Molecular Genetics and Otolaryngology

Michael E. Prater, MD
Faculty Advisor: Shawn D. Newlands, MD, PhD
The University of Texas Medical Branch
Department of 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 inheritence
  - Work "lost" until early 1900's
- Charles Darwin, 1859
  - "The Origin of Species"
  - Jean Baptiste Lemarck

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: Trimsomies 13, 18, Klinefelter's Syndrome, Turner's Syndrome
  - phenotypically obvious
  - usually incompatible with life

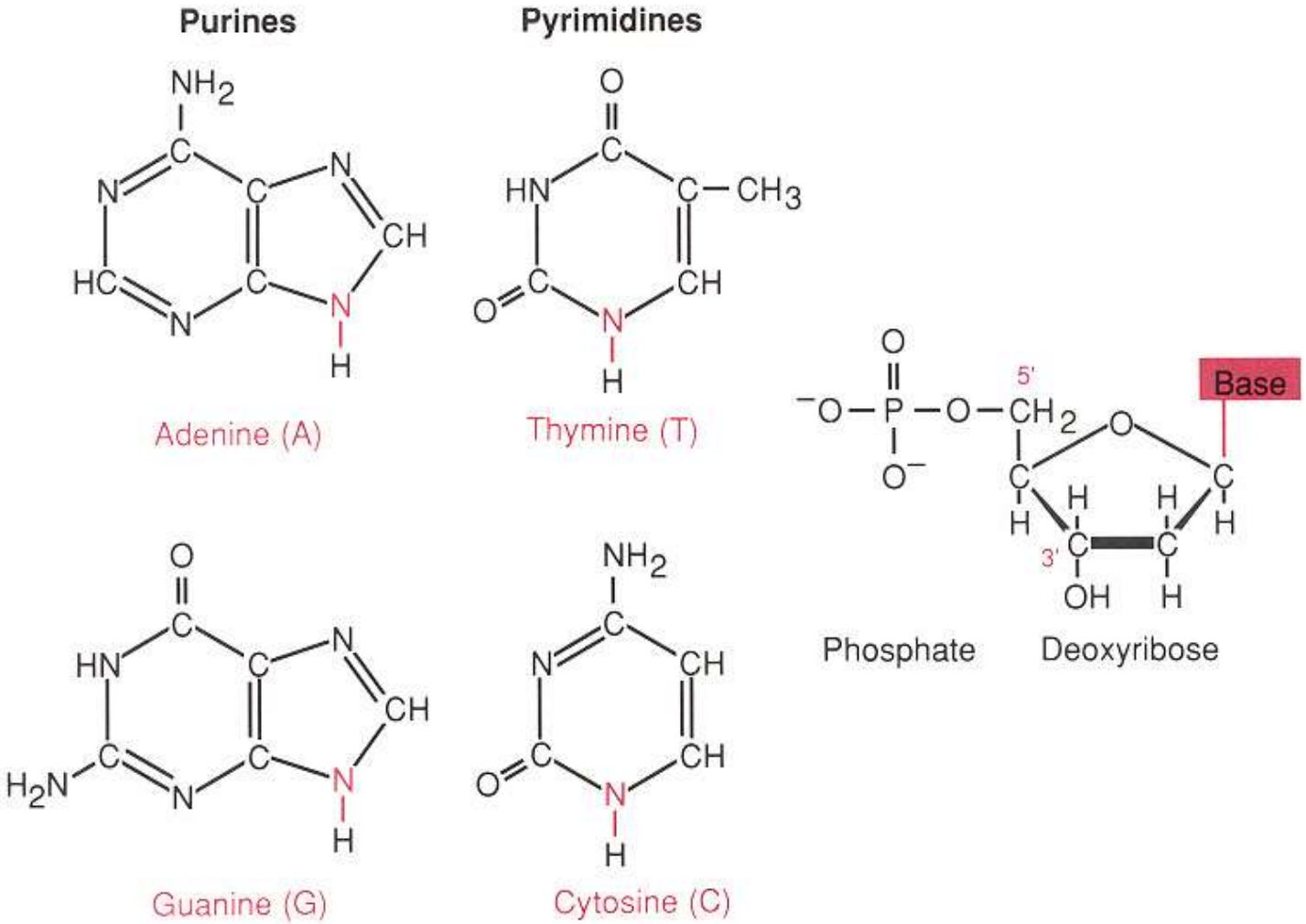
Classification, continued

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

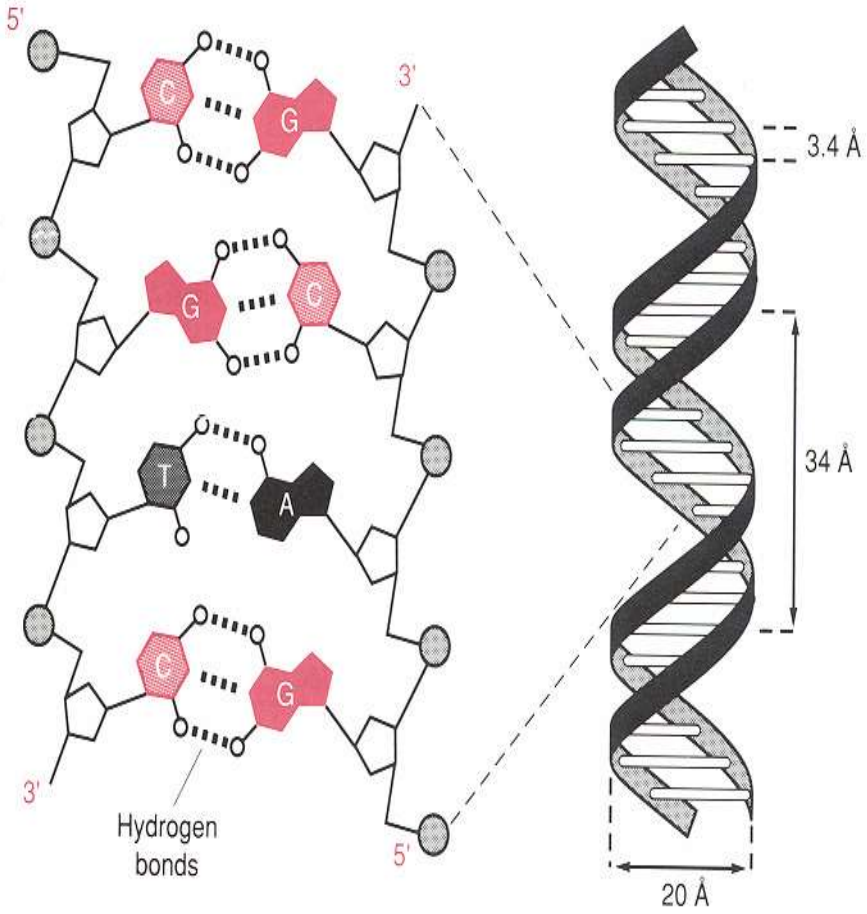
Chromosomal Structure

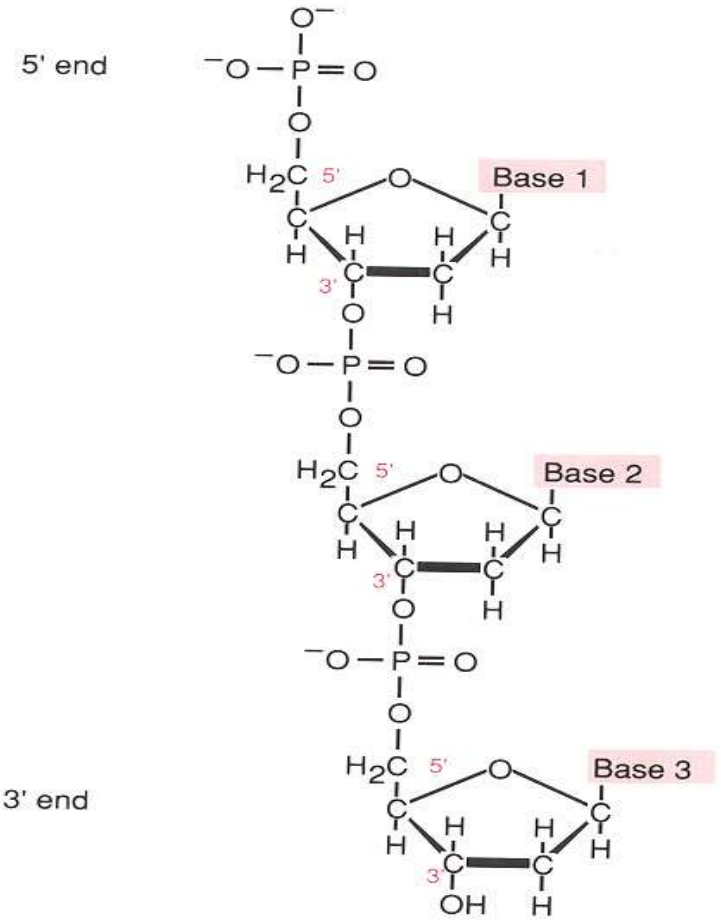
- 23 pairs of chromosomes
- approximately 7 million base pairs
- 100,000 genes
- DNA:
  - five carbon sugar (deoxyribose; ribose in RNA)
  - nitrogen base (purines, pyrimidines)
  - 3’5’ phosphate linkage
  - hydrogen bonded double strand

DNA Bases

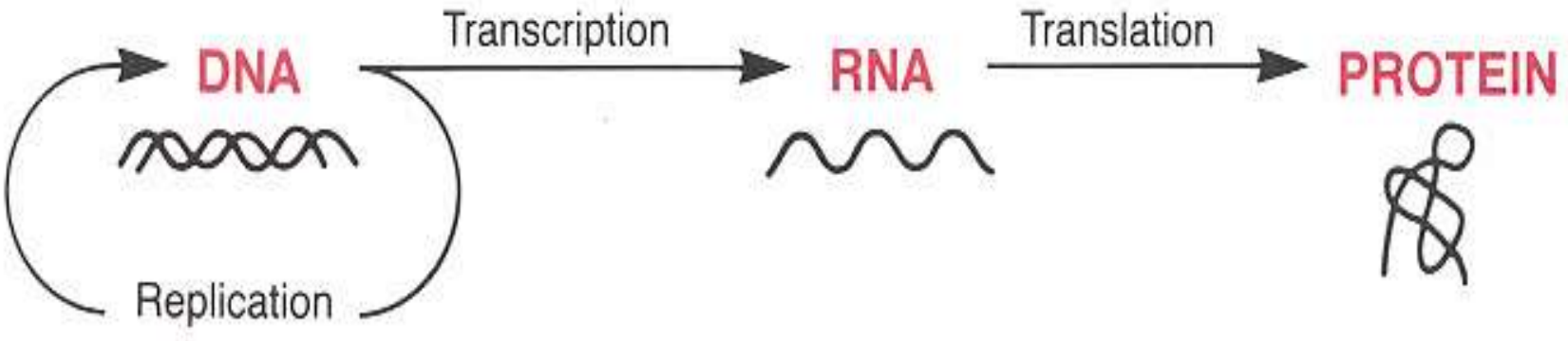

Purines
Pyrimidines
Adenine (A)
Thymine (T)
Base
Phosphate
Deoxyribose
Guanine (G)
Cytosine (C)

DNA Bases


5'
3'
C
G
G
C
T
A
C
G
3'
5'
Hydrogen bonds
3.4 Å
34 Å
20 Å


5' end
Base 1
Base 2
Base 3
3' end
OH

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/chromotography 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 with inhibits DNA synthesis
  - endonuclease which cleaves at nucleotide site
  - electrophoresis/chromotography
  - radioactive tagging/antibodies

Genetic Mutations

- Defn: Permanent chagne 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

Gentic 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 froms, 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 familiar forms
    - familial MTC associated with MEN 2A and 2B
      - MEN 2A: pheo, hyperparthyroid, 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 inENT, 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.