Genetics

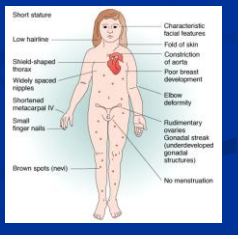
Chromosome and gene aberrations

Chromosomal abnormalities
Structural
Numeric

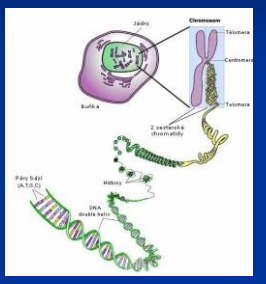
Gene mutatios
Rare alleles
Polymorphisms

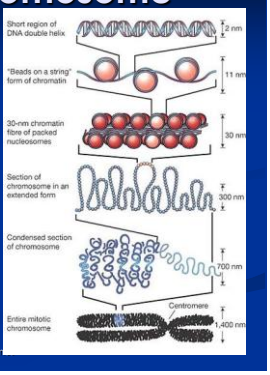
Chromosomal aberrations

- aneuploidy (a difference in chromosome number)
  - meiotic non-disjunction
  - later → somatic mozaicism
  - monosomy
    - gonosomal
      - Turner´s sy. (45, X0)
  - trisomy
    - autosomal
      - Down´s sy. (47, XX/XY + 21)
      - Edwards´s sy. (47, XX/XY +18)
      - Patau´s sy. (47, XX/XY +13)
    - gonosomal
      - Klinefelter´s sy. (47, XXY)
- polyploidy -letal

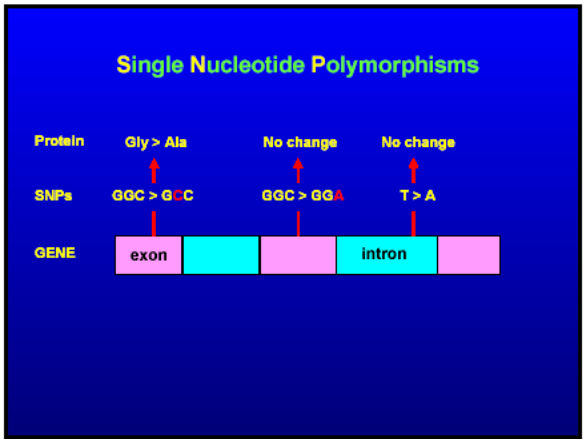


Chromatin × chromosome





- In non dividing cells is chromatin dispersed freely in nucleus
- In dividing cells is chromatin organized to visible chromosom



Gene mutation - types

- Normal state
- DNA
  - ATGCAGGTGACCTCAGTG
  - TACGTCCACTGGAGTCAC
- RNA
  - AUGCAGGUGACCUCAGUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- **Mutation „missense“**
- DNA
  - ATGCAG**C**TGACCTCAGTG
  - TACGTC**G**ACTGGAGTCAC
- RNA
  - AUGCAG**C**UGACCUCAGUG
- PROTEIN
  - Met-Gln-**Leu**-Thr-Ser-Val
- **Examples-hemoglobin S in sickle cell anemia- heterozygote advantage**

Gene mutation - types

- Normal state
- DNA
  - ATGCAGGTGACCTCAGTG
  - TACGTCCACTGGAGTCAC
- RNA
  - AUGCAGGUGACCUCAGUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- **Mutation „nonsense“**
- DNA
  - ATGCAGGTGACCT**G**AGTG
  - TACGTCCACTGGA**C**TCAC
- RNA
  - AUGCAGGUGACCU**G**AGUG
- PROTEIN
  - Met-Gln-Val-Thr-Stop
  - **Examples: β^0 thalasemia**

Gene mutations- types

- Normal state
- DNA
  - ATGCAGGTGACCTCAGTG
  - TACGTCCACTGGAGTCAC
- RNA
  - AUGCAGGUGACCUCAGUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- Mutation type of trinucleotide expanse
- DNA
  - ATG$(CAGCAGCAG)_{20}$CAGGTGACCTCAGTG
  - TAC$(GTCGTCGTC)_{20}$GTCCACTGGAGTCAC
- RNA
  - AUG$(CAGCAGCAG)_{20}$CAGGUGACCUCAGUG
- PROTEIN
  - Met-$(Gln\text{-}Gln\text{-}Gln)_{20}$Gln-Val-Thr-Ser-Val
- **Examples: Huntington´s disease**

Gene mutations- types

- Normal state
- DNA
  - ATGCAGGTGACCTCAGTG
  - TACGTCCACTGGAGTCAC
- RNA
  - AUGCAGGUGACCUCAGUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- **Mutation, type „frameshift"**
- DNA
  - ATGCAGGTG**A**ACCTCAGTG
  - TACGTCCAC**T**TGGAGTCAC
- RNA
  - AUGCAGGUG**A**ACCUCAGUG
- PROTEIN
  - Met-Gln-Val-**Asn-Leu-Ser**
  - **Examples:**
  - **Duchenn´s muscular dystrophy, β⁰ thalasemia, Tay-Sachs´s disease**

Gene mutations- types

- Normal state
- DNA
  - ATGCAGGTGACCTCA GTG
  - TACGTCCACTGGAGTC AC
- RNA
  - AUGCAGGUGACCUCA GUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- **Mutation: type „insertion"**
- DNA
  - ATGCAGGTG-**3000 bp-** ACCTCAGTG
  - TACGTCCAC-**3000 bp-** TGGAGTCAC
- RNA
  - AUGCAGGUG-**3000 bp-** ACCUCAGUG
- PROTEIN
  - Met-Gln-Val----------?
  - **Examples: Hemophilia *A***

Gene mutations- types

- Normal state
- DNA
  - ATGCAGGTGACCTCAGTG
  - TACGTCCACTGGAGTCAC
- RNA
  - AUGCAGGUGACCUCAGUG
- PROTEIN
  - Met-Gln-Val-Thr-Ser-Val

- **Mutation: type „deletion"**
- DNA
  - ATGCAGGTG
  - TACGTCCAC
- RNA
  - AUGCAGGUG
- PROTEIN
  - Met-Gln-Val
- **Examples:**
- **small-cystic fibrosis**
- **large: Duchenn´s muscular dystrophy**

Four basic types of heredity

	dominant	recessive
Autosomal	autosomal dominant (AD)	autosomal recessive (AR)
X-linked	X-dominant (XD)	X-recessive (XR)

Monogenic disorders

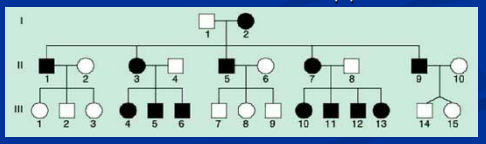
- Determined by one locus allele.
- Variant allele which had arosen sometimes in the history replaces original („wild") allele on one (heterozygote) or both (homozygote) chromosomes.
- Monogenic disorders have a characteristic transfer of the genotypes in families.
- Rare alleles are associated with monogenic disorders as a „big" factor.

Monogenic disorders

- Clinical manifestations are observed usually in childhood.
- Less than 10% are manifested after puberty and only 1% after reproductive age.
- Prevalence about 0.36%; in 6-8% hospitalizod children some monogenic disorder is suspected.

Mitochondrial heredity

- mtDNA is transfered by mother (after fertilization, only maternal mitochondria are conserved).
- Active process in paternal mitochondria elimination is supposed.



Complex (multifactorial, multigene) diseases

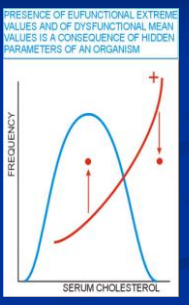
- *Every disease has its own genetic predisposition with different impact to clinical manifestation and/or other phenotypes of the disease.*

Complex (multifactorial, multigene) diseases

- They may be interactions of certain gene variants and certain environmental factors (and their combinations) which could be responsible for predisposition for many
- biological processes
- evolutional adaptations and/or
- complex diseases.

Mortality

- Can be explained by progressive disequilibrium between individual genome and environmental factors
- Genome and environmental factors can be changed by different rate
- Genome is more inertial



Genome stability vs. genome variability

- *Genome stability* endangered during life time by many repeated DNA replications is preserved by different mechanisms.
- *Genome variability* seems to be a source of surviving potencial in changing environmental context.

Genome stability

- Since our genomes are constantly exposed to exogenously-derived (e.g. UV radiation) and endogenously-derived (e.g. metabolically generated reactive oxygen species) DNA damaging agents, an impaired ability to detect and/or respond appropriately to these efects can impact on the maintenance of genetic stability.

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Genome stability

- There are many examples of human Mendelian disorders defective in the repair of or response to DNA damage.
- The importance of these pathways is demonstrated by the increase in cancer predisposition and developmental abnormalities associated with these conditions.

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Genome instability-copy number variants (CNVs)

- Recent studies have revealed that DNA segments in sizes from kilobases to megabases can vary in copy number among individuals in a population.
- These changes in copy number are the result of duplications, deletions, insertions, inversions and complex combinations of rearrangements, and are termed collectively copy number variants (CNVs).

van Attikum H, Gasser SM. Trends Cell Biol. 2009 May;19(5):207-17.

Copy number variants

- The changes in gene copy number are associated with different phenotypes in humans. Perhaps, the most well known example of this is the *trisomy 21 causative of Down syndrome.*
- An increased expression of the genes on chromosome 21 results directly or indirectly in a clinically heterogeneous disorder incorporating *cognitive impairment, facial dysmorphology, growth retardation, cancer predisposition, microcephaly, heart and skeletal abnormalities*

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

Genomic disorders

- Genomic disorders represent a clinically diverse group of conditions caused by *gain, loss or re-orientation of a genomic region containing dosage-sensitive genes.*
- Determining how the *copy number variation* (CNV) affects human variation and contributes to the aetiology and progression of various genomic disorders represents important questions for the future.

O´Driscoll M: Curr Genomics. 2008 May; 9(3): 137–146

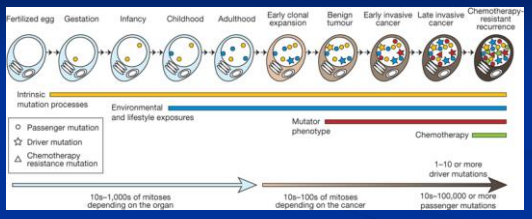
Gene mutations as a cause of variability in genes

- Rare alleles (prevalence less than 1% in population as a result of selection pressure and/or „recent" mutation). These mutations represent „great genetic factors" causing *monogenic diseases* – subjects of clinical genetics.
- Polymorphisms (prevalence more than 1% in population, smaller genetic factors in interactions with environmental factors conditioning *complex diseases* - subjects of personalized medicine).

Gene mutations as a cause of variability in genes

- *Mutations in somatic cells*
  - ✓ are generating in somatic cells during the lifetime
  - ✓ are cell and/or tissue specific, without transfer to offspring
- *Mutations in germ cells*
  - ✓ they become components of genetic predisposition
  - ✓ they are present in all cells of the individual
  - ✓ they are transfered to offspring

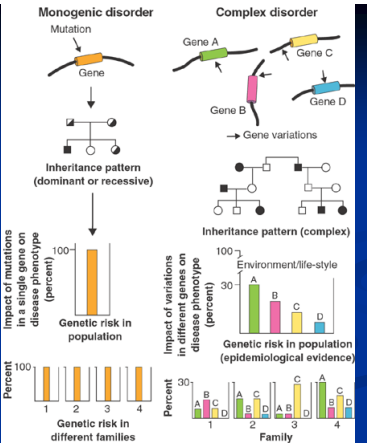
Somatic cell mutations: an example Sporadic colorectal cancer



The lineage of mitotic cell divisions from the fertilized egg to a single cell within a cancer showing the timing of the somatic mutations acquired by the cancer cell and the processes that contribute to them.

MR Stratton *et al. Nature* **458**, 719-724 (2009)

nature



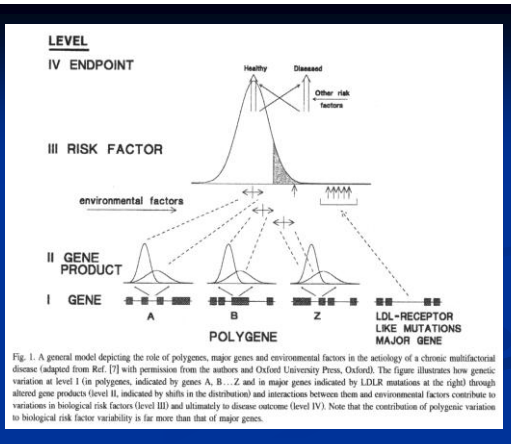


Fig. 1. A general model depicting the role of polygenes, major genes and environmental factors in the aetiology of a chronic multifactorial disease (adapted from Ref. [7] with permission from the authors and Oxford University Press, Oxford). The figure illustrates how genetic variation at level I (in polygenes, indicated by genes A, B....Z and in major genes indicated by LDLR mutations at the right) through altered gene products (level II, indicated by shifts in the distribution) and interactions between them and environmental factors contribute to variations in biological risk factors (level III) and ultimately to disease outcome (level IV). Note that the contribution of polygenic variation to biological risk factor variability is far more than that of major genes.

Candidate gene and its association with disease

- ✓ The question is simplier in mendelistic diseases in which a change function of a gene can be easier indentified.
- *Two main possibilities for this strategy:*
- ***Linkage analysis***
  - ✓ needs examination of genealogy
  - ✓ is evaluating common occurrence of genetic marker and disease in related individuals
- ***Association study***

Genetic studies

- *Candidate gene selection strategy.*
  - ✓ Etiopatogenetic (pathophysiological) approach using for candidate gene selection
- *Genome- wide analysis*
  - ✓ Based on analysis of large sequences of genome

Association studies

are evaluating common occurrence of genetic marker and disease in unrelated individuals

Types of association studies

- *case-control* (healthy-ill)
- *case-case* (severity of disease, early onset of disease, risk factors for disease including gender
- *genotype-phenotype (e.g.biochemical parameters)*

DNA markers

- Thus, it is possible to associate alleles of many polymorphisms with clinical manifestation of the disease and/or with some phenotypes of a disease.
- Therefore, a certain genotype and/or allele of the polymorphism can represent statistically higher (lower) risk for the disease (odds ratio).

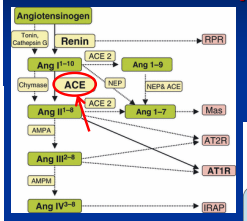
↓

Odds ratio (OR):

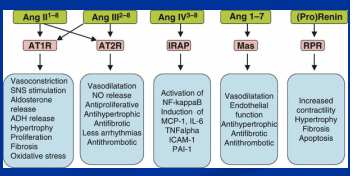
Number of patients with risk genotype x number of healthy individuals with different genotype
Number of healthy with risk genotype x number of patients with other than risk genotype

⇒ DNA marker does not have to be causative for the disease. Definitelly, the most important characteristic of *clinically useful* DNA marker must be its high statistic asscociation with a disease and/or its phenotype.

Association of a disease with candidate gene variants: an example: I/D ACE



Fyhrquist F and Saijonmaa O, Journal of Internal Medicine 264: 224-236, 2008



RPR, renin/prorenin receptor; Mas, mas oncogene, receptor for Ang 1–7; AT2R, angiotensin type 2 receptor AT1R, angiotensin type 1 receptor, IRAP, insulin-regulated aminopeptidase; Ang IV receptor AMPA, aminopeptidase A; AMPM, aminopeptidase M; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral endopeptidase.

Zygote → Embryo → Adult organism

- How does a single egg or zygote become a complete organism with many different tissues and differentiated cells?
- How can this happen, when the zygote undergoes many rounds of mitosis – mitosis is supposed to produce identical daughter cells?

How do Organisms Control the Level of Gene Expression?

- Cells must only express genes when needed
- Gene expression (transcription, translation) takes up large amounts of cellular energy and resources
- Cells live frugal lifestyles – they conserve energy and resources
- So genes will only be expressed when their products are needed.

How do Eukaryotes Control the Level of Gene Expression?

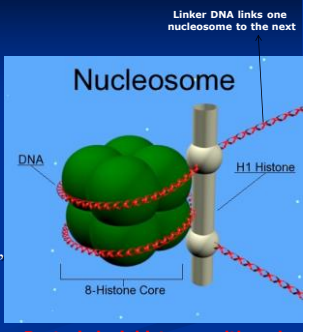
- Cells of more complex organisms turn on and turn off genes based on the functions of the cells – hence cells differentiate
- Eukaryotes control genes at almost every level:
  - Regulation of Chromatin Structure
  - Regulation at the transcriptional level
  - Regulation at a post-transcriptional level
  - Regulation at a translational level
  - Regulation at a post-translational level

Regulation of Chromatin Structure

- Histone acetylation prevents DNA from winding tightly around histones, allowing easy access to promoter sites (Deacetylation does the opposite)
- DNA methylation causes DNA to wind tightly around histones, preventing easy access to gene promoters (Demethylation does the opposite)

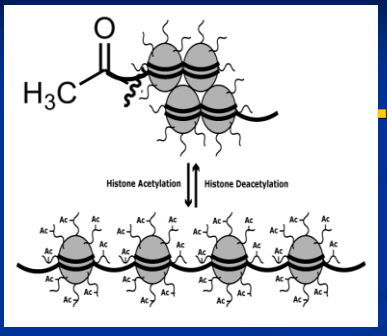
Histones

- Histone subunits are:
  - 2 units of H2A
  - 2 units of H2B
  - 2 units of H3
  - 2 units of H4
- Histone H1 is not in the core, but acts as a clamp and keeps the linker DNA in place
- Histones are positively charged, so DNA which is negatively charged, wraps around them

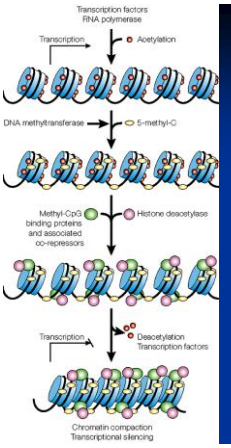


Bacteria lack histones, although some archea have them

Histone



- Histones have tails that get actylated by HAT enzymes (Histone Acetyl Transferases)
- Histone acetylation makes their (+) charge more neutral, so DNA interaction is reduced



DNA Methylation in conjunction with Histone deacetylation

Epigenetic Inheritance

- Modification of chromatin does not change the DNA, only its expression.
- However, this modification pattern *IS* inherited (remember genomic imprinting?)
- Scientists now believe that certain environmental factors may play a part in promoting chromatin modification that causes expression or suppression of certain genes – e.g. one twin gets schizophrenia and another doesn't. Certain cancers may also be caused that way

Epigenetic effects

- Transcriptionally active chromatin is predominantly unmethylated and has high levels of acetylated histone tails.
- Most mammalian transcription factors have GC-rich binding sites and many have CpGs in their DNA recognition elements.
- Binding by several of these factors is impeded or abolished by methylation of CpG.

Regulation at the transcriptional level

- Enhancers (proximal and distal)
- Silencers
- Transcription factors at promoters
  - General transcription factors
  - Specific transcription factors
  - All these play a role in regulating gene expression.
  - Enhancers increase the rate of a gene's expression and silencers decrease it.
  - Transcription factors are needed if the gene is to be expressed at all.

Regulation at post-transcriptional level

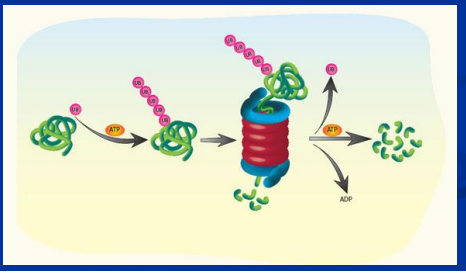
- RNA processing – alternative splicing allows certain proteins to be made instead of others (all from the same gene)
- mRNA Degradation – cytoplasmic nucleases degrade mRNAs so polypeptide synthesis stops. More mRNA is made later, if necessary
- 5' caps and 3' tails can be removed or changed and this will prevent translation

Pre-translational Regulation

- Certain proteins in the cytoplasm can bind to the mRNA's 5' UTR and prevent ribosomes from binding
- Any change in mRNA shape will prevent ribosome binding
- Decreased length of poly-A tail will prevent translation

Post-translational Regulation

- Proteins can be ubiquitinzed and degraded in a proteasome



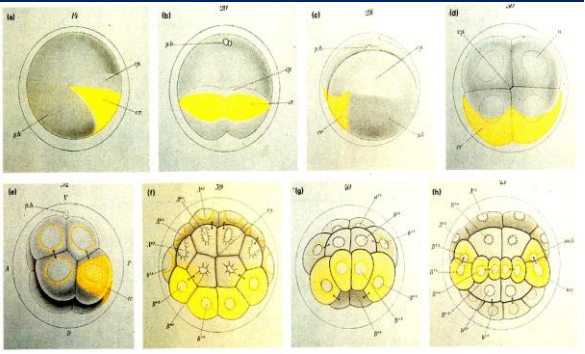
Non-protein-coding RNAs and Gene Regulation

- MicroRNAs or miRNAs are small non-coding RNAs that were
  - Transcribed from DNA
  - Complexed with a number of proteins
  - These miRNAs have several bases that are complementary to some protein-coding mRNAs
  - The miRNA-protein complex can bind to these protein-coding mRNAs and prevent them from being translated
  - Nucleases eventually degrade the dsRNA

Cytoplasmic Determinants

- Certain molecules such as maternal mRNAs, transcription factors and other proteins are localized in specific cytoplasmic regions of the unfertilized egg or zygote
- These molecules affect cell fate decisions by segregating into different embryonic cells and controlling distinct gene activities in these cells (specialized transcription factors will only turn on certain genes).
- Cytoplasmic determinants are also found in some post-embryonic cells, where they produce cytoplasmic asymmetry.
- In dividing cells, this leads to asymmetric cell division in which each of the daughter cells differentiates into a different cell type. *Also called* localized cytoplasmic determinants or morphogenetic determinants.

Cytoplasmic Determinants and Cell Fate



Pharmaco-genetic

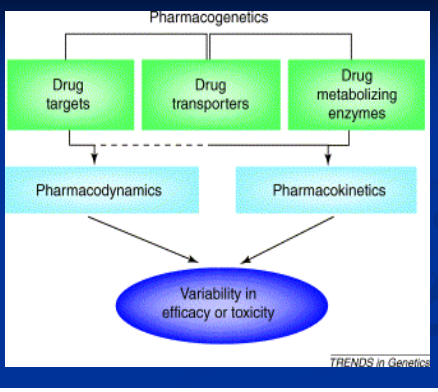
Pharmacogenetics & Pharmacogenomics

- Pharmacogenetics: The role of genetics in drug responses.
  - F. Vogel. 1959
- Pharmacogenomics: The science that allows us to predict a response to drugs based on an individuals genetic makeup.
  - Felix Frueh, Associate Director of Genomics, FDA

Courtesy Felix W. Frueh

Pharmacogenetics & Pharmacogenomics

- Pharmacogenetics: study of individual gene-drug interactions, usually one or two genes that have dominant effect on a drug response (SIMPLE relationship)
- Pharmacogenomics: study of genomic influence on drug response, often using high-throughput data (sequencing, SNP chip, expression, proteomics - COMPLEX interactions)



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GENETIC POLYMORPHISMS

- Transporters
- Plasma protein binding
- Metabolism

- Receptors
- Ion channels
- Enzymes
- Immune molecules

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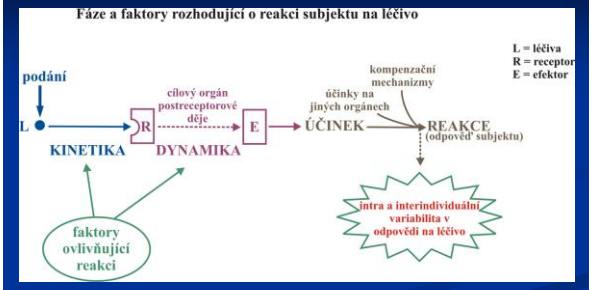

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Relation to genes

- Almost every pathway of drug metabolism, its transport or activation is influenced by genetic variability.
  - Clinical variability in the response
  - The risk of side effects
  - Genotype specific dosage
  - Polymorphic targets drug

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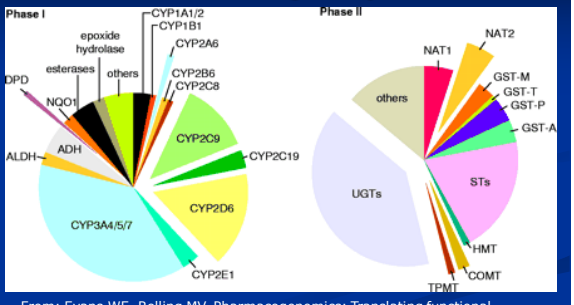
Genetic polymorphisms in drug metabolizing enzymes



From: Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 286:487-491, 1999.

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10 questions in polygenic disorders

- ✓ How important are genetic influences in the most common forms of multigene diseases?
- ✓ What is the influence of the environment on the onset of the disease?
- ✓ Which are the most promising approaches to the determination of genetic factors leading to the onset of disease?
- ✓ Which genes have already been selected as candidate genes?
- ✓ Which paths contribute to genetic susceptibility for the disease?
- ✓ How many genes are involved in susceptibility to disease?
- ✓ Are the most common forms of polygenic diseases associated with frequent or rare genetic variability in the population? (hypothesis frequent variations / frequent genetic disease vs. heterogeneous model)
- ✓ Why alleles that are associated with the disease were not eliminated from the population?
- ✓ The importance for the disease-environment interaction genes and genes-genes?
  What are the implications for pharmacogenetics?

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Pharmacogenetics: A Case Study

Individuals respond differently to the anti-leukemia drug 6-mercaptopurine.

Most people metabolize the drug quickly. Doses need to be high enough to treat leukemia and prevent relapses.

Others metabolize the drug slowly and need lower doses to avoid toxic side effects of the drug.

A small portion of people metabolize the drug so poorly that its effects can be fatal.

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The diversity in responses is due to variations in the gene for an enzyme called TPMT, or thiopurine methyltransferase.

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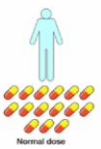
The diversity in responses is due to variations in the gene for an enzyme called TPMT, or thiopurine methyltransferase.

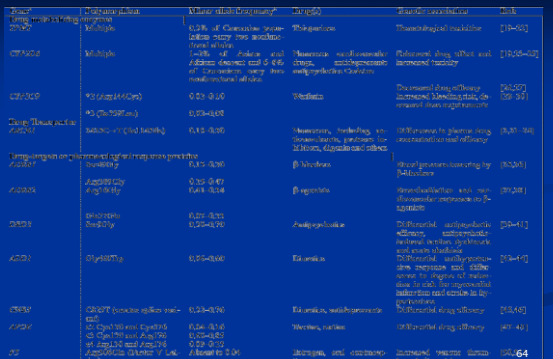
After a simple blood test, individuals can be given doses of medication that are tailored to their genetic profile.



Normal dose

Dose for an extra slow metabolizer (TPMT deficient)

Clinically relevant genetic polymorphisms in relation to the side effects of drugs



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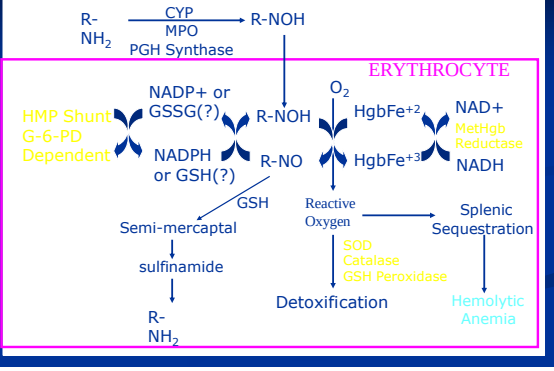
Clinically relevant genetic polymorphisms in relation to the effectiveness of drugs

Disease	Treatment	Comment	References
MS, APL	all trans-retinoic acid	Patients with PLZF/RARA fusion are not responsive to retinoids.	[9]
Glioma	carmustine, BCNU	Only tumors with CpG methylation of the promoter of the O^6-methylguanine-DNA-methyltransferase gene respond to treatment with alkylating substances.	[22]
Asthma	5-lipoxygenase inhibitors	ALOX5 promoter genotype influences response to treatment; individuals with two non-wild type alleles show no response to 5-LOX inhibitors.	[23]
	β_2 adrenergic agents	Gly16-allele of β_2 adrenergic receptor is associated with much stronger bronchodilator desensitization than Arg16.	[24]
Depression	imipramine	Fast metabolizers do not reach therapeutic drug levels with normal dosage.	[15]

65

Glucose-6-phosphate dehydrogenase activity

Effects >100 million worldwide



Drugs and Chemicals Unequivocally Demonstrated to Precipitate Hemolytic Anemia in Subjects with G6PD Deficiency

Acetanilide	Nitrofurantoin	Primaquine
Methylene Blue	Sulfacetamide	Nalidixic Acid
Naphthalene	Sulfanilamide	Sulfapyridine
Sulfamethoxazole		

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INCIDENCE OF G6PD DEFICIENCY IN DIFFERENT ETHNIC POPULATIONS

Ethnic Group	*Incidence(%)*
Asiatics	
Chinese	2
Filipinos	13
Indians-Parsees	16
Javanese	13
Micronesians	<1
Iranians	8
Greeks	0.7-3
Persia	15

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Cytochrome Oxidase P450 Enzymes

- 57 Different active genes
- 17 Different families
- CYP1, CYP2 and CYP3 are primarily involved in drug metabolism.
- CYP2A6, CYP2B6, CYP2C9 ,CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are responsible for metabolizing most clinically important drugs

Polymorphic Cytochrome P-450s

CYP2B6

Selected Substrates	Location	Poor Metabolizer Incidence
bupropion cyclophosphamide efavirenz methadone ifosfamide	Chromosome 19	3-4% of Caucasians

CYP2C9

Selected Substrates	Location	Poor Metabolizer Incidence
NSAIDs celecoxib diclofenac ibuprofen naproxen piroxicam Oral Hypoglycemic Agents tolbutamide glipizide ARBs irbesartan losartan fluvastatin warfarin phenytoin	Chromosome 10	1-3% Caucasians

CYP2C19

Selected Substrates	Location	Poor Metabolizer Incidence
Proton pump (-) amitriptyline cyclophosphamide diazepam indomethacin phenytoin phenobarbital progesterone voriconazole	Chromosome 10	2-4% African-Americans 3-5% Caucasians 15-20% Asians

CYP2D6

Selected Substrates	Location	Poor Metabolizer Incidence
antidepressants beta-blockers antipsychotics chlorpheniramine codeine dextromethorphan ondansetron lidocaine promethazine tamoxifen tramadol	Chromosome 22	5-10% Caucasians

Effect of Metabolic Rate on Drug Dosage

Drug	Poor Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Poor efficacy Possible accumulation of prodrug
Active drug, inactivated by metabolism (example is omeprazole)	Good efficacy Accumulation of active drug can produce adverse reactions May need lower dose

Drug	Ultra-rapid Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Good efficacy, rapid effect
Active drug, inactivated by metabolism (example is omeprazole)	Poor efficacy Need greater dose or slow release formulation

Debrisoquine phenotype in subjects with different *CYP2D6* genotypes

Genotype	# of Subjects	Metabolic Ratio
CYP2D6wt/$(CYP2D6L)_2$	9	0.33
CYP2D6wt/CYP2D6wt	12	1.50
CYP2D6wt/CYP2D6(A or B)	9	2.14
CYP2D6B/CYP2D6B	6	48.84

Data from: Agundez JG et al. *Clin Pharmacol Ther* 57:265, 1995.

Codeine and Cytochrome P450 CYP2D6

- Codeine is a commonly used opioid
  - Codeine is a prodrug
  - It must be metabolized into morphine for activity
- Cytochrome P450 allele CYP2D6 is the metabolizing enzyme in the liver
- 7% of Caucasians are missing one copy of the Cytochrome P450 CYP2D6 gene
  - codeine does not work effectively in these individuals

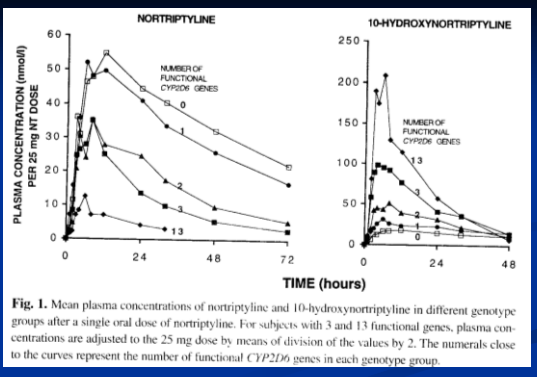

Fig. 1. Mean plasma concentrations of nortriptyline and 10-hydroxynortriptyline in different genotype groups after a single oral dose of nortriptyline. For subjects with 3 and 13 functional genes, plasma concentrations are adjusted to the 25 mg dose by means of division of the values by 2. The numerals close to the curves represent the number of functional *CYP2D6* genes in each genotype group.

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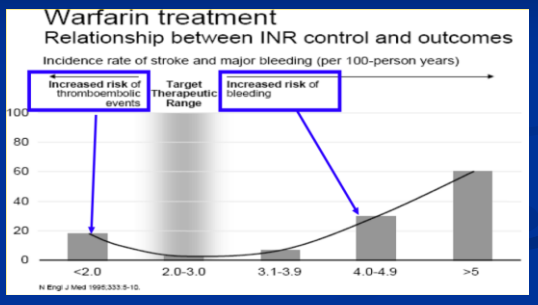
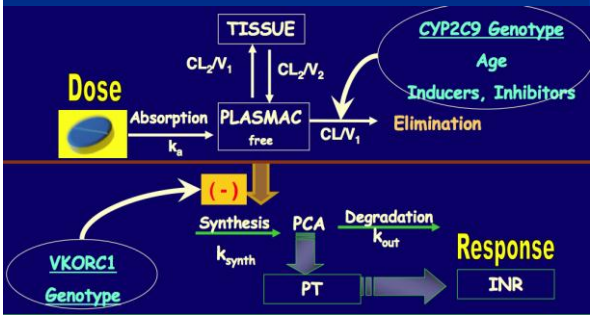
Why Maintaining Warfarin Therapeutic Range is Critical



European Atrial Fibrillation Trial Study Group, N Engl J Med 1995;333:5–10.

Warfarin Levels Depend on Two Enzymes – CYP2C9 & VKORC1



Estimated Warfarin Dose (mg/day) Based on Genotypes

CYP2C9 genotype

VKORC1 genotype	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	6	5	4	4	3.5	3
GA	5	4	3	3	2.5	2
AA	3	2.5	2	2	2	1.5

‹Kim MJ, Huang S-M, Meyer U, Rahman, A, Lesko LJ,

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CYP2C9 ACTIVITA

Prescribed Daily Warfarin Dose and CYP2C9 Genotype

Warfarin Dose*	Genotype
5.63 (2.56)	*1/*1
4.88 (2.57)	*1/*2
3.32 (0.94)	*1/*3
4.07 (1.48)	*2/*2
2.34 (0.35)	*2/*3
1.60 (0.81)	*3/*3

From: Higashi MK, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 287:1690-1698, 2002.

78

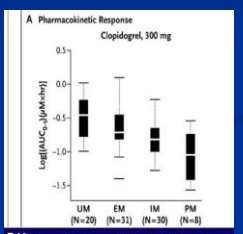
Frequency of VKORC1 Alleles in Various Populations

-1639 G>A	AA	AG	GG
Caucasians (N=297)	19%	56%	25%
Spanish (N=105)	32%	40%	28%
Chinese (N=104)	80%	18%	2%
African Americans (N=159)	0%	21%	79%

Asians may need a lower dose

Sconce et al. Blood 2005, Yuan et al. Human Mol Genetics 2005, Schelleman et al. Clin Pharmacol Ther 2007, Montes et al Br J Haemat 2006

Another Anticoagulant Clopidogrel (Plavix) and CYP2C19 Alleles



PM: with two reduced function alleles
IM: one reduced function allele
EM: no variant alleles;
UM: one or two *17

Interaction with drogs metabolized and/or reactingi with CYP2C9

17, 20, 21

Competition	Enzyme inductor	Enzyme inhibitor
ASA a většina NSAID	rifampicin	fluvoxamin (ostatní SSRI slabí)
fenobarbital, fenytoin	fenobarbital, fenytoin	omeprazol
S-warfarin	karbamazepin	inhibitory HMG-CoA reduktázy
losartan		tolbutamid
tolbutamid		cimetidin (slabý)
sulfonamidy, dapson		azolová antimykotika (slabá)
diazepam, tenazepam		ritonavir
fluoxetin, moclobemid		desethylamiodaron
zidovudin		

20. Topinková E et al: Postgrad Med 2002; 5:477-82
21. Naganuma M et al: J Cardiovasc Pharmacol Ther 2001; 6:636-7

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GENETIC POLYMORPHISMS, MATERNAL SMOKING AND LOW BIRTH WEIGHT (LBW)

65% of all infant deaths occur among LBW infants, while LBW infants account for 7.6% of all live births

Reduction in birth wgt among smoking women

Genotype	Weight Reduction
CYP1A1 AA	252 g
CYP1A1 Aa/aa	520 g
GST1 AA/Aa	285 g
GST1 aa	642 g

Data from: Wang X, et al. *JAMA* 287:195-2002, 2002.

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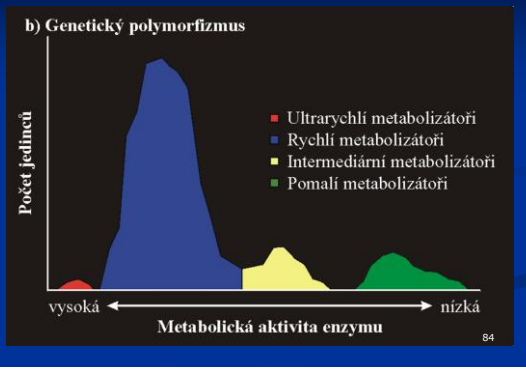
Metabolic rate

- According to the activity of the enzyme may be a population divided into four main groups - poor metabolisers (PM), intermediate metabolizers (IM), efficient metabolizers (EM), and ultra-fast metabolizers (UM).
- Most individuals among the white population - extensive metabolizers (EM) - the drugs are metabolized by the expected rate.
- 5-10% of individuals are genetically determined poor metabolisers (PM) - the slow degradation of substances metabolised and are at a higher incidence of adverse events.
- Intermediate metabolizers (IM) are represented in 10-15% and in long term treatment in response – comparable to PM.
- Ultra-fast metabolizers (UM) – metabolization is intensive; clinically unresponsive to the usual doses of drugs - 5-10%.



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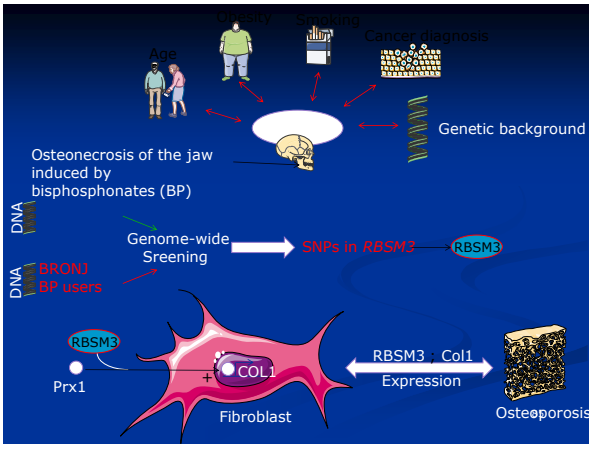

Methotrexate in RA

- Effectiveness of treatment of rheumatoid arthritis (RA) by methotrexate (MTX) 46% - 65% (ACR20)
- During treatment with MTX side effects may occur. At least one in 72.9% of patients, severe in 30% of patients.
  - gastrointestinal toxicity (nausea, vomiting, diarrhea, 20% - 65%
  - Hepatotoxicity 10% - 43%
  - oral ulceration 37%
  - alopecia to 4%
  - pulmonary toxicity 2.1% - 8%
  - Bone marrow suppression light 12%
  - pancytopenia 0.8%

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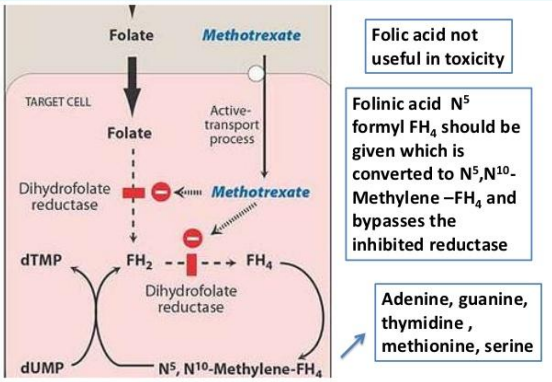
Methotrexate



Folic acid not useful in toxicity

Folinic acid N^5 formyl FH_4 should be given which is converted to N^5,N^{10}-Methylene $-FH_4$ and bypasses the inhibited reductase

Adenine, guanine, thymidine , methionine, serine

Methotrexate

Table 1. Pharmacogenetics of MTX transporters*

Gene	Polymorphism	Amino acid substitution in enzyme	Biochemical effects	Clinical effects	Reference
RFC-1	G80A	Histidine to arginine at codon 27	May affect transcriptional activity of RFC1 gene and MTX entry into cell	May affect response to MTX	48, 72
ABCB1	C3435T	No amino acid substitution	May affect MTX entry into cell	May affect response to MTX	55

* MTX = methotrexate; RFC-1 = reduced folate carrier 1; ABCB1 = ATP binding casette transporter B1.

Table 2. Pharmacogenetics of MTHFR*

Gene	Polymorphism	Amino acid substitution in enzyme	Biochemical effects	Clinical effects (ref.)
MTHFR	C677T	Alanine to valine	Thermolabile MTHFR with decreased activity; increased plasma homocysteine	May increase the following: GI toxicity (60); hepatic and GI toxicity, alopecia, stomatitis, and rash (62,63); headache, lethargy (74). No effect on toxicity (62); no effect on efficacy or toxicity (71)
MTHFR	A1298C	Glutamine to alanine	May decrease MTHFR activity and increase plasma homocysteine	May affect MTX efficacy (63); may increase susceptibility to RA and decrease MTX toxicity (62). No effect on efficacy or toxicity (71)

* MTHFR = methylenetetrahydrofolate reductase; GI = gastrointestinal; MTX = methotrexate; RA = rheumatoid arthritis.

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Ranganathan P, McLeod HL. A&R 20

Methotrexate

Table 3. MTX pathway pharmacogenetics

Gene	Role in MTX pathway	Polymorphism	Effects on gene product/enzyme	Clinical effects	Reference
ATIC	Conversion of AICAR to 10-formyl-AICAR; target of MTX	C347G	May decrease ATIC activity and affect AICAR accumulation and adenosine release	May affect MTX efficacy and toxicity	72, 74
TYMS	Conversion of dUMP to dTMP; target of MTX	5′-UTR 28-bp repeat	May increase TYMS enzyme activity	May affect MTX efficacy and toxicity	71, 74
		3′-UTR 6-bp deletion	May decrease TYMS mRNA stability and expression	May affect MTX efficacy	71

* MTX = methotrexate; ATIC = aminoimidazole carboxamide ribonucleotide transformylase; AICAR = aminoimidazole carboxamide ribonucleotide; TYMS = thymidylate synthase; 5′-UTR = 5′-untranslated region.

Table 4. Other genes with potential pharmacogenetic implications in the MTX pathway*

Gene	Role in MTX pathway	Polymorphism	Effects on gene product/enzyme	Postulated clinical effects	Reference
GGH	Conversion of long-chain MTXPGs to short-chain MTXPGs by removal of glutamates	C452T	Decreased binding affinity of GGH for MTXPGs	May affect MTX efficacy	76
		C401T	Affects MTXPG levels	–	47
DHFR	Reduction of DHF to THF; target of MTX	3′-UTR T721A and C829T	May increase DHFR expression	May affect MTX efficacy	77
MS	Methylation of homocysteine to methionine	A2756G	May decrease MS activity; increase homocysteine levels	May affect MTX toxicity	79, 80
MTRR	Methylation of cobalamin cofactor required for the action of MS	A66G	May decrease MTRR activity; increase homocysteine levels	May affect MTX toxicity	81, 82

* MTX = methotrexate; GGH = γ-glutamyl hydrolase; MTXPGs = methotrexate polyglutamates; DHFR = dihydrofolate reductase; DHF = dihydrofolate; THF = tetrahydrofolate; 3′-UTR = 3′-untranslated region; MS = methionine synthase; MTRR = methionine synthase reductase.

Ranganathan P, McLeod HL. A&R 20

MDR1

- MDR1 (ATP-binding cassette B1/multidrug resistance 1) is an efflux pump that transports toxic endogenous substances, drugs and xenobiotics out of cells.
- It is known to affect susceptibility to many hematopoietic malignancies.
- ABCB1/MDR1 polymorphisms may either change the protein expression or alter its function, suggesting a possible association between ABCB1/MDR1 single nucleotide polymorphisms (SNP) and clinical aspects of T-cell lymphoma.
- Therefore, association of two polymorphisms in the gene with clinical staging and therapy was evaluated.

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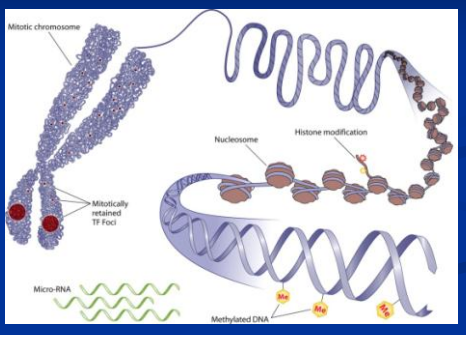
Epigenetics



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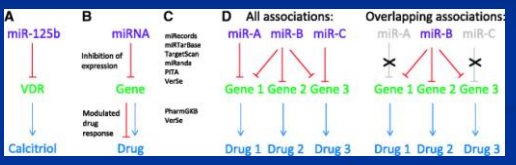
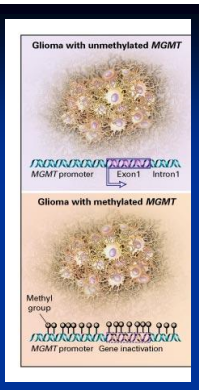
(A) An example of an experimentally verified miRNA pharmacogenomic set. miR-125 b inhibits vitamin D receptor (VDR) expression.



Rukov J L et al. Brief Bioinform 2013;bib.bbs082

Briefings in Bioinformatics



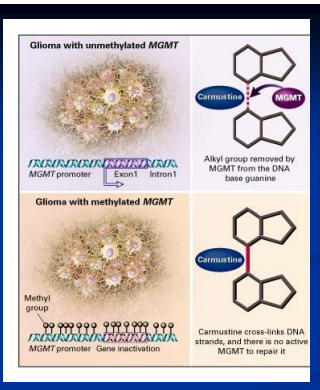
Why are some gliomas resistant to nitrosourea alkylating agents?

Evidence suggests this may be the result of an epigenetic phenomenon – one that does not involve a change in DNA sequence.

MGMT – methylguanine-DNA methyltransferase
Methylation of the promoter region of MGMT may silence the gene

From: Esteller M, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *NEJM* 243:1350-1354, 2000.

93



From: Esteller M, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *NEJM* 243:1350-1354, 2000.

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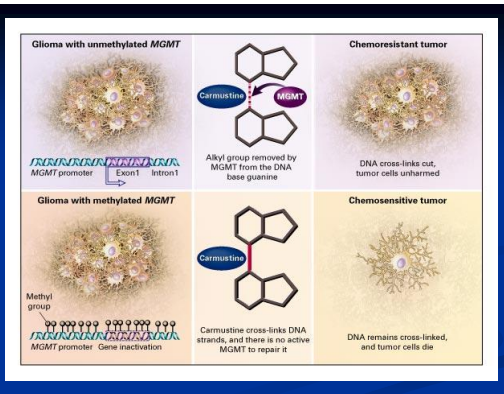

From: Esteller M, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *NEJM* 243:1350-1354, 2000.

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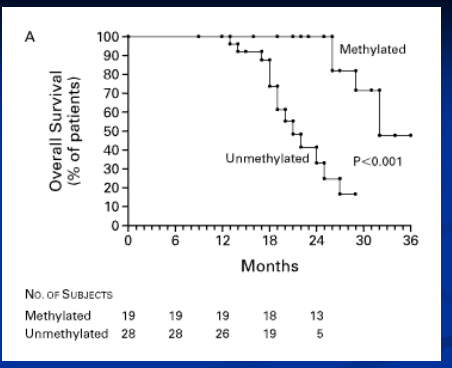

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Genetic Analysis Permits

- More rapid determination of stable therapeutic dose.
- Better prediction of dose than clinical methods alone.
- Applicable to the 70-75% of patients not in controled anticoagulation centers.
- Reduces between 4,500 and 22,000 serious bleeding events annually.
- Genetic testing now required by

Personalized Drugs

- Herceptin (breast cancer, target: Her2/neu)
- Erbitux (colorectal cancer, target: EGFR)
- Tarceva (lung cancer, target: EGFR)
- Strattera (attention-deficit/hyperactivity disorder, Metabolism: P4502D6)
- 6-MP (leukemia, Metabolism: TPMT)
- Antivirals (i.e. resistance based on form of HIV)

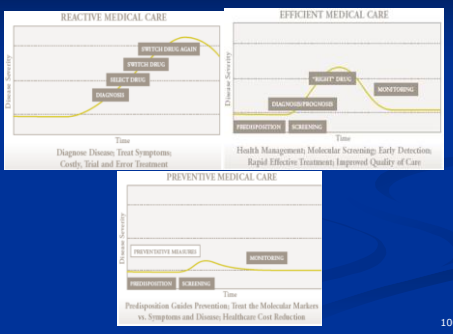
- etc. and the list is growing rapidly ...

FDA Requires Genetic Tests for Certain Therapies

List of FDA Required or Recommended Biomarker Tests in Drug Labels

Biomarker	Test[13]	Drug Example	User Prevalence (%) (n=36.1 million)
CYP2C9	Recommended	Warfarin	2.0896
EGFR	Required	Cetuximab	0.0001
G6PD deficiency	Recommended	Dapsone	0.0257
G6PD deficiency	Recommended	Rasburicase	0.0000
HER2/neu overexpression	Required	Trastuzumab	0.0003
TPMT variants	Recommended	Azathioprine	0.1168
TPMT variants	Recommended	Mercaptopurine	0.0541
TPMT variants	Recommended	Thioguanine	0.0012
UGT1A1 variants	Recommended	Irinotecan	0.0002
Urea cycle enzyme deficiency	Recommended	Valproic acid	0.48
Total			2.768

CYP = cytochrome P450; EGFR = human epidermal growth factor receptor; G6PD = glucose-6-phosphate dehydrogenase; HER2/neu = human epidermal growth factor receptor 2; TPMT = thiopurine S-methyltransferase.



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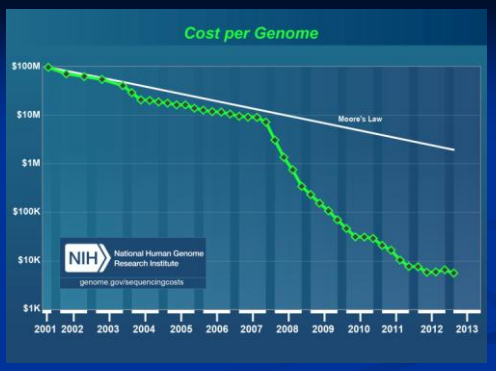

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Thank you for your attention

„I just need a closer look..."