MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Humangenetik Innsbruck

Medical Genetics
Rare diseases

Johannes Zschocke
Institute of Human Genetics

Human genetics

Mechanisms and principles by which genetic information influences health and disease in humans

Medical genetics = application of human genetics in medicine

Gregor Mendel
Versuche über Pflanzenhybriden
1865

Theodor Boveri & Walter Sutton
Chromosome theory
1902-1904

Thomas Hunt Morgan
Chromosomes in Drosophila
from 1908

James Watson, Francis Crick
Molecular structure of Nucleic Acids 1953

The Human Genome
2001

Human Genetics

The science of the hereditary basis of health and disease in humans

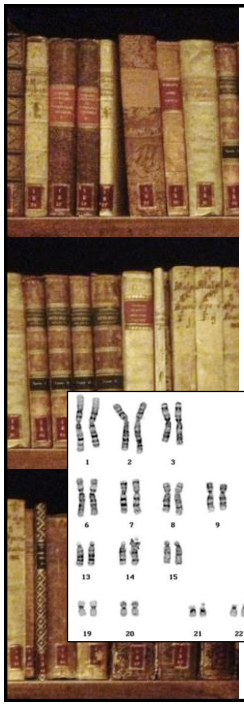
- Understanding how inherited (genetic) information determines...
  - normal functions of the human organism
  - the development of disease in humans
- Diagnosing genetic diseases and risk factors in individuals
  - clinically
  - special (genetic) laboratory tests
- Helping people to live with genetic diseases and risk factors
  - specific treatment, management
  - genetic counselling

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Humangenetik Innsbruck

Human genome basics

Johannes Zschocke
Institute of Human Genetics



Human genome

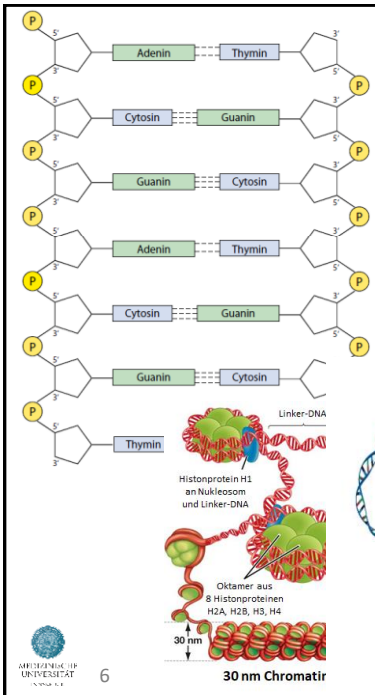
2 x 23 = 46 Chromosomes

3,096,649,726 Nukleotide
(„Golden Path Length“)

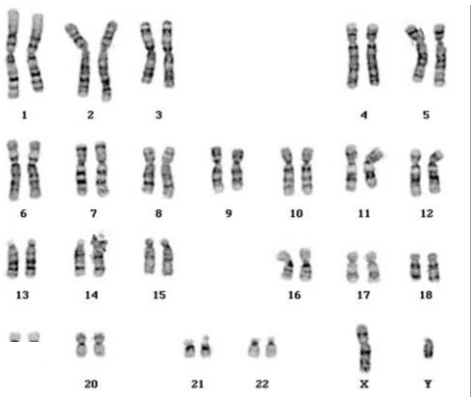
20,442 Protein coding genes
23,982 Non-protein coding genes
15,228 Pseudogenes

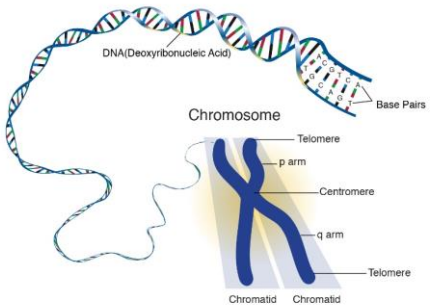
6,768,792 structural variants
714,562,852 short/small variants

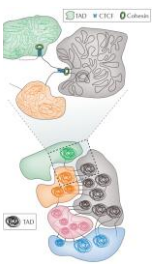
Ensembl genome browser, www.ensembl.org
Database version 104.38 (März 2021)



Human genome

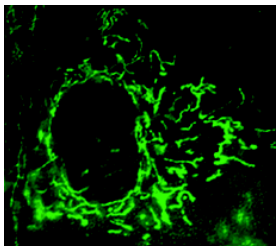


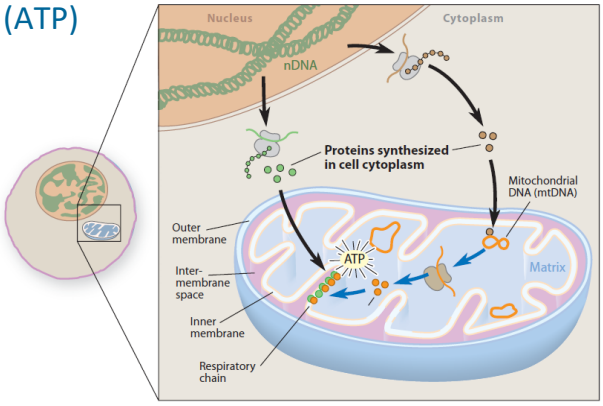




Mitochondria

- Organelle/Network within the cell
- Important function:
  generation of chemical energy (ATP)



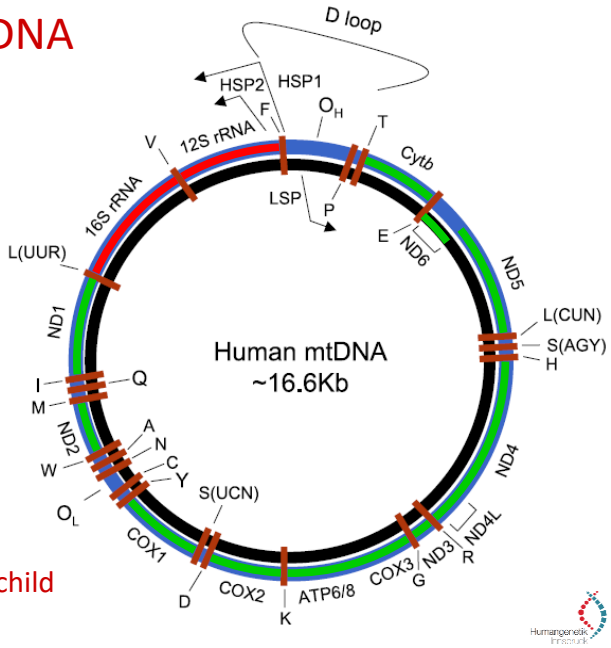


mtDNA

Special features
- Resembles bacterial genome
- Polycistronic, no introns
- Mitochondrial transcription/translation

Circular DNA
- 2 rRNA genes
- 13 mRNA genes
- separated by 22 tRNA genes

Numbers
- 2-10 copies/mitochondrion
- >1000 copies/cell
- >100.000 copies/ovary



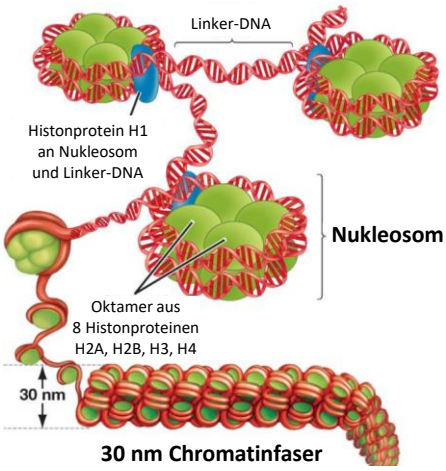
Maternal inheritance: only from mother to child

Chromatin

Komplex of DNA und associated proteins in the nucleus

- Euchromatin
  - Open structure during interphase
  - Accessible for transcription factors etc.
  - Contains active genes
- Heterochromatin
  - Condensed also during interphase
  - Transcriptionally inactive
  - Constitutional/facultative

Nucleosome

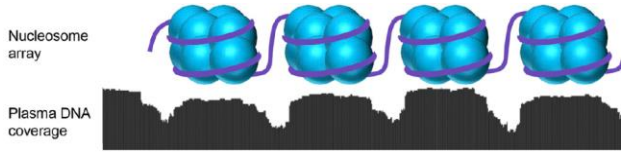


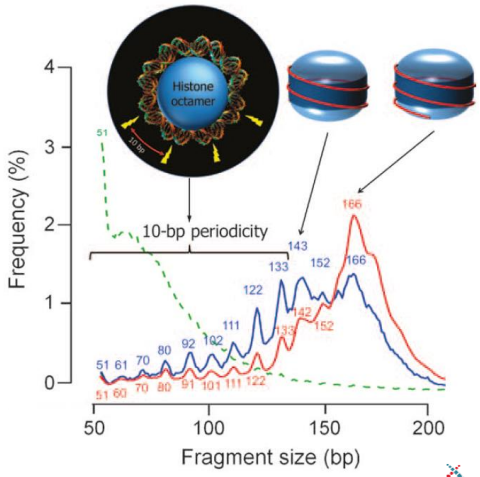
- Basic structure of the chromosomes
- DNA wound around histon proteins
  - Protein: Octamer (H2A, H2B, H3, H4)
  - Linked through histone H1
  - Regulation by histone modification
- Separates DNA in units of ca. 167 bp

 https://beyondthedish.files.wordpress.com/2013/07/chromatin1.jpg

Cell free DNA in plasma

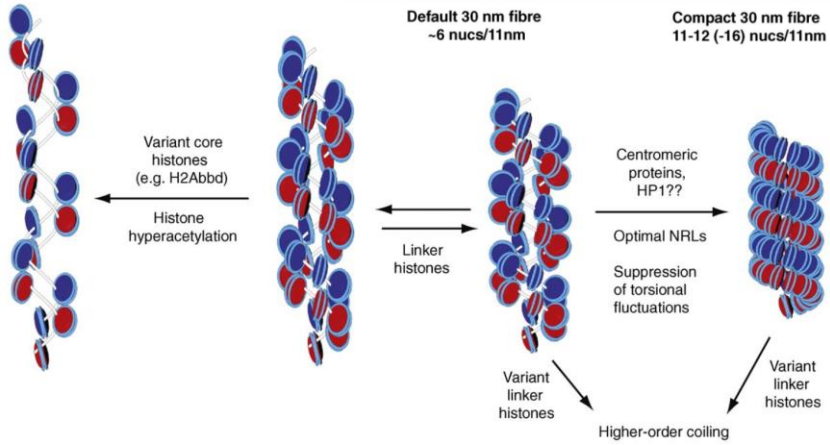
- Released during cell death
- Nucleosomes provide limited protection from degradation
  - cfDNA in plasma: initially cut in linker region
- Useful for the analysis of
  - Cell free tumor DNA
  - Cell free placental DNA





Chromatin fibre

- Variable structure dependent e.g. on linker size, scaffolding proteins and epigenetic modification



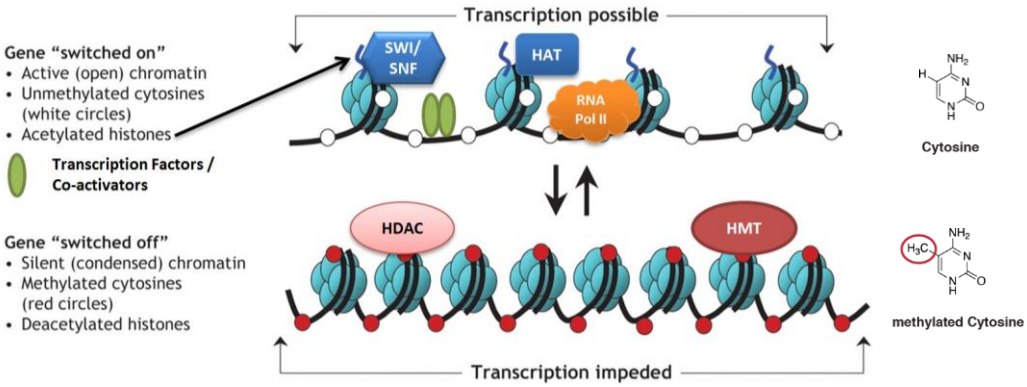
 Bassett et al., Current Opinion in Genetics & Development 2009;19:159–165

Chromatin remodelling

DNA methylation

Histone modification: acetyl transferases (HAT), deacetylases (HDAC), methylases (HMT)

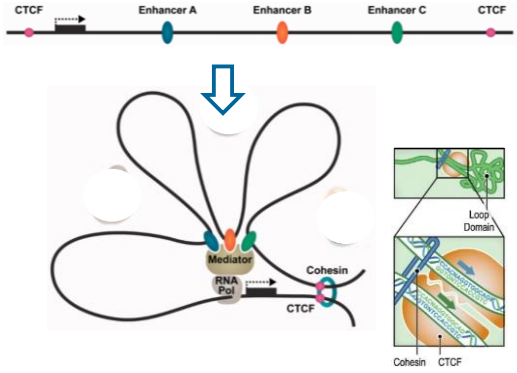
ATP-dependent chromatin remodelling complexes: z.B. SWI/SNF



 Wikipedia, adaptiert nach Luong, P. Basic Principles of Genetics, 2009

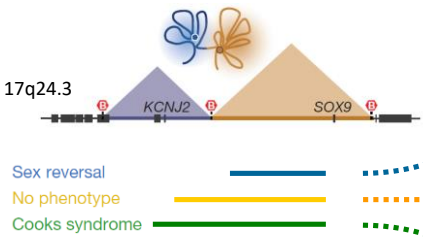
Topologically associated domains

- Evolutionary highly conserved
- Binding sites of protein CTCF function as insulators
- Connection of
  - Promotors
  - Enhancers
  - Binding proteins (mediators)
  - Transcription factors, polymerases
- Deletion, Insertion, Inversion etc. →
  - Monogenic diseases
  - Tumors



 Spielmann et al., Hum Mol Genet. 2016;25(R2):R157-R165, Rao et al., Cell 2015;162:687-688

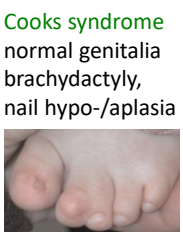
Duplications in the region of *KCNJ2/SOX9*



♀→♂
Sex Reversal

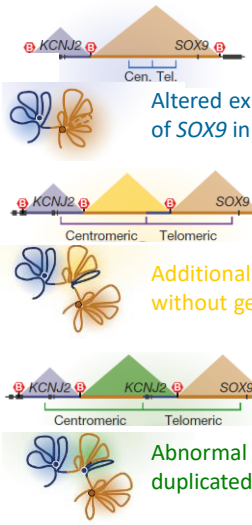
Normal

Cooks syndrome
normal genitalia
brachydactyly,
nail hypo-/aplasia



SOX9 = important target of SRY

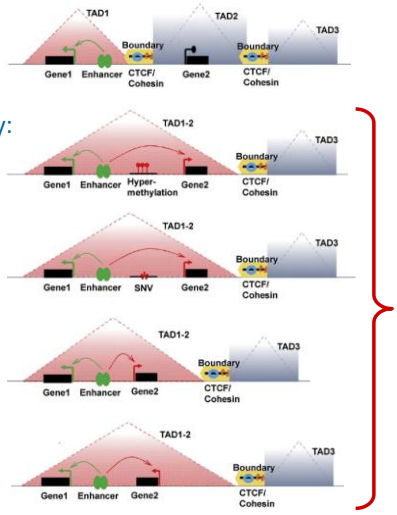
- ↑ Anti-Müller hormone → central role in ♂ sex differentiation
- Loss of function: campomelic dysplasia with ♂→♀ sex reversal



15 Franke et al., Nature 2016;538:265-269

Exampes of TAD mutations

- Wild type
- Loss of TAD boundary:
  - Epigenetic inactivation
  - Point mutation
  - Deletion
  - Inversion

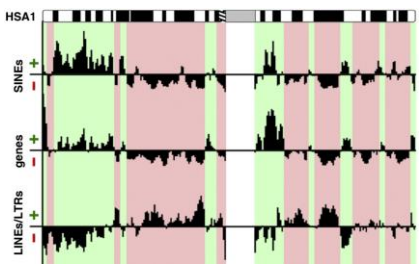


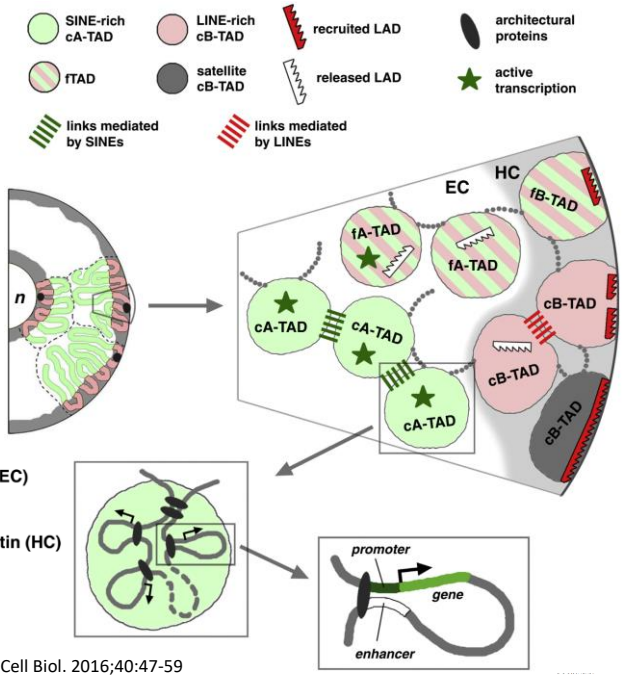
Abnormal expression of gene 2

16 Krumm & Duan, Semin Cell Dev Biol. 2018, in press

Compartments

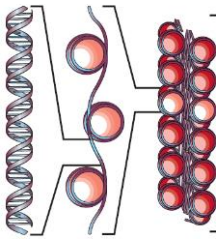
- Active (A): euchromatin
  - Inner regions of the nucleus (cA)
  - Gene rich, early replication
- Inactive (B): heterochromatin
  - Consitutional/facultative (cB/fB)
  - At nuclear membrane or nucleolus,
  - Gene poor, late replication

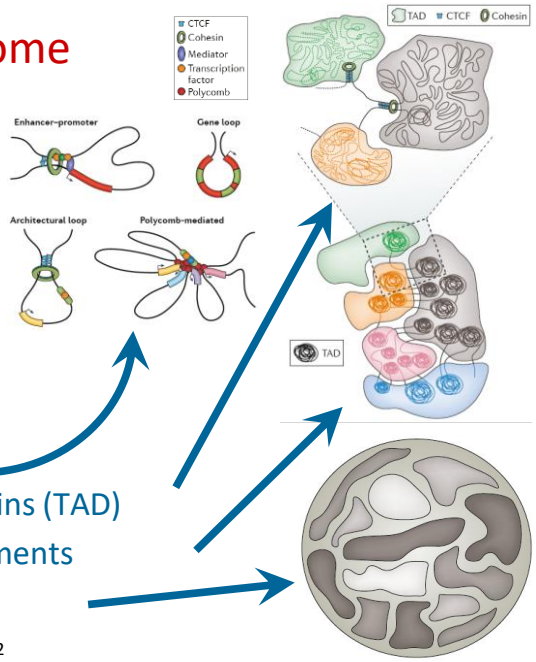




Solvei et al., Curr Opin Cell Biol. 2016;40:47-59

Organisation of the genome



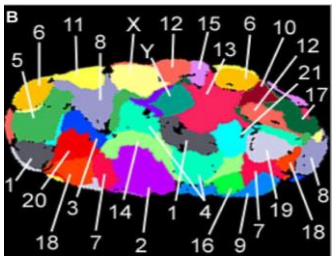


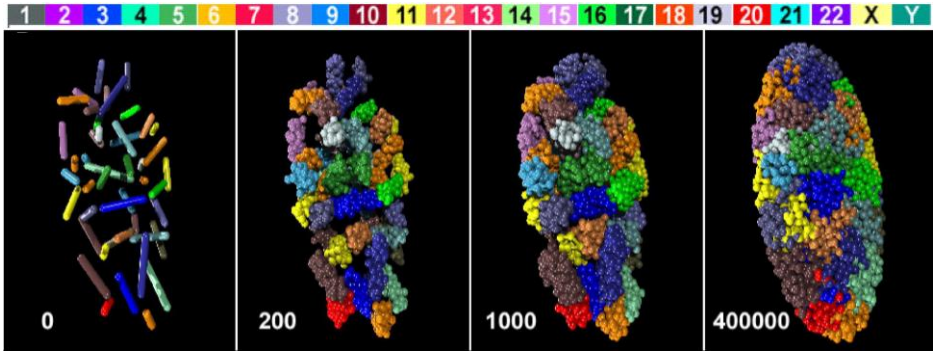
- DNA double strand
- Nucleosome, Chromatin fibre
- Chromatin loops
- Topologically associated domains (TAD)
- Active/inactive (A/B) compartments
- Chromosomal territories

Bonev & Cavalli Nat Rev Genet. 2016 Dec;17(12):772

Chromosomes in the nucleus

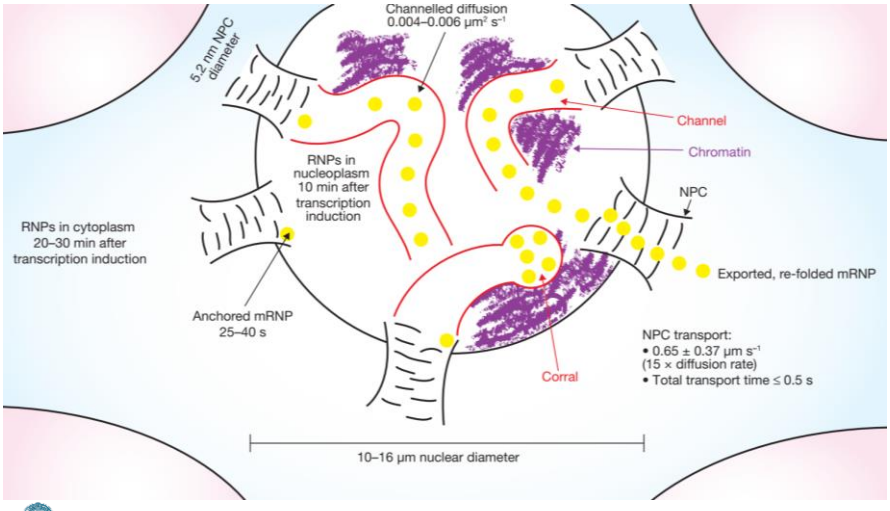
Organization in specific domains

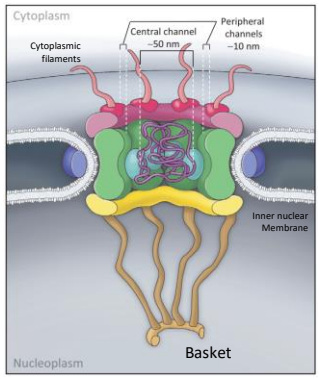




Bolzer et al., PLOS Biol. 2005

mRNA transport through nucleoplasma und nuclear pores

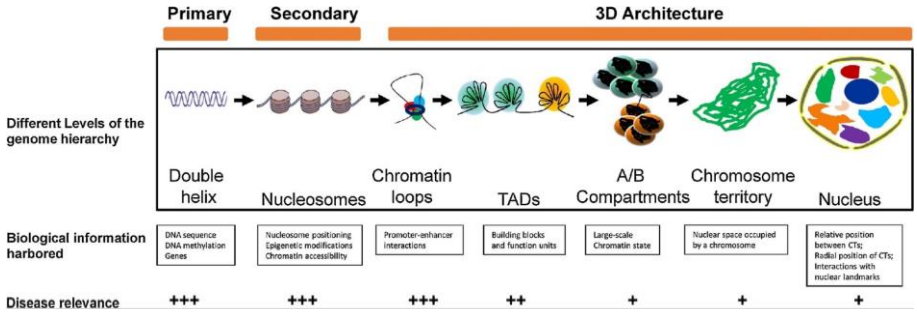




Nuclear pore complex

Noble & Wente, Nat Cell Biol 2010;12:525-7, Katta et al., Trends Cell Biol. 2014 Apr;24(4):221-9

Structural organisation of the genome



 Krumm & Duan, Semin Cell Dev Biol. 2018, in press

NATURE|Vol 441|25 May 2006

WHAT IS A GENE?

The idea of genes as beads on a DNA string is fast fading. Protein-coding sequences have no clear beginning or end and RNA is a key part of the information package, reports **Helen Pearson**.

'Gene' is not a typical four-letter word. It is not offensive. It is never bleeped out of TV shows. And

Laurence Hurst at the University of Bath, UK.

"All of that information seriously challenges our conventional definition of a gene," says

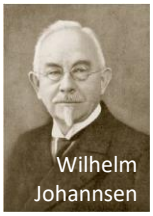
viously unimagined scope of RNA.

The one gene, one protein idea is coming under particular assault from researchers who

Gene

- One gene – one enzyme
- „central dogma“ of molecular biology

1909



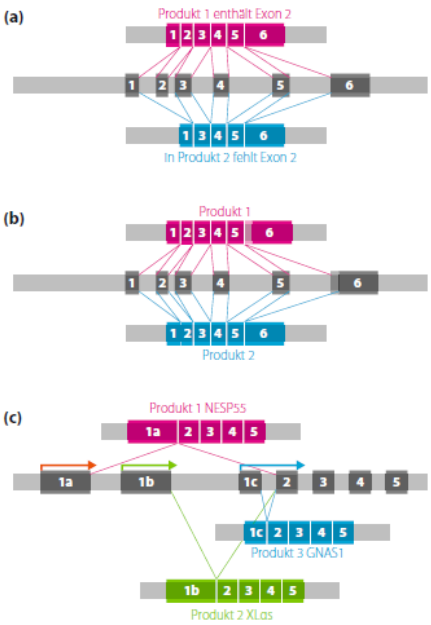
Nobelpreis 1958:
Gene kontrollieren einzelne Stoffwechselschritte


George W. Beadle


Edward L. Tatum

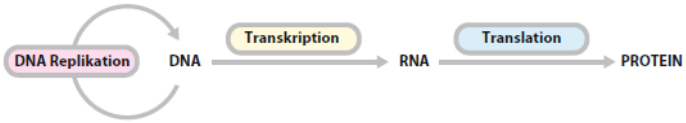
Gene

- Definition problems:
  - Not all genes code for proteins (→ ncRNAs)
  - „One gene“ can code for different Proteins
  - „One gene“ can overlap with another gene (or lie within)
  - Neighbouring genes can code for a third „fusion gene“



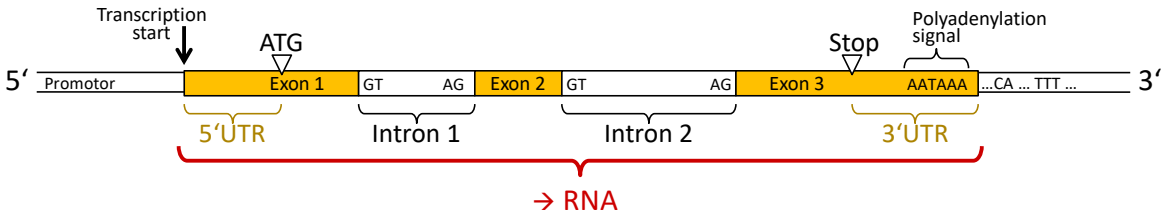
Gene

- Localized region of the genomic sequence that contains the information for a specific gene product / a specific function
- Transcribed (working copy RNA) and (not always) translated into protein



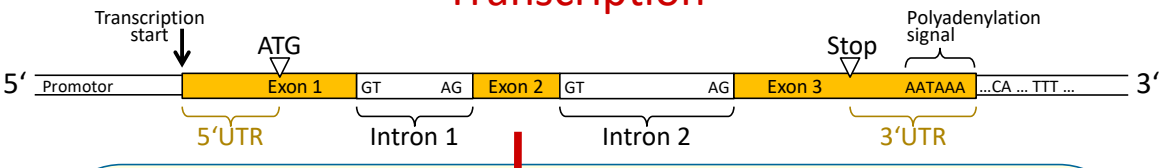
Gen

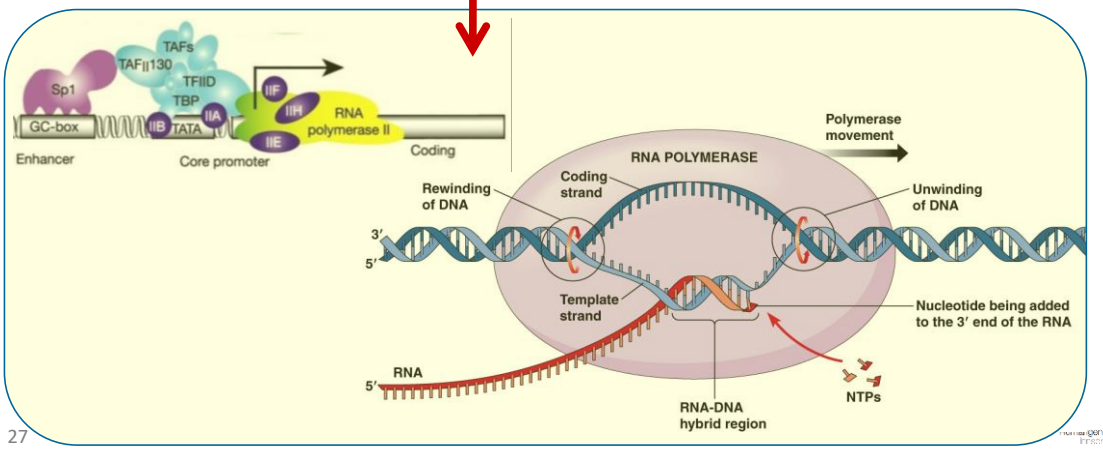
- Localized region of the genomic sequence that contains the information for a specific gene product / a specific function



- Promotor
- Start codon (ATG)
- Exons/Introns (EXONgt.....agEXON)
- Stop codon (TAA/TAG/TGA)
- 3‘-Sequences

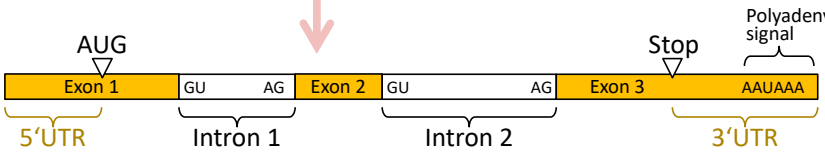
Transcription





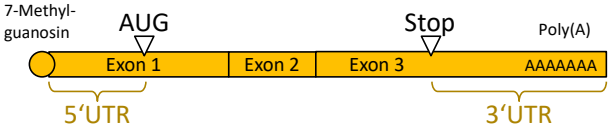
RNA processing

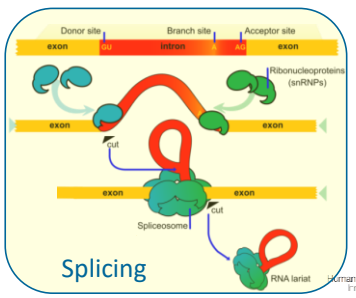
Primary transcript



Splicing, capping, polyadenylation

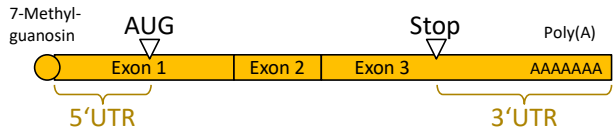
Mature transcript

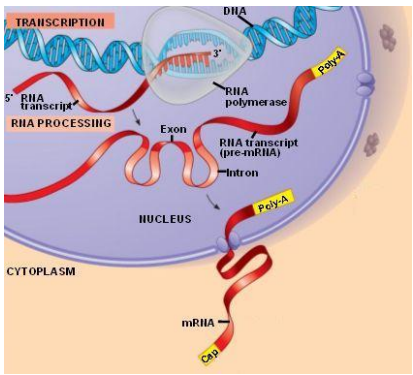




Export from the nucleus

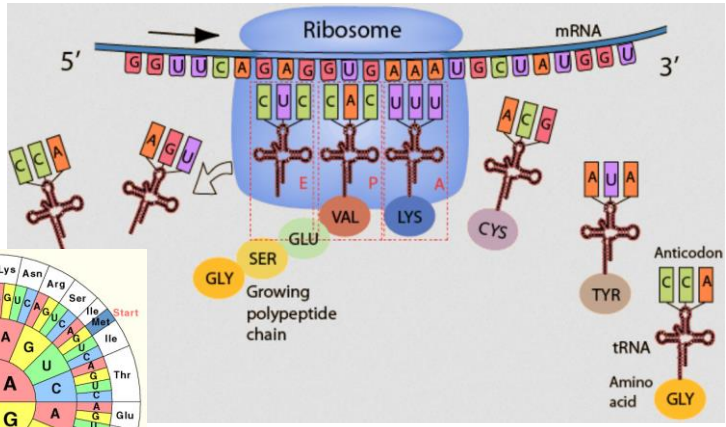
Mature transcript

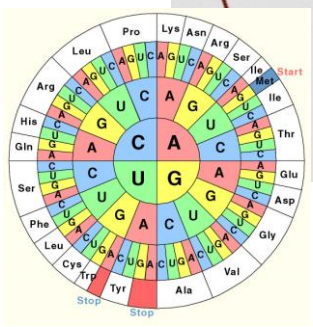




Transfer into the cytosol for translation into protein

Translation



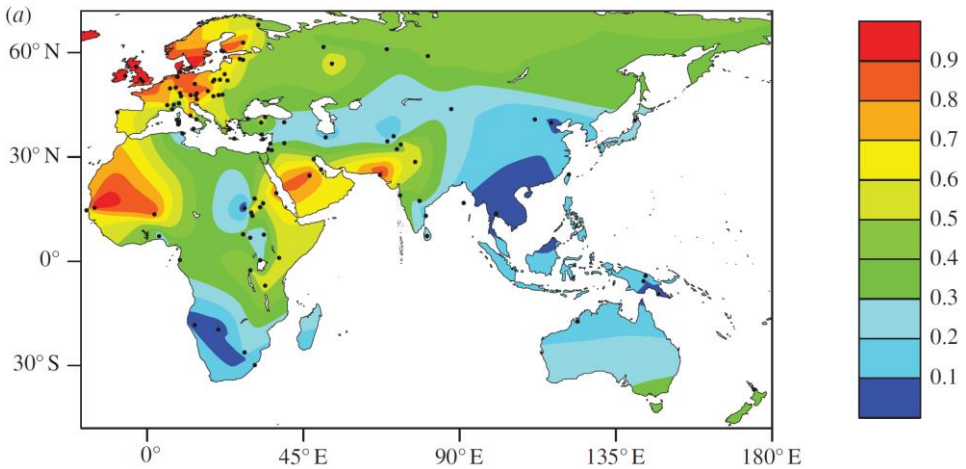


tRNA Wobble:
Only ca. 30 cytosolic tRNAs
Last base often irrelevant

Who of you
can drin
plenty of milk?

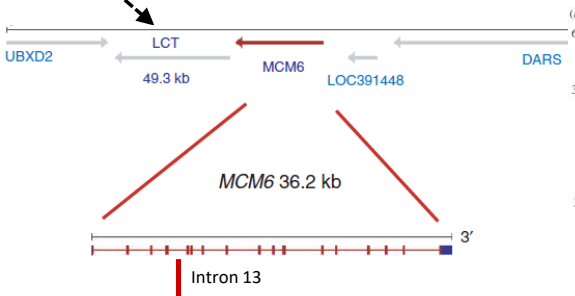
Lactose tolerance

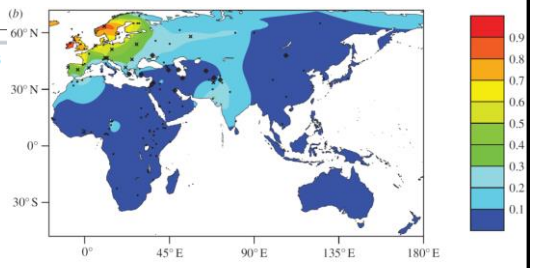
Inherited persistence of lactase activity



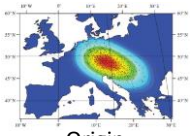
LCT = lactase gene
Chromosome 2q21

Lactose tolerance





Allele frequency
-13910C>T



Origin
ca. 5-10.000 BC

.....AAGATAATGTAGCCCCTG.....
T
-13910C>T

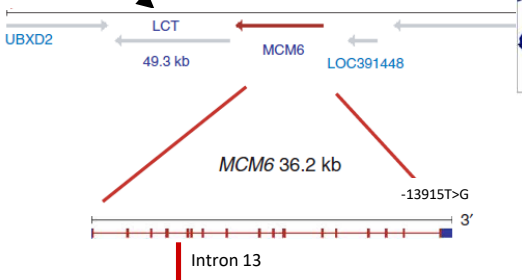
Variant causes persistence of lactase activity in adulthood.

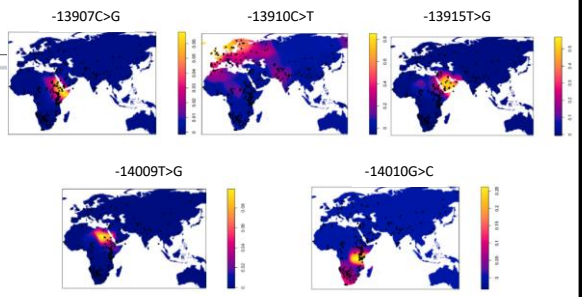
14.000 nucleotides before *LCT* gene

Common in Europeans

LCT = lactase gene
Chromosome 2q21

Lactose tolerance





Allele frequencies

...CGTAAGTTACCA.....AAGATAATGTAGCCCCTG.....
CG G T G
-14010G>C
-14009T>G
-13915T>G
-13910C>T
-13907C>G

Variants cause persistence of lactase activity in adulthood.

14.000 nucleotides before *LCT* gene

Common in some African/Asian regions

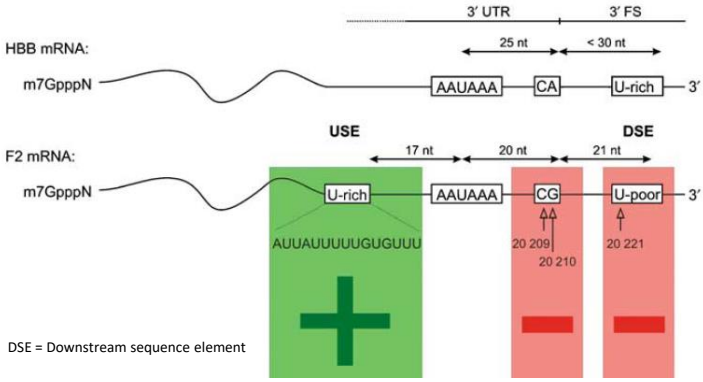
Common in Europeans

Lactose tolerance

- Most adults world-wide cannot drink milk
- Evolution takes place in humans
- The regulation of gene functions is complex
- Regional difference of prevalent functional variants

Prothrombin variant c.*97G>A (20210G>A)

- Clotting factor II, helps to stop bleeding
- Improved („corrected“) 3´ processing of the transcript
  → increased amount of prothrombin, risk of thrombosis



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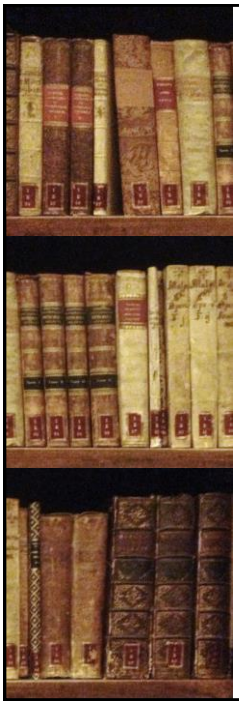
MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Humangenetik Innsbruck

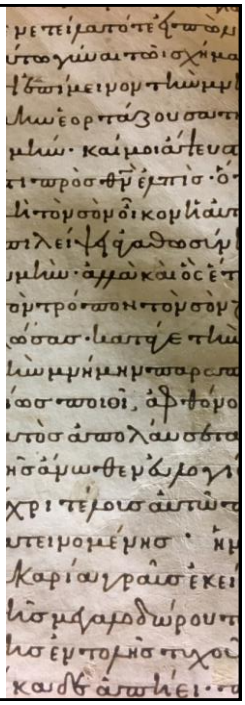
Genetic variability

Johannes Zschocke
Institut für Humangenetik, MUI



Genetic/genomic variability

- Single nucleotide variants
  - SNPs,
    rare variants/mutations
- Small deletions/duplications
- Tandem repeats
  - STRs (short tandem repeats , 2-6 nt)
- Larger monogenic deletions
- Structural chromosomal variants
  - Copy number variants (CNV)
    - Deletion/duplication/multiplication
    - Including microdeletions/-duplications
  - Other structural variants
- Numerical chromosomal variants



Genetic/genomic variability

- Single nucleotide variants
  - SNPs,
    rare variants/mutations
- Small deletions/duplications
- Tandem repeats
  - STRs (short tandem repeats , 2-6 nt)
- Larger monogenic deletions

Monogenic effects

- Structural chromosomal variants
  - Copy number variants (CNV)
    - Deletion/duplication/multiplication
    - Including microdeletions/-duplications
  - Other structural variants
- Numerical chromosomal variants

Many genes, gene dosis-effects

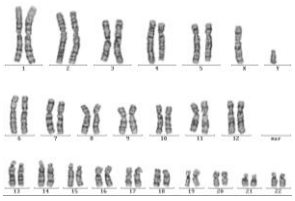
Cytogenetics

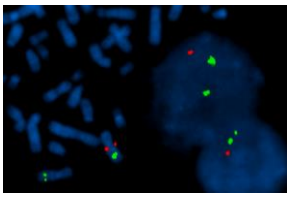
Classical cytogenetics

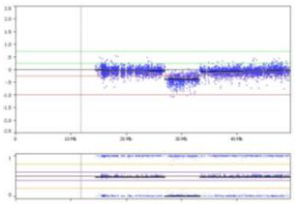
- High resolution chromosome analysis
  - Microscopic („prophase chromosomes“)
  - Undirected analysis of the whole genome
  - Resolution 5-10 Mb (Mb = Million bases)

Molecular cytogenetics

- FISH (Fluorescence in situ hybridisation)
  - Microscopic („submicroscopic“)
  - Targeted analysis of specific areas
  - Resolution 2-10 kb (kb = 1000 bases)
- Genome-wide array analysis
  - „Molecular chromosome analysis“
  - Undirected analysis of the whole genome
  - Resolution 2-100 kb (in principle unlimited)
  - No identification of balanced aberrations

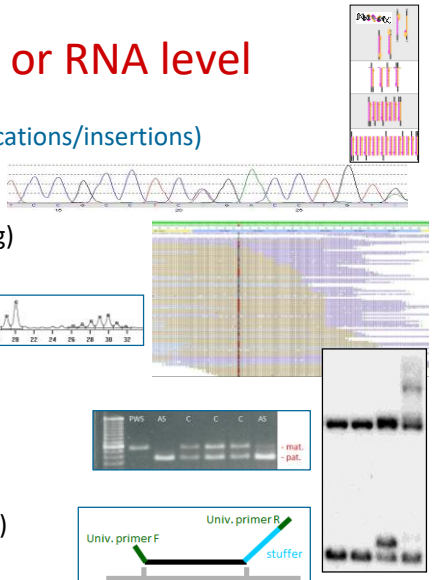






Molecular genetics = analysis on DNA or RNA level

- Sequence analysis (nucleotide variants, small deletions/duplications/insertions)
  - PCR amplification + Sanger sequencing
  - Targeted genotyping (DNA-Array etc.)
  - Massive parallel sequencing (next generation sequencing)
- Variable repeat sequences
  - PCR amplification + fragment analysis
  - Southern Blot
- Epigenetic alteration (DNA methylatio)
  - Analysis after bisulfite treatment of DNA
- Large deletions and duplications, genomic quantification
  - Quantitative PCR (single targets)
  - Multiplex ligation-dependent probe amplification (MLPA)
  - Massive parallel sequencing + quantitative evaluation
- Others
  - Linkage analysis, autozygosity mapping (DNA array)



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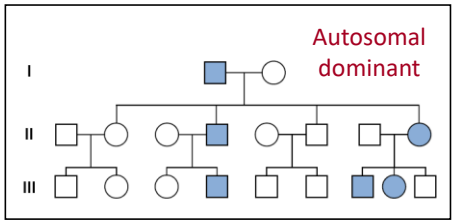
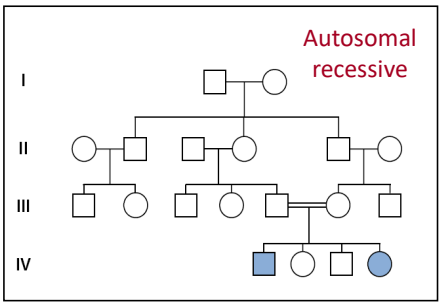
Humangenetik Innsbruck

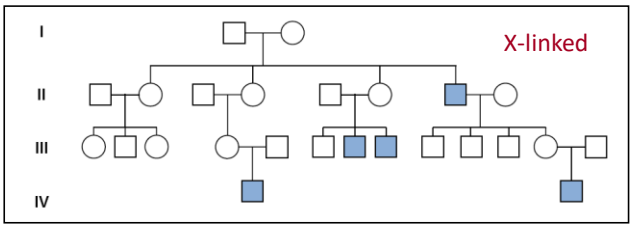
Inheritance patterns

Johannes Zschocke
Institut für Humangenetik, MUI





= affected



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Genotype: NN NM MM

Phenotype:

M is dominant

M is recessive

Phenotype N | Phenotype M

44

Versuche

über

Pflanzen-Hybriden,

von

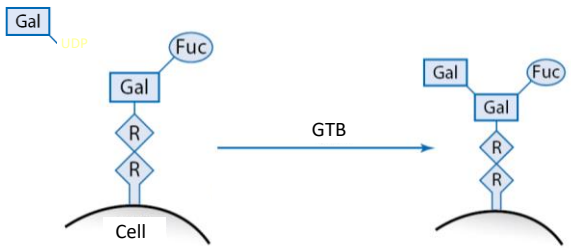
Gregor Mendel.

Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant,* and those which become latent in the process *recessive.*

Brünn, 1866.

Aus Georg Gastl's Buchdruckerei, Postgasse Nr. 446.

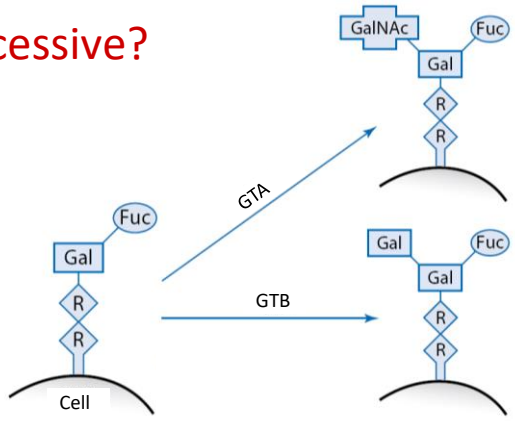
Dominant or recessive?



α1,3-Galactosyltransferase (GTB)

Transfer of Gal from UDP-Gal to β-Gal
in α-Fuc-1,2-Gal terminated structures

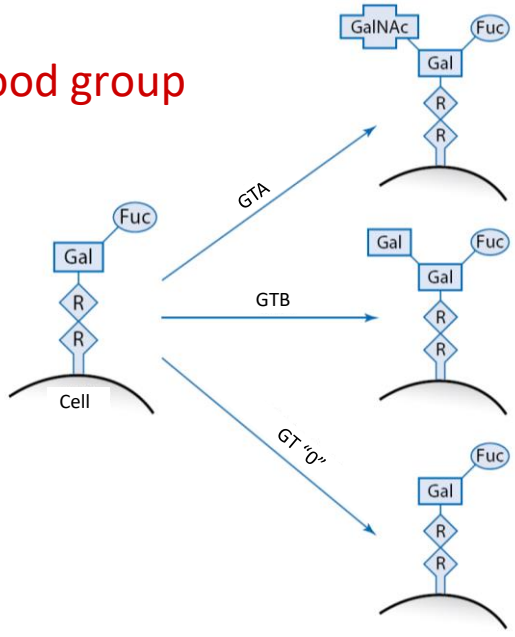
Dominant or recessive?



α1,3,N-Acetylgalactosaminyltransferase (GTA)

Transfer of GalNAc from UDP-GalNAc to β-Gal
in α-Fuc-1,2-Gal terminated structures

AB0 blood group system

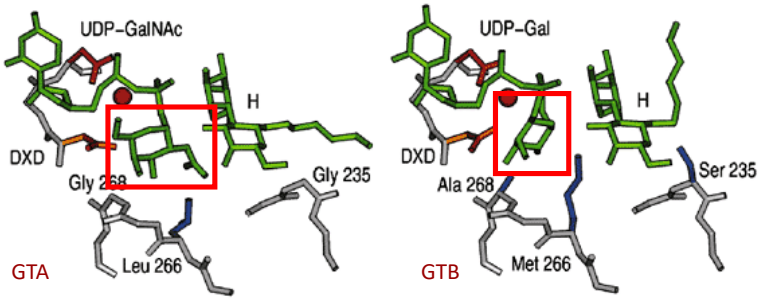


A agglutinogen

B agglutinogen

Neither A nor B agglutinogen

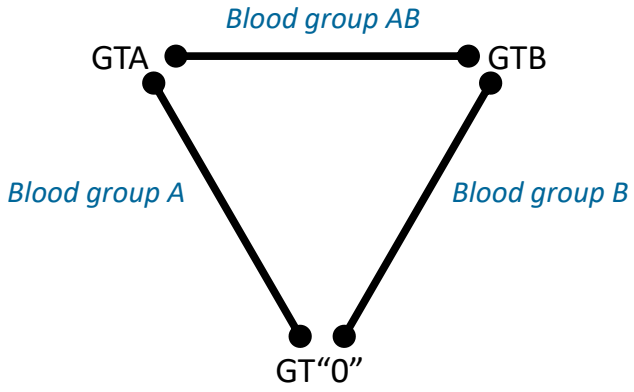
ABO gene variants



- [G176, G235, L266, A268]: GTA function → A agglutinogen
- [R176, S235, M266, G268]: GTB function → B agglutinogen
- Frameshift-Deletion: Non-functional protein → No agglutinogen

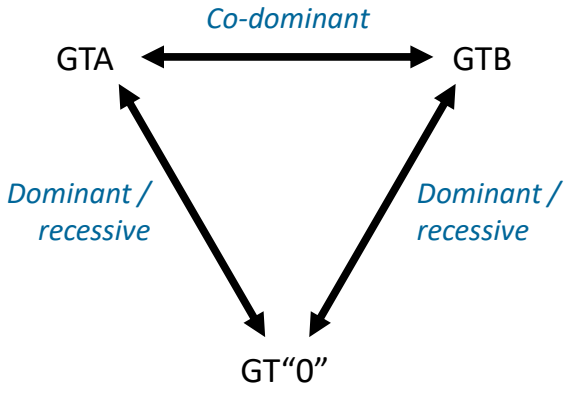
… „one gene – two enzymes“

Heterozygosity for *ABO* gene variants



Function competes with function, and dominates over non-function

ABO gene: co-dominant inheritance

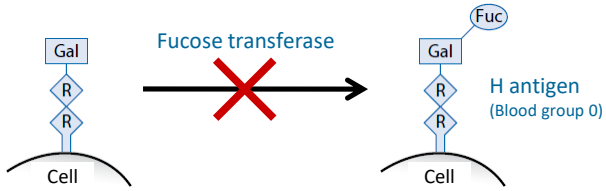


Function competes with function, and dominates over non-function

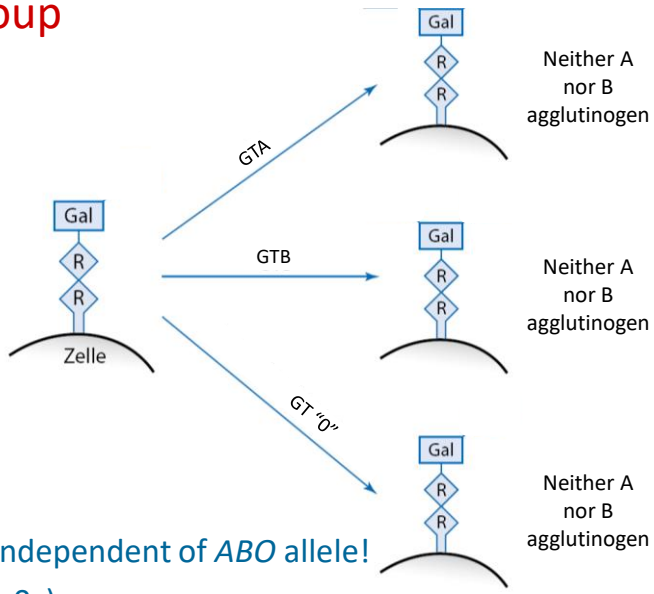
The terms dominant and recessive
describe the
functional relationship of different alleles
of the same gene in a
(compound) heterozygous organism

Epistasis

- One gene suppresses the phenotypic manifestation of another gene
  - (more general: gene-gene-interaction)
  - Dominant and recessive mechanisms possible
- Example: Bombay blood group:
  - Autsomal recessive deficiency of fucose transferase
  - Clinically irrelevant; exception: transfusions
  - Antibodies against blood group antigens A, B and H!



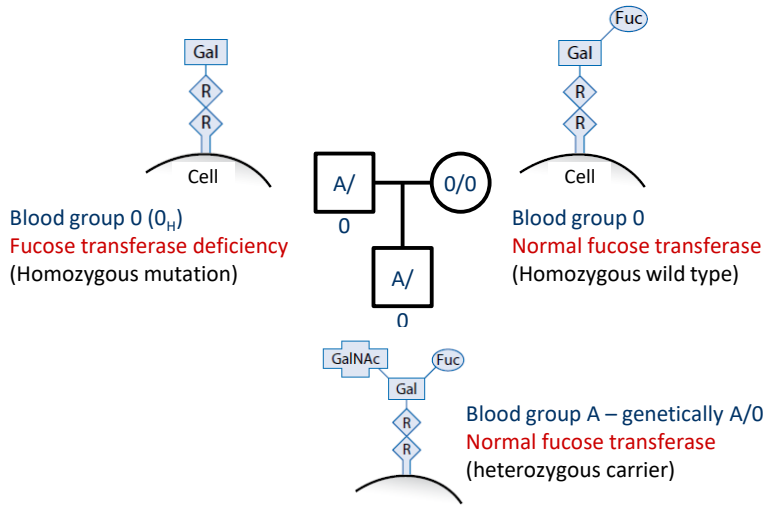
Bombay blood group



Blutgruppe 0 independent of *ABO* allele!

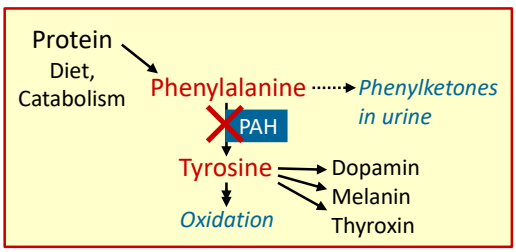
(→blood group 0_H)

Family with Bombay blood group



Phenylketonuria (PKU)

- Disorder of amino acid metabolism
- Mutations in the *PAH* gene; autosomal recessive inheritance
- Untreated severe intellectual disability
  - Spasticity, epilepsy, microcephaly
  - Fair complexion
- Dietary treatment
- Newborn screening



Genotype

homozygous wild type	heterozygous	homozygous mutation

Enzymatic Phenotype

100 % activity	50 % activity	No activity

Metabolic Phenotype

Normal ? Abnormal

Clinical Phenotype

Genotype

Enzymatic Phenotype

Metabolic Phenotype

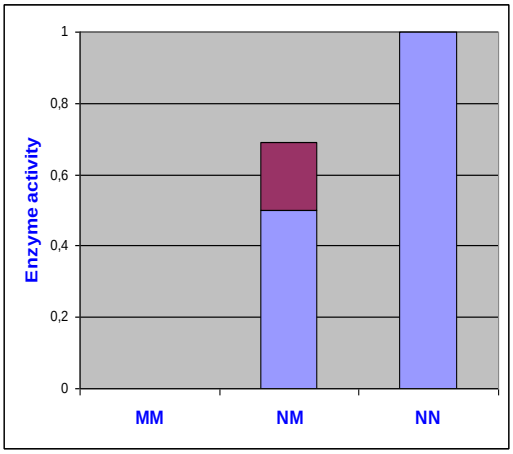
Clinical Phenotype

Enzyme activity
Metabolic flux
Substrate / product concentration

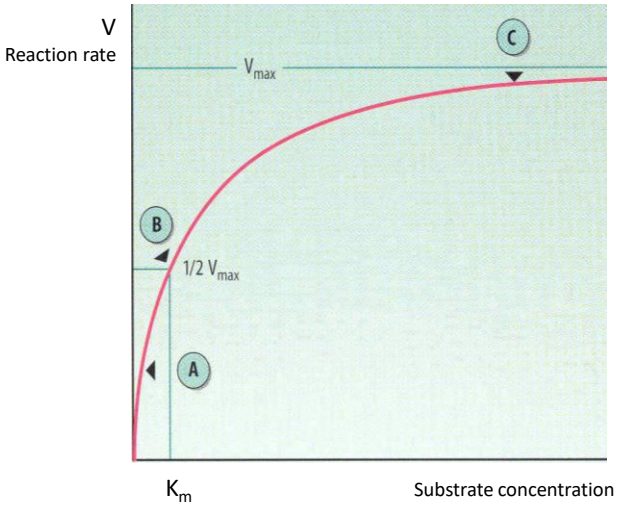
Enzyme activity

↑ Gene expression

↑ Enzyme activation



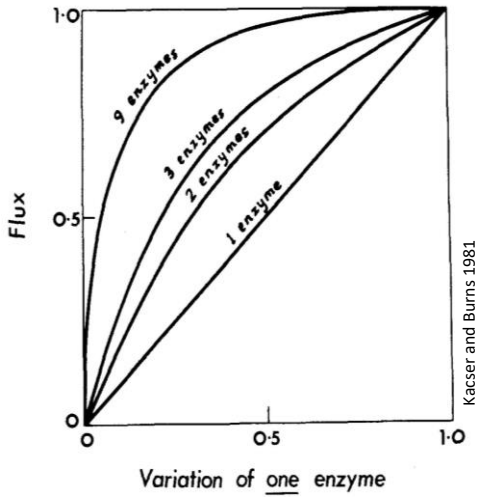
Enzyme kinetics



Metabolic Flux

Reduced activity of a single enzyme:

Impact on flux depends on the number of enzymes in the pathway



Kacser and Burns 1981

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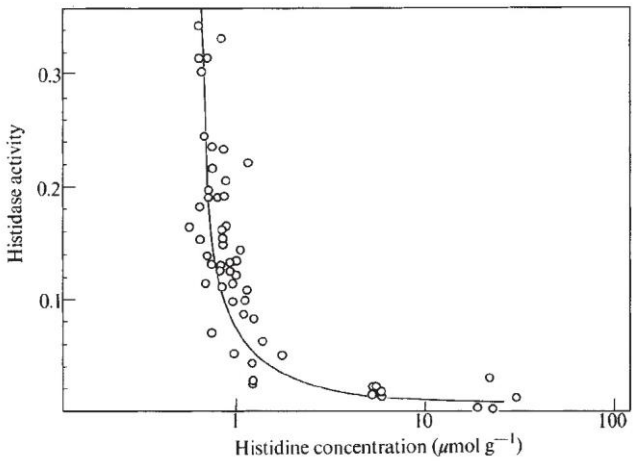
Substrate concentration



Kacser et al. 1973

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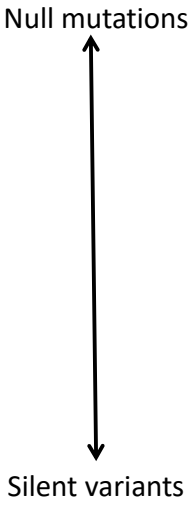
Different mutations have different functional effects



Spectrum of severity

Classical PKU

Mild PKU

Mild hyperphenylalaninaemia (MHP)

- Marked residual PAH activity
- Phe levels consistentl <600 µmol/l
- No treatment necessary

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Genotypes in mild hyperphenylalaninaemia (MHP)

Patient	MHP mutation	PKU mutation	Phe values (µmol/l)	
			MW	Range
1	p.V245A	p.L194P	237	164-328
2	p.T380M	p.R408W	276	166-395
3	p.T380M	p.R261Q	252	158-353
4	p.T380M	p.I65T	345	248-534
5	p.T380M	p.I65T	346	241-439
6	p.T380M	p.F299C	250	216-360
7	p.E390G	p.Y277D	307	214-429

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Mild mutations dominate over severe mutations

Genotype

Mutation 1
„null mutation“

Mutation 2
„mild“

No functional protein

Protein/enzyme

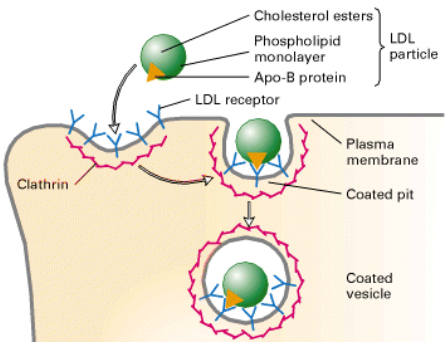
Only mutation 2

Phenotype

Residual activity of mutation 2

Familial hypercholesterolaemia

- Heterozygous LDL receptor mutations
  - Impaired uptake of cholesterol from blood into liver
    → LDL cholesterol in macrophages/monocytes
    → migration into vessel wall, proliferation
    → Atherosclerosis
  - Autosomal dominant, prevalence 1:250
- Clinical features
  - Xanthomas, Xanthelsmas, Arcus corneae
  - Increased LDL cholesterol in blood
  - Early cardiovascular complications
    e.g. heart attack before age 50 years
- Therapy
  - Cholesterol lowering diet
  - Statins, other medication



Homozygous LDLR deficiency

- Prevalence 1:1.000.000?
  - Certainly higher!
- Features
  - LDLR cholesterol >600 mg/dl
  - Early xanthomas etc.
  - Cardiovascular complications in childhood (e.g. myocardial infarction age 5-10 years)
- Therapy
  - Lipid apharesis
  - Liver transplantation
- Which term to use for the inheritance?

LDLR deficiency: semidominant inheritance

	Controls	Sister	Mother	Father	Patient 1	Patient 2
Cholesterol (mg/dl)	< 200	280	290	355	1140	1250
LDLR Mutation p.W556R	Wild type	heterozygous			homozygous	

Genotype NN NM MM

Phenotype:
N and M are semidominant

Most monogenic disorders represent a
spectrum of phenotypes
from normal via attenuated to severe
(and sometimes prenatally fatal)

In dominant disorders,
clinical symptoms are a regular feature
in the heterozygote.

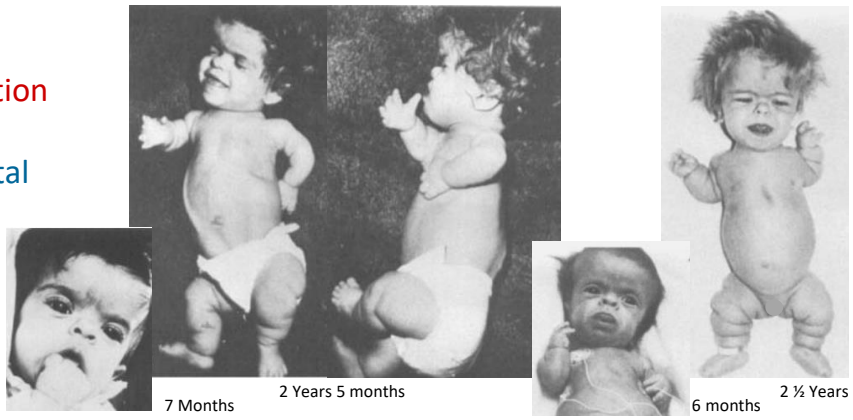
Genotype	NN	NM	MM
Phenotype:			
N and M are semidominant			not observed

Achondroplasia

- Monogenic skeletal dysplasia
  = disorder of skeletal growth
  - Dysproportionate small stature
  - Large prominant skull
  - Normal intelligence
- Activating mutation p.Gly380Arg in *FGFR3*
  - FGFR3 controls cell division of certain cartilage cells
  - Mutation → constant activation (gain of function)
- Autosomal dominant
  - Prevalence 1:20.000

Eleanor Simmonds,
Paralympics multiple
gold medal winner (UK)

Homozygous achondroplasia mutation p.Gly380Arg in *FGFR3* is usually pre-/perinatal lethal



Genotype	NN	NM	MM
Phenotype:			
N and M are semidominant			not observed

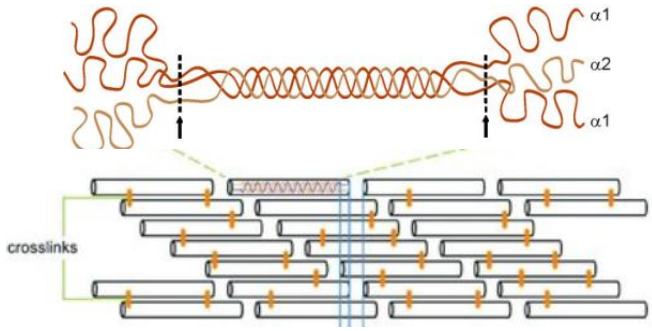
Pauli et al., Am J Med Genet. 1983;16:459-73.

Collagen I

- Main component of connective tissue e.g. skin, bone, tendon, cornea, etc.
- Fibrillary structure: two α1 and one α2 chains (Gly-X-Y-polypeptide)

Genes:

- *COL1A1*, 17q21.31-q22: Precursor of α1(I) chain
- *COL1A2*, 7q22.1: Precursor of α2(I) chain



Osteogenesis imperfecta

Collagen I deficiency, „brittle bone disease“

- Type I („mild“):
  - Slender long bones, fractures after inadequate trauma
  - Normal stature, no deformities,
  - Blue sclera (usually)

Osteogenesis imperfecta

Collagen I deficiency, „brittle bone disease“

- Typ II (perinatal lethal)
  - Numerous intrauterine fractures
  - Abnormal short bent extremities
  - Multiple rib fractures

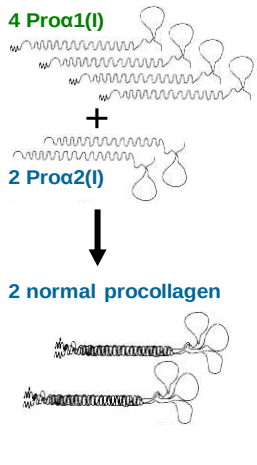
Molecular genetics of osteogenesis imperfecta types 1+2

Both types are caused by heterozygous mutations in the *COL1A* genes

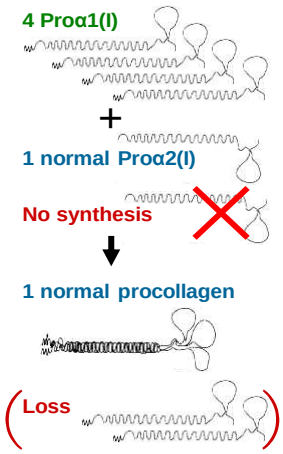
- One type is usually caused by a null mutation that completely removes the protein
- The other type is usually caused by a missense mutation that produces a stable protein

Which type of mutation is found in which type of osteogenesis imperfecta?

Osteogenesis imperfecta

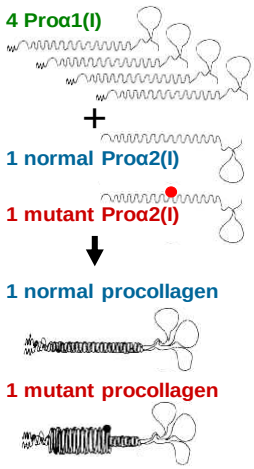


Normal



Type I: „mild“

Null mutations (instable protein instabile mRNA etc.)



Type II: „severe“

Point mutation at Gly position of the triple helix domain

Dominant negative effect

Malfunction of the mutant protein
interrupts the function of the normal protein

Example: structural proteins, multimer channels

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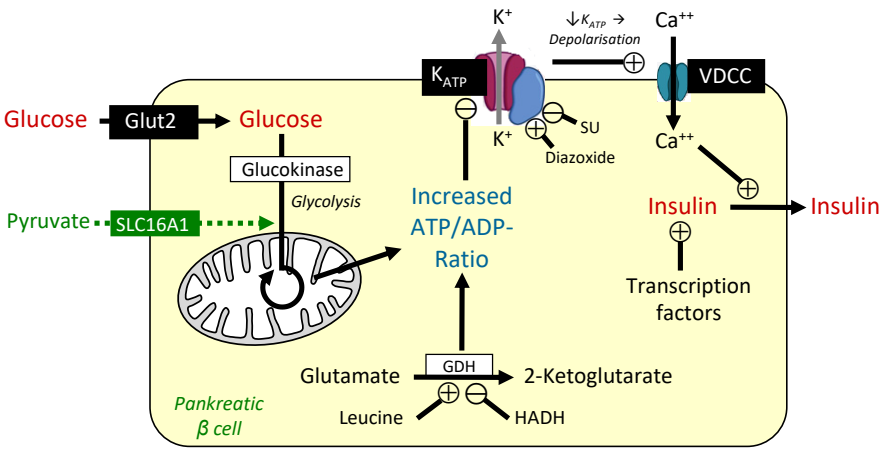
Glucose/insulin regulation



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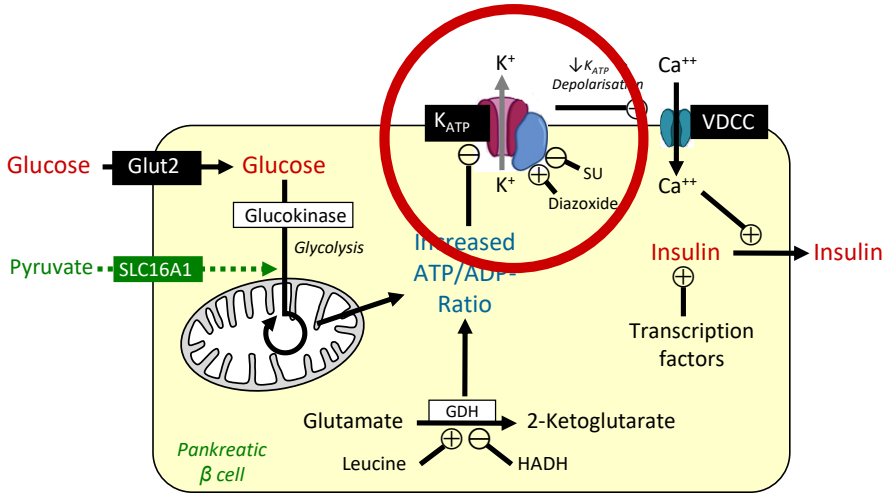
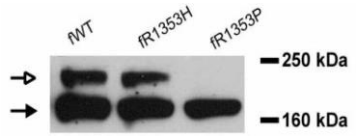
Hyperinsulinism caused by K_{ATP} channel mutations

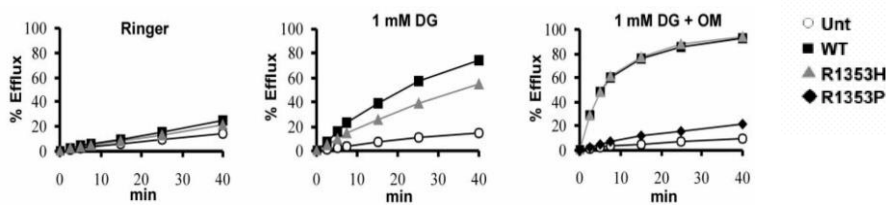


K_{ATP} channel mutations

ABCC8 mutation p.R1353H: dominant hyperinsulinism

ABCC8 mutation p.R1353P: recessive hyperinsulinism





Magge et al., J Clin Endocrinol Metab. 2004;89:4450-6.

K_{ATP} channel mutations

p.R1353H: stable SUR1 protein

Heterozygous

1 x

4 x

6 x

4 x

1 x

Dominant negative effect

Homozygous

16 x

Mutant channel

p.R1353P: instable SUR1 protein

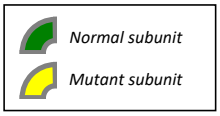
Heterozygous

8 x

Reduced amount of normal channel → healthy

Homozygous

No channel



Dominant and recessive in Medical Genetics

- The terms refer to the *functional relationship of different alleles in biallelic genes with regard to the physical manifestation*, elucidated by comparison with the normal (wild type) state.
- This definition differs from the original designation by Gregor Mendel, who used the terms specifically for non-quantitative traits in which heterozygotes (hybrids) and one type of homozygotes had more or less identical phenotypes.
- "Mendelian" and "monogenic" are not synonymous, as frequently used in the medical and non-medical literature.
- Different pathomechanisms by which heterozygous variants may or may not cause phenotypic manifestations → genetic diagnosis, genetic counselling.