Medical Genetics

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Why medical genetics

- **Genome role in diagnostics, therapy and prevention**

  = application in medical practice

  It is possible to implement into practice only what I know and what I have in mind

What you should already know

- **What is a gene**

  genes: structural
  for functional RNAs

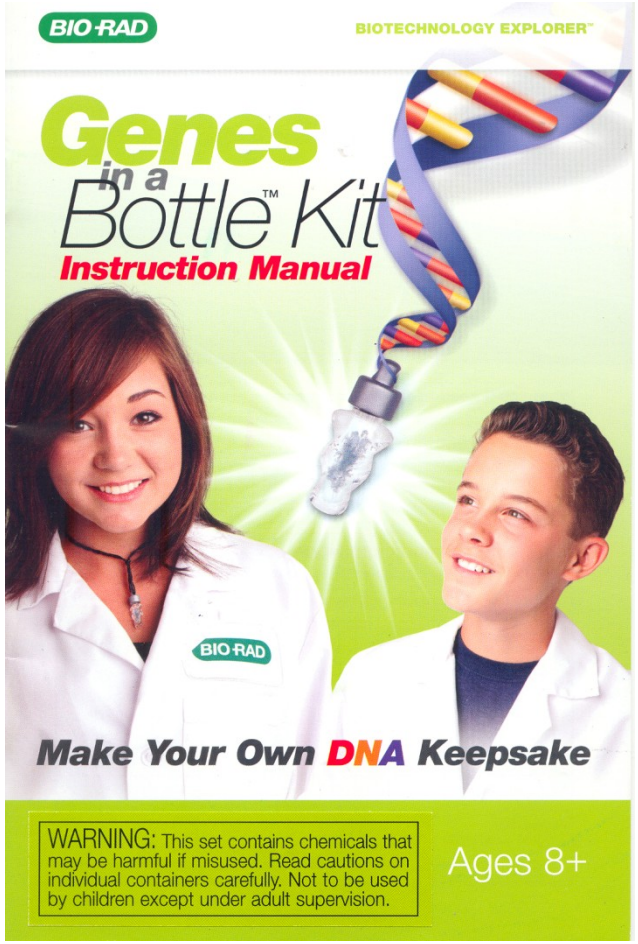
- housekeeping genes
- gene expression
- exones, introns, non-transcribed regions, promotors

- **Informational macromolecules**

- **Transcription, alternative splicing, translation**

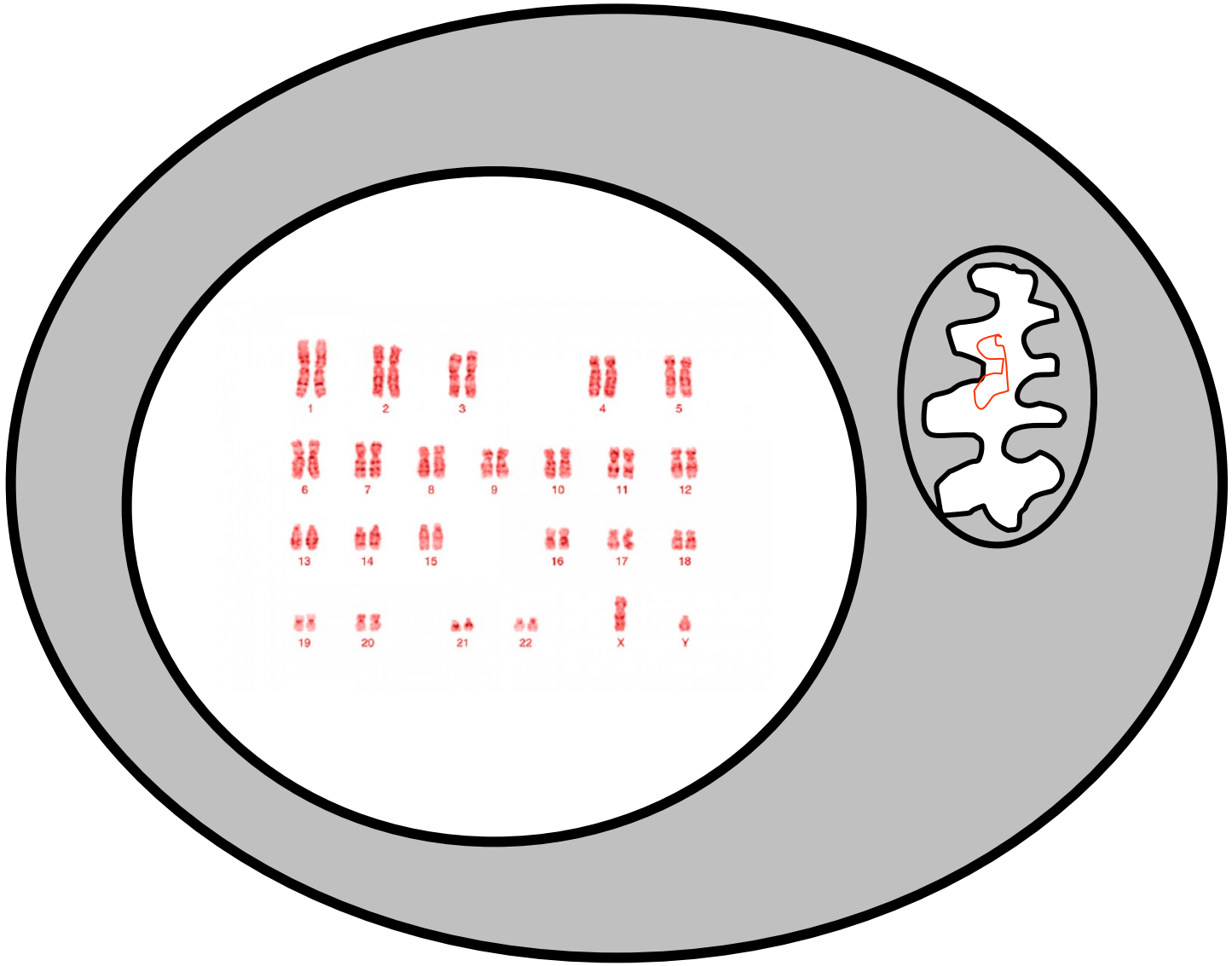
- **Chromosomes**

What is a DNA?



cold ethanol + salt + detergent
> 1 m DNA

Věda je zábava, Svojtka & Co, 2015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Kočárek, 2013

Cavendish Laboratory and The Eagle Pub

Watson + Crick + Wilkins + Franklin

?
Using results without permit
Eugnenics
Black men's IQ

Cavendish Laboratory and The Eagle Pub

Watson + Crick + Wilkins + Franklin

Terminology

- **Genetics:** study of genes, genetic variation, and heredity in living organism
- **Genome:** complete set of DNA within a single cell of an organism
- **Genomics:** focuses on the structure, function, evolution, and mapping of genomes

Terminology

Genome is more than just a sum of genes

- **Genetics**
- **Genome**
- **Genomics**

Terminology

- **Genetics**
- **Genome**
- **Genomics**

- Structural
(DNA, chromosomes)
- Functional
(RNA, gene expression)
- Comparative

Terminology

- Genetics
- Genome
- Genomics
- Microbiome
- Transcriptome
- Epigenetics

Terminology

- **Genetics**
- **Genome**
- **Genomics**
- **Microbiome**
- **Transcriptome**
- **Epigenetics**

Community of microorganisms inhabiting a particular environment

Terminology

- **Genetics**
- **Genome**
- **Genomics**
- **Microbiome**
- **Transcriptome**
- **Epigenetics**

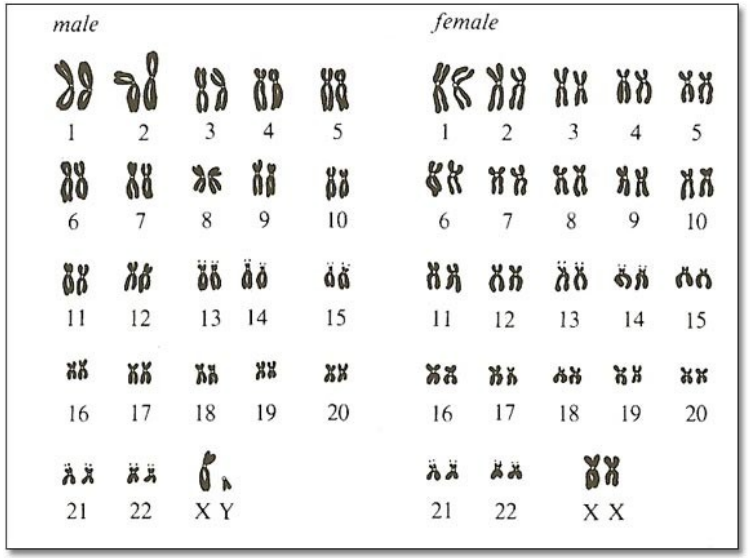
Set of all RNA molecules in one cell or a population of cells in certain time

Terminology

- **Genetics**
- **Genome**
- **Genomics**
- **Microbiome**
- **Transcriptome**
- **Epigenetics**

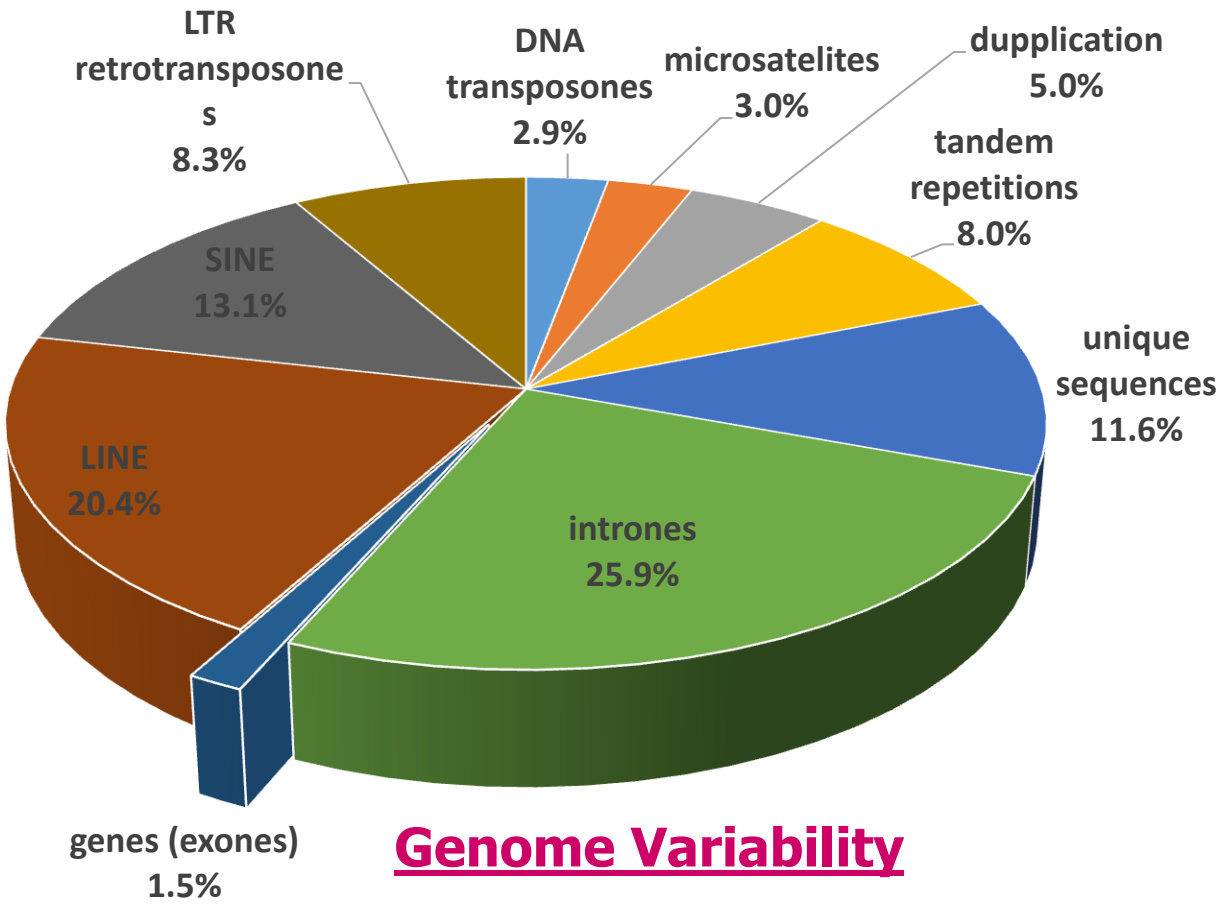
Study of heritable changes in gene function that do not involve changes in the DNA sequence

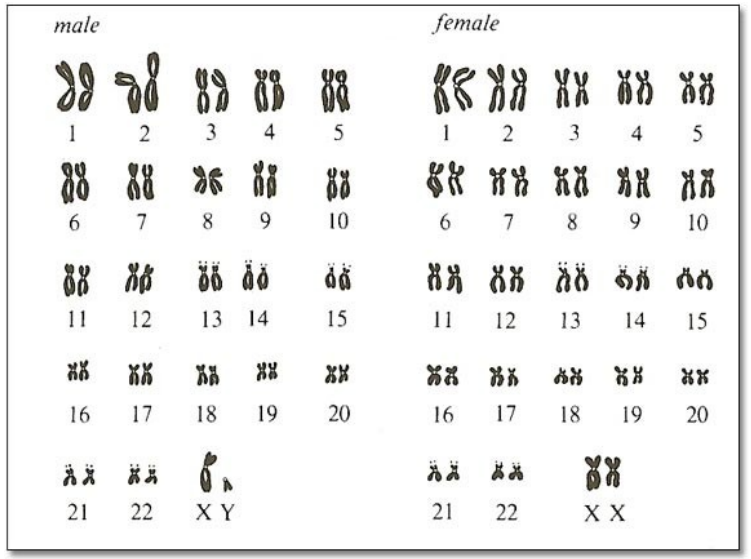
What is a genome?



Human genome:
3.2×10^9 bp,
~ 20,000 genes

What is a genome?





Genome Variability

- **Nucleotide polymorphism**
  - **Single Nucleotide Polymorphisms - SNP**
- **Structural variations**
  - **Copy Number Variations – CNV**
  - **Short Tandem Repeats – STR (2-5)**

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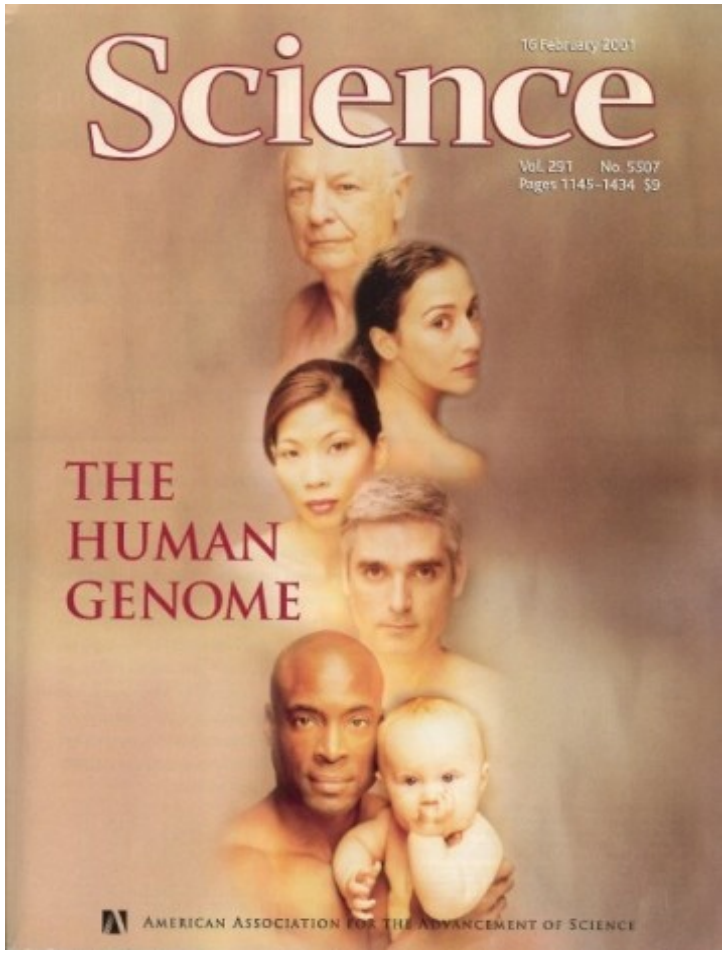
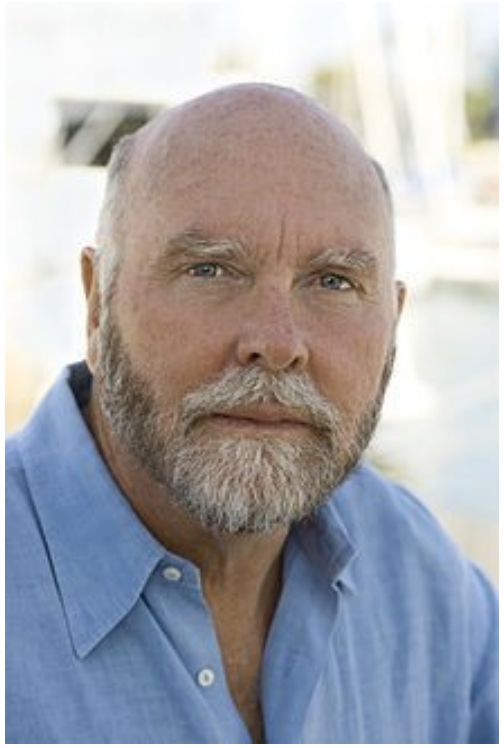
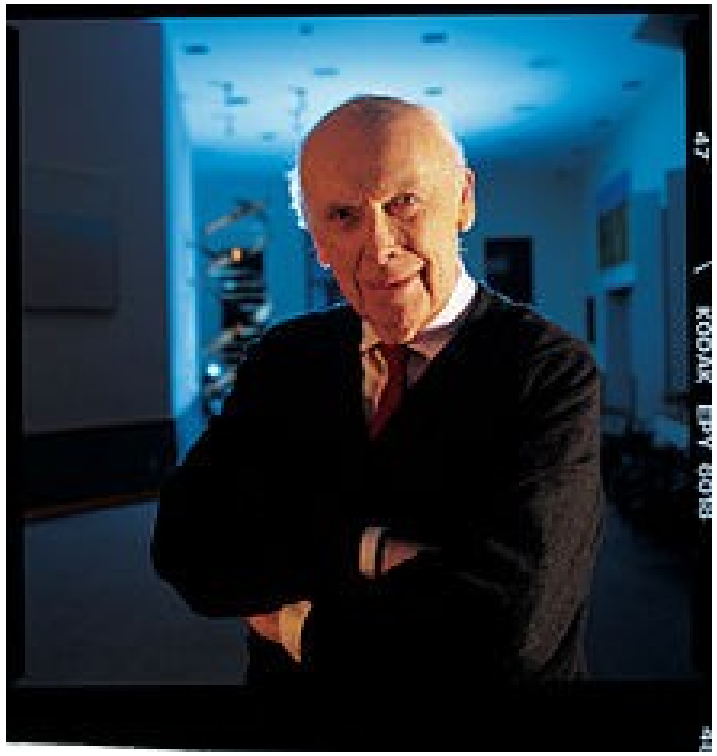


Human genome was published in 2001

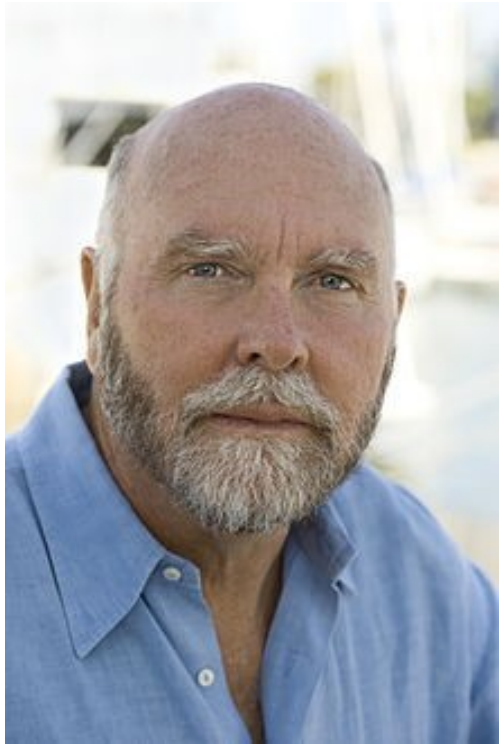


C. Venter



J. D. Watson

Individual sequences of human genomes were published in 2007 and 2008



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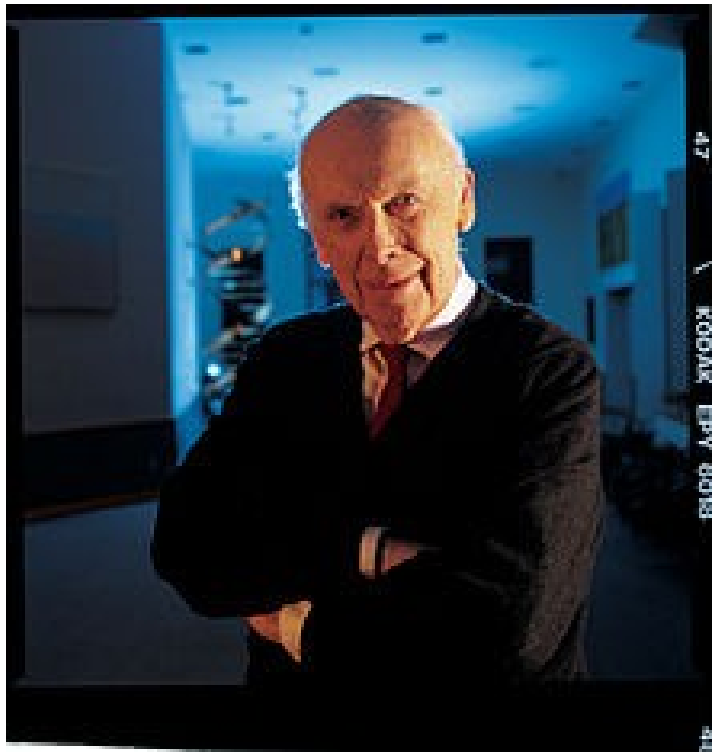
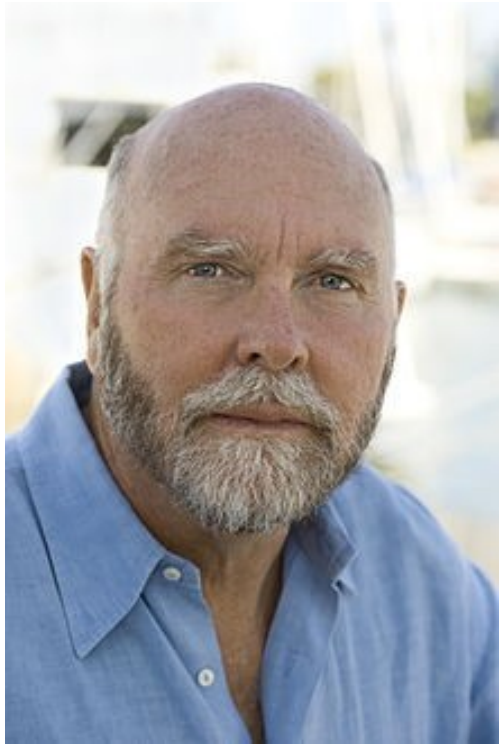


J. D. Watson

Individual sequences of human genomes were published in 2007 and 2008

Difference in 7648 amino acid substitutions



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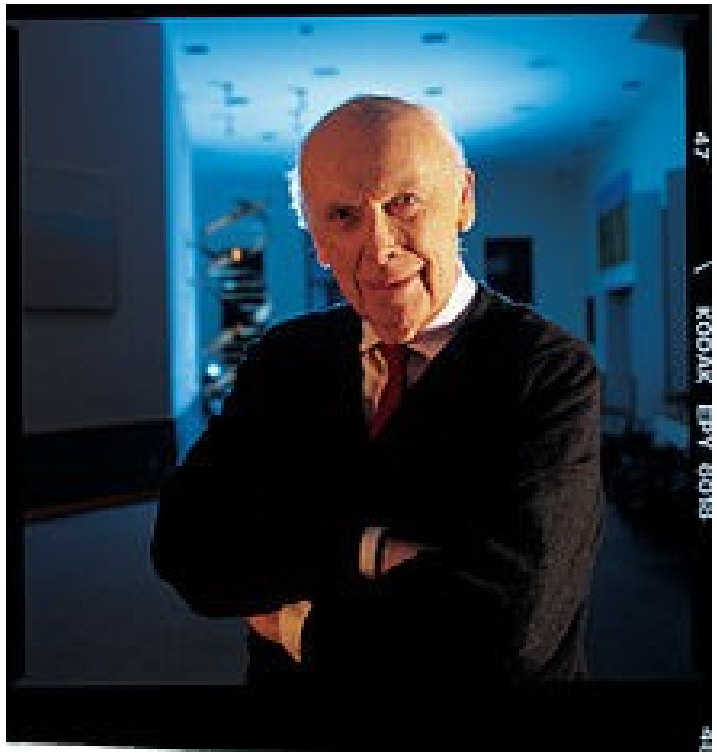
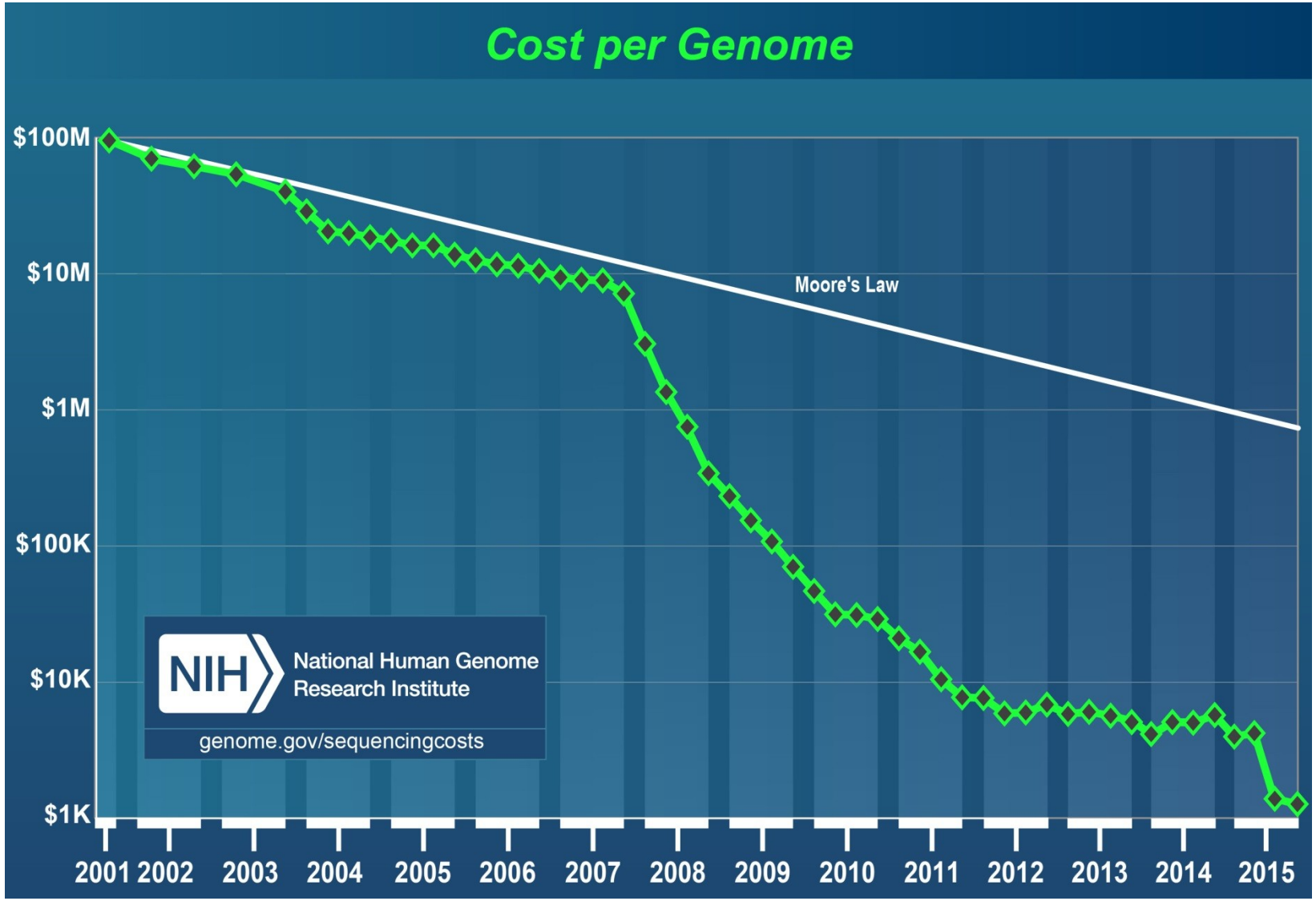


J. D. Watson

Individual sequences of human genomes were published in 2007 and 2008

The 1000 genome project published in 2010



Moore's law (1965): „The number of transistors (hence the processing power) that can be squeezed onto a silicon chip of a given size will double every 18 months".

Postgenomic era

- **Genomes were described**
- **Ongoing genomes annotations**

Genetics today

from phenotype to genotype

from genotype to phenotype

Modern techniques of genome analysis

Whole-Genome Sequencing vs. Whole-Exome Sequencing

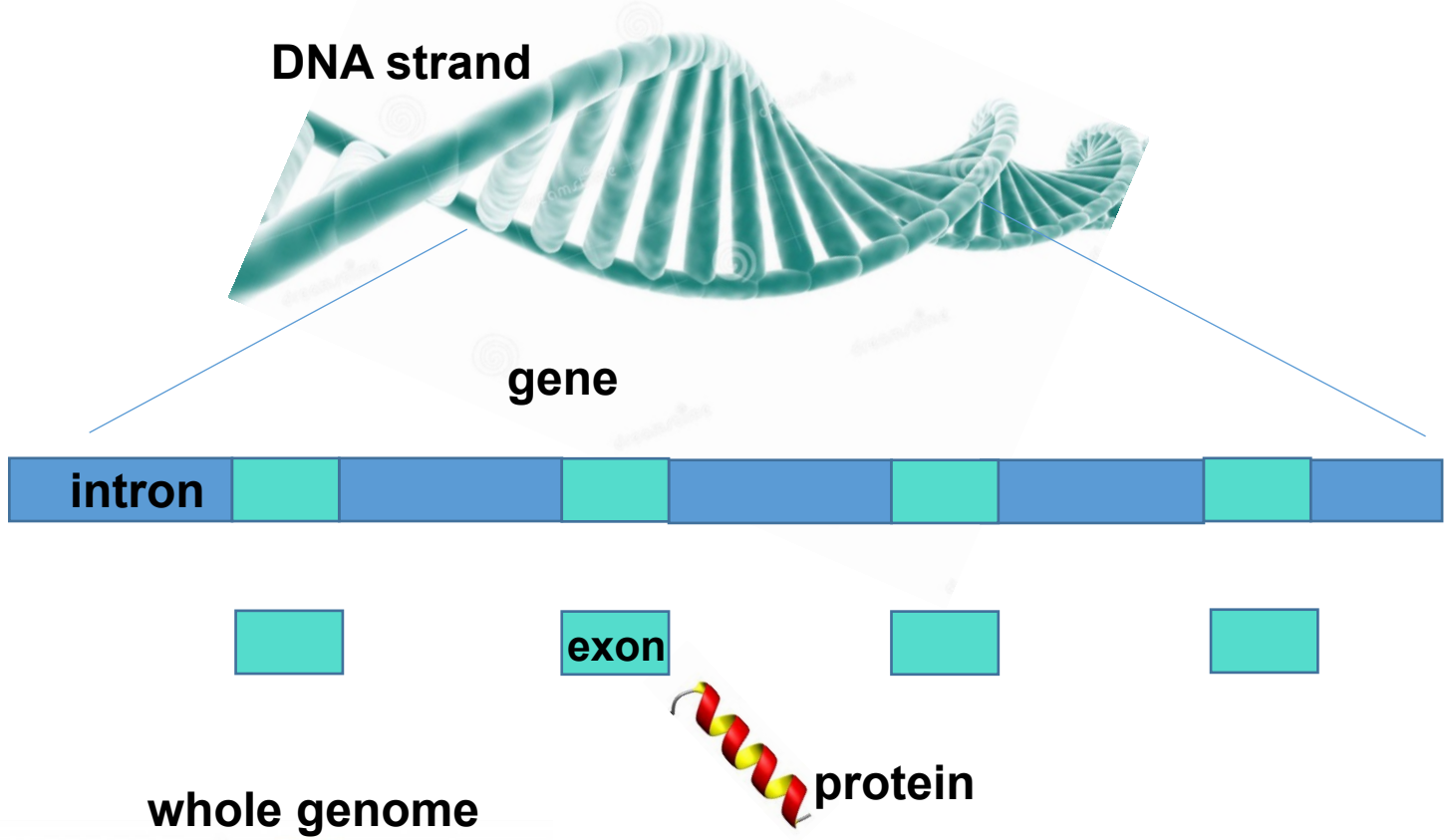
- human Genome

= 3.2 * 10^9 bp

~ 20 000 genes

- **Exome** = < 1.5% of human genome

contains ~ 85% of known disease causing mutations



whole genome

all exons = exome
< 1.5% of whole genome

NGS – flexibility

whole genome

3 200 000 000 bp
30 x coverage

exome

20 000 genes
100 x coverage

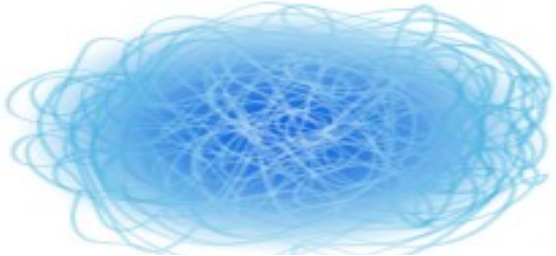
targeted genes or hotspots

< 100 genes
≥ 1000 x coverage

Whole-Genome Sequencing

Generating a Person's Genome Sequence

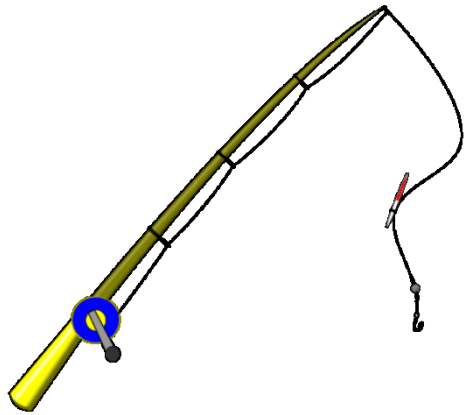
Whole-Exome Sequencing



Genomic DNA

Break genome into small pieces

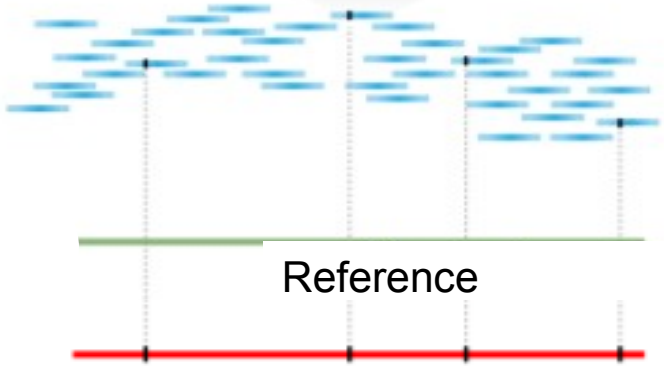
Capture library

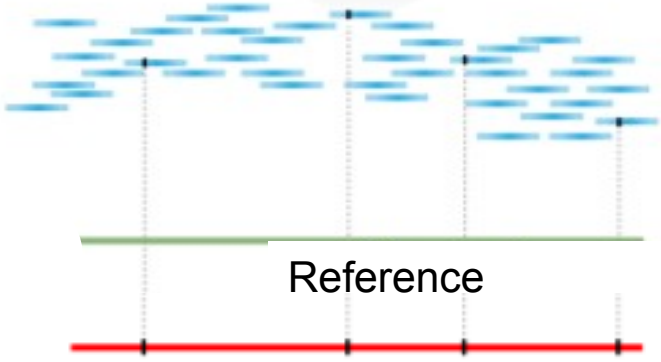


DNA

Generate millions of sequence reads

Align sequence reads to establish reference sequence





Deduce starting sequence and identity differences from reference sequence

Mutation
vs. human genome variability

Mutation
vs. human genome variability

- Every 1000^{th} base could be mutated $\Rightarrow$ 3.2 x 10^6 variants
- One men has approx. 0.5 x 10^6 variants
- Exome analysis (1.5% of genome) $\Rightarrow$ tens thousands of variants

Which of the found variants is the disease causing one?

Mutation
vs. human genome variability

- Every 1000th base could be mutated $\Rightarrow$ 3.2 x 10^6 variants
- One men has approx. 0.5 x 10^6 variants
- Exome analysis (1.5% of genome) $\Rightarrow$ [illegible]s thousands of variants

Which of the found variants is the one?

mutation frequency
1.1 – 1.3 x 10^{-8}

Mutation
vs. human genome variability

- Every 1000th base could be mutated $\Rightarrow$ 3.2 x 10^6 variants
- One men has approx. 0.5 x 10^6 variants
- Exome analysis (1.5% of genome) $\Rightarrow$ tens thousands of variants

mutation
x polymorfisms

d variants is the disease causing

Mutation
vs. human genome variability

Mutations: **spontaneous** vs. **induced**

gene vs. **chromosomal**

Mutations: **missense**
nonsense (terminating triplet)
same sense
frameshift

Mutation
vs. human genome variability

Single nucleotide polymorphisms (SNPs)

cgcgcggcctcctccttgtggccatcctggtcctcctaaaccacctggac

cgcgcggcctcctccttgtggtcatcctggtcctcctaaaccacctggac

Insertions/deletions (indels)

cgcgcggcctcctccttgtggccatcctggtcctcctaaaccacctggac

cgcgcggcctcctccttgtgg-------ctggtcctcctaaaccacctggac

Mutation
vs. human genome variability

Microsatelites (STR)

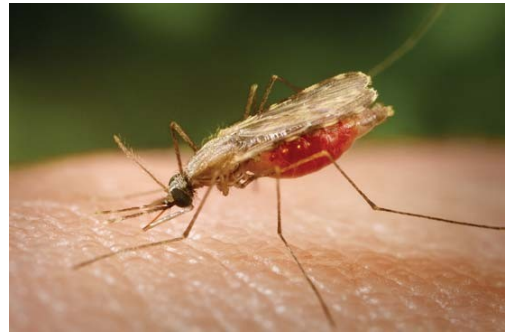
cgcgcggcctcctccttgtgg**cacacacacaca**catcctggtcctcctaaaccacctgga

cgcgcggcctcctccttgtgg**cacacacaca**catcctggtcctcctaaaccacctgga

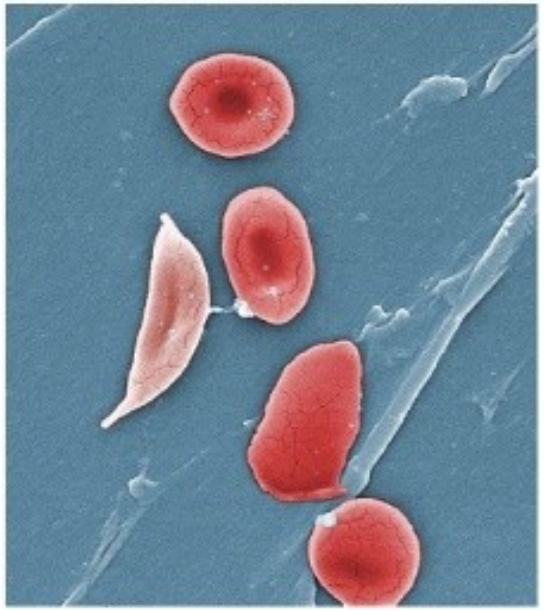
Copy number variants (CNV)

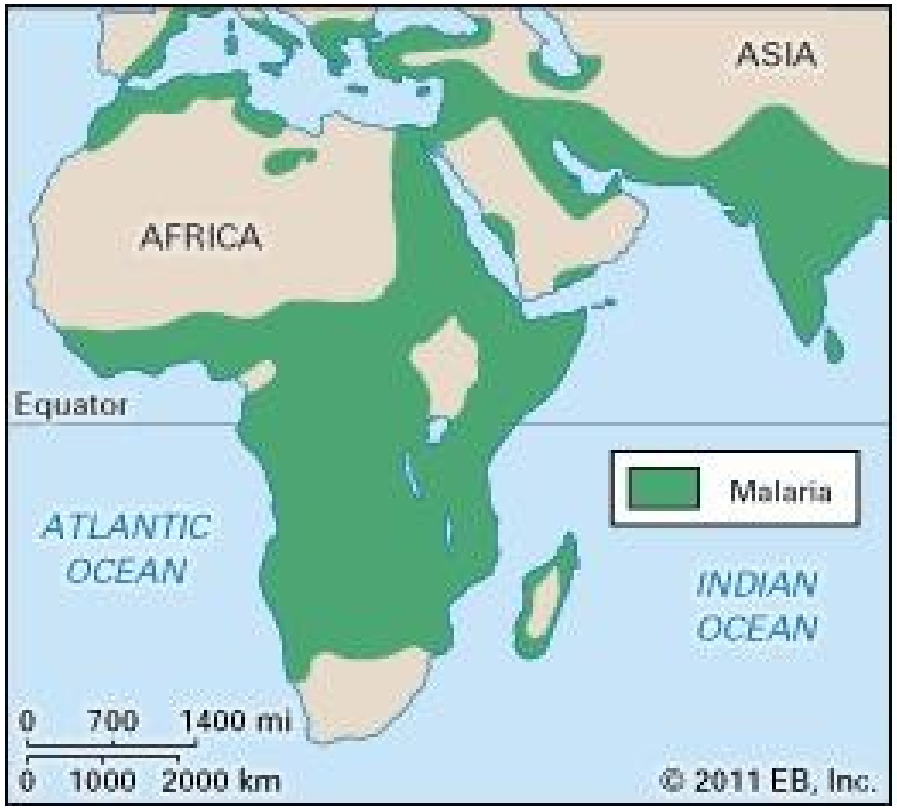
>1 kb – 1MGb

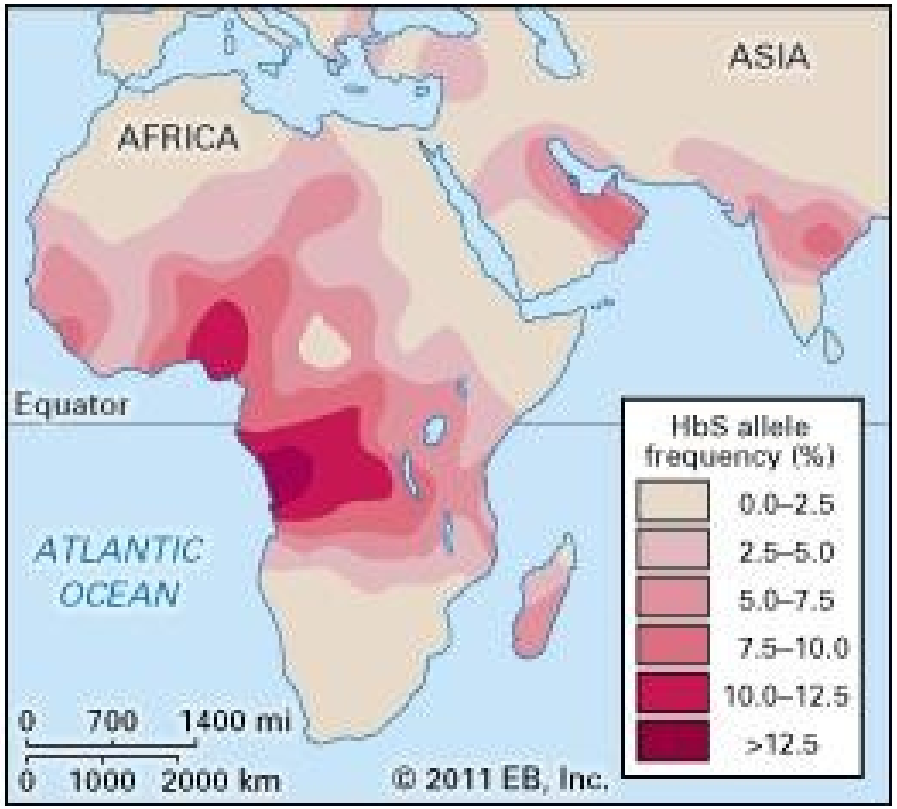
Mutation
vs. human genome variability



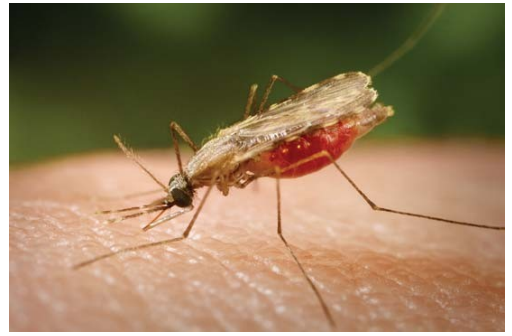
Sickle-cell anemia



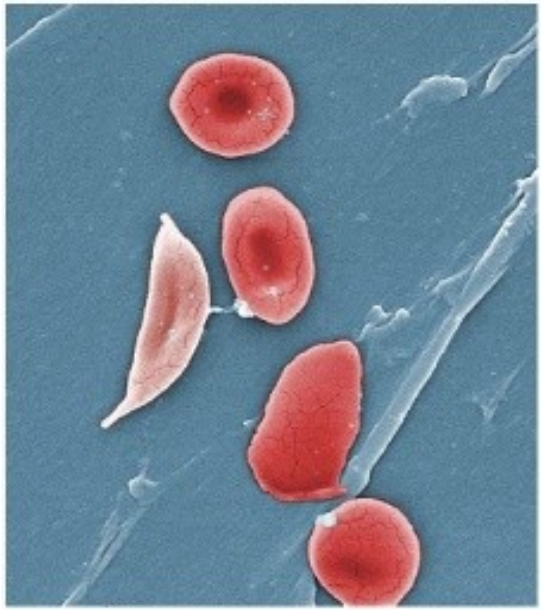




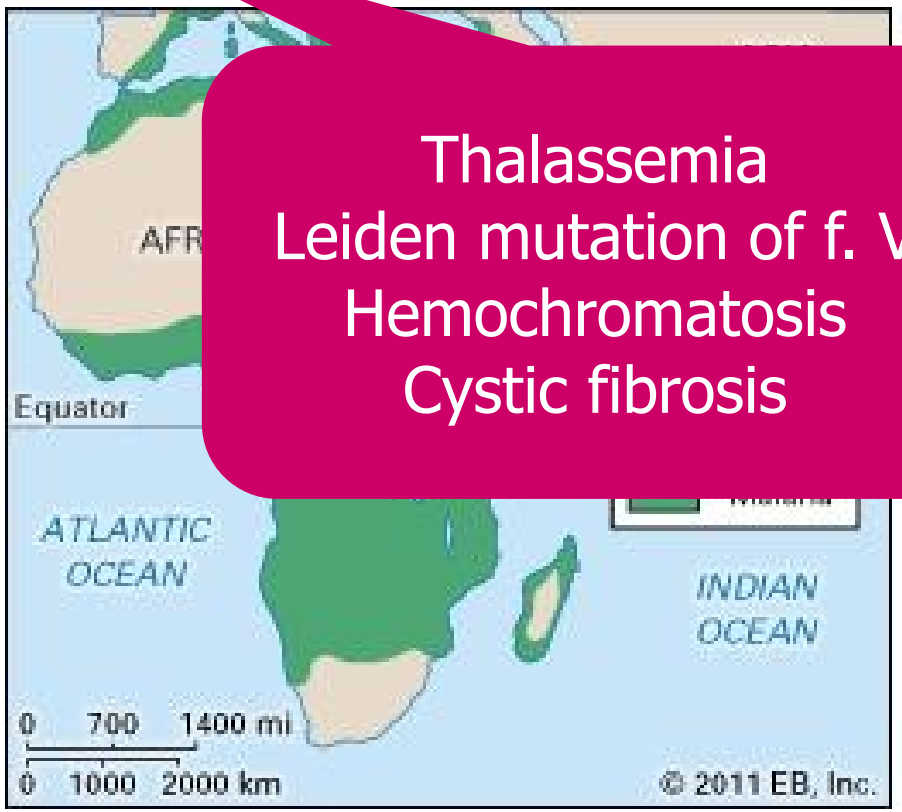
Mutation
vs. human genome variability

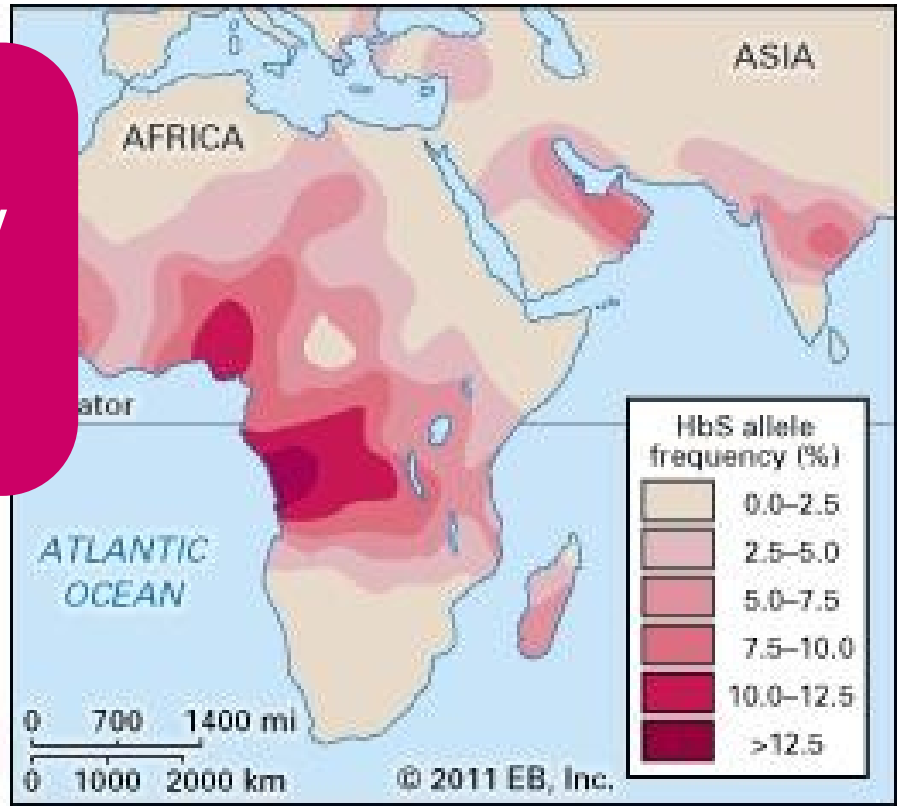


Sickle-cell anemia



Thalassemia
Leiden mutation of f. V
Hemochromatosis
Cystic fibrosis

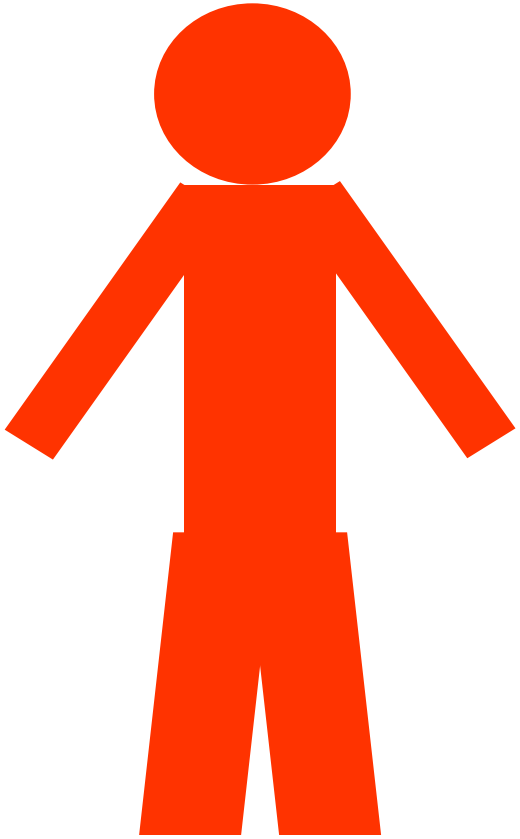




Positive mutations

Germinal vs. somatic mutations

Germinal vs. somatic mutation

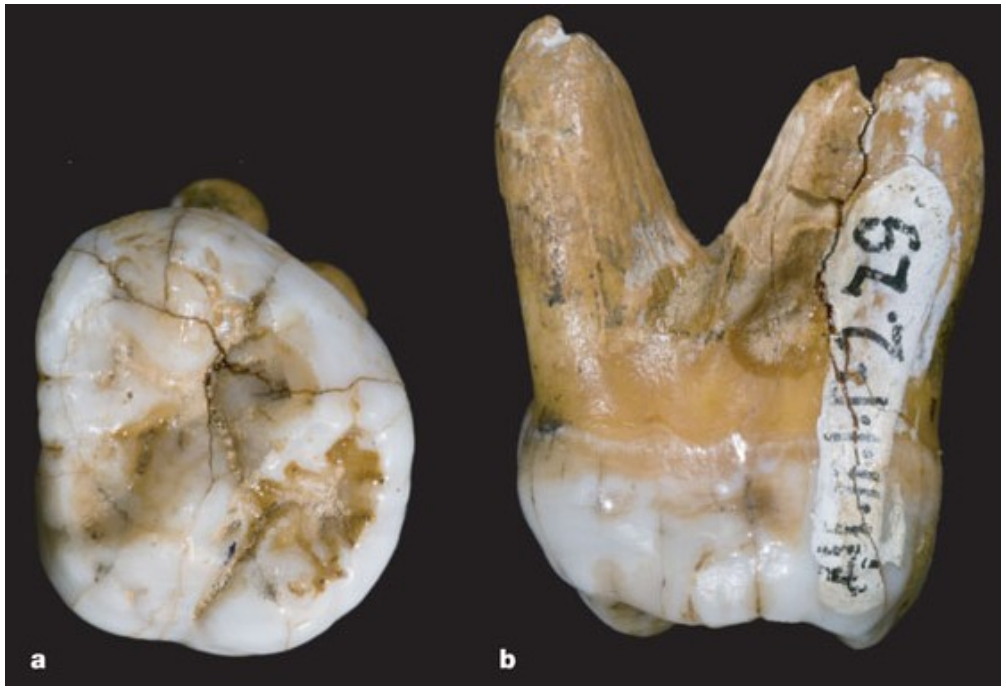


Germinal mutation

Somatic mutation

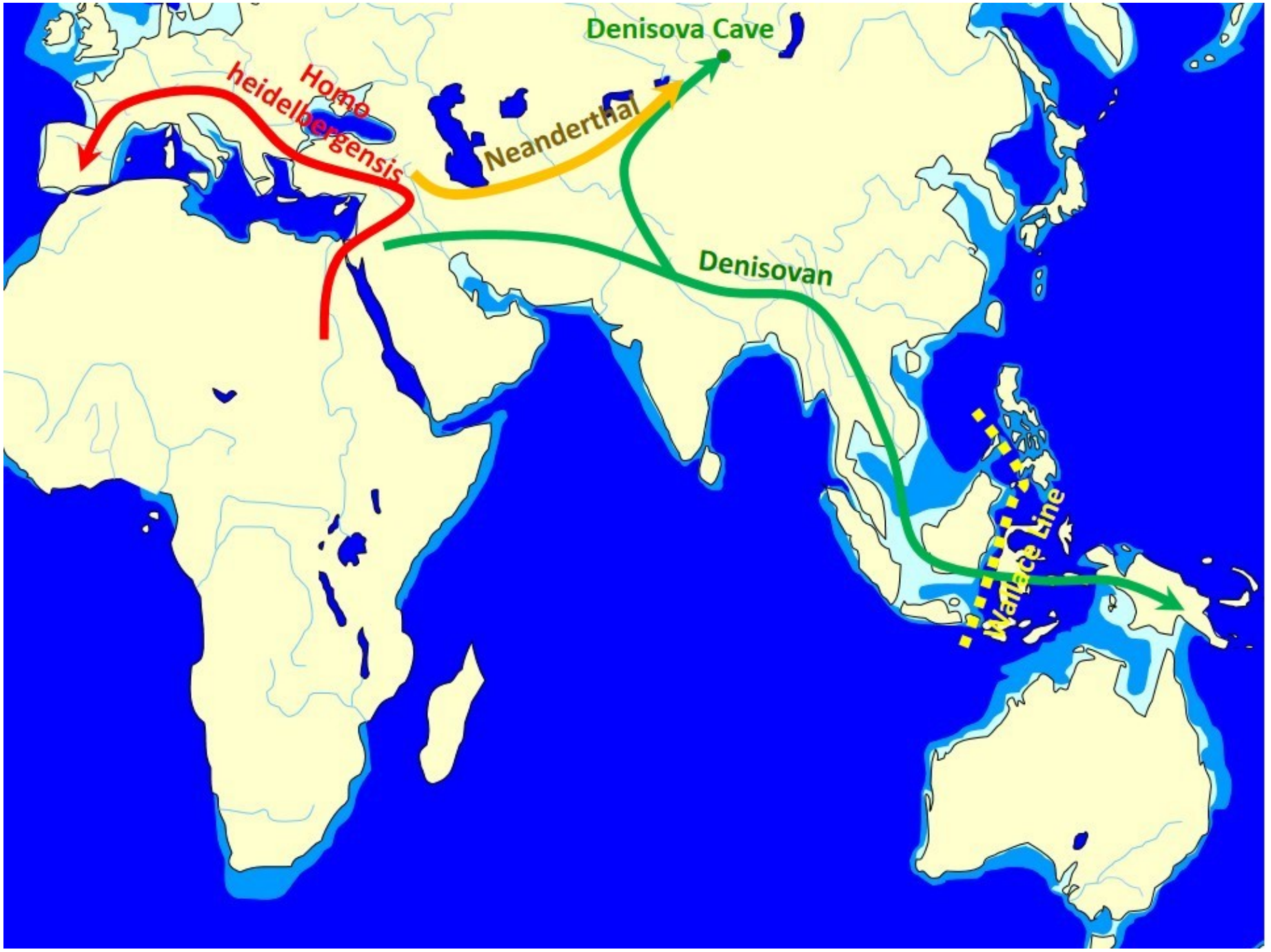
Are we Homo sapiens?

Are we Homo sapiens?



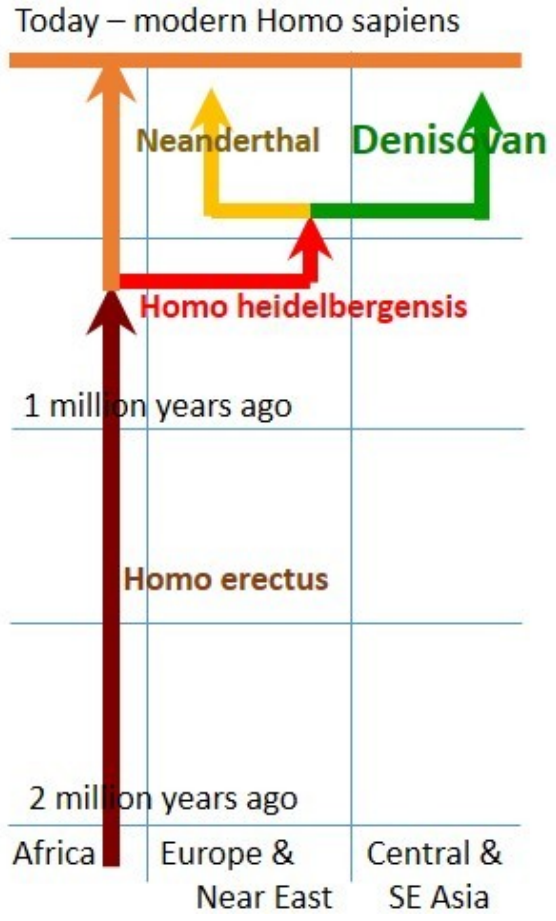
Denisova hominins, 41 000 years ago
mtDNA

Reich a kol.
Nature 2010



Key

Maximum Sea Level during the Ice Age



Are we Homo sapiens?

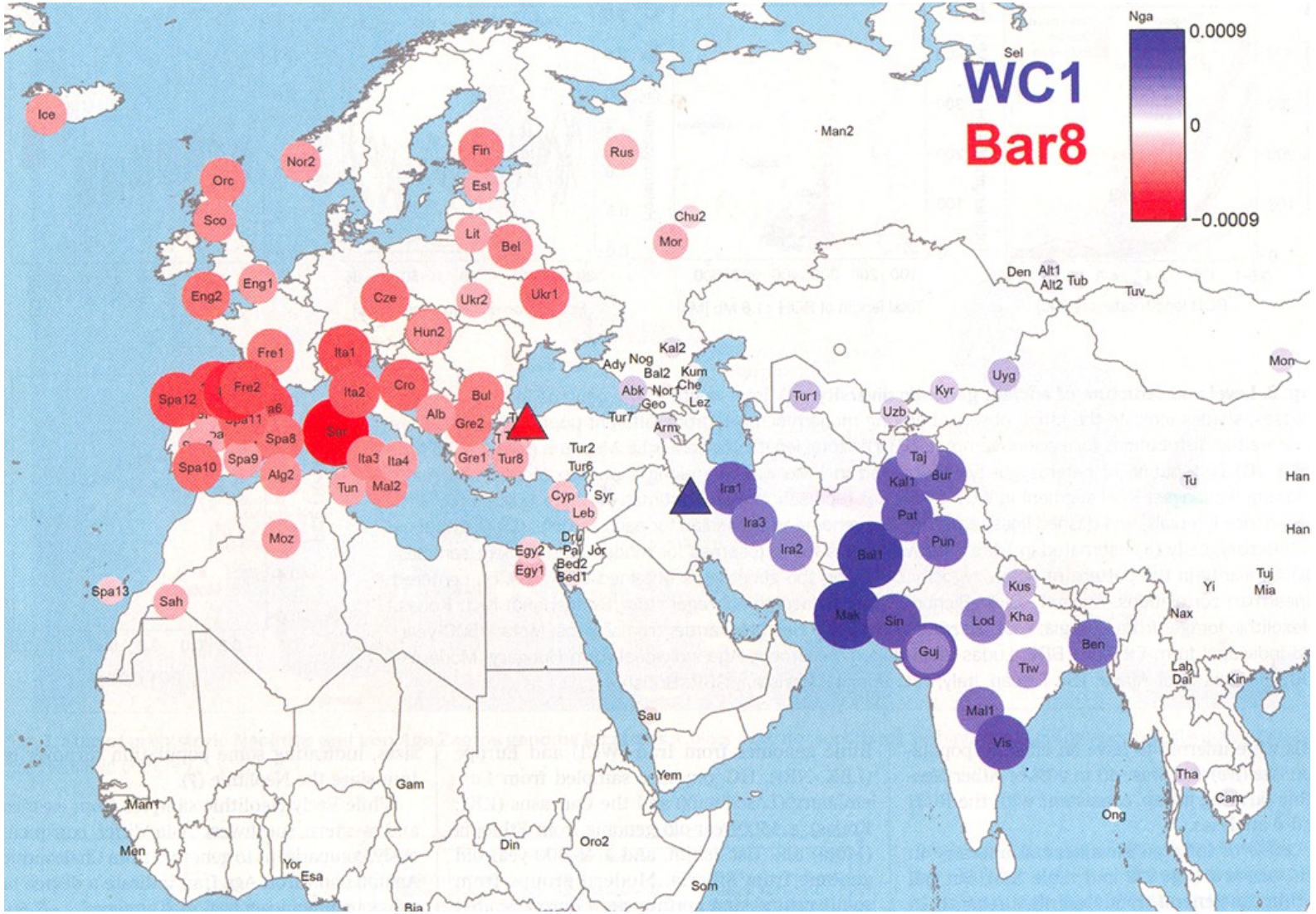
Europe 1 – 3% of the genome
Some diseases of Neanderthal origin – depression, skin disorders?

Ancient genomes analysis

Genome from the Younger Stone Age and Iron Age - Zagros, Iran

Broushaki a kol., Science 2017

Two ancient genomes in modern humans



Broushaki a kol., Science 2017

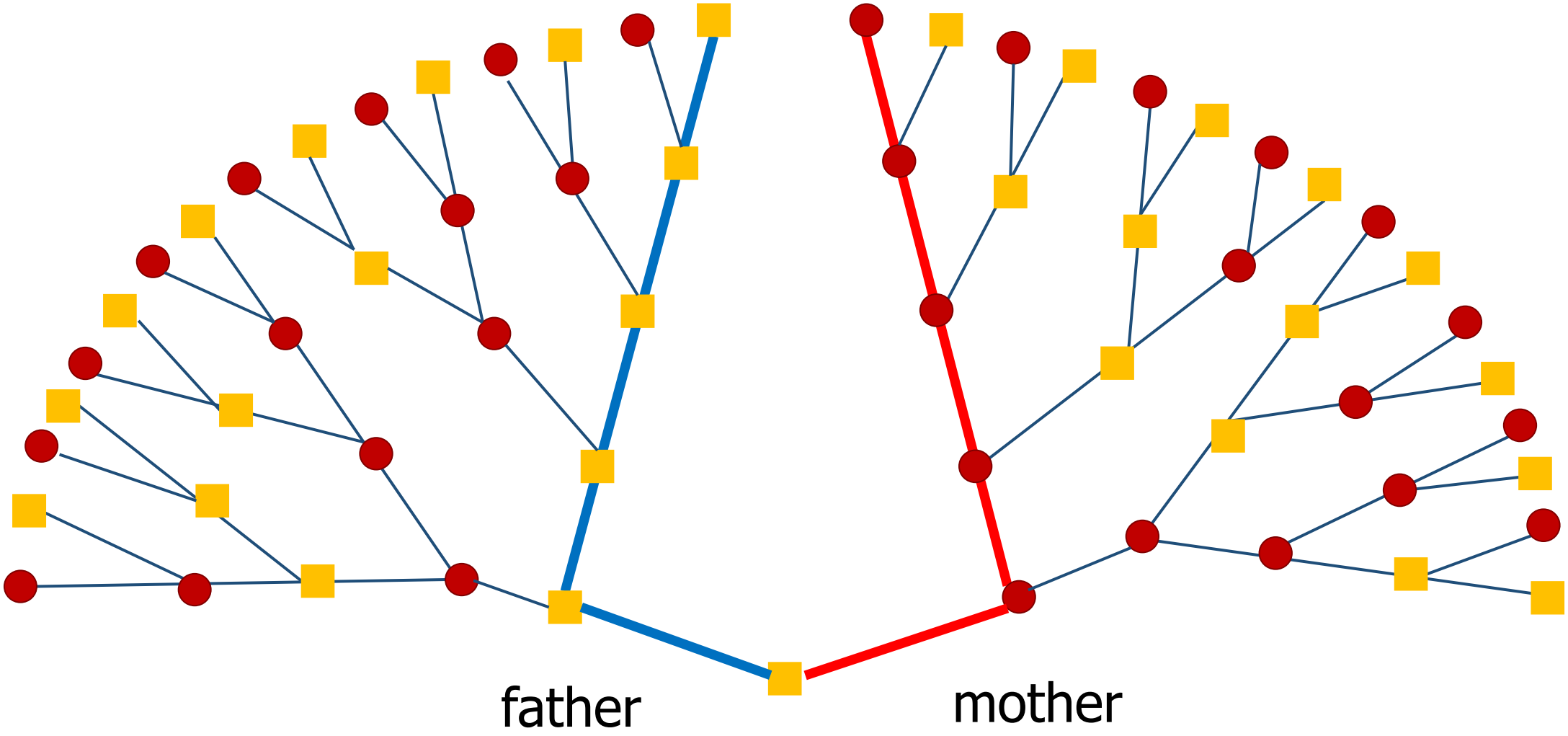
Famous ancient genomes

Ötzi

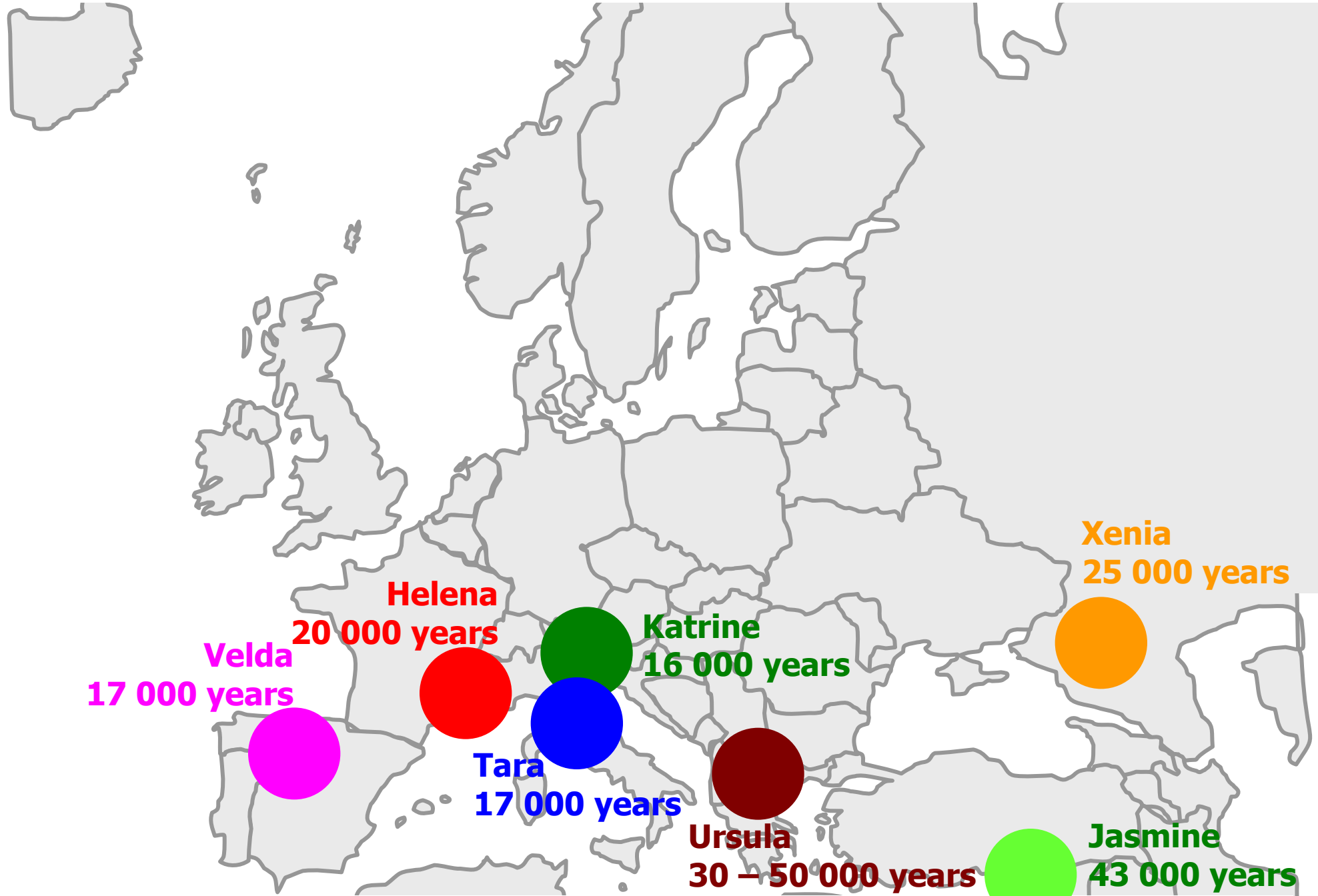
Cheddar man

Origin based on mitochondrial DNA

Mitochondrial and Y-inheritance



The seven daughters of Eve



The seven daughters of Eve

- **mitochondrial Eve (140 000 years ago in Ethiopia)**
- **7 main mitochondrial haplotypes in Europe**
- **29 haplotypes worldwide**
- **Results are not as accurate as from other methods – mtDNA is very similar**

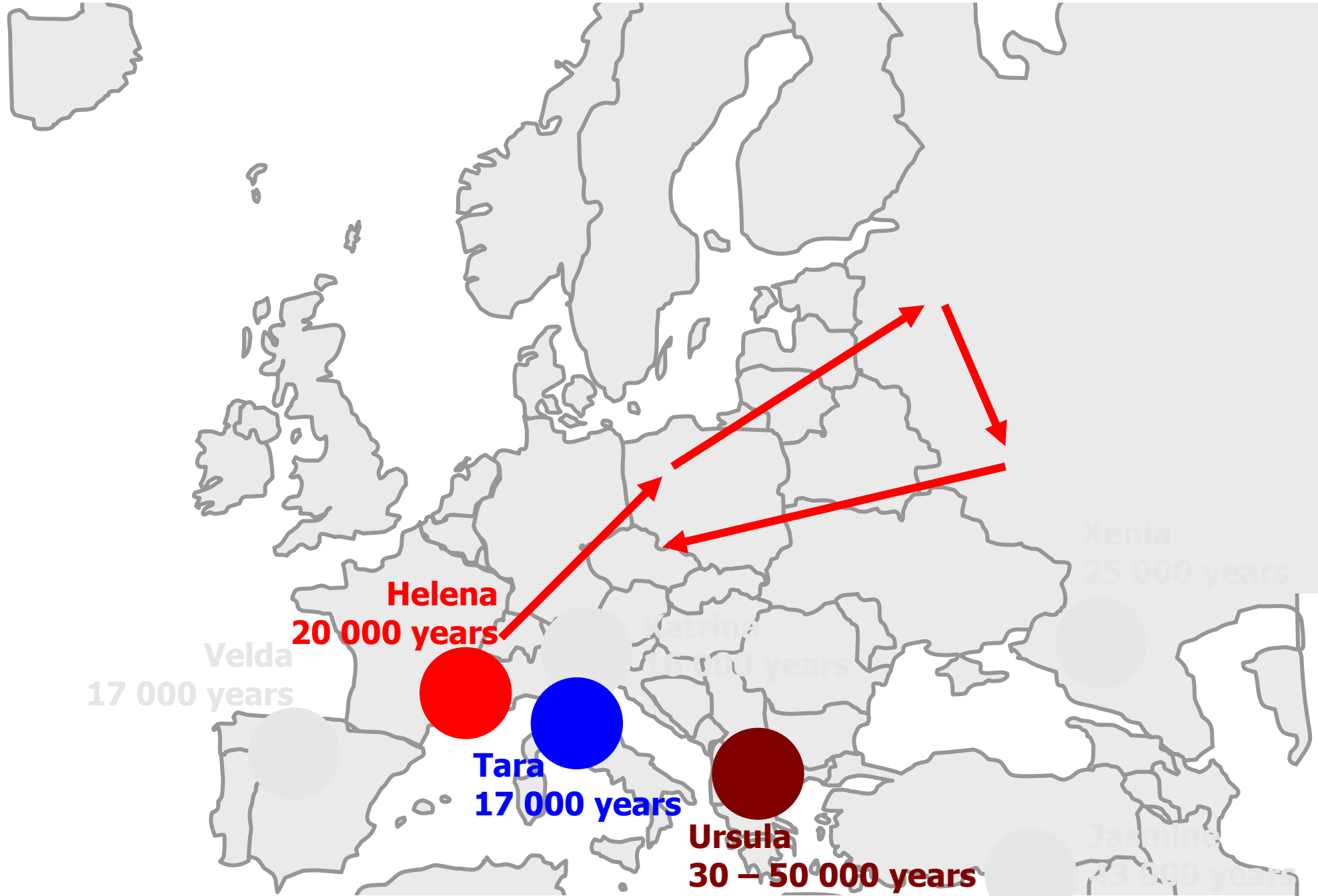
offspring of the mitochondrial Eve may still live

Bryan Sykes, 2004

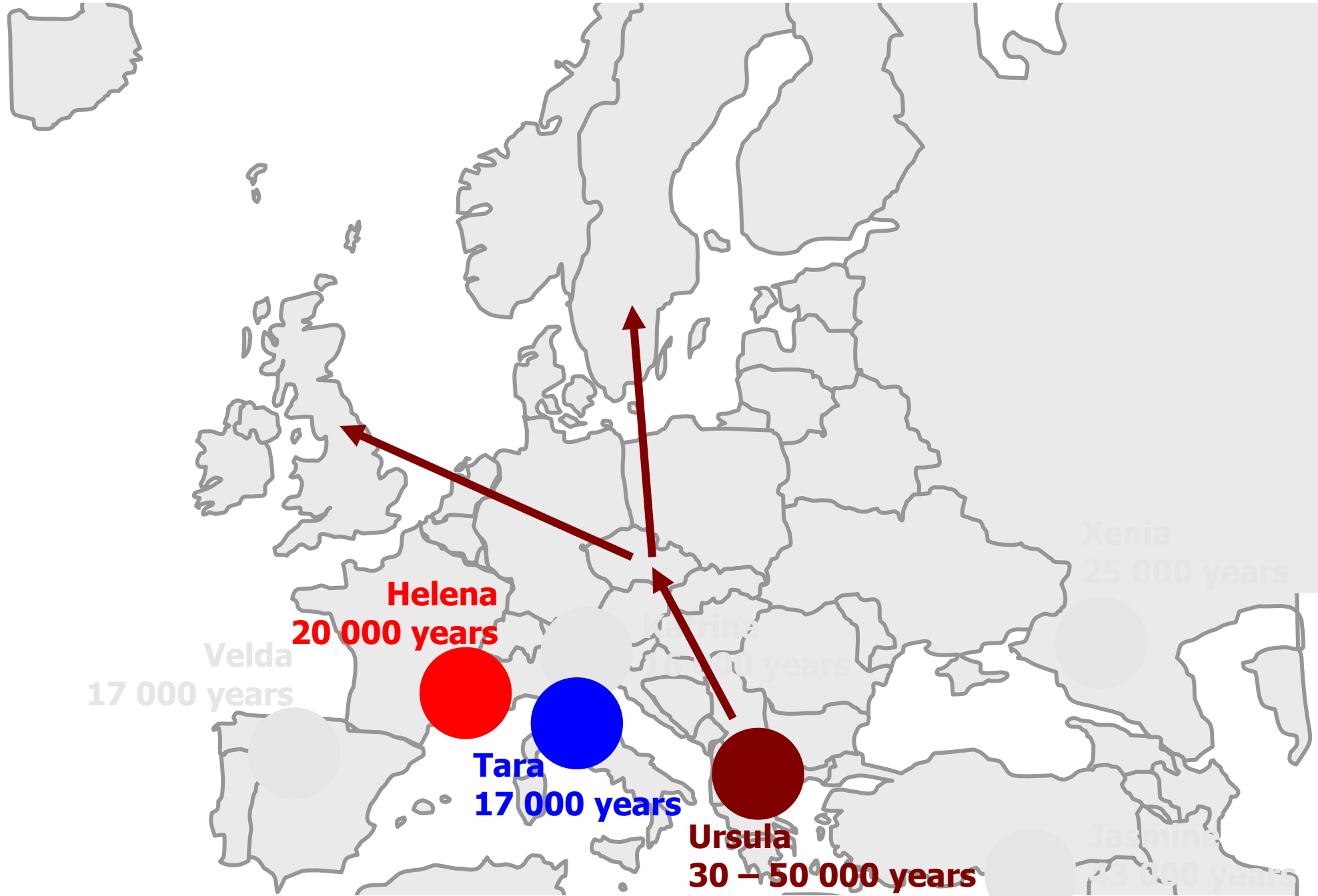
The seven daughters of Eve in the Czech population

- **Helena 43.51% (dominant lineage from Polland and european part of Russia)**
- **Ursula 17.6% (mainly UK and Scandinavia)**
- **Tara 11.17%**
- **Jasmine 8.78%**
- **Katrine 5.89% (Ashkenazi Jews)**
- **Velda 4%**
- **Xenia 3%**

The seven daughters of Eve after the Ice Age



The seven daughters of Eve after the Ice Age



The role of genome in the disease onset

- **Mendelian hereditary diseases 8%**
- **Multifactorial 90%**
- **Others 2%**

The role of genome in the disease onset

- **Mendelian hereditary diseases 8%**
- **Multifactorial 90%**
- **Others 2%**

⇒ **genetic background plays almost always a role in the disease onset**

Inheritance types

Inheritance types

Mendelian
monogenic: one gene ⇒ one feature

X-linked and Y-linked (sex-linked disorders)

Polygenic
several genes ⇒ one feature

Mitochondrial

Environmental factors

What is the procedure of hereditary diseases tracing?

- **family studies:**
  pedigree
  monozygous twins
  odds ratio
  relative risk

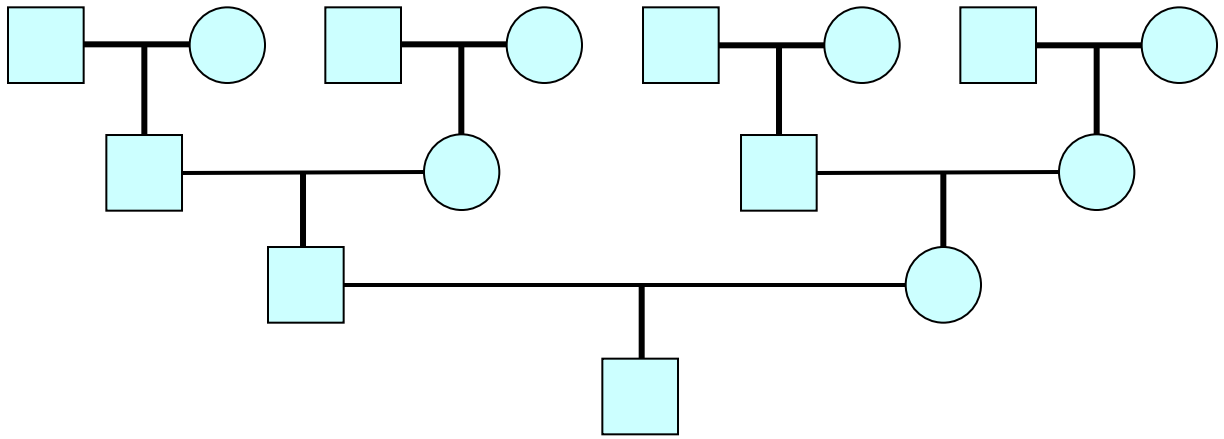
- **disease frequency in population**

- **molecular biology methods**

- **genetic linkeage and functional tests**

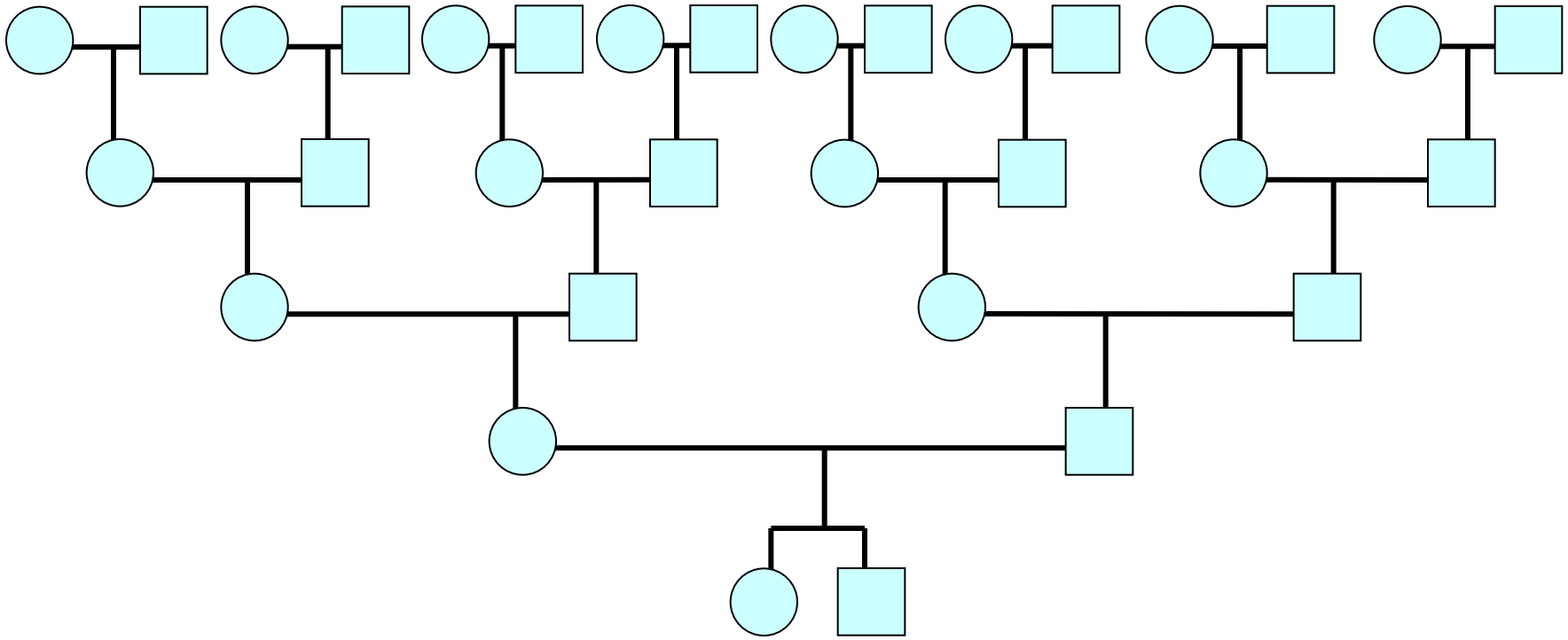
Common ancestor

- two common ancestors in previous generation: parents
- 4 grandparents, 8 great-grandparents
- the number of ancestors in generation n is 2^n

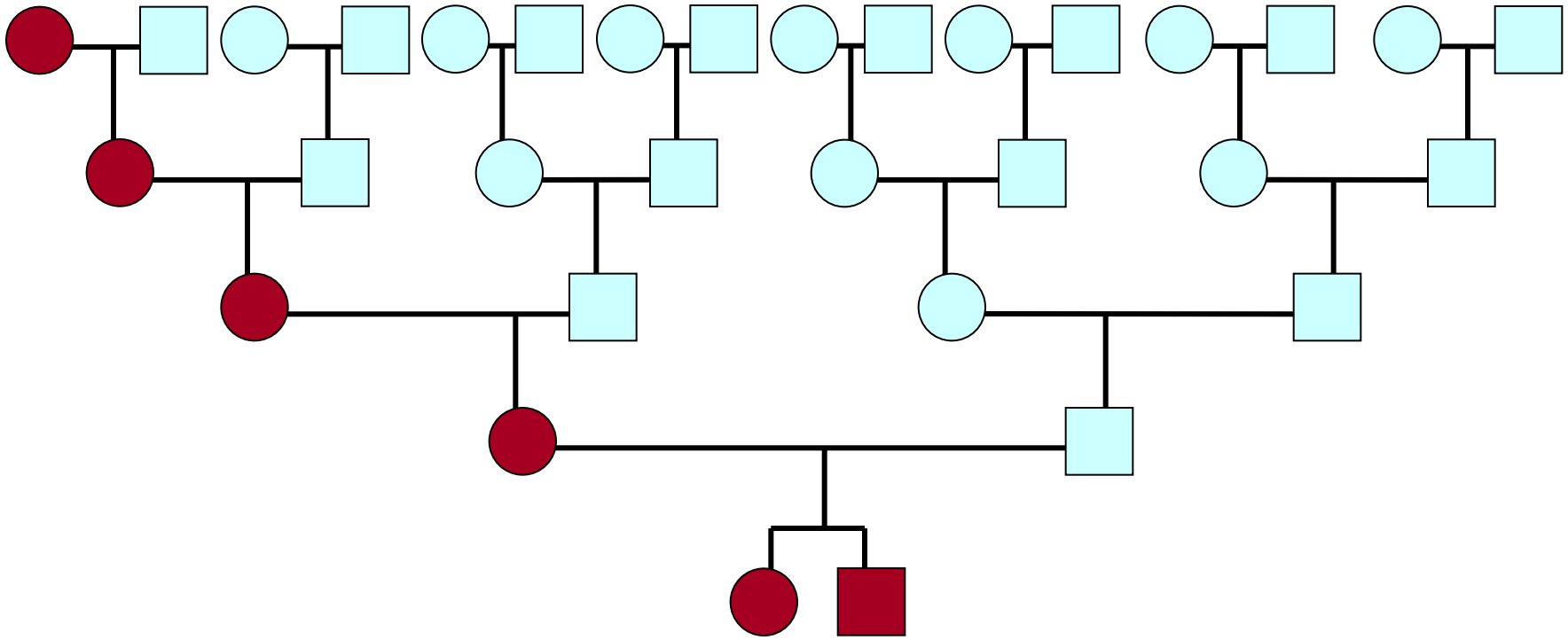


- 40^{th} generation (1000 years back): $2^{40} = 1.09 \times 10^{12}$
- so many people didn't live on this planet (7.0×10^9)

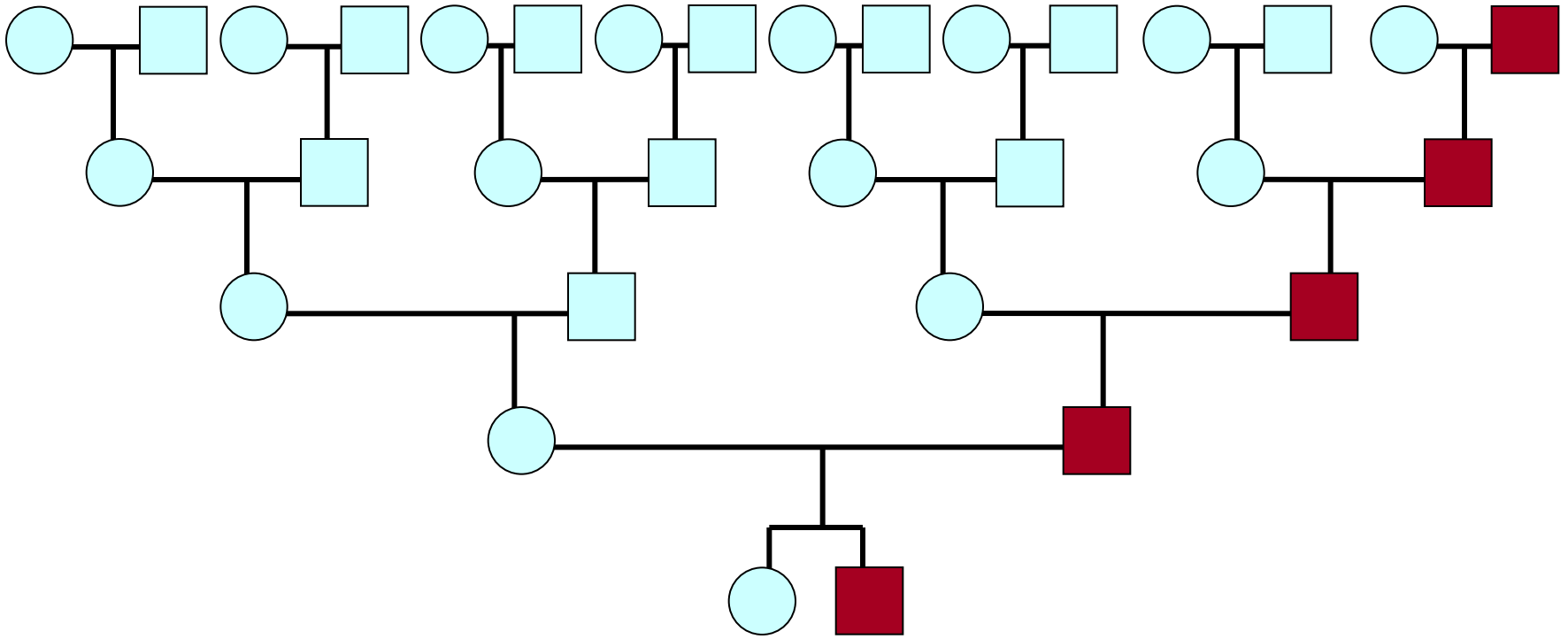
Pedigree



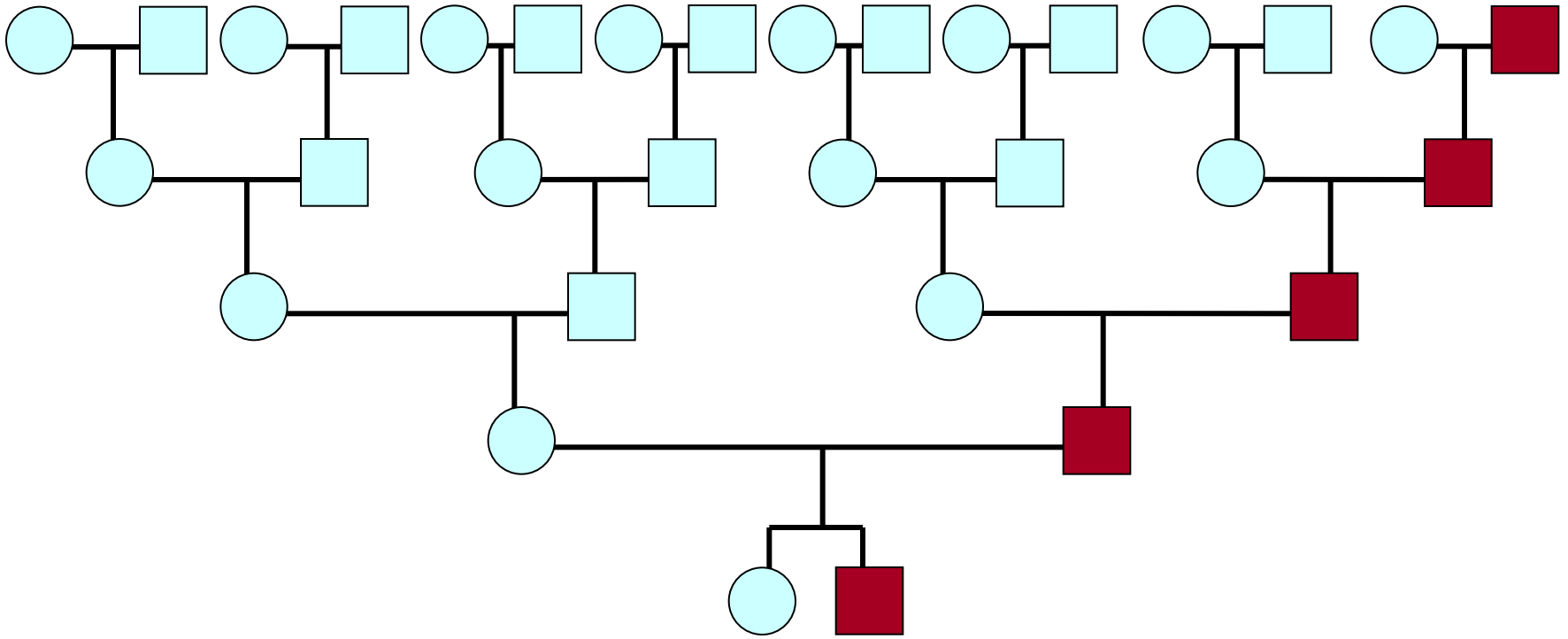
Mitochondrial inheritance



Y-chromosome inheritance

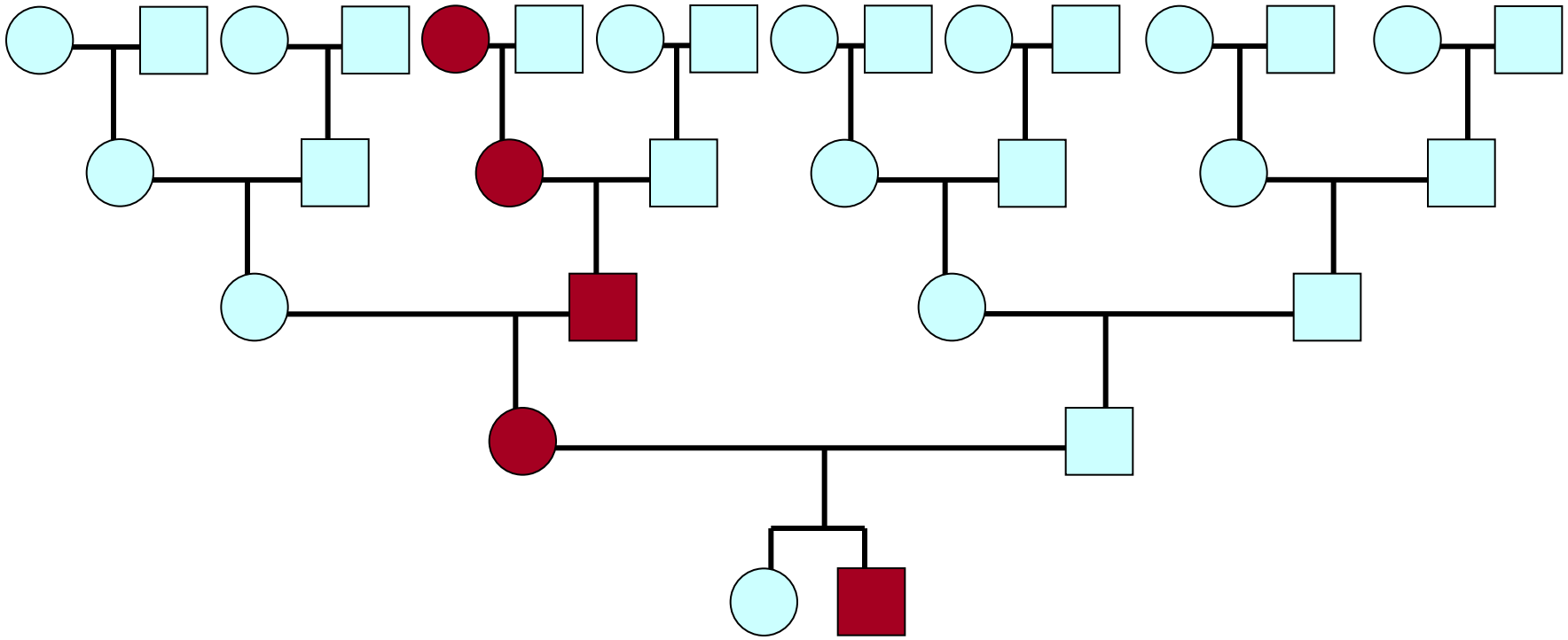


Y-chromosome inheritance

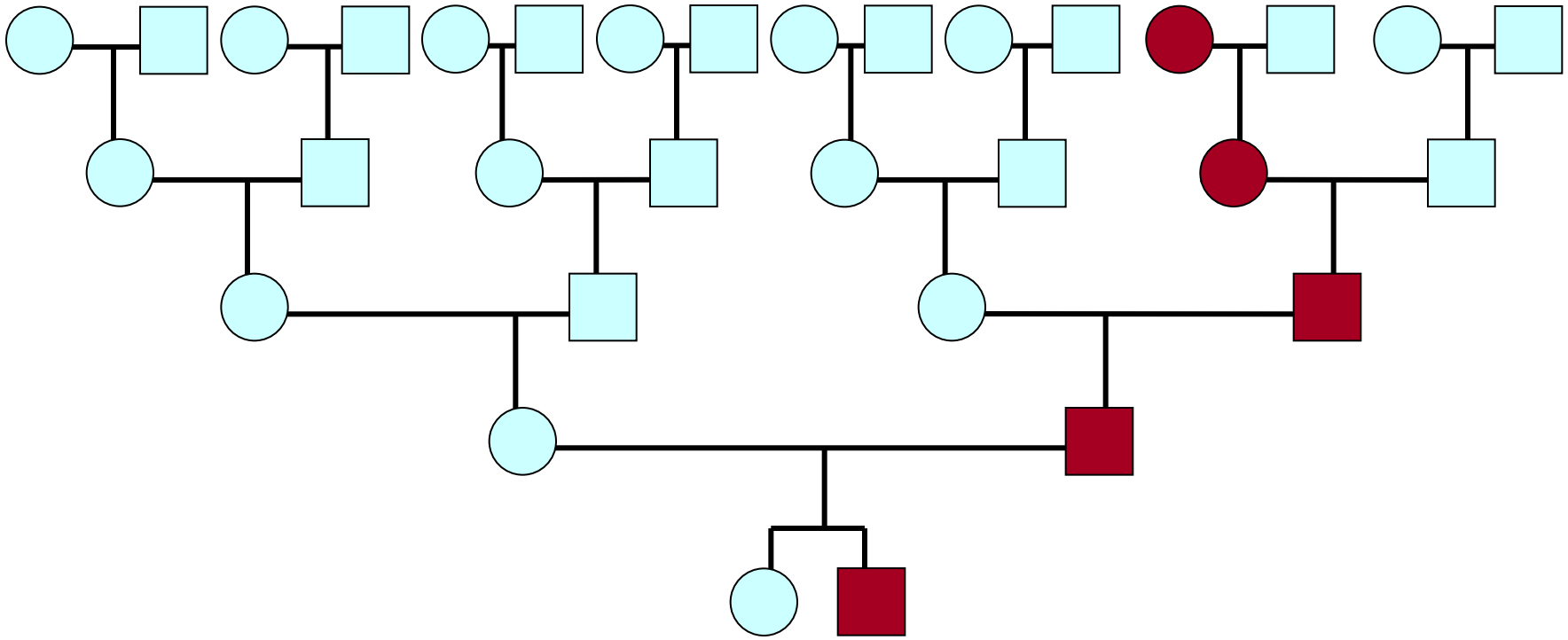


What Y-chromosome carries on?

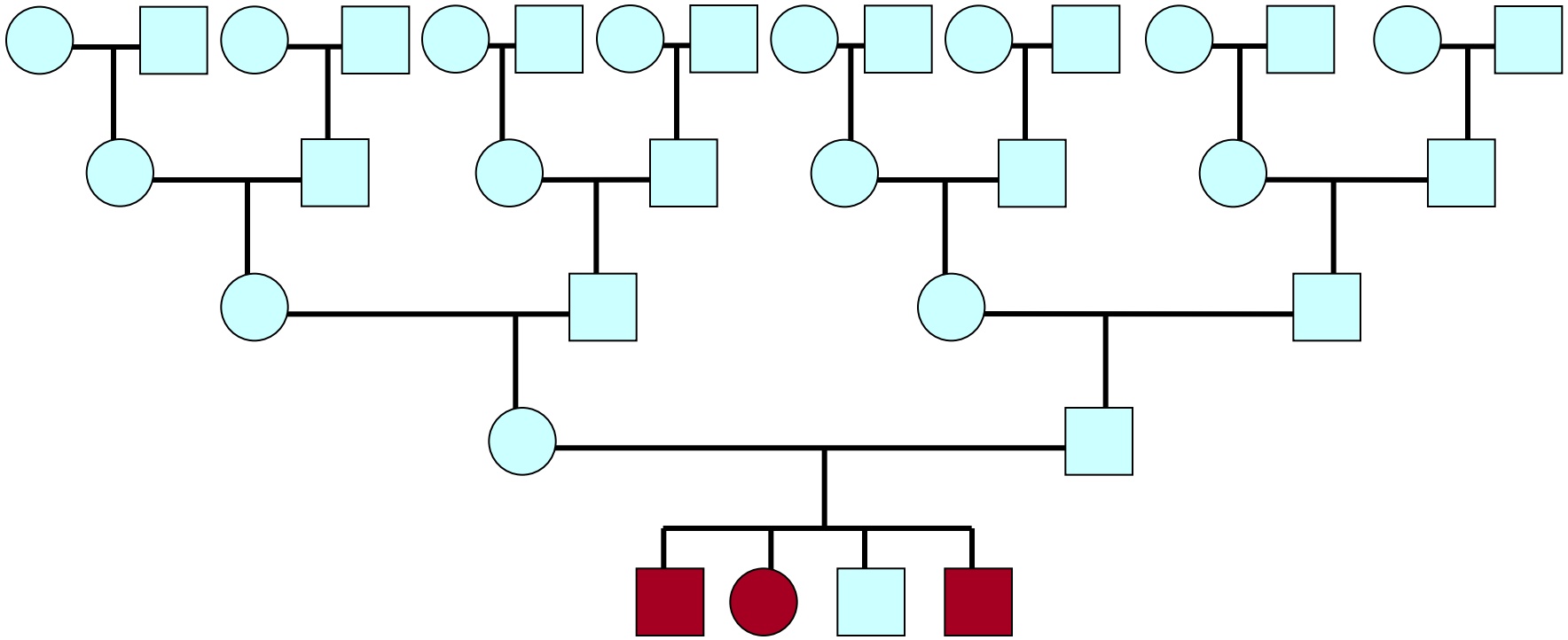
Autosomal dominant inheritance



Autosomal dominant inheritance



Autosomal recessive inheritance

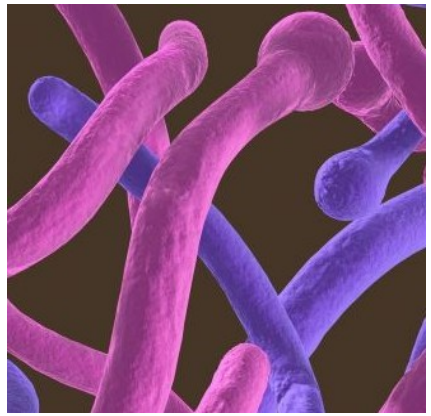


Environmental factors










Monogenic disorders

How many monogenic disorders exist?

≈ 1 000

≈ 10 000

≈ 100 000

How many monogenic disorders exist?

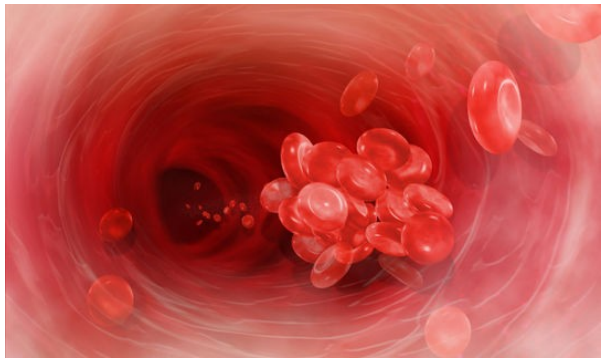
≈ 1 000

≈ 10 000

≈ 100 000

Recessive disorders

- hemochromatosis (1:10)
- mutation of factor V Leiden (1:20)
- cystic fibrosis (1:25)
- spinal muscular atrophy (1:40)



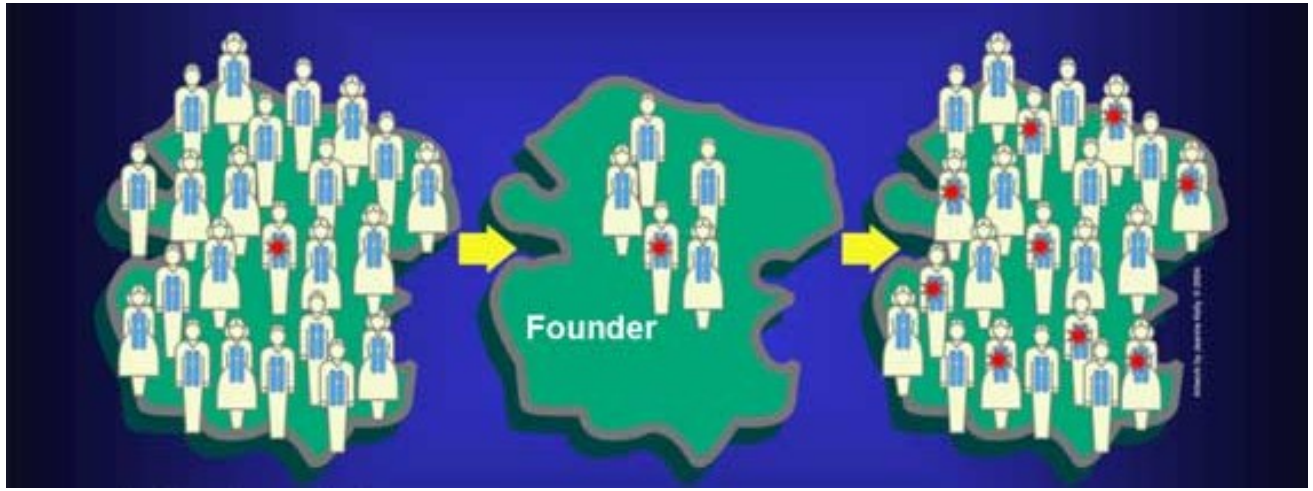
Dominant disorders

- deafness
- polydactyly
- Huntington's Chorea
- Li-Fraumeni syndrome
- breast and ovarian cancer

Origin by mutation type

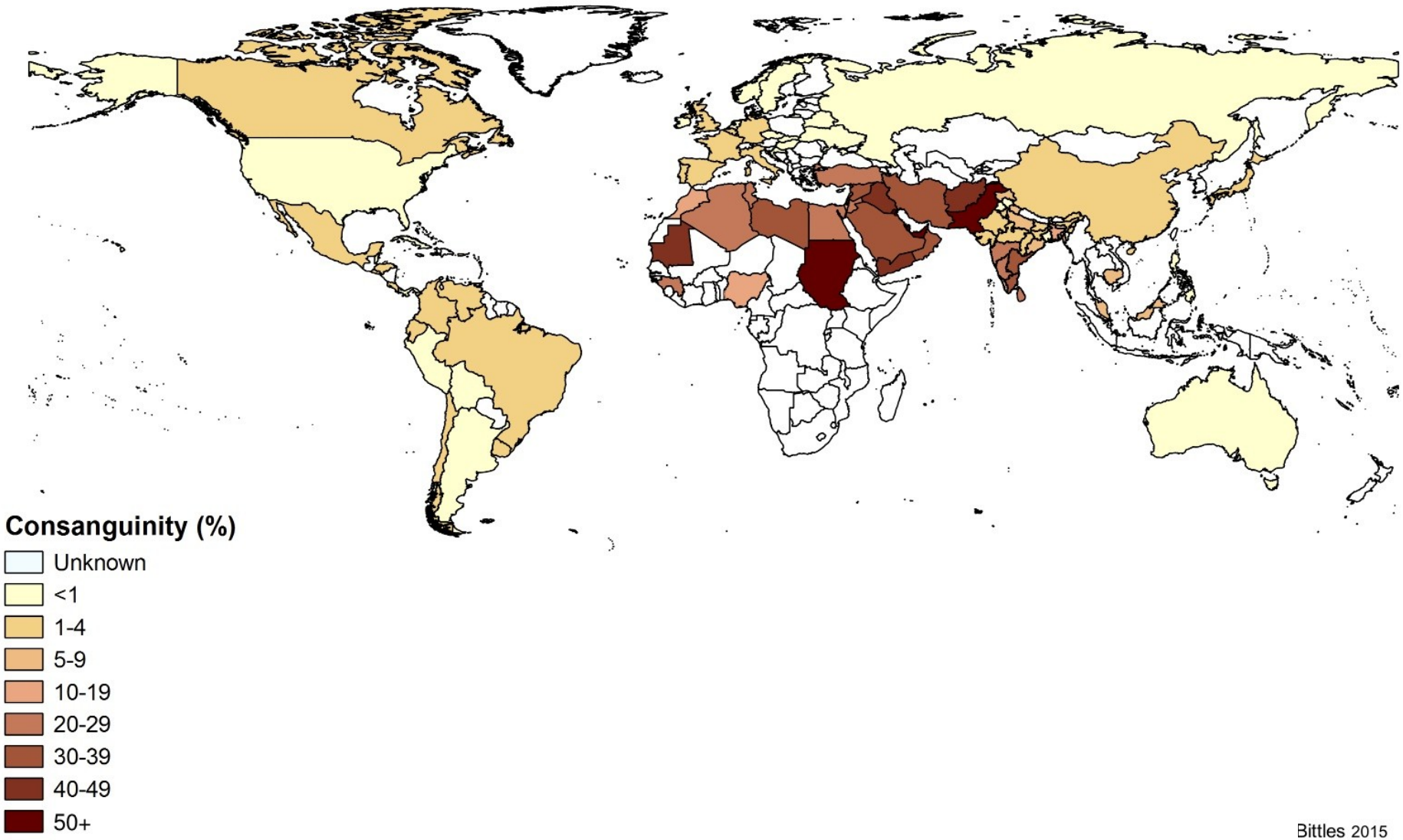
- **Founder effect**

- **Small closed populations:**
  Ashkenazi Jews
  franco-Canadiens
  Iceland
  surroundings od Maracaibo lake…

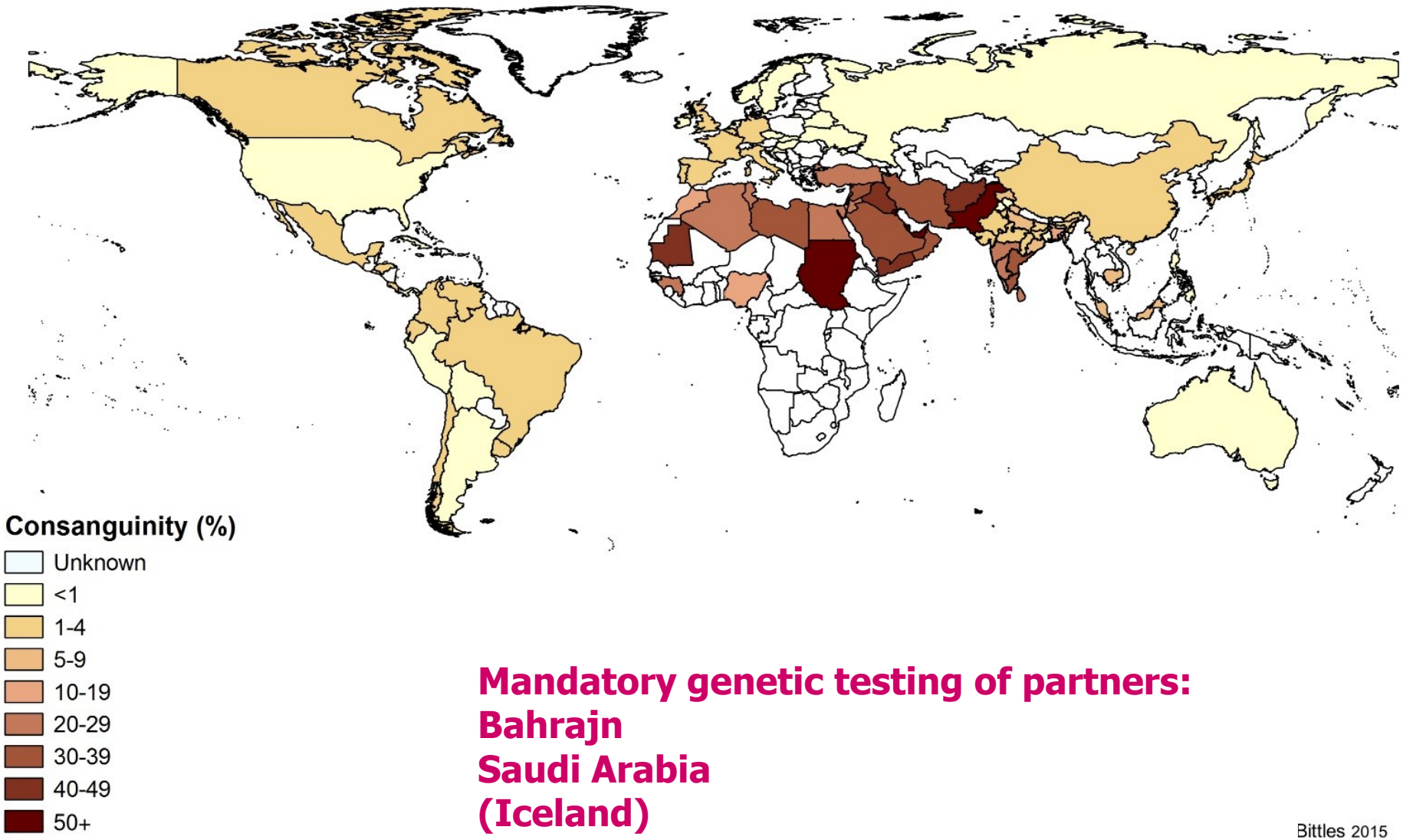


- **Marriages of relatives**

Consanguinity map



Consanguinity map



Mandatory genetic testing of partners:
Bahrajn
Saudi Arabia
(Iceland)

Bittles 2015

Consanguinity example

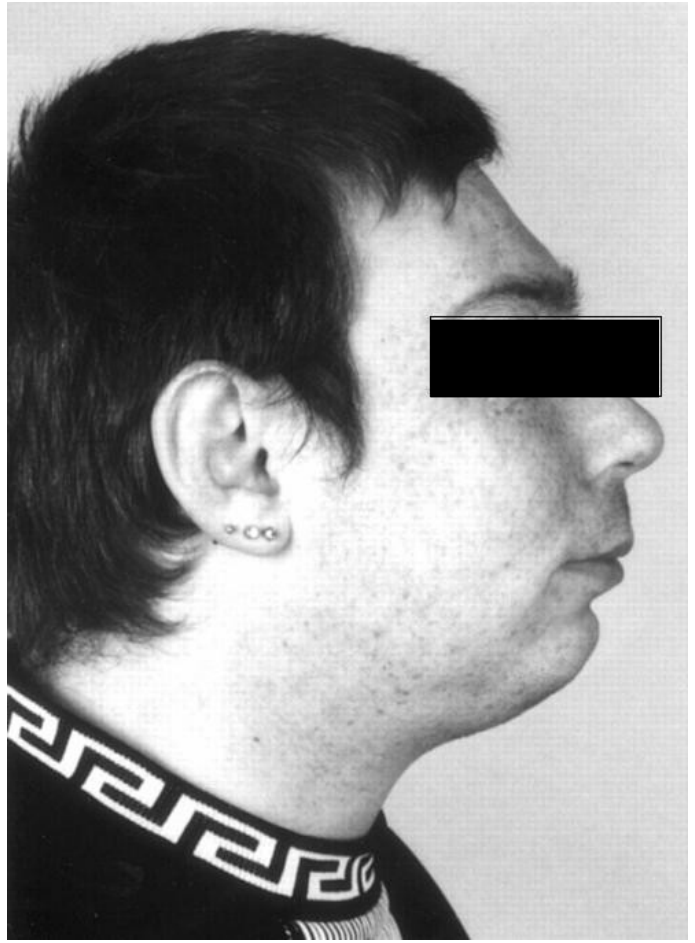
Consanguinity example

Homozygous mutation
BLM gene
c.1642C>T, p.(Gln548*)

Genetic diseases in Czech population

Nijmegen breakage syndrome = Seeman syndrome (NBS)

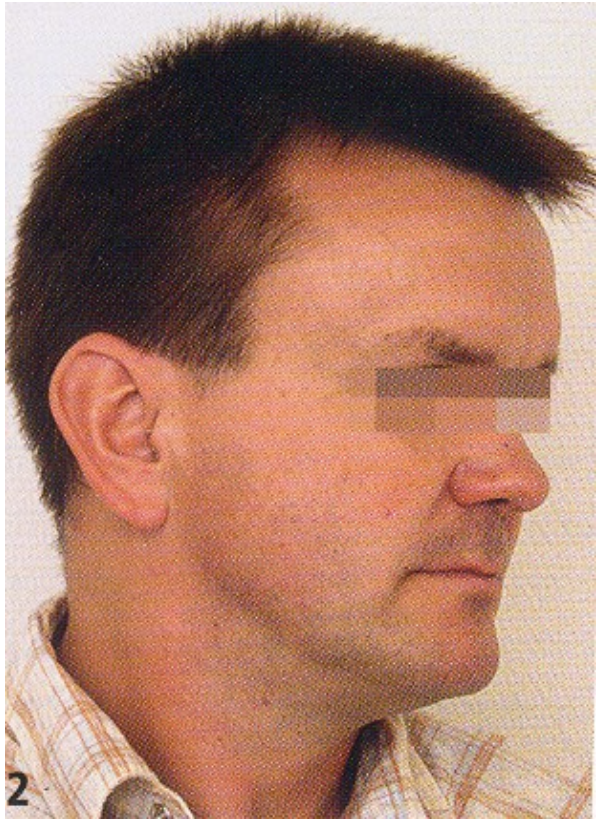
NBN gene for nibrin in 8q21
Heterozygotes 1:130-150
Common ancestor

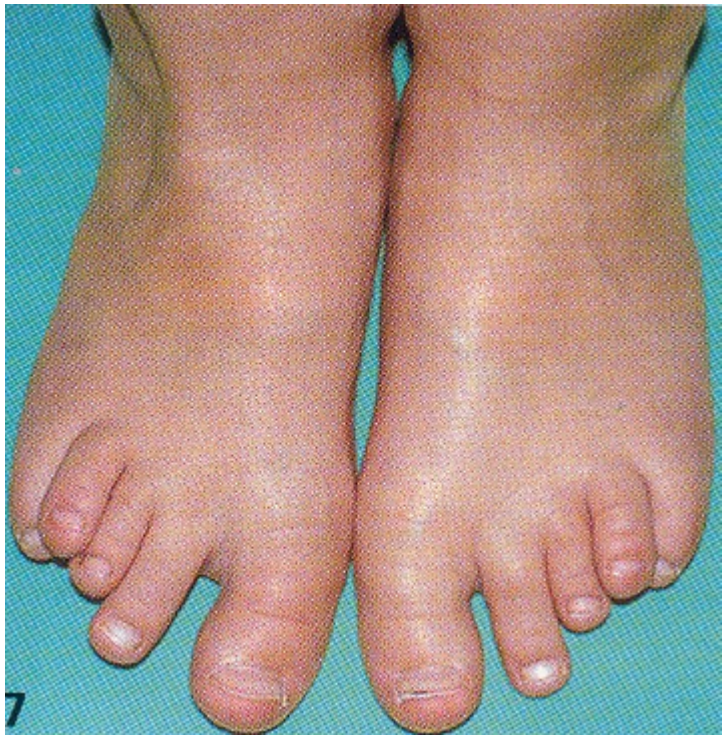


Seemanová, 1985

Czech dysplasia

COL2A1 gene
absence of ocular and orofacial anomalies
shortening of third and/or fourth toes





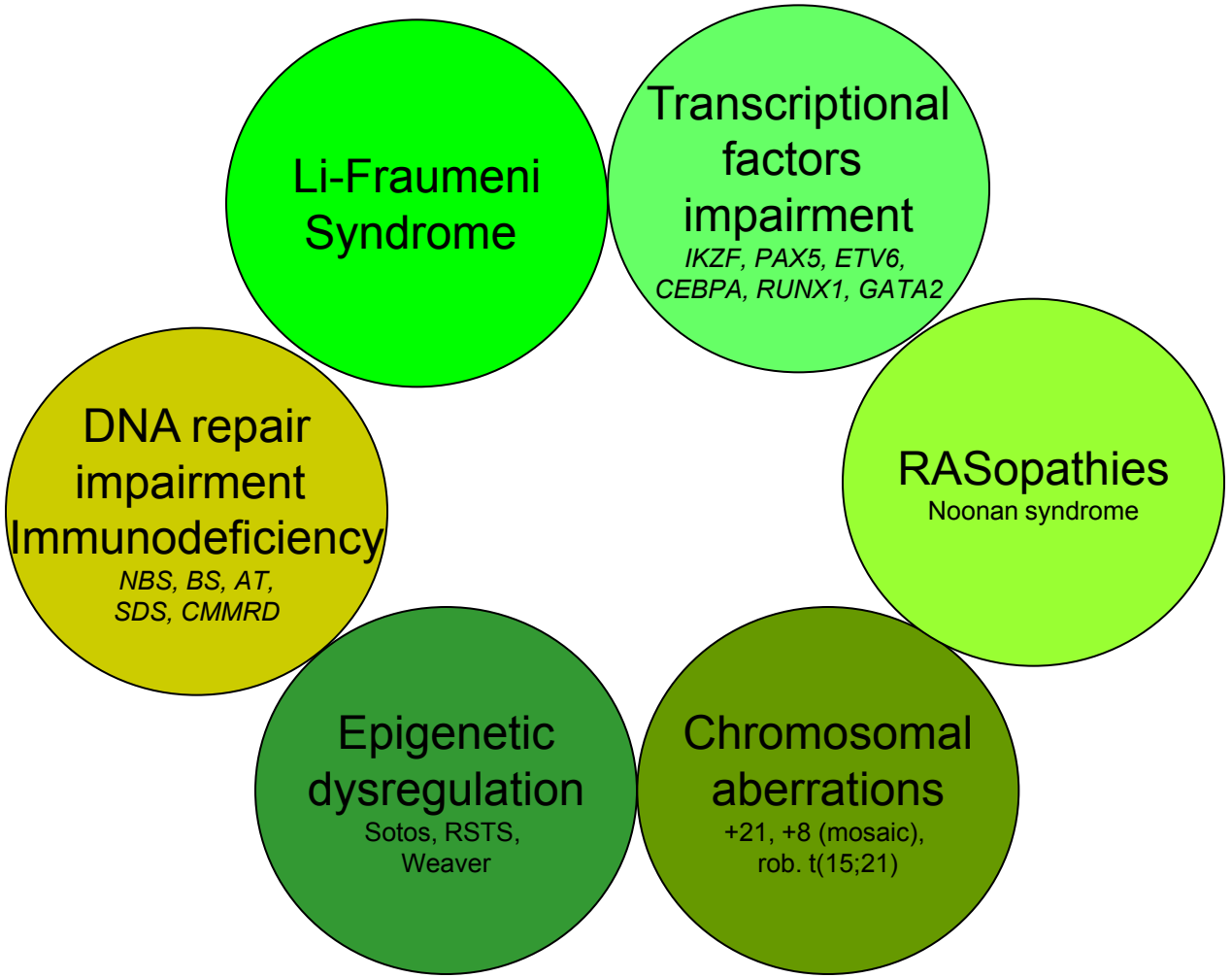
Neumann, 2003

How do we search for new genetic disorders?

Molecular biology methods

- Analysis of known disease-associated genes
- Comparing genetic information of healthy and affected family members
- Looking for new variants and “new” genes
- Verification by functional tests

DNA variants and disorders



- **genes together with non-coding regions**

Twins

- Genetic vs. nongenetic influences
  - **monozygotic**: 100% of identical alelleles
  - **dizygotic**: twins/siblings 50% of identical alelleles

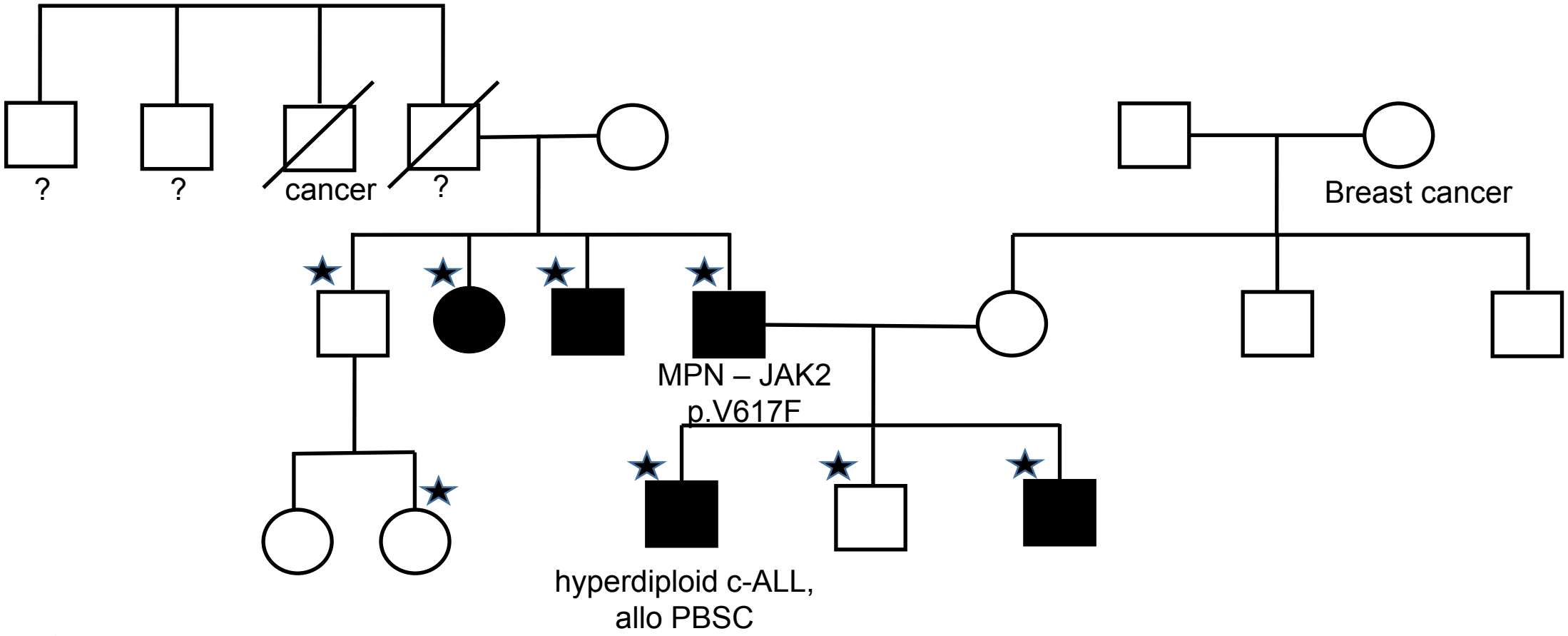
- Genetic influence: (concordance in MZ and DZ twins):

  Diabetes mellitus
  Schizophrenia
  Lupus
  Cleft
  Sclerosis multiplex

Are monozygotic twins genetically identical?

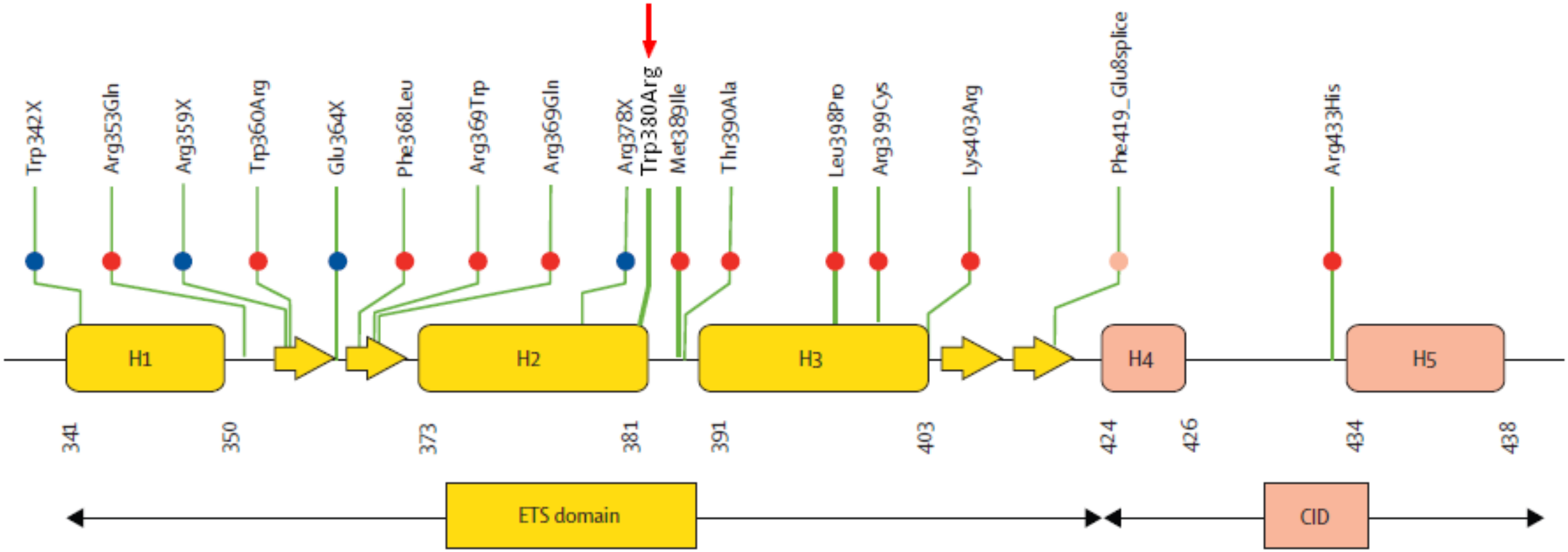
Clinical cases

Clinical case: thrombocytopenia



ETV6

- Exome sequencing – exome comparison of healthy and affected family members
- variant in gene *ETV6*: p.W380R



Adjusted from Moriyama et al., 2015

Bioinformatics and biostatistics

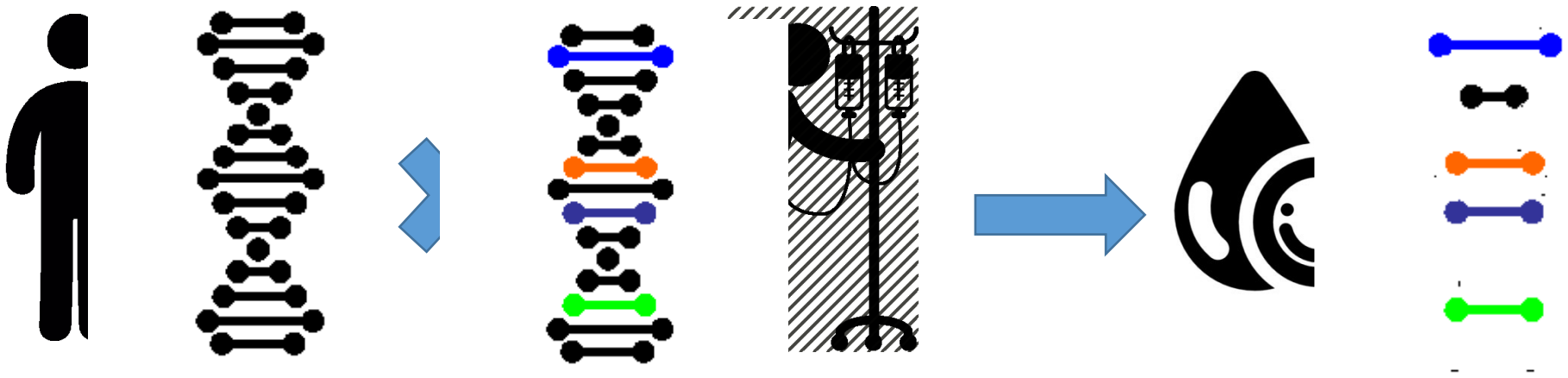
- Mapping on a reference sequence (BWA-mem)
- build-up of two variant files (Samtools mpileup):

1. file – affected family members
2. file – healthy family members

Exclusion of population and familial variants (VarScan):
Selection of variants present only in affected family members

Identification of potentionally causal variants:
variant anotation (Annovar)
filters: coverage > 20
left only exonic, ncRNA exonic, downstream and upstream variants
left out synonymous variants
excluded variants annotated in dbSNP dtb. with rsXXXXX ID

Bioinformatics and biostatistics



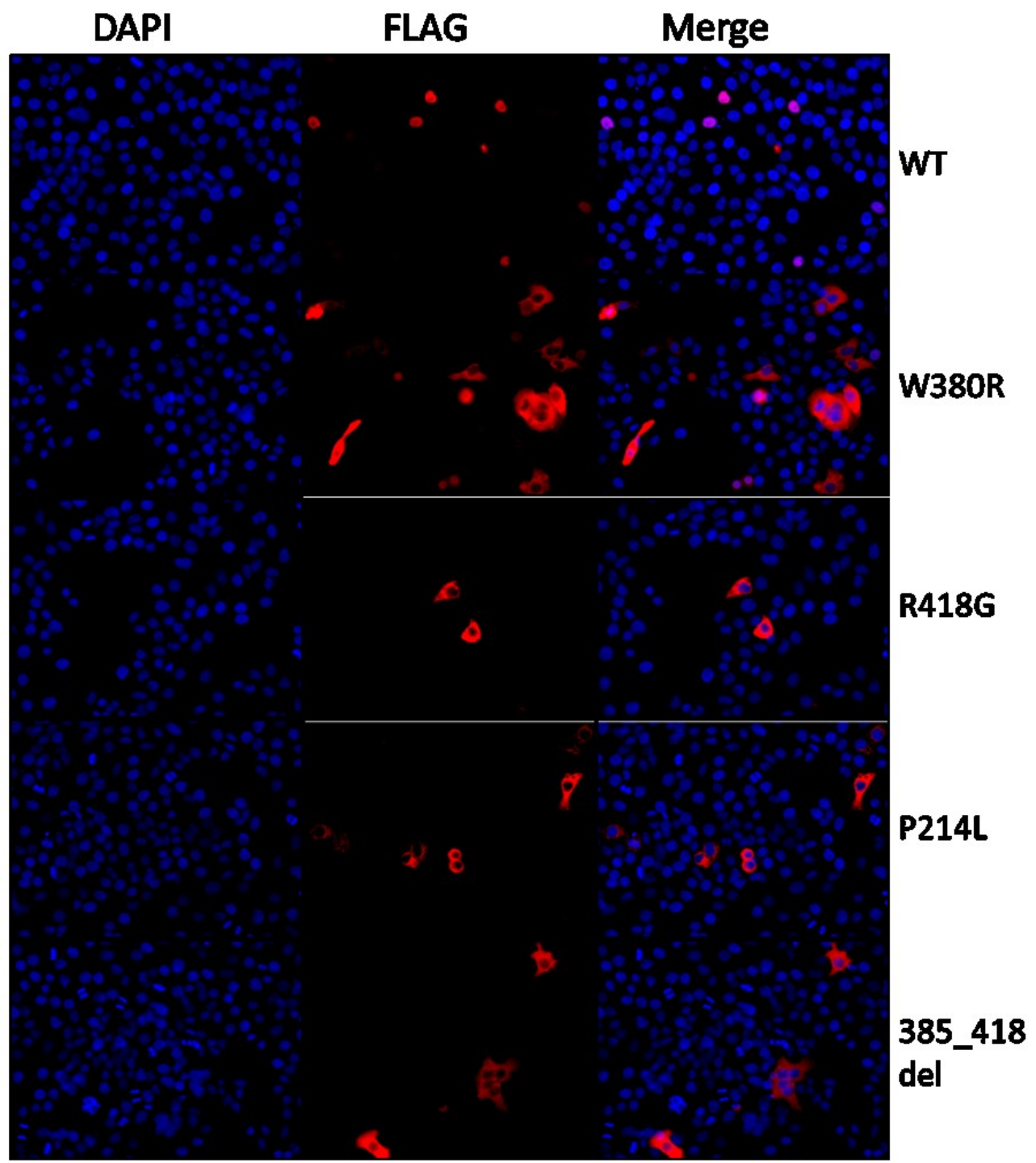
Annotated mutations

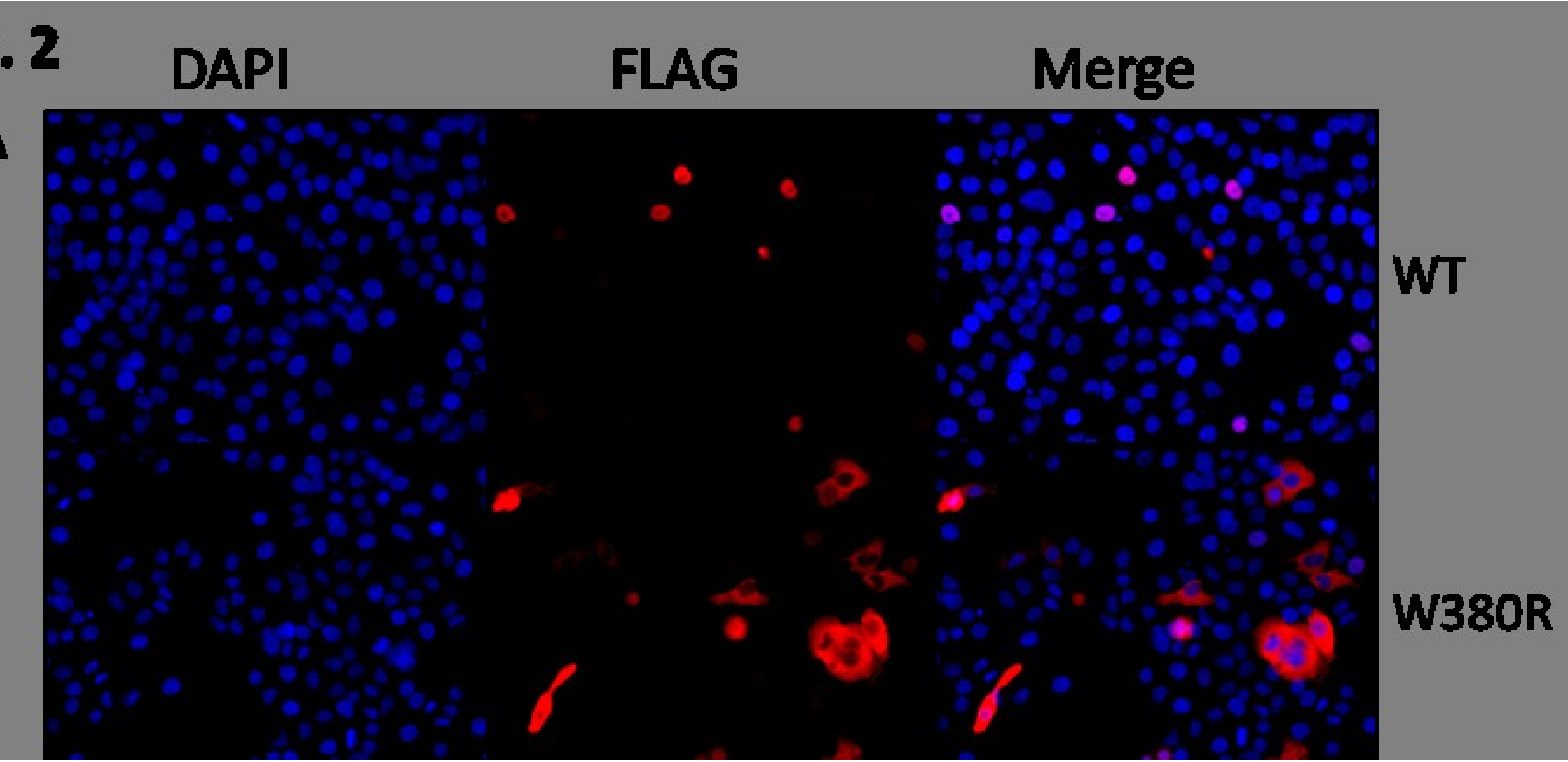
Variant frequency in population

Variant effect on the protein structure

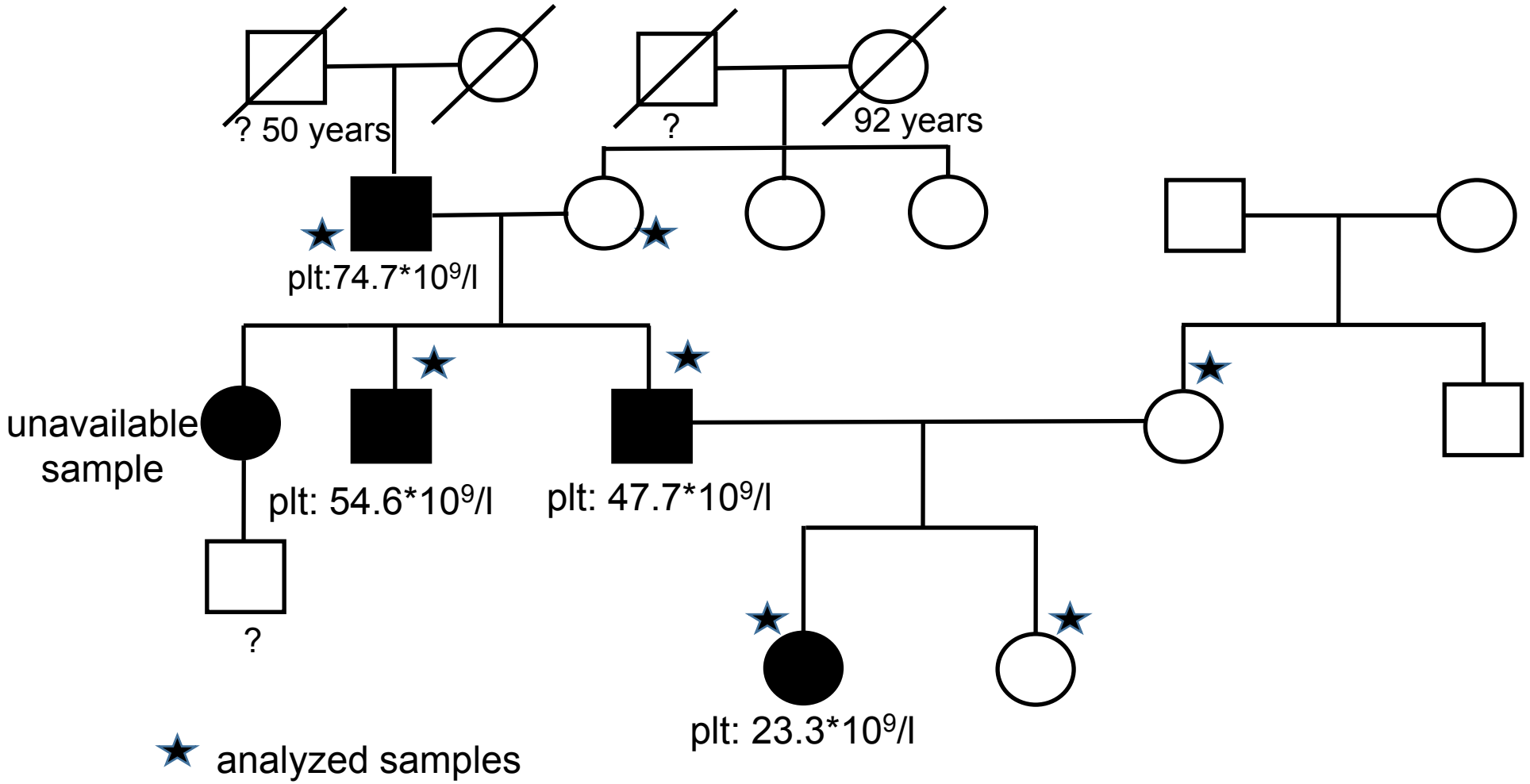
Functional analysis of *ETV6* :

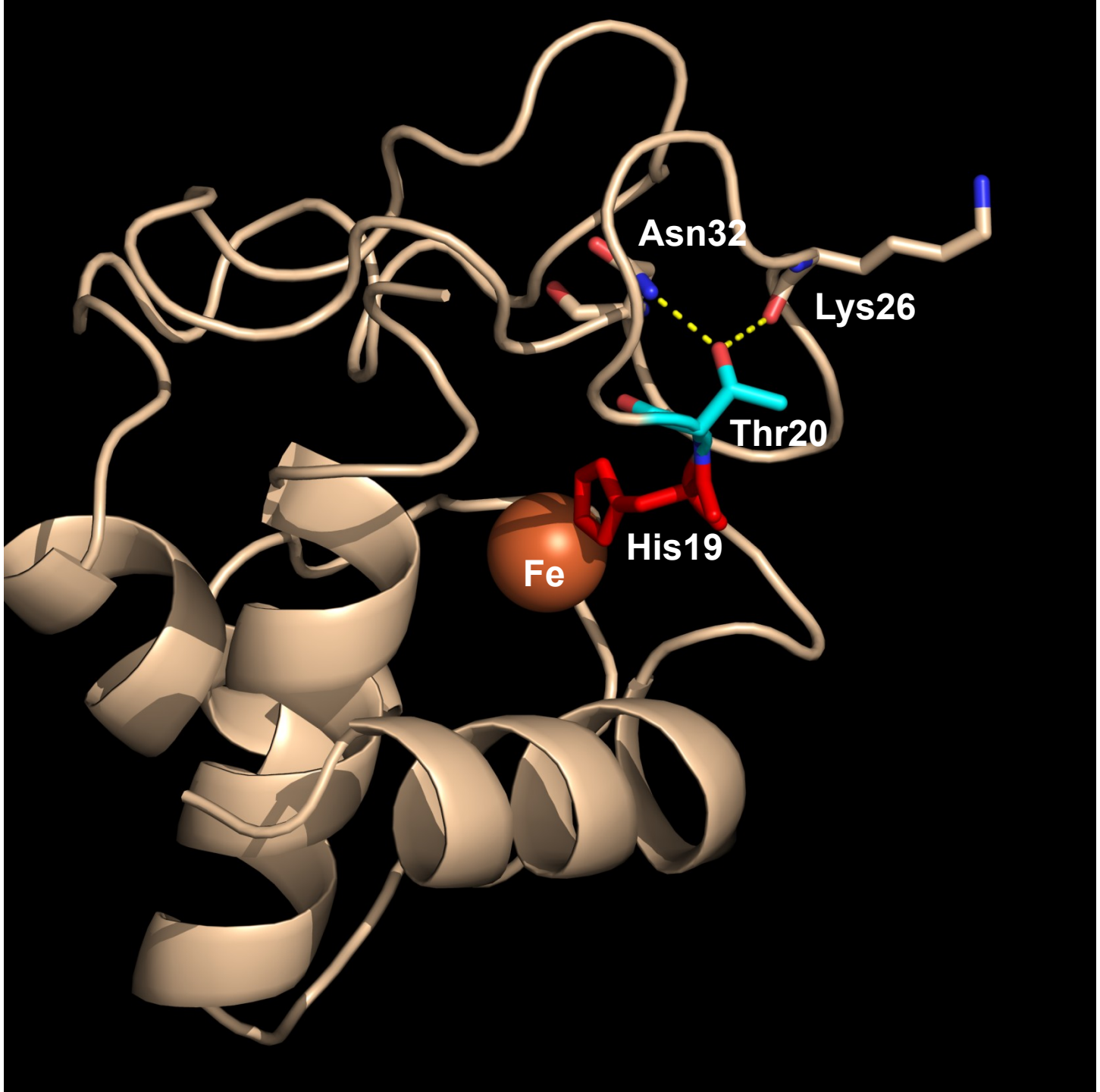
fluorescence microscopy




. 2
DAPI
FLAG
Merge
WT
W380R

CYCS: exon 2, p.T20I





CYCS: p.T20I

What are the skills of clinical geneticist?

- **complex examination**

- **gene/s analysis indication**
  - exome sequencing
  - genome sequencing
  - functional tests

- **results interpretation**
  (from practitioners to clinical geneticists)

- **therapeutic and preventive intervenation proposal**
  - respecting wishes of affected individuals together with ethical aspects

What are the skills of clinical geneticist?

- **complex examination**

- **gene/s analysis indication**
  - exome sequencing
  - genome sequencing
  - functional tests

- **results interpretation**
  (from practitioners to clinical geneticists)

Complex information analysis – complex is more than the sum of individual parts

- **therapeutic and preventive intervenation proposal**
  - respecting the desire of affected individuals together with ethical aspects

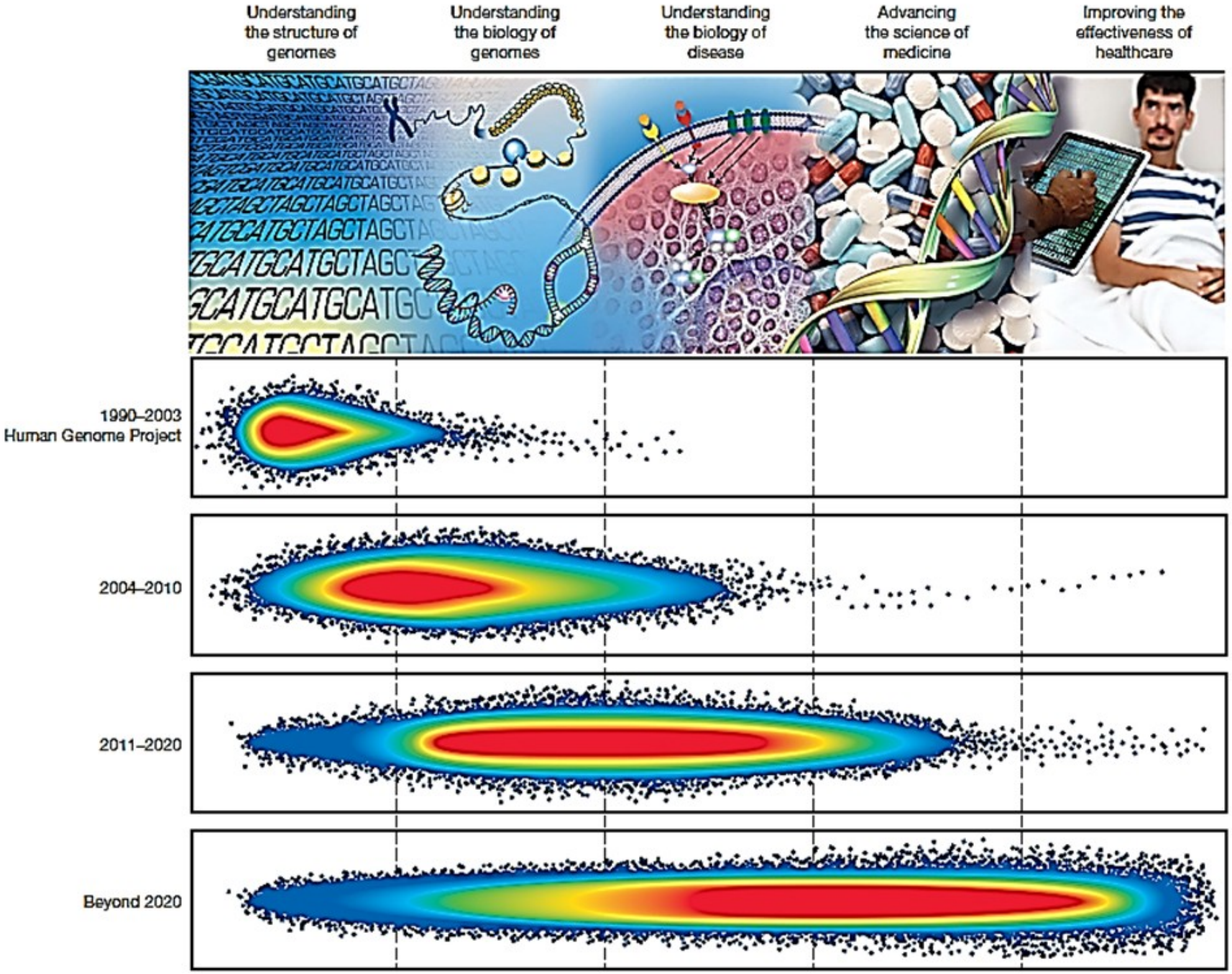
Why genetics?

- **Disease diagnostics:**
  - prenatal
  - preimplantational
  - genetic counselling

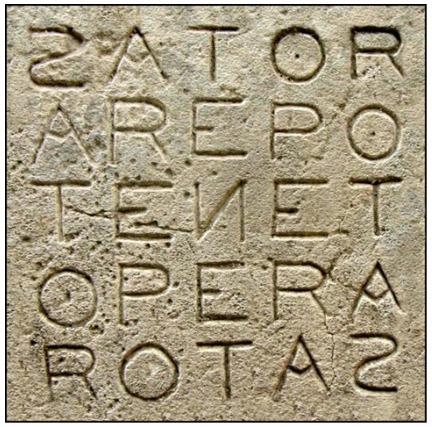
- **Therapy:**
  - pharmacogenetics
  - pharmacogenomics
  - immunogenetics

- **Prevention**

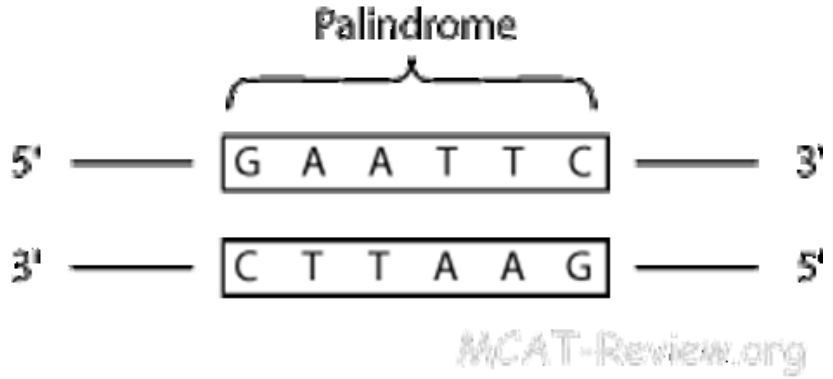
- **Gene therapy, genome editing**

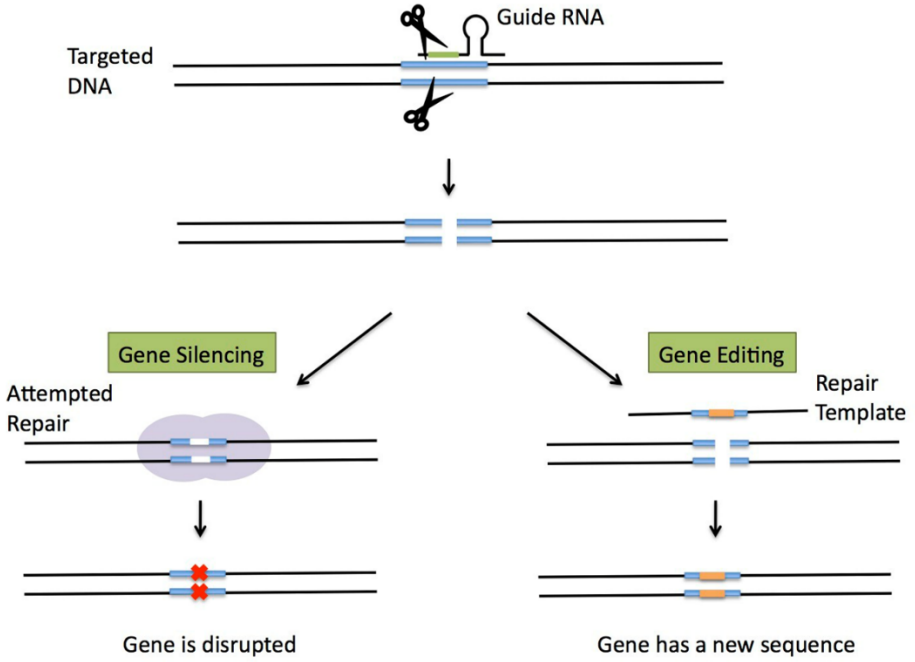

Understanding the structure of genomes
Understanding the biology of genomes
Understanding the biology of disease
Advancing the science of medicine
Improving the effectiveness of healthcare
1990–2003 Human Genome Project
2004–2010
2011–2020
Beyond 2020

CRISPR-Cas9



Pompeie, 79AD





Made-to-order children?

"Your weight problem is partly genetic and partly Boston Cream pie."