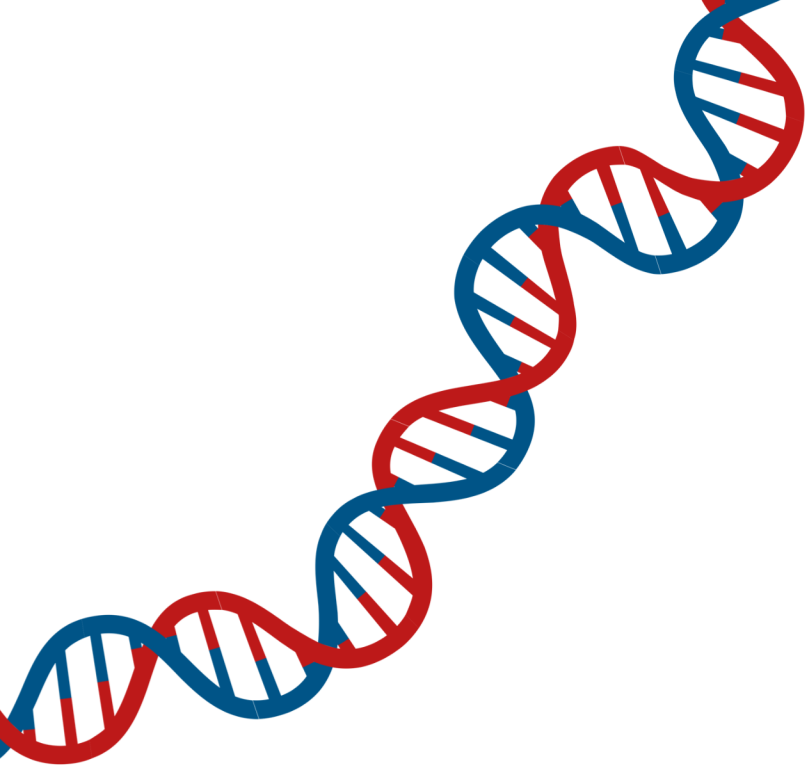


A Clinician's Guide to the Human Genome

Unraveling Genomic Variants for Patient Care

Created by:
Maryam Vaseghi-Shanjani (MSc)
Emilie Théberge (MSc)
Dr. Phillip Richmond (PhD)

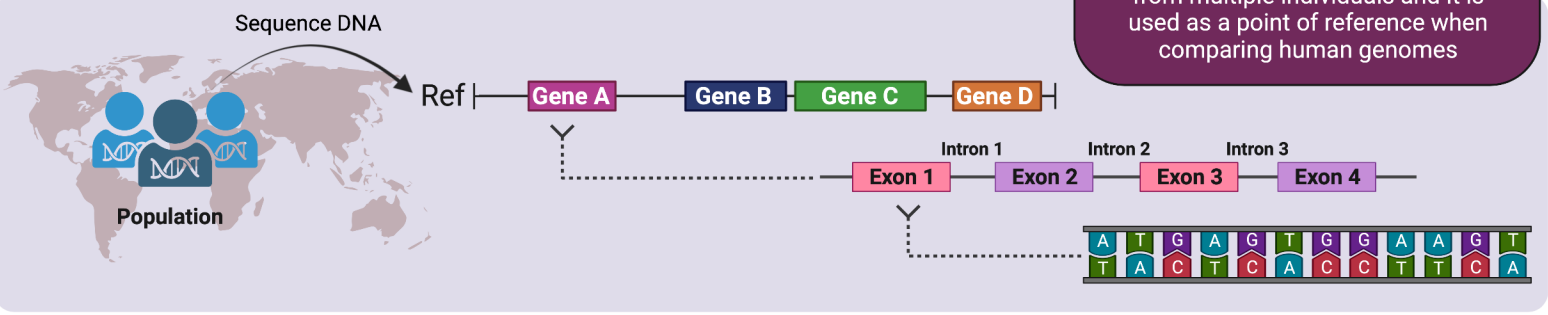
THE UNIVERSITY OF BRITISH COLUMBIA

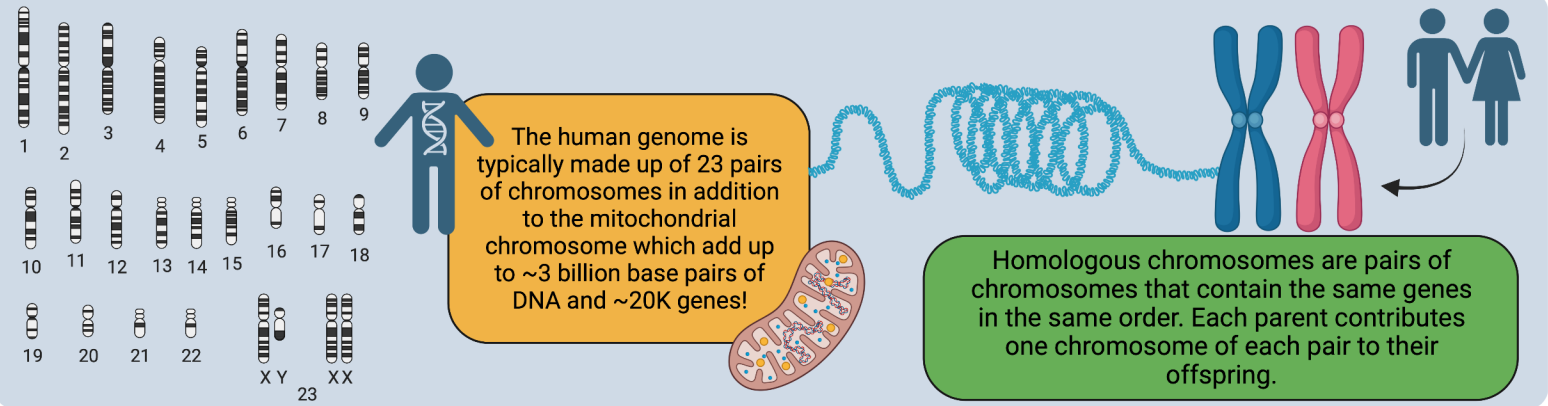
Genome BritishColumbia

BC Children's Hospital Research Institute

Understanding the Human Genome and Genomic Variants

The reference genome is assembled from multiple individuals and it is used as a point of reference when comparing human genomes



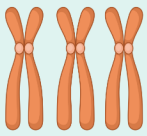


The human genome is typically made up of 23 pairs of chromosomes in addition to the mitochondrial chromosome which add up to ~3 billion base pairs of DNA and ~20K genes!

Homologous chromosomes are pairs of chromosomes that contain the same genes in the same order. Each parent contributes one chromosome of each pair to their offspring.

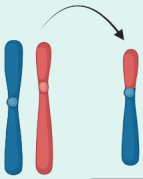
Genomic variants are differences in the DNA sequence of an individual compared to a reference genome and these variants come in different shapes and sizes, here are a few examples of them:

Chromosomal Variants



Aneuploidy

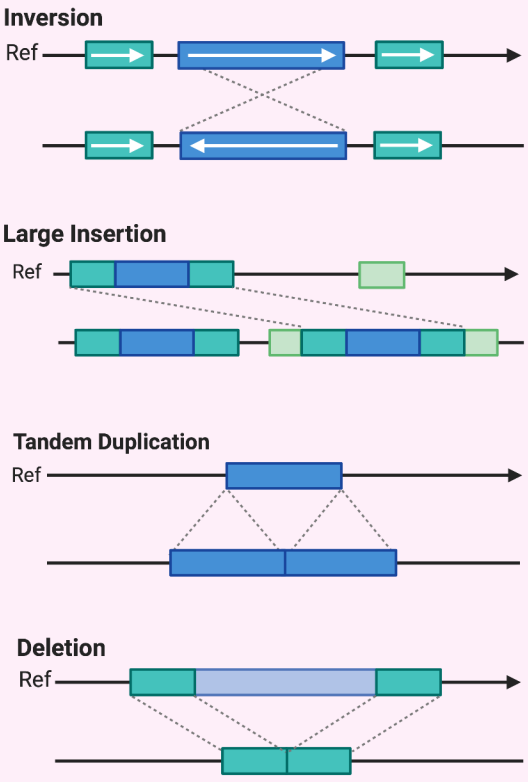
Aneuploidy is a condition in which the affected individual has an abnormal number of chromosomes



Chromosomal Translocation

Chromosomal translocation occurs when a part of one chromosome breaks off and attaches to another chromosome

Large Structural Variants



Small Genomic Variants

Single Nucleotide Variant (SNVs)

Ref A C G C T G A

A C G T T G A

Insertion

A C G C T G A

A C G T G C T G A

Deletion

A C G C T G A C T

A C G _ _ _ A C T

Repeat Expansion Variant

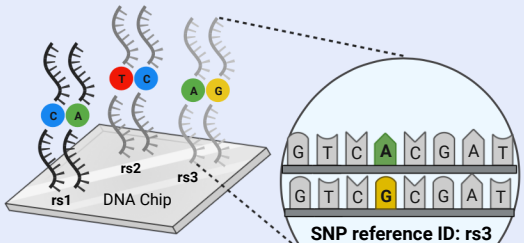
A C G C T G A

A C G C C G C C G C C T G A

These variations can have a range of effects, from having no noticeable impact to causing disease.

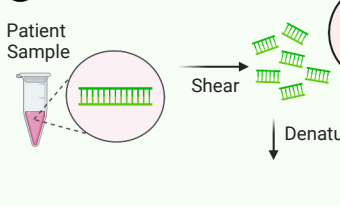
Technologies for the Detection of Genomic Variants

DNA Microarray



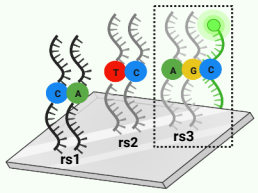
The chip's surface has thousands of synthetic single-stranded DNA probes representing reference genome sequences or known common single nucleotide polymorphisms (SNPs) in the population

1 DNA Preparation & Tagging



When patient DNA is washed over the chip, it binds to a sequence match and emits measurable fluorescence

2 Chip Hybridization

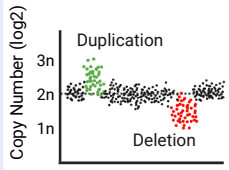


Detect single nucleotide polymorphisms (SNPs)

rs	Chr	Pos	Allele 1	Allele 2	Patient
rs3	12	100	A	G	G / G

Based on the fluorescence intensity for the allele 1 and 2 probes for each SNP, the patient genotype is determined

Detect copy number variants (CNVs)



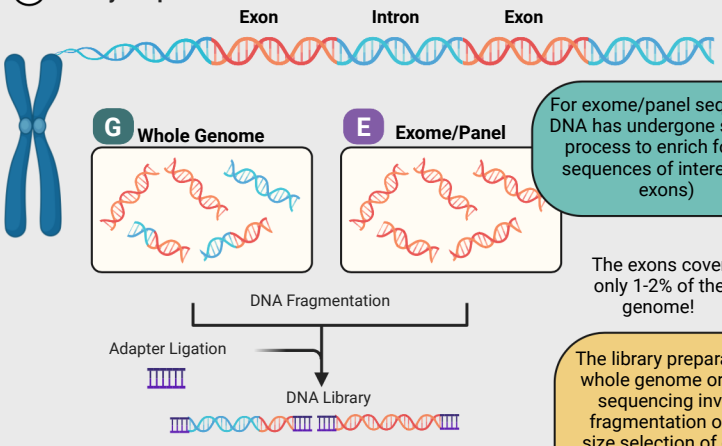
Comparing the signal intensity of each probe to reference samples enables inference of DNA copy number, which can inform deletions and duplications

Can detect large CNVs (>50 kb) and known pre-targetted SNPs represented by the probes

Unable to detect new or rare variants that are not represented by a probe on the DNA chip

Next Generation Sequencing

1 Library Preparation

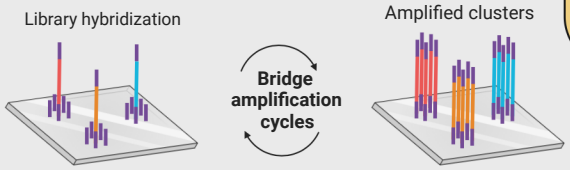


For exome/panel sequencing, DNA has undergone selection process to enrich for DNA sequences of interest (e.g. exons)

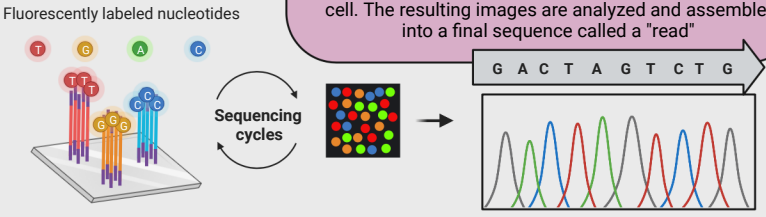
The exons cover only 1-2% of the genome!

The library preparation for whole genome or exome sequencing involves fragmentation of DNA, size selection of desired fragments, adapter ligation, and PCR amplification to create a library of fragments ready for sequencing

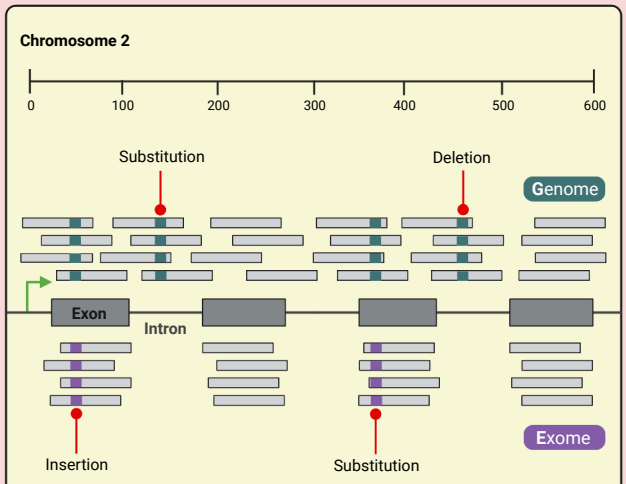
2 DNA library bridge amplification



3 DNA library sequencing



The DNA library is loaded onto a sequencing platform where the individual DNA fragments are sequenced in parallel one base per cycle using fluorescently-labeled nucleotides which emit a color at each spot on the flow cell. The resulting images are analyzed and assembled into a final sequence called a "read"



The millions of individual reads from the sequencer are aligned to the reference genome and variants are called by identifying sequence differences between the sample and the reference genome

	Detect	Chr.	Pos.	Ref.	Alt.
Variant 1	G E	2	55	A	AC
Variant 2	G	2	130	C	T
Variant 3	G E	2	380	G	A
Variant 4	G	2	470	T	_

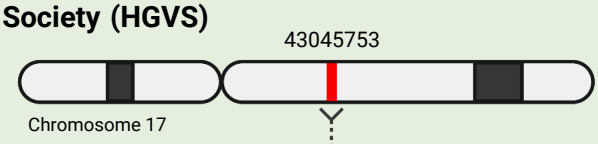
Exome sequencing is generally less expensive than whole genome sequencing, but it may miss variants in non-coding regions that could be relevant to disease

Exome sequencing can detect novel rare SNVs and Indels over exonic regions. Genome sequencing can detect novel and rare SNVs, Indels, large CNVs and simple structural variants in both exonic/intronic and intergenic regions

Both technologies have difficulty accurately characterizing complex structural variants such as inversions, translocations, large insertions, and repeat expansions in part due to the limited length of the sequencing reads

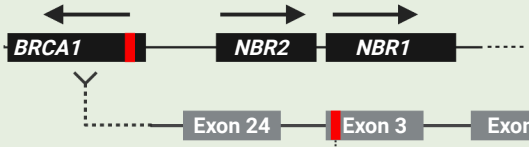
Interpreting Genomic Variants

Reporting variants with standardized nomenclature: Human Genome Variatio n Society (HGVS)

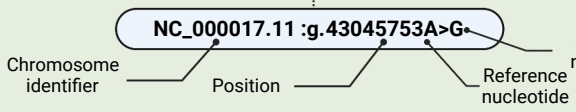


Patient has a *de novo* pathogenic variant in *BRCA1*
NM_007294.4:c.5516T>C

BRCA1 is on chromosome 17 and is on the "minus" strand; *NBR2* and *NBR1* are on the "plus" strand.

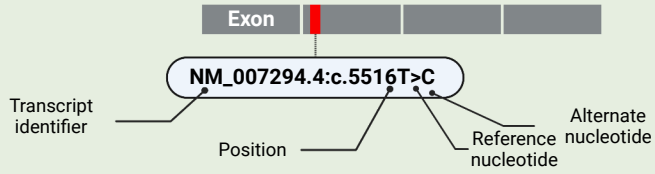


Genomic Position: "g."



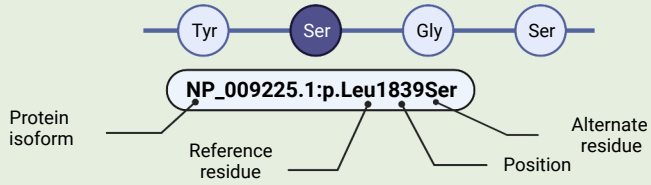
Genomic position is based on the "plus" strand and it starts at position 1 on the chromosome.

Coding Position: "c."



Coding position refers to the nucleotide positions within the exonic coding sequence of a gene, starting with "1" at the ATG start codon and extending through until the stop codon. For genes on the "minus" strand, the nucleotides are the complement of the genomic sequence.

Protein Position: "p."



Protein position denotes amino acid positions in a protein, from N- to C-terminal, reflecting changes due to change in the gene's coding region.

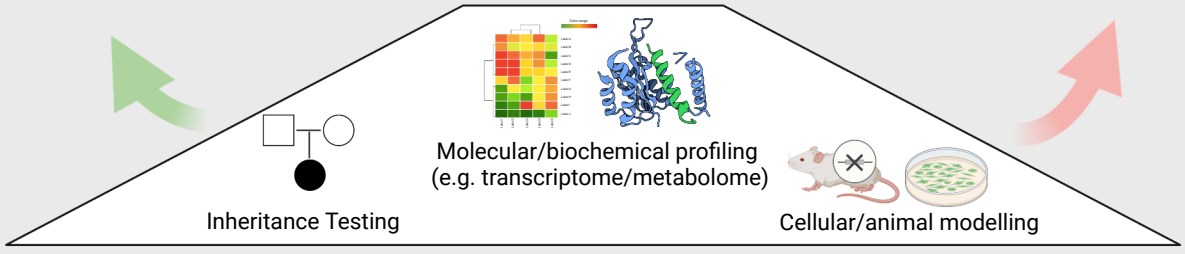
! The exact positions may differ between genome builds and transcript variants; for this example, here we use the GRCh38 (hg38) human reference genome assembly and the NM_007294.3 RefSeq transcript for *BRCA1*, which is the MANE select transcript model. Learn more at: https://www.ncbi.nlm.nih.gov/refseq/MANE/

Each variant is assessed based on the following characteristics: whether it has previously been identified as causing disease in a clinical database, its frequency within the population, the potential it has to damage gene function or expression, and how it segregates with disease occurrence in a familial context.

Clinical Databases	Population Databases	*In silico* Predictions	Inheritance
• Has the variant been previously identified as causing disease? • Is the gene of relevance to the disease in question?	• What is the frequency of variant allele in the healthy population? • Is this gene commonly mutated within the population with impactful variants?	• Does the variant alter the protein sequence or introduce a stop codon? • Is the base pair evolutionarily conserved across species?	• Is the variant *de novo*? • Does the variant segregate with the phenotype? • If the condition is compound heterozygous, are the variants present on different alleles ?
ClinVar, OMIM	gnomAD, UKBB	CADD, SpliceAI	Pedigree, Trio Sequencing

Through this process, candidate variants are categorized on a scale ranging from benign to pathogenic. However, despite these efforts, many variants may still remain classified as "VUS". To further categorize these as either benign or pathogenic, additional evidence is required. Techniques such as familial segregation analysis, cellular/animal modeling, and molecular/biochemical profiling can provide the necessary information to clarify the status of a VUS as either benign or pathogenic.

Benign | Likely Benign | **V**ariant of **U**ncertain **S**ignificance (VUS) | Likely Pathogenic | Pathogenic



Created with Biorender.com

Common Disease Risk via Genome-wide Association Studies and Polygenic Risk Scores

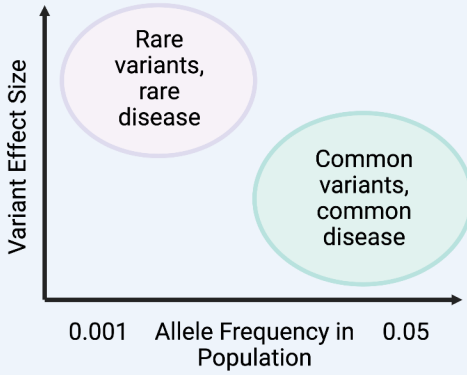
Common diseases caused by common variants

Complex diseases result from the cumulative effects of 100s to 1000s of common genetic variants of relatively small effect sizes interacting with the environment over time to manifest the trait.

For example: Alzheimer's disease, asthma, coronary artery disease, obesity

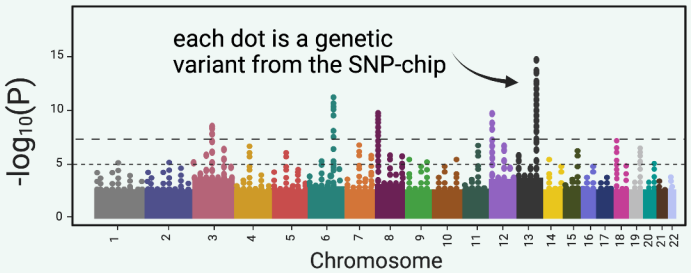
Generally, a "common" variant is seen in >5% of the population.

Variant frequencies differ by genetic ancestry, so what is considered "common" is relative to the referent population.



How do researchers find common variants associated with disease?
Genome-Wide Association Studies (GWAS)!

1. Genetic variant information is collected from a large population sample (early days 10,000-100,000; now 100,000-1 million)
2. Each person is labeled as either a case (have disease) or control (no disease)
3. Effect sizes and associated p values for all genetic variants are calculated based on how often the variants are present in cases and not in controls



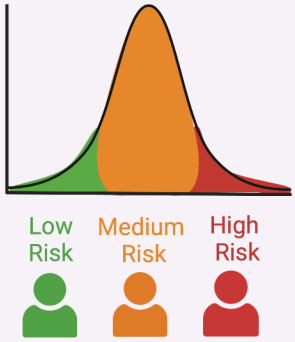
Higher negative log-transformed p value (y axis) means higher probability that this variant is associated with the disease

Clinical applications using GWAS

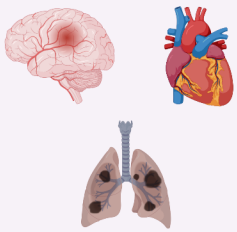
Many stakeholders are hoping to use information from GWAS to inform population-based screening for common complex diseases to help guide primary prevention interventions.

You can calculate an individual's relative risk of developing disease using GWAS results:

Polygenic Risk Scores (PRS) computing methods calculate a score integrating the number and effect sizes of the risk variants they carry, adding them up to estimate overall risk from many genetic risk variants.



Risk assessment studies for many common diseases are suggesting that individuals in the highest PRS risk group (i.e., 10th percentile) would benefit the most from preventive interventions.

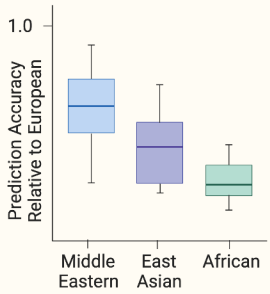


Researchers can also identify potential drug targets using GWAS results:

Applying gene network analyses, they can identify molecular pathways shared by many variants, and in what tissue(s) these pathways are expressed.

Concerns about the relative utility and generalizability of PRSs.

Many GWASs are made from mostly "White European" population samples. PRSs made from these GWASs are less accurate in assessing risk for individuals with non-European genetic ancestry.



Many efforts are underway internationally to develop diverse-ancestry-informed GWAS.

Several common diseases show pronounced sex differences in prevalence, age of onset, presentation (pathology), comorbid risk factor profiles, etc.

However, many GWASs regress sex out as a covariate (ignore within the model) and exclude X- and Y-chromosome genes from analysis. This does not allow for the genetic variants encoding mechanisms behind these sex differences to be identified.

More sex-disaggregated GWAS need to be done to identify which disease pathways are sex-specific and shared between both sexes.