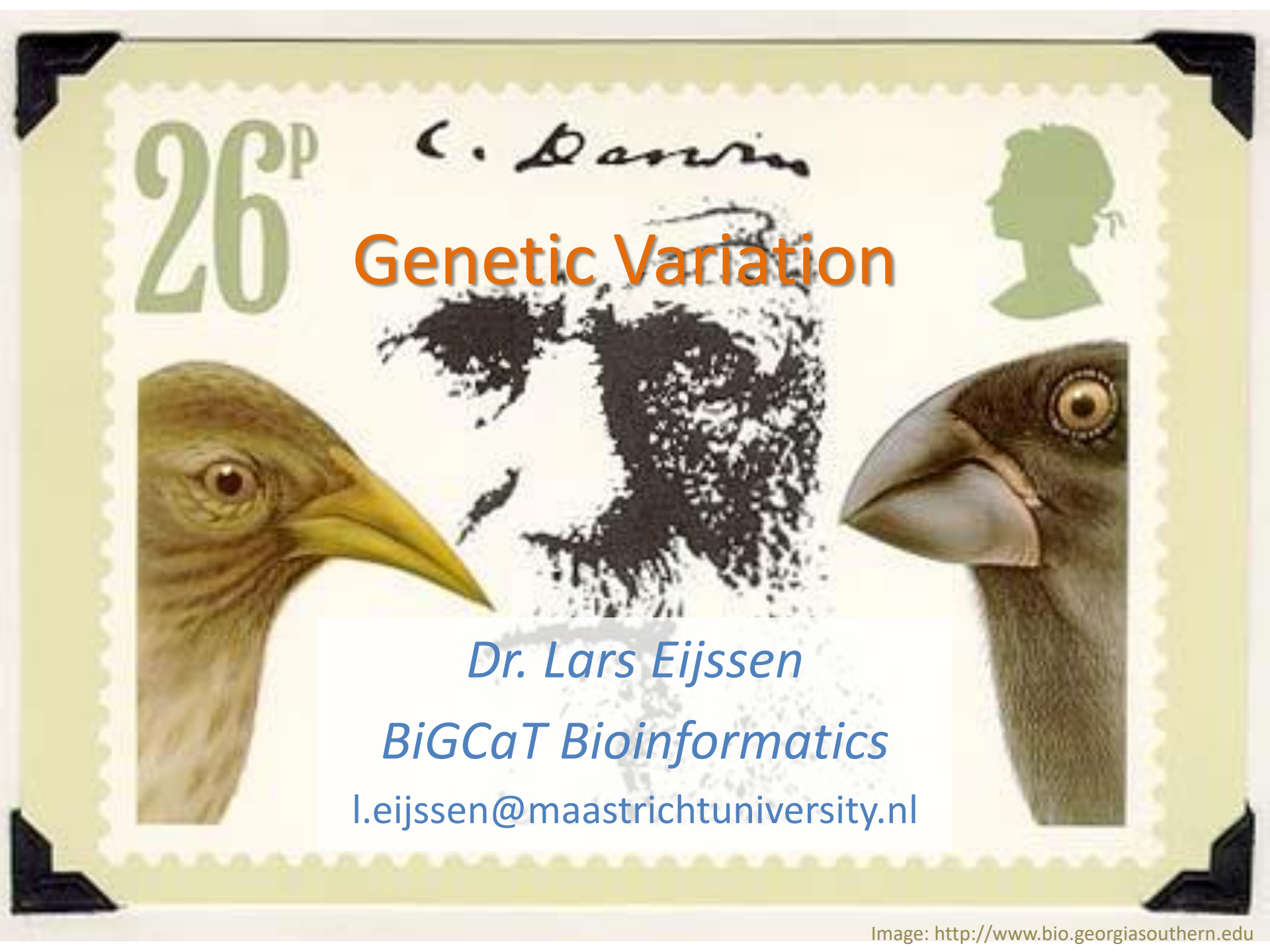

Genetic Variation

Dr. Lars Eijssen

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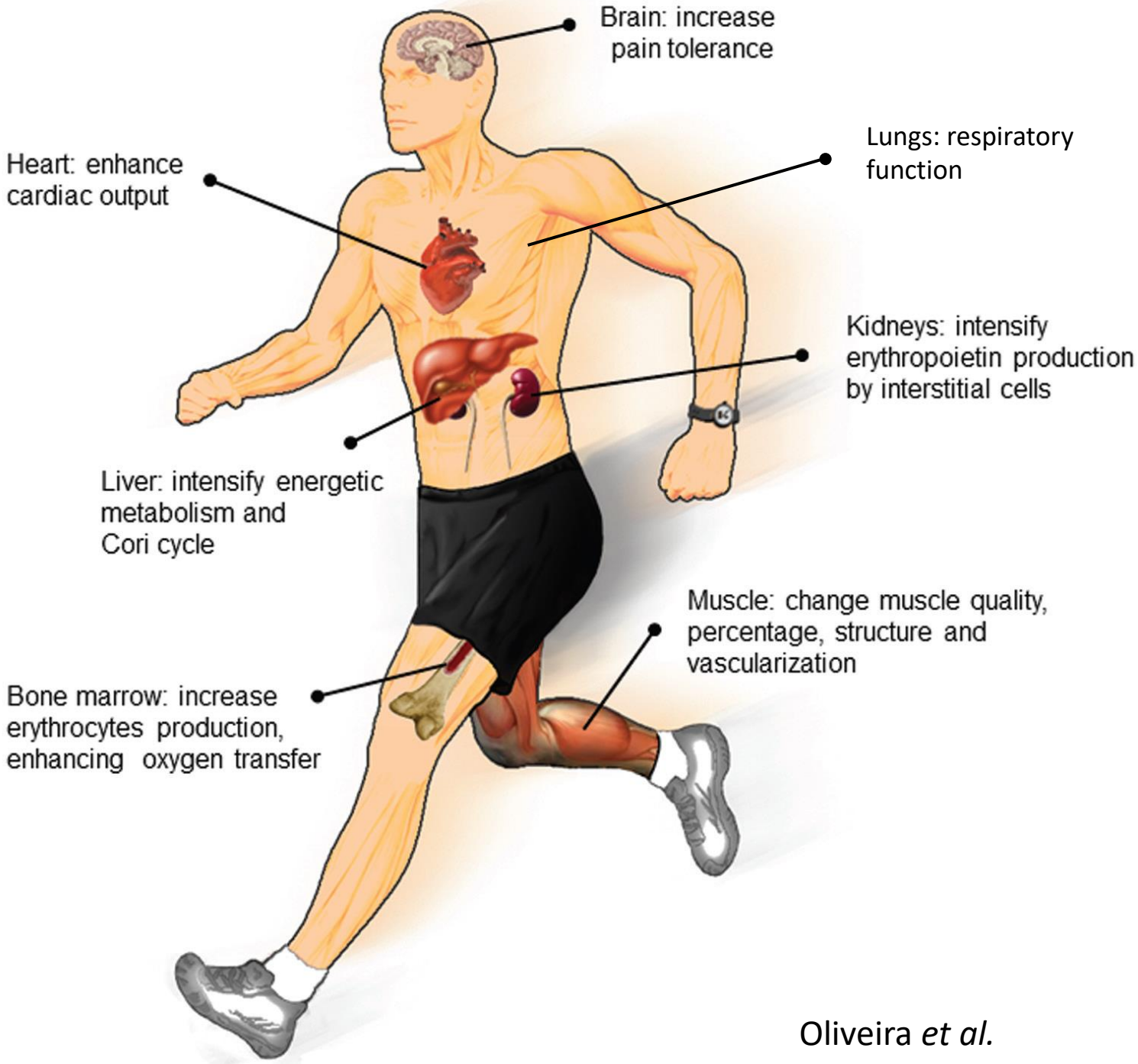
Image: http://www.bio.georgiasouthern.edu

Genetic variations and athletic performance?



Oliveira *et al.*

"Athleticogenomics"

IJPH - Year 9, Volume 8, Number 3, 2011

ITALIAN JOURNAL OF PUBLIC HEALTH

Athleticogenomics and elite athletes: a review of the state of the art and a possible relationship with inflammatory response

Alessandra Colombini[1], Giovanni Lombardi[1], Giuseppe Banfi[1,2], Marisa Arpesella[3], Gabriele Pelissero[3,4]

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Abstract

Background: Recent research in athleticogenomics has begun to reveal how particular genetic polymorphisms may influence athletic status and confer an individual predisposition for better sports performances. This is of particular interest for elite athletes because it could help to assess an athlete's potential, to enhance specific training protocols for selected performances, to monitor the individual response to training load and recovery and, finally, aid in the prevention of accidents.

Methods: Using a topics search in the PubMed database, the search strategy included studies examining the relationship between the presence of polymorphisms in genes influencing selected physiological parameters and the elite athletic status. English written case (elite athletes) -control (general population) studies were selected.

Results: 26 research articles concerning polymorphic genes involved in muscle physiology, cell respiratory

ITALIAN JOURNAL OF PUBLIC HEALTH

Table 1. The main polymorphic genes in nuclear DNA analyzed in elite athletes and the more significant case-control studies of their association with sports performance. (Continue)

Genes encoding for adrenergic receptors or for a G protein-coupled receptor subunit							
Gene	***Location***	***Polymorphism***	***Number of subjects***		***P Value (genotype/ allele frequency)***	***Reference***	***Year***
			Elite athletes	***Controls***			
ADRA2A (alpha2a adrenergic receptor)	10q24–q26	Dra I RFLP	140	141	0.037	29	2000
ADRB2 (beta2 adrenergic receptor)	5q31–q32	Arg16Gly polymorphism	303	297	0.03	30	2007
GNB3 (guanine nucleotide binding protein β polypeptide 3)	12p13	exon 10 C825T polymorphism	155	234	0.046	31	2009

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doi: 10.1590/S0100-879X2011007500145

The use of genes for performance enhancement: doping or therapy?

R.S. Oliveira, T.F. Collares, K.R. Smith, T.V. Collares and F.K. Seixas

"Gene doping"

ORIGINAL ARTICLE

Endocrine Care

Doping Test Results Dependent on Genotype of Uridine Diphospho-Glucuronosyl Transferase 2B17, the Major Enzyme for Testosterone Glucuronidation

Jenny Jakobsson Schulze, Jonas Lundmark, Mats Garle, Ilona Skilving, Lena Ekström, and Anders Rane

Department of Laboratory Medicine, Karolinska Institutet at Division of Clinical Pharmacology, Karolinska University Hospital, SE-141 86 Stockholm, Sweden

Context: Testosterone abuse is conventionally assessed by the urinary testosterone/epitestosterone (T/E) ratio, levels above 4.0 being considered suspicious. The large variation in testosterone glucuronide (TG) excretion and its strong association with a deletion polymorphism in the uridine diphospho-glucuronosyl transferase (UGT) 2B17 gene challenge the accuracy of the T/E ratio test.

Objective: Our objective was to investigate whether genotype-based cutoff values will improve the sensitivity and specificity of the test.

Design: This was an open three-armed comparative study.

Participants: A total of 55 healthy male volunteers with either two, one, or no allele [*insertion/insertion*, *insertion/deletion*, or deletion/deletion (*del/del*)] of the *UGT2B17* gene was included in the study.

Intervention: A single im dose of 500 mg testosterone enanthate was administered.

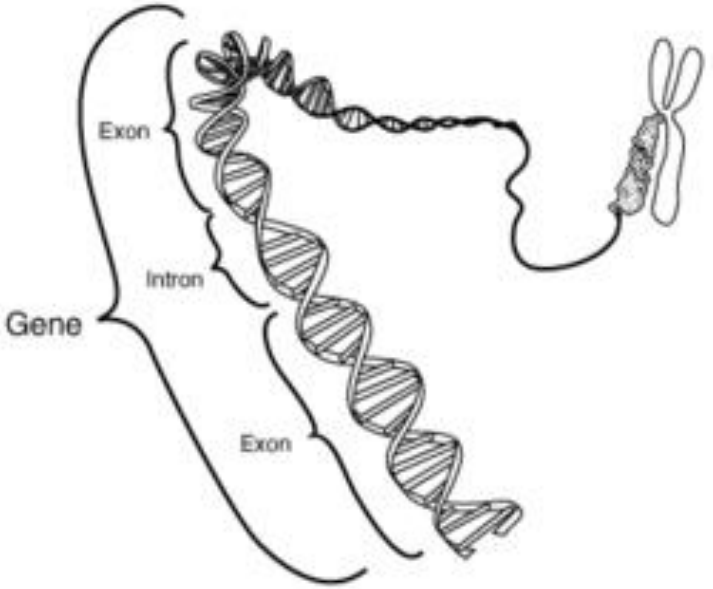
Contents

1. Basics of genetic variation
2. Technology to measure variation
3. Linking SNPs to traits
4. SNPs in genome browsers

1. Basics of genetic variation

Genetic variations

- In human beings, 99.9 percent of the bases are the same.
- Remaining 0.1 percent makes a person unique.
  - Different attributes / characteristics / traits
    - how a person looks
    - diseases he or she develops
- Most of those variations are in non-coding regions
  - This does not necessarily mean they have no effect!

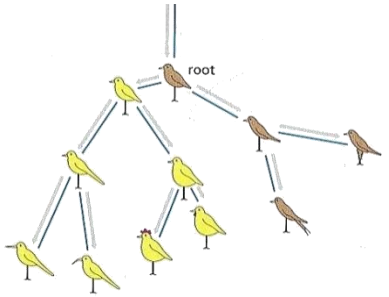


Genetic variations (II)

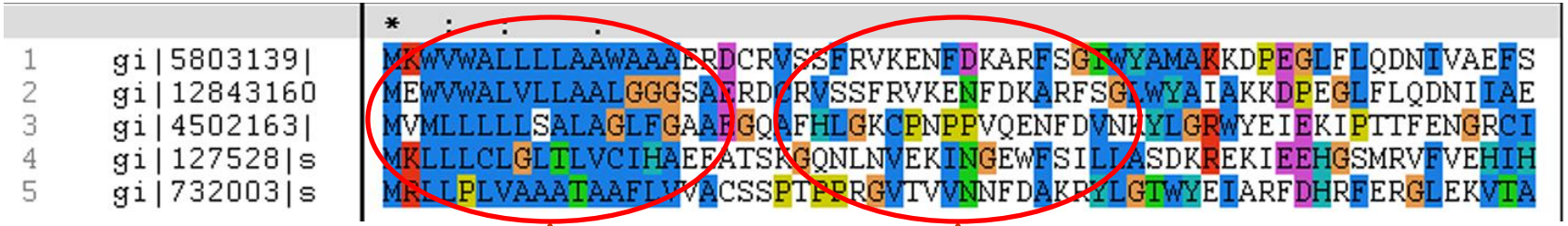
- Variations can be:
  - Harmless (change in phenotype)
  - Harmful (diabetes, cancer, heart disease, Huntington's disease, and hemophilia)
  - Latent (variations found in coding and regulatory regions, are not harmful on their own, and the change in each gene only becomes apparent under certain conditions e.g. susceptibility to lung cancer)
- A variation is called a mutation if a disadvantageous effect on disease is proven
  - This is far from easy

Genetic variations and evolution

- (Advantageous) variations drive evolution

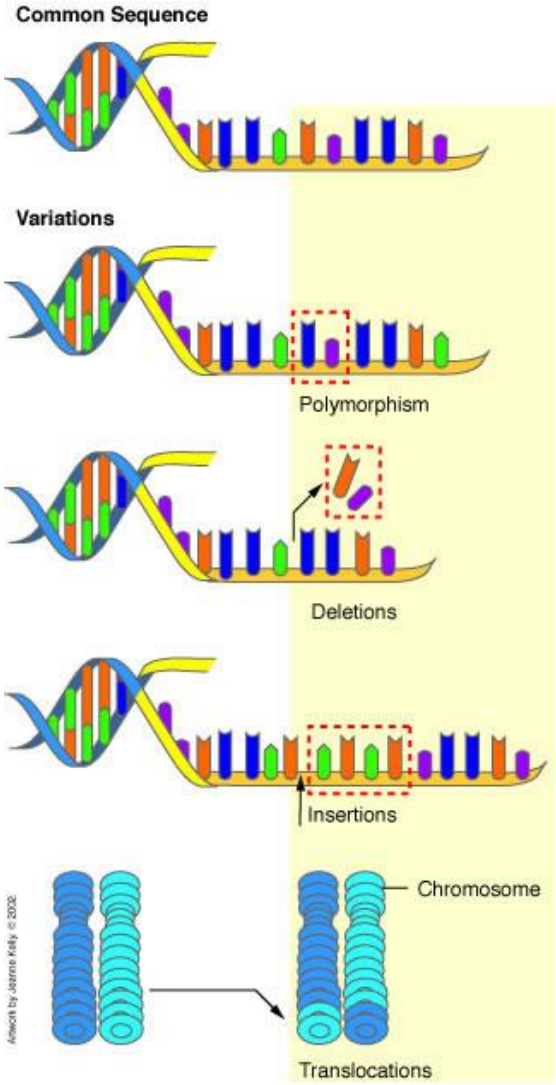


- The less variation in a region – or the more *conserved* the region is – the more important we can assume it to be
  - Higher evolutionary pressure
  - Functional domains of proteins, regulatory elements in promotors, …



- fairly conserved region
- poorly conserved region

Types of genetic variations



Other variations in genes

- Apart from variations in the sequence of the genes, other (inheritable) variations occur
  - These are called **epi**genetic variations

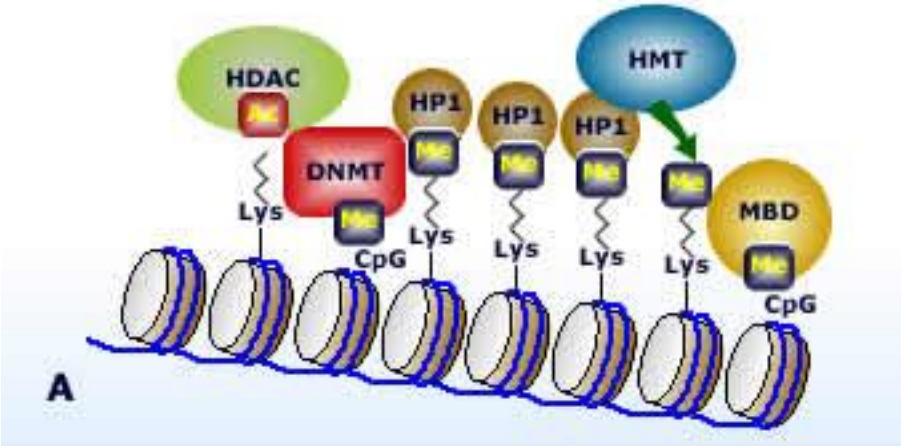


Image: http://www.avernes.fr

Single Nucleotide Polymorphisms (SNP)

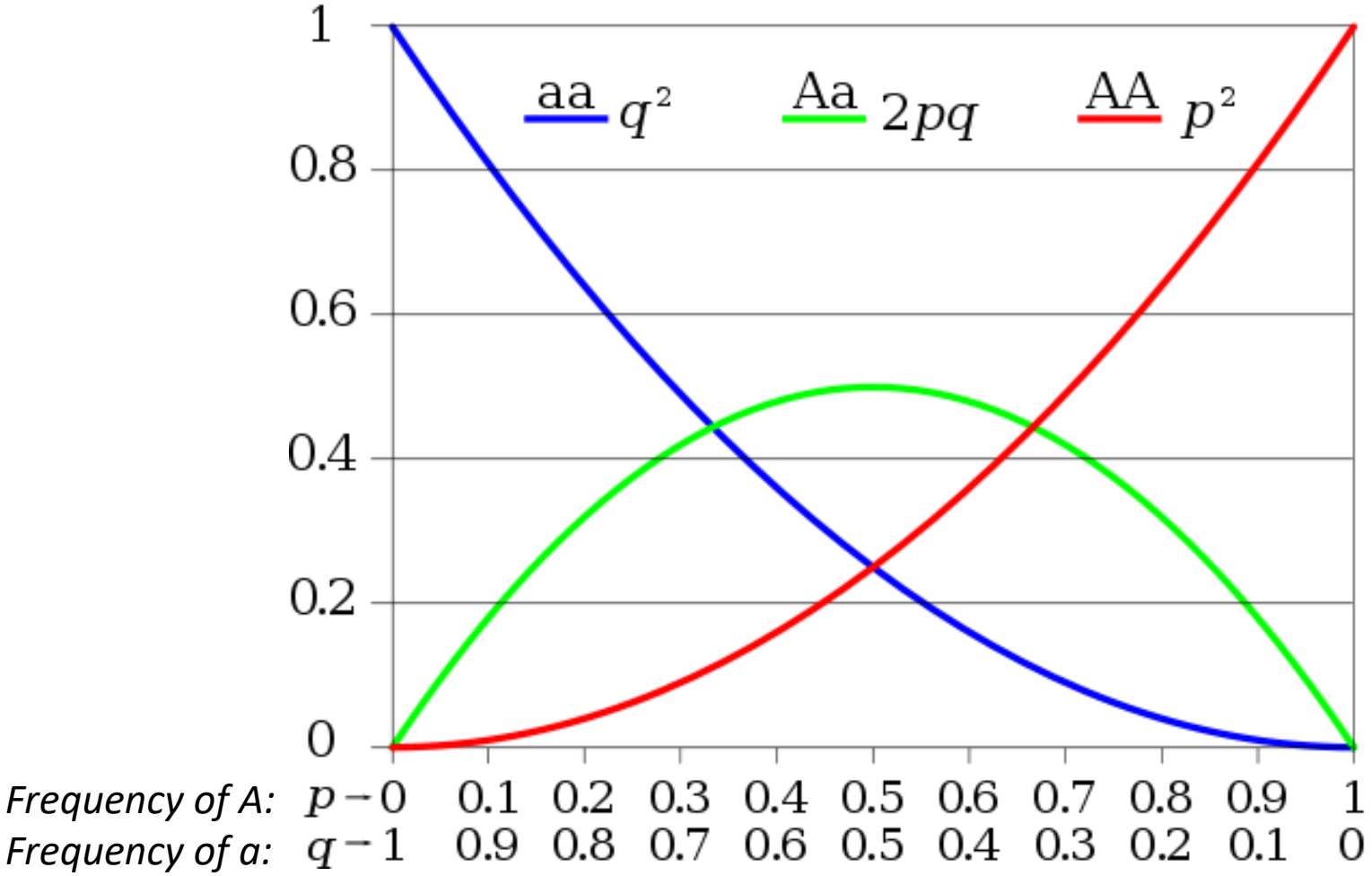
- A SNP (single nucleotide polymorphism) is defined as a single base change in a DNA sequence *that occurs in a significant proportion* (more than 1 percent) of a large population

- Currently, dbSNP at NCBI (build 132) has about 7 million human SNPs (4.5 million validated refSNPs)
  - The minimal frequency criterion is not used

To refresh memory

- The two copies of a gene we have (each of both chromosomes) are called **alleles**
- In case of a SNP the nucleotide at a position can be different between the alleles of an individual
- The combination of alleles one has, is called the **genotype**
- The related trait one has is called the **phenotype**
- **Allele frequency**: the frequency of occurence of a specific allele
- **Penetrance**: the number of people with a certain genotype that also develop the associated phenotype

Hardy-Weinberg principle

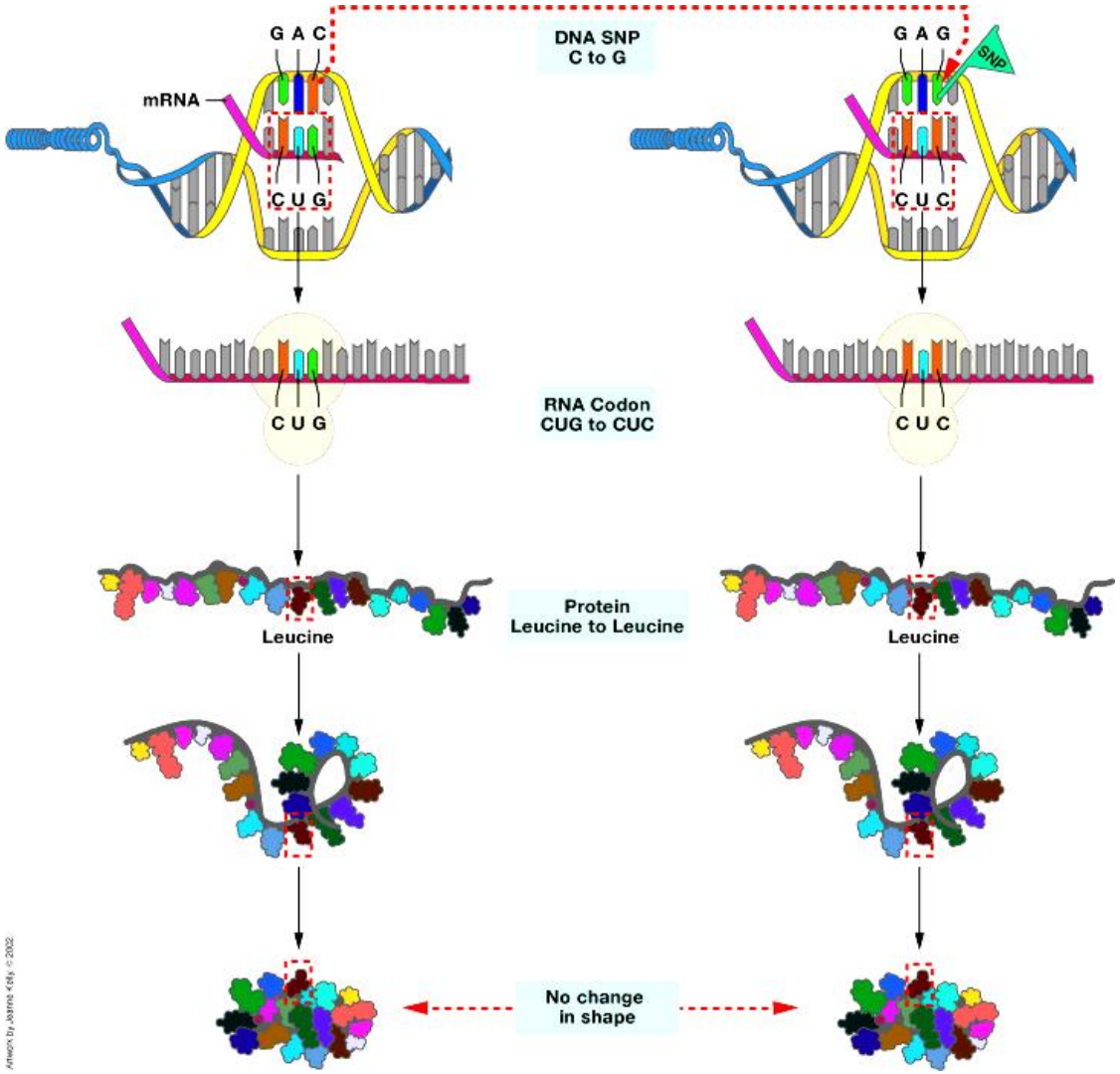


For SNPs we can check whether the observerd allele frequencies correspond to the expected frequencies in HW equilibrium; if not there may be evolutionary selection for certain genotypes

SNP facts

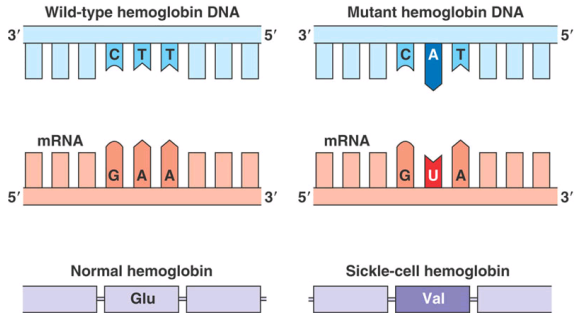
- SNPs are found in
  - coding and (mostly) non-coding regions.
- Occur with a very high frequency
  - about 1 in 1000 bases to 1 in 100 to 300 bases.
- The abundance of SNPs and the ease with which they can be measured make these genetic variations significant.
- SNPs close to a particular gene acts as a **genetic marker** for that gene.
- SNPs in coding regions alter the protein sequence made by that coding region:
  - **Synonymous** SNP: no protein sequence alteration
  - **Non-synonymous** SNP: protein sequence alteration -> aka as **missense** mutation.

SNPs may / may not alter protein structure



Functional effects of SNPs

- When amino acid (AA) changes, is the change relevant?
  - Type of AA
  - Site of the change
  - Functional domain
  - Conservation in other species
  - Truncating mutation



http://avonapbio.pbworks.com

- When is a variation a mutation?
  - In fact functional proof is needed (functional testing protein, enzymes, protein-protein binding, cellular localization, make KO cells, substitute defect)

Functional effects of SNPs

- Also non-coding SNPs may have an effect:
  - Effect on target sites of Transcription Factors (regulation of transcription)
  - Effect on target sites of miRNAs (regulation of transcript decay)
  - effect on splice donor or acceptor sites (regulation of – alternative – splicing)
  - Other…

Predicting functional effects of SNPs

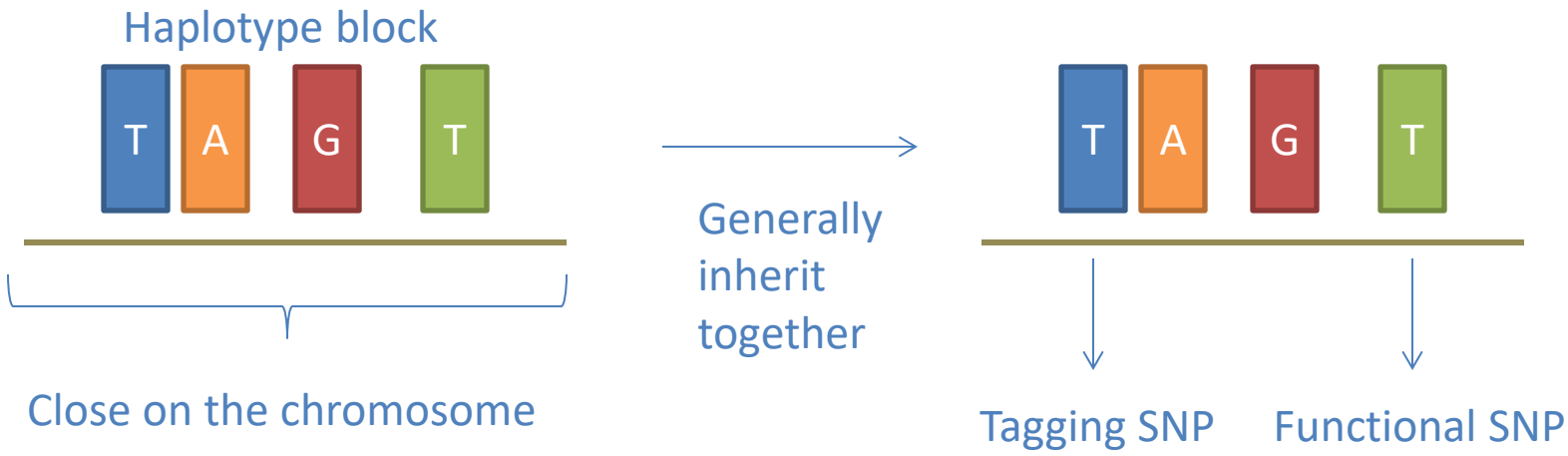
- **SIFT** is a tool that predicts whether an amino acid change affects protein function, based on the conservation of amino acid residues in closely related sequences

- **Polyphen** uses annotations (e.g. is the position in a known binding site), 3D-structural (if available) and sequence alignment information to predict the effect of an amino acid change

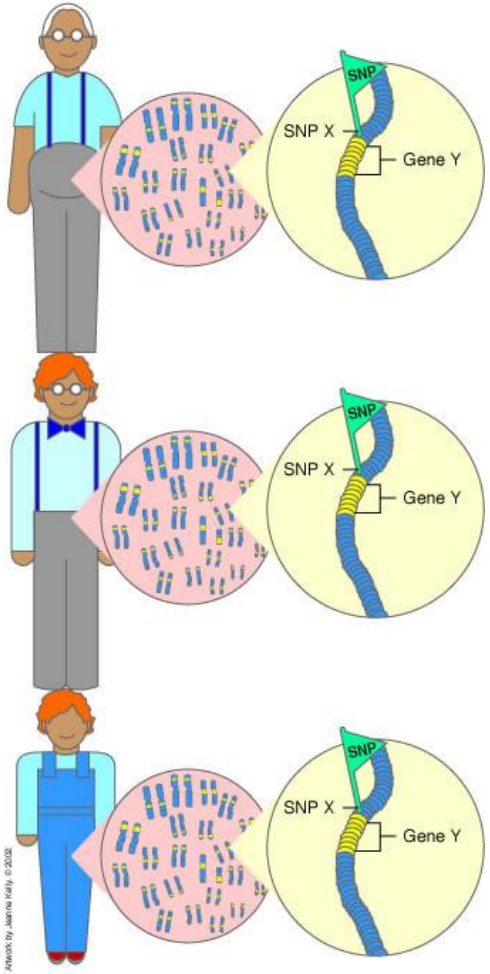
→ Both only work for non-synonymous coding SNPs

Haplotypes

- The combination of a number of SNPs one has is called a **haplotype**
- SNPs that are close on the genome form haplotype blocks, as they inherit as a group due to the mechanism of cross-over and recombination
- A SNP that is informative for a haplotype block is called a tagging SNP
- A tagging SNP may have no direct function, but still be predictive as it may correlate with functional SNPs



SNPs act as gene markers



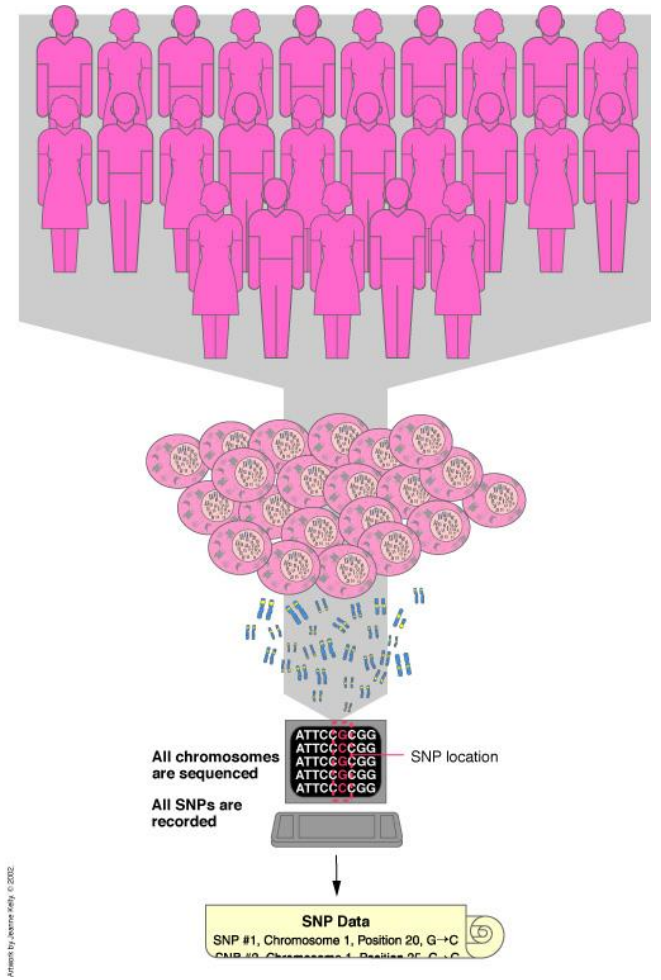
More on markers

- Several types of **genetic polymorphic markers** can be used to identify chromosomes, alleles, samples:
  - SNPs
    - SNPs with more than 2 alleles are of special interest as markers
  - Tandem repeats (microsatellites)
    - These can vary in number of repeats (repeat length)
  - Longer variabele sequences of DNA
    - Satellites

SNP Maps

- Sequence genomes of a large number of people
- Compare the base sequences to discover SNPs
- Generate a single map of the human genome containing all possible SNPs → SNP maps
- Examples:
  - Hapmap project
  - 1000 Genomes

SNP Maps (II)


All chromosomes are sequenced
All SNPs are recorded
SNP location
SNP Data
SNP #1, Chromosome 1, Position 20, G→C

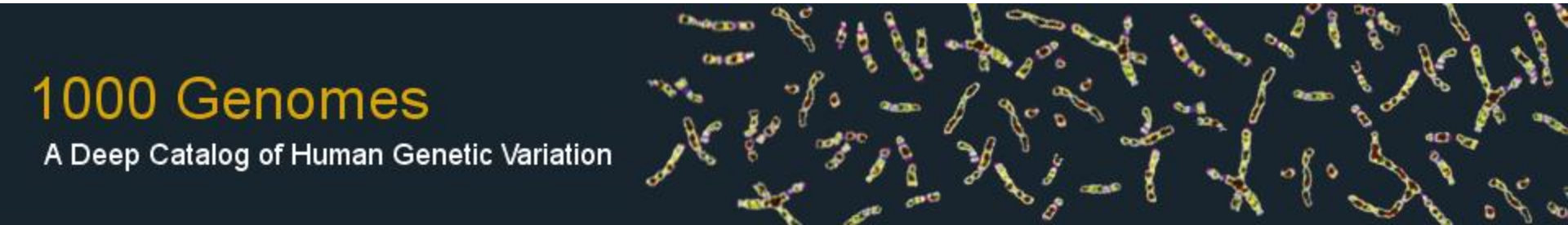
HAPMAP

- Millions of SNPs selected and measured in hundreds of people (minor allele frequency > 1%)

- From several populations all over the world
  - Nigeria (YRI)
  - Americans from European origin (CEU)
  - Tokyo residents (JPT)
  - Beijing residents (CHB)

1000 Genomes

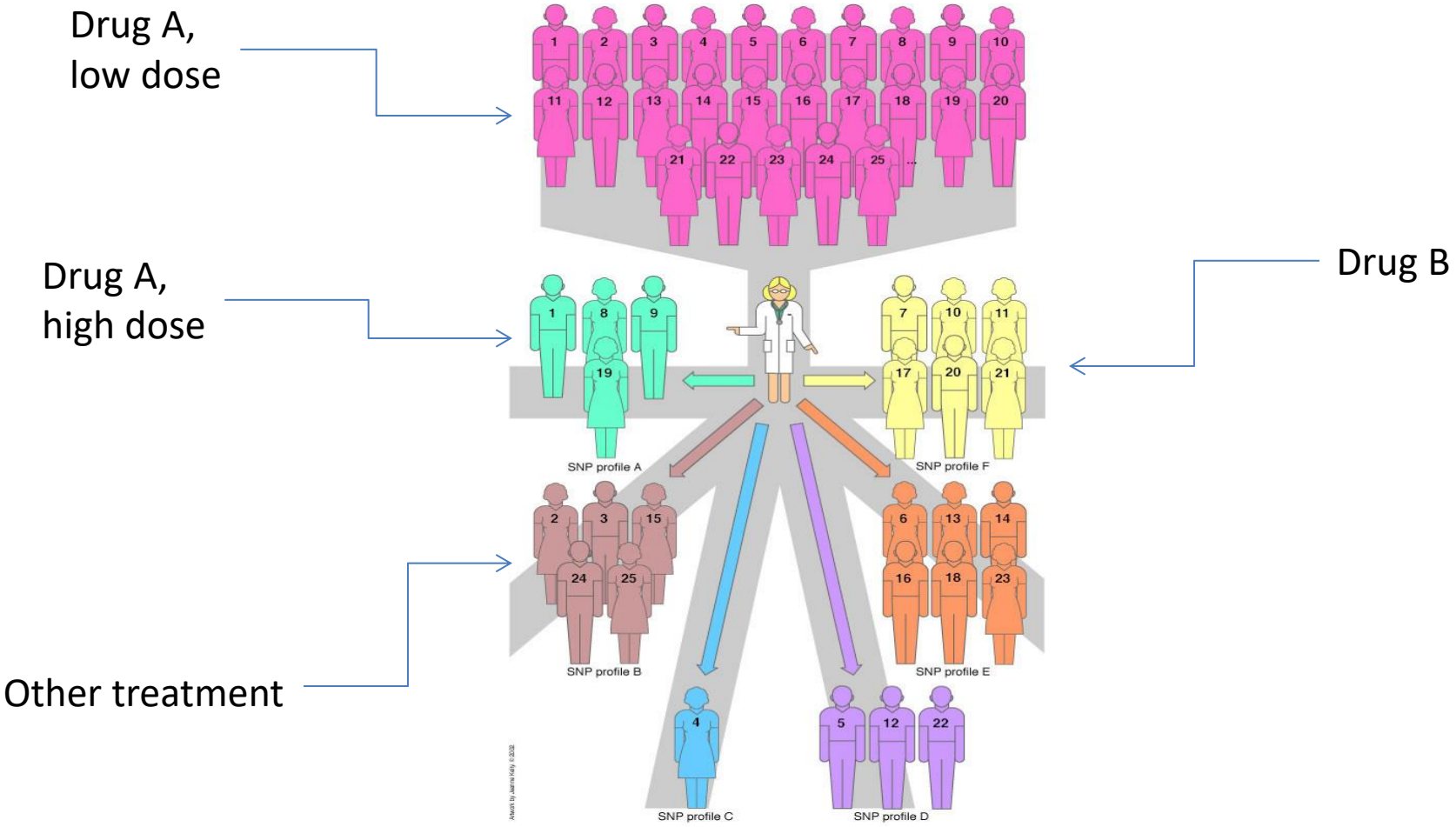
- Sequence the full DNA of many people
  - This also reveals all polymorphisms with frequency > 1%
- 2,500 people sequenced
- Haplotype block information used to complete predictions



SNP Profiles

- Genome of each individual contains distinct SNP pattern.
- People can be grouped based on the SNP profile.
- SNPs Profiles important for identifying response to Drug Therapy:
  - Correlations might emerge between certain SNP profiles and specific responses to treatment.
  - **Personalized medicine**

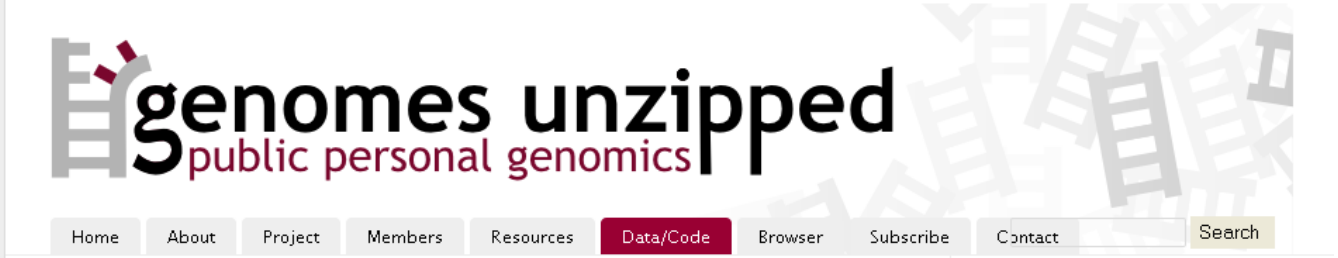
SNP Profiles (II)



SNP Profiles (II)

- You can already get your own SNP profile (for selected SNPs)
  - 23andme – deCODEme – Navigenics – …
    - Costs: few hundred Euros
    - Personalised health risk estimates
    - Ancestry
  - Knome even determines DNA sequence
    - Costs: few thousand Euros

Collection of 23andme data



Data/Code

Genomes Unzipped is a collaborative online project aiming to provide genetic testing customers with the knowledge and tools they need to make the most of their own genetic data. As part of the project our members are taking commercial genetic tests and making the raw data publicly available for others to download, analyse and reuse. There are currently three ways for users to access our genotype data.

1: Our Genome Browser

You can download our genotype data for particular parts of the genome, using the "Download Genotypes" link on the Genome Browser. Download genotype data for a particular SNP by clicking on it.

2: Download Complete Data

You can download all genotypes for particular individuals from our Amazon S3 genotype bucket, from the following locations:

23andMe v2 data:

- Daniel MacArthur (DGM001)
- Luke Jostins (LXJ001)
- Dan Vorhaus (DBV001)
- Caroline Wright (CFW001)
- Kate Morley (KIM001)
- Vincent Plagnol (VXP001)

About

Genomes Unzipped is a group blog providing expert, independent commentary on the personal genomics industry.

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£100M for whole patient genomes - revolutionising genetic diagnostics or squandering NHS cash?

Crowd-funding personalized bioscience

Response to "Exaggerations and errors in the promotion of genetic ancestry testing"

Exaggerations and errors in the promotion of genetic ancestry testing

Categories

Admin (7)

Resources for analyzing your own genome

1. SNPedia: a community-annotated wiki containing information on traits associated with single nucleotide polymorphisms.
2. Promethease: a freely downloadable tool for analysing 23andMe, deCODEme or Navigenics raw data with information from SNPedia.
3. Catalogue of genome-wide association studies: produced by the National Human Genome Research Institute.
4. DIYgenomics: A website providing apps and tools to compare predicted disease risk from different personal genomics companies.
5. Ensembl and UCSC: genome browsers containing information on genes, variants and associated phenotypes. Can be somewhat complex to use.
6. SNPtips: a FireFox extension that allows you to rapidly check your 23andMe genotype for any SNP you see mentioned on a web page.

Personalized medicine

- **Concept**: use information about a patient's genome, epigenome, proteome, etc. to adapt medical care:
  - select between different medications
  - optimize drug dosage
  - provide a specific therapy

- This **omics-centered approach** is not yet in widespread use clinically.

- **Important unanswered question**: does personalized medicine really offer any significant advantages over the traditional combination of clinical approaches (medical history, family history, data from imaging, laboratory, and other tests)

Personalized food

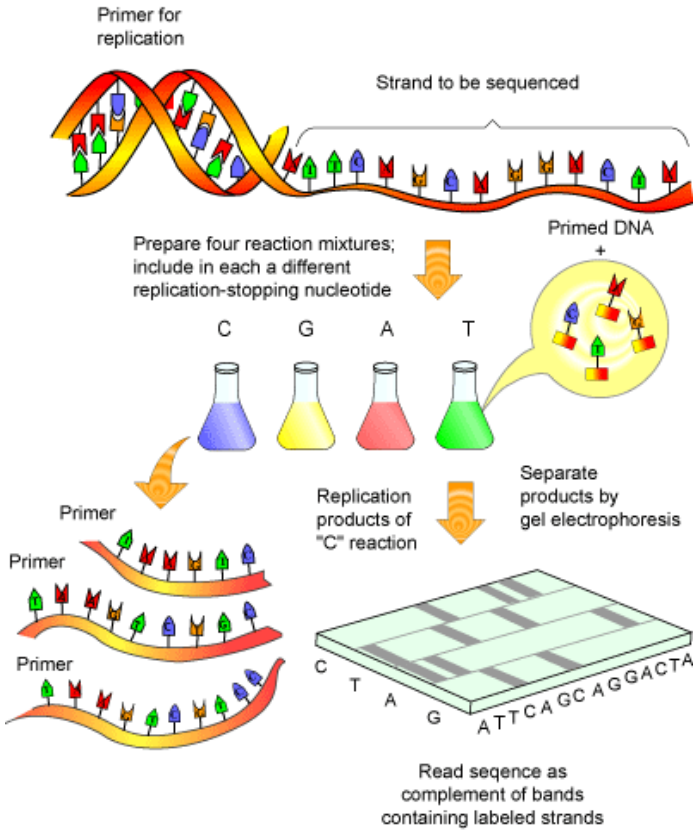
- Same concept as personalized medicine: use omics information of an individual or specific group of individuals to “optimize” nutrition
- Optimization on product level:
  - “You should eat more kiwi’s”
  - “You shouldn’t eat kiwi’s at all”

- Optimization on molecular level:
  - “You should take extra vitamin B12”
  - “Taking extra vitamin B12 is dangerous for you”
- Big business for food companies

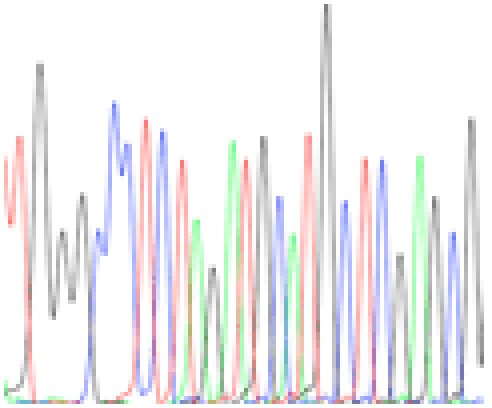
2. Technology to measure variation

Measuring SNPs

- Sanger sequencing
- terminates the chain with incorporation of a ddNT



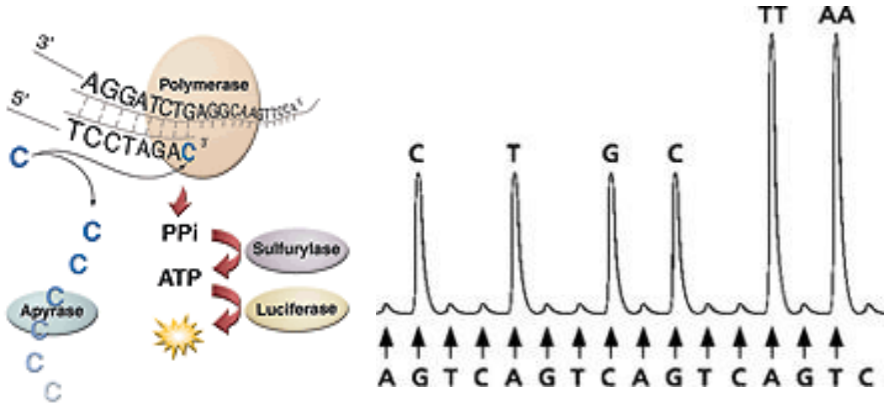
http://www.scq.ubc.ca



http://www.mrc-lmb.cam.ac.uk

Measuring SNPs

- SNPs can be measured using several technologies
  - Pyrosequencing
    - detects pyrophosphate (light)



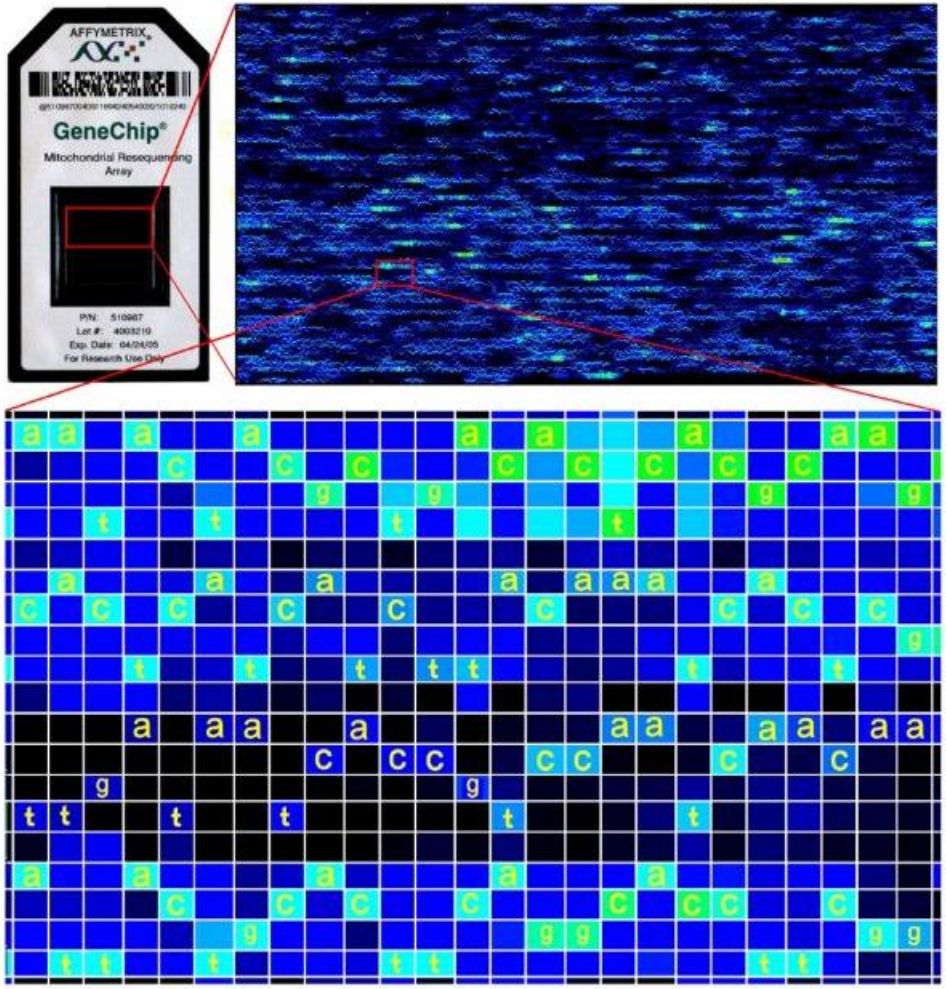
Images from: http://www.har.mrc.ac.uk (left) and http://www.ercim.eu (right)

Marker sets

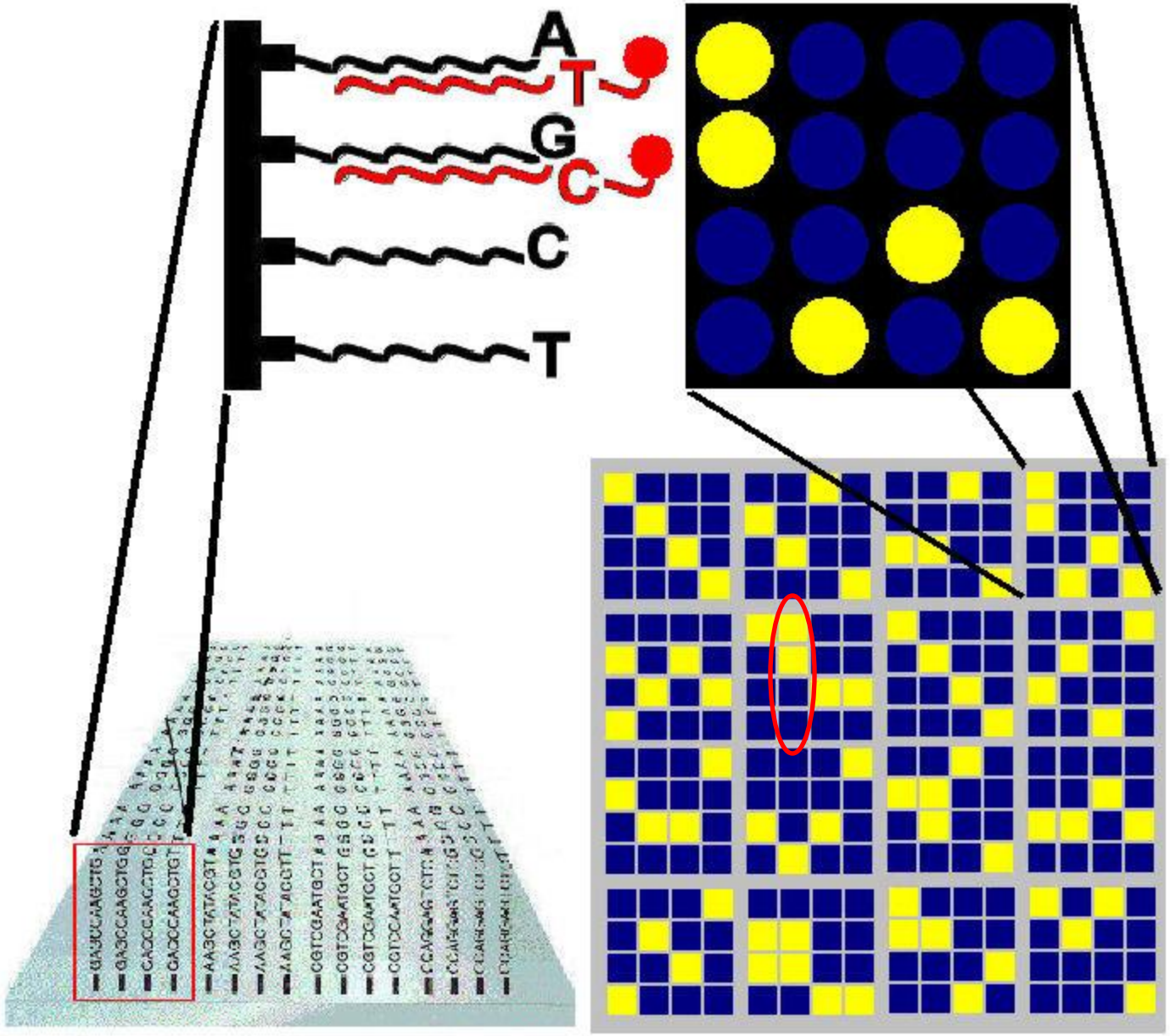
- A way to measure genetic variation on a more global scale is the construction of genetic markers sets
  - Covering the whole genome or part of it
  - Often, microsatellite markers are used for construction of these sets
  - Regions in which changes are observed can be explored further by sequencing or SNPs
- Marker sets are also used in forensics or in paternity determination
- Less used in other applications with the development of large scale technologies

Large scale measurement of SNPs

- Affymetrix SNP chip
- 500,000 or 1M SNPs
- Genome wide studies
- Data analysis?



(SM Carr *et al.* 2008. Comp. Biochem. Physiol. D, 3:11)



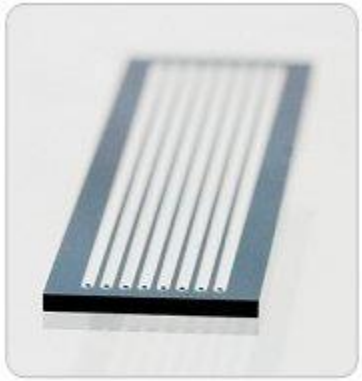
(after SM Carr *et al.* 2008. Comp. Biochem. Physiol. D, 3:11)
http://www.mun.ca/biology/scarr/DNA_Chips.html

Resequencing chips

- Another type of chip allows sequencing genes or genomic regions of interest
  - One can design the chips depending on the genes of interest
  - As such one can measure all known mutations related to a disease, also the yet unknown SNPs (in these genes)

Sequencing the whole genome

- Next Generation Sequecing (NGS) has made it possible to sequence the whole genome of an organism
  - In principle, all variations between individuals can be determined
  - In any case: massive amounts of data are generated (Gbs per sample)

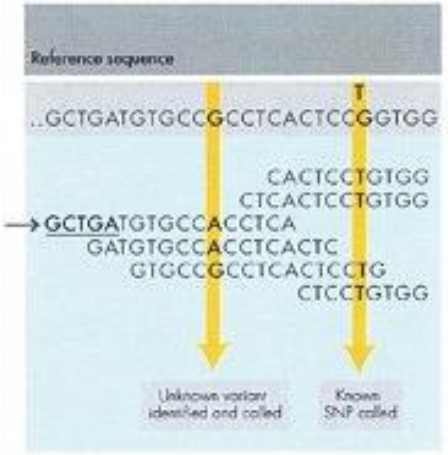


Up to eight samples can be loaded onto the flow cell for simultaneous analysis on the Illumina Genome Analyzer.

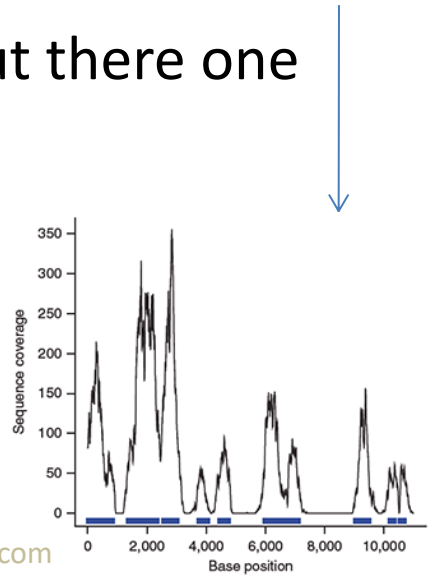
http://seqanswers.com

Sequencing the whole genome

- Data analysis is not that easy
  - Aligning
  - Calling (‘peak’ calling)
  - Real changes or sequencing errors
    - Error file
    - Same issue with ‘regular’ sequencing, but there one can evaluate by eyesight
  - How many fold coverage is needed?



http://seqanswers.com



http://www.genomics.agilent.com

3. Linking SNPs to traits

Traits

- A trait is just a characteristic
  - Length, weights, eye color, sex, …
- Traits can be discrete (sex, …) or continuous (weight, …)
- Discrete = ‘quantitative’
- Continuous = ‘qualitative’

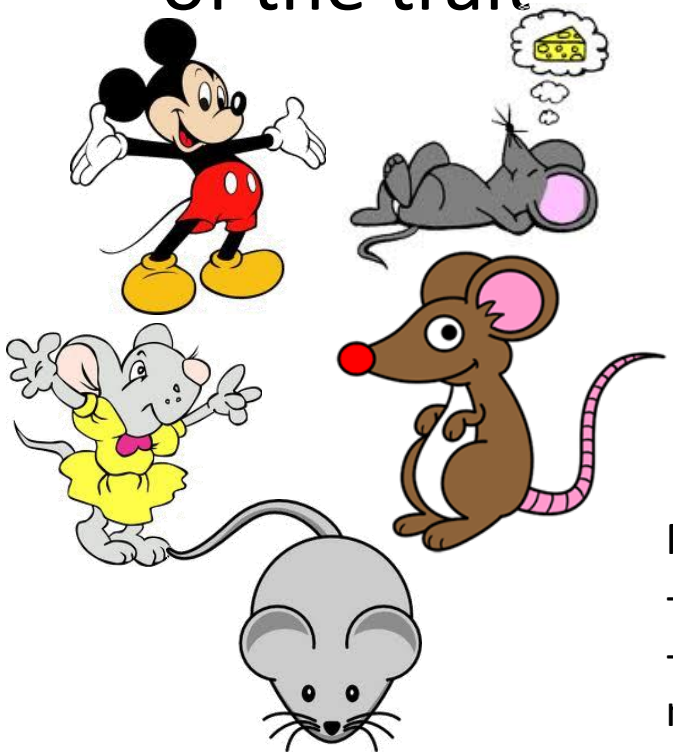
http://phe.rockefeller.edu

Heritability

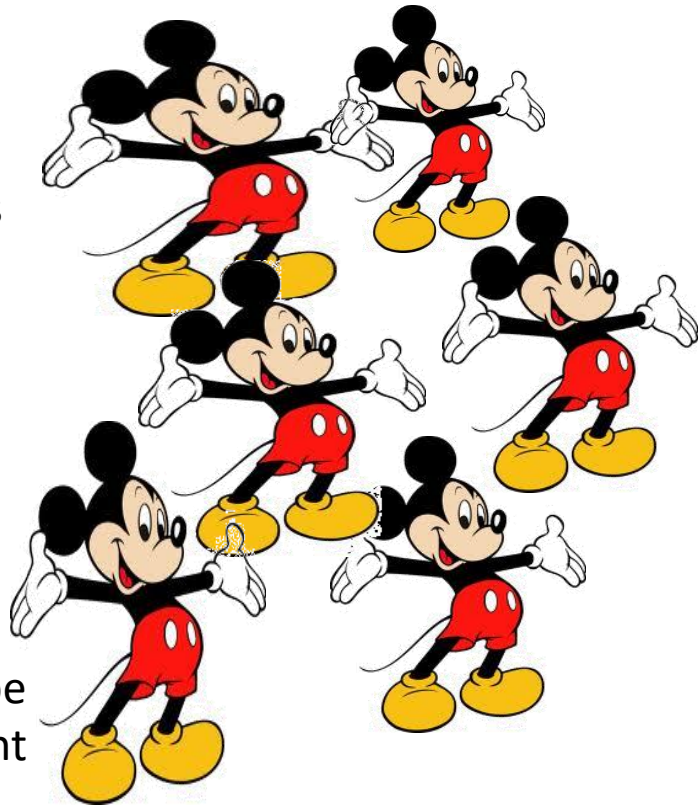
- This term is often MISinterpreted
- The heritability of a trait means how much of its variation can be explained by genetic variation
  - …in the population in which it is measured
  - Thus high heritability does not mean that the trait is genetically determined in general

Heritability

- The more genetically uniform the population (e.g. inbred strains) the [ ] the heritability of the trait



All kinds of mice
-genetic variation contributes to differences in phenotype
-environment does too



Mickey clones
-no genetic variation
-all differences in phenotype must be due to environment

Images: various sources

Heritability

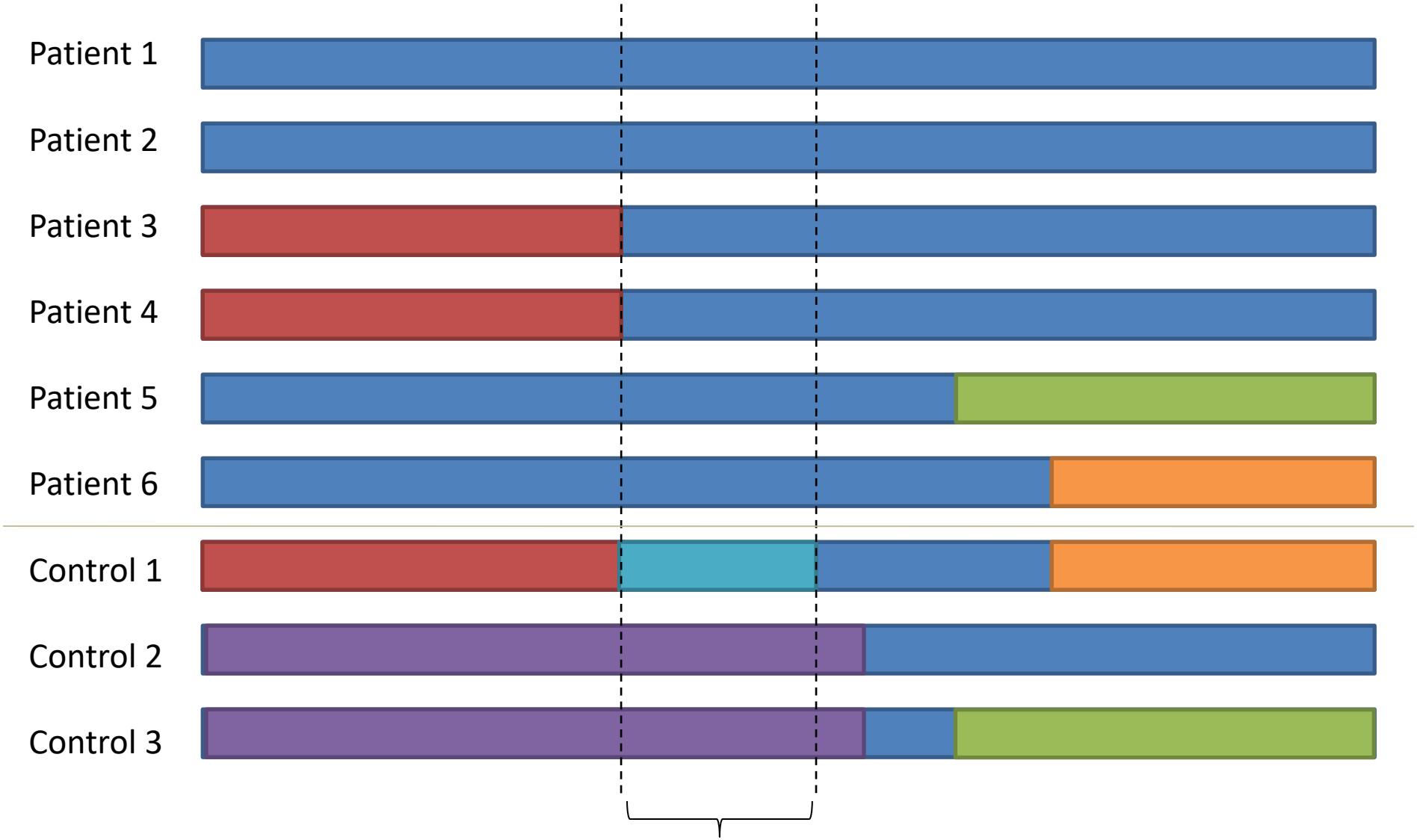
- Heritability estimates in your population can tell you whether you can find possible genetic factors contributing to a phenotype using the population of choice

Genome wide association studies

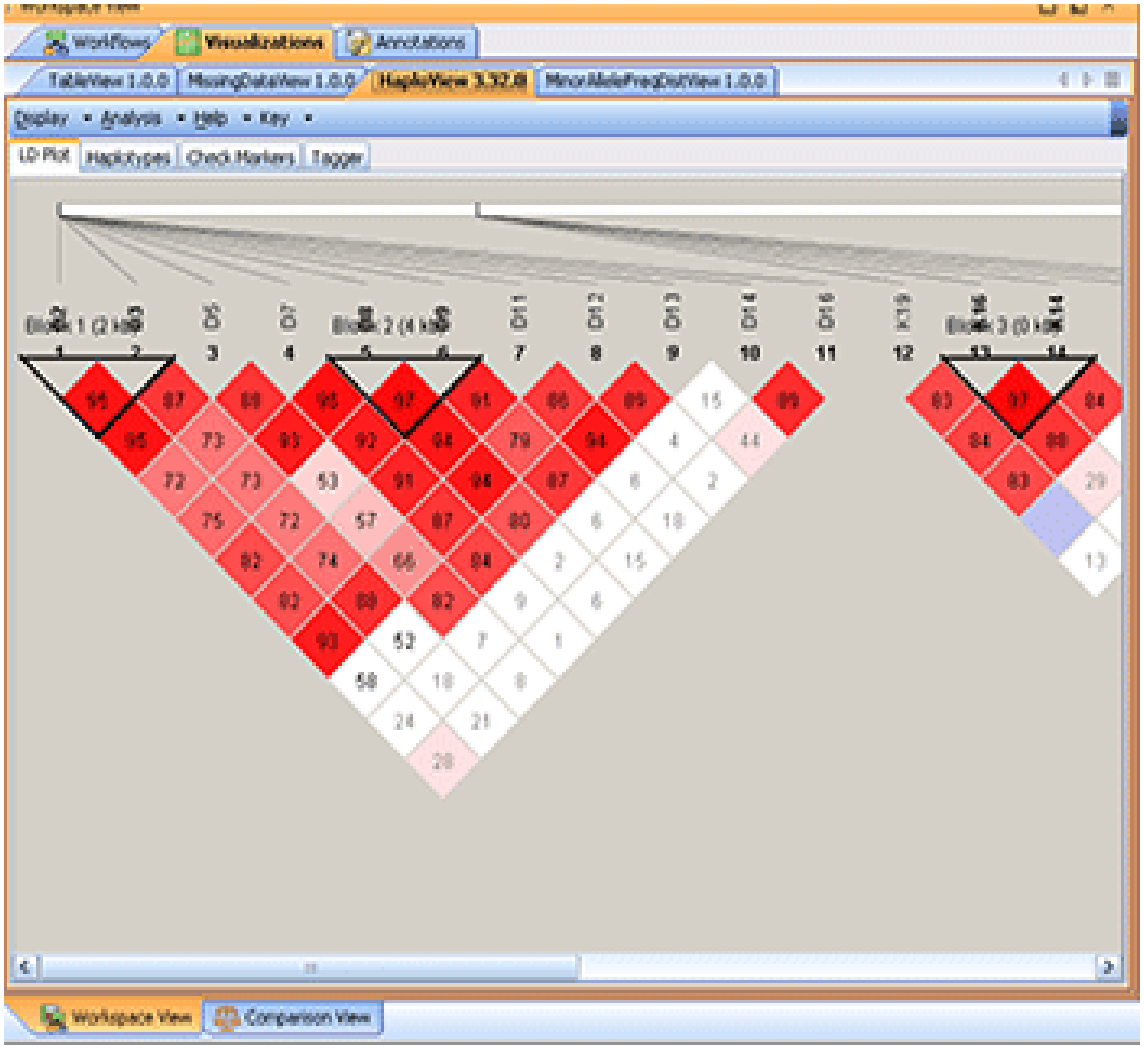
- **GWAS** (‘association’) tries to link SNPs to traits (diseases) in a genome wide way
- Makes use of *unrelated* individuals
  - So no family members
- Tries to find which allelic variants, correlate with the phenotype of interest
- If a complete haplotype goes together with the phenotype, this is considered association
  - Tools such as HaploView[1] can assist

[1] www.broad.mit.edu/mpg/**haploview**/

Each color indicates a different haplotype in the study population



Region of interest (determine in more detail, or check genes it contains)


WorkFlow
Visualizations
Annotations
TableView 1.0.0
MissingDataView 1.0.0
HapAssocView 3.32.0
MinorAlleleFreqDistView 1.0.0
Display
Analysis
Help
Key
LD Plot
Haplotypes
Check Markers
Tagger
Workspace View
Comparison View

Linkage studies

- Linkage makes use of related individuals
  - family members
- Adantage is higher power as compared to GWAS
- But one needs large enough families with enough (informative) ‘cross overs’ and preferably several generations
- Principle is the same as with GWAS, using markers or SNPs

When linkage, when association?

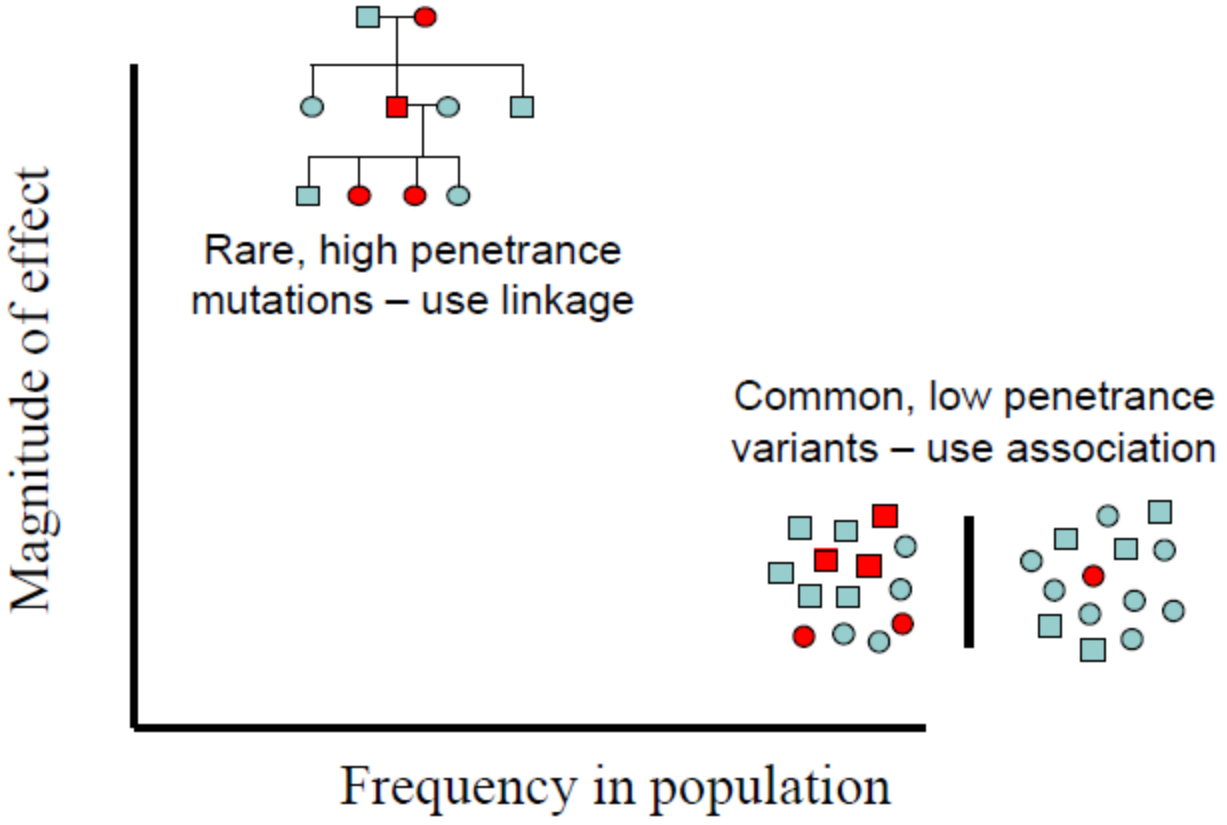
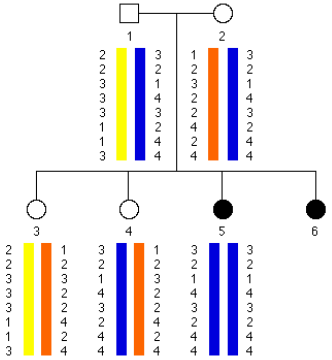
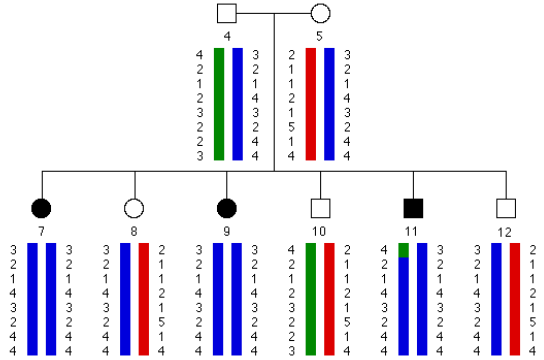


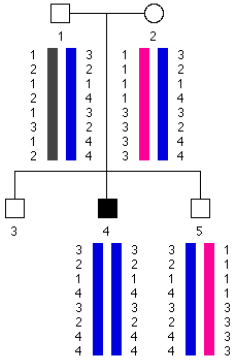
Image: University of Michigan School of Public Health



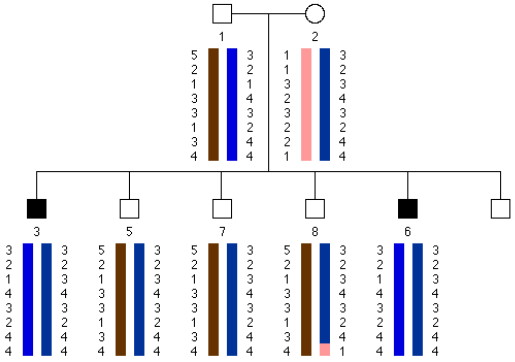
Pedigree 101



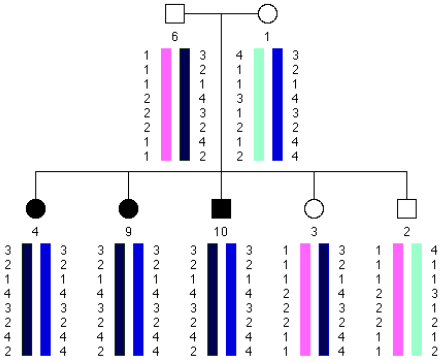
Pedigree 102



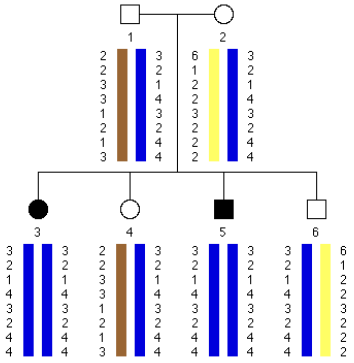
Pedigree 103



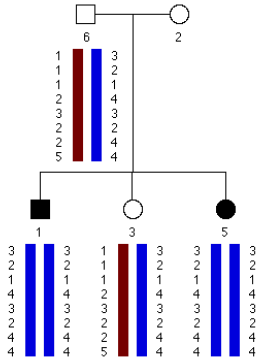
Pedigree 104



Pedigree 105



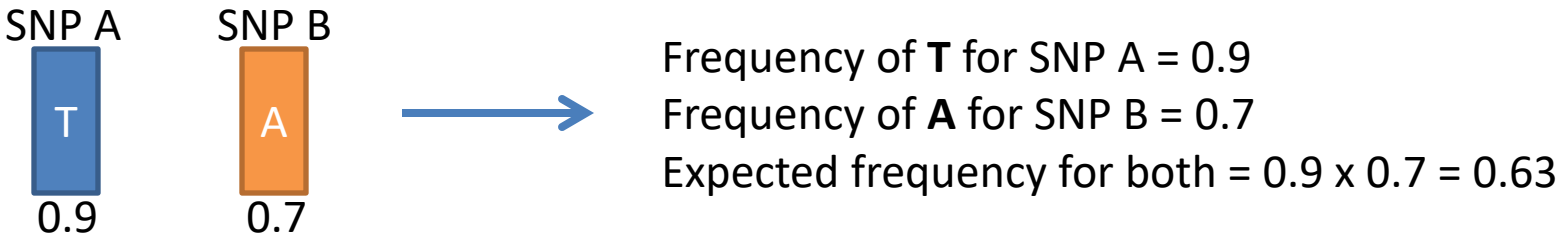
Pedigree 106



Pedigree 107

Computations

- The **linkage disequilibrium** (**D** or **LD**) indicates the deviation of a haplotype's frequency from its expected frequency
  - The expected frequency for each combination of SNP A and SNP B is the multiplication of the separate frequencies



  - When this does **not** hold the SNPs are in a haplotype block, also called 'in linkage disequilibrium'

Computations (II)

- Disadvantage of the LD: it has a range that depends on the allele frequencies
  → difficult to compare between haplotype blocks

- **D'** is an adapted scaled score, to correct for the influence of allele frequences
  - It ranges between -1 and +1
  - Disadvantages: small sample size or low allele frequences tend to overestimate the score

Computations (III)

- An alternative way of correcting is computing the correlation coefficient (**r**) between loci, also sometimes given as its square (**r^2**)
  - more stringent than D'
  - r^2 ranges between 0 and 1

Computations (IV)

- The LOD score (^{10}log of the odds) indicates the likelihood of obtaining the data given that the loci are indeed linked, versus obtaining the data by chance
  - A score higher than 3 (which means a 1000:1 odds) is considered evidence of linkage

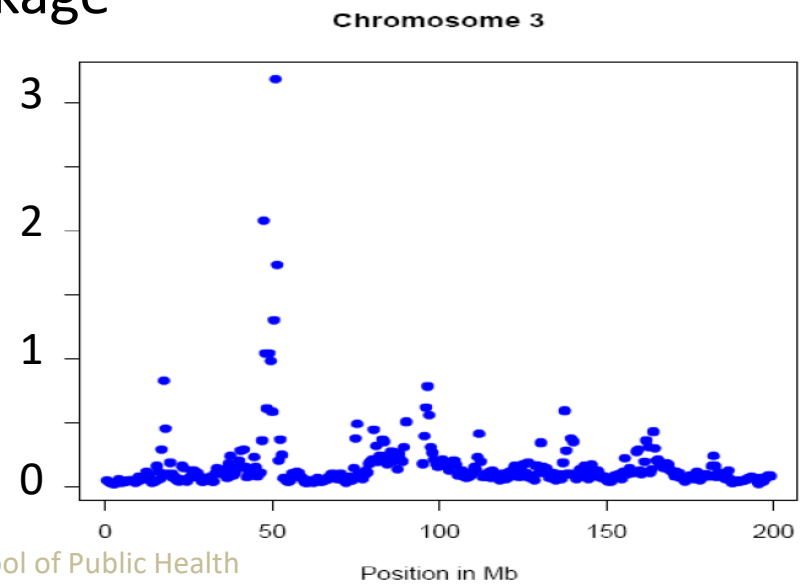


Image: adapted from University of Michigan School of Public Health

Limitations

- A very large sample size is needed but also population uniformity
  - Trade-off
- To most common diseases, many SNPs/genes contribute for a few percent each
  - Difficult to detect
- Often many genes in haplotype blocks, which one is linked to the disease (contains the relevant SNP(s))
- Rare alleles make sampling even more difficult

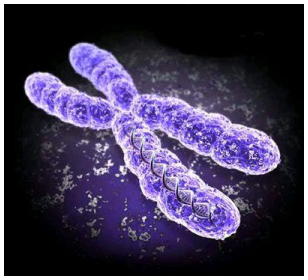
  → often discrepancy even between large studies

Strategies

- Populations to use to increase power:
  - Families (linkage more power)
- Simplify by candidate gene approach
  - Rank the genes in a linked genomic region by their likelihood of involvement in the disease, based on knowledge on their biological functions and/or literature
- Scan globally, and scan regions of interest in more detail

What's more?

- Always realise that the SNPs linked to the phenotype are not (neccesarily) the functional or causing SNPs, they are just close enough to be markers
- Now we only discussed genetic contributions to a phenotype
- Other aspects to study:
  - Genes modifying the effects of other genes (epistatis)
  - Gene-environment interactions



- ADE models can help in estimating genetic and environmental contributions (here twins are of interest)
- Specific study of interactions is very difficult
  - Even more possibilities
  - Even smaller effects

Images: http://theosophical.wordpress.com (left) and http://www.foodfacts.info (right)

4. SNPs in genome browsers

SNP IDs

- A standard ID for SNPs is the dbSNP ID
  - also called “rs number”
  - example: rs4986852
  - Standardised, unique, stable

- An alternative for disease related SNPs is the OMIM variation ID
  - example: 113705.0011
  - Standardised, unique, stable

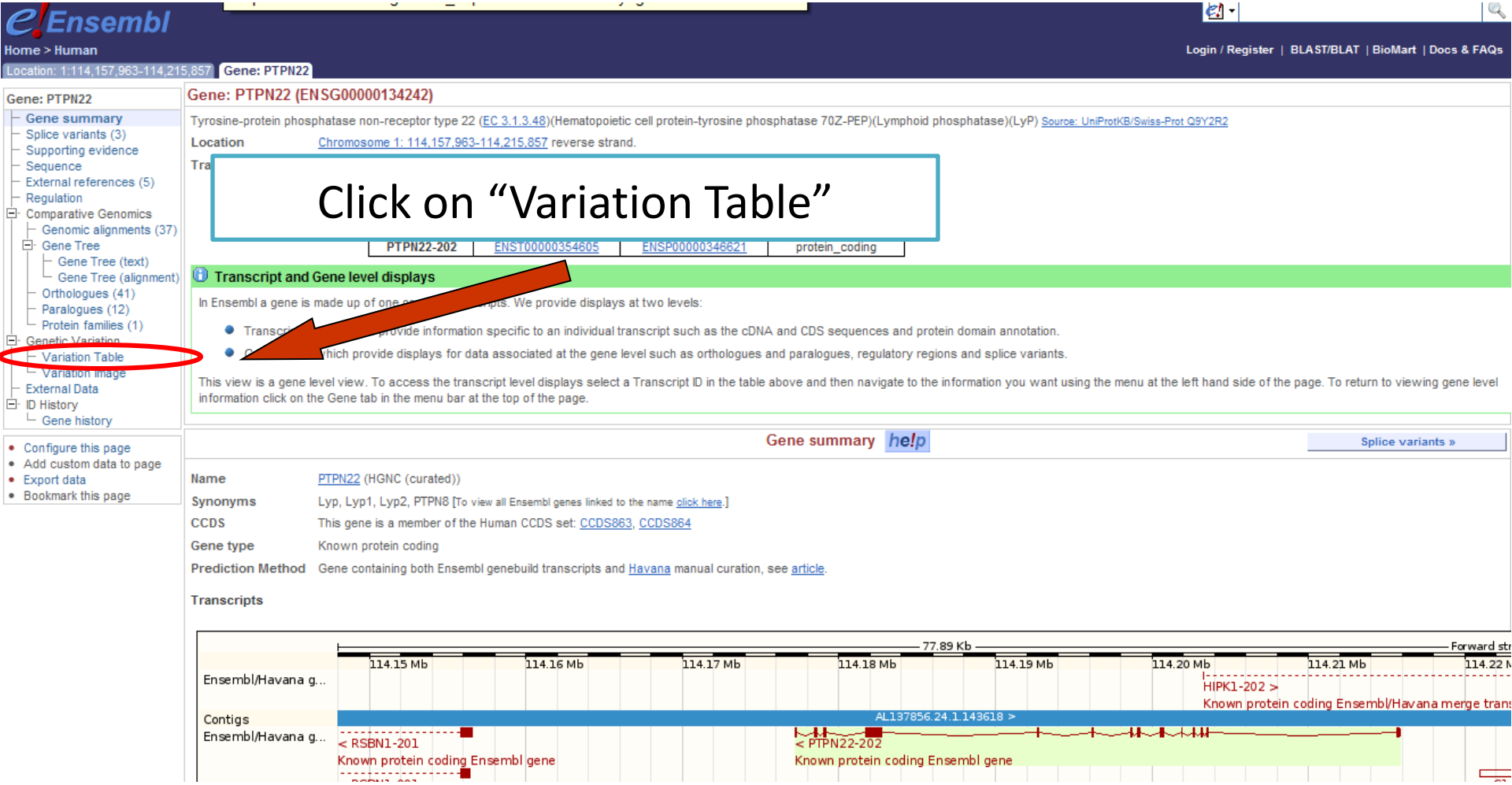
- A final possibility is the
  - For non-coding or coding SNPs: variation
    - Example: BRCA1, 2978G>A
  - For coding SNPs (also): mutation
    - Example: BRCA1, SER1040ASN
  - Easier to interpret, but not stable

Investigating SNPs in NCBI (Entrez SNP)

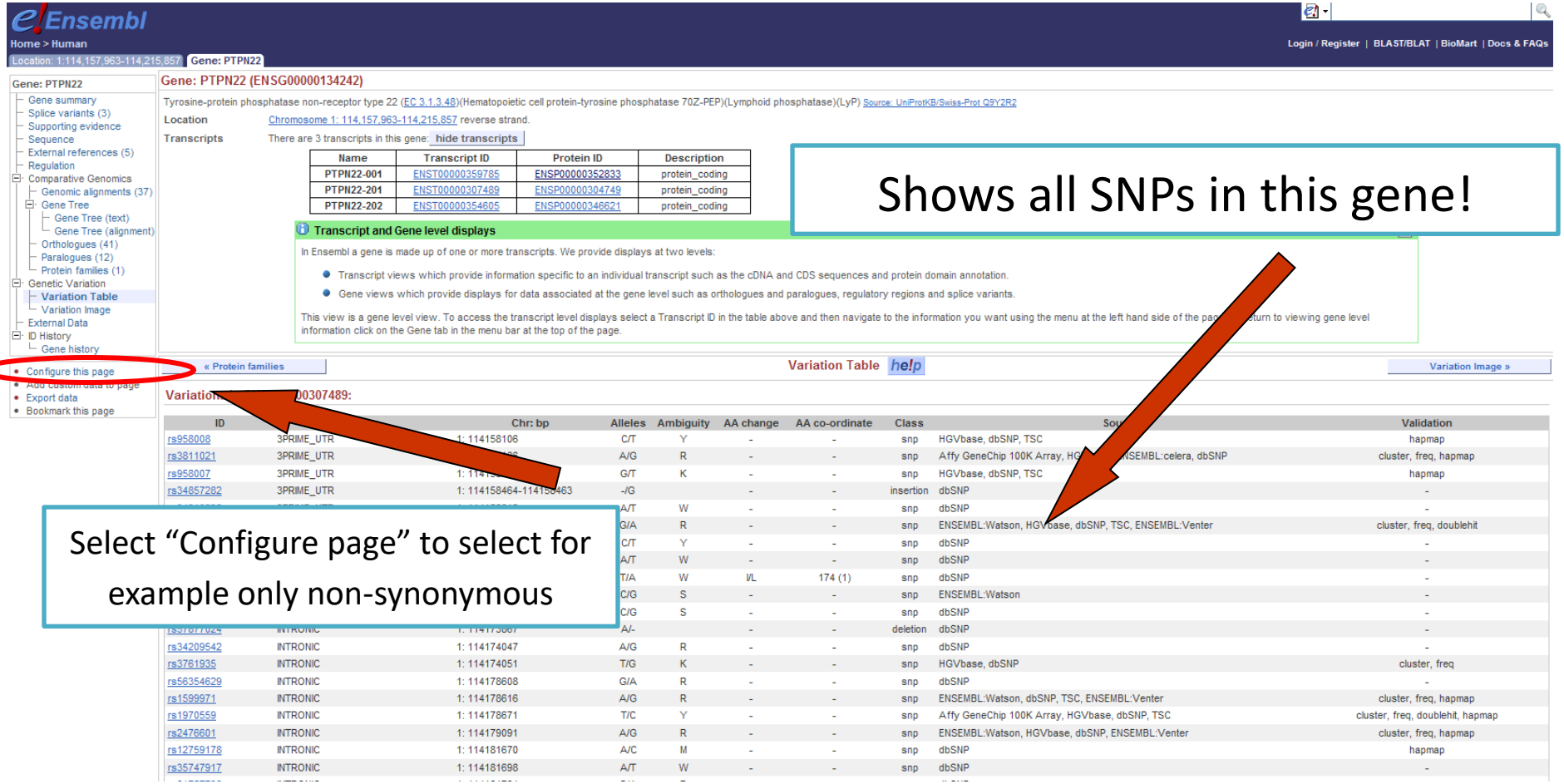
gene model (contig mRNA transcript):	Contig Label	Contig	mrna	protein	mrna orientation	transcript	snp count
	GRCh37	NT_010783.15	NM_007294.3	NP_009225.1	forward	plus strand	53, coding

Region	Chr. position	mRNA pos	dbSNP rs# cluster id	Hetero-zygosity	Validation	MAF	Allele origin	3D	Linkout	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos	PubMed
	41197708	5811	rs116185952	0.222						missense	C	Pro [P]	2	1860	
										contig reference	A	His [H]	2	1860	
	41197716	5803	rs28897699	0.013				Yes		missense	T	His [H]	3	1857	
										contig reference	G	Gln [Q]	3	1857	
	41197816	5703	rs70953662	N.D.				Yes		missense	A	Asn [N]	2	1824	
										contig reference	T	Ile [I]	2	1824	
	41199693	5666	rs1800751	N.D.				Yes		missense	G	Ala [A]	1	1812	
										contig reference	C	Pro [P]	1	1812	
	41199694	5665	rs4438367	N.D.				Yes		synonymous	A	Gln [Q]	3	1811	
										contig reference	G	Gln [Q]	3	1811	
	41203086	5558	rs1800757	N.D.				Yes		missense	T	Ser [S]	1	1776	
										contig reference	C	Pro [P]	1	1776	
	41209082	5496	rs76171189	N.D.						frame shift			2	1755	
										contig reference	C	Ser [S]	2	1755	
	41215360	5414	rs34570933	N.D.				Yes		frame shift		Met [M]	1	1728	
										frame shift	A	Asn [N]	1	1728	

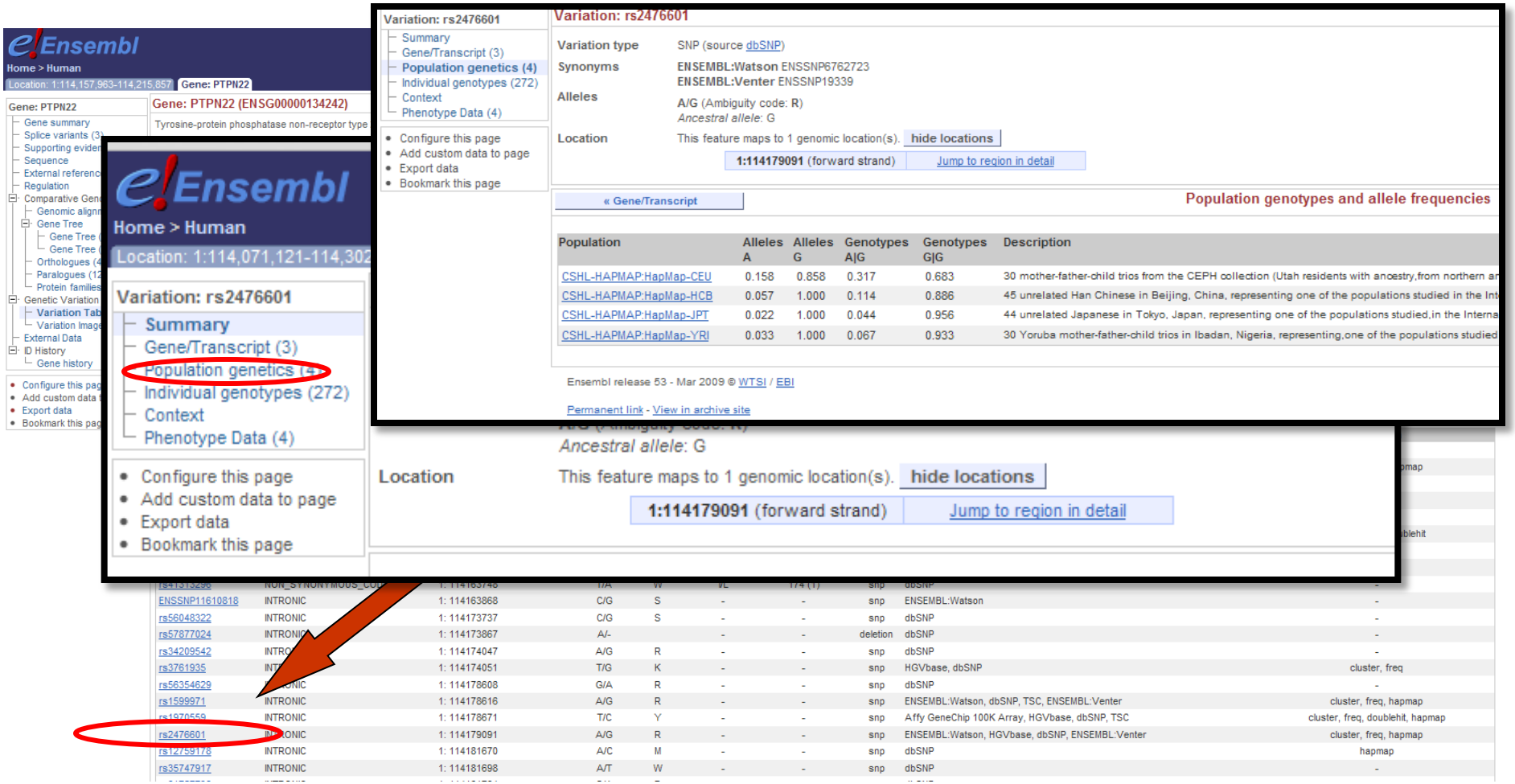
Investigating SNPs in Ensembl



Investigating SNPs in Ensembl



Investigating SNPs in Ensembl



Afternoon session

- You will look into SNPs related to athletic performance
  - Look up information in NCBI (dbSNP) and Ensembl
  - Look into personal SNPs profiles, what does the data look like, how can we predict risks using the data
  - Look up information about SNPs and their characteristic in populations in the HapMap genome browser