
بسم الله الرحمن الرحيم

State of Genetic Testing

How is genetic testing done? What do the results mean?

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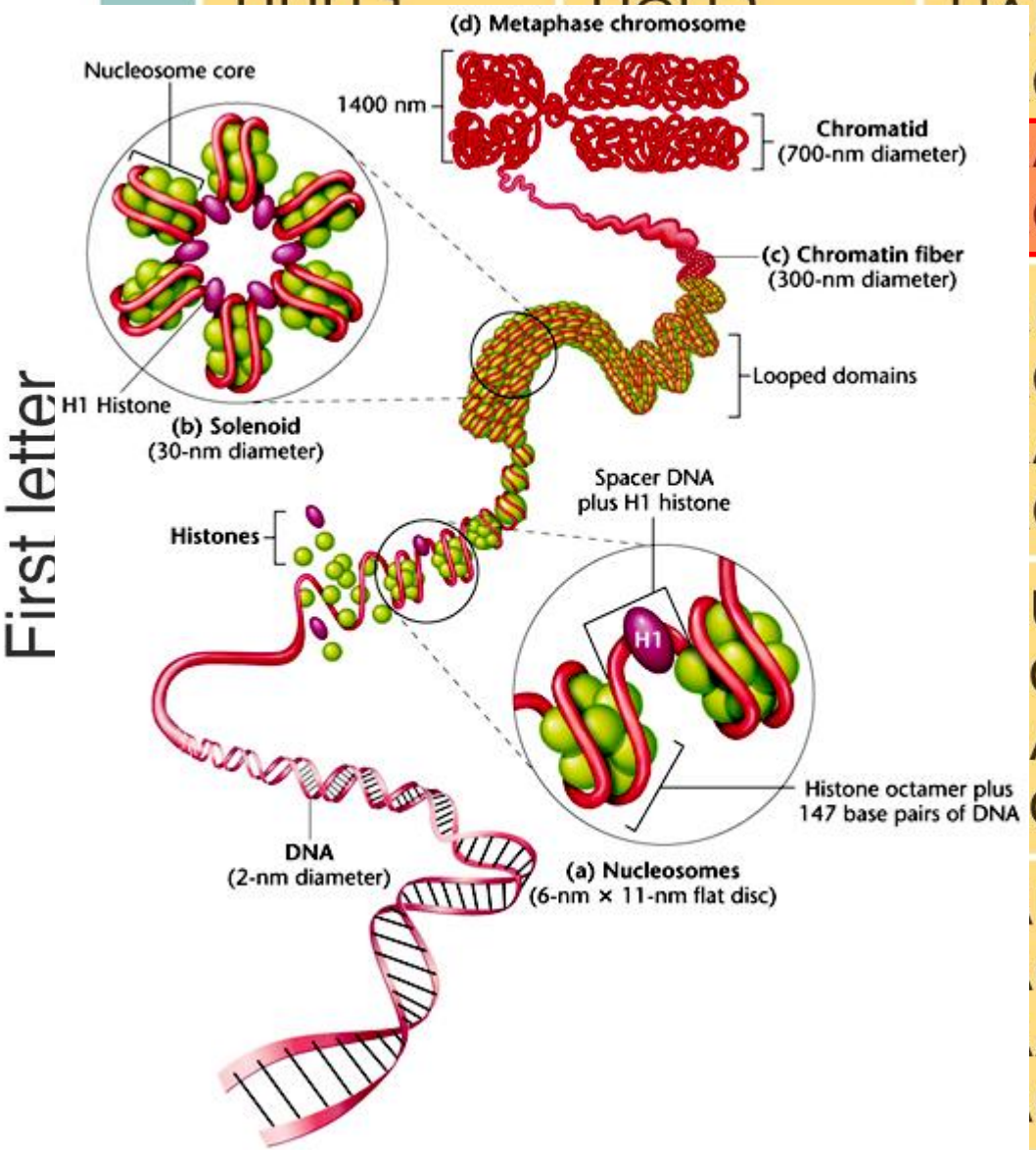
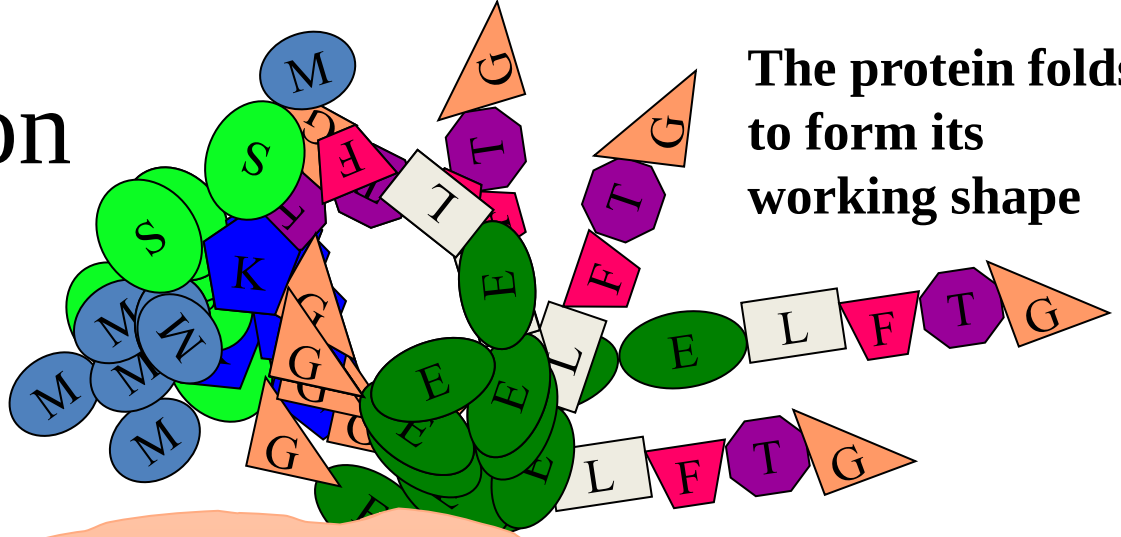
Second letter

First letter	U	C	A	G	Third letter
U	UUU, UUC } Phe	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr	UGU, UGC } Cys	U
	UUA, UUG } Leu		UAA Stop	UGA Stop	C
			UAG Stop	UGG Trp	A
					G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His	CGU, CGC, CGA, CGG } Arg	U
			CAA, CAG } Gln		C
					A
					G
A	AUU, AUC, AUA } Ile	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn	AGU, AGC } Ser	U
			AAA, AAG } Lys	AGA, AGG } Arg	C
					A
					G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp	GGU, GGC, GGA, GGG } Gly	U
			GAA, GAG } Glu		C
					A
					G

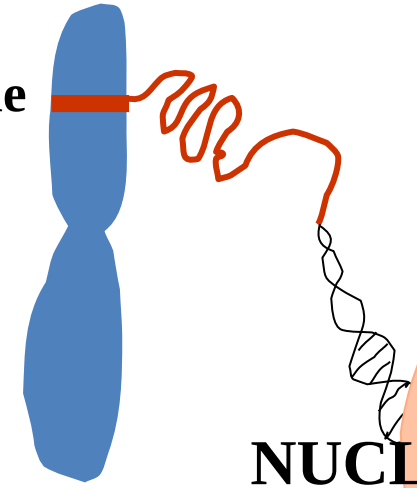


Gene Expression



The protein folds to form its working shape

Gene

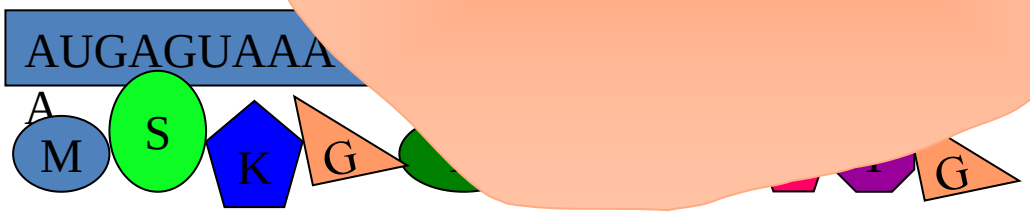


NUCLEUS

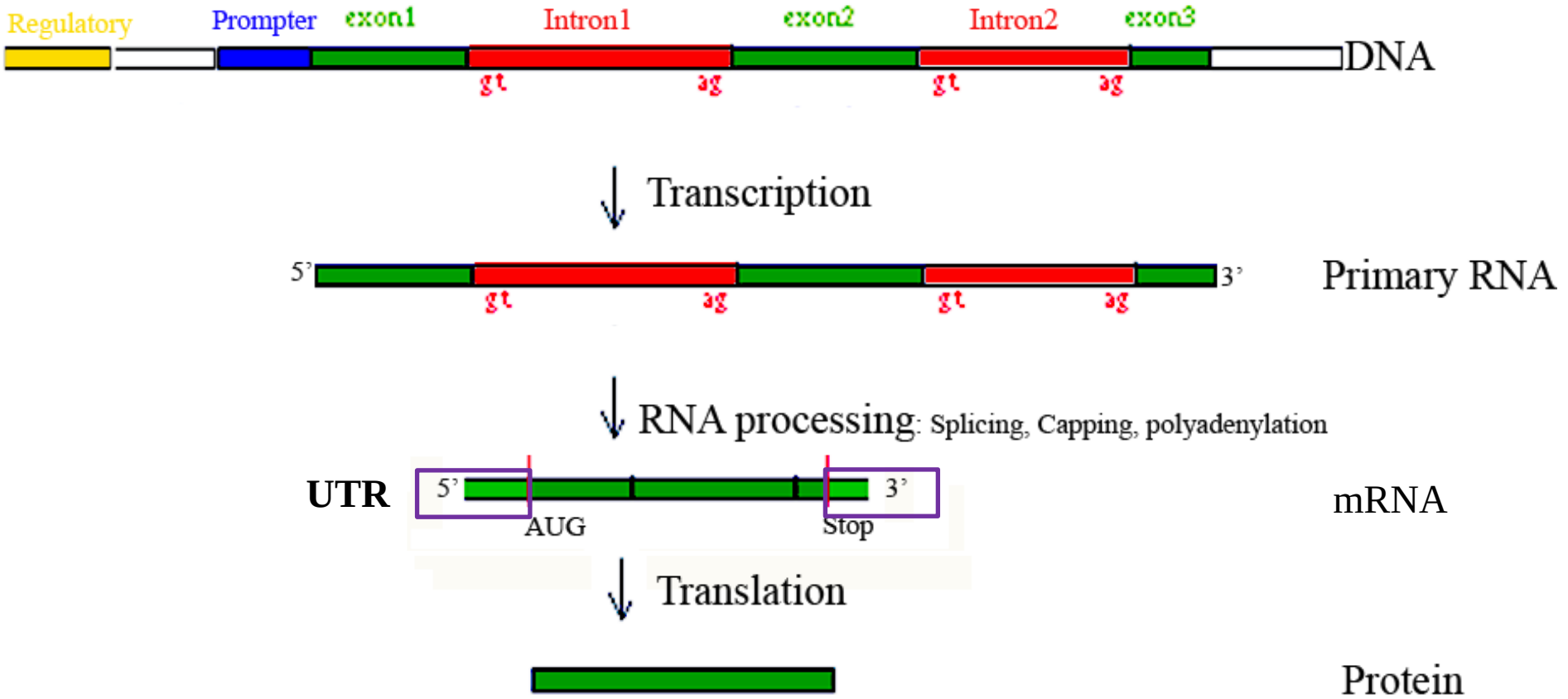
CELL

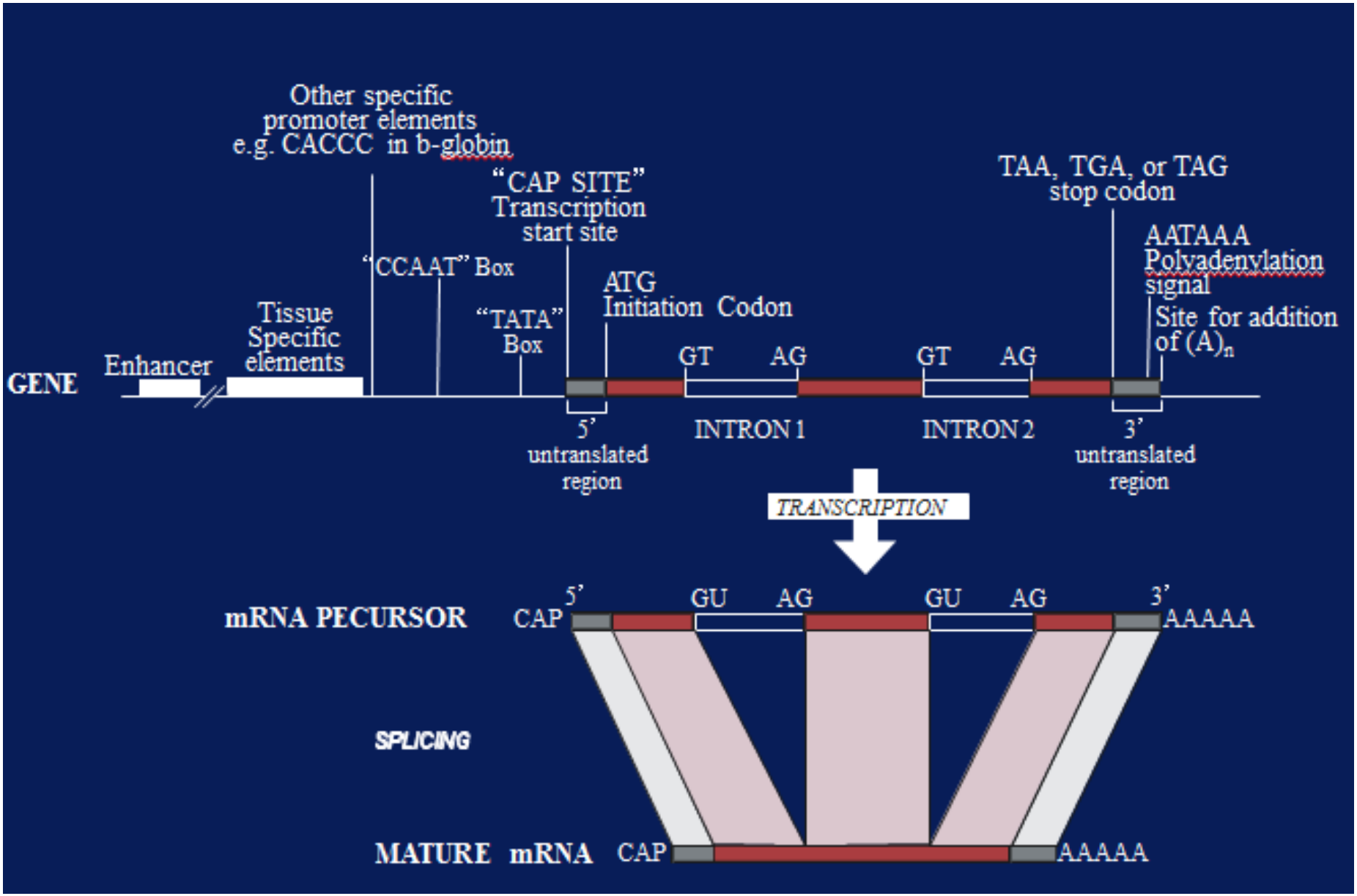
Cell machinery copies the code aking an mRNA olecule. This ves into the plasm.

osomes read the ode and accurately in Amino acids together to make a protein



Central Dogma, Gene Structure



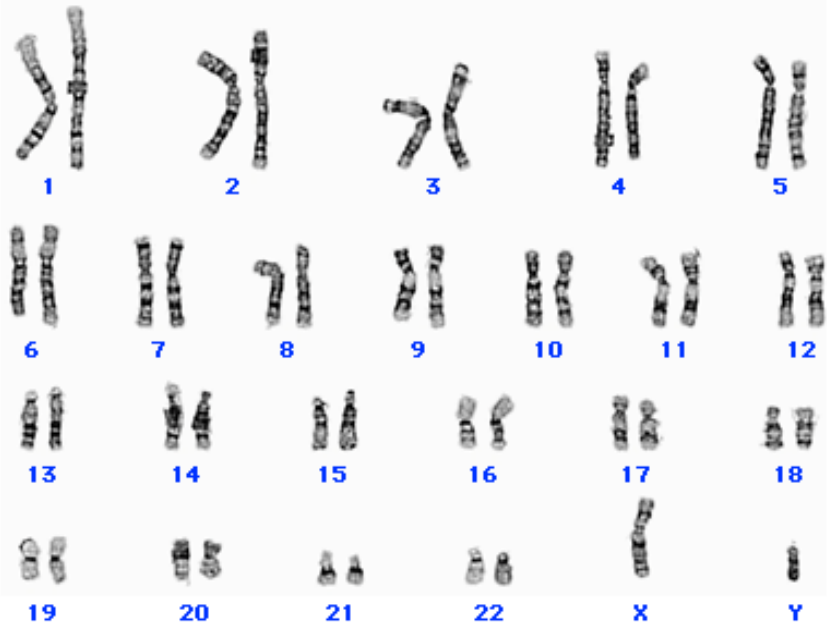

Other specific promoter elements e.g. CACCC in b-globin
"CAP SITE" Transcription start site
TAA, TGA, or TAG stop codon
AATAAA Polyadenylation signal
"CCAAT" Box
ATG Initiation Codon
Site for addition of (A)n
Tissue Specific elements
"TATA" Box
GT
AG
GT
AG
GENE
Enhancer
5' untranslated region
INTRON 1
INTRON 2
3' untranslated region
TRANSCRIPTION
mRNA PECURSOR
5'
GU
AG
GU
AG
3'
CAP
AAAAA
SPLICING
MATURE mRNA
CAP
AAAAA

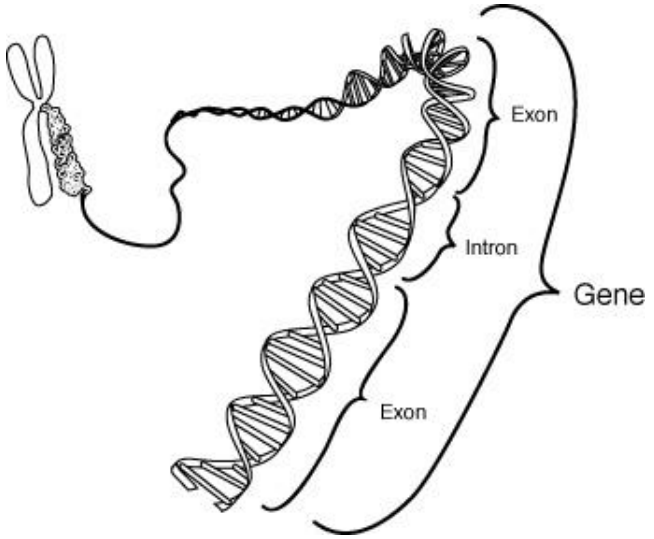
Genetic variation

- Makes us unique
  - “variants”
- Is the basis for evolution
- Is the basis for disease

Genetic Disorders

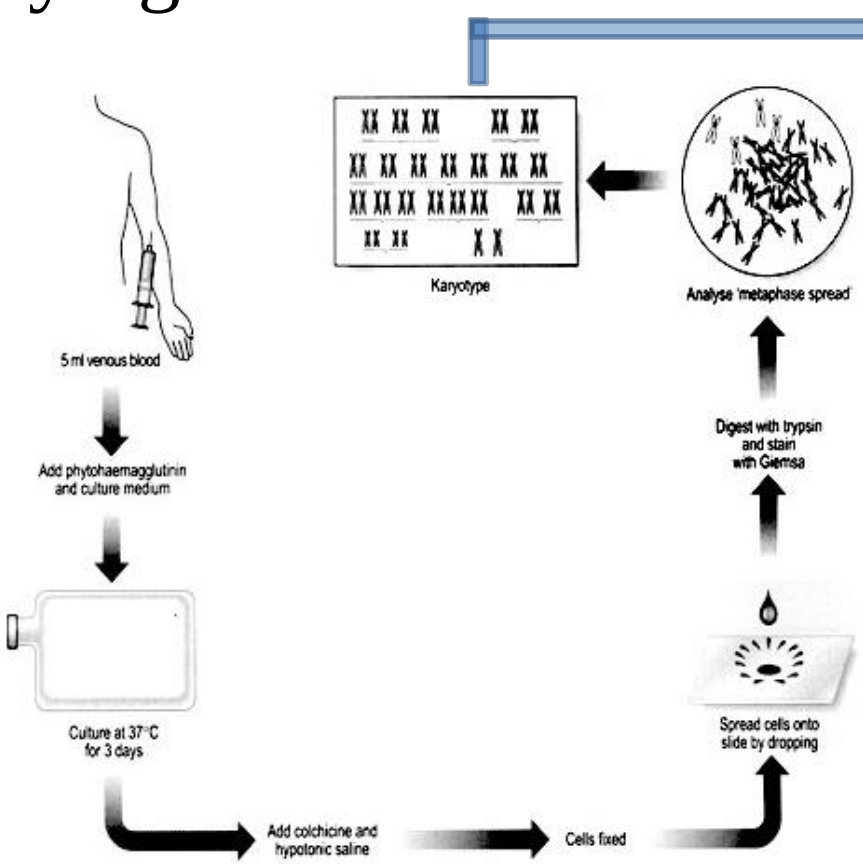
1. **Single Gene Inheritance** (Mendelian inheritance)
    - Cystic Fibrosis, Marfan Syn., Familial Hypercholestrolemia
2. **Chromosomal disorders** (Cytogenetics)
    - Trisomy 21
3. **Multifactorial Disorders**
    - Combination of Genetic and environmental factors (Common disease: Diabetes Mellitus, CAD





Cytogenetic tests

- Cytogenetics:



Chromosome Analysis

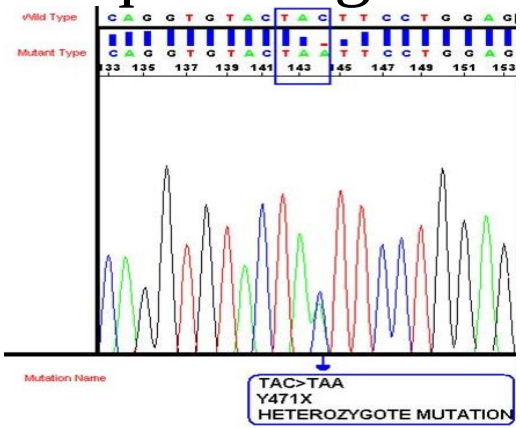
Report

Molecular tests

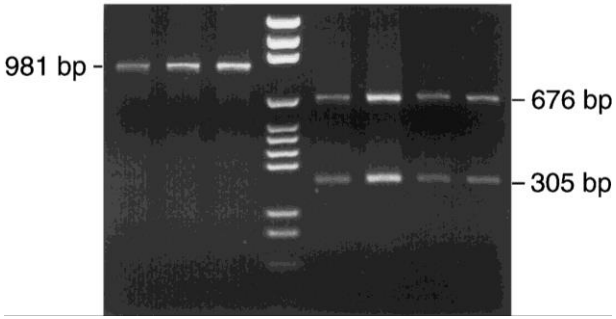
- Molecular Genetics:
  - PCR
  - Sanger Sequencing
  - WES

PCR

Sequencing

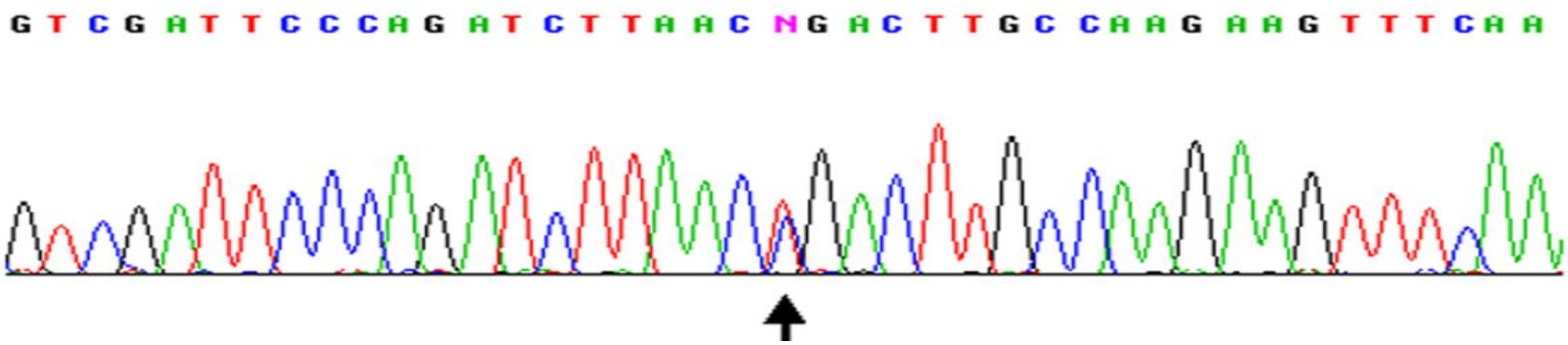


Electrophoresis



Report

Mutation Nomenclature



c.1552C>T

Base position

"c." for coding DNA sequence

Original base

Replacement

Deletion: 197delAG

Insertion: 2552insT

Inversion: 76_83inv

p.R518X

Original aa

aa position

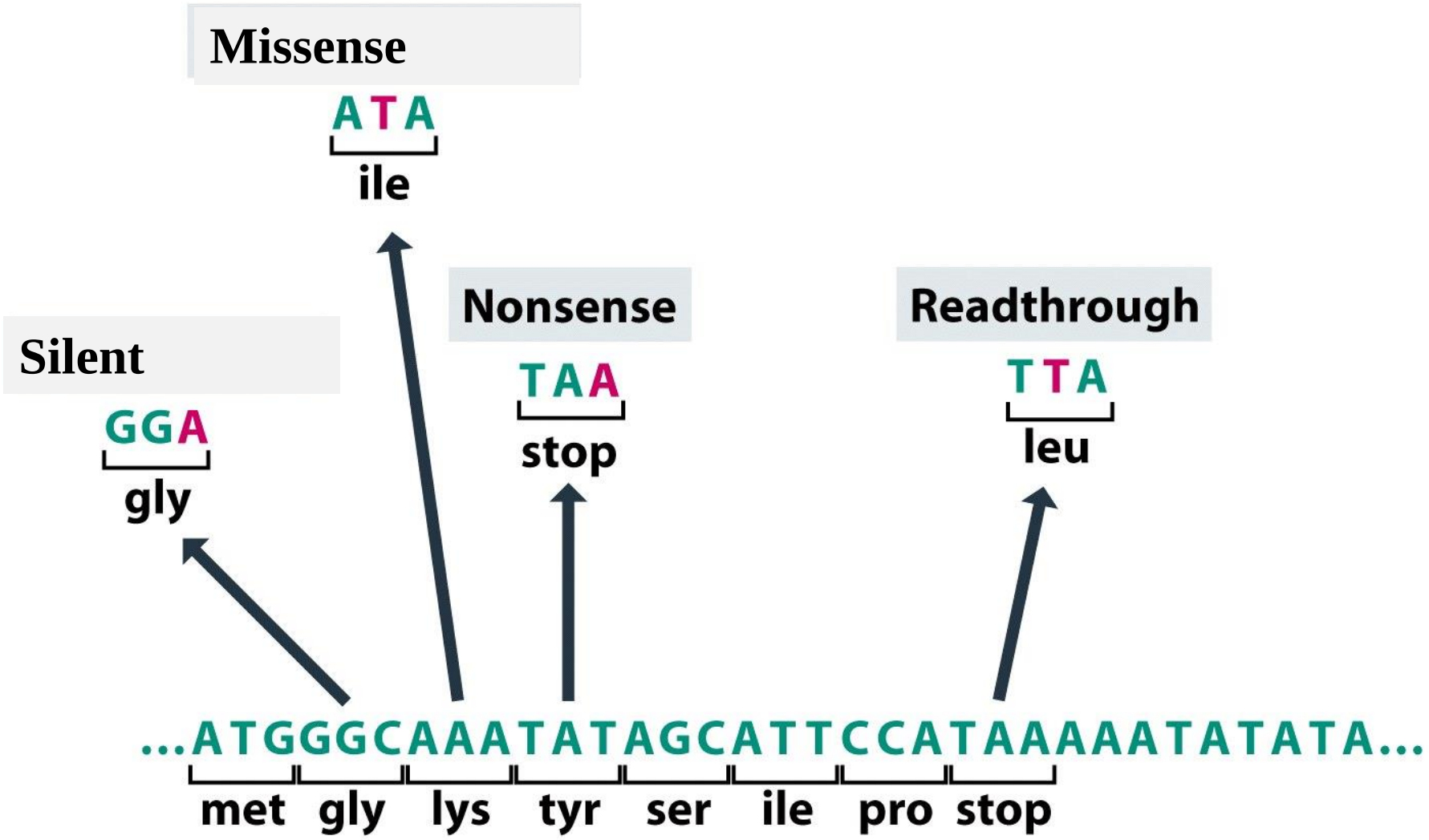
Silent: p.Arg197Arg

Missense: p.Arg197Trp

Nonsense: p.Arg197Ter

Replacement

del508F in CFTR gene


Missense
ATA
ile
Silent
GGA
gly
Nonsense
TAA
stop
Readthrough
TTA
leu
...ATGGGCAAATATAGCATTCCATAAAAATATATA...
met gly lys tyr ser ile pro stop

Allelic heterogeneity

Locus heterogeneity

Loss of function

Gain of function

Dominant negative

Haploinsuficiency

Pleiotropy

Reduced penetrance
Variable expressivity

Variant classification

Varaint, mutation, polymorphism

The American College of Medical Genetics and Genomics (ACMG):

(1) pathogenic

(2) likely pathogenic

(3) uncertain significance

(4) likely benign

(5) benign

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause	Patient's phenotype or FH highly specific for			

Rules for Combining Criteria to Classify Sequence Variants

Pathogenic:

1. 1 Very Strong (PVS1) *AND*
   - ≥1 Strong (PS1–PS4) *OR*
   - ≥2 Moderate (PM1–PM6) *OR*
   - 1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5) *OR*
   - ≥2 Supporting (PP1–PP5)
2. ≥2 Strong (PS1–PS4) *OR*
3. 1 Strong (PS1–PS4) *AND*≥3 Moderate (PM1–PM6) *OR*
   - 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*
   - 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)

Likely Pathogenic:

1. 1 Very Strong (PVS1) *AND* 1 Moderate (PM1–PM6) *OR*
2. 1 Strong (PS1–PS4) *AND* 1–2 Moderate (PM1–PM6) *OR*
3. 1 Strong (PS1–PS4) *AND* ≥2 Supporting (PP1–PP5) *OR*
4. ≥3 Moderate (PM1–PM6) *OR*
5. 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*
6. 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)

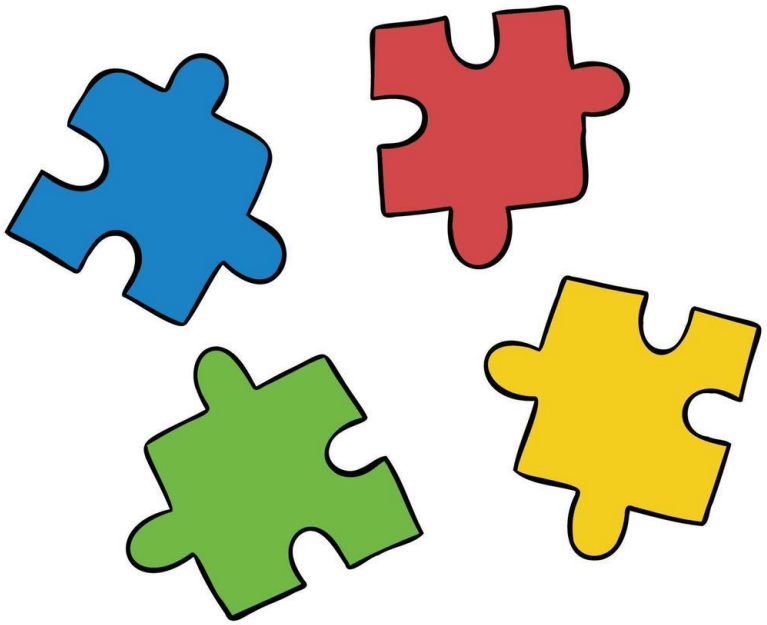
Benign:

1. 1 Stand-Alone (BA1) *OR*
2. ≥2 Strong (BS1–BS4)

Likely Benign:

1. 1 Strong (BS1–BS4) and 1 Supporting (BP1–BP7) *OR*
2. ≥2 Supporting (BP1–BP7)

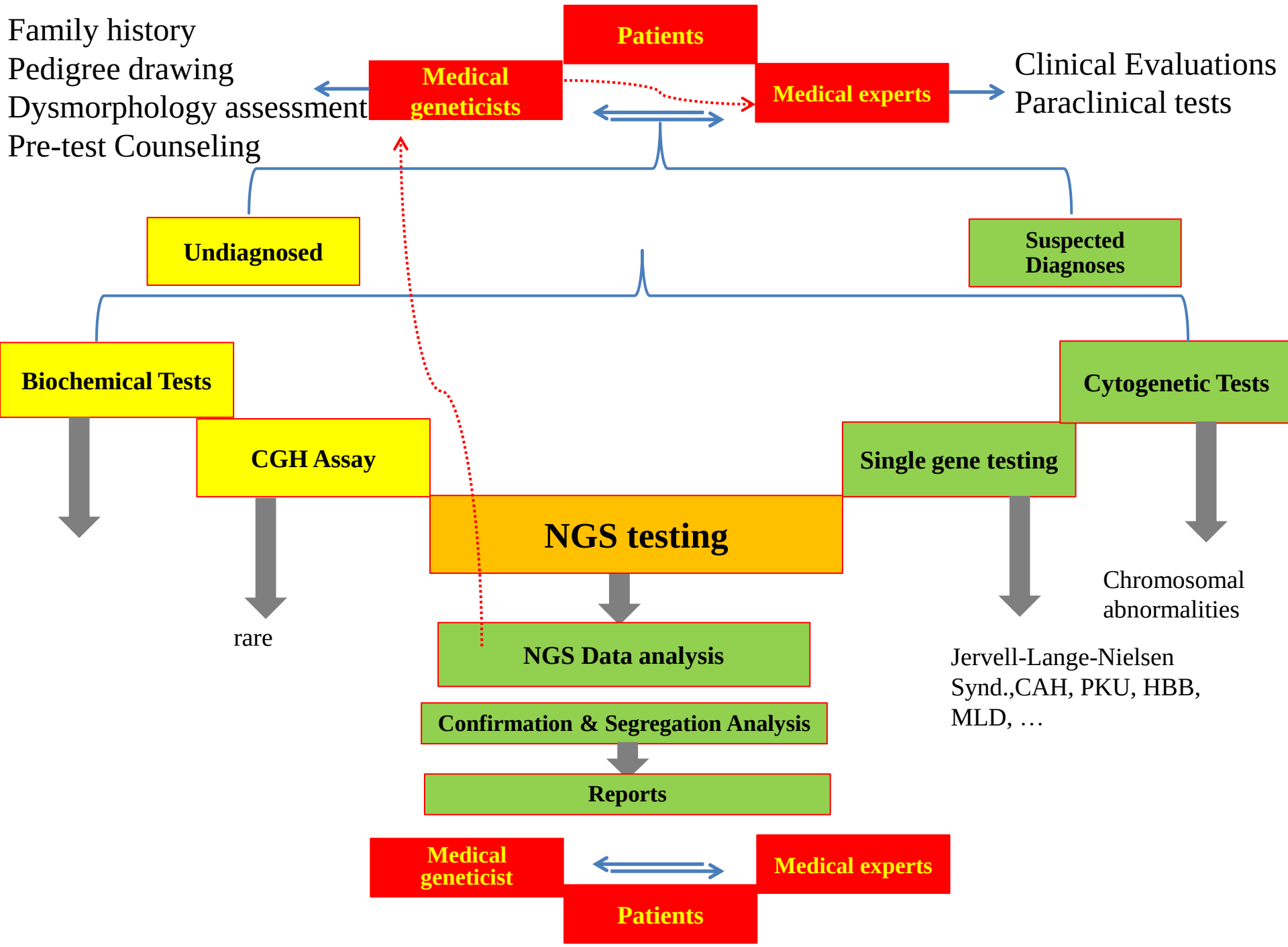
وضعیت جاری



- پزشک:
  - درخواست تست
  - گرفتن جواب
- بیمار:
  - پذیرش تست
  - ارجاع به پزشک معالج
- آزمایشگاه:
  - دریافت نسخه پزشکی توسط واحد پذیرش و پذیرش بیمار
  - انجام آزمایش
  - جوابدهی

فرآیند تشخیص بیماری ژنتیک

- بیمار
- پزشک (های) متخصص
- مشاوره ژنتیک، pre-test، post-test
- آزمایشگاه:


Family history
Pedigree drawing
Dysmorphology assessment
Pre-test Counseling
Patients
Medical geneticists
Medical experts
Clinical Evaluations
Paraclinical tests
Undiagnosed
Suspected Diagnoses
Biochemical Tests
Cytogenetic Tests
CGH Assay
Single gene testing
NGS testing
rare
Chromosomal abnormalities
NGS Data analysis
Jervell-Lange-Nielsen Synd.,CAH, PKU, HBB, MLD, …
Confirmation & Segregation Analysis
Reports
Medical geneticist
Medical experts
Patients

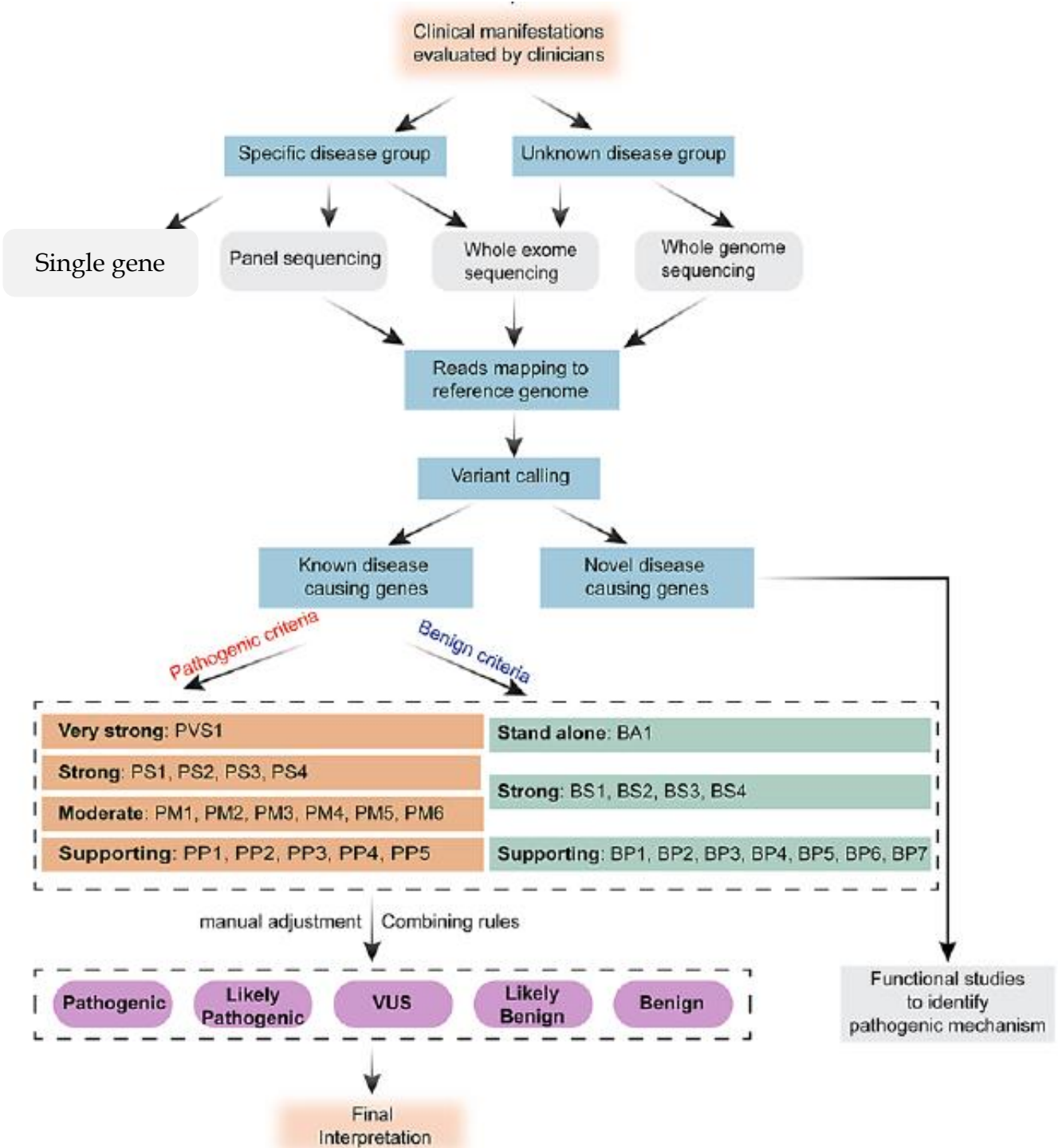
Genetic Testing

- Essential principles of genetic testing
  - modes of inheritance
  - different testing methodologies,
  - interpretation of variants

Genetic Models of Cardiac disease

- Syndromic
  - Chromosomal Syndromes
    - Aneuploidy syndromes; e.g. *Down syndrome*, *Turner syndrome*
    - Abnormal chromosomal structural syndromes; e.g. *Williams-Beuren*, *DiGeorge syndrome*
  - Single gene mutation syndromes; e.g. *Holt-Oram*, *Noonan*, *Costello syndromes*

- Nonsyndromic
  - single gene & polygenes
    - Cardiomyopathies
    - CHD


Clinical manifestations evaluated by clinicians
Specific disease group
Unknown disease group
Single gene
Panel sequencing
Whole exome sequencing
Whole genome sequencing
Reads mapping to reference genome
Variant calling
Known disease causing genes
Novel disease causing genes
Pathogenic criteria
Benign criteria
Very strong: PVS1
Strong: PS1, PS2, PS3, PS4
Moderate: PM1, PM2, PM3, PM4, PM5, PM6
Supporting: PP1, PP2, PP3, PP4, PP5
Stand alone: BA1
Strong: BS1, BS2, BS3, BS4
Supporting: BP1, BP2, BP3, BP4, BP5, BP6, BP7
manual adjustment
Combining rules
Pathogenic
Likely Pathogenic
VUS
Likely Benign
Benign
Functional studies to identify pathogenic mechanism
Final Interpretation

Genetic testing

- **Sanger sequencing (gene-by-gene)**
  - CAH (CYP21A2)
  - PKU (PAH)
  - CF (CFTR)
  - Galactosemia (GALT, GALK1, GALE)
  - Gaucher (GBA), Fabry disease(GLA)
  - Niemann-Pick disease? (SMPD1 : A,B; NPC1,2: C)
- **Next generation sequencing (NGS)**
  - **Gene-panel**
  - **Whole-exome sequencing (WES)**
    - ~1–2% of the genome
    - ~85% of recognized disease-causing mutations
    - exome coverage 90 and 95%
    - A month
  - **Whole-genome sequencing (WGS)**

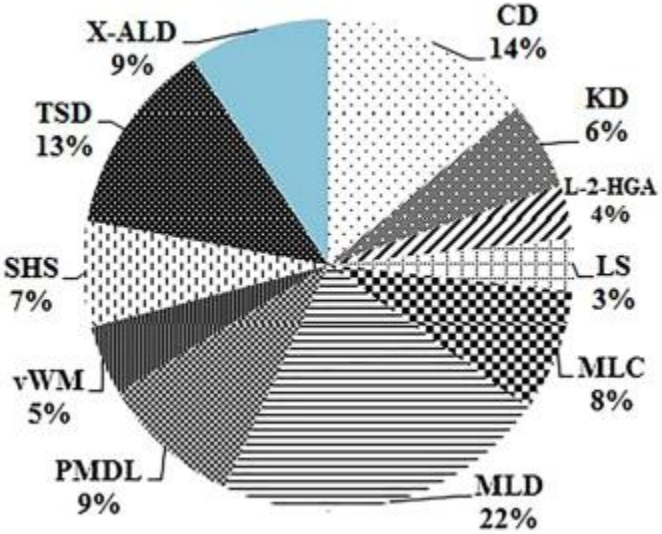
Genetic testing

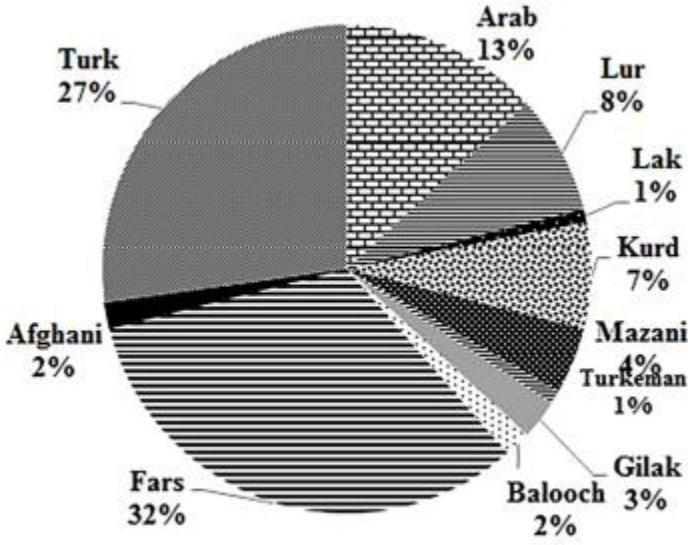
Non-sequencing approaches			
Allele-specific PCR	Quick, cheap, accurate	Pre-specified variants only	Testing a single variant in a large family (more likely Sanger sequencing now)
Array comparative genomic hybridization	Cheap screening for SVs/CNVs High-resolution (compared with cytogenetic approaches)	Insensitive to other variant classes	Screening for structural variants including aneuploidy, e.g. in structural congenital heart disease
Droplet digital PCR	Low cost, high-sensitivity, detection of genome dose for SV/CNV detection at a pre-specified locus	Scalability limited by multiplexing of pre-specified PCR amplicons targeting regions of interest	Confirmation of putative CNVs detected in high-throughput sequence data
DNA SNP arrays	Genome wide Relatively cheap	Pre-specified variants only Accuracy poor for many rarer variants	Recreational ancestry analysis Polygenic risk Pharmacogenetics

Types of Genetic Test Results

- **Positive**
  - the test found a genetic change known to cause disease
- **Negative**
  - the test did not find a genetic change known to cause disease
- **Uncertain**
  - a variant of unknown or uncertain significance means there isn't enough information about that genetic change to determine whether it is benign (normal) or pathogenic (disease causing)

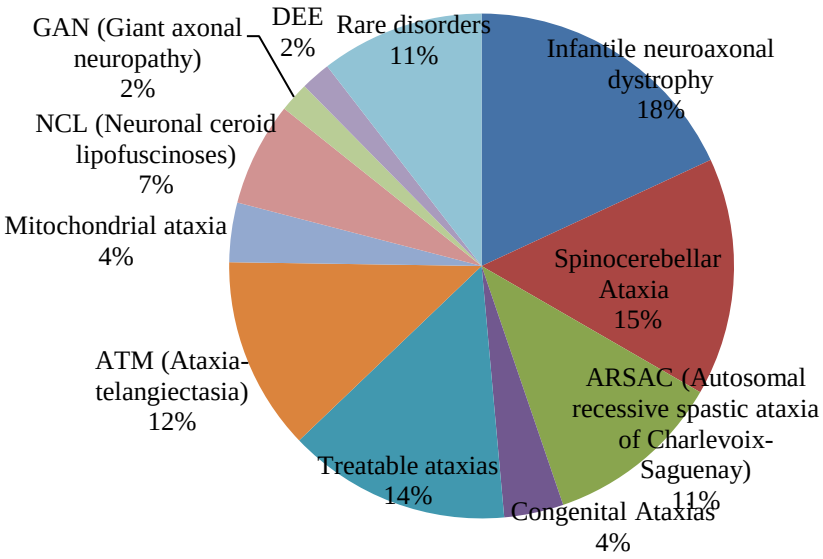
- 152 children based on clinical and neuroradiological findings

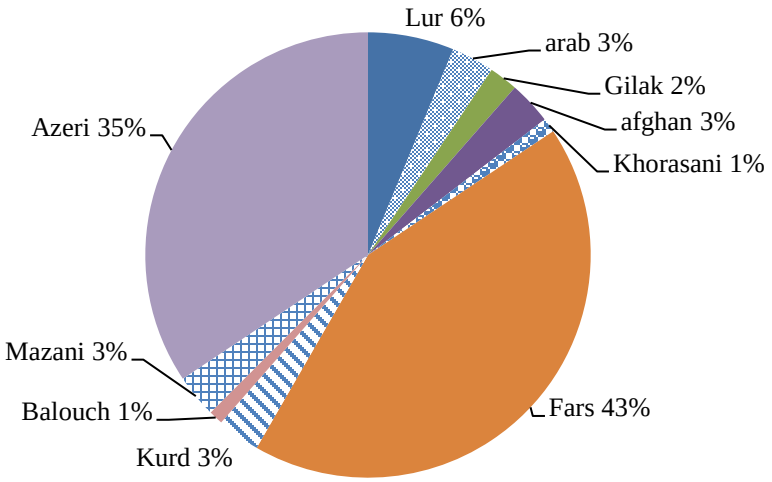




Mahdieh et al., Sci Rep 2021

The genetic basis of early-onset hereditary ataxia in Iran





168 patients from different parts of Iran
diagnostic rate of 70%

Mahdieh et al., submitted