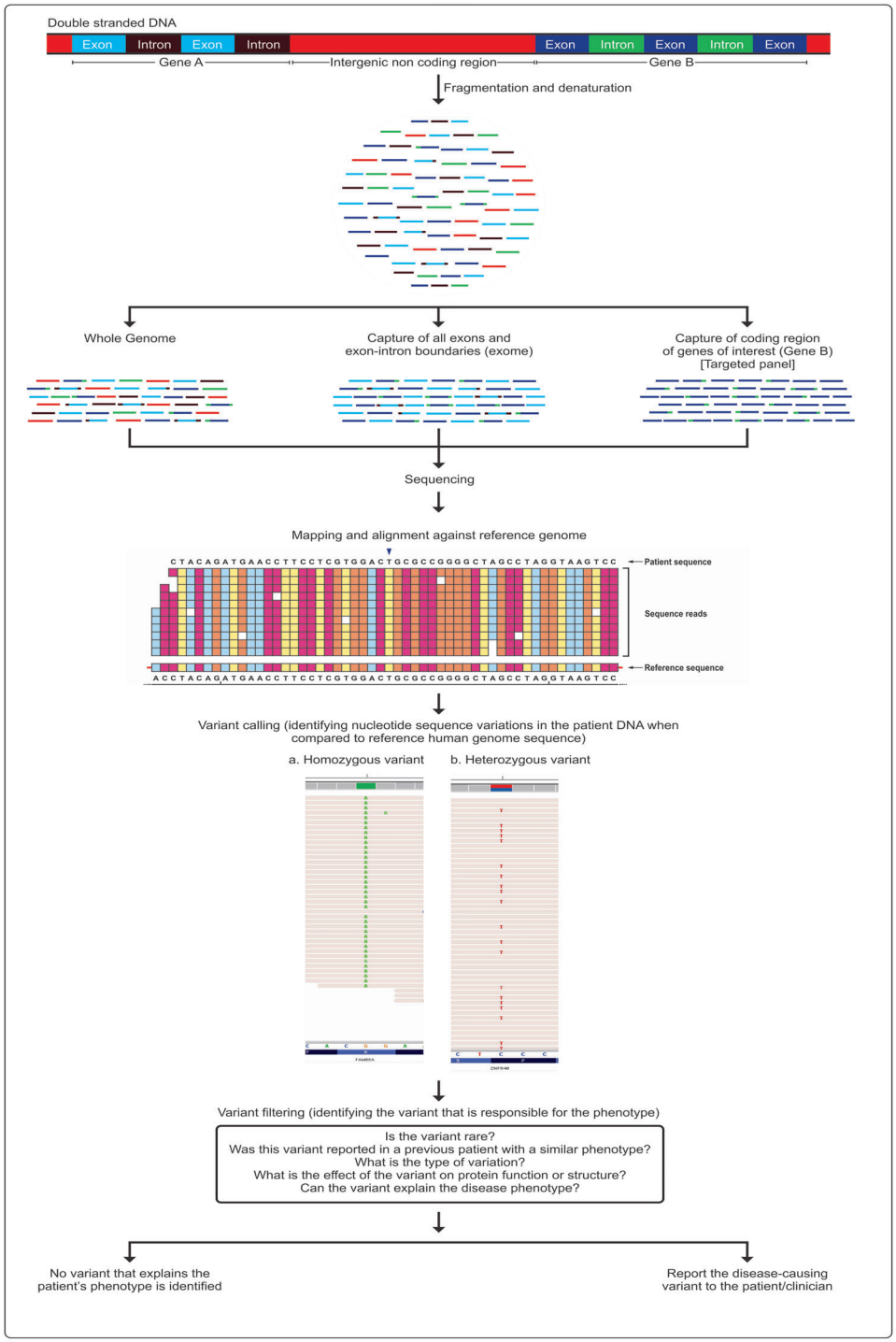

Web Fig. I *Steps in NGS: Double stranded DNA is fragmented into smaller segments and denatured. In whole genome sequencing, all these fragments (exons, introns, non-coding intergenic segments) are sequenced. In exome sequencing, capture kits that selectively capture the exome (all exons and flanking introns) are used and those fragments are sequenced. In a targeted panel, capture kits that selectively capture the coding portion of the genes of interest are used (in this example, capture kit for Gene B). Once sequencing is done, mapping and alignment of reads against a reference genome is done. The next step is variant calling, which detects variants in the subject against the reference sequence. A homozygous variant is seen as a change in almost all reads whereas a heterozygous change is seen in nearly half of the total number of reads. The final step is variant filtering, interpretation and reporting. From a list of variants, pathogenic and benign variants are identified by several filtering approaches.*

Web Table I Popular Databases used in Interpretation of Copy Number Variants

Database	*Key features*
DECIPHER (DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources) *https://decipher.sanger.ac.uk*	Interactive web based free browser where the patient's variant is displayed along with normal and pathogenic variants in that locus
DGV (Database of Genomic Variants) *http://dgv.tcag.ca/dgv/app/home*	Database of common structural variations in healthy individuals
ISCA (International Standards for Cytogenomic Arrays) *http://dbsearch.clinicalgenome.org/search/*	Database of pathogenic, likely pathogenic, uncertain, likely benign, and benign CNVs

Other databases include UCSC genome browser (University of California, Santa Cruz), ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations) and OMIM (Online Mendelian Inheritance in Man).

Web Table II Databases Used in Exome or Genome Data Analysis

Name of database	*Description*	*Website*
Population database of variants		
Genome Aggregation Databases (gnomAD)	Disease specific or population specific exome and genome data from unrelated individuals	*https://gnomad.broadinstitute.org*
The International Genome Sample Resource (IGSR) (1000 Genomes Project)	Database of genetic variants with a frequency of more than 1%	*http://www.internationalgenome.org*
The Exome Aggregation Consortium (ExAC)	Exome sequencing data from disease specific and population genetic studies	*http://exac.broadinstitute.org*
Databases of disease causing variants		
The Human Gene Mutation Database (HGMD)	All known published disease-causing variants	*http://www.hgmd.cf.ac.uk/ac/index.php*
ClinVar	Clinical description and variants	*https://www.ncbi.nlm.nih.gov/clinvar/*
Leiden Open Variation Database (LOVD)	Variant database	*http://www.lovd.nl*
Online Mendelian Inheritance in Man (OMIM)	Human diseases and variants	*https://www.omim.org*

Web Table III Clinical Scenarios where Genomic Testing is Useful

Scenario 1: A non-consanguineous couple with a five-years-old girl with autism wanted to know the risk of recurrence of autism in subsequent pregnancies. Chromosomal microarray was opted as the first tier test in this child. No pathogenic copy number variant causing autism was identified. They were offered exome/genome sequencing as well, but did not opt for it in view of high cost and low yield. Only an empiric risk of recurrence of 10% was provided to the family. Since exact genetic etiology was not identified in the child, prenatal diagnosis could not be offered.

Scenario 2: A three-years-old boy born to third degree consanguineous parents had spastic diplegia and was being treated as cerebral palsy. There was no history of any adverse perinatal events. In the absence of a perinatal insult, exome sequencing done for this child, identified a biallelic pathogenic variant c.700G>C (p.Asp234His) in *ARG1* causing arginase deficiency (MIM#207800). Parents were heterozygous carriers for the same variant. The child was advised supportive care. The parents were counseled about the recurrence risk of 25% of this condition in every pregnancy and prenatal diagnosis was offered by chorionic villus sampling.

Scenario 3: A four-years-old girl had developmental delay, repetitive hand wringing movements and hyperventilation. DNA methylation analysis for Angelman syndrome and sequencing of *MECP2* gene for Rett syndrome were normal. Exome sequencing identified a de novo heterozygous disease-causing variant, c.1512insA (p.Ser505Glu*8) in TCF4 gene, causing Pitt Hopkins syndrome (MIM#610954). Since the parents did not have this variant, they were counseled about very low risk of recurrence (usually less than one percent) in subsequent pregnancies.

Scenario 4: Six-years-old girl, who was the first child of non-consanguineous parents, was evaluated for developmental delay and intellectual disability. Chromosomal microarray and fragile X mutation analysis did not reveal disease- causing variants. Exome sequencing of the child was performed. A heterozygous novel variant c.3817C>A, p.(His1273Asn) in HIVEP2 gene causing autosomal dominant mental retardation type 43(MIM#616977) was reported. The variant was interpreted as VUS (variant of unknown significance). It was noted that the parents were not tested for this variant. On testing them, the same variant was observed in heterozygous state in her father who had normal intellect. Hence this variant was re-classified as a benign variant in HIVEP2. Exome sequencing was performed in parents to complete the trio (parents-child) and a novel biallelic compound heterozygous variant in a novel gene in the proband was identified (suggesting the possibility of a hitherto unknown disease with intellectual disability and its genetic cause). Further validation of these findings by more patients with similar condition and experiments are awaited to provide definitive genetic counseling and prenatal diagnosis to the family.

Scenario 5: A 12-years-old girl with multiple fractures was diagnosed to have osteogenesis imperfecta (OI). She did not have blue sclera or dentinogenesis imperfecta. Her radiographs showed hyperplastic callus and calcified interosseus membrane in forearm. Since this pointed to a specific diagnosis of osteogenesis imperfecta type V (MIM#610967), instead of ordering a panel test covering all genes causing OI, Sanger sequencing of only the particular region of *IFITM5* was done. A de novo heterozygous pathogenic variant c.-14C>T was identified in this gene. This variant was not identified in her parents. Clinical and radiological examination is useful even in genomic era.