Medical Genetics and Genetic Diseases

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Chapter 1

Medical Genetics

Medical genetics is the specialty of medicine that involves the diagnosis and management of hereditary disorders. Medical genetics differs from Human genetics in that human genetics is a field of scientific research that may or may not apply to medicine, but medical genetics refers to the application of genetics to medical care. For example, research on the causes and inheritance of genetic disorders would be considered within both human genetics and medical genetics, while the diagnosis, management, and counseling of individuals with genetic disorders would be considered part of medical genetics.

In contrast, the study of typically non-medical phenotypes such as the genetics of eye color would be considered part of human genetics, but not necessarily relevant to medical genetics (except in situations such as albinism). *Genetic medicine* is a newer term for medical genetics and incorporates areas such as gene therapy, personalized medicine, and the rapidly emerging new medical specialty, predictive medicine.

Scope

Medical genetics encompasses many different areas, including clinical practice of physicians, genetic counselors, and nutritionists, clinical diagnostic laboratory activities, and research into the causes and inheritance of genetic disorders. Examples of conditions that fall within the scope of medical genetics include birth defects and dysmorphology, mental retardation, autism, metabolic and mitochondrial disorders, skeletal dysplasia, connective tissue disorders, cancer genetics, teratogens, and prenatal diagnosis. Medical genetics is increasingly becoming relevant to many common diseases. Overlaps with other medical specialties are beginning to emerge, as recent advances in genetics are revealing etiologies for neurologic, endocrine, cardiovascular, pulmonary, ophthalmologic, renal, psychiatric, and dermatologic conditions.

Subspecialties

In some ways, many of the individual fields within medical genetics are hybrids between clinical care and research. This is due in part to recent advances in science and technology that have enabled an unprecedented understanding of genetic disorders.

Clinical genetics

Clinical genetics is the practice of clinical medicine with particular attention to hereditary disorders. Referrals are made to genetics clinics for a variety of reasons, including birth defects, developmental delay, autism, epilepsy, short stature, and many others. Examples of genetic syndromes that are commonly seen in the genetics clinic include chromosomal rearrangements, Down syndrome, DiGeorge syndrome (22q11.2 Deletion Syndrome), Fragile X syndrome, Marfan syndrome, Neurofibromatosis, Turner syndrome, and Williams syndrome.

Metabolic/biochemical genetics

Metabolic (or biochemical) genetics involves the diagnosis and management of inborn errors of metabolism in which patients have enzymatic deficiencies that perturb biochemical pathways involved in metabolism of carbohydrates, amino acids, and lipids. Examples of metabolic disorders include galactosemia, glycogen storage disease, lysosomal storage disorders, metabolic acidosis, peroxisomal disorders, phenylketonuria, and urea cycle disorders.

Cytogenetics

Cytogenetics is the study of chromosomes and chromosome abnormalities. While cytogenetics historically relied on microscopy to analyze chromosomes, new molecular technologies such as array comparative genomic hybridization are now becoming widely used. Examples of chromosome abnormalities include aneuploidy, chromosomal rearrangements, and genomic deletion/duplication disorders.

Molecular genetics

Molecular genetics involves the discovery of and laboratory testing for DNA mutations that underlie many single gene disorders. Examples of single gene disorders include achondroplasia, cystic fibrosis, Duchenne muscular dystrophy, hereditary breast cancer (BRCA1/2), Huntington disease, Marfan syndrome, Noonan syndrome, and Rett syndrome. Molecular tests are also used in the diagnosis of syndromes involving epigenetic abnormalities, such as Angelman syndrome, Beckwith-Wiedemann syndrome, Prader-willi syndrome, and uniparental disomy.

Mitochondrial genetics

Mitochondrial genetics concerns the diagnosis and management of mitochondrial disorders, which have a molecular basis but often result in biochemical abnormalities due to deficient energy production.

There exists some overlap between medical genetic diagnostic laboratories and molecular pathology.

Genetic Counseling

Genetic counseling is the process of providing information about genetic conditions, diagnostic testing, and risks in other family members, within the framework of nondirective counseling. Genetic counselors are non-physician members of the medical genetics team who specialize in family risk assessment and counseling of patients regarding genetic disorders. The precise role of the genetic counselor varies somewhat depending on the disorder.

History

Although genetics has its roots back in the 19th century with the work of the Bohemian monk Gregor Mendel and other pioneering scientists, human genetics emerged later. It started to develop, albeit slowly, during the first half of the 20th century. Mendelian (single-gene) inheritance was studied in a number of important disorders such as albinism, brachydactyly (short fingers and toes), and hemophilia. Mathematical approaches were also devised and applied to human genetics. Population genetics was created.

Medical genetics was a late developer, emerging largely after the close of World War II (1945) when the eugenics movement had fallen into disrepute. The Nazi misuse of eugenics sounded its death knell. Shorn of eugenics, a scientific approach could be used and was applied to human and medical genetics. Medical genetics saw an increasingly rapid rise in the second half of the 20th century and continues in the 21st century.

Current practice

The clinical setting in which patients are evaluated determines the scope of practice, diagnostic, and therapeutic interventions. For the purposes of general discussion, the typical encounters between patients and genetic practitioners may involve:

- Referral to an out-patient genetics clinic (pediatric, adult, or combined) or an in-hospital consultation, most often for diagnostic evaluation.
- Specialty genetics clinics focusing on management of inborn errors of metabolism, skeletal dysplasia, or lysosomal storage diseases.
- Referral for counseling in a prenatal genetics clinic to discuss risks to the pregnancy (advanced maternal age, teratogen exposure, family history of a genetic disease), test results (abnormal maternal serum screen, abnormal ultrasound), and/or options for prenatal diagnosis (typically amniocentesis or chorionic villus sampling).
- Multidisciplinary specialty clinics that include a clinical geneticist or genetic counselor (cancer genetics, cardiovascular genetics, craniofacial or cleft lip/palate, hearing loss clinics, muscular dystrophy/neurodegenerative disorder clinics).

Diagnostic evaluation

Each patient will undergo a diagnostic evaluation tailored to their own particular presenting signs and symptoms. The geneticist will establish a differential diagnosis and recommend appropriate testing. Increasingly, clinicians use SimulConsult, paired with the National Library of Medicine Gene Review articles, to narrow the list of hypotheses (known as the differential diagnosis) and identify the tests that are relevant for a particular patient. These tests might evaluate for chromosomal disorders, inborn errors of metabolism, or single gene disorders.

Chromosome studies

Chromosome studies are used in the general genetics clinic to determine a cause for developmental delay/mental retardation, birth defects, dysmorphic features, and/or autism. Chromosome analysis is also performed in the prenatal setting to determine whether a fetus is affected with aneuploidy or other chromosome rearrangements. Finally, chromosome abnormalities are often detected in cancer samples. A large number of different methods have been developed for chromosome analysis:

- Chromosome analysis using a karyotype involves special stains that generate light and dark bands, allowing identification of each chromosome under a microscope.
- Fluorescence in situ hybridization (FISH) involves fluorescent labeling of probes that bind to specific DNA sequences, used for identifying aneuploidy, genomic deletions or duplications, characterizing chromosomal translocations and determining the origin of ring chromosomes.
- Chromosome painting is a technique that uses fluorescent probes specific for each chromosome to differentially label each chromosome. This technique is more often used in cancer cytogenetics, where complex chromosome rearrangements can occur.
- Array comparative genomic hybridization is a new molecular technique that involves hybridization of an individual DNA sample to a glass slide or microarray chip containing molecular probes (ranging from large ~200kb bacterial artificial chromosomes to small oligonucleotides) that represent unique regions of the genome. This method is particularly sensitive for detection of genomic gains or losses across the genome but does not detect balanced translocations or distinguish the location of duplicated genetic material (for example, a tandem duplication versus an insertional duplication).

Basic metabolic studies

Biochemical studies are performed to screen for imbalances of metabolites in the bodily fluid, usually the blood (plasma/serum) or urine, but also in cerebrospinal fluid (CSF). Specific tests of enzyme function (either in leukocytes, skin fibroblasts, liver, or muscle) are also employed under certain circumstances. In the US, the newborn screen incorporates biochemical tests to screen for treatable conditions such as galactosemia and

phenylketonuria (PKU). Patients suspected to have a metabolic condition might undergo the following tests:

- Quantitative amino acid analysis is typically performed using the ninhydrin reaction, followed by liquid chromatography to measure the amount of amino acid in the sample (either urine, plasma/serum, or CSF). Measurement of amino acids in plasma or serum is used in the evaluation of disorders of amino acid metabolism such as urea cycle disorders, maple syrup urine disease, and PKU. Measurement of amino acids in urine can be useful in the diagnosis of cystinuria or renal Fanconi syndrome as can be seen in cystinosis.
- Urine organic acid analysis can be either performed using quantitative or qualitative methods, but in either case the test is used to detect the excretion of abnormal organic acids. These compounds are normally produced during bodily metabolism of amino acids and odd-chain fatty acids, but accumulate in patients with certain metabolic conditions.
- The acylcarnitine combination profile detects compounds such as organic acids and fatty acids conjugated to carnitine. The test is used for detection of disorders involving fatty acid metabolism, including MCAD.
- Pyruvate and lactate are byproducts of normal metabolism, particularly during anaerobic metabolism. These compounds normally accumulate during exercise or ischemia, but are also elevated in patients with disorders of pyruvate metabolism or mitochondrial disorders.
- Ammonia is an end product of amino acid metabolism and is converted in the liver to urea through a series of enzymatic reactions termed the urea cycle. Elevated ammonia can therefore be detected in patients with urea cycle disorders, as well as other conditions involving liver failure.
- Enzyme testing is performed for a wide range of metabolic disorders to confirm a diagnosis suspected based on screening tests.

Molecular studies

- DNA sequencing is used to directly analyze the genomic DNA sequence of a particular gene. In general, only the parts of the gene that code for the expressed protein (exons) and small amounts of the flanking untranslated regions and introns are analyzed. Therefore, although these tests are highly specific and sensitive, they do not routinely identify all of the mutations that could cause disease.
- DNA methylation analysis is used to diagnose certain genetic disorders that are caused by disruptions of epigenetic mechanisms such as genomic imprinting and uniparental disomy.
- Southern blotting is an early technique basic on detection of fragments of DNA separated by size through gel electrophoresis and detected using radiolabeled probes. This test was routinely used to detect deletions or duplications in conditions such as Duchenne muscular dystrophy but is being replaced by high-resolution array comparative genomic hybridization techniques. Southern blotting is still useful in the diagnosis of disorders caused by trinucleotide repeats.

- Short tandem repeats are unique markers that can be used to determine haplotypes and are used in identity testing for maternal cell contamination.

Treatments

Each cell of the body contains the hereditary information (DNA) wrapped up in structures called chromosomes. Since genetic syndromes are typically the result of alterations of the chromosomes or genes, there is no treatment currently available that can correct the genetic alterations in every cell of the body. Therefore, there is currently no "cure" for genetic disorders. However, for many genetic syndromes there is treatment available to manage the symptoms. In some cases, particularly inborn errors of metabolism, the mechanism of disease is well understood and offers the potential for dietary and medical management to prevent or reduce the long-term complications. In other cases, infusion therapy is used to replace the missing enzyme. Current research is actively seeking to use gene therapy or other new medications to treat specific genetic disorders.

Management of Metabolic disorders

In general, metabolic disorders arise from enzyme deficiencies that disrupt normal metabolic pathways. For instance, in the hypothetical example:

```
    A ---> B ---> C ---> D          AAAA ---> BBBBBB ---> CCCCCCCCCC ---
> (no D)
       X      Y      Z                    X           Y              (no
Z)
```

Compound "A" is metabolized to "B" by enzyme "X", compound "B" is metabolized to "C" by enzyme "Y", and compound "C" is metabolized to "D" by enzyme "Z". If enzyme "Z" is missing, compound "D" will be missing, while compounds "A", "B", and "C" will build up. The pathogenesis of this particular condition could result from lack of compound "D", if it is critical for some cellular function, or from toxicity due to excess "A", "B", and/or "C". Treatment of the metabolic disorder could be achieved through dietary supplementation of compound "D" and dietary restriction of compounds "A", "B", and/or "C" or by treatment with a medication that promoted disposal of excess "A", "B", or "C". Another approach that can be taken is enzyme replacement therapy, in which a patient is given an infusion of the missing enzyme.

- Diet

Dietary restriction and supplementation are key measures taken in several well-known metabolic disorders, including galactosemia, phenylketonuria (PKU), maple syrup urine disease, organic acidurias and urea cycle disorders. Such restrictive diets can be difficult for the patient and family to maintain, and require close consultation with a nutritionist who has special experience in metabolic disorders. The composition of the diet will change depending on the caloric needs of the growing child and special attention is needed during a pregnancy if a woman is affected with one of these disorders.

- Medication

Medical approaches include enhancement of residual enzyme activity (in cases where the enzyme is made but is not functioning properly), inhibition of other enzymes in the biochemical pathway to prevent buildup of a toxic compound, or diversion of a toxic compound to another form that can be excreted. Examples include the use of high doses of pyridoxine (vitamin B6) in some patients with homocystinuria to boost the activity of the residual cystathione synthase enzyme, administration of biotin to restore activity of several enzymes affected by deficiency of biotinidase, treatment with NTBC in Tyrosinemia to inhibit the production of succinylacetone which causes liver toxicity, and the use of sodium benzoate to decrease ammonia build-up in urea cycle disorders.

- Enzyme replacement therapy

Certain lysosomal storage diseases are treated with infusions of a recombinant enzyme (produced in a laboratory), which can reduce the accumulation of the compounds in various tissues. Examples include Gaucher disease, Fabry disease, Mucopolysaccharidoses and Glycogen storage disease type II. Such treatments are limited by the ability of the enzyme to reach the affected areas (the blood brain barrier prevents enzyme from reaching the brain, for example), and can sometimes be associated with allergic reactions. The long-term clinical effectiveness of enzyme replacement therapies vary widely among different disorders.

Other examples

- Angiotensin receptor blockers in Marfan syndrome & Loeys-Dietz
- Bone marrow transplantation
- Gene therapy

Career paths and training

There are a variety of career paths within the field of medical genetics, and naturally the training required for each area differs considerably. It should be noted that the information included in this section applies to the typical pathways in the United States and there may be differences in other countries. US Practitioners in clinical, counseling, or diagnostic subspecialties generally obtain board certification through the American Board of Medical Genetics.

Career	Degree	Description	Training
Clinical Geneticist	MD or MD/PhD	A **Clinical geneticist** is typically a physician who evaluates patients in the office or as a hospital consultation. This process includes a medical history, family history (pedigree), a detailed physical examination,	College (4 yrs) → Medical school (4 yrs) → Primary residency (2-3 yrs) → Residency in Clinical genetics (2 yrs). Some Clinical geneticists also obtain a PhD degree (4-7

		reviewing objective data such as imaging and test results, establishing a differential diagnosis, and recommending appropriate diagnostic tests.	yrs). A new residency track offers a 4 yr primary residency in Clinical genetics immediately after finishing Medical school.
Genetic Counselor	MS	A **Genetic counselor** specializes in communication of genetic information to patients and families. Genetic counselors often work closely with Clinical geneticists or other physicians (such as Obstetricians or Oncologists) and often convey the results of the recommended tests.	College (4 yrs) → Graduate program in Genetic counseling (2 yrs).
Metabolic nurse and/or nutritionist	BA/BS, MS, RN	One of the critical aspects of the management of patients with metabolic disorders is the appropriate nutritional intervention (either restricting the compound that cannot be metabolized, or supplementing compounds that are deficient as the result of an enzyme deficiency). The metabolic nurse and nutritionist play important roles in coordinating the dietary management.	College (4 yrs) → Nursing school or graduate training in nutrition.
Biochemical Diagnostics	PhD, MD, or MD/PhD	Individuals who specialize in **Biochemical genetics** typically work in the diagnostic laboratory, analyzing and interpreting specialized biochemical tests that measure amino acids, organic acids, and enzyme activity. Some Clinical Geneticists are also board certified in Biochemical Genetics.	College (4 yrs) → Graduate school (PhD, usually 4–7 years) and/or Medical school (MD, 4 years)
Cytogenetic Diagnostics	PhD, MD, or MD/PhD	Individuals who specialize in **Cytogenetics** typically work in the diagnostic laboratory, analyzing and interpreting karyotypes, FISH, and comparative genomic hybridization tests. Some Clinical Geneticists are also board certified in Cytogenetics.	College (4 yrs) → Graduate school (PhD, usually 4–7 years) and/or Medical school (MD, 4 years)

Molecular Diagnostics	PhD, MD, or MD/PhD	Individuals who specialize in **Molecular genetics** typically work in the diagnostic laboratory, analyzing and interpreting specialized genetic tests that look for disease-causing changes (mutations) in the DNA. Some examples of molecular diagnostic tests include DNA sequencing and Southern blotting.	College (4 yrs) → Graduate school (PhD, usually 4–7 years) and/or Medical school (MD, 4 years)
Research Geneticist	PhD, MD, or MD/PhD	Any researcher who studies the genetic basis of human disease or uses model organisms to study disease mechanisms could be considered a Research Geneticist. Many of the clinical career paths also include basic or translational research, and thus individuals in the field of medical genetics often participate in some form of research.	College (4 yrs) → Graduate school (PhD, usually 4–7 years) and/or Medical school (MD, 4 years) → Post-doctoral research training (usually 3+ years)
Laboratory Technician	BS or MS	Technicians in the diagnostic or research labs handle samples and run the assays at the bench. Often these individuals are promoted to supervisory positions.	College (4 yrs), may have higher degree (MS, 2+ years)

Ethical, legal and social implications

Genetic information provides a unique type of knowledge about an individual and his/her family, fundamentally different than a typically laboratory test that provides a "snapshot" of an individual's health status. The unique status of genetic information and inherited disease has a number of ramifications with regard to ethical, legal, and societal concerns.

Societies

The more empirical approach to human and medical genetics was formalized by the founding in 1948 of the American Society of Human Genetics. The Society first began annual meetings that year (1948) and its international counterpart, the International Congress of Human Genetics, has met every 5 years since its inception in 1956. The Society publishes the American Journal of Human Genetics on a monthly basis.

Medical genetics is now recognized as a distinct medical specialty in the U.S. with its own approved board (the American Board of Medical Genetics) and clinical specialty college (the American College of Medical Genetics). The College holds an annual

scientific meeting, publishes a monthly journal, Genetics in Medicine, and issues position papers and clinical practice guidelines on a variety of topics relevant to human genetics.

Research

The broad range of research in medical genetics reflects the overall scope of this field, including basic research on genetic inheritance and the human genome, mechanisms of genetic and metabolic disorders, translational research on new treatment modalities, and the impact of genetic testing

Basic genetics research

Basic research geneticists usually undertake research in universities, biotechnology firms and research institutes.

Allelic architecture of disease

Sometimes the link between a disease and an unusual gene variant is more subtle. The genetic architecture of common diseases is an important factor in determining the extent to which patterns of genetic variation influence group differences in health outcomes. According to the common disease/common variant hypothesis, common variants present in the ancestral population before the dispersal of modern humans from Africa play an important role in human diseases. Genetic variants associated with Alzheimer disease, deep venous thrombosis, Crohn disease, and type 2 diabetes appear to adhere to this model. However, the generality of the model has not yet been established and, in some cases, is in doubt. Some diseases, such as many common cancers, appear not to be well described by the common disease/common variant model.

Another possibility is that common diseases arise in part through the action of combinations of variants that are individually rare. Most of the disease-associated alleles discovered to date have been rare, and rare variants are more likely than common variants to be differentially distributed among groups distinguished by ancestry. However, groups could harbor different, though perhaps overlapping, sets of rare variants, which would reduce contrasts between groups in the incidence of the disease.

The number of variants contributing to a disease and the interactions among those variants also could influence the distribution of diseases among groups. The difficulty that has been encountered in finding contributory alleles for complex diseases and in replicating positive associations suggests that many complex diseases involve numerous variants rather than a moderate number of alleles, and the influence of any given variant may depend in critical ways on the genetic and environmental background. If many alleles are required to increase susceptibility to a disease, the odds are low that the necessary combination of alleles would become concentrated in a particular group purely through drift.

Population substructure in genetics research

One area in which population categories can be important considerations in genetics research is in controlling for confounding between population substructure, environmental exposures, and health outcomes. Association studies can produce spurious results if cases and controls have differing allele frequencies for genes that are not related to the disease being studied, although the magnitude of this problem in genetic association studies is subject to debate. Various methods have been developed to detect and account for population substructure, but these methods can be difficult to apply in practice.

Population substructure also can be used to advantage in genetic association studies. For example, populations that represent recent mixtures of geographically separated ancestral groups can exhibit longer-range linkage disequilibrium between susceptibility alleles and genetic markers than is the case for other populations. Genetic studies can use this admixture linkage disequilibrium to search for disease alleles with fewer markers than would be needed otherwise. Association studies also can take advantage of the contrasting experiences of racial or ethnic groups, including migrant groups, to search for interactions between particular alleles and environmental factors that might influence health.

Chapter 2

Genetic Disorder

Genetic disorder	
MeSH	D030342

A **genetic disorder** is an illness caused by abnormalities in genes or chromosomes. While some diseases, such as cancer, are due in part to genetic disorders, they can also be caused by environmental factors. Most disorders are quite rare and affect one person in every several thousands or millions. Some types of recessive gene disorders confer an advantage in the heterozygous state in certain environments.

Single gene disorder

Prevalence of some single gene disorders

Disorder	Prevalence (approximate)
Autosomal dominant	
Familial hypercholesterolemia	1 in 500
Polycystic kidney disease	1 in 1250
Hereditary spherocytosis	1 in 5,000
Marfan syndrome	1 in 4,000
Huntington disease	1 in 15,000
Autosomal recessive	
Sickle cell anemia	1 in 625 (African Americans)
Cystic fibrosis	1 in 2,000 (Caucasians)
Tay-Sachs disease	1 in 3,000 (American Jews)
Phenylketonuria	1 in 12,000
Mucopolysaccharidoses	1 in 25,000
Glycogen storage diseases	1 in 50,000
Galactosemia	1 in 57,000

X-linked	
Duchenne muscular dystrophy	1 in 7,000
Hemophilia	1 in 10,000

Values are for liveborn infants

A **single gene disorder** is the result of a single mutated gene. There are estimated to be over 4000 human diseases caused by single gene defects. Single gene disorders can be passed on to subsequent generations in several ways. Genomic imprinting and uniparental disomy, however, may affect inheritance patterns. The divisions between recessive and dominant types are not "hard and fast" although the divisions between autosomal and X-linked types are (since the latter types are distinguished purely based on the chromosomal location of the gene). For example, achondroplasia is typically considered a dominant disorder, but children with two genes for achondroplasia have a severe skeletal disorder that achondroplasics could be viewed as carriers of. Sickle-cell anemia is also considered a recessive condition, but heterozygous carriers have increased resistance to malaria in early childhood, which could be described as a related dominant condition. When a couple where one partner or both are sufferers or carriers of a single gene disorder and wish to have a child they can do so through IVF which means they can then have PGD (pre-implantation genetic diagnosis) to check whether the fertilised egg has had the genetic disorder passed on.

Autosomal dominant

Only one mutated copy of the gene will be necessary for a person to be affected by an autosomal dominant disorder. Each affected person usually has one affected parent. There is a 50% chance that a child will inherit the mutated gene. Conditions that are autosomal dominant sometimes have reduced penetrance, which means that although only one mutated copy is needed, not all individuals who inherit that mutation go on to develop the disease. Examples of this type of disorder are Huntington's disease, neurofibromatosis type 1, Marfan syndrome, hereditary nonpolyposis colorectal cancer, and hereditary multiple exostoses, which is a highly penetrant autosomal dominant disorder. Birth defects are also called congenital anomalies.

Autosomal recessive

Two copies of the gene must be mutated for a person to be affected by an autosomal recessive disorder. An affected person usually has unaffected parents who each carry a single copy of the mutated gene (and are referred to as carriers). Two unaffected people who each carry one copy of the mutated gene have a 25% chance with each pregnancy of having a child affected by the disorder. Examples of this type of disorder are cystic fibrosis, sickle-cell disease, Tay-Sachs disease, Niemann-Pick disease, spinal muscular atrophy, and Roberts syndrome. Certain other phenotypes, such as wet versus dry earwax, are also determined in an autosomal recessive fashion.

X-linked dominant

X-linked dominant disorders are caused by mutations in genes on the X chromosome. Only a few disorders have this inheritance pattern, with a prime example being X-linked hypophosphatemic rickets. Males and females are both affected in these disorders, with males typically being more severely affected than females. Some X-linked dominant conditions such as Rett syndrome, incontinentia pigmenti type 2 and Aicardi syndrome are usually fatal in males either in utero or shortly after birth, and are therefore predominantly seen in females. Exceptions to this finding are extremely rare cases in which boys with Klinefelter syndrome (47,XXY) also inherit an X-linked dominant condition and exhibit symptoms more similar to those of a female in terms of disease severity. The chance of passing on an X-linked dominant disorder differs between men and women. The sons of a man with an X-linked dominant disorder will all be unaffected (since they receive their father's Y chromosome), and his daughters will all inherit the condition. A woman with an X-linked dominant disorder has a 50% chance of having an affected fetus with each pregnancy, although it should be noted that in cases such as incontinentia pigmenti only female offspring are generally viable. In addition, although these conditions do not alter fertility per se, individuals with Rett syndrome or Aicardi syndrome rarely reproduce.

X-linked recessive

X-linked recessive conditions are also caused by mutations in genes on the X chromosome. Males are more frequently affected than females, and the chance of passing on the disorder differs between men and women. The sons of a man with an X-linked recessive disorder will not be affected, and his daughters will carry one copy of the mutated gene. A woman who is a carrier of an X-linked recessive disorder (X^RX^r) has a 50% chance of having sons who are affected and a 50% chance of having daughters who carry one copy of the mutated gene and are therefore carriers. X-linked recessive conditions include the serious diseases Hemophilia A, Duchenne muscular dystrophy, and Lesch-Nyhan syndrome as well as common and less serious conditions such as male pattern baldness and red-green color blindness. X-linked recessive conditions can sometimes manifest in females due to skewed X-inactivation or monosomy X (Turner syndrome).

Y-linked

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. Because females inherit an X chromosome from their fathers, female offspring of affected fathers are *never* affected.

Since the Y chromosome is relatively small and contains very few genes, there are relatively few Y-linked disorders. Often the symptoms include infertility, which may be circumvented with the help of some fertility treatments. Examples are male infertility and hypertrichosis pinnae.

Mitochondrial

This type of inheritance, also known as maternal inheritance, applies to genes in mitochondrial DNA. Because only egg cells contribute mitochondria to the developing embryo, only mothers can pass on mitochondrial conditions to their children. An example of this type of disorder is Leber's hereditary optic neuropathy.

Multifactorial and polygenic (complex) disorders

Genetic disorders may also be complex, multifactorial, or polygenic, meaning that they are likely associated with the effects of multiple genes in combination with lifestyle and environmental factors. Multifactorial disorders include heart disease and diabetes. Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person's risk of inheriting or passing on these disorders. Complex disorders are also difficult to study and treat because the specific factors that cause most of these disorders have not yet been identified.

On a pedigree, polygenic diseases do tend to "run in families", but the inheritance does not fit simple patterns as with Mendelian diseases. But this does not mean that the genes cannot eventually be located and studied. There is also a strong environmental component to many of them (e.g., blood pressure).

- asthma
- autoimmune diseases such as multiple sclerosis
- cancers
- ciliopathies
- cleft palate
- diabetes
- heart disease
- hypertension
- inflammatory bowel disease
- mental retardation
- mood disorder
- obesity
- refractive error
- infertility

Prognosis and treatment of genetic disorders

Genetic disorders rarely have effective treatments, though gene therapy is being tested as a possible treatment for some genetic diseases, including some forms of retinitis pigmentosa

- Gauchers disease is a genetic disease affecting metabolism. It is more treatable then most other genetic diseases, and can be treated with enzyme replacement therapy, medication miglustat, and bone marrow transplantion.

Chapter 3

Inborn Error of Metabolism

Inborn error of metabolism	
ICD-10	E70.-E90.
ICD-9	270-279
MedlinePlus	002438
eMedicine	emerg/768
MeSH	D008661

Inborn errors of metabolism comprise a large class of genetic diseases involving disorders of metabolism. The majority are due to defects of single genes that code for enzymes that facilitate conversion of various substances (substrates) into others (products). In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds. Inborn errors of metabolism are now often referred to as **congenital metabolic diseases** or **inherited metabolic diseases**.

The term *inborn error of metabolism* was coined by a British physician, Archibald Garrod (1857–1936), in the early 20th century (1908). He is known for work that prefigured the "one gene-one enzyme" hypothesis, based on his studies on the nature and inheritance of alkaptonuria. His seminal text, *Inborn Errors of Metabolism* was published in 1923.

Major categories of inherited metabolic diseases

Traditionally the inherited metabolic diseases were categorized as disorders of carbohydrate metabolism, amino acid metabolism, organic acid metabolism, or lysosomal storage diseases. In recent decades, hundreds of new inherited disorders of metabolism have been discovered and the categories have proliferated. Following are some of the major classes of congenital metabolic diseases, with prominent examples of each class. Many others do not fall into these categories. ICD-10 codes are provided where available.

- Disorders of carbohydrate metabolism

    - E.g., glycogen storage disease
- Disorders of amino acid metabolism
    - E.g., phenylketonuria, maple syrup urine disease, glutaric acidemia type 1
- Disorders of organic acid metabolism (organic acidurias)
    - E.g., alcaptonuria
- Disorders of fatty acid oxidation and mitochondrial metabolism
    - E.g., medium chain acyl dehydrogenase deficiency (glutaric acidemia type 2)
- Disorders of porphyrin metabolism
    - E.g., acute intermittent porphyria
- Disorders of purine or pyrimidine metabolism
    - E.g., Lesch-Nyhan syndrome
- Disorders of steroid metabolism
    - E.g., congenital adrenal hyperplasia
- Disorders of mitochondrial function
    - E.g., Kearns-Sayre syndrome
- Disorders of peroxisomal function
    - E.g., Zellweger syndrome
- Lysosomal storage disorders
    - E.g., Gaucher's disease
    - E.g., Niemann Pick disease

Incidence

In a study in British Columbia, the overall incidence of the inborn errors of metabolism were estimated to be 70 per 100,000 live births or 1 in 1,400 births, overall representing more than approximately 15% of single gene disorders in the population.

Type of inborn error	Incidence	
Disease involving amino acids (e.g. PKU), organic acids, primary lactic acidosis, galactosemia, or a urea cycle disease	24 per 100 000 births	1 in 4,200
Lysosomal storage disease	8 per 100 000 births	1 in 12,500
Peroxisomal disorder	~3 to 4 per 100 000 of births	~1 in 30,000
Respiratory chain-based mitochondrial disease	~3 per 100 000 births	1 in 33,000
Glycogen storage disease	2.3 per 100 000 births	1 in 43,000

Manifestations and presentations

Because of the enormous number of these diseases and wide range of systems affected, nearly every "presenting complaint" to a doctor may have a congenital metabolic disease as a possible cause, especially in childhood. The following are examples of potential manifestations affecting each of the major organ systems:

- Growth failure, failure to thrive, weight loss
- Ambiguous genitalia, delayed puberty, precocious puberty
- Developmental delay, seizures, dementia, encephalopathy, stroke
- Deafness, blindness, pain agnosia
- Skin rash, abnormal pigmentation, lack of pigmentation, excessive hair growth, lumps and bumps
- Dental abnormalities
- Immunodeficiency, thrombocytopenia, anemia, enlarged spleen, enlarged lymph nodes
- Many forms of cancer
- Recurrent vomiting, diarrhea, abdominal pain
- Excessive urination, renal failure, dehydration, edema
- Hypotension, heart failure, enlarged heart, hypertension, myocardial infarction
- Hepatomegaly, jaundice, liver failure
- Unusual facial features, congenital malformations
- Excessive breathing (hyperventilation), respiratory failure
- Abnormal behavior, depression, psychosis
- Joint pain, muscle weakness, cramps
- Hypothyroidism, adrenal insufficiency, hypogonadism, diabetes mellitus

Diagnostic techniques

Dozens of congenital metabolic diseases are now detectable by newborn screening tests, especially the expanded testing using mass spectrometry. This is an increasingly common way for the diagnosis to be made and sometimes results in earlier treatment and a better outcome. There is a revolutionary GC/MS based technology with an integrated analytics system, which has now made it possible to test a newborn for over 100 genetic metabolic disorders.

Because of the multiplicity of conditions, many different diagnostic tests are used for screening. An abnormal result is often followed by a subsequent "definitive test" to confirm the suspected diagnosis.

Common screening tests used in the last sixty years:

- Ferric chloride test (turned colors in reaction to various abnormal metabolites in urine)
- Ninhydrin paper chromatography (detected abnormal amino acid patterns)
- Guthrie bacterial inhibition assay (detected a few amino acids in excessive amounts in blood) The dried blood spot can be used for multianalyte testing using Tandem Mass Spectroscopy (MS/MS). This given an indication for a disorder. The same has to be further confirmed by enzyme assays, GC/MS or DNA Testing.
- Quantitative measurement of amino acids in plasma and urine
- Urine organic acid analysis by Gas chromatography-mass spectrometry
- Plasma acylcarnitines analysis by mass spectrometry

- Urine purines and pyrimidines analysis by Gas chromatography-mass spectrometry

Specific diagnostic tests (or focused screening for a small set of disorders):

- Tissue biopsy or necropsy: liver, muscle, brain, bone marrow
- Skin biopsy and fibroblast cultivation for specific enzyme testing
- Specific DNA testing

Treatment

In the middle of the 20th century the principal treatment for some of the amino acid disorders was restriction of dietary protein and all other care was simply management of complications. In the last two decades, enzyme replacement, gene transfer, and organ transplantation have become available and beneficial for many previously untreatable disorders. Some of the more common or promising therapies are listed:

- Dietary restriction
  - E.g., reduction of dietary protein remains a mainstay of treatment for phenylketonuria and other amino acid disorders
- Dietary supplementation or replacement
  - E.g., oral ingestion of cornstarch several times a day helps prevent people with glycogen storage diseases from becoming seriously hypoglycemic.
- Vitamins
  - E.g., thiamine supplementation benefits several types of disorders that cause lactic acidosis.
- Intermediary metabolites, compounds, or drugs that facilitate or retard specific metabolic pathways
- Dialysis
- Enzyme replacement E.g. Acid-alpha glucosidase for Pompe disease
- Gene transfer
- Bone marrow or organ transplantation
- Treatment of symptoms and complications
- Prenatal diagnosis and avoidance of pregnancy or abortion of an affected fetus

Resources

For clinicians and scientists in the field of inborn errors of metabolism, good resources include books by Scriver, Fernandes, Clarke, Blau (diagnosis), Blau (treatment), Lyon, Nyhan, Hoffmann, and Zschocke. Other resources include genetests, orphanet, OMIM, Metab-L, societies such as the SSIEM, the SIMD and links therein. For medical students and clinicians looking for overviews of the field, such reviews can be found on pubmed and in good pediatric textbooks (e.g. articles by Saudubray, Ellaway, Raghuveer or Burton, and textbooks by Hay or Behrman).

For patients, their families or other individuals seeking good information and support groups, the National Institutes of Health offers the office of rare diseases, genetics home reference, medlineplus and health information. The National Human Genome Research Institute hosts an information center, a section for patients and the public and additional educational resources. Support groups can be found at NORD, Genetic Alliance and Orphanet.

Chapter 4

Angelman Syndrome

Angelman syndrome	
ICD-10	Q93.5
ICD-9	759.89
OMIM	105830
DiseasesDB	712
MeSH	D017204

Angelman syndrome (AS) is a neuro-genetic disorder characterized by intellectual and developmental delay, sleep disturbance, seizures, jerky movements (especially hand-flapping), frequent laughter or smiling, and usually a happy demeanor.

AS is a classic example of genomic imprinting in that it is usually caused by deletion or inactivation of genes on the maternally inherited chromosome 15 while the paternal copy, which may be of normal sequence, is imprinted and therefore silenced. The sister syndrome, Prader-Willi syndrome, is caused by a similar loss of paternally inherited genes and maternal imprinting. AS is named after a British pediatrician, Dr. Harry Angelman, who first described the syndrome in 1965. An older, alternative term for AS, **happy puppet syndrome**, is generally considered pejorative and stigmatizing so it is no longer the accepted term, though it is sometimes still used as an informal term of diagnosis. People with AS are sometimes known as "angels", both because of the syndrome's name and because of their youthful, happy appearance.

History

"Boy with a Puppet" or "A child with a drawing" by Giovanni Francesco Caroto

Dr. Harry Angelman, a pediatrician working in Warrington (then in Lancashire) first reported three children with this condition in 1965. Angelman later described his choice of the title "Puppet Children" to describe these cases as being related to an oil painting he had seen while vacationing in Italy:

The history of medicine is full of interesting stories about the discovery of illnesses. The saga of Angelman's syndrome is one such story. It was purely by chance that nearly thirty years ago (e.g., circa 1964) three handicapped children were admitted at various times to my children's ward in England. They had a variety of disabilities and although at first sight they seemed to be suffering from different conditions I felt that there was a common cause for their illness. The diagnosis was purely a clinical one because in spite of technical investigations which today are more refined I was unable to establish scientific proof that the three children all had the same handicap. In view of this I hesitated to write

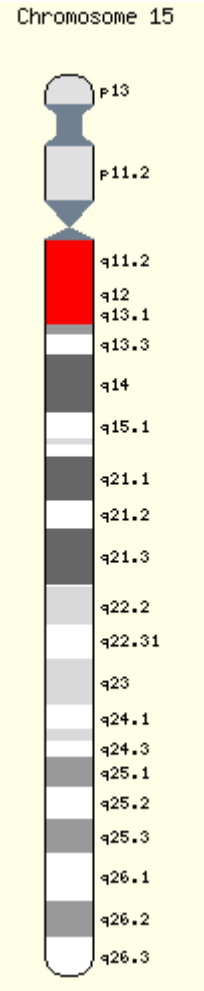
about them in the medical journals. However, when on holiday in Italy I happened to see an oil painting in the Castelvecchio Museum in Verona called . . . a Boy with a Puppet. The boy's laughing face and the fact that my patients exhibited jerky movements gave me the idea of writing an article about the three children with a title of Puppet Children. It was not a name that pleased all parents but it served as a means of combining the three little patients into a single group. Later the name was changed to Angelman syndrome.

Case reports from the United States first began appearing in the medical literature in the early 1980s. In 1987, it was first noted that around half of the children with AS have a small piece of chromosome 15 missing (*chromosome 15q partial deletion*).

Prevalence

Though the prevalence of Angelman syndrome is not precisely known, there are some estimates. The best data available are from studies of school age children, ages 6–13 years, living in Sweden and from Denmark where the diagnosis of AS children in medical clinics was compared to an 8 year period of about 45,000 births. The Swedish study showed an AS prevalence of about 1/20,000 and the Danish study showed a minimum AS prevalence of about 1/10,000.

Pathophysiology



Chromosome 15

Angelman syndrome is caused by the loss of the normal maternal contribution to a region of chromosome 15, most commonly by deletion of a segment of that chromosome. Other causes include uniparental disomy, translocation, or single gene mutation in that region. A healthy person receives two copies of chromosome 15, one from the mother, the other from the father. However, in the region of the chromosome that is critical for Angelman syndrome, the maternal and paternal contribution express certain genes very differently. This is due to gender-related epigenetic imprinting; the biochemical mechanism is DNA methylation. In a normal individual, the maternal allele is expressed and the paternal allele is silenced. If the maternal contribution is lost or mutated, the result is Angelman syndrome. (When the paternal contribution is lost, by similar mechanisms, the result is Prader-Willi syndrome.) It should be noted that the methylation test that is performed for Angelman syndrome (a defect in UBE3A) is actually looking for the gene's neighbour SNRPN (which has the opposite pattern of methylation).

Angelman syndrome can also be the result of mutation of a single gene. This gene (*UBE3A*, part of the ubiquitin pathway) is present on both the maternal and paternal chromosomes, but differs in the pattern of methylation (imprinting). The paternal silencing of the *UBE3A* gene occurs in a brain region-specific manner; in the hippocampus and cerebellum, the maternal allele is almost exclusively the active one. The most common genetic defect leading to Angelman syndrome is an ~4Mb (mega base) maternal deletion in chromosomal region 15q11-13 causing an absence of *UBE3A* expression in the paternally imprinted brain regions. *UBE3A* codes for an E6-AP ubiquitin ligase, which chooses its substrates very selectively and the four identified E6-AP substrates have shed little light on the possible molecular mechanisms underlying the human Angelman syndrome mental retardation state.

Initial studies of mice that do not express maternal *UBE3A* show severe impairments in hippocampal memory formation. Most notably, there is a deficit in a learning paradigm that involves hippocampus-dependent contextual fear conditioning. In addition, maintenance of long-term synaptic plasticity in hippocampal area CA1 *in vitro* is disrupted in *Ube3a*$^{-/-}$ mice. These results provide links amongst hippocampal synaptic plasticity *in vitro*, formation of hippocampus-dependent memory *in vivo*, and the molecular pathology of Angelman syndrome.

Clinical features

The following list features of Angelman syndrome and their relative frequency in affected individuals.

Consistent (100%)

- Developmental delay, functionally severe
- Speech impairment, no or minimal use of words; receptive and non-verbal communication skills higher than verbal ones
- Movement or balance disorder, usually ataxia of gait and/or tremulous movement of limbs
- Behavioral uniqueness: any combination of frequent laughter/smiling; apparent happy demeanor; easily excitable personality, often with hand flapping movements; hypermotoric behavior; short attention span

Frequent (more than 80%)

- Delayed, disproportionate growth in head circumference, usually resulting in microcephaly (absolute or relative) by age 2
- Seizures, onset usually < 3 years of age
- Abnormal EEG, characteristic pattern with large amplitude slow-spike waves

Associated (20 - 80%)

- Strabismus

- Hypopigmented skin and eyes
- Tongue thrusting; suck/swallowing disorders
- Hyperactive tendon reflexes
- Feeding problems during infancy
- Uplifted, flexed arms during walking
- Prominent mandible
- Increased sensitivity to heat
- Wide mouth, wide-spaced teeth
- Sleep disturbance
- Frequent drooling, protruding tongue
- Attraction to/fascination with water
- Excessive chewing/mouthing behaviors
- Flat back of head
- Smooth palms

Neurophysiology

One of the more notable features of Angelman Syndrome (AS) is the syndrome's pathognomonic neurophysiological findings. The electroencephalogram (EEG) in AS is usually very abnormal, and more abnormal than clinically expected. Three distinct interictal patterns are seen in these patients. The most common pattern is a very large amplitude 2–3 Hz rhythm most prominent in prefrontal leads (**A**). Next most common is a symmetrical 4–6 Hz high voltage rhythm (**B**). The third pattern, 3–6 Hz activity punctuated by spikes and sharp waves in occipital leads, is associated with eye closure (**C**). Paroxysms of laughter have no relation to the EEG, ruling out this feature as a gelastic phenomenon (Williams 2005).

Diagnosis

The diagnosis of Angelman syndrome is based on:

- A history of delayed motor milestones and then later a delay in general development, especially of speech
- Unusual movements including fine tremors, jerky limb movements, hand flapping and a wide-based, stiff-legged gait.
- Characteristic facial appearance (but not in all cases).
- A history of epilepsy and an abnormal EEG tracing.
- A happy disposition with frequent laughter
- A deletion or inactivity on chromosome 15

Diagnostic criteria for the disorder were initially established in 1995 in collaboration with the Angelman syndrome Foundation (USA); these criteria have undergone revision in 2005.

Treatment and care

There is currently no cure available. The epilepsy can be controlled by the use of one or more types of anticonvulsant medications. However, there are difficulties in ascertaining the levels and types of anticonvulsant medications needed to establish control, because AS is usually associated with having multiple varieties of seizures, rather than just the one as in normal cases of epilepsy. Many families use melatonin to promote sleep in a condition which often affects sleep patterns. Many individuals with Angelman syndrome sleep for a maximum of 5 hours at any one time. Mild laxatives are also used frequently to encourage regular bowel movements and early intervention with physiotherapy is important to encourage joint mobility and prevent stiffening of the joints.

Those with the syndrome are generally happy and contented people who like human contact and play. People with AS exhibit a profound desire for personal interaction with others. Communication can be difficult at first, but as a child with AS develops, there is a definite character and ability to make themselves understood. People with AS tend to develop strong non-verbal skills to compensate for their limited use of speech. It is widely accepted that their understanding of communication directed to them is much larger than their ability to return conversation. Most afflicted people will not develop more than 5-10 words, if any at all.

Seizures are a consequence, but so is excessive laughter, which is a major hindrance to early diagnosis.

Actor Colin Farrell, author Ian Rankin, professional baseball player Dave Henderson, and professional hockey player Peter McDuffe have sons with AS.

Prognosis

The severity of the symptoms associated with Angelman syndrome varies significantly across the population of those affected. Some speech and a greater degree of self-care are possible among the least profoundly affected. Unfortunately, walking and the use of simple sign language may be beyond the reach of the more profoundly affected. Early and continued participation in physical, occupational (related to the development of fine-motor control skills), and communication (speech) therapies are believed to improve significantly the prognosis (in the areas of cognition and communication) of individuals affected by AS. Further, the specific genetic mechanism underlying the condition is thought to correlate to the general prognosis of the affected person. On one end of the spectrum, a mutation to the UBE3A gene is thought to correlate to the least affected, whereas larger deletions on chromosome 15 are thought to correspond to the most affected.

The clinical features of Angelman syndrome alter with age. As adulthood approaches, hyperactivity and poor sleep patterns improve. The seizures decrease in frequency and often cease altogether and the EEG abnormalities are less obvious. Medication is typically advisable to those with seizure disorders. Often overlooked is the contribution

of the poor sleep patterns to the frequency and/or severity of the seizures. Medication may be worthwhile in order to help deal with this issue and improve the prognosis with respect to seizures and sleep. Also noteworthy are the reports that the frequency and severity of seizures temporarily escalate in pubescent Angelman syndrome girls but do not seem to affect long-term health.

The facial features remain recognizable but many adults with AS look remarkably youthful for their age.

Puberty and menstruation begin at around the average age. Sexual development is thought to be unaffected, as evidenced by a single reported case of a woman with Angelman syndrome conceiving a female child who also had Angelman syndrome.

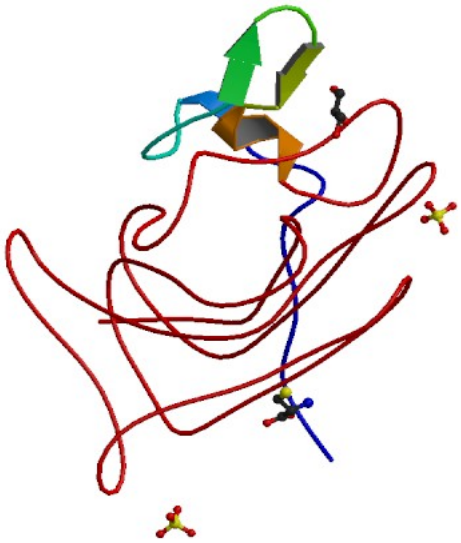
The majority of those with AS achieve continence by day and some by night. Angelman syndrome is not a degenerative syndrome. Many people with AS improve their living skills with support.

Dressing skills are variable and usually limited to items of clothing without buttons or zippers. Most adults are able to eat with a knife or spoon and fork and can learn to perform simple household tasks. General health is fairly good and life-span near average. Particular problems which have arisen in adults are a tendency to obesity (more in females), and worsening of scoliosis if it is present. The affectionate nature which is also a positive aspect in the younger children may also persist into adult life where it can pose a problem socially, but this problem is not insurmountable.

Chapter 5

Haemophilia

Haemophilia



Deficiency in coagulation factor VIII is the most common cause of haemophilia.

ICD-10	D66.-D68.
ICD-9	286
OMIM	306700 306900 264900
DiseasesDB	5555 5561 29376
MedlinePlus	000537
eMedicine	med/3528
MeSH	D025861

Haemophilia (also spelled **hemophilia** in North America, from the Greek *haima* αἷμα 'blood' and *philia* φιλος 'love') is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor VIII deficiency) is the most common form of the disorder, occurring at about 1 in 5,000–10,000 male births. Haemophilia B (factor IX deficiency) occurs at about 1 in about 20,000–34,000 male births.

Like most recessive sex-linked, X chromosome disorders, haemophilia is more likely to occur in males than females. This is because females have two X chromosomes while males have only one, so the defective gene is guaranteed to manifest in any male who carries it. Because females have two X chromosomes and haemophilia is rare, the chance of a female having two defective copies of the gene is very low, so females are almost exclusively asymptomatic carriers of the disorder. Female carriers can inherit the defective gene from either their mother or father, or it may be a new mutation. Only under rare circumstances do females actually have haemophilia.

Haemophilia lowers blood plasma clotting factor levels of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. A haemophiliac does not bleed more intensely than a normal person, but can bleed for a much longer time. In severe haemophiliacs even a minor injury can result in blood loss lasting days or weeks, or even never healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Signs and symptoms

Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". Patients with more severe haemophilia suffer more severe and more frequent bleeds, while patients with mild haemophilia typically suffer more minor symptoms except after surgery or serious trauma. Moderate haemophiliacs have variable symptoms which manifest along a spectrum between severe and mild forms.

Prolonged bleeding and re-bleeding are the diagnostic symptoms of haemophilia. Internal bleeding is common in people with severe haemophilia and some individuals with moderate haemophilia. The most characteristic type of internal bleed is a joint bleed where blood enters into the joint spaces. This is most common with severe haemophiliacs and can occur spontaneously (without evident trauma). If not treated promptly, joint bleeds can lead to permanent joint damage and disfigurement. Bleeding into soft tissues such as muscles and subcutaneous tissues is less severe but can lead to damage and requires treatment.

Children with mild to moderate haemophilia may not have any signs or symptoms at birth especially if they do not undergo circumcision. Their first symptoms are often frequent and large bruises and haematomas from frequent bumps and falls as they learn to walk.

Swelling and bruising from bleeding in the joints, soft tissue, and muscles may also occur. Children with mild haemophilia may not have noticeable symptoms for many years. Often, the first sign in very mild haemophiliacs is heavy bleeding from a dental procedure, an accident, or surgery. Females who are carriers usually have enough clotting factors from their one normal gene to prevent serious bleeding problems, though some may present as mild haemophiliacs.

Complications

Severe complications are much more common in severe and moderate haemophiliacs. Complications may be both directly from the disease or from its treatment:

- **Deep internal bleeding**, e.g. deep-muscle bleeding, leading to swelling, numbness or pain of a limb.
- **Joint damage** from haemarthrosis, potentially with severe pain, disfigurement, and even destruction of the joint and development of debilitating arthritis.
- **Transfusion transmitted infection** from blood transfusions that are given as treatment.
- **Adverse reactions** to clotting factor treatment, including the development of an immune inhibitor which renders factor replacement less effective.
- **Intracranial haemorrhage** is a serious medical emergency caused by the buildup of pressure inside the skull. It can cause disorientation, nausea, loss of consciousness, brain damage, and death.

Life expectancy

Like most aspects of the disorder, life expectancy varies with severity and adequate treatment. People with severe haemophilia who don't receive adequate, modern treatment have greatly shortened lifespans and often do not reach maturity. Prior to the 1960s when effective treatment became available, average life expectancy was only 11 years. By the 1980s the life span of the average haemophiliac receiving appropriate treatment was 50–60 years. Today with appropriate treatment, males with haemophilia typically have a near normal quality of life with an average lifespan approximately 10 years shorter than an unaffected male.

Since the 1980s the primary leading cause of death of people with severe haemophilia has shifted from haemorrhage to HIV/AIDS acquired through treatment with contaminated blood products. The second leading cause of death related to severe haemophilia complications is intracranial haemorrhage which today accounts for one third of all deaths of patients with haemophilia. Two other major causes of death include: hepatitis infections causing cirrhosis and, obstruction of air or blood flow due to soft tissue haemorrhage.

Causes

- Haemophilia A is a recessive X-linked genetic disorder involving a lack of functional clotting Factor VIII and represents 80% of haemophilia cases.
- Haemophilia B is a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. It comprises approximately 20% of haemophilia cases.
- Haemophilia C is an autosomal genetic disorder (i.e. *not* X-linked) involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive: heterozygous individuals also show increased bleeding.

Genetics

Females possess two X-chromosomes, males have one X and one Y chromosome. Since the mutations causing the disease are X-linked, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. However, the Y-chromosome in men has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and he will develop the illness.

Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence haemophilia is far more common among males than females. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness.

A mother who is a carrier has a 50% chance of passing the faulty X chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father.

Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition.

As with all genetic disorders, it is of course also possible for a human to acquire it spontaneously through mutation, rather than inheriting it, because of a new mutation in

one of their parents' gametes. Spontaneous mutations account for about 33% of all cases of haemophilia A. About 30% of cases of haemophilia B are the result of a spontaneous gene mutation.

If a female gives birth to a haemophiliac child, either the female is a carrier for the disease or the haemophilia was the result of a spontaneous mutation. Until modern direct DNA testing, however, it was impossible to determine if a female with only healthy children was a carrier or not. Generally, the more healthy sons she bore, the higher the probability that she was not a carrier.

If a male is afflicted with the disease and has children with a female who is not even a carrier, his daughters will be carriers of haemophilia. His sons, however, will not be affected with the disease. The disease is X-linked and the father cannot pass haemophilia through the Y chromosome. Males with the disorder are then no more likely to pass on the gene to their children than carrier females, though all daughters they sire will be carriers and all sons they father will not have haemophilia (unless the mother is a carrier).

Severity

There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, patients with haemophilia often have some level of active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia, and those with mild haemophilia have between 5-40% of normal levels of active clotting factor.

Diagnosis

Haemophilia A can be mimicked by von Willebrand disease.

- von Willebrand Disease could significantly affect as many as 1 in 10,000 people.
- von Willebrand Disease type 2A, where decreased levels of von Willebrand Factor can lead to premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 2A is inherited in an autosomal dominant fashion.
- von Willebrand Disease type 2N, where von Willebrand Factor cannot bind Factor VIII, autosomal recessive inheritance. (i.e.; both parents need to give the child a copy of the gene).
- von Willebrand Disease type 3, where lack of von Willebrand Factor causes premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 3 is inherited in an autosomal recessive fashion.

Additionally, severe cases of vitamin K deficiency can present similar symptoms to haemophilia. This is due to the fact that vitamin K is necessary for the human body to produce several protein clotting factors. This vitamin deficiency is rare in adults and older children but is common in newborns. Infants are born with naturally low levels of vitamin K and do not yet have the symbiotic gut flora to properly synthesise their own

vitamin K. Bleeding issues due to vitamin K deficiency in infants is known as "haemorrhagic disease of the newborn", to avoid this complication newborns are routinely injected with vitamin K supplements.

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected
Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier syndrome	unaffected	unaffected	prolonged	decreased

Management

Commercially produced factor concentrates such as "Advate", a recombinant Factor VIII produced by Baxter International, come as a white powder in a vial which must be mixed with sterile water prior to intravenous injection.

Though there is no cure for haemophilia, it can be controlled with regular infusions of the deficient clotting factor, i.e. factor VIII in haemophilia A or factor IX in haemophilia B. Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some haemophiliacs develop antibodies (inhibitors) against the replacement factors given to them, so the amount of the factor has to be increased or non-human replacement products must be given, such as porcine factor VIII.

If a patient becomes refractory to replacement coagulation factor as a result of circulating inhibitors, this may be partially overcome with recombinant human factor VII (NovoSeven), which is registered for this indication in many countries.

In early 2008, the US Food and Drug Administration (FDA) approved Xyntha (Wyeth) anti-haemophilic factor, genetically engineered from the genes of Chinese hamster ovary cells. Since 1993 (Dr. Mary Nugent) recombinant factor products (which are typically cultured in Chinese hamster ovary (CHO) tissue culture cells and involve little, if any human plasma products) have been available and have been widely used in wealthier western countries. While recombinant clotting factor products offer higher purity and safety, they are, like concentrate, extremely expensive, and not generally available in the developing world. In many cases, factor products of any sort are difficult to obtain in developing countries.

In Western countries, common standards of care fall into one of two categories: prophylaxis or on-demand. Prophylaxis involves the infusion of clotting factor on a regular schedule in order to keep clotting levels sufficiently high to prevent spontaneous bleeding episodes. On-demand treatment involves treating bleeding episodes once they arise. In 2007, a clinical trial was published in the *New England Journal of Medicine* comparing on-demand treatment of boys (< 30 months) with haemophilia A with prophylactic treatment (infusions of 25 IU/kg body weight of Factor VIII every other day) in respect to its effect on the prevention of joint-diseases. When the boys reached 6 years of age, 93% of those in the prophylaxis group and 55% of those in the episodic-therapy group had a normal index joint-structure on MRI. Prophylactic treatment, however, resulted in average costs of $300,000 per year. The author of an editorial published in the same issue of the *NEJM* supports the idea that prophylactic treatment not only is more effective than on demand treatment but also suggests that starting after the first serious joint-related haemorrhage may be more cost effective than waiting until the fixed age to begin. This study resulted in the first (October 2008) FDA approval to label any Factor VIII product to be used prophylactically. As a result, the factor product used in the study (Bayer's Kognate) is now labelled for use to prevent bleeds, making it more likely that insurance carries in the US will reimburse consumers who are prescribed and use this product prophylactically. Despite Kognate only recently being "approved" for this use in the US, it and other factor products have been well studied and are often prescribed to treat Haemophilia prophylactically to prevent bleeds, especially joint bleeds.

Preventive exercises

It is recommended that people affected with haemophilia do specific exercises to strengthen the joints, particularly the elbows, knees, and ankles. Exercises include elements which increase flexibility, tone, and strength of muscles, increasing their ability to protect joints from damaging bleeds. These exercises are recommended after an internal bleed occurs and on a daily basis to strengthen the muscles and joints to prevent new bleeding problems. Many recommended exercises include standard sports warm-up and training exercises such as stretching of the calves, ankle circles, elbow flexions, and quadriceps sets.

Alternative medicine

While not a replacement for traditional treatments, preliminary scientific studies indicate that hypnosis and self-hypnosis can be effective at reducing bleeds and the severity of bleeds and thus the frequency of factor treatment. Herbs which strengthen blood vessels and act as astringents may benefit patients with haemophilia, however there are no peer reviewed scientific studies to support these claims. Suggested herbs include: Bilberry (Vaccinium myrtillus), Grape seed extract (Vitis vinifera), Scotch broom (Cytisus scoparius), Stinging nettle (Urtica dioica), Witch hazel (Hamamelis virginiana), and yarrow (Achillea millefolium).

Contraindications

Anticoagulants such as Heparin and Warfarin are contraindicated for people with haemophilia as these can aggravate clotting difficulties. Also contraindicated are those drugs which have "blood thinning" side effects. For instance, medications which contain aspirin, ibuprofen, or naproxen sodium should not be taken because they are well known to have the side effect of prolonged bleeding.

Also contraindicated are activities with a high likelihood of trauma, such as motorcycling and skateboarding. Popular sports with very high rates of physical contact and injuries such as American football, hockey, boxing, wrestling, and rugby should be avoided by people with haemophilia. Other active sports like soccer, baseball, and basketball also have a high rate of injuries, but have overall less contact and should be undertaken cautiously and only in consultation with a doctor.

Epidemiology

Haemophilia is rare, with only about 1 instance in every 10,000 births (or 1 in 5,000 male births) for haemophilia A and 1 in 50,000 births for haemophilia B. About 18,000 people in the United States have haemophilia. Each year in the US, about 400 babies are born with the disorder. Haemophilia usually occurs in males and less often in females. It is estimated that about 2500 Canadians have haemophilia A, and about 500 Canadians have haemophilia B.

History

"About seventy or eighty years ago, a woman by name of Smith, settled in the vicinity of Plymouth, New Hampshire, and transmitted the following idiosyncrasy to her descendants. It is one, she observed, to which her family is unfortunately subject, and had been the source not only of great solicitude, but frequently the cause of death. If the least scratch is made on the skin of some of them, as mortal a hemorrhagy will eventually ensue as if the largest wound is inflicted. (…) So assured are the members of this family of the terrible consequences of the least wound, that they will not suffer themselves to be bled on any consideration, having lost a relation by not being able to stop the discharge occasioned by this operation."

John C. Otto, 1803

Scientific discovery

The first written account of haemophilia occurred in the 2nd century in the Babylonian Talmud. In it Rabbi Judah haNasi, redactor of the Mishneh, wrote: "If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child." This passage refers to both the prolonged bleeding caused by circumcision and to the maternal inheritance of the disease. The first medical professional to describe a disease was Albucasis. In the tenth century he described families whose males died of bleeding after only minor traumas. While many other such descriptive and practical references to the disease appear throughout historical writings, scientific analysis did not begin until the start of the nineteenth century.

In 1803, Dr. John Conrad Otto, a Philadelphian physician, wrote an account about "a hemorrhagic disposition existing in certain families" in which he called the affected males "bleeders." He recognised that the disorder was hereditary and that it affected mostly males and was passed down by healthy females. His paper was the second paper to describe important characteristics of an X-linked genetic disorder (the first paper being a description of colour blindness by John Dalton who studied his own family). Otto was able to trace the disease back to a woman who settled near Plymouth in 1720. The idea that affected males could pass the trait onto their unaffected daughters was not described until 1813 when John Hay published an account in The New England Journal of Medicine.

A Finnish Doctor in 1924 discovered a heredity bleeding disorder similar to Haemophilia localised in a group of islands (called the "Aland Islands") which are located to the southwest of Finland. This bleeding disorder is called "Von Willebrand Disease".

The term "haemophilia" is derived from the term "haemorrhaphilia" which was used in a description of the condition written by Friedrich Hopff in 1828, while he was a student at the University of Zurich. In 1937, Patek and Taylor, two doctors from Harvard, discovered anti-haemophilic globulin. In 1947, Pavlosky, a doctor from Buenos Aires, found haemophilia A and haemophilia B to be separate diseases by doing a lab test. This test was done by transferring the blood of one haemophiliac to another haemophiliac. The fact that this corrected the clotting problem showed that there was more than one form of haemophilia.

European royalty

Queen Victoria passed haemophilia on to some of her descendants

Haemophilia has featured prominently in European royalty and thus is sometimes known as "the royal disease". Queen Victoria passed the mutation to her son Leopold and, through some of her daughters, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei Nikolaevich, son of Nicholas II, was a descendant of Queen Victoria through his mother Empress Alexandra and suffered from haemophilia.

Ryan White was an American haemophiliac who became infected with HIV/AIDS through contaminated blood products.

It was claimed that Rasputin was successful at treating the Tsarevich's haemophilia. At the time, a common treatment administered by professional doctors was to use aspirin, which worsened rather than lessened the problem. It is believed that, by simply advising against the medical treatment, Rasputin could bring visible and significant improvement to the condition of Alexei.

In Spain, Queen Victoria's youngest daughter, Princess Beatrice, had a daughter Victoria Eugenie of Battenberg, who later became Queen of Spain. Two of her sons were haemophiliacs and both died from minor car accidents: Her eldest son, Prince Alfonso of Spain, Prince of Asturias, died at the age of 31 from internal bleeding after his car hit a telephone booth. Her youngest son, Infante Gonzalo, died at age 19 from abdominal bleeding following a minor car accident where he and his sister hit a wall while avoiding a cyclist. Neither appeared injured or sought immediate medical care and Gonzalo died two days later from internal bleeding.

Blood contamination issues

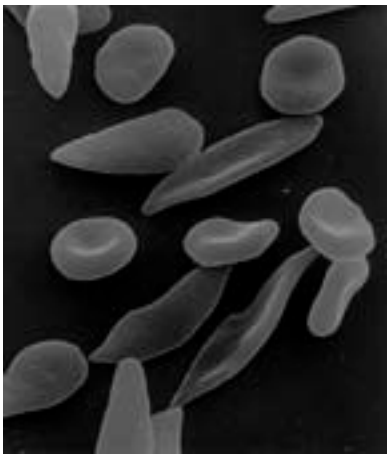
Prior to 1985, there were no laws enacted within the U.S. to screen blood. As a result, many haemophilia patients who received untested and unscreened clotting factor prior to 1992 were at an extreme risk for contracting HIV and hepatitis C via these blood products. It is estimated that more than 50% of the haemophilia population, over 10,000 people, contracted HIV from the tainted blood supply in the United States alone.

As a direct result of the contamination of the blood supply in the late 1970s and early/mid 1980s with viruses such as hepatitis and HIV, new methods were developed in the production of clotting factor products. The initial response was to heat-treat (pasteurise) plasma-derived factor concentrate, followed by the development of monoclonal factor concentrates, which use a combination of heat treatment and affinity chromatography to inactivate any viral agents in the pooled plasma from which the factor concentrate is derived. The Lindsay Tribunal in Ireland investigated, among other things, the slow adoption of the new methods.

Chapter 6

Sickle-Cell Disease

Sickle-cell anaemia



Normal and sickle-shaped red blood cells

ICD-10	D57.
ICD-9	282.6
OMIM	603903
DiseasesDB	12069
MedlinePlus	000527
eMedicine	med/2126 oph/490 ped/2096 emerg/26 emerg/406
MeSH	*C15.378.071.141.150.150*
GeneReviews	Sickle-cell disease

Sickle-cell disease (**SCD**), or **sickle-cell anaemia** (or **anemia**; **SCA**) or **drepanocytosis**, is an autosomal co-dominant genetic blood disorder characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various complications. The sickling occurs because of a mutation in

the haemoglobin gene. Life expectancy is shortened, with studies reporting an average life expectancy of 42 in males and 48 in females.

Sickle-cell disease, usually presenting in childhood, occurs more commonly in people (or their descendants) from parts of tropical and sub-tropical regions where malaria is or was common. One-third of all indigenous inhabitants of Sub-Saharan Africa carry the gene, because in areas where malaria is common, there is a fitness benefit in carrying only a single sickle-cell gene (sickle cell trait). Those with only one of the two alleles of the sickle-cell disease, while not more resistant, are more tolerant of infection and thus show less severe symptoms when infected.

The prevalence of the disease in the United States is approximately 1 in 5,000, mostly affecting Americans of Sub-Saharan African descent, according to the National Institutes of Health. In the United States, about 1 in 500 African-American children born will have sickle-cell anaemia.

Sickle-cell anaemia is the name of a specific form of sickle-cell disease in which there is homozygosity for the mutation that causes HbS. Sickle-cell anaemia is also referred to as "HbSS", "SS disease", "haemoglobin S" or permutations thereof. In heterozygous people, who have only one sickle gene and one normal adult haemoglobin gene, it is referred to as "HbAS" or "sickle cell trait". Other, rarer forms of sickle-cell disease include sickle-haemoglobin C disease (HbSC), sickle beta-plus-thalassaemia (HbS/β^{+}) and sickle beta-zero-thalassaemia (HbS/β^{0}). These other forms of sickle-cell disease are compound heterozygous states in which the person has only one copy of the mutation that causes HbS and one copy of another abnormal haemoglobin allele.

The term *disease* is applied, because the inherited abnormality causes a pathological condition that can lead to death and severe complications. Not all inherited variants of haemoglobin are detrimental, a concept known as genetic polymorphism.

Signs and symptoms

Sickle-cell disease may lead to various acute and chronic complications, several of which are potentially lethal.

Sickle cell crisis

The term "sickle cell crisis" is used to describe several independent acute conditions occurring in patients with sickle cell disease. Sickle cell disease results in anaemia and crisis that could be of many types including The vaso-occlusive crisis, aplastic crisis, sequestration crisis, hyper haemolytic crisis and others. Most episodes of sickle cell crises last between five and seven days.

Vaso-occlusive crisis

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ, resulting in ischaemia, pain, necrosis and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia (PCA) devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis or lungs are considered an emergency and treated with red-blood cell transfusions. Diphenhydramine is sometimes effective for the itching associated with the opioid use. Incentive spirometry, a technique to encourage deep breathing to minimise the development of atelectasis, is recommended.

Splenic sequestration crisis

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected. It is usually infarcted before the end of childhood in individuals suffering from sickle-cell anaemia. This autosplenectomy increases the risk of infection from encapsulated organisms; preventive antibiotics and vaccinations are recommended for those with such asplenia.

- *Splenic sequestration crises*: are acute, painful enlargements of the spleen. The sinusoids and gates would open at the same time resulting in sudden pooling of the blood into the spleen and circulatory defect leading to sudden hypovolaemia. The abdomen becomes bloated and very hard. Splenic sequestration crises is considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient, they continue for 3–4 hours and may last for one day.

Aplastic crisis

Aplastic crises are acute worsenings of the patient's baseline anaemia, producing pallor, tachycardia, and fatigue. This crisis is triggered by parvovirus B19, which directly affects erythropoiesis (production of red blood cells) by invading the red cell precursors and multiplying in them and destroying them. Parvovirus infection nearly completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of sickle-cell patients results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion.

Haemolytic crisis

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with co-existent G6PD deficiency. Management is supportive, sometimes with blood transfusions.

Other

One of the earliest clinical manifestations is dactylitis, presenting as early as six months of age, and may occur in children with sickle trait. The crisis can last up to a month. Another recognised type of sickle crisis is the acute chest syndrome, a condition characterised by fever, chest pain, difficulty breathing, and pulmonary infiltrate on a chest X-ray. Given that pneumonia and sickling in the lung can both produce these symptoms, the patient is treated for both conditions. It can be triggered by painful crisis, respiratory infection, bone-marrow embolisation, or possibly by atelectasis, opiate administration, or surgery.

Complications

Sickle-cell anaemia can lead to various complications, including:

- Overwhelming post-(auto)splenectomy infection (OPSI), which is due to functional asplenia, caused by encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Daily penicillin prophylaxis is the most commonly used treatment during childhood, with some haematologists continuing treatment indefinitely. Patients benefit today from routine vaccination for *H. influenzae*, *S. pneumoniae*, and *Neisseria meningitidis*.
- Stroke, which can result from a progressive narrowing of blood vessels, preventing oxygen from reaching the brain. Cerebral infarction occurs in children and cerebral haemorrhage in adults.
- Cholelithiasis (gallstones) and cholecystitis, which may result from excessive bilirubin production and precipitation due to prolonged haemolysis.
- Avascular necrosis (aseptic bone necrosis) of the hip and other major joints, which may occur as a result of ischaemia.
- Decreased immune reactions due to hyposplenism (malfunctioning of the spleen).
- Priapism and infarction of the penis.
- Osteomyelitis (bacterial bone infection); the most common cause of osteomyelitis in sickle cell disease is *Salmonella* (especially the non-typical serotypes Salmonella typhimurium, Salmonella enteritidis, Salmonella choleraesuis and Salmonella paratyphi B), followed by *Staphylococcus aureus* and Gram-negative enteric bacilli perhaps because intravascular sickling of the bowel leads to patchy ischaemic infarction.
- Opioid tolerance, which can occur as a normal, physiologic response to the therapeutic use of opiates. Addiction to opiates occurs no more commonly among individuals with sickle-cell disease than among other individuals treated with opiates for other reasons.

- Acute papillary necrosis in the kidneys.
- Leg ulcers.
- In eyes, background retinopathy, proliferative retinopathy, vitreous haemorrhages and retinal detachments, resulting in blindness. Regular annual eye checks are recommended.
- During pregnancy, intrauterine growth retardation, spontaneous abortion, and pre-eclampsia.
- Chronic pain: Even in the absence of acute vaso-occlusive pain, many patients have chronic pain that is not reported.
- Pulmonary hypertension (increased pressure on the pulmonary artery), leading to strain on the right ventricle and a risk of heart failure; typical symptoms are shortness of breath, decreased exercise tolerance and episodes of syncope.
- Chronic renal failure due to Sickle cell nephropathy—manifests itself with hypertension (high blood pressure), proteinuria (protein loss in the urine), haematuria (loss of red blood cells in urine) and worsened anaemia. If it progresses to end-stage renal failure, it carries a poor prognosis.

Heterozygotes

The heterozygous form (sickle cell trait) is almost always asymptomatic, and the only usual significant manifestation is the renal concentrating defect presenting with isosthenuria.

Pathophysiology

Sickle-cell anaemia is caused by a point mutation in the β-globin chain of haemoglobin, causing the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine at the sixth position. The β-globin gene is found on the short arm of chromosome 11. The association of two wild-type α-globin subunits with two mutant β-globin subunits forms haemoglobin S (HbS). Under low-oxygen conditions (being at high altitude, for example), the absence of a polar amino acid at position six of the β-globin chain promotes the non-covalent polymerisation (aggregation) of haemoglobin, which distorts red blood cells into a sickle shape and decreases their elasticity.

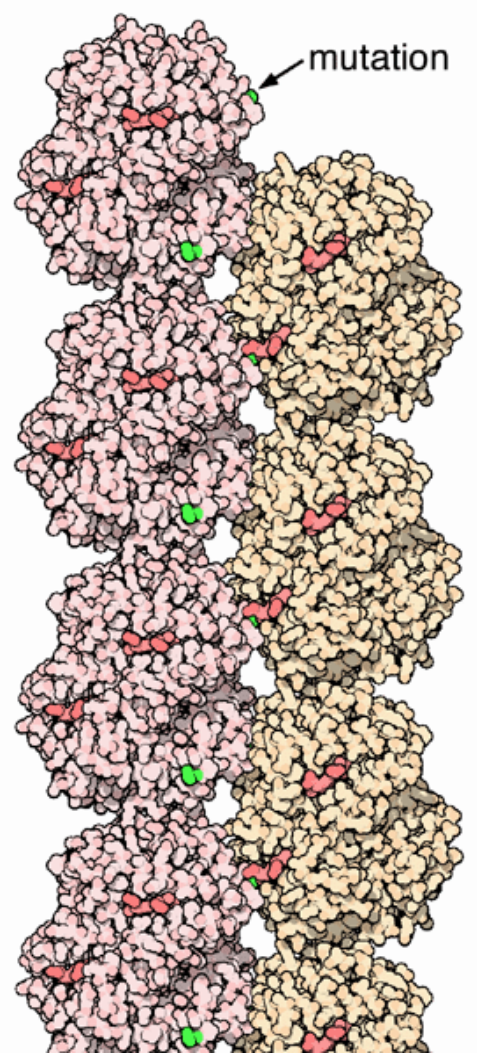
The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. Normal red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. In sickle-cell disease, low-oxygen tension promotes red blood cell sickling and repeated episodes of sickling damage the cell membrane and decrease the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischaemia.

The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells inside the spleen, because of their misshape. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction. Healthy red blood cells typically live 90–120 days, but sickle cells only survive 10–20 days.

Normally, humans have Haemoglobin A, which consists of two alpha and two beta chains, Haemoglobin A2, which consists of two alpha and two delta chains and Haemoglobin F, consisting of two alpha and two gamma chains in their bodies. Of these, Haemoglobin A makes up around 96-97% of the normal haemoglobin in humans.

In normal Haemoglobin A, glutamic acid is on the 6th position of the beta chain, while in sickle-cell disease, this glutamic acid is replaced by valine leading to the formation of sickle cells. This happens due to a one point mutation. This leads to polymerization of the two beta chains and therefore their appearance as puzzle pieces (or lock and key); which means they fit into each other forming a longitudinal polymer that would lead to the cell becoming deformed and very rigid leading to vessel occlusion. This process of polymerization can be activated by infections, hypoxia, acidosis, physical exercise, vasoocclusion due to cold as well as hypertonic dehydration.

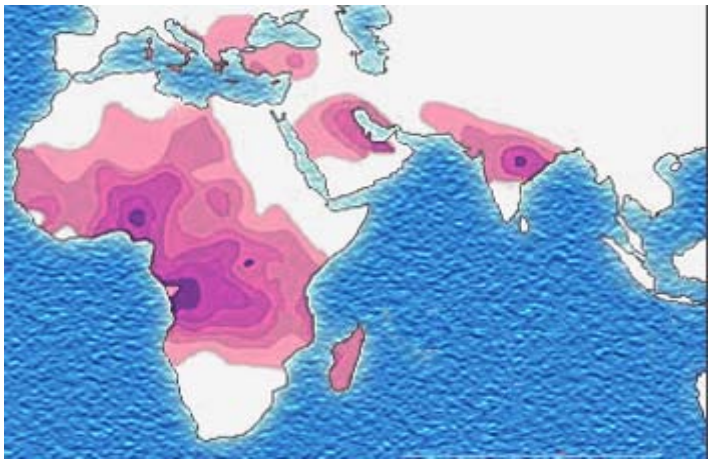
Genetics



A single amino acid change causes haemoglobin proteins to form fibres

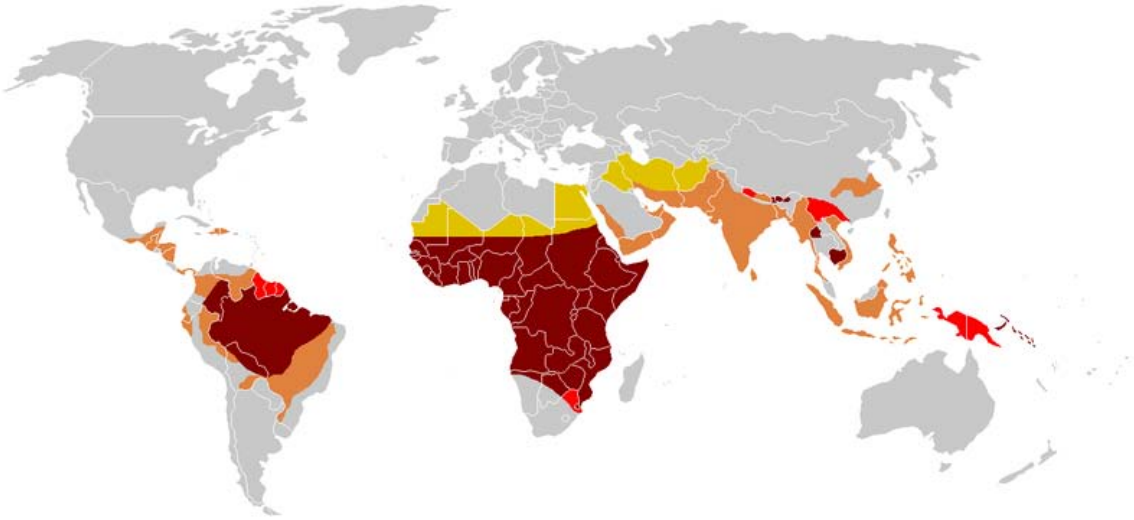
Sickle-cell gene mutation probably arose spontaneously in different geographic areas, as suggested by restriction endonuclease analysis. These variants are known as Cameroon, Senegal, Benin, Bantu and Saudi-Asian. Their clinical importance springs from the fact that some of them are associated with higher HbF levels, e.g., Senegal and Saudi-Asian variants, and tend to have milder disease.

In people heterozygous for HgbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the haemoglobin. In people homozygous for HgbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. Under normal circumstances, these painful crises occur about 0.8 times per year per patient. The sickle-cell disease occurs when the seventh amino acid (if the initial methionine is counted), glutamic acid, is replaced by valine to change its structure and function.



Distribution of the sickle-cell trait shown in pink and purple

Historical distribution of malaria (no longer endemic in Europe) shown in green



Modern distribution of malaria

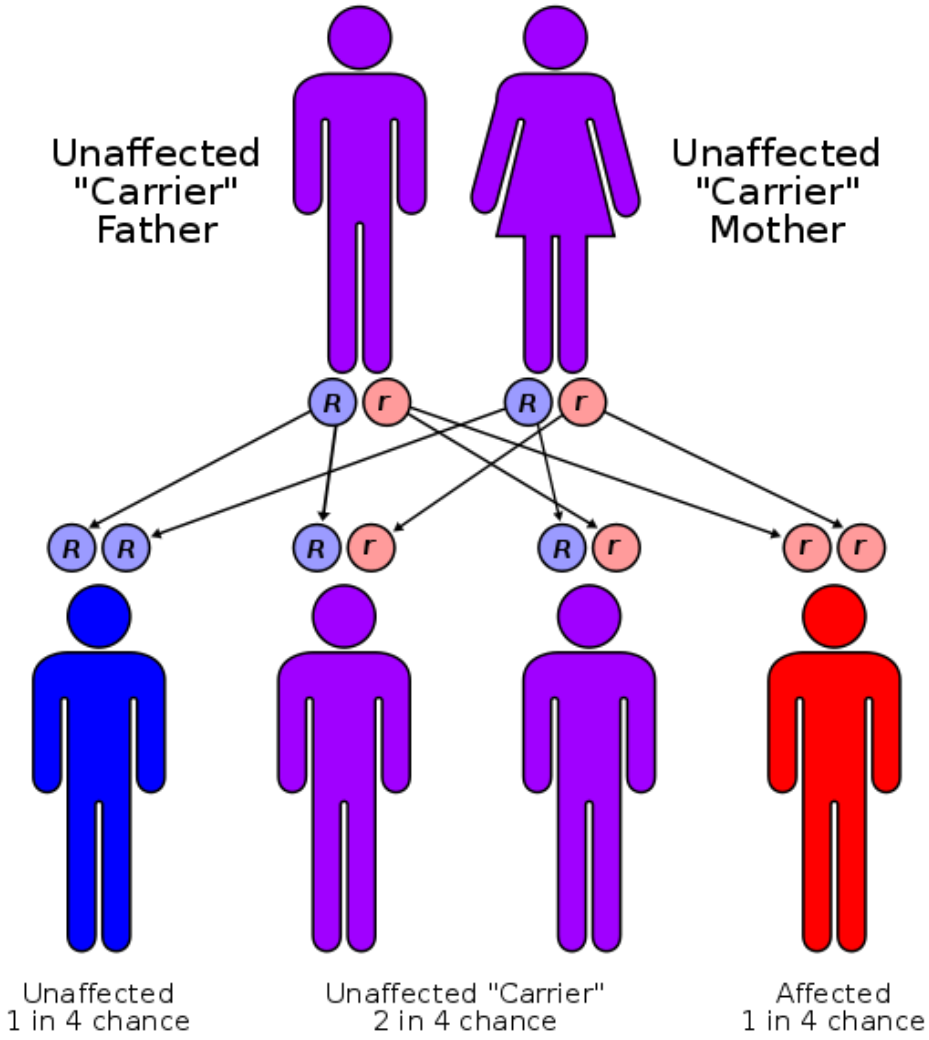
The gene defect is a known mutation of a single nucleotide (A to T) of the β-globin gene, which results in glutamic acid being substituted by valine at position 6. Haemoglobin S with this mutation are referred to as HbS, as opposed to the normal adult HbA. The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GUG codon mutation. This is normally a benign mutation, causing *no* apparent effects on the secondary, tertiary, or quaternary structure of haemoglobin in conditions of normal oxygen concentration. What it does allow for, under conditions of low oxygen concentration, is the polymerization of the HbS itself. The deoxy form of haemoglobin exposes a hydrophobic patch on the protein between the E and F helices. The hydrophobic residues of the valine at position 6 of the beta chain in haemoglobin are able to associate with the hydrophobic patch, causing haemoglobin S molecules to aggregate and form fibrous precipitates.

The allele responsible for sickle-cell anaemia is autosomal recessive and can be found on the short arm of chromosome 11. A person that receives the defective gene from both father and mother develops the disease; a person that receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier. If two parents who are carriers have a child, there is a 1-in-4 chance of their child developing the disease and a 1-in-2 chance of their child's being just a carrier. Since the gene is incompletely recessive, carriers can produce a few sickled red blood cells, not enough to cause symptoms, but enough to give resistance to malaria. Because of this, heterozygotes have a higher fitness than either of the homozygotes. This is known as heterozygote advantage.

Due to the adaptive advantage of the heterozygote, the disease is still prevalent, especially among people with recent ancestry in malaria-stricken areas, such as Africa, the Mediterranean, India and the Middle East. Malaria was historically endemic to southern Europe, but it was declared eradicated in the mid-20th century, with the exception of rare sporadic cases.

The malaria parasite has a complex life cycle and spends part of it in red blood cells. In a carrier, the presence of the malaria parasite causes the red blood cells with defective haemoglobin to rupture prematurely, making the plasmodium unable to reproduce. Further, the polymerization of Hb affects the ability of the parasite to digest Hb in the first place. Therefore, in areas where malaria is a problem, people's chances of survival actually increase if they carry sickle-cell trait (selection for the heterozygote).

In the USA, where there is no endemic malaria, the prevalence of sickle-cell anaemia among blacks is lower (about 0.25%) than in West Africa (about 4.0%) and is falling. Without endemic malaria from Africa, the sickle cell mutation is purely disadvantageous and will tend to be selected out of the affected population. Another factor limiting the spread of sickle-cell genes in North America is the absence of cultural proclivities to polygamy.



Sickle-cell disease is inherited in the autosomal recessive pattern

Inheritance

Sickle-cell conditions are inherited from parents in much the same way as blood type, hair colour and texture, eye colour, and other physical traits. The types of haemoglobin a person makes in the red blood cells depend on what haemoglobin genes are inherited from his parents. If one parent has sickle-cell anaemia (SS) and the other has sickle-cell trait (AS), there is a 50% chance of a child's having sickle-cell disease (SS) and a 50% chance of a child's having sickle-cell trait (AS). When both parents have sickle-cell trait (AS), a child has a 25% chance (1 of 4) of sickle-cell disease (SS), as shown in the diagram.

Epidemiology

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, India and the Middle-East. Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle cell disease has now overtaken more familiar genetic conditions such as hemophilia and cystic fibrosis.

Africa

Three quarters of sickle-cell cases occur in Africa. A recent WHO report estimated that around 2% of newborns in Nigeria were affected by sickle cell anaemia, giving a total of 150,000 affected children born every year in Nigeria alone. The carrier frequency ranges between 10% and 40% across equatorial Africa, decreasing to 1–2% on the north African coast and <1% in South Africa.

Europe

France

In Europe, the highest prevalence of the disease has been observed in France. As a result of population growth in African-Caribbean regions of overseas France, and now immigration essentially from North and sub-Saharan Africa to mainland France, sickle cell disease has become a major health problem in France. SCD has become the most common genetic disease in this country, with an overall birth prevalence of 1/2,415 in mainland France, ahead of phenylketonuria (1/10,862), congenital hypothyroidism (1/3,132), congenital adrenal hyperplasia (1/19,008) and cystic fibrosis (1/5,014) for the same reference period. In 2007, 28.45% of all newborns in mainland France in 2007 had at least one parent originated from a region defined "at risk" (mainly Africa and Overseas departments and territories of France) and were screened for SCD. The Paris metropolitan district (Île-de-France) is the region that accounts for the largest number of at-risk. Indeed, nearly 56% of all newborns in this area in 2007 had at least one parent originated from a region defined as "at-risk" and were screened for SCD. The second largest number of at-risk is in Provence-Alpes-Côte d'Azur at nearly 42% and the lowest number is in Brittany at 4.40%.

Region	% of newborns at risk for SCD based on ethnic origin among all newborns in France (2007)
Île-de-France	55.68
Provence-Alpes-Côte d'Azur	41.91
Languedoc-Roussillon	34.78
Alsace	29.29
Midi-Pyrénées	27.77
Rhône-Alpes-Pays de Savoie	27.67
Picardie	19.92
Franche-Comté	17.90
Bourgogne	17.01
Lorraine	16.14
Champagne-Ardenne	15.36
Limousin	15.16
Nord-Pas-de-Calais	14.27
Centre Val de Loire	14.03
Auvergne	12.84
Aquitaine	12.29
Normandie	11.61
Pays de la Loire/Poitou-Charentes	11.20
Bretagne	4.40
Metropolitan France	28.45

United Kingdom

In United Kingdom, more than 200 babies are born annually with SCD.

Middle East

About 6,000 children are born annually with SCD, at least 50% of these in Saudi Arabia.

India

Sickle cell disease is prevalent in many parts of India, where the prevalence has ranged from 9.4 to 22.2% in endemic areas.

Diagnosis

In HbSS, the full blood count reveals haemoglobin levels in the range of 6–8 g/dL with a high reticulocyte count (as the bone marrow compensates for the destruction of sickle cells by producing more red blood cells). In other forms of sickle-cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies).

Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle haemoglobin can also be demonstrated with the "sickle solubility test". A mixture of haemoglobin S (Hb S) in a reducing solution (such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution.

Abnormal haemoglobin forms can be detected on haemoglobin electrophoresis, a form of gel electrophoresis on which the various types of haemoglobin move at varying speeds. Sickle-cell haemoglobin (HgbS) and haemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography (HPLC). Genetic testing is rarely performed, as other investigations are highly specific for HbS and HbC.

An acute sickle-cell crisis is often precipitated by infection. Therefore, a urinalysis to detect an occult urinary tract infection, and chest X-ray to look for occult pneumonia should be routinely performed.

People who are known carriers of the disease often undergo genetic counselling before they have a child. A test to see if an unborn child has the disease takes either a blood sample from the fetus or a sample of amniotic fluid. Since taking a blood sample from a fetus has greater risks, the latter test is usually used.

After the mutation responsible for this disease was discovered in 1979, the U.S. Air Force required black applicants to test for the mutation. It dismissed 143 applicants because they were carriers, even though none of them had the condition. It eventually withdrew the requirement, but only after a trainee filed a lawsuit.

Management

Folic acid and penicillin

Children born with sickle-cell disease will undergo close observation by the paediatrician and will require management by a haematologist to assure they remain healthy. These

patients will take a 1 mg dose of folic acid daily for life. From birth to five years of age, they will also have to take penicillin daily due to the immature immune system that makes them more prone to early childhood illnesses.

Painful (vaso-occlusive) crisis

Most people with sickle-cell disease have intensely painful episodes called vaso-occlusive crises. The frequency, severity, and duration of these crises, however, vary tremendously. Painful crises are treated symptomatically with analgesics; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia (PCA) devices are commonly used in this setting. Diphenhydramine is also an effective agent that is frequently prescribed by doctors in order to help control any itching associated with the use of opioids.

Acute chest crisis

Management is similar to vaso-occlusive crisis, with the addition of antibiotics (usually a quinolone or macrolide, since wall-deficient ["atypical"] bacteria are thought to contribute to the syndrome), oxygen supplementation for hypoxia, and close observation. Should the pulmonary infiltrate worsen or the oxygen requirements increase, simple blood transfusion or exchange transfusion is indicated. The latter involves the exchange of a significant portion of the patients red cell mass for normal red cells, which decreases the percent of haemoglobin S in the patient's blood.

Hydroxyurea

The first approved drug for the causative treatment of sickle-cell anaemia, hydroxyurea, was shown to decrease the number and severity of attacks in a study in 1995 (Charache *et al.*) and shown to possibly increase survival time in a study in 2003 (Steinberg *et al.*). This is achieved, in part, by reactivating fetal haemoglobin production in place of the haemoglobin S that causes sickle-cell anaemia. Hydroxyurea had previously been used as a chemotherapy agent, and there is some concern that long-term use may be harmful, but this risk has been shown to be either absent or very small and it is likely that the benefits outweigh the risks.

Bone marrow transplants

Bone marrow transplants have proven to be effective in children.

History

This collection of clinical findings was unknown until the explanation of the sickle cells in 1910 by the Chicago cardiologist and professor of medicine James B. Herrick (1861–1954), whose intern Ernest Edward Irons (1877–1959) found "peculiar elongated and

sickle-shaped" cells in the blood of Walter Clement Noel, a 20-year-old first-year dental student from Grenada, after Noel was admitted to the Chicago Presbyterian Hospital in December 1904 suffering from anaemia.

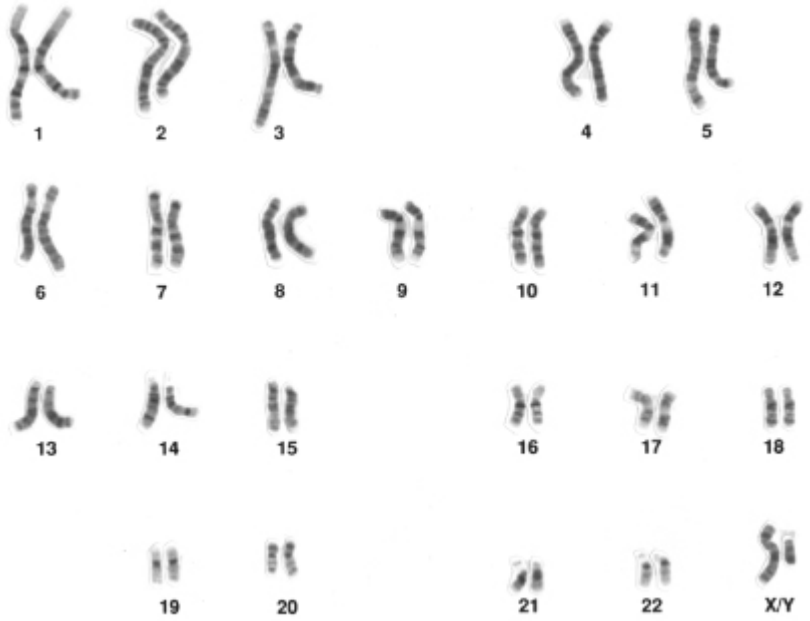
Noel was readmitted several times over the next three years for "muscular rheumatism" and "bilious attacks". Noel completed his studies and returned to the capital of Grenada (St. George's) to practice dentistry. He died of pneumonia in 1916 and is buried in the Catholic cemetery at Sauteurs in the north of Grenada. Herrick's published account included illustrations, but the earliest available slide showing sickle cells is that of a 1918 autopsy from a soldier with sickle trait, initially reviewed only 92 years later.

The disease was named "sickle-cell anaemia" by Verne Mason in 1922, then a medical resident at Johns Hopkins Hospital. However, some elements of the disease had been recognized earlier: A paper in the *Southern Journal of Medical Pharmacology* in 1846 described the absence of a spleen in the autopsy of a runaway slave. The African medical literature reported this condition in the 1870s, when it was known locally as *ogbanjes* ("children who come and go") because of the very high infant mortality rate caused by this condition. A history of the condition tracked reports back to 1670 in one Ghanaian family. Also, the practice of using tar soap to cover blemishes caused by sickle-cell sores was prevalent in the black community.

Linus Pauling and colleagues were the first, in 1949, to demonstrate that sickle-cell disease occurs as a result of an abnormality in the haemoglobin molecule. This was the first time a genetic disease was linked to a mutation of a specific protein, a milestone in the history of molecular biology, and it was published in their paper "Sickle Cell Anemia, a Molecular Disease".

Chapter 7

Full Genome Sequencing



An image of the 46 chromosomes, making up the diploid genome of human male. (The mitochondrial chromosome is not shown.)

Full genome sequencing (FGS), also known as **whole genome sequencing**, **complete genome sequencing**, or **entire genome sequencing**, is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. This entails sequencing all of an organism's chromosomal DNA as well as DNA contained in the mitochondria and for plants the chloroplast as well. Almost any biological sample—even a very small amount of DNA or ancient DNA—can provide the genetic material necessary for full genome sequencing. Such samples may include saliva, epithelial cells, bone marrow, hair (as long as the hair contains a hair follicle), seeds, plant leaves, or anything else that has DNA-containing cells. Because the sequence data that is produced can be quite large (for example, there are approximately six billion base pairs in each human diploid genome), genomic data is stored electronically and requires a large amount of computing power and storage capacity. Full genome sequencing would have

been nearly impossible before the advent of the microprocessor, computers, and the Information Age.

Full genome sequencing should thus not be confused with DNA profiling. The latter only determines the likelihood that genetic material came from a particular individual or group and does not contain additional information on genetic relationships, origin or susceptibility to specific diseases. It is also distinct from SNP genotyping which covers less than 0.1% of the genome. Almost all truly complete genomes are of microbes; the term "full genome" is thus sometimes used loosely to mean "greater than 95%".

In general, knowing the complete DNA sequence of an individual's genome does not, on its own, provide useful clinical information, but this may change over time as a large number of scientific studies continue to be published detailing clear associations between specific genetic variants and disease.

The first nearly complete human genomes sequenced were J. Craig Venter's (Caucasian at 7.5-fold average coverage), James Watson's (Caucasian male at 7.4-fold), a Han Chinese (YH at 36-fold), a Yoruban from Nigeria (at 30-fold), a female leukemia patient (at 33 and 14-fold coverage for tumor and normal tissues), and Seong-Jin Kim (Korean at 29-fold). Other full genomes have been sequenced but not published, and as of June 2009, commercialization of full genome sequencing is in an early stage and growing rapidly.

Older techniques

An ABI PRISM 3100 Genetic Analyzer. Such capillary sequencers automated the early efforts of sequencing genomes.

Sequencing of nearly an entire human genome was first accomplished in 2000 partly through the use of shotgun sequencing technology. While full genome shotgun sequencing for small (4000–7000 base pair) genomes was already in use in 1979, broader application benefited from pairwise end sequencing, known colloquially as *double-barrel shotgun sequencing*. As sequencing projects began to take on longer and more complicated genomes, multiple groups began to realize that useful information could be obtained by sequencing both ends of a fragment of DNA. Although sequencing both ends of the same fragment and keeping track of the paired data was more cumbersome than sequencing a single end of two distinct fragments, the knowledge that the two sequences were oriented in opposite directions and were about the length of a fragment apart from each other was valuable in reconstructing the sequence of the original target fragment.

The first published description of the use of paired ends was in 1990 as part of the sequencing of the human HPRT locus, although the use of paired ends was limited to closing gaps after the application of a traditional shotgun sequencing approach. The first theoretical description of a pure pairwise end sequencing strategy, assuming fragments of constant length, was in 1991. In 1995 Roach et al. introduced the innovation of using fragments of varying sizes, and demonstrated that a pure pairwise end-sequencing strategy would be possible on large targets. The strategy was subsequently adopted by The Institute for Genomic Research (TIGR) to sequence the entire genome of the bacterium *Haemophilus influenzae* in 1995, and then by Celera Genomics to sequence the entire fruit fly genome in 2000, and subsequently the entire human genome. Applied Biosystems, now called Life Technologies, manufactured the automated capillary sequencers utilized by both Celera Genomics and The Human Genome Project.

While capillary sequencing was the first approach utilized to successfully sequence a nearly full human genome, it is too expensive and requires too long of a turn-around-time to be utilized for commercial purposes. Because of this, shotgun sequencing technology, even though it is still relatively 'new', since 2005 is being displaced by technologies like pyrosequencing, SMRT sequencing, and nanopore technology.

New techniques

One possible way to accomplish the cost-effective high-throughput sequencing necessary to accomplish full genome sequencing is by using Nanopore technology, which is a patented technology held by Harvard University and Oxford Nanopore Technologies and licensed to biotechnology companies. To facilitate their full genome sequencing initiatives, Illumina licensed nanopore sequencing technology from Oxford Nanopore Technologies and Sequenom licensed the technology from Harvard University. Another possible way to accomplish cost-effective high-throughput sequencing is by utilizing fluorophore technology. Pacific Biosciences is currently using this approach in their SMRT (single molecule real time) DNA sequencing technology. Complete Genomics has developed DNA Nanoball (DNB) technology that arranges DNA on self-assembling arrays. Complete Genomics' sequencing technology combines its DNB arrays with its proprietary cPAL™ read technology. Pyrosequencing is a method of DNA sequencing based on the sequencing by synthesis principle. The technique was developed by Pål

Nyrén and his student Mostafa Ronaghi at the Royal Institute of Technology in Stockholm in 1996, and is currently being used by 454 Life Sciences in their effort to deliver an affordable, fast and highly accurate full genome sequencing platform.

Race to commercialization

In October 2006, the X Prize Foundation, working in collaboration with the J. Craig Venter Science Foundation, established the Archon X Prize for Genomics, intending to award US$10 million to "the first Team that can build a device and use it to sequence 100 human genomes within 10 days or less, with an accuracy of no more than one error in every 100,000 bases sequenced, with sequences accurately covering at least 98% of the genome, and at a recurring cost of no more than US$10,000 per genome." However, higher accuracy rates (or confirmatory methods) are desirable for some clinical applications. An error rate of 1 in 100,000 bases, out of a total of six billion bases in the human diploid genome, would mean about 60,000 errors per genome, which is a significant number of false positives and negatives. For the latter it is not known where the errors occur. The error rates required for widespread clinical use, such as Predictive Medicine is currently set by over 1400 clinical single gene sequencing tests (for example, errors in BRCA1 gene for breast cancer risk analysis). As of October 2010, the Archon X Prize for Genomics remains unclaimed.

In 2007, Applied Biosystems started selling a new type of sequencer called SOLiD System in 2008. Current SOLiD chemistries enable users to sequence 60 gigabases per run.

In 2008 and 2009, both public and private companies have emerged that are now in a competitive race to be the first mover to provide a full genome sequencing platform that is commercially robust for both research and clinical use, including Illumina, Knome, Sequenom, 454 Life Sciences, Pacific Biosciences, Complete Genomics, Intelligent Bio-Systems, Genome Corp., ION Torrent Systems, and Helicos Biosciences. These companies are heavily financed and backed by venture capitalists, hedge funds, investment banks and, in the case of Illumina, Sequenom and 454, heavy re-investment of revenue into research and development, mergers and acquisitions, and licensing initiatives.

In the race to commercialize full genome sequencing, companies have made claims about being able to offer a service at a specific time for a specific price that have turned out to not be true. Intelligent Bio-Systems stated in November 2007 that by the end of 2008 they would release a platform capable of a providing a US$5,000 full genome sequence, but, as of May 2010, no such platform has been released.

Pacific Biosciences stated that they would start selling their full genome sequencers in early 2010. While they did not disclose the cost to sequence a single genome, they did state they may not release their second-generation machine capable of a US$1,000 genome until 2013. Complete Genomics, however, stated that they will be able to provide a US$5,000 full genome sequencing service by the summer of 2009. Complete Genomics

demonstrated in a peer-reviewed paper that was published online in *Science* in Nov. 2009 that it could sequence a human genome for a consumables cost of approximately US$1700. The accuracy, precision, and reproducibility of both Pacific Biosciences and Complete Genomics technology, however, is still unknown. Complete Genomics clearly demonstrated the accuracy, precision and reproducibility of its sequencing technology in the *Science* paper referenced above.

Knome provides full genome (98% genome) sequencing services for US$39,500 for whole genome sequencing and interpretation for consumers. It's US$29,500 for whole genome sequencing and analysis for researchers depending on their requirements.

As of January 2009, there are no indications that any of these companies have been hindered by the global recession. And thus, the race appears to be proceeding forward at full speed.

In early February 2009, Complete Genomics released a full sequence of a human genome that was sequenced using their service. The data indicates that Complete Genomics' full genome sequencing service accuracy is just under 99.999%, meaning that just one in every one hundred thousand variants was called incorrectly. This means that their full sequence of the human genome will contain approximately 80,000–100,000 false positive errors in each genome. However, this accuracy rate was based on Complete Genomics' sequence that was completed utilizing a 90× depth of coverage (each base in the genome was sequenced 90 times) while their commercialized sequence is reported to be only 40×, so the accuracy may be substantially lower unless they can find some way to improve it before their first service release planned for the summer 2009. This accuracy rate may be acceptable for research purposes, and clinical use would require confirmation by other methods of any reportable alleles. Complete Genomics announced in Dec. 2010 that for the last 500 complete human genomes that it had sequenced, an average of over 98 percent of the genome was read at 10-fold or greater coverage. In addition, its software made high confidence calls of an average of over 95 percent of the genome and over 94 percent of the exome.

In March 2009, it was announced that Complete Genomics has signed a deal with the Broad Institute to sequence cancer patients' genomes and will be sequencing five full genomes to start. In April 2009, Complete Genomics announced that it plans to sequence 1,000 full genomes between June 2009 and the end of the year and that they plan to be able to sequence one million full genomes *per year* by 2013. Complete Genomics sequenced 50 genomes in 2009. Since then, it has significantly increased the throughout in its genome sequencing center and was able to sequence and analyze 300 complete human genomes in Q3 2010. Complete Genomics plans to officially launch in June 2009, although it is unknown if their lab will have received CLIA-certification by that time. Complete Genomics announced its R&D human genome sequencing service in October 2008 and its commercial sequencing service in May 2010. The company does not produce clinical data and as such its genome center does not require CLIA certification.

In June 2009, Illumina announced that they were launching their own Personal Full Genome Sequencing Service at a depth of 30× for US$48,000 per genome. This is still expensive for widespread consumer use, but the price may decrease substantially over the next few years as they realize economies of scale and given the competition with other companies such as Complete Genomics. Jay Flatley, Illumina's President and CEO, stated that "during the next five years, perhaps markedly sooner," the price point for full genome sequencing will fall from US$48,000 to under US$1,000. Illumina has already signed agreements to supply full genome sequencing services to multiple direct-to-consumer personal genomics companies.

In August 2009, the founder of Helicos Biosciences, Dr. Stephen Quake, stated that using the company's Heliscope Single Molecule Sequencer he sequenced his own full genome for less than US$50,000. He stated that he expects the cost to decrease to the US$1,000 range within the next two to three years.

In August 2009, Pacific Biosciences secured an additional US$68 million in new financing, bringing their total capitalization to US$188 million. Pacific Biosciences said they are going to use this additional investment in order to prepare for the upcoming launch of their full genome sequencing service in 2010. Complete Genomics followed by securing another US$45 million in a fourth round venture funding during the same month. Complete Genomics has also made the claim that it will sequence 10,000 full genomes by the end of 2010. Since then, it has significantly increased the throughout in its genome sequencing center and was able to sequence and analyze 300 complete human genomes in Q3 2010.

GE Global Research is also part of this race to commercialize full genome sequencing as they have been working on creating a service that will deliver a full genome for US$1,000 or less.

In September 2009, the President of Halcyon Molecular announced that they will be able to provide full genome sequencing in under 10 minutes for less than US$100 per genome. This is, to date, the most ambitious promise of any full genome sequencing company.

In the same month, Complete Genomics announced that it had sequenced, analyzed and delivered data from 14 human genomes to customers since March 2009.

In October 2009, IBM announced that they were also in the heated race to provide full genome sequencing for under US$1,000, with their ultimate goal being able to provide their service for US$100 per genome. IBM's full genome sequencing technology, which uses nanopores, is known as the "DNA Transistor".

In November 2009, Complete Genomics published a peer-reviewed paper in *Science* demonstrating its ability to sequence a complete human genome for US$1,700. If true, this would mean the cost of full genome sequencing has come down exponentially within just a single year from around US$100,000 to US$50,000 and now to US$1,700. This consumables cost was clearly detailed in the *Science* paper. However, Complete

Genomics has previously released statements that it was unable to follow through on. For example, the company stated it would officially launch and release its service during the "summer of 2009", provide a "US$5,000" full genome sequencing service by the "summer of 2009", and "sequence 1,000 genomes between June 2009 and the end of 2009" – all of which, as of November 2009, have not yet occurred. Complete Genomics launched its R&D human genome sequencing service in October 2008 and its commercial service in May 2010. The company sequenced 50 genomes in 2009. Since then, it has significantly increased the throughput of its genome sequencing factory and was able to sequence and analyze 300 genomes in Q3 2010.

Also in November 2009, Complete Genomics announced that it was beginning a large-scale human genome sequencing study of Huntington's disease (up to 100 genomes) with the Institute for Systems Biology.

In March 2010, Pacific Biosciences said they have raised more than US$256 million in venture capital money and that they will be shipping their first ten full genome sequencing machines by the end of 2010. The company reported that the market initially will be researchers and academic institutions and then will rapidly turn into clinical applications that will be applicable to every single person in the world. Pacific Biosciences also stated that their second-generation machine, which is scheduled for release in 2015, will be capable of providing a full genome sequence for a person in just 15 minutes for less than US$100. Several other technologies have similar goals. Meanwhile, full genome sequencing might revolutionize medicine at even current prices by providing a clinician with a full genome for each one of his or her patients. However, some critics have stated that even if they are supplied with a full genome sequence of a patient, they would not know how to analyze or make use of that data. Since then, new resources have begun to address this.

Also in March 2010, Complete Genomics' customers began publishing papers describing research breakthroughs that they have made using data it has provided. Examples included the Institute for Systems Biology's project to sequence a family of four and verify the gene responsible for Miller Syndrome, a rare craniofacial disorder[x] and Genentech's work to sequence and compare a patient's primary lung tumor and adjacent normal tissue[y].

In June 2010, Illumina lowered the cost of its individual sequencing service to US$19,500 from US$48,000. The company is offering a discounted price of US$9,500 for people with serious medical conditions who could potentially benefit from having their genomes decoded.

Complete Genomics charges approximately US$10,000 to sequence a complete human genome and offers discounts for large orders. This service includes sample quality control, library preparation, sequencing, mapping, assembly and data analysis.

Helicos Biosciences, Pacific Biosciences, Complete Genomics, Illumina, Sequenom, ION Torrent Systems, Halcyon Molecular, IBM, and GE Global appear to all be going head to head in the race to commercialize full genome sequencing.

Disruptive technology

Full genome sequencing provides information on a genome that is orders of magnitude larger than that provided by the current leader in sequencing technology, DNA arrays. For humans, DNA arrays currently provides genotypic information on up to one million genetic variants, while full genome sequencing will provide information on all six billion bases in the human genome, or 3,000 times more data. Because of this, full genome sequencing is considered disruptive to the DNA array markets as the accuracy of both range from 99.98% to 99.999% (in non-repetitive DNA regions) and their consumables cost of US$5000 per 6 billion base pairs is competitive (for some applications) with DNA arrays (US$500 per 1 million basepairs). Agilent, another established DNA array manufacturer, is working on targeted (selective region) genome sequencing technologies. It is thought that Affymetrix, the pioneer of array technology in the 1990s, has fallen behind due to significant corporate and stock turbulence and is currently not working on any known full genome sequencing approach. It is unknown what will happen to the DNA array market once full genome sequencing becomes commercially widespread, especially as companies and laboratories providing this disruptive technology start to realize economies of scale. It is postulated, however, that this new technology may significantly diminish the total market size for arrays and any other sequencing technology once it becomes commonplace for individuals and newborns to have their full genomes sequenced.

Sequencing versus analysis

Full genome sequencing provides raw data on all six billion letters in an individual's DNA. However, it does not provide an analysis of what that data means or how that data can be utilized in various clinical applications, such as in medicine to help prevent disease. As of 2010 the companies that are working on providing full genome sequencing provide clinical CLIA certified data (Illumina) and analytical services for the interpretation of the full genome data (Knome). Nevertheless there is plenty of room for researchers or companies to improve such analyses and make it useful to physicians and patients.

Societal impact

Inexpensive, time-efficient full genome sequencing will be a major accomplishment not only for the field of Genomics, but for the entire human civilization because, for the first time, individuals will be able to have their entire genome sequenced. Utilizing this information, it is speculated that health care professionals, such as physicians and genetic counselors, will eventually be able to use genomic information to predict what diseases a person may get in the future and attempt to either minimize the impact of that disease or avoid it altogether through the implementation of personalized, preventive medicine. Full

genome sequencing will allow health care professionals to analyze the entire human genome of an individual and therefore detect all disease-related genetic variants, regardless of the genetic variant's prevalence or frequency. This will enable the rapidly emerging medical fields of Predictive Medicine and Personalized Medicine and will mark a significant leap forward for the clinical genetic revolution. Full genome sequencing is clearly of great importance for research into the basis of genetic disease. However, it should be recognized that despite advancements in genome sequencing technology, incomplete understanding of the significance of individual variants or combinations of variants will limit the widespread usefulness of full genome sequencing in medicine until its clinical utility can be demonstrated.

Illumina's CEO, Jay Flatley, stated in February 2009 that "A complete DNA read-out for every newborn will be technically feasible and affordable in less than five years, promising a revolution in healthcare" and that "by 2019 it will have become routine to map infants' genes when they are born." This potential use of genome sequencing is highly controversial, as it runs counter to established ethical norms for predictive genetic testing of asymptomatic minors that have been well established in the fields of medical genetics and genetic counseling. The traditional guidelines for genetic testing have been developed over the course of several decades since it first became possible to test for genetic markers associated with disease, prior to the advent of cost-effective, comprehensive genetic screening. It is established that norms, such as in the sciences and the field of genetics, are subject to change and evolve over time. It is unknown whether traditional norms practiced in medical genetics today will be altered by new technological advancements such as full genome sequencing.

Today, parents have the legal authority to obtain testing of any kind for their children. Currently available newborn screening for childhood diseases allows detection of rare disorders that can be prevented or better treated by early detection and intervention. Specific genetic tests are also available to determine an etiology when a child's symptoms appear to have a genetic basis. Full genome sequencing, however, reveals a large amount of information (such as carrier status for autosomal recessive disorders, genetic risk factors for complex adult-onset diseases, and other predictive medical and non-medical information) that is currently not completely understood, not clinically useful during childhood, and may not necessarily be wanted by the individual upon reaching adulthood. Despite the theoretical (and currently unproven) benefits of predicting disease risk in childhood, genetic testing also introduces potential harms (such as discovery of non-paternity, genetic discrimination, and psychological impacts). The established ethical guidelines for predictive genetic testing of asymptomatic minors thus has more to do with protecting this vulnerable population and preserving the individual's privacy and autonomy to know or not to know their genetic information, than with the technology that makes this possible. While parents may have legal authority to obtain such testing, the mainstream opinion of professional medical genetics societies is that presymptomatic testing should be offered to minors only when they are competent to understand the relevancy of genetic screening so as to allow them to participate in the decision about whether or not it is appropriate for them.

In the book *Outsmart Your Genes*, by Brandon Colby, MD, Dr. Colby discusses the rapidly emerging field of predictive medicine and comprehensive genetic testing. The book appears to be written for the lay-person and provides specific examples of how genetic testing may be utilized to lower a person's risk of a disease they are found to be genetically predisposed to, such as cancer, Alzheimer's disease and other neurological diseases such as stroke, heart disease, and autoimmune diseases, as well as a number of genetic testing pop-culture references such as to Sergei Grinkov, David Bloom, and the NFL.

Chapter 8

Genetic Testing

Genetic Testing : Gene tests (also called DNA-based tests), the newest and most sophisticated of the techniques used to test for genetic disorders, involve direct examination of the DNA molecule itself. Other genetic tests include biochemical tests for such gene products as enzymes and other proteins and for microscopic examination of stained or fluorescent chromosomes. Genetic tests are used for several reasons, including:

- carrier screening, which involves identifying unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to be expressed
- preimplantation genetic diagnosis
- prenatal diagnostic testing
- newborn screening
- presymptomatic testing for predicting adult-onset disorders such as Huntington's disease
- presymptomatic testing for estimating the risk of developing adult-onset cancers and Alzheimer's disease
- confirmational diagnosis of a symptomatic individual
- forensic/identity testing

Genetic testing allows the genetic diagnosis of vulnerabilities to inherited diseases, and can also be used to determine a child's paternity (genetic father) or a person's ancestry. Normally, every person carries two copies of every gene (with the exception of genes related to sex-linked traits, which are only inherited from the mother by males), one inherited from their mother, one inherited from their father. The human genome is believed to contain around 20,000 - 25,000 genes. In addition to studying chromosomes to the level of individual genes, genetic testing in a broader sense includes biochemical tests for the possible presence of genetic diseases, or mutant forms of genes associated with increased risk of developing genetic disorders. Genetic testing identifies changes in chromosomes, genes, or proteins. Most of the time, testing is used to find changes that are associated with inherited disorders. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. Several hundred genetic tests are currently in use, and more are being developed.

Since genetic testing may open up ethical or psychological problems, genetic testing is often accompanied by genetic counseling.

Types

Genetic testing is "the analysis of, chromosomes (DNA), proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes." It can provide information about a person's genes and chromosomes throughout life. Available types of testing include:

- Newborn screening: Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. The routine testing of infants for certain disorders is the most widespread use of genetic testing—millions of babies are tested each year in the United States. All states currently test infants for phenylketonuria (a genetic disorder that causes mental illness if left untreated) and congenital hypothyroidism (a disorder of the thyroid gland).
- Diagnostic testing: Diagnostic testing is used to diagnose or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical mutations and symptoms. Diagnostic testing can be performed at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disease.
- Carrier testing: Carrier testing is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.
- Prenatal testing: Prenatal testing is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them decide whether to abort the pregnancy. It cannot identify all possible inherited disorders and birth defects, however.
- Preimplantation genetic diagnosis: Genetic testing procedures that are performed on human embryos prior to the implantation as part of an in vitro fertilization procedure.
- Predictive and presymptomatic testing: Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that increase a person's chances of developing disorders with a genetic basis, such as certain types of cancer. For example, an individual with a mutation in *BRCA1* has a 65% cumulative risk of breast cancer. Presymptomatic testing can determine

whether a person will develop a genetic disorder, such as hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder and help with making decisions about medical care.

- Forensic testing: Forensic testing uses DNA sequences to identify an individual for legal purposes. Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).
- Parental testing: This type of genetic test uses special DNA markers to identify the same or similar inheritance patterns between related individuals. Based on the fact that we all inherit half of our DNA from the father, and half from the mother, DNA scientists test individuals to find the match of DNA sequences at some highly differential markers to draw the conclusion of relatedness.
- Research testing: Research testing includes finding unknown genes, learning how genes work and advancing our understanding of genetic conditions. The results of testing done as part of a research study are usually not available to patients or their healthcare providers.
- Pharmacogenomics: type of genetic testing that determines the influence of genetic variation on drug response.

Medical procedure

Genetic testing is often done as part of a genetic consultation and as of mid-2008 there were more than 1,200 clinically applicable genetic tests available. Once a person decides to proceed with genetic testing, a medical geneticist, genetic counselor, primary care doctor, or specialist can order the test after obtaining informed consent.

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a fetus during pregnancy), or other tissue. For example, a medical procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. Alternatively, a small amount of saline mouthwash may be swished in the mouth to collect the cells. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorder. The laboratory reports the test results in writing to a person's doctor or genetic counselor.

Routine newborn screening tests are done on a small blood sample obtained by pricking the baby's heel with a lancet.

Interpreting results

The results of genetic tests are not always straightforward, which often makes them challenging to interpret and explain. When interpreting test results, healthcare

professionals consider a person's medical history, family history, and the type of genetic test that was done.

A positive test result means that the laboratory found a change in a particular gene, chromosome, or protein of interest. Depending on the purpose of the test, this result may confirm a diagnosis, indicate that a person is a carrier of a particular genetic mutation, identify an increased risk of developing a disease (such as cancer) in the future, or suggest a need for further testing. Because family members have some genetic material in common, a positive test result may also have implications for certain blood relatives of the person undergoing testing. It is important to note that a positive result of a predictive or presymptomatic genetic test usually cannot establish the exact risk of developing a disorder. Also, health professionals typically cannot use a positive test result to predict the course or severity of a condition.

A negative test result means that the laboratory did not find a dangerous copy of the gene, chromosome, or protein under consideration. This result can indicate that a person is not affected by a particular disorder, is not a carrier of a specific genetic mutation, or does not have an increased risk of developing a certain disease. It is possible, however, that the test missed a disease-causing genetic alteration because many tests cannot detect all genetic changes that can cause a particular disorder. Further testing may be required to confirm a negative result.

In some cases, a negative result might not give any useful information. This type of result is called uninformative, indeterminate, inconclusive, or ambiguous. Uninformative test results sometimes occur because everyone has common, natural variations in their DNA, called polymorphisms, that do not affect health. If a genetic test finds a change in DNA that has not been associated with a disorder in other people, it can be difficult to tell whether it is a natural polymorphism or a disease-causing mutation. An uninformative result cannot confirm or rule out a specific diagnosis, and it cannot indicate whether a person has an increased risk of developing a disorder. In some cases, testing other affected and unaffected family members can help clarify this type of result.

Risks and limitations

The physical risks associated with most genetic tests are very small, particularly for those tests that require only a blood sample or buccal smear (a procedure that samples cells from the inside surface of the cheek). The procedures used for prenatal testing carry a small but real risk of losing the pregnancy (miscarriage) because they require a sample of amniotic fluid or tissue from around the fetus.

Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. People may feel angry, depressed, anxious, or guilty about their results. In some cases, genetic testing creates tension within a family because the results can reveal information about other family members in addition to the person who is tested. The possibility of genetic discrimination in employment or insurance is also a concern. Some individuals avoid genetic testing out of fear it will

affect their ability to purchase insurance or find a job. Health insurers do not currently require applicants for coverage to undergo genetic testing, and when insurers encounter genetic information, it is subject to the same confidentiality protections as any other sensitive health information. In the United States, the use of genetic information is governed by the Genetic Information Nondiscrimination Act (GINA).

Genetic testing can provide only limited information about an inherited condition. The test often can't determine if a person will show symptoms of a disorder, how severe the symptoms will be, or whether the disorder will progress over time. Another major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed.

A genetics professional can explain in detail the benefits, risks, and limitations of a particular test. It is important that any person who is considering genetic testing understand and weigh these factors before making a decision.

Direct-to-Consumer genetic testing

Direct-to-Consumer (DTC) genetic testing is a type of genetic test that is accessible directly to the consumer without having to go through a health care professional. Usually, to obtain a genetic test, health care professionals such as doctors acquire the permission of the patient and order the desired test. DTC genetic tests, however, allow consumers to bypass this process and order one themselves. There are a variety of DTC tests, ranging from testing for breast cancer alleles to mutations linked to cystic fibrosis. Benefits of DTC testing are the accessibility of tests to consumers, promotion of proactive healthcare and the privacy of genetic information. Possible additional risks of DTC testing are the lack of governmental regulation and the potential misinterpretation of genetic information.

Controversy

DTC genetic testing has been controversial due to outspoken opposition within the scientific community. Critics of DTC testing argue against the risks involved, the unregulated advertising and marketing claims, and the overall lack of governmental oversight.

DTC testing involves many of the same risks associated with any genetic test. One of the more obvious and dangerous of these is the possibility of severe misreading of test results. Without professional guidance, consumers can potentially misinterpret genetic information, causing them to be deluded about their personal health.

Some advertising for direct-to-consumer genetic testing has been criticized as conveying an exaggerated and inaccurate message about the connection between genetic information and disease risk, utilizing emotions as a selling factor. An advertisement for a BRCA-predictive genetic test for breast cancer stated: “There is no stronger antidote for fear than information.”

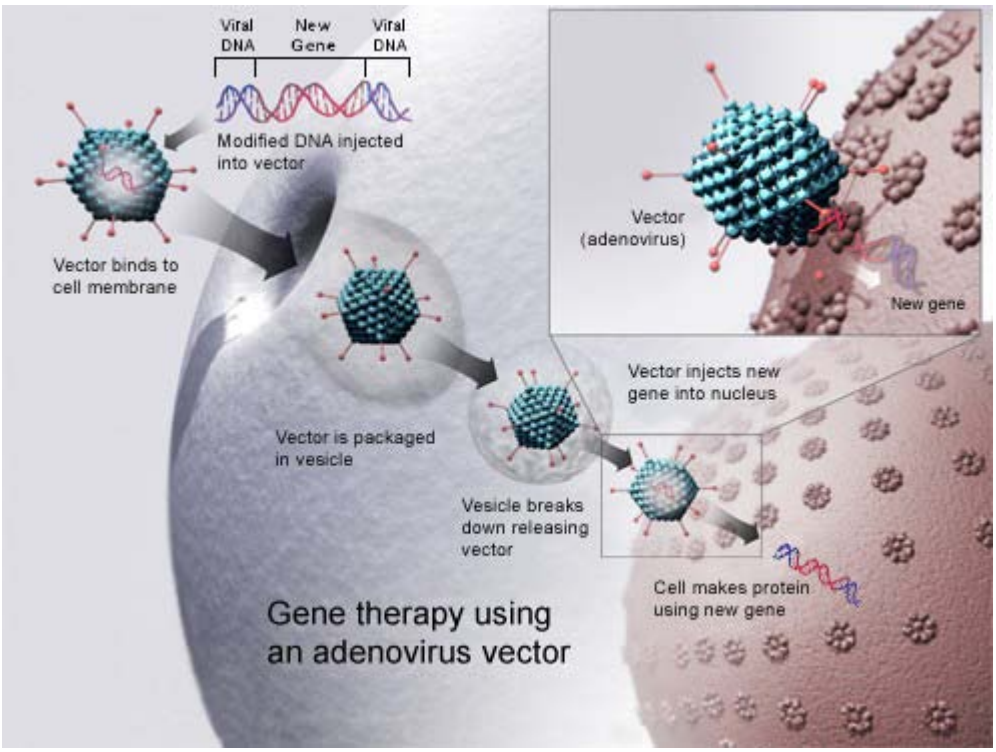
Government regulation in the United States

Currently, the U.S. has no strong Federal regulation moderating the DTC market. Though there are several hundred tests available, only a handful are approved by the Food and Drug Administration (FDA); these are sold as at-home test kits, and are therefore considered "medical devices" over which the FDA may assert jurisdiction. Other types of DTC tests require customers to mail in DNA samples for testing; it is difficult for the FDA to exercise jurisdiction over these types of tests, because the actual testing is completed in the laboratories of providers. As of 2007, the FDA had not yet officially substantiated with scientific evidence the claimed accuracy of the majority of direct-to-consumer genetic tests.

With regard to genetic testing and information in general, legislation in the United States called the Genetic Information Nondiscrimination Act prohibits group health plans and health insurers from denying coverage to a healthy individual or charging that person higher premiums based solely on a genetic predisposition to developing a disease in the future. The legislation also bars employers from using individuals' genetic information when making hiring, firing, job placement, or promotion decisions. The legislation, the first of its kind in the U.S., was passed by the United States Senate on April 24, 2008, on a vote of 95-0, and was signed into law by President George W. Bush on May 21, 2008. It went into effect on November 21, 2009.

Chapter 9

Gene Therapy



Gene therapy

Gene therapy is the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat disease. It is a technique for correcting defective genes that are responsible for disease development. The most common form of gene therapy involves the insertion of functional genes into an unspecified genomic location in order to replace a mutated gene, but other forms involve directly correcting the mutation or modifying normal gene that enables a viral infection. Although the technology is still in its infancy, it has been used with some success.

Gene therapy using an Adenovirus vector. A new gene is inserted into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

Approach

Scientists have taken the logical step of trying to introduce genes directly into human cells, focusing on diseases caused by single-gene defects, such as cystic fibrosis, haemophilia, muscular dystrophy and sickle cell anemia. However, this has proven more difficult than modifying bacteria, primarily because of the problems involved in carrying large sections of DNA and delivering them to the correct site on the gene. Today, most gene therapy studies are aimed at cancer and hereditary diseases linked to a genetic defect. Antisense therapy is not strictly a form of gene therapy, but is a related, genetically-mediated therapy.

The most common form of genetic engineering involves the insertion of a functional gene at an unspecified location in the host genome. This is accomplished by isolating and copying the gene of interest, generating a construct containing all the genetic elements for correct expression, and then inserting this construct into a random location in the host organism. Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases, engineered I-CreI homing endonucleases, or nucleases generated from TAL effectors. An example of gene-knockout mediated gene therapy is the knockout of the human CCR5 gene in T-cells in order to control HIV infection. This approach is currently being used in several human clinical trials.

The biology of human gene therapy remains complex and many techniques need further development. Many diseases and their strict genetic link need to be understood more fully before gene therapy can be used appropriately. The public policy debate surrounding the possible use of genetically engineered material in human subjects has been equally complex. Major participants in the debate have come from the fields of biology, government, law, medicine, philosophy, politics, and religion, each bringing different views to the discussion.

Types of gene therapy

Gene therapy may be classified into the two following types:

Germ line gene therapy

In the case of germ line gene therapy, germ cells, i.e., sperm or eggs, are modified by the introduction of functional genes, which are integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations. This new approach, theoretically, should be highly effective in counteracting genetic disorders and hereditary diseases. However, many jurisdictions prohibit this for application in human beings, at least for the present, for a variety of technical and ethical reasons.

Somatic gene therapy

In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring or later generations.

Vectors in gene therapy

Viruses

All viruses bind to their hosts and introduce their genetic material into the host cell as part of their replication cycle. This genetic material contains basic 'instructions' of how to produce more copies of these viruses, hijacking the body's normal production machinery to serve the needs of the virus. The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells becoming infected. Some types of viruses insert their genes into the host's genome, but do not actually enter the cell. Others penetrate the cell membrane disguised as protein molecules and enter the cell.

There are two main types of virus infection: lytic and lysogenic. Shortly after inserting its DNA, viruses of the lytic cycle quickly produce more viruses, burst from the cell and infect more cells. Lysogenic viruses integrate their DNA into the DNA of the host cell and may live in the body for many years before responding to a trigger. The virus reproduces as the cell does and does not inflict bodily harm until it is triggered. The trigger releases the DNA from that of the host and employs it to create new viruses. HIV is a lysogenic infection. Some scientists believe that if they find the origin of its trigger, they will be able to stop the virus from ever reproducing throughout the body.

Retroviruses

The genetic material in retroviruses is in the form of RNA molecules, while the genetic material of their hosts is in the form of DNA. When a retrovirus infects a host cell, it will introduce its RNA together with some enzymes, namely reverse transcriptase and integrase, into the cell. This RNA molecule from the retrovirus must produce a DNA copy from its RNA molecule before it can be integrated into the genetic material of the host cell. The process of producing a DNA copy from an RNA molecule is termed reverse transcription. It is carried out by one of the enzymes carried in the virus, called reverse transcriptase. After this DNA copy is produced and is free in the nucleus of the host cell, it must be incorporated into the genome of the host cell. That is, it must be inserted into the large DNA molecules in the cell (the chromosomes). This process is done by another enzyme carried in the virus called integrase.

Now that the genetic material of the virus has been inserted, it can be said that the host cell has been modified to contain new genes. If this host cell divides later, its descendants will all contain the new genes. Sometimes the genes of the retrovirus do not express their information immediately.

One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur. This problem has recently begun to be addressed by utilizing zinc finger nucleases or by including certain sequences such as the beta-globin locus control region to direct the site of integration to specific chromosomal sites.

Gene therapy trials using retroviral vectors to treat X-linked severe combined immunodeficiency (X-SCID) represent the most successful application of gene therapy to date. More than twenty patients have been treated in France and Britain, with a high rate of immune system reconstitution observed. Similar trials were restricted or halted in the USA when leukemia was reported in patients treated in the French X-SCID gene therapy trial. To date, four children in the French trial and one in the British trial have developed leukemia as a result of insertional mutagenesis by the retroviral vector. All but one of these children responded well to conventional anti-leukemia treatment. Gene therapy trials to treat SCID due to deficiency of the Adenosine Deaminase (ADA) enzyme continue with relative success in the USA, Britain, Italy and Japan.

Adenoviruses

Adenoviruses are viruses that carry their genetic material in the form of double-stranded DNA. They cause respiratory, intestinal, and eye infections in humans (especially the common cold). When these viruses infect a host cell, they introduce their DNA molecule into the host. The genetic material of the adenoviruses is not incorporated (transient) into the host cell's genetic material. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene. The only difference is that these extra genes are not replicated when the cell is about to undergo cell division so the descendants of that cell will not have the extra gene. As a result, treatment with the adenovirus will require readministration in a growing cell population although the absence of integration into the host cell's genome should prevent the type of cancer seen in the SCID trials. This vector system has been promoted for treating cancer and indeed the first gene therapy product to be licensed to treat cancer, Gendicine, is an adenovirus. Gendicine, an adenoviral p53-based gene therapy was approved by the Chinese FDA in 2003 for treatment of head and neck cancer. Advexin, a similar gene therapy approach from Introgen, was turned down by the US FDA in 2008.

Concerns about the safety of adenovirus vectors were raised after the 1999 death of Jesse Gelsinger while participating in a gene therapy trial. Since then, work using adenovirus vectors has focused on genetically crippled versions of the virus.

Adeno-associated viruses

Adeno-associated viruses, from the parvovirus family, are small viruses with a genome of single stranded DNA. The wild type AAV can insert genetic material at a specific site on chromosome 19 with near 100% certainty. But the recombinant AAV, which does not contain any viral genes and only the therapeutic gene, does not integrate into the genome. Instead the recombinant viral genome fuses at its ends via the ITR (inverted terminal repeats) recombination to form circular, episomal forms which are predicted to be the primary cause of the long term gene expression. There are a few disadvantages to using AAV, including the small amount of DNA it can carry (low capacity) and the difficulty in producing it. The production problem however has recently been solved by Amsterdam Molecular Therapeutics. This type of virus is being used, however, because it is non-pathogenic (most people carry this harmless virus). In contrast to adenoviruses, most people treated with AAV will not build an immune response to remove the virus and the cells that have been successfully treated with it. Several trials with AAV are on-going or in preparation, mainly trying to treat muscle and eye diseases; the two tissues where the virus seems particularly useful. However, clinical trials have also been initiated where AAV vectors are used to deliver genes to the brain. This is possible because AAV viruses can infect non-dividing (quiescent) cells, such as neurons in which their genomes are expressed for a long time.

Envelope protein pseudotyping of viral vectors

The viral vectors described above have natural host cell populations that they infect most efficiently. Retroviruses have limited natural host cell ranges, and although adenovirus and adeno-associated virus are able to infect a relatively broader range of cells efficiently, some cell types are refractory to infection by these viruses as well. Attachment to and entry into a susceptible cell is mediated by the protein envelope on the surface of a virus. Retroviruses and adeno-associated viruses have a single protein coating their membrane, while adenoviruses are coated with both an envelope protein and fibers that extend away from the surface of the virus. The envelope proteins on each of these viruses bind to cell-surface molecules such as heparin sulfate, which localizes them upon the surface of the potential host, as well as with the specific protein receptor that either induces entry-promoting structural changes in the viral protein, or localizes the virus in endosomes wherein acidification of the lumen induces this refolding of the viral coat. In either case, entry into potential host cells requires a favorable interaction between a protein on the surface of the virus and a protein on the surface of the cell. For the purposes of gene therapy, one might either want to limit or expand the range of cells susceptible to transduction by a gene therapy vector. To this end, many vectors have been developed in which the endogenous viral envelope proteins have been replaced by either envelope proteins from other viruses, or by chimeric proteins. Such chimera would consist of those parts of the viral protein necessary for incorporation into the virion as well as sequences meant to interact with specific host cell proteins. Viruses in which the envelope proteins have been replaced as described are referred to as pseudotyped viruses. For example, the most popular retroviral vector for use in gene therapy trials has been the lentivirus Simian immunodeficiency virus coated with the envelope proteins, G-protein, from Vesicular

stomatitis virus. This vector is referred to as VSV G-pseudotyped lentivirus, and infects an almost universal set of cells. This tropism is characteristic of the VSV G-protein with which this vector is coated. Many attempts have been made to limit the tropism of viral vectors to one or a few host cell populations. This advance would allow for the systemic administration of a relatively small amount of vector. The potential for off-target cell modification would be limited, and many concerns from the medical community would be alleviated. Most attempts to limit tropism have used chimeric envelope proteins bearing antibody fragments. These vectors show great promise for the development of "magic bullet" gene therapies.

Replication-Competent Vectors

A replication-competent vector called ONYX-015 is used in replicating tumor cells. It was found that in the absence of the E1B-55Kd viral protein, adenovirus caused very rapid apoptosis of infected, p53(+) cells, and this results in dramatically reduced virus progeny and no subsequent spread. Apoptosis was mainly the result of the ability of EIA to inactivate p300. In p53(-) cells, deletion of E1B 55kd has no consequence in terms of apoptosis, and viral replication is similar to that of wild-type virus, resulting in massive killing of cells.

A replication-defective vector deletes some essential genes. These deleted genes are still necessary in the body so they are replaced with either a helper virus or a DNA molecule.

Cis and trans-acting elements

Replication-defective vectors always contain a “transfer construct”. The transfer construct carries the gene to be transduced or “transgene”. The transfer construct also carries the sequences which are necessary for the general functioning of the viral genome: packaging sequence, repeats for replication and, when needed, priming of reverse transcription. These are denominated cis-acting elements, because they need to be on the same piece of DNA as the viral genome and the gene of interest. Trans-acting elements are viral elements, which can be encoded on a different DNA molecule. For example, the viral structural proteins can be expressed from a different genetic element than the viral genome.

Herpes Semplex Virus

Herpes Semplex Virus is a human neurotropic virus. This is mostly examined for gene transfer in the nervous system. The wild type HSV-1 virus is able to infect neurons. Infected neurones are not rejected by the immune system. Though the latent virus is not transcriptionally apparent, it does possess neurone specific promoters that can continue to function normally. Antibodies to HSV-1 are common in humans, however complications due to herpes infection are somewhat rare.

Non-viral methods

Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses.

Injection of Naked DNA

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection. In addition to trials with plasmids, there have been trials with naked PCR product, which have had similar or greater success. Cellular uptake of naked DNA is generally inefficient. Research efforts focusing on improving the efficiency of naked DNA uptake have yielded several novel methods, such as electroporation, sonoporation, and the use of a "gene gun", which shoots DNA coated gold particles into the cell using high pressure gas.

Physical Methods to Enhance Delivery

Electroporation

Electroporation is a method that uses short pulses of high voltage to carry DNA across the cell membrane. This shock is thought to cause temporary formation of pores in the cell membrane, allowing DNA molecules to pass through. Electroporation is generally efficient and works across a broad range of cell types. However, a high rate of cell death following electroporation has limited its use, including clinical applications.

More recently a newer method of electroporation, termed electron-avalanche transfection, has been used in gene therapy experiments. By using a high-voltage plasma discharge, DNA was efficiently delivered following very short (microsecond) pulses. Compared to electroporation, the technique resulted in greatly increased efficiency and less cellular damage.

Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells.

Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells.

Magnetofection

In a method termed magnetofection, DNA is complexed to a magnetic particles, and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer.

Chemical Methods to Enhance Delivery

Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA, and therefore expression of the gene. A further strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression. Additionally, single stranded DNA oligonucleotides have been used to direct a single base change within a mutant gene. The oligonucleotide is designed to anneal with complementarity to the target gene with the exception of a central base, the target base, which serves as the template base for repair. This technique is referred to as oligonucleotide mediated gene repair, targeted gene repair, or targeted nucleotide alteration.

Lipoplexes and polyplexes

To improve the delivery of the new DNA into the cell, the DNA must be protected from damage and (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors. However, in spite of the facts that there is little toxicity associated with them, that they are compatible with body fluids and that there was a possibility of adapting them to be tissue specific; they are complicated and time consuming to produce so attention was turned to the cationic versions.

Cationic lipids, due to their positive charge, were first used to condense negatively charged DNA molecules so as to facilitate the encapsulation of DNA into liposomes. Later it was found that the use of cationic lipids significantly enhanced the stability of lipoplexes. Also as a result of their charge, cationic liposomes interact with the cell membrane, endocytosis was widely believed as the major route by which cells uptake

lipoplexes. Endosomes are formed as the results of endocytosis, however, if genes can not be released into cytoplasm by breaking the membrane of endosome, they will be sent to lysosomes where all DNA will be destroyed before they could achieve their functions. It was also found that although cationic lipids themselves could condense and encapsulate DNA into liposomes, the transfection efficiency is very low due to the lack of ability in terms of "endosomal escaping". However, when helper lipids (usually electroneutral lipids, such as DOPE) were added to form lipoplexes, much higher transfection efficiency was observed. Later on, it was figured out that certain lipids have the ability to destabilize endosomal membranes so as to facilitate the escape of DNA from endosome, therefore those lipids are called fusogenic lipids. Although cationic liposomes have been widely used as an alternative for gene delivery vectors, a dose dependent toxicity of cationic lipids were also observed which could limit their therapeutic usages.

The most common use of lipoplexes has been in gene transfer into cancer cells, where the supplied genes have activated tumor suppressor control genes in the cell and decrease the activity of oncogenes. Recent studies have shown lipoplexes to be useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.

Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions. One large difference between the methods of action of polyplexes and lipoplexes is that polyplexes cannot release their DNA load into the cytoplasm, so to this end, co-transfection with endosome-lytic agents (to lyse the endosome that is made during endocytosis, the process by which the polyplex enters the cell) such as inactivated adenovirus must occur. However, this isn't always the case, polymers such as polyethylenimine have their own method of endosome disruption as does chitosan and trimethylchitosan.

Dendrimers

A dendrimer is a highly branched macromolecule with a spherical shape. The surface of the particle may be functionalized in many ways and many of the properties of the resulting construct are determined by its surface.

In particular it is possible to construct a cationic dendrimer, i.e. one with a positive surface charge. When in the presence of genetic material such as DNA or RNA, charge complimentarity leads to a temporary association of the nucleic acid with the cationic dendrimer. On reaching its destination the dendrimer-nucleic acid complex is then taken into the cell via endocytosis.

In recent years the benchmark for transfection agents has been cationic lipids. Limitations of these competing reagents have been reported to include: the lack of ability to transfect a number of cell types, the lack of robust active targeting capabilities, incompatibility with animal models, and toxicity. Dendrimers offer robust covalent construction and extreme control over molecule structure, and therefore size. Together these give compelling advantages compared to existing approaches.

Producing dendrimers has historically been a slow and expensive process consisting of numerous slow reactions, an obstacle that severely curtailed their commercial development. The Michigan based company Dendritic Nanotechnologies discovered a method to produce dendrimers using kinetically driven chemistry, a process that not only reduced cost by a magnitude of three, but also cut reaction time from over a month to several days. These new "Priostar" dendrimers can be specifically constructed to carry a DNA or RNA payload that transfects cells at a high efficiency with little or no toxicity.

Hybrid methods

Due to every method of gene transfer having shortcomings, there have been some hybrid methods developed that combine two or more techniques. Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. Other methods involve mixing other viral vectors with cationic lipids or hybridising viruses.

Major developments in gene therapy

1970s and earlier

In 1972 Friedmann and Roblin authored a paper in Science titled "Gene therapy for human genetic disease?" They cite Rogers S for proposing "that exogenous 'good'" DNA be used to replace the defective DNA in those who suffer from genetic defects. They also cite the first attempt to perform gene therapy as [New York Times, 20 September 1970].

1990s

The first approved gene therapy case in the United States took place on September 14, 1990, at the National Institute of Health. It was performed on a four year old girl named Ashanti DeSilva. It was a treatment for a genetic defect that left her with an Immune System deficiency. The effects were only temporary, but successful (Boylan 313).

New gene therapy approach repairs errors in messenger RNA derived from defective genes. This technique has the potential to treat the blood disorder thalassaemia, cystic fibrosis, and some cancers. Researchers at Case Western Reserve University and Copernicus Therapeutics are able to create tiny liposomes 25 nanometers across that can carry therapeutic DNA through pores in the nuclear membrane.

Sickle cell disease is successfully treated in mice.

in 1992 Doctor Claudio Bordignon working at the Vita-Salute San Raffaele University, Milan, Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary diseases. In 2002 this work led to the publication of the first successful gene therapy treatment for adenosine deaminase-deficiency (SCID). The success of a multi-center trial for treating children with SCID

(severe combined immune deficiency or "bubble boy" disease) held from 2000 and 2002 was questioned when two of the ten children treated at the trial's Paris center developed a leukemia-like condition. Clinical trials were halted temporarily in 2002, but resumed after regulatory review of the protocol in the United States, the United Kingdom, France, Italy, and Germany.

In 1993 Andrew Gobea was born with severe combined immunodeficiency (SCID). Genetic screening before birth showed that he had SCID. Blood was removed from Andrew's placenta and umbilical cord immediately after birth, containing stem cells. The allele that codes for ADA was obtained and was inserted into a retrovirus. Retroviruses and stem cells were mixed, after which they entered and inserted the gene into the stem cells' chromosomes. Stem cells containing the working ADA gene were injected into Andrew's blood system via a vein. Injections of the ADA enzyme were also given weekly. For four years T-cells (white blood cells), produced by stem cells, made ADA enzymes using the ADA gene. After four years more treatment was needed.

The 1999 death of Jesse Gelsinger in a gene therapy experiment resulted in a significant setback to gene therapy research in the United States. The pivotal event resulted in the FDA's suspension of several clinical trials as ethical and procedural practices in the field were reevaluated.

2000s

In 2003 a University of California, Los Angeles research team inserted genes into the brain using liposomes coated in a polymer called polyethylene glycol. The transfer of genes into the brain is a significant achievement because viral vectors are too big to get across the blood-brain barrier. This method has potential for treating Parkinson's disease.

RNA interference or gene silencing may be a new way to treat Huntington's disease. Short pieces of double-stranded RNA (short, interfering RNAs or siRNAs) are used by cells to degrade RNA of a particular sequence. If a siRNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced.

Scientists at the National Institutes of Health (Bethesda, Maryland) have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells. This study constitutes one of the first demonstrations that gene therapy can be effective in treating cancer.

In March 2006 an international group of scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells. The study, published in Nature Medicine, is believed to be the first to show that gene therapy can cure diseases of the myeloid system.

In May 2006 a team of scientists led by Dr. Luigi Naldini and Dr. Brian Brown from the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) in Milan, Italy reported

a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene. Similar to organ transplantation, gene therapy has been plagued by the problem of immune rejection. So far, delivery of the 'normal' gene has been difficult because the immune system recognizes the new gene as foreign and rejects the cells carrying it. To overcome this problem, the HSR-TIGET group utilized a newly uncovered network of genes regulated by molecules known as microRNAs. Dr. Naldini's group reasoned that they could use this natural function of microRNA to selectively turn off the identity of their therapeutic gene in cells of the immune system and prevent the gene from being found and destroyed. The researchers injected mice with the gene containing an immune-cell microRNA target sequence, and the mice did not reject the gene, as previously occurred when vectors without the microRNA target sequence were used. This work will have important implications for the treatment of hemophilia and other genetic diseases by gene therapy.

In November 2006 Preston Nix from the University of Pennsylvania School of Medicine reported on VRX496, a gene-based immunotherapy for the treatment of human immunodeficiency virus (HIV) that uses a lentiviral vector for delivery of an antisense gene against the HIV envelope. In the Phase I trial enrolling five subjects with chronic HIV infection who had failed to respond to at least two antiretroviral regimens, a single intravenous infusion of autologous CD4 T cells genetically modified with VRX496 was safe and well tolerated. All patients had stable or decreased viral load; four of the five patients had stable or increased CD4 T cell counts. In addition, all five patients had stable or increased immune response to HIV antigens and other pathogens. This was the first evaluation of a lentiviral vector administered in U.S. Food and Drug Administration-approved human clinical trials for any disease. Data from an ongoing Phase I/II clinical trial were presented at CROI 2009.

On 1 May 2007 Moorfields Eye Hospital and University College London's Institute of Ophthalmology announced the world's first gene therapy trial for inherited retinal disease. The first operation was carried out on a 23 year-old British male, Robert Johnson, in early 2007. Leber's congenital amaurosis is an inherited blinding disease caused by mutations in the RPE65 gene. The results of the Moorfields/UCL trial were published in New England Journal of Medicine in April 2008. They researched the safety of the subretinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene, and found it yielded positive results, with patients having modest increase in vision, and, perhaps more importantly, no apparent side-effects.

In September 2009, the journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys using gene therapy, a hopeful precursor to a treatment for color blindness in humans. In November 2009, the journal Science reported that researchers succeeded at halting a fatal brain disease, adrenoleukodystrophy, using a vector derived from HIV to deliver the gene for the missing enzyme.

A paper by Komáromy *et* al. published in April 2010, deals with gene therapy for a form of achromatopsia in dogs. Achromatopsia, or complete color blindness, is presented as an

ideal model to develop gene therapy directed to cone photoreceptors. Cone function and day vision have been restored for at least 33 months in two young dogs with achromatopsia. However, the therapy was less efficient for older dogs.

Problems and ethics

For the safety of gene therapy, the Weismann barrier is fundamental in the current thinking. Soma-to-germline feedback should therefore be impossible. However, there are indications that the Weismann barrier can be breached. One way it might possibly be breached is if the treatment were somehow misapplied and spread to the testes and therefore would infect the germline against the intentions of the therapy.

Some of the problems of gene therapy include:

- Short-lived nature of gene therapy – Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.
- Immune response – Anytime a foreign object is introduced into human tissues, the immune system has evolved to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a possibility. Furthermore, the immune system's enhanced response to invaders that it has seen before makes it difficult for gene therapy to be repeated in patients.
- Problems with viral vectors – Viruses, the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient —toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.
- Multigene disorders – Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. Multigene or multifactorial disorders such as these would be especially difficult to treat effectively using gene therapy.
- Chance of inducing a tumor (insertional mutagenesis) - If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.

Deaths have occurred due to gene therapy, including that of Jesse Gelsinger.

Chapter 10

Canavan Disease

Canavan disease	
ICD-10	E75.2
ICD-9	330.0
OMIM	271900
DiseasesDB	29780
MedlinePlus	001586
MeSH	D017825

Canavan disease, also called **Canavan-Van Bogaert-Bertrand disease**, **aspartoacylase deficiency** or **aminoacylase 2 deficiency**, is an autosomal recessive degenerative disorder that causes progressive damage to nerve cells in the brain. Canavan disease is also one of the most common degenerative cerebreal diseases of infancy. This disease is one of a group of genetic disorders called leukodystrophies.

Leukodystrophies are characterized by degeneration of myelin in the phospholipid layer insulating the axon of a neuron. The gene associated with the disorder is located on human chromosome 17.

History

Canavan disease was first described in 1931 by Myrtelle Canavan.

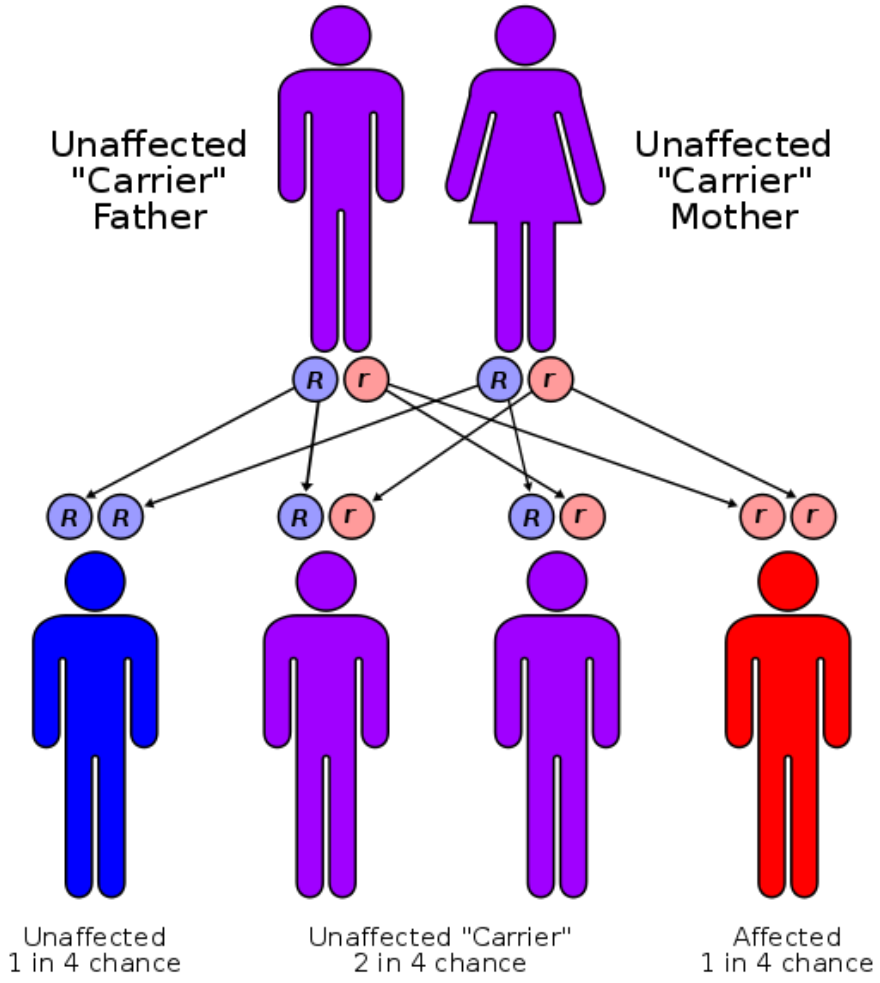
The discovery of the gene for Canavan disease, and subsequent events, generated considerable controversy. In 1987 the Greenbergs, a family with two children affected by Canavan disease, donated tissue samples to Dr Reuben Matalon, a researcher looking for the Canavan gene. He successfully identified the gene in 1993, and developed a test for it that would enable antenatal counselling of couples at risk of having a child with Canavan disease. For a while the Canavan Foundation offered free genetic testing with the test. However, in 1997, Dr Matalon's employer, the Miami Children's Hospital, patented the gene and started claiming royalties on the genetic test, forcing the Canavan Foundation to

withdraw their testing. A subsequent lawsuit brought by the Canavan Foundation against the Miami Children's Hospital was resolved with a sealed out-of-court settlement. The case is sometimes cited in arguments about the appropriateness of patenting genes.

Prevalence

Although Canavan disease may occur in any ethnic group, it affects persons of Eastern European Jewish ancestry more frequently. About 1/40 individuals of Eastern European (Ashkenazi) Jewish ancestry are carriers.

Pathophysiology



Canavan disease has an autosomal recessive pattern of inheritance

Canavan disease is inherited in an autosomal recessive fashion. When both parents are carriers, there is a 25% chance of having an affected child. Genetic counseling and genetic testing is recommended for families with two parental carriers.

Canavan disease is caused by a defective *ASPA* gene which is responsible for the production of the enzyme aspartoacylase. This enzyme breaks down the concentrated brain molecule *N*-acetyl aspartate. Decreased aspartoacylase activity prevents the normal breakdown of N-acetyl aspartate, and the lack of breakdown somehow interferes with growth of the myelin sheath of the nerve fibers in the brain. The myelin sheath is the fatty covering that surrounds nerve cells and acts as an insulator, which allows for efficient transmission of nerve impulses.

Symptoms

Symptoms of Canavan disease, which appear in early infancy and progress rapidly, may include mental retardation, loss of previously acquired motor skills, feeding difficulties, abnormal muscle tone (i.e., floppiness or stiffness), poor head control, and megalocephaly (abnormally enlarged head). Paralysis, blindness, or seizures may also occur.

Treatment

There is no cure for Canavan disease, nor is there a standard course of treatment. Treatment is symptomatic and supportive, but there is an experimental treatment using lithium citrate. When a person has Canavan Disease, his or her levels of N-acetyl aspartate are chronically elevated. The lithium citrate has proven that, in a rat genetic model of Canavan Disease, the lithium citrate significantly decreased the levels of N-acetyl aspartate. When tested on a human, the subject reversed during a two week wash-out period after withdrawal of lithium. The investigation reported that the N-acetyl aspartate levels decreased in regions of the brain tested and magnetic resonance spectroscopic values that are more characteristic of normal development and myelination. With this evidence it is suggested that a larger controlled trial of lithium may be warranted as supportive therapy for children with Canavan disease by significantly decreasing the elevated amounts of N-acetyl aspartate.

In addition, there are experimental trials of gene therapy. A healthy gene is cloned to take over for the defective one that causes Canavan disease.

Prognosis

Death usually occurs before age 4 without treatment. Some children may survive into their twenties via newer gene therapy treatments which have extended their life expectancy. In some cases, this helps to temporarily stop the progression of the disease.

Current research

Research involving triacetin supplementation has shown promise in a mouse model. Triacetin, which can be enzymatically cleaved to form acetate, enters the brain more readily than the negatively charged acetate.

A team of researchers headed by Paola Leone are currently at the University of Medicine and Dentistry of New Jersey, in Camden, New Jersey. The brain gene therapy is conducted at Cooper University Hospital. The procedure involves the insertion of six catheters into the brain that deliver a solution containing 600 billion to 900 billion engineered virus particles. The virus, a modified version of AAV, is designed to replace the aspartoacylase enzyme. Children treated with this procedure to date have shown marked improvements, including the growth of myelin with decreased levels of the n-acetyl-aspartate toxin.

Chapter 11

Color Blindness

Color blindness

An 1895 illustration of normal vision and various kinds of color blindness

ICD-10	H53.5
ICD-9	368.5
DiseasesDB	2999
MeSH	D003117

Color blindness or **color vision deficiency** is the decreased ability to perceive differences between some of the colors that others can distinguish. It is most often of genetic nature, but may also occur because of some eye, nerve, or brain damage, or exposure to certain chemicals. The English chemist John Dalton published the first scientific paper on this subject in 1798, "Extraordinary facts relating to the vision of colours", after the realization of his own color blindness. Because of Dalton's work, the condition was often called **daltonism**, although this term is now used for a type of color blindness called deuteranopia.

Color blindness is usually classed as a mild disability, but in certain situations, color blind individuals have an advantage over those with normal color vision. There are some studies which conclude that color blind individuals are better at penetrating certain color camouflages and it has been suggested that this may be the evolutionary explanation for the surprisingly high frequency of congenital red–green color blindness.

Background

The average human retina contains two kinds of light cells: the rod cells (active in low light) and the cone cells (active in normal daylight). Normally, there are three kinds of cones, each containing a different pigment, which are activated when the pigments absorb light. The spectral sensitivities of the cones differ; one is maximally sensitive to short wavelengths, one to medium wavelengths, and the third to long wavelengths, with their peak sensitivities in the blue, yellowish-green, and yellow regions of the spectrum, respectively. The absorption spectra of all three systems cover the visible spectrum. These receptors are often called S cones, M cones, and L cones, for short, medium, and long wavelength; but they are also often referred to as blue cones, green cones, and red cones, respectively.

Although these receptors are often referred to as "blue, green, and red" receptors, this terminology is not very accurate, especially as the "red" receptor actually has its peak sensitivity in the yellow region. The sensitivity of normal color vision actually depends on the overlap between the absorption spectra of the three systems: different colors are recognized when the different types of cone are stimulated to different degrees. Red light, for example, stimulates the long wavelength cones much more than either of the others, and reducing the wavelength causes the other two cone systems to be increasingly stimulated, causing a gradual change in hue.

Many of the genes involved in color vision are on the X chromosome, making color blindness more common in males than in females because males have only one X chromosome, while females have two. Because this is an X-linked trait about 1% of women have an 4th color cone and can be considered tetrachromats although it is not clear that this provides an advantage in color discrimination.

Classification

By cause

The colors of the rainbow as viewed by a person with no color vision deficiencies

The colors of the rainbow as viewed by a person with protanopia

The colors of the rainbow as viewed by a person with deuteranopia

The colors of the rainbow as viewed by a person with tritanopia

Color vision deficiencies can be classified as acquired or inherited.

- Acquired
- Inherited: There are three types of inherited or congenital color vision deficiencies: monochromacy, dichromacy, and anomalous trichromacy.

    - Monochromacy, also known as "total color blindness," is the lack of ability to distinguish colors; caused by cone defect or absence. Monochromacy occurs when two or all three of the cone pigments are missing and color and lightness vision is reduced to one dimension.

    - Rod monochromacy (achromatopsia) is an exceedingly rare, nonprogressive inability to distinguish any colors as a result of absent or nonfunctioning retinal cones. It is associated with light sensitivity (photophobia), involuntary eye oscillations (nystagmus), and poor vision.
    - Cone monochromacy is a rare total color blindness that is accompanied by relatively normal vision, electoretinogram, and electrooculogram.

    - Dichromacy is a moderately severe color vision defect in which one of the three basic color mechanisms is absent or not functioning. It is hereditary and, in the case of Protanopia or Deuteranopia, sex-linked, affecting predominantly males. Dichromacy occurs when one of the cone pigments is missing and color is reduced to two dimensions.

    - Protanopia is a severe type of color vision deficiency caused by the complete absence of red retinal photoreceptors. It is a form of dichromatism in which red appears dark. It is hereditary, sex-linked, and present in 1% of males.
    - Deuteranopia is a color vision deficiency in which the green retinal photoreceptors are absent, moderately affecting red–green hue discrimination. It is a form of dichromatism in which there are only two cone pigments present. It is likewise hereditary and sex-linked.
    - Tritanopia is a very rare color vision disturbance in which there are only two cone pigments present and a total absence of blue retinal receptors.

    - Anomalous trichromacy is a common type of inherited color vision deficiency, occurring when one of the three cone pigments is altered in its spectral sensitivity. This results in an impairment, rather than loss, of trichromacy (normal three-dimensional color vision).

    - Protanomaly is a mild color vision defect in which an altered spectral sensitivity of red retinal receptors (closer to green receptor response) results in poor red–green hue discrimination. It is hereditary, sex-linked, and present in 1% of males.

- Deuteranomaly, caused by a similar shift in the green retinal receptors, is by far the most common type of color vision deficiency, mildly affecting red–green hue discrimination in 5% of males. It is hereditary and sex-linked.
- Tritanomaly is a rare, hereditary color vision deficiency affecting blue–yellow hue discrimination. Unlike most other forms, it is not sex-linked.

By clinical appearance

Based on clinical appearance, color blindness may be described as total or partial. Total color blindness is much less common than partial color blindness. There are two major types of color blindness: those who have difficulty distinguishing between red and green, and those who have difficulty distinguishing between blue and yellow.

- Total color blindness
- Partial color blindness
    - Red–green
    - Dichromacy (protanopia and deuteranopia)
    - Anomalous trichromacy (protanomaly and deuteranomaly)
    - Blue–yellow
    - Dichromacy (tritanopia)
    - Anomalous trichromacy (tritanomaly)

Causes

Evolutionary arguments

Any recessive genetic characteristic that persists at a level as high as 5% is generally regarded as possibly having some advantage over the long term. At one time the U.S. Army found that color blind people could spot "camouflage" colors that fooled those with normal color vision. Humans have a higher percentage of color blindness than macaque monkeys according to recent research.

Another possible advantage might result from the presence of a tetrachromic female. Owing to X-chromosome inactivation, females who are heterozygous for anomalous trichromacy ought to have at least four types of cone in their retinae. It is possible that this affords them an extra dimension of color vision, by analogy to New World monkeys where heterozygous females gain trichromacy in a basically dichromatic species.

Genetics

Color blindness can be inherited. It is most commonly inherited from mutations on the X chromosome but the mapping of the human genome has shown there are many causative mutations – mutations capable of causing color blindness originate from at least 19 different chromosomes and 56 different genes (as shown online at the Online Mendelian Inheritance in Man (OMIM) database at Johns Hopkins University).

Some of the inherited diseases known to cause color blindness are:

- cone dystrophy
- cone-rod dystrophy
- achromatopsia (aka rod monochromatism, aka stationary cone dystrophy, aka cone dysfunction syndrome)
- blue cone monochromatism,
- Leber's congenital amaurosis.
- retinitis pigmentosa (initially affects rods but can later progress to cones and therefore color blindness)

Inherited color blindness can be congenital (from birth), or it can commence in childhood or adulthood. Depending on the mutation, it can be stationary, that is, remain the same throughout a person's lifetime, or progressive. As progressive phenotypes involve deterioration of the retina and other parts of the eye, certain forms of color blindness can progress to legal blindness, i.e., an acuity of 6/60 or worse, and often leave a person with complete blindness.

Color blindness always pertains to the cone photoreceptors in retinas, as the cones are capable of detecting the color frequencies of light.

About 8 percent of males, but only 0.5 percent of females, are color blind in some way or another, whether it is one color, a color combination, or another mutation. The reason males are at a greater risk of inheriting an X linked mutation is because males only have one X chromosome (XY, with the Y chromosome being significantly shorter than the X chromosome), and females have two (XX); if a woman inherits a normal X chromosome in addition to the one which carries the mutation, she will not display the mutation. Men do not have a second X chromosome to override the chromosome which carries the mutation. If 5% of variants of a given gene are defective, the probability of a single copy being defective is 5%, but the probability that two copies are both defective is $0.05 \times 0.05 = 0.0025$, or just 0.25%.

Other causes

Other causes of color blindness include brain or retinal damage caused by shaken baby syndrome, accidents and other trauma which produce swelling of the brain in the occipital lobe, and damage to the retina caused by exposure to ultraviolet light. Most ultraviolet light damage is caused during childhood and this form of retinal degeneration

is the leading cause of blindness in the world. Damage often presents itself later on in life.

Color blindness may also present itself in the spectrum of degenerative diseases of the eye, such as age-related macular degeneration, and as part of the retinal damage caused by diabetes.

Types

There are many types of color blindness. The most common are red–green hereditary photoreceptor disorders, but it is also possible to acquire color blindness through damage to the retina, optic nerve, or higher brain areas. Higher brain areas implicated in color processing include the parvocellular pathway of the lateral geniculate nucleus of the thalamus, and visual area V4 of the visual cortex. Acquired color blindness is generally unlike the more typical genetic disorders. For example, it is possible to acquire color blindness only in a portion of the visual field but maintain normal color vision elsewhere. Some forms of acquired color blindness are reversible. Transient color blindness also occurs (very rarely) in the aura of some migraine sufferers.

The different kinds of inherited color blindness result from partial or complete loss of function of one or more of the different cone systems. When one cone system is compromised, dichromacy results. The most frequent forms of human color blindness result from problems with either the middle or long wavelength sensitive cone systems, and involve difficulties in discriminating reds, yellows, and greens from one another. They are collectively referred to as "red–green color blindness", though the term is an over-simplification and is somewhat misleading. Other forms of color blindness are much more rare. They include problems in discriminating blues from yellows, and the rarest forms of all, complete color blindness or *monochromacy*, where one cannot distinguish any color from grey, as in a black-and-white movie or photograph.

Congenital

Congenital color vision deficiencies are subdivided based on the number of primary hues needed to match a given sample in the visible spectrum.

Monochromacy

Monochromacy is the condition of possessing only a single channel for conveying information about color. Monochromats possess a complete inability to distinguish any colors and perceive only variations in brightness. It occurs in two primary forms:

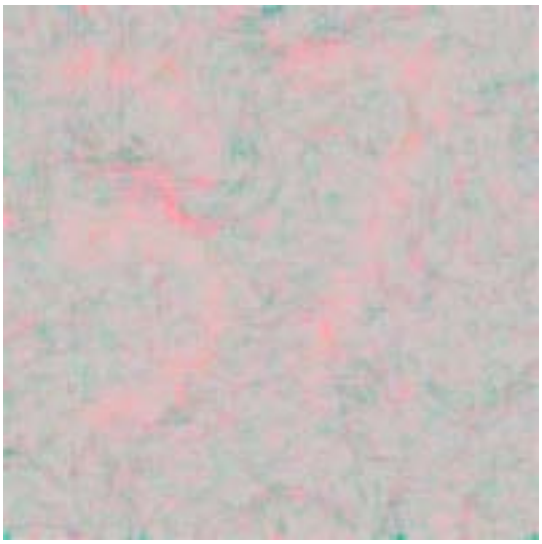
1. **Rod monochromacy**, frequently called *achromatopsia*, where the retina contains no cone cells, so that in addition to the absence of color discrimination, vision in lights of normal intensity is difficult. While normally rare, achromatopsia is very common on the island of Pingelap, a part of the Pohnpei state, Federated States of Micronesia, where it is called *maskun*: about 10% of the population there has it,

and 30% are unaffected carriers. The island was devastated by a storm in the 18th century, and one of the few male survivors carried a gene for achromatopsia; the population is now several thousand.

2. **Cone monochromacy** is the condition of having both rods and cones, but only a single kind of cone. A cone monochromat can have good pattern vision at normal daylight levels, but will not be able to distinguish hues. **Blue cone monochromacy (X chromosome)** is caused by a complete absence of L and M cones (red and green). It is encoded at the same place as red–green color blindness on the X chromosome. Peak spectral sensitivities are in the blue region of the visible spectrum (near 440 nm). People with this condition generally show nystagmus ("jiggling eyes"), photophobia (light sensitivity), reduced visual acuity, and myopia (nearsightedness). Visual acuity usually falls to the 20/50 to 20/400 range.

Dichromacy

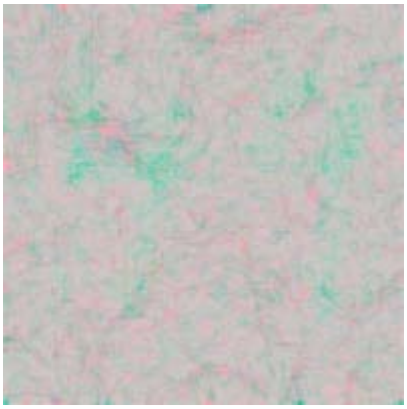
Protanopes, deuteranopes, and tritanopes are dichromats; that is, they can match any color they see with some mixture of just two spectral lights (whereas normally humans are trichromats and require three lights). These individuals normally know they have a color vision problem and it can affect their lives on a daily basis. Protanopes and deuteranopes see no perceptible difference between red, orange, yellow, and green. All these colors, that seem so different to the normal viewer, appear to be the same color for this two percent of the population. The terms protanopia, deuteranopia, and tritanopia come from Greek, and literally mean "inability to see (*anopia*) with the first (*prot-*), second (*deuter-*), or third (*trit-*) [cone]", respectively.



Test for protanopia
This image contains the number 37

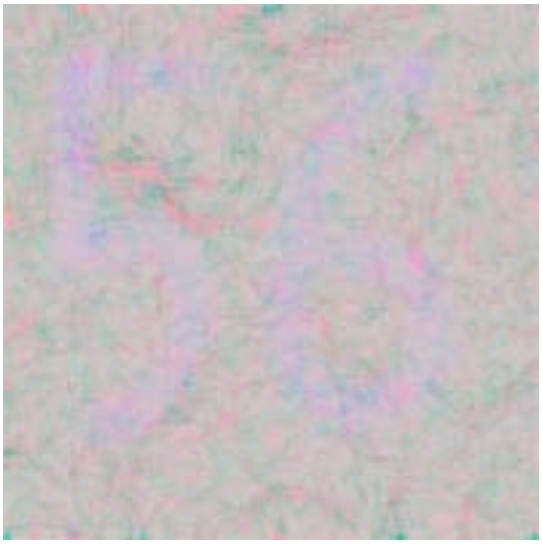
- **Protanopia** (1% of males): Lacking the long-wavelength sensitive retinal cones, those with this condition are unable to distinguish between colors in the green–

yellow–red section of the spectrum. They have a neutral point at a greenish wavelength around 492 nm – that is, they cannot discriminate light of this wavelength from white. For the protanope, the brightness of red, orange, and yellow are much reduced compared to normal. This dimming can be so pronounced that reds may be confused with black or dark gray, and red traffic lights may appear to be extinguished. They may learn to distinguish reds from yellows and from greens primarily on the basis of their apparent brightness or lightness, not on any perceptible hue difference. Violet, lavender, and purple are indistinguishable from various shades of blue because their reddish components are so dimmed as to be invisible. E.g., pink flowers, reflecting both red light and blue light, may appear just blue to the protanope. Very few people have been found who have one normal eye and one protanopic eye. These *unilateral dichromats* report that with only their protanopic eye open, they see wavelengths below the neutral point as blue and those above it as yellow. This is a rare form of color blindness.



Test for deuteranopia
This image shows a number 49, but someone who is deuteranopic may not be able to see it.

- **Deuteranopia** (1% of males): Lacking the medium-wavelength cones, those affected are again unable to distinguish between colors in the green–yellow–red section of the spectrum. Their neutral point is at a slightly longer wavelength, 498 nm. The deuteranope suffers the same hue discrimination problems as the protanope, but without the abnormal dimming. Similarly, violet, lavender, purple, and blue, seem to be too many names to use logically for hues that all look alike to him. This is one of the rarer forms of colorblindness making up about 1% of the male population, also known as *Daltonism* after John Dalton. (Dalton's diagnosis was confirmed as deuteranopia in 1995, some 150 years after his death, by DNA analysis of his preserved eyeball.) Deuteranopic unilateral dichromats report that with only their deuteranopic eye open, they see wavelengths below the neutral point as blue and those above it as yellow.



Test for tritanopia
This image shows the number 56, but someone who is tritanopic may not be able to see it.

- **Tritanopia** (less than 1% of males and females): Lacking the short-wavelength cones, those affected are unable to distinguish colors along the blue–yellow dimension. This form of color blindness is not sex-linked.

Anomalous trichromacy

Those with protanomaly, deuteranomaly, or tritanomaly are trichromats, but the color matches they make differ from the normal. They are called anomalous trichromats. In order to match a given spectral yellow light, protanomalous observers need more red light in a red/green mixture than a normal observer, and deuteranomalous observers need more green. From a practical standpoint though, many protanomalous and deuteranomalous people have very little difficulty carrying out tasks that require normal color vision. Some may not even be aware that their color perception is in any way different from normal.

Protanomaly and deuteranomaly can be diagnosed using an instrument called an anomaloscope, which mixes spectral red and green lights in variable proportions, for comparison with a fixed spectral yellow. If this is done in front of a large audience of males, as the proportion of red is increased from a low value, first a small proportion of the audience will declare a match, while most will see the mixed light as greenish; these are the deuteranomalous observers. Next, as more red is added the majority will say that a match has been achieved. Finally, as yet more red is added, the remaining, protanomalous, observers will declare a match at a point where normal observers will see the mixed light as definitely reddish.

- **Protanomaly** (1% of males, 0.01% of females): Having a mutated form of the long-wavelength (red) pigment, whose peak sensitivity is at a shorter wavelength than in the normal retina, protanomalous individuals are less sensitive to red light

than normal. This means that they are less able to discriminate colors, and they do not see mixed lights as having the same colors as normal observers. They also suffer from a darkening of the red end of the spectrum. This causes reds to reduce in intensity to the point where they can be mistaken for black. Protanomaly is a fairly rare form of color blindness, making up about 1% of the male population. Both protanomaly and deuteranomaly are carried on the X chromosome.

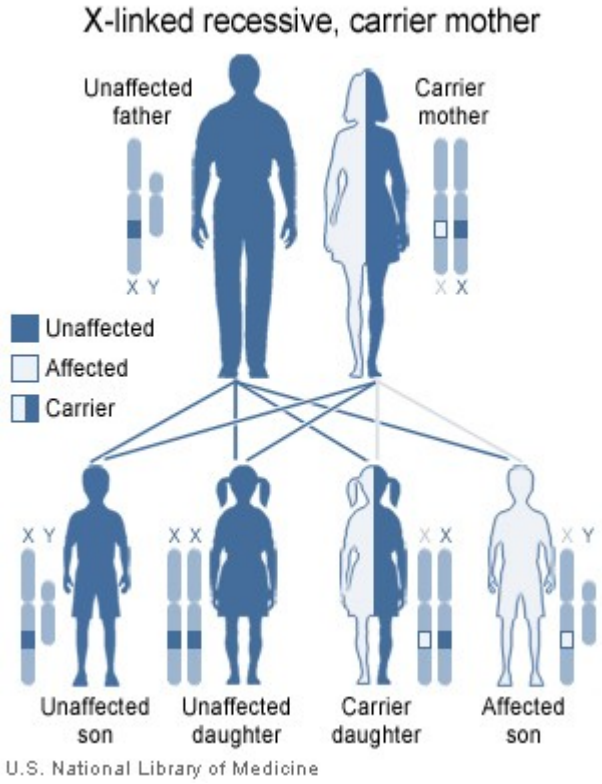
- **Deuteranomaly** (most common — 6% of males, 0.4% of females): Having a mutated form of the medium-wavelength (green) pigment. The medium-wavelength pigment is shifted towards the red end of the spectrum resulting in a reduction in sensitivity to the green area of the spectrum. Unlike protanomaly the intensity of colors is unchanged. This is the most common form of color blindness, making up about 6% of the male population. The deuteranomalous person is considered "green weak". For example, in the evening, dark green cars appear to be black to Deuteranomalous people. Similar to the protanomates, deuteranomates are poor at discriminating small differences in hues in the red, orange, yellow, green region of the spectrum. They make errors in the naming of hues in this region because the hues appear somewhat shifted towards red. One very important difference between deuteranomalous individuals and protanomalous individuals is deuteranomalous individuals do *not* have the loss of "brightness" problem.
- **Tritanomaly** (equally rare for males and females [0.01% for both]): Having a mutated form of the short-wavelength (blue) pigment. The short-wavelength pigment is shifted towards the green area of the spectrum. This is the rarest form of anomalous trichromacy color blindness. Unlike the other anomalous trichromacy color deficiencies, the mutation for this color blindness is carried on chromosome 7. Therefore it is equally prevalent in both male & female populations. The OMIM gene code for this mutation is 304000 “Colorblindness, Partial Tritanomaly”.

Total color blindness

Achromatopsia is strictly defined as the inability to see color. Although the term may refer to acquired disorders such as color agnosia and cerebral achromatopsia, it typically refers to congenital color vision disorders (i.e. more frequently rod monochromacy and less frequently cone monochromacy).

In color agnosia and cerebral achromatopsia, a person cannot perceive colors even though the eyes are capable of distinguishing them. Some sources do not consider these to be true color blindness, because the failure is of perception, not of vision. They are forms of visual agnosia.

Red–green color blindness



X-linked recessive inheritance

Those with protanopia, deuteranopia, protanomaly, and deuteranomaly have difficulty with discriminating red and green hues. It is sex-linked: genetic red–green color blindness affects males much more often than females, because the genes for the red and green color receptors are located on the X chromosome, of which males have only one and females have two. Females (46, XX) are red–green color blind only if *both* their X chromosomes are defective with a similar deficiency, whereas males (46, XY) are color blind if their single X chromosome is defective.

The gene for red–green color blindness is transmitted from a color blind male to all his daughters who are heterozygote carriers and are usually unaffected. In turn, a carrier woman has a fifty percent chance of passing on a mutated X chromosome region to each of her male offspring. The sons of an affected male will not inherit the trait from him, since they receive his Y chromosome and not his (defective) X chromosome. Should an affected male have children with a carrier or colorblind woman, their daughters may be colorblind by inheriting an affected X chromosome from each parent.

Because one X chromosome is inactivated at random in each cell during a woman's development, it is possible for her to have four different cone types, as when a carrier of protanomaly has a child with a deuteranomalic man. Denoting the normal vision alleles by P and D and the anomalous by p and d, the carrier is PD pD and the man is Pd. The daughter is either PD Pd or pD Pd. Suppose she is pD Pd. Each cell in her body expresses

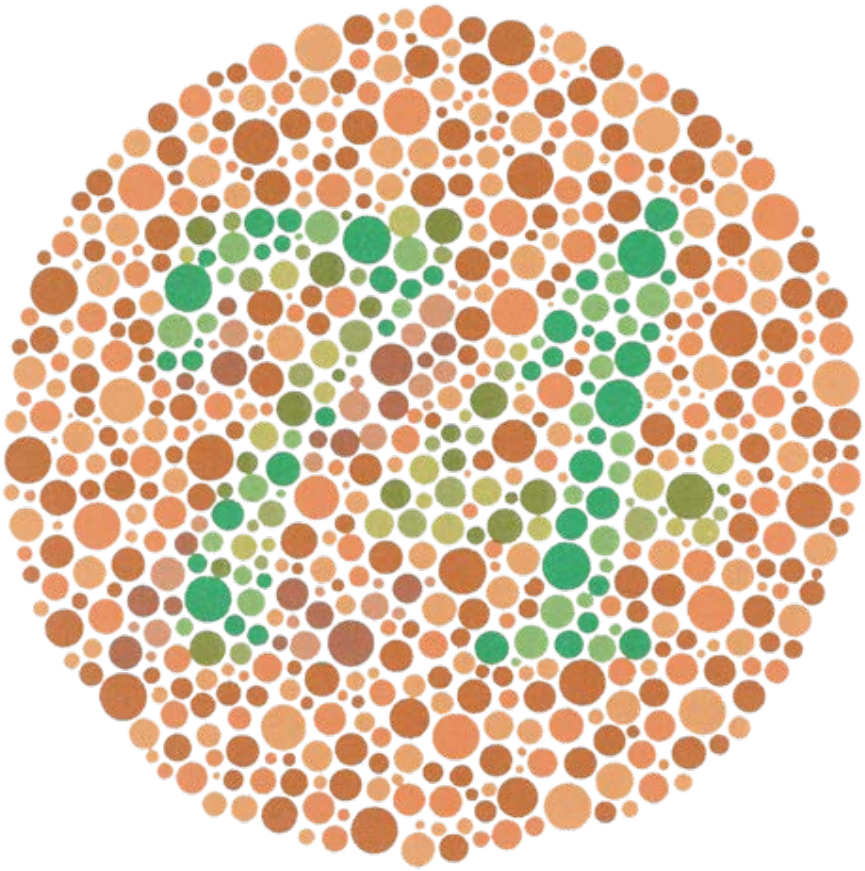
either her mother's chromosome pD or her father's Pd. Thus her red–green sensing will involve both the normal and the anomalous pigments for both colors. Such females are tetrachromats, since they require a mixture of four spectral lights to match an arbitrary light.

Blue–yellow color blindness

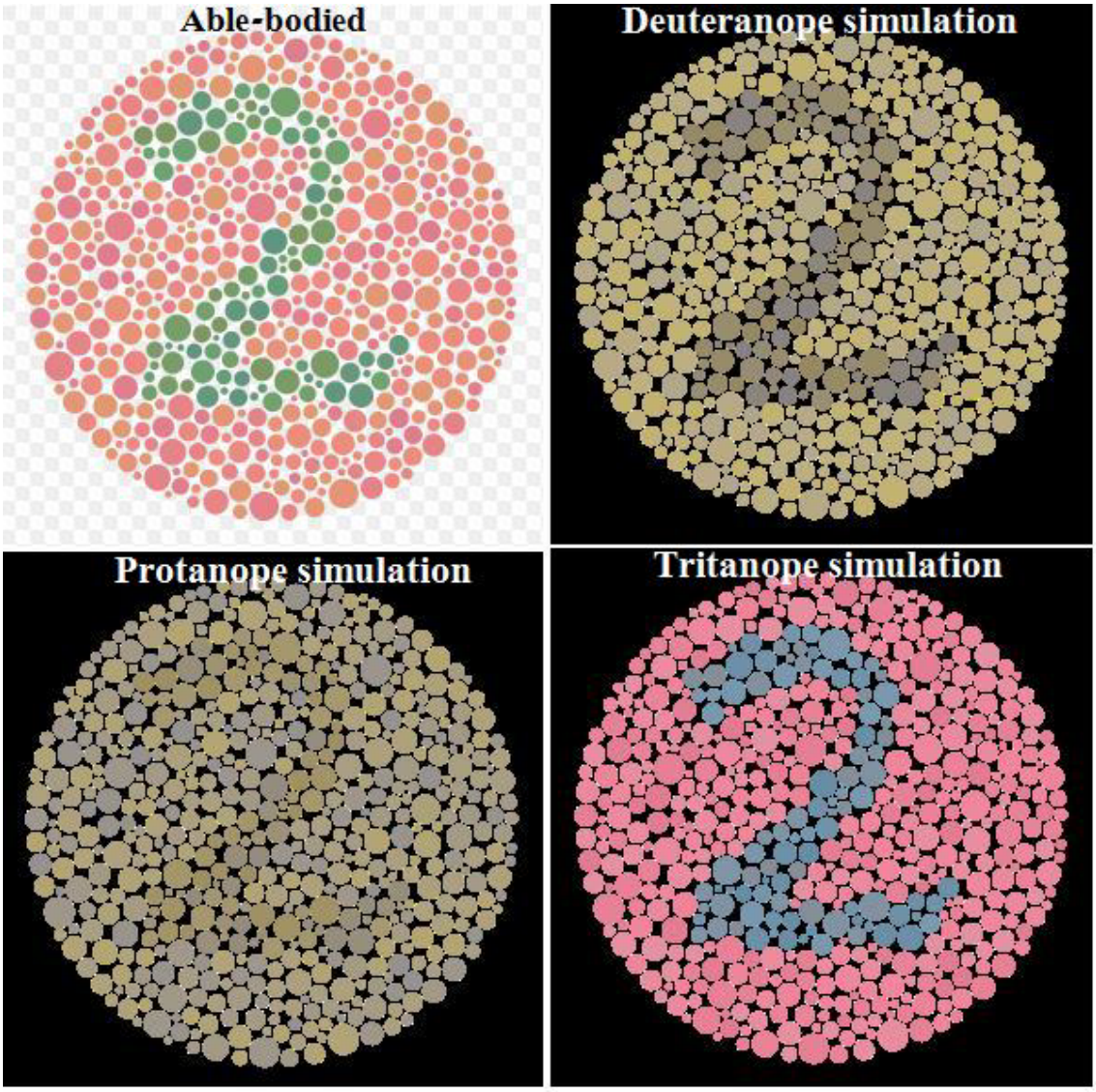
Those with tritanopia and tritanomaly have difficulty discriminating blueish versus yellowish hues.

Color blindness involving the inactivation of the short-wavelength sensitive cone system (whose absorption spectrum peaks in the bluish-violet) is called **tritanopia** or, loosely, blue–yellow color blindness. The tritanopes neutral point occurs near a yellowish 570 nm; green is perceived at shorter wavelengths and red at longer wavelengths. Mutation of the short-wavelength sensitive cones is called **tritanomaly**. Tritanopia is equally distributed among males and females. Jeremy H. Nathans (with the Howard Hughes Medical Institute) proved that the gene coding for the blue receptor lies on chromosome 7, which is shared equally by males and females. Therefore it is not sex-linked. This gene does not have any neighbor whose DNA sequence is similar. Blue color blindness is caused by a simple mutation in this gene.

Diagnosis



Example of an Ishihara color test plate. The numeral "74" should be clearly visible to viewers with normal color vision. Viewers with dichromacy or anomalous trichromacy may read it as "21", and viewers with achromatopsia may not see numbers.



An Ishihara test image as seen by subjects with normal color vision and by those with a variety of color deficiencies

The Ishihara color test, which consists of a series of pictures of colored spots, is the test most often used to diagnose red–green color deficiencies. A figure (usually one or more Arabic digits) is embedded in the picture as a number of spots in a slightly different color, and can be seen with normal color vision, but not with a particular color defect. The full set of tests has a variety of figure/background color combinations, and enable diagnosis of which particular visual defect is present. The anomaloscope, described above, is also used in diagnosing anomalous trichromacy.

Because the Ishihara color test contains only numerals, it may not be useful in diagnosing young children, who have not yet learned to use numerals. In the interest of identifying these problems early on in life, alternative color vision tests were developed using only symbols (square, circle, car).

Besides the Ishihara color test, the US Navy and US Army also allow testing with the Farnsworth Lantern Test. This test allows 30% of color deficient individuals, whose deficiency is not too severe, to pass.

Most clinical tests are designed to be fast, simple, and effective at identifying broad categories of color blindness. In academic studies of color blindness, on the other hand, there is more interest in developing flexible tests to collect thorough datasets, identify copunctal points, and measure just noticeable differences.

Management

There is generally no treatment to cure color deficiencies. However, certain types of tinted filters and contact lenses may help an individual to better distinguish different colors. Optometrists can supply a singular red-tint contact lens to wear on the non-dominant eye. This may enable the wearer to pass some color blindness tests, but they have little practical use. The effect of wearing such a device is akin to wearing red/blue 3D glasses and can take some time getting used to as certain wavelengths can "jump" out and be overly represented. Additionally, computer software and cybernetic devices have been developed to assist those with visual color difficulties such as an eyeborg, a "cybernetic eye" that allows individuals with color blindness to hear sounds representing colors.

The GNOME desktop environment provides colorblind accessibility using the gnome-mag and the libcolorblind software. Using a gnome applet, the user may switch a color filter on and off choosing from a set of possible color transformations that will displace the colors in order to disambiguate them. The software enables, for instance, a color blind person to see the numbers in the Ishihara test.

In September 2009, the journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys, which normally have only dichromatic vision, using gene therapy.

Epidemiology

Color blindness affects a significant number of people, although exact proportions vary among groups. In Australia, for example, it occurs in about 8 percent of males and only about 0.4 percent of females. Isolated communities with a restricted gene pool sometimes produce high proportions of color blindness, including the less usual types. Examples include rural Finland, Hungary, and some of the Scottish islands. In the United States, about 7 percent of the male population – or about 10.5 million men – and 0.4 percent of the female population either cannot distinguish red from green, or see red and green differently from how others do (Howard Hughes Medical Institute, 2006). It has been found that more than 95 percent of all variations in human color vision involve the red and green receptors in male eyes. It is very rare for males or females to be "blind" to the blue end of the spectrum.

Prevalence of color blindness

	Males	Females	Total	References
Overall	—	—	—	
Overall (United States)	—	—	—	
Red–green (Overall)	7 to 10%	—	—	
Red–green (Caucasians)	8%	—	—	
Red–green (Asians)	5%	—	—	
Red–green (Africans)	4%	—	—	
Monochromacy	—	—	—	
Rod monochromacy (dysfunctional, abnormally shaped or no cones)	0.00001%	0.00001%	—	
Dichromacy	2.4%	0.03%	1.30%	
Protanopia (red deficient: L cone absent)	1% to 1.3%	0.02%	—	
Deuteranopia (green deficient: M cone absent)	1% to 1.2%	0.01%	—	
Tritanopia (blue deficient: S cone absent)	0.001%	0.03%	—	
Anomalous Trichromacy	6.3%	0.37%	—	
Protanomaly (red deficient: L cone defect)	1.3%	0.02%	—	
Deuteranomaly (green deficient: M cone defect)	5.0%	0.35%	—	
Tritanomaly (blue deficient: S cone defect)	0.01%	0.01%	—	

Society and culture

Design implications of color blindness

Color codes present particular problems for those with color deficiences as they are often difficult or impossible for them to perceive.

Good graphic design avoids using color coding or using color contrasts alone to express information; this not only helps color blind people, but also aids understanding by normally sighted people.

Designers need to take into account that color-blindness is highly sensitive to differences in material. For example, a red–green colorblind person who is incapable of distinguishing colors on a map printed on paper may have no such difficulty when viewing the map on a computer screen or television. In addition, some color blind people find it easier to distinguish problem colors on artificial materials, such as plastic or in acrylic paints, than on natural materials, such as paper or wood. Third, for some color blind people, color can only be distinguished if there is a sufficient "mass" of color: thin

lines might appear black while a thicker line of the same color can be perceived as having color.

When the need to process visual information as rapidly as possible arises, for example in an emergency situation, the visual system may operate only in shades of gray, with the extra information load in adding color being dropped. This is an important possibility to consider when designing, for example, emergency brake handles or emergency phones.

Occupations

Color blindness may make it difficult or impossible for a person to engage in certain occupations. Persons with color blindness may be legally or practically barred from occupations in which color perception is an essential part of the job (*e.g.,* mixing paint colors), or in which color perception is important for safety (*e.g.,* operating vehicles in response to color-coded signals). This occupational safety principle originates from the Lagerlunda train crash of 1875 in Sweden. Following the crash, Professor Alarik Frithiof Holmgren, a physiologist, investigated and concluded that the color blindness of the engineer (who had died) had caused the crash. Professor Holmgren then created the first test using different-colored skeins to exclude people from jobs in the transportation industry on the basis of color blindness.

Driving motor vehicles

Some countries (e.g. Bulgaria, Romania and Turkey) have refused to grant individuals with color blindness driving licenses. In Romania, there is an ongoing campaign to remove the legal restrictions that prohibit colorblind citizens from getting drivers' licenses.

The usual justification for such restrictions is that drivers of motor vehicles must be able to recognize color-coded signals, such as traffic lights or warning lights.

Piloting aircraft

While many aspects of aviation depend on color coding, only a few of them are critical enough to be interfered with by some milder types of color blindness. Some examples include color-gun signaling of aircraft that have lost radio communication, color-coded glide-path indications on runways, and the like. Some jurisdictions restrict the issuance of pilot credentials to persons who suffer from color blindness for this reason. Restrictions may be partial, allowing color-blind persons to obtain certification but with restrictions, or total, in which case color-blind persons are not permitted to obtain piloting credentials at all.

In the United States, the Federal Aviation Administration requires that pilots be tested for normal color vision as part of the medical certification that is prerequisite to obtaining a pilot's license. If testing reveals color blindness, the applicant may be issued a license with restrictions, such as no night flying and no flying by color signals—such a

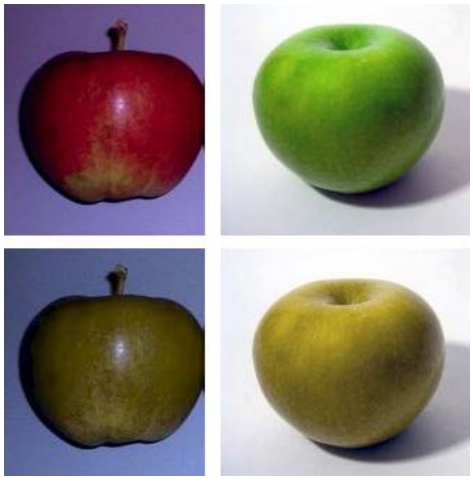
restriction effectively prevents a pilot from working for an airline. The government allows several types of tests, including medical standard tests (*e.g.,* the Ishihara, Dvorine, and others) and specialized tests oriented specifically to the needs of aviation. If an applicant fails the standard tests, he or she will receive a restriction on their medical certificate that states- "Not valid for night flying or by color signal control." He/she may apply to the FAA to take a specialized test, administered by the FAA. Typically, this test is the "color vision light gun test." For this test an FAA inspector will meet the pilot at an airport with an operating control tower, and the color signal light gun will be shone at the pilot from the tower, and he or she must identify the color. If he passes, he may be issued a waiver, which states that the color vision test is no longer required during medical examinations. He will then receive a new medical certificate with the restriction removed. This was once a Statement of Demonstrated Ability (SODA), but the SODA was dropped, and converted to a simple waiver (letter) early in the 2000s.

Research published in 2009 carried out by the City University of London's Applied Vision Research Centre, sponsored by the UK's Civil Aviation Authority and the US Federal Aviation Administration, has established a more accurate assessment of colour deficiencies in pilot applicants' red–green and yellow–blue colour range which could lead to a 35% reduction in the number of prospective pilots who fail to meet the minimum medical threshold.

Art

Inability to distinguish color does not necessarily preclude the ability to become a celebrated artist. The expressionist painter Clifton Pugh, three-time winner of Australia's Archibald Prize, on biographical, gene inheritance and other grounds has been identified as a protanope. Nineteenth century French artist Charles Méryon became successful by concentrating on etching rather than painting after he was diagnosed as having a red–green deficiency.

Misconceptions and compensations



Simulation of the normal (above) and dichromatic (below) perception of red and green apples.

Color blindness is not the swapping of colors by the observer — grass is never red, and stop signs are never green. The color impaired do not learn to call red "green" and vice versa. However, dichromats often confuse red and green items. For example, they may find it difficult to distinguish a Braeburn apple from a Granny Smith and in some cases, the red and green of a traffic light without other clues (e.g., shape or location). The vision of dichromats may also be compared to images produced by a color printer that has run out of the ink in one of its three color cartridges (for protanopes and deuteranopes, the red cartridge, and for tritanopes, the yellow cartridge).

Dichromats tend to learn to use texture and shape clues and so are often able to penetrate camouflage that has been designed to deceive individuals with color-normal vision.

Traffic light colors are confusing to some dichromats as there is insufficient apparent difference between the red/amber traffic lights, and that of sodium street lamps; also the green can be confused with a grubby white lamp. This is a risk factor on high-speed undulating roads where angular cues can't be used. British Rail color lamp signals use more easily identifiable colors: the red is really blood red, the amber is quite yellow and the green is a bluish color. Most British road traffic lights are mounted vertically on a black rectangle with a white border (forming a "sighting board") and so dichromats simply look for the position of the light within the rectangle — top, middle or bottom. In the Eastern provinces of Canada horizontally-mounted traffic lights are generally differentiated by shape to facilitate identification for those with color blindness.

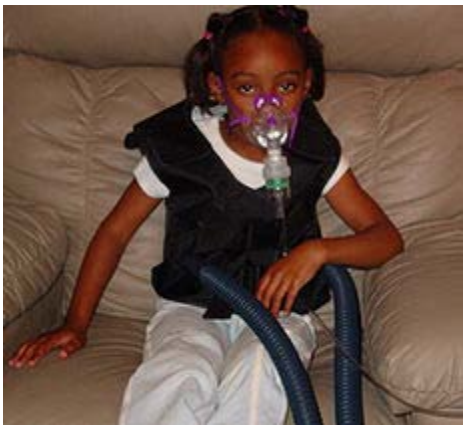
Horizontal traffic light in Halifax, NS Canada

Color blindness very rarely means complete monochromatism. In almost all cases, color blind people retain blue–yellow discrimination, and most color-blind individuals are anomalous trichromats rather than complete dichromats. In practice this means that they often retain a limited discrimination along the red–green axis of color space, although their ability to separate colors in this dimension is severely reduced.

Chapter 12

Cystic Fibrosis

Cystic fibrosis



A breathing treatment for cystic fibrosis, using a mask nebuliser and a ThAIRapy Vest

ICD-10	E84.
ICD-9	277.0
OMIM	219700
DiseasesDB	3347
MedlinePlus	000107
eMedicine	ped/535
MeSH	D003550

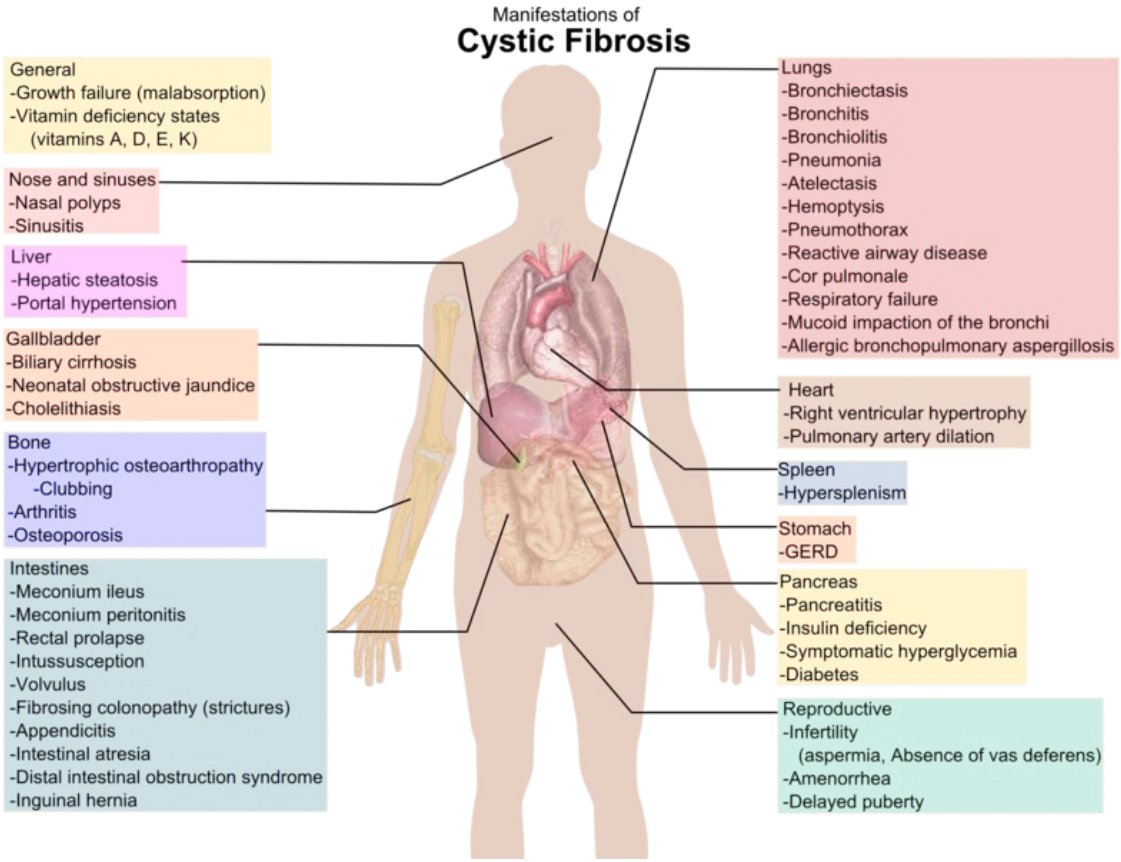
Cystic fibrosis (also known as **CF** or **mucoviscidosis**) is a common recessive genetic disease which affects the entire body, causing progressive disability and often early death. The name *cystic fibrosis* refers to the characteristic scarring (fibrosis) and cyst formation within the pancreas, first recognized in the 1930s. Difficulty breathing is the most serious symptom and results from frequent lung infections that are treated with, though not cured by, antibiotics and other medications. A multitude of other symptoms,

including sinus infections, poor growth, diarrhea, and infertility result from the effects of CF on other parts of the body.

CF is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). This gene is required to regulate the components of sweat, digestive juices, and mucus. Although most people without CF have two working copies of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither gene works normally. Therefore, CF is considered an autosomal recessive disease.

CF is most common among Caucasians; one in 25 people of European descent carry one gene for CF. Approximately 30,000 Americans have CF, making it one of the most common life-shortening inherited diseases. Individuals with cystic fibrosis can be diagnosed before birth by genetic testing, or by a sweat test in early childhood. Ultimately, lung transplantation is often necessary as CF worsens.

Signs and symptoms



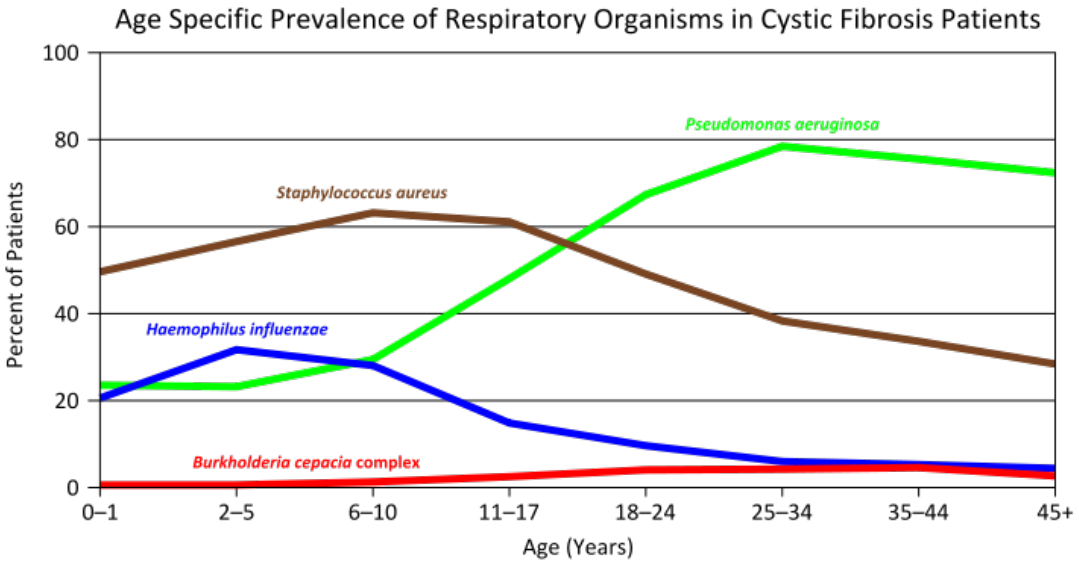
A diagram showing clinical manifestations of cystic fibrosis

The hallmark symptoms of cystic fibrosis are salty tasting skin, poor growth and poor weight gain despite a normal food intake, accumulation of thick, sticky mucus, frequent chest infections and coughing or shortness of breath. Males can be infertile due to

congenital absence of the vas deferens. Symptoms often appear in infancy and childhood, such as bowel obstruction due to meconium ileus in newborn babies. As the child grows, he or she will need to exercise to release mucus in the alveoli. Ciliated epithelial cells in the patient have a mutated protein that leads to abnormally viscous mucus production. The poor growth in children typically presents as an inability to gain weight or height at the same rate as their peers and is occasionally not diagnosed until investigation is initiated for poor growth. The causes of growth failure are multi-factorial and include chronic lung infection, poor absorption of nutrients through the gastrointestinal tract, and increased metabolic demand due to chronic illness.

In rare cases, cystic fibrosis can manifest itself as a coagulation disorder. Young children are especially sensitive to vitamin K malabsorptive disorders because only a very small amount of vitamin K crosses the placenta, leaving the child with very low reserves. Because factors II, VII, IX, and X (clotting factors) are vitamin K–dependent, low levels of vitamin K can result in coagulation problems. Consequently, when a child presents with unexplained bruising, a coagulation evaluation may be warranted to determine whether there is an underlying disease.

Lung and sinus



Respiratory infections in CF patients varies according to age

Green = *Pseudomonas aeruginosa*
Brown = *Staphylococcus aureus*
Blue = *Haemophilus influenzae*
Red = *Burkholderia cepacia* complex

Lung disease results from clogging of the airways due to mucus build-up, decreased mucociliary clearance and resulting inflammation. Inflammation and infection will cause injury and structural changes to the lungs, leading to a variety of symptoms. In the early

stages, incessant coughing, copious phlegm production, and decreased ability to exercise are common. Many of these symptoms occur when bacteria that normally inhabit the thick mucus grow out of control and cause pneumonia. In later stages, changes in the architecture of the lung such as pathology in the major airways (bronchiectasis) further exacerbate difficulties in breathing. Other symptoms include coughing up blood (hemoptysis), high blood pressure in the lung (pulmonary hypertension), heart failure, difficulties getting enough oxygen to the body (hypoxia), and respiratory failure requiring support with breathing masks such as bilevel positive airway pressure machines or ventilators. *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* are the three most common organisms causing lung infections in CF patients. In addition to typical bacterial infections, people with CF more commonly develop other types of lung disease. Among these is allergic bronchopulmonary aspergillosis, in which the body's response to the common fungus *Aspergillus fumigatus* causes worsening of breathing problems. Another is infection with *Mycobacterium avium* complex (MAC), a group of bacteria related to tuberculosis, which can cause a lot of lung damage and does not respond to common antibiotics.

Mucus in the paranasal sinuses is equally thick and may also cause blockage of the sinus passages, leading to infection. This may cause facial pain, fever, nasal drainage, and headaches. Individuals with CF may develop overgrowth of the nasal tissue (nasal polyps) due to inflammation from chronic sinus infections. Recurrent sinonasal polyps can occur in as many as 10% to 25% of CF patients. These polyps can block the nasal passages and increase breathing difficulties.

Cardiorespiratory complications are the most common cause of death (~80%) in patients followed by most CF centers in the United States.

Gastrointestinal

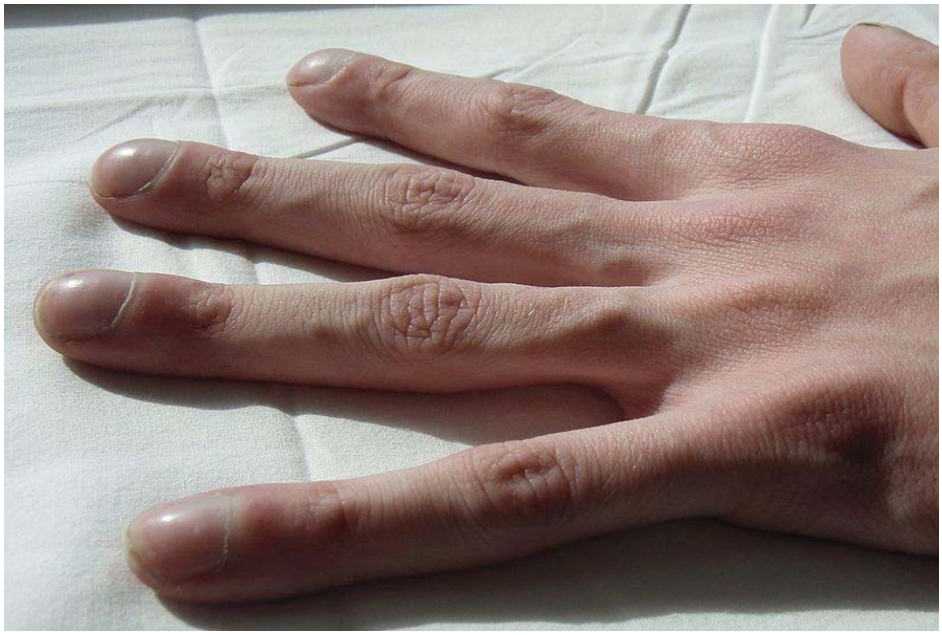
Prior to prenatal and newborn screening, cystic fibrosis was often diagnosed when a newborn infant failed to pass faeces (meconium). Meconium may completely block the intestines and cause serious illness. This condition, called meconium ileus, occurs in 5–10% of newborns with CF. In addition, protrusion of internal rectal membranes (rectal prolapse) is more common, occurring in as many as 10% of children with CF, and it is caused by increased fecal volume, malnutrition, and increased intra–abdominal pressure due to coughing.

The thick mucus seen in the lungs has a counterpart in thickened secretions from the pancreas, an organ responsible for providing digestive juices which help break down food. These secretions block the exocrine movement of the digestive enzymes into the duodenum and result in irreversible damage to the pancreas, often with painful inflammation (pancreatitis). The pancreatic ducts are totally plugged in more advanced cases, usually seen in older children or adolescents. This causes atrophy of the exocrine glands and progressive fibrosis. The lack of digestive enzymes leads to difficulty absorbing nutrients with their subsequent excretion in the feces, a disorder known as malabsorption. Malabsorption leads to malnutrition and poor growth and development

because of calorie loss. Resultant hypoproteinemia may be severe enough to cause generalized edema. Individuals with CF also have difficulties absorbing the fat-soluble vitamins A, D, E, and K. In addition to the pancreas problems, people with cystic fibrosis experience more heartburn, intestinal blockage by intussusception, and constipation. Older individuals with CF may develop distal intestinal obstruction syndrome when thickened feces cause intestinal blockage. Exocrine pancreatic insufficiency occurs in the majority (85% to 90%) of patients with CF. It is mainly associated with "severe" CFTR mutations, where both alleles are completely nonfunctional (e.g. ΔF508/ΔF508). It occurs in 10% to 15% of patients with one "severe" and one "mild" CFTR mutation where there still is a little CFTR activity, or where there are two "mild" CFTR mutations. In these milder cases, there is still sufficient pancreatic exocrine function so that enzyme supplementation is not required. There are usually no other GI complications in pancreas-sufficient phenotypes, and in general, such individuals usually have excellent growth and development. Despite this, idiopathic chronic pancreatitis can occur in a subset of pancreas-sufficient individuals with CF, and is associated with recurrent abdominal pain and life-threatening complications.

Thickened secretions also may cause liver problems in patients with CF. Bile secreted by the liver to aid in digestion may block the bile ducts, leading to liver damage. Over time, this can lead to scarring and nodularity (cirrhosis). The liver fails to rid the blood of toxins and does not make important proteins such as those responsible for blood clotting. Liver disease is the third most common cause of death associated with CF.

Endocrine



Clubbing of the fingers in a person with cystic fibrosis

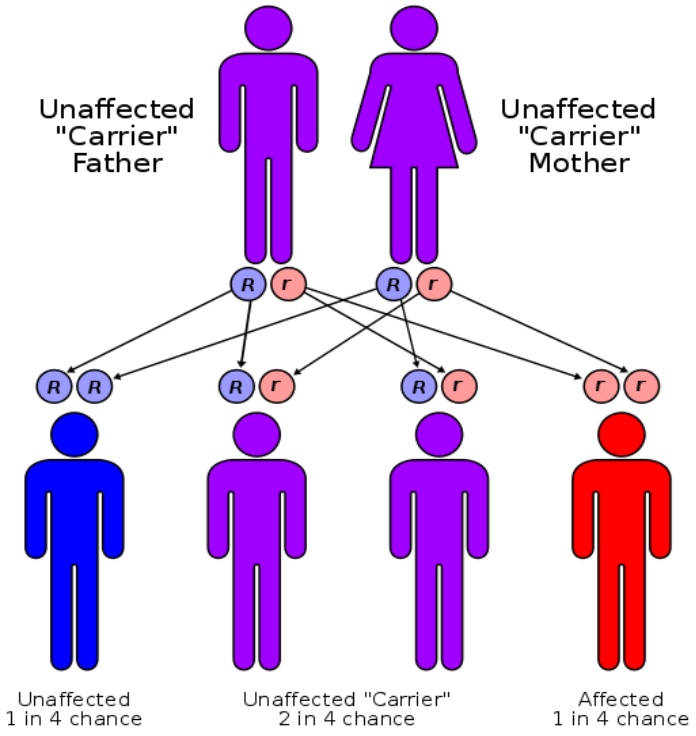
The pancreas contains the islets of Langerhans, which are responsible for making insulin, a hormone that helps regulate blood glucose. Damage of the pancreas can lead to loss of

the islet cells, leading to a type of diabetes that is unique to those with the disease. This cystic fibrosis related diabetes (CFRD) shares characteristics that can be found in Type 1 and Type 2 diabetics, and is one of the principal non-pulmonary complications of CF. Vitamin D is involved in calcium and phosphate regulation. Poor uptake of vitamin D from the diet because of malabsorption can lead to the bone disease osteoporosis in which weakened bones are more susceptible to fractures. In addition, people with CF often develop clubbing of their fingers and toes due to the effects of chronic illness and low oxygen in their tissues.

Infertility

Infertility affects both men and women. At least 97% of men with cystic fibrosis are infertile, but not sterile and can have children with assisted reproductive techniques. These men make normal sperm but are missing the tube (vas deferens), which connects the testes to the ejaculatory ducts of the penis. Many men found to have congenital absence of the vas deferens during evaluation for infertility have a mild, previously undiagnosed form of CF. Some women have fertility difficulties due to thickened cervical mucus or malnutrition. In severe cases, malnutrition disrupts ovulation and causes amenorrhea.

Cause



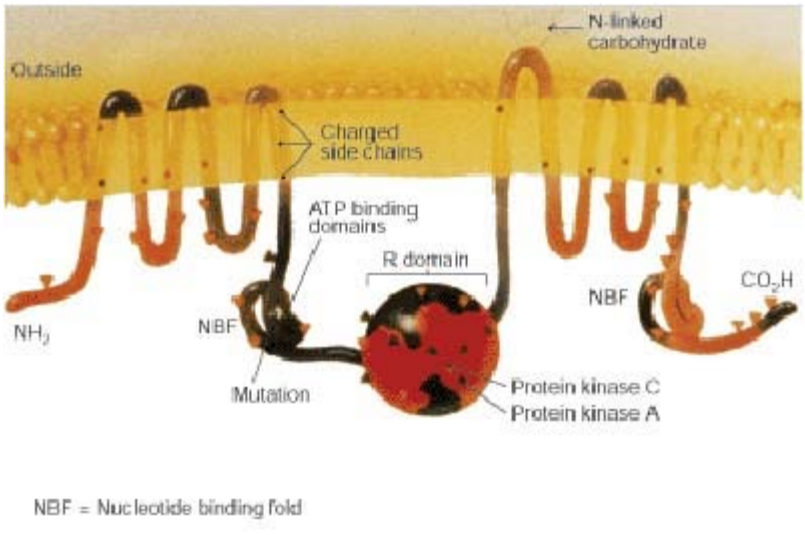
Cystic fibrosis has an autosomal recessive pattern of inheritance

CF is caused by a mutation in the gene cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, ΔF508, is a deletion (Δ) of three nucleotides that results in a loss of the amino acid phenylalanine (F) at the 508th (508) position on the protein. This mutation accounts for two-thirds (66-70%) of CF cases worldwide and 90 percent of cases in the United States; however, there are over 1,400 other mutations that can produce CF. Although most people have two working copies (alleles) of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither allele can produce a functional CFTR protein. Thus, CF is considered an autosomal recessive disease.

The CFTR gene, found at the q31.2 locus of chromosome 7, is 230,000 base pairs long, and creates a protein that is 1,480 amino acids long. Structurally, CFTR is a type of gene known as an ABC gene. The product of this gene (the CFTR) is a halide anion channel important in creating sweat, digestive juices and mucus. This protein possesses two ATP-hydrolyzing domains which allows the protein to use energy in the form of ATP. It also contains two domains comprising 6 alpha helices apiece, which allow the protein to cross the cell membrane. A regulatory binding site on the protein allows activation by phosphorylation, mainly by cAMP-dependent protein kinase. The carboxyl terminal of the protein is anchored to the cytoskeleton by a PDZ domain interaction.

In addition, there is increasing evidence that genetic modifiers besides CFTR modulate the frequency and severity of the disease. One example is mannan-binding lectin, which is involved in innate immunity by facilitating phagocytosis of microorganisms. Polymorphisms in one or both mannan-binding lectin alleles that result in lower circulating levels of the protein are associated with a threefold higher risk of end-stage lung disease, as well as an increased burden of chronic bacterial infections.

Pathophysiology



Molecular structure of the CFTR protein

There are several mechanisms by which mutations cause problems with the CFTR protein. ΔF508, for instance, creates a protein that does not fold normally and is degraded by the cell. Several different mutations result in proteins that are too short because production is ended prematurely. Less common mutations produce proteins that do not use energy normally, do not allow chloride, iodide and thiocyanate to cross the membrane appropriately, or are degraded at a faster rate than normal. Mutations may also lead to fewer copies of the CFTR protein being produced.

The protein created by this gene is anchored to the outer membrane of cells in the sweat glands, lungs, pancreas, and other affected organs. The protein spans this membrane and acts as a channel connecting the inner part of the cell (cytoplasm) to the surrounding fluid. This channel is primarily responsible for controlling the movement of halogens from inside to outside of the cell; however, in the sweat ducts it facilitates the movement of chloride from the sweat into the cytoplasm. When the CFTR protein does not work, chloride and thiocyanate are trapped inside the cells in the airway and outside in the skin. Then hypothiocyanite, OSCN, cannot be produced by immune defense system. Because chloride is negatively charged, this creates a difference in the electrical potential inside and outside the cell causing cations to cross into the cell. Sodium is the most common cation in the extracellular space and the combination of sodium and chloride creates the salt, which is lost in high amounts in the sweat of individuals with CF. This lost salt forms the basis for the sweat test.

How this malfunction of cells in cystic fibrosis causes the clinical manifestations is not well understood. One theory suggests that the lack of halogen and pseudohalogen (mainly, chloride, iodide and thiocyanate) exodus through the CFTR protein leads to the accumulation of more viscous, nutrient-rich mucus in the lungs that allows bacteria to hide from the body's immune system. Another theory proposes that the CFTR protein failure leads to a paradoxical increase in sodium and chloride uptake, which, by leading to increased water reabsorption, creates dehydrated and thick mucus. Yet another theory focuses on abnormal chloride movement *out* of the cell, which also leads to dehydration of mucus, pancreatic secretions, biliary secretions, etc. These theories all support the observation that the majority of the damage in CF is due to blockage of the narrow passages of affected organs with thickened secretions. These blockages lead to remodeling and infection in the lung, damage by accumulated digestive enzymes in the pancreas, blockage of the intestines by thick faeces, etc.

Chronic infections

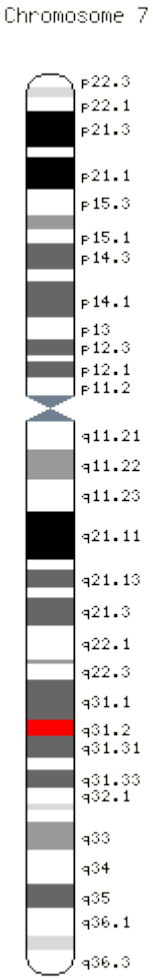
The lungs of individuals with cystic fibrosis are colonized and infected by bacteria from an early age. These bacteria, which often spread among individuals with CF, thrive in the altered mucus, which collects in the small airways of the lungs. This mucus leads to the formation of bacterial microenvironments known as biofilms that are difficult for immune cells and antibiotics to penetrate. Viscous secretions and persistent respiratory infections repeatedly damage the lung by gradually remodeling the airways which makes infection even more difficult to eradicate.

Over time, both the types of bacteria and their individual characteristics change in individuals with CF. In the initial stage, common bacteria such as *Staphylococcus aureus* and *Hemophilus influenzae* colonize and infect the lungs. Eventually, *Pseudomonas aeruginosa* (and sometimes *Burkholderia cepacia*) dominates. By 18 years of age, 80% of patients with classic CF harbor *P. aeruginosa*, and 3.5% harbor *B. cepacia*. Once within the lungs, these bacteria adapt to the environment and develop resistance to commonly used antibiotics. *Pseudomonas* can develop special characteristics that allow the formation of large colonies, known as "mucoid" *Pseudomonas*, which are rarely seen in people that do not have CF.

One way in which infection has spread is by passage between different individuals with CF. In the past, people with CF often participated in summer "CF Camps" and other recreational gatherings. Hospitals grouped patients with CF into common areas and routine equipment (such as nebulizers) was not sterilized between individual patients. This led to transmission of more dangerous strains of bacteria among groups of patients. As a result, individuals with CF are routinely isolated from one another in the healthcare setting and healthcare providers are encouraged to wear gowns and gloves when examining patients with CF to limit the spread of virulent bacterial strains.

CF patients may also have their airways chronically colonized by filamentous fungi (such as *Aspergillus fumigatus*, *Scedosporium apiospermum*, *Aspergillus terreus*) and/or yeasts (such as *Candida albicans*); other filamentous fungi less commonly isolated include *Aspergillus flavus* and *Aspergillus nidulans* (occur transiently in CF respiratory secretions), and *Exophiala dermatitidis* and *Scedosporium prolificans* (chronic airway-colonizers); some filamentous fungi like *Penicillium emersonii* and *Acrophialophora fusispora* are encountered in patients almost exclusively in the context of CF. Defective mucociliary clearance characterizing CF is associated with local immunological disorders. In addition, the prolonged therapy with antibiotics and the use of corticosteroid treatments may also facilitate fungal growth. Although the clinical relevance of the fungal airway colonization is still a matter of debate, filamentous fungi may contribute to the local inflammatory response, and therefore to the progressive deterioration of the lung function, as often happens with allergic broncho-pulmonary aspergillosis (ABPA) - the most common fungal disease in the context of CF, involving a Th2-driven immune response to Aspergillus.

Diagnosis and monitoring



The location of the CFTR gene on chromosome 7

Cystic fibrosis may be diagnosed by many different categories of testing including those such as, newborn screening, sweat testing, or genetic testing. As of 2006 in the United States, 10 percent of cases are diagnosed shortly after birth as part of newborn screening programs. The newborn screen initially measures for raised blood concentration of immunoreactive trypsinogen. Infants with an abnormal newborn screen need a sweat test in order to confirm the CF diagnosis. In many cases, a parent makes the diagnosis because the infant tastes salty. Trypsinogen levels can be increased in individuals who have a single mutated copy of the *CFTR* gene (carriers) or, in rare instances, in individuals with two normal copies of the *CFTR* gene. Due to these false positives, CF screening in newborns can be controversial. Most states and countries do not screen for CF routinely at birth. Therefore, most individuals are diagnosed after symptoms (e.g. sinopulmonary disease and GI manifestations) prompt an evaluation for cystic fibrosis. The most commonly used form of testing is the sweat test. Sweat-testing involves application of a medication that stimulates sweating (pilocarpine). In order to deliver the medication through the skin, iontophoresis is used to, whereby one electrode is placed

onto the applied medication and an electric current is passed to a separate electrode on the skin. The resultant sweat is then collected on filter paper or in a capillary tube and analyzed for abnormal amounts of sodium and chloride. People with CF have increased amounts of sodium and chloride in their sweat. In opposite, people with CF have less thiocyanate and hypothiocyanite in their saliva (Minarowski et al.) and mucus (Banfi et al.). CF can also be diagnosed by identification of mutations in the CFTR gene.

A multitude of tests are used to identify complications of CF and to monitor disease progression. X-rays and CAT scans are used to examine the lungs for signs of damage or infection. The examination of the sputum is required to isolate organisms which may be causing an infection or colonising the lower respiratory tract so that effective antimicrobial therapy can be provided. Culture for organisms such as *Burkholderia (previously Pseudomonas) cepacia* is required for candidates of Lung transplantation as persistent bacterial colonisation reduces the chances of survival.

Pulmonary function tests measure how well the lungs are functioning, and are used to measure the need for and response to antibiotic therapy. Blood tests can identify liver abnormalities, vitamin deficiencies, and the onset of diabetes. DXA scans can screen for osteoporosis and testing for fecal elastase can help diagnose insufficient digestive enzymes.

In individuals with a mild mutation in the CFTR gene the sweat test may be near normal (i.e. a chloride concentration of less than 60mM/L). As an adjunct to diagnosis, the nasal transepithelial potential difference (TEPD) may be used. Due to abnormalities in the CFTR gene in exocrine glands, chloride secretion is reduced and sodium and water reabsorption is increased. The net effect of the preceding is a more negative baseline resulting in a higher than normal TEPD that can be used as an ancillary or necessary form of diagnosis for mild mutations.

People with CF may be listed in a disease registry that allows researchers and doctors to track health results and identify candidates for clinical trials.

Prenatal

Couples who are pregnant or who are planning a pregnancy can themselves be tested for CFTR gene mutations to determine the degree of risk that their child will be born with cystic fibrosis. Testing is typically performed first on one or both parents and, if the risk of CF is found to be high, testing on the fetus can then be performed. The American College of Obstetricians and Gynecologists (ACOG) recommends testing for couples who have a personal or close family history of CF, and they recommend that carrier testing be offered to all Caucasian couples and be made available to couples of other ethnic backgrounds.

Because development of CF in the fetus requires each parent to pass on a mutated copy of the CFTR gene and because CF testing is expensive, testing is often performed initially on one parent. If that parent is found to be a carrier of a CFTR gene mutation, the other

parent is then tested to calculate the risk that their children will have CF. CF can result from more than a thousand different mutations, and as of 2006 it is not possible to test for each one. Testing analyzes the blood for the most common mutations such as ΔF508—most commercially available tests look for 32 or fewer different mutations. If a family has a known uncommon mutation, specific screening for that mutation can be performed. Because not all known mutations are found on current tests, a negative screen does not guarantee that a child will not have CF. In addition, because the mutations tested are necessarily those most common in the highest risk groups, testing in lower risk ethnicities is less successful because the mutations commonly seen in these groups are less common in the general population. These couples may therefore consider testing through labs that offer CF screens with a high number of mutations tested.

Couples at high risk for having a child with CF will often opt to perform further testing before or during pregnancy. In vitro fertilization with preimplantation genetic diagnosis offers the possibility to examine the embryo prior to its placement into the uterus. The test, performed three days after fertilization, looks for the presence of abnormal CF genes. If two mutated CFTR genes are identified, the embryo is not used for embryo transfer and an embryo with at least one normal gene is implanted.

During pregnancy, testing can be performed on the placenta (chorionic villus sampling) or the fluid around the fetus (amniocentesis). However, chorionic villus sampling has a risk of fetal death of 1 in 100 and amniocentesis of 1 in 200; a recent study has indicated this may be much lower, approximately 1 in 1,600. In any case, the benefits must be determined to outweigh these risks prior to going forward with testing. Alternatively, some couples choose to undergo third party reproduction with egg or sperm donors.

Economically, for carrier couples of cystic fibrosis, when comparing preimplantation genetic diagnosis (PGD) with natural conception (NC) followed by prenatal testing and abortion of affected pregnancies, PGD provides net economic benefits up to a maternal age of approximately 40 years, after which NC, prenatal testing and abortion has higher economic benefit.

Management

While there are no cures for cystic fibrosis there are several treatment methods. The management of cystic fibrosis has improved significantly over the past 70 years. While infants born with cystic fibrosis 70 years ago would have been unlikely to live beyond their first year, infants today are likely to live well into adulthood. Recent advances in the treatment of cystic fibrosis have meant that a cystic fibrosis person can live a fuller life less encumbered by their condition. The cornerstones of management are proactive treatment of airway infection, and encouragement of good nutrition and an active lifestyle. Management of cystic fibrosis continues throughout a patient's life, and is aimed at maximizing organ function, and therefore quality of life. At best, current treatments delay the decline in organ function. Because of the wide variation in disease symptoms treatment typically occurs at specialist multidisciplinary centers, and is tailored to the individual. Targets for therapy are the lungs, gastrointestinal tract (including pancreatic

enzyme supplements), the reproductive organs (including assisted reproductive technology (ART)) and psychological support.

The most consistent aspect of therapy in cystic fibrosis is limiting and treating the lung damage caused by thick mucus and infection, with the goal of maintaining quality of life. Intravenous, inhaled, and oral antibiotics are used to treat chronic and acute infections. Mechanical devices and inhalation medications are used to alter and clear the thickened mucus. These therapies, while effective, can be extremely time-consuming for the patient. One of the most important battles that CF patients face is finding the time to comply with prescribed treatments while balancing a normal life.

In addition, therapies such as transplantation and gene therapy aim to cure some of the effects of cystic fibrosis. Gene therapy aims to introduce normal CFTR to airway. Theoretically this process should be simple as the airway is easily accessible and there is only a single gene defect to correct. There are two CFTR gene introduction mechanisms involved, the first use of a viral vector (adenovirus, adeno-associated virus or retro virus) and secondly the use of liposome. However there are some problems associated with these methods involving efficiency (liposomes insufficient protein) and delivery (virus provokes an immune response).

Antibiotics

Many CF patients are on one or more antibiotics at all times, even when they are considered healthy, in order to prophylactically suppress infection. Antibiotics are absolutely necessary whenever pneumonia is suspected or there has been a noticeable decline in lung function, and are usually chosen based on the results of a sputum analysis and the patient's past response. Many bacteria common in cystic fibrosis are resistant to multiple antibiotics and require weeks of treatment with intravenous antibiotics such as vancomycin, tobramycin, meropenem, ciprofloxacin, and piperacillin. This prolonged therapy often necessitates hospitalization and insertion of a more permanent IV such as a peripherally inserted central catheter (PICC line) or Port-a-Cath. Inhaled therapy with antibiotics such as tobramycin, colistin, and cayston is often given for months at a time in order to improve lung function by impeding the growth of colonized bacteria. Oral antibiotics such as ciprofloxacin or azithromycin are given to help prevent infection or to control ongoing infection. The aminoglycoside antibiotics (e.g. tobramycin) used can cause hearing loss, damage to the balance system in the inner ear or kidney problems with long-term use. In order to prevent these side-effects, the amount of antibiotics in the blood are routinely measured and adjusted accordingly.

Other treatments for lung disease

Several mechanical techniques are used to dislodge sputum and encourage its expectoration. In the hospital setting, chest physiotherapy (CPT) is utilized; a respiratory therapist percusses an individual's chest with his or her hands several times a day, to loosen up secretions. Devices that recreate this percussive therapy include the ThAIRapy Vest and the intrapulmonary percussive ventilator (IPV). Newer methods such as

Biphasic Cuirass Ventilation, and associated clearance mode available in such devices, integrate a cough assistance phase, as well as a vibration phase for dislodging secretions. These are portable and adapted for home use. Physiotherapy is essential to help manage an individual's chest on a long term basis, and can also teach techniques for the older child and teenager to manage themselves at home. Aerobic exercise is of great benefit to people with cystic fibrosis. Not only does exercise increase sputum clearance but it also improves cardiovascular and overall health.

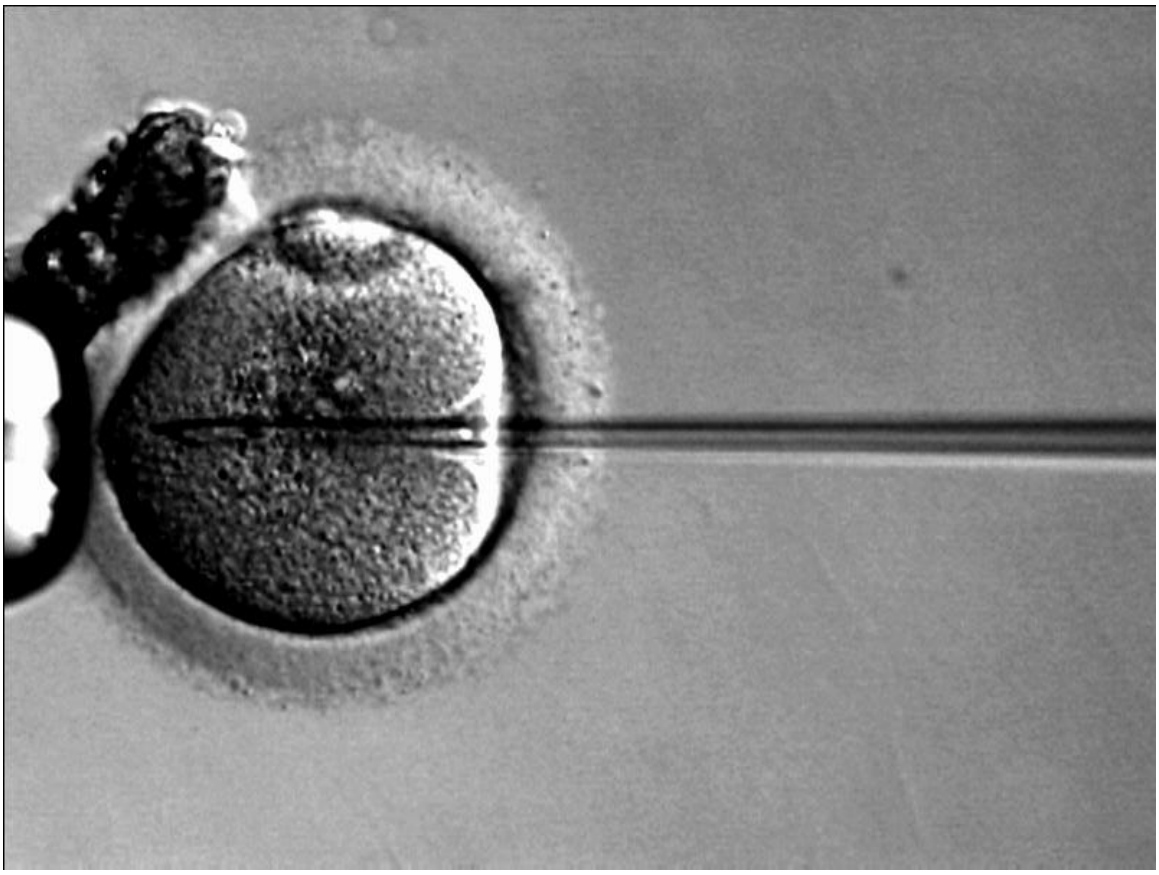
Aerosolized medications that help loosen secretions include dornase alfa and hypertonic saline. Dornase is a recombinant human deoxyribonuclease, which breaks down DNA in the sputum, thus decreasing its viscosity. N-Acetylcysteine may also decrease sputum viscosity, but research and experience have shown its benefits to be minimal. Salbutamol and ipratropium bromide are inhaled to increase the size of the small airways by relaxing the surrounding muscles.

As lung disease worsens, mechanical breathing support may become necessary. Individuals with CF may need to wear special masks at night that help push air into their lungs. These machines, known as bilevel positive airway pressure (BiPAP) ventilators, help prevent low blood oxygen levels during sleep. BiPAP may also be used during physical therapy to improve sputum clearance. During severe illness, a tube may be placed in the throat (a procedure known as a tracheostomy) to enable breathing supported by a ventilator.

Transplantation

Lung transplantation often becomes necessary for individuals with cystic fibrosis as lung function and exercise tolerance declines. Although single lung transplantation is possible in other diseases, individuals with CF must have both lungs replaced because the remaining lung might contain bacteria that could infect the transplanted lung. A pancreatic or liver transplant may be performed at the same time in order to alleviate liver disease and/or diabetes. Lung transplantation is considered when lung function declines to the point where assistance from mechanical devices is required or patient survival is threatened. This point typically occurs when lung function declines to approximately 20 to 30 percent, however there is a small time frame when transplantation is feasible as the patient must be healthy enough to endure the procedure.

Treatment of other aspects



Intracytoplasmic sperm injection can be used to provide fertility for men with cystic fibrosis

Newborns with CF (CYSTIC FIBROSIS) typically require surgery, whereas adults with distal intestinal obstruction syndrome typically do not. Treatment of pancreatic insufficiency by replacement of missing digestive enzymes allows the duodenum to properly absorb nutrients and vitamins that would otherwise be lost in the feces. Even so, most individuals with CF are advised take additional amounts of vitamins A, D, E, and K and eat high-calorie meals. So far, no large-scale research involving the incidence of atherosclerosis and coronary heart disease in adults with cystic fibrosis has been conducted. This is likely due to the fact that the vast majority of people with cystic fibrosis do not live long enough to develop clinically significant atherosclerosis or coronary heart disease.

Diabetes is the most common non-pulmonary complication of CF. It mixes features of type 1 and type 2 diabetes, and is recognized as a distinct entity, cystic fibrosis-related diabetes (CFRD). While oral anti-diabetic drugs are sometimes used, the only recommended treatment is the use of insulin injections or an insulin pump, and, unlike in type 1 and 2 diabetes, dietary restrictions are not recommended.

Development of osteoporosis can be prevented by increased intake of vitamin D and calcium, and can be treated by bisphosphonates, although adverse effects can be an issue. Poor growth may be avoided by insertion of a feeding tube for increasing calories through supplemental feeds or by administration of injected growth hormone.

Sinus infections are treated by prolonged courses of antibiotics. The development of nasal polyps or other chronic changes within the nasal passages may severely limit airflow through the nose, and over time reduce the patient's sense of smell. Sinus surgery is often used to alleviate nasal obstruction and to limit further infections. Nasal steroids such as fluticasone are used to decrease nasal inflammation. Female infertility may be overcome by assisted reproduction technology, particularly embryo transfer techniques. Male infertility caused by absence of the vas deferens may be overcome with testicular sperm extraction (TEST), collecting sperm cells directly from the testicles. If the collected sample contains too few sperm cells to likely have a spontaneous fertilization, intracytoplasmic sperm injection can be performed. Third party reproduction is also a possibility for women with CF.

Prognosis

The improved prognosis of cystic fibrosis, combined with earlier diagnosis through screening, has already started to result in a change in attitude. The hitherto scrawny, ill, infected CF infant who will die before adult life is increasingly being replaced by a fit individual, who has only ever had minimal if any symptoms, who happens to have a problem called CF. Many factors will influence the prognosis of a person with cystic fibrosis. These factors include treatment compliance, efficacy of treatment, and access to health care.

Life expectancy for people with CF depends largely upon access to health care. In 1959, the median age of survival of children with cystic fibrosis was six months. In the United States, the life expectancy for infants born in 2008 with CF is 37.4 years, based upon data compiled by the Cystic Fibrosis Foundation. The median survival age in Canada has increased from 24 in 1982 to 47.7 in 2007, based on data compiled by the Canadian Cystic Fibrosis Foundation.

The U.S. Cystic Fibrosis Foundation compiles lifestyle information about American adults with CF. In 2008, the foundation reported that 92% had graduated from high school and 66% had at least some college education. Employment data revealed 15% of adults were disabled and 7% were unemployed. Marital information showed that 54.8% of adults were single and 40.1% were married or living with a partner. In 2008, 240 American women with CF were pregnant.

Epidemiology

Mutation	Frequency worldwide
ΔF508	66%-70%
G542X	2.4%
G551D	1.6%
N1303K	1.3%
W1282X	1.2%
All others	27.5%

Cystic fibrosis is the most common life-limiting autosomal recessive disease among people of European heritage. In the United States, approximately 30,000 individuals have CF; most are diagnosed by six months of age. Canada has approximately 3,000 citizens with CF. Approximately 1 in 25 people of European descent, and one in 30 of Caucasian Americans, is a carrier of a cystic fibrosis mutation. Although CF is less common in these groups, approximately 1 in 46 Hispanics, 1 in 65 Africans and 1 in 90 Asians carry at least one abnormal CFTR gene.

Although technically a rare disease, cystic fibrosis is ranked as one of the most widespread life-shortening genetic diseases. It is most common among nations in the Western world. An exception is Finland, where only one in 80 people carry a CF mutation. In the United States, 1 in 4,000 children are born with CF. In 1997, about 1 in 3,300 caucasian children in the United States was born with cystic fibrosis. In contrast, only 1 in 15,000 African American children suffered from cystic fibrosis, and in Asian Americans the rate was even lower at 1 in 32,000.

Cystic fibrosis is diagnosed in males and females equally. For unclear reasons, males tend to have a longer life expectancy than females some recent studies suggest this gender gap may no longer exist in younger patients with access to excellent health care facilities., while a recent study from Ireland identified a link between the female hormone oestrogen and worse CF outcomes

The distribution of CF alleles varies among populations. The frequency of ΔF508 carriers has been estimated to be 1:200 in northern Sweden, 1:143 in Lithuanians, and 1:38 in Denmark. No ΔF508 carriers were found among 171 Finns and 151 Saami people. ΔF508 does occur in Finland, but it is a minority allele there. Cystic fibrosis is known to occur in only 20 families (pedigrees) in Finland.

Theories about prevalence

The ΔF508 mutation is estimated to be up to 52,000 years old. Numerous hypotheses have been advanced as to why such a lethal mutation has persisted and spread in the human population. Other common autosomal recessive diseases such as sickle-cell anemia have been found to protect carriers from other diseases, a concept known as

heterozygote advantage. Resistance to the following have all been proposed as possible sources of heterozygote advantage:

- Cholera: With the discovery that cholera toxin requires normal host CFTR proteins to function properly, it was hypothesized that carriers of mutant CFTR genes benefited from resistance to cholera and other causes of diarrhea. Further studies have not confirmed this hypothesis.
- Typhoid: Normal CFTR proteins are also essential for the entry of Salmonella typhi into cells, suggesting that carriers of mutant CFTR genes might be resistant to typhoid fever. No *in vivo* study has yet confirmed this. In both cases, the low level of cystic fibrosis outside of Europe, in places where both cholera and typhoid fever are endemic, is not immediately explicable.
- Diarrhea: It has also been hypothesized that the prevalence of CF in Europe might be connected with the development of cattle domestication. In this hypothesis, carriers of a single mutant CFTR chromosome had some protection from diarrhea caused by lactose intolerance, prior to the appearance of the mutations that created lactose tolerance.
- Tuberculosis: Another possible explanation is that carriers of the gene could have some resistance to TB.

History

National Library of Medicine photo of Dorothy Hansine Andersen. Andersen first described cystic fibrosis in 1938.

Although the entire clinical spectrum of CF was not recognized until the 1930s, certain aspects of CF were identified much earlier. Indeed, literature from Germany and Switzerland in the 18th century warned *Wehe dem Kind, das beim Kuß auf die Stirn salzig schmekt, er ist verhext und muss bald sterbe* or "Woe is the child who tastes salty from a kiss on the brow, for he is cursed, and soon must die," recognizing the association between the salt loss in CF and illness.

In the 19th century, Carl von Rokitansky described a case of fetal death with meconium peritonitis, a complication of meconium ileus associated with cystic fibrosis. Meconium ileus was first described in 1905 by Karl Landsteiner. In 1936, Guido Fanconi published a paper describing a connection between celiac disease, cystic fibrosis of the pancreas, and bronchiectasis.

In 1938 Dorothy Hansine Andersen published an article, "Cystic Fibrosis of the Pancreas and Its Relation to Celiac Disease: a Clinical and Pathological Study," in the *American Journal of Diseases of Children*. She was the first to describe the characteristic cystic fibrosis of the pancreas and to correlate it with the lung and intestinal disease prominent in CF. She also first hypothesized that CF was a recessive disease and first used pancreatic enzyme replacement to treat affected children. In 1952 Paul di Sant' Agnese discovered abnormalities in sweat electrolytes; a sweat test was developed and improved over the next decade.

In 1988 the first mutation for CF, ΔF508 was discovered by Francis Collins, Lap-Chee Tsui and John R. Riordan on the seventh chromosome. Subsequent research has found over 1,000 different mutations that cause CF.

Because mutations in the CFTR gene are typically small, classical genetics techniques had been unable to accurately pinpoint the mutated gene. Using protein markers, gene-linkage studies were able to map the mutation to chromosome 7. Chromosome-walking and -jumping techniques were then used to identify and sequence the gene. In 1989 Lap-Chee Tsui led a team of researchers at the Hospital for Sick Children in Toronto that discovered the gene responsible for CF. Cystic fibrosis represents the first genetic disorder elucidated strictly by the process of reverse genetics.

Research

Gene therapy has been explored as a potential cure for cystic fibrosis. Ideally, gene therapy attempts to place a normal copy of the CFTR gene into affected cells. Transferring the normal CFTR gene into the affected epithelium cells would result in the production of functional CFTR in all target cells, without adverse reactions or an inflammation response. Studies have shown that to prevent the lung manifestations of cystic fibrosis, only 5–10% the normal amount of CFTR gene expression is needed. Multiple approaches have been tested for gene transfer, such as liposomes and viral vectors in animal models and clinical trials. However, both methods were found to be relatively inefficient treatment options. The main reason is that very few cells take up the vector and express the gene, so the treatment has little effect. Additionally, problems

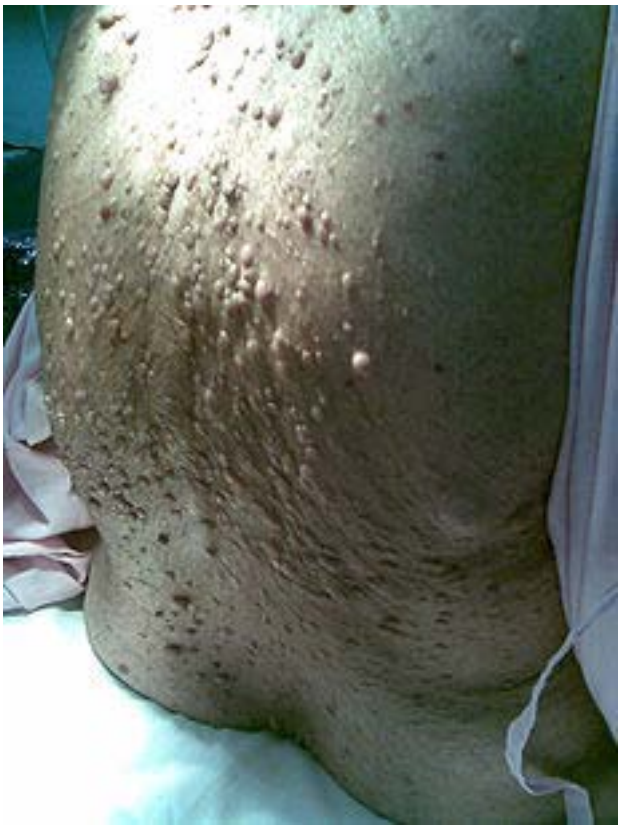
have been noted in cDNA recombination, such that the gene introduced by the treatment is rendered unusable.

Another approach is to develop drugs that will get the ribosome to overcome the stop codon and synthesize a full-length CFTR protein. About 10% of CF result from a premature stop codon in DNA, leading to early termination of protein synthesis and truncated proteins. Aminoglycoside antibiotics interfere with DNA synthesis and error-correction. In some cases, they can cause the cell to overcome the stop codon, insert a random amino acid, and express a full-length protein. The aminoglycoside gentamicin has been used to treat lung cells from CF patients in the laboratory to induce the cells to grown full-length proteins.

Chapter 13

Neurofibromatosis

Neurofibromatosis



Back of an elderly woman with Neurofibromatosis.

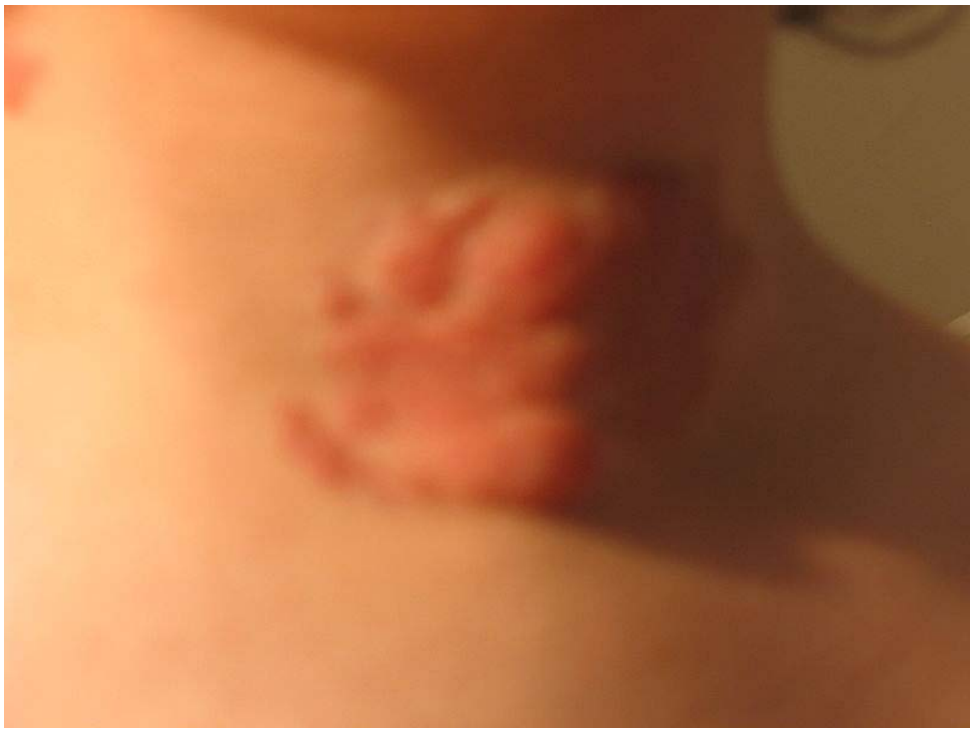
ICD-10	Q85.0
ICD-9	237.7
ICD-O:	9540/0
eMedicine	derm/287
MeSH	D017253

Neurofibromatosis (commonly abbreviated **NF**; neurofibromatosis type 1 is also known as **von Reklinghausen disease**) is a genetically-inherited disorder in which the nerve tissue grows tumors (i.e., neurofibromas) that may be benign or may cause serious damage by compressing nerves and other tissues. The disorder affects all neural crest cells (Schwann cells, melanocytes, endoneurial fibroblasts). Cellular elements from these cell types proliferate excessively throughout the body forming tumors; melanocytes also function abnormally in this disease resulting in disordered skin pigmentation and "cafe-au-lait" spots. The tumors may cause bumps under the skin, colored spots, skeletal problems, pressure on spinal nerve roots, and other neurological problems.

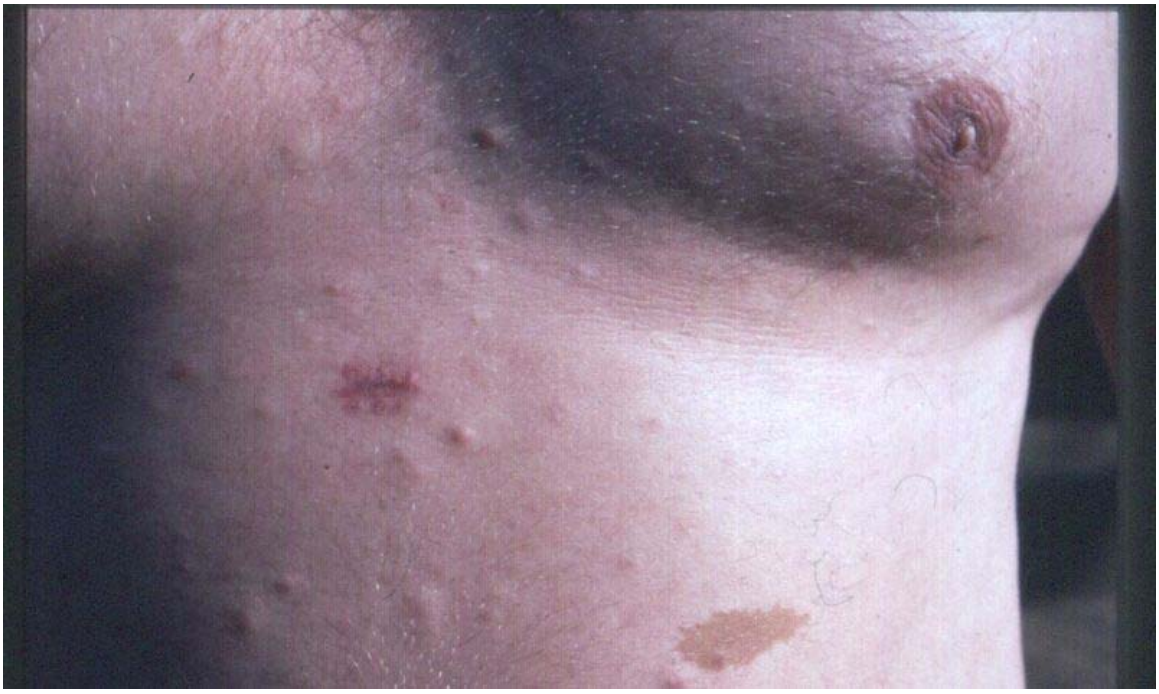
Neurofibromatosis is an autosomal dominant disorder, which means that it affects males and females equally and is dominant (only one copy of the affected gene is needed to get the disorder). Therefore, if only one parent has neurofibromatosis, his or her children have a 50% chance of developing the condition as well. The severity in affected individuals, however, can vary (this is called variable expressivity). Moreover, in around half of cases there is no other affected family member because a new mutation has occurred.

Classification

Neurofibromatosis type 1 (NF 1)



plexiform neurofibroma on the neck of a patient; plexiform neurofibromas are a cause of morbidity in the affected individuals.



Patient with multiple small cutaneous neurofibromas and a 'café au lait spot' (bottom of photo, to the right of centre). A biopsy has been taken of one of the lesions

Neurofibromatosis type 1 (also known as "von Recklinghausen disease") is the most common form of NF, accounting for up to 90% of the cases. NF 1 has a disorder frequency of 1 in 3,000 making it more common than neurofibromatosis type 2, with a frequency of 1 in 45,000 people. It occurs following the mutation of neurofibromin on chromosome 17q11.2. Neurofibromin is a tumor suppressor gene whose function is to inhibit the p21 ras oncoprotein. In absence of this tumor suppressor's inhibitory control on the ras oncoprotein, cellular proliferation is erratic, and uncontrolled resulting in unbalanced cellular proliferation, and tumor development. The diagnosis of NF1 is made if any two of the following seven criteria are met:

- Two or more neurofibromas on or under the skin **or** one plexiform neurofibroma (a large cluster of tumors involving multiple nerves); Neurofibromas are the subcutaneous bumps that are characteristic of the disease and increase in number with age.
- Freckling of the groin or the axilla (arm pit).
- Café au lait spots (pigmented, light brown macules located on nerves, with a smooth edges("coast of California") birthmarks). Six or more measuring 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals.
- Skeletal abnormalities, such as sphenoid dysplasia or thinning of the cortex of the long bones of the body (i.e. bones of the leg, potentially resulting in bowing of the legs)
- Lisch nodules (hamartomas of iris), freckling in the iris.
- Tumors on the optic nerve, also known as an optic glioma.

- Macrocephaly in 30-50% of the pediatric population without any hydrocephalus.
- Epilepsy (seizures)
- Juvenile posterior lenticular opacity

NF 1 also increases the risk of tumor development, particularly, meningiomas, gliomas and pheochromocytomas.

Neurofibromatosis type 2 (NF 2)

Neurofibromatosis type 2 (also called "central neurofibromatosis") is the result of mutation of the Merlin (also known as "schwannomin") in chromosome 22q12. It accounts for only 10% of all cases of NF, and its frequency is lower than NF1. It is also caused by a mutation in a tumor suppressor gene (NF2 or Merlin). The normal function of Merlin is not well understood. The disorder manifests in the following fashion:

- bilateral acoustic neuromas (tumors of the vestibulocochlear nerve or cranial nerve 8 (CN VIII) also known as schwannoma) often leading to hearing loss. In fact, the hallmark of NF 2 is hearing loss due to acoustic neuromas around the age of twenty.
- the tumors may cause:
    - headaches
    - balance problems, and peripheral vertigo often due to schwannoma and involvement of the inner ear.
    - facial weakness/paralysis due to involvement or compression of the facial nerve (cranial nerve 7 or CN VII)
    - patients with NF2 may also develop other brain tumors, as well as spinal tumors.
    - Deafness and Tinnitus.

NF 2 increases the risk of meningiomas and ependymomas.

Schwannomatosis

Schwannomatosis - mutation in both chromosomes 17 and 22

1. Multiple Schwannomas occur.
2. The Schwannomas develop on cranial, spinal and peripheral nerves.
3. Chronic pain, and sometimes numbness, tingling and weakness.
4. About 1/3 of patients have segmental Schwannomatosis, which means that the Schwannomas are limited to a single part of the body, such as an arm, a leg or the spine.
5. Unlike the other forms of NF, the Schwannomas do not develop on vestibular nerves, and as a result, no loss of hearing is associated with Schwannomatosis.
6. Patients with Schwannomatosis do not have learning disabilities related to the disorder.

One must keep in mind, however, that neurofibromatosis can occur in or affect any of the organ systems, whether that entails simply compressing them (from tumor growth) or in fact altering the organs in some fundamental way. This disparity in the disorder is one of many factors that makes it difficult to diagnose, and eventually find a prognosis for.

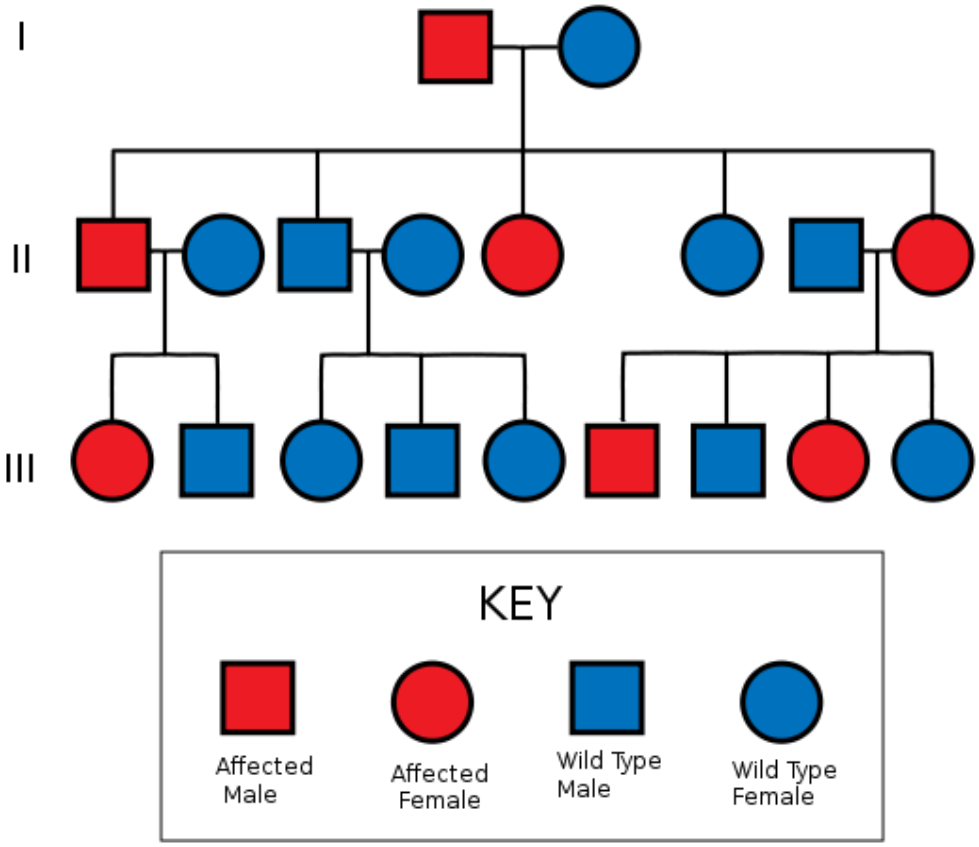
Signs and symptoms

Patients with Neurofibromatosis can be affected in many different ways. Morbidity is often due to plexiform neuromas, optic gliomas, or acoustic neuromas, but mortality can also be associated with malignant transformation of the neuromas such as neurofibrosarcomas (often there is a malignant transformation in less than 3% of the cases of NF1). There is a high incidence of learning disabilities or cognitive deficit in patients with NF, particularly NF-1, however severe retardation is not part of the syndrome. Due to the tumor generating nature of the disorder and its involvement of the nervous system and also due to early onset macrocephaly in the pediatric population, there is often an increased chance of development of epilepsy in those affected. Neurofibromatosis also increases the risk of leukemia particularly in children; Children with NF-1 have 200 to 500 times the normal risk of developing leukemia compared to the general population. Since the tumors grow where there are nerves, they can also grow in areas that are visible causing considerable social suffering for those affected. The tumors can also grow in places that can cause other medical issues that may require them to be removed for the patient's safety. Affected individuals may need multiple surgeries (such as reduction surgery, or Gamma knife surgery), depending on where the tumors are located. For instance those affected with NF 2 might benefit from a surgical decompression of the vestibular tumors to prevent deafness.

Related disorders

Neurofibromatosis is considered a member of the *neurocutaneous syndromes* (*phakomatoses*). In addition to the types of neurofibromatosis, the phakomatoses also include tuberous sclerosis, Sturge-Weber syndrome and von Hippel-Lindau disease. This grouping is an artifact of an earlier time in medicine, before the distinct genetic basis of each of these diseases was understood.

Genetics



NF-1 and NF-2 may be inherited in an autosomal dominant fashion, as well as through random mutation.

Neurofibromatosis type 1 is due to mutation on chromosome 17q11.2 , the gene product being Neurofibromin (a GTPase activating enzyme (GAP)). Neurofibromatosis type 2 is due to mutation on chromosome 22q, the gene product is Merlin, a cytoskeletal protein.

Both NF-1 and NF-2 are autosomal dominant disorders, meaning that only one copy of the mutated gene need be inherited in order to pass the disorder. A child of a parent with NF-1 or NF-2 and an unaffected parent will have a 50%-100% chance of inheriting the disorder, depending on whether the affected parent is heterozygous (*Aa*) or homozygous (*AA*) for the trait ("*A*" depicts the affected dominant allele, while "*a*" depics the recessive allele).

Complicating the question of heritability is the distinction between genotype and phenotype, that is, between the genetics and the actual manifestation of the disorder. In the case of NF1, no clear links between genotype and phenotype have been found, and the severity and the specific nature of the symptoms may vary widely among family members with the disorder. This is a good example of the phenomenon of variable expressivity: the differing severities of disease in different individuals with the same genotype. In the case of NF-2, however, manifestations are similar among family members; a strong genotype-phenotype correlation is believed to exist. Both NF-1 and NF-2 can also appear to be spontaneous de-novo mutations, with no family history. These cases account for about one half of neurofibromatosis cases.

Similar to polydactyly, NF is also a autosomally dominant mutation, that is not prevalent in the society. Neurofibromatosis-1 is found in approximately 1 in 2,500-3,000 live births (carrier incidence 0.0004, gene frequency 0.0002) and is more common than NF-2.

Pathophysiology

The gene affected in NF-1, is located on the long arm of the chromcome 17 (q11.2). It encodes for a protein called Neurofibromin, otherwise known as "the tumor suppressor" protein. This protein is a negative regulator of the Ras kinase pathway (p21 oncoprotein). Neurofibromatosis alters or weakens this protein (due to deletion, missense mutation, or nonsense mutations) allowing rapid, radical growth of cells all over the body, especially around the nervous system. The essential problem is the inability to inactivate GTP due to a defective GTP-ase (Neurofibromin). This leads to the common symptoms for neurofibromatosis - clumpings of the tumors, called neurofibromas and schwannomas. Less is known about the NF-2 linked gene and its product Merlin. However, it is on the long arm of the chromosome 22q(11.1-13.1) and codes for the protein Merlin.

Treatment

Because there is no cure for the condition itself, the only therapy for patients with neurofibromatosis is a program of treatment by a team of specialists to manage symptoms or complications. Surgery may be needed when the tumors compress organs or other structures. Less than 10% of people with neurofibromatosis develop cancerous growths; in these cases, chemotherapy may be successful.

Although there's no cure for NF, the "Neurofibromatosis Association" is optimistic that there will be an effective treatment within the next five to ten years. For families with NF, genetic screening and counselling is available.

History

Neurofibromatosis (or von Recklinghausen disease) was first described in 1882 by the German pathologist, Friedrich Daniel von Recklinghausen (December 2, 1833-August 26, 1910). As a young scientist, Recklinghausen was the student of the then renowned Rudolf Virchow in Berlin. Recklinghausen was successful in generating some of the most

descriptive medical observations of his time, making him the first person to describe and coin the term "hemachromatosis" (*Hämochromatose, Tageblatt der Naturforschenden Versammlung*). Recklinghausen is now known for his contributions to staining methods and most importantly for his important paper on neurofibromatosis published in 1881, to honor Rudolf Virchow's 25 year jubilee in which he describes neurofibromatosis. Recognized as a distinguished histopathologists, and a great scientist to this date, he lends his name to the syndrome, which he himself elucidated.

Chapter 14

Phenylketonuria

Phenylketonuria	
ICD-10	E70.0
ICD-9	270.1
OMIM	261600 261630
DiseasesDB	9987
MedlinePlus	001166
eMedicine	ped/1787 derm/712
MeSH	D010661

Phenylketonuria (**PKU**) is an autosomal recessive metabolic genetic disorder characterized by a deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH).[:541] This enzyme is necessary to metabolize the amino acid phenylalanine ('Phe') to the amino acid tyrosine. When PAH is deficient, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which is detected in the urine.

Since its discovery, there have been many advances in its treatment. It can now be managed by the patient with little or no side-effects other than the inconvenience of managing the treatment. If, however, the condition is left untreated, it can cause problems with brain development, leading to progressive mental retardation, brain damage, and seizures. In the past, PKU was treated with a low-phenylalanine diet. Latter-day research now has shown that diet alone may not be enough to prevent the negative effects of phenylalanine levels. Optimal treatment involves lowering blood Phe levels to a safe range and monitoring diet and cognitive development. Lowering of phenylalanine levels to a safe range may be achieved by combining a low-phenylalanine diet with protein supplements. There is currently no cure for this disease; however, some treatments are available with varying success rates. In general, PKU is detected through newborn screening and diagnosed by a geneticist. PKU clinics around the world provide care for PKU patients to optimize phe levels, dietary intake, and cognitive outcomes.

History

Phenylketonuria was discovered by the Norwegian physician Ivar Asbjørn Følling in 1934 when he noticed that hyperphenylalaninemia (HPA) was associated with mental retardation. In Norway, this disorder is known as **Følling's disease**, named after its discoverer. Dr. Følling was one of the first physicians to apply detailed chemical analysis to the study of disease. His careful analysis of the urine of two affected siblings led him to request many physicians near Oslo to test the urine of other affected patients. This led to the discovery of the same substance that he had found in eight other patients. The substance found was subjected to much more basic and rudimentary chemical analysis (taste). He conducted tests and found reactions that gave rise to benzaldehyde and benzoic acid, which led him to conclude the compound contained a benzene ring. Further testing showed the melting point to be the same as phenylpyruvic acid, which indicated that the substance was in the urine. His careful science inspired many to pursue similar meticulous and painstaking research with other disorders.

Screening and presentation

Blood is taken from a two-week old infant to test for phenylketonuria

PKU is normally detected using the HPLC test, but some clinics still use the Guthrie test, part of national biochemical screening programs. Most babies in developed countries are screened for PKU soon after birth.

If a child is not screened during the routine newborn screening test (typically performed 6 –14 days after birth, using samples drawn by Neonatal heel prick), the disease may present clinically with seizures, albinism (excessively fair hair and skin), and a "musty odor" to the baby's sweat and urine (due to phenylacetate, one of the ketones produced).

In most cases, a repeat test should be done at approximately 2 weeks of age to verify the initial test and uncover any phenylketonuria that was initially missed.

Untreated children are normal at birth, but fail to attain early developmental milestones, develop microcephaly, and demonstrate progressive impairment of cerebral function. Hyperactivity, EEG abnormalities and seizures, and severe learning disabilities are major clinical problems later in life. A "musty or mousy" odor of skin, hair, sweat and urine (due to phenylacetate accumulation); and a tendency to hypopigmentation and eczema are also observed.

In contrast, affected children who are detected and treated are less likely to develop neurological problems or have seizures and mental retardation, though such clinical disorders are still possible.

Pathophysiology

Classical PKU is caused by a mutated gene for the enzyme phenylalanine hydroxylase (PAH), which converts the amino acid phenylalanine to other essential compounds in the body. Other non-PAH mutations can also cause PKU. This is an example of genetic heterogeneity.

Classical PKU

The PAH gene is located on chromosome 12 in the bands 12q22-q24.1. More than four hundred disease-causing mutations have been found in the PAH gene. PAH deficiency causes a spectrum of disorders including classic phenylketonuria (PKU) and hyperphenylalaninemia (a less severe accumulation of phenylalanine).

PKU is known to be an autosomal recessive genetic disorder. This means that both parents must have at least one mutated allele of the PAH gene. The child must inherit both mutated alleles, one from each parent. Therefore, it is not impossible for a parent with the disease to have a child without it if the other parent possesses one functional allele of the gene for PAH. Yet, a child from two parents with PKU will inherit two mutated alleles every time, and therefore the disease.

Phenylketonuria can exist in mice, which have been extensively used in experiments into an effective treatment for PKU. The macaque monkey's genome was recently sequenced, and it was found that the gene encoding phenylalanine hydroxylase has the same sequence that, in humans, would be considered the PKU mutation.

Tetrahydrobiopterin-deficient hyperphenylalaninemia

A rarer form of hyperphenilalaninemia occurs when PAH is normal but there is a defect in the biosynthesis or recycling of the cofactor tetrahydrobiopterin (BH_4) by the patient. This cofactor is necessary for proper activity of the enzyme. The coenzyme (called biopterin) can be supplemented as treatment.

Levels of dopamine can be used to distinguish between these two types. Tetrahydrobiopterin is required to convert phenylalanine to tyrosine, but it is also required to convert tyrosine to L-DOPA (via the enzyme tyrosine hydroxylase), which in turn is converted to dopamine. Low levels of dopamine lead to high levels of prolactin. By contrast, in classical PKU, prolactin levels would be relatively normal. Tetrahydrobiopterin deficiency can be caused by defects in four different genes. These types are known as HPABH4A, HPABH4B, HPABH4C, and HPABH4D.

Metabolic pathways

The enzyme phenylalanine hydroxylase normally converts the amino acid phenylalanine into the amino acid tyrosine. If this reaction does not take place, phenylalanine accumulates and tyrosine is deficient. Excessive phenylalanine can be metabolized into phenylketones through the minor route, a transaminase pathway with glutamate. Metabolites include phenylacetate, phenylpyruvate and phenethylamine. Elevated levels of phenylalanine in the blood and detection of phenylketones in the urine is diagnostic.

Phenylalanine is a large, neutral amino acid (LNAA). LNAAs compete for transport across the blood-brain barrier (BBB) via the large neutral amino acid transporter (LNAAT). If phenylalanine is in excess in the blood, it will saturate the transporter. Excessive levels of phenylalanine tend to decrease the levels of other LNAAs in the brain. However, as these amino acids are necessary for protein and neurotransmitter synthesis, phenylalanine buildup hinders the development of the brain, causing mental retardation.

Treatment

If PKU is diagnosed early enough, an affected newborn can grow up with normal brain development, but only by managing and controlling phenylalanine (Phe) levels through diet, or a combination of diet and medication. When phenylalanine cannot be metabolized by the body, abnormally high levels accumulate in the blood and are toxic to the brain. When left untreated, complications of PKU include severe mental retardation, brain function abnormalities, microcephaly, mood disorders, irregular motor functioning, and behavioral problems such as ADHD.

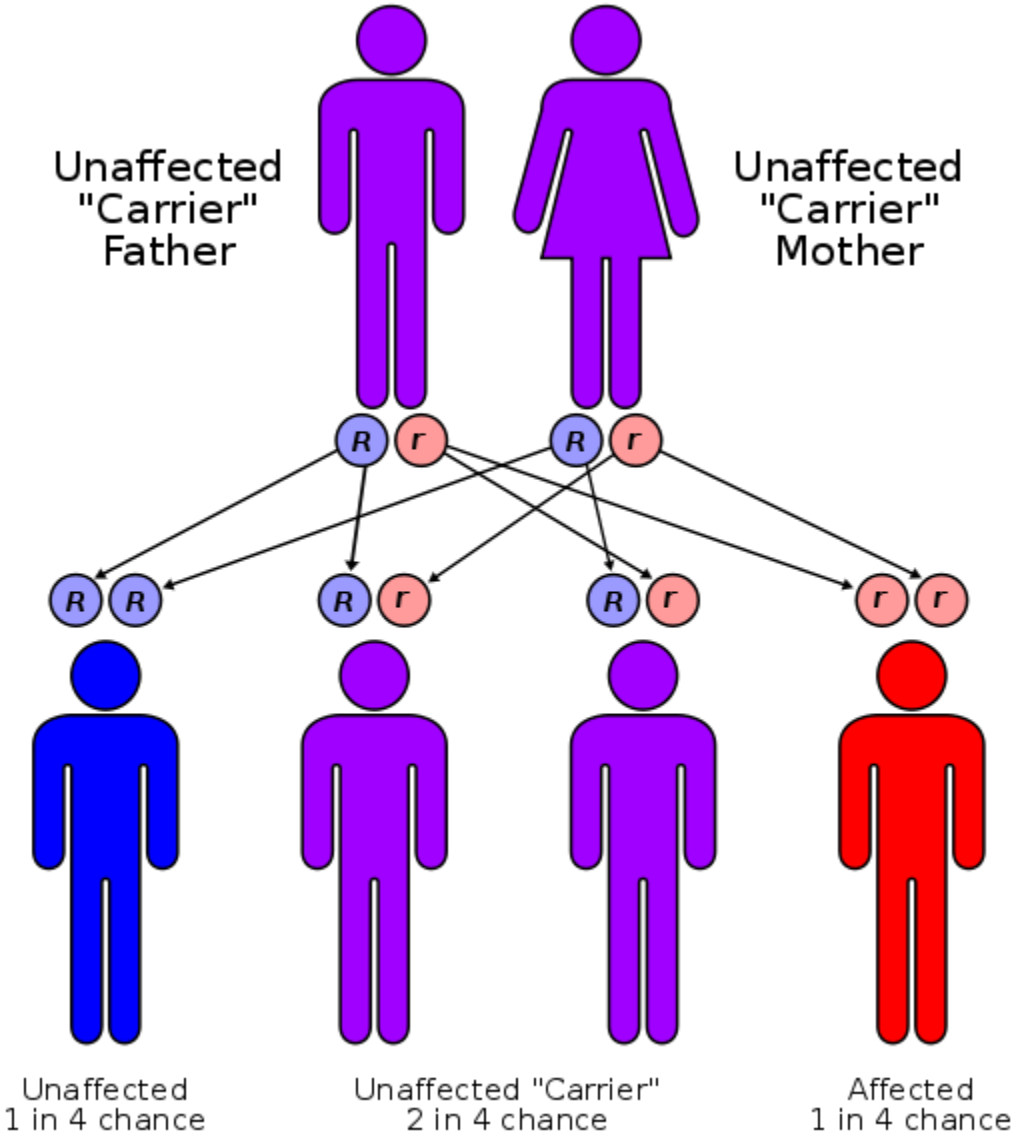
All PKU patients must adhere to a special diet low in phenylalanine for at least the first 16 years of their lives. This requires severely restricting or eliminating foods high in phenylalanine, such as meat, chicken, fish, eggs, nuts, cheese, legumes, cow milk and other dairy products. Starchy foods such as potatoes, bread, pasta, and corn must be monitored. Infants may still be breastfed to provide all of the benefits of breastmilk, but the quantity must also be monitored and supplementation for missing nutrients will be required. Many diet foods and diet soft drinks that contain the sweetener aspartame must also be avoided, as aspartame consists of two amino acids: phenylalanine and aspartic acid.

Supplementary infant formulas are used in these patients to provide the amino acids and other necessary nutrients that would otherwise be lacking in a low-phenylalanine diet. As the child grows up, these can be replaced with pills, formulas, and specially formulated foods. (Since phenylalanine is necessary for the synthesis of many proteins, it is required for appropriate growth but levels must be strictly controlled in PKU patients). In addition, tyrosine, which is normally derived from phenylalanine, must be supplemented.)

The oral administration of tetrahydrobiopterin (or BH4) (a cofactor for the oxidation of phenylalanine) can reduce blood levels of this amino acid in certain patients. The company BioMarin Pharmaceutical has produced a tablet preparation of the compound sapropterin dihydrochloride (Kuvan),which is a form of tetrahydrobiopterin. Kuvan is the first drug that can help BH4-responsive PKU patients (defined among clinicians as about 1/2 of the PKU population) lower Phe levels to recommended ranges. Working closely with a dietitian, some PKU patients who respond to Kuvan may also be able to increase the amount of natural protein they can eat. After extensive clinical trials, Kuvan has been approved by the FDA for use in PKU therapy. Some researchers and clinicians working with PKU are finding Kuvan a safe and effective addition to dietary treatment and beneficial to patients with PKU.

There are several other therapies currently under investigation, including gene therapy, large neutral amino acids, and enzyme substitution therapy with phenylalanine ammonia lyase (PAL). In the past, PKU-affected people were allowed to go off diet after approximately 8, then 18 years of age. Today most physicians recommend that PKU patients must manage their Phe levels throughout life.

Maternal phenylketonuria



Phenylketonuria is inherited in an autosomal recessive fashion

For women affected with PKU, it is essential for the health of their child to maintain low-phenylalanine levels before and during pregnancy. Though the developing fetus may only be a carrier of the PKU gene, the intrauterine environment can have very high levels of phenylalanine, which can cross the placenta. The result is that the child may develop congenital heart disease, growth retardation, microcephaly and mental retardation. PKU-affected women themselves are not at risk from additional complications during pregnancy.

In most countries, women with PKU that wish to have children are advised to lower their blood phenylalanine levels (typically to between 2 and 6 micromol/deciliter) before they

become pregnant, and carefully control their phenylalanine levels throughout the pregnancy. This is achieved by performing regular blood tests and adhering very strictly to a diet, in general monitored on a day-to-day basis by a specialist metabolic dietitian. In many cases, as the fetus' liver begins to develop and produce PAH normally, the mother's blood phenylalanine levels will drop, requiring an increased phenylalanine intake to remain within the safe range of 2-6 micromol/dL. The mother's daily phenylalanine intake may double or even triple by the end of the pregnancy, as a result. When maternal blood phenylalanine levels fall below 2 micromol/dL, anecdotal reports indicate that the mothers may suffer adverse effects including headaches, nausea, hair loss, and general malaise. When low phenylalanine levels are maintained for the duration of pregnancy, there are no elevated levels of risk of birth defects compared with a baby born to a non-PKU mother. Babies with PKU may drink breast milk, while also taking their special metabolic formula. Some research has indicated that an exclusive diet of breast milk for PKU babies may alter the effects of the deficiency, though during breastfeeding the mother must maintain a strict diet to keep their phenylalanine levels low. More research is needed. US scientist have recently announced (June 2010) that they will be conducting thorough investigation on the mutation of genes in the human genome. Their top priority is Phenylketonuria as it has become increasingly common, due to the fact that sufferers often live past the age of sixty and often bear children (carriers of the recessive gene).

Incidence

The incidence of PKU is about 1 in 15,000 births, but the incidence varies widely in different human populations from 1 in 4,500 births among the population of Ireland to 1 in 13,000 births in Norway to fewer than one in 100,000 births among the population of Finland. Turkey, at 1 in 2600, has the highest incidence rate in the world. The illness is also more common in Italy and China, as well as in Yemeni populations.

Chapter 15

Prader–Willi Syndrome

Prader-Willi syndrome

ICD-10	Q87.1
ICD-9	759.81
OMIM	176270
DiseasesDB	104
eMedicine	ped/1880
MeSH	D011218

Prader–Willi syndrome (abbreviated **PWS**) is a rare genetic disorder in which seven genes (or some subset thereof) on chromosome 15 (q 11-13) are deleted or unexpressed (chromosome 15q partial deletion) on the paternal chromosome. It was first described in 1956 by Andrea Prader (1919-2001), Heinrich Willi (1900-1971), Alexis Labhart (1916), Andrew Ziegler, and Guido Fanconi of Switzerland. The incidence of PWS is between 1 in 25,000 and 1 in 10,000 live births. The paternal origin of the genetic material that is

affected in the syndrome is important because the particular region of chromosome 15 involved is subject to parent of origin imprinting, meaning that for a number of genes in this region only one copy of the gene is expressed while the other is silenced through imprinting. For the genes affected in PWS it is the paternal copy that is usually expressed, while the maternal copy is silenced. This means that while most people have a single working copy of these genes, people with PWS have no working copy. PWS has the sister syndrome Angelman syndrome in which maternally derived genetic material is affected in the same genetic region.

Signs and symptoms

Clinical features and signs
Holm *et al.* (1993) describe the following features and signs as pretest indicators of PWS, although not all will be present.

In utero:

- Reduced fetal movement
- Frequent abnormal fetal position
- Occasional polyhydramnios (excessive amniotic fluid)

At birth:

- Often breech or caesarean births
- Lethargy
- Hypotonia
- Feeding difficulties (due to poor muscle tone affecting sucking reflex)
- Difficulties establishing respiration
- Hypogonadism

Infancy:

- Failure to thrive (continued feeding difficulties)
- Delayed milestones/intellectual delay
- Excessive sleeping
- Strabismus
- Scoliosis (often not detected at birth)

Childhood:

- Speech delay
- Poor physical coordination
- Hyperphagia (over-eating) from age 2 – 8 years. Note change from feeding difficulties in infancy
- Excessive weight gain
- Sleep disorders

- Scoliosis

Adolescence:

- Delayed puberty
- Short stature
- Obesity
- Extreme flexibility

Adulthood:

- Infertility (males and females)
- Hypogonadism
- Sparse pubic hair
- Obesity
- Hypotonia
- Learning disabilities/borderline intellectual functioning (but some cases of average intelligence)
- Prone to diabetes mellitus
- Extreme flexibility

General physical appearance (adults)

- Prominent nasal bridge
- Small hands and feet with tapering of fingers
- Soft skin, which is easily bruised
- Excess fat, especially in the central portion of the body
- High, narrow forehead
- Almond-shaped eyes with thin, down-turned lids
- Light skin and hair relative to other family members
- Lack of complete sexual development
- Frequent skin picking
- Striae
- Delayed motor development

Neuro-cognitive

Individuals with PWS are at risk of learning and attention difficulties. Curfs and Frym (1992) conducted research into the varying degrees of learning disability found in Prader Willi Syndrome (PWS). Their results were as follows:

- 5%: IQ above 85 (average to low average intelligence)
- 27%: IQ 70 – 85 (borderline intellectual functioning)
- 39%: IQ 50 – 70 (mild intellectual disability)

- 27%: IQ 35 – 50 (moderate intellectual disability)
- 1%: IQ 20 – 35 (severe intellectual disability)
- <1%: IQ <20 (profound intellectual disability)

Cassidy found that 40% of individuals with PWS have borderline/low average intelligence, a figure higher than that found in Curfs and Frym's study (32%). However, both studies suggest that most individuals (50–65%) fall within the mild/borderline/low average intelligence range.

Children with PWS show an unusual cognitive profile. They are often strong in visual organization and perception, including reading and vocabulary, but their spoken language (sometimes affected by hypernasality) is generally poorer than their comprehension. A marked skill in completing jigsaw puzzles has been noted, although this may be an effect of increased practise.

Auditory information processing and sequential processing are relatively poor, as are arithmetic and writing skills, visual and auditory short term memory and auditory attention span. These sometimes improve with age, but deficits in these areas remain throughout adulthood.

Behavioral

Prader–Willi syndrome is also frequently associated with an extreme and insatiable appetite, often resulting in morbid obesity. There is currently no consensus as to the cause for this particular symptom, although genetic abnormalities in chromosome 15 disrupt the normal functioning of the hypothalamus. Given that the hypothalamus regulates many basic processes, including appetite, there may well be a link. However, no organic defect of the hypothalamus has been discovered on post mortem investigation.

Prader–Willi syndrome patients have high ghrelin levels, which are thought to directly contribute to the increased appetite, hyperphagia, and obesity seen in this syndrome. Cassidy states the need for a clear delineation of behavioural expectations, the reinforcement of behavioural limits and the establishment of regular routines.

The main mental health difficulties experienced by people with PWS include compulsive behaviour (usually manifested in skin-picking) and anxiety. Psychiatric symptoms, for example, hallucinations, paranoia and depression have been described in some cases and affect approximately 5–10% of young adults. Psychiatric and behavioural problems are the most common cause of hospitalization.

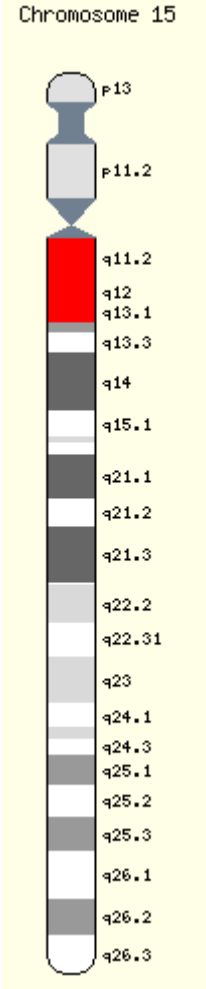
Endocrine

There are several aspects of PWS that support the concept of growth hormone deficiency in individuals with PWS. Specifically, individuals with PWS have short stature, are obese

with abnormal body composition, have reduced fat free mass (FFM), have reduced LBM and total energy expenditure, and have decreased bone density.

PWS is characterized by hypogonadism. This is manifested as undescended testes in males and benign premature adrenarche in females. Testes may descend with time or can be managed with surgery or testosterone replacement. Adrenarche may be treated with hormone replacement therapy.

Genetics



PWS is caused by the deletion of the paternal copies of the imprinted SNRPN and necdin genes along with clusters of snoRNAs: SNORD64, SNORD107, SNORD108 and two copies of SNORD109, 29 copies of SNORD116 (HBII-85) and 48 copies of SNORD115 (HBII-52). These are on chromosome 15 located in the region 15q11-13. This so-called PWS/AS region may be lost by one of several genetic mechanisms which, in the majority of instances occurs through chance mutation. Other less common mechanisms include; uniparental disomy, sporadic mutations, chromosome translocations, and gene deletions. Due to imprinting, the maternally inherited copies of these genes are virtually silent, only the paternal copies of the genes are expressed. PWS results from the loss of paternal

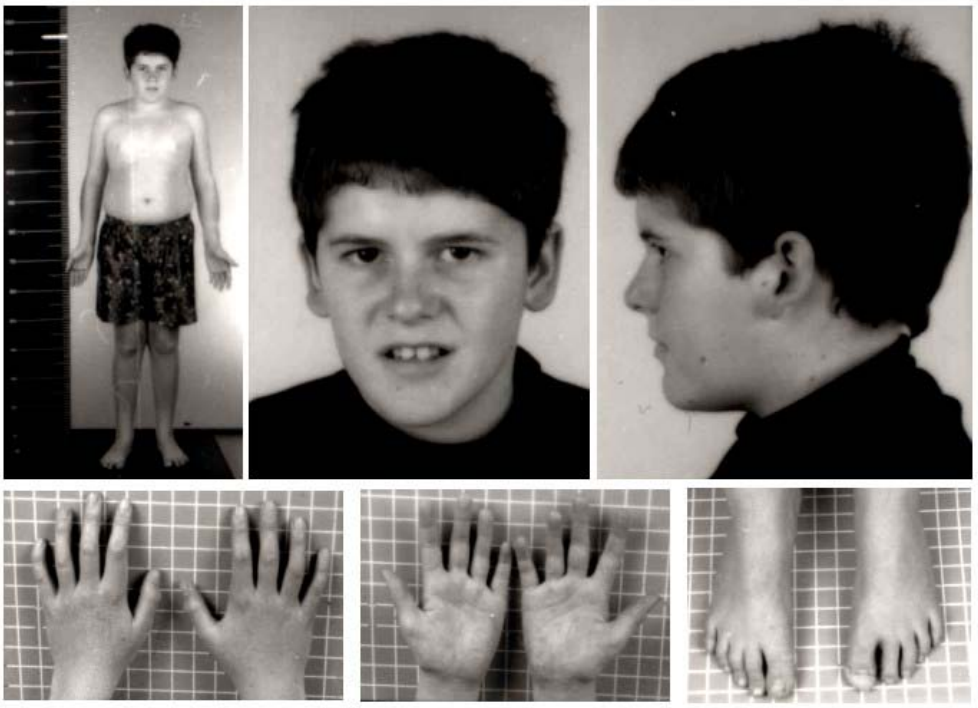
copies of this region. Deletion of the same region on the maternal chromosome causes Angelman syndrome (AS). PWS and AS represent the first reported instances of imprinting disorders in humans.

The risk to the sibling of an affected child of having PWS depends upon the genetic mechanism which caused the disorder. The risk to siblings is <1% if the affected child has a gene deletion or uniparental disomy, up to 50% if the affected child has a mutation of the imprinting control region, and up to 25% if a parental chromosomal translocation is present. Prenatal testing is possible for any of the known genetic mechanisms.

A microdeletion in one family of the snoRNA HBII-52 has excluded it from playing a major role in the disease.

Studies of human and mouse model systems have shown that deletion of the 29 copies of the C/D box snoRNA SNORD116 (HBII-85) has been shown to be the primary cause of Prader–Willi syndrome.

Diagnosis



Prader-Willi syndrome phenotype at 15 years of age. Note absence of typical PWS facial features and presence of mild truncal obesity.

PWS affects approximately 1 in 10,000 to 1 in 25,000 newborns. There are more than 400,000 people who live with PWS around the world. It is traditionally characterized by hypotonia, short stature, hyperphagia, obesity, behavioral issues (specifically OCD-like

behaviors), small hands and feet, hypogonadism, and mild mental retardation. However, with early diagnosis and early treatment (such as with growth hormone therapy), the prognosis for persons with PWS is beginning to change. Like autism, PWS is a spectrum disorder and so symptoms can range from mild to severe, and may change throughout the person's lifetime. Various organ systems are affected.

Traditionally, Prader-Willi Syndrome was diagnosed by clinical presentation. Currently, the syndrome is diagnosed through genetic testing; testing is recommended for newborns with pronounced hypotonia. Early diagnosis of PWS allows for early intervention as well as the early prescription of growth hormone. Daily recombinant growth hormone (GH) injections are indicated for children with PWS. GH supports linear growth and increased muscle mass, and may lessen food preoccupation and weight gain.

The mainstay of diagnosis is genetic testing, specifically DNA-based methylation testing to detect the absence of the paternally contributed Prader–Willi syndrome/Angelman syndrome (PWS/AS) region on chromosome 15q11-q13. Such testing detects over 97% of patients. Methylation-specific testing is important to confirm the diagnosis of PWS in all individuals, but especially those who are too young to manifest sufficient features to make the diagnosis on clinical grounds or in those individuals who have atypical findings. Because PWS infants have a higher rate of difficulties at birth (including breech delivery and respiratory delay) birth-related injuries and oxygen deprivation may complicate the genetic handicaps, resulting in atypical PWS.

Differential diagnosis

Prader–Willi syndrome is often misdiagnosed as a variety of other syndromes due to many in the medical community's unfamiliarity with PWS. Sometimes it is misdiagnosed as Down syndrome, simply because of the relative frequency of Down syndrome compared to PWS. Also, marked obesity can occur in Down syndrome due to behavioral problems. Adding to the confusion, parents of children who already carry a diagnosis of Prader–Willi syndrome may tell friends, family, and even physicians and nurses that their child has Down syndrome because more people have heard of that condition. It is thought that 75% of those with PWS are undiagnosed.

Treatment

Prader–Willi syndrome has no cure; however, several treatments are in place to lessen the condition's symptoms. During infancy, subjects should undergo therapies to improve muscle tone. Speech and occupational therapy are also indicated. During the school years, children benefit from a highly structured learning environment as well as extra help. The largest problem associated with the syndrome is severe obesity.

Prescription of daily recombinant growth hormone injections are indicated for children with PWS. GH supports linear growth and increased muscle mass, and may lessen food preoccupation and weight gain.

Because of severe obesity, obstructive sleep apnea is a common sequela, and a positive airway pressure machine is often needed.

Society and culture

Prader–Willi syndrome appeared in the UK media in July 2007 when Channel 4 aired a program *Can't Stop Eating*, surrounding the everyday lives of two Prader-Willi patients, Joe and Tamara.

An individual with Prader-Willi Syndrome featured in the episode entitled "Dog Eat Dog" of the television series *CSI: Crime Scene Investigation* (aired on November 24, 2005).

Another individual with Prader–Willi syndrome, Ethan Starkweather, was on *Extreme Makeover: Home Edition* originally aired on Mothers Day 2010.

Actress and neuroscientist Mayim Bialik wrote a thesis on Prader–Willi syndrome for her Ph.D, which was completed in 2008.

On TLC's *My Deadly Appetite* (aired in December 2010), a patient named Will was treated for the condition (as well as others featured).

Chapter 16

1p36 Deletion Syndrome

1p36 deletion syndrome

A toddler showing facial symptoms of the syndrome.

OMIM	607872
DiseasesDB	34535

1p36 deletion syndrome (also known as **monosomy 1p36**) is a congenital genetic disorder characterized by moderate to severe intellectual disability, delayed growth, hypotonia, seizures, limited speech ability, malformations, hearing and vision impairment, and distinct facial features. The symptoms may vary, depending on the exact location of the chromosomal deletion.

The condition is caused by a genetic deletion (loss of a segment of DNA) on the outermost band on the short arm (p) of chromosome 1. It is one of the most common deletion syndromes. It is estimated that the syndrome occurs in one in every 5,000 to 10,000 births. Knowledge of the disorder has increased a great deal over the last decade, mainly because more patients have been accurately diagnosed and described in international medical literature.

Characteristics

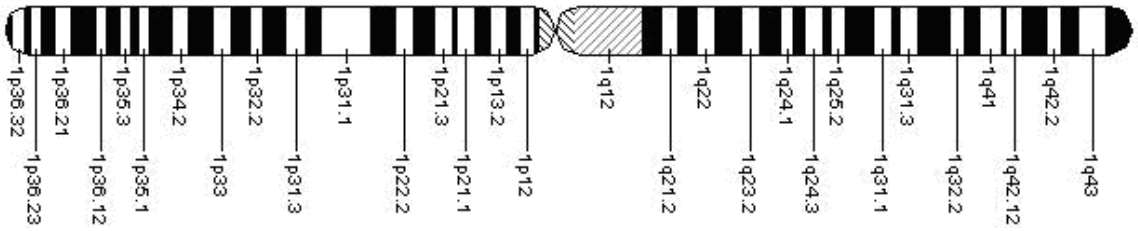
The facial features of 1p36 deletion syndrome have been considered to be characteristic, although few patients have been diagnosed solely on the basis of facial appearance. These features may include microcephaly, small, possibly slanted, deep-set eyes, a flat nose and nasal bridge, anomalous, low-set and small ears, a small mouth with down-turned corners and a pointed chin. Distinguishing features in another study were a large or late-closing anterior fontanelle (up to 85% of patients) and facial asymmetry.

History

The first cases of 1p36 deletion syndrome were described in the 1980s. However, since many of these individuals also had other chromosomal imbalances, symptoms varied widely. The reason it took so long to recognize the condition as a distinct chromosome deletion syndrome is that the deletions causing the disorder are too small to be detected in a routine chromosomal analysis. FISH (fluorescent *in situ* hybridization) and DNA-based technology known as MLPA (multiple ligation probe amplification) used in testing have aided in diagnosing an increasing number of cases since the 1990s.

Genetics

1p36 Deletion Syndrome is a congenital genetic disorder caused by the deletion of the most distal light band of the short arm of chromosome 1. Chromosome 1 is the largest human chromosome and represents about 8 percent of the total DNA in human cells. The "p" stands for the short or 'petite' arm of the chromosome. '36' stands for the location of the deletion on the chromosome.



Human chromosome 1

The breakpoints for 1p36 Deletion Syndrome have been variable and are most commonly found from 1p36.13 to 1p36.33. 40 percent of all breakpoints occur 3 to 5 million base pairs from the telomere. The size of the deletion ranges from approximately 1.5 million base pairs to greater than 10 million. Studies have suggested that the larger the deletion, the more severe the symptoms exhibited in the individual, but this has not been proven definitively.

Most deletions in chromosome 1p36 are new mutations, that occur before fertilization, during the formation of gametes (eggs or sperm). There have also been reports of patients

with 1p36 deletion syndrome whose parents have a balanced or symmetrical translocation. This means a portion of one chromosome is transferred to another chromosome, so the parent has the "36" portion of chromosome 1 attached in an alternate location. When this occurs, cell division creates gametes that are missing a piece of 36.

In new mutations, the mechanism causing chromosome breakage is unknown. Deletions of paternal origin (father) are larger than the deletions deriving from the maternal (mother) chromosome. The majority of deletions are maternally derived. There do not seem to be differences in the clinical manifestations (the symptoms or observable conditions which are seen as a result of 1p36) based on whether the deletion is on the paternal or maternal chromosome.

Health concerns

Developmental delay

Most young children with 1p36 deletion syndrome have delayed development. They sit up, walk and talk later than typical children. Speech is severely affected, with many patients learning only a few words. It was originally thought that the degree of the delay and the ability to acquire complex speech was somewhat dependent on deletion size. Reports of a milder learning disability in children with smaller deletions have suggested that there may be a correlation between deletion size and mental ability; however, this requires further investigation and research. Recent research by Dr Lisa Shaffer has shown however that there is no correlation between deletion size and degree of developmental delay. This suggests that the most genetically potent area of the 1P36 chromosome occurs at the terminal end of the chromosome.

Behavioral differences

Many children with 1p36 deletion syndrome have behavioral problems. Some of these include temper outbursts, banging or throwing objects, striking people, screaming episodes, and self-injurious behavior (wrist biting, head striking/banging). Autistic behavior has also been noted in some children. Also, some parents have described behaviors such as a love of water, although there have not been any studies into this yet.

Feeding difficulties

Many children with 1p36 deletion syndrome have oropharyngeal dysphagia which is characterized by difficulty in initiating a swallow. Some of the other feeding issues include poor sucking and swallowing, reflux, and vomiting in infancy. Many require nasalgastric or gastric tubes to ensure they are receiving sufficient nutrition.

Brain abnormalities

Brain imaging has documented cerebral atrophy, which is a loss of neurons in the brain and the connections between them. Also documented were problems with the ventricular

system in the brain, such as ventricular asymmetry and ventricular enlargement. Hydrocephalus has also been noted in children with 1p36 deletion syndrome. This is basically too much fluid within the brain. Hyperreflexia, which is defined as overactive or overresponsive reflexes in the body, was also found to be common. Many children also have epilepsy which is a disorder of the brain that results in recurrent, unprovoked seizures.

Microcephaly

Microcephaly is a disorder in which the circumference of the head is smaller than average for the person's age and gender. Most children with microcephaly also have a small brain and mental retardation. Some of the most common signs and symptoms associated with microcephaly are seizures, poor feeding, high pitched cry, mental retardation, developmental delay, and increased movement of arms and legs.

Vision problems

Vision abnormalities in children with 1p36 have been wide-ranging, including:

- **Strabismus**: A condition in which the two eyes do not point in the same direction when the patient is looking at a distant object.
- **Sixth nerve palsy**: Double vision
- **Refractive errors**: Refractive errors include nearsightedness, farsightedness, astigmatism (a warping of the curvature of the cornea) and presbyopia (the inability to maintain a clear image or focus as objects are moved closer). These disorders of the eye can be corrected with glasses or contacts.
- **Hypermetropia**: A condition where the eye is too small and eyes have to over focus to see clearly; also called farsightedness.
- **Cataracts**: A cataract is an opacity or cloudiness in the natural lens of the eye.
- **Nystagmus**: A condition characterized by the repetitive oscillations (vibration) of the eyes. Parents of children with nystagmus often refer to this as "jerking" "or "jiggling" eyes.
- **Lacrimal defects**: The lacrimal glands in the eye secrete tears.
- **Visual Inattentiveness**: Defined as an absence of attentive visual behavior such as fixation and following movements.

Distinct facial features

Children with 1p36 deletion syndrome are all unique individuals, but do have some common distinct facial features such as:

- **Large anterior fontanelle/Frontal bossing**: The anterior fontanelle is the "soft spot" towards the front of the top of an infant's head between the growing skull bones. Frontal bossing simply means a prominent forehead.
- **Small and pointed chin**
- **Flat nose and/or nasal bridge**

- **Low-set, small ears/Ear asymmetry**: Ears are abnormally low set on the head and may be small. They may not be the same shape or size, or not lined up.
- **Deep set eyes**
- **Thickened ear helices**: Ear helices are the outer rings of cartilage of the ears.
- **Short, narrow and slanting palpebral fissures**: Palpebral fissures are the gaps between the upper and lower eyelids, or the opening of the eyes.
- **Midface Hypoplasia**: This is where the middle of the face is underdeveloped, leading to a concave-looking face. The bridge of the nose looks sunken in and the eyes are set widely apart and often protrude out of the sockets.
- **Small mouth with down-turned corners**
- **Orofacial clefting**: This is a relatively common birth defect in which the fetus develops with deformities of the upper lip, gum, and roof of the mouth. Children with 1p36 have been noted to have orofacial clefting involving the lip and/or palate or uvula (the small piece of flesh hanging down inside the mouth at the back of the palate).

Growth abnormalities

There are many growth abnormalities associated with 1p36 deletion syndrome. One common problem is delayed growth or difficulty in gaining weight. Even though some of the children may eat well, they still may not grow normally. In contrast, some children may develop hyperphagia, which is overeating, and may become obese. These children clinically resemble children with Prader-Willi syndrome. Developmental delay has also been severe in the patients with the Prader-Willi like characteristics.

- **Hypotonia**: Hypotonia is a decreased or low muscle tone. This may explain the delayed motor skills in children with 1p36.
- **Hypothyroidism**: Hypothyroidism is insufficient production of the thyroid hormone. Symptoms include weight gain, constipation, dry skin, and sensitivity to the cold. Around one third of children with the syndrome have this low thyroid function, which is also called underactive thyroid, and leads to slow metabolism and fatigue.
- **Heart defects**:
    - **Infantile dilated cardiomyopathy**: Dilated cardiomyopathy (DCM) is a disease of the heart muscle that causes the heart to become enlarged, and to pump less strongly. This causes fluid to build up in the lungs, which therefore become congested, and results in a feeling of breathlessness. Children with DCM due to their 1p36 deletion syndrome typically do not worsen over time, though some of them may need to continue taking medication.
    - **Patent ductus arteriosus**: This is the most common structural heart defect in children with 1p36. It is a condition in which the connecting blood vessel between the pulmonary artery and the aorta in fetal circulation stays open in the newborn. The defect often corrects itself within several months of birth, but may require the infusion of chemicals, the placement of "plugs" via catheters, or surgical closure.

    - **Tetralogy of Fallot**: Tetralogy of Fallot is a ventricular septal defect, a hole between the two bottom chambers (ventricles) of the heart. These defects can cause less blood flow to the lungs, the mixing of oxygen-rich and oxygen-poor blood inside the heart, and low levels of oxygen in the blood. When oxygen levels are low, the baby's skin, fingertips, or lips have a bluish tint. This condition is called cyanosis.
- **Increased Risk for Neoplasia**: Chromosome 1p36 alterations, mostly deletions, have been reported to occur in various types of neoplastic growths or tumors which may be benign or malignant. The 1p36 region contains a number of tumor-suppressor genes, which are genes that act to prevent cell growth. The deletion of one or more of these genes can cause malignancy (cancer). Some of the neoplams involved in the 1p36 are neuroblastoma, prostate cancer, lung cancer, melanoma, hepatoma, cervical cancer, breast cancer, colorectal cancer, ovarian cancer, and non-Hodgkin lymphoma. This is not to say that the children with 1p36 deletion syndrome will get these cancers, but this is a theory that has been put forth.
- **Genital hypoplasia**: Genital hypoplasia is the underdevelopment of the genital areas. Some of the genital problems in children with 1p36 are:
    - **Cryptorchidism**: This is the failure of one or both of the testicles to descend into the scrotum.
    - **Shawl Scrotum**: A condition in which the scrotum tends to surround the penis.
    - **Small Genitalia**

Dilation of the renal collecting system

The collecting system is the structure that collects urine directly from the kidney tissue and routes it by way of the ureter to the bladder. Structural renal abnormalities are rare in both sexes.

Hearing loss

Hearing loss affects approximately two thirds of 1p36 deletion patients. It can be of different types. Sensorineural hearing loss is a type of hearing impairment caused by damage that occurs to the inner ear (cochlea) or to the nerve used for hearing (vestibulocochlear nerve). Conductive hearing loss is a hearing loss associated with the functioning of the outer or middle ear. This type is most common in children with 1p36 deletion syndrome. It ranges from mild loss at various frequencies, to severe loss at all frequencies.

Puberty

Puberty in children with 1p36 deletion syndrome can be early, normal, or delayed.

Spinal deformities

Only a few spinal deformities have been seen in children with 1p36. The deformities found are:

- **Kyphoscoliosis**: Spinal deformity combining a sideways curvature with a hunching forward of the upper part of the spine.
- **Postural Kyphosis**: Also called postural "round back". This was found secondary to hypotonia in some children with 1p36.

Treatments and therapy

Although 1p36 Deletion Syndrome can be debilitating in many ways, patients do respond to various treatments and therapies. These include the following:

American Sign Language: Because few individuals with Monosomy 1p36 develop complex speech, an alternate form of communication is critical to development. Most patients can learn basic signs to communicate their needs and wants. This also appears to reduce frustration and may reduce self-injurious tendencies. Children with hearing loss will often qualify for locally sponsored sign language classes.

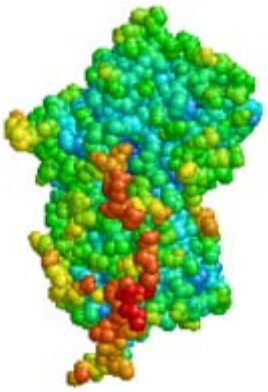
Music: Music has been shown to aid children with 1p36 deletion in various developmental areas. It serves as an excellent auditory stimulus and can teach listening skills. Songs with actions help the child to develop coordination and motor skills.

Physical Therapy: Due to low muscle tone, patients with 1p36 Deletions take a great deal of time to learn to roll over, sit up, crawl and walk. However, regular physical therapy has shown to shorten the length of time needed to achieve each of those developmental milestones.

Chapter 17

Alpha 1-Antitrypsin Deficiency

Alpha 1-antitrypsin deficiency



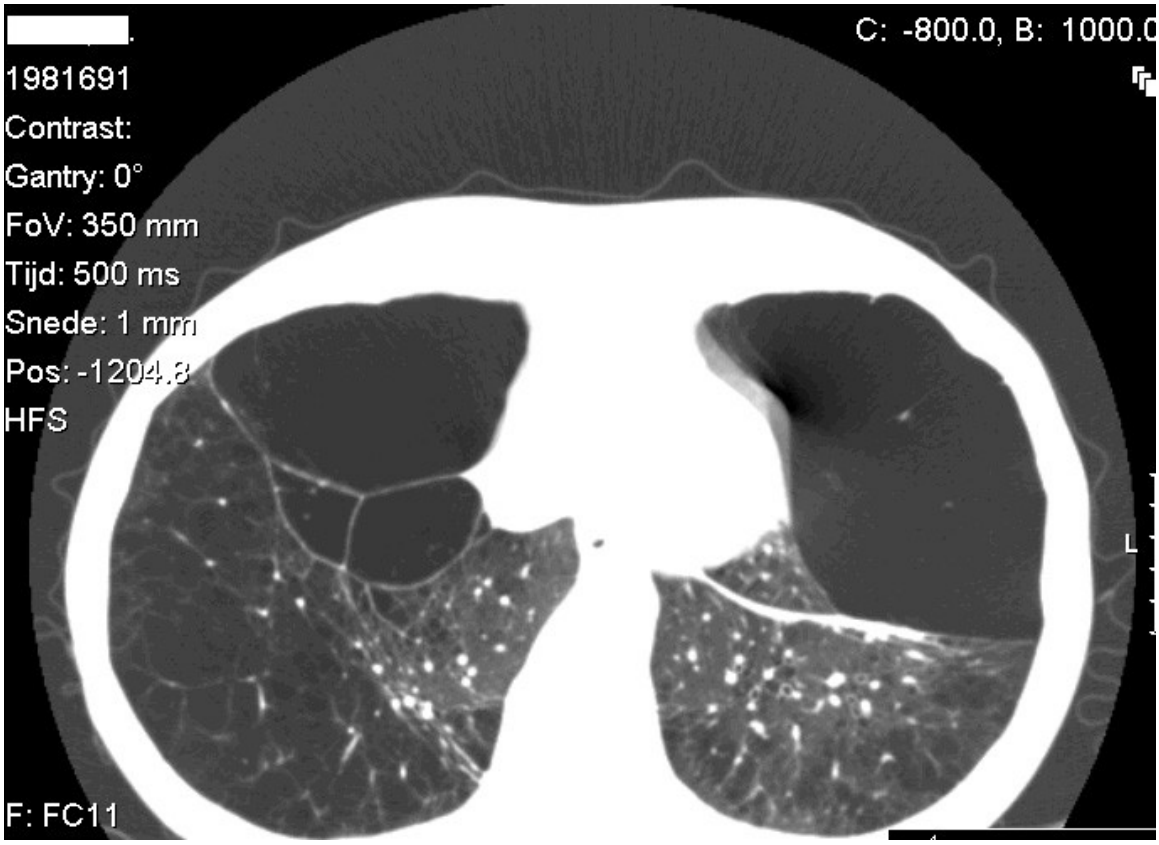
Structure of Alpha 1-antitrypsin

ICD-10	E88.0
ICD-9	273.4
OMIM	107400
DiseasesDB	434
MedlinePlus	000120
eMedicine	med/108
MeSH	D019896
GeneReviews	Alpha1-Antitrypsin Deficiency

Alpha 1-antitrypsin deficiency (**α1-antitrypsin deficiency**, **A1AD** or simply **Alpha-1**) is an autosomal codominant genetic disorder caused by defective production of alpha 1-antitrypsin (A1AT), leading to decreased A1AT activity in the blood and lungs, and deposition of excessive abnormal A1AT protein in liver cells. There are several forms and degrees of deficiency, principally depending on whether the sufferer has one or two copies of the affected gene. Severe A1AT deficiency causes panacinar emphysema or

COPD in adult life in many people with the condition (especially if they are exposed to cigarette smoke), as well as various liver diseases in a minority of children and adults, and occasionally more unusual problems. It is treated by avoidance of damaging inhalants, by intravenous infusions of the A1AT protein, by transplantation of the liver or lungs, and by a variety of other measures, but it usually produces some degree of disability and reduced life expectancy.

Signs and symptoms



Computed tomography of the lung showing emphysema and bullae in the lower lung lobes of a subject with type ZZ alpha-1-antitrypsin deficiency. There is also increased lung density in areas with compression of lung tissue by the bullae.

Symptoms of alpha-1 antitrypsin deficiency include shortness of breath, wheezing, rhonchi, and rales. The patient's symptoms may resemble recurrent respiratory infections or asthma that does not respond to treatment. Individuals with A1AD may develop emphysema during their thirties or forties even without a history of significant smoking, though smoking greatly increases the risk for emphysema. A1AD also causes impaired liver function in some patients and may lead to cirrhosis and liver failure (15%). It is a leading cause of liver transplantation in newborns.

Associated conditions

α_1-antitrypsin deficiency has been associated with a number of diseases:

- Cirrhosis
- COPD
- Pneumothorax
- Asthma
- Wegener's granulomatosis
- Pancreatitis
- Gallstones
- Bronchiectasis
- Pelvic organ prolapse
- Primary sclerosing cholangitis
- Autoimmune hepatitis
- Emphysema, predominantly involving the lower lobes and causing bullae
- Cancer
    - Hepatocellular carcinoma (liver)
    - Bladder carcinoma
    - Gallbladder cancer
    - Lymphoma
    - Lung cancer

Pathophysiology

Alpha 1-antitrypsin (A1AT) is produced in the liver, and one of its functions is to protect the lungs from the neutrophil elastase enzyme, which can disrupt connective tissue. Normal blood levels of alpha-1 antitrypsin are 1.5-3.5 g/l. In individuals with PiSS, PiMZ and PiSZ phenotypes, blood levels of A1AT are reduced to between 40 and 60% of normal levels. This is usually sufficient to protect the lungs from the effects of elastase in people who do not smoke. However, in individuals with the PiZZ phenotype, A1AT levels are less than 15% of normal, and patients are likely to develop panacinar emphysema at a young age; 50% of these patients will develop liver cirrhosis, because the A1AT is not secreted properly and instead accumulates in the liver. A liver biopsy in such cases will reveal PAS-positive, diastase-resistant granules.

Cigarette smoke is especially harmful to individuals with A1AD. In addition to increasing the inflammatory reaction in the airways, cigarette smoke directly inactivates alpha 1-antitrypsin by oxidizing essential methionine residues to sulfoxide forms, decreasing the enzyme activity by a factor of 2000.

Diagnosis

A1AT deficiency remains undiagnosed in many patients. Patients are usually labelled as having COPD without an underlying cause. It is estimated that about 1% of all COPD patients actually have A1AT deficiency. Thus, testing should be performed for all

patients with COPD, asthma with irreversible air-flow obstruction, unexplained liver disease, or necrotizing panniculitis. The initial test performed is serum A1AT level. A low level of A1AT confirms the diagnosis and further assessment with A1AT protein phenotyping and A1AT genotyping should be carried out subsequently.

As protein electrophoresis is imprecise, A1AT is analysed by isoelectric focusing (IEF) in the pH range 4.5-5.5, where the protein migrates in a gel according to its isoelectric point or charge in a pH gradient. Normal A1AT is termed M, as it is migrates toward the center of such an IEF gel. Other variants are less functional, and are termed A-L and N-Z, dependent on whether they run proximal or distal to the M band. The presence of deviant bands on IEF can signify the presence of alpha 1-antitrypsin deficiency. Since the number of identified mutations has exceeded the number of letters in the alphabet, subscripts have been added to most recent discoveries in this area, as in the Pittsburgh mutation described above. As every person has two copies of the A1AT gene, a heterozygote with two different copies of the gene may have two different bands showing on electrofocusing, although heterozygote with one null mutant that abolishes expression of the gene will only show one band. In blood test results, the IEF results are notated as in PiMM, where Pi stands for protease inhibitor and "MM" is the banding pattern of that patient. Other detection methods include use of enzyme-linked-immuno-sorbent-assays in vitro and radial immunodiffusion. Alpha 1-antitrypsin levels in the blood depend on the genotype. Some mutant forms fail to fold properly and are, thus, targeted for destruction in the proteasome, whereas others have a tendency to polymerise, being retained in the endoplasmic reticulum. The serum levels of some of the common genotypes are: PiMM: 100% (normal) PiMS: 80% of normal serum level of A1AT PiSS: 60% of normal serum level of A1AT PiMZ: 60% of normal serum level of A1AT PiSZ: 40% of normal serum level of A1AT PiZZ: 10-15% (severe alpha 1-antitrypsin deficiency) PiZ is caused by a glutamate to lysine mutation at position 342 PiS is caused by a glutamate to valine mutation at position 264 Other rarer forms have been described; in all there are over 80 variants.

Treatment

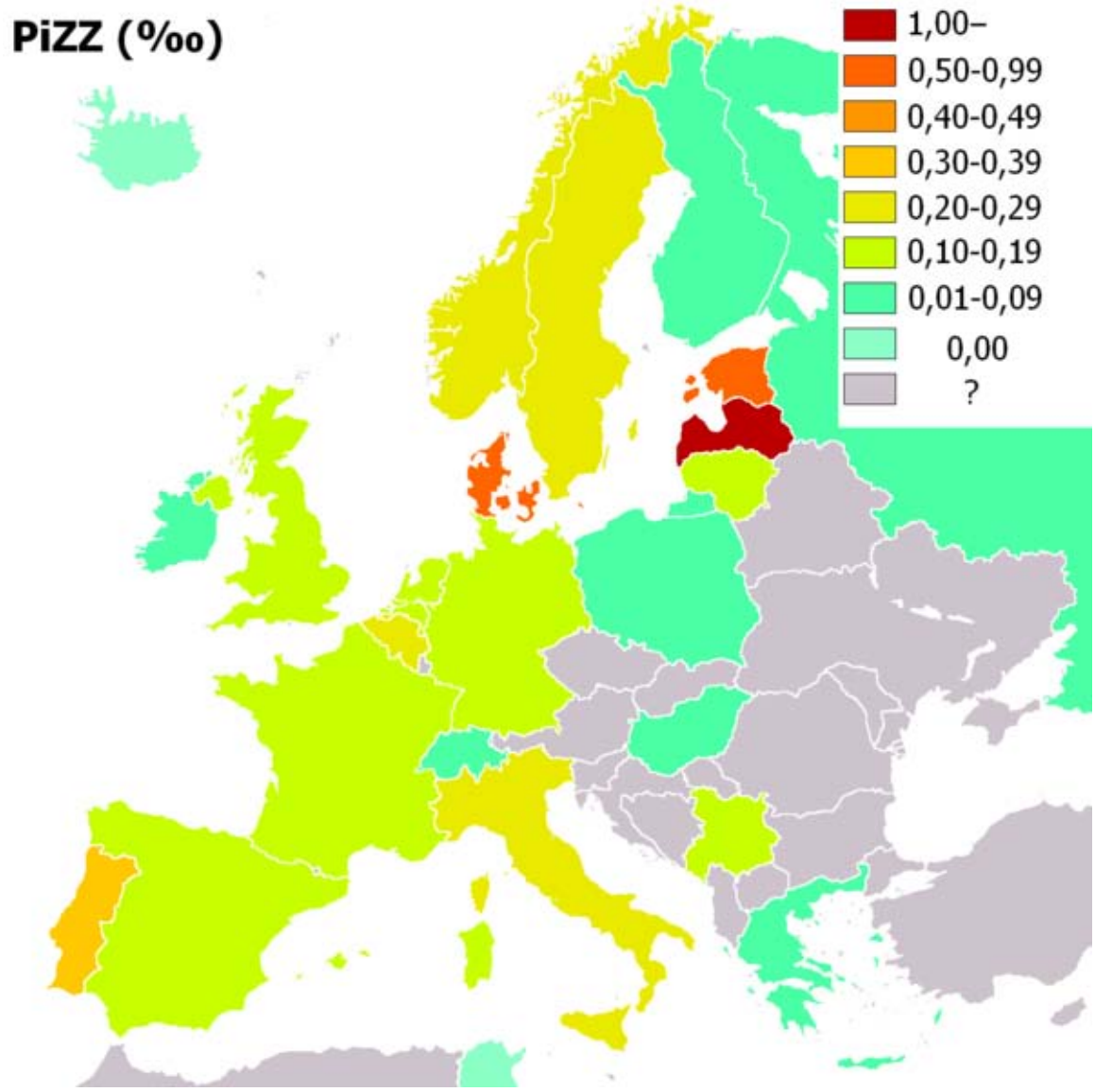
In the United States, Canada, and several European countries, lung-affected A1AD patients may receive intravenous infusions of alpha-1 antitrypsin, derived from donated human plasma. This augmentation therapy is thought to arrest the course of the disease and halt any further damage to the lungs. Long-term studies of the effectiveness of A1AT replacement therapy are not available. It is currently recommended that patients begin augmentation therapy only after the onset of emphysema symptoms.

Augmentation therapy is not appropriate for liver-affected patients; treatment of A1AD-related liver damage focuses on alleviating the symptoms of the disease. In severe cases, liver transplantation may be necessary.

As α_1-antitrypsin is an acute phase reactant, its transcription is markedly increased during inflammation elsewhere in response to increased interleukin-1 and 6 and TNFα production.

Treatments currently being studied include recombinant and inhaled forms of A1AT. Other experimental therapies are aimed at the prevention of polymer formation in the liver.

Epidemiology



Distribution of PiZZ in Europe

People of northern European, Iberian and Saudi Arabian ancestry are at the highest risk for A1AD. Four percent carry the PiZ allele; between 1 in 625 and 1 in 2000 are homozygous.

History

A1AD was discovered in 1963 by Carl-Bertil Laurell (1919–2001), at the University of Lund in Sweden. Laurell, along with a medical resident, Sten Eriksson, made the discovery after noting the absence of the α_1 band on protein electrophoresis in five of 1500 samples; three of the five patient samples were found to have developed emphysema at a young age.

The link with liver disease was made six years later, when Sharp *et al.* described A1AD in the context of liver disease.

Chapter 18

Chronic Granulomatous Disease

Chronic granulomatous disease

Superoxide

ICD-10	D71.
ICD-9	288.1
OMIM	306400 233690 233700
DiseasesDB	2633
MedlinePlus	001239
eMedicine	ped/1590 derm/719
MeSH	D006105

Chronic granulomatous disease (CGD) (also known as "Bridges–Good syndrome," "Chronic granulomatous disorder," and "Quie syndrome") is a diverse group of hereditary diseases in which certain cells of the immune system have difficulty forming the reactive oxygen compounds (most importantly, the superoxide radical) used to kill certain ingested pathogens. This leads to the formation of granulomata in many organs. CGD affects about 1 in 200,000 people in the United States, with about 20 new cases diagnosed each year.

This condition was first discovered in 1954, and in 1957 described as "a fatal granulomatosus of childhood". The underlying cellular mechanism that causes chronic

granulomatous disease was discovered in 1967, and research since that time has further elucidated the molecular mechanisms underlying the disease.

Classification

Chronic granulomatous disease is the name for a genetically heterogeneous group of immunodeficiencies. The core defect is a failure of phagocytic cells to kill organisms that they have engulfed because of defects in a system of enzymes that produce free radicals and other toxic small molecules. There are several types, including chronic X-linked disease, chronic b-negative disease, X-linked cytochrome b-positive disease, x-linked variant disease, and atypical granulomatous disease.

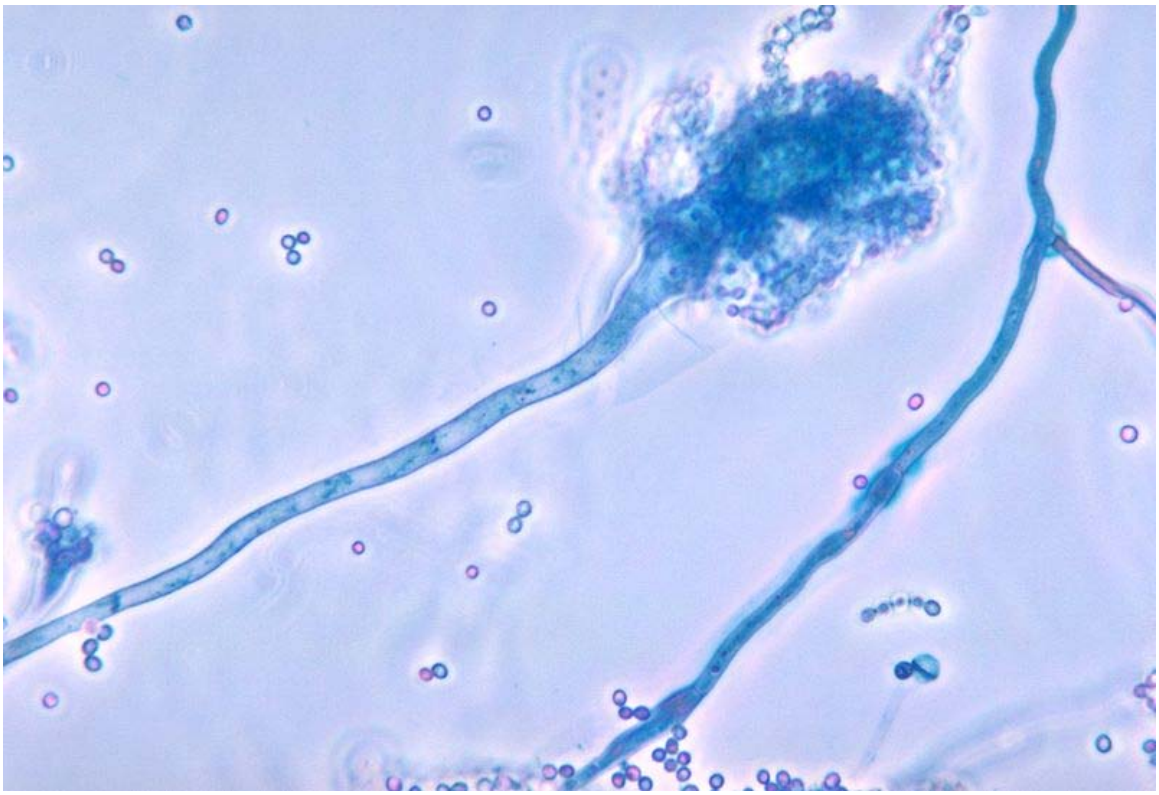
Symptoms

Classically, patients with chronic granulomatous disease will suffer from recurrent bouts of infection due to the decreased capacity of their immune system to fight off disease-causing organisms. The recurrent infections they acquire are specific and are, in decreasing order of frequency:

- pneumonia
- abscesses of the skin, tissues, and organs
- suppurative arthritis
- osteomyelitis
- bacteremia/fungemia
- superficial skin infections such as cellulitis or impetigo

Most people with CGD are diagnosed in childhood, usually before age 5. Early diagnosis is important since these people can be placed on antibiotics to ward off infections before they occur.

Atypical infections



Microscopic image of the fungus, *Aspergillus fumigatus*, an organism that commonly causes disease in people with chronic granulomatous disease.

People with CGD are sometimes infected with organisms that usually do not cause disease in people with normal immune systems. Among the most common organisms that cause disease in CGD patients are:

- bacteria (particularly those that are catalase-positive)
  - *Staphylococcus aureus*.
  - *Serratia marcescens*.
  - *Salmonella* species.
  - *Klebsiella* species.
  - *Pseudomonas cepacia, a.k.a. Burkholderia cepacia.*
  - *Nocardia*.
- fungi
  - *Aspergillus* species. Aspergillus has a propensity to cause infection in people with CGD and of the Aspergillus species, *Aspergillus fumigatus* seems to be most common in CGD.
  - *Candida* species.

Patients with CGD can usually resist infections of catalase-negative bacteria. Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide in many organisms. In organisms that lack catalase (catalase-negative), normal metabolic functions will cause an

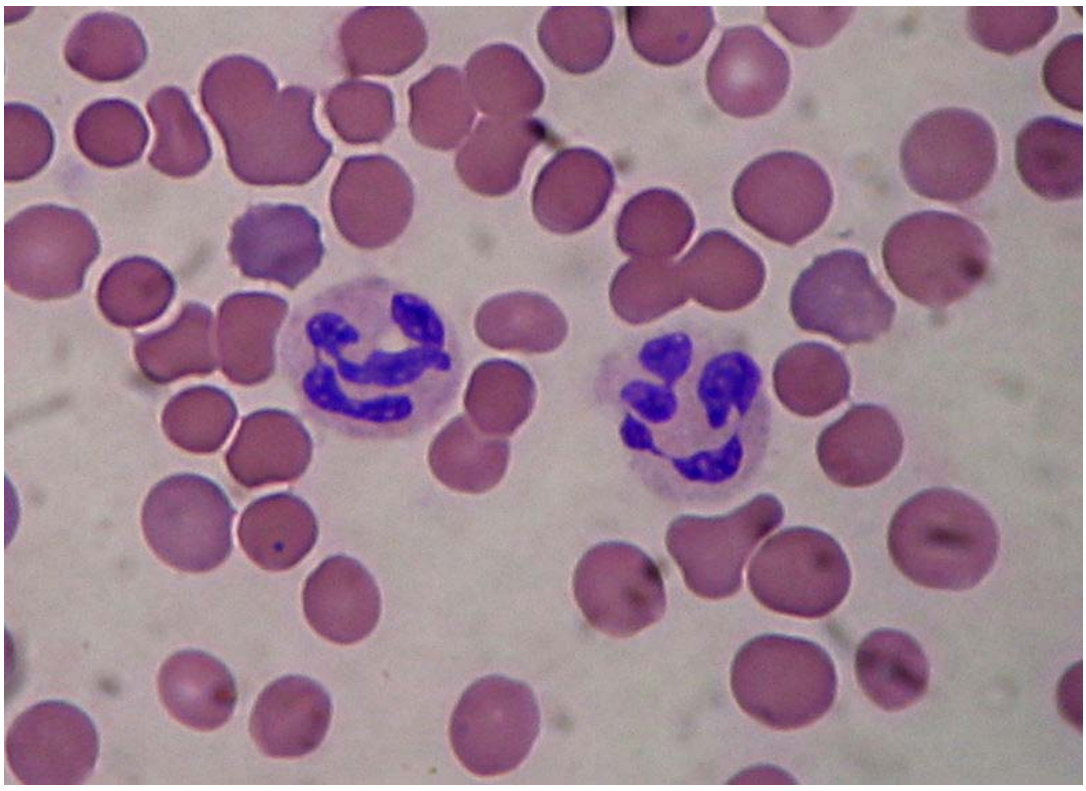
accumulation of hydrogen peroxide which the host's immune system can use to fight off the infection. In organisms that have catalase (catalase-positive), the enzyme breaks down any hydrogen peroxide that was produced through normal metabolism. Therefore hydrogen peroxide will not accumulate, leaving the patient vulnerable to catalase-positive bacteria.

Genetics

Most cases of chronic granulomatous disease are transmitted as a mutation on the X chromosome and are thus called an "X-linked trait". The affected gene on the X chromosome codes for the gp91 protein p91-PHOX (*p* is the weight of the protein in kDa; the *g* means glycoprotein). CGD can also be transmitted in an autosomal recessive fashion (via CYBA and NCF1) and affects other PHOX proteins. The type of mutation that causes both types of CGD are varied and may be deletions, frame-shift, nonsense, and missense.

A low level of NADPH, the cofactor required for superoxide synthesis, can lead to CGD. This has been reported in women who are homozygous for the genetic defect causing glucose-6-phosphate dehydrogenase deficiency (G6PD), which is characterised by reduced NADPH levels.

Pathophysiology



Two neutrophils among many red blood cells. Neutrophils are one type of cell affected by chronic granulomatous disease.

Phagocytes (i.e., neutrophils, monocytes, and macrophages) require an enzyme to produce reactive oxygen species to destroy bacteria after they ingest the bacteria in a process called phagocytosis, a process known as the respiratory burst. This enzyme is termed "phagocyte NADPH oxidase" (*PHOX*). The initial step in this process involves the one-electron reduction of molecular oxygen to produce superoxide anion, a free radical. Superoxide then undergoes a further series of reactions to produce products such as hydrogen peroxide (through the action of superoxide dismutase), hydroxyl radical and hypochlorite (bleach - through the action of myeloperoxidase on hydrogen peroxide). The reactive oxygen species this enzyme produces are toxic to bacteria and help the phagocyte kill them once they are ingested. Defects in one of the four essential subunits of this enzyme can all cause CGD of varying severity, dependent on the defect. There are over 410 known possible defects in the PHOX enzyme complex that can lead to chronic granulomatous disease.

Diagnosis

The nitroblue-tetrazolium (NBT) test is the original and most widely-known test for chronic granulomatous disease. It is negative in CGD, and positive in normal individuals. This test depends upon the direct reduction of NBT by superoxide free radical to form an insoluble formazan. This test is simple to perform and gives rapid results, but only tells whether or not there is a problem with the PHOX enzymes, not how much they are affected. A similar test uses dihydrorhodamine (DHR); whole blood is stained with DHR, incubated, and stimulated produce superoxide radicals which reduce DHR to rhodamin in cells with normal function. An advanced test called the cytochrome C reduction assay tells physicians how much superoxide a patient's phagocytes can produce. Once the diagnosis of CGD is established, a genetic analysis may be used to determine exactly which mutation is the underlying cause.

Treatment

Management of chronic granulomatous disease revolves around two goals: 1) diagnose the disease early so that antibiotic prophylaxis can be given to keep an infection from occurring, and 2) educate the patient about his or her condition so that prompt treatment can be given if an infection occurs.

Antibiotics

Physicians often prescribe the antibiotic trimethoprim-sulfamethoxazole to prevent bacterial infections. This drug also has the benefit of sparing the normal bacteria of the digestive tract. Fungal infection is commonly prevented with itraconazole, although a newer drug of the same type called voriconazole may be more effective. The use of this drug for this purpose is still under scientific investigation.

Immunomodulation

Interferon, in the form of interferon gamma-1b (Actimmune) is approved by the Food and Drug Administration for the prevention of infection in CGD. It has been shown to prevent infections in CGD patients by 70% and to reduce their severity. Although its exact mechanism is still not entirely understood, it has the ability to give CGD patients more immune function and therefore, greater ability to fight off infections. This therapy has been standard treatment for CGD for several years.

Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation from a matched donor is curative although not without significant risk.

Prognosis

There are currently no studies detailing the long term outcome of chronic granulomatous disease with modern treatment. Without treatment children often die in the first decade of life. Available data indicates that X linked CGD is more severe, with most treated patients dying in the third or fourth decade of life.

Epidemiology

CGD affects about 1 in 200,000 people in the United States, with about 20 new cases diagnosed each year.

Chronic granulomatous disease affects all people of all races, however, little information on prevalence outside of the United States is available. One survey in Sweden reported an incidence of 1 in 220,000 people.

History

This condition was first described in 1954 by Janeway, who reported five cases of the disease in children. In 1957 it was further characterized as "a fatal granulomatosus of childhood". The underlying cellular mechanism that causes chronic granulomatous disease was discovered in 1967, and research since that time has further elucidated the molecular mechanisms underlying the disease. Use of antibiotic prophylaxis, surgical abscess drainage, and vaccination lead to the term "fatal" being dropped from the name of the disease as children survived into adulthood. The oldest person to suffer from Chronic Granulomatos Disease was Mr. Jackie Ray Johnson of Fredericksburg, Virginia who died in 2002 at the age of 63.

Research

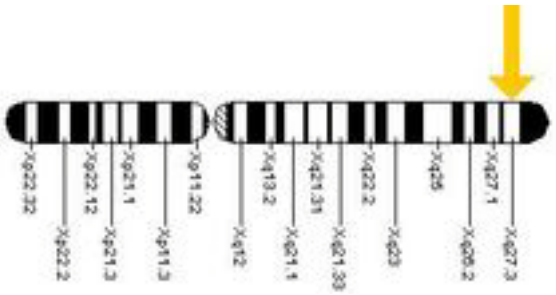
Gene therapy is currently being studied as a possible treatment for chronic granulomatous disease. CGD is well-suited for gene therapy since it is caused by a mutation in single gene which only affects one body system (the hematopoietic system). Viruses have been used to deliver a normal gp91 gene to rats with a mutation in this gene, and subsequently the phagocytes in these rats were able to produce oxygen radicals.

In 2006, two human patients with X-linked chronic granulomatous disease underwent gene therapy and blood cell precursor stem cell transplantation to their bone marrow. Both patients recovered from their CGD, clearing pre-existing infections and demonstrating increased oxidase activity in their neutrophils. However, long-term complications and efficacy of this therapy are unknown.

Chapter 19

Fragile X Syndrome

Fragile X syndrome



Location of FMR1 gene

ICD-10	Q99.2
ICD-9	759.83
OMIM	309550
DiseasesDB	4973
eMedicine	ped/800
MeSH	D005600

Fragile X syndrome (FXS), or **Martin–Bell syndrome**, is a genetic syndrome which results in a spectrum of characteristic physical and intellectual limitations and emotional and behavioral features which range from severe to mild in manifestation.

The syndrome is associated with the expansion of a single trinucleotide gene sequence (CGG) on the X chromosome, and results in a failure to express the protein coded by the FMR1 gene, which is required for normal neural development. There are four generally accepted states of the chromosome region involved in Fragile X syndrome which relate to the length of the repeated CGG sequence; Normal (29–31 CGG repeats) (not affected by the syndrome), Premutation (55–200 CGG repeats)(not affected by the syndrome), Full Mutation (more than 200 CGG repeats)(affected), and Intermediate or Gray Zone Alleles (40–60 repeats).

Martin and Bell in 1943, described a pedigree of X-linked mental disability, without considering the macroorchidism (larger testicles). In 1969 Herbert Lubs first sighted an unusual "marker X chromosome" in association with mental disability. In 1970 Frederick Hecht coined the term "fragile site".

Renpenning's syndrome is not synonymous with the syndrome. In Renpenning's syndrome, there is no fragile site on the X chromosome. Renpenning's cases have short stature, moderate microcephaly, and neurological (brain) disorders.

Escalante's syndrome is synonymous with the fragile X syndrome. This term has been used in South American countries.

Fragile X is the most common known single gene cause of autism and the most common inherited cause of intellectual disability.

Signs and symptoms

Prominent characteristics of the syndrome include an elongated face, large or protruding ears, and low muscle tone.

Aside from intellectual disability, prominent characteristics of the syndrome include an elongated face, large or protruding ears, flat feet, larger testes (macroorchidism), and low muscle tone. Speech may include cluttered speech or nervous speech. Behavioral

characteristics may include stereotypic movements (e.g., hand-flapping) and atypical social development, particularly shyness, limited eye contact, memory problems, and difficulty with face encoding.

Some individuals with the fragile X syndrome also meet the diagnostic criteria for autism. Most females who have the syndrome experience symptoms to a lesser degree because of their second X-chromosome; however, they can develop symptoms just as severe as their male counterparts. While full mutation males tend to present with severe intellectual disability, the symptoms of full mutation females run the gamut of minimally affected to severe intellectual disability, which may explain why females are underdiagnosed relative to males.

In short, similarities between X-linked recessive inheritance and fragile X are:

1. Males are predominantly affected;
2. Females (mothers) are obligatory carriers (*i.e.*, are conclusively proven to be carriers) if a male child is affected, but not necessarily if female children are affected, as a female child with one fragile and one normal X chromosome may have inherited the fragile chromosome from the father.

A difference is that females may also have clinical symptoms.

Physical phenotype

- Prominent ears (one or both)
- Long face (vertical maxillary excess)
- High-arched palate (related to the above)
- Hyperextensible finger joints
- Double-jointed thumbs
- Flat feet
- soft skin
- Larger testes in men (macroorchidism)
- Low muscle tone

Social interaction

FXS is characterized by social anxiety, including gaze aversion, prolonged time to commence social interaction, and challenges forming peer relationships. Social anxiety in individuals with FXS is related to challenges with face encoding. Face encoding is the ability to recognize a face that one has seen before.

Individuals with FXS show decreased activation in the prefrontal regions of the brain. These regions are associated with social cognition. A child with FXS is likely to have hyperactivity, anxiety, and social deficits. Individuals with fragile X-associated tremor/ataxia syndrome (FXTAS) are likely to experience dementia, mood and/or anxiety disorders. Males with the FMR1 premutation and clinical evidence of FXTAS were

found to have increased occurrence of somatization, obsessive–compulsive disorder, interpersonal sensitivity, depression, phobic anxiety and psychoticism.

Females with FXS show a high frequency of avoidant behavior, mood disorder, and habit disorder. Females are significantly more withdrawn and depressed as compared to normal individuals. The size of DNA insertion was related to IQ, severity of attention problems, and withdrawal symptoms. Females with FXS are most vulnerable to social anxiety, social avoidance, withdrawal, and depression, so special attention should be paid.

Mental age is positively correlated and autistic behavior is negatively correlated with sadness in a particular study. The results shows that there are different behavioral profiles for young children than there are for older aged children which implies that temperament and problem behaviors are not rooted in early temperament.

Working memory

From their 40s onward, males with FXS begin developing progressively more severe problems in performing tasks that require the central executive of working memory. Working memory involves the temporary storage of information 'in mind', while processing the same or other information. Phonological memory (or verbal working memory) deteriorates with age in males, while visual-spatial memory is not found to be directly related to age. Males often experience an impairment in the functioning of the phonological loop. The CGG length is significantly correlated with central executive and the visual–spatial memory through regression analysis. However, in a premutation individual, CGG length is only significantly correlated with the central executive, not with either phonological memory or visual–spatial memory.

Intellectual development

Current evidence shows that individuals with premutation have difficulties with mathematics, anxiety, attention, and/or executive functions. Premutation is the stage where the CGG sequence in the FMR-1 gene expands to contain between 54 and 230 repeats. There is also a decrease in measures of executive cognitive functioning, working memory and information processing speed. The relative weaknesses observed in performance IQ can be partly attributed to slowed motor performance as a result of intention tremor. Children with FXS have an intellectual learning rate which is 2.2 times slower than unaffected children.

There is overlap of behavioral and clinical symptomology between autism and FXS. The commonalities include social and communication skills, though the degree to which these two syndromes share the processes and stages of development and medical causes for the disease (etiology) is not known. Research has shown that phenotype ‘commonalities’ reflected different developmental pathways that diverge over time and across syndromes.

Using this information will permit early and specialized interventions. These earlier interventions will allow for optimal development and show educational, clinical, and

adaptive benefits in the patient. When both autism and FXS are present a severe language deficit and lower IQ is observed as compared to children with only FXS.

Hypersensitivity and repetitive behavior

Children with fragile X have very short attention spans, are hyperactive, and show hypersensitivity to visual, auditory, tactile, and olfactory stimuli. These children have difficulty in large crowds due to the loud noises and this can lead to tantrums due to hyperarousal. Children with FXS pull away from light touch and can find textures of materials to be irritating. Transitions from one location to another can be difficult for children with FXS. Behavioral therapy can be used to decrease the child's sensitivity in some cases.

Perseveration is a common communicative and behavioral characteristic in FXS. Children with FXS may repeat a certain ordinary activity over and over. In speech, the trend is not only in repeating the same phrase but also talking about the same subject continually. Cluttered speech and self-talk are commonly seen. Self-talk includes talking with oneself using different tones and pitches.

Visual orientation

Eye problems have not been found to develop in accordance with mental age in individuals with fragile X. But patients with the syndrome have showed delayed voluntary orienting. The group differences in reflexive orienting between individuals with Down and fragile X syndrome at the low mental age level reinforce the practice of separating etiologies and highlight the contribution of basic attentional processes in the study of people with mental limitations.

Ophthalmologic problems include strabismus (lazy eye). This requires early identification to avoid amblyopia. Surgery and/or patching are usually necessary to treat strabismus if diagnosed early. Refractive errors in patients with fragile X are also common.

Fertility

According to a study of patients with fragile X premutation undergoing preimplantation genetic diagnosis (PGD), there is a positive correlation between number of CGG repeats and estradiol level, retrieved oocytes and two-pronuclear zygotes. Premutation carriers with <100 CGG repeats suffer from impaired ovarian response and decreased fertilization rate.

Diagnosis

Fragile X syndrome was originally diagnosed by culturing cells in a folate deficient medium and then assessing the cultures for X-chromosome breakage by cytogenetic analysis of the long arm of the X chromosome. This technique proved unreliable for both diagnosis and carrier testing.

The fragile X abnormality is now directly determined by analysis of the number of CGG repeats and their methylation status using restriction endonuclease digestion and Southern blot analysis.

Not everyone with fragile X syndrome has the same signs and symptoms. Even affected people in the same family don't show the same symptoms. The signs and symptoms fall into six categories:

- Intelligence and learning
- Physical
- Social and emotional
- Speech and language
- Sensory
- Disorders commonly associated or sharing features with Fragile X

Autism and Fragile X syndrome

Fragile X syndrome can cause a child to have autism or an Autism Spectrum Disorder (ASD) though not all children with fragile X syndrome have autism or an ASD.

For between 2% and 6% of all children diagnosed with autism, the cause is the Fragile X gene mutation. Approximately one-third of all children diagnosed with fragile X syndrome also have some degree of autism. Fragile X syndrome is the most common known single gene cause of autism.

From Dr. Randi Hagerman's statement to the United States House of Representatives Subcommittee on Health and Environment: "...Fragile X represents a portal through which we hope to view and treat a wide variety of other disorders of brain development and function. All children with autism...should be tested for Fragile X."

Causes

Fragile X syndrome is a genetic disorder caused by mutation of the FMR1 gene on the X chromosome. Mutation at that site is found in 1 out of about every 2000 males and 1 out of about every 259 females. (Incidence of the disorder itself is about 1 in every 3600 males and 1 in 4000–6000 females.)

Normally, the FMR1 gene contains between 6–55 (29 in Robbins–Kumar pathology textbooks) repeats of the CGG codon (trinucleotide repeats). In people with the fragile X syndrome, the FMR1 allele has over 230–4000 repeats of this codon.

Expansion of the CGG repeating codon to such a degree results in a methylation of that portion of the DNA, effectively silencing the expression of the FMR1 protein.

This methylation of the FMR1 locus in chromosome band Xq27.3 is believed to result in constriction of the X chromosome which appears 'fragile' under the microscope at that point, a phenomenon that gave the syndrome its name.

Mutation of the FMR1 gene leads to the transcriptional silencing of the fragile X-mental retardation protein, FMRP. In normal individuals, FMRP is believed to regulate a substantial population of mRNA: FMRP plays important roles in learning and memory, and also appears to be involved in development of axons, formation of synapses, and the wiring and development of neural circuits.

Transmission

Fragile X syndrome is an X-linked dominant condition with variable expressivity and possibly reduced penetrance.

Because males normally have only one copy of the X chromosome, those males with significant trinucleotide expansion at the FMR1 locus are symptomatic. They are intellectually disabled and may show various physical features of the fragile X syndrome.

Females have two X chromosomes and thus have an increased probability of having a working FMR1 allele. Females carrying one X chromosome with an expanded FMR1 gene can have some signs and symptoms of the disorder or be normal. Although the extra X chromosome can serve as a backup, only one X chromosome is active in each cell due to X-inactivation.

Males with the fragile X cannot transmit it to any of their sons (since males contribute a Y chromosome, not an X, to their male offspring), but will transmit the premutation to all of their daughters, as males contribute their X to all of their daughters. Males never transmit their full mutation (males with full mutations in their blood have premutations in their sperm), and expansion to full mutations never occurs through paternal transmission.

Females carrying one copy of the fragile X can transmit it to their sons or daughters; in this case each child has a 50% chance of inheriting the fragile X. Sons who receive the fragile X are at high risk of intellectual disability. Daughters who receive the fragile X may appear normal or they may be intellectually disabled, usually to a lesser degree than boys with the syndrome. The transmission of fragile X often increases with each passing generation. This seemingly anomalous pattern of inheritance is referred to as the Sherman paradox.

Treatment

Although several medications have been proposed to treat fragile X syndrome, none of them are supported by good evidence. While there is no current cure for the syndrome, there is hope that further understanding of its underlying causes would lead to new therapies. Currently, the syndrome can be treated through behavioral therapy, special education, and when necessary, treatment of physical abnormalities. Persons with the

fragile X syndrome in their family histories are advised to seek genetic counseling to assess the likelihood of having children who are affected, and how severe any impairments may be in affected descendants.

Research

Recent studies have focused on a number of critical areas. The role of FMRP's RNA partners, many of which have now been validated through in vitro assays, is of primary importance. Also being examined is the function the various domains of FMRP, an RNA-binding protein, which is still relatively unknown. One hypothesis is that many symptoms are caused by unchecked activation of mGluR5, a metabotropic glutamate receptor, which was found in a 2007 study to contribute significantly to the pathogenesis of the disease; this suggests that mGluR5 blockers could be used to treat fragile X syndrome.