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REVIEW ARTICLE

Is the Y chromosome all that is required for sex determination?

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The gender identity of a person is the final result of genetic, hormonal and morphologic sex. Over a long period sex determination, and, specifically, male sex determination, has been correlated to the presence of the Y chromosome, which in turn has been the karyotype signal of the testes. However, research has provided data to convince that this theory is only part of the truth. In addition to the Y chromosome, a multitude of other genes influence sex determination and are able to cause male to female sex-reversal and vice versa. It is of great interest that these genes are located in more than one autosomal chromosomes or even in the X chromosome. It has become obvious that sex determination, according to the genetic sex, is a complicated matter that not only requires the presence of Y chromosome. This fact triggered extensive research of the Y chromosome and led to great insight into its structure, origin, evolution and eventual fate in humans. *Hippokratia 2007; 11 (3): 120-123*

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The gender identity of a person is the final result of genetic, hormonal and morphologic sex. It also includes all behaviour, such as body gestures and mannerisms, way of speech, sexual preferences and content of dreams. Particularly, gender identity is the result of the genetic sex, the gonadal sex, the internal genitalia, the external genitalia, the secondary sexual characteristics that appear in puberty and the social gender that is attributed in accordance with the norms and the beliefs of the social body.

Prenatally, sexual differentiation follows a specific sequence. First, is determined the genetic sex and follows the gonadal differentiation, under the control of the genetic sex and the hormonal signals. Subsequently, it is developed the internal duct system and then it is formed the external genitalia. There is evidence that the embryonic brain is also sexually differentiated. The influence of the hormones on the central nervous system has a potential effect on the patterns of the hormone secretion and sexual behaviour on the adult[1-5].

Normal male sex differentiation

In human embryos, the gonads begin development during the fifth week of gestation as protuberances overlying the mesonephric ducts. The migration of primordial cells into these gonadal ridges occurs between weeks 4 and 6 of gestation. At 6 weeks of gestation (4 weeks after ovulation) the gonads are indifferent, but bipotential, possessing both cortical and medullary areas and capable of differentiation into either testes or ovaries. They are composed of germ cells, special epithelia (potential granulosa/Sertoli cells), mesenchyme (potential theca/Leydig cells) and the mesonephric duct system. Wolffian and Móllerian ducts exist side by side [6]. Subsequent sexual differentiation requires direction by various genes, with a single gene determinant on the Y chromosome, the testes-determining factor (TDF), necessary for testicular differentiation, beginning at 6-7 weeks of gestation[7].

Testicular differentiation begins first with Sertoli cells that aggregate to form spermatogenic cords, then seminiferous tubules followed by Leydig cell formation a week later. The Sertoli cells are the sites of the sex region of the Y chromosome (SRY) expression. Therefore, the Sertoli cells orchestrate the development of primordial germ cells into testis. The molecular sequence of events may start with the activation of the SRY, following the early expression of the steroidogenic factor-1 (SF-1). SRY expression raises SF-1 and SOX9 gene activity to a critical level in the Sertoli cells, causing male differentiation and activation of the antimóllerian hormone (AMH) gene.

Human chorionic gonadotropin (hCG) stimulation produces Leydig cell hypertrophy and peak fetal testosterone levels are seen at 15-18 weeks of pregnancy[8]. It has been suggested that hCG stimulates steroidogenesis in the early fetal testes, so that androgen production will ensue and masculine differentiation can be accomplished[9]. However, normal masculine differentiation occurs in mouse models lacking luteinising hormone receptors (LH-r) and molecular evidence indicates that fetal Leeydig cells respond to adrenocorticotropin hormone (ACTH) as well as hCG[10].

AMH is produced by Sertoli cells and after testicu-

lar differentiation and is responsible for the ipsilateral regression of the móllerian ducts that proceeds in a craniocaudal direction and is complete by 8 weeks, before the emerge of testosterone (T) and stimulation of the wolffian ducts[11]. Testosterone is secreted by the fetal testes soon after Leydig cell formation and rapidly rises to peak concentrations at 15-18 weeks of pregnancy. This testosterone secretion stimulates development of the wolffian duct system into epididymis, vas deferens and seminal vesicles. As hCG declines, the LH assumes control of Leydig cell testosterone secretion.

The wolffian ducts receive testosterone signals directly from nearby Leydig cells as well as the general fetal circulation. This local paracrine effect is essential to the stimulation of ipsilateral differentiation into epididymis, vas deferens and seminal vesicles. The wolffian do not form dihydrotestosterone (DHT), so the direct high concentration of testosterone is crucial for normal development[12]. The internal genitalia possess the intrinsic tendency to feminize. In the absence of a Y chromosome and a functional testis, the lack of AMH allows retention of the móllerian system. In the absence of testosterone, the wolffian system regresses.

In the bipotential state, the external genitalia consist of a genital tubercle, a urogenital sinus and two lateral labioscrotal swellings. Unlike the internal genitalia where both duct systems initially coexist, the external genitalia are neutral primordia able to develop into either male female structures depending on gonadal steroid hormone signals. Normally, this differentiation is under the active influence of androgen from the Leydig cells of the testis. The genital tubercle forms the penis, labioscrotal folds fuse to form a scrotum and folds of the urogenital sinus form the penile urethra. The testis begins androgen secretion by 8-9 weeks. Masculinization of the external genitalia is manifest one week later and is completed by 14 weeks. To achieve this morphologic changes, external genitalia target tissue cells must convert T to DHT by the intracellular enzyme 5α-reductase. DHT mediates temporal hairline recession, growth of facial and body hair, development of acne and development of the external genitalia and prostate[13].

The importance of the Y chromosome

In mammals, the genetic sex is established at fertilization with the inheritance of an X or Y chromosome of their father. This paternal pattern of sex determination was showed in studies of sexual ambiguity in humans[14,15]. This fact triggered extensive research of the Y chromosome and led to great insight into its structure, origin, evolution and eventual fate in humans[16-18]. The Y chromosome ability to determine sex, and specifically to create males with its presence led to the speculation that there was a testis determining factor (TDF), which was later identified as the gene in the sex-determining region of Y chromosome (SRY), because its absence in XY mice leads to female development[19,20].

Both the X and the Y chromosomes evolved from autosomal ancestors in a 300 million years time line[10]. Most of the ancestral genes on the Y chromosome deteriorated, leaving only a limited number of currently active genes. The male-specific region of the Y chromosome (MSY) area encompasses almost all of the active genes.

The Y chromosome presents two regions, the pseudoautosomal and the non-recombining region (NRY) or better called male-specific region (MSY), according to the existence of X-Y crossing over during meiosis I. The MSY region comprises 95% of its length and it is flanked on both sides by pseudoautosomal regions.

The SRY is a single exon gene located in the small arm of the Y chromosome in the MSY region. It is expressed in the genital ridge only during the time that testicular cords form. It is deleted or mutated in cases of human XY females. It is present in 46,XX males and it can sex-reverse XX mice into males[21-24].

Sequencing of the human *SRY* gene revealed that it encoded a protein containing a region of 80 aminoacids that shares homology with the abundant nuclear protein, high mobility group of the family of the transcription factors (HMG1) (24). The SRY protein can bind DNA in a sequence-specific manner[25] and, like HMG1, it can induce a bend in DNA[26]. The action of SRY is thought to trigger differentiation of the Sertoli cell lineage in the testis.

Other genetic factors, which contribute to male sex differentiation

Apart from the SRY gene, there is a number of other genes that contribute dramatically to the sex determination, as it has been proven from sex reversal disorders. The Wilm's tumor-associated gene (WT1), which is located in the short arm of the chromosome 11, is comprised of 10 exons and can give rise to 24 different protein isoforms. The +KTS and –KTS isoforms arise from an alternative splicing event that incorporates or omits, respectively, three aminoacids.

A research team[27] conducted an isoform-specific knockout. Both male and female mice lacking the WT1 (-KTS) isoform had reduced gonadal size, but cells in the gonads of male mice showed male-specific marker gene expression, although reduced. Mice lacking WT1 (+KTS) had severe kidney defects and male mice were completely XY sex-reversed.

Consistent with the latter study, XY patients with Frasier syndrome develop as females and display urogenital malformations. Analysis of these patients has showed that donor splice mutations in WT1 with loss of the +KTS isoform are associated with this syndrome[28]. According to the findings of a study, mutations in WT1 (+KTS) could lead to sex reversal because of a deficiency in the precursors of Sertoli cells[29].

Steroidogenic factor 1 (SF1) is a member of the subfamily of nuclear receptors, the orphan receptors, for which no activating ligand has been found. SF1 gene expression is specifically associated with the gonad and

the adrenal as they arise and is a good marker for these cells[30]. The gene that encodes for SF1 is located in the long arm of the chromosome 9. Mutation in this gene provoke male to female sex reversal[31].

The gene named SOX9 encodes a transcription factor that contains a SRY-related HMG box[32]. It is located in the long arm of the chromosome 17. Expression studies in mice revealed that SOX9 is present in both sexes genital ridges, but after the initial development, is upregulated in males and switched off in females[33]. SOX9 is expressed in Sertoli cells throughout life, but not in the ovary. This characteristic is consistent with the upregulation of SOX9 being a direct effect of SRY action. It also suggests that is involved in determination of Sertoli cell fate. Significantly, heterozygotes for mutations in SOX9 show male to female sex reversion[34,35].

According to the latter study, the protein has to reach a critical threshold to be effective, thus an additional copy of SOX9 can stimulate female to male sex reversal. SRY and SOX9 are functionally related, perhaps ensuring the male development. SOX9 is also necessary for the activation of gene which encodes for the anti-Mullerian hormone (AMH).

Another sex reversal event is observed when the Dosage Sensitive Sex Reversing Adrenal Hypoplasia Congenita critical region on the X (DAX1) gene is partially duplicated in males. The DAX1 is located on the short arm of the X chromosome[36]. The single X chromosome in the male is active, but a translocated region from the female X (or a partial duplication of the male X) results in two active alleles of the *DAX1* gene when only one occurs normally. This gene dosage feminising effect is sufficient to counteract the effect of SRY[37].

The action of DAX1 gene has resulted to its characterization as an anti-testes gene. Another anti-testes gene is the WNT4. In humans, its duplication causes male to female sex-reversal[38]. This effect indicates that both DAX1 and WNT4 can override the effect of SRY.

Finally, there is one more gene that interferes with sex determination, the Doublesex and Mab-3 Related Transcription factor1 (DMTR1). DMTR1 lies on the short arm of the chromosome 9. Mutation or partial deletion of this gene provokes male to female sex reversal [39,40]. Two functioning copies of DMRT1 are therefore required to support testis development in mammals[41].

Conclusions

Over a long period sex determination, and specifically male sex determination, has been correlated to the presence of the Y chromosome, which in turn has been the karyotype signal of the testes. However, research has provided data to convince that this theory is only part of the truth. In addition to the Y chromosome, a multitude of other genes influence sex determination and are able to cause male to female sex-reversal and vice versa. It is of great interest that these genes are located in more than one autosomal chromosomes or even in the X chromosome.

It has become obvious that sex determination, according to the genetic sex, is a complicated matter that not only requires the presence of Y chromosome. But, is only the genetic sex that defines gender? What about the internal genitalia, which after all specify the reproductive ability of a person? It is known that testes formation require the presence of two hormones: testosterone and AMH. So, a mutation of their gene or of their receptor gene results in feminization of the internal genitalia despite the presence of the Y chromosome.

And what about the first impression of the embryo sex? Impressions can be deceptive? Well, external genitalia are very important for the gender determination, as it can be inferred from the sex confusions that are registered just after the labour. The development of the male external genitalia depends on dehydrotestosterone (DHT). Two molecules of testosterone are converted to DHT by the intracellular enzyme 5α-reductase. Mutation of the enzyme or its receptor leads to DHT deficiency and, subsequently, to female external genitalia, in spite of the Y chromosome. DHT contributes, also, to the morphologic sex, as it formed by the secondary sexual characteristics of the male.

References

1. Dittmann RW, Kappes ME, Kappes MH. Sexual behavior in adolescent and adult females with congenital adrenal hyperplasia. Psychoneuroendocrinology 1992; 17: 153-155
2. Kuhnle U, Bollinger M, Schwarz HP, Knorr D. Partnership and sexuality in adult female patients with congenital adrenal hyperplasia. First results of a cross-sectional quality-of-life evaluation. J Steroid Biochem Mol Biol 1993; 45: 123-127
3. Rubinow DR, Schmidt PJ. Androgens, brain, and behavior. Am J Psychiatr 1996; 153: 974- 984
4. Berenbaum SA, Bailey JM. Effects on gender identity of prenatal androgens and genital appearance: evidence from girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2003; 88: 1102-1106
5. Servin A, Nordestrom A, Larsson A, Bohlin G. Prenatal androgens and gender-typed behaviour: a study of girls with mild and severe forms of congenital adrenal hyperplasia. Dev Psychol 2003; 39: 440-444
6. Van Wagenen G, Simpson ME. Embryology of the ovary and testis, Homo Sapiens and Mucaca Mulatta. Yale University Press, New Haven, Connecticut, 1965
7. Sinclair AH, Berta P, Hawkins JR, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990; 346: 240-246
8. Wilson JD, Griffin JE, George FW, Leshin M. The role of gonadal steroids in sexual differentiation. Recent Prog Horm Res 1981; 37: 1-5
9. Ravidovici J, Jaffe RB. Development and regulation of growth and differentiated function of human and subhuman primate fetal gonads. Endocr Rev 1990; 11: 532-536
10. O'Shaughnessy PJ, Fleming LM, Jackson G. et al. Adrenocortocotropic hormone directly stimulates testosterone production by the fetal and neonatal mouse testis. Endocrinology 2003; 144: 3279-3285
11. Taguchi O, Cunha GR, Lawrence WD, Robboy SJ. Timing and irreversibility of móllerian duct inhibition in the embryonic reproductive tract of the human male. Dev Biol 1984; 106: 394-398
12. Siiteri PK, Wilson JD. Testosterone formation and metabolism

during male sexual differentiation in human embryo. J Clin Endocrinol Metab 1974; 38: 113-118
13. Speroff L, Fritz MA. Normal and abnormal sexual differentiation. In: Clinical gynaecologic endocrinology and infertility. Lippincott Williams & Wilkins, Philadelphia, 2005
14. Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sexchromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1959; 1: 711-713
15. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature 1959; 183: 302-303
16. Lahn BT, Page DC. Functional coherence of the human Y chromosome. Science 1997; 278: 675-679
17. Lahn BT, Page DC. Four evolutionary strata on the human X chromosome. Science 1999; 286: 964-967
18. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003; 423: 825-837
19. Berta P, Hawkins JR, Sinclair AH, et al. Genetic evidence equating SRY and the testes-determining factor. Nature 1990; 348: 448-452
20. Koopman, P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for *Sry.* Nature1991; 351: 117–121
21. Jager RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. Nature 1990; 348: 448-452
22. Moore CCD, Grumbah MM. Sex determination and gonadogenesis: a transcription cascade of sex chromosome and autosome genes. Seminars Perinatol 1992; 16: 266-231
23. Muller J, Schwartz M, Skakkebaek NE. Analysis of the sex-determining region of the Y chromosome (SRY) in sex reversed patients: point-mutation in SRY causing sex-reversion in a 46,XY female. J Clin Endocrinol Metab 1992; 75: 331-337
24. Sinclair AH, Berta P, Palmer MS, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990; 190; 346: 240–244
25. Harley VR, Jackson DI, Hextall PJ, et al. DNA binding activity of recombinant SRY from normal males and XY females. Science 1992; 255: 453-456
26. Pontiggia A, Whitfield S, Goodfellow PN, Lovell-Badge R, Bianchi ME. Evolutionary conservation in the DNA-binding and -bending properties of HMG-boxes from SRY proteins of primates. Gene 1995; 154: 277-280
27. Hammes A, Guo JK, Lutsch G, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell 2001; 106: 319-329
28. Barbaux SP, Niaudet MC, Gubler JP, et al. Donor splicesite mutations in WT1 are responsible for Frasier syndrome. Nat Genet 1997; 17: 467–470
29. Karl J, Capel B. Sertoli cells of the mouse testis originate from the coelomic epithelium. Dev Biol 1998; 203: 323–333
30. Shinoda K, Lei H, Yoshii H, et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 1995; 204: 22–29
31. Lou X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and for male sexual differentiation. Cell 1994; 77: 481-485
32. Bowles J, Schepers G, Koopman P. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. Dev Biol 2000; 227: 239-255
33. Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P. A male-specific role for Sox9 in vertebrate sex determination. Development 1996; 122: 2813–2822
34. Foster JW, Dominguez-Steglich MA, Guioli S, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an *SRY* related gene. Nature 1994; 372: 525–530
35. Wagner T, Wirth J, Meyer J, et al. Autosomal sex reversal campomelic dysplasia are caused by mutations in and around the *SRY*-related gene sox9. Cell 1994; 79: 1111–1120
36. Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R. Dax1 antagonizes Sry action in mammalian sex determination. Nature 1998; 391: 761-767
37. Meeks JJ, Weiss J, Jameson JL. *Dax1* is required for testis determination. Nat Genet 2003; 34: 32-33
38. Jordan BK, Mohammed M, Ching ST, et al. Up-regulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. Am J Hum Genet 2001; 68: 1102-1109
39. Saccone G, Pane A, Polito LC. Sex determination in flies, fruitflies and butterflies. Genetica 2002; 116: 15-23
40. Shan Z, Nanda I, Wang Y, et al. Sexspecific expression of an evolutionarily conserved male regulatory gene, DMRT1, in birds. Cytogenet Cell Genet 2000; 89: 252-257
41. Nanda I, Zend-Ajusch E, Shan Z, et al. Conserved synteny between the chicken Z sex chromosome and human chromosome 9 includes the male regulatory gene DMRT1: a comparative review on avian sex determination. Cytogenet Cell Genet 2000; 89: 67-78