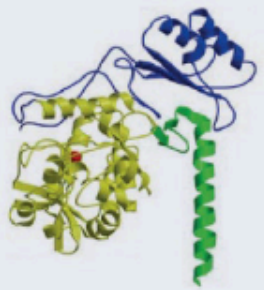
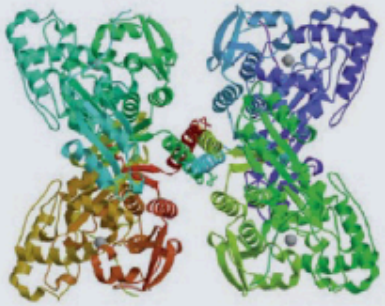
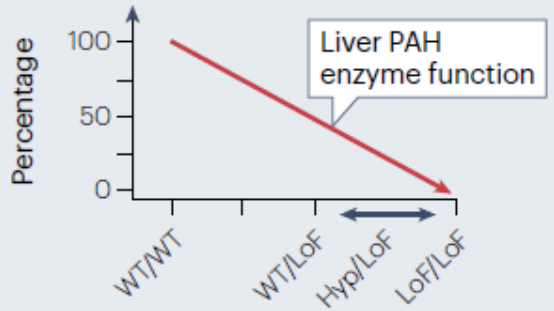
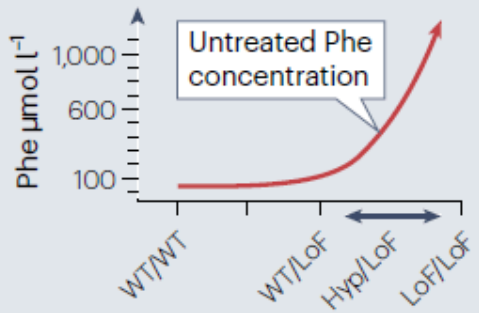
Module 3: Human genetic variation

Level	Variable functions and effects		Abnormalities in PAH deficiency	Treatment strategies in PAH deficiency
Genotype				
DNA ↓	Genetic and epigenetic variants		Variants in the *PAH* gene	Potential gene therapy
Transcript				
↓ mRNA ↓	Transcript quantity and sequence		Abnormal transcript amount and/or sequence	Potential mRNA replacement therapy
Different phenotype levels				
↓ Protein ↓	Protein quantity, structure and function		Abnormal amount, stability, activity and/or regulation of PAH enzyme	Pharmaceutical supplementation of the cofactor tetrahydrobiopterin stabilizes mutant proteins

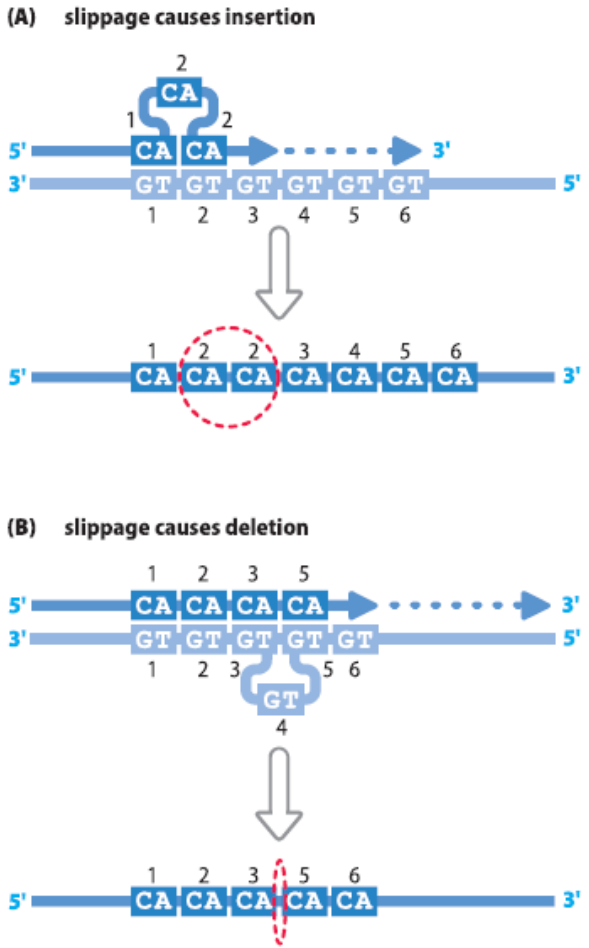
Multimer	Structural interactions		Some variants interfere with active tetramer formation	
Enzyme	Molecular function		Heterozygous variants cause reduced enzyme activity	
Cell metabolism	Regulatory and/or compensatory effects		Increased levels of Phe and its metabolites and reduced levels of Tyr and its products	Dietary reduction of Phe and supplementation with Tyr to prevent abnormalities
	Cellular function			
	Biochemical and network function			Exogenous Phe ammonia lyase

Organ	Organ function		Increased levels of Phe interferes with transport processes at the blood–brain barrier	Supplementation with large neutral amino acids
Organism	Clinical manifestation		Progressive intellectual disability	Symptomatic therapy

Origins of sequence variation - mutation mechanisms

1. DNA Replicatin Errors

- DNA polymerases occasionally insert the wrong nucleotide resulting a in **base mismatch**
- **Replicate slippage**: Occasionally misalignment between template and newly synthesized strands occur in regions of short tandem oligonucleotide repeats



Origins of sequence variation - mutation mechanisms

2. Chromosome segregation and recombination errors

- Errors in chromosome segregation changes chromosomal DNA copy number
- Sometimes chromatids can misalign during recombination and subsequent crossovers results in duplication/deletion

3. Exogenous chemical damage to DNA

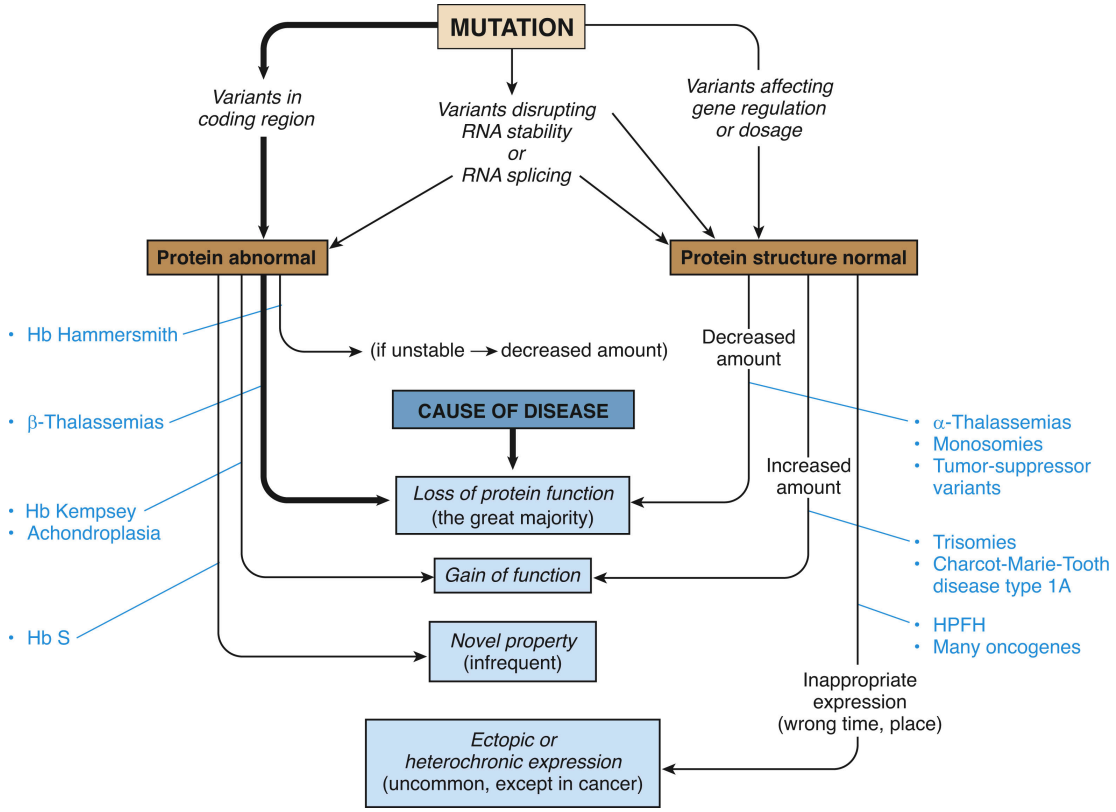
- **Mutagens**, such as radiation and harmful chemicals are external agents that can induce mutation.

Origins of sequence variation - mutation mechanisms

4. Endogenous chemical damage to DNA

- Base deletion through hydrolytic damage
- Oxidative damage due to reactive oxygen species (ROS) causing breakage of DNA strands or base modification.
    - ROS are generated in different cellular pathways and play important roles in intercelullular and intracellular signalling paths.
- Aberrant DNA methylation: sometimes methyltransferase can inappropriately methylate DNA to produce harmful bases

Genetic mechanisms of pathogenesis



- Variants in the coding region result in structurally abnormal proteins that have a loss or gain of function or a novel property that causes disease.
- Variants in noncoding sequences include (1) those that alter the stability or splicing of the messenger RNA (mRNA) and (2) those that disrupt regulatory elements or change **gene dosage**.
- Variants in regulatory elements alter the abundance of the mRNA or the time or cell type in which the gene is expressed.

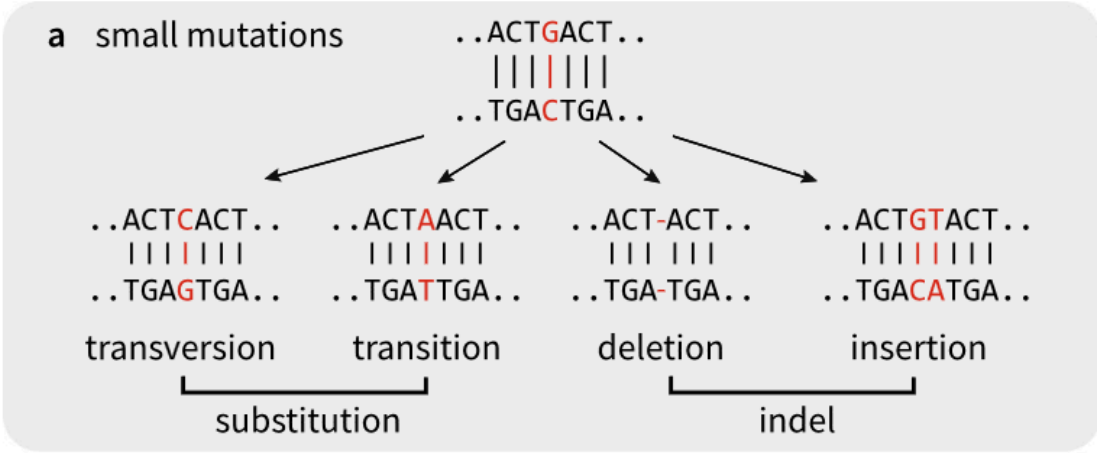
Types of Variation in Human Genetic Disease - Nucleotide Substitutions

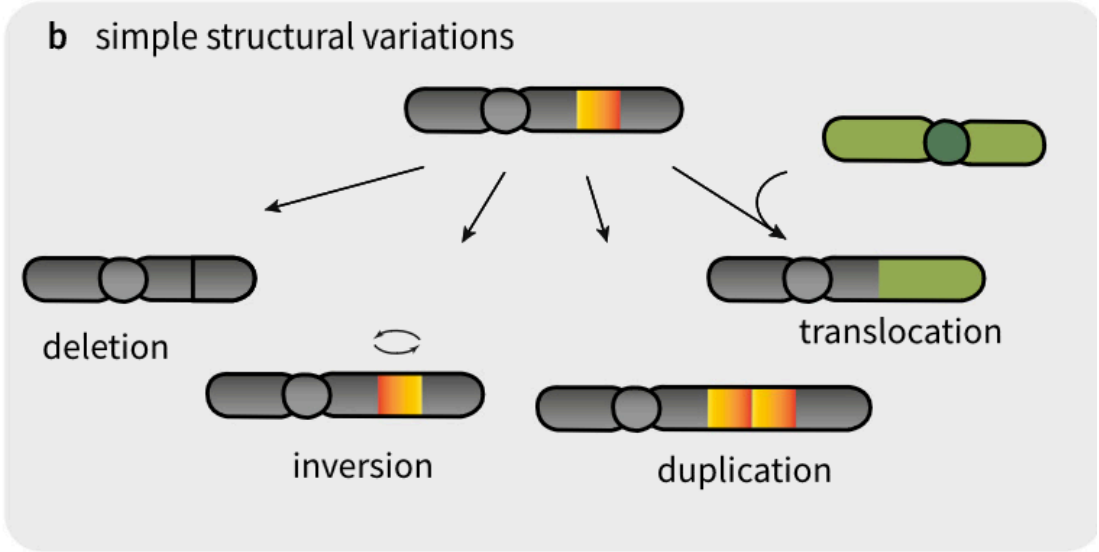
Type of Variation	Percentage
Missense variants (amino acid substitutions)	40%
Nonsense variants (premature stop codons)	10%
RNA processing variants (destroy consensus splice sites, cap sites, and polyadenylation sites or create cryptic sites)	10%
Splice-site variants leading to frameshift mutations and premature stop codons	10%
Long-range regulatory variants	Rare

Types of Variation in Human Genetic Disease - Deletions and Insertions

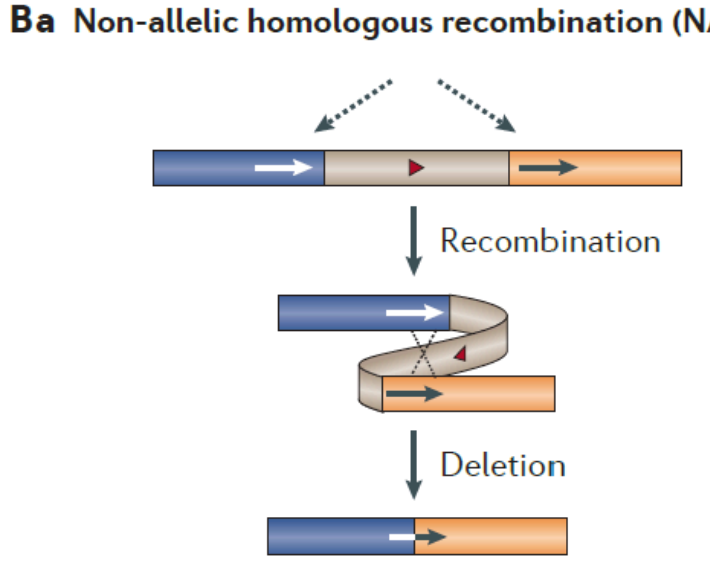
Type of Variation	Percentage
Addition or deletions of a small number of bases	25%
Larger gene deletions, inversions, fusions, and duplications (may be mediated by DNA sequence homology either within or between DNA strands)	5%
Insertion of a LINE or Alu element (disrupting transcription or interrupting the coding sequence)	Rare
Dynamic variants (expansion of trinucleotide or tetranucleotide repeat sequences)	Rare

Types of genetic mutations





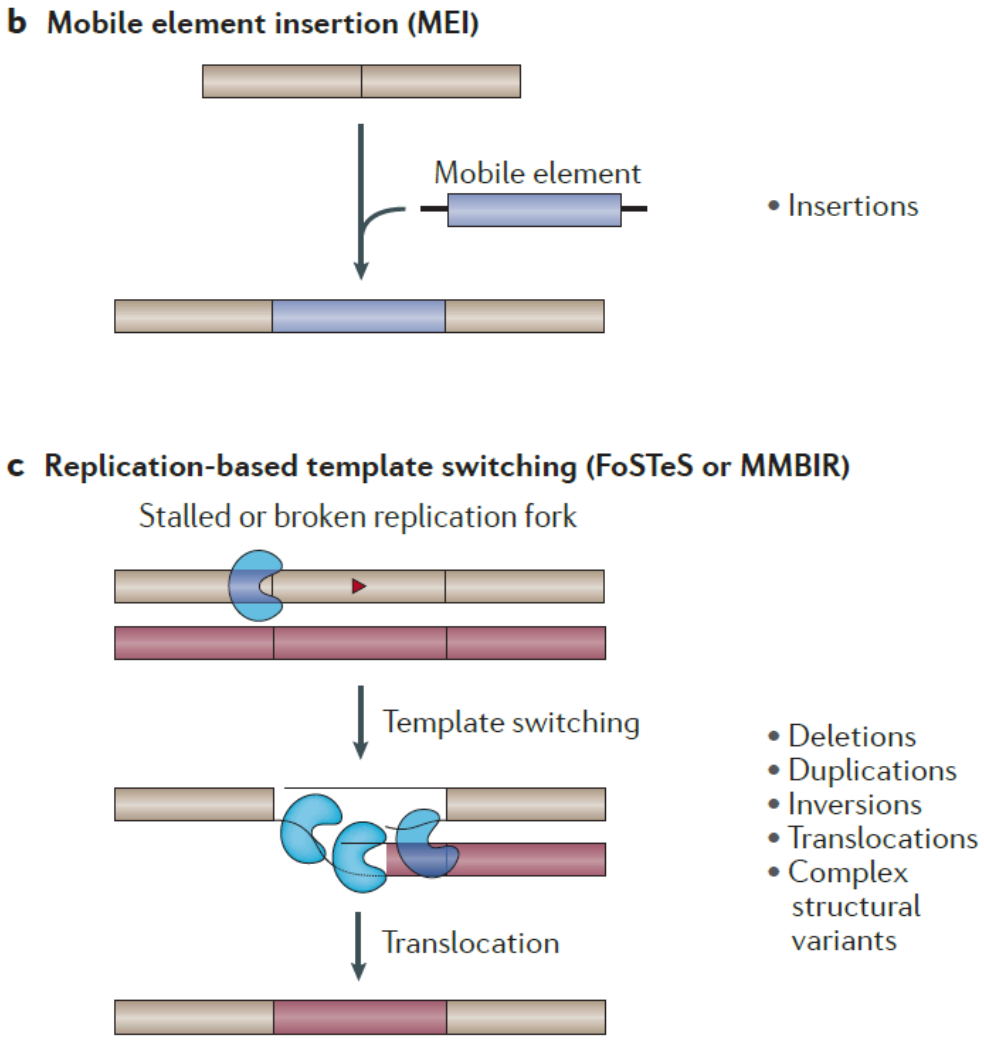
Mechanisms



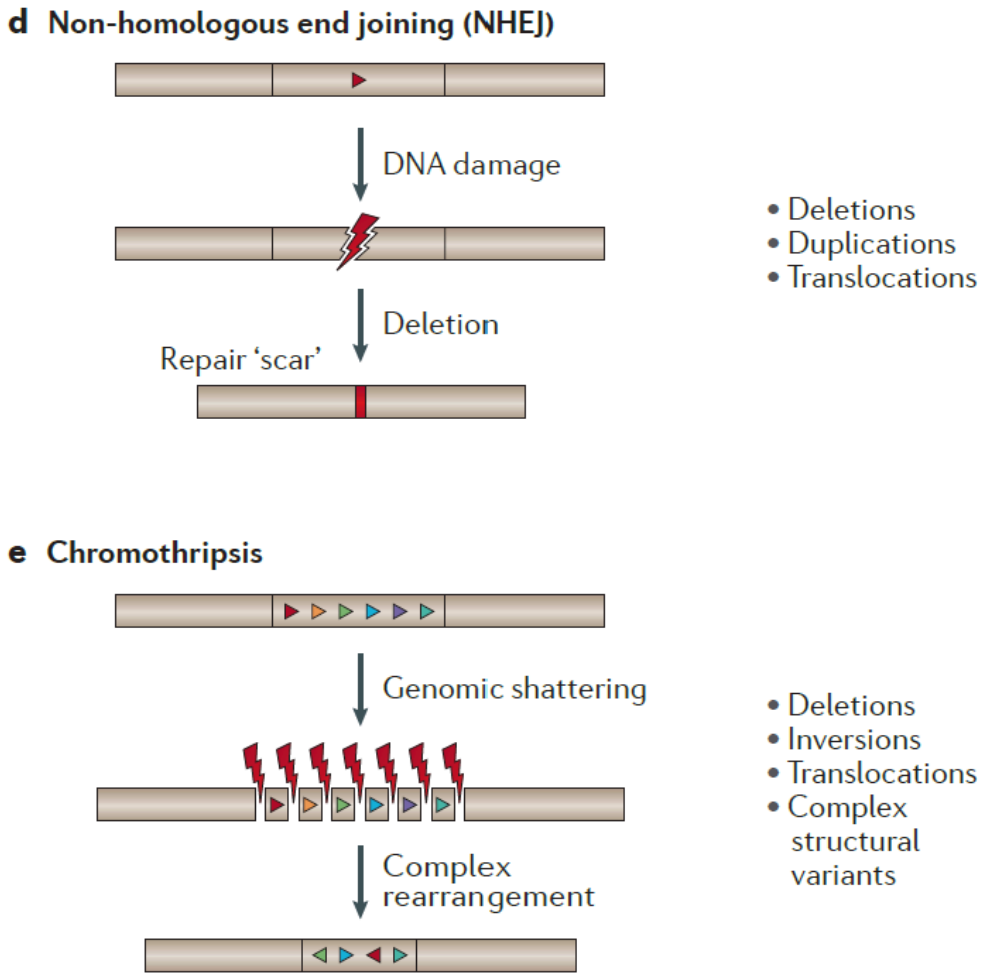
Structural variant types

- Deletions
- Duplications
- Inversions
- Translocations

Recurrent structural variants can result from **non-allelic homologous recombination (NAHR)** which involves recombination between long highly similar low-copy-number repeats (blue and orange segments).

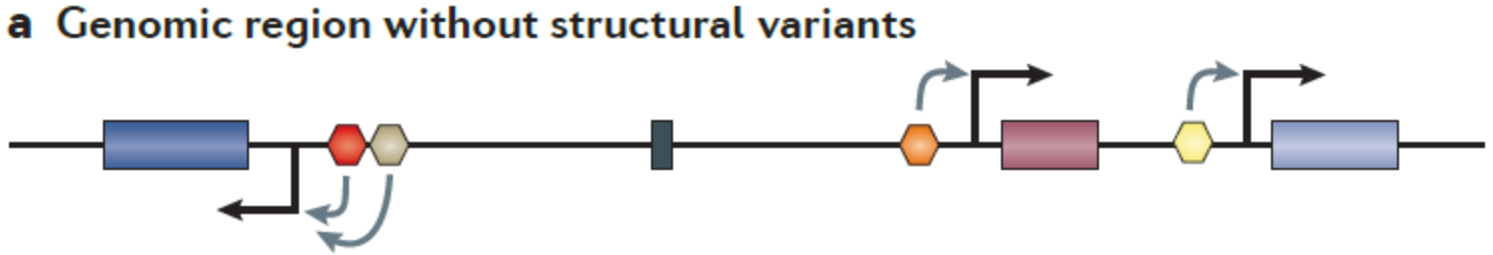


- Novel genomic insertions can involve **mobile element insertion** of transposable elements by retrotransposition.
- DNA-replication-associated template-switching events, involving the **fork-stalling and template switching (FoSTeS)** and **microhomology-mediated break-induced replication (MMBIR) mechanisms**, can lead to simple or complex structural variants, frequently involving duplications.



- **Non-homologous end joining (NHEJ)** is a process that repairs DNA double-strand breaks in the absence of extensive sequence homology and is often accompanied by the addition or deletion of several nucleotides in the form of a 'repair-scar' (small red bar).
- **Chromothripsis** is a phenomenon that involves chromosome shattering leading to numerous breakpoints, followed by error-prone DNA repair. This mechanism can lead to rare catastrophic rearrangements in cancer cells and also in the context of germline DNA rearrangements.

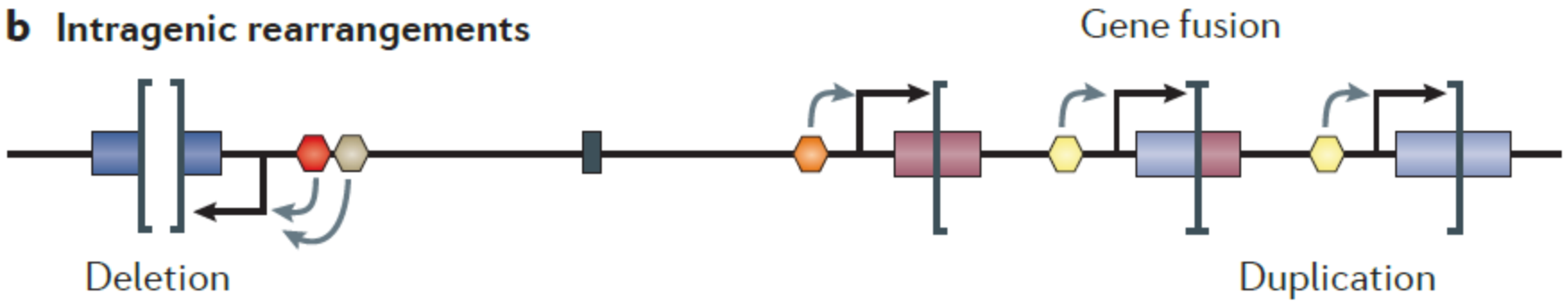
Functional consequences of structural variants



Genes (boxes) are regulated by the collective and combinatorial input of regulatory elements, including tissue-specific enhancers (hexagons, with different colours indicating tissue specificity, and arrows pointing to the target gene) and insulators (black rectangles), which block the activity of regulatory elements.

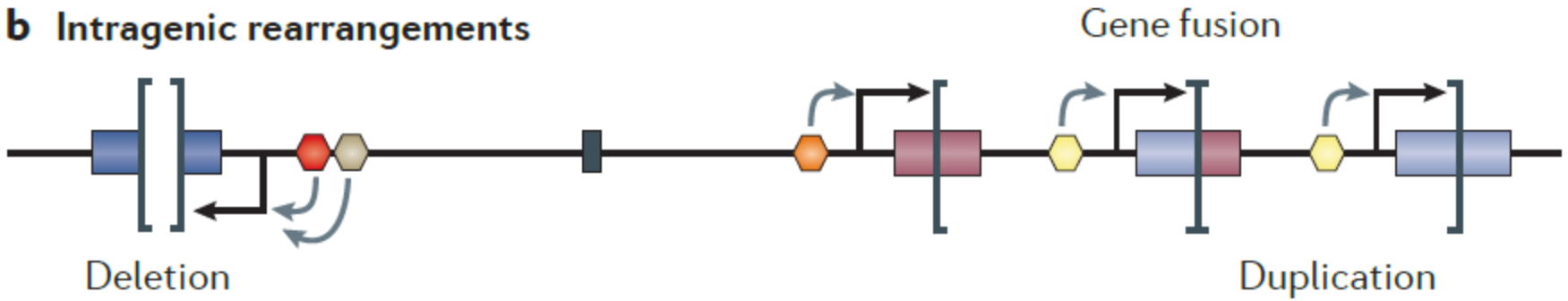
Functional consequences of structural variants

Structural variants (shown by square brackets) can have phenotypic consequences by altering coding regions.



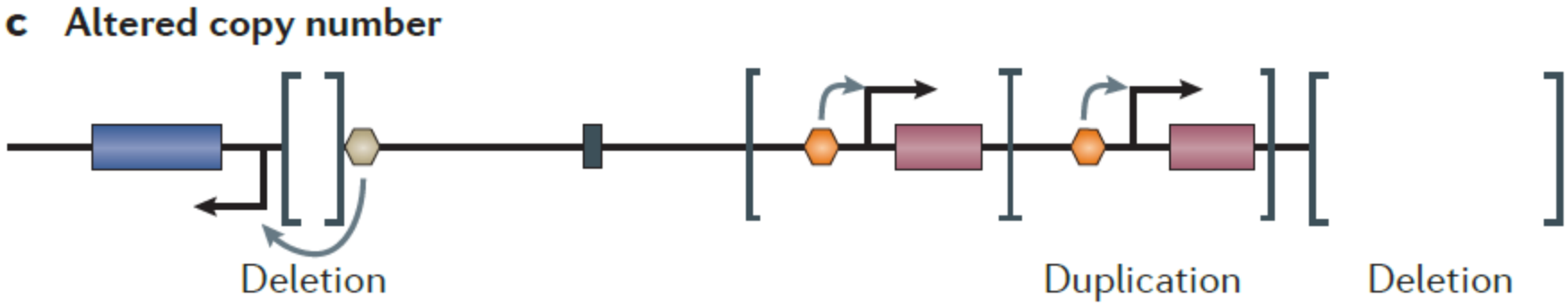
- SVs can remove part of a coding region or fuse different coding regions after a duplication, resulting in aberrant transcripts.
- When the breakpoint of a deletion, insertion, or tandem duplication is located within a functional gene, it may interrupt the gene and cause a **loss of function** by inactivating a gene.

Functional consequences of structural variants



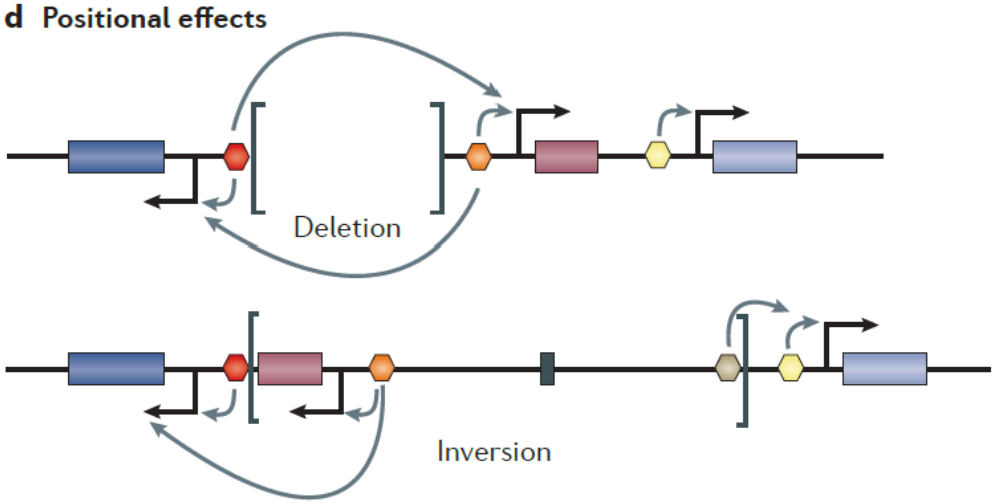
- **Gene fusion** caused by genomic rearrangements between different genes or their regulatory sequences can generate a **gain-of-function** mutation.
- This mechanism is prominent among cancers associated with specific somatic chromosomal translocations.

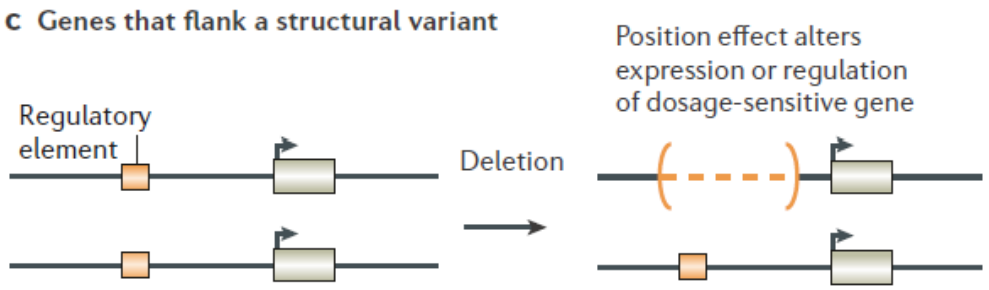
Functional consequences of structural variants



Deletions or duplications can lead to altered doses of otherwise functionally intact elements, resulting in altered regulatory input (left) or altered gene copy number (right).

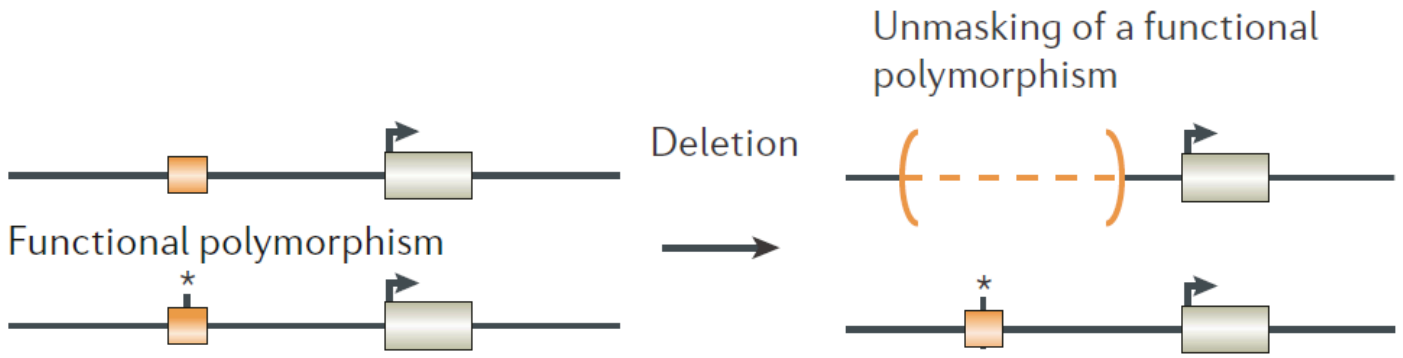
Functional consequences of structural variants





SVs flanking genes can affect expression through position effects. A deletion of important regulatory elements can alter gene expression; similar effects could result from inversion or translocation of such elements.

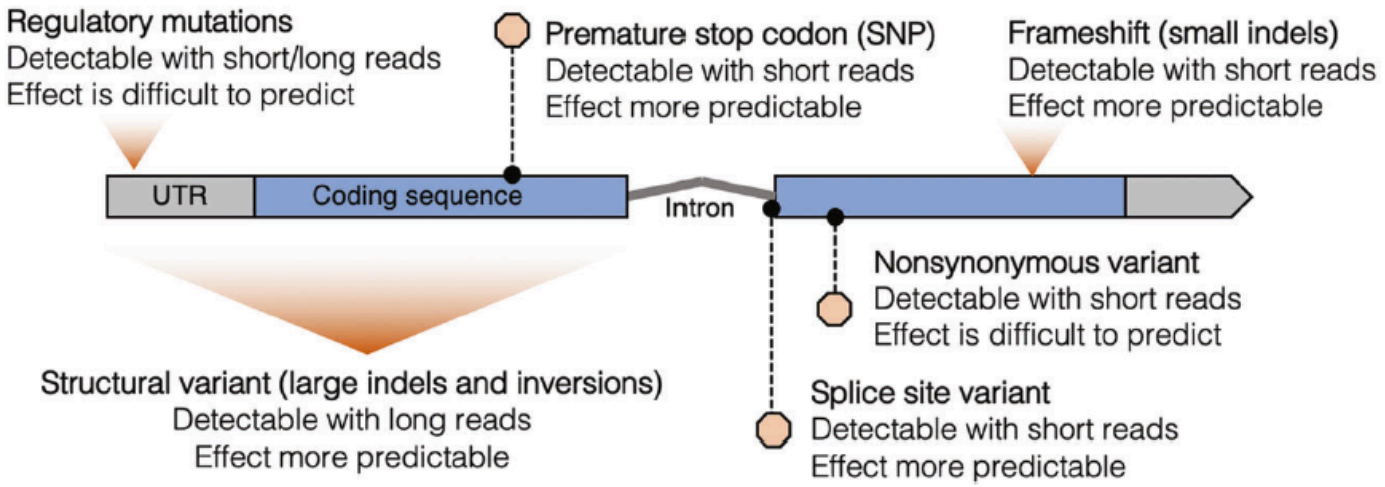
Functional consequences of structural variants



- The deletion of a functional element could unmask a functional polymorphism within an effector, which could have consequences for gene function.

Loss of Function Variants

- Gene dosage: many variants have primarily quantitative ‘dosage’ consequences for the transcript and protein, that is, they cause a loss, reduction or increase of the gene product without introducing novel functional characteristics.
- Loss of function (LoF) variant: the complete loss of a protein encoded from the allele, due to loss of the allele, unstable mRNA or unstable and inactive protein.



Gain of Function Variants

1. Variants that increase the production of a normal protein.

- The most common variants of this type are due to increased gene dosage, which generally results from duplication of part or all of a chromosome (e.g. Down Syndrome)

2. Variants that improves the normal function of a protein. (rare occurrence)

- Example: a missense variant that creates hemoglobin Kempsey locks hemoglobin into its high oxygen affinity state, thereby reducing oxygen delivery to tissues.

Variable expression of complex diseases

Type	Definition	Example
Allelic heterogeneity	The occurrence of more than one allele at a locus	βThalassemia
Locus heterogeneity	The association of more than one locus with a clinical phenotype	Thalassemia can result from variants in either the α-globin or β-globin genes
Clinical or phenotypic	The association of more than one phenotype with variants at a locus	Sickle cell disease and β-thalassemia each result from distinct β-globin gene variants

Case Study Example: α-Thalassemia

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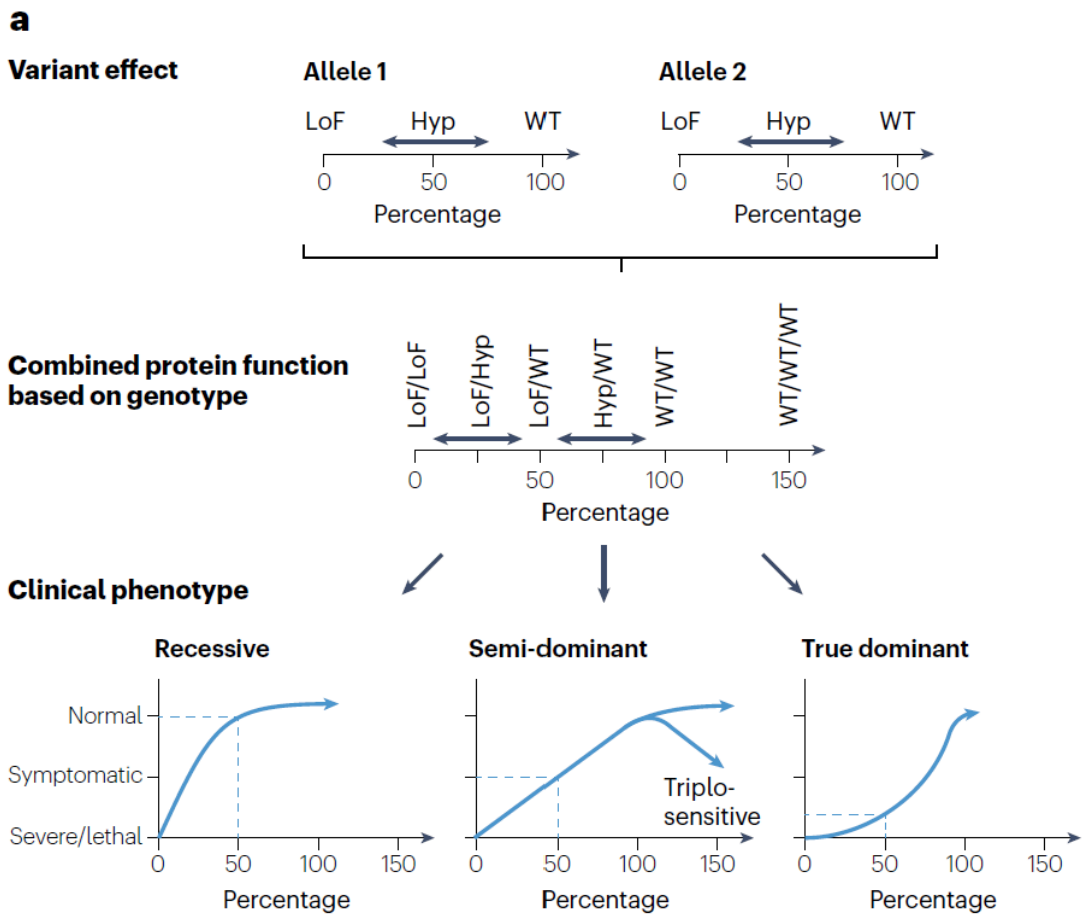
Disease or trait name	Phenotype	Number of normal α-gene copies	Genotype constellation
	Normal	4	αα/αα
α-Thalassaemia minima (trait)	Asymptomatic	3	αα/α–
α-Thalassaemia minor (trait)	Mild microcytic anaemia	2	αα/-- (*cis* functional) α–/α– (*trans* functional)
HbH disease	Microcytic anaemia, hepatosplenomegaly Hb electrophoresis: HbH = β4 elevated	1	α–/--
Hb Bart's hydrops fetalis syndrome	Fetal oedema, prenatal or postnatal lethal Hb electrophoresis: Hb Bart's = γ4	0	--/--



- Heterozygosity for a loss of function (LoF) variant (LoF/WT, typically 50% protein function) is often asymptomatic, reflecting recessive inheritance
- **Hypomorphic (Hyp) variants**: genetic variants that reduce but do not completely abolish the function of the encoded protein.
- Autosomal dominant diseases caused by quantitative variants are generally semi-dominant, with more severe consequences or lethality in the homozygous state.