

Abnormality of the X Chromosome in Human

46,XY Female Siblings with Dysgenetic Ovaries

Abstract. An abnormal extra band was found on the short arm of the X chromosome in a 46,XY female and in her 46,XY female fetal sibling. Despite presence of the intact Y chromosome, there was no evidence of testicular differentiation in either subject. Production of H-Y antigen was suppressed in both subjects. The data suggest that development of the mammalian testis requires a normal function of the X chromosome.

In mammals the initially undifferentiated gonad develops as a testis in the presence of the Y chromosome and as an ovary in the absence of the Y chromosome. Yet presence of the Y does not guarantee testicular differentiation, because XY females are known in several mammalian species. Perhaps the most instructive example is the Scandinavian wood lemming, *Myopus schisticolor*, in which there is a skewed sex ratio with a preponderance of females (1). Roughly half of these female wood lemmings have a male (XY) karyotype, yet they are grossly normal with fertile ovaries. Since XY female wood lemmings are uniformly H-Y negative (2), it might seem at first glance that XY sex reversal in this species is due merely to an abnormality of

the Y chromosome. The curious fact is that XY female wood lemmings produce only X-bearing eggs. They do not transmit the Y. Indeed the condition is inherited as an X-linked trait. It appears that a gene on the X chromosome of this species can suppress normal synthesis of H-Y antigen thereby promoting ovarian development in the XY gonadal primordium (3).

Extreme evolutionary conservatism of the mammalian X chromosome (4) implies existence of a similar gene in humans. However, two X chromosomes are needed to support differentiation of the normal human ovary. Whereas X0 gonads of the rodent develop as fertile ovaries, X0 gonads of the human initially develop as ovaries but degenerate, and

are represented at around the time of birth by undifferentiated "streaks" devoid of follicles (5). With respect to the number of X chromosomes that are present, XY embryos resemble those with the X0 karyotype. Thus in the functional absence of the testis-determining portion of the Y, the gonads of human XY embryos could be expected to initiate a program of ovarian differentiation, but could not be expected to retain a normal ovarian architecture.

These observations connote a human analog of the wood lemming condition: X-linked suppression of testis-determining H-Y genes in 46,XY phenotypic females with streak gonads (gonadal dysgenesis) (6). In fact there are numerous reports of familial XY gonadal dysgenesis in man. In several of these cases, the pedigree suggests an X-linked mode of inheritance (7). Here we describe failure of testicular differentiation in two 46,XY female sibs, both exhibiting a striking abnormality of the short arm of the X chromosome. The case provides rather more direct evidence of a critical role of the X chromosome in primary sex determination of the mammal in general and of man in particular.

Chromosome studies on a profoundly retarded girl with multiple congenital abnormalities revealed a karyotype of 46,Xp⁺Y in peripheral blood and skin fibroblast metaphases (Xp⁺ represents additional material on the short arm of the X; see Fig. 1, III-2). The Giemsa banding pattern of the abnormal X suggested duplication of the p21 and p22 bands, that is, 46,dup(X)(p21→pter)Y. The Y chromosome was morphologically normal and indistinguishable from the Y chromosome of the father (Fig. 1, II-4) and paternal grandfather (Fig. 1, I-3). Evidently, the aberrant X chromosome was derived from the mother. The proband's maternal grandmother (Fig. 1, I-2), mother (Fig. 1, II-2), and a younger phenotypically normal sister (Fig. 1, III-3) all had a karyotype of 46,XXp⁺. The proband died at 5 years of age. Autopsy disclosed female internal genitalia and microscopic ovarian remnants consisting of ovarian stroma and degenerating primordial follicles. No testicular tissue was found. Complete details of the case will appear elsewhere (8).

Amniocentesis, performed on the mother during a subsequent pregnancy, revealed cells with a karyotype identical to that of the proband (Fig. 1, III-4). Diagnosis of a grossly abnormal female fetus was confirmed after termination of the pregnancy at 20 weeks of gestation. Multiple anomalies were found similar to

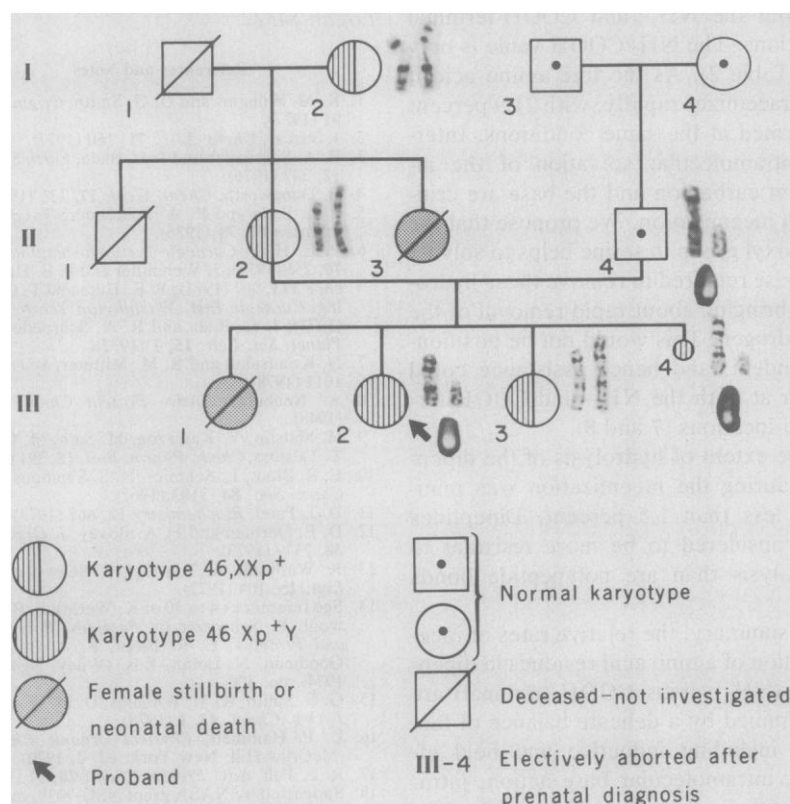


Fig. 1. Pedigree showing segregation of the Xp⁺ chromosome. The Giemsa-banded sex chromosomes and quinacrine-stained Y chromosome are depicted next to the relevant family members. Note the extra band (black) on the abnormal chromosome in comparison with the band on the normal X of the maternal grandmother (I-2), mother (II-2), and normal female sibling (III-3). The extra band is found on the single X chromosome of the XY female proband (III-2) and XY female fetus (III-4).

those of the proband. Internal genitalia were those of a normal female fetus; normal fetal ovaries were present bilaterally; histological sections failed to reveal any trace of testicular tissue.

The following points are worth emphasizing. (i) Serological tests indicated absence of H-Y antigen in skin fibroblasts of the fetus and in blood leukocytes and skin fibroblasts of the proband; (ii) cytogenetic analysis failed to provide evidence for autosome-to-X translocation in any members of the family; and (iii) computer-based videodensitometric analysis of the abnormal chromosome (performed in the Department of Medical Genetics, Mayo Clinic) indicated the likelihood of a duplication of densitometric X bands p12 and 3.

The foregoing observations are consistent with the notion that testicular development is secondary to function of genes on the Y chromosome and on Xp. If X-linked regulatory genes normally served to prevent excess production of H-Y antigen, duplication of these genes might be expected to reduce production of H-Y below a certain critical threshold required for testicular differentiation. Alternatively, the extra bands might interfere with a structural element thereby thwarting production altogether (9). In either event the result would be subnormal expression of the testis-inducer. The XY gonad would now differentiate as an ovary; the human ovary would degenerate in the absence of the second X. We assume that the disparate gonadal histologies of proband and fetus represent different phases of the same developmental anomaly.

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3. K. Fredga, A. Gropp, H. Winking, F. Frank, *Hereditas* **85**, 101 (1977). More recently these authors identified two different G-banded X chromosomes in the wood lemming, one of which is present in "sex-reversed" females. They suggest three sex chromosome constitutions in females of that species: XX, X*X, and X*

- Y, where X* represents a chromosome with a mutant short arm (X*p) that blocks testicular differentiation; E. W. Herbst, K. Fredga, F. Frank, H. Winking, A. Gropp, *Chromosoma* **69**, 185 (1978). Remarkably X*p is shorter than Xp; see text and compare with Fig. 1.
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8. For complete details of the case, see R. Bernstein, T. Jenkins, B. Dawson, J. Wagner, G. De-

wald, G. C. Koo, S. S. Wachtel, *J. Med. Genet.*, in press.

9. It may be argued that structural testis-determinants are situated on the X chromosome and activated by genes on the Y; see, for example, J. L. Hamerton, *Nature (London)* **219**, 910 (1968); R. A. McFeely, W. C. D. Hare, J. D. Biggers, *Cytogenetics* **6**, 242 (1967). If H-Y structural genes are X-situated, then failure of H-Y synthesis in the present study could be due to a position effect resulting from duplication of a segment of Xp.
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Lactate Dehydrogenases of Atlantic Hagfish: Physiological and Evolutionary Implications of a Primitive Heart Isozyme

Abstract. *Isozymes of lactate dehydrogenase from heart and muscle of Atlantic hagfish show less functional divergence than those from other fishes and higher vertebrates. The enzyme from hagfish heart (B_4) displays a higher Michaelis constant for pyruvate and lower substrate inhibition at moderate pyruvate concentrations than heart isozymes from other species. These properties support the hypothesis that the ancestral vertebrate lactate dehydrogenase was a muscle (A_4)-type enzyme and also suggest a role for the B_4 enzyme in the unusual physiology of hagfish cardiac tissue which functions under sustained hypoxic conditions.*

Most vertebrates have two predominant forms of the tetrameric enzyme lactate dehydrogenase (LDH). One of the enzymes (H_4 , B_4) is the primary molecular form in heart and other aerobically respiring tissues, while the other (M_4 , A_4) is the primary form in skeletal muscle tissue (1-3). Muscle-type isozymes of LDH retain activity in the presence of high pyruvate concentrations and have a moderately high Michaelis constant (K_m) for this substrate. This form of the enzyme functions primarily in the reduction of pyruvate to lactate under conditions of limiting oxygen supply (1-5). Typical heart-type isozymes of LDH are severely inhibited by pyruvate concentrations within the physiological range and display a relatively low K_m for this substrate. Substrate inhi-

bition of the heart (B_4) isozyme is considered to promote aerobic reduction to lactate (1-5). The observed distribution of A_4 and B_4 isozymes in vertebrate tissues is generally consistent with this hypothesis (3, 4). Substantial difference in K_m for pyruvate between A_4 and B_4 isozymes within species is a characteristic also shared by widely divergent vertebrate taxa (5, 6).

Lactate dehydrogenases of Atlantic hagfish (*Myxine glutinosa*) provide an excellent system for examining both evolution and physiological roles of LDH isozymes. The hagfish has two forms of LDH which correspond to the A_4 and B_4 isozymes of higher vertebrates in both relative electrophoretic mobility and tissue distribution (7, 8). The phylogenetic position of these animals in class Agnatha (jawless fishes) places them among the most primitive living vertebrates. Among living vertebrates, hagfish are the only ones that have multiple hearts and a partially open circulatory system with sinuses reminiscent of those found in the invertebrate phyla. The two primary pumps, the branchial and portal vein hearts, are composed of true vertebrate cardiac muscle which shows a myogenic origin of rhythmic heartbeat (9). Like their Agnathan relatives, the lampreys (family Petromyzontidae), hagfish lack a coronary circulation for delivery of well-oxygenated blood to cardiac tissue (10). Both hearts pump mixed venous blood which, even when fully oxygenated, has

Table 1. Effect of preliminary incubation with the acetylpyridine analog of NAD^+ on the activity of Atlantic hagfish lactate dehydrogenases. Enzyme was first incubated for 10 minutes at 25°C in the presence of $3.3 \times 10^{-4}M$ pyruvate or $1.0 \times 10^{-2}M$ pyruvate, and $1.43 \times 10^{-6}M$ acetylpyridine analog of NAD^+ . Enzyme activity of micromoles of NADH oxidized per minute was then determined in the presence of natural cofactor under conditions described (14). The ratio reported is the activity at $3.3 \times 10^{-4}M$ pyruvate divided by activity at $1.0 \times 10^{-2}M$ pyruvate.

Isozyme	Activity ratio
Muscle (A_4)	1.01
Heart (B_4)	2.99