Chapter 1
Pedigree Analysis

The fully decoded human genome has been estimated to contain about thirty thousand genes held within the three billion or so nucleotides in each of our cells. A genetic disorder is a disease caused by abnormalities in an individual's gene or chromosomal set. But due to the infrequently reproduce and also fetal development process that spans a lifetime; it becomes more difficult to study genetic disorders in humans. So the method devised by geneticist to study the process is through analysis of the genetic history of a family or a "Pedigree analysis".Pedigree analysis tries to deduce inferences about a pedigree on the basis of partial information. The information available may be of three types: the genealogical structure (relatedness among the member of the family), the phenotypic expression of each member, and tries to infer the genetic-or other-mechanism underlying the distribution of phenotypes over the members of the pedigree. Also it is then used to make inferences about the information that is missing. Difficulty arises when information about some link in the pedigree is missing the inference about the phenotype of an unborn child(in case of miscarriage) or information on phenotype of the member's of pedigree is missing like in case if the the ancestral origin of specific alleles are unknown. The mode of inheritance is also a typical problem related to genetic epidemiology. These inheritance patterns are present in the genetic material during zygote formation or at the time of cell division. It may be expressed at the time of birth or during the late course of the life of an individual. As we know every human on Earth shares 99.9% of the same genetic code; only 0.1% of the genetic makeup differs.This 0.1% variation, therefore, gives rise to a disease condition in an individual.The disease can be a mutation in single gene of nucleus or mitochondrial genome or can be present in a cluster of genes giving rise to a complex disorders. The complex disorders have a genetic component along with an attached environmental factors too which helps in its progression.

The inheritance patterns trace the genetically encoded traits, condition and disease to an offspring passed to him by his previous generation. The study of inheritance of genetic disease process is not straightforward in humans because of the infrequent reproduction process and delay in expression of the diseased genes. So instead of experimental biology, genetic counsellor have built their interest in studying pedigree analysis .The pedigree gives

the aggregation of the occurrence of more cases of a given disorder in close relatives of the proband than in control families. The process includes building an information chart about the genetic history of a family or a "genetic family tree". This tree not only includes genotype of a particular family but also phenotypes of a particular genetic trait from one generation inherited by the next generation. Thus pedigree is a pictorial description of a family tree generally used by genetic counsellors to determine whether the disease is indeed inherited in his/her family or not; if it is, then the very next step is to establish the mode of inheritance. The inheritance pattern which is known from pedigree along with the diagnosis of the proband help the clinicalsion to provide accurate risk information related to the family. This includes risk information for future generations or relatives who are currently unaffected, but who are at risk for developing the disorder based on family historical information. In the studies aimed at identifying genes that cause a particular genetic disorder, researchers must collect detailed information on relatives participating in the study, particularly those relatives who are affected with the disorder. Then a comparison is made between genes of affected individuals with the genes of those who did not inherit the disorder to identify the specific mutations responsible through high throughput methods. A pedigree can help identify which family members should be included in mutation analysis because including only those members who are affected or are at-risk of carry that particular mutation will save time and money. Not only this the linkage maps between various markers or haplotypes can also be show pictorially in a pedigree. The investigator can trace the history of some variant phenotype back, through the history of the family and can draw up a family tree, or pedigree, using the standard symbols. The clues in the pedigree have to be interpreted differently depending on whether one of the contrasting phenotypes is a rare disorder or whether both phenotypes of a pair are common forms of a polymorphism.

Some genetic changes are very rare; others are common in the population. Genetic changes that occur in more than 1 percent of the population are called polymorphisms. They are responsible for creating the uniqueness among individual. Though most of them are neutral polymorphisms having no negative effects on an individual's health, some of them have heavy risk associated with them. They belong to two broad categories. One of them includes those which are inherited from a parent and other is those which are acquired during an individual's lifetime. Sometimes the egg and sperm cells contain certain sets of mutations which are passed to the zygote during its formation .This type of mutation are localized in every cell of the body. Mutations that occur just after fertilization are called denovo

mutations. Denovo mutations may explain genetic disorders in which an affected progeny has a mutation in every cell, but has no family history of the disorder associated with that.

1.1 The Relationship of Genes and Traits

An allele is a particular version of a given DNA sequence. There can be more than two possible alleles for a given gene locus but only one will be expressed at a time, in a given individual. Multiple alleles can mean many different possible combinations for individuals like the allele for eye colour. The alleles confer traits, by expressing gene products, which are either mRNA and protein, or a functional RNA. Traits result from complex interactions (1) among the products of genes; (2)between genes and regulatory proteins expressed by other genes; (3) between genes and proteins, and environmental factors such as nutrients, temperature, etc.; (4) chance effects during development. Even after so much of complexity the reason because of which Mendelian inheritance seems to "work" in many situation is because the phenotype (appearance of trait) is constant, for a given genotype (genes affecting a trait).Alleles at a single gene locus can act by the following 3 ways .

(1) Each allele contributing to the phenotype in a dominant fashion. In case of Incomplete dominance; the hybrid of both the alleles produces a lesser degree of the dominant phenotype than the pure-breeding dominant.
(2)Either dominant or recessive results in death before birth, a class of progeny will be absent from the offspring; condition is called Lethality.
(3) One allele (or pair of recessive alleles) at one gene locus can affect other phenotypic traits; this effect is called Pleiotropic.

1.2 Types of Inheritance pattern

Genetic disorders may also be a single gene defect or a complex, multifactorial, or polygenic defect. The single gene defect inheritance pattern can be studied under autosomal, X-linked or mitochondrial categories. The autosomal disorders can belong to any of the dominant or recessive phenotypes. The mitochondrial system, the critical organelle is involved in the power generation activity .Thus mitochondrial diseases are worse when the defective mitochondria are present in the muscles, cerebrum or nerves because of the fact that these organs require more amount of energy than other parts of the body. Although these diseases

vary greatly in presentation from individual to individual, several major categories of disease conditions have been defined based on the common phenotypic features, symptoms, and signs associated with the particular mutations that tend to cause them.

1.2.1 Monogenic Disorder Inheritance

The disease condition arises by mutations in the DNA sequence of one single gene. Genes code for proteins, the molecules that carry out most vital functions of the body, even make up the majority of cellular structures. When a gene is mutated so that its protein product can no longer carry out its normal function, a disorder can result. There are more than 6,000 known single-gene disorders, which occur in about 1 out of every 200 births [1]. The single gene disorders follow Mendelian pattern inheritance and construction of an accurate family pedigree will serve as an informational framework for human genetic disease research. The genetic characters belonging to this category are those whose presence or absence depends on the genotype at a particular gene locus. The chromosomes in the body can be divided into autosomes and sex chromosome, so the basic categorization of single gene disorder includes autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive and Y linked disorder.

1.2.1.1 Autosomal Dominant Inheritance

The disease condition in which only one abnormal allele of a gene is present .The affected male or female have equal probability of passing the disease to offspring. In a typical pedigree for an AD disease, individuals in multiple generations will be affected, no generations will be skipped. A heterozygous affected parent and an unaffected parent have, on average, an equal number of affected and unaffected children; i.e., risk of occurrence for each child of an affected parent is 50%, because the affected individual can be heterozygous for the trait that is having one normal copy of gene and one affected copy, thus the offspring have 50% chances of inheriting the disorder from affected parents. Also the unaffected children of an affected parent do not transmit the trait to their descendants. A typical pedigree of an autosomal dominant trait has been shown in Figure 1.1.

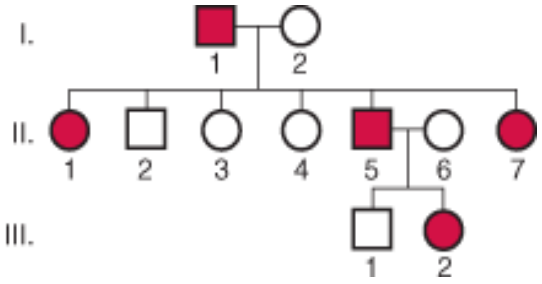

Figure 1.1 Shows typical pedigree having Autosomal dominant mode of inheritance.

1.2.1.2 Autosomal Recessive Inheritance

The two copies of a mutant allele are needed to manifest an autosomal recessive trait. Generally, AR disorders are seen in one or more siblings in a family, but not in parents or children of patients. Heterozygous carriers are usually clinically normal but may exhibit biochemical abnormalities. An example of a pedigree is shown in Figure1.2. If normal parents have an affected child, both parents are heterozygotes. On average, one fourth of their children are affected, half are heterozygotes, and one fourth are normal. Therefore, among the children, the chance of not developing the disorder (that is, being normal or a carrier) is 3/4, and among the unaffected children, the chance of being a carrier is 2/3. All children of an affected parent and a genetically typically normal parent are phenotypically normal heterozygotes. On average, half the children of an affected parent and a heterozygotic parent are affected, and half are heterozygotes. All children of 2 affected parents are affected. Males and females are equally likely to be affected. Heterozygotes are phenotypically normal but carry the abnormal gene.

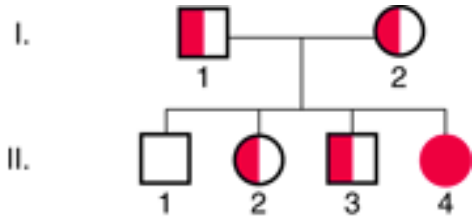

Figure 1.2 Shows typical pedigree having Autosomal recessive mode of inheritance.

The relatives are more likely to carry the same mutant allele, so mating between close relatives (consanguinity) increases the likelihood of increasing the risk of recessive trait. In parent-child or brother-sister unions (incest), the risk of having abnormal children is increased because so much of their genetic material is the same. In certain populations, the

percentage of heterozygotes (carriers) is high because of a founder effect, the group started with few members, one of whom was a carrier or sometimes it may have a selective advantage .If the trait results in a defect of a specific protein, heterozygotes usually have a reduced amount of that protein.

1.2.1.3 X-Linked Dominant Inheritance

X-linked dominant traits are carried on the X chromosome. These rare disorders are usually affecting the males; some X-linked dominant disorders are often lethal in males. Females who carry only one abnormal allele are affected, but less severely. A typical pedigree in Figure1.3 shows that affected males transmit the trait to all of their daughters but to none of their sons. Affected heterozygous females transmit the trait to half of their children, regardless of sex. The affected homozygous females transmit the trait to all of their children. Because females can be heterozygous or homozygous, more females have the trait than males. The difference between the sexes is even larger if the disorder is lethal in males.

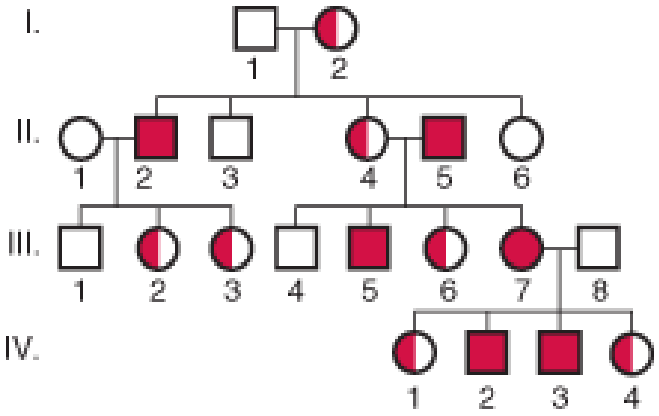

Figure 1.3 Shows typical pedigree having X-linked dominant mode of inheritance.

X-linked dominant inheritance may be difficult to differentiate from autosomal dominant inheritance by studying only inheritance patterns. Large pedigrees are required; with particular attention to children of affected males because male-to-male transmission rules out X-linkage (males pass only their Y chromosomes to their sons).

1.2.1.4 X-Linked Recessive Inheritance

X-linked recessive traits are carried on the X chromosome. Thus, nearly all affected people are male because females are heterozygous they have one normal copy of the involved gene .The Figure 1.4 has a typical pedigree showing the traits of X-linked recessive pattern of inheritance. The rules of inheritance defines that nearly all affected people are male. The heterozygous females are usually phenotypically normal but, as carriers, transmit the abnormal gene to 1/2 of their children. The 50% of sons of a carrier female are affected, and 50% of the daughters are carriers. An affected male never transmits the trait to his sons but all his daughters are carriers. No daughters of a carrier female and a normal father are affected, but half are carriers. Occasionally, females who are heterozygous for X-linked mutations show some expression, but they are rarely affected as severely as affected males.

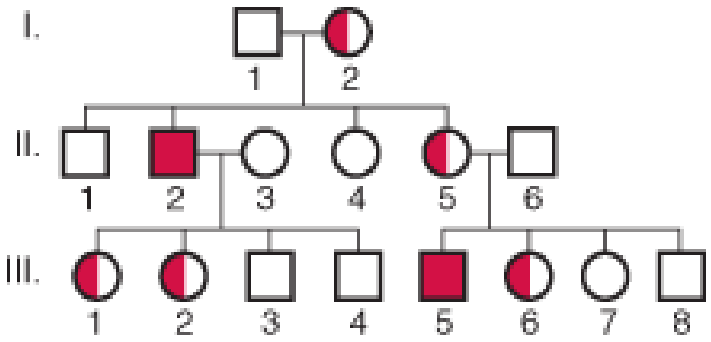

Figure 1.4 Shows typical pedigree having X-linked Recessive mode of inheritance.

1.2.1.5 Y-Linked disorder Inheritance

Y-linked disorders are caused by mutations on the Y chromosome. Because males inherit a Y chromosome from their fathers, every son of an affected father will be affected (Figure1.5). Because females inherit an X chromosome from their fathers, female offspring of affected fathers are never affected. Although a few Y-linked characters are present but no such Y-linked diseases are known, apart from disorders of male sexual function. Possibly such a disease may exist undiscovered, but this is unlikely for two reasons. First, the pedigree pattern would be strikingly noticeable, especially in societies that trace family through the male line, yet they have not been noted .Secondly, the Y-chromosome cannot carry any genes whose function is important for health, because females are perfectly normal without any Y-linked genes. Thus any Y-linked genes must code either for non-essential characters or for

male-specific functions and defects are unlikely to cause diseases apart from defects of male sexual function. Genes present as functional copies on both the Y and the X might prove an exception to this argument.

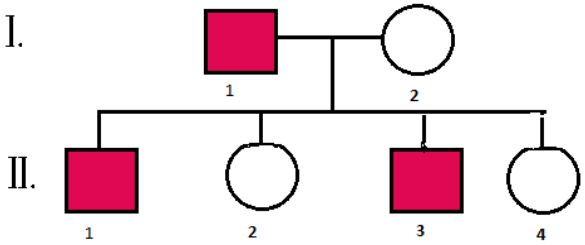

Figure 1.5 Shows typical pedigree having Y-linked mode of inheritance.

1.2.2 Complex Disorders Inheritance

Complex disorders are those which do not exhibit simple Mendelian inheritance patterns but are **multifactorial** (i.e., influenced by multiple genetic and environmental factors). Such disorders may be **oligogenic** (influenced by a small number of genetic loci) or **polygenic** (influenced by many loci). Given an adequate study sample, the extent to which a trait is inherited can be estimated. For example, in one study, sisters and mothers of women with a particular type of ICP were found to have ~12 times greater risk of developing ICP than women in the general population [4]; however, both genetic and environmental factors could contribute to this increased relative risk.

A number of **susceptibility loci** may exist for a disorder; a susceptibility locus is one at which mutation increases the risk of developing the disease but does not lead inevitably to disease. **Modifier loci** may influence the phenotype of a disorder; in cystic fibrosis (CF), a modifier locus for meconium ileus has been mapped [5]. Many traits in body are spread across continuum, rather than just been influenced by single gene locus. Inheritance of such quantitative traits is called polygenic inheritance. This disease condition is considered to be influenced by the combination of environmental factors and mutations in multiple genes. Thus a genetic mutation may predispose an individual to a particular disease, but other genetic factors along with environmental factors contribute to susceptibility of that individual developing the disease. Effect of one gene on multiple traits is called **pleiotropic**. In several congenital syndromes, a flaw in one gene causes widespread problems for a person. It

becomes more complicated to analyse its inheritance pattern as compared to single-gene or chromosomal disorders .There may also be a case in which numerous genetic alterations may prompt same disease condition .This situation is called **genetic hetrozygosity.** The multifactorial disease run in families and show great level of familial aggregation but no clear pattern of inheritance can be judged without carrying out extensive study on a large family. The associated gene has a strong environmental component attached to them. This makes it difficult even more difficult to determine an individual's risk of inheriting or passing on these disorders to the further generations.

1.2.3 Chromosomal Aberration Inheritance

Chromosome abnormalities are due to a disruption in the normal set of 46 chromosomes that is inherited from parents. Individuals may have more (polyploidy) or fewer chromosomes number than this defined set. Other may be other abnormalities too as a result of structural changes in the chromosomes. These changes occur at the time of cell division when a section of chromosomes gets detached from its actual location, this part may get lost completely causing **deletions**; or may get re-attach in the erroneous way called **inversions** or it may attach onto another chromosome that is the case of **Robertsonian translocation**. Alternatively, two sections can become detached and swap their positions causing a **Reciprocal translocation**. When a cell does not accurately copy its DNA, a mistake or variation in codon may arise. The different DNA sequence is likely to result in a different protein being made and if that protein is vital or crucial for the operations of the human body, then a disease may occur.

1.2.4 Mitochondrial Inheritance

In addition to the alterations in genes carried on the nuclear chromosomes, mitochondrial mutations are a significant cause of human genetic disease. The human cell contains two sets of genome one belongs to nucleus and the other to the mitochondria. The disorder mutation inherited from non-chromosomal DNA of mitochondria is called Mitochondrial inheritance. This type of inheritance, also known as maternal inheritance, because of the fact that only egg cells contribute mitochondria to the developing embryo, so only mothers can pass on

mitochondrial conditions to their children. Thus a mitochondrial inherited condition can affect both sexes, but is passed on only by affected mothers (Figure 1.6) gives a recognizable pedigree pattern. Mitochondria are small round or rod-like organelles involved in cellular respiration and found in the cytoplasm of plant and animal cells. It is called power house of the cell and has the activity of producing ATP using electron transport chain. In each cell of the body, mitochondrion may contain 5 to 10 circular pieces of DNA. Since the function of energy generation is carried out by the expression of these circular DNA, mutation in them may cause defect in organ requiring high amount of energy such as heart, skeletal muscles, liver, kidney etc.

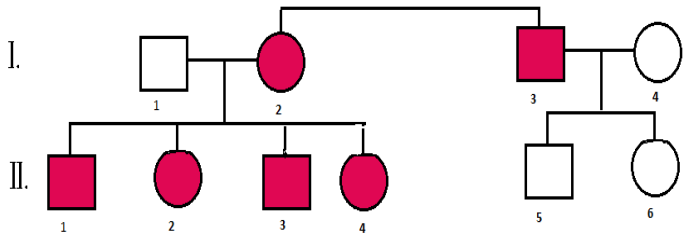

Figure 1.6 Shows typical pedigree having Mitochondrial mode of inheritance.

The mitochondrial genome is small but highly mutable compared to nuclear DNA, probably because mitochondrial DNA replication is more error-prone and the number of replications is much higher. In many genetic diseases in which mitochondrial defects occur do not demonstrate mitochondrial inheritance. This is because many proteins essential for normal function of mitochondria are encoded by the nuclear genome. Navajo Neurohepatopathy may be an example of such a disorder; which involves depletion of mitochondrial DNA, and the primary defect in disease is due to improper nuclear regulation of mtDNA copy number [1].

1.3 Penetrance and Expressivity

The disorders that present at different rates or with different symptoms in males versus females can be due to mutation in autosomal genes. Such disorders are **sex-influenced** or **sex-limited**; intrahepatic cholestasis of pregnancy (ICP) is a sex-limited disorder. Another important feature of a genetic disease is its **penetrance**—i.e., the proportion of people with a disease-causing genotype who actually develop the disease. Penetrance can be **complete** (i.e., all patients with a disease-causing genotype develop the disease) or **incomplete**, and also

may be age-dependent; BRIC due to mutation in *ATP8B1* exhibits incomplete, **age-dependent** penetrance [2].

In a disorder with **variable expressivity**, patients differ in the severity and/or pattern of disease expressions. Sometimes variable expressivity is due to mutations with effects of differing severity on protein function, but in other cases, substantial variability is seen even between patients possessing the same disease mutation. Both BRIC and Alagille syndrome have substantially variable expressivity [2, 3].

Several other features should be kept in mind when considering the pattern of inheritance of a genetic disorder:

1. A **new mutation** may have occurred. For example, a new mutation for a dominant disease may occur in a parent, so that a child is affected, although the parent was not. If the possibility of new mutation is not considered, the disorder in that family might be believed to be recessive. In Alagille syndrome, as in many AD disorders that reduce reproductive success, a many mutations are 'de novo' [3]. Such a new mutation can occur in a single germ cell in the parent, or the parent can be **mosaic** for it— i.e., a proportion of the parent's cells carry the mutation. In a dominant disorder, parental **germline mosaicism** for a new disease mutation means that multiple children in the family can suffer from an AD disorder, although neither parent is affected.
2. Some disorders show **expectancy**, in which the age of onset decreases, and/or disease severity increases (on average), with each successive generation. Expectancy is seen most typically in disorders caused by expansion of a tri-nucleotide repeat, such as Huntington Disorder, as the repeat can further expand in successive generations.
3. **Imprinting** is said to occur when inheritance of the same mutation has a different effect on the child, depending upon whether it was inherited from the mother or father.

1.4 Developing a pedigree

The pedigree building process begins by collecting the available family history of the proband and proceeds with the frequency of visit between doctor and the proband .The individual is interviewed about ethnicity, consanguineous relationships, number of siblings, their dates of birth, dates of death, cause of death, and pregnancies-dead or alive. Minimum of three generation of medical history record of a family is required .Detailed information

about general health problems, such as cancer and heart disease, and their specific symptoms, onset, and age at which it was diagnosed gives the age at which expression of the gene is generally observed. The information pertaining to specific genetic disease for which the family was referred is gathered. These targeted questions help genetic counsellors and researchers identify the underlying facts of spread of disease in the family, its severity, possible variable expression, reduced penetrance of the gene, or genetic anticipation, age of onset etc. Because accurate diagnoses are essential for accurate genetic counselling and research studies, all the live members of the family are diagnosed. Further, pedigree updates can be obtained over time during follow-up genetic counselling sessions or research interviews.

While studying the features of a proband, the researcher comes cross certain novel polymorphisms seen exceptionally without warning or any previous history of the disease in the family. In such cases individual rarely reproduce, so their chances of transferring the mutant genes is fairly less. The mutation have different effects on pedigrees, depending on the mode of inheritance. For those with dominant expression, the turnover of diseased genes is faster, because they are constantly exposed to the process of selection.The recessive mutations are by their virtue hard to study, they are only epressed if an individual with heterozygous genotype mates an individual with homozygous genotype of the recessive allele. Half the progeny becomes carriers, but their evolutionary fitness is not affected. So until carriers run into each other, the disease is able to spread in the population without any selection pressure from the environment. The recessive pattern pedigrees are not significantly affected by new mutations because they are generally manifested in homozygotes.

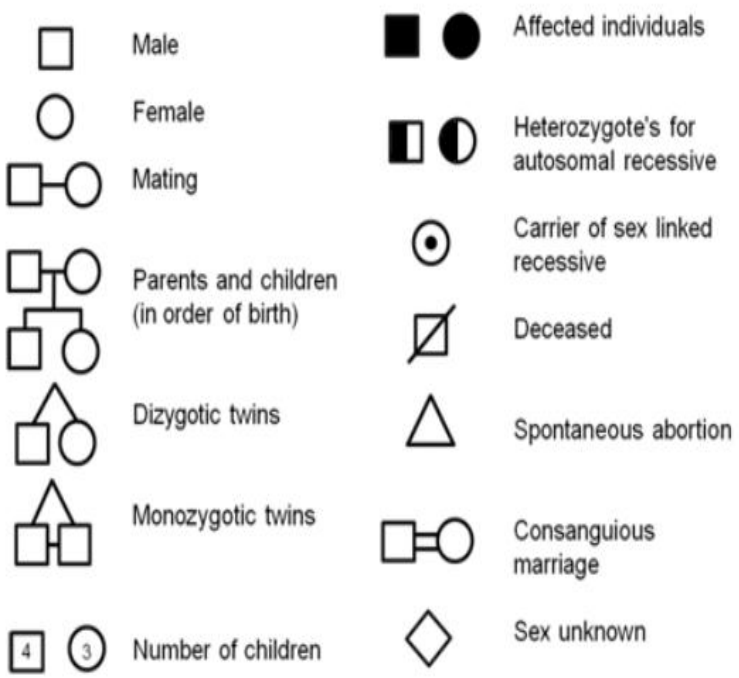

Figure 1.7 Shows Symbol used for building pedigree.

With the information gathered about limited members of human families, it is rarely possible to be completely certain of the mode of inheritance of a character simply by inspecting a single pedigree. In recessive pedigrees the proportion of affected children often seems to be greater than 1 in many. This is because families are assertive that both parents are carriers but it becomes difficult to trace back the history. For many of the rarer conditions, the stated mode of inheritance is no more than an informed guess. Assigning modes of inheritance to a defect is an essential task, because that is the basis of the risk estimates used in genetic counselling.

Sometimes, a disorder's mode of inheritance can be evaluated statistically using a formal segregation analysis. In other cases, especially for rare disorders, and given today's typically smaller families, too few patients and family members are available to permit statistically definitive segregation analysis; nevertheless, it is often possible to identify the most likely mode of inheritance. Knowledge of the mode of inheritance of a disorder can help greatly in identifying the genetic etiology of the disease; for example, if a disorder demonstrates X-linked inheritance, only a genetic screen needs to be performed for the X-chromosome, rather than for the entire nuclear genome.

1.5 Localizing a Disease Gene

The **Simple sequence repeats (**SSRs), genetic markers most frequently used in mapping studies. These are widely used because of their distributed, size, tandem repeated sequence. The most commonly employed SSRs include di-nucleotide and tetra-nucleotide repeats. The number of copies of the repeat unit varies between alleles, so that alleles may differ in length. The inheritance pattern can be assessed by amplifying the repeat from genomic DNA using the polymerase chain reaction (PCR), with unique primers flanking the repeat, and then electrophoresing the PCR products to separate the alleles by size. A major advantage of SSRs for genetic mapping is that they are highly polymorphic and consequently, very informative in genetic analyses. The use of **single nucleotide polymorphisms** (SNPs; genomic sites at which a single base varies between alleles) has been increasing. An advantage of SNPs is that they are extremely common; 2 chromosomes differ from each other at ˜1 bp in 1,300. Also, SNPs have a lower mutation rate than do SSRs.

The genotyping data can be interpreted using a range of statistical or empiric analysis; the most appropriate in a specific situation depends upon characteristics of the disorder, the type and size of sample available, and characteristics of the population(s) from which the sample is derived. The Genetic mapping data can be evaluated using linkage analysis and/or population genetic mapping.

1.5.1 Evaluating Genetic Mapping Data Using Linkage Analysis

Linkage analysis is a prevailing, family-based approach to disease mapping. It makes use of the fact that specific copies of the genomic region containing the disease gene are co-inherited with the disease within a family; this reflects lack of recombination between the disease mutation and neighboring genetic markers, due to their close proximity. Within a family, individuals who share a disease will typically share alleles at markers near the disease gene. The particular alleles co-inherited with the disease often differ between families, reflecting allelic heterogeneity or ancestral genetic recombination events. Results of linkage analysis are reported as LOD scores representing the relative likelihood that a disease locus and a genetic marker are genetically linked (with a recombination fraction theta), rather than that they are genetically unlinked. A LOD score of at least +3.3 is typically considered evidence of linkage from a genome-wide screen. A LOD score of −2 or below excludes

disease linkage to a region. Linkage analysis permitted mapping of the CF gene [6, 7].The **parametric linkage** analysis measuring recessive conditions in consanguineous families is done using Homozygosity mapping .In such a family, patient(s) are likely to be homozygous by descent for a single disease mutation, and for alleles at nearby genetic markers, i.e., both the mutation and marker alleles were inherited from a single ancestor shared by their mother and father. Homozygosity mapping identifies segments of homozygous DNA in patients.

If the mode of inheritance of a disorder is also unknown, a **nonparametric** linkage approach, such as **affected sib pair** analysis, can be performed. Regions that are shared by affected siblings or other relatives more often than expected by chance are identified; such approaches are useful in mapping susceptibility loci for complex traits.

1.5.2 Localizing a Disease Gene Using Fine-Mapping

Often the original mapping of a disease locus to a region is too indefinite to permit immediate detection of the actual disease gene, mainly if the region contains several genes, and none of them are in particular promising from a functional viewpoint. The location of a disease gene is genetically further refined through study of additional patients, and/or use of additional genetic markers. Often a combination of analytic approaches and populations is used for study of a single disease.

1.5.3 Candidate Gene Identification

Once a disease gene has been mapped, genes within the candidate region are to be identified. In the past, this required much laborious and clever laboratory work, but it has become vastly easier. With the accessibility of the human genome sequence, a large catalog of the genes in a given region can be obtained without performing any experiments through the use of publically available databases. The steps to identify a candidate gene are as follows.

1. The genomic region of interest can be selected, and a list of known or predicted genes in the region displayed.
2. Sequence from the region can be used to scan databases of transcribed sequences.

A gene that encodes a functionally uncharacterized protein can be evaluate by evaluating its homologous proteins that have known functions consistent with the disease phenotype, and/or whether it is expressed in the tissue(s) most affected by the disease.

1.6 Genetic Polymorphisms and Mutations: Types and Identification

Most of the disorders fall in the category of single base pair substitution, in which a single base pair of DNA is replaced with another base pair. Deletions, inversions and insertions (including duplications and repeat expansions) also occur. Not all mutations are deleterious. The mutations that change the DNA sequence leading to development of, or increased susceptibility to, disease are deleterious. Another category is of Neutral polymorphisms. These are the mutations that don't change the apparent function of gene even after change in DNA sequence. When it is unclear whether a sequence change has any functional consequence, it can conservatively be referred to as a variant. The table 1.1 shows various categories of mutation along with its sub types.

Category	**Mutation Type**
Single base-pair change	Missense (altered amino acid)
	Nonsense (stop codon)
	Splicing (prevention of normal splicing, induction of abnormal splicing)
Deletion	Frameshift (change of reading frame)
	In-frame (removal of amino acids)
	Gene deletion
	Micro-deletion/contiguous gene syndrome (loss or disruption of multiple genes)
Insertion (including duplication and repeat expansion)	Frameshift In-frame (addition of amino acids) Regulatory Splicing
Inversion	Disruption of normal gene structure
Chromosomal abnormalities	Changes in chromosome number (polyploidy, trisomy, etc.) or structure (translocations, deletions, inversions, duplications)

Table 1.1 Shows various categories of mutation along with its sub types.

Several laboratory methods can be used for identifying mutations. Regardless of the method used for mutation detection, findings in patients should be compared with those in a control sample, to help distinguish disease-causing mutations from neutral polymorphisms. If a particular disease mutation is found to occur frequently in patients, a specific assay for its efficient detection can be developed. The functional implications of a mutation can be projected based upon the sequence change induced; nevertheless, functional studies are extremely valuable in confirming, refining, or rejecting previous predictions. There are a variety of ways in which a disease mutation in a gene can ultimately affect the function of the encoded protein.

1.6.1 Transcription

A mutation may occur in a promoter or enhancer element of a gene, and prevent transcription of the gene, or alter the levels, timing, and/or tissue distribution of expression. Like in case of congenital superior oblique muscle palsy ,in which C76G in the 5′-UTR of the exon 1 and C-9A in the promoter region on the same strand. Also G153A in the 5′-UTR of exon 1 alters the gene expression [8].

1.6.2 mRNA stability and translation

The mutation may affect mRNA levels by decreasing the stability of the transcript. Mutation in a polyadenylation site may prevent polyadenylation of the transcript, and lead to decreased stability of the mRNA, and/or inhibition of its translation. The presence of a sequence change that leads to premature termination of translation (such as occurrence of a nonsense mutation, or an insertion, deletion, or splicing mutation) that leads to a frameshift.

1.6.3 Protein stability or localization

A mutation can have no influence on transcript levels, but alter the stability or localization of the protein. A mutation causing abnormal folding of the protein marking ubiquitination and finally resulting in degradation may also be caused.

1.6.4 Protein function

The normal levels of a protein may be produced, and the protein may be delivered to its proper location, but a change in the amino acid sequence of the protein may prevent it from functioning properly; its function may be partially or completely destroyed. Sometimes these mutations instead of cause a ‘gain of function’, may lead to loss-of-function mutations in which, in heterozygous individuals, the mutated protein interferes with the function of the normal versions of the protein; generally, this effect occurs with multimeric proteins.