

Female Development in Mammals by *Wnt-4* Signalling

Although differentiation of the testes and of male internal and external genitalia have long been known to be actively guided processes, it has been assumed that differentiation of the ovary and of female internal genitalia are "innate" processes that occur by default. Vainio et al demonstrated that in the mouse, ovarian and Müllerian duct differentiation are active processes influenced by the product of *Wnt-4*, a member of a family of extracellular, cysteine-rich glycoprotein signaling molecules (OMIM 603490). The latter influence a number of developmental processes, including normal renal development in the mouse.

Wnt-4 also is expressed in the embryonic gonadal ridge, the undifferentiated fetal gonad, the fetal ovary (although downregulated in the fetal testis), and in mesenchyme of the Müllerian but not the Wolffian ducts. By crossbreeding animals heterozygous for inactivation of *Wnt-4*, these investigators developed fetuses and newborns that were homozygous for this defect (*Wnt-4*^{-/-}).

The male offspring were phenotypically normal, but the female offspring were virilized. The ovary was deformed and the Müllerian ducts were absent. The single gonadal duct resembled an epididymis. The external genitalia of the female offspring were normal. The *Wnt-4*^{-/-} female gonad appeared to synthesize and secrete androgens as it expressed both *HSD3B* and *CYP17*. The homozygous fetal ovary had far fewer oocytes than the normal ovary, suggesting that *Wnt-4* is necessary for oocyte maintenance. The investigators conclude that *Wnt-4* is important for normal fetal female sexual differentiation in the

mouse and, by inference, in other mammals, possibly including humans.

Vainio S, et al. *Nature* 1999;397:405-409.

Editor's comment: *This study demonstrates the essential role that Wnt-4 plays in Müllerian duct differentiation and ovarian function. It is of interest that in the Wnt-4^{-/-} females, Müllerian duct inhibitor factor from the Sertoli cells was not necessary for regression of the Müllerian ducts!*

It has been suggested that DAX1 (dosage sensitive sex reversal on the X chromosome—Xp21) may be important for ovarian differentiation. The role of DAX1 in sexual differentiation has been demonstrated by the sex reversal of 46,XY males in patients with duplication of this gene, thus suggesting that this transcription factor is involved in ovarian determination and repression of testicular function. Yu et al (Role of Ahch in Gonadal Development and Gametogenesis. Nature Genet 1998;20:353-357) generated a mouse model in which Dax1 has been inactivated. In these animals, ovarian differentiation and fertility are normal, but spermatogenesis and testosterone secretion in the male are disrupted. Thus, Dax1 affects testicular function in a dose-dependent manner; normally, it supports spermatogenesis and Leydig cell function, but when duplicated leads to inhibition of the testis-determining effects of SRY and SOX9. Embryologic sex differentiation becomes more complex as we learn more and more about gene control. We used to be able to explain sexual differentiation on a hormonal basis, but we cannot do this in 1999.

Allen W. Root, MD

COL9A3: A Third Locus for Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) refers to an autosomal dominant clinical phenotype characterized by mild to moderate short stature associated with painful joints and precocious osteoarthritis. Historically, MED has been divided into the severe (or Fairbank) form and the mild (or Ribbing) form, although the phenotypes merge to form a gradient of severity. MED is a genetically heterogeneous condition.

MED mutations were first found in the gene encoding cartilage oligomeric matrix protein, COMP. This locus, which also harbors mutations responsible for pseudoachondroplasia, was designated *EDM1*. It was clear soon after the *EDM1* locus was identified that, in some families, MED does not map to the *COMP-EDM1* locus.

A second locus, designated *EDM2*, was identified in 1996 through positional cloning. It encodes the alpha 2 chain of type IX collagen, an extracellular matrix protein of cartilage and skeletal growth plate. This led to the view that type IX collagen and COMP interact functionally in growing bones and that disturbances of this interaction occur in pseudoachondroplasia and MED.

The existence of a third *EDM* locus was anticipated from linkage studies that excluded *EDM1* and *EDM2* in some families with MED phenotypes. The genes encoding the other 2 chains of type IX collagen, *COL9A1* and *COL9A3*, were the strongest candidates. *COL9A3* has now been identified as *EDM3*. Paasilta et al first established linkage of a relatively mild form of MED to a genetic marker in *COL9A3*. They next demonstrated a mutation predicted to disrupt splicing of *COL9A3* mRNA transcripts, leading to deletion of 12 amino acids near the amino-terminal end of the collagen chain. The mutation resembles the *COL9A2* mutation found in other cases of MED. Not surprisingly, the manifestations are similar in the families with type IX collagen mutations.

Paasilta P, et al. *Am J Hum Genet* 1999;64:1036-1044.

Editor's comment: *It is now evident that genes encoding cartilage matrix proteins are a very rich source of mutations that cause human chondrodysplasias. This reflects the importance of these proteins to endochondral bone growth. Since these proteins interact with each other to form a functional extracellular matrix, it makes sense that disturbances of different elements of this matrix lead to relatively similar clinical*

phenotypes. However, the mechanisms by which such disturbances interfere with bone growth remain poorly understood. Potential mechanisms include: (1) disturbances of the mechanical properties of cartilage, which must serve as a template for bone growth; (2) disturbances in the diffusion, sequestration, and/or presentation of growth factors; and (3) disturbances of direct interactions of the matrix with cartilage cells. As emphasis evolves in the post genome era from finding gene loci and detecting mutations to elucidating how mutations act, the mechanisms relevant to cartilage matrix protein mutations should be unveiled.

William A. Horton, MD

2nd Editor's comment: MED must be thought of clinically in the presence of unexplained short stature (normal or

abnormal proportions) and/or joint pains (particularly of the knees). Osteoarthritis results and frequently necessitates hip and knee replacement in adult life. In the current 4-generation family described by Paasilta et al, none of the 8 affected adults were outside the normal range for height. All had knee pain, often dating to childhood, and some had hip pain. A few had involvement of other joints. Clinical investigation should include radiologic examination, particularly of the knees and hips. X-ray studies of adults may or may not show abnormalities after epiphyseal fusion has occurred. Family history and investigation of short children and/or children with knee pain in the family may prove the diagnosis in the adult with suspected MED (by familial association).

Robert M. Blizzard, MD

10 Years of Genomics, Chromosome 21, and Down Syndrome

Despite being the smallest of human chromosomes, chromosome 21 occupies a prominent place in human genetics. The long arm of this chromosome is approximately 37 mb in length and constitutes about 1% of the human genome. Trisomy for chromosome 21 is the most common aneuploidy at birth in humans; it results in the most common form of mental retardation, occurring in 1/700 live births. To celebrate the 10th birthday of the journal *Genomics*, Stylianos Antonarakis has written a comprehensive review covering the last decade of progress involving chromosome 21 and Down syndrome. The review covers diverse topics, including a comparison of different types of chromosome 21 maps; genes that may be responsible for the clinical phenotype in Down syndrome; genes that contribute to the pathogenesis of other disorders, including mouse models of Down syndrome; and the mechanisms that cause trisomy 21. The review also provides a good reference

list of achievements in this area and insight into future progress that can be expected.

Antonarakis SE. *Genomics* 1998;51:1-16.

Editor's comment: This 16-page article is an excellent review, especially for nongeneticists and geneticists who do not work in this field. It not only provides considerable information about chromosome 21 but also serves as a good tutorial on gene mapping strategies. Especially interesting is a discussion of how the many different types of genetic maps of chromosome 21 are related and how they are constructed. This review article is highly recommended to all our readers.

William A. Horton, MD

Familial Defects in X-Inactivation

The molecular mechanism by which X-inactivation occurs is beginning to be elucidated. The process of X-inactivation is under the control of the X-inactivation center (XIC), which initiates and proliferates inactivation along the X chromosome. The active X chromosome *Xist* gene encodes and produces RNAs that coat the opposite X chromosome and seems to keep it inactive. Lee et al have shown there also is a *Tsix* element, which apparently lies within the XIC region and is expressed on the active X chromosome. High transcription levels of *Tsix* and *Xist* appear to be mutually exclusive. Interestingly, the transcript seems to overlap the *Xist* genes. How *Xist* and *Tsix* interrelate is not yet clear.

Naumova et al studied X-inactivation in normal women in families that are not known to have any genetic disease. They found quantitative differences among families with strong sister-to-sister correlations as to the degree of skewing from the expected 50% inactivation of each X chromosome. Interestingly, there is a lack of correlation between mothers and daughters. Lymphocytes were used for these studies, and it is certainly possible that other tissues might yield other

results. The sister-to-sister correlation is consistent not only with a hereditary aspect of X-inactivation but also with the possibility that their phenotype is controlled by cis-acting gene. Also intriguing is the possibility that familial X-inactivation skewing involves differences in *Xist* and *Tsix* expression.

Heard E, et al. *Nature Genet* 1999;21:343-344.

Lee JT, et al. *Nature Genet* 1999;21:400-404.

Naumova AK, et al. *Eur J Hum Genet* 1998;1018:552-562.

Editor's comment: X-inactivation is beginning to be unraveled. It is a wonderful model for gene control, identifying the mechanisms that may apply to both genomic imprinting and time-specific expression in tissues. The interesting correlation between sibs, but not mothers and daughters, in skewed X-inactivation families suggests that there is "cross talk" between the 2 X chromosomes. We all have been taught to expect 50-50 inactivation of the X chromosomes, but that appears to have been a generalization rather than a reality.

Judith G. Hall, MD