Applications of Genetics to Cardiovascular Medicine

Pradeep Natarajan and Kiran Musunuru

Naturally occurring human genetic variation has served for decades to elucidate the root causes of disease, including **cardiovascular disease**.

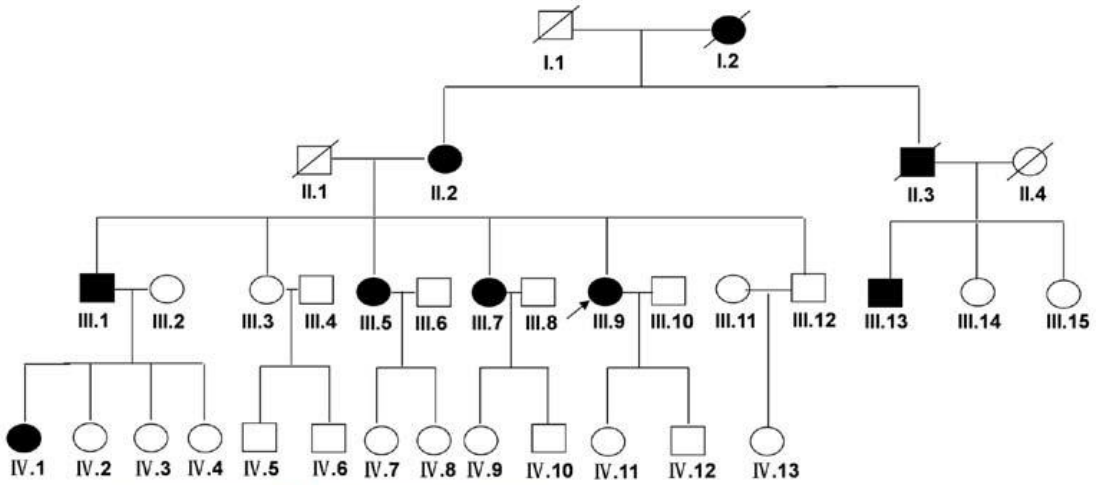
Exponential technologic advances in computation, data science, and assay development have recently enabled population-based analyses, broad clinical profiling, and direct-to- consumer genetic testing in millions of people. Because germline genetic variation is established at conception and persists for the lifetime, genetics offers a robust tool for causal inference for broader preventive and therapeutic insights.

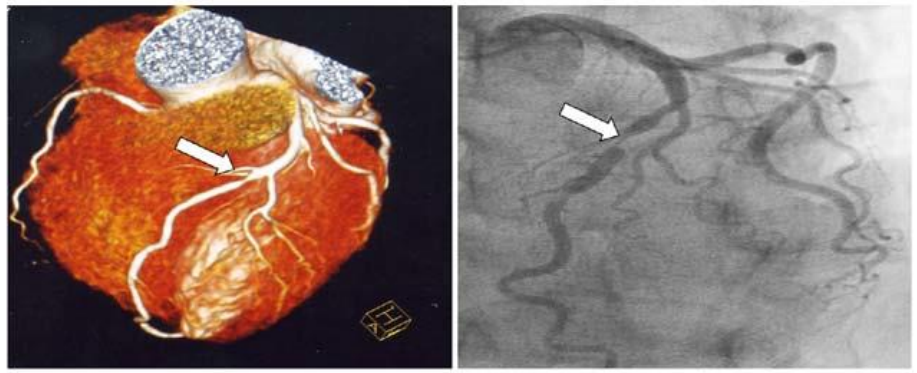
Heritability

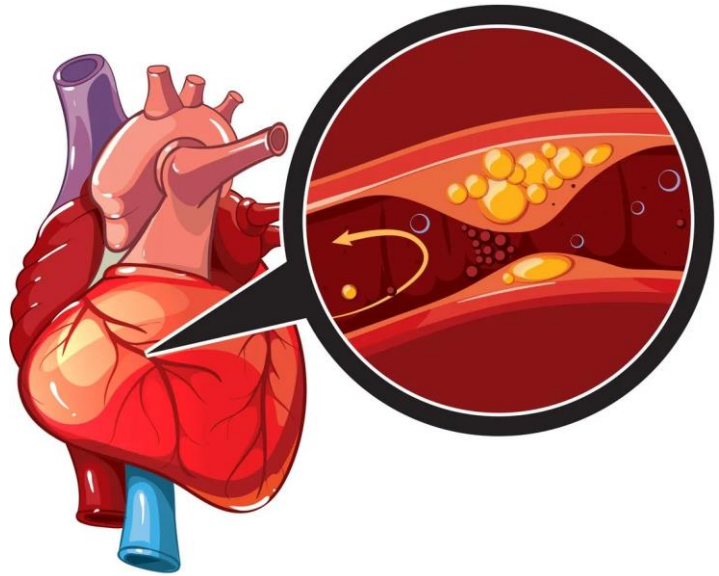
Heritability refers to the fraction of interindividual variability in risk for disease attributable to additive genetic variation.

Many cardiovascular diseases, including CAD, aggregate within families.

- It is estimated that CAD is **40% to 60%** heritable, based on the family-based methods or statistical genetics approaches.



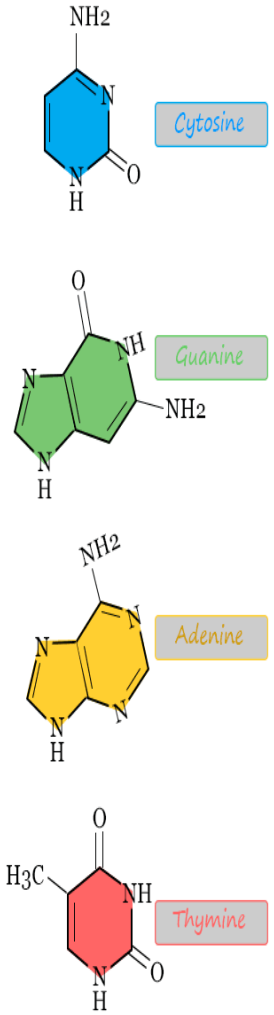




Genetic Variation

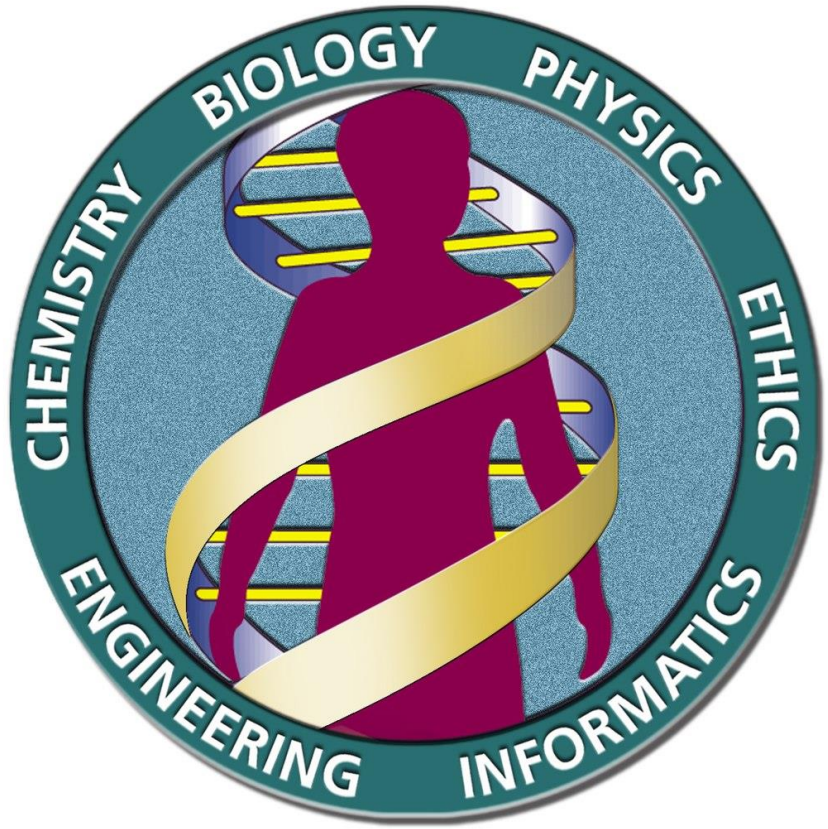
- Among individuals, 99.9% of the 6.4 billion base pairs are the same; genetic analyses leverage the 0.1% differences to understand trait or disease variation.

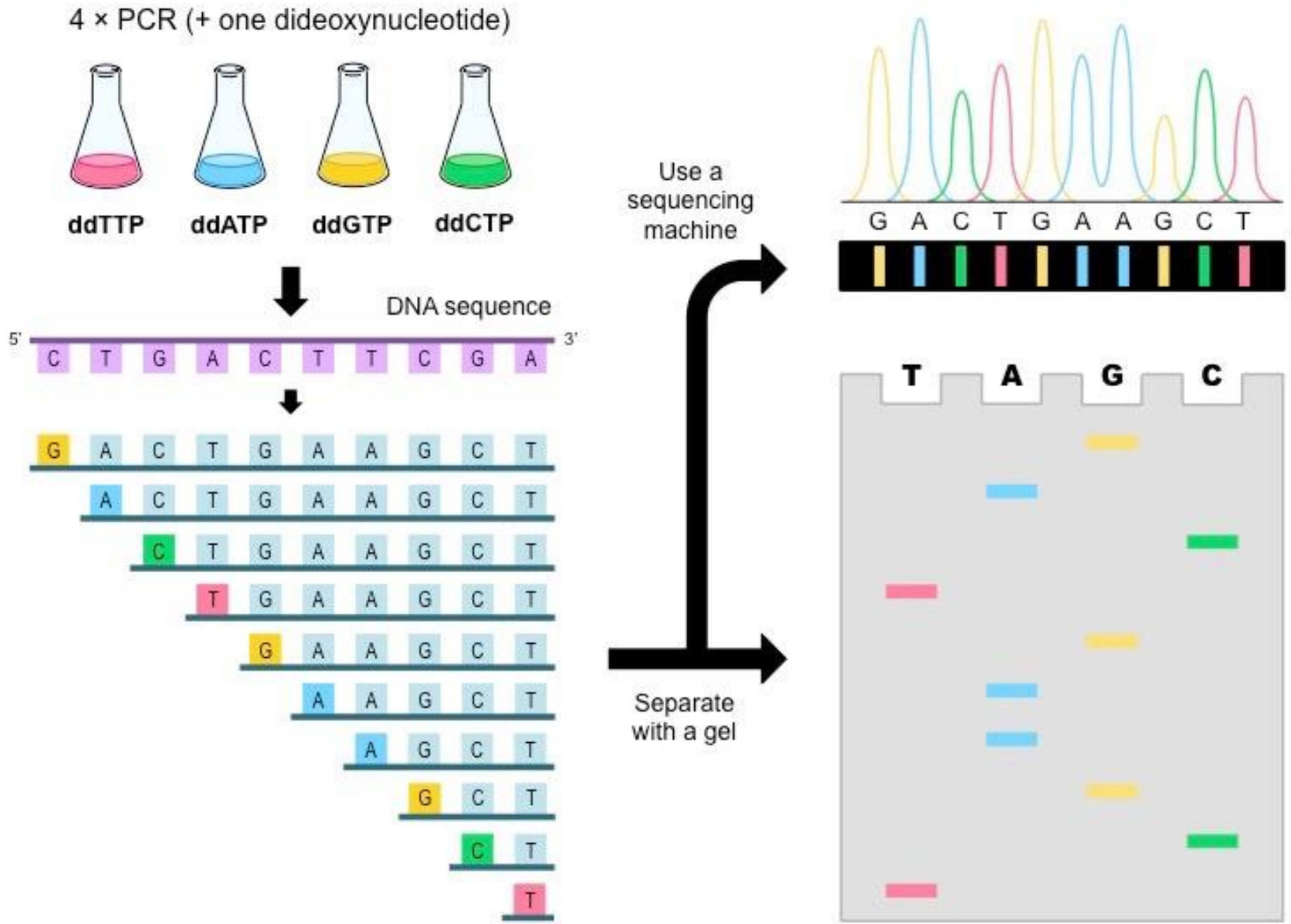
DNA
Deoxyribonucleic acid



Nucleobases of DNA

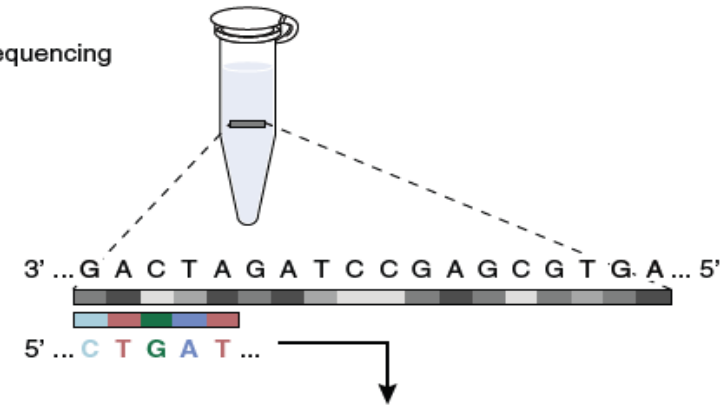
FIG. 1.6 Frederick Sanger, inventor of the most widely used method of DNA sequencing, and a double Nobel Laureate. With permission from Victor A McKusick: Mendelian Inheritance in Man, 12th ed. 1998 Johns Hopkins University Press.



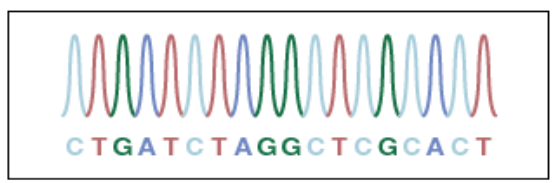

4 × PCR (+ one dideoxynucleotide)
ddTTP
ddATP
ddGTP
ddCTP
DNA sequence
5'
3'
C T G A C T T C G A
G A C T G A A G C T
A C T G A A G C T
C T G A A G C T
T G A A G C T
G A A G C T
A A G C T
A G C T
G C T
C T
T
Use a sequencing machine
Separate with a gel
G A C T G A A G C T
T A G C

First generation sequencing (Sanger)

1. Genomic DNA
2. Fragmented DNA
3. Cloning and amplification
4. Sequencing

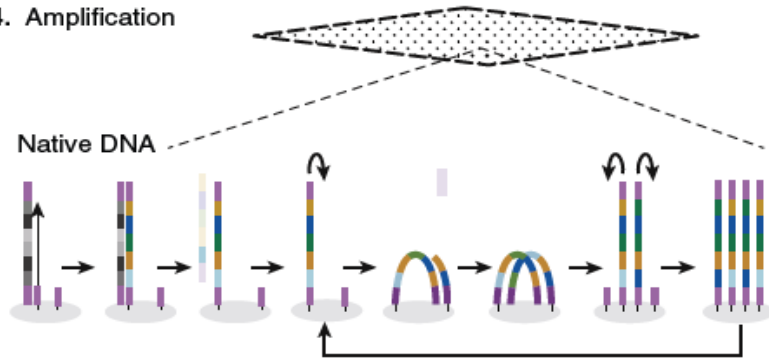


5. Detection

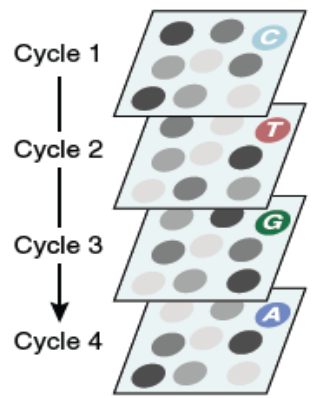


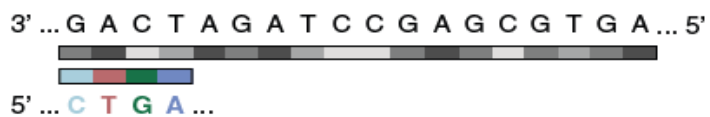
Second generation sequencing (massively parallel)

1. Genomic DNA
2. Fragmented DNA
3. Adaptor ligation
4. Amplification



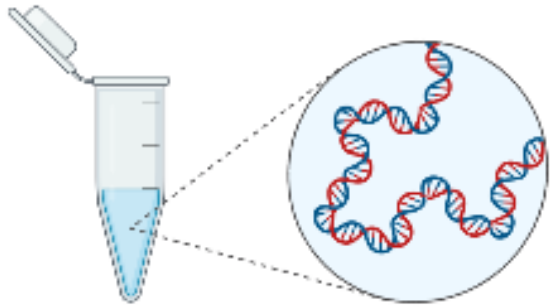
5. Detection



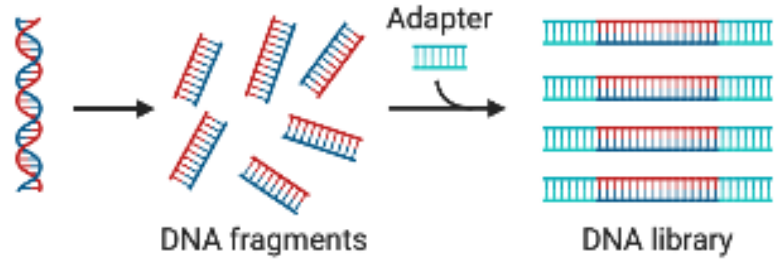


Next Generation Sequencing Workflow

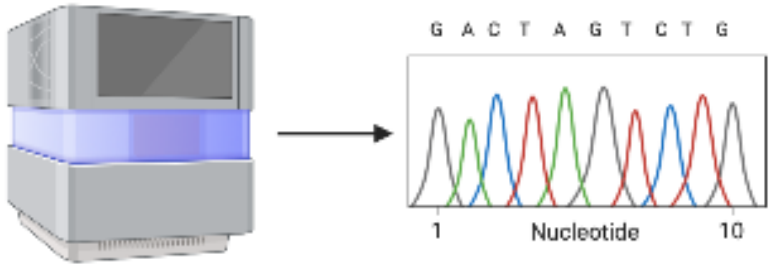
Step 1:
DNA extraction



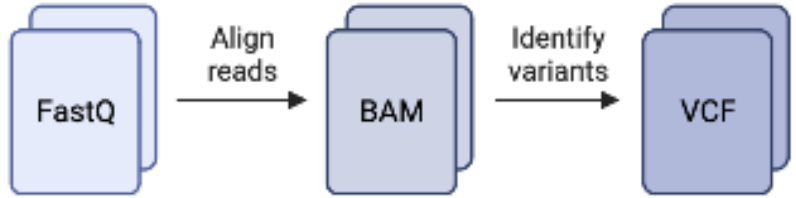
Step 2:
Library preparation



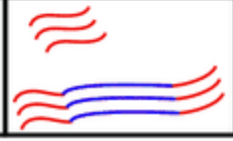
Step 3:
Sequencing



Step 4:
Analysis



Common genetic variation influencing phenotypes tends to occur within **noncoding regulatory elements**. **Coding sequence** is less tolerant of genetic variation, and single base pair changes may lead to substantial phenotypic changes.

Variant Location	Transcript Map	Transcript Product	Transcript description	Potential Outcome
Coding (standard interpretation)			Synonymous/ Missense/ Nonsense	Homeostasis/ Altered Product/ Loss of function
Isoform specific/ Noncoding regulatory			Isoform loss/alteration Altered translation	Aberrant expression patterns
Promoter/Enhancer/ Looping/cis-regulatory lncRNA			Over/ Under expression	Aberrant expression patterns
Splice Donor/Acceptor Branchpoint			Skipped exon/ Retained intron	Altered product Nonsense Mediated Decay

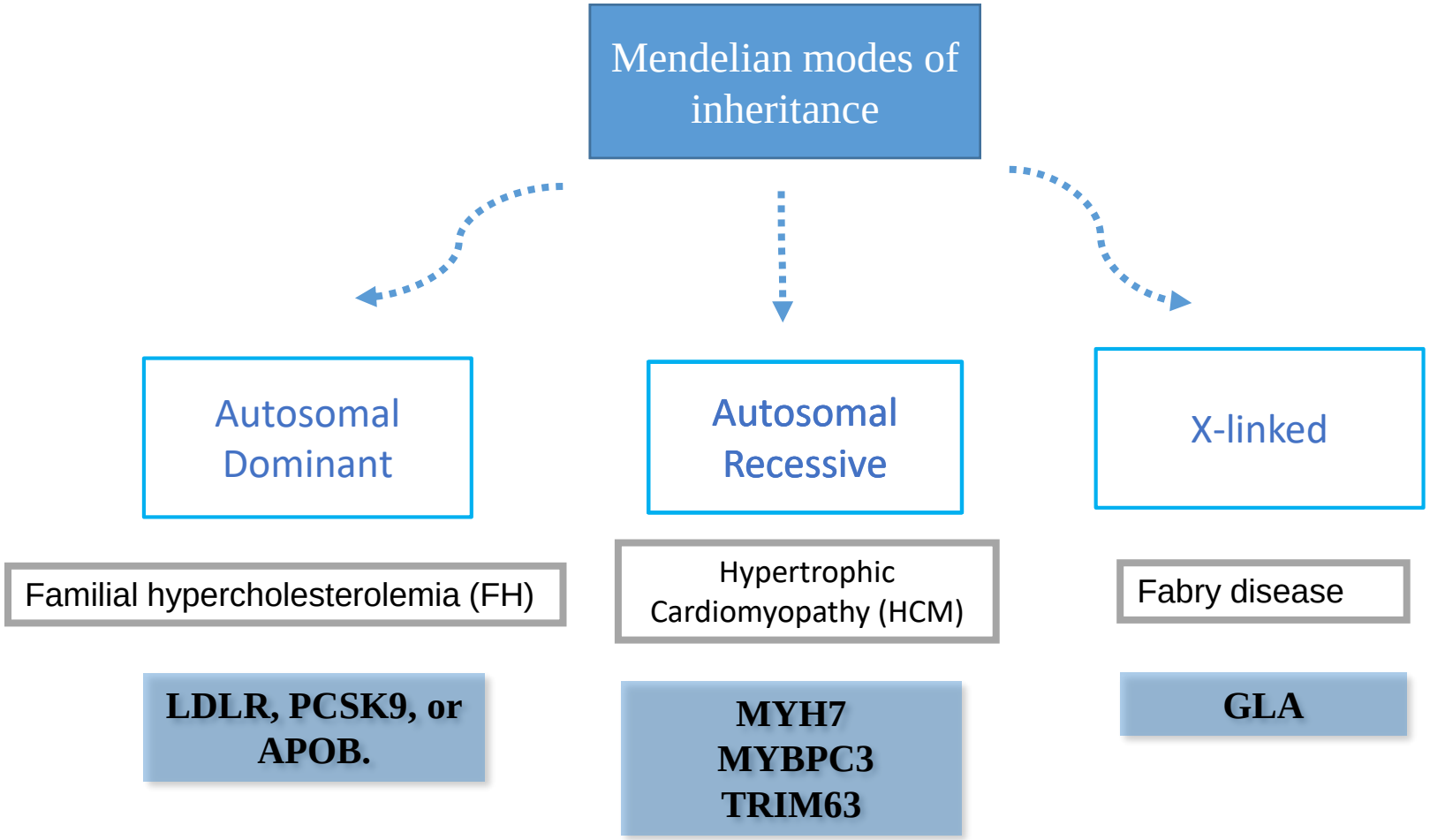
Genetic Architecture

The “genetic architecture” of a disease refers to the number and magnitude of genetic risk factors that exist in each patient and in the population, as well as their frequencies and interactions.

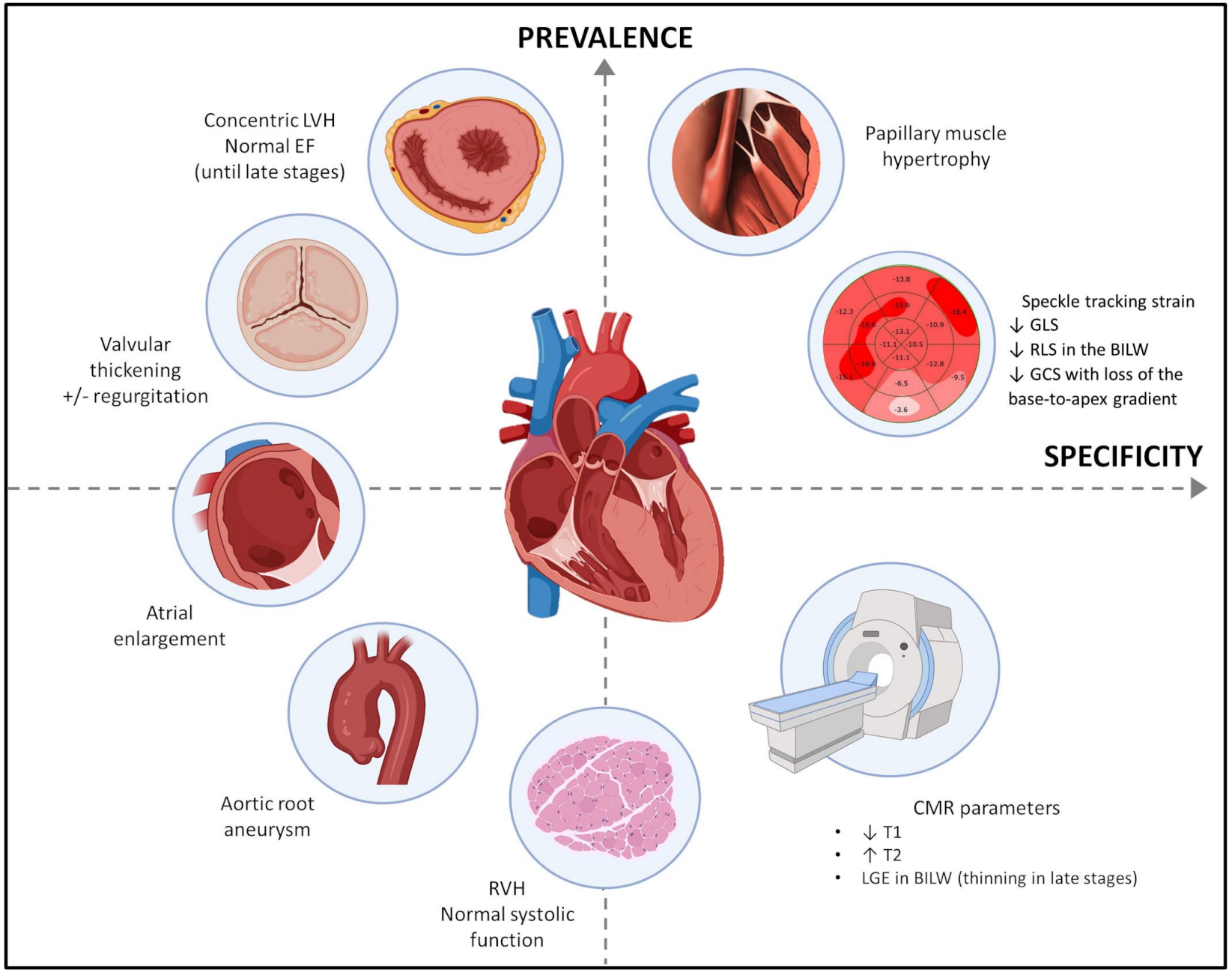
Single gene (*monogenic*)

Few genes (*oligogenic*)

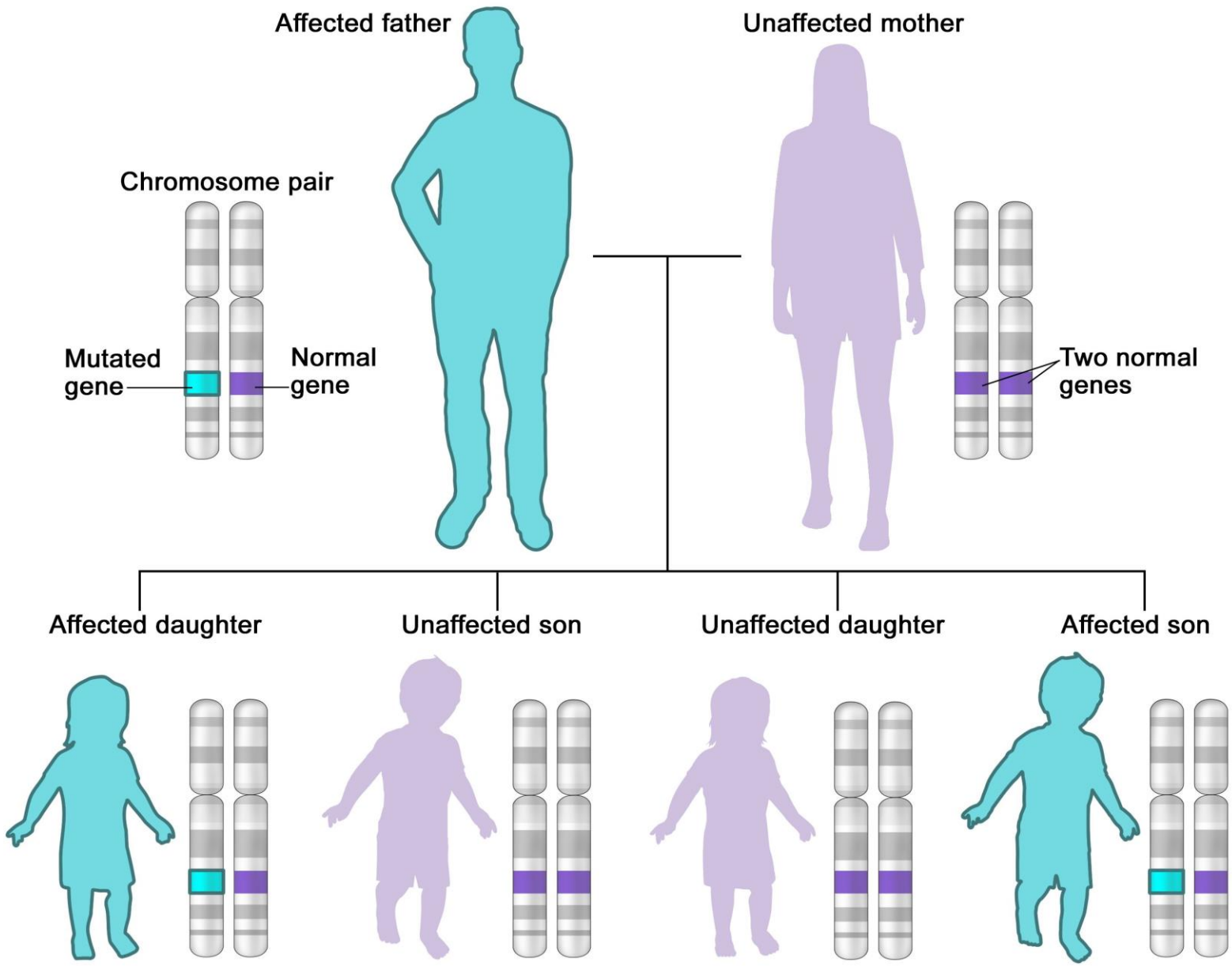
Several genes (*polygenic*)


Mendelian modes of inheritance
Autosomal Dominant
Autosomal Recessive
X-linked
Familial hypercholesterolemia (FH)
Hypertrophic Cardiomyopathy (HCM)
Fabry disease
LDLR, PCSK9, or APOB.
MYH7
MYBPC3
TRIM63
GLA

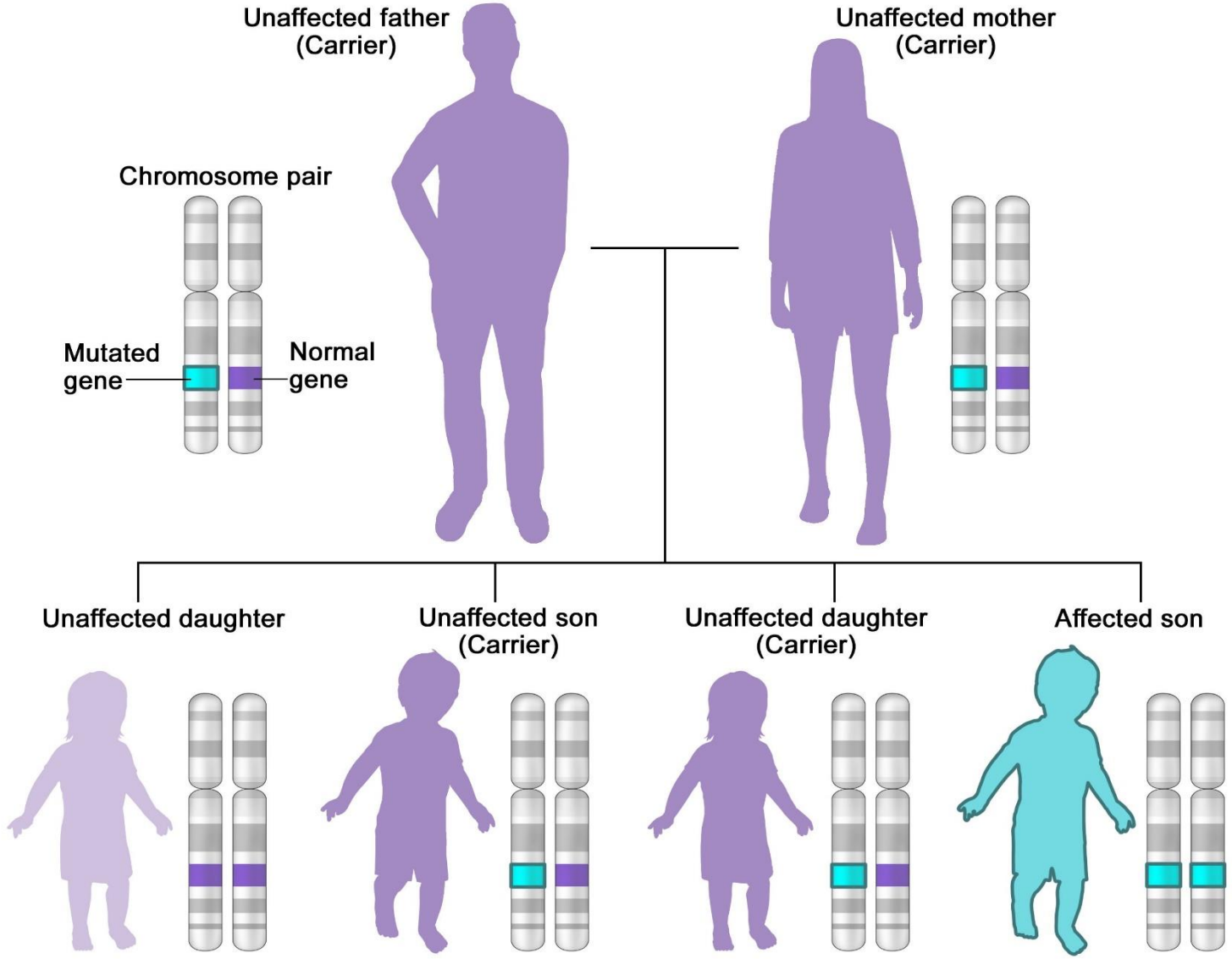
Fabry Disease Cardiomyopathy



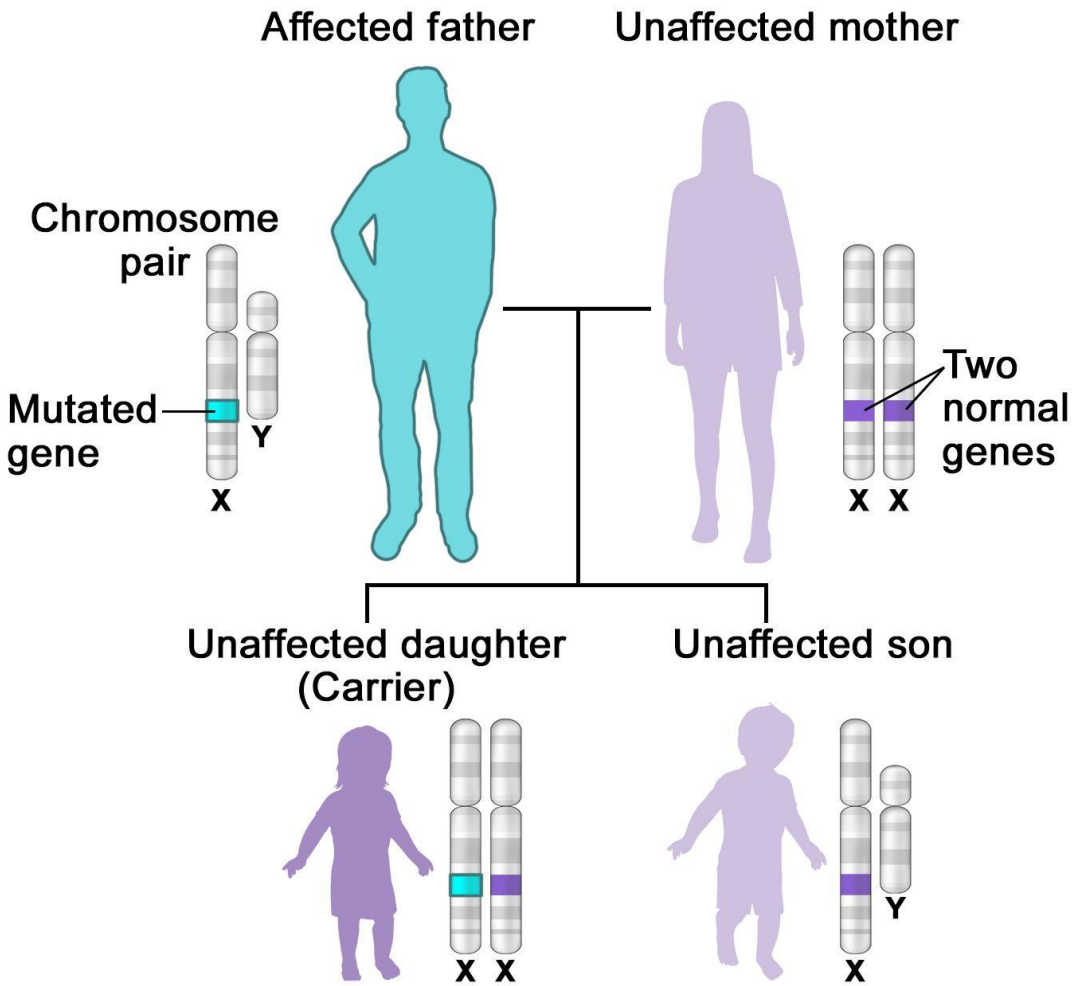
Autosomal Dominant Inheritance

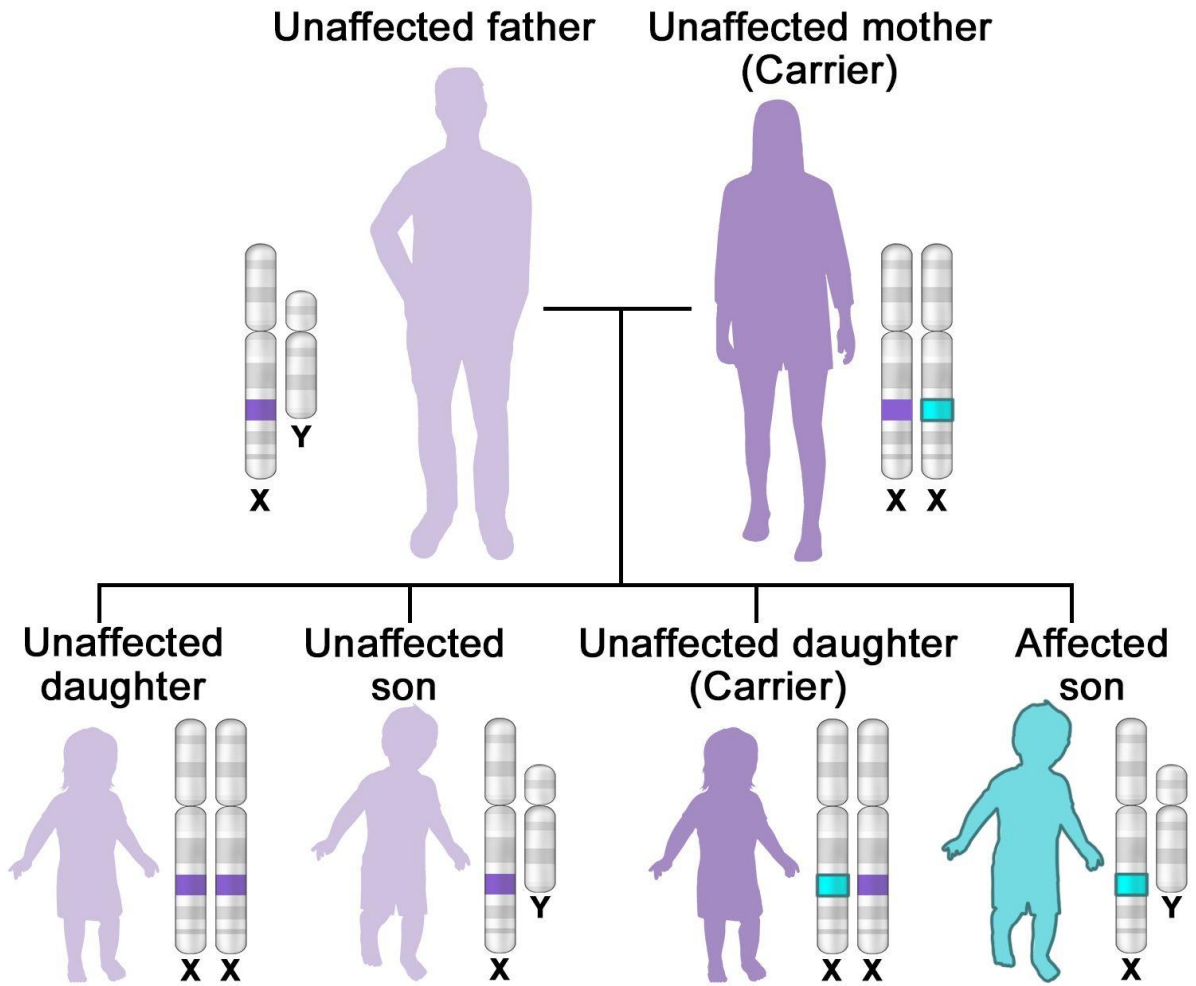


Autosomal Recessive Inheritance



X-Linked Recessive Inheritance





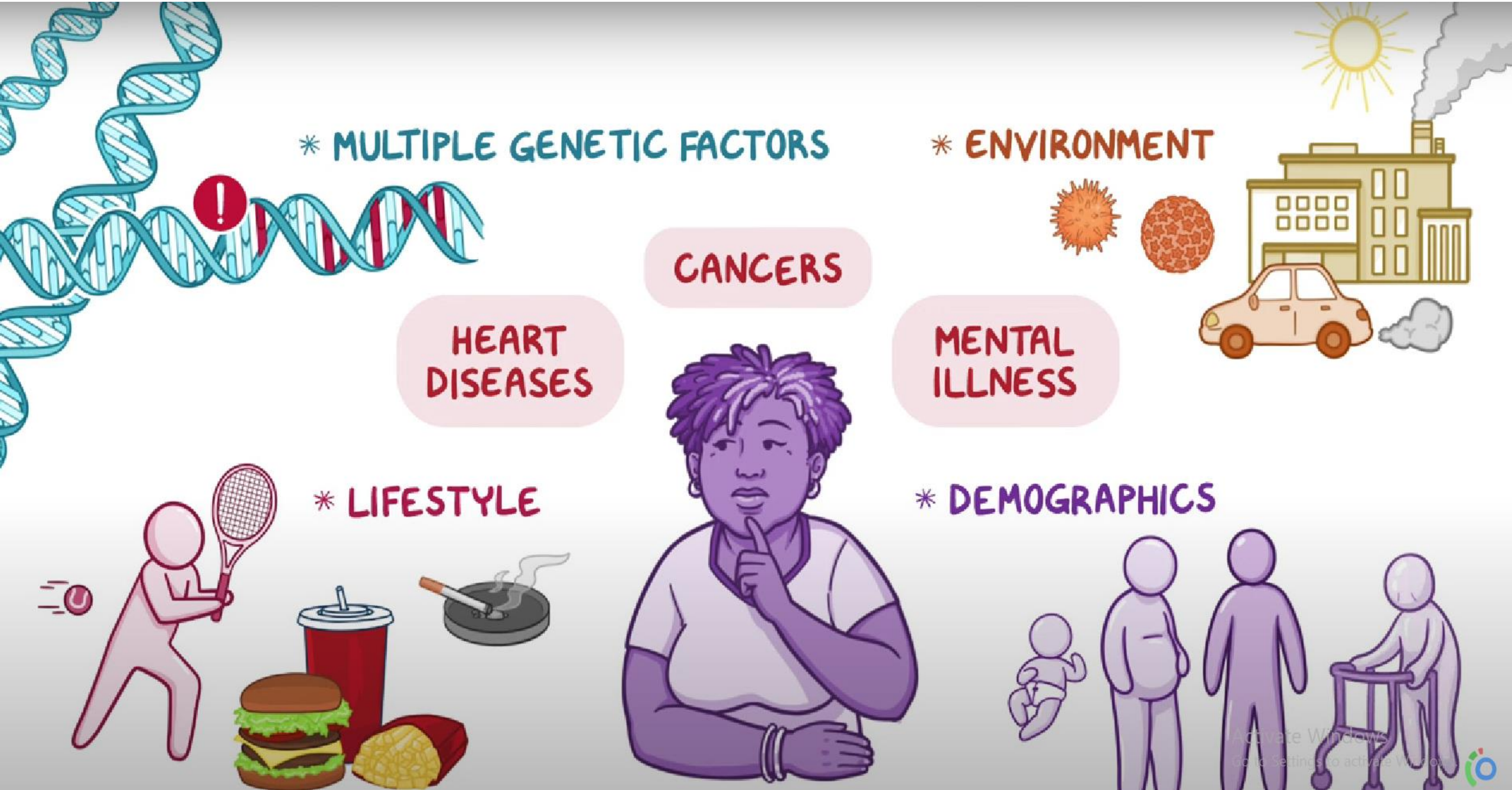
- Traits with higher degrees of heritability are mo suitable for **gene discovery** studies and genetic risk prediction.
- Remaining contributors to disease risk variability include environmental influences, nonadditive genetic influences (epistasis), nonadditive genotype/environment effects, errors in estimations of relatedness or disease, and random chance.

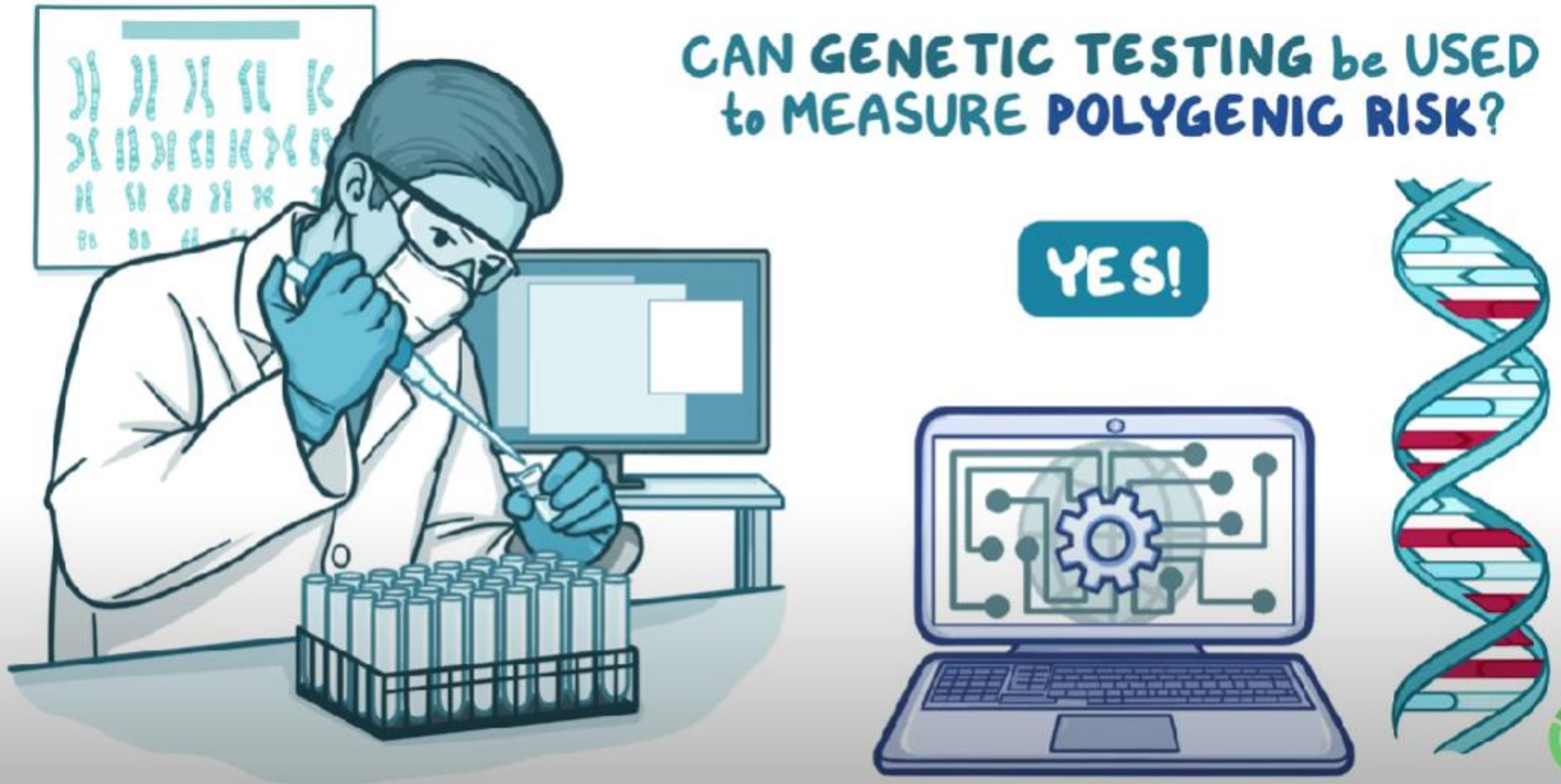
OUR HEALTH is DICTATED by a COMBINATION of MANY FACTORS

MONOGENIC CONDITIONS

* a SINGLE GENETIC VARIANT in a SINGLE GENE
  - CYSTIC FIBROSIS
  - SICKLE CELL ANEMIA
  - HUNTINGTON DISEASE

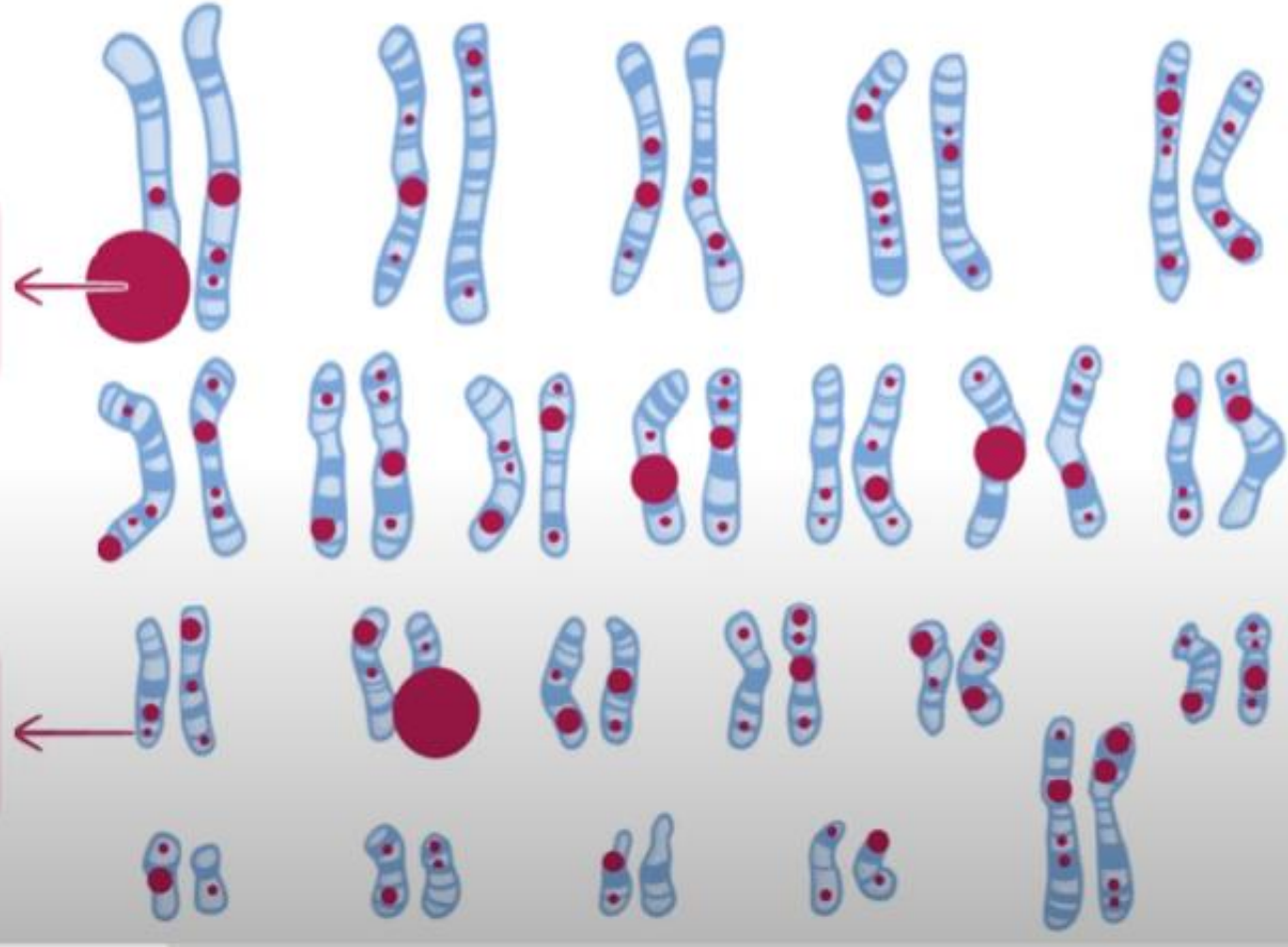
* a GENETIC TEST OFTEN GIVES a CLEAR ANSWER:


* MULTIPLE GENETIC FACTORS
* ENVIRONMENT
CANCERS
HEART DISEASES
MENTAL ILLNESS
* LIFESTYLE
* DEMOGRAPHICS


CAN GENETIC TESTING be USED
to MEASURE POLYGENIC RISK?
YES!

POLYGENIC CONDITIONS

* HUNDREDS to THOUSANDS of GENETIC VARIANTS may INFLUENCE the CONDITION



LARGER EFFECT

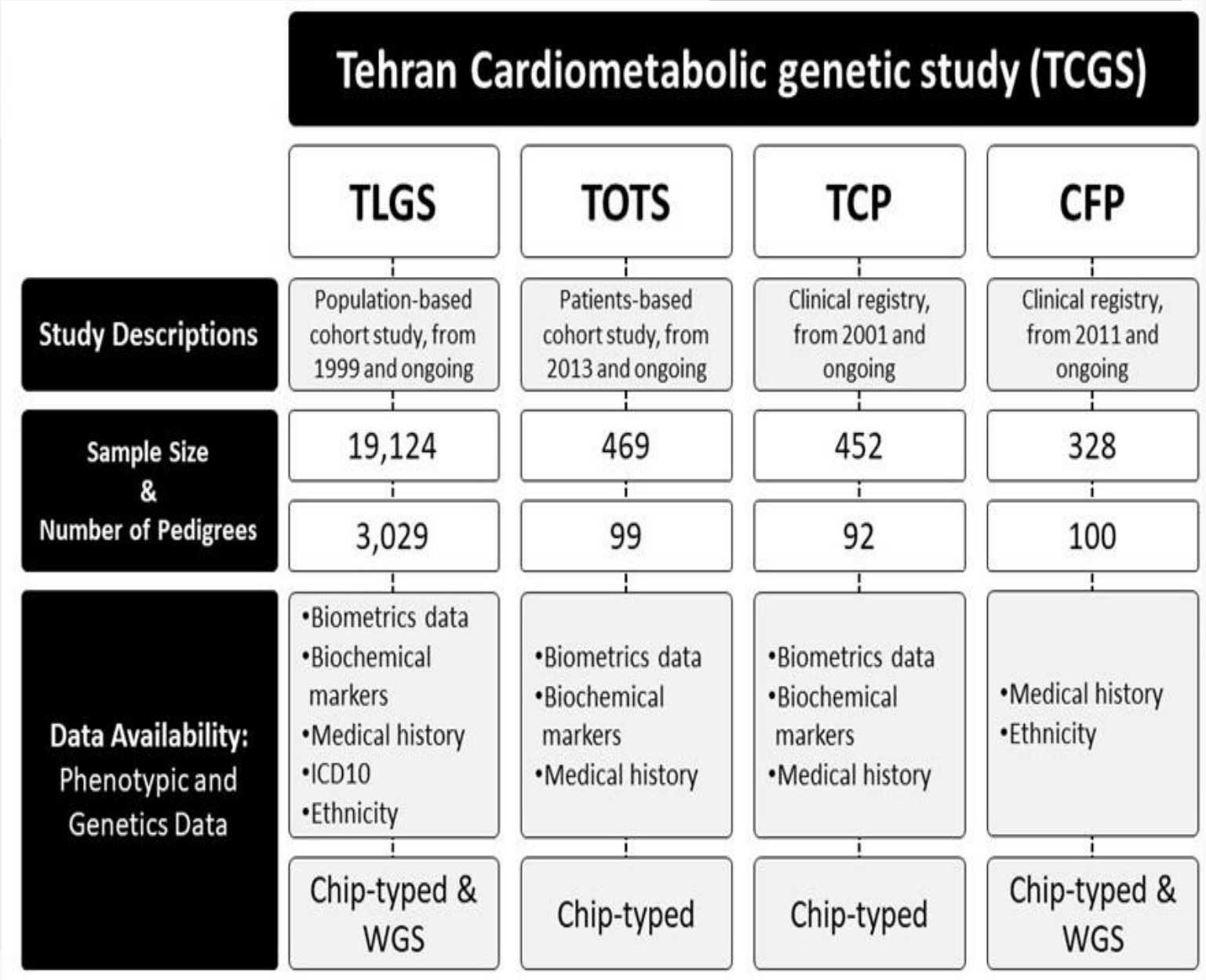
VERY SMALL EFFECT

ADDITIVE EFFECT: SIGNIFICANT IMPACT

ABOUT TCGS

The Tehran cardiometabolic genetic study (TCGS) is a large population-based cohort study that conducts periodic follow-ups.

TCGS has created a comprehensive database comprising 20,367 participants born between 1911 and 2015 selected from four main ongoing studies in a family-based longitudinal framework.



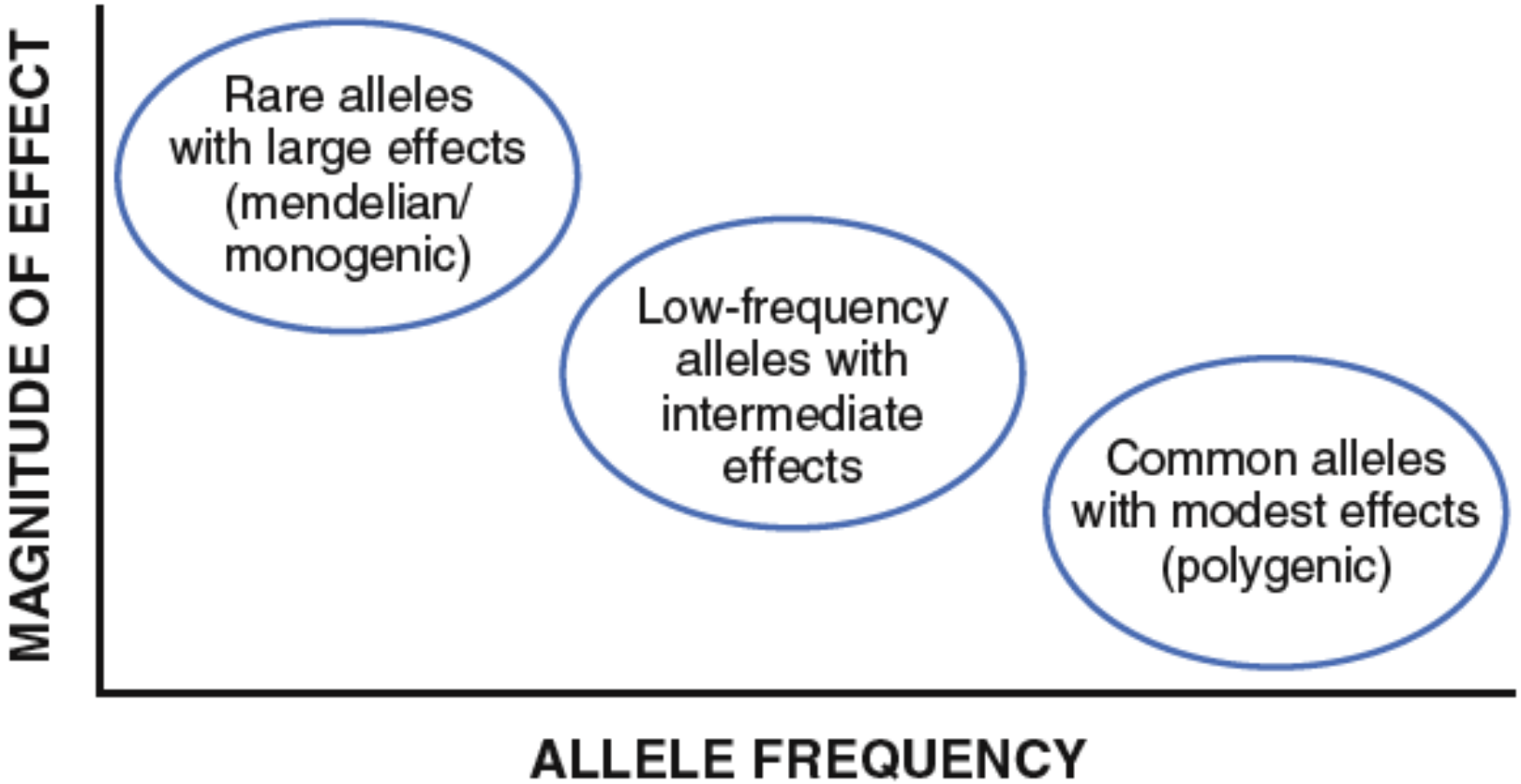


FIGURE 7.1 Relationship between allele frequency and effect magnitude of associated variants. Genome-wide assay studies, typically conducted with genome-wide genotyping arrays, typically identify common alleles with modest effects. Array coverage and imputation better enable the detection of lower frequency variants with intermediate effects. Rare alleles with larger effects are only detectable through genetic sequencing. Whole exome sequencing will detect the full allelic spectrum in coding regions, and whole genome sequencing will detect the full allelic spectrum across the genome.

GENE DISCOVERY

Family-Based Studies

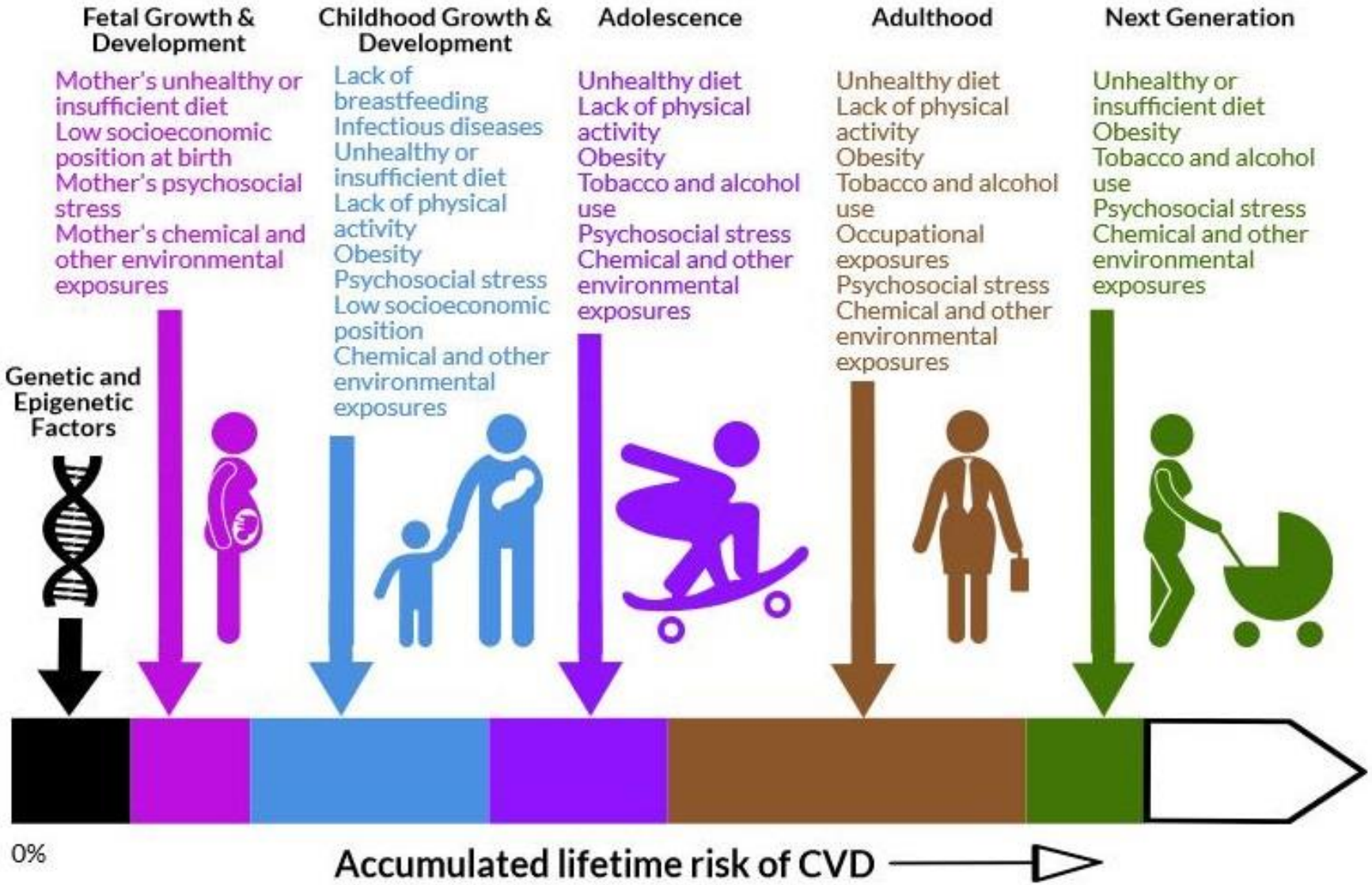
Case-Control and Population-Based Studies

Family-Based Studies

- classic mendelian inheritance patterns → monogenic factor
- polygenic or environmental factors- *Phenocopy*
- novel syndromes or phenotypes- Recruitment of multiple family members both with and without the phenotype allows for elimination of genotypes inconsistent with mendelian segregation.

phenocopy and reduced penetrance

Life Course Model of Cardiovascular Health



Individual risks vary according to each person's life experiences and exposure history.

Comprehensive Cardiovascular NGS Panel

Panel Description

Print Order

Diseases Targeted:

Cardiovascular Disease | Cardiomyopathy | Arrhythmia | Congenital Heart Defects | Familial Hypercholesterolemia

Genes:

A2ML1, ABCC9, ABL1, ACADVL, ACTA2, ACTB, ACTC1, ACTG1, ACTN2, ACVR2B, ACVRL1, ADA2, ADAMTS10, ADAMTS17, AGL, AKAP9, ALMS1, ANK2, ANKRD1, APOA5, APOB, B4GALT7, BAG3, BBS10, BCOR, BGN, BMPR2, BRAF, C1R, C1S, CACNA1C, CACNA2D1, CACNB2, CALM1, CALM2, CALM3, CALR3, CASQ2, CAV1, CAV3, CAVIN4, CBL, CBS, CCDC103, CCDC39, CCDC40, CHD7, CHRM2, COL1A1, COL3A1, COL5A1, COL5A2, COX15, CPT1A, CPT2, CRELD1, CRYAB, CSRP3, CTF1, CTNNA3, DEPDC5, DES, DMD, DNAAF1, DNAAF2, DNAAF3, DNAAF4, DNAAF5, DNAH11, DNAH5, DNAI1, DNAI2, DNAJC11, DNAJC19, DNAL1, DOLK, DSC2, DSG2, DSP, DTNA, EFEMP2, ELAC2, ELN, EMD, ENG, EYA4, FBN1, FBN2, FGD1, FHL1, FHL2, FKRP, FKTN, FLNA, FLNC, FOXE3, FOXH1, FXN, GAA, GATA4, GATA6, GATAD1, GDF1, GJA1, GJA5, GLA, GPC3, GPD1L, GYG1, HAMP, HAND1, HCN4, HFE, HJV, HRAS, ILK, INVS, IPO8, JAG1, JPH2, JUP, KAT6B, KCNA5, KCND3, KCNE1, KCNE2, KCNE3, KCNE5, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNK3, KCNQ1, KCNQ2, KCNQ3, KCNT1, KRAS, LAMA4, LAMP2, LDB3, LDLR, LDLRAP1, LEFTY2, LMNA, LOX, LTBP3, LZTR1, MAP2K1, MAP2K2, MAT2A, MED12, MED13L, MFAP5, MIB1, MKS1, MMP21, MRPL3, MTO1, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLK, MYLK2, MYOM1, MYOZ2, MYPN, NDUFAF1, NDUFB11, NEBL, NEK8, NEXN, NF1, NKX2-5, NKX2-6, NME8, NODAL, NOTCH1, NOTCH2, NPHP3, NPPA, NR2F2, NRAS, NSD1, OFD1, PCDH19, PCSK9, PDLIM3, PKD1L1, PKP2, PLN, PLOD1, PRDM16, PRKAG2, PRKG1, PRRT2, PSEN2, PTPN11, RAF1, RANGRF, RASA1, RBM20, RIT1, RRAS, RYR2, SCN10A, SCN1A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SCN8A, SCN9A, SDHA, SGCD, SHOC2, SKI, SLC22A5, SLC25A20, SLC2A1, SLC2A10, SLC40A1, SLMAP, SMAD2, SMAD3, SMAD4, SMAD6, SMAD9, SNTA1, SOS1, SOS2, SYNE1, TAB2, TAZ, TBX1, TBX20, TBX5, TCAP, TFR2, TGFB2, TGFB3, TGFBR1, TGFBR2, TMEM43, TMEM70, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TRDN, TRPM4, TTC8, TTN, TTR, TXNRD2, VCL, YWHAE, ZFPM2, ZIC3 **(264 genes)**

Coverage:

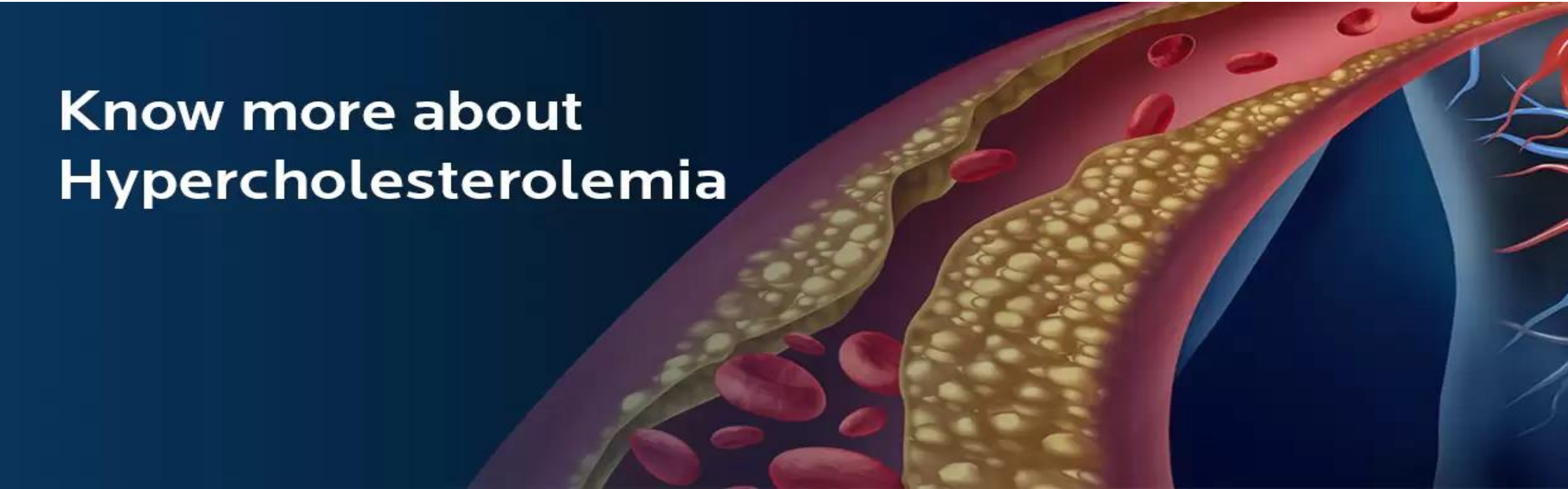
96% at 20x

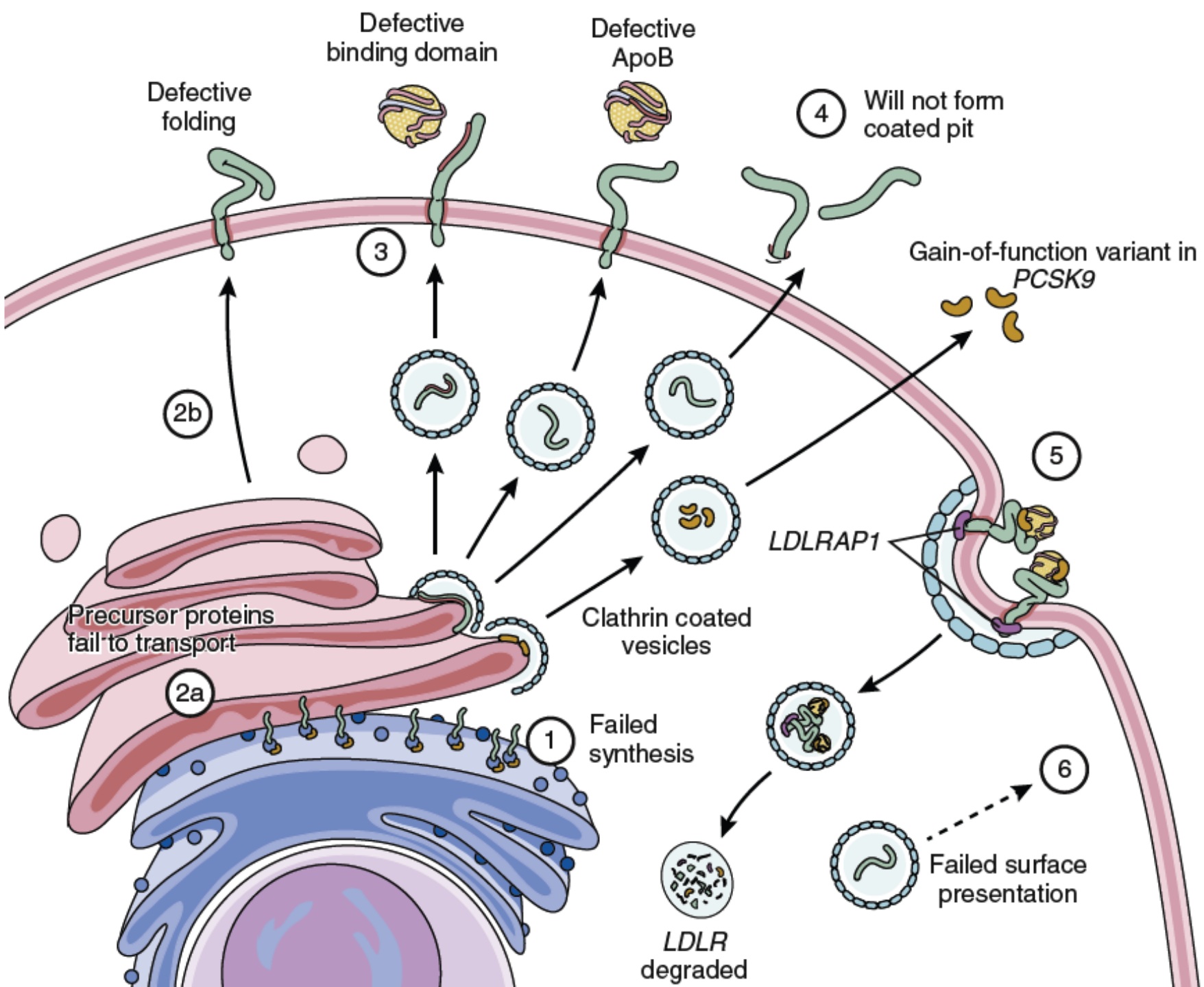
Specimen Requirements:

Blood (two 4ml EDTA tubes, lavender top) or Extracted DNA (3ug in EB buffer) or Buccal Swab or Saliva (kits available upon request)

Hypercholesterolemia and Coronary Artery Disease

- FH afflicts approximately 1 in 300 individuals, manifesting as severely elevated blood cholesterol levels and increased risk for early-onset myocardial infarction.




Defective binding domain
Defective ApoB
Defective folding
4
Will not form coated pit
3
Gain-of-function variant in PCSK9
2b
5
LDLRAP1
Precursor proteins fail to transport
Clathrin coated vesicles
2a
1
Failed synthesis
6
Failed surface presentation
LDLR degraded

However, a single pathogenic variant in the canonical FH-genes (*LDLR, APOB or PCSK9*) is identified in only **15–50%** of phenotypical FH patients (classified by clinical scoring systems)

in the early 2000s, linkage and cloning analyses of families with autosomal recessive FH prioritized a large region on chromosome 1. Ultimately, homozygous mutations in LDLRAP1 (previously known as ARH, autosomal recessive hypercholesterolemia). LDLRAP1 encodes LDL receptor adaptor protein 1, which is required for endocytosis of the LDL receptor.

At present, over **3100** common genetic variants have been shown to be associated with LDL-C levels

PRS is constructed by summing the number of alleles from trait-affecting variants an individual has, weighted by their effect size as reported in the GWAS.

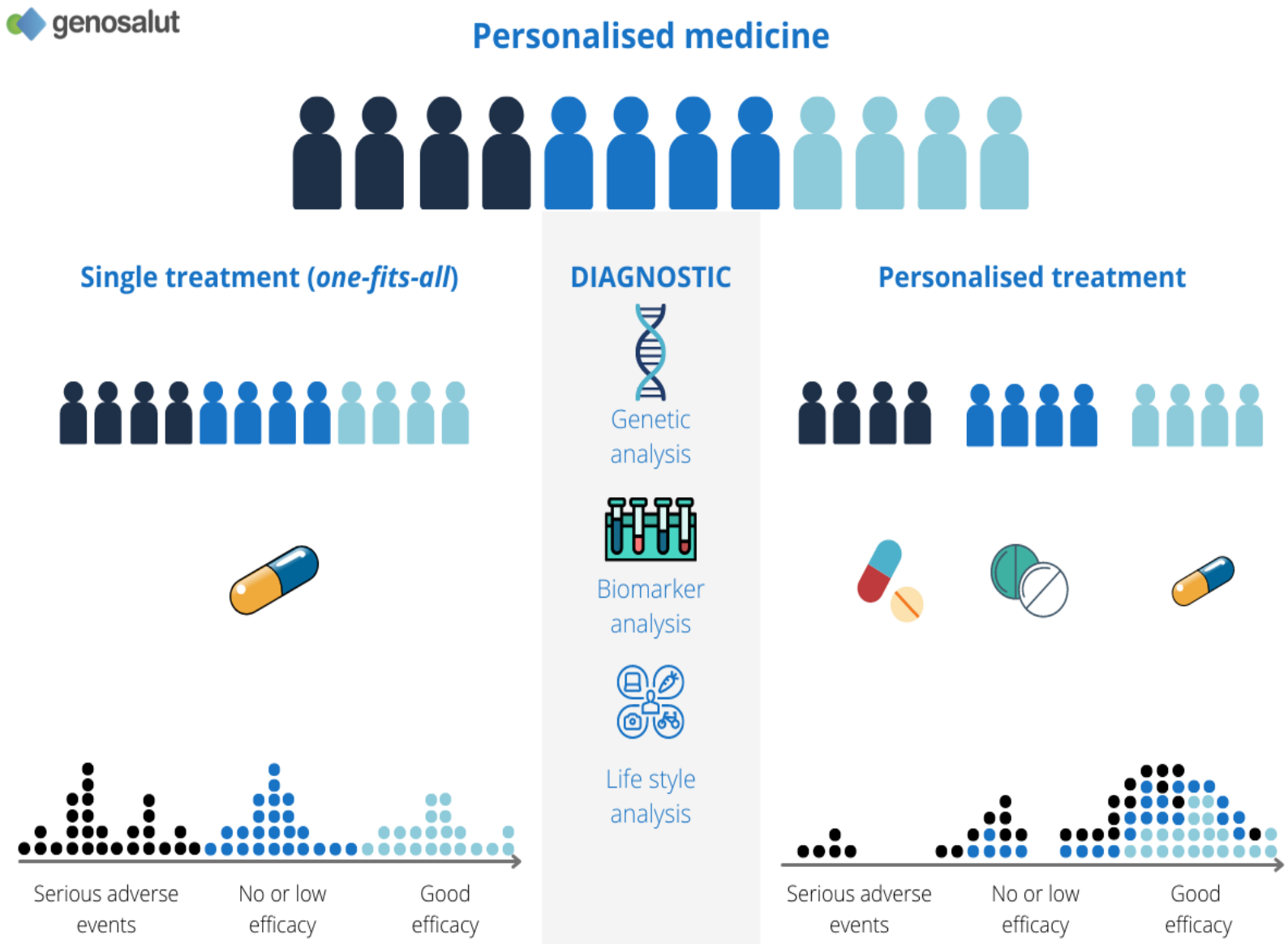
LDLR Mutations:
Patients with mutations in the LDLR gene have a more severe form of FH and may respond less effectively to statins compared to those with mutations in other genes like APOB or PCSK9.

APOB Mutations:
Statins are generally more effective in patients with APOB mutations compared to those with LDLR mutations.

PCSK9 Mutations:
Patients with gain-of-function mutations in PCSK9 may also have a different response to statins. While statins can still lower LDL cholesterol levels, these patients may require additional therapies, such as PCSK9 inhibitors, for optimal management due to their unique genetic profile.

For patients who do not respond adequately to statins due to their genetic makeup, other medications such as **ezetimibe or PCSK9 inhibitors** may be recommended.

As a result, precision medicine, by incorporating established biomarkers, functional tests, imaging, and new genomics and omics developments for each population, aims to provide the "**right treatment to the right patient at the right time**"



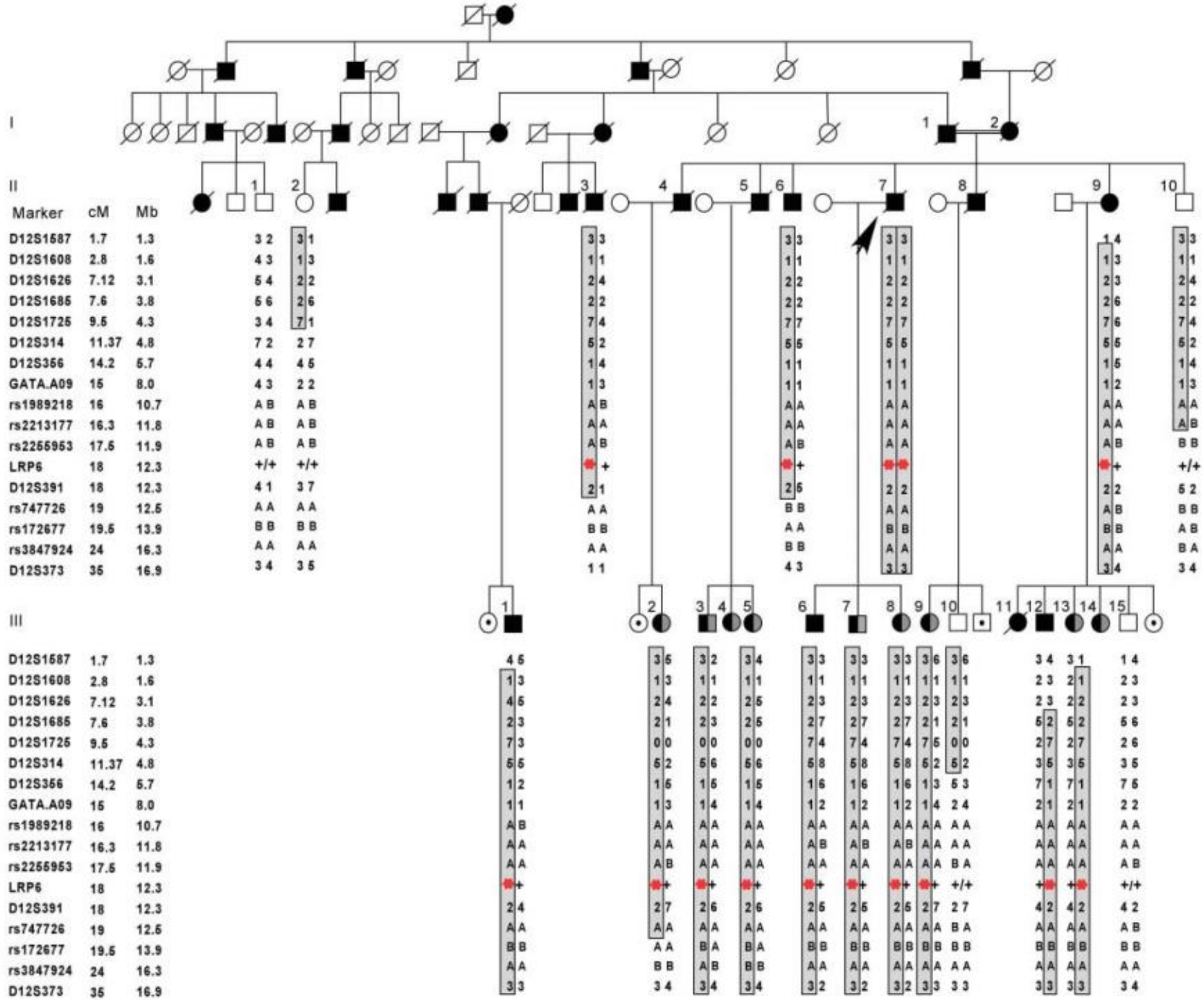
Metabolic Syndrome and Coronary Artery Disease

- In 2007, researchers studied a **large Iranian family** with early coronary artery disease (CAD) and metabolic syndrome.
- They found a specific genetic change (missense variant) in the **LRP6 gene** that disrupted a key signaling pathway called **Wnt signaling**.

LRP6 Mutation in a Family with Early Coronary Disease and Metabolic Risk Factors

Arya Mani,[1*] Jayaram Radhakrishnan,[1] He Wang,[2] Alaleh Mani,[3] Mohammad-Ali Mani,[4] Carol Nelson-Williams,[1] Khary S. Carew,[1] Shrikant Mane,[1] Hossein Najmabadi,[5] Dan Wu,[2] Richard P. Lifton[1*]

Coronary artery disease (CAD) is the leading cause of death worldwide and is commonly caused by a constellation of risk factors called the metabolic syndrome. We characterized a family with autosomal dominant early CAD, features of the metabolic syndrome (hyperlipidemia, hypertension, and diabetes), and osteoporosis. These traits showed genetic linkage to a short segment of chromosome 12p, in which we identified a missense mutation in *LRP6*, which encodes a co-receptor in the Wnt signaling pathway. The mutation, which substitutes cysteine for arginine at a highly conserved residue of an epidermal growth factor–like domain, impairs Wnt signaling in vitro. These results link a single gene defect in Wnt signaling to CAD and multiple cardiovascular risk factors.



Marker	cM	Mb
D12S1587	1.7	1.3
D12S1608	2.8	1.6
D12S1626	7.12	3.1
D12S1685	7.6	3.8
D12S1725	9.5	4.3
D12S314	11.37	4.8
D12S356	14.2	5.7
GATA.A09	15	8.0
rs1989218	16	10.7
rs2213177	16.3	11.8
rs2256953	17.5	11.9
LRP6	18	12.3
D12S391	18	12.3
rs747726	19	12.5
rs172677	19.5	13.9
rs3847924	24	16.3
D12S373	35	16.9

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D12S373	35	16.9

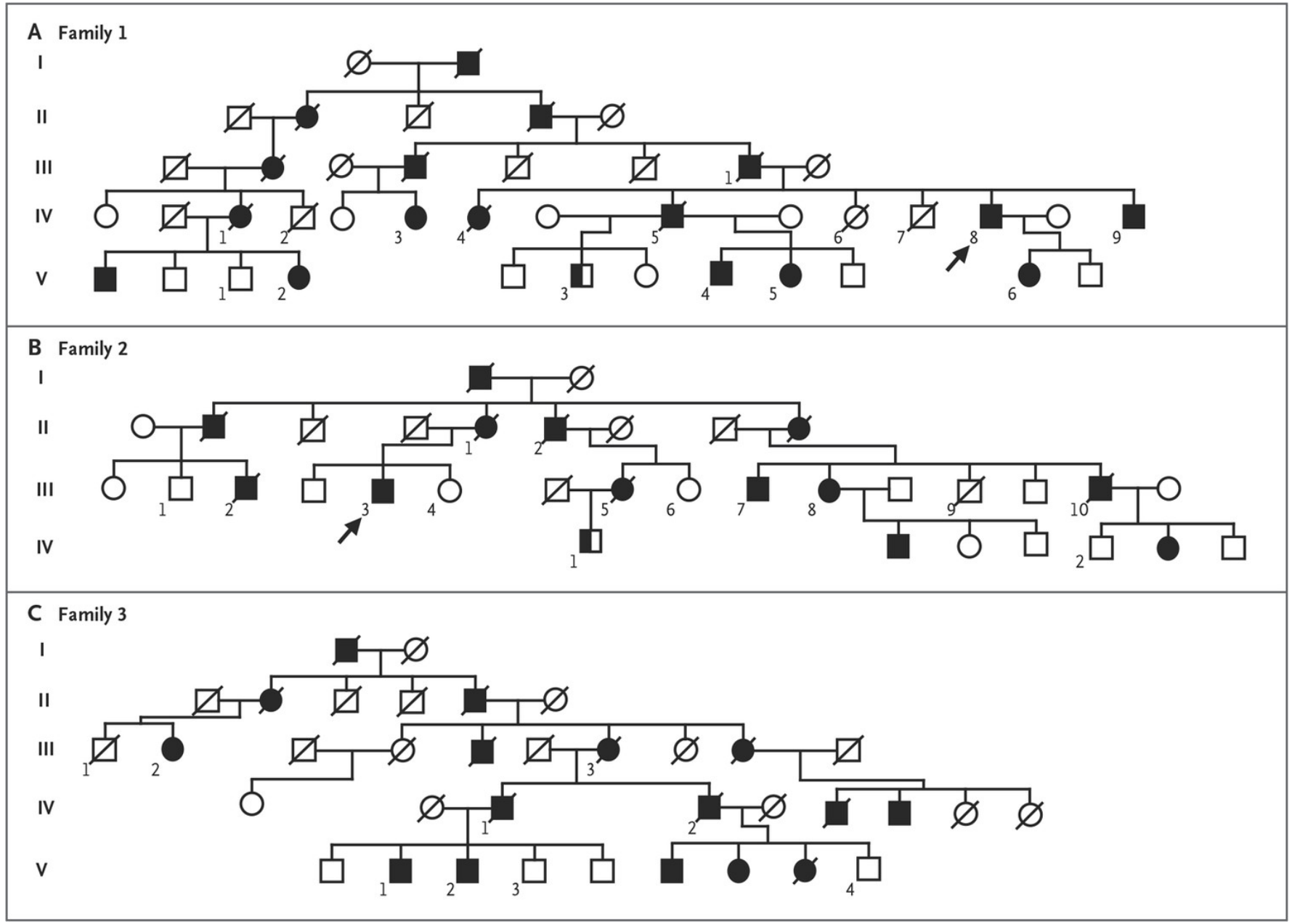
the same team examined three additional large Iranian families with similar health issues.

ORIGINAL ARTICLE

A Form of the Metabolic Syndrome Associated with Mutations in *DYRK1B*

Authors: Ali R. Keramati, M.D., Mohsen Fathzadeh, Ph.D., Gwang-Woong Go, Ph.D., Rajvir Singh, Ph.D., Murim Choi, Ph.D., Saeed Faramarzi, M.D., Shrikant Mane, Ph.D., +9, and Arya Mani, M.D. Author Info & Affiliations

Published May 15, 2014 | N Engl J Med 2014;370:1909-1919 | DOI: 10.1056/NEJMoa1301824 | VOL. 370 NO. 20


A Family 1
I
II
III
IV
V
B Family 2
I
II
III
IV
C Family 3
I
II
III
IV
V

- They used linkage analysis to narrow down a specific area on **chromosome 19** that was linked to these conditions.
- By performing whole exome sequencing in this region, they discovered another missense variant in the **DYRK1B gene** that was consistently found in all three families.

These findings indicate a role for *DYRK1B* in adipogenesis and glucose homeostasis and associate its altered function with an inherited form of the metabolic syndrome.


Thank You