica*, in which gonad volumes averaged less than 0.01 mm^3 and which showed a highly significant difference in favor of the left gonad (28). In newborn opossums, this difference was reduced.

SEX DIFFERENCES IN DEVELOPMENTAL RATE OF EARLY EMBRYOS

Side by side with the search for sex-determining genes that characterized the last decades of the past century, there appeared an impressive, if less publicized, body of data that contradict the view that the switch directing the indifferent gonad into the testicular pathway is the first step of sexual differentiation and that all differences between males and females derive from it (29). Evidence of an increased growth rate of male compared with female fetuses prior to the time of gonadal differentiation has been reported in rats (30) as well as in human fetuses studied by ultrasound, which showed that at 8 to 12 menstrual weeks female fetuses were 1 day behind their male counterparts in crown-rump length and parietal diameter (31). The data suggested that at

least part of the difference between growth rates of male and female fetuses is determined by the sex chromosomes. A similar sex difference is shown in preimplantation mouse embryos, and Burgoyne *et al.* (32) have presented evidence that this is caused by both an accelerating effect of the Y chromosome and a retarding effect of the X chromosome.

In the mouse, a sex difference was established already at the blastocyst stage (33), and the same applies to bovine embryos (34,35). In human embryos produced *in vitro,* male embryos had on average more cells than female embryos on day 2 after insemination, and the male embryos showed evidence of higher metabolic activity (36). Evidence of increased metabolic activity had previously been reported in male preimplantation bovine embryos (37).

In addition to the very early growth differences between XX and XY embryos that are now known to occur, it has been reported that the Y chromosomal genes *Sry* and *Zfy* are expressed at the two-cell stage in mouse embryos (38) and that their human homologues are expressed in the zygote (39). *Sry* expression has also been found in mouse blastocysts (40). Although the significance of these findings is still controversial (41), there is now ample evidence demonstrating that the process of sex differentiation begins long before the stage of genital ridge formation. Clearly, the topic is in need of reevaluation.

Sry AND CELL PROLIFERATION

In a morphometric study of fetal mouse gonads carrying the *Steel* mutation Sl/Sl^{d}, which causes the gonads to be virtually devoid of germ cells, McCoshen (42) found that the growth of the somatic cells of the testis, but not of the ovary, was independent of the presence of germ cells and that XY gonadal somatic cells possess an inherent capacity to grow at an accelerated rate compared with XX gonadal somatic cells. This led to the suggestion that either there are additional Y-chromosomal genes involved in gonadal growth or the testis-determining gene itself exerts a growth influence even prior to sex cord differentiation.

The latter suggestion has now been shown to be correct in the laboratory of Blanche Capel (43). To investigate whether the rapid size increase of the rudimentary testis is dependent on the presence of *Sry,* the authors used 5′-bromo-2′-deoxyuridine (BrdU) incorporation to label dividing cells. The results showed that the size increase was accompanied by a dramatic increase in cell proliferation in XY gonads, which was absent in XX gonads and appeared to be independent of the number of X chromosomes. The increase in cell proliferation began less than 24 h after the onset of *Sry* expression in cells giving rise to Sertoli cells. The increase was not seen in B6 XY^{POS} mice, in which testis development is inhibited, but was present in B6 XX mice carrying a transgenic copy of *Sry* and forming testes. The authors concluded that a high level of cell proliferation is tightly correlated with testicular differentiation.

These results represent a decisive step forward in the study of sex determination, which has ramifications in at least two different areas.

EVOLUTIONARY QUESTIONS AND A FEW POSSIBLE ANSWERS

The perceived function of *SRY* as a testis-determining factor led to the expectation that the gene was an integral part of the sex-determining process, and, therefore, its absence in nonmammalian vertebrates came as a surprise (44). It has also emerged during the past few decades that genotypic sex-determining mechanisms are by no means universal among fish, amphibians, and reptiles. In some species of reptiles, including snakes, some lizards, and a minority of turtles, sex is determined by the genotype, whereas in other species sex is determined by the temperature of incubation (45). Whereas genotypic sex determination is clearly a necessity in mammals and birds on account of their homoiothermy, it appears to be a luxury in reptiles.

In reptiles, temperature is an important factor influencing developmental rates and can also affect vertebrae number and some morphometric traits (46). In the turtle *Emys orbicularis,* gonads of embryos incubated at the male-producing temperature of 25°C initially grow faster than those of embryos incubated at the female-producing temperature of 30°C; subsequently, ovarian growth accelerates in conjunction with aromatase activity (47). The comparative evidence suggests that *SRY* is an internalized factor that accelerates developmental rates, and the puzzling question why *SRY* is confined to mammals may be simply explained as an adaptation to the reproductive biology of mammals, in which both sexes are exposed to the estrogenic environment of the uterus (48,49).

The sex chromosome mechanism of birds, ZZ males and ZW females, appears to be the opposite of that in mammals, and bilateral asymmetry plays a

major role in the differentiation of the female gonad (50). It has recently been shown that the Z chromosome of chickens carries an orthologue of the human *DMRT1* gene, which is located on chromosome 9 and has been implicated in XY sex reversal if one homologue is deleted (51,52). The question of whether birds, likewise, require two doses of *DMRT1* is particularly intriguing, since developmental studies in chick embryos have shown that initially ZZ gonads grow faster than ZW gonads (50). Could this be due to a dosage effect of *DMRT1*, and might this gene act in a similar way to *SRY* by enhancing cell proliferation?

Ongoing studies on sex determination in non-mammalian vertebrates will undoubtedly throw light on the human situation.

CONCLUSIONS AND OUTLOOK

It is evident that the sum of the research findings of recent years requires a modification in the current dogma of mammalian sex determination, which was based on gonadal histology and tended to ignore quantitative criteria. The finding that *Sry* functions by increasing cell proliferation in the fetal mouse gonad emphasizes the significance of cell growth in gonadal differentiation and suggests that the differentiation of Sertoli cells may be dependent on a critical cell number. This relationship should help in the elucidation of the etiology of true hermaphroditism, where the evidence suggests an interaction between genotype and epigenetic factors.

It has also become apparent that sex differences in growth begin soon after fertilization, which could be of particular significance in view of recent evidence of an effect of fetal growth rate on disease in later life (46,53). It remains to be determined whether sex-determining genes are involved in early differences in growth. Nevertheless, it can no longer be maintained that all differences between males and females in later life are the result of gonadal hormones.

As we enter the second century of genetics, the aim will be to look beyond simple switch mechanisms by unraveling the relationship between genes and epigenetic factors on cell growth during development and its effect on the phenotype at later stages. This knowledge should also increase the opportunity for providing the best possible conditions for optimum cell growth during development.

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