HST.161 Molecular Biology and Genetics in Modern Medicine
Fall 2007

Harvard-MIT Division of Health Sciences and Technology
HST.161: Molecular Biology and Genetics in Modern Medicine, Fall 2007
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HST 161

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Lecture 1 Part 1

Goals of Medical Genetics

- Identify patterns of DNA sequence variation which contribute to or cause human disease

- Use this knowledge to understand the underlying molecular basis of pathology

- Use this knowledge to provide diagnostic insight and information to patients and their families

- Use this knowledge to develop treatments and cures for human disease

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Evidence Supporting a Genetic Basis for a Clinical Observation

- Family studies reveal a Mendelian inheritance pattern.

- Chromosomal analysis correlates a specific pattern of clinical symptoms with a specific chromosomal aberration.

- Relative frequency of a specific pattern of clinical symptoms in genetically related individuals is higher than less related or unrelated individuals.

- Specific DNA sequence differences or karyotype variations are observed in clones of somatic cells correlated with a specific clinical phenotype (for example--tumor cells)

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What do we mean by the statement

"There is a **Genetic Basis** for a Clinical Observation?"

Evidence Supporting a Genetic Basis for a Clinical Observation

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Family studies reveal a Mendelian inheritance pattern

The observation of **a Mendelian inheritance pattern for phenotype in a single family** means that there is *one and only one site of DNA sequence variation in the genome* responsible for that phenotype *in that family*

Family studies reveal a Mendelian inheritance pattern

Direct molecular genetic tests can be carried out to identify the relevant site of genetic variation associating genetic variation and clinical phenotype via a cause and effect relationship

Chromosomal analysis correlating a specific pattern of clinical symptoms with a specific chromosomal aberration

- Clinical symptom patterns can be stereotypically associated with specific chromosome monosomies, trisomies
- Specific deletions, duplications and inversions occur repetitively in the human population and are often associated with specific clinical symptom patterns

Relative frequency of a specific pattern of clinical symptoms in genetically related individuals is higher than less related or unrelated individuals

- Clinical symptoms may occur more frequently in the presence of specific genotypes--higher genotype relative risk

- Genetic inheritance pattern-- "complex trait"

- Case control or population based studies are used to identify higher risk genotypes

Somatic Cell Genetics

A clone of cells is observed which shows phenotypic characteristics associated with disease and exhibit a particular pattern of genetic changes characteristic of that disease state

Human Genetic Variation and Medical Genetics

Goal--Identify sites of genetic variation which cause or contribute to the causes of human disease

Human Genetic Variation

- Single base pair differences (SNPs) and differences in small numbers of adjacent base pairs
- Insertion or deletion of a DNA sequence including duplication of a DNA sequence and expansion or contraction in the numbers of copies of a repeated sequence
- Rearrangement of DNA sequences including chromosomal inversion and translocation
- Variation in chromosome number including trisomy, partial monosomy, variation in sex chromosome number
- Epigenetic variation including variation in DNA methylation and imprinting

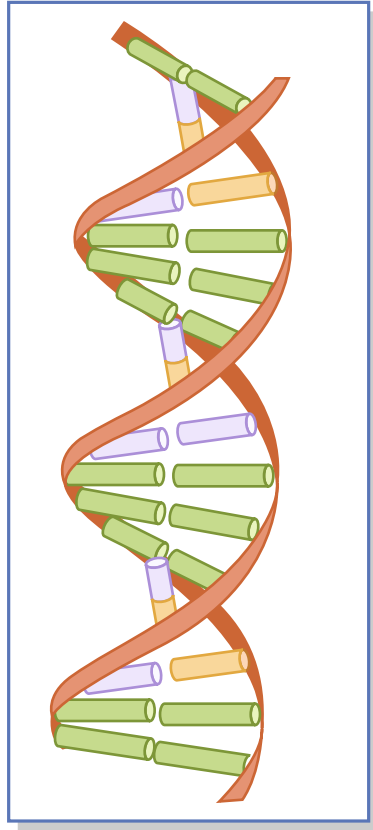# Human Genetic Information is Encoded in the Structure of the DNA Molecules of the Human Genome

Figure by MIT OpenCourseWare.

Image removed due to copyright restrictions.
Human chromosomes.

- Whole genome *haploid* =3 billion base pairs

- 23 linear DNA molecules (each a single chromosome)

  (also one small circular molecule--mitochondrial DNA)

- Average chromosome=150 million base pairs of DNA

Some Key Features of DNA

- Made up of units of deoxyribose (a five Carbon sugar) and four basic building blocks termed nucleotides connected in a linear array by phosphate groups
- Purines

A = adenine   G = guanine

- Pyrimidines

C =cytosine   T = thymine

Images removed due to copyright restrictions.
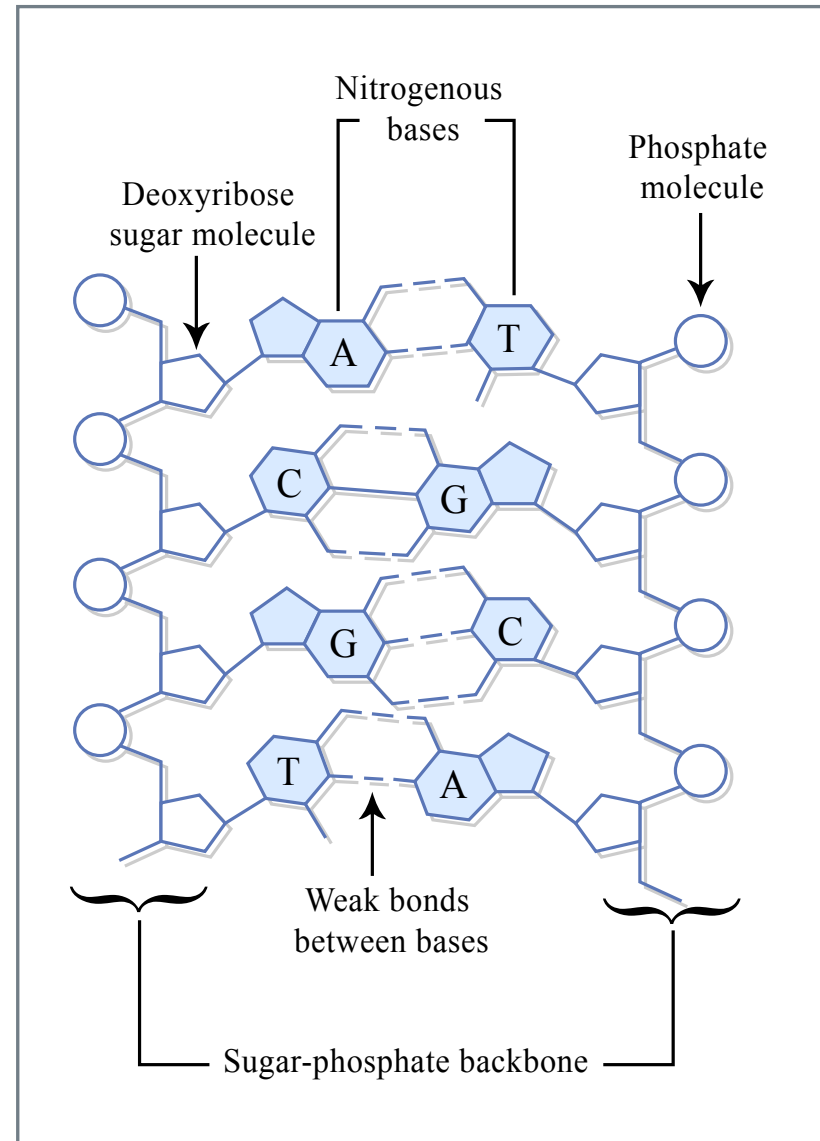Atomic models of the 4 nucleic acids.

Figure by MIT OpenCourseWare.

Some Key Features of DNA

¥ Sugar-phosphate linkages are the structural basis for polymer formation

¥ Linear DNA chain (single strand) is accompanied by a complementary DNA chain to form a double stranded helical molecule

¥ Each DNA strand has a 5'--3' orientation. The two strands of a double helix are ANTIPARALLEL.

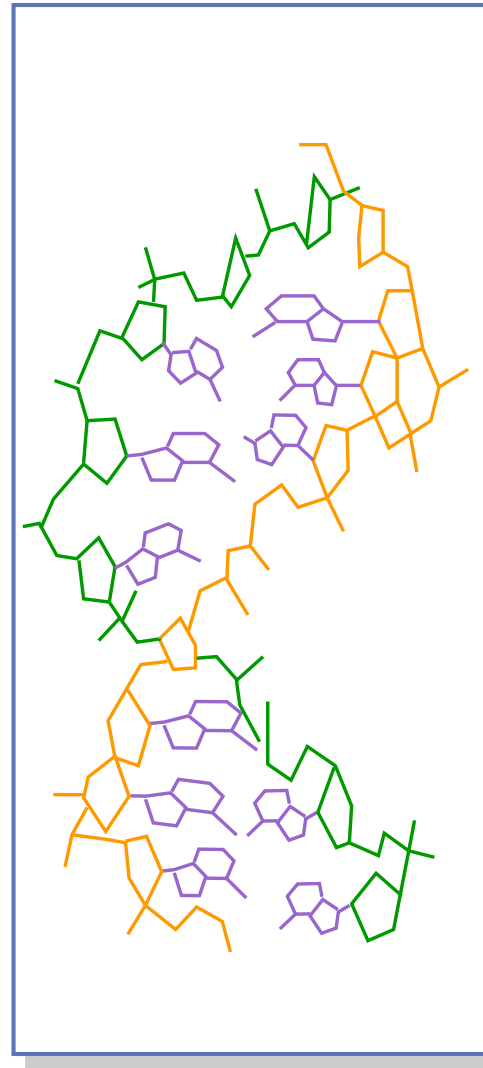Image of DNA structure removed due to copyright restrictions.

Figure by MIT OpenCourseWare.

Some Key Features of DNA

- In living cells DNA is normally double stranded, A is always opposite T and G is always opposite C. The hydrogen bonds formed by these base pairs cause the double stranded structure to be more stable than the separated single strands.

- Information is encoded by the order of the nucleotide building blocks in the linear DNA polymer.

- Information encoded in DNA is expressed through the production of RNA molecules which either act directly to perform a biochemical function or act as messenger RNA to direct the synthesis of polypeptide chains

Image removed due to copyright restrictions. Wireframe and space-filling models of DNA.

Differences between RNA and DNA

- In RNA the same building blocks are used except that ribose replaces deoxyribose and uracil replaces thymine

- RNA is less stable than DNA because the 2' OH of ribose in RNA can attack the phosphodiester linkage via the formation of a glycol intermediate to break the RNA chain. The 2' position of the DNA chain is an H which can't carry out this reaction

- Deamination of cytosine will lead to uracil not thymine making it possible for DNA repair enzymes to distinguish a damaged base;

- However, 5- methyl cytosine is deaminated to thymidine which is not recognized as a damaged base by repair enzymes so genomic sites which can be methylated to form 5 methyl cytosine have higher mutation rates than other bases in the human genome

Images removed due to copyright restrictions.
Chemical differences between RNA and DNA.

Physical Properties of DNA

- DNA has the characteristics of a long thread or rope.
- When DNA is prepared in solution, shearing forces will break a long DNA molecule.
- It is therefore extremely difficult to prepare large DNA molecules intact in the test tube.
- Most DNA analysis of human DNA is therefore done with DNA which is broken by shear forces to some extent and is therefore significantly less than 100,000 base pairs in length.
- In the cell, the intact DNA molecule may be up to a thousand times this length.

Some Important Chemical and Physical Properties of DNA

- Each human diploid cell contains approximately 10^{-11} gm of DNA corresponding to 6×10^9 base pairs

- Each base pair is stacked approximately 3.4 Angstrom above the next. The helix turns completely after approximately 10 base pairs.

- Each human cell contains approximately 2 meters of DNA

- DNA must be tightly packaged to fit into the nucleus of a cell

- Repulsion of negatively charged phosphate groups makes it difficult to pack DNA tightly without first neutralizing charge

- A positively charged family of small proteins, the histones, neutralize the negative charge of the phosphates in most eukaryotic cells.

Figure by MIT OpenCourseWare.

Histones, neutralization of negative charge and packaging of DNA in chromatin

Figure by MIT OpenCourseWare.

- Repulsion of negatively charged Phosphate groups makes it difficult to pack DNA tightly without first neutralizing charge
- A positively charged family of small proteins, the histones, neutralize the negative charge of the phosphates in most eukaryotic cells.
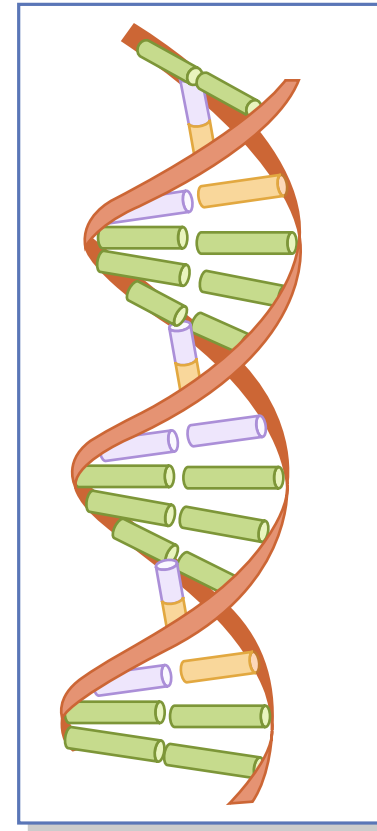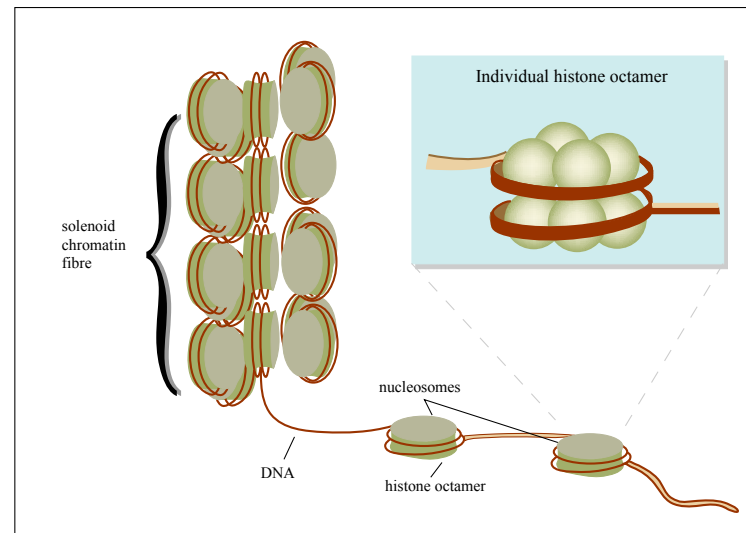- The tight association between histones and DNA allows an orderly structure termed the nucleosome to be formed.

Images removed due to copyright restrictions.
Molecular model of DNA coiled around histones.

DNA (LADDER) IS COILED AROUND THE OUTSIDE OF THE
NUCLEOSOME--HISTONES (RIBBON) ARE ON THE INSIDE

Nucleosome subunits are linked together like beads on a string to form chromatin structure

Image removed due to copyright restrictions.
DNA coiled around histones.

Packaging of DNA into Coils and Supercoils is Necessary to Fit DNA into the Nucleus

- DNA in the nucleus is tightly wound into coils and "supercoils" which allow better packaging but also affect the structure of the DNA
- To access the DNA for information transfer and to replicate it, it is necessary to unwind the coils
- Specialized enzymes are necessary to unwind the coiled and supercoiled DNA

Packaging of DNA in Chromatin and in Chromosome

Image removed due to copyright restrictions.
Illustration of DNA packaging into a chromosome.

Duplicating DNA

- Each time a cell divides, the division must be preceded by the replication of the cellular DNA.

- The synthesis of new DNA molecules involves the process of semi-conservative replication.

- Each DNA strand in the cell must unwind and the double helix must temporarily divide into two single strands.

Duplicating DNA

- The enzymatic machinery of the cell then catalyzes the synthesis of a new complementary strand for each of the separated strands

- G-C & A-T base pairing rules are used to determine the new base to be added

- Bases in the new strand can only be added in a 5' to 3' direction.

Mutation--An Event which Causes Human DNA Sequence Variation

- Error in DNA replication

- Damage to DNA and errors in DNA repair

- Errors in chromosome segregation during mitosis

- Errors in chromosome segregation and synapsis during meiosis

- Also errors in epigenetic marking of DNA can have important phenotypic (clinical) consequence even though the primary sequence of DNA is not altered.

Mutations Can occur in Germ Cells and Somatic Cells

- Consequence of mutation in a germ cell--mutation is transferred to all cells of the zygote fertilized by mutant gamete
- Consequence of mutation in a somatic cell--development of a clone of mutant cells in an organism

Quality Control is Built in to Human DNA Metabolism

- Proofreading and error checking during DNA replication
- Checkpoints following key steps in the cell cycle including start and end of DNA synthesis and steps of mitosis and meiosis
- Repair of damaged DNA bases

Mosaicism and Chimerism

- Genetic events which occur after conception cause mosaicism; these include non-disjunction at mitosis and new mutations

- Mixing of the cells of two genetically distinct organism is termed chimerism; can occur rarely through fusion of two embryos--but frequently through exchange of hematopoietic stem cells between mother and fetus

Methods for Detecting Variation in Human DNA

- Technique
- PCR+Sequencing
- Southern blotting
- FISH-interphase
- FISH-metaphase
- Karyotyping

- Size range detected
- 1-500 base pairs
- 500 bp-20 kbp
- 20 kbp- 1 megabase
- 1 mB-10 mB
- 3 mB-200 mB

How to Detect Variation in DNA sequence Among Individuals in a Specific Gene

- Amplify a specific sequence in the genome by polymerase chain reaction (PCR)

- Determine sequence of amplified DNA by chain terminating sequencing reaction

- Once site of variation has been determined in one or more individuals, develop an assay for variation at that site which can be carried out on large numbers of individuals (genotyping assay)

Copying DNA in a the Laboratory Using a Purified DNA Polymerase

- DNA polymerases absolutely require a free 3'end to add onto

- A chemically synthesized oligonucleotide primer is used to direct the synthesis of the DNA polymerase

- DNA synthesis directed by oligonucleotide primers are the basis of amplification by polymerase chain reaction (PCR) and dideoxynucleotide (Sanger) DNA sequencing

PCR CAN BE USED TO AMPLIFY A SPECIFIC HUMAN DNA SEQUENCES UP TO 2000 BASE PAIRS LONG ROUTINELY (AND LARGER LENGTHS UP TO APPROXIMATELY 10,000 BASE PAIRS WITH ADDITIONAL EFFORT)

TWO OLIGONUCLEOTIDE PRIMERS
~20 BASE PAIRS LONG MUST
BIND ACCURATELY TO THE
CORRECT SITES IN HUMAN DNA
TO AMPLIFY THE SEQUENCES
IN BETWEEN

Image removed due to copyright restrictions.
Illustration of how DNA is amplified exponentially through PCR.

PCR PRODUCT IS USED TO IDENTIFY VARIATION IN DNA SEQUENCE

Image removed due to copyright restrictions.
Peaks of fluorescence from tagged nucleotides are graphed to determine the sequence of a piece of DNA.

Sequencing DNA

- DNA to be sequenced must contain a primer binding sequence immediately adjacent to area of DNA to be sequenced

- Primer binding sequence may be present because it is adjacent to site in vector into which DNA to be sequenced has been inserted

- Primer binding sequence may be introduced adjacent to DNA to be sequenced as part of primer sequence in a PCR reaction

DNA Sequencing Reaction

- Template DNA
- DNA polymerase
- Oligonucleotide Primer
- Deoxynucleotide triphosphates (dATP, dCTP, dGTP, TTP)
- One chain terminating nucleotide triphosphate (for example: dideoxyCTP)
- Reporter group (fluorescent or radioactive) attached either to primer or chain terminating nucleotide

Slides removed due to copyright restrictions.
Illustrations of DNA sequencing process.

Massively parallel sequencing technologies

- Grow millions of "colonies" of short DNA molecules each immobilized to a solid support

- Carry out a stepwise set of DNA synthesis reactions which add a single flourescent base at a time to a primer site

- Use optical imaging to read the identity of the added base at each position on the solid support

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Nucleic Acid Hybridization

- Many laboratory based procedures for analyzing DNA are based on the increased thermodynamic stability of the complementary double stranded structure over the stability of the separated single strands. The process by which two complementary DNA strands are allowed to come together in the laboratory is termed nucleic acid hybridization or reannealing.

- Phosphate groups are negatively charged leading to a repulsive force between the two strands of a DNA double helix

- As the temperature is increased the energy of the hydrogen bonds is eventually unable to overcome the force of the negative charge from the phosphate atoms and the double stranded DNA molecule "melts" into two complementary single strands. The temperature at which this occurs is termed the melting temperature or T_m for a given DNA double stranded (also termed duplex) molecule

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NUCLEIC ACID HYBRIDIZATION IS A FUNDAMENTAL TOOL FOR ANALYSIS OF DNA STRUCTURE

Image removed due to copyright restrictions.
When denatured and allowed to re-nature, DNA strands will anneal only to complementary strands.

Slides removed due to copyright restrictions.
Diagram of how to probe DNA for specific sequences.

HOW ALLELE SPECIFIC OLIGNUCLEOTIDE HYBRIDIZATION IS USED TO DETECT VARIANT HUMAN ALLELES

Image removed due to copyright restrictions.

Oligonucleotides can be created with different point mutations to see which one will anneal to the mutated gene, since only identical strands can anneal to each other. This will identify the mutation.

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A A   G G   A A   A A

G A   A A

A A G A G A   A A G A   G A G A G A A A   G A A A
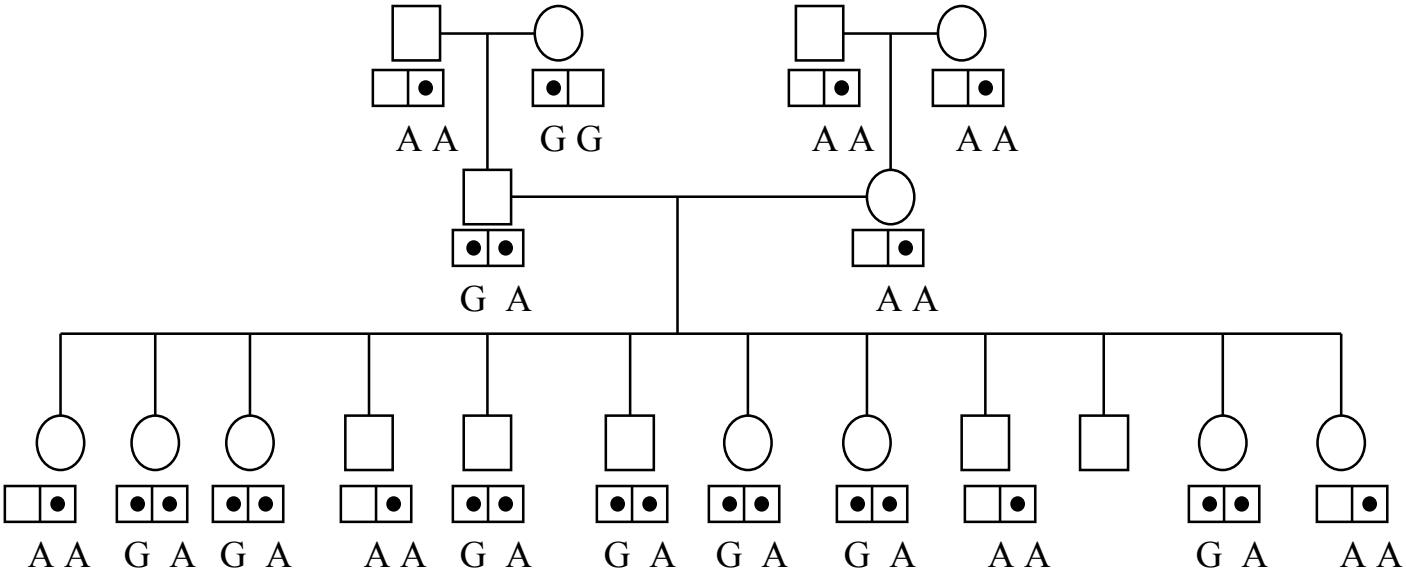
Binding of oligonucleotides which differ by a single nucleotide has the sensitivity to detect single base differences in individuals in a family or a population; these differences always inherit in a Mendelian pattern except in the exceptional circumstance in which there has been an error in meiosis

DNA chips an be used to carry out many such genotyping assays in parallel

A **DNA chip** is a small piece of silicon glass (~1 cm^2) to which a large number of synthetic, single-stranded **DNA oligonucleotides** *("oligos")* have been chemically bonded [left]. Oligos function as **DNA probes**: they "*stick*" (anneal) selectively only to those **DNA** molecules whose nucleotide sequences are exactly complementary: **T** pairs with **A**, and **G** with **C**. They can therefore be used to **identify the presence of specific DNA sequence differences** in a heterogeneous mixture of genes

Images removed due to copyright restrictions.

DNA chips can be used as **Variant Detector Arrays (VDAs)** to look for **DNA** sequences that differ by **single nucleotide polymorphisms** ("*SNPs*"). In this example, the **DNA** sequences of the four **oligos** highlighted in the first bloc differ only at the last position. To determine which alleles are present, **genomic DNA** from an individual is isolated, fragmented, tagged with a fluorescent dye, and applied to the chip. The **genomic DNA** fragments anneal only to those **oligos** to which they are perfectly complementary: in this case, the allele with the **~T~ SNP** allele binds to the∧ **~~A oligo**, and the allele with the **~C~ SNP** allele binds to the **~~G oligo**. A computer reads the position of the two fluorescent tags and identifies the individual as a **C / T heterozygote**. [The *single* spots in the other three columns indicate that the individual is *homozygous* at the three corresponding **SNP** positions].

Images removed due to copyright restrictions.

Current chip designs allow the reading of up to a million sites of variation in the genome simultaneously

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Dimensions of the human genome

- Whole genome *haploid* =3 billion base pairs
- 23 linear DNA molecules (each a single chromosome) (also one small circular molecule-- mitochondrial DNA)
- Average chromosome=150 megabases
- Human genetic map=3000 centimorgans
- 1 megabase ~= 1 centimorgan
- Average human gene~= ~10 ,000 base pairs

Repeat Sequences Are Interspersed in the Human Genome Including in Introns and Promoters

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Image removed due to copyright restrictions.

Chart of different types of interspersed repeats in the human genome, including LINEs, SINEs, retrovirus-like elements, and DNA transposon fossils.

Interspersed repetitive DNA sequences make many molecular biology procedures more difficult for mammalian genomes

- Southern blotting
- PCR
- Fluorescent in situ hybridization (FISH)
- Comparative genomic hybridization

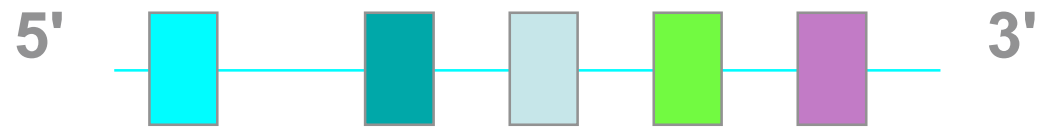# Gene Duplication is an Important Element in the Human Genome

- Gene Duplication is the Evolutionary Source of Genes with Closely Related Functional Properties (Gene Families)
- Gene duplication can lead to unequal crossing over at meiosis--consequences include deletion and inversion
- Gene duplication can lead to the presence of gene copies which have lost function--pseudogenes

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Gene Families such as the Receptor Tyrosine Kinases have Evolved through Gene Duplication
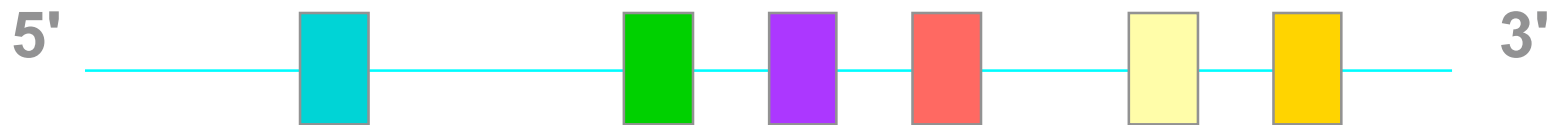
Globin Gene Organization

ζ   ψζ   ψα   α2   α1

5'   3'
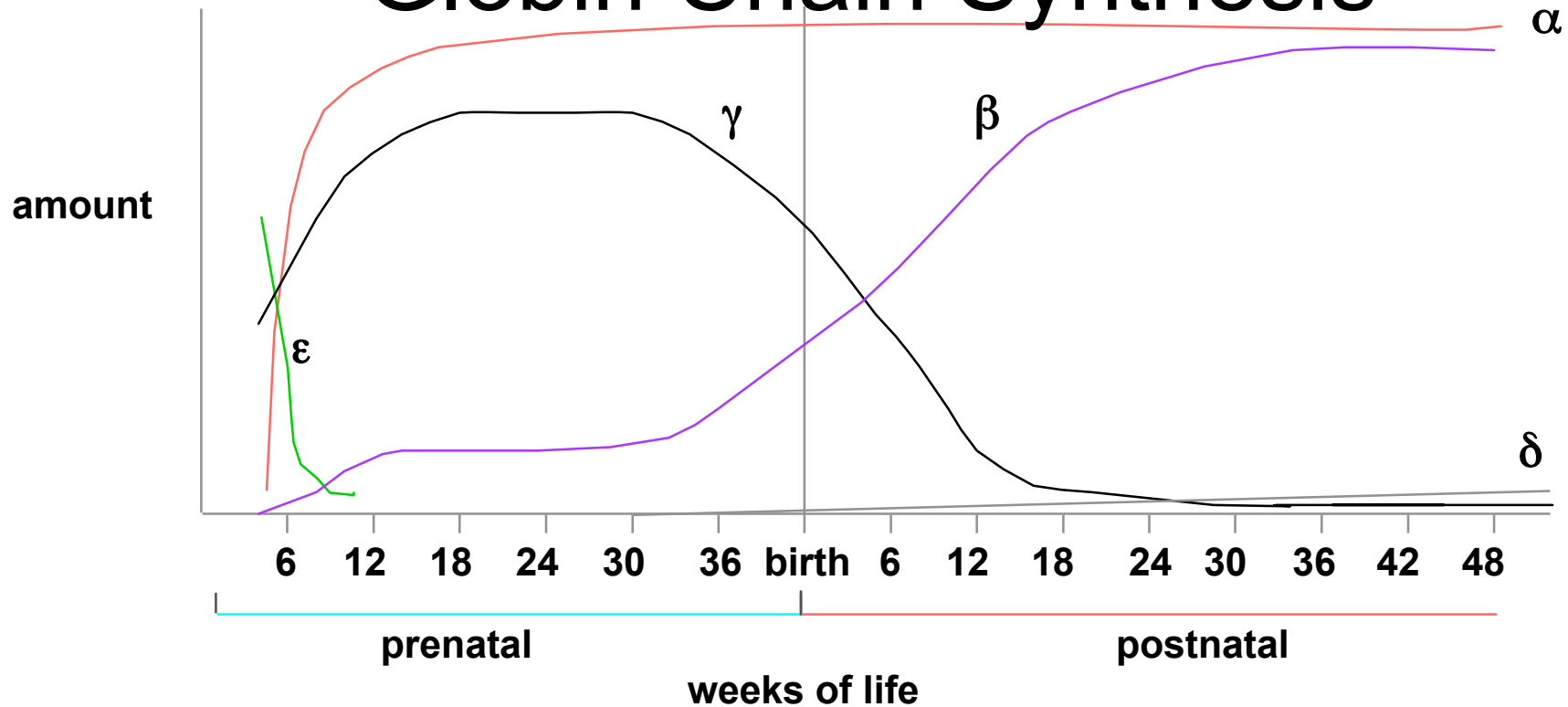
Alpha cluster on chromosome 16

ε   **G**γ   **A**γ   ψβ   δ   β

5'   3'

Beta cluster on chromosome 11

Globin Chain Synthesis

amount

α

β

γ

ε

δ

birth

6 12 18 24 30 36 birth 6 12 18 24 30 36 42 48

prenatal

postnatal

weeks of life

$\alpha_2\varepsilon_2$ embryonic hemoglobin

$\alpha_2\gamma_2$ fetal hemoglobin

$\alpha_2\beta_2$ hemoglobin A

$\alpha_2\delta_2$ hemoglobin A2

Globin Gene Duplications

- The α globin gene has been recently duplicated in the human genetic lineage; both copies of the α globin gene are functionally similar

- Ancient duplications are responsible for the presence of embryonic and fetal globin genes ζ (embryonic α globin) ε (embryonic β globin) and the two $G\gamma$ and $A\gamma$ (fetal β globin) genes

- The δ globin gene is an older duplicated adult β globin gene which has diverged from adult β globin and is expressed at low levels in adult red blood cells

- Some duplications have led to the presence of non-functional pseudogenes such as $\psi\beta$, $\psi\alpha$ and $\psi\zeta$

Unequal crossover between the two α globin genes can lead to deletion of one of the α globin genes and α thalassemia; α thalassemia mutations have an extremely high frequency in East Asian populations

Image removed due to copyright restrictions.
Illustration of allele crossover.

Slides removed due to copyright restrictions.

Fig. 3 from Blake, Derek J. "Function and Genetics of Dystrophin and Dystrophin-Related Proteins in Muscle." *Physiological Reviews* 82 (2002): 291-329.

THE DYSTROPHIN PROTEIN HELPS TO ATTACH THE MUSCLE FIBER TO THE EXTRACELLULAR MATRIX

THE DYSTROPHIN PROTEIN CONTAINS A SERIES OF REPEAT UNITS WHICH RESEMBLE THE PROTEIN SPECTRIN THE REPEAT UNITS ARE THE CONSEQUENCE OF DUPLICATION OF THE DNA SEQUENCE WHICH ENCODES THIS UNIT
DYSTROPHIN PROTEIN WITH A SMALLER NUMBER OF SPECTRIN LIKE REPEAT UNITS CAN STILL FUNCTION

What Is The Frequency Of Human Mutation?

Point mutations, the alteration of one or a small number of base pairs at a particular site in DNA, can occur as frequently as 1 in 20,000 per human generation at a specific site but generally occur at 1 per 10^8 or less per human generation at most sites

Mutations due to unequal crossing over and other forms of genetic exchange can occur at frequencies as high as 1 in 7,000 per human generation

Anueploidy due to errors in chromosome segregation at meiosis occur in as many as 1 in 3 conceptions

ACHONDROPLASIA

- FREQUENT RECURRENT NEW MUTATION AT A SINGLE SITE IN THE GENOME

Image removed due to copyright restrictions.
Photograph of a young girl with achondroplasia.

- Short stature

  Age = 4 years old

  Height = 20 mos

- Midface hypoplasia

- Trident hand

- Curvature of the spine

Image removed due to copyright restrictions.
Fig. 2 from Haworth, J. C., and A. E. Chudley. "Dwarfs in Art." *Clinical Genetics* 59 (2001): 84-87.

If one parent has achondroplasia, and one does not there is a 50/50 chance that their child will have achondroplasia

If both parents have achondroplasia, there is a 2 out of 3 chance their child will have achondroplasia

Image removed due to copyright restrictions.
Photograph of an achondroplasiac couple; wife is pregnant.

ACHONDROPLASIA

- Incidence aproximately 1 in 20,000
- Automsomal dominant inheritance pattern with 100% penetrance--so whenever a mutant gamete is transmitted from parent to child, achondroplasia phenotype is observed
- New mutatons almost always occur in the transmission of gametes from an unaffected father in almost all cases
- Age of father important risk factor
- Mutation almost always at the same nucleotide in the DNA of the FGFR3 gene

Image removed due to copyright restrictions.
Diagram of male gametogenesis.

Number of Cell Divisions in the Male Primary Germ Cells

- AGE   CHROMOSOME REPLICATIONS
- 15   35
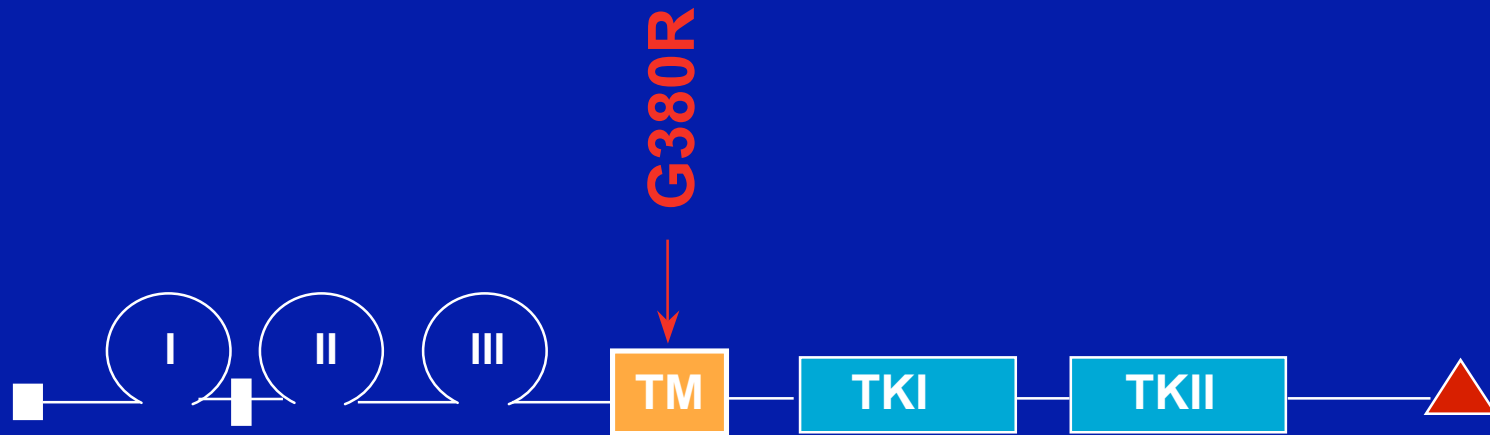- 20   150
- 30   380
- 40   610
- 50   840

MUTATION IN FGFR3 WHICH CAUSES ACHONDROPLASIA

G380R

I   II   III   TM   TKI   TKII
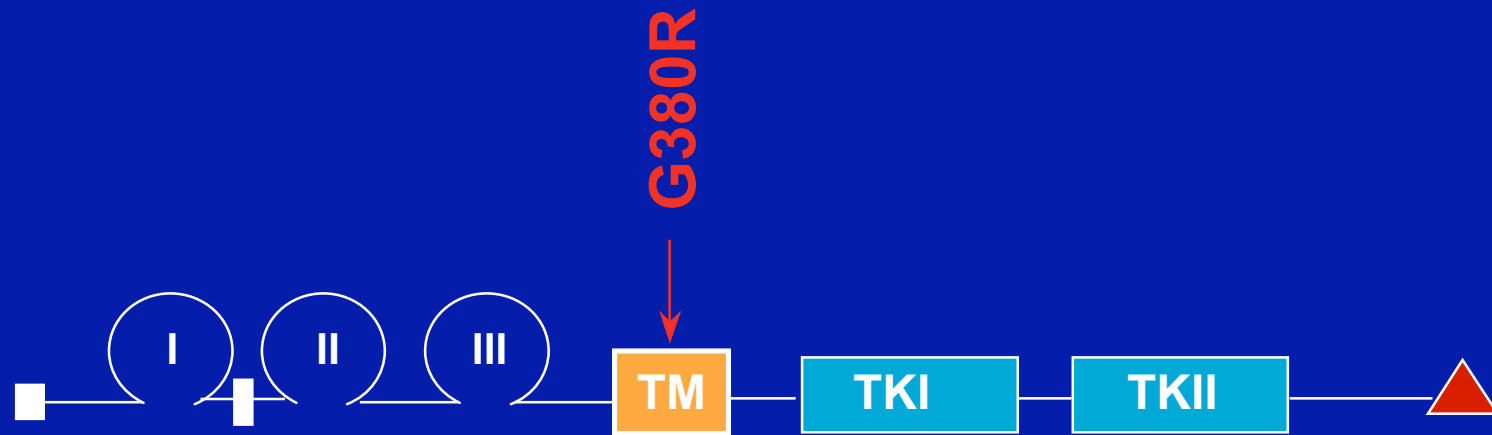
PROTEIN:- GSVYA G ILSY G VGFFLFILVVAAVTC -

R

MUTATION IN FGFR3 WHICH CAUSES ACHONDROPLASIA

G380R

DNA: GGC ATC CTC AGC TAC GGG GTG GGC TTC TTC

TAC AGG
ATG TCC

CpG DINUCLEOTIDES IN THE HUMAN GENOME

- CpG dinucleotides are the most frequent site of point mutation in humans

- Presence of methyl cytosine in DNA allows conversion to thymine by deamination

- Thymine is a normal base and so it is not removed by DNA repair enzymes

Gene Duplications are Common in Mammalian Genomes

- Tandemly duplicated genes can undergo unequal crossover to create mutant phenotypes with high frequency
- Pseudogenes can serve as the source for mutations which inactivate genes by gene conversion

DNA Sequences which are duplicated next to each other on a chromosome (tandemly repeated) can frequently undergo changes due to misalignment during recombination events
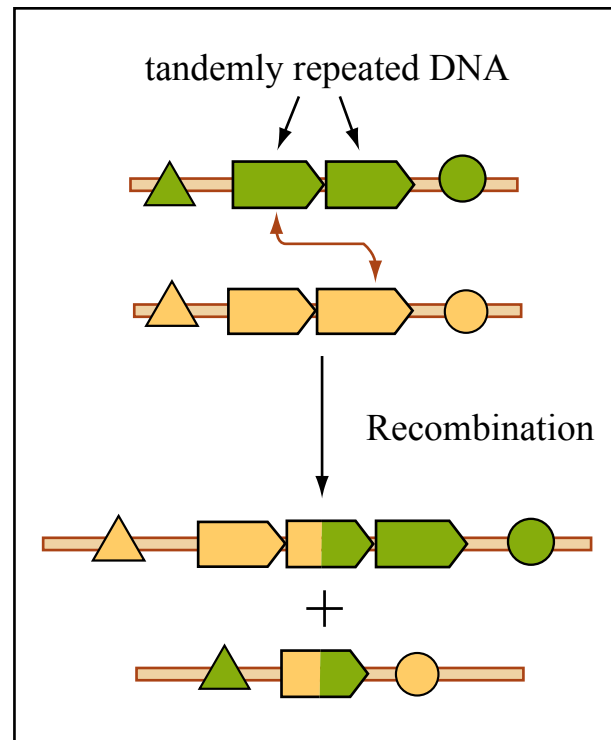

Figure by MIT OpenCourseWare.

Red-green colorblindness

- Initial development of discrimination between red and green was a consequence of duplication of an opsin gene on the X chromosome
- High frequency of red-green colorblindness in the human population is a consequence of frequent incorrect pairing at meiosis leading to many new mutations
- These mutations are then transmitted in families

Glucocorticoid remediable aldosteronism

Image removed due to copyright restrictions.

- Promoter for 11- β-hydroxylase which controls production of cortisone is placed by unequal croosing over in front of gene controlling synthesis of aldosterone (aldosterone synthase)
- Results in hypertension

Globin Gene Organization

ζ   ψζ   ψα   α2   α1

Alpha cluster on chromosome 16

ε   **G**γ   **A**γ   ψβ   δ   β
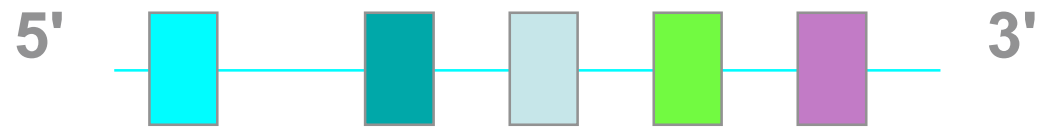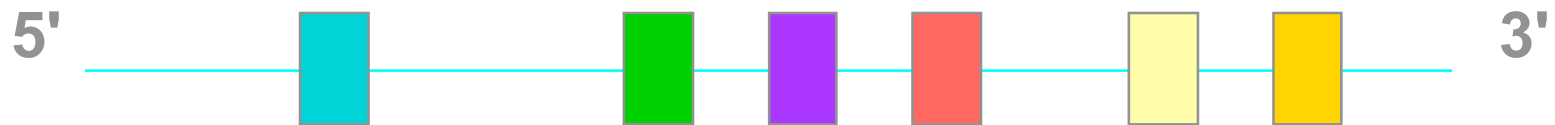
Beta cluster on chromosome 11

Unequal crossover between the two α globin genes can lead to deletion of one of the α globin genes and α thalassemia; α thalassemia mutations have an extremely high frequency in East Asian populations

Image removed due to copyright restrictions.

21-hydroxylase deficiency

- 21-hydroxylase deficiency (also known as congenital adrenal hyperplasia) is a disorder of steroid metabolism. People with a shortage of the enzyme 21-hydroxylase cannot convert cholesterol to cortisol and aldosterone, steroid hormones that regulate stress responses and blood pressure, respectively. When the precursors of cortisol and aldosterone build up in the adrenal glands, they are converted to androgens. Elevated levels of androgens can affect growth and development in both males and females.

- There are three types of 21-hydroxylase deficiency. Two types are classic forms, known as the simple virilizing and salt-loss types.

- Simple virilizing 21-hydroxylase deficiency causes a buildup of potent androgens that leads to the masculinization of external genitalia in females at birth. The development of the reproductive organs (uterus and ovaries) in these patients is normal.

- Salt-loss 21-hydroxylase deficiency results from an extremely severe loss of enzyme activity. In these cases, so little aldosterone is produced that the kidneys do not reabsorb sodium (in the form of salt).

- In the third type of 21-hydroxylase deficiency, known as the nonclassic form, there are moderate levels of functional 21-hydroxylase enzyme. Both males and females with the nonclassic type can display signs and symptoms of androgen excess after birth.

- The incidence of the classic forms of 21-hydroxylase deficiency is 1 in 15,000 live births. The prevalence of the nonclassic form of 21-hydroxylase deficiency is estimated to be 1 in 100 individuals.

- Mutations in the active copy of the 21-hydroxylase gene occur through genetic exchange with a pseudogene for 21-hydroxylase nearby the active gene.

MISALIGNMENT OF DUPLICATED SEQUENCES IN AN OPPOSITE ORIENTATION ON A CHROMOSOME CAN LEAD TO AN INVERSION OF A DNA SEQUENCE

- Example : Factor VIII gene inversion is most frequent cause of hemophilia A

- 1 in 4000 males is born with an absence or low level of clotting Factor VIII--~40% of cases of hemophilia A are due to Factor VIII gene inversion

Genetic Recombination: Inversion in Factor VIII gene

Image removed due to copyright restrictions.
Recombination follows the length of the DNA, moving from
the telomeric to the centromeric end, bridging the cross and
following along the strand.

Duplicated sequences can lead to deletion of millions of base pairs of DNA--Example--22q deletion syndrome

~1 in 4,000 live births is a child with 22q deletion syndrome hemizygous for a region of chromosome 22 up to 3 megabases in length

The deletions which cause 22q deletion syndrome are most frequently caused by unequal crossover between repeat sequences at positions A and D which are ~ 3 megabases apart

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DUCHENNE MUSCULAR DYSTROPHY

X linkage-- boys inherit a defective gene copy from from their unaffected (but mosaic) mothers

1 in 3,500 boys affected

30% of cases are new mutations

First signs at 2-3 years, wheelchair bound by 10 years

Often die from pneumonia or cardiac problems in teens

30% show mental retardation

Beckers muscular dystrophy-similar but much less severe

AN EARLY SIGN OF DUCHENNE MUSCULAR DYSTROPHY IS THE INABILITY TO RISE TO A STANDING POSITION WITHOUT USING THE ARMS FOR ASSISTANCE BECAUSE LEG MUSCLES ARE WEAK

Parents may notice that their child stumbles more frequently, waddles, has difficulty going up stairs, and toe walks (walks on the toes without the heels hitting the floor). Toddlers may develop a swayed back to compensate for weakening hip-area muscles. Children may struggle to get up from a sitting position or have a hard time pushing things, like a wagon or a tricycle.

Image removed due to copyright restrictions. Photograph of a young boy with Duchenne's, supporting himself by leaning down and holding onto his knees.

Phenotype to Genotype 1: DMD

Many children also develop enlarged calf muscles, a condition called calf pseudohypertrophy, as muscle tissue is destroyed and replaced by nonmuscle tissue.

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Photographs of a boy with Duchenne's with noticeably enlarged calf muscles.

DYSTROPHIN GENE IS THE LARGEST HUMAN GENE ~2.4 MEGABASES LONG (0.1% of human genome)

APPROXIMATELY 70% OF DUCHENNE MUSCULAR DYSTROPHY PATIENTS SHOW DELETIONS

DELETIONS IN EXON 47-52 AREA OF THE GENE MOST COMMON

REMAINDER OF DUCHENNE MUSCULAR DYSTROPHY HAVE POINT MUTATIONS WHICH CAUSE CHAIN TERMINATION, ABNORMAL SPLICING OR OTHER LOSS OF FUNCTION OF THE DYSTROPHIN PROTEIN

Image removed due to copyright restrictions.
Fig. 3 from Blake, Derek J. "Function and Genetics of Dystrophin and Dystrophin-Related Proteins in Muscle." *Physiological Reviews* 82 (2002): 291-329.

THE DYSTROPHIN PROTEIN HELPS TO ATTACH THE MUSCLE FIBER TO THE EXTRACELLULAR MATRIX

THE DYSTROPHIN PROTEIN CONTAINS A SERIES OF REPEAT UNITS WHICH RESMBLE THE PROTEIN SPECTRIN

DYSTROPHIN PROTEIN WITH A SMALLER NUMBER OF SPECTRIN LIKE REPEAT UNITS CAN STILL FUNCTION

Mutations in the dystrophin gene

- Becker and Duchenne muscular dystrophy
    - BMD is a less-severe disease (patients are still walking after 16 yrs)
    - DMD is a more-severe disease (patients are not walking at 12 yrs)

- both can be caused by massive deletions in the dystrophin gene (as well as other types of mutations)

- the severity is not necessarily correlated with the size of the deletion

Image removed due to copyright restrictions.
Diagram of dystrophin structure.

The relationship of the reading frame of the exons which are brought together by the deletion is crucial in determining whether a functional protein will be produced;
If the exons brought together are in the same reading frame, then a truncated protein will be produced and Becker's will result--but if they are in different reading frames no functional protein will be produced and the result will be Duchenne dystrophy

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Mendelian Inheritance

- DNA sequence variants inherit in a Mendelian inheritance pattern essentially 100% of the time

- Medically significant "traits" sometimes inherit in a Mendelian inheritance pattern

Mendelian Inheritance Patterns

- High genotype relative risk
- Unique pattern of clinical findings
- High penetrance

Genotype

- The genetic constitution of an individual, or in a specific case the alleles at specific genetic loci.

Phenotype

- The observed expression of a particular genotype.

Penetrance

- The proportion of individuals with a given genotype who express the phenotype under consideration.

- Penetrance has numerical value between 0 and 1.

- When penetrance is significantly less than 100% it is referred to as incomplete.

Age Dependent Penetrance

- The proportion of individuals with a given genotype who express the phenotype under consideration as a function of age.

- Age dependent penetrance can have a numerical value which rises as a function of age

Expressivity

- The extent or severity of the phenotype in individuals of equivalent genotype.
- Expressivity can be referred to as variable but does not have a quantitative value.

Genotype Relative Risk

- The ratio of individuals with a given genotype who exhibit a phenotype relative to an alternative genotype.

- Example: the number of individuals who get deep vein thrombosis who carry the Factor V Leiden allele compared to the frequency in the population of individuals who carry the normal allele at the Factor V locus.

Implication of a Mendelian Inheritance Pattern for a Medically Significant Trait

- There must exist a single site in the genome where a specific difference in DNA sequence is present which is the primary cause of the difference between affected and unaffected individuals.

Slides removed due to copyright restrictions.
Excerpts from textbook appendix on Mendelian pedigree patterns.

Family experience-- Autosomal dominant

- Family members are likely to be aware of the disease gene and its consequences because parents and close relatives are affected

Family experience--autosomal recessive

- Parents are unlikely to be aware of the disease gene and its consequences unless they are a member of a community with high allele frequency for gene

Family experience--X linked recessive

- Family members are often not aware of the disease gene and its consequences because many cases are new mutations; when other family members are affected they may often be cousins or second cousins

Family experience--maternal inheritance

- Family members often are affected variably because of heteroplasmy so the effects of the disease gene may be difficult for family members to understand clearly