

Molecular Genetics and Otolaryngology



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Introduction



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

History



- Gregor Mendel, 1865
 - “Mendel’s Laws” of autosomal inheritance
 - Work “lost” until early 1900’s
- Charles Darwin, 1859
 - “The Origin of Species”
 - Jean Baptiste Lamarck

History, continued



- Francis Galton (Charles Darwin's cousin)
 - The "father" of modern genetics
 - rediscovered Mendel's laws
 - "nature versus nurture"
 - "inborn errors of metabolism" responsible for biological abnormalities

History, Continued



- James Watson and Francis Crick
 - DNA discovered in 1940's
 - Determined double helix in 1953
 - Nobel Prize in 1962
- Human Genome Project
 - Begun in 1990
 - Goal is to identify every human gene by 2005
 - 9% completed as of 1999

Classification of Disorders



■ Single Gene Defects

- | Usually single critical error in the genetic code
- | Usually phenotypically obvious
- | Examples: NF I and II, osteogenesis imperfecta, cystic fibrosis

Classification, continued



■ Chromosomal disorders

- | not due to single defect
- | usually due to deficiency in number of genes within chromosome
- | classic example is Down Syndrome (Trisomy 21)
- | other examples: Trisomies 13, 18, Klinefelter's Syndrome, Turner's Syndrome
- | phenotypically obvious
- | usually incompatible with life

Classification, continued



■ Multifactorial inheritance

- | multiple single code defects
- | usually form a pattern
- | classic examples: cleft lip/palate, neural tube defects
- | possible example: head and neck cancer?

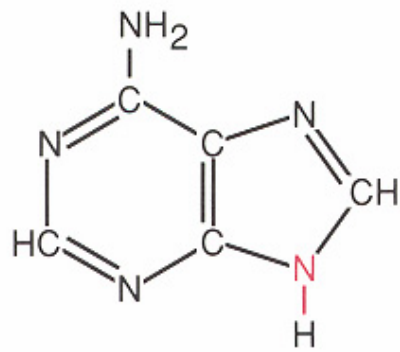
Chromosomal Structure



- 23 pairs of chromosomes
- approximately 7 million base pairs
- 100,000 genes
- DNA:
 - | five carbon sugar (deoxyribose; ribose in RNA)
 - | nitrogen base (purines, pyrimidines)
 - | 3'5' phosphate linkage
 - | hydrogen bonded double strand

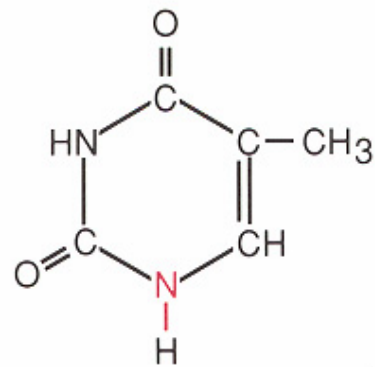
DNA Bases

Purines

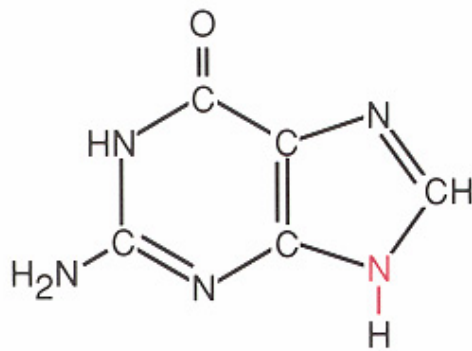


Adenine (A)

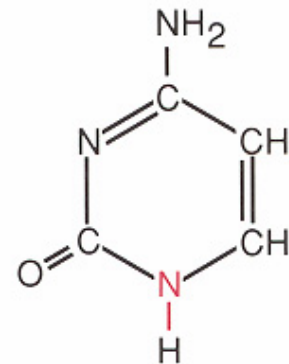
Pyrimidines



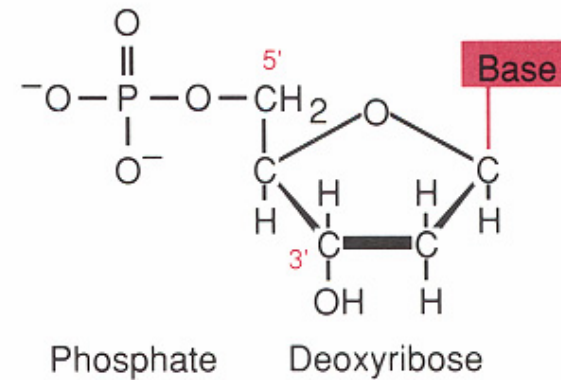
Thymine (T)



Guanine (G)



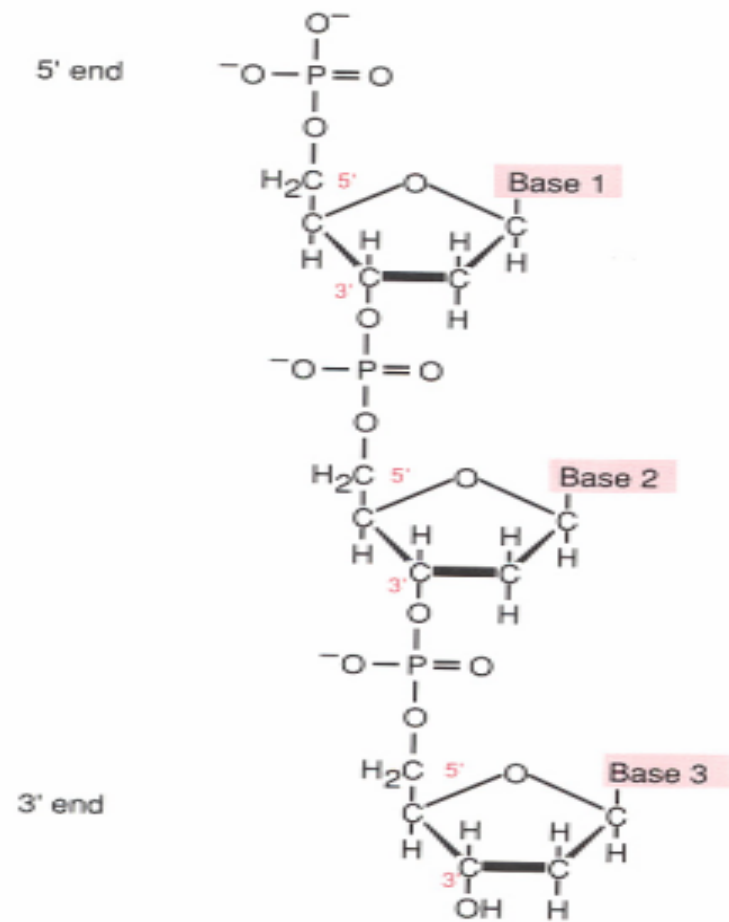
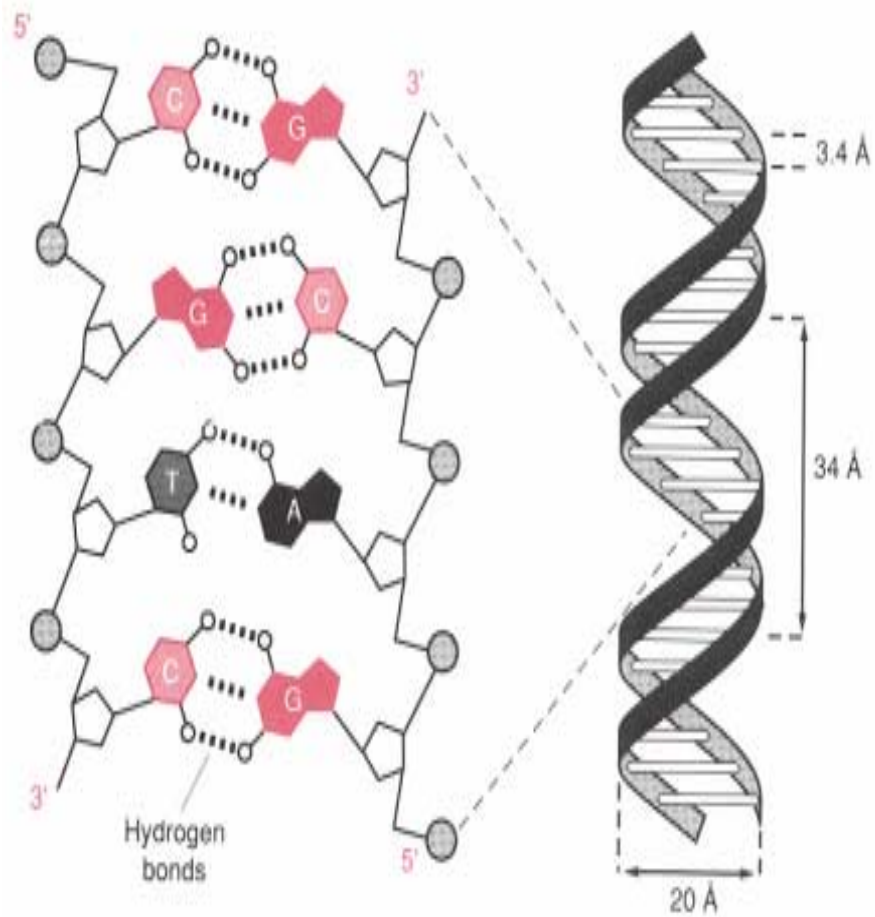
Cytosine (C)



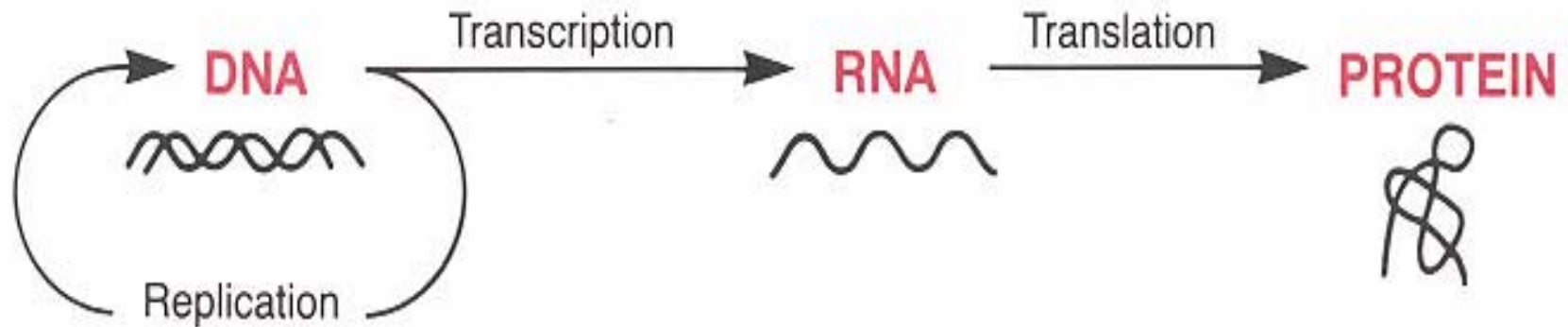
Phosphate

Deoxyribose

DNA Bases



Transcription



■ The Central Dogma

Tools of Genetics



- Revolutionary changes since late 1970's
 - | restriction enzymes
 - | recombinant DNA
 - | vectors
 - | probes
 - | PCR
 - | DNA sequence analysis
 - | protein analysis

Tools of Genetics, cont.



■ Restriction Endonucleases

- | enzymes which cleave DNA at specific sites
- | almost always palindromic
- | hundreds of known endonucleases

■ Recombinant DNA

- | an DNA fragment is combined with a known piece of DNA to form a plasmid
- | plasmid inserted in vector (bacterium, virus, yeast)
- | vector cultured and isolated

Tools, continued



- Identification of recombinant fragments
 - “Blotting” - southern, northern, western
 - | electrophoresis/chromatography of fragment
 - | hybridization with known radioactive fragment
 - | antibodies to known fragments may be used

Tools, continued



- Polymerase Chain Reaction (PCR)
 - | simplest, most rapid, most effective
 - | enzymatic amplification of desired fragment
 - | DNA fragment formed by endonuclease
 - | known "primer" is annealed to fragment
 - | steps repeated approximately 30 times
 - | yields more than a billion copies of desired DNA fragment

Tools, continued



■ DNA Sequence Analysis

- Fred Sanger, Nobel Prize 1980

 - also won Nobel Prize in 1958 for protein analysis

- nucleotide analog which inhibits DNA synthesis

- endonuclease which cleaves at nucleotide site

- electrophoresis/chromatography

- radioactive tagging/antibodies

Genetic Mutations



- Defn: Permanent change in nucleotide sequence
- occur in somatic cells or germline cells
 - | only germline cells inherited
- somatic mutations believed responsible for many medical problems
 - | many cancers, ?CAD

Genetic Mutations, cont.



■ Genome Mutations

- | missegregation of chromosome
 - results in aneuploidy
 - Down Syndrome classic example
 - 1:50 meiotic divisions
 - usually incompatible with life

Genetic Mutations, cont.



■ Chromosome mutations

- | usually involve translocations and rearrangements
- | 1:1000 meiotic divisions
- | almost uniformly incompatible with life

■ Gene mutations (single gene defects)

- | DNA replicates 20 bases/sec/polymerase
- | Only one defect per ten million copies
- | Repair enzymes repair 99.9% of defects
- | Less than one defect per 10 billion bases!

Genetics and Cancer



- Tumor cells are clone of abnormally dividing cell
 - | usually from single/multiple point mutations
 - | rarely from translocations
- Protooncogenes
 - | normal growth genes
- Oncogenes
 - | a protooncogene which has undergone somatic mutation and is oncogenic

Genetics/Cancer, cont.



■ Tumor Suppressor Genes

- | genes that regulate cell growth/genomic expression
- | p53, Bcl-2 are classic examples
- | p53:
 - arrests growth in G1 (growth 1) phase
 - allows repair of DNA defects
 - induces apoptosis (programmed cell death)
 - found in 40% of HNSCCa
 - have NOT shown correlation with prognosis

Genetics/Cancer, cont.



■ Bcl-2 tumor suppressor gene

- | normal Bcell lymphoma/leukemia gene (Bcl-2)
- | prevents apoptosis (programmed cell death)
- | somatic mutations present HNSCC, usually resulting in overexpression
- | Friedman's study:
 - retrospective study of Stage I/II HNSCCa
 - overexpression of Bcl-2 lead to 50% cure versus 90% in normal expression
 - others unable to reproduce (see Gallo)

Treatment



- Most disease treated at phenotypic level
 - | medicines
 - | surgery
 - | genetic counseling
- Molecular level
 - | gene therapy

Treatment, continued



■ Gene Therapy

- | attempted modification of abnormal cell function
- | involves transfer of functioning genes
- | gene therapy via addition
 - more practical
 - insertion into cell (not necessarily into genome) of functioning gene
- | gene therapy via replacement
 - theoretical
 - goal is to replace abnormal gene with inserted gene

Treatment, continued



■ Gene therapy, continued

| Transfer strategies

- recombinant DNA in vector
 - viral versus bacterium
 - retroviral vectors with reverse transcriptase
- not inserted into host genome

| problems:

- inability to maintain expression
- under/overexpression
- adenine deaminase deficiency (ADA)

Genetic Disease in ENT



■ Cystic Fibrosis

- | chromosome 7q, spans 250,000 bases
- | 70% have deletion of phenylalanine at position 508 (point mutation)
 - frameshift versus point mutation
- | most common fatal autosomal disease in whites
- | phenotypic expression results from failure of membrane transport (Cl, Na) and from exocrine function (pancreas)
- | Tx at phenotypic level

Genetic Dz in ENT, cont.



■ Cleft Lip and Palate

- | one of the most common malformations
- | CL and P genetically distinct from isolated CL
- | failure of fusion of frontal process with maxillary process at 35 days gestation
- | classically described as multifactorial, although single gene forms, chromosomal forms (Trisomy 13) teratogenic forms (rubella, thalidomide) are known

Genetic Dz in ENT, cont.



■ Human papilloma virus

- | strains 16, 18 and 31 carcinogenic in GU tract
- | exact role in HNSCCa not fully known, although 46% of post mortem specimens contained HPV strains
- | E6 HPV protein binds to p53 forming mutation which suppresses gene function in vivo

Genetic Dz in ENT, cont.



■ Thyroid carcinoma

| Medullary thyroid carcinoma (MTC)

- neoplasm of parafollicular C cells (ultimobranchial body)
- produce calcitonin
- sporadic and familial forms
- familial MTC associated with MEN 2A and 2B
 - MEN 2A: pheo, hyperparathyroid, MTC
 - MEN 2B: pheo, MTC, Marfan's, NFI
- RET protooncogene associated with familial forms
 - 10p

| Aggressive papillary CA associated with aneuploidy

- noninvasive dz uniformly diploid

Genetic Dz in ENT, cont.



■ Salivary Gland Neoplasms

- | Aggressive adenoid cystic Ca associated with aneuploidy
 - all patients with aneuploidy recurred after resection versus only 2/14 with diploid genome (Sugano)
- | Salivary gland adenocarcinoma with overexpression of Bcl-2 were more difficult to resect, recurred more frequently and metastasized more frequently (Sugano)

Genetic Dz in ENT, cont.



■ Acoustic Neuroma

- | 5% are familial and associated with NF II
- | often bilateral
- | NF II defect on 22p
- | therapy at phenotypic level

Genetic Dz in ENT, cont.



■ Congenital Hearing Loss

- | 60% of congenital hearing loss is genetic
- | most associated with phenotypic anomaly
- | Waardenburg Syndrome
 - autosomal dominant - variable penetrance
 - dystopia canthorum, hyperchromatic iris, white forelock and SNHL
 - PAX3 locus of chromosome 2
 - treatment at phenotypic level

Genetic Dz in ENT, cont.



■ Congenital hearing loss, continued

| Usher's Syndrome

- autosomal recessive
- five different classifications (Usher's Types I through V)
 - all subtypes on different chromosomes
- associated with retinitis pigmentosum
- therapy at phenotypic level

Genetic Dz in ENT, cont.



- Congenital Hearing Loss, continued
 - Pendred's Syndrome
 - | autosomal recessive with variable penetrance
 - | located on chromosome 7q
 - | associated with thyroid goiter and carcinoma
 - | tx at phenotypic level

Genetic Dz in ENT, cont.



■ Congenital hearing loss, cont.

■ Alport's Syndrome

- | two forms: X linked, autosomal recessive
 - X linked on 5p, produces mutant alpha 5 protein
 - recessive form on 2p, produces mutant Type IV collagen
- | treatment at phenotypic level

Genetic Dz in ENT, cont.



■ Head and Neck Cancer

- heavily associated with p53 underexpression, Bcl-2 overexpression, HPV types 16, 18 and 31
- None of these proven prognostic
- Ultimate goal: gene therapy to correct somatic mutation

Future Directions and Conclusion



- Rapidly expanding field
- Ultimate goal: correction of somatic defect which would correct phenotypic abnormality. Would eliminate surgical intervention.