

TITLE: Molecular Genetics And Otolaryngology
SOURCE: Grand Rounds Presentation,
The University of Texas Medical Branch in Galveston, Department of
Otolaryngology
DATE: October 1999
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Genetics is a diverse subject concerned with variation in living organisms. Medical genetics is concerned with human genetic variation of medical significance, and one of the foremost aims of medical genetics is to understand the basis for mutations and genetic disease and to use that information to improve treatment. Within medical genetics there are numerous areas of interest, each of which is progressing rapidly. Major areas of specialization include, but are by no means limited to:

Chromosomal analysis
Cytogenetics
Molecular biology and genetics
Biochemical genetics
Clinical genetics
Population genetics
Genetic epidemiology
Developmental genetics
Immunogenetics
Genetic counseling
Fetal genetics

History of Medical Genetics

Genetics in medicine did not always occupy such a place of prominence. Although now fully recognized, prior to Mendel's principles of heredity in 1865, inheritance was considered an insignificant part of the life process. Mendel's laws went unappreciated and unrecognized until rediscovered in the early twentieth century. Mendel himself became disillusioned and abandoned experimental research.

Charles Darwin's the *Origin of Species* emphasized the hereditary nature of evolution, but at that time (1859), heredity was believed to be environmentally acquired (see Jean Baptiste Lamarck). The next "pioneers" of medical genetics did not occur until the early 1900's, when Francis Galton (Charles Darwin's cousin!) and Archibald Garrod recognized the works of Mendel and postulated evolution occurs via "nature versus nurture" and that "inborn errors of metabolism" lead to biological abnormalities. Little progress was made until 1953 when James Watson and Francis Crick described the molecular basis of DNA. They were awarded the Nobel Prize in 1962.

In 1990 the "Human Genome Project" was started, with the goal of identifying every human gene by the year 2005. Of note, as of October, 1999, they were 9% completed.

Classification of Genetic Disorders

Genetic disorders may be classified into one of three types: single gene disorders, chromosome disorders and multifactorial disorders. Although virtually all disease progresses as a combination of environment and genetics ("nature versus nurture"), the genetic contribution is now believed to play the most significant role.

Single gene defects are caused by mutant genes, usually a single critical error in the genetic code. Such disorders are usually phenotypically obvious, and include neurofibromatosis types I and II, osteogenesis imperfecta and cystic fibrosis. More than 4000 single gene disorders have been described.

Chromosome disorders are not due to a single genetic mistake but are due to an excess or deficiency in the number of genes contained within an entire chromosome. The most common example is Down Syndrome (Trisomy 21), which is an extra normal copy of chromosome 21. Other examples include Trisomy 13, Turner syndrome and Klinefelter syndrome. These disorders are usually fatal in utero, and are believed to account for more than half of all spontaneous abortions.

Multifactorial inheritance is responsible for a wide range of disorders. Unlike single gene disorders or chromosome disorders, these disorders are believed due to multiple single genetic mistakes which result in a characteristic pattern. Common anomalies in cleft lip and palate and neural tube defects. Clinical diseases are also represented in this group, and include some forms of cancer, coronary artery disease and diabetes mellitus.

Chromosome Structure and Function

The human genome contains approximately 7 billion base pairs of DNA organized into 23 pairs of chromosomes. By current estimates, these base pairs code 100,000 genes and proteins, which control all aspects of life.

DNA itself is a polymeric nucleic acid composed of three subunits: a five carbon sugar (deoxyribose – ribose in RNA), a nitrogen base and a phosphate group. The base pairs are purines and pyrimidines and include adenine, guanine, thymine and cytosine. The base pairs are connected by phosphodiester bonds in the 5'3' direction. Each single strand is connected via hydrogen bonds to a complementary strand in the 3'5' direction. In the intact human chromosome, these nucleotide chains may stretch millions of nucleotides long.

Although genetic information is contained within DNA, RNA is required for the synthesis of proteins. DNA is used as a template for RNA and proteins are translated from the RNA. This is known as the “central dogma” of molecular biology.

Tools of Molecular Genetics

The tools of molecular genetics have undergone revolutionary changes since the late 1970's, and include the identification and use of restriction enzymes, cloning for recombinant DNA, vectors, probes, polymerase chain reaction, DNA sequence analysis and protein analysis.

Molecular cloning requires the use of restriction endonucleases to cleave a DNA strand at a specific site. For example, *EcoRI* cleaves DNA at a palindromic site on each DNA strand. There are hundreds of restriction endonucleases able to form countless DNA fragments. The specific DNA fragments are then combined with a known DNA strand, usually from a bacterium, using DNA ligase. The combined fragment is then inserted into a rapidly dividing bacterium, which are placed in culture medium to form large amounts of the desired “recombinant” DNA. A vector is DNA that can use the host bacterium (or yeast, or virus) to autonomously replicate. This allows a virtually endless supply of the recombinant DNA fragment.

Once a specific recombinant DNA strand has been isolated, the structure of the fragment is determined using Southern Blotting. Southern Blotting involves electrophoresis of the recombinant DNA followed by hybridization with known radioactive phosphorylated DNA fragment. Northern blotting is the corresponding method of isolated RNA fragments.

Of all the techniques available for the analysis of DNA and RNA, polymerase chain reaction (PCR) is the simplest, most rapid and most effective. It is based on the enzymatic amplification of a sequence of an unknown DNA fragment. The DNA fragment is formed by the action of DNA polymerase on a “primer” strand of DNA. The primer strand is a portion of the unknown DNA which is paired with a known fragment of a short oligonucleotide. The procedure is rapidly repeated. After 30 rounds of amplification, more than a billion DNA copies may be formed.

Once the recombinant DNA is isolated, DNA sequence analysis may be performed. The most common method is the Sanger procedure, named after Fred Sanger, who was awarded the Nobel Prize for his work in 1980 (his second, his first was in 1958 for protein sequence analysis). The method involves using a nucleotide analog during DNA synthesis which inhibits DNA polymerase. Electrophoresis is then used to determine fragment size. Depending upon the nucleotide analog, the sequential identification of each DNA fragment may be determined.

Detection of specific proteins is usually performed via Western Blot Analysis. This technique is similar to the Southern and Northern Blotting techniques used in DNA and RNA analysis. It involves the detection of specific protein extracts which are obtained via electrophoresis. Chromatography separates the proteins based upon size. A known antibody is washed over the protein. The antibody may be radioactive or fluorescent for identification.

Genetic Variation and Mutation

A mutation is defined as any permanent change in the nucleotide sequence of DNA. They may be classified into three broad categories: genome mutations, chromosome mutations and gene mutation.

Mutations may occur in somatic or germline cells, but only germline mutations are inherited. Somatic mutations, however, are responsible for many medical problems, including a significant proportion of cancers, and perhaps, coronary artery disease. It is this reason that cancer and coronary artery disease are often considered “genetic” diseases.

Genome mutations are missegregation of the chromosome and usually result in aneuploidy. The classic example is Down Syndrome (Trisomy 21). These are the most common of all human mutations, and occur an estimated one per 50 meiotic divisions. Most of these mutations are incompatible with life.

Chromosome mutations are the next most common cause of genetic variation and mutation. They usually involve chromosomal translocations and rearrangement, and occur approximately once per 1000 meiotic divisions. These anomalies are usually incompatible with life.

Gene mutations are base pair substitutions, insertions or deletions in the DNA sequence. DNA replication progresses at a rate of nearly 20 base pairs per second per site of DNA polymerase. Despite this rapid speed, there is only one point mutation per ten million copied base pairs. Additionally, there are numerous “repair” enzymes which scan the “size” of the double helix and repair identified abnormalities. These repair enzymes are effective 99.9% of the time. Thus, a point mutation occurs less than one in 10 billion base pairs. Since there are approximately one billion base pairs, this translates to less than one base pair point mutation per cell division.

Genetics and Cancer

The field of cancer genetics is rapidly growing. Currently it is believed a tumor is a clone of cells derived from a single somatic mutation. These mutations are either single point mutations, multiple point mutations or translocations. The genes that cause these cancers are of two types: oncogenes and tumor suppressor genes. Oncogenes facilitate malignant transformation whereas tumor suppressor genes block tumor growth.

Oncogenes are genes that affect normal cell growth. They are believed to be derived from normal growth genes, called protooncogenes, which have undergone a somatic mutation. This can lead to uncontrolled cell growth. Oncogenes have been identified by recombinant DNA techniques. Suspected oncogenes have been isolated and transferred into mice, thus generating malignant foci. To date, more than 50 oncogenes have been identified.

Tumor suppressor genes are responsible for blocking abnormal growth. Function of these genes are lost only when both alleles undergo a somatic mutation. This is more commonly known as the “two hit hypothesis” of cancer. In contrast, oncogenes are formed when only one allele of a protooncogene is altered.

P53 Tumor Suppressor Gene. The p53 tumor suppressor gene arrests the cell cycle in the G1 (growth –1) phase thus allowing repair of genetic injury. It also induces apoptosis (programmed cell death). It is the most commonly demonstrated abnormality in head and neck cancer.

Somatic mutation of the p53 tumor suppressor gene is postulated to induce SCC of the head and neck. Cigarettes and alcohol are known to cause mutations in this gene. In a study by Davidson in 1993, mutations of the p53 gene were found in 40% of head and neck cancer. Despite these findings, mutations in the p53 gene have not been conclusively shown to have prognostic abilities in SCC of the head and neck.

Bcl-2 Tumor Suppressor Gene. The normal B-cell lymphoma/leukemia –2 gene (Bcl-2) tumor suppressor gene functions to prevent apoptosis (programmed cell death). Studies by Friedman have identified this gene as a highly sensitive marker for predicting prognosis in squamous cell carcinoma of the head and neck. Friedman did a retrospective study of early (T1N0, T2N0) SCC of the head and neck and found overexpression of Bcl-2 correlated with a 50% cure rate versus a 90% cure rate in those with normal gene expression. Other studies, however, particularly those by Gallo, have failed to confirm these findings.

The Treatment of Genetic Disease

Genetic disease can be treated at many levels. Most diseases are treated at the “phenotypic” level by medical or surgical intervention.

Gene transfer therapy is the attempted modification of abnormal cell function by “transferring” normal functioning genes into the genome. An area of intense research at present, the goal is to transfer functional copies of DNA into a patient, leading to permanent correction of the mutant phenotype. There are two types of gene transfer therapy: gene addition and gene replacement. In general gene addition is more practical, as it is the expression of a normal allele that is required. Gene replacement, in contrast, has never been accomplished in vivo.

The goal of gene therapy is the improvement of a patient’s health by correction of the mutant phenotype. This requires delivery of the recombinant gene into the malfunctioning somatic cells. General transfer strategies include either gene transfer via a plasmid (usually through a bacterium), or more commonly, through a retroviral vector. Viral vectors use recombinant RNA and reverse transcriptase to function. It is therefore not necessary to transfer the recombinant nucleotide directly into the host genome.

Current problems in gene transfer therapy are primarily the inability to maintain a high expression of the transferred gene in the target cell. Conversely, the second most common problem is overexpression of the transferred gene in the target cell. These seemingly opposite problems are areas of intense research.

Genetic Disease in Otolaryngology

Cystic Fibrosis. Cystic fibrosis is the most common fatal autosomal disease in caucasians. The basic phenotypic defect is a failure of exocrine function, including those of the upper and lower airway as well as the pancreas. The CF gene has been isolated to chromosome 7q and spans about 250,000 bases. Recent studies have shown the defect probably involves failure of cyclic AMP activated chloride channels, which also leads to membrane sodium transport discrepancies. The first CF mutation is a deletion of phenylalanine at position 508, but this is found in only 70% of all people. The remainder are believed to suffer other point and/or frameshift mutations.

Current CF therapy is unfortunately limited to phenotypic medical and surgical therapy. Current areas of research include gene transfer therapy.

Cleft Lip and Palate. Cleft lip with or without cleft palate is one of the most common congenital malformations. Cleft lip with cleft palate (CL and P) is distinct from isolated cleft palate. They originate as a failure of fusion of the frontal process with the maxillary process at about 35 days gestation.

Although classically presented as a multifactorial disease, there are single gene forms, chromosomal disorders (particularly Trisomy 13) and teratogenic forms (Rubella, thalidomide exposure). The multifactorial nature of the disease is represented by the unusual inheritance pattern.

Human Papilloma Virus. The exact role of HPV in head and neck cancer is not yet known, although it is well documented to be carcinogenic to the genitourinary tract. It has also been shown that binding of the E6 HPV protein to the p53 tumor suppressor gene leads to markedly decreased p53 action and subsequently tumorigenesis in vitro.

HPV oncogenic types 16, 18 and 31 have been associated with squamous cell carcinoma of the tongue, tonsil, larynx and pharynx in 46% of post mortem specimens. There was no statistical correlation towards survival in these specimens.

Thyroid Carcinoma. Medullary thyroid carcinoma (MTC) is a neoplasm of the parafollicular C cells of the thyroid gland. They are derived from the ultimobranchial body and secrete calcitonin. Two types exist, the sporadic and the familial forms. The familial forms are hereditary and are seen in the MEN II syndromes. MEN IIA involves pheochromocytoma and hyperparathyroidism, whereas MEN IIB is associated with neurofibromatosis and Marfan's habitus.

Familial MTC is associated with the a mutation in the RET protooncogene. More than 90% of all people with this protooncogene develop MTC. The RET protooncogene is located on the short arm of chromosome 10.

Papillary thyroid carcinoma is also associated with genetic abnormalities, with more aggressive lesions associated with aneuploidy. Noninvasive disease is uniformly diploid.

Salivary Gland Neoplasms. Prognostic significance has long been associated with genetic markers in salivary gland tumors. Adenoid cystic carcinoma was studied in a series of twenty patients. All patients with DNA aneuploidy recurred after resection, whereas only 2 of 14 DNA diploid lesions recurred. Of note, aneuploid lesions are usually more radiosensitive. Similarly, salivary gland adenocarcinoma neoplasms which showed expression of c-erb B2 oncogene were more difficult to resect, recurred more frequently and had more frequent nodal metastases.

Acoustic Neuroma. Approximately 5% of all acoustic neuromas are familial, and are associated with neurofibromatosis Type II. The chromosome has been shown to be a deletion of the short arm of chromosome 22.

Congenital Hearing Loss. Approximately 60% of all congenital hearing loss is associated with a genetic and/or phenotypical abnormality. Recently, several chromosomal abnormalities have been identified.

Waardenberg's Syndrome is an autosomal dominant disorder with variable penetrance. Clinical manifestations include dystopia canthorum, hyperchromatic iris,

white forelock and sensorineural hearing loss. The genetic locus has been identified and named the PAX3 locus of chromosome 2. Currently, all therapy is at the phenotypic level.

Usher's Syndrome is an autosomal recessive disorder associated with retinitis pigmentosa. It exists in five different classifications, all of which have been isolated on different chromosomes. All therapy is currently at the phenotypic level.

Pendred's Syndrome is an autosomal recessive disorder with variable penetrance that has recently been mapped to the long arm of chromosome 7. It is associated with thyroid goiter and thyroid carcinoma. Treatment is currently at the phenotypic level.

Alport's Syndrome is either an X linked or an autosomal recessive disease. It is characterized by deafness and nephritis. The X linked variation is located on the short arm of the chromosome and produces the alpha 5 protein gene. The recessive form is located on the short arm of chromosome 2 and codes for collagen type IV. Treatment is at the phenotypic level.

Future Directions and Conclusion

Molecular genetics is a rapidly expanding field with nearly limitless possibilities for the treatment of head and neck diseases. Therapy of genetic disease of the head and neck is currently limited to the pharmacologic and surgical treatment of phenotypic expressions. Gene therapy, if theory is proven practical, could eliminate disease at the molecular level, thus obviating the need for pharmacologic or surgical treatment.

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