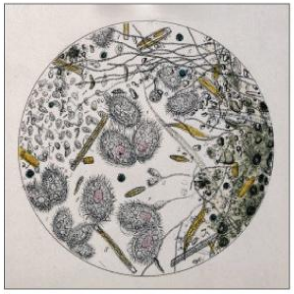
Practical applications of modern methods of DNA analysis

Detection and identification of microorganisms

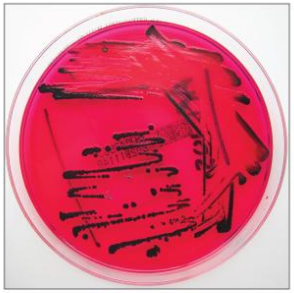
- Classical microbiology relies on culturing of microorganisms
- Modern methods allow direct detection and identification of microorganisms.

circa 1600:
Microscope invented

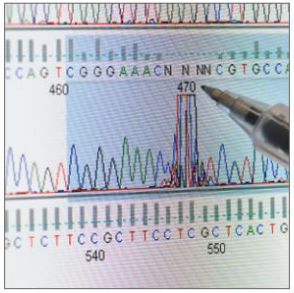


mid-1600s:
First microbes described

1800s:
Connection made between microbes and disease



1800s – Present:
Culture, staining, and microscopy used to study microbes that can be cultured



1990s:
DNA sequencing becomes available, allowing study of microbes that cannot be cultured

PCR detection of pathogens

- Total DNA is isolated from a specimen.
- PCR is performed using pathogen-specific primers
- PCR reaction products are analyzed by gel electrophoresis
- If pathogen is present in the specimen an amplified DNA band of specific molecular mass should appear on the electrophophoregram.

- Theoretically a single bacteria or virus could be detected using PCR.
- Closely related microorganisms could be distinguished by targeting strain-specific DNA regions.
- More precise methods utilize restriction analysis of the PCR fragment or its sequencing.

Microbiomics

- Microbiota - A collection or community of microbes. Microbiota is studied for environmental, agricultural and biomedical applications.
- In order to study microbiota total DNA from the specimen is sequenced using next-generation methods
- If specimen comes from human then human DNA sequences are excluded.
- Microorganisms are detected and identified by presence of specific DNA sequences called DNA markers.

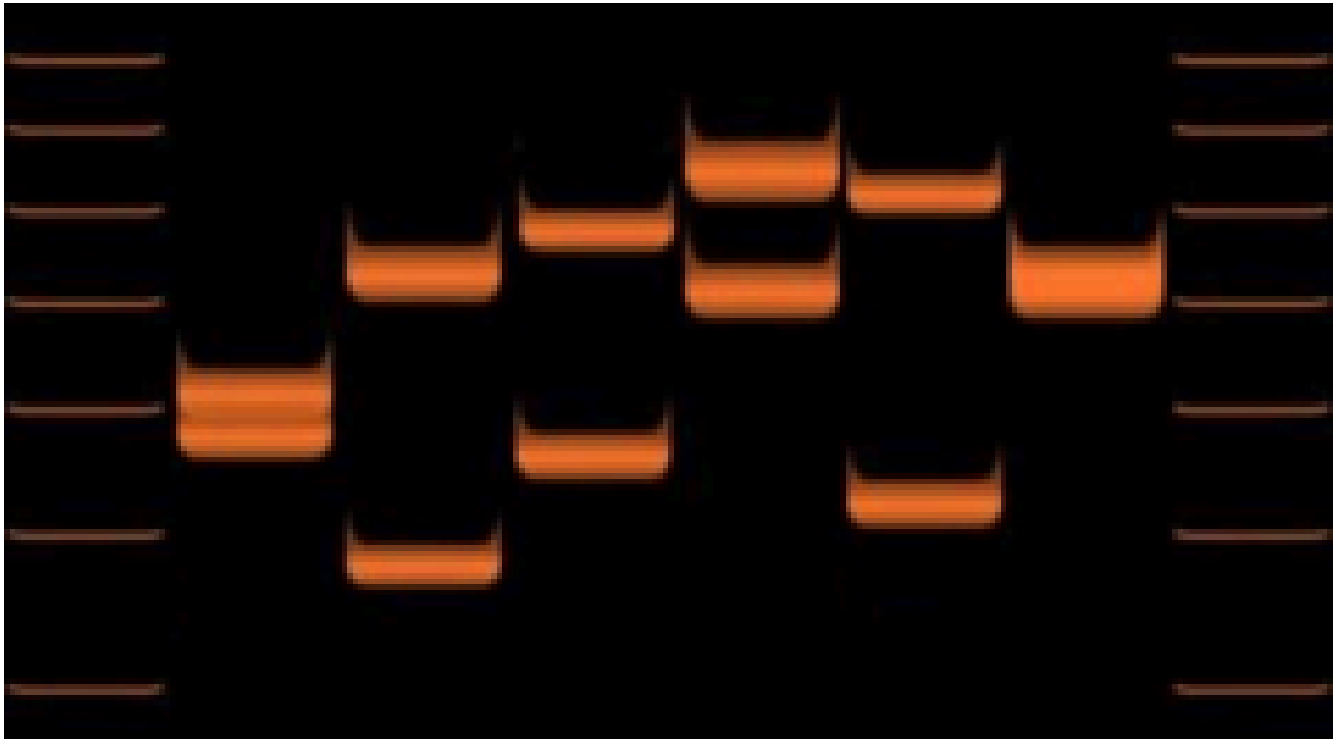
Genotyping

- Members of the same species have genetic variations. The process of determining differences in the genetic make-up (genotype) of an individual is called genotyping
- Genotyping is used for medical and forensic purposes

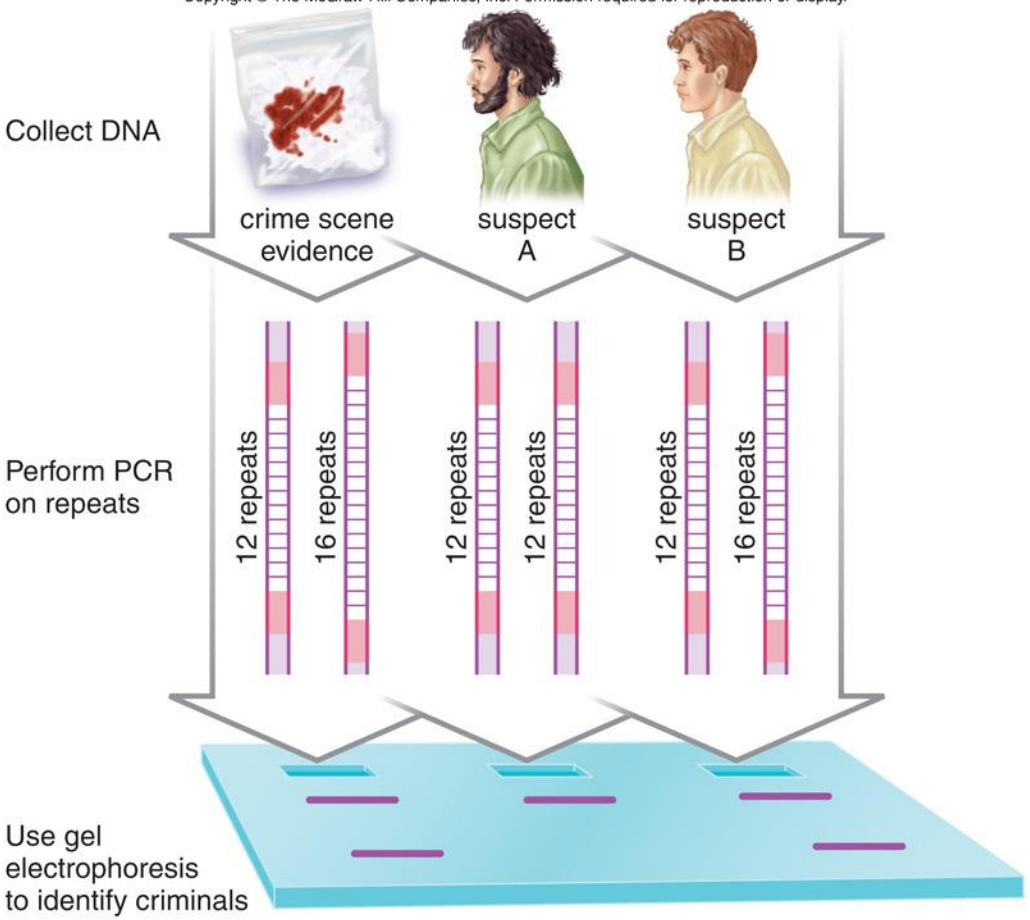
DNA profiling (fingerprinting)

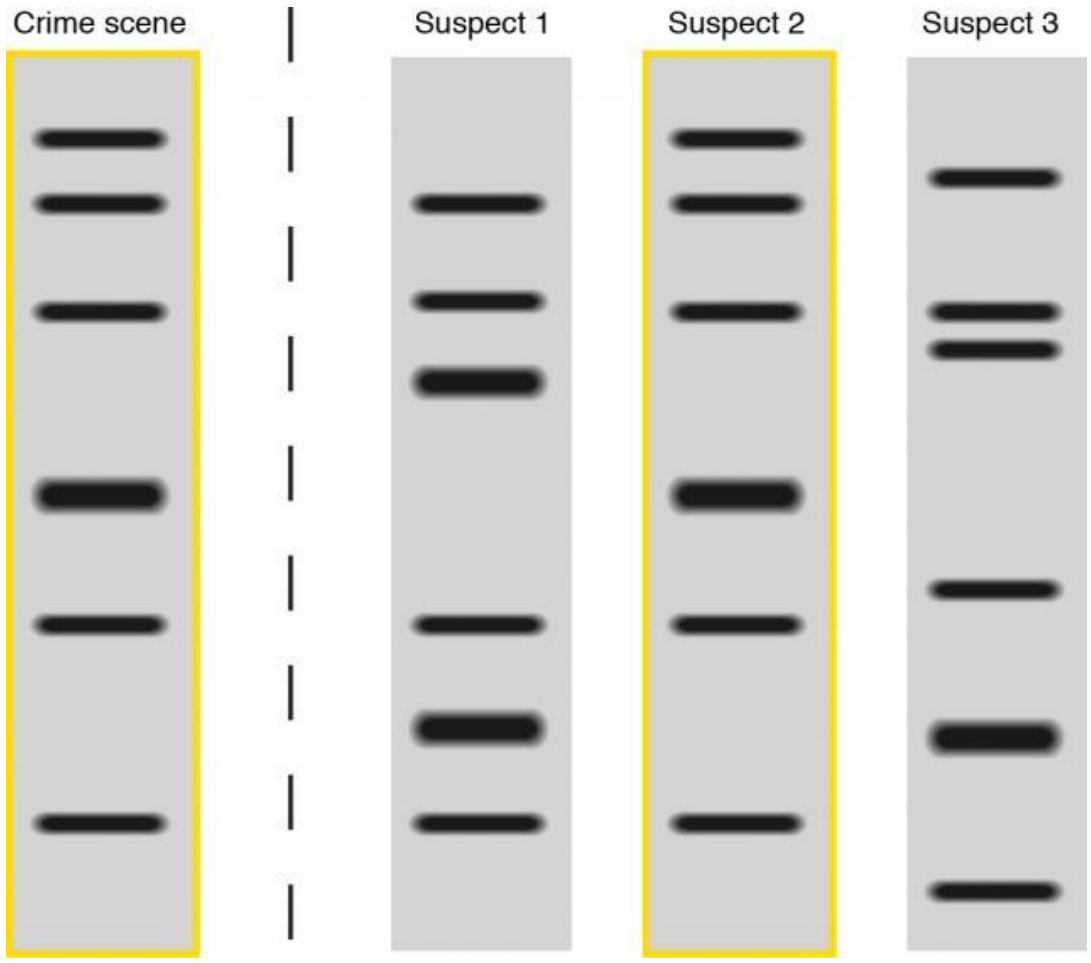
- DNA profiling is the process of determining an individual's DNA characteristics, called a DNA profile, that is very likely to be different in unrelated individuals, thereby being as unique to individuals as are fingerprints.
- Used as a forensic technique in criminal investigations to identify an unidentified person or whose identity needs to be confirmed, or to place a person at a crime scene or to eliminate a person from consideration
- Also used in in parentage testing and in genealogical research

- DNA profiling uses repetitive ("repeat") sequences that are highly variable, called variable number tandem repeats (VNTRs), in particular short tandem repeats (STRs), also known as microsatellites, and minisatellites.



Variations of VNTR allele lengths in 6 individuals.




Crime scene
Suspect 1
Suspect 2
Suspect 3

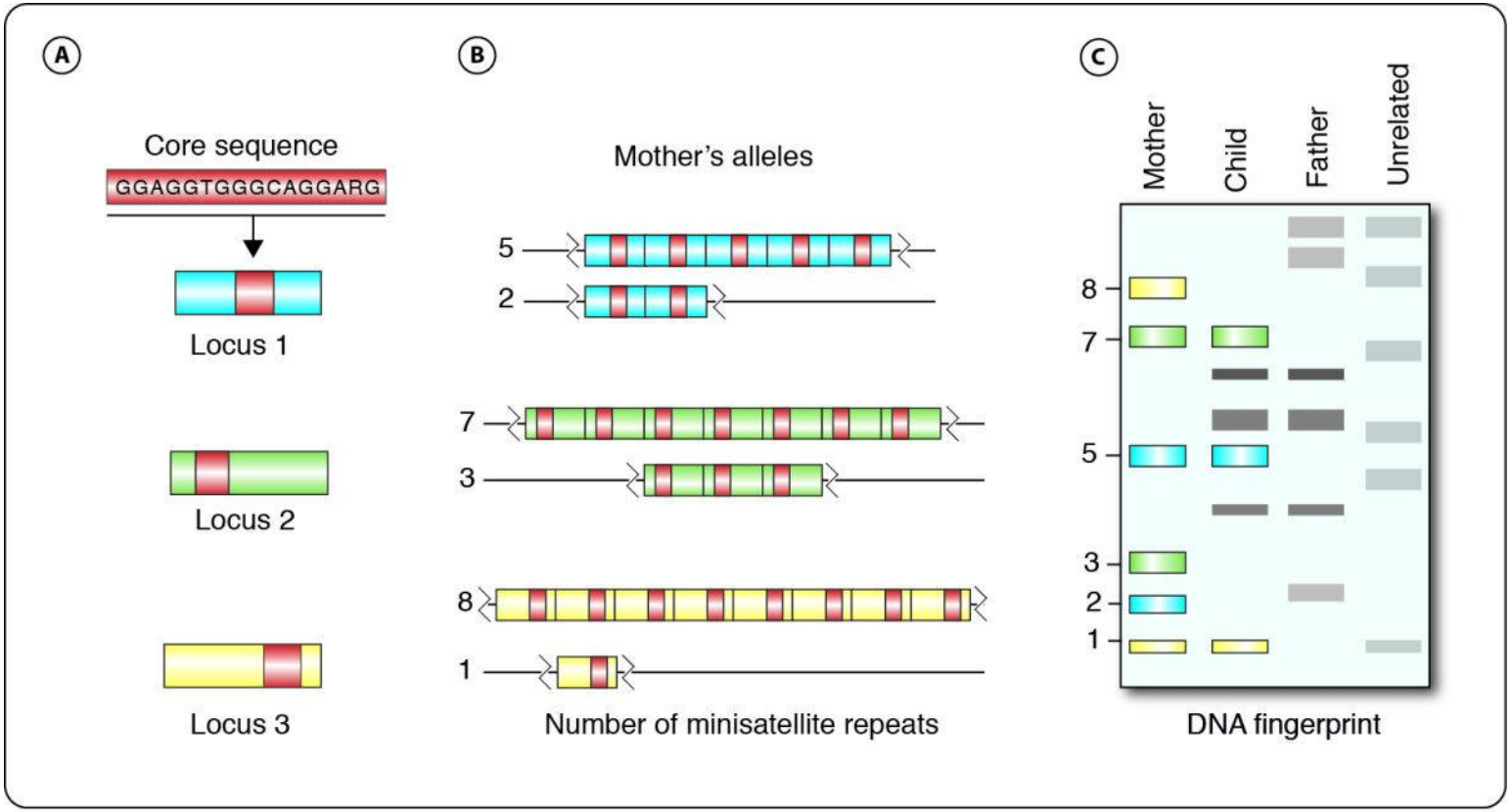
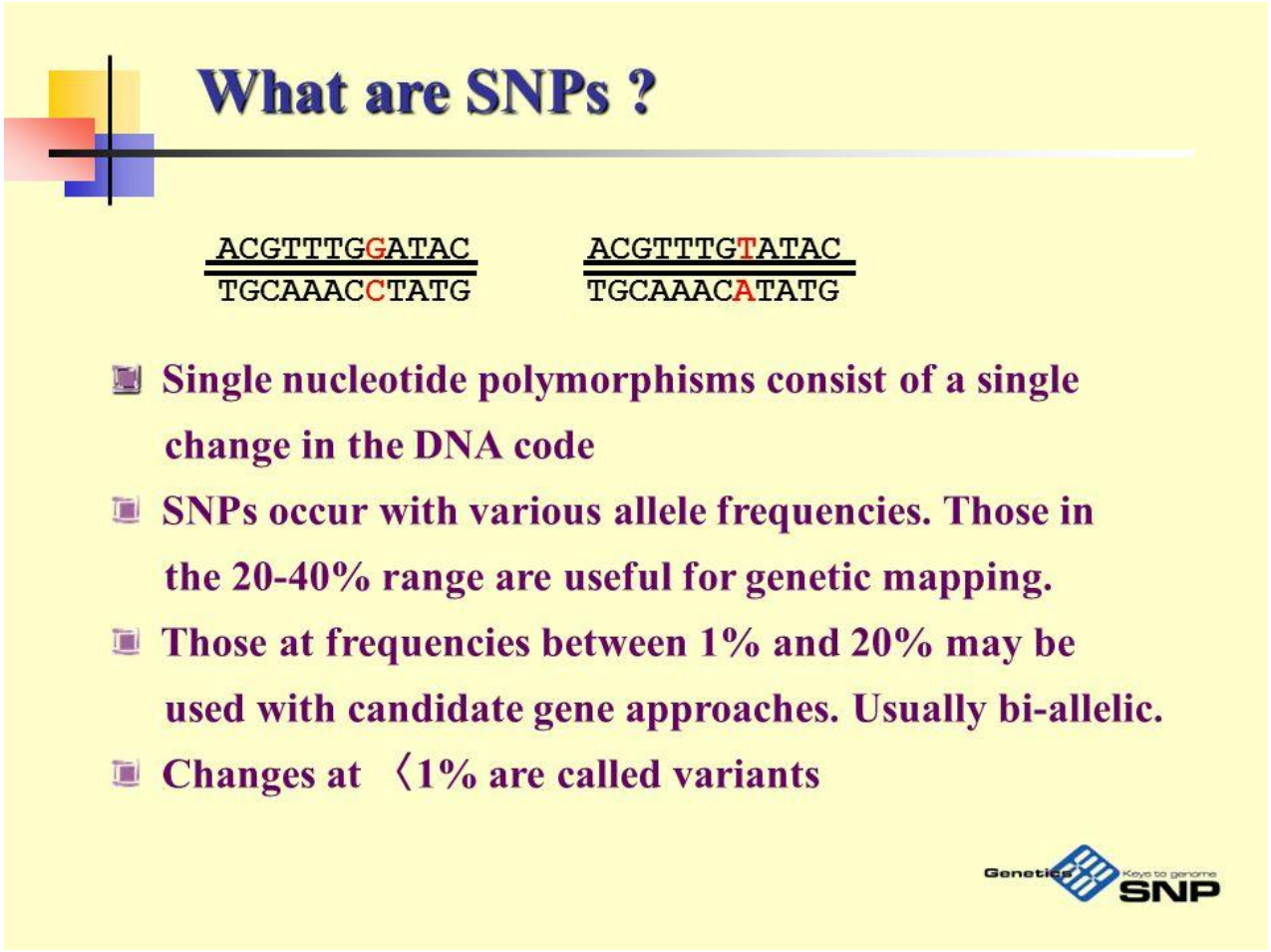
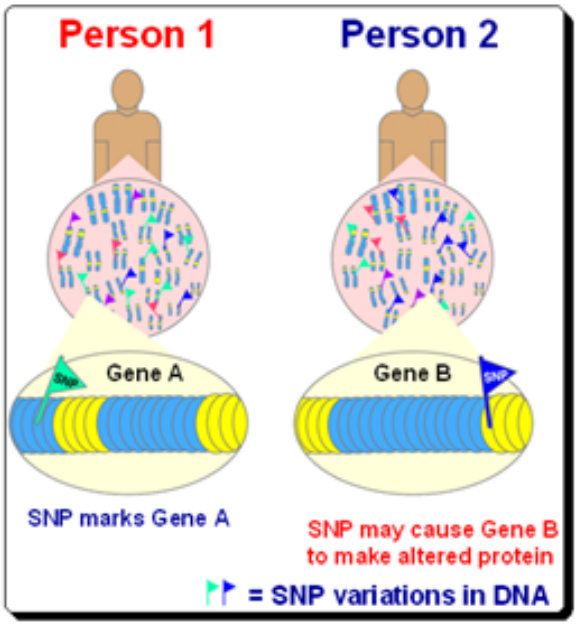


Fig 1. Chambers et al. 2013 I Investigative Genetics

Single-nucleotide polymorphisms (SNPs)



WHY SNPs ARE SO IMPORTANT ?

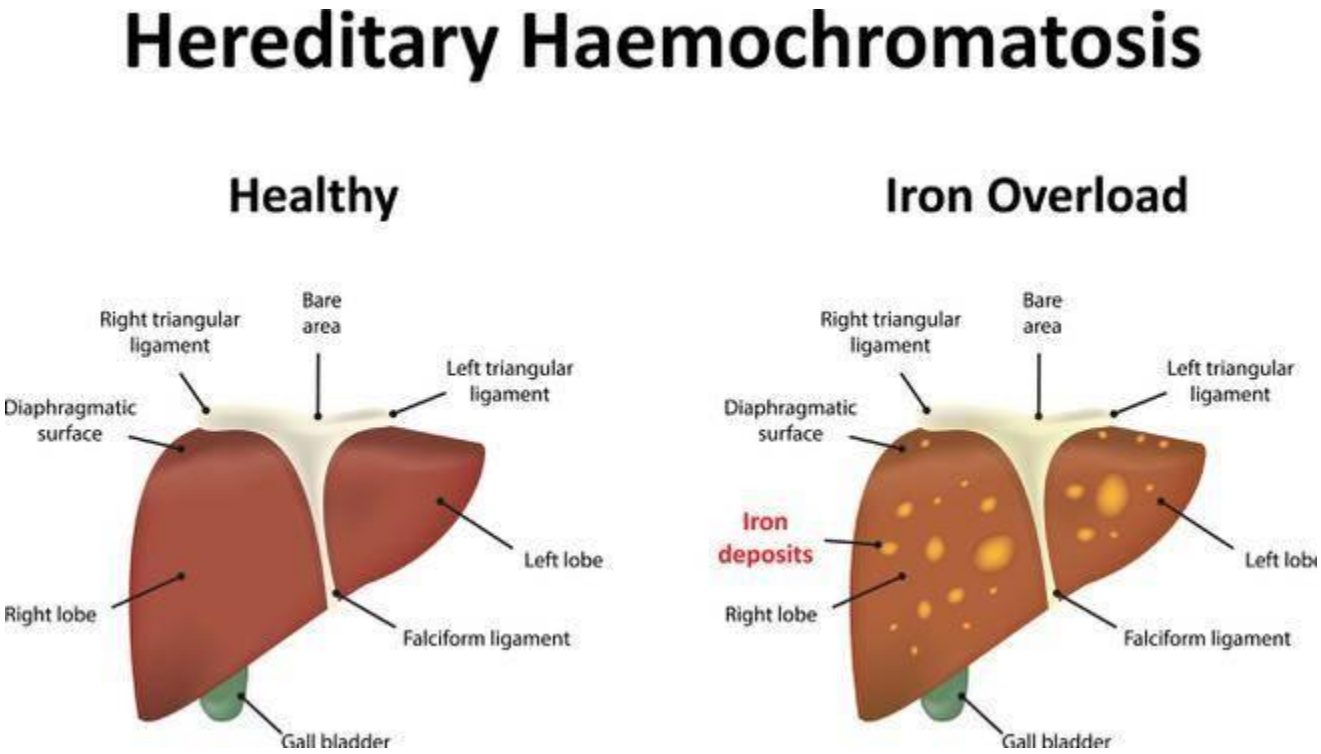


1. SNPs can cause silent, harmless, harmful, or latent effects.
2. Most SNPs occur in noncoding regions and do not alter genes.
3. The remaining SNPs occur in coding regions. They could alter the protein made by that coding region, which in turn could influence a person's health.

"SNP is the key enabler in the realization of the concept of personalized medicine".

So, let us have a look how proteins are changed in presence of SNPs

Example – hereditary hemochromatosis

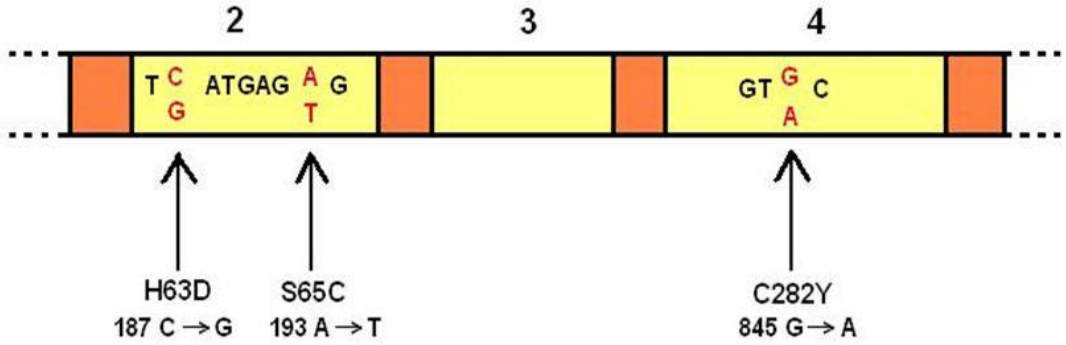


Genetic basis

- Principal HFE gene defect was first described in 1996,
- Tightly linked to the HLA-A locus on chromosome 6p
- **G-to-a missense mutation** ;
- **Cysteine → tyrosine at 282 (C282Y)**
  - **C282Y homozygotes account for 80%-85%**
- Histidine → aspartate at 63 (h63d)
- Serine → cysteine at 65 (s65c)

- Methodology: DNA is isolated from peripheral blood and is analyzed for two (2) mutations in the HFE gene, which have been associated with hereditary hemochromatosis. The analysis is performed by restrictionanalysis of PCR-amplified segments of the HFE gene:
- C282Y mutation is detected by restriction with Rsa1, and
- the H63D mutation is detected with Mbo1.

HFE gene mutations



5′...GATC...3′
3′...CTAG...5′

MboI