The Genetic Testing in Endocrine Disorders

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Jun 2024

Outline

- Some facts about the human genome
- Different types of DNA sequence variants
- Next Generation Sequencing (NGS) and levels of DNA testing
- Clinical Indications of Genetic Testing in Endocrine Diseases
- Conclusions

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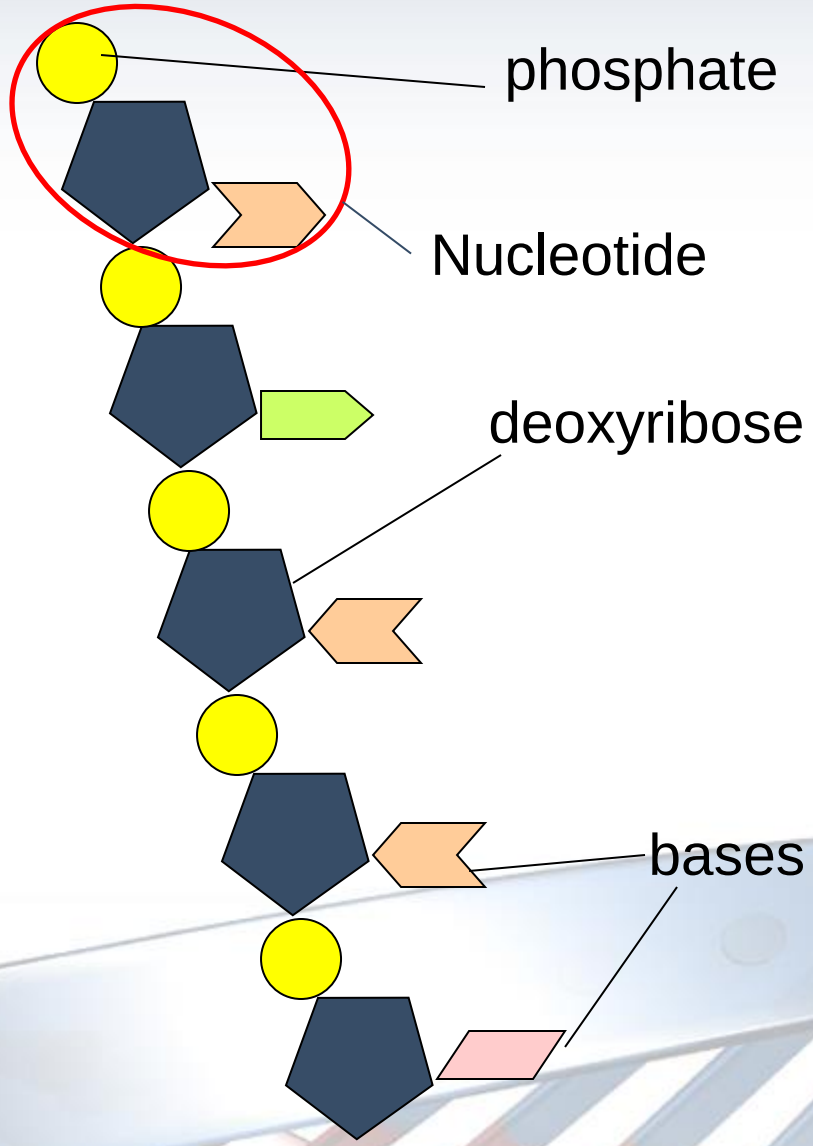
What is DNA?

- DNA is the code to our life
- The backbone of the molecule is alternating phosphates and deoxyribose sugar
- The teeth are nitrogenous bases
- Chromosomes are made up of DNA and our genes are located on these chromosomes

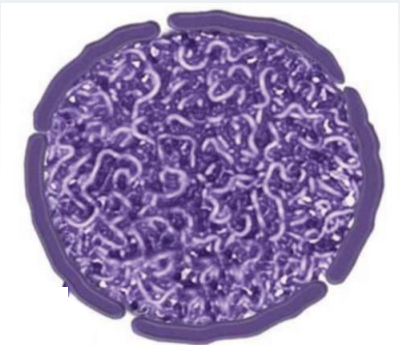


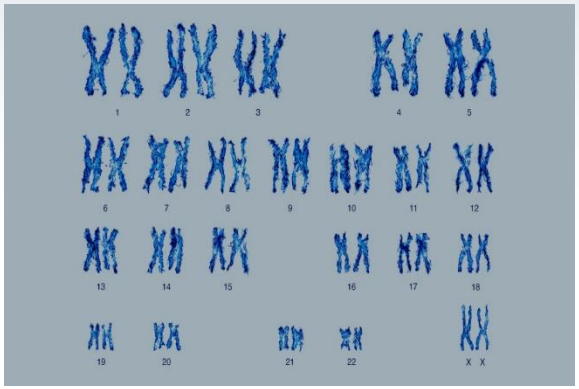
Our genome is a long encrypted code

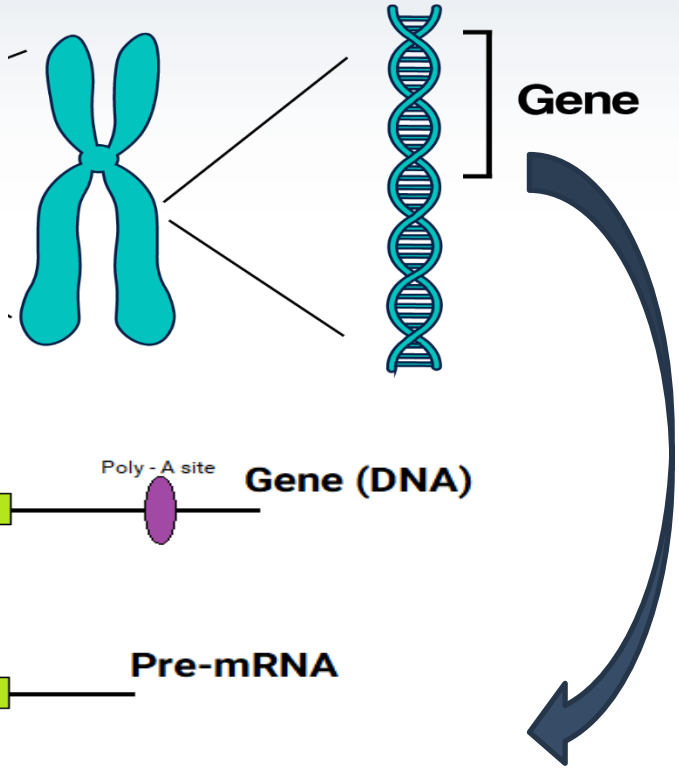
- The genome is made up of 4 letters: ATCG
- The length of our genome is 3,000,000,000 bases

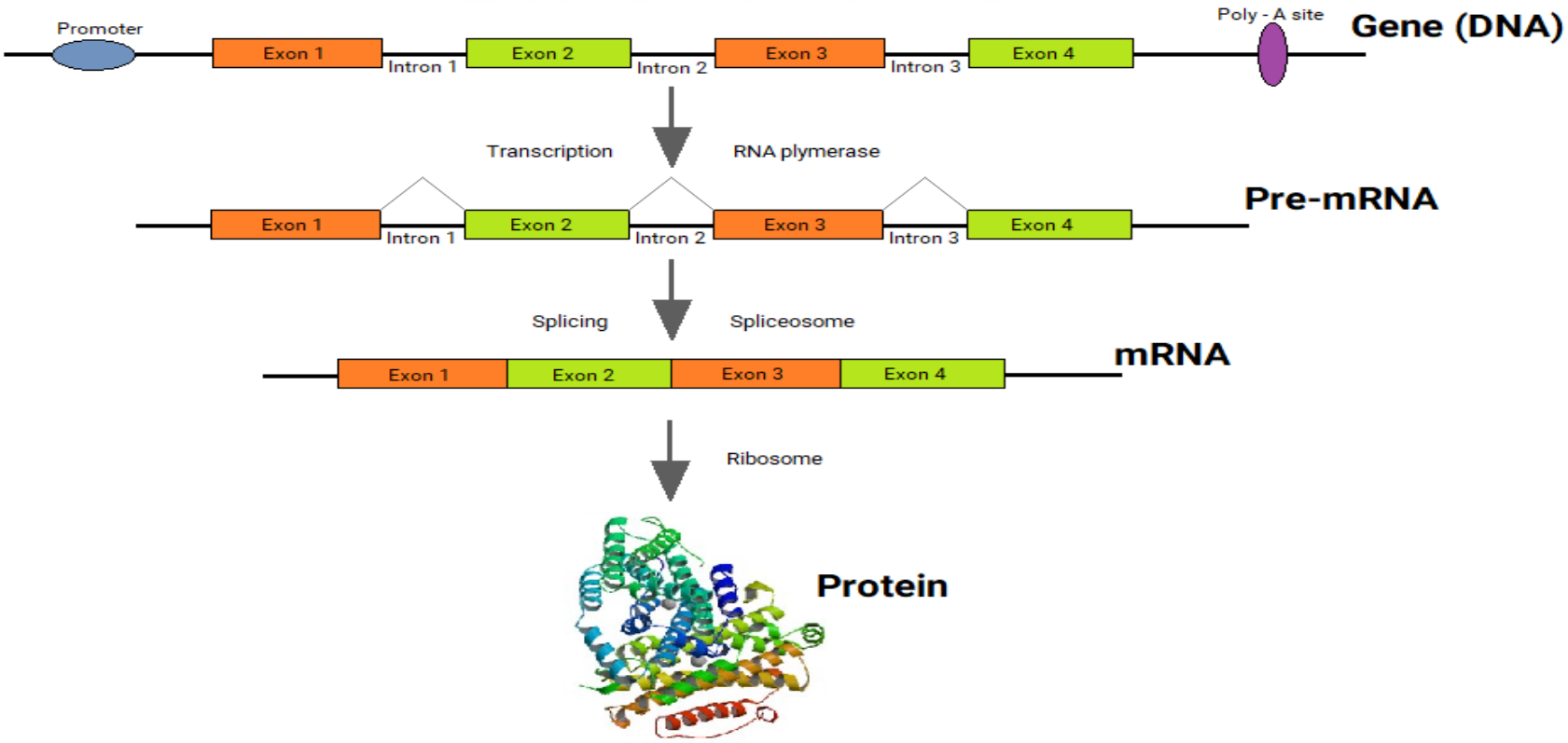
- The actual size of human DNA in ONE cell is 3 meters long!!!

DNA is the source of life…









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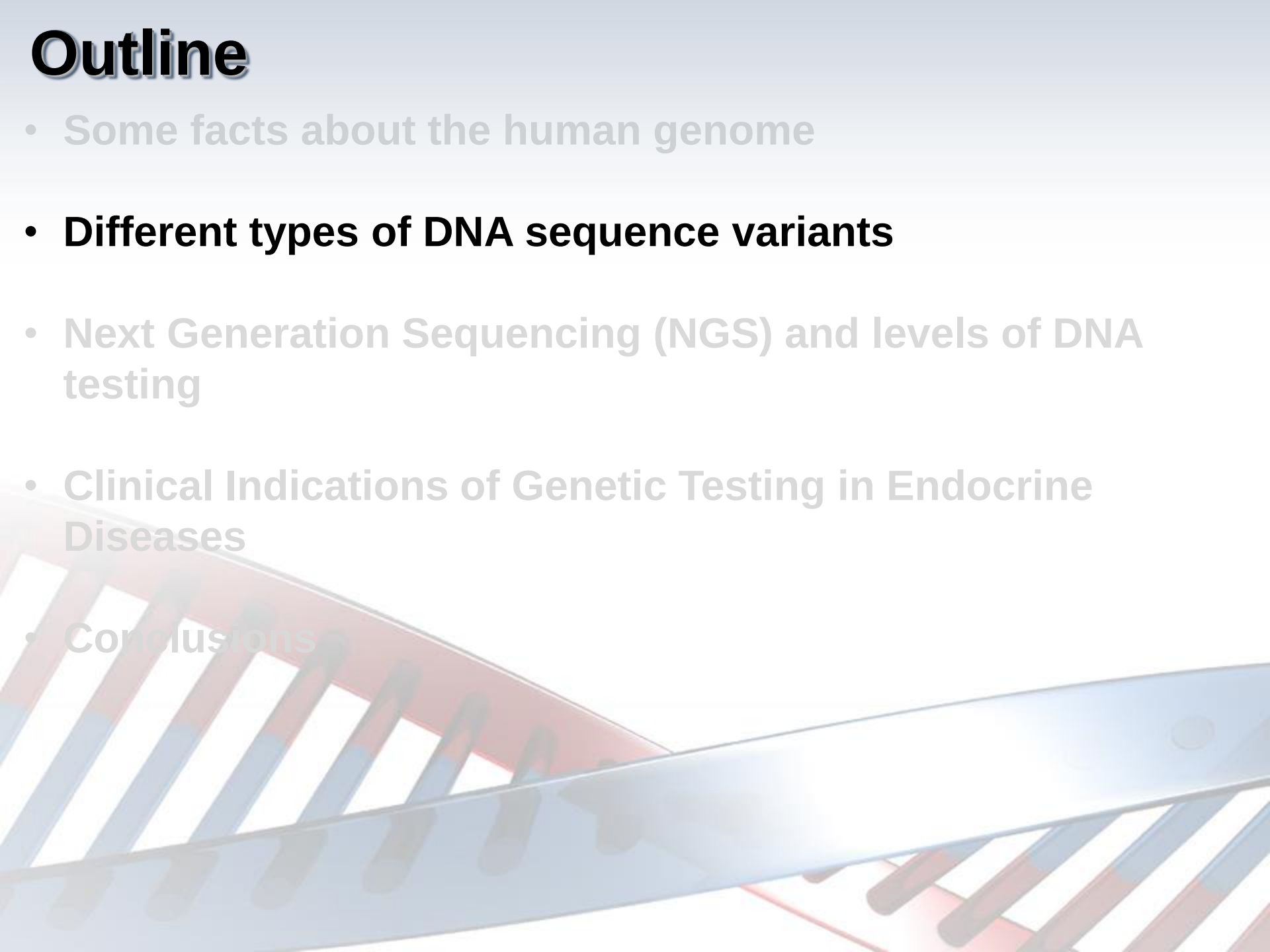
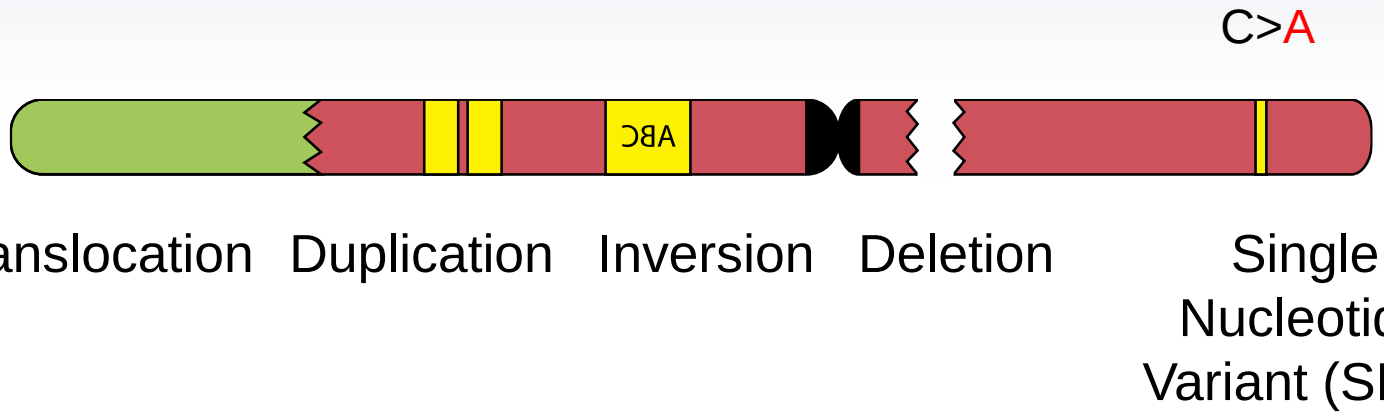
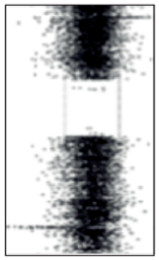
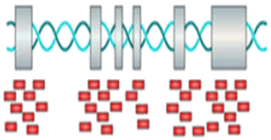
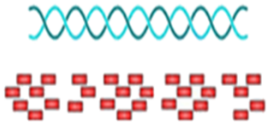


What are different types of DNA variation



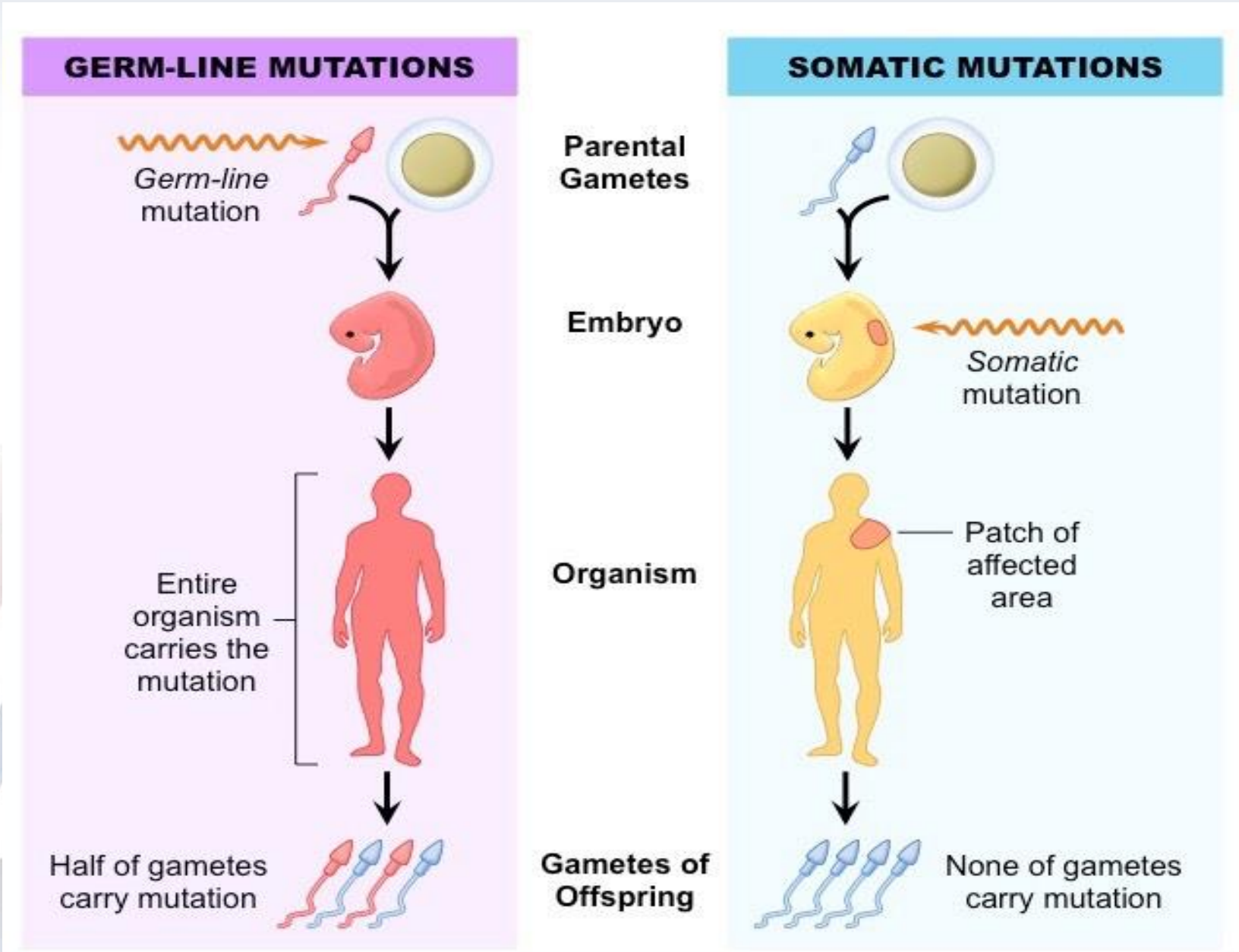
Polymorphism	Mutation
Low to high frequency in the population	Very rare in the population
Mild effect on the protein	Deleterious or activating effect on the protein
Mostly NOT disease causing	Could be disease causing
No natural selection for or against	Natural selection affect the frequency

Molecular techniques for prenatal genetic diagnosis

	Karyotyping	Microarray (Array CGH)	Whole Exome Sequencing	Whole Genome Sequencing
				
Resolution	5-10 Mb	25Kb-1 Mb	1 bp	1 bp
Variants Detected	Variants >5 Mb	Copy number variants	Coding regions	Majority of variants
Diagnostic yield	Low →			High

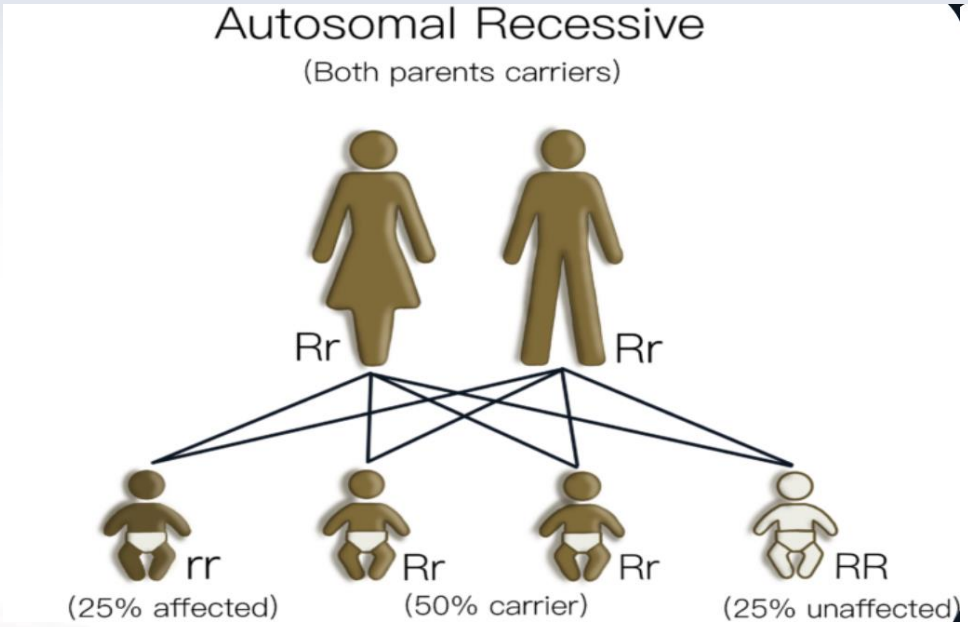
Derived from Write et al Nat Rev Genet 2018

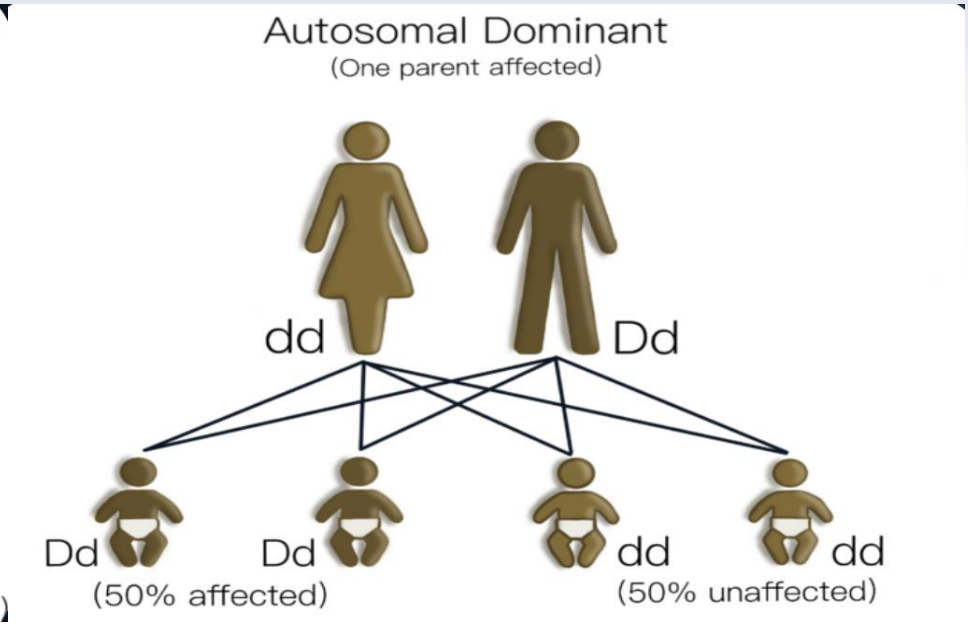
Germline vs Somatic mutations

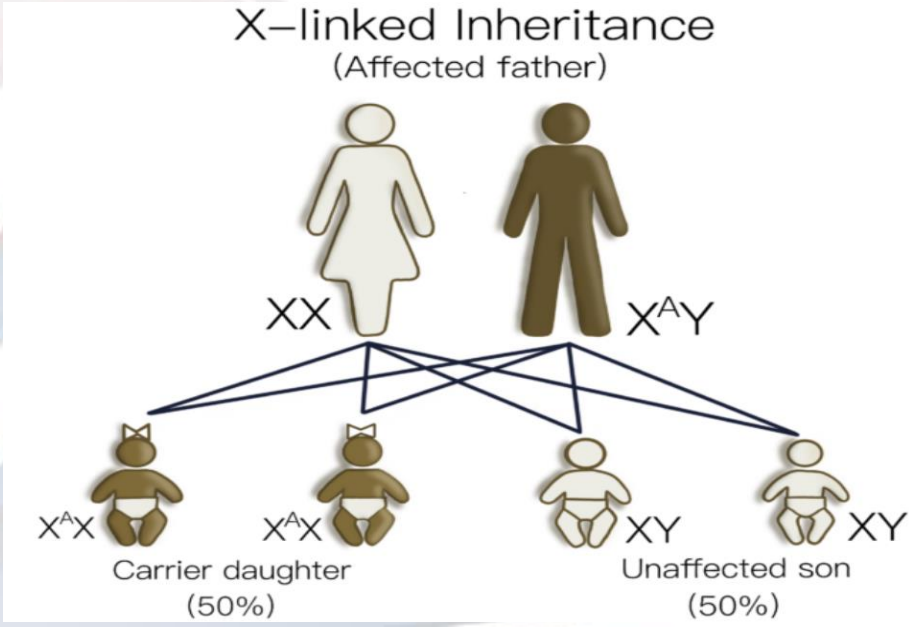


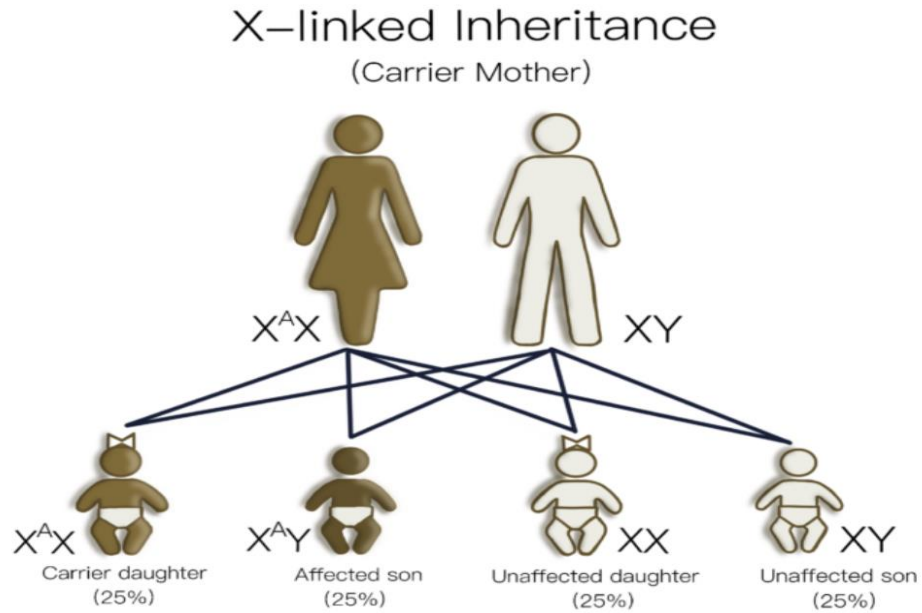
https://ib.bioninja.com.au/

Common Patterns of Gene Inheritance









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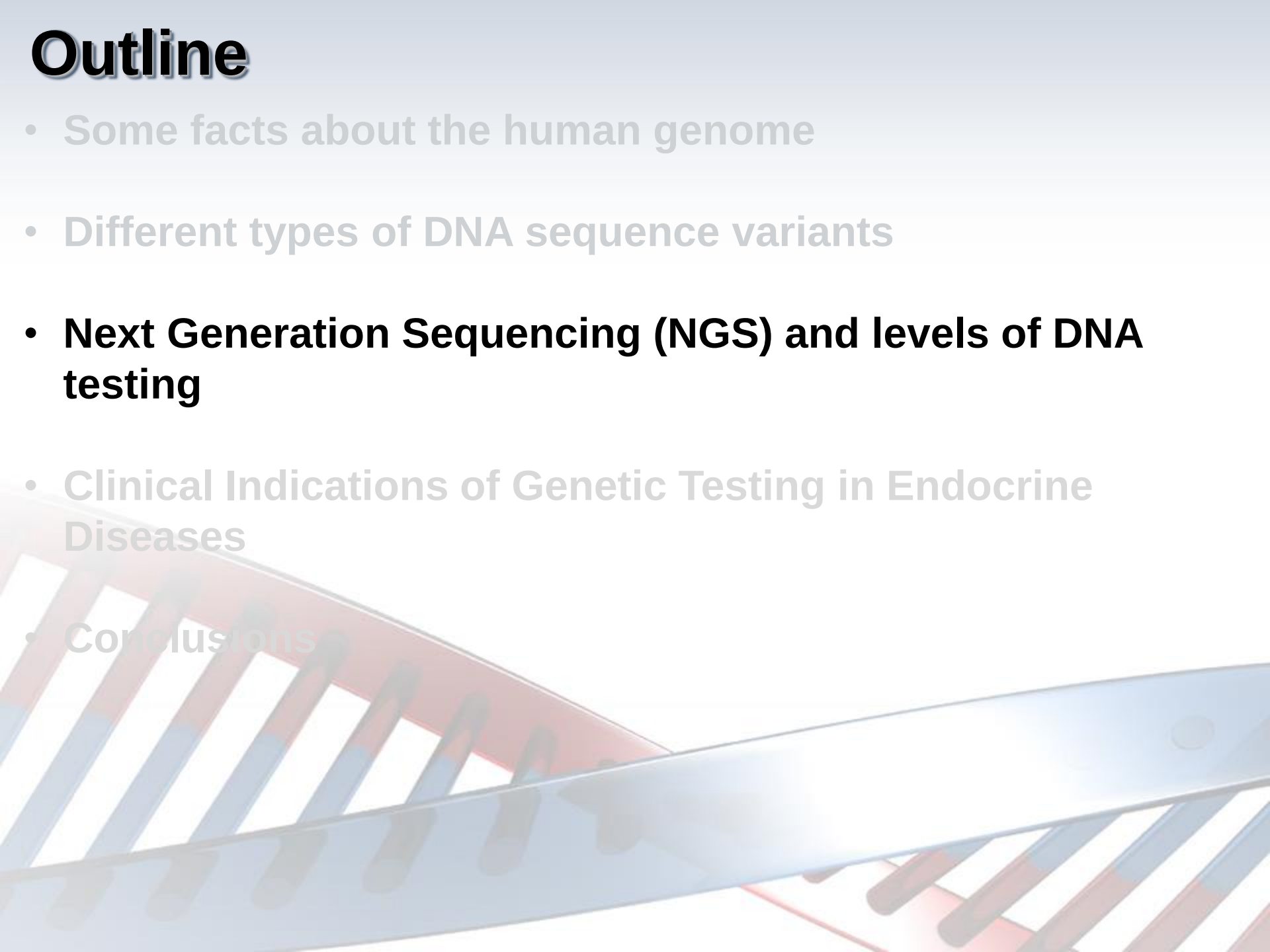
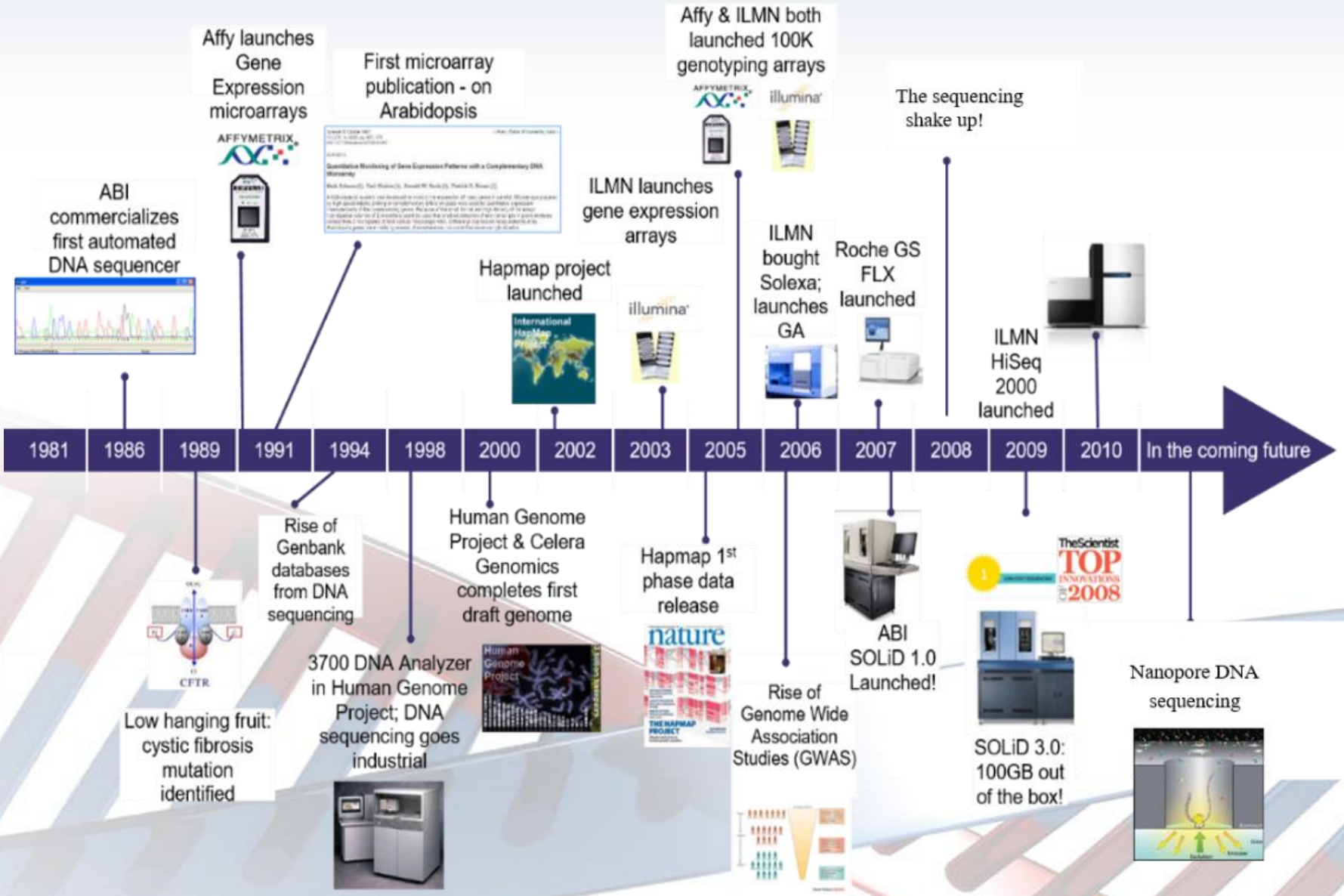


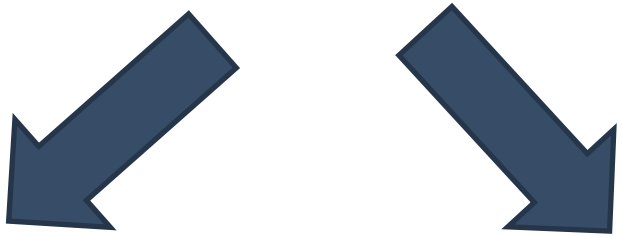
History of DNA sequencing



Ref: http://2016.igem.org/Team:UESTC-software/Description

Next Generation Sequencing (NGS)

Two Most Commonly Used Companies



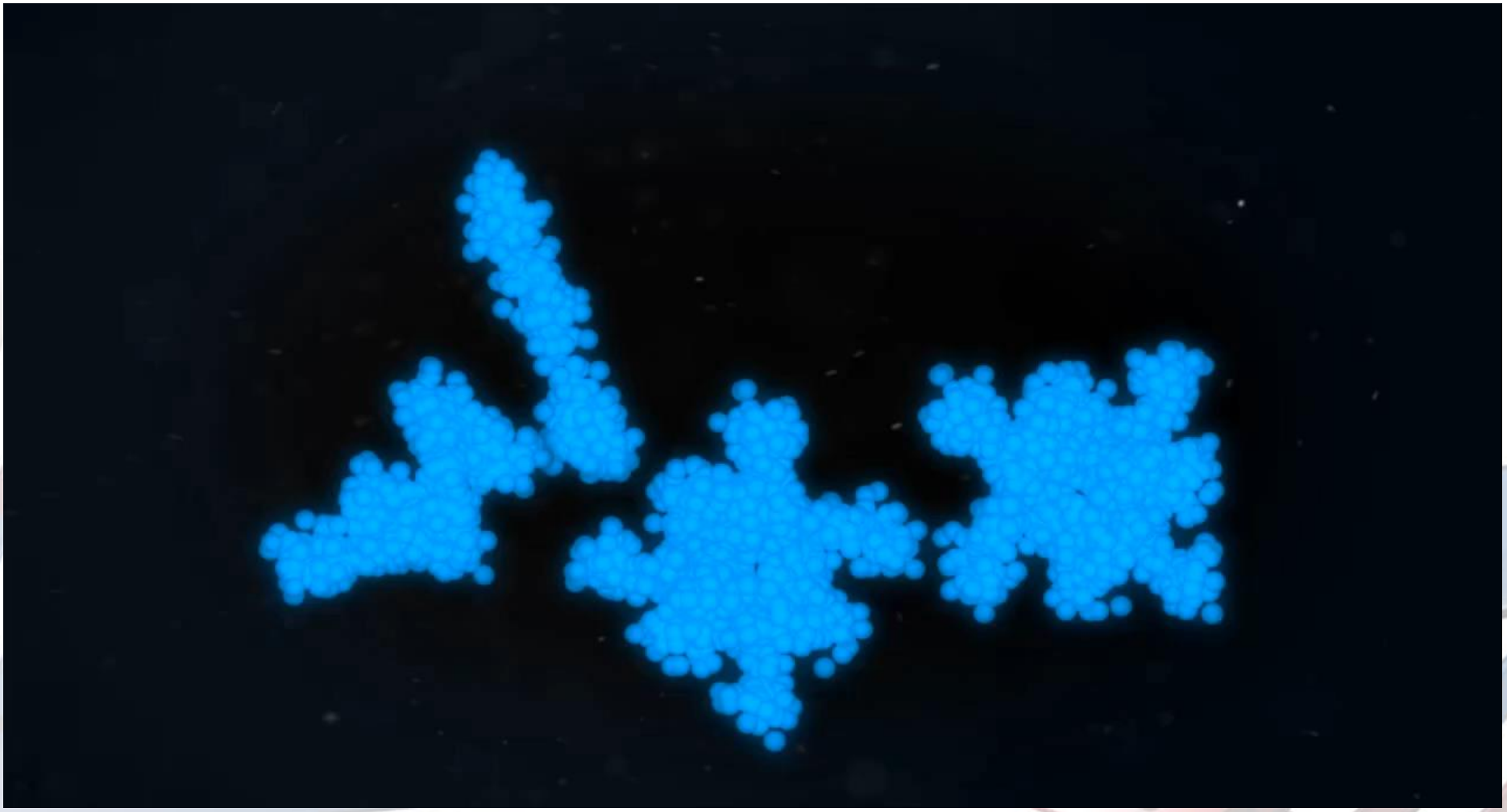
ion torrent

by life technologies™

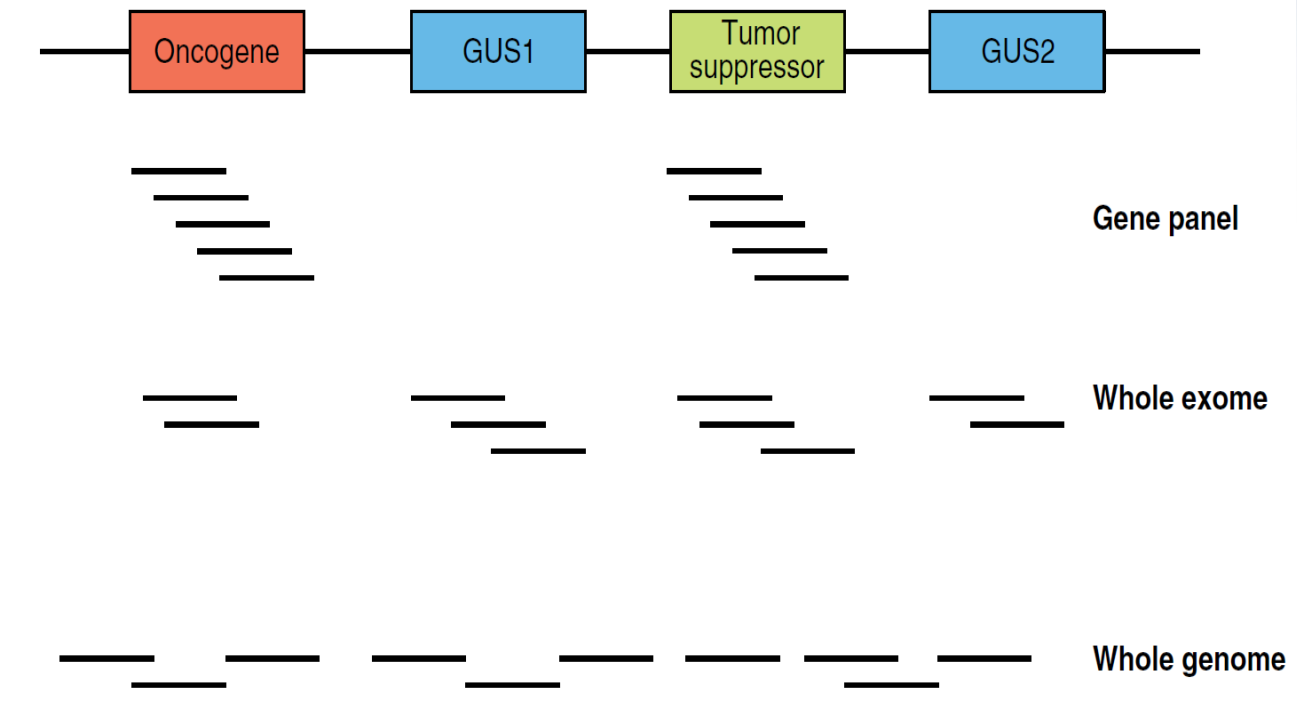
illumina®

Illumina Sequencing

- Started in 2006
- Sequencing by synthesis technology



Different types of tests are available



Test	Advantages	Disadvantages
Gene Panel	Cost/coverage	Missing translocation/limited number of genes
Whole Exome	All pt coding genes/detecting CNV	Missing translocations/higher cost
Whole Genome	All the genome/detecting CNVs, translocations	Expensive/no good coverage for mutations

NGS data analysis is the main challenge in interpreting the results

- NGS data analysis requires expertise in computational programming and medical genetics.

- The analysis of each case could result in three types of variants:
  - Benign/Likely Benign
  - Pathogenic/ Likely Pathogenic
  - Variants of unknown significance (VUS)

- The classification of variants is based on several factors:
  - Previous studies and/or cosegregation with the disease
  - Computational prediction algorithms
  - Frequency in normal populations:
    - 1000 Genomes (n=2000)
    - gnomAD (n=200,000)
    - A unique database of Iranian Patients (IRExome)

NGS Report Template

Whole Exome Sequencing Analysis
Post-Sanger Report

Case ID: KG-00-WES-11-0411	Referrer: XXX
Patient Name: XXX	Date Sample Received:
Sample Type: Blood	Date of report: Date of report of Sanger:

Result

Major Finding

Gene	Protein	cDNA	Zygosity	Class	Matching phenotype
CYP21A2	p.Ile173Asn	NM_000500.9 c.518T>A	Female: Hom Male: Ukn.	Likely Pathogenic	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency (AR)

Interpretation:

Based on the clinical history of the patient, XXX was born of a non-consanguineous marriage. She is 23 years old and suffers from CAH.

The whole exome sequencing (WES) analysis was performed based on the phenotype. We discovered a homozygous **Likely Pathogenic** variant in ***CYP21A2*** gene that can cause **"Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency"** transmitted in an autosomal recessive pattern and **is correlated with the patient's phenotype.**

We also found several heterozygous mutations as the secondary finding that may be disease causing.

NGS Report Template

Recommendation:	- Genetic counseling is recommended.. - It is highly recommended to assess the male sample via Sanger sequencing for the variant in *CYP21A2* gene. - Given the fact that novel genes are being constantly discovered and added to the OMIM database, the re-analysis of the data is highly recommended upon the pregnancy.
Method:	Exome raw data (FastQ files) was analyzed by our computational biology company, **Palindrome**. Briefly, reads were mapped to GRCh38 using BWA, followed by base recalibration, variant calling, and genotyping using GATK4. Finally, variants were funneled through PalinVar (Palindrome's pipeline for variant filtration) to retain those of clinical significance. The shortlisted annotated variants were further analyzed for interpretation of pathogenic variants. The pathogenicity of the variants is determined based on the latest criteria published by ACMG (PMID: 25741868).

NGS Report Template

Secondary finding					
Gene	**Protein**	**cDNA**	**Zygosity**	**Class**	**Matching phenotype**
C8B	p.Arg428Ter	NM_000066.4 c.1282C>T	Female: Het Male: Ukn.	Pathogenic	C8 deficiency, type II (AR)
LRBA	Splice site	NM_001367550.1 c.2767-1G>A	Female: Het Male: Ukn.	Likely Pathogenic	Immunodeficiency, common variable, 8, with autoimmunity (AR)
ESR1	p.Arg394His	NM_001385570.1 c.1181G>A	Female: Het Male: Ukn.	Likely Pathogenic	Estrogen resistance (AR)
MMP14	Splice site	NM_004995.4 c.850+2C>T	Female: Het Male: Ukn.	Likely Pathogenic	Winchester syndrome (AR)
NARS1	p.Asp356Ala	NM_004539.4 c.1067A>C	Female: Het Male: Ukn.	Likely Pathogenic	Neurodevelopmental disorder with microcephaly, impaired language, and gait abnormalities (AR)

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Clinical Indications of Genetic Testing in Endocrine Diseases

- Clinical Diagnosis
- Prognosis and Therapeutic guidance
- Family testing for surveillance and management
- Family planning

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Polyglandular Autoimmune (PGA) Syndrome Type I

- PGA-I is characterized by the triad of hypoparathyroidism, Addison disease, and chronic mucocutaneous candidiasis.
- PGA-I is due to mutations in the autoimmune regulatory gene (AIRE) which has both AD and AR pattern of inheritance.
- The AIRE gene encodes a transcription factor involved in the presentation of tissue-restricted antigens during T-cell development in the thymus.
- So far, about 60 different mutations have been described of which arginine substitution at position 257 and 13 base pair deletion in exon 8 are the most common.
- Common mutations can be tested via Sanger sequencing while the whole gene analysis should be performed via NGS.

Clinical Indications of Genetic Testing in Endocrine Diseases

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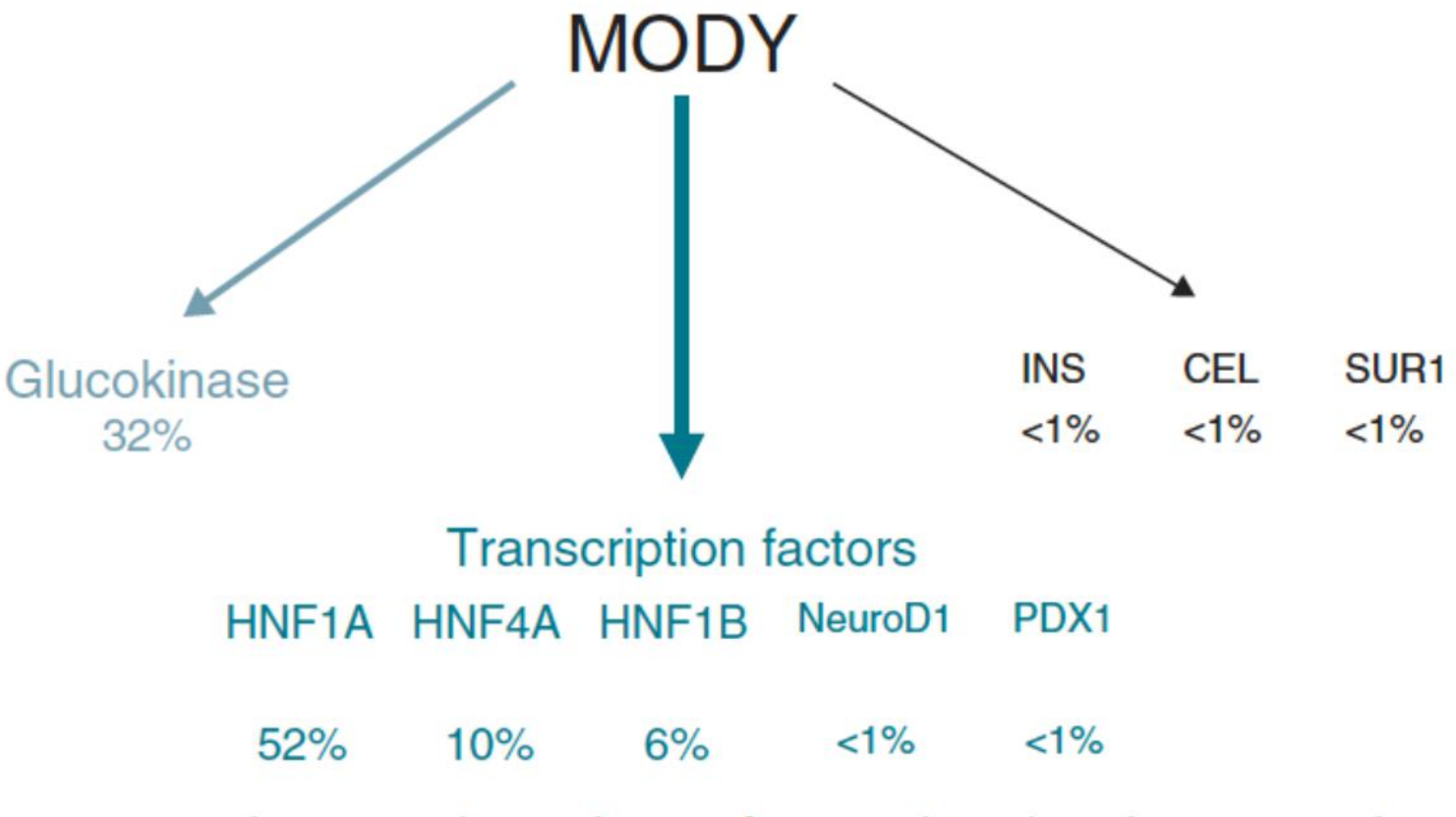
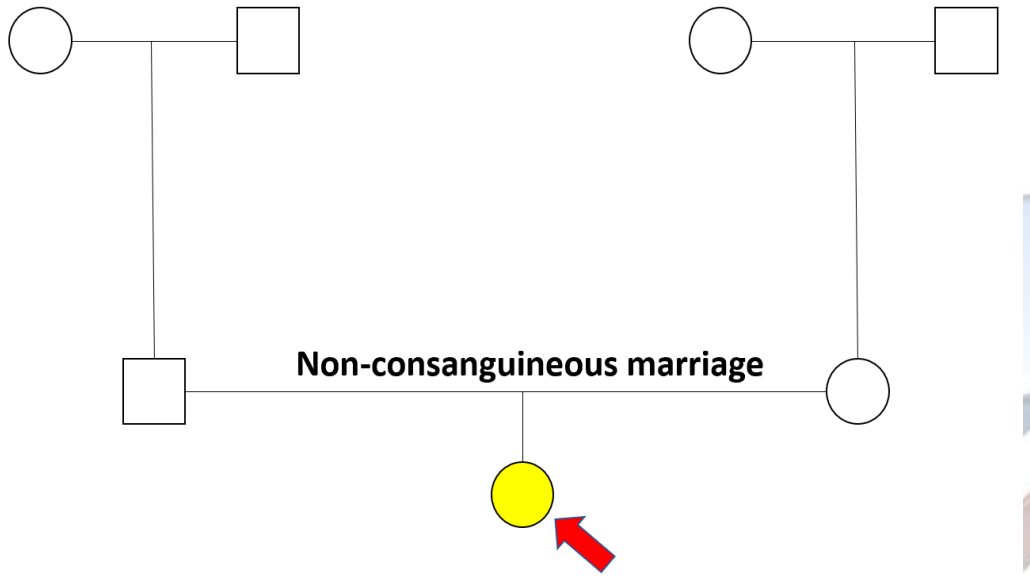
The genetic heterogeneity of MODY

- Maturity-onset diabetes of the young (MODY) is a group of inherited disorders of non-autoimmune diabetes mellitus which usually present in adolescence or young adulthood.
- 14 genes have been so far identified that are causing MODY



Case presentation

- A 7 year old female with no family history of early onset diabetes was referred for genetic testing due to neonatal diabetes.
- NGS was performed and a heterozygous Pathogenic mutation (p.Arg201His) was identified in *KCNJ11* gene.
- The mutation was then confirmed to be *de novo.*
- The patient is responsive to Glibenclamide due to this mutation.



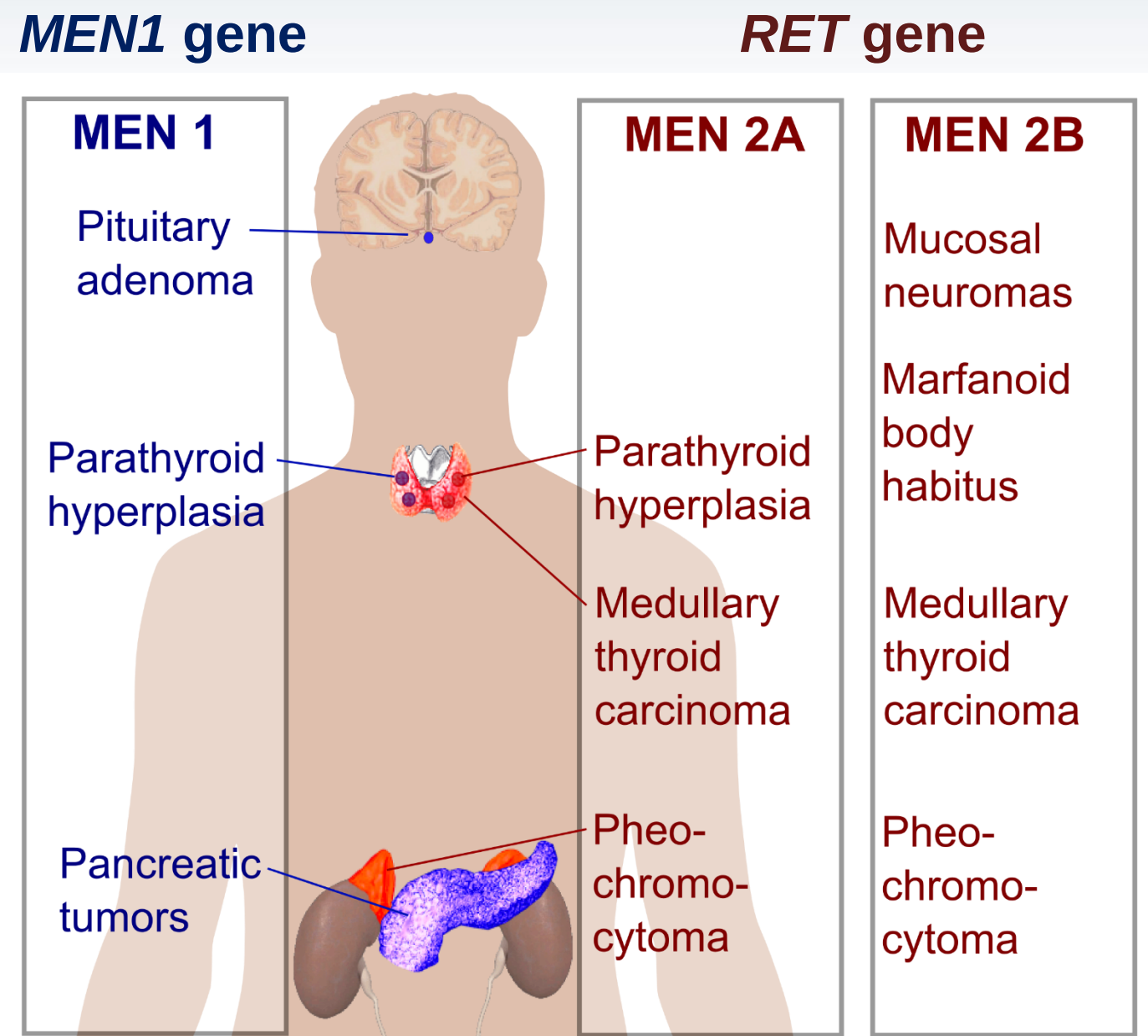
MODY: Management by Genetic Cause

Gene	Pathophysiology	Treatment				References
		None	Diet	OAD	Insulin	
GCK	β-cell dysfunction (glucose-sensing defect)	Except possibly in pregnancy [1]				Stride et al [2014], Chakera et al [2015]
HNF1A	β-cell dysfunction; mainly insulin secretory defect			Low-dose sulfonylureas or meglitinides; GLP-1 agonists also used	May be required [2]	Shepherd et al [2003], Tuomi et al [2006], Østoft et al [2014], Bacon et al [2016b]
HNF1B	β-cell dysfunction			A minority respond to sulfonylureas.	Commonly needed	Dubois-Laforgue et al [2017]
HNF4A	β-cell dysfunction (mainly insulin secretory defect)			Sensitive to sulfonylureas		Pearson et al [2005]
INS	β-cell dysfunction		X	X	X [3]	Molven et al [2008], Boesgaard et al [2010]
KCNJ11	ATP-sensitive potassium channel dysfunction		X	Sulfonylureas	X	Bonnefond et al [2012], Liu et al [2013]
KLF11	Decreased glucose sensitivity of β-cells			X	X	Neve et al [2005]
NEUROD1	β-cell dysfunction		X	X	X	Malecki et al [1999], Kristinsson et al [2001]

Clinical Indications of Genetic Testing in Endocrine Diseases

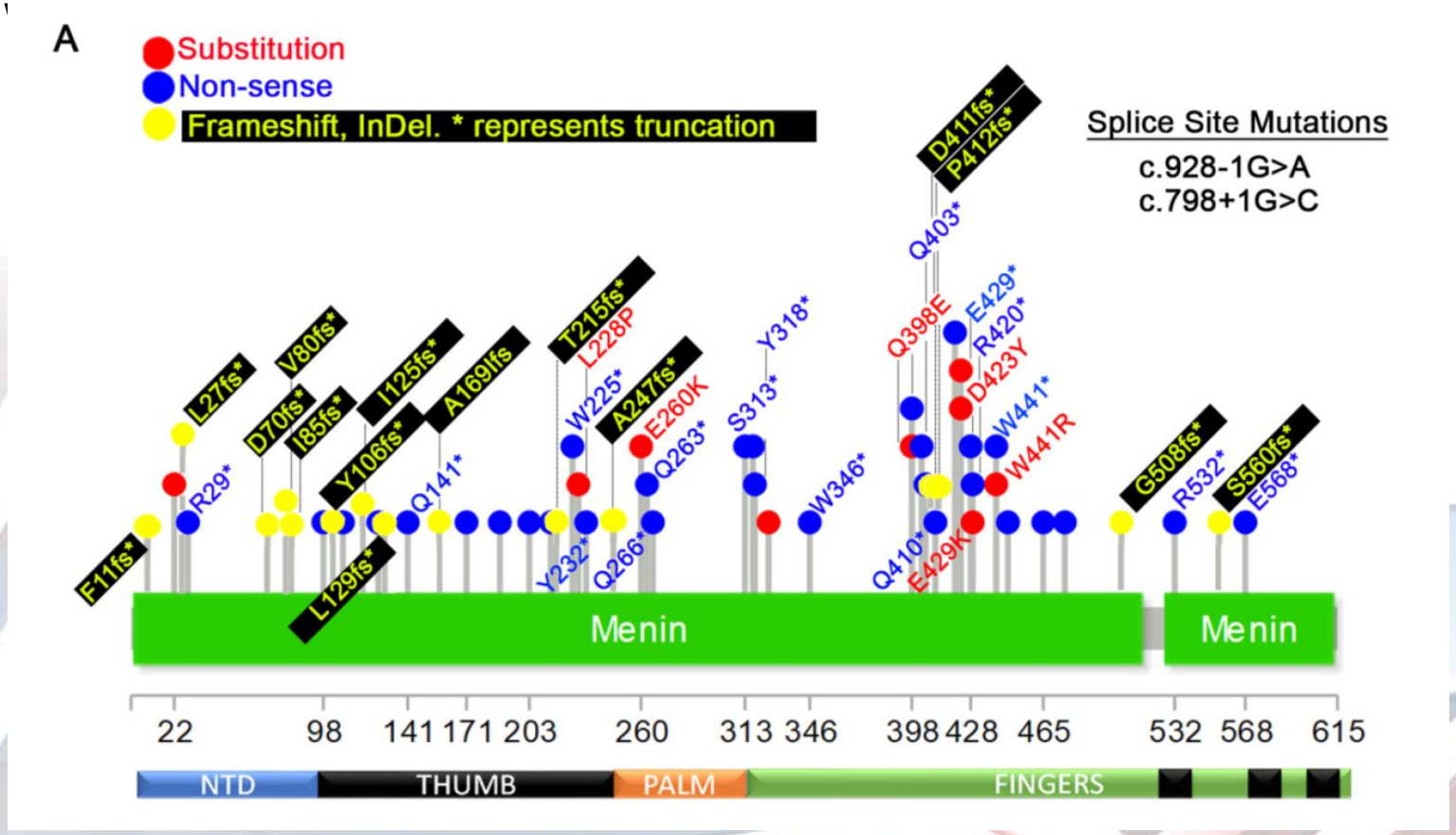
- Clinical Diagnosis
- Prognosis and Therapeutic guidance
- **Family testing for surveillance and management**
- Family planning

Multiple Endocrine Neoplasia



MEN Type 1

- MEN1 is inherited in an autosomal dominant manner due to the mutations in *MEN1* gene. Approximately 90% of individuals diagnosed with MEN1 have an affected parent.



Devi D Nelakurti et al. Cancers (Basel). 2020

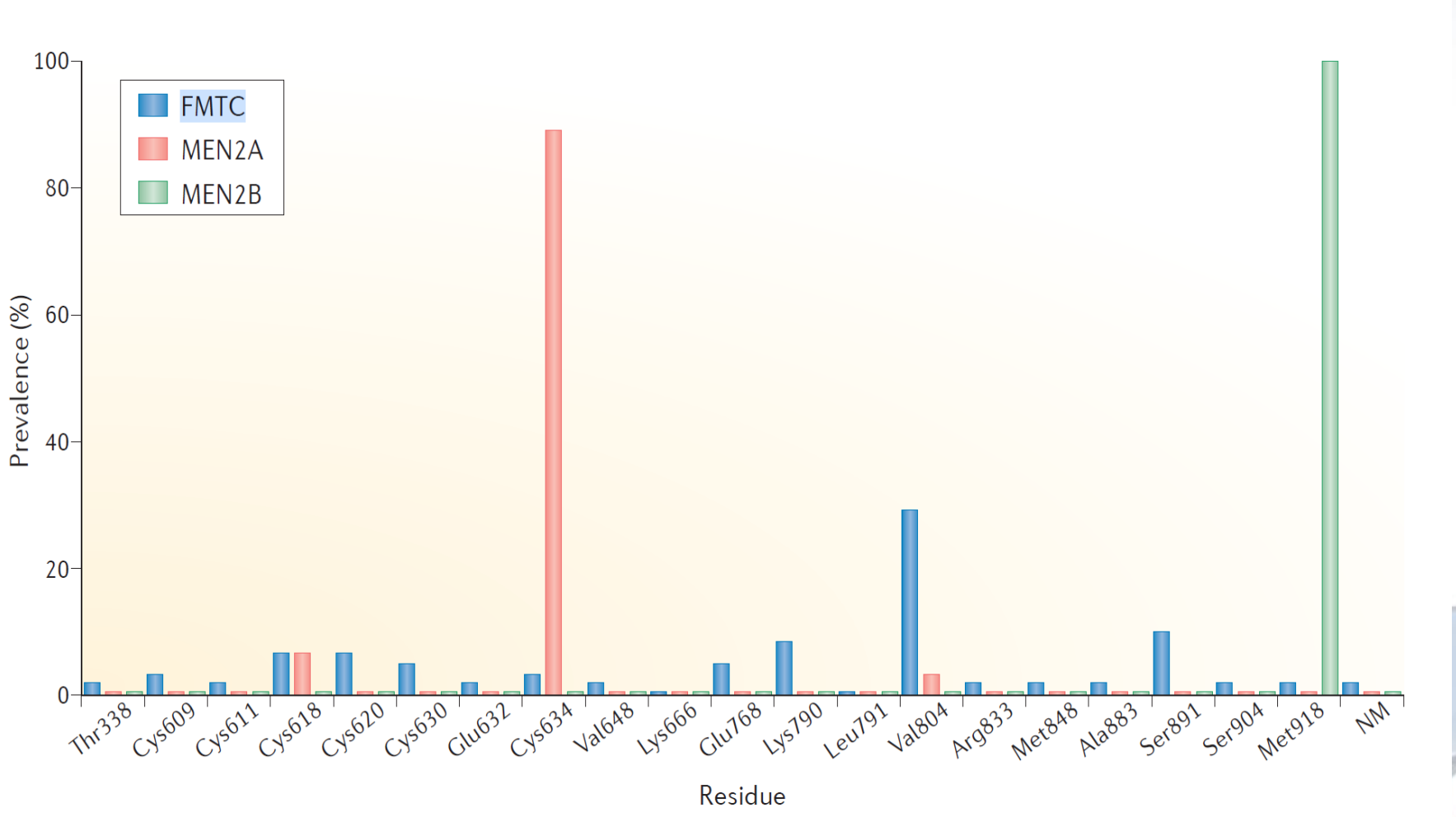
MEN Type 2

- MEN2 includes the following phenotypes: MEN2A, familial medullary thyroid carcinoma (FMTC, which may be a variant of MEN2A), and MEN2B. All three phenotypes involve high risk for development of medullary carcinoma of the thyroid (MTC); MEN2A and MEN2B involve an increased risk for pheochromocytoma.

- All MEN2 phenotypes are inherited in an autosomal dominant manner due to the mutations in *RET* gene. Up to 95% of individuals diagnosed with MEN2A and 50% of individuals diagnosed with MEN2B have an affected parent.

- Approximately 5%-9% of individuals with MEN2A and 50% of individuals with MEN2B have the disorder as the result of a de novo germline pathogenic variant.

MEN2: Distribution of mutation in RET



Cristina Romei et al. Nat Rev Endocrinol. 2016

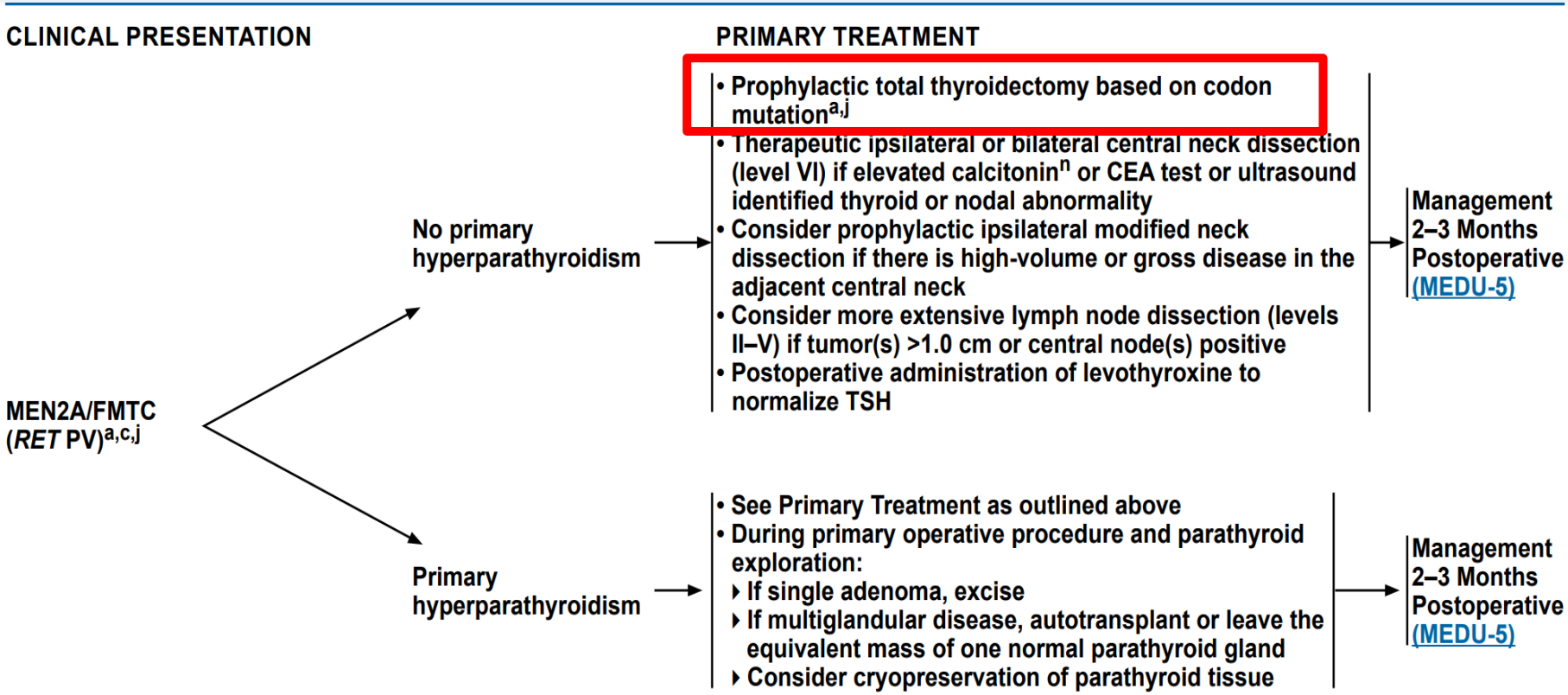
Genetic testing in MEN and Clinical Guidelines

- For MEN1, the whole *MEN1* gene sequence analysis is recommended via NGS while in MEN2, the hotspot exons (10, 11, and 13-16) should be first analyzed via Sanger sequencing and if nothing is found, the NGS is recommended.

- NCCN (2024.3) recommends RET gene screening for all MTC cases.

- The clinical guideline for positive cases with Pathogenic/Likely Pathogenic variants in RET:

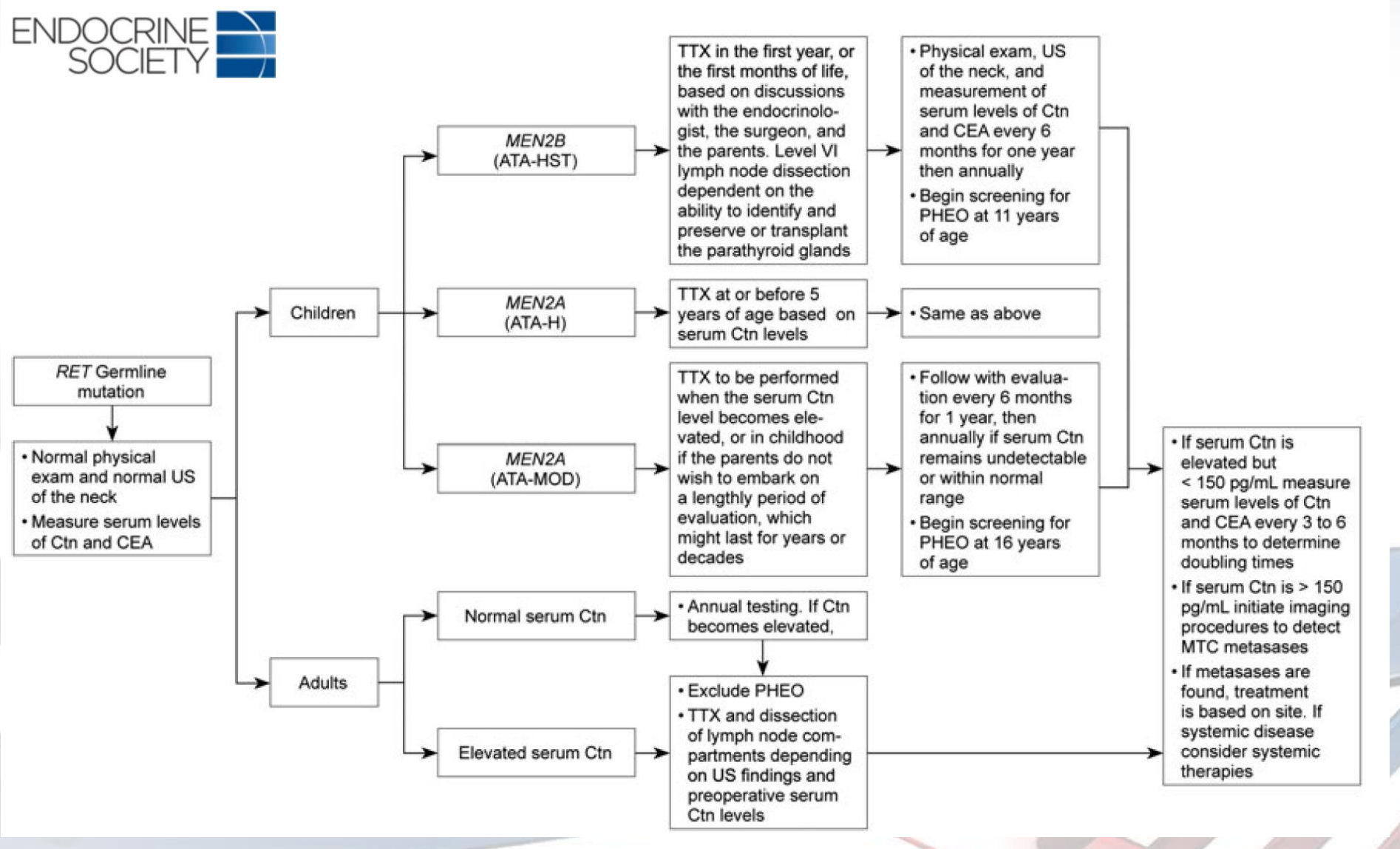
Genetic testing in MEN and Clinical Guidelines



**The timing of prophylactic thyroidectomy generally depends on the aggressiveness of the inherited RET PV. Codon M918T mutations are considered highest risk and codon 634 and A883F mutations are considered high risk, with MTC usually presenting at a younger age, whereas other RET PVs associated with MEN2A or FMTC are generally moderate risk.

Genetic testing in MEN and Clinical Guidelines

- Endocrine Society guideline (2015):



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Pheochromocytoma and Paraganglioma

- Hereditary paraganglioma-pheochromocytoma (PGL/PCC) syndromes are characterized by heterozygous mutations in 7 genes transmitted in an autosomal dominant manner and should be assessed by NGS.

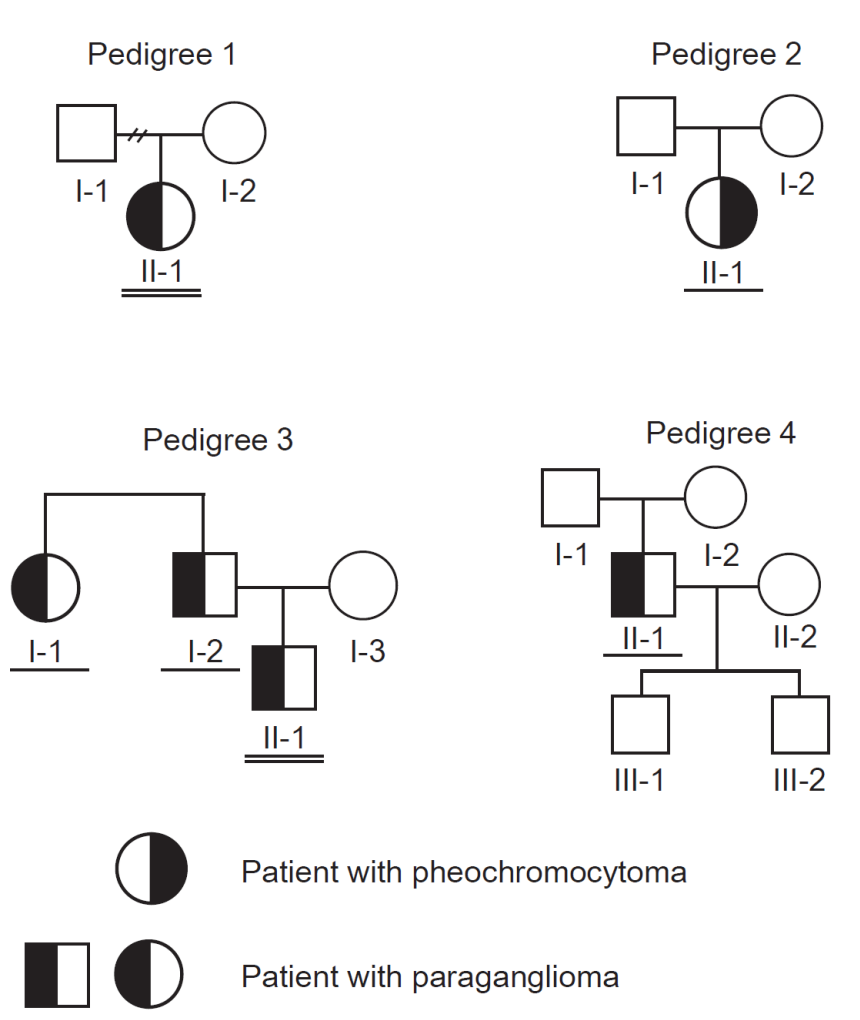
Gene [1, 2]	Proportion of Hereditary PGL/PCC Syndromes Attributed to Pathogenic Variants in Gene [3]	Proportion of Pathogenic Variants [4] Detectable by Method	
		Sequence analysis [3, 5]	Gene-targeted deletion/duplication analysis [3, 6]
MAX	~4%	~90%	~10%
SDHA	~4%	~98%	1 reported
SDHAF2	~1%	~100%	None reported
SDHB	50%-55% [7]	~85%-95%	~5%-15%
SDHC	~8%	~85%	~15%
SDHD	~20%-25% [8]	90%-95%	5%-10%
TMEM127	~5% [3]	~100%	None reported
Unknown [9]	NA		

Pheochromocytoma and Paraganglioma case presentation

- A recurrent mutation (IVS2-2A>C) was identified in 4 unrelated families with PGG/PCC

- The mutation in the Pedigrees 1,2 and 4 is de novo and it is familial in Pedigree 3.

- The risk of recurrence is 50% in the next offspring for familial cases while it is >1% for de novo cases.



Mie Yamanaka et al. Tohoku J Exp Med. 2018

Congenital adrenal hyperplasia (CAH)

- Congenital Adrenal Hyperplasia (CAH) is a group of genetic disorders that affect the adrenal glands.
- There are several genes associated with different forms of CAH:
  - CYP21A2: 21-hydroxylase def
  - CYP11B1: 11-hydroxylase def
  - CYP17A1: 17-hydroxylase def
  - HSD3B2: 3-beta-hydroxysteroid dehydrogenase def
  - STAR: lipoid adrenal hyperplasia
  - CYPOR: PORD

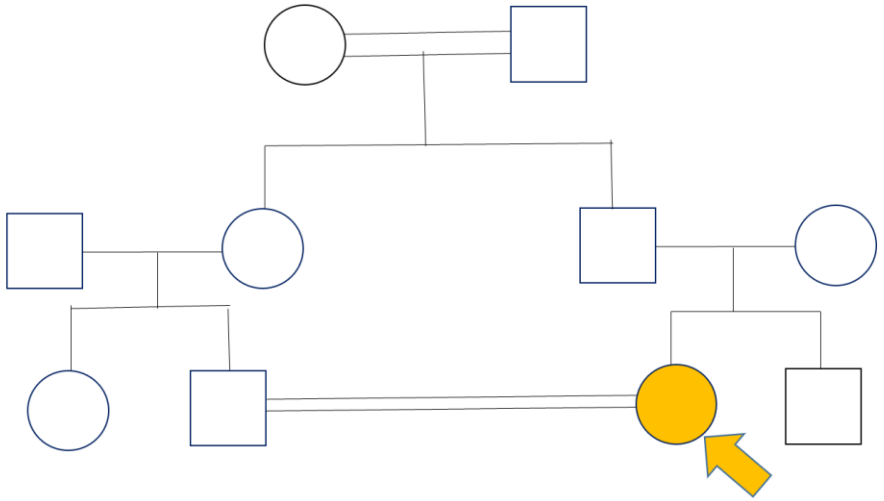
- The CYP genes have highly homology pseudogene which makes them complicated to assess in CAH patients.
- A combination of NGS sequencing and MLPA technique should be used to screen for all point mutations and gene deletions.

CAH Case Presentation

- The proband is a 35 year old female born of a consanguineous marriage. She was diagnosed with CAH at the age of 1.
- The NGS was performed for the patient and a homozygous pathogenic mutation was identified in *CYP21A2*

- What is the approach of family planning for this family?

  - Sanger seq of the male
  - Prenatal screening
  - Screening of other family members

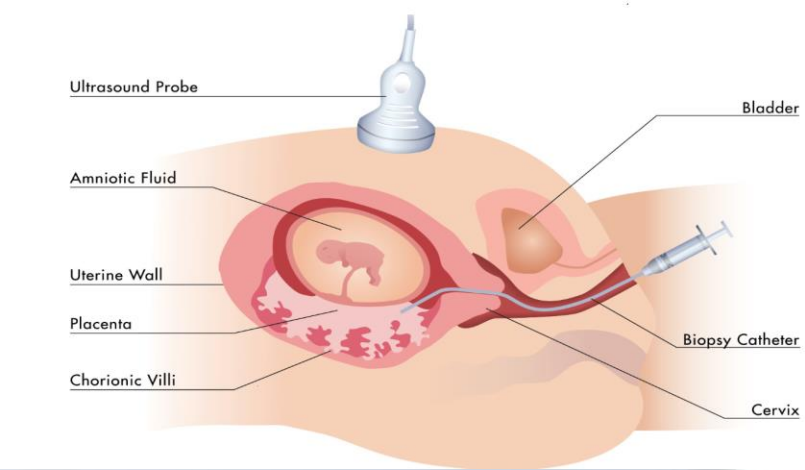


Family Planning Approaches

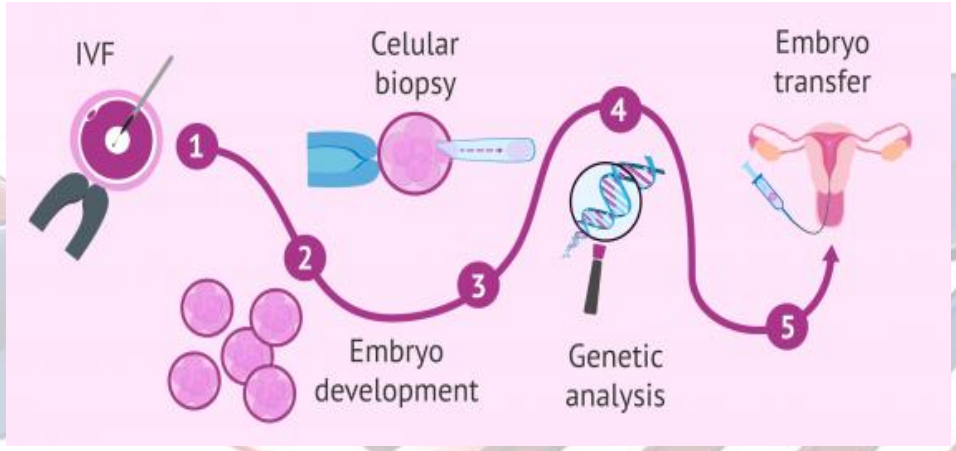
The Family with an identified disease causing

Prenatal Diagnosis (PND)

Preimplantation genetic diagnosis (PGD



www.prenatalscreeningontario.ca



www.invitra.com

Summary and Conclusions

- Endocrine disorders with a genetics basis includes a spectrum of diseases from MODY to MEN syndromes.
- Genetic testing could aid determine diagnosis, prognosis and therapeutic decisions.
- Genetic counseling is highly recommended to specify which tests could benefit the patients and their families.
- Family planning methods including PND and PGD are available for families with an pre-identified mutation.


THANK YOU!

Multi Gene testing in Iranian Patients

- wewr