

Know Your Chromosomes

2. The Strong Holds of Family Trees

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We continue the series with a discussion on family trees or pedigree analysis and the mapping of genes through linkage studies.

Kings, canine breeders and geneticists worry about pedigree. A cytogeneticist while dealing with a suspected genetic disorder asks the patient for a family history and draws a *pedigree chart* (Figure 1) which shows if there are other family members having the same disorder and whether there is any sex bias in its occurrence, i.e. does the disorder occur in males more often than in females or vice versa.

Chromosome Sets and Gene Locations

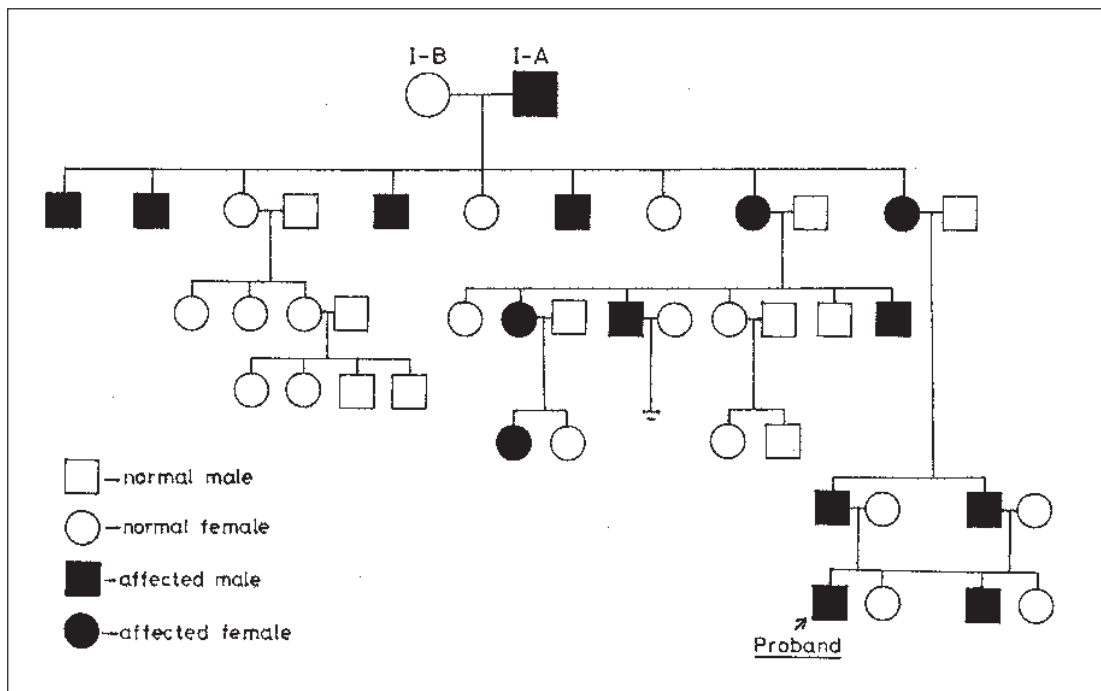
We know that most organisms including humans, are diploid which means that each gene is present in at least two copies. In fact, some genes in the human genome are present in multiple copies. You can well imagine that when one copy gets bad the other one can take over just as two musicians can fill in for each other in a concert. If one stops singing, the concert does not have to stop but can continue, perhaps as well as before. A similar situation occurs in the case of most genes on chromosomes 1 to 22, which are the autosomes. But the X and Y chromosomes in men are like single musicians singing in a concert; if this musician stops singing, the concert comes to an end. The X chromosome in the female still enjoys the luxury of having a second copy but males have only one X chromosome. They are haploid for X and Y chromosomes. Now does it make sense to say that any defect in a gene located on the X chromosome is expressed in males more often than in females? For most genes located on the autosomes we see no such sex bias.

There are about 357 loci mapped on chromosome 1 so far (1st September, 1995). The word loci (singular *locus*) is used instead of

Terms italicized in the main text are explained in the glossary on page

39.





genes because there can be a disease state mapped on a given chromosome, for which the specific gene has not yet been identified. *Locus* is the term for a chromosomal region where a trait is mapped. There may be more than one gene in the region responsible for the trait. For instance, there is a locus termed neuroblastoma on the short arm of chromosome 1 in band 32. The gene position is denoted as 1p32. The information known in this case is that a defect in this region results in an increased susceptibility to neuroblastoma. This band itself comprises about 3 million base pairs of DNA capable of housing 50-100 genes. Therefore the trait leading to neuroblastoma can be due to a defect in one or more genes in the region. On the other hand, 'Maple syrup urine disease-type II' can be called a gene because it is known that the gene codes for a polypeptide chain of a multiple subunit enzyme called alpha-keto acid dehydrogenase. In this particular example the gene sequence is also known. But often the terms locus and gene are used as synonyms.

Pedigrees Help Determine the Mode of Inherit-

Figure 1 Pedigree analysis of Antithrombin deficiency in a family with thrombophlebitis. This analysis shows that AT3 deficiency is an autosomal dominant disorder. (Taken from E.W. Lovein et al in *Cytogenetics and Cell Genetics* (1978) 22, 319-323).

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Chromosome 1 is one in which the largest number of loci have been mapped. It is also the first human autosome on which a gene locus was mapped. As mentioned in the previous article (*Resonance*, Vol.1, No.1) there are several approaches to mapping genes. In this case I will use the example of antithrombin III (AT3) and describe gene mapping by the classical genetic approach.

Antithrombin is an α -globulin protein found in the *plasma*. It acts as the principal inhibitor of thrombin and other coagulation factors in blood. Deficiency of AT3 leads to disorders related to inappropriate blood clotting like 'thrombophlebitis' and 'acute aortic thrombosis'. Patients die prematurely because of blood clotting in vital arteries and veins. There are several families where this deficiency occurs. One such pedigree or family chart is shown in *Figure 1*. When the male marked as '*proband*' came to the doctor's attention, the family history of the patient and his parents as well as the AT3 phenotype in all available family members was documented. An analysis of the data presented in *Figure 1* reveals the following features:

Deficiency of AT3 leads to disorders related to inappropriate blood clotting like 'thrombophlebitis' and 'acute aortic thrombosis'.

- a) Both males and females in the family are affected.
- b) The father I-A, but not all his children has the disease. Only about 50% of the children of an affected parent have the disorder.
- c) When a child is affected, we see that at least one of the parents has the disease.
- d) When both the parents are normal, none of the children are affected.

Based on these observations one can draw certain conclusions. This disorder is not sex chromosome linked because both males and females are affected. Only one of the two AT3 genes that the father I-A has is defective, as there are normal children among his progeny. Genetically he is said to be *heterozygous* for AT3 as against his wife who is *homozygous* for normal AT3. The observations (c) and (d) would lead one to conclude that it is a *dominant* disorder. This means that whenever an individual has one defective copy of the gene and



the other is normal, he or she expresses the disorder. In technical terms the individual shows the disease *phenotype*. Thus, we conclude that AT3 deficiency is an autosomal dominant disorder.

Mapping by Linkage

Having determined the mode of inheritance, the next task was to localize AT3 to a specific chromosome. In experimental animals one can interbreed individuals with selected phenotypes. For instance, one can set up a mating between a brown eyed female with AT3 deficiency and black eyed male with normal AT3. Similar matings using several pairs of animals with different combinations of eye colour and AT3 gene can be set up. Let us assume that with respect to the phenotype of the AT3 gene (normal versus defective) and eye colour (brown versus black), the offspring always receives the parental combination of the two phenotypes; i.e. whichever offspring inherits AT3 deficiency also inherits brown eyes. In the example described here one concludes that AT3 and eye colour genes are close together and are not separated by *genetic recombination*. This is an instance of *genetic linkage* which means that AT3 is closely linked to the eye colour gene. Assuming that one knows the chromosomal location of brown eyes one could then map AT3 to the same chromosome. Of course, this approach is not possible in humans who marry with total disregard to the needs of geneticists!! In spite of this, the large size of human populations and the fact that individuals who show deviations from normal development and physiology are brought to medical attention, make it possible to obtain information to map human chromosomes. To identify the exact position of the gene on the chromosome other approaches are required, and these will be discussed in subsequent articles.

In the specific case of AT3, linkage studies were done with several known traits or markers in various families that had been kept on record with different investigators. It was found that a blood group called 'Duffy' is inherited along with AT3 more often than expected by chance alone. Duffy is an antigen on red blood cells like A, B or the Rh factor. It was originally detected in a patient named Duffy.

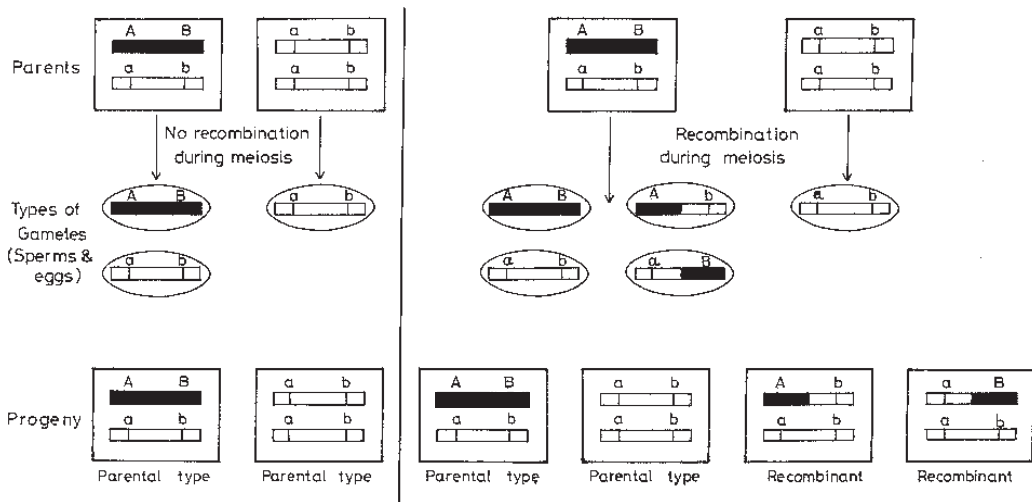
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Genetic Recombination

Recombination is the process of exchange of genetic material between *homologous chromosomes*. Recombination during meiosis results in the formation of gametes (sperms and eggs) with combinations of genes different from those present on parental chromosomes. The progeny of such matings will generate individuals with combinations of traits not present in the parents. The frequency with which recombinant individuals occur in a pedigree, provides an estimate

of the distance between the genes. The greater the distance between two genes, the higher their recombination frequency; 50% being maximum, in which case the genes will behave as if they were on two different chromosomes. To map a new gene based on recombination frequency one should have a large number of known genes on each chromosome so that this parameter of distance and *recombination frequency* can be reliably assessed.



A and B are two traits that have two *alleles* (forms) A, a; B, b.
Each box represents an individual.

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The Duffy blood group represented as Fy had been mapped to chromosome 1. In this particular case it was seen that in several pedigrees or families, Duffy blood group was present in those individuals who also had an abnormal looking chromosome 1. Localization of Duffy to chromosome 1 was based on this observation and the linkage of Duffy to another locus previously mapped



to this chromosome. This was the only approach used to map genes on human chromosomes till modern methods of molecular biology were developed and applied. In the case of AT3 these methods have helped in localizing it to a defined band on chromosome 1 and in identifying the protein product encoded by the gene. The first description of the disorder was made around the year 1965, it was mapped to chromosome 1 in 1978 and the gene sequence was identified in 1983. One can imagine the effort required to complete these analyses which involve chromosomal mapping, identifying the gene, sequencing it and understanding its function! But methods of disease management, principally by the injection of anticoagulants, have not changed much over the decades. What good is it to dissect the disease process to its ultimate molecular basis when one cannot correct it? It is hoped that a molecular understanding would lead us to the correction of gene defects at the DNA level. I will discuss some of these aspects in articles at the end of this series.

As I mentioned before there are more than 300 genes mapped on chromosome 1. There are source materials that provide all the information known about these genes. What I have done here is to provide an abbreviated map which indicates the genes known to cause specific disorders (*Figure 2*) and the biochemical pathways they affect (*Table 1*).

The challenging route from chromosomal location to identifying the gene and pinning down the functional defect is too intricate to describe in one article. Future articles will deal with this process, one aspect at a time. Just as hybrid plants and animals have been created for improving farm products, hybrid cells have served as one of the essential tools for genetic analysis. We will examine this approach in the next article.

Glossary

Allele: One of an array of possible forms of a given gene, which can be distinguished by their differing effects on the manifestation of a genetic trait.

Dominant and Recessive allele: An allele that expresses its effects even in the

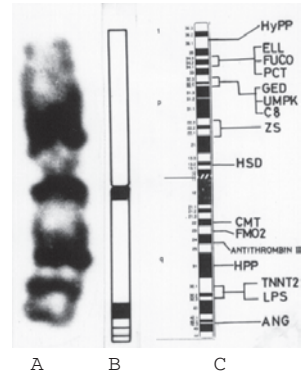


Figure 2 A diagrammatic representation of chromosome 1, as seen under a light microscope after giemsa banding (A), a diagrammatic representation indicating the bands used as landmarks for chromosome identification (B), schematic showing the position of genes listed in Table 1(C).

The author would like to thank Sanjeev Khosla for help in accessing databases on Internet.



Table 1 A Listing of Representative Loci Mapped to Chromosome 1.

Gene/disorder	Chromosomal Location	Mode of Inheritance
1. Fucosidosis (FUCO) The disorder is due to deficiency of fucosidase enzyme leading to accumulation of the sugar fucose in tissues. Neurological deterioration, growth retardation, seizures and early death are the manifestations of the disease.	1p 34	Autosomal recessive
2. UDP-galactose-4-epimerase deficiency (GED) The enzyme epimerase is involved in the interconversion of galactose and glucose. Deficiency leads to seizures, requires external galactose.	1p	32 Autosomal recessive
3. Liver, Alkaline Phosphatase, Hypophosphatasia (HyPP) Enzyme acts as a lipid anchored ectophosphatase. Seizures due to accumulation of pyridoxal-5'-phosphate which interferes with neurotransmitter levels. One of the forms is fatal in infancy. Adult form leads to skeletal abnormalities and premature shedding of teeth.	1p 36	Autosomal recessive
4. Porphyria cutanea tarda (PCT) Disease results in light sensitive skin lesions, fragile skin due to deficiency of uroporphyrinogen decarboxylase, an enzyme involved in biosynthesis of heme. A rare instance where an enzyme deficiency is dominant in its effect.	1p 34	Autosomal dominant
5. Complement component-8 C8 deficiency (C8) Deficiency leads to defect in immune system resulting in frequent bacterial infections specially Neisseria and meningococcus.	1p 32	Autosomal dominant
6. Uridine monophosphate Kinase (UMPK) UMPK catalyses conversion of uridine monophosphate to uridine diphosphate. Deficiency leads to an impairment of effective immune reactions resulting in susceptibility to infections.	1p 32	Autosomal recessive
7. Peroxisomal membrane protein. PK MP1 Zellweger syndrome (ZS) Peroxisomes are single membrane bound organelles of cells, involved in oxidation of fatty acids. Deficiency	1p 22-p21	Autosomal recessive



of FXMP1 leads to absence of peroxisomes in liver and kidney and to early death.

Gene/disorder	Chromosomal Location	Mode of Inheritance
8. 3-β-hydroxy steroid dehydrogenase delta isomerase (HSD)	1p 13.1	Autosomal recessive
This enzyme is involved in the biosynthesis of testosterone. Defect in the enzyme leads to excretion and incomplete masculinization in males and mild virilism in females.		
9. Elliptocytosis (ELL)	1p 34.2-p33	Autosomal recessive
The disorder is due to a defective membrane protein of red blood cells. Results in elliptic shape of RBCs, anaemia and gall stones. Removal of spleen relieves the symptoms.		
10. Charcot-Marie-Tooth Disease (CMT)	1q 22	Autosomal dominant
This locus codes for a myelin protein. Mutation in the gene leads to neural disorders like CMT, and sensorineural polyopathy.		
11. Flavin containing mono oxygenase 2 (FMO2)	1q 23-25	Autosomal recessive
This enzyme mediates oxidation of amino-trimethylaminase (TMA) derived from diet. Defect leads to excretion of TMA in urine, fishy odour of the body, anaemia and psychosocial problems.		
12. Hypokalemic periodic paralysis (HPP)	1q 31-32	Autosomal dominant
The gene codes for a calcium channel protein that controls entry and exit of calcium ions. The defective gene leads to weakness and paralytic attacks accompanied by low levels of potassium in blood.		
13. Cardiac Troponin-T2 (TNNT2)	1q 32	Autosomal recessive
Troponin is a muscle protein. This specific variety is made only in heart muscles. Defect in the gene manifests as cardiomyopathy.		
14. Lip-pit syndrome (LPS)	1q 32	Autosomal dominant
No specific gene is identified. But a deletion or loss of DNA sequences from this region results in cleft palate and deformities of the upper lip.		
15. Angiotensinogen (ANG)	1q 42-q43	Autosomal recessive(?)
The gene codes for angiotensinogen which is a precursor of angiotensin II involved in elevating blood pressure.		

The abbreviations given in parentheses are used to indicate the gene loci in Figure 2.



Suggested reading

Daniel L Hartl. Human Genetics. Harper and Row Publishers New York, Cambridge, London. 1983.

Morroe W. Strickberger. Genetics. MacMillan Publishing Company New York, Collier Mac Millan Publishers, London. 1976.

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presence of a different form of the same gene is called a dominant allele. The allele whose effects are overridden by the dominant allele is said to be recessive.

Genotype: The specific allelic constitution of genes of an organism or a cell.

Genetic linkage: This means that two or more genes in question are located on the same chromosome. The proximity of the genes is estimated by recombination frequency.

Heterozygosity: A condition of having one or more pairs of dissimilar alleles.

Homozygosity: This means having identical rather than different alleles in the corresponding loci of homologous chromosomes.

Homologous chromosomes: Chromosomes that pair during meiosis. Homologous chromosomes contain the same linear sequence of genes and as a consequence, each gene is present in at least two copies.

Locus: (plural loci) The position that a gene occupies on a chromosome.

Pedigree analysis: Analysis of ancestral history or genealogical register or a family tree drawn to show the inheritance patterns for specific phenotypic characters.

Phenotype: The detectable manifestations of a *genotype* in conjunction with the environment.

Plasma: Fluid portion of the blood made up of proteins.

Proband: The person who is brought to the attention of the clinician for a potential genetic disorder. Starting from him/her the family tree is traced.

Recombination: The process that generates progeny with combination of alleles on a given chromosome, different from those that occur in the parents.

Recombination frequency: The proportion or percentage of products of recombination. This frequency is used as a guide in assessing the relative distances between loci on a genetic map. The proportion or percentage of products of recombination. This frequency is used as a guide in assessing the relative distances between loci on a genetic map.

