

Know Your Chromosomes

5. The Uniqueness of Sex Chromosomes

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The pattern of inheritance of genes linked to the sex chromosomes in humans have their own signature due to the presence of a single copy of X and Y chromosomes in males and 2 copies of the X chromosome in females. However nature has adopted ingenious methods to equalize the copy number of most genes that map to the X chromosome. This in turn has contributed to the understanding of regulatory phenomena involving whole chromosomes.

We have come a long way from the belief prevalent during Aristotle's time that the sex of an unborn child is determined by the direction of the wind, to a stage where we are trying to gain a total understanding of the basis of sex determination. The change occurred with the work of Edmund B Wilson and his book *The Cell in Development and Heredity* published in late 1800. Historically, the X and Y chromosomes constitute the earliest examples of the involvement of chromosomes and therefore of genes, in animal development. Often the female is thought to be at fault when the sex of a child is not what the family desires. But genetically speaking females produce only one type of egg with respect to the sex chromosome constitution, whereas males produce two types of sperms, one bearing the X-chromosome and the other the Y-chromosome. Which one of the sperms fertilizes the egg depends on chance. Therefore in mammals (including humans) males are the heterogametic sex and females the homogametic sex, thus bearing upon the saying that "It needs a man to make a girl".

Sex Linked Inheritance

The X and Y chromosomes referred to as sex chromosomes,

Previous articles of this series were:

1. Nature's way of packing genes, January 1996.
2. The strong holds of family trees, March 1996.
3. Hybrid cells and human genetics, June 1996.
4. The paths to disorder are many, October 1996.

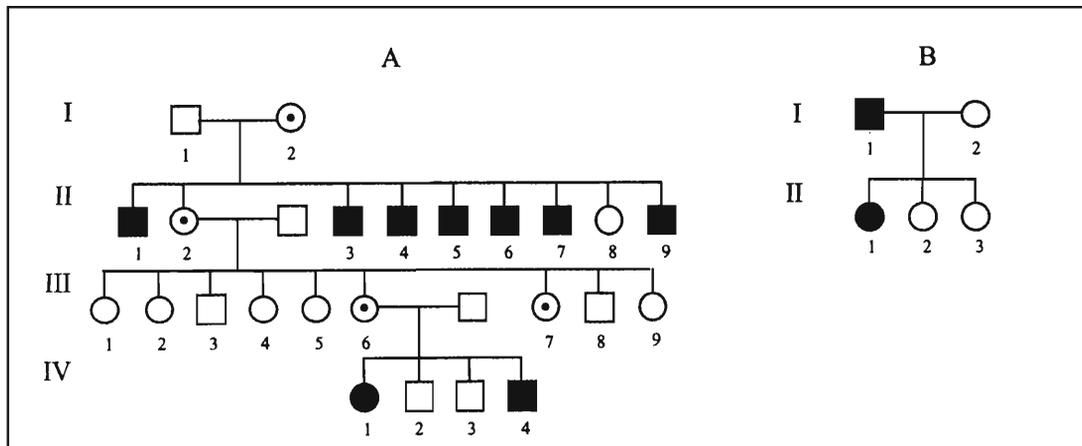


Figure 1 Pedigree with X-linked recessive disorder. A – is Lesch–Nyhan syndrome family drawn based on the data of Nyhan, B – is hemophilia in a family described by Coleman R and coworkers. Female IV –1 in A and II-1 in B are very rare instances where a female expresses an X-linked recessive disorder in spite of carrying a dominant normal allele. In A, IV–1 has inherited the disease from a normal mother, in B II-1 inherits the disease from an affected hemizygous father.

have several unique features. As we know, humans are diploid, each autosome is present as a pair of homologous chromosomes, the X and Y chromosomes are in single copy in human males. Therefore males are haploid or hemizygous for sex chromosomes. This has interesting implications on the pattern of inheritance.

To illustrate the point let us consider the pattern of inheritance of disorders caused by genes located on the X-chromosome. (X-linked disorder, *Figure 1*). These pedigrees trace the inheritance of two diseases. First the Lesch–Nyhan syndrome, which is due to the deficiency of an enzyme called hypoxanthine–guanine phosphoribosyl transferase (HGPRT) that is involved in the metabolism of purines like guanine. This disease leads to neurological anomalies, gout and a compulsive tendency to self-mutilation. The other is haemophilia and is due to deficiency of a blood clotting factor. What interests us in these pedigrees is that more males have the disorder while females are very rarely affected. (Individual IV–1 in *Figure 1A* and II-1 in *Figure 1B*). This kind of a 'sex bias' in the inheritance pattern is the first clue

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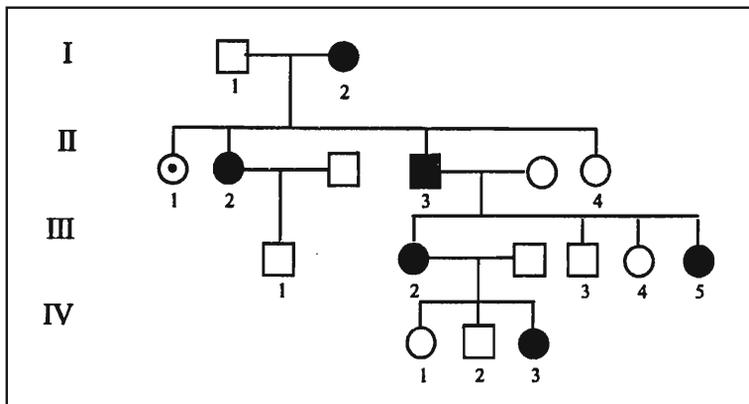


Figure 2 A modified pedigree with X-linked dominant vitamin D-resistant rickets based on the data from Winters and co-workers. The point of interest is female III-4 who inherits the mutant X-chromosome from her affected father but does not manifest the disease.

to a sex linked genetic disorder especially a recessive one. The pattern of inheritance of a sex linked dominant disorder is shown in the pedigree depicted in *Figure 2*. In contrast to a recessive disorder, both sons and daughters of an affected, heterozygous mother manifest the disease and when the father is affected all his daughters have the disorder. In extremely rare instances, inspite of dominant inheritance, a normal daughter is born of a diseased father, as individual III-4 in *Figure 2*. This figure shows a family transmitting vitamin D-resistant rickets, a rare X-linked dominant disorder. This results in skeletal deformities similar to rickets but is due to an enzyme deficiency and not vitamin-D. The two rare instances of a heterozygous female expressing a recessive disorder and the normal phenotype of a female carrying a dominant defect bring us to another interesting phenomenon operating on X-chromosomes.

The sex chromatin or the Barr body represents a chromosome that is highly condensed or compacted.

Nature Attempts to Balance its Endowment to Men and Women

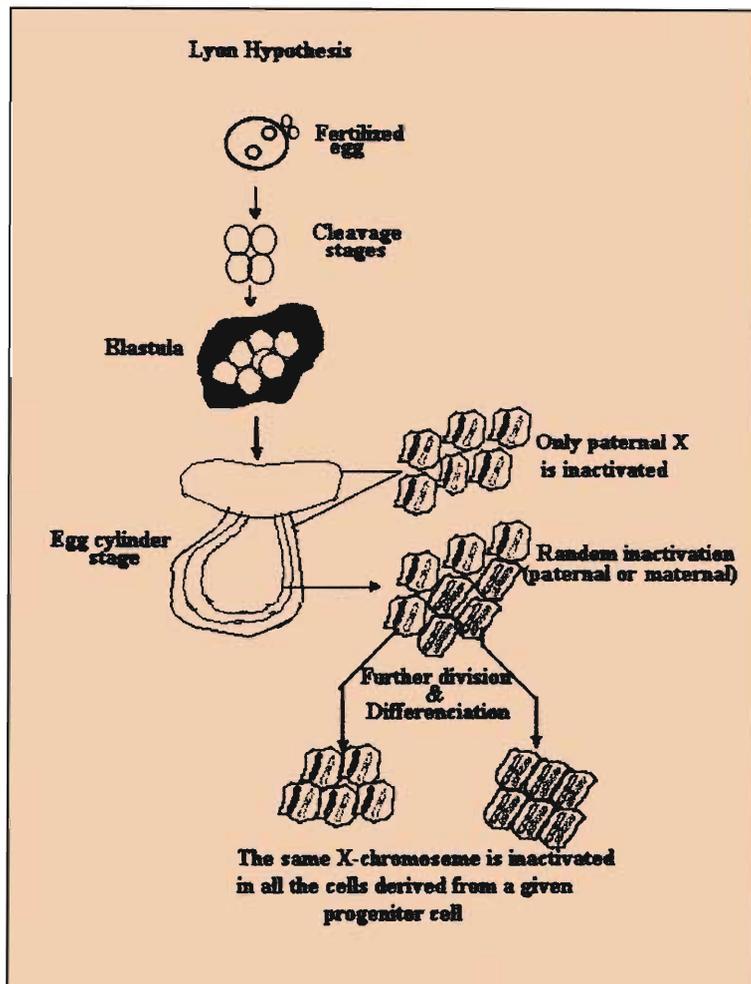
In the 1960s two scientists Barr and Bertam, discovered a darkly staining structure in nuclei derived from nerve cells of female

cats but not male cats. This came to be called the sex *chromatin* or the *Barr body*. It represents a chromosome that is highly condensed or compacted. While it is generally found in all women, in a condition described as *Turner syndrome* such a structure is absent and the karyotype of these women shows that they have only one X chromosome (XO). As a corollary, in females whose karyotype shows the presence of more than two X-chromosomes there is more than one Barr body and males who have XXY chromosomal constitution also have a Barr body. Irrespective of the presence of Y chromosome a Barr body is found whenever there is more than one X-chromosome. One finds that the number of Barr bodies in a cell is $(n-1)$ where n is the number of X-chromosomes. It was also known that mice with only one X-chromosome were normal females, thus suggesting that one X-chromosome is sufficient for female development. Considering all these observations Mary F Lyon from England put forward the well known *Lyon hypothesis*, which addresses the issue that females have twice the number of X-linked genes as the males but the same number of autosomal genes. Lyon proposed that to equalize the effective chromosome dosage between the sexes, one of the two X-chromosomes in females is inactivated. This means that none of the genes from that chromosome is transcribed. She also proposed that this process is random meaning that in some cells the X-chromosome inherited from the father is inactivated while in others the maternal X-chromosome is inactivated. But once an X-chromosome becomes inactive, the same X-chromosome continues to be inactive in all the descendants of that cell (*Figure 3*).

How does this explain the unexpected expression of a sex linked recessive disorder in a heterozygous female, or the absence of a sex linked dominant disorder in a female carrying the defective gene? In the female IV-1 *Figure 1A* and II-1 in *Figure 1B*, if the normal X-chromosome is inactivated the X-chromosome that bears a mutation is kept active; effectively

According to *Lyon hypothesis*, to equalize the effective chromosome dosage between the sexes, one of the two X-chromosomes in females is inactivated.

Figure 3 X Chromosome inactivation is non – random in extraembryonic tissues (only paternal X inactivated), but random in the embryo; either maternal (red) or paternal (blue) is inactivated. But once inactivation occurs, it is clonally maintained. Inactivated X are shown as filled bars.



Calico cats are females having one X chromosome carrying the orange allele and the other X chromosome carrying the black allele for coat colour.

this would lead to the absence of the protein and therefore she has the disorder. Similarly, in the female III-4 in *Figure 2*, if the X-chromosome carrying the dominant defective gene is inactivated, she will be normal inspite of having a dominant disorder genetically. In rare instances haemophilia, an X-linked recessive disorder due to deficiency of a blood coagulation factor, has been observed in females (*Figure 1B*). All these instances can be explained by non-random inactivation of X-chromosomes wherein for reasons not clear at present, the same X-chromosome is inactivated in all the cells of that individual.



This phenomenon referred to as *dosage compensation* is seen in several systems and it provides yet another example where nature not only responds to the presence or absence of functional genes but is also sensitive to quantitative differences in gene dosage. Readers may recollect that a similar dose difference leads to Down Syndrome due to trisomy 21 (*Resonance* October, 1996).

Random X–chromosome inactivation results in the interesting patterns of coat colour seen in mice and cats. This is described as the *calico cat* (featured in the cover page of this issue). The coat colour genes responsible for orange and black colours map to the X–chromosome. Calico cats are females having one X chromosome carrying the orange allele and the other X chromosome carrying the black allele for coat colour. Each patch indicates the presence of a set of melanocytes or pigment cells in the hair follicles which are clonally derived from one precursor cell where an irrevocable but random inactivation of one of the two X chromosomes was brought about during early embryonic stages. This phenotype of coat colour substantiates the randomness of X–inactivation as proposed by Mary Lyon. The white patches are due to another gene at a locus called the spotting locus involved in melanocyte migration.

Some Genes on the X–Chromosome Escape Inactivation

Earlier in this article I mentioned that humans with single X–chromosomes are females with associated abnormalities (Turner syndrome). This poses an apparent paradox: males with single X–chromosome are normal and females are also physiologically haploid for X–chromosome because of the inactivation of one of the two X–chromosomes; why then are females with an XO constitution abnormal? The reason seems to lie in the fact that not all the genes on the X–chromosome are inactivated. There are some genes that escape inactivation, for example steroid sulphatase gene (STS). Does this mean that females have two

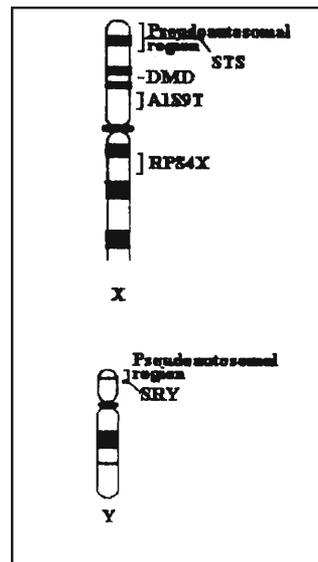


Figure 4 Diagrammatic representation of X and Y chromosomes. A1S9T is a locus involved in DNA replication, RPS4X is a gene coding for a ribosomal protein, S4. Both A1S9T and RPS4X escape X–inactivation like the pseudo-autosomal region. DMD is the gene for dystrophin, mutations in which result in Duchenne Muscular distrophy.

	Drosophila		Human		C.elegans	
	Female	Male	Female	Male	Hermaphrodite	Male
Chromosome constitution	XX	XY	XX	XY	XX	XO
Activity level of each X chromosome (in arbitrary units)	50	50	100	50	0	50
Dosage compensated by	higher activity in male		silencing one X in females		reducing activity of both Xs in hermaphrodite	
Result	Equal levels of activity of X linked genes in males and females					

Figure 5 Varied strategies of dosage compensation lead to the same net effect in worms, insects and mammals.

copies of functional STS gene and males have only one? It turns out that a copy of the STS gene is present on the Y–chromosome. Thus for all the genes known to escape inactivation there is a Y–linked copy. Therefore these genes would behave genetically as autosomal genes and hence are appropriately called *pseudoautosomal genes* (Figure 4). Persons with Turner syndrome (XO) have only one copy of pseudoautosomal genes whereas normal men and women have two copies of these genes. Yet another example of dose difference resulting in a genetic disorder. In mice there are no genes known so far, to escape inactivation, one entire X–chromosome is inactivated in females. Therefore XO mice are normal fertile females. Equalising gene dosage between the two sexes is seen in some insects and worms, but is achieved by different means (Figure 5). For example the fruitfly *Drosophila* (XX female and XY male) achieves dosage compensation by doubling the transcriptional activity of a single X–chromosome in males while the nematode *C. elegans* accomplishes dosage compensation by reducing the transcriptional activity of both the X chromosomes in females or hermaphrodites. (In this worm XX individuals are commonly hermaphrodites, having both male and female sexual organs and XO individuals are males).

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Genes that Map to X and Y Chromosomes

There are about 237 genes mapped to the X-chromosome so far. The haploid state of X-chromosome in males provides an advantage in mapping X-linked genes. Though X and Y chromosomes are called sex chromosomes, the nature of genes mapping to the X-chromosome varies. They include genes coding for enzymes involved in normal metabolism of the cell like the Glucose 6-phosphate dehydrogenase, DNA polymerase and dystrophin (a muscle protein). Some well known genetic disorders mapping to X-chromosome are Duchenne Muscular Dystrophy (DMD), haemophilia, Becker Muscular Dystrophy (BMD), retinitis pigmentosum type 2 & 3 and colour blindness. There are about 45 X-linked loci identified with mental retardation. In most of these cases the gene responsible for mental retardation is not known. A gene designated as Tfm (testicular feminisation) which codes for a receptor for the male hormone androgen maps to the X chromosome. In the absence of the receptor the hormone cannot act. This results in feminisation of XY individuals.

The Y-chromosome is also haploid and is unique to males. It is observed that an embryo that carries a Y-chromosome develops into a male irrespective of the number of X-chromosomes it bears. Thus the Y-chromosome is a dominant male determinant. It took nearly 30 years to identify the male determining gene on the Y-chromosome even though we knew that the Y-chromosome is a dominant male determinant. Individuals with abnormalities relating to sex determination, such as XX males and XY females, were crucial in the identification of the male determining gene on the X-chromosome. We do not understand all the molecular genetic mechanisms of male determination completely, but a gene designated as sex – determining region Y (SRY) has been identified in both humans and mice. Introduction of this gene into chromosomally female zygotes (XX mice) transforms them into males.

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Table 1 Frequency of numerical anomalies of chromosomes.

Chromosome	Frequency of occurrence in spontaneous abortions (%)	Probability of survival to birth (%)
2	1.1	0
16	7.5	0
21	2.3	22.1
XXY	0.2	55.3 (1/700)*
XXX	0.1	70.0 (1/1000)+
XYY	-	100.0 (1/800)*
XO	8.6	0.3 (1/2500)+

The fractions in parenthesis indicate the incidence relative to male births (*) and female births (+). Data taken from P A Jacobs and T J Hassold. *Advances in Genetics*, 1995.

The Y-chromosome is the one on which the least number of genes have been mapped. There are only 18 loci mapped to the Y-chromosome. Some of these genes are involved in fertility in males. There is a gene on the Y-chromosome which codes for a protein factor identified on the cell surface only in males. This is the H-Y antigen and for many years it was thought to be involved in male determination. The identification of a female mouse carrying an active H-Y antigen gene translocated from the Y-chromosome to the X chromosome disproved this hypothesis. There are several syndromes with numerical abnormalities in sex chromosomes. Screening of new born babies and abortuses tells us that the probability of birth of fetuses with numerical sex chromosomes anomalies is higher than those with numerical autosomal anomalies (except Turner Syndrome, *Table 1*). This may be related to dosage compensation mechanisms of X-chromosome and the fact that Y-chromosome contains very few genes necessary for basic metabolism.

The discussion in this series of articles has illustrated that the

distribution of genes on different chromosomes is not related to their function except in certain instances, genes occur in clusters similar to globin gene clusters (*Resonance*, October 1996). However it is interesting to note that most genes which map to the X-chromosome in humans are also found on the X chromosome in mice. These correlations are believed to have evolutionary significance.

In the next and the last part of this series I will discuss some of the implications of knowing our chromosomes.

Acknowledgement

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Suggested Reading

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And Science, we should insist, better than any other discipline, can hold up to its students and followers an ideal of patient devotion to the search for objective truth, with vision unclouded by personal or political motive.

Sir Henry Hallett Dalt

