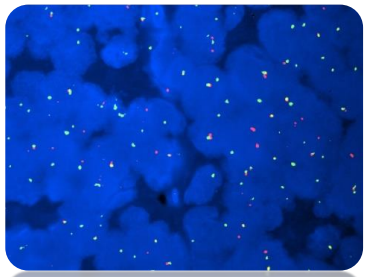
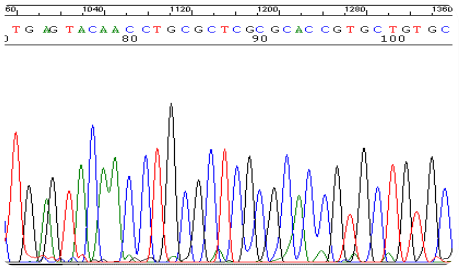
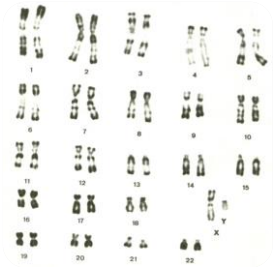






Medical Genetics and Genomics

2023

Pr Ouldim Karim
Médecin généticien
Professeur de Génétique Médicale
et d'oncogénétique

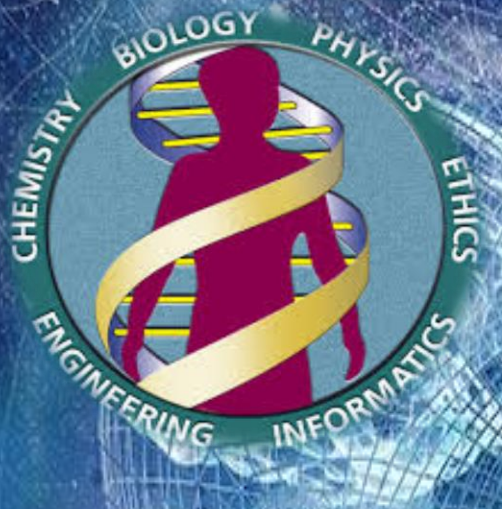
Cours de Génétique Médicale

1ère année médecin 2023 / 2024

Faculté de médecine et de Pharmacie d'Errachidia

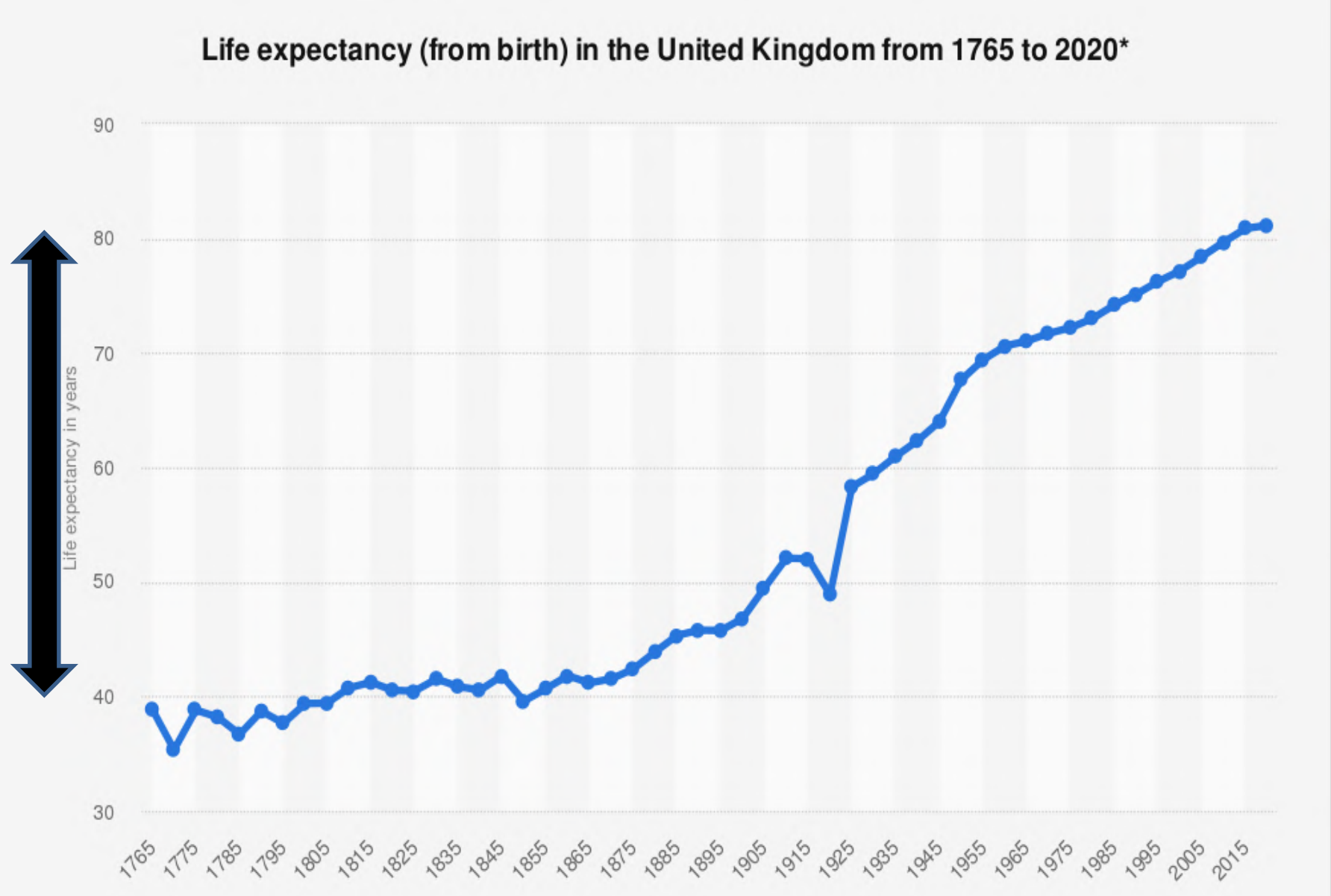
1. Les acides nucléiques et Génome Humain
2. Réplication et systèmes de réparation de l'ADN
3. Transcription
4. Traduction
5. Contrôle de l'expression génique
6. Cytogénétique classique et moléculaire
7. Types et mécanismes des anomalies chromosomiques
8. Techniques d'analyse de l'ADN
9. Mutations et leurs conséquences en pathologie humaine
10. Mode de transmission des Maladies héréditaires

Medical Genetics & Personalized *Medicine*



Precision medicine (PM) has been defined as an approach that uses a person's genetics, environment, and lifestyle to help determine the best approach to prevent or treat disease

Biotechnology Research & Innovation

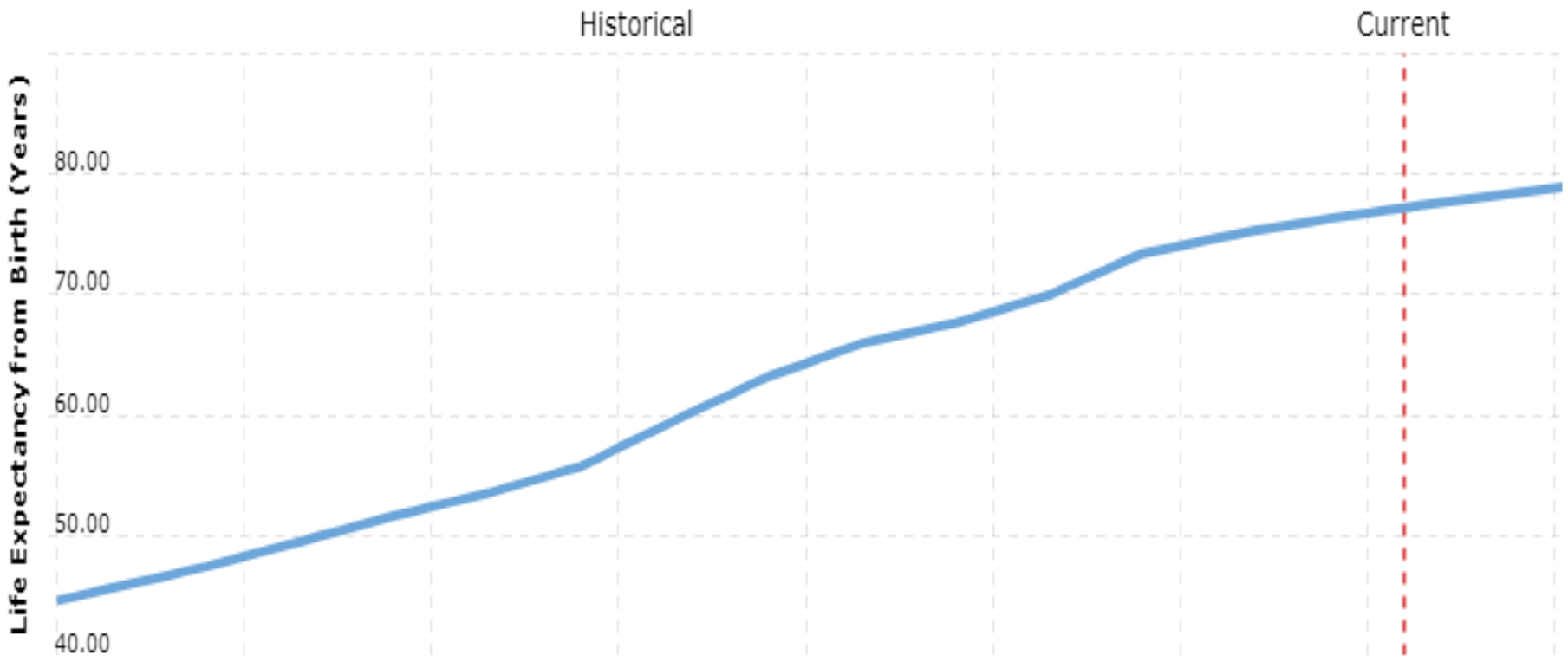


Precision medicine (PM)

Morocco Life Expectancy 1950-2022

From: 1950 To: 2030

Zoom: 10Y 20Y 30Y 40Y 50Y All



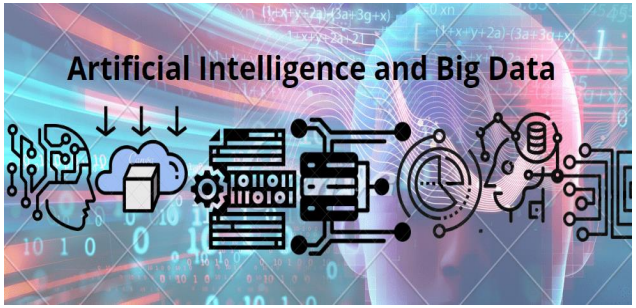
Precision medicine

Precision medicine promises improved health by accounting for individual variability in genes, environment, and lifestyle.

Precision medicine will continue to transform healthcare in the coming decade as it expands in key areas:

Huge cohorts

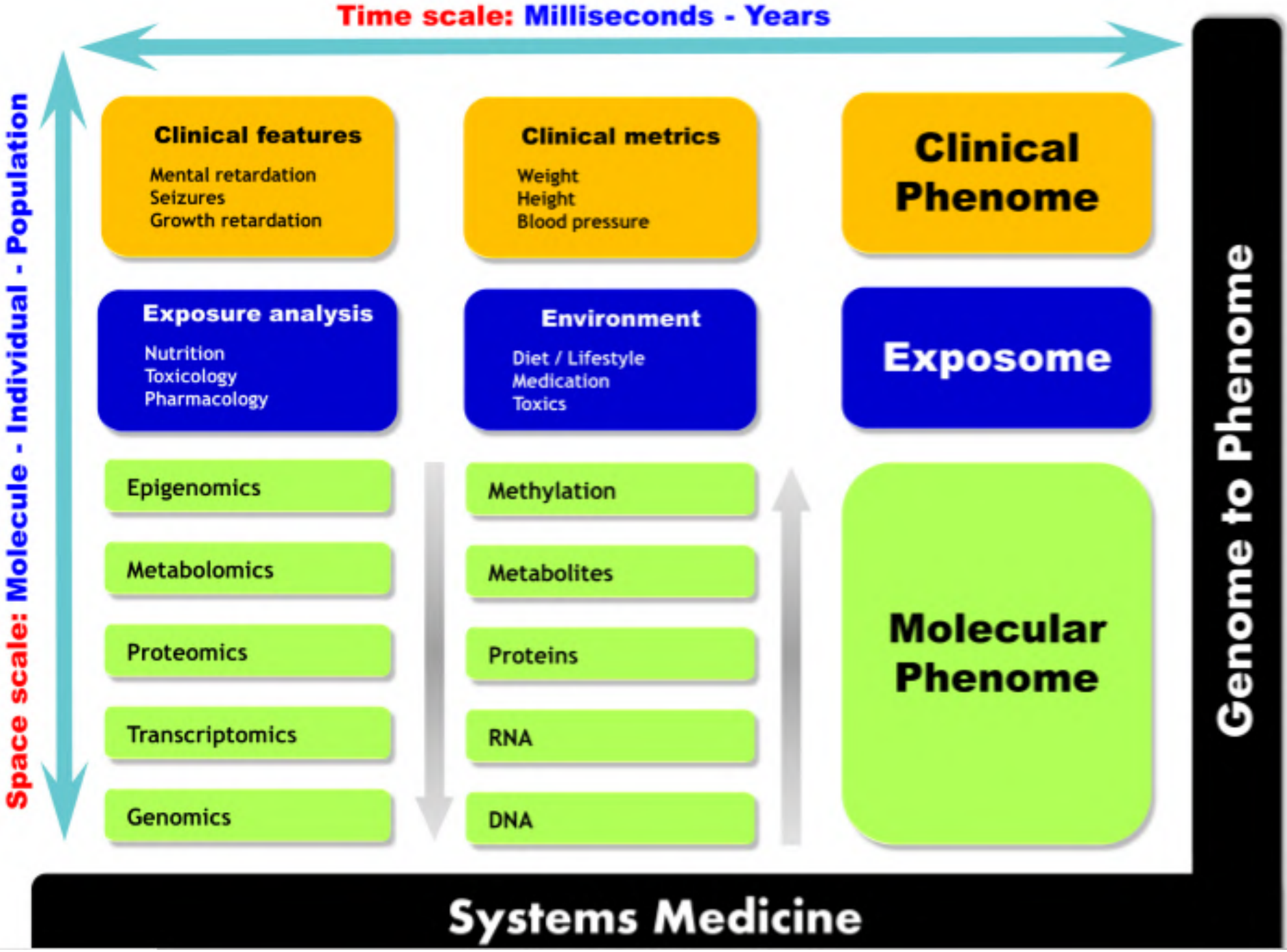
Big Data
Artificial intelligence (AI)

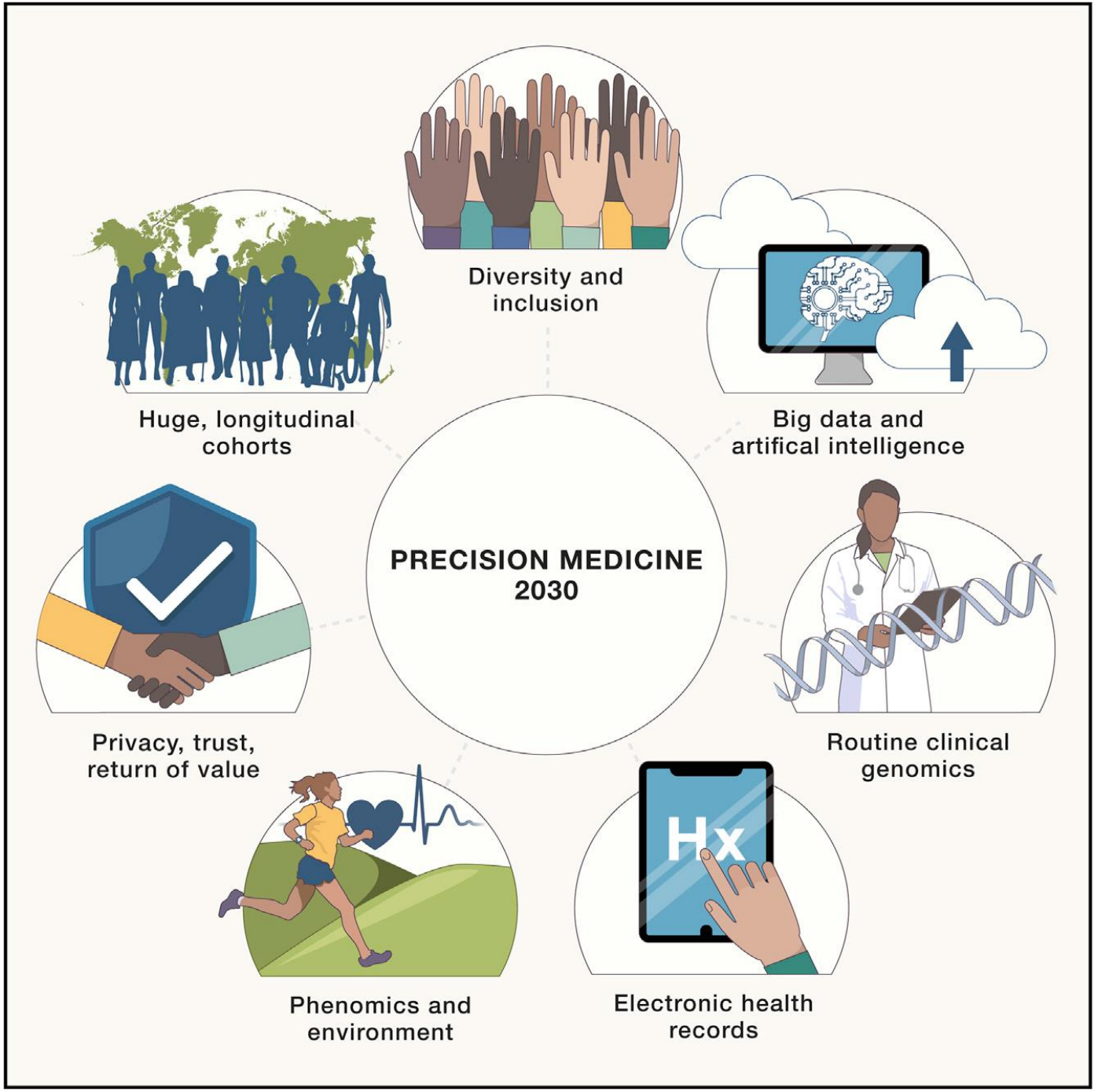


Routine clinical genomics

Phenomics and environment

Joshua C Denny 1, Francis S Collins 2 Precision medicine in 2030-seven ways to transform healthcare 2021 Mar 18;184(6):1415-1419.

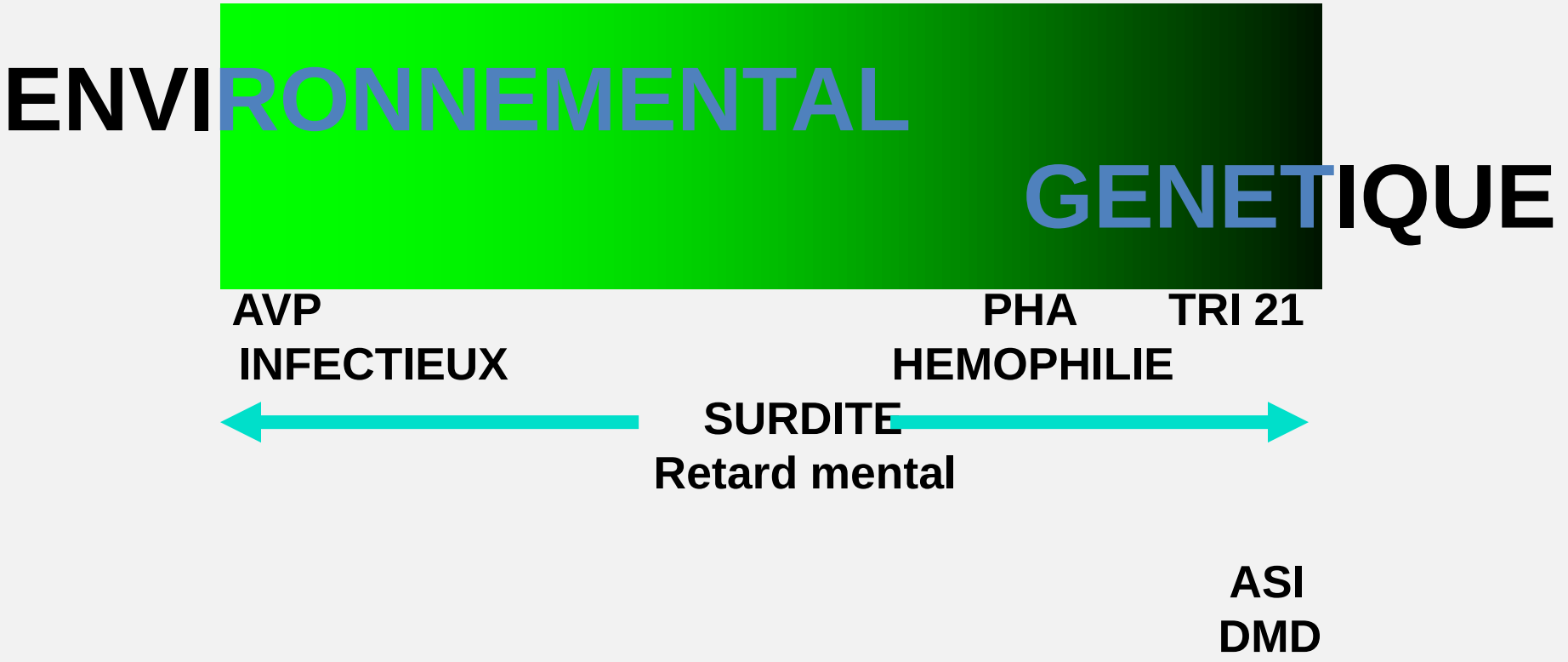

Time scale: Milliseconds - Years
Space scale: Molecule - Individual - Population
Clinical features
Mental retardation
Seizures
Growth retardation
Clinical metrics
Weight
Height
Blood pressure
Clinical Phenome
Exposure analysis
Nutrition
Toxicology
Pharmacology
Environment
Diet / Lifestyle
Medication
Toxics
Exposome
Epigenomics
Metabolomics
Proteomics
Transcriptomics
Genomics
Methylation
Metabolites
Proteins
RNA
DNA
Molecular Phenome
Genome to Phenome
Systems Medicine



Joshua C Denny 1, Francis S Collins 2 Precision medicine in 2030-seven ways to transform healthcare 2021 Mar 18;184(6):1415-1419.

PATHOLOGIE

Gènes et Environnement



ROYAUME DU MAROC

Indice synthétique de fécondité (nombre d'enfants par femme)

	1994	2004	2014
Urbain	2,6	2,1	2,0
Ensemble	3,3	2,5	2,2

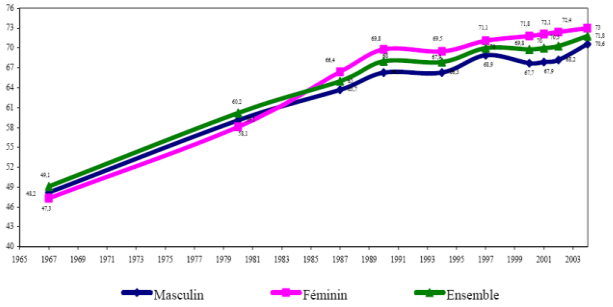
Source : RGPH 1994, 2004 et 2014 (échantillon 2%); HCP.

Age moyen au premier mariage (en années)

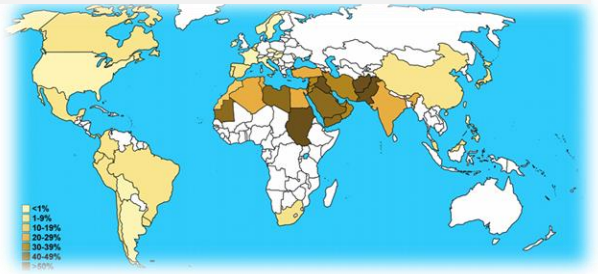
		1994	2004	2014
Urbain	Femmes	26,9	27,1	26,4
	Hommes	31,2	32,2	32,1
	Ecart (H-F)	5,1	5,5	5,7
Rural	Femmes	24,2	25,5	24,9
	Hommes	28,3	29,5	30,3
	Ecart (H-F)	4,1	4,0	5,4
Ensemble	Femmes	25,8	26,3	25,8
	Hommes	30,0	31,2	31,4
	Ecart (H-F)	4,2	4,9	5,6

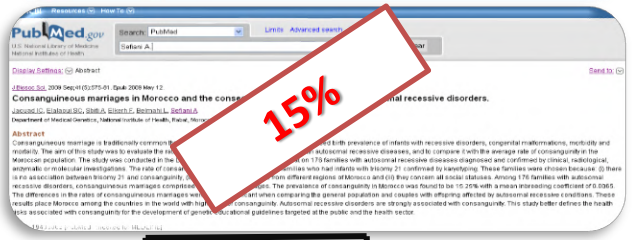
Source : RGPH 1994, 2004 et 2014 (échantillon 2%); HCP.

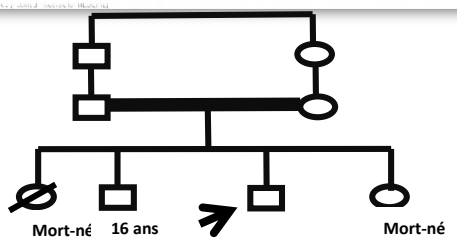
Evolution par sexe de l'espérance de vie a la naissance



Mariages consanguins





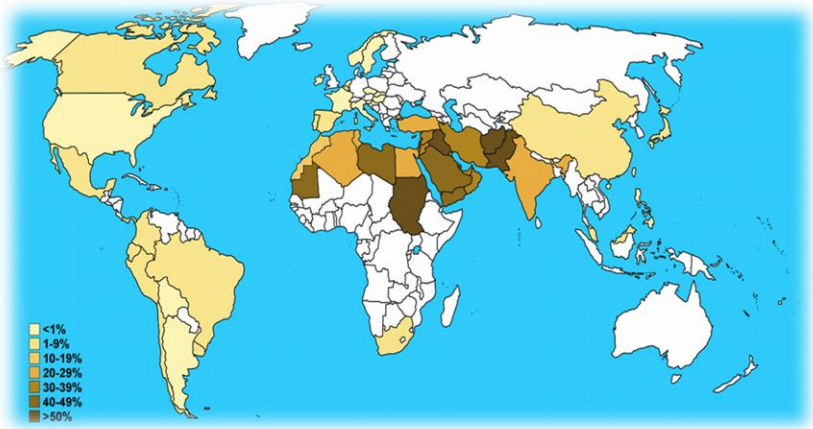


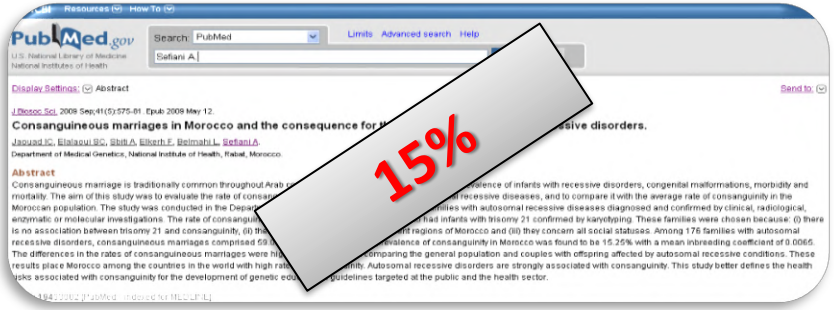
EPIDEMIOLOGIE / POPULATION MAROCAINE

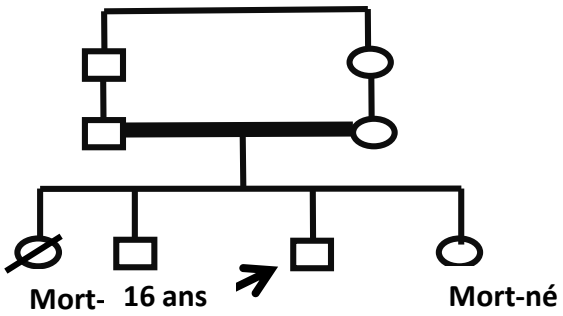
	2007	2008	2009	2010	2011	2012	2016 (estimation)
Accouchements en milieu surveillé	380067	412316	439934	469954	498187	505239	550000
Césariennes	36 421	40 877	45 461	48 280	49 180	55 022	60 000

Facteurs de risque

Mariages consanguins







Procréation en âge avancé

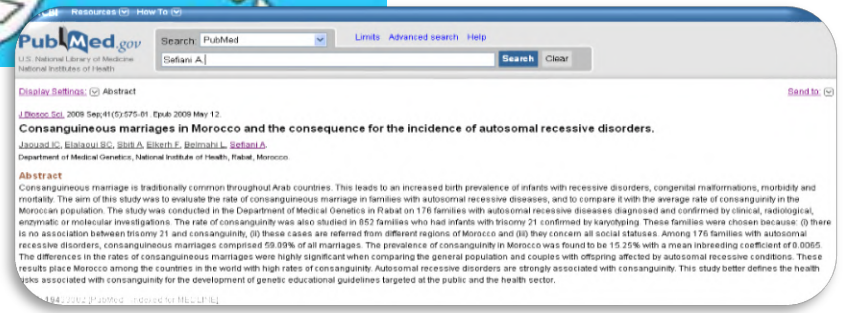
Age maternel : trisomie 21, 13,18

Age paternel : Maladie génétique autosomique dominante

Age Maternel	Risque de Trisomie 21
20	1/1500
25	1/1350
30	1/900
35	1/380
37	1/240
39	1/150
41	1/85
43	1/50
45	1/28

CONSANGUINEOUS MARRIAGES IN MOROCCO

Morocco : **Mediterranean countries**



-). Consanguineous marriages are **culturally favoured**.
- The practice is frequent in all Moroccan populations, **which are grouped according to cultural or linguistic differences**; it is the result of a mixing of Arabs who speak Arabic and non-Arabs (northern–central Berbers who speak Tarifit and southern Moroccan Berbers who speak Tamazigh

The prevalence of consanguinity in Morocco was found to be *15.25%* with a mean inbreeding coefficient of 0.0065.

Estimation du nombre de nouveaux cas de cancers au Maroc (RGCC) 2004 - 2030

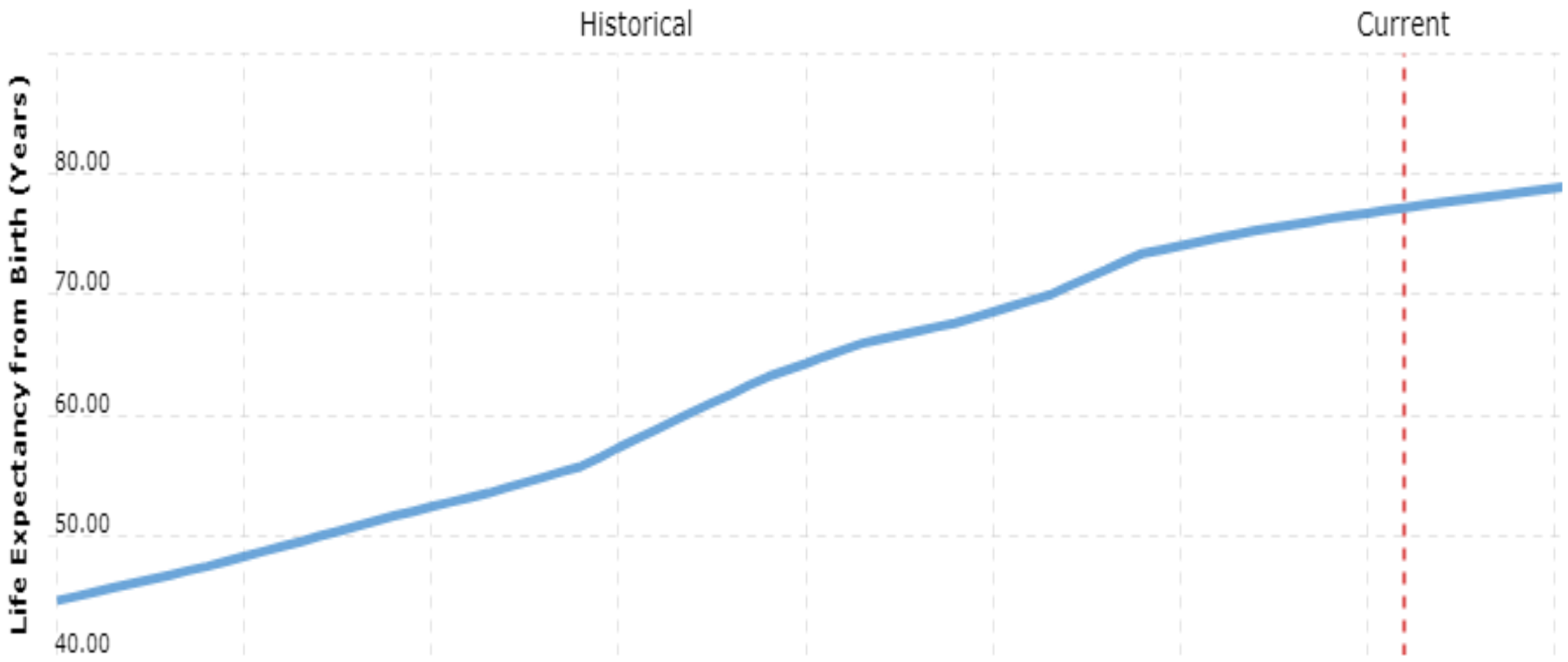
FONDATION LALLA SALMA
PRÉVENTION ET TRAITEMENT DES CANCERS
مؤسسة للا سلمى للوقاية وعلاج السرطان

وزارة الصحة
Ministère de la Santé

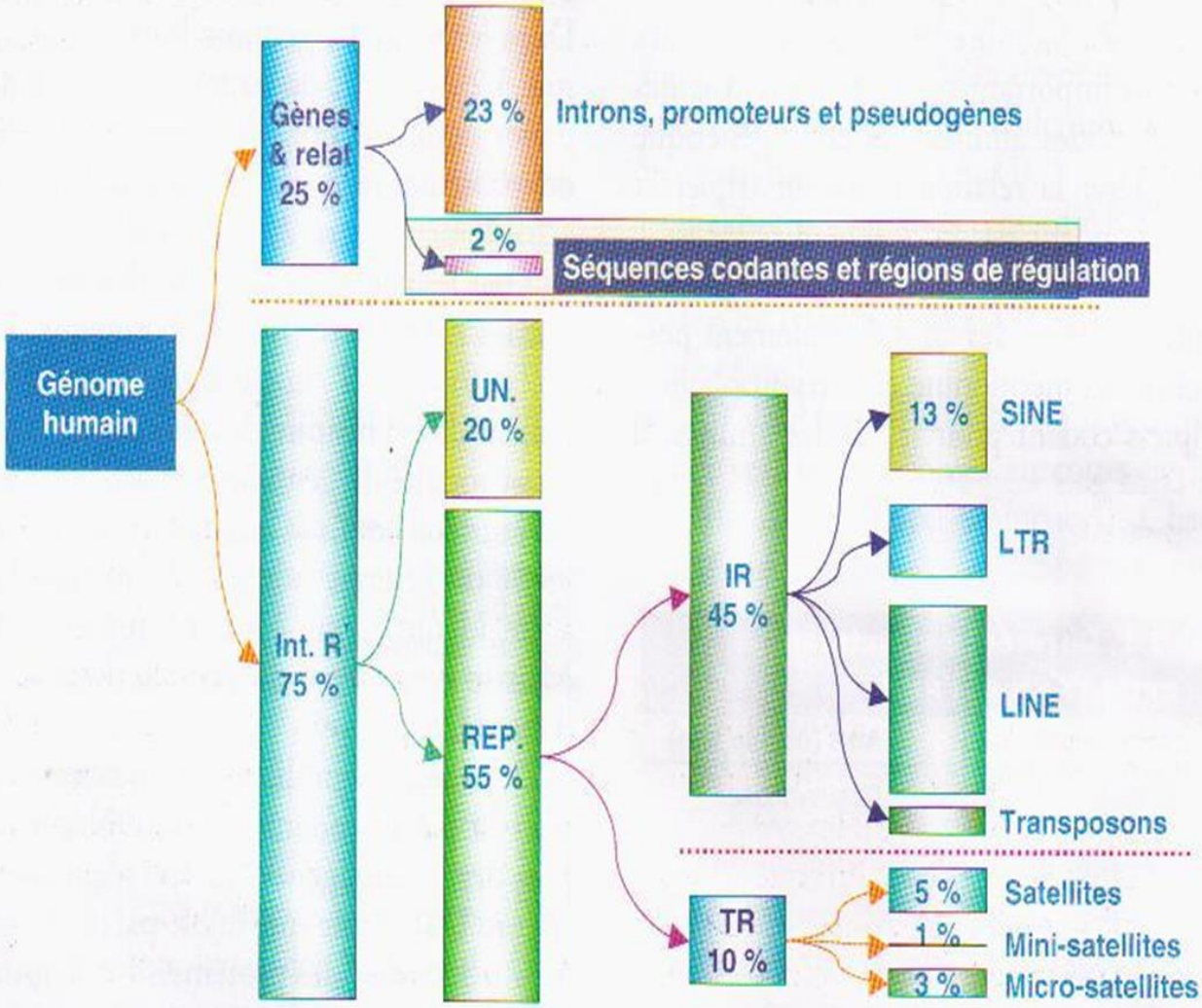
Morocco Life Expectancy 1950-2022

From: 1950 To: 2030

Zoom: 10Y 20Y 30Y 40Y 50Y All



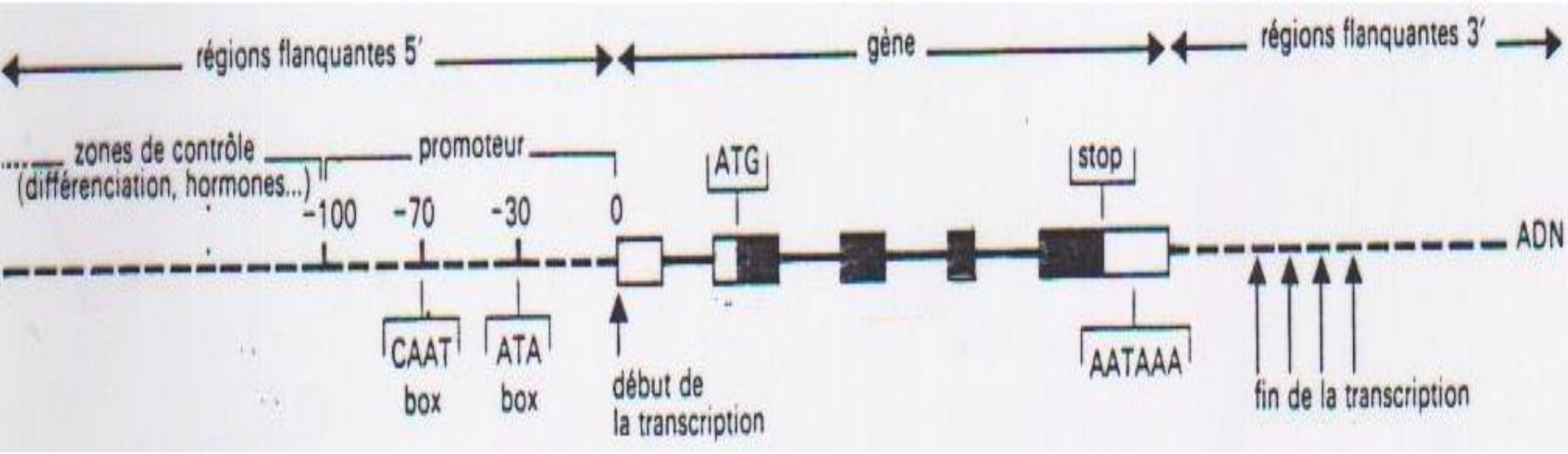
Description et composition générale du génome humain



Composition générale du génome humain. Le pourcentage représente la quantité de séquences par rapport à la séquence totale connue du génome. Gènes & relat : gènes et séquences associées ; Int. R : régions intergéniques ; Un. : séquences intergéniques uniques ; Rep. : séquences intergéniques répétitives ; IR : séquences intergéniques répétitives dispersées ; TR : séquences intergéniques répétées en tandem.

Anatomie d'un gène

Le gène d'un eucaryote est morcelé en fragments codants : **les exons** (dont la taille varie en moyenne entre 50 et 200 pb), séparés en général par des séquences non codantes : les introns. En amont du gène, se trouve une **séquence régulatrice** et le **promoteur.**



Structure d'un gène codant une protéine chez les eucaryotes

A quelques exceptions près, tous les gènes des eucaryotes possèdent des introns. Le nombre et la taille des introns varient d'un gène à un autre.

Les gènes de l'ADN mitochondrial sont sans introns.

Structure des gènes

Anatomie d’un gène

Promoteur :

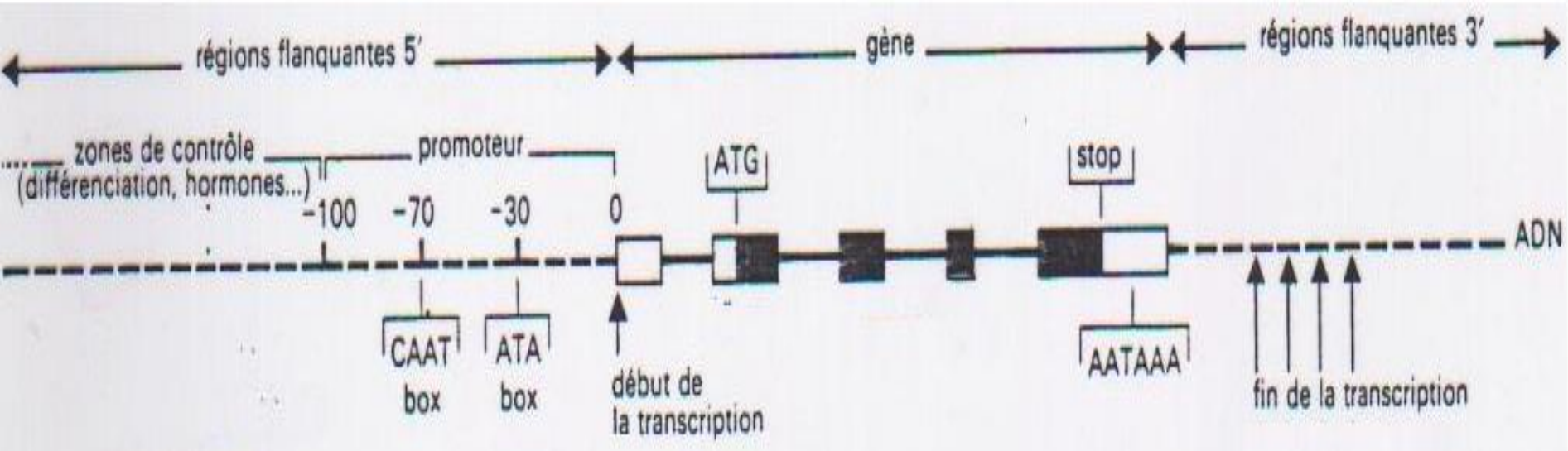
En amont du gène en 5', se trouve la région promotrice ou promoteur et la séquence régulatrice de la transcription du gène.

Gène :

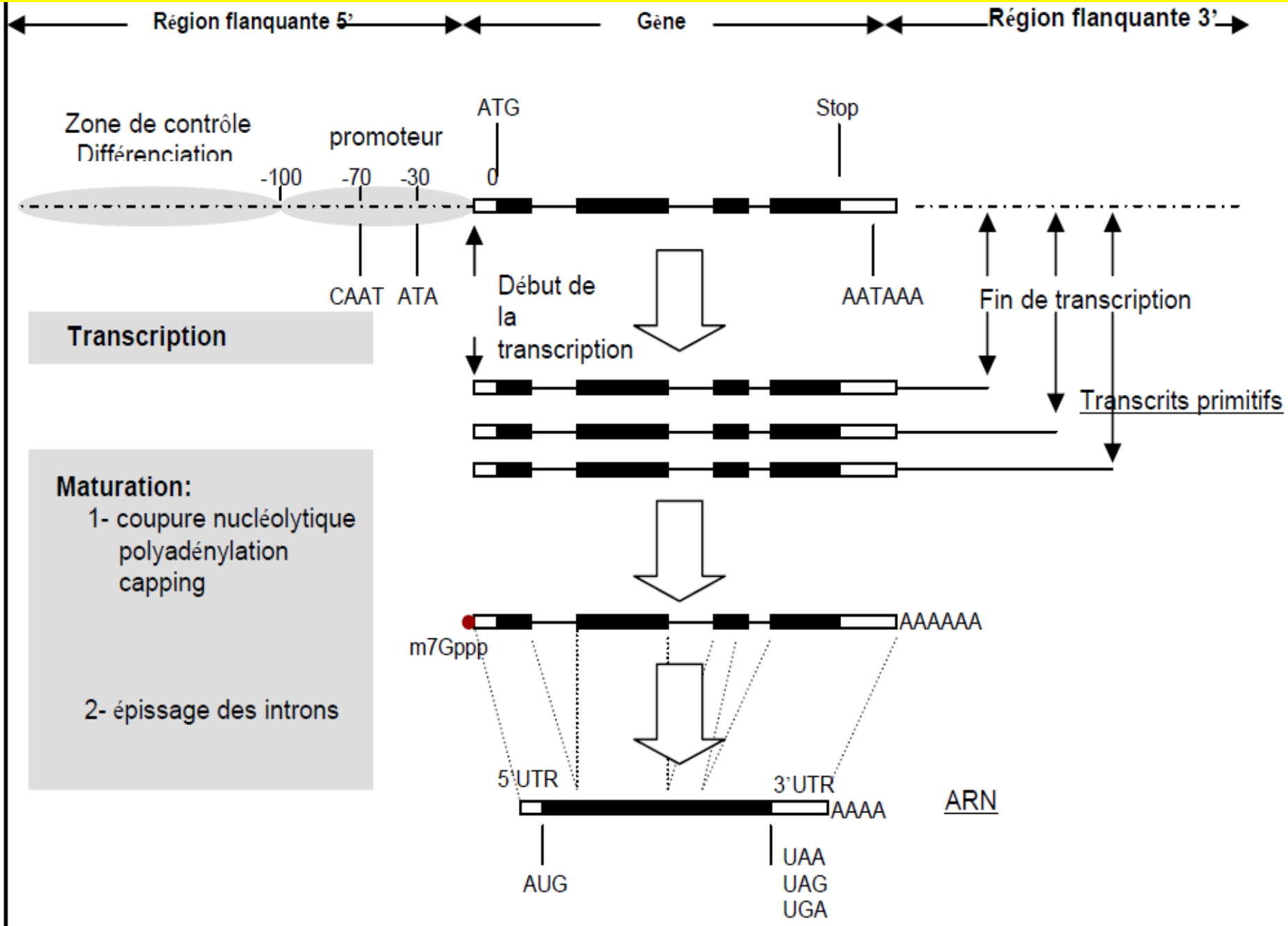
Un gène est une entité discontinue dans laquelle les parties codantes (**Exons**) sont en général séparées entre elles par des parties non codantes (**Introns**) éliminées au cours de la maturation de l'ARNm.

Introns

Certains introns jouent un rôle important dans la **régulation de l'expression d'un gène**.



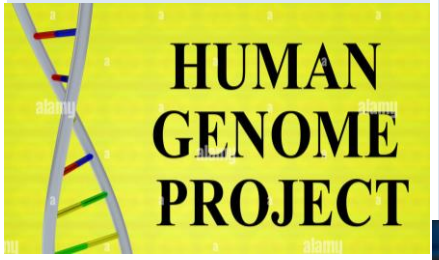
GENE ET SON EXPRESSION



Human genomic data past 30 years

1990–2000

Launch of the « Human Genome Project and related endeavors ».



2000–2010

- Law
- Ethics
- Research infrastructures (biobanks)
- Citizenship and ‘public goods’

2010–2020

Genetic privacy in response of large international research consortia and big data.

2020……2050….2100….

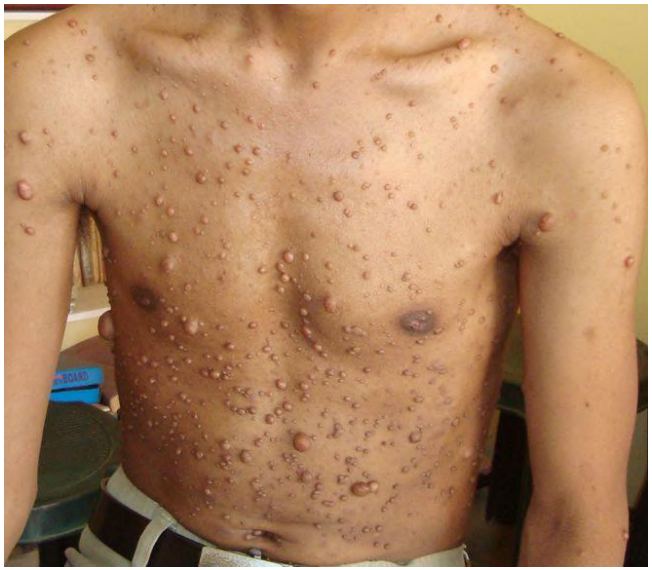
- **Big Data**
- **Artificial intelligence (AI)**
- **Gene and cell therapies**
- **Nanotechnology**

Bartha Maria Knoppers , Michael J S Beauvais Three decades of genetic privacy: a metaphoric journey Hum Mol Genet2021 Oct 1;30(R2):R156-R160. doi: 10.1093/hmg/ddab164.

Genetic variations

Diversity

Diseases



Génétique Médicale

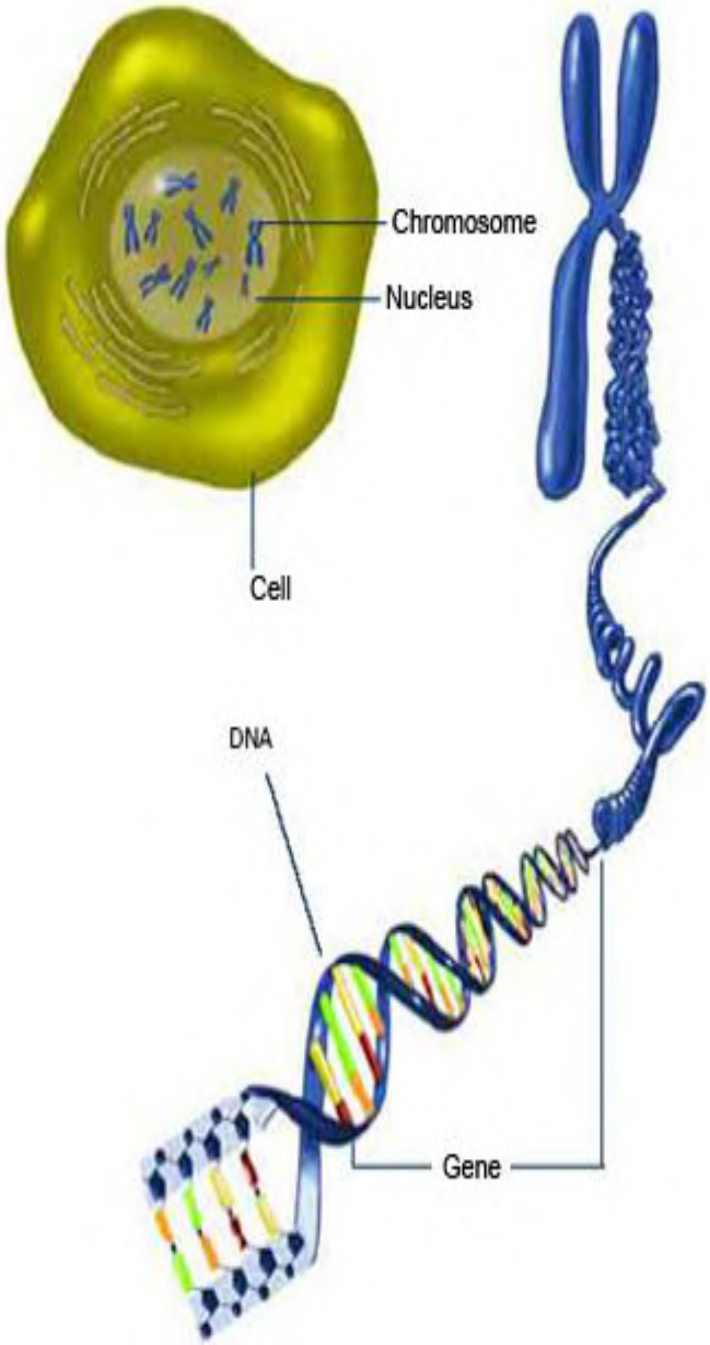
- **Clinique :**
  - Consultation de génétique médicale (conseil génétique, dysmorphologie, endocrinogénétique, néphrogénétique, neurogénétique, maladies osseuses constitutionnelles, dermatogénétique, immunogénétique…)
  - Consultation d'oncogénétique
- **Cytogénétique conventionnelle et moléculaire constitutionnelle post-natale et CGHarrays**
- **Génétique moléculaire itaires :**
  - Biologie molécualire : PCR derivées, RT PCR…..
  - Séquençage classique
  - séquençage de nouvelle génération *(Next Generation Sequencing « NGS »)*

PANELS DE GENES / EXOME /WGS

- **Métagenomique (recherche)**

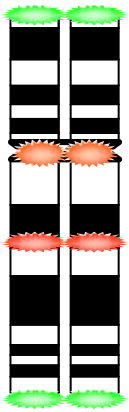
Plateau technique + Expertise

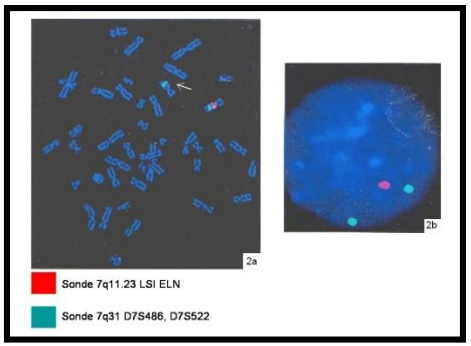
Explorations en génétiques



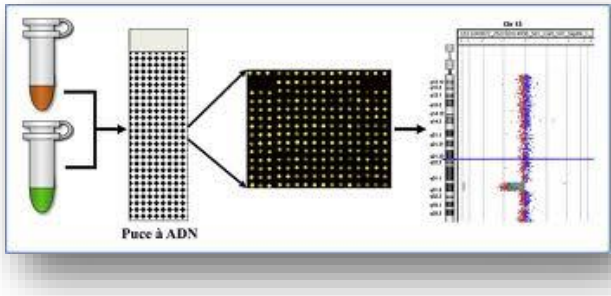
Chromosome analysis

FISH





ACPA CGHarrays



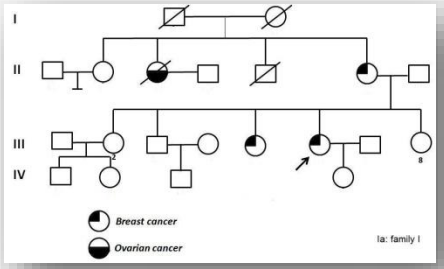
DNA sequencing

ACTGACTGACTG

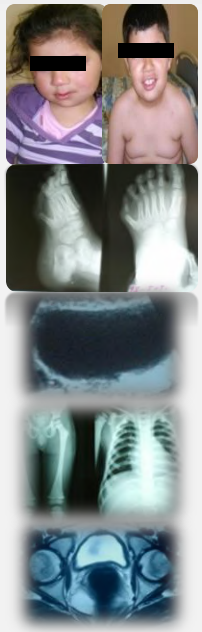
Medical Genetics and *oncogenetics*

Clinical Genetics

Genetic consuling

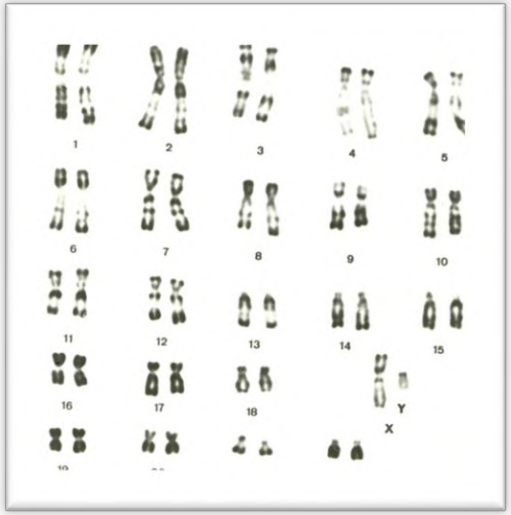


Clinical Diagnosis

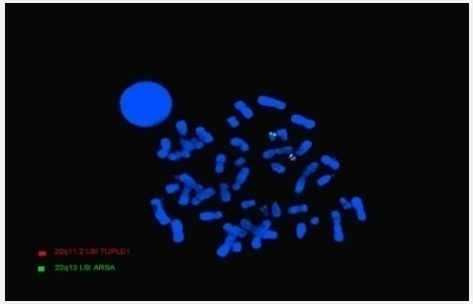


Cytogenetics

Karyotype

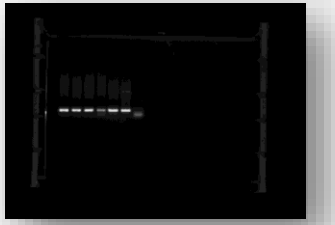


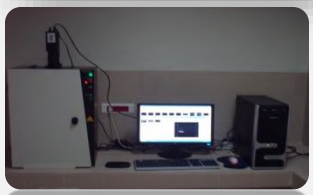
Molecular Cytogenetics



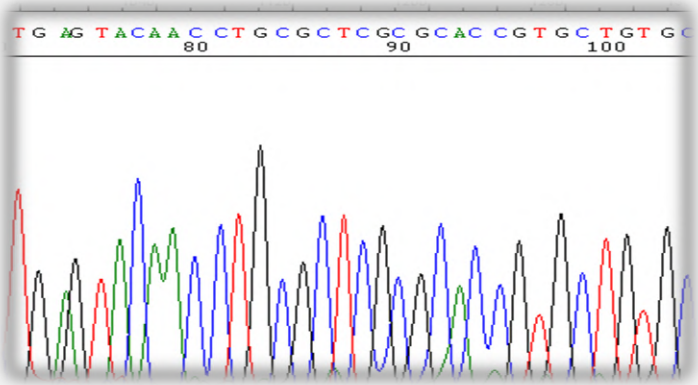
Molecular diagnosis

PCR



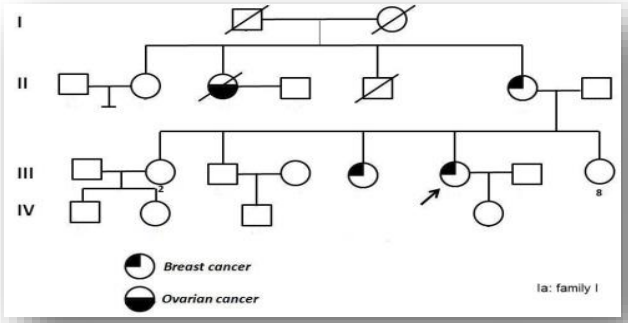


Sequence analysis

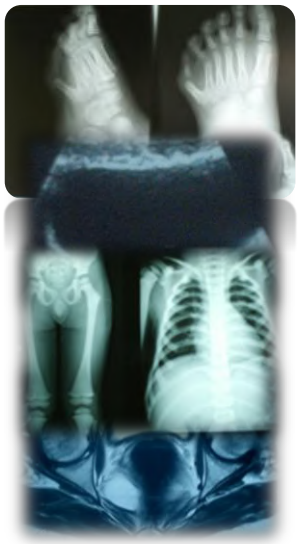


Clinical Genetics

Genetic consuling

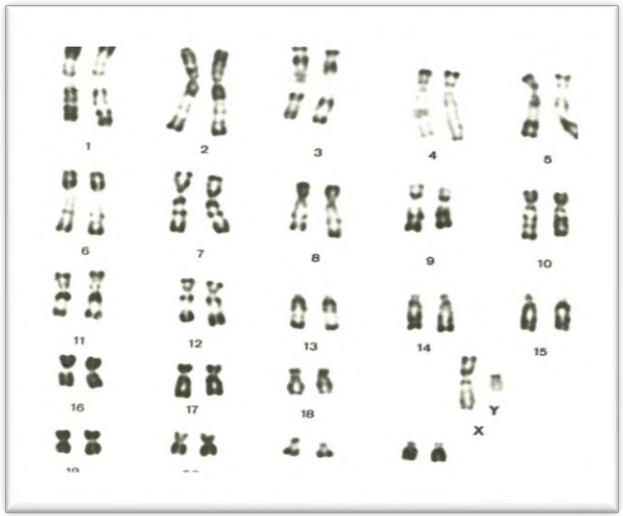


Clinical Diagnosis

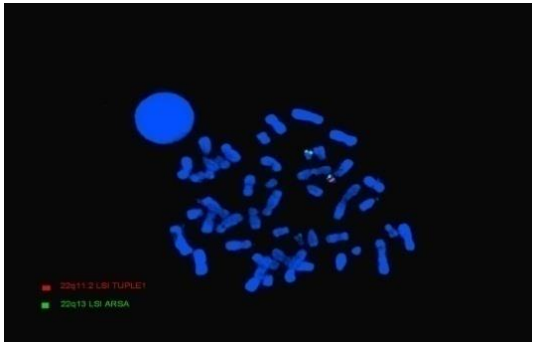


Cytogenetics

Karyotype



Molecular Cytogenetics

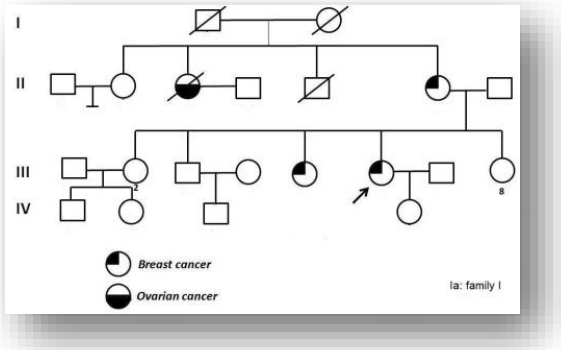


Medical Genetics and *oncogenetics*

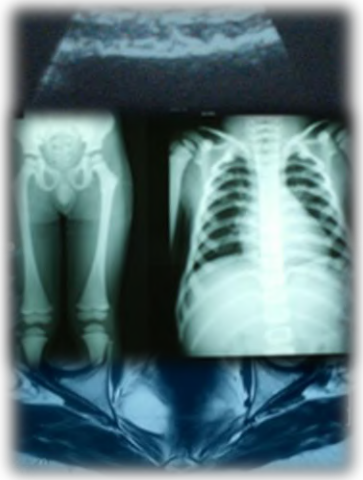
Postnatal karyotype	**(+)**
Onco-hématology Karyotype	**(+)**
Chromosome breakage analysis : Fanconi Anaemia	**(+)**
FISH postnatal (CEP X, LSI SRY,22q11.2,WBS, …)	**(+)**
FISH oncology solid tumor (HER-2, EGFR, TOPO2A, 1p36,EWSR)	**(+)**
FISH oncology hematology (BCR/ABL)	**(+)**
DNA extraction-Blood	**(+)**
DNA extraction -Tumor	**(+)**
Simplex PCR	**(+)**
Multiplex PCR	**(+)**
PCR sequencing	**(+)**

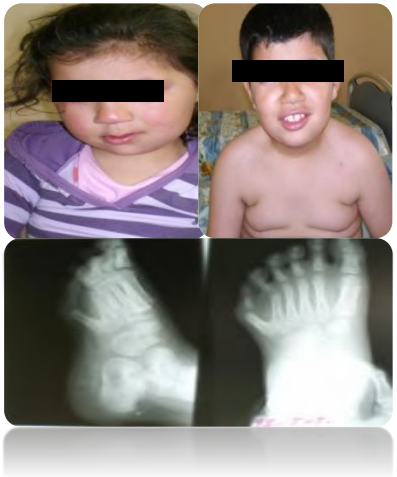
CLINCAL GENETICS

GENETIC CONSULING



DIAGNOSIS





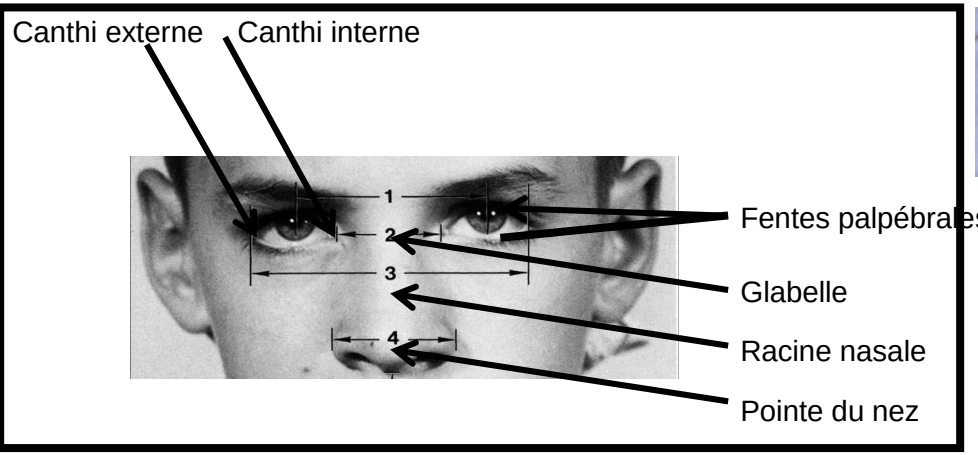
Dysmorphology, neurogenetic,

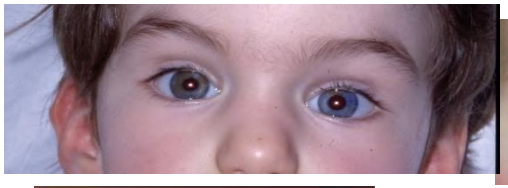
dermatogenetic, nephrogenetic….

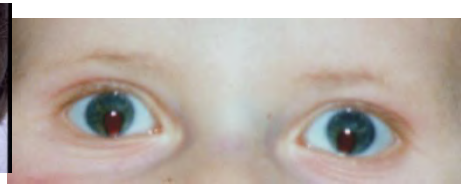
CONSULTATION GENETIQUE

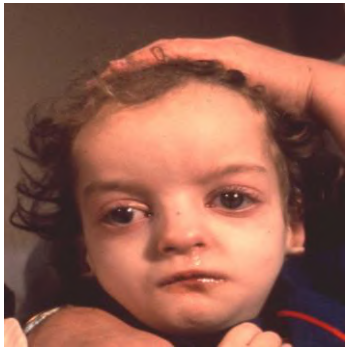
1. Anamnèse (arbre généalogique)
2. Recueil de tous les documents
3. Examen clinique du sujet atteint
4. Diagnostic moléculaire : ADN
5. Conseil génétique + Estimation du risque.
6. Prévention et la faisabilité /conductrices d'hémophilie
7. Eventuelle du diagnostic prénatal DPN/Preimplantaoire DPI
8. Le génotypes: important pour la prise en charge +++

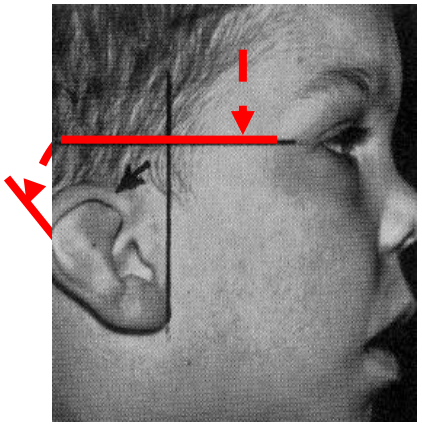
DYSMORPHOLOGIE

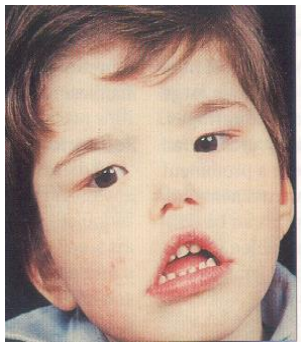


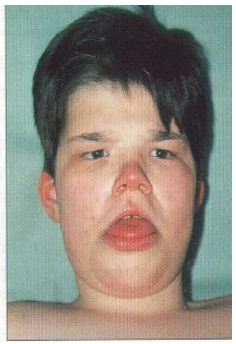


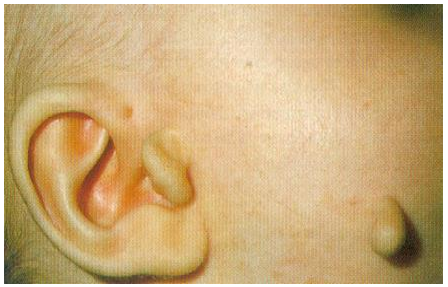


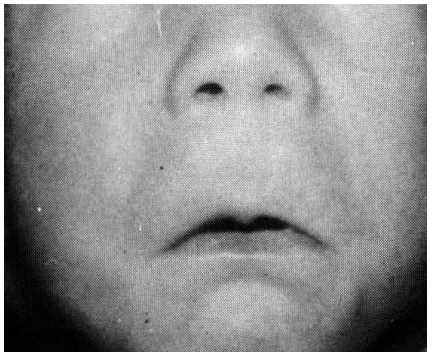


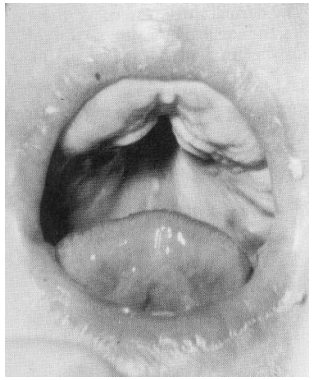


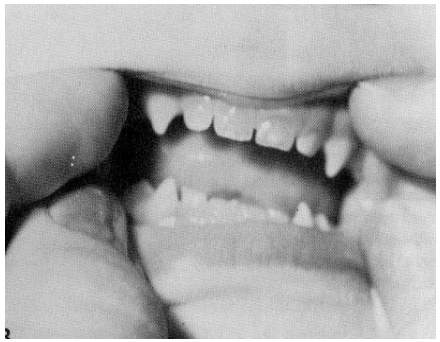


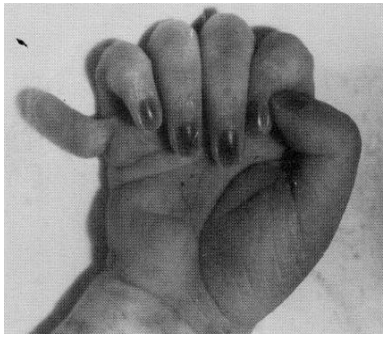


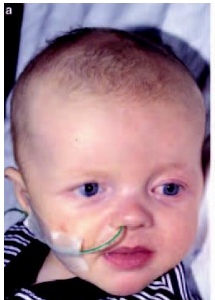


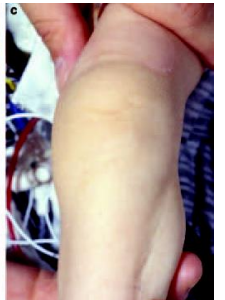








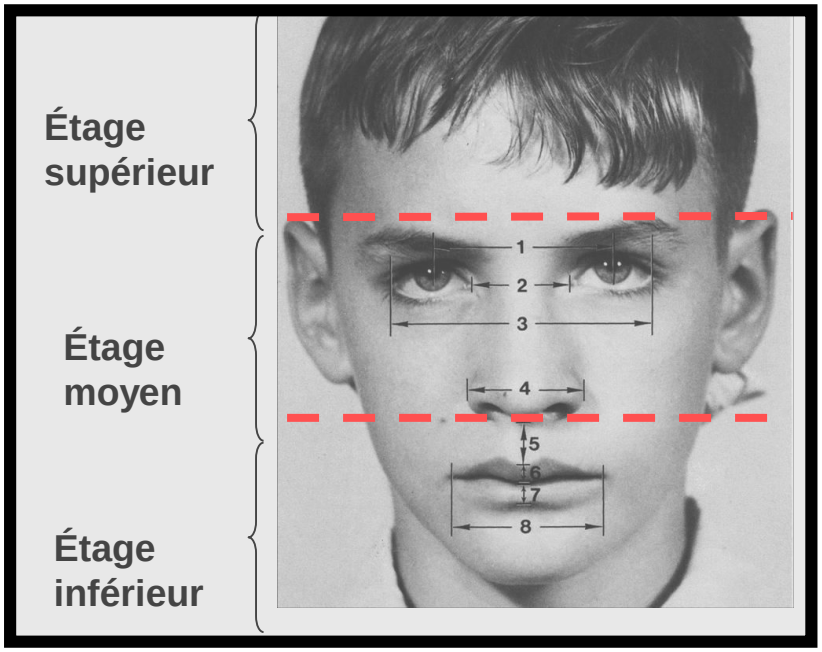


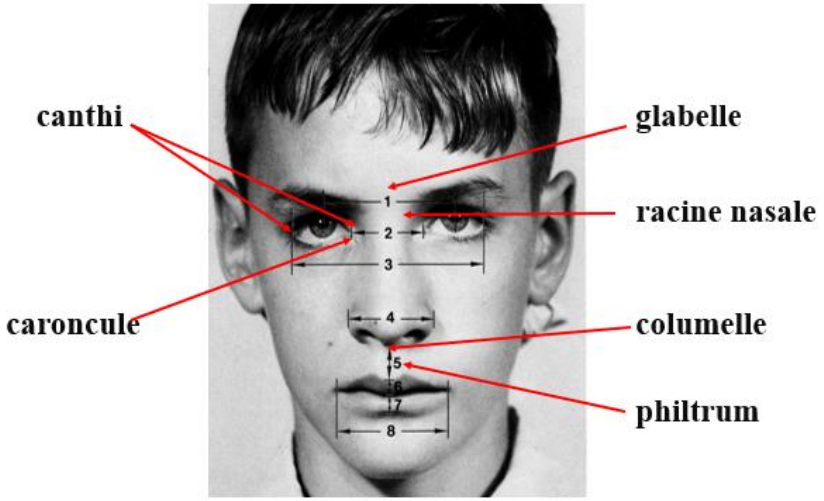


Examen de la face

Deux étapes :

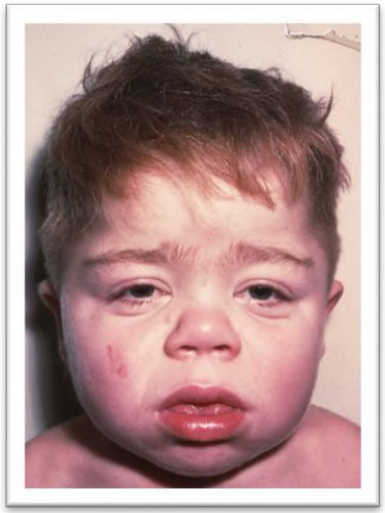
- **Aspect général**
- **Examen minutieux en subdivisant la face en trois étages :**
  - **Supérieure**
  - **Moyen**
  - **Inférieure**



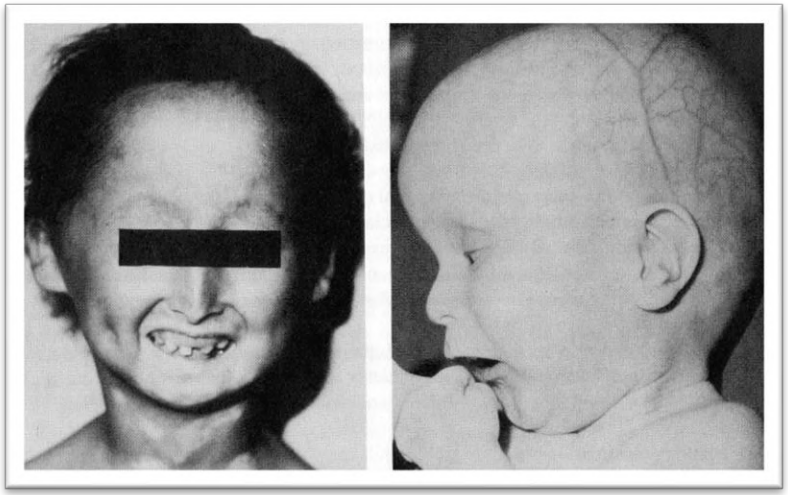


Examen en dysmorphologie

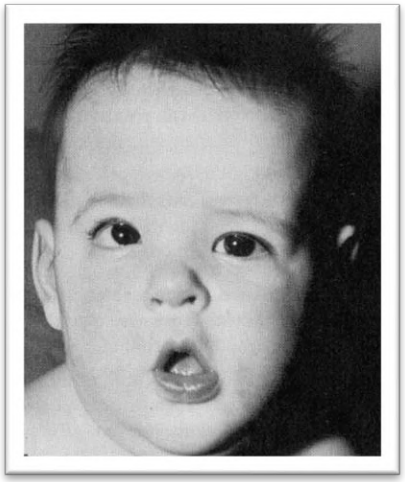
Examen de la face / Aspect général de la face



Epais

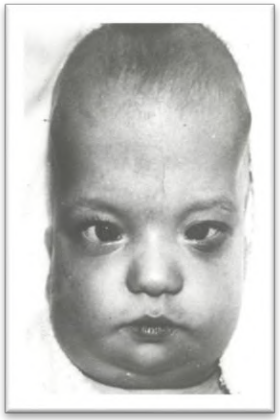


Vieillot

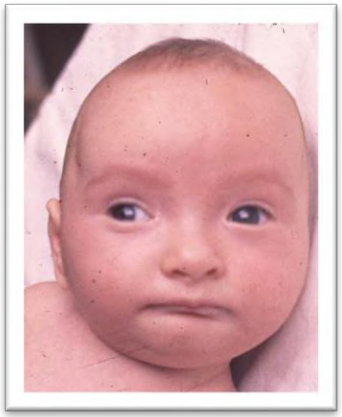


Asymétrique

Triangulaire



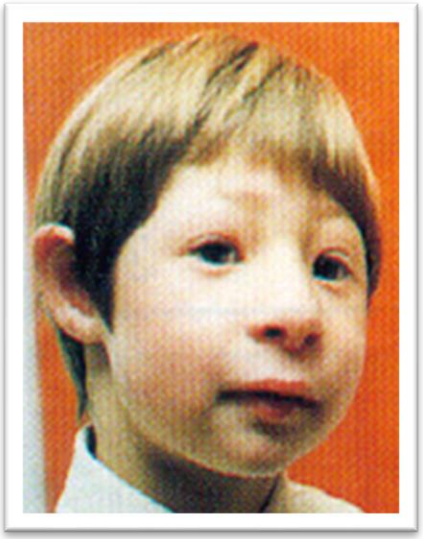
Allongé



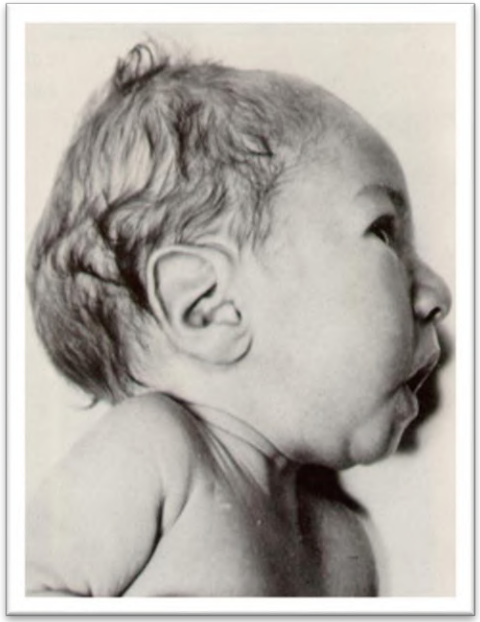
Rond

Examen de la face : Etage supérieur de la face:

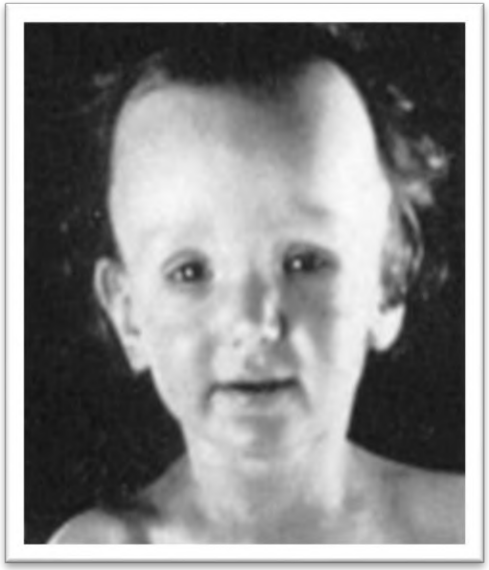
Forme du crâne



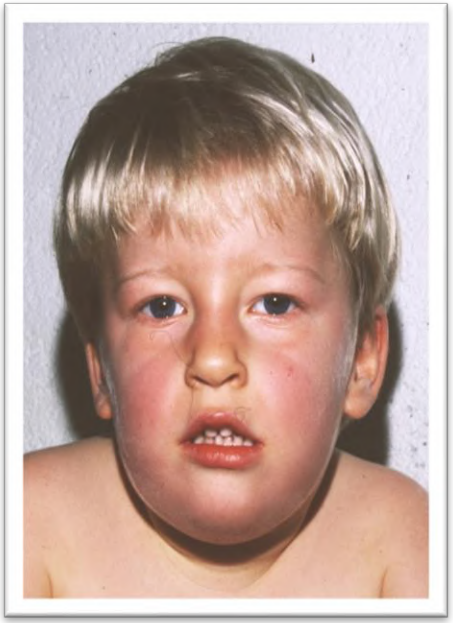
Microcéphalie



Front fuyant

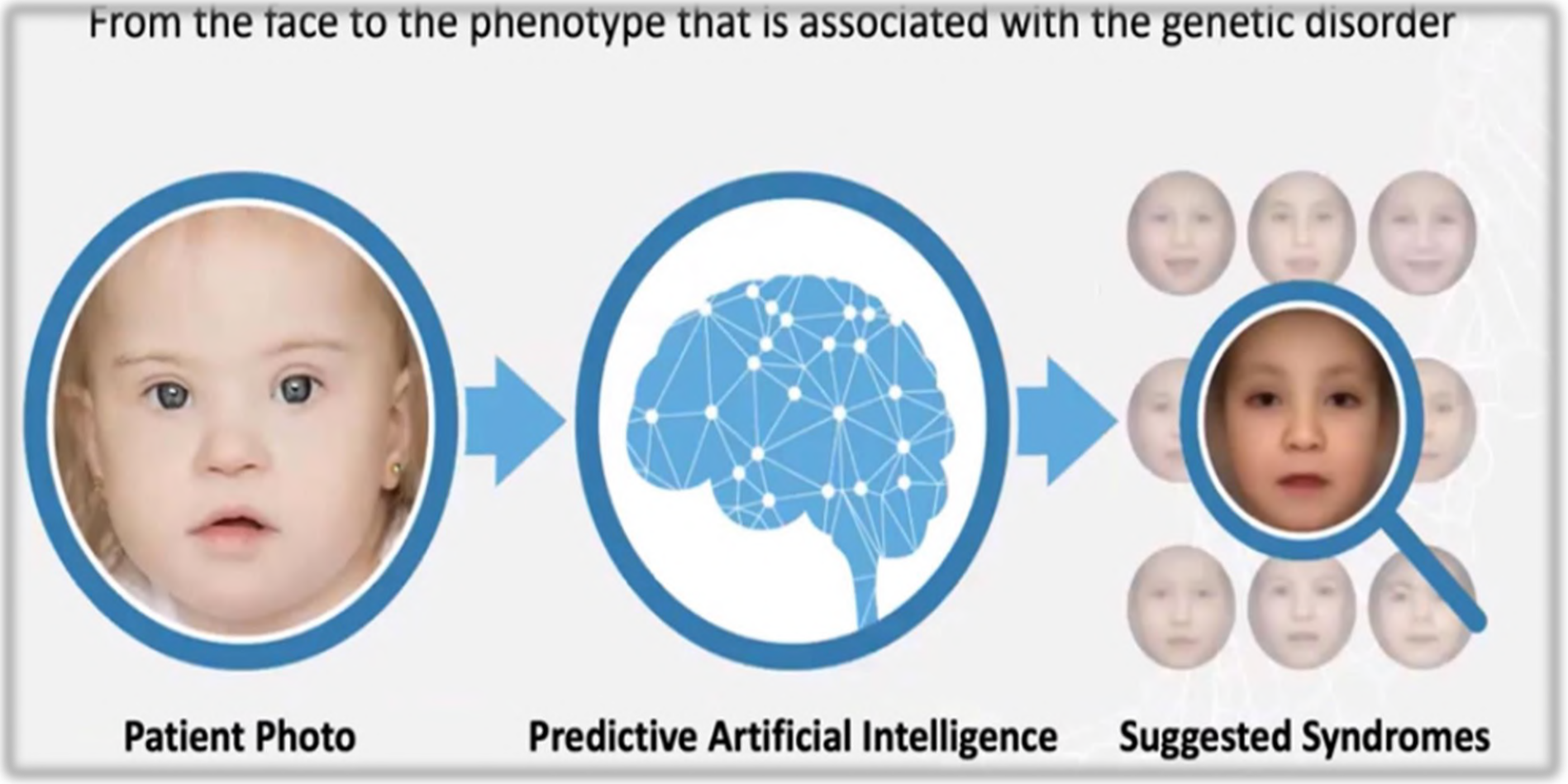


Macrocéphalie
Bosses frontales



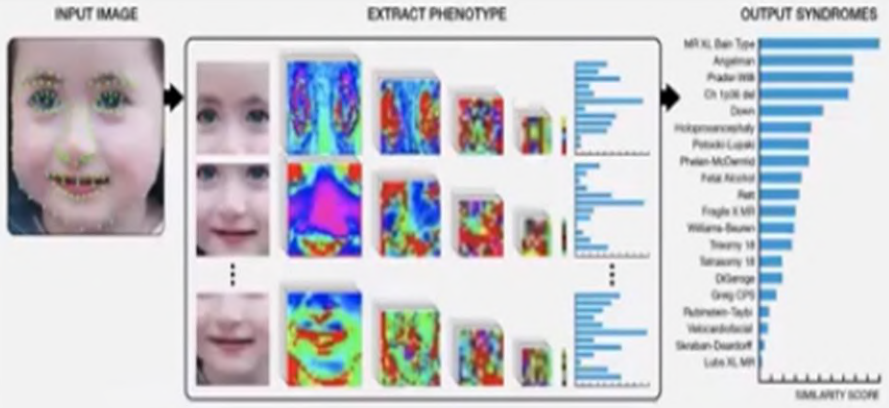
Rétraction
temporale

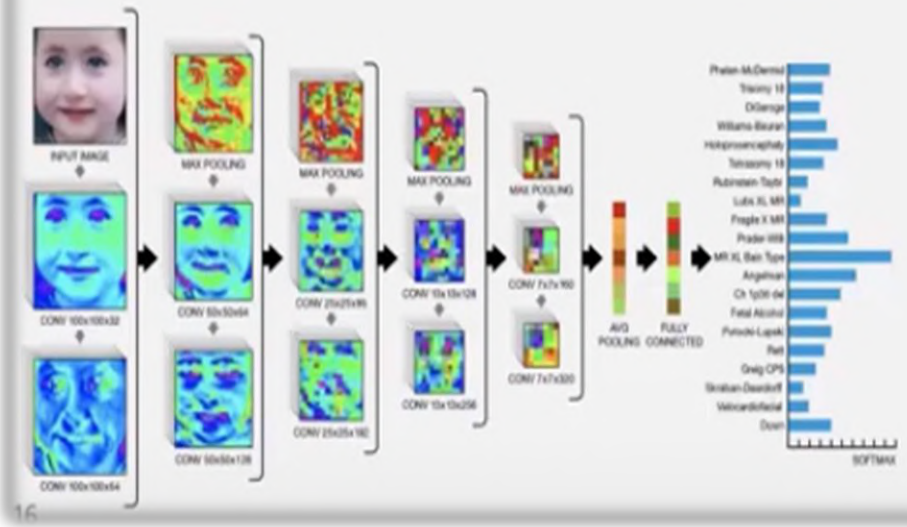
Intelligence artificielle
Dysmorphologie



Intelligence artificielle

Dysmorphologie

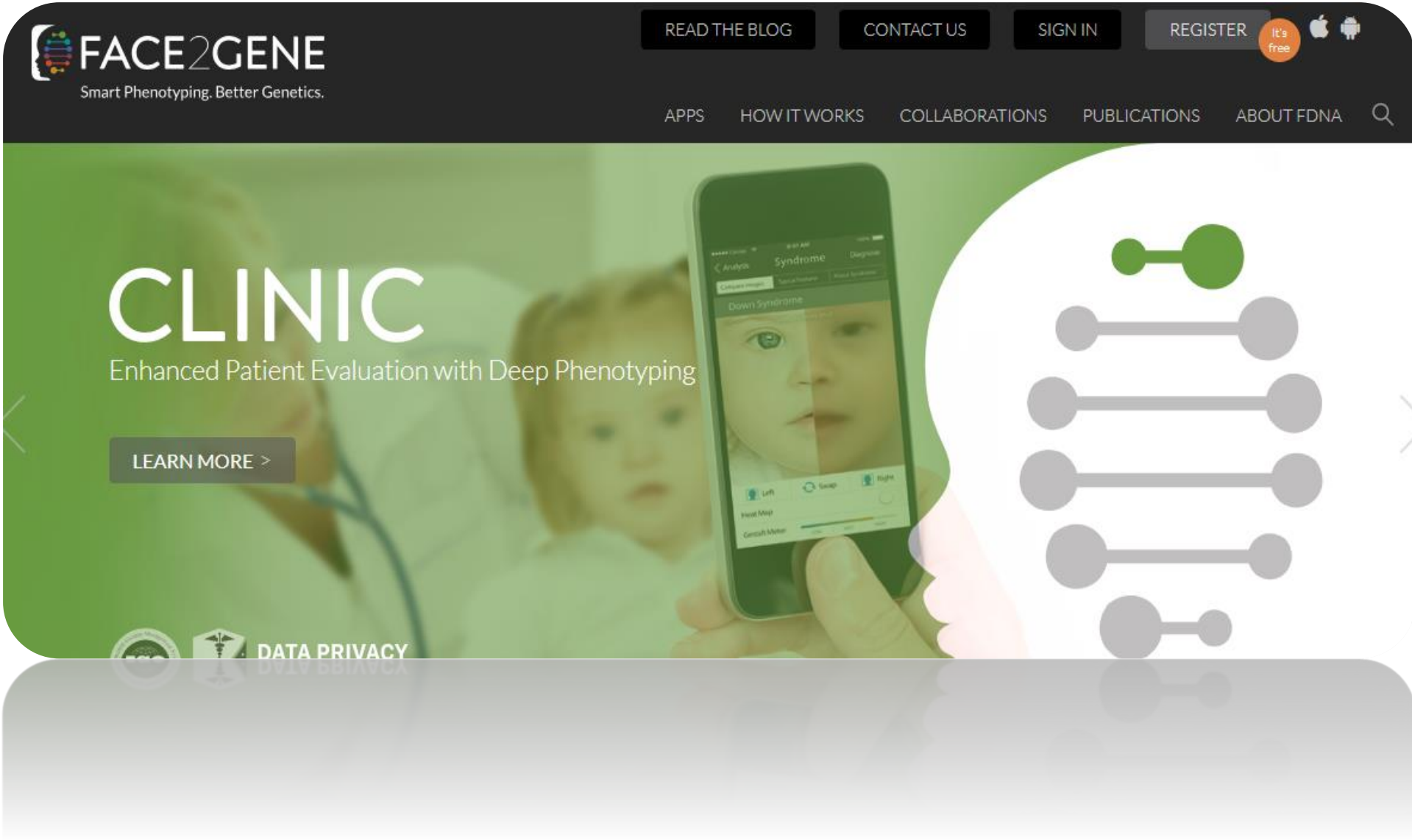




- Deep convolutional neural network (DCNN) approach
- Transfer learning approach to allow learning from a relatively small database
- Community driven: Uploaded images are analyzed in a non-identifiable manner, data is used to further train syndrome recognition

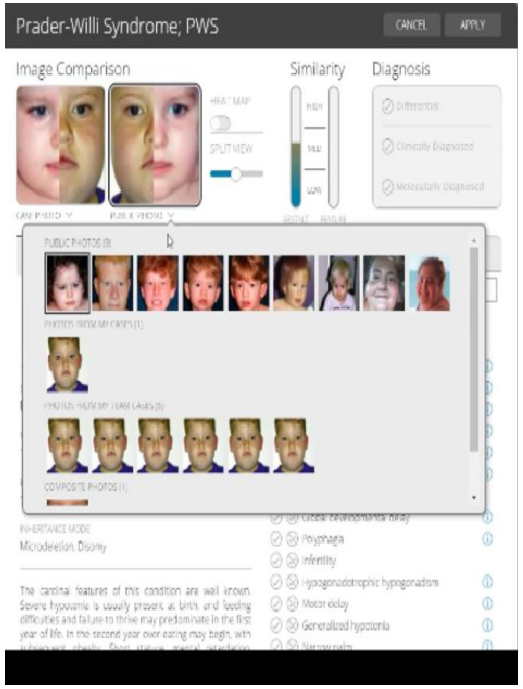
FDNA

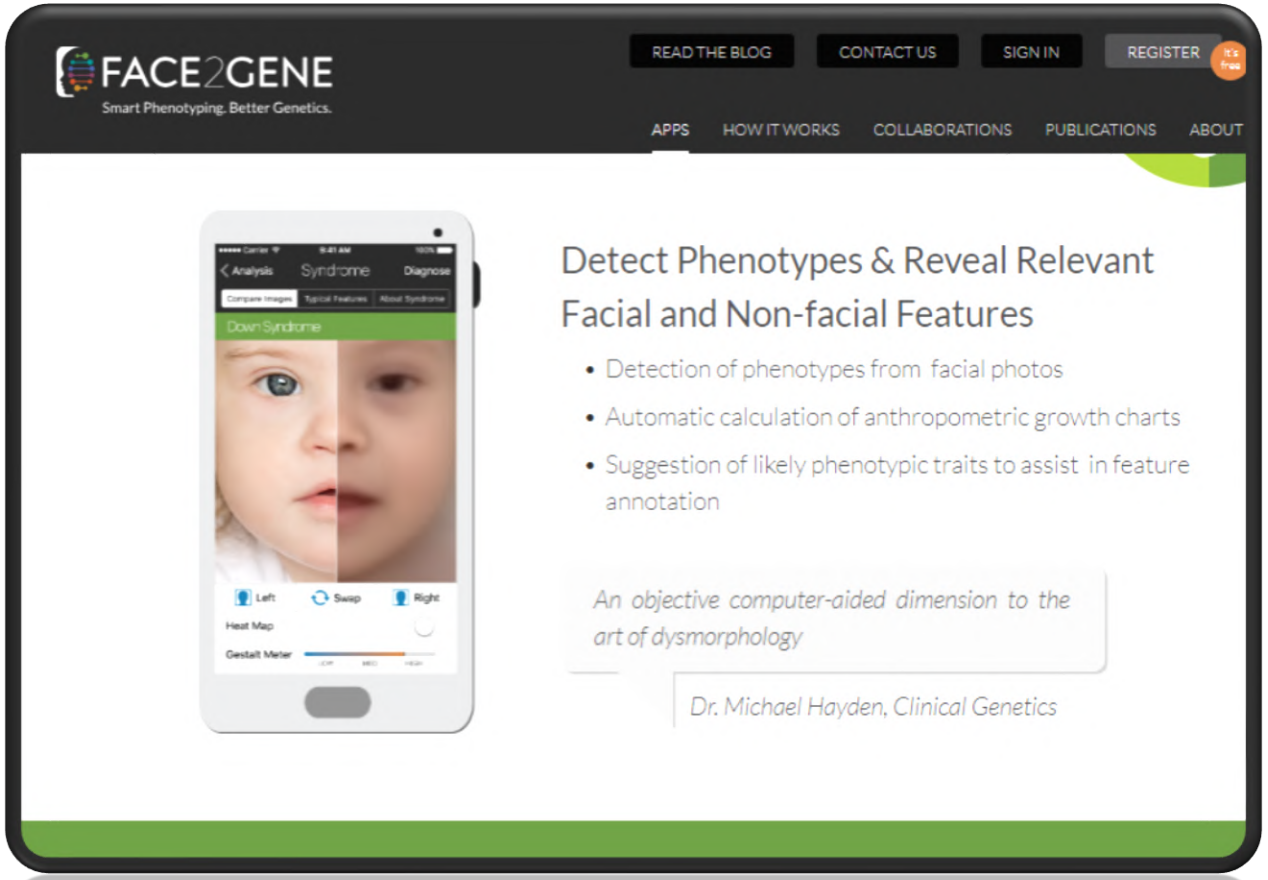
Intelligence artificielle Dysmorphologie



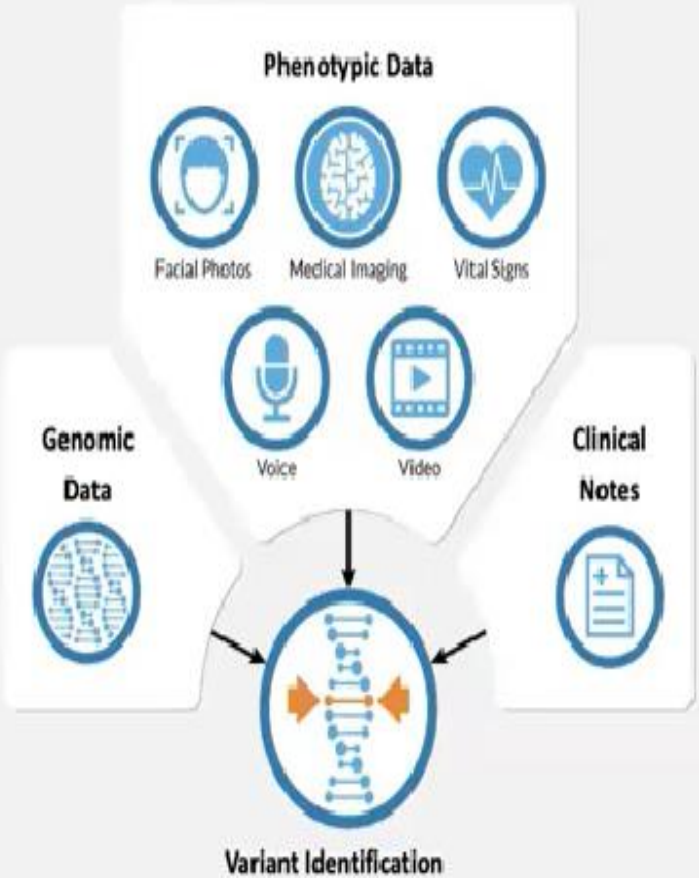
Intelligence artificielle

Dysmorphologie 2019

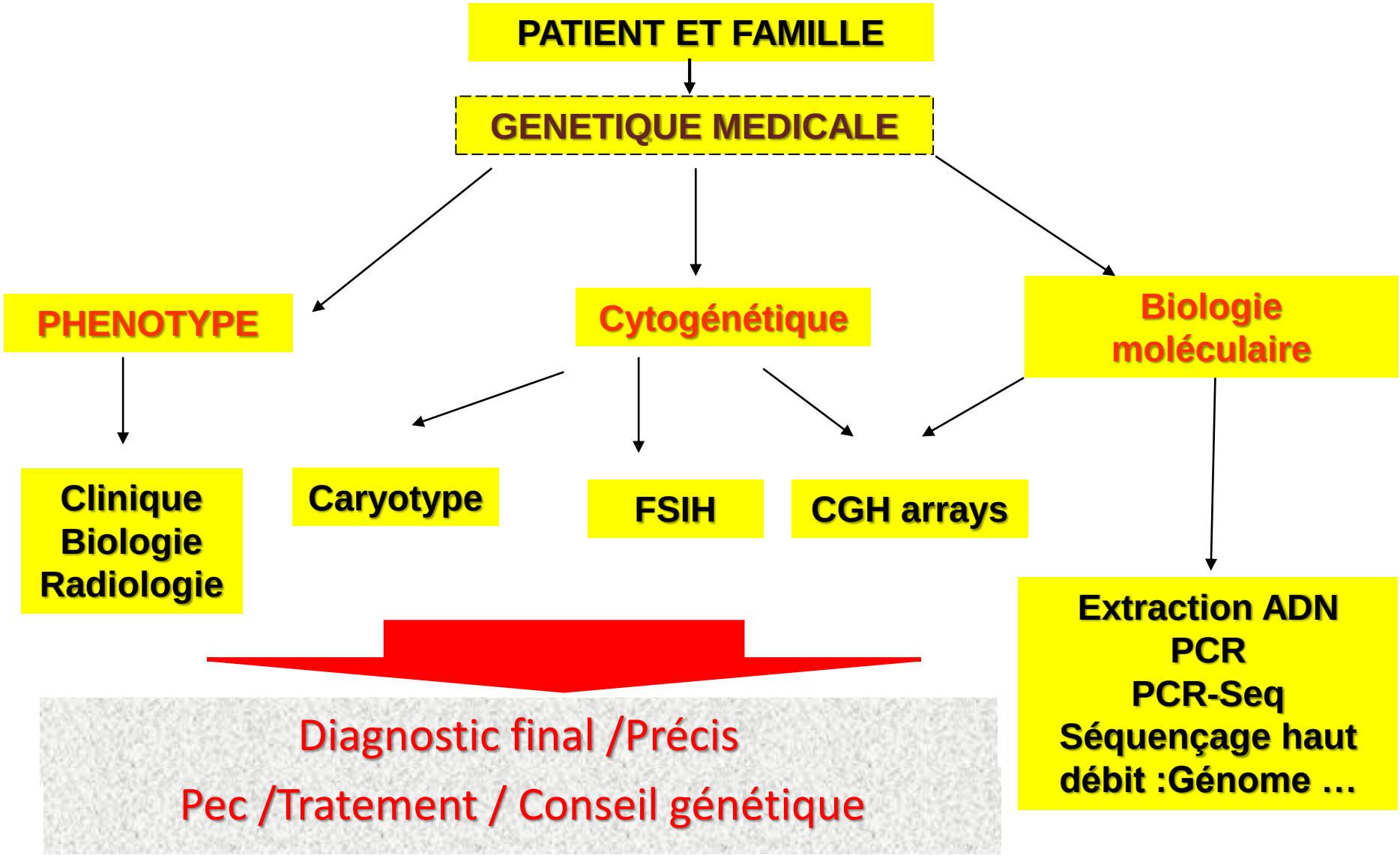




Intelligence artificielle
Dysmorphologie et Gènes 2021



GENETIQUE MEDICALE



Diagnostic final /Précis

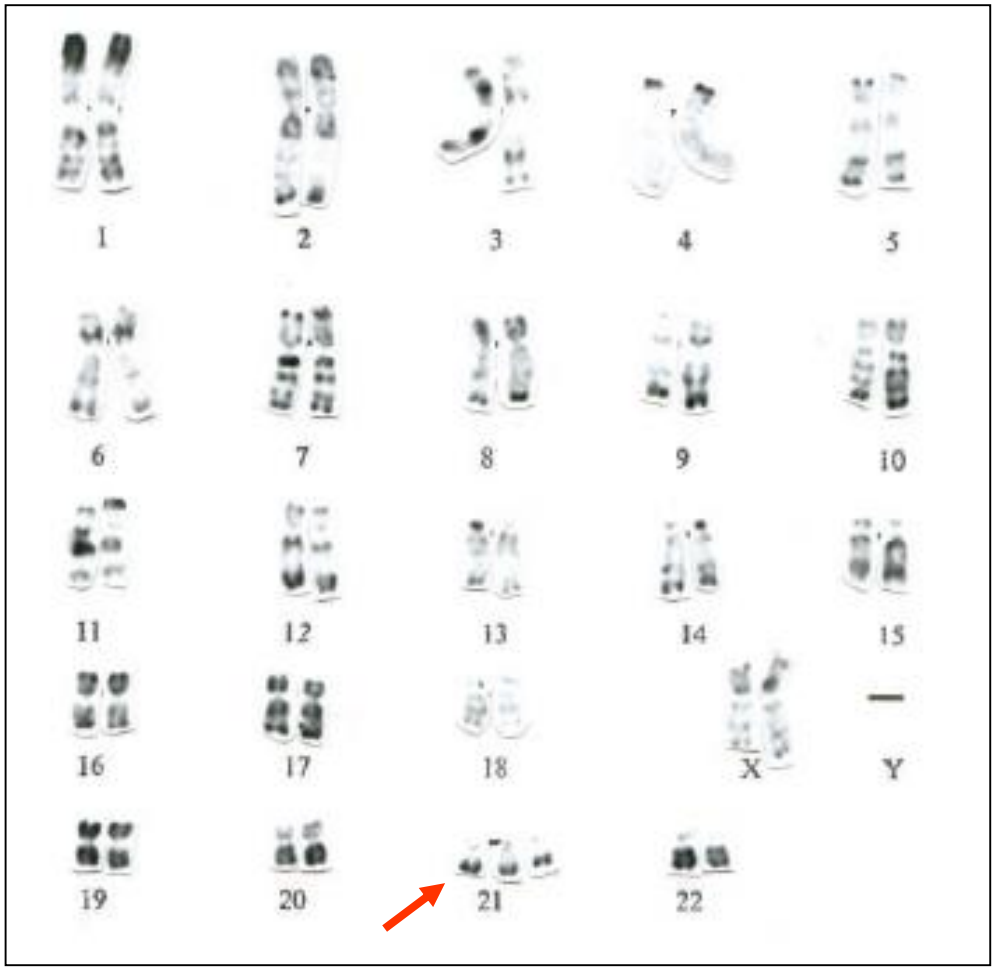
Conseil génétique

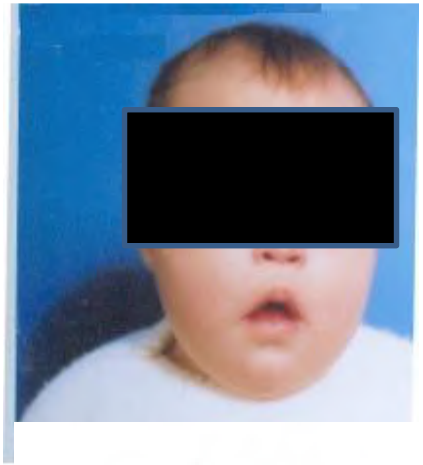
Modalités de prévention et la faisabilité

Diagnostic prénatal invasif et non invasif DPN / DPNI

Diagnostic Preimplantaoire DPI

VOTRE DIAGNOSTIC ?

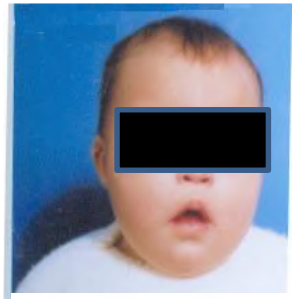


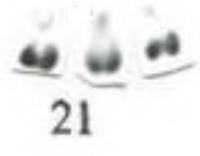


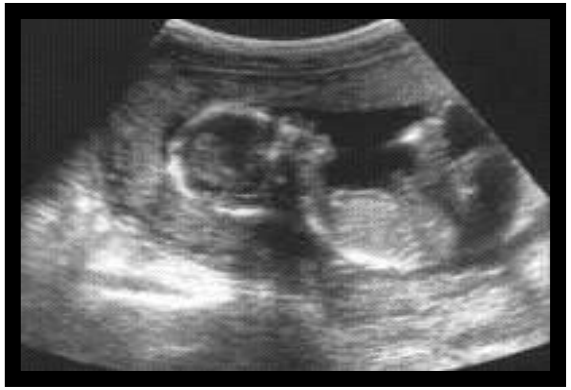
Trisomie 21 libre

La trisomie 21 : Anomalie génétique la plus fréquente au Maroc

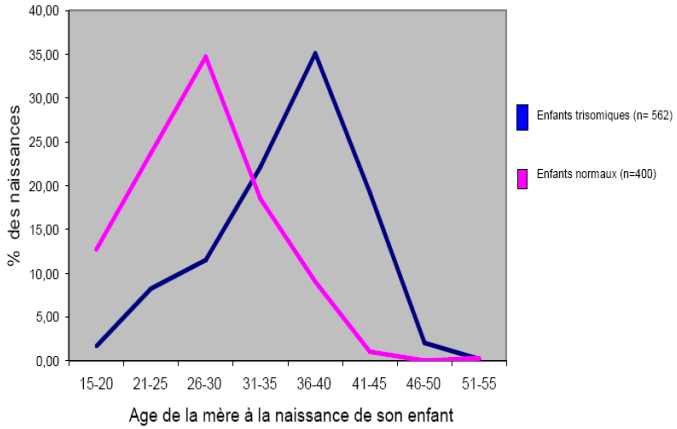
- **Un enfant trisomique naît pour 700 naissances vivantes (1.3 ‰).**
- **La fréquence de la trisomie 21 à la conception est 7.3 ‰ dont seul 1.3 ‰ arrivent à terme et 6‰ sont à l'origine de fausses couches spontanés.**
- 3 garçons / 2 filles







La clarté nucale

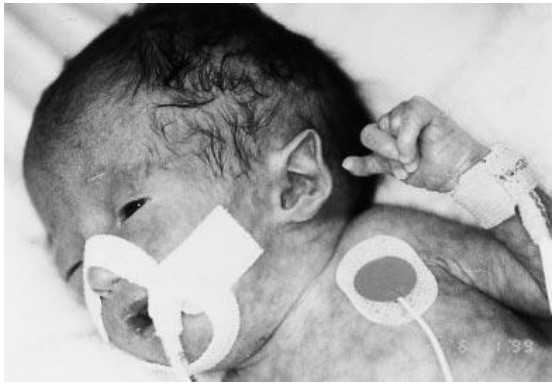


Age Maternel	Risque de Trisomie 21
20	1/1500
25	1/1350
30	1/900
35	1/380
37	1/240
39	1/150
41	1/85
43	1/50
45	1/28

LA TRISOMIE 18

Fréquence : 1/ 8 000 naissances

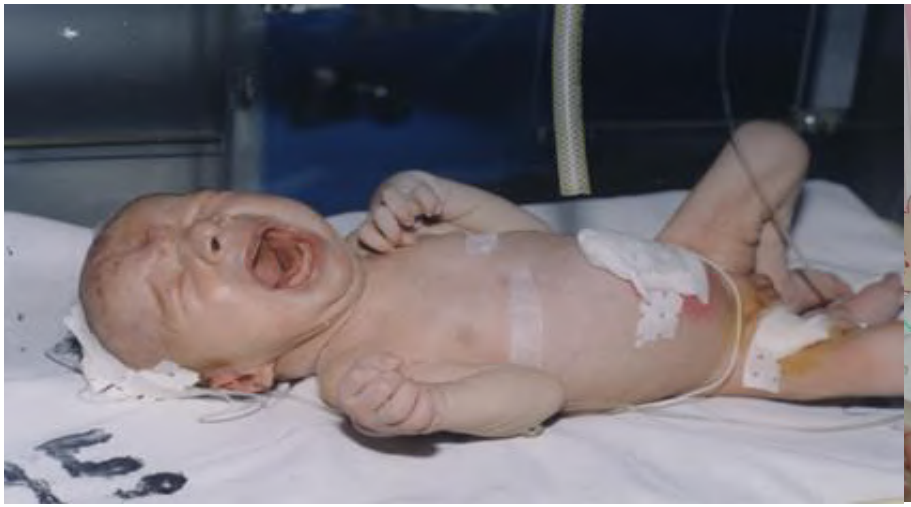
Pronostic vital est très mauvais puisque la majorité des enfants atteints décèdent avant l'âge d'un an

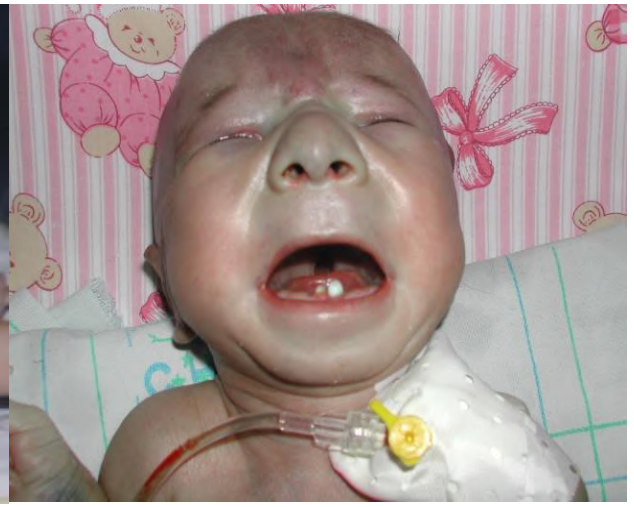


LA TRISOMIE 13

Fréquence : 1/ 4 000 à 1/ 10 000

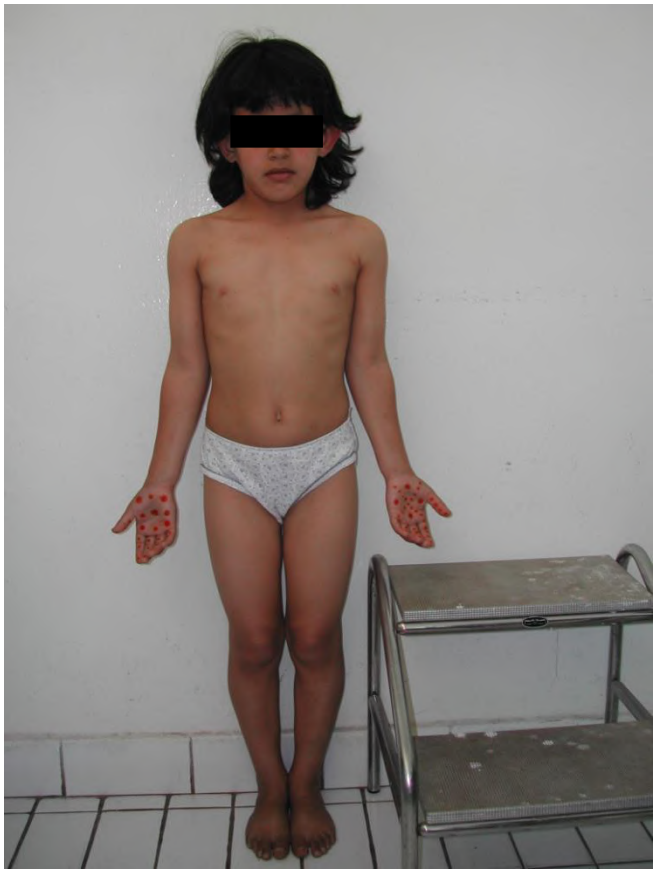
Moyenne de survie 4 mois.





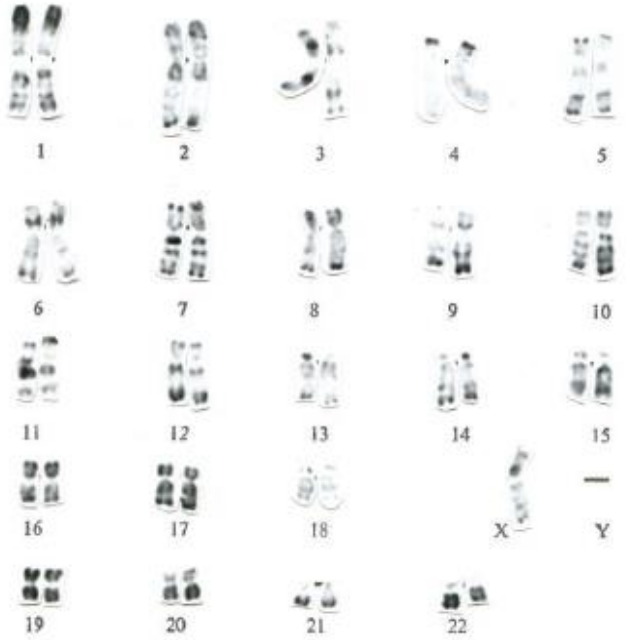
Fille avec Retard statural

Caryotype 45,X en bandes R

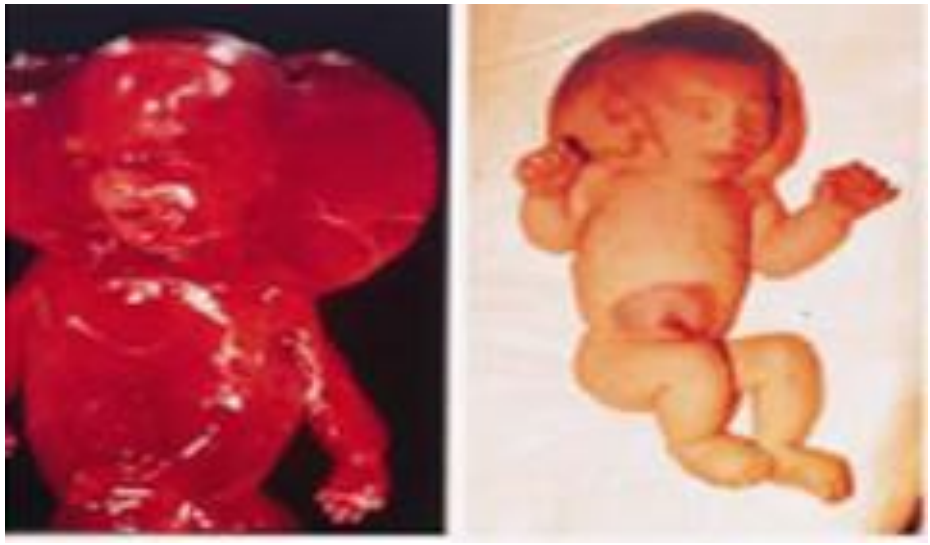


LE SYNDROME DE TURNER

1 sur 2 500 nouveau-né fille



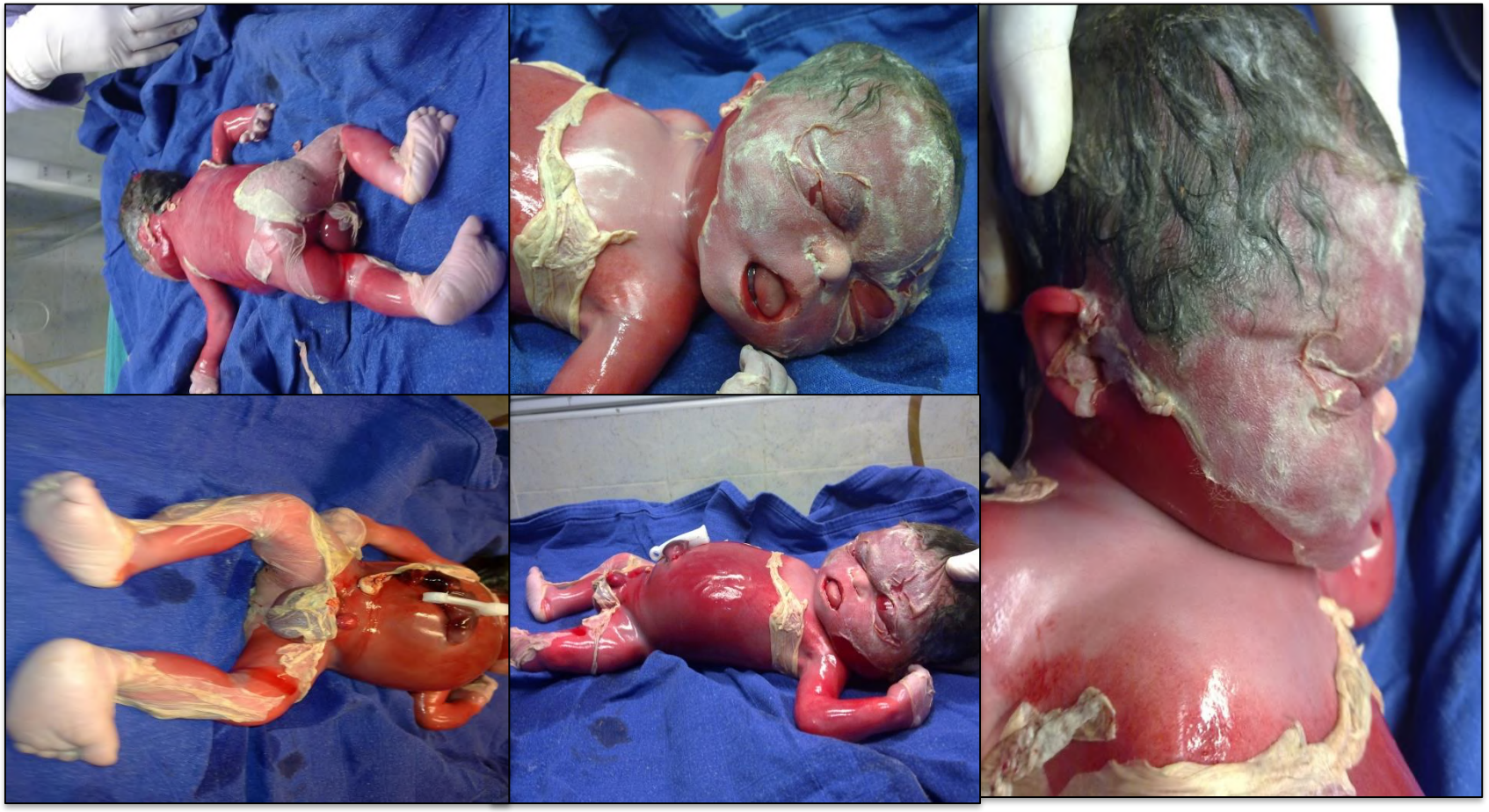
Caryotype 45,X en bandes R



Triploïdie

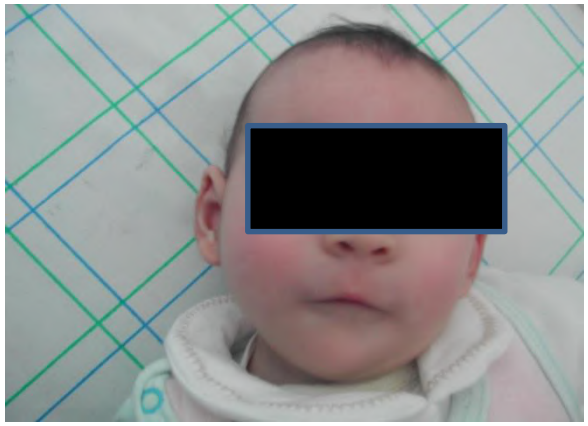
Accidents de la fécondation.

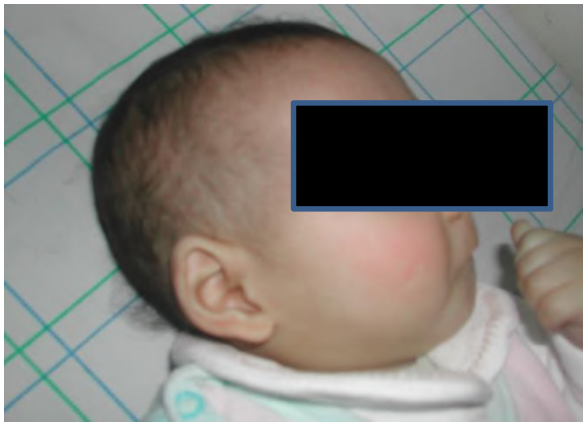
69,XYY



LA MONOSOMIE 5p-

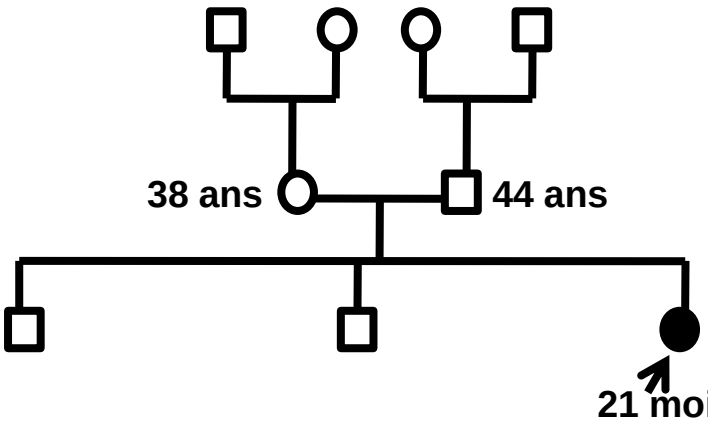
Incidence de la maladie est de 1 /20000 à 1/50000.



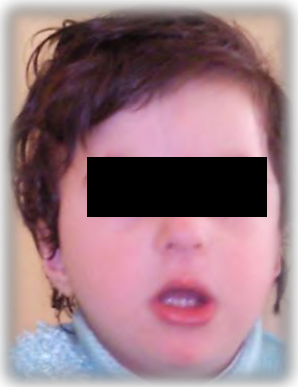


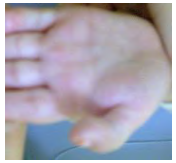
Évolution :

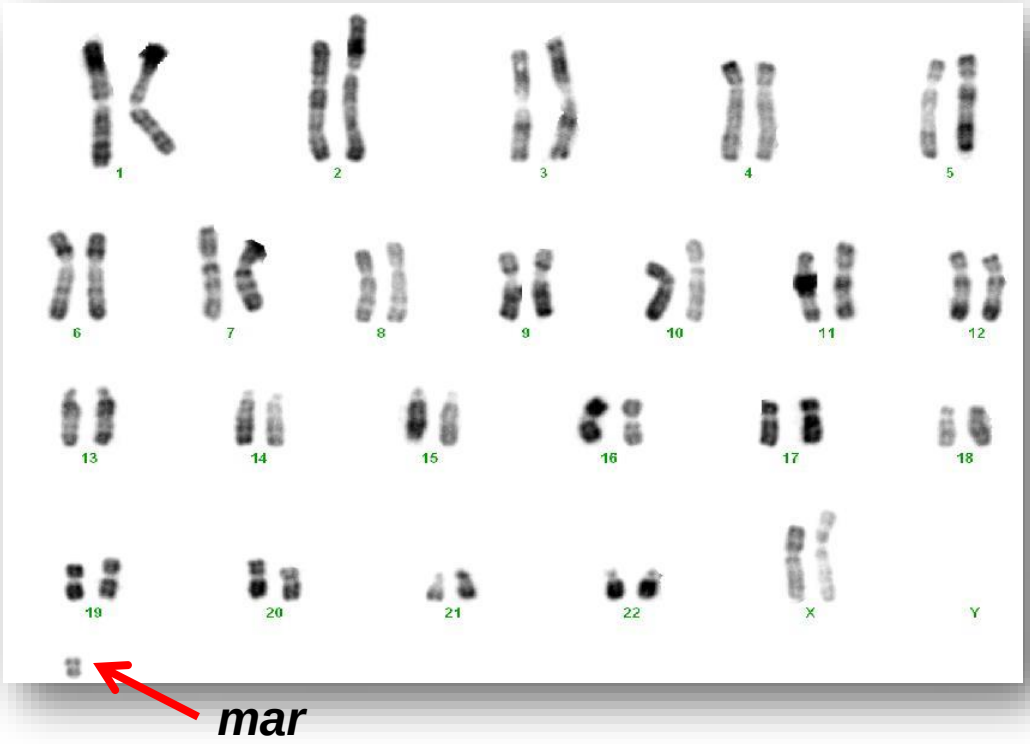
la létalité est faible, à l'âge adulte ils demeurent hypotrophiques et de taille inférieure à la normale.



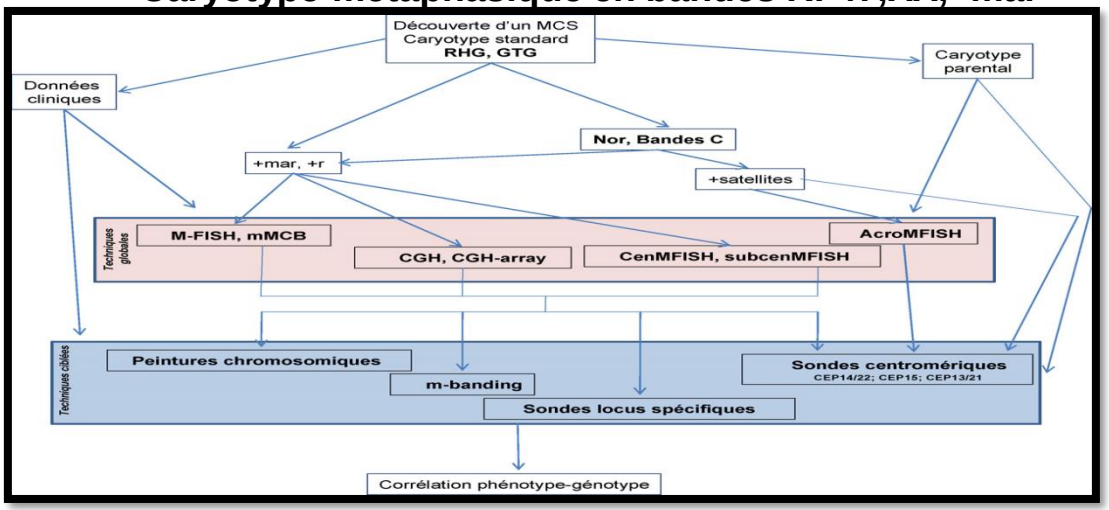
Arbre généalogique de la famille de H.A







Caryotype métaphasique en bandes R: 47,XX,+mar



LE PREMIER MARQUEUR CHROMOSOMIQUE SURNUMÉRAIRE AU CHU HASSANII DE FES

Anomalies chromosomiques de nombre

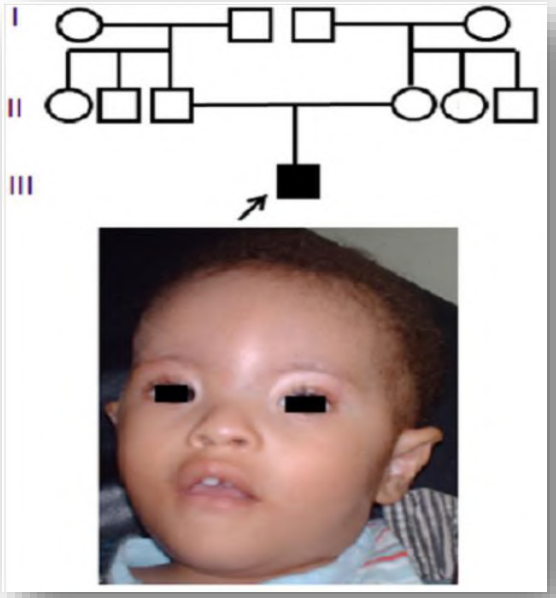
:

Trisomie 21 libre

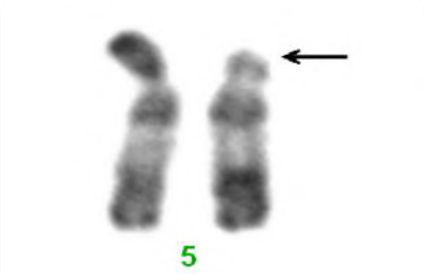
Trisomie 18 libre

Trisomie 13libre

Syndrome du
« Cri de chat »



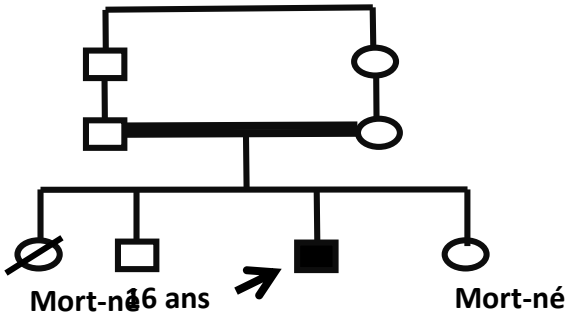
Arbre généalogique et aspect facial de notre patient présentant de Le syndrome du Cri du Chat

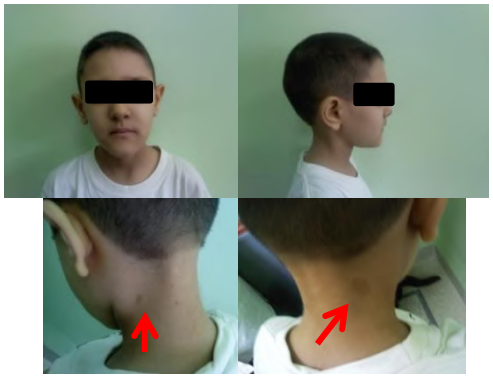


Le caryotype partiel métaphasique en bandes R de notre patient a mis en évidence la délétion 5p-:
46,XY,del(5)(p13) (La flèche indique le niveau de la délétion)

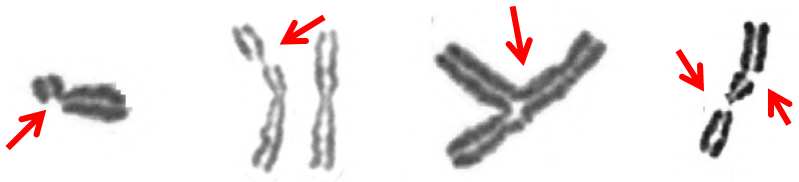
Anémie de Fanconi

Phénotype	Observation 1	
Age (ans)	11	
Dysmorphie facile	+	
Retard staturo-pondérale	Poids	Taille
	-2 DS	-2DS
Anémie	+	
Pancytopénie,	+	
Aplasie médullaire	-	
Malformations du pouce	-	
Taches café au lait cutané	+	





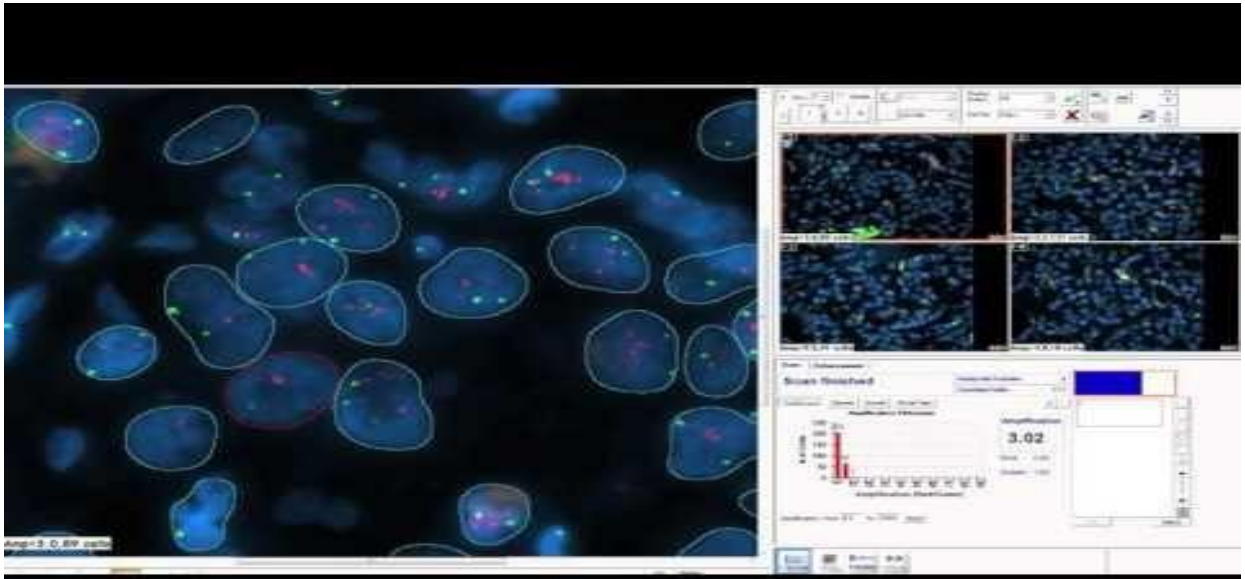
Résultats	Observation 1
Caryotype métaphasique (bandes R)	46,XY
Nombre de mitoses observées	58
Nombre de cassures	22
Nombre d'images radiales	2
Résultats	Grande instabilité chromosomique après culture sous *Mitomycine C* en comparaison avec un témoin normal



Différents aspects cytogénétiques d'une instabilité chromosomique après culture sous *Mitomycine C*

Caryotype partiel 46,X,i(X)(q10)

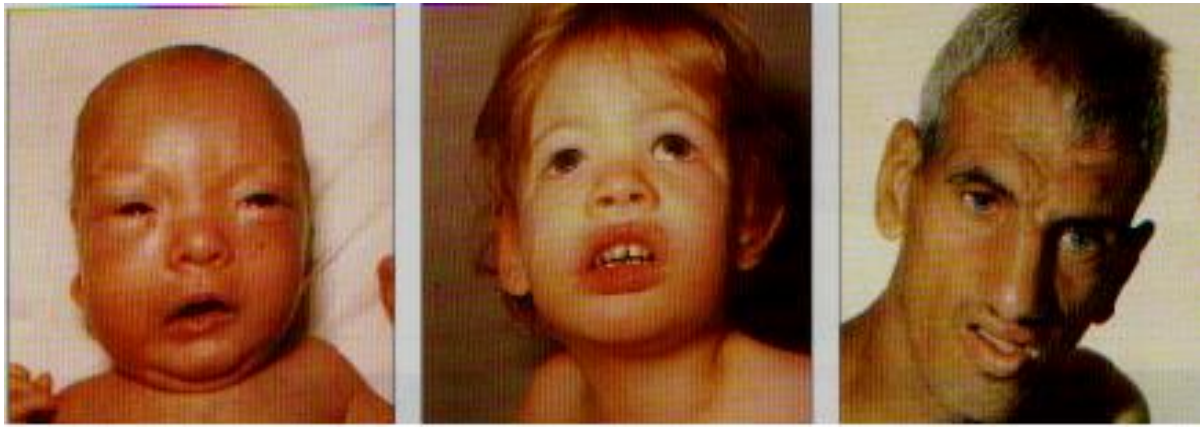
Caryotype partiel 47,XXY

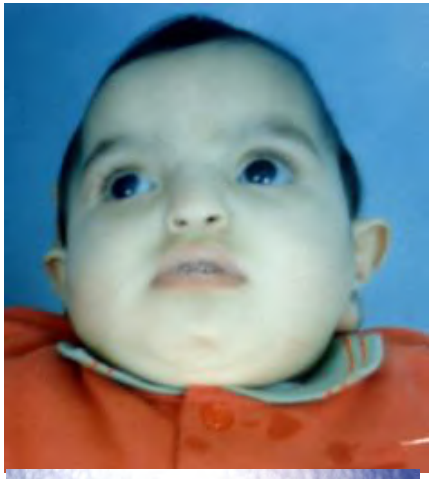

3.02

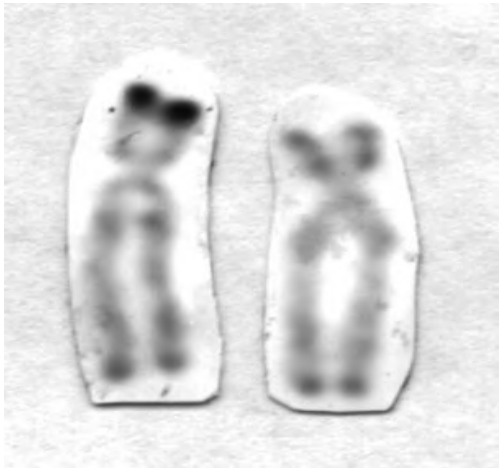
LA MONOSOMIE 4p-

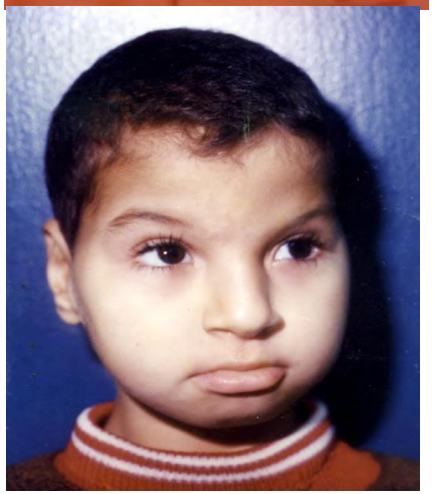
Dysmorphie + Retard mental

Durée de vie : peut aller jusqu'à 20 ans.

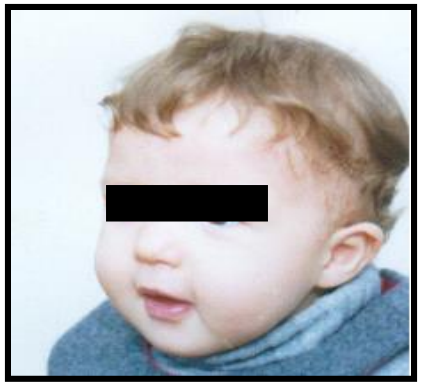


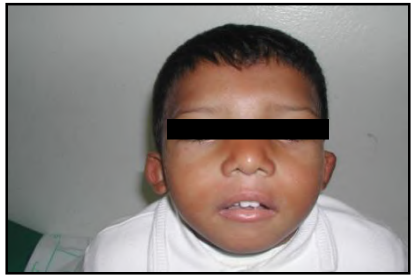


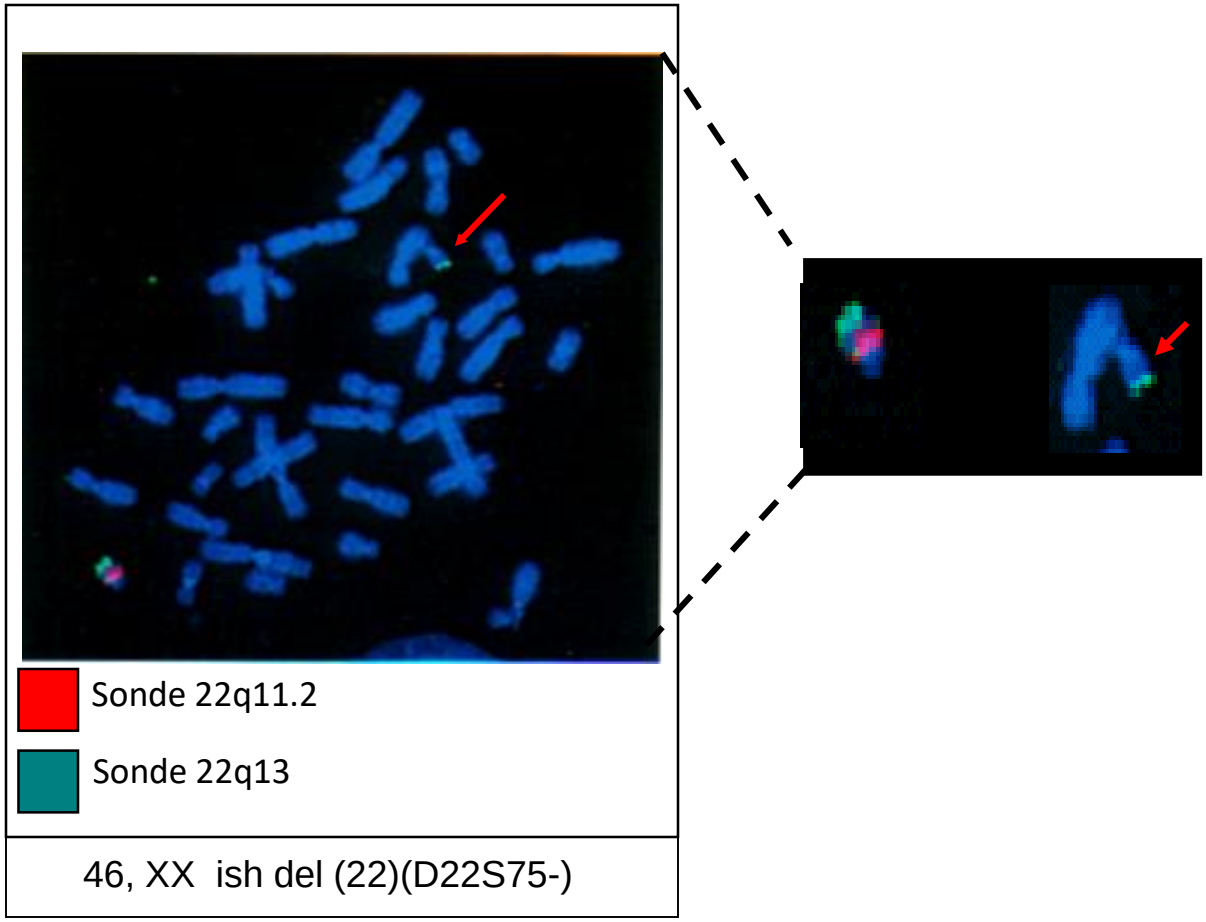




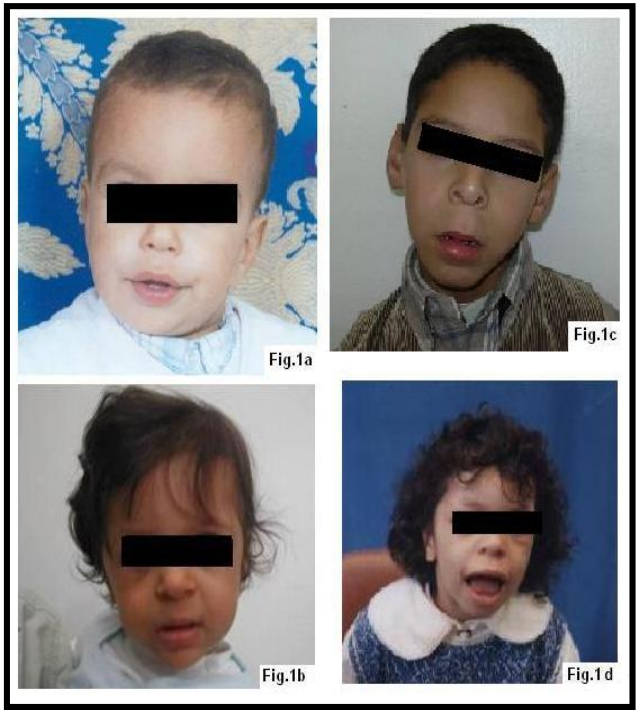
Les syndromes microdélétionnels :
Syndrome de la délétion 22q11.2

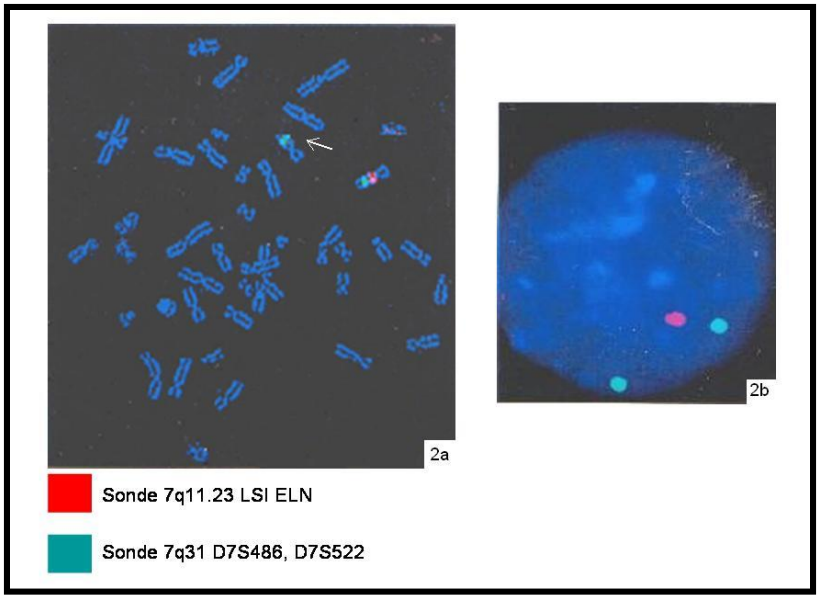






Les syndromes microdélétionnels : Syndrome de Williams et Beuren



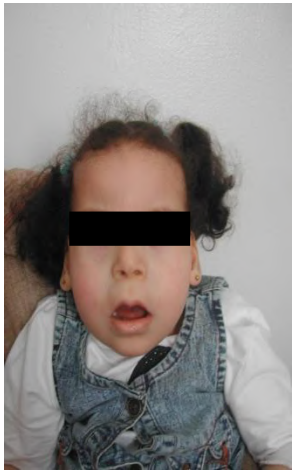


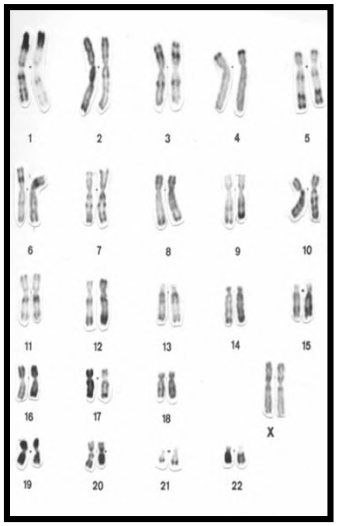
46,XY,ish del(7)(q11.23q11.23)(ELN-)

LE SYNDROME DE PALLISTER-KILLIAN
ou la Tétrasomie 12p

Première observation marocaine

« FISH sur cellules buccales »





46,XX

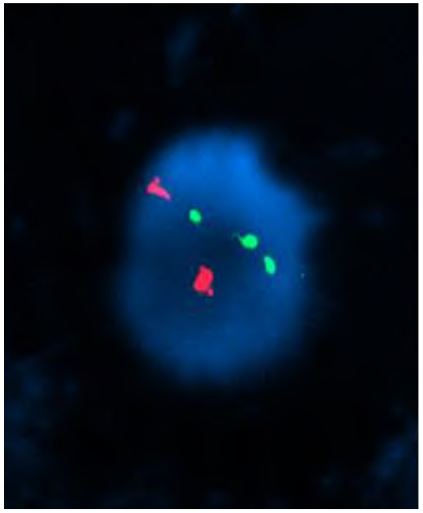


Image de la FISH réalisée avec la sonde centromérique du chromosomes 12 (couleur verte) et du chromosome 7 (couleur rouge) sur les cellules buccales.

Présence de 3 signaux verts signant la présence de 3 centromères pour le chromosome 12.

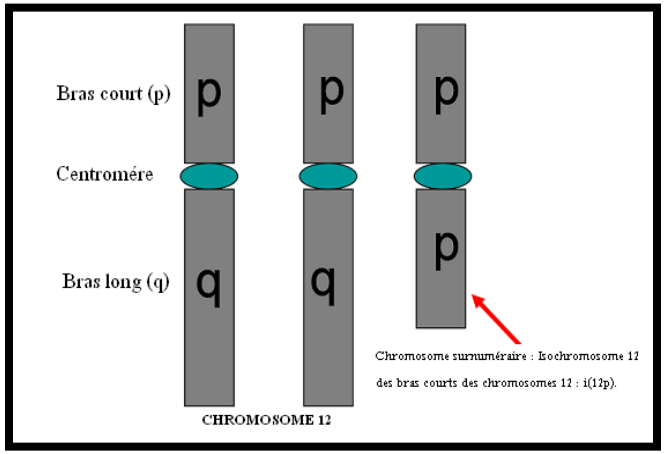
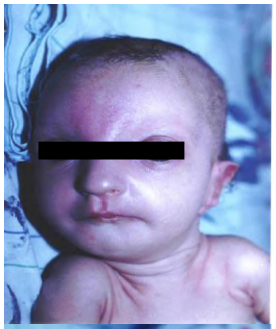
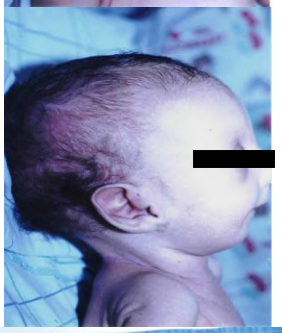
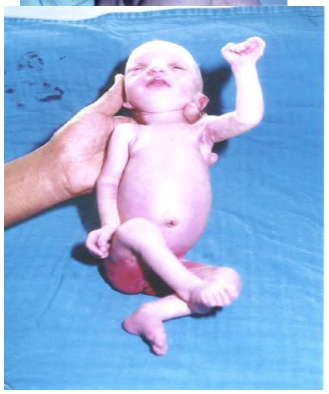


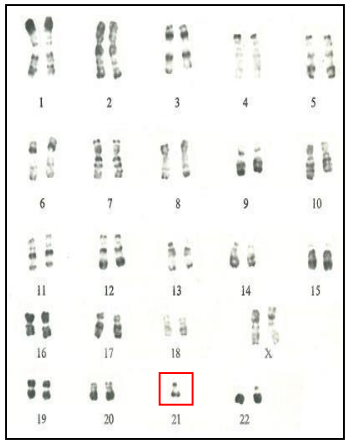
Schéma illustrant l'aspect des chromosomes 12 normaux en métaphase ainsi que l'aspect du chromosome surnuméraire : Isochromosome 12 des bras courts des chromosomes 12 : i(12p).

Étude des translocations cryptiques : Translocation (4;21) *de Novo*

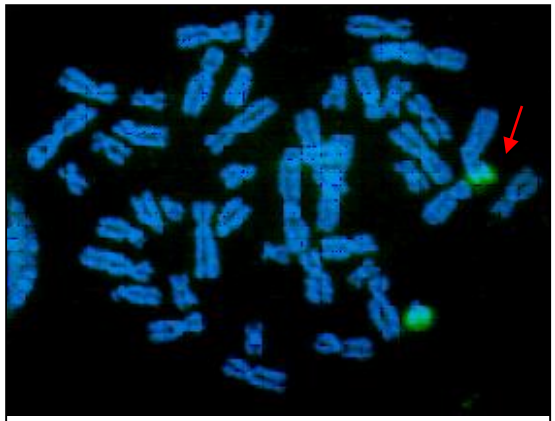




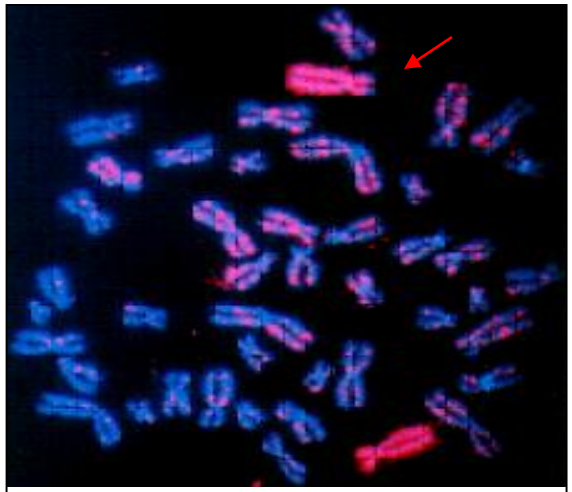




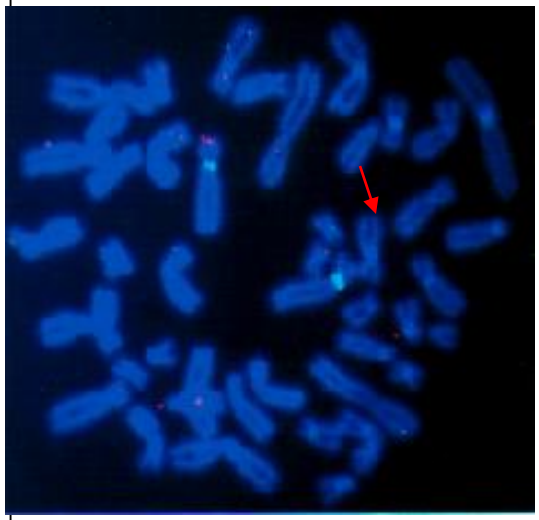
Monosomie 21



Whole chromosome painting (WCP21)



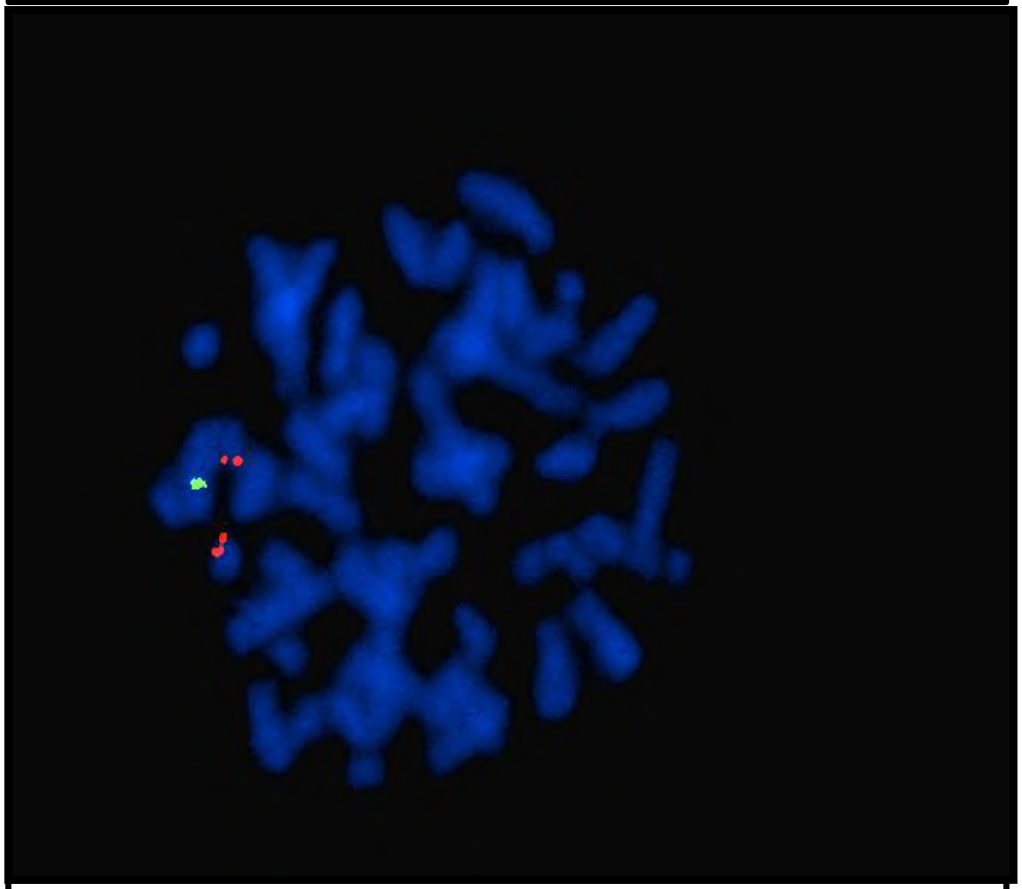
Whole chromosome painting (WCP4)



CEP 4

Translocation (4;21) *de Novo* avec une délétion de la région critique responsable du syndrome de Wolf-Hirschhorn 'WHSCR : «Wolf-Hirschhorn syndrome critical région»'

MedicalGenetics and oncogenetics

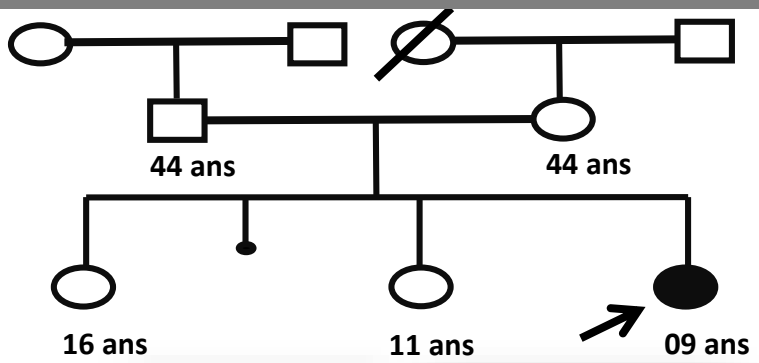


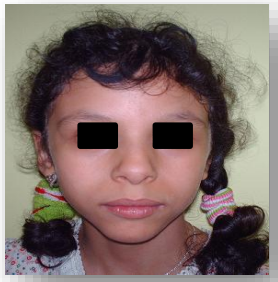
■ *Yp11.3 LSI SRY*

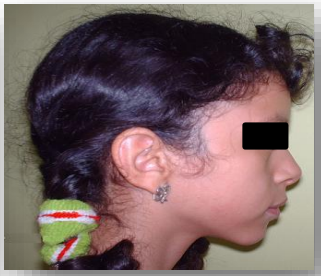
■ *CEPX*

Observation 6

Le syndrome de Williams-Beuren (SWB)

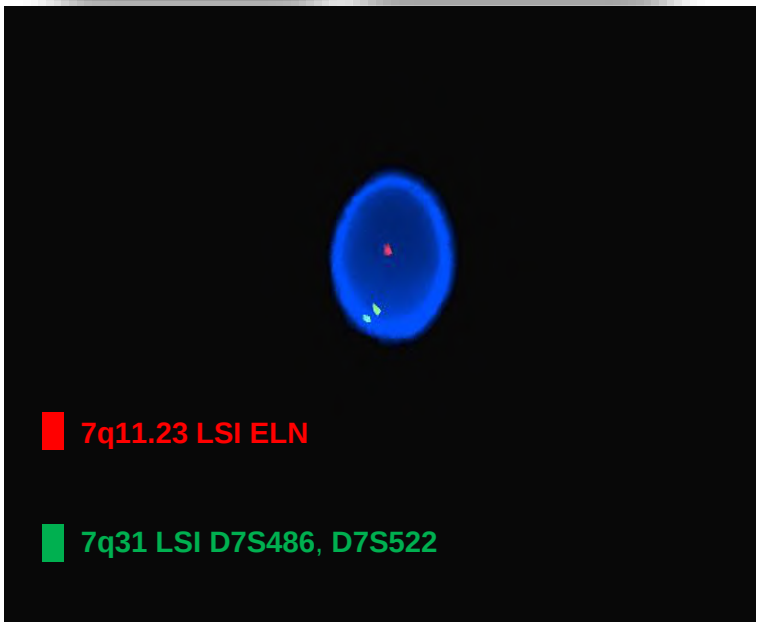


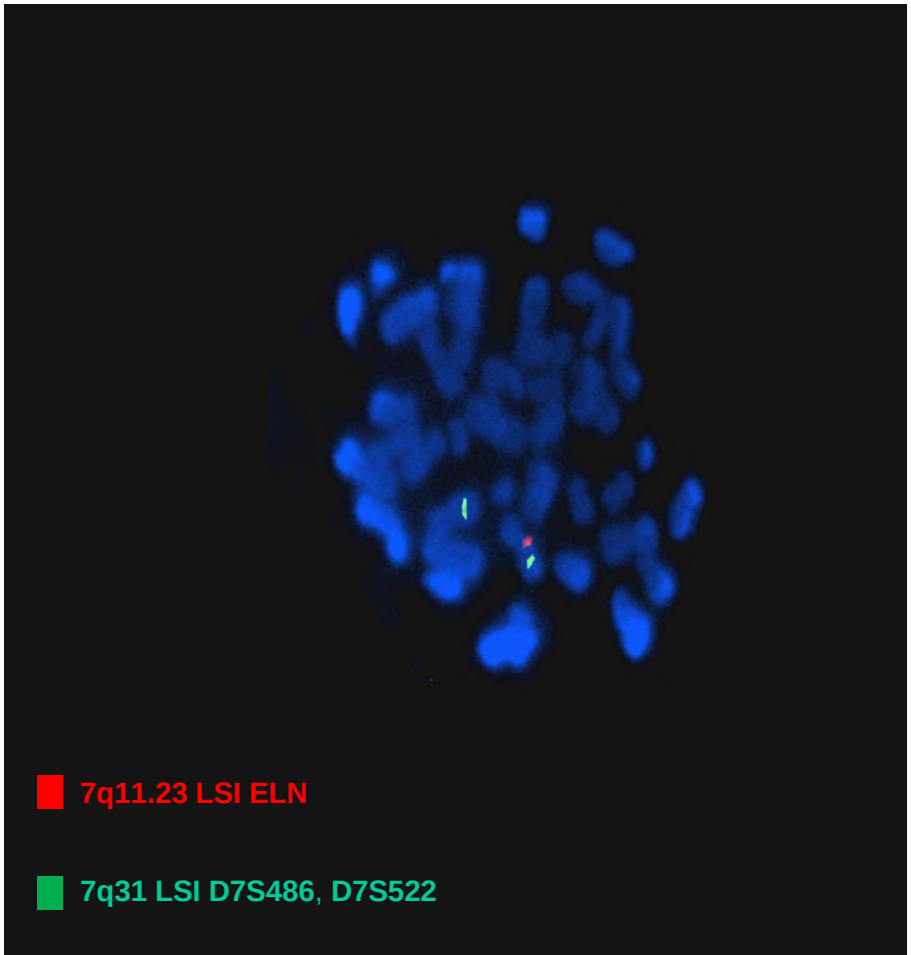




46,XX.ish del(7)(q11.23q11.23)(ELN-)[11]

nuc ish(ELN×1)(D7S522×2)[80]

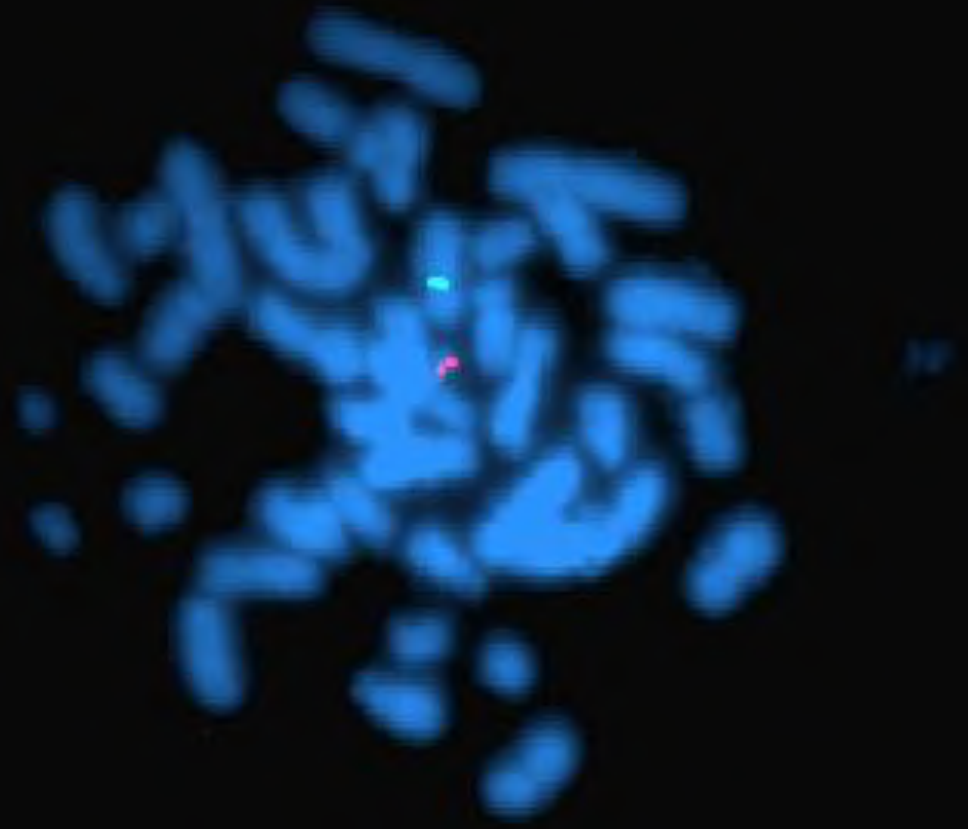




Exploration par FISH : trouble de différenciation sexuelle

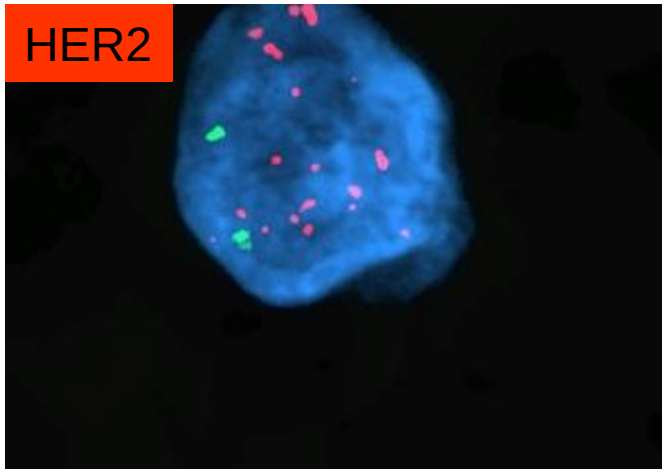
Laboratoire de Génétique Médicale,

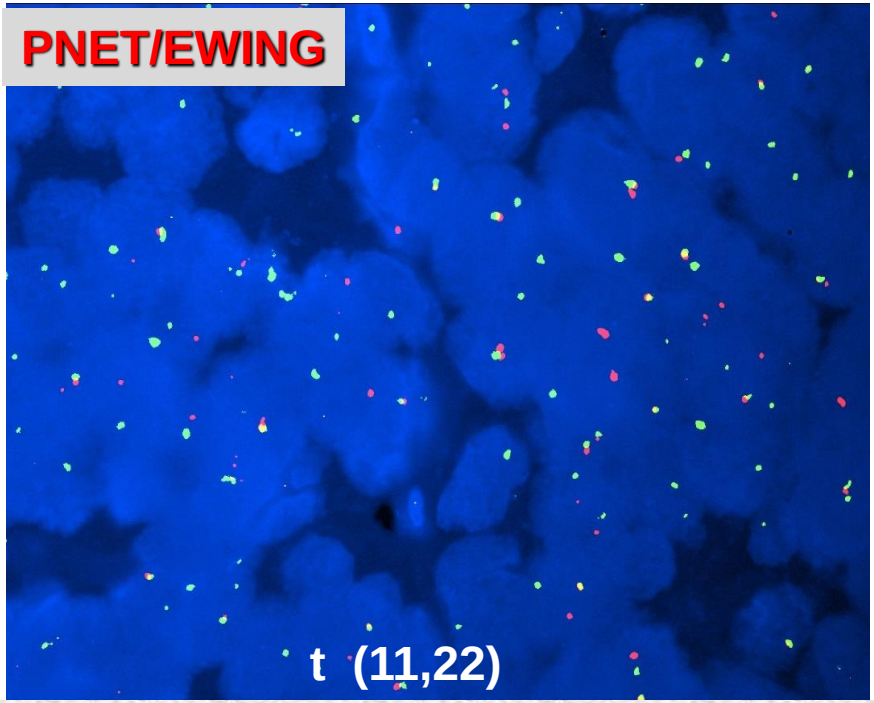
SRY/CEPX

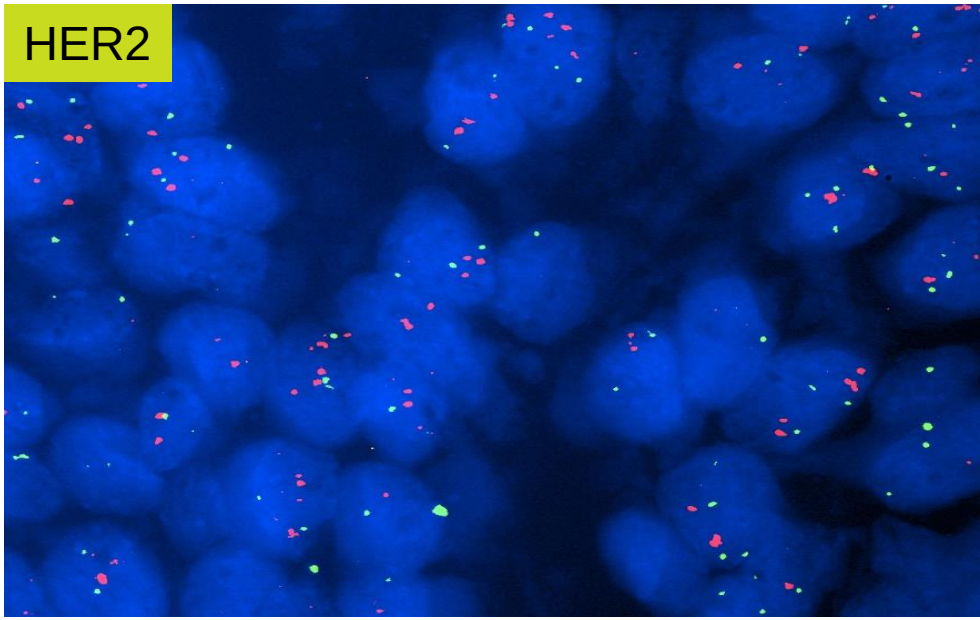


■ *Yp11.3 LSI SRY*
■ *CEPX*

FISH oncology solid tumor : Breast cancer





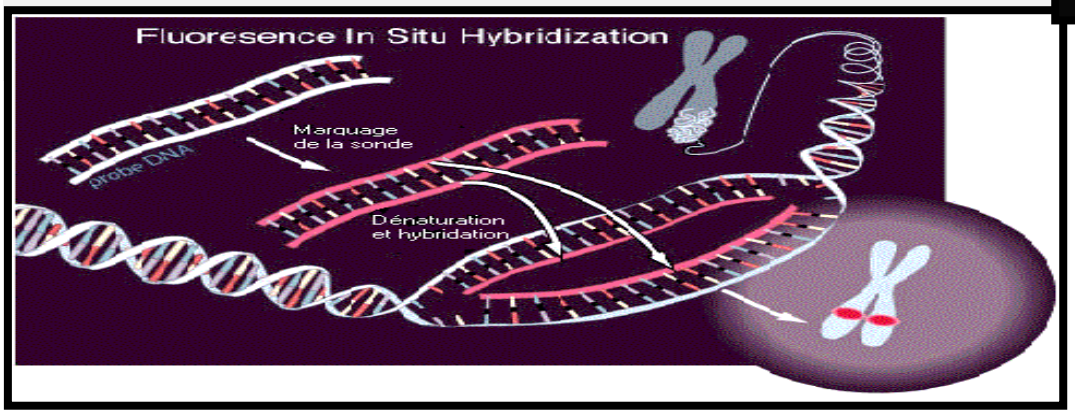


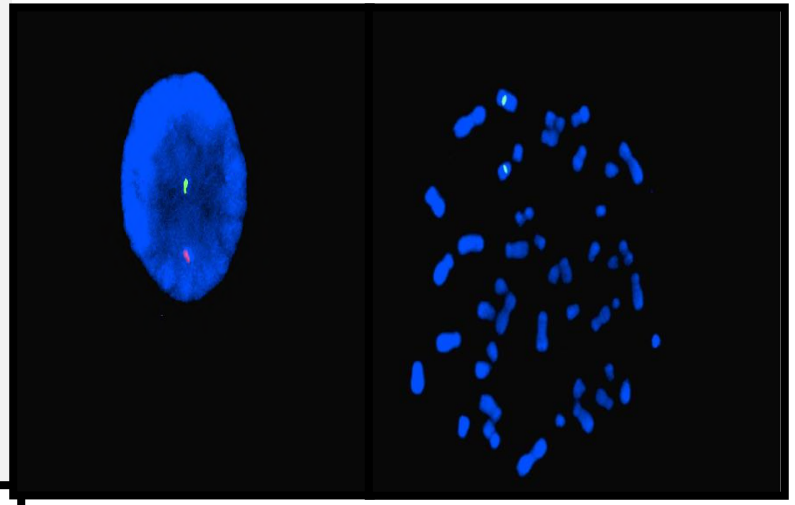
Couples with infertility

General considerations on genetic testing

Molecular cytogenetics (fluorescence in situ hybridization,FISH)

- Occasionally utilized in fertility diagnostics
- Characterization of:
  - Chromosome translocations
  - Y chromosomal abnormalities.



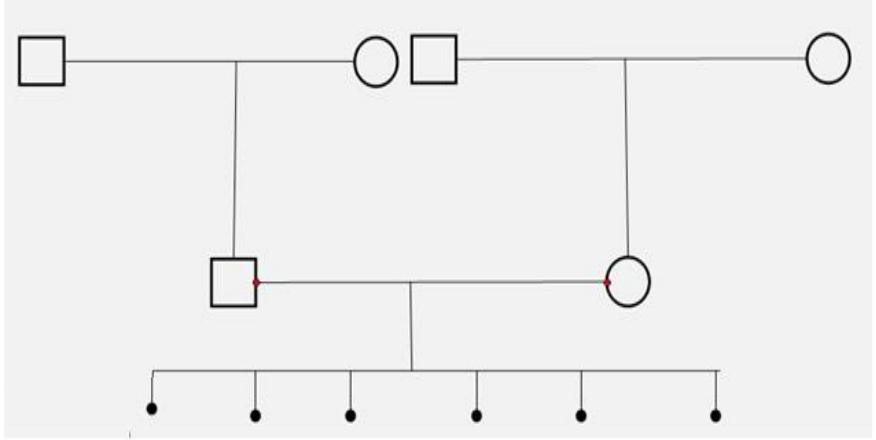


Yp11.3 LSI SRY

CEPX

Couples with infertility /recurrent miscarriages

Chromosome analysis / *FISH :* Chromosome translocations



CARYOTYPE CONSTITUTIONNEL POST NATAL – SANGUIN

Nom /Prénom

Code Patient 160801104124300

Prélèvement du 31/05/2016 Edition du 07/06/2016

Indication Maladie abortive

Médecin prescripteur

RESULTAT

Formule chromosomique	46,XY,t(3;18)(q28;q22)
Nombre de Mitoses examinées	50
Nombre de Mitoses classées	15
Type et nombres de bandes	RHG, 400

Hybridation in situ / FISH

Sondes utilisées WCP3 ; WCP18 ; Tel3q

COMMENTAIRE :

Présence sur toutes les mitoses observées d'une translocation équilibrée entre le bras long d'un chromosome 3 et le bras long d'un chromosome 18.

Compte-rendu après le complément d'analyse par FISH

La FISH confirme la translocation t(3;18)

ish t(3;18)(wcp3+,wcp18+,wcp18+,wcp3-,tel3q+)

Aucune autre paire chromosomique n'est impliquée dans ce remaniement.

Dr. Ouldim Karim

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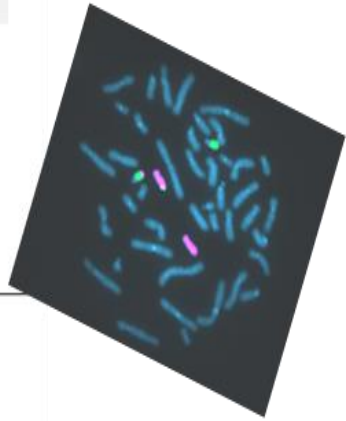
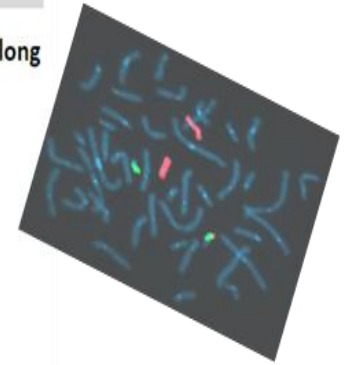
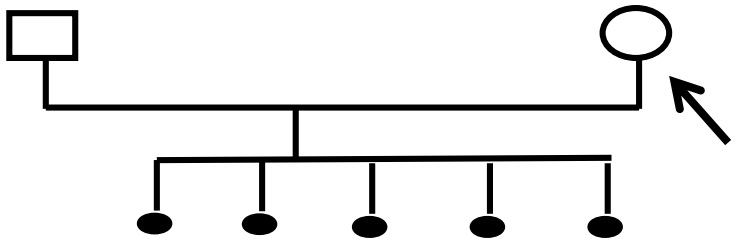




Couples with recurrent abortions

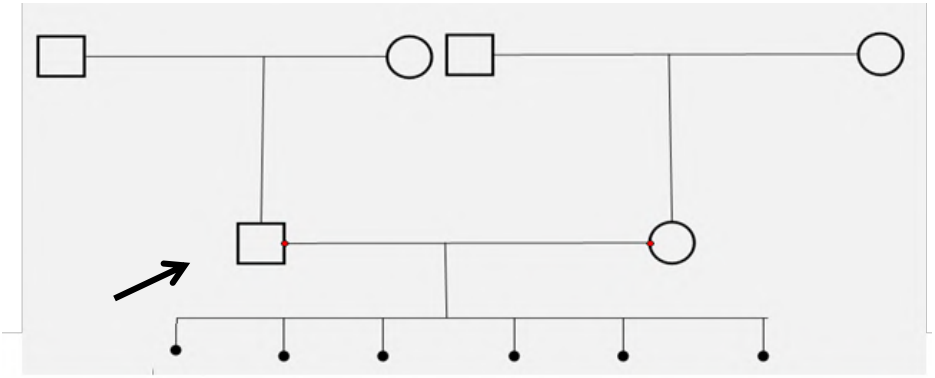
3% to carry a balanced chromosomal aberrations in one parent

2016



Caryotype postnatal constitutionnel	46,XX,t(1;7)(p35;q11)	46,XY

En cours DPI



Formule chromosomique	46,XY,t(3;18)(q28;q22)
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Sondes utilisées WCP3 ; WCP18 ; Tel3q

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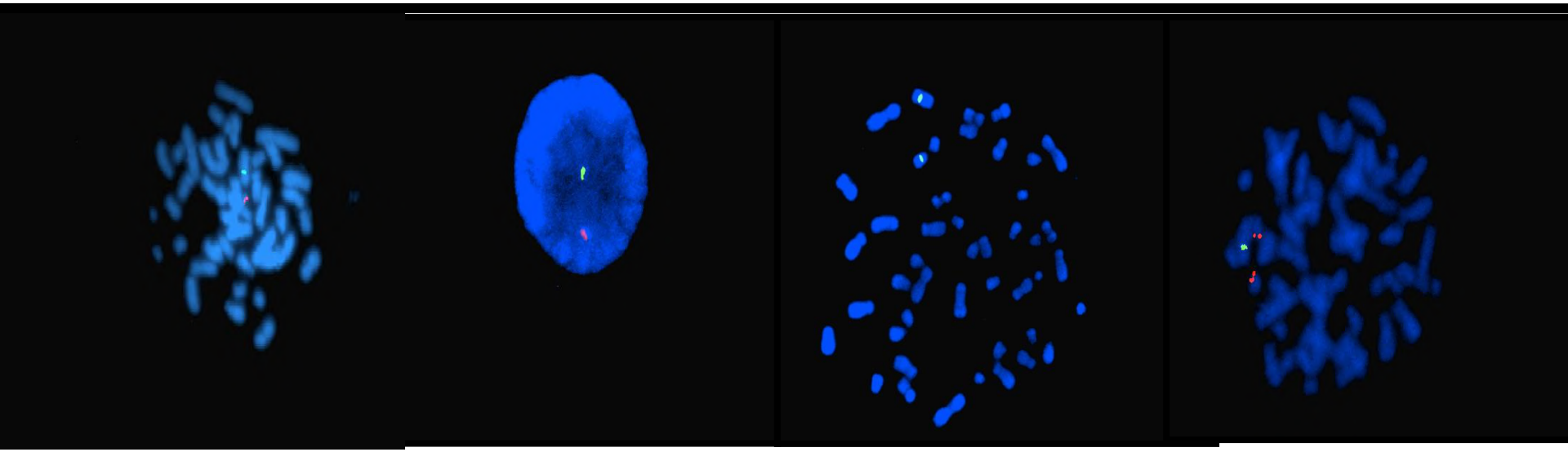
La FISH confirme la translocation t(3;18)

ish t(3;18)(wcp3+,wcp18+,wcp18+,wcp3-,tel3q+,

Aucune autre paire chromosomique n'est impliquée dans ce remaniement.

Cytogénétique classique et moléculaire		Diagnostic
Caryotype métaphasique en bandes R	FISH *SRY/CEP X*	
mos 45,X[37]/46,XX[4]/46,XY[2] nuc ish(SRY×1),(CEPX×1)[10]/(CEPX×2)[20]/(CEPX×1)[100]		Turner Syndrome with chromosome Y
47,XY,mar nuc ish(SRY×2),(CEPX×1)[100]		XYY



Yp11.3 LSI SRY

CEPX

Couples with infertility /recurrent miscarriages

Femme XY

FISH	Hybridation in situ en fluorescence	Post-natal

Nom /Prénom

Code Patient 180803104558MA

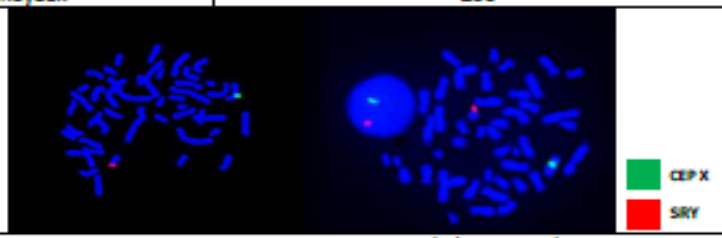
Analyse faite à partir : culot cellulaire 03/08/2016 Edition du 09/08/2016

Indication *Aménorrhée primaire*

Médecin prescripteur

RESULTAT

Caryotype métaphasique (RHG)	46,XY
Sonde utilisée	SRY/CEP X FISH Probe Kit.CE marked ; Vysis
Nombre de Mitoses	10
Nombre de noyaux	200



Formule

Cytogénétique moléculaire

46,XY.ish(SRY+,CEP X+)[10]

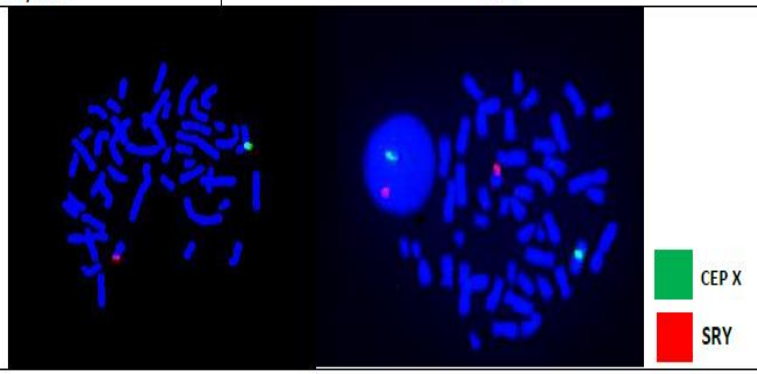
nuc ish(SRY,CEPX)x1 [200]

COMMENTAIRE ET CONCLUSION

Présence du SRY sur toutes les mitoses et noyaux observés.

Dr. K. Ouldim

Caryotype métaphasique (RHG)	46,XY
Sonde utilisée	SRY/CEP X FISH Probe Kit.CE marked ; Vysis
Nombre de Mitoses	10
Nombre de noyaux	200



Formule

Cytogénétique moléculaire

46,XY.ish(SRY+,CEP X+)[10]

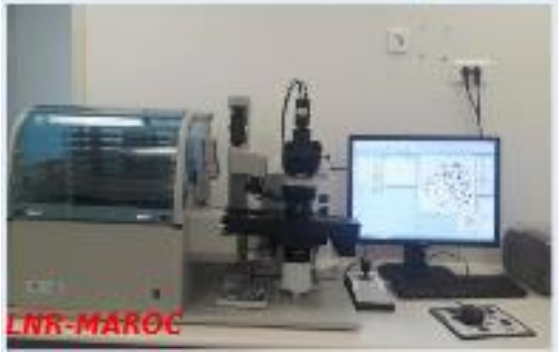
nuc ish(SRY,CEPX)x1 [200]

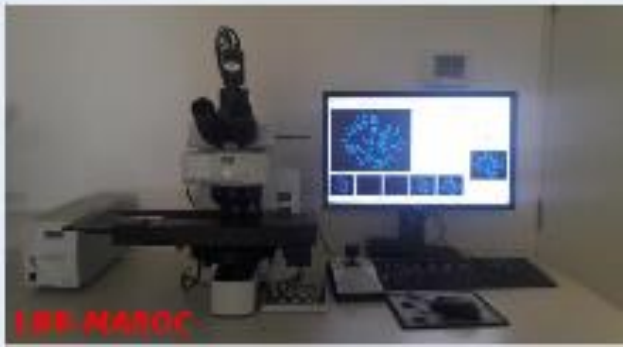
Délai des résultats caryotype ET FISH = 5 - 10 jours

Préparateur des chromosomes en métaphase de façon automatique des échantillons de culture cellulaire

Choc, fixation, étalement, recherche automatique des métaphases, capture automatisée des images de FISH

Culture cellulaire





LABORATOIRE NATIONAL DE REFERENCE

Laboratoire National de Référence, LNR
Génétique Médicale / Cytogénétique

FISH	Hybridation in situ en fluorescence	Post-natal

Nom /Prénom

Code Patient 1808031046568MA

Analyse faite à partir : culot cellulaire 03/08/2016 — Edition du 09/08/2016

Indication *Aménorrhée primaire*

Médecin prescripteur

RESULTAT

Caryotype métaphasique (RHG)	46,XY
Sonde utilisée	SRY/CEP X FISH Probe Kit.CE marked ; Vysis
Nombre de Mitoses	10
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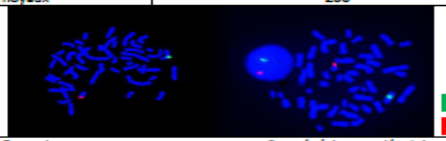


Formule — Cytogénétique moléculaire

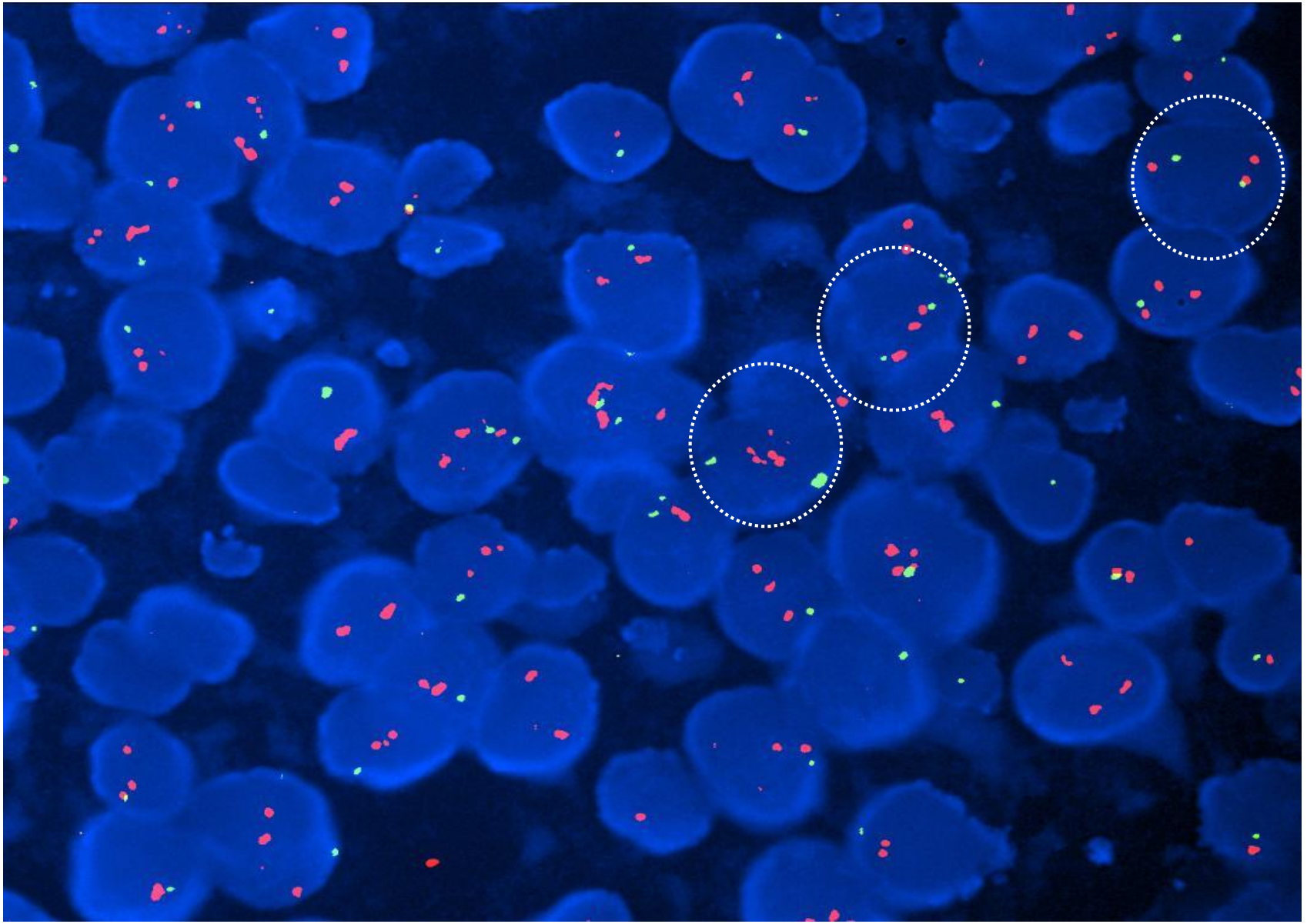
46,XY.ish(SRY+,CEP X+)[10]
nuc ish(SRY,CEPX)x1 [200]

COMMENTAIRE ET CONCLUSION

Présence du SRY sur toutes les mitoses et noyaux observés.

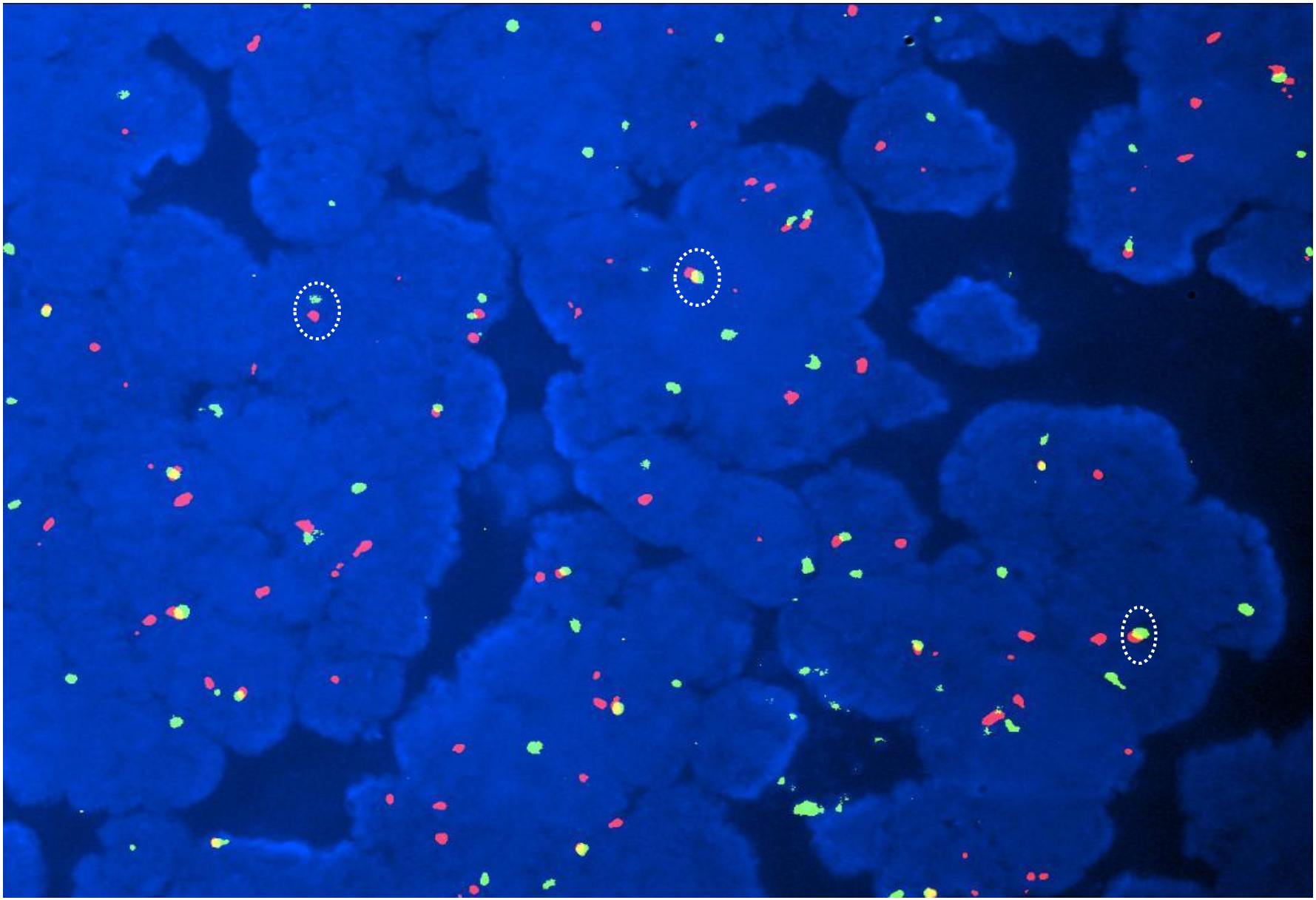
Dr. K. Ouldim

Cas d'une amplification du gène Her-2 observé par FISH sur microscope à fluorescence



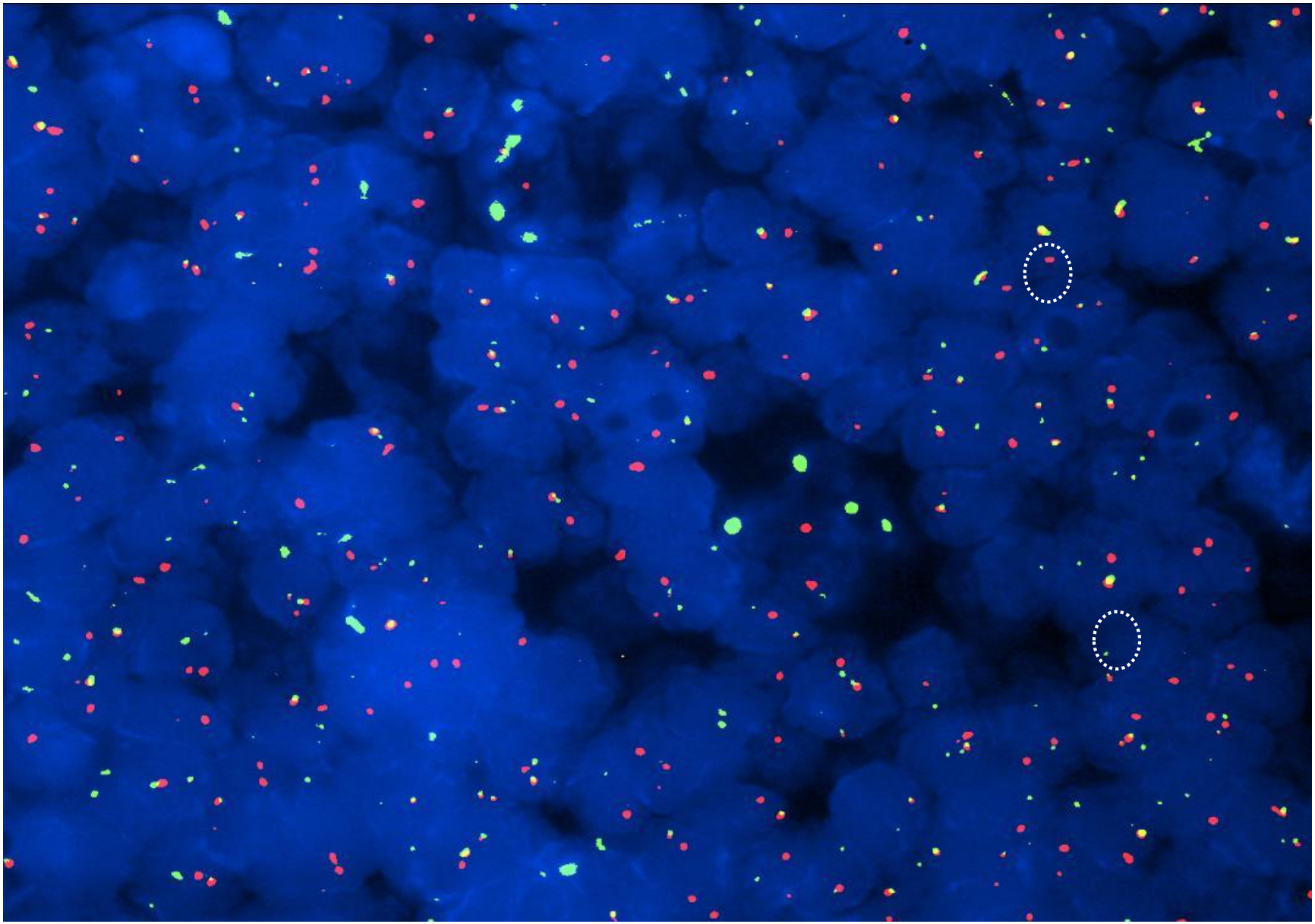
Sarcome d'Ewing

Cas d'une translocation t(22q12) au niveau de la région du gène EWSR1



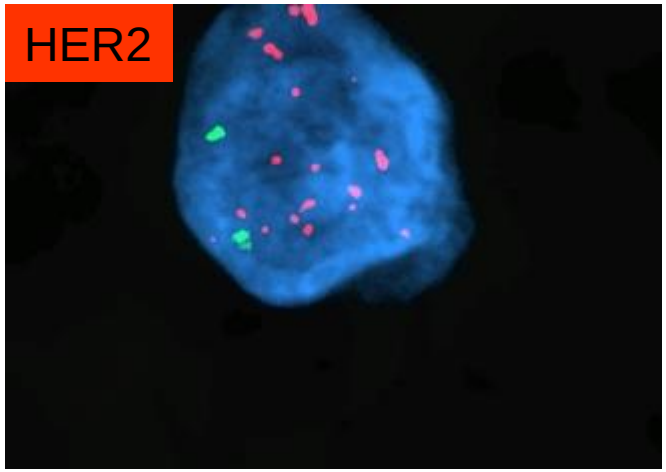
Sonde LSI EWSR1, Break Apart Rearrangement

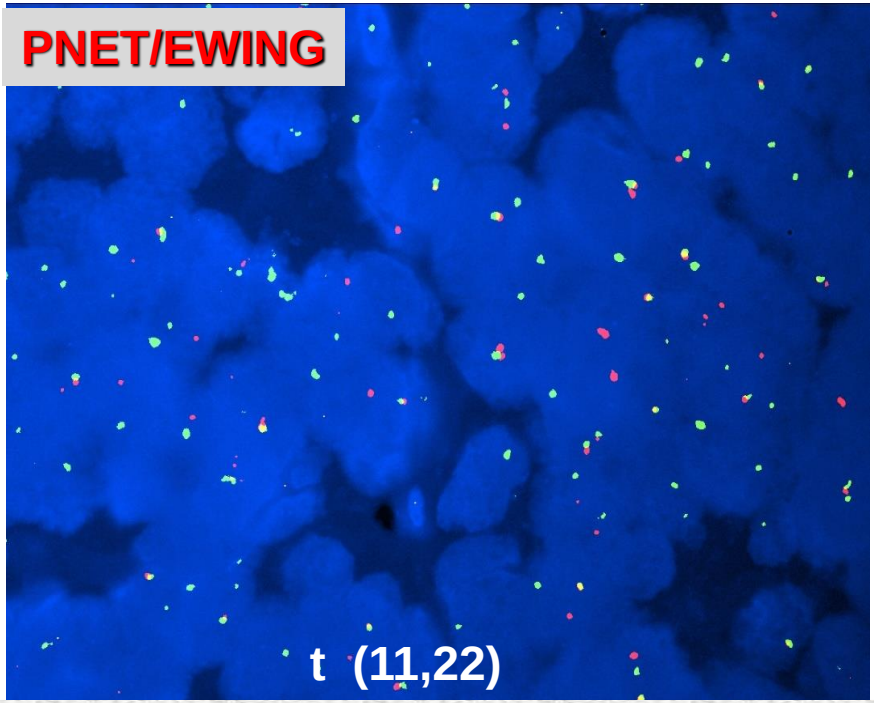
Translocation t(11;14)(q32;q13) causant un lymphome du manteau

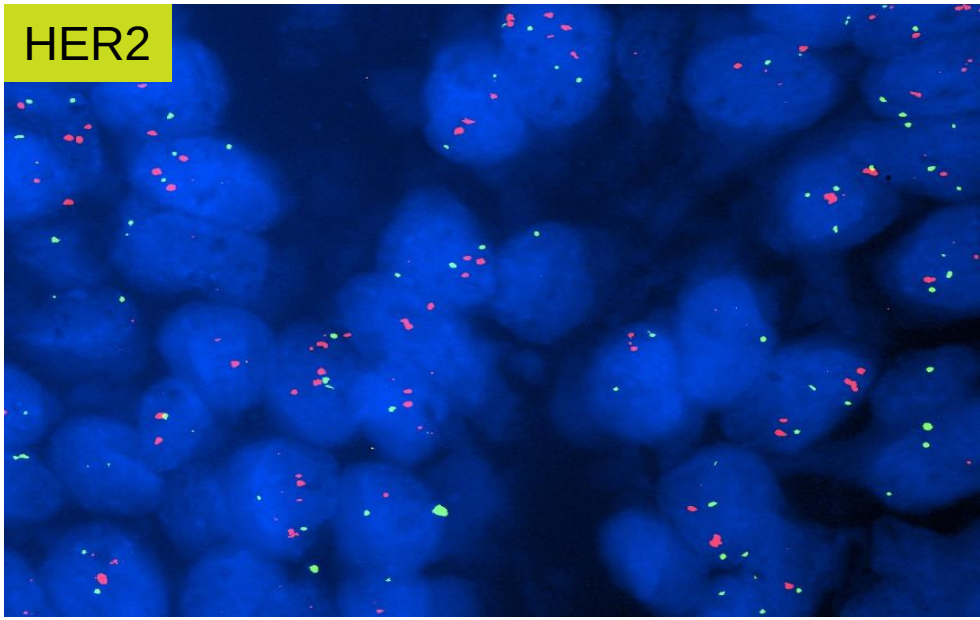


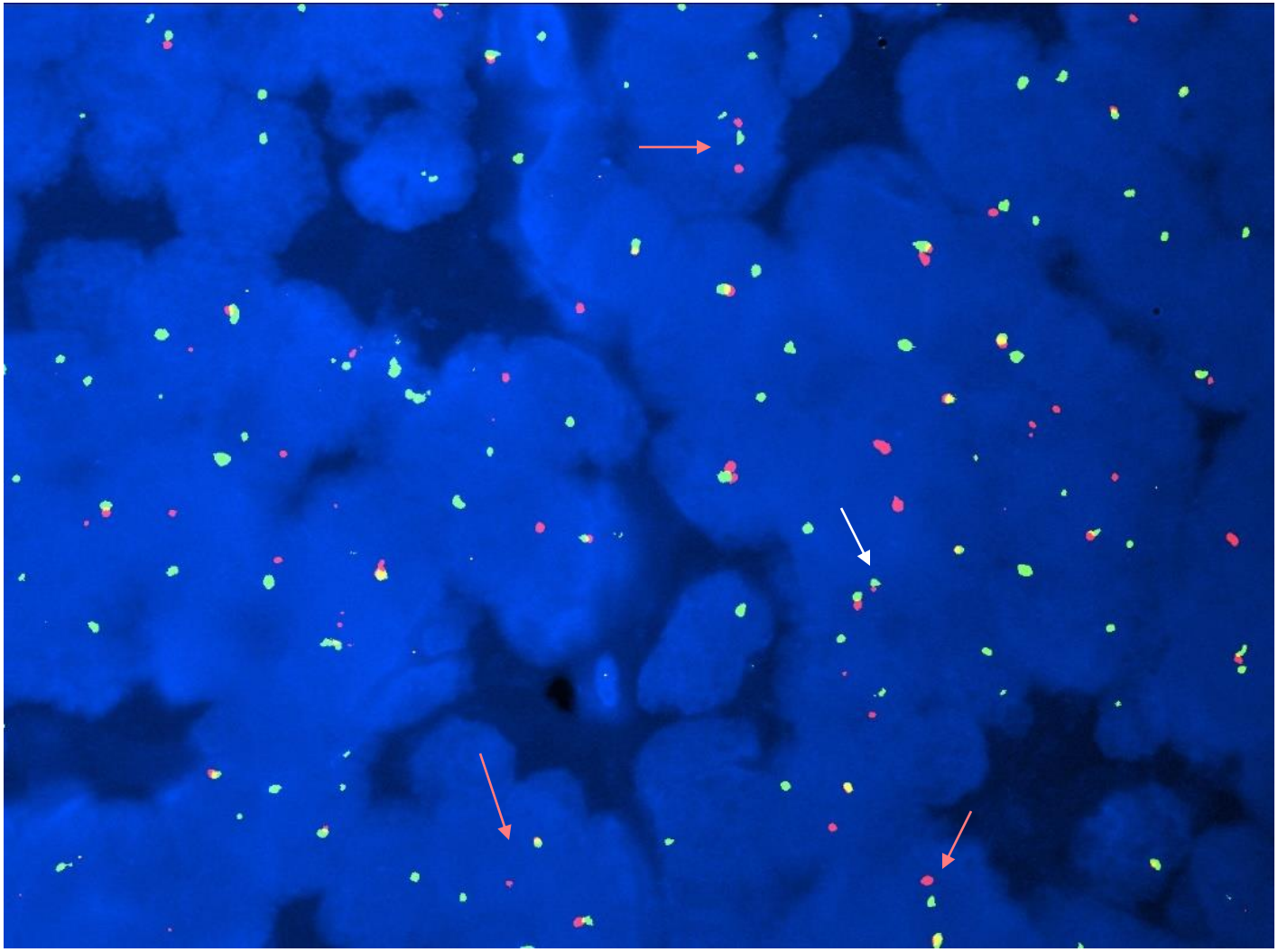
Sondes LSI 1GH et CCND1/MYEOV, Dual fusion

FISH oncology solid tumor : Breast cancer









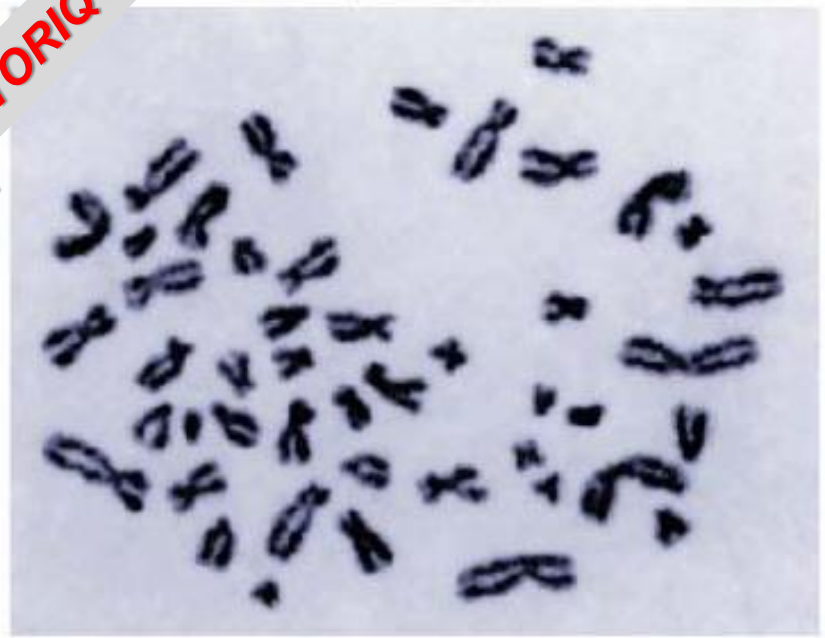
SONDE BREAK APART EWSR 1 AU MOINS 30% des cellules

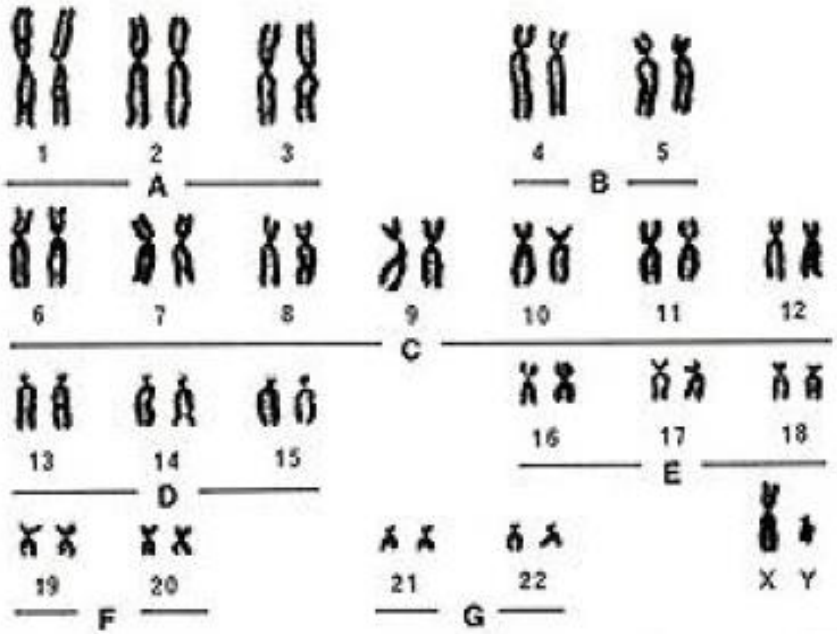
1956 Caryotype humain 46,XY (Choc hypotonique)

Tjio

Levan

HISTORIQUE





1959 Trisomie 21 Lejeune

Chromosomes et cancers

HISTORIQUE

3 évènements majeurs

- **Chromosome Ph1 et LMC**
  1960 : NOWELL et HUNGERFORD

- **Techniques de bandes**
  1970 : CASPERSSON QFQ
  1972 : Janet ROWLEY t(9;22)
  1980 : SANDBERG
  répertoire des anomalies

- **Découverte des oncogènes** (C-src)
  1976 : D. STEHELIN

  puis **gènes suppresseurs de tumeur**

hémopathies malignes

Anomalies chromosomiques en onco-hématologie

- **Acquises**

- **Clonales** toutes les cellules possèdent la même anomalie primaire.

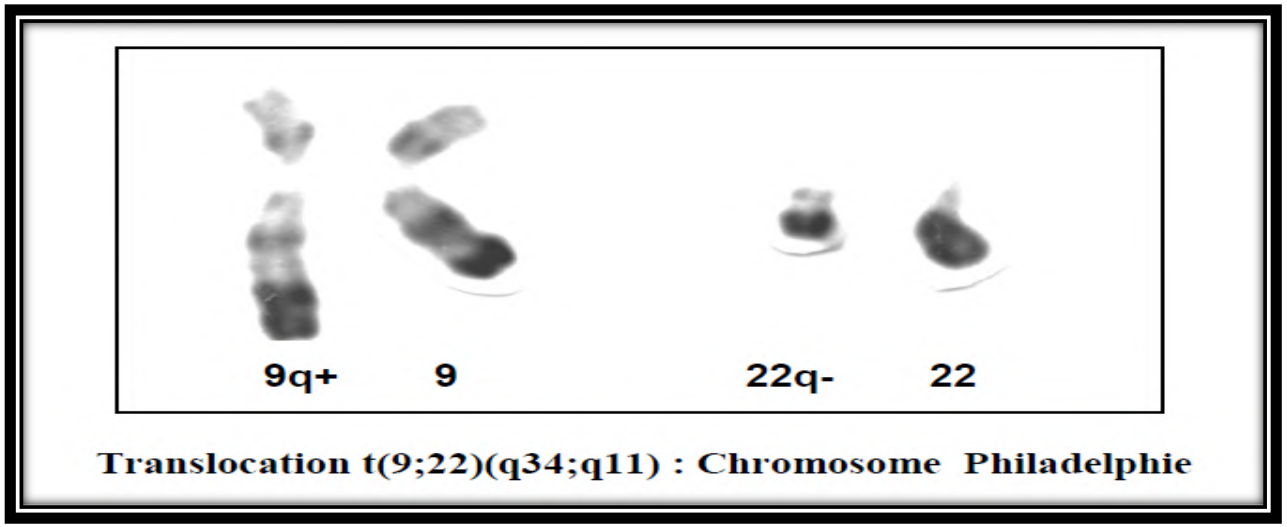
- **Limitées aux cellules malignes**

- **Non aléatoires** Retrouvées plus souvent que ne le voudrait le hasard, plus ou moins spécifiques d'un type de leucémie

- **Primaires ou secondaires**

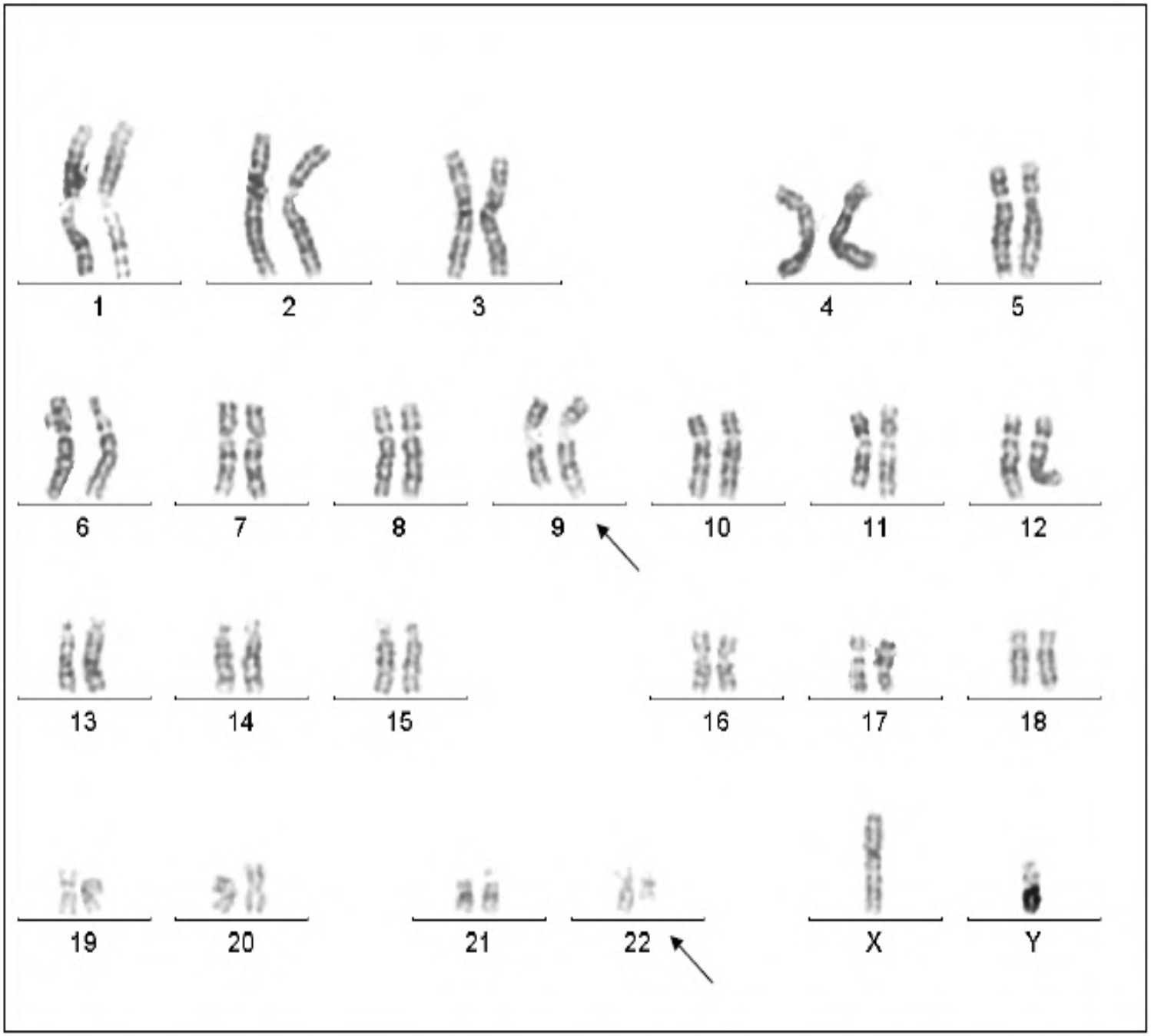
La leucémie myéloïde chronique (LMC)

- La LMC a bénéficié très tôt d'un marqueur biologique : le chromosome de Philadelphie ,(Ph1).
- Il fut dès lors reconnu comme marqueur spécifique (non pathognomonique) dans la LMC.
- Il s'agit d'une translocation réciproque entre les chromosomes 9 et 22, cassés respectivement en 9q34 et 22q11.
- Il apparaît parfois sous une forme variante (moins de 10% des cas) se traduisant par des translocations complexes impliquant un ou plusieurs chromosomes en plus du chromosome 9 et du chromosome 22



Translocation t(9;22)(q34;q11) : Chromosome Philadelphie

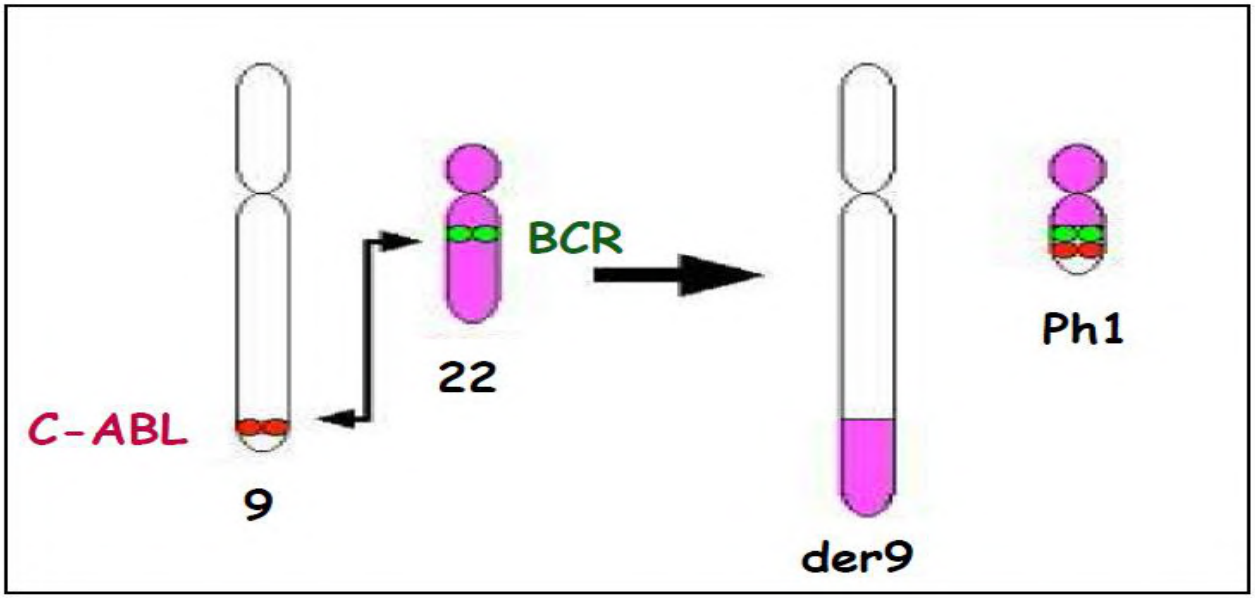
Association quasi constante à la t(9;22)



La leucémie myéloïde chronique (LMC)

L'équivalent moléculaire du chromosome Ph1 est le **gène de fusion BCR-ABL**,

transcrit en un ARNm hybride et traduit en une **protéine de 210 KDa** à forte activité **tyrosine kinase,** jouant un rôle dans la **leucémogénèse**



La leucémie myéloïde chronique (LMC)

Leucémie Myéloïde Chronique (LMC) et Caryotype

t(9 ;22) (q34 ;q11) ou les variants (4-8 %)

(Batty N ,Blood 112:1108,2008)

Anomalies clonales additionnelles (ACA)(5-10 %)

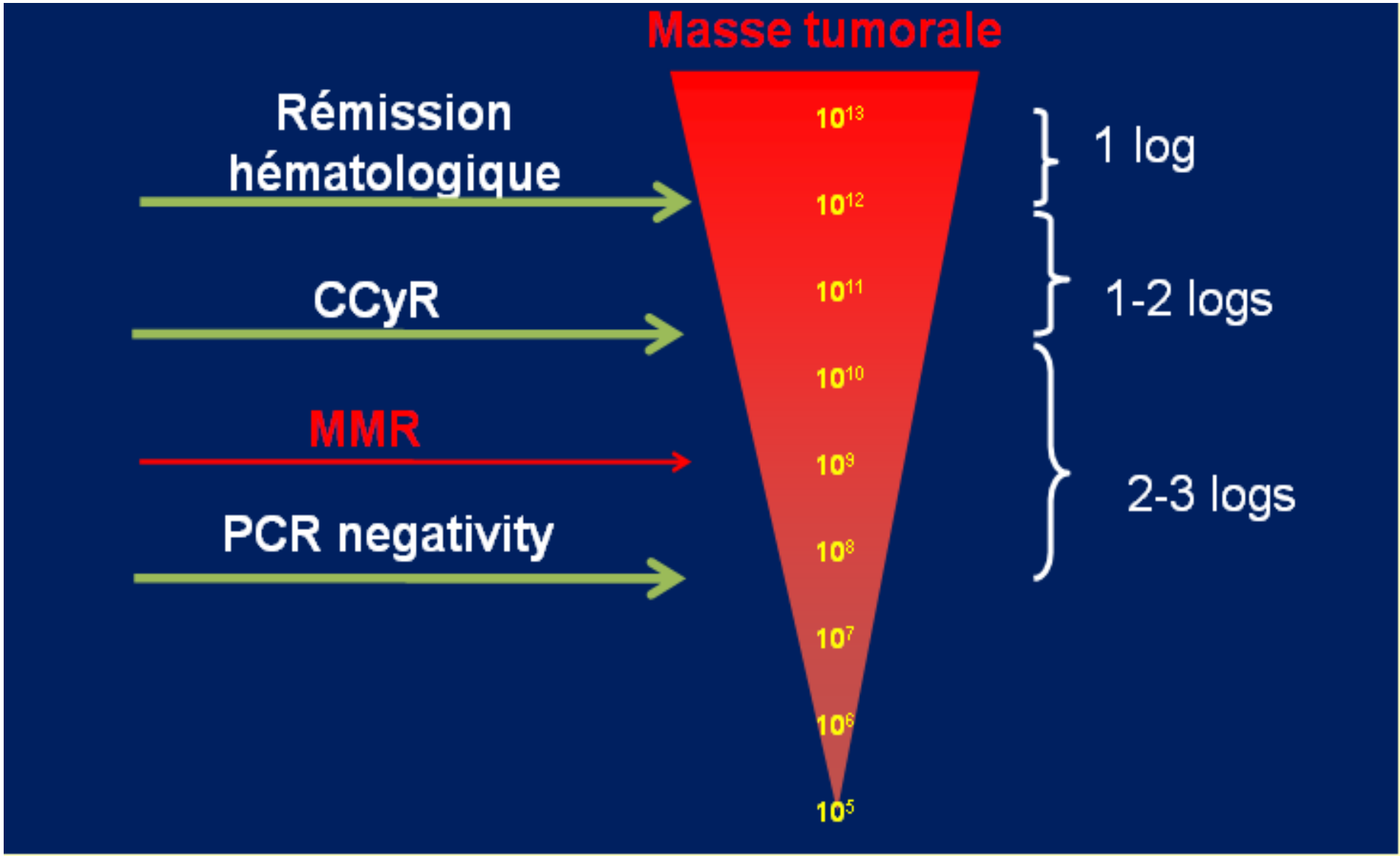
(Marin D, Blood 112 : 4437-4444,2008)

Translocation (9;22) isolée (90-95%)

Rarement le caryotype est normal (< 10%) : LMC

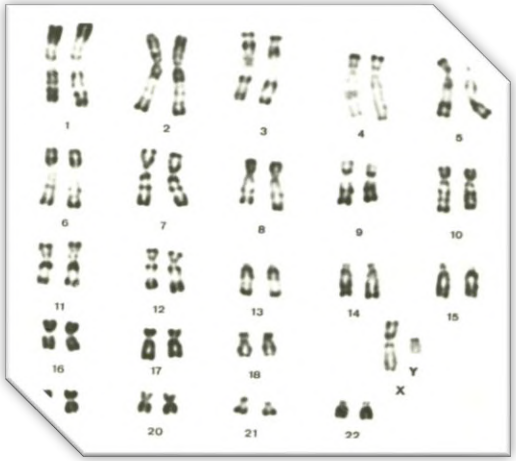
Ph1 négatif ⇒ FISH ou biologie moléculaire

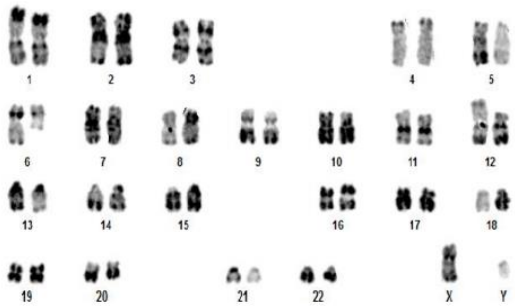
Objectifs du Traitement de la LMC



Oncohématologie (Cytogénétique conventionell, FISH)

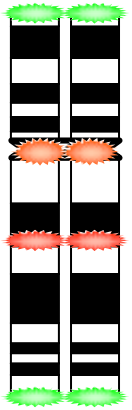
Cytogénétique conventionnelle

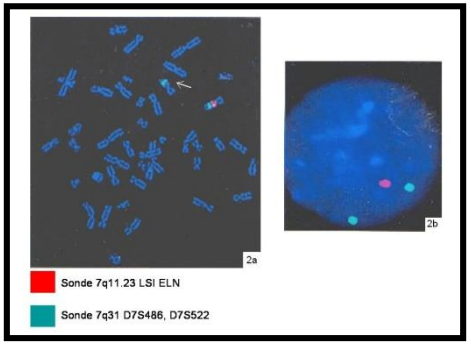


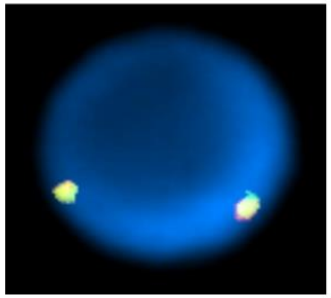


46,XY,der (6)t(6;?)(q15;?), add(12)(p11), t(12;21) (p12;q12),der(16)t(16;?)(p11;?)

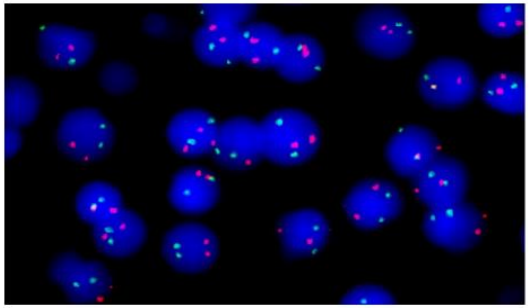
FISH





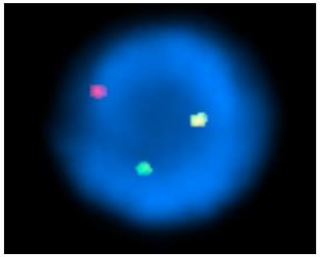


Cellule normale
La sonde: ETV6 Break Apart, double couleur



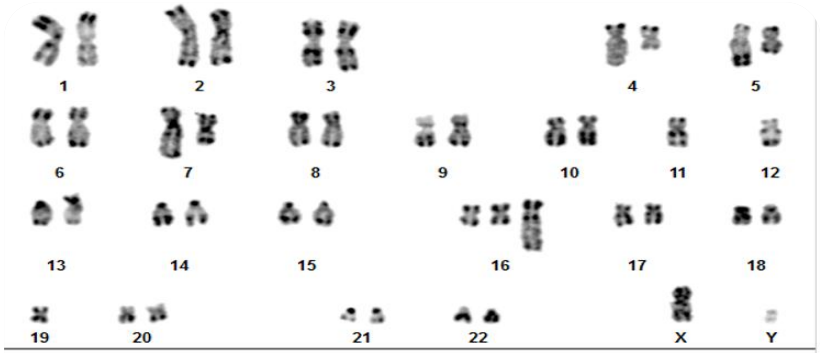
Réarrangement Bi-allélique du locus ETV6

t(12;21)(p13;q22)



t(12;21)(p13;q22)

Anomalies chromosomiques identifiées au LNR
44,XX,der(1)t(1;?)(p21;?),-3,der(5)t(5;?)(q14;?), der(6)t(6;?)(p21;?),-9,-17, 19,der(22)t(12;22)(q13;p12),+mar1,+mar2[cp17]/46,XX[3]
46,XY,inv(3)(q21q26)[28]/46,XY[2]
46,XY,der(8)(q23?),der(16)(q22?q23?)[20]
47,XY,+8[27]
46,XX,t(8;21)(q22;q22)[21]
46,XY,t(11 ;14)(q13,q32)[4]/46,XY[26]
46,XX,der(2)t(2;?)(p25;?)[5]/47,XX,+4[2]/46,XX[23]
47,XY,+13[13]
45,X,der(X)t(X;?),+iso(3)(q10),der(9)t(9;?),t(11;14)(q13;q32),t(13;14)(p11;q24),+add(19)t(19;?)(q13;?),-21x2[cp15]/46,XX[10]
46,XY,iso(7)(q10),der(19)t(1;19)(q23;p13)[6]/ 45,sdl,del(3)(p14?)[2]/46,XY[6]
47,XY,+8[2]/46,idem,-7[26]/46,XY[2]
46,XY,t(12;21)(p12;q12)[6]/46,idem,der(6)t(6;?)(q15;?),add(12)(p11),der(16)t(16;?)(p11;?)[11]
46,XX,t(9;22)(q34;q11)[20]
48,XY,+8,+21[2]/46,XY[18]
41,X,-X,-5,der(8)t(8;?)(q24;?)-13,-14,-17,-21,-22,+mar1,+mar2[17]/46,XX[3]
46,XX,der(9)(p)?[25]
44~45,XX,-5,-7,der(12)t(12;?)(p12;?),-14,-16,+mar1,+mar2, +mar3[cp15]
46,XX,del(5)(q13q34)[28]
46,XX,t(9;22)(q34;q11)[30]
44-45,XY,der(2)t(2 ;?)(p25 ;?),inv(3)(q21q26),del(4)(q21qter),del(5)(q13q31),del(7)(q22qter),-13,der(16),-21,-22,+mar1,+mar2[19]/46,XY[1]
46,XY,t(15;17)(q24;q21)[8]/46,XY[17]
46,XY,add(4)(p16),der(9)t(9;16)(q34 ;q22),der(16)inv16(p13q22)t(9;16)(q34q22)[21]/46,XX[1]
46,XX,t(12;21)[21]
46,XY,t(15;17)(q24;q21)[1]/46,XY[24]
52,XX,+X,+2,+4 t(9;22)(q34;q11),+14,+16,+21[6]/46,XX[24]



M

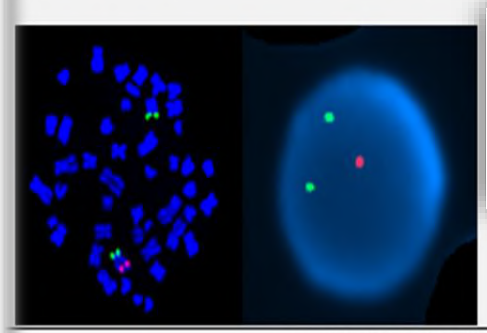
44-45,XY,der(2)t(2 ;?)(p25 ;?),inv(3)(q21q26), del(4)(q21qter),del(5)(q13q31),del(7)(q22qter), der(7)t(7 ;4)(q22 ;q21qter),der(12)t(12 ;?)(p12 ;?), -13,der(16),-21,-22,+mar1,+mar2[19]/46,XY[1]

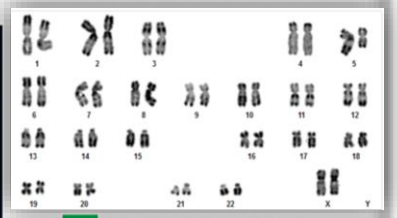
Type de prélèvement : *Moelle*

Indication : *SMD*

RESULTAT

Formule chromosomique	46,XX,del(5)(q13q34)[28]
Nombre de mitoses examinées	28
Nombre de mitoses classées	28
Type et nombres de bandes	RHG, 250
Type de Sondes	LSI EGR1/D5S23, D5S721 Dual Color Probe Set CE marked Vysis





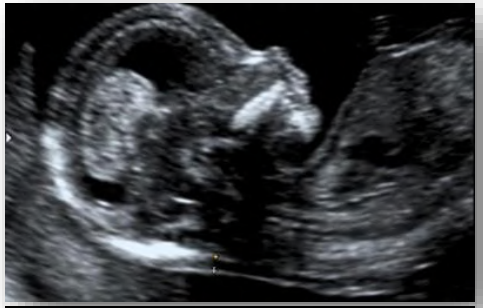
LSI D5S23, D5S721

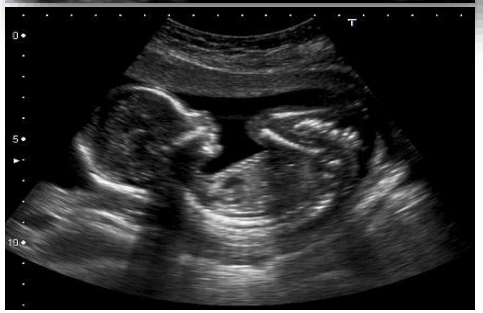
LSI EGR1

nuc ish (EGR-1x1) [195/200]

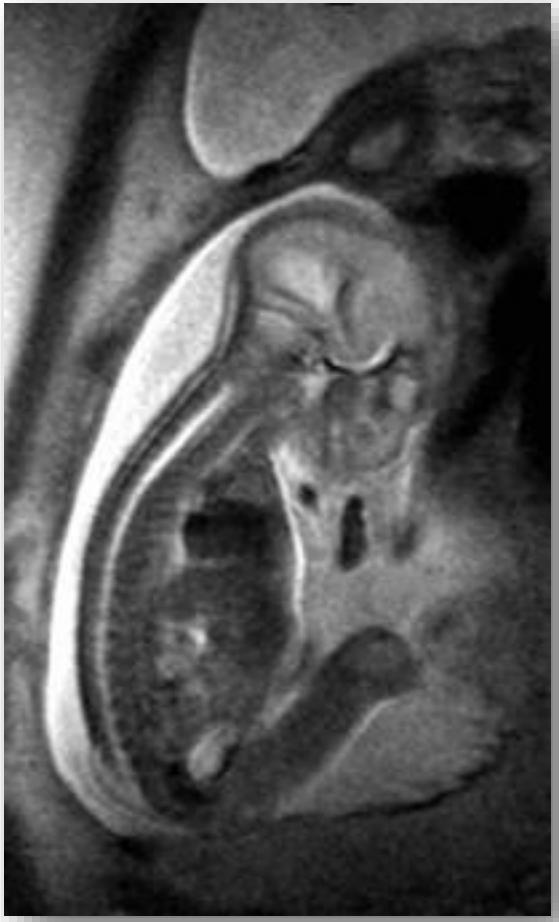
Le Diagnostic Prénatal
Imagerie fœtale ET Biologie

Echographique





IRM



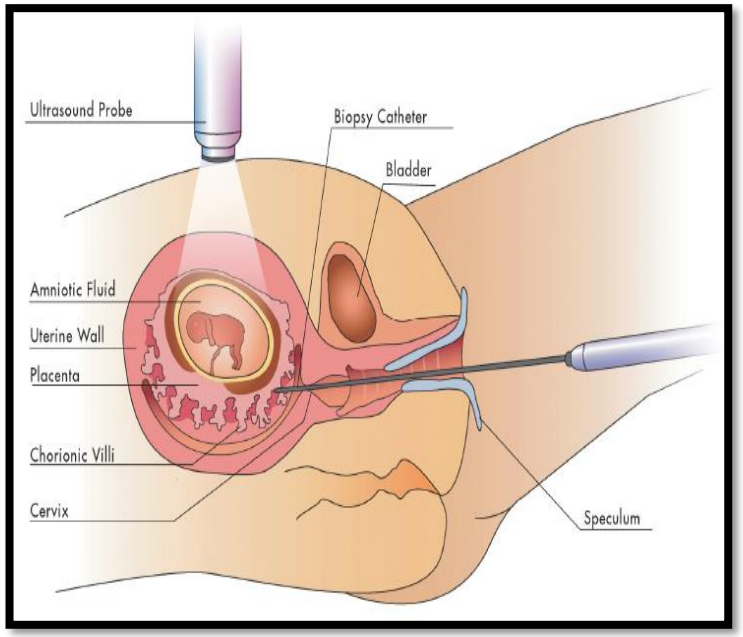
Signes d'appel biologiques

Marqueurs sériques maternels:

Hormones et produits foetaux en circulation maternelle

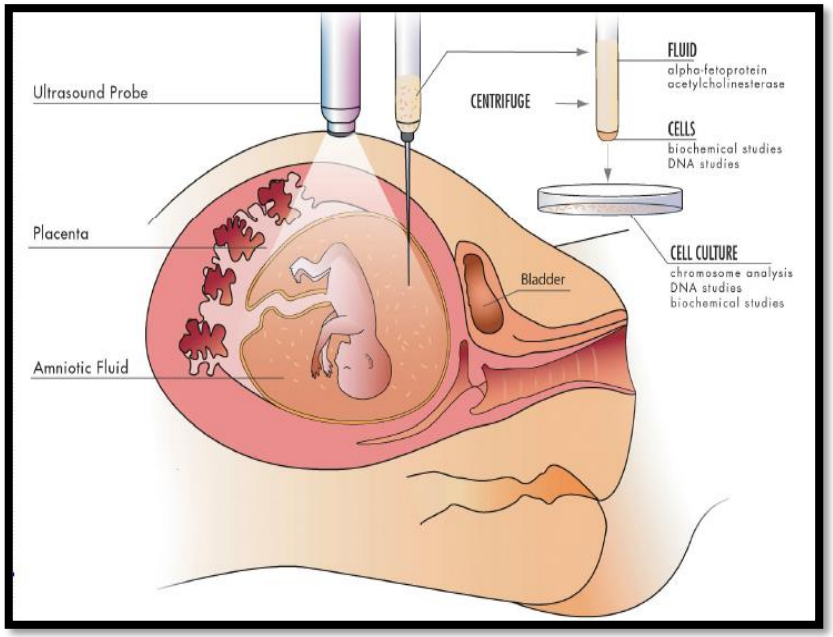
Techniques de prélèvements de tissus foetaux

Biopsie du trophoblaste



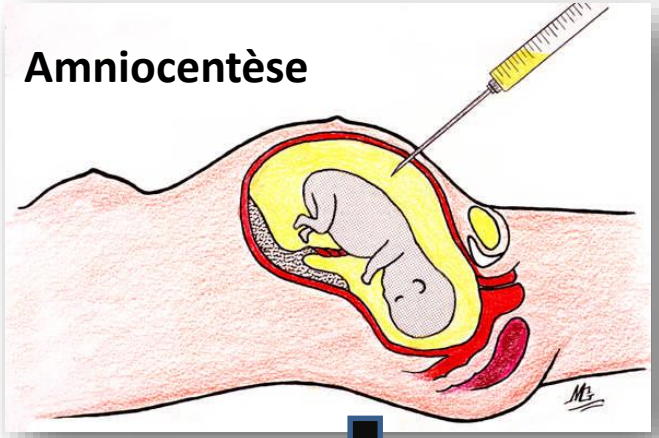
12 – 14 SA

Amniocentèse



14 – 17 SA

Applications en prénatal



Amniocytes non cultivés

24-48H

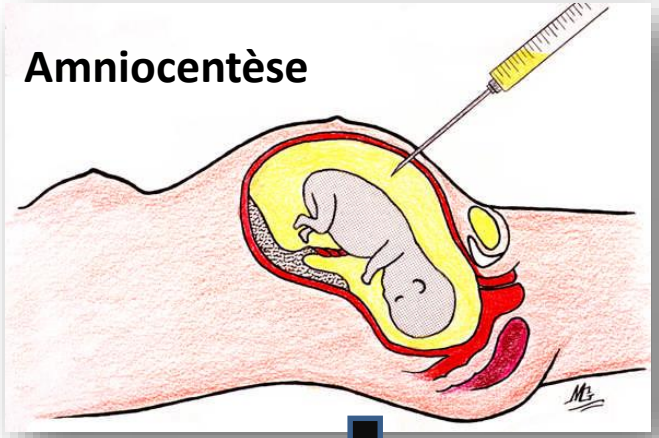
21

14

Trisomie 13 + + +

Analyse spécifique des chromosomes **X,Y,13,18,21**

Applications en prénatal



Amniocytes non cultivés

24-48H

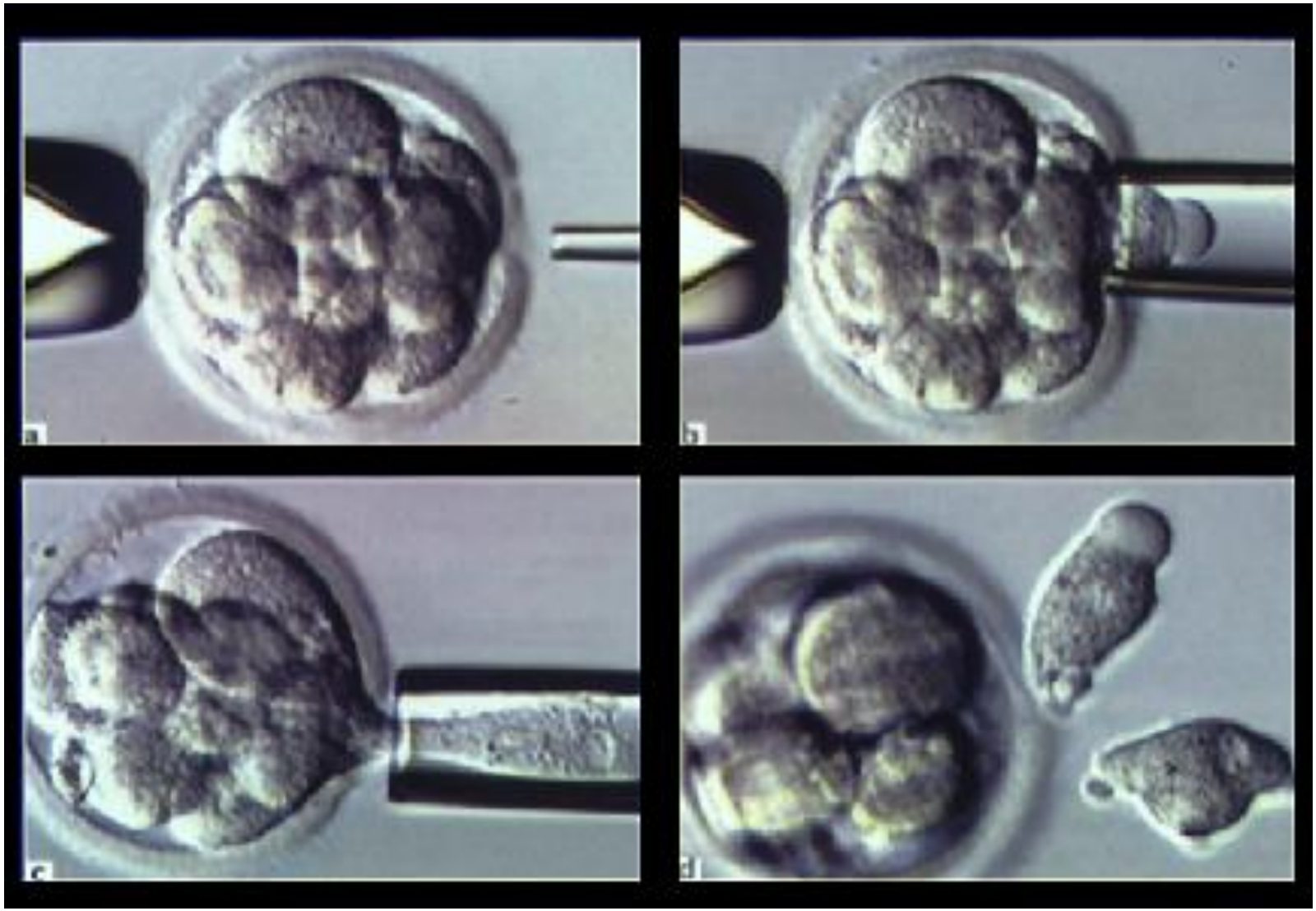
21

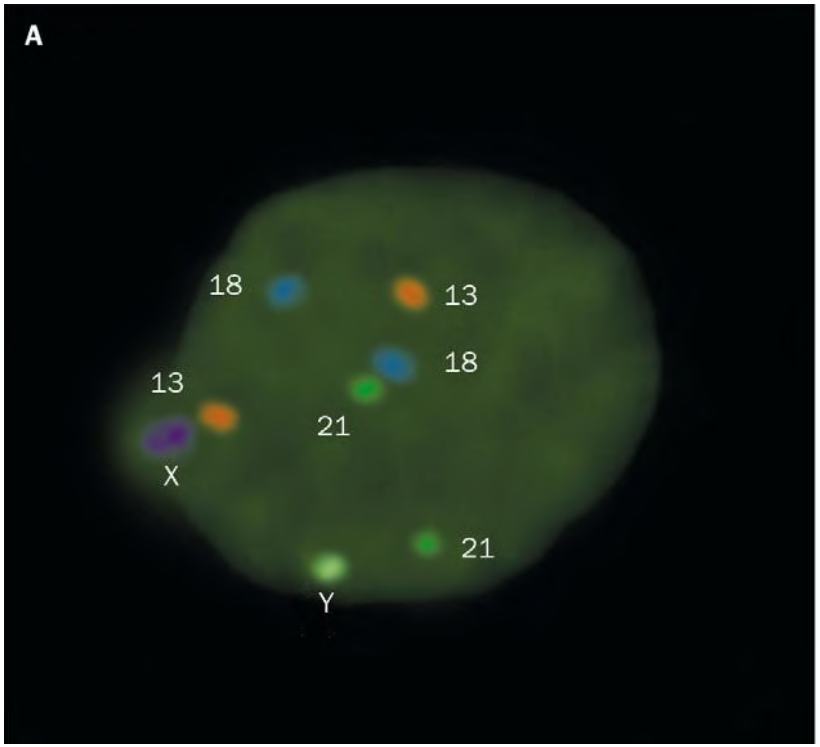
14

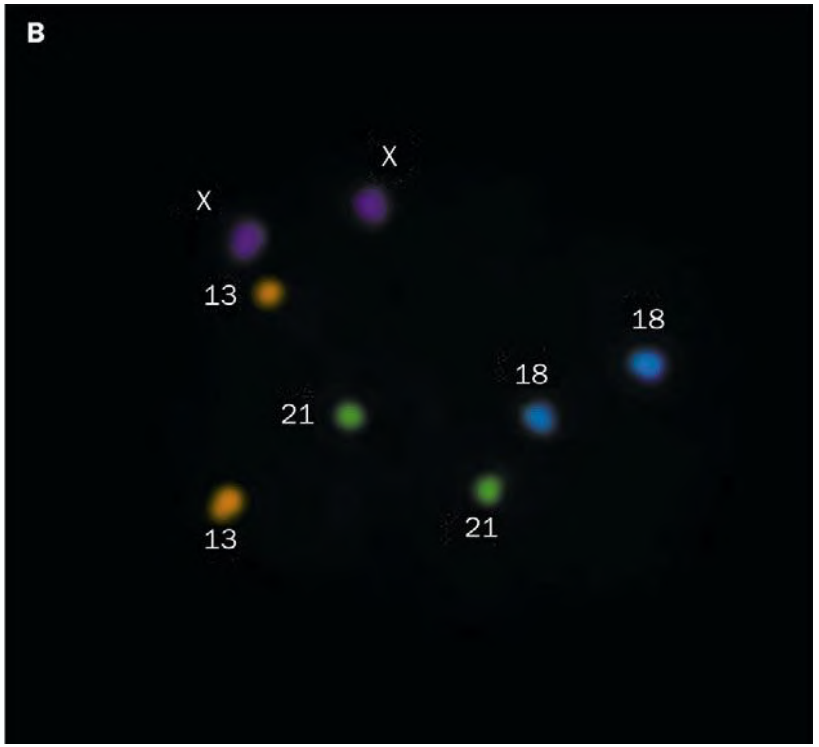
Trisomie 13 + + +

Analyse spécifique des chromosomes **X,Y,13,18,21**

Blastomère

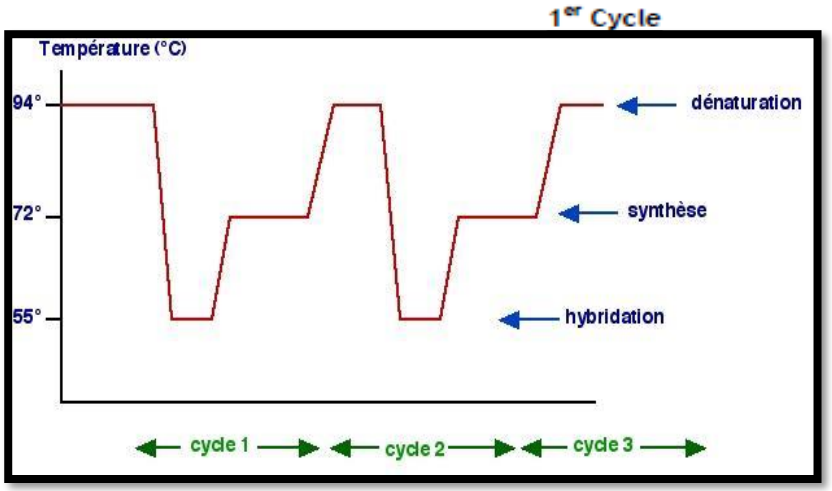


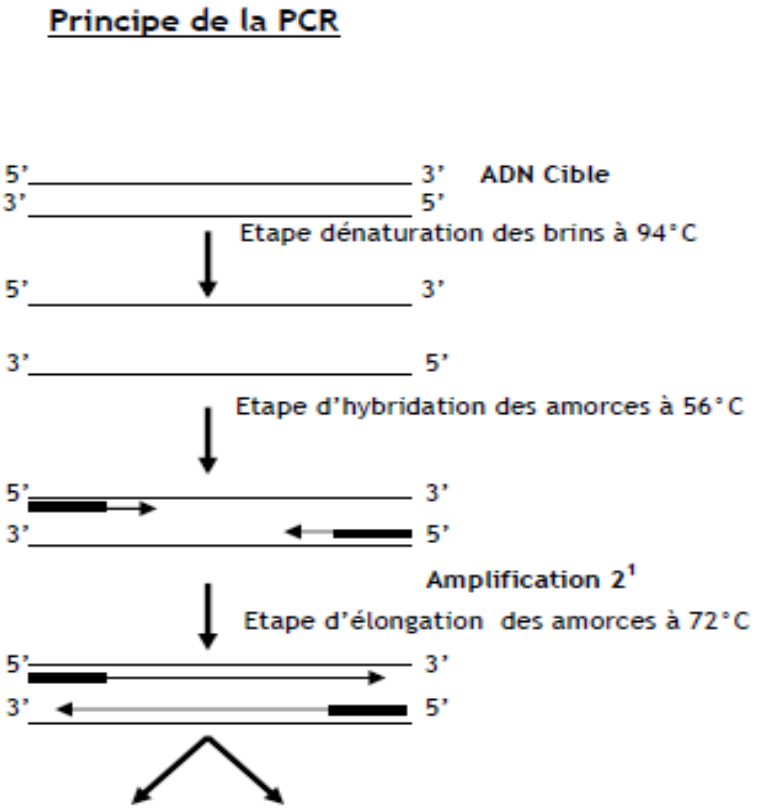




Preimplantation genetic diagnosis, THE LANCET • Vol 363 • May 15, 2004

REACTION DE POLYMERISATION EN CHAINE (PCR)



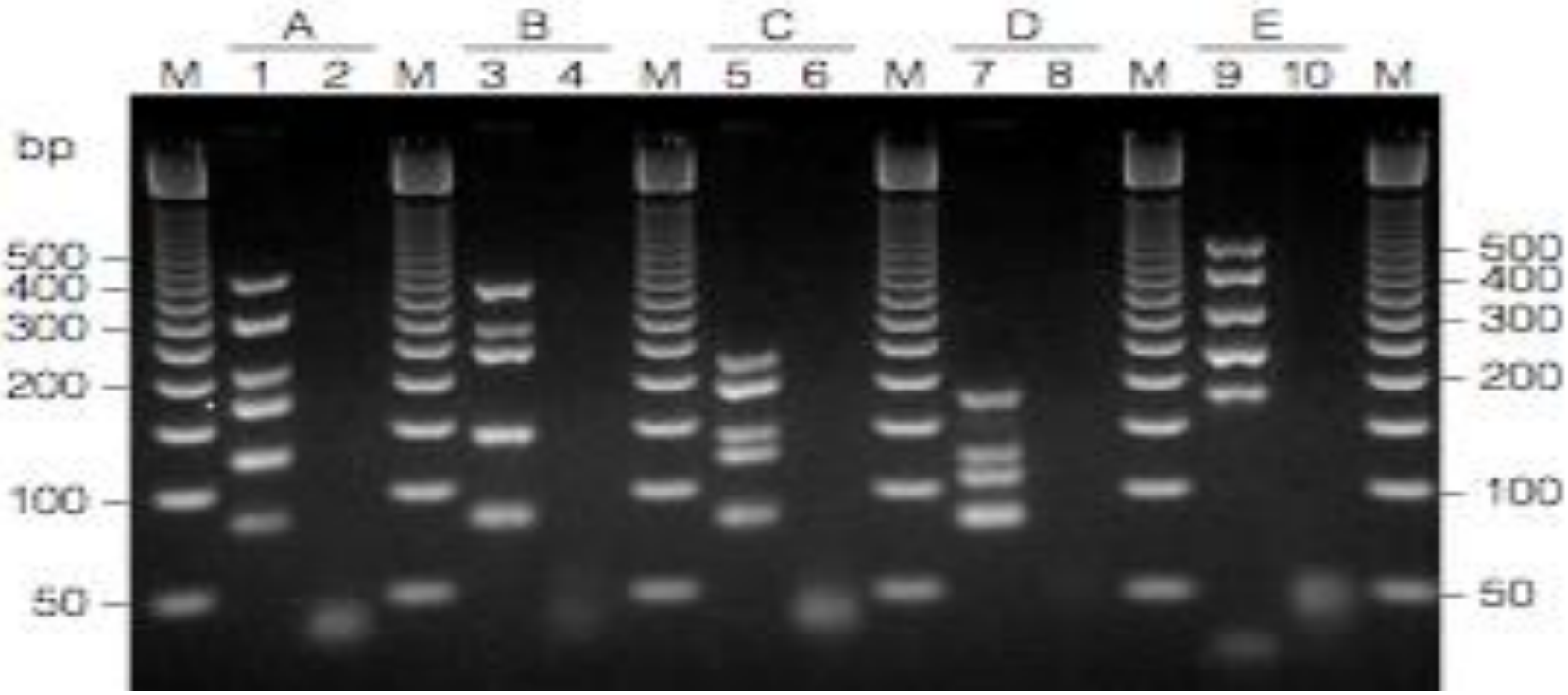


- Chaque brin néo-synthétisé sert de **matrice** pour une nouvelle synthèse au cycle suivant.

- Après **n** cycles on aura une amplification exponentielle de la séquence d'ADN cible (2n copies où n représente le nombre de cycles effectués).

➢L'analyse moléculaire du bras long du chromosome Y permet de mettre en évidence des **microdélétions des régions AZF** (azoospermia factor)

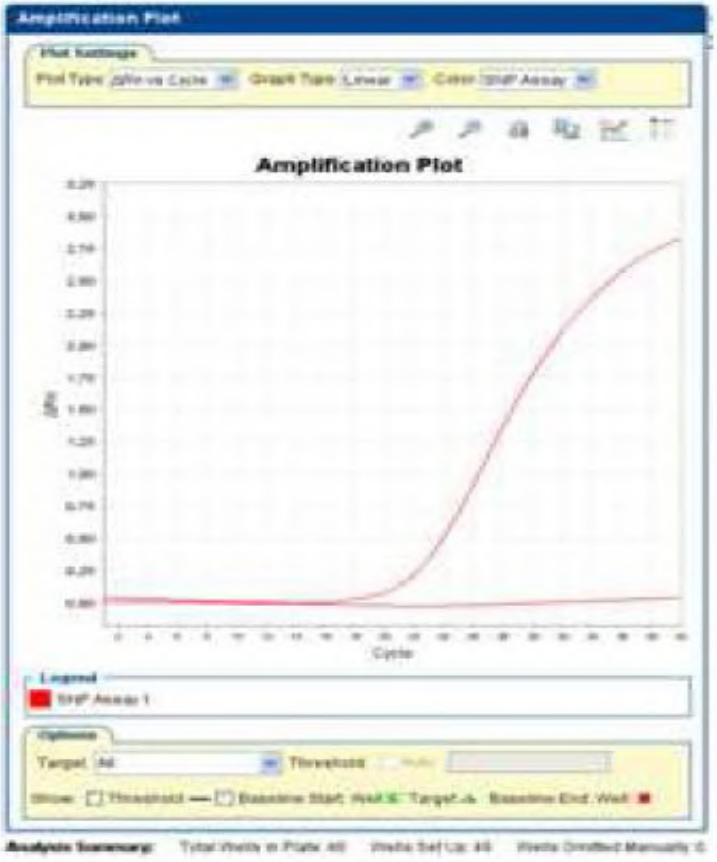
Azoospermia factor

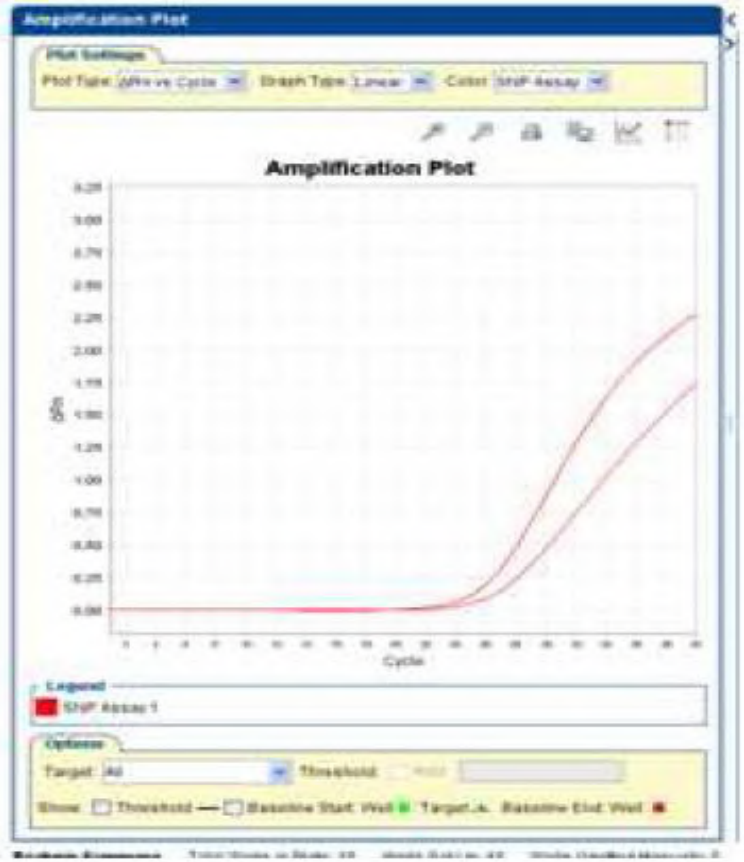


Example of amplification of male genomic DNA. AZF Multiplex PCR

LA PCR EN TEMPS REEL

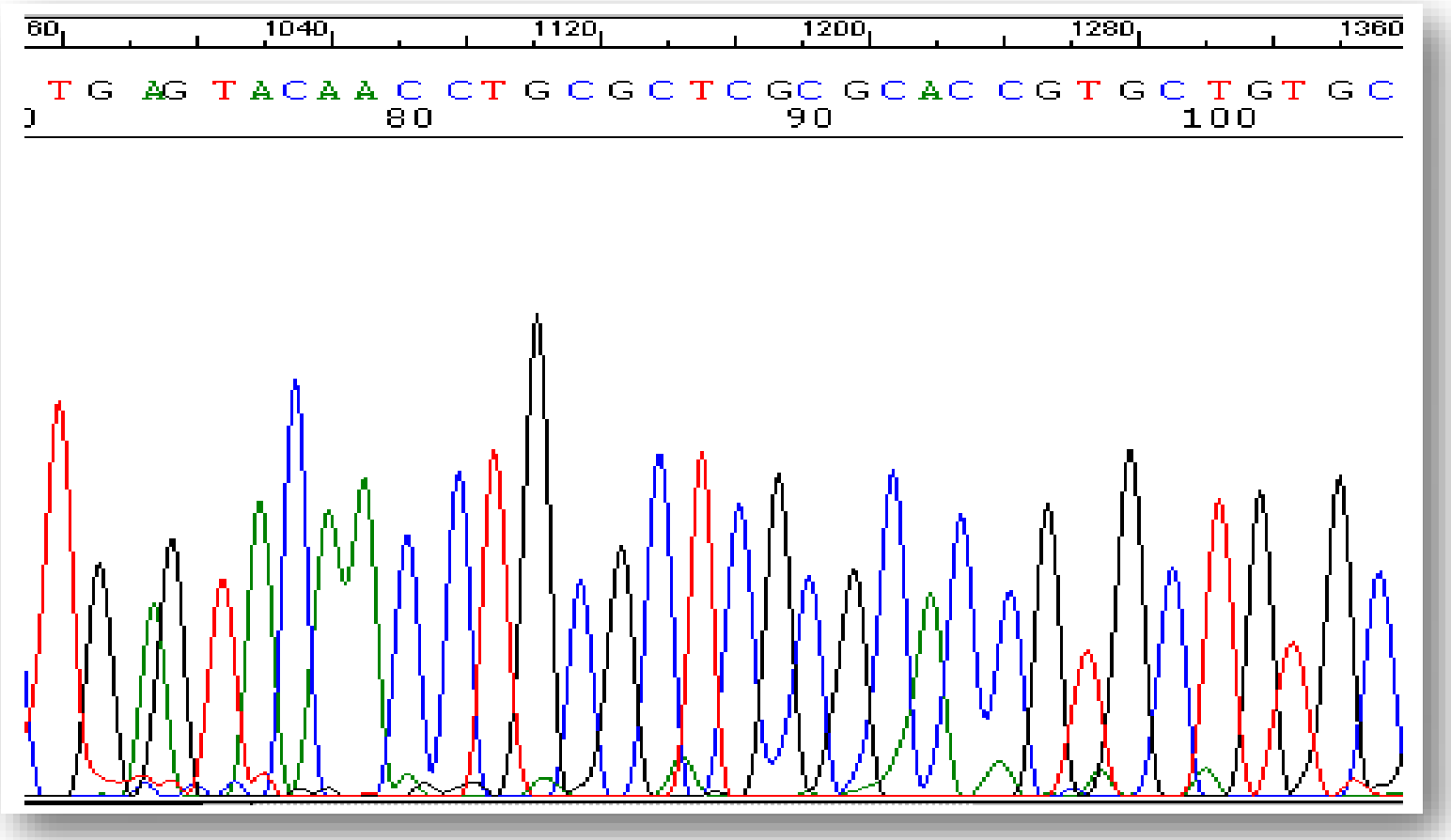
La PCR en temps réel utilise le principe de base de la PCR classique, avec pour différence une amplification mesurée non pas en final mais tout au long de la réaction, donc en **temps réel**.



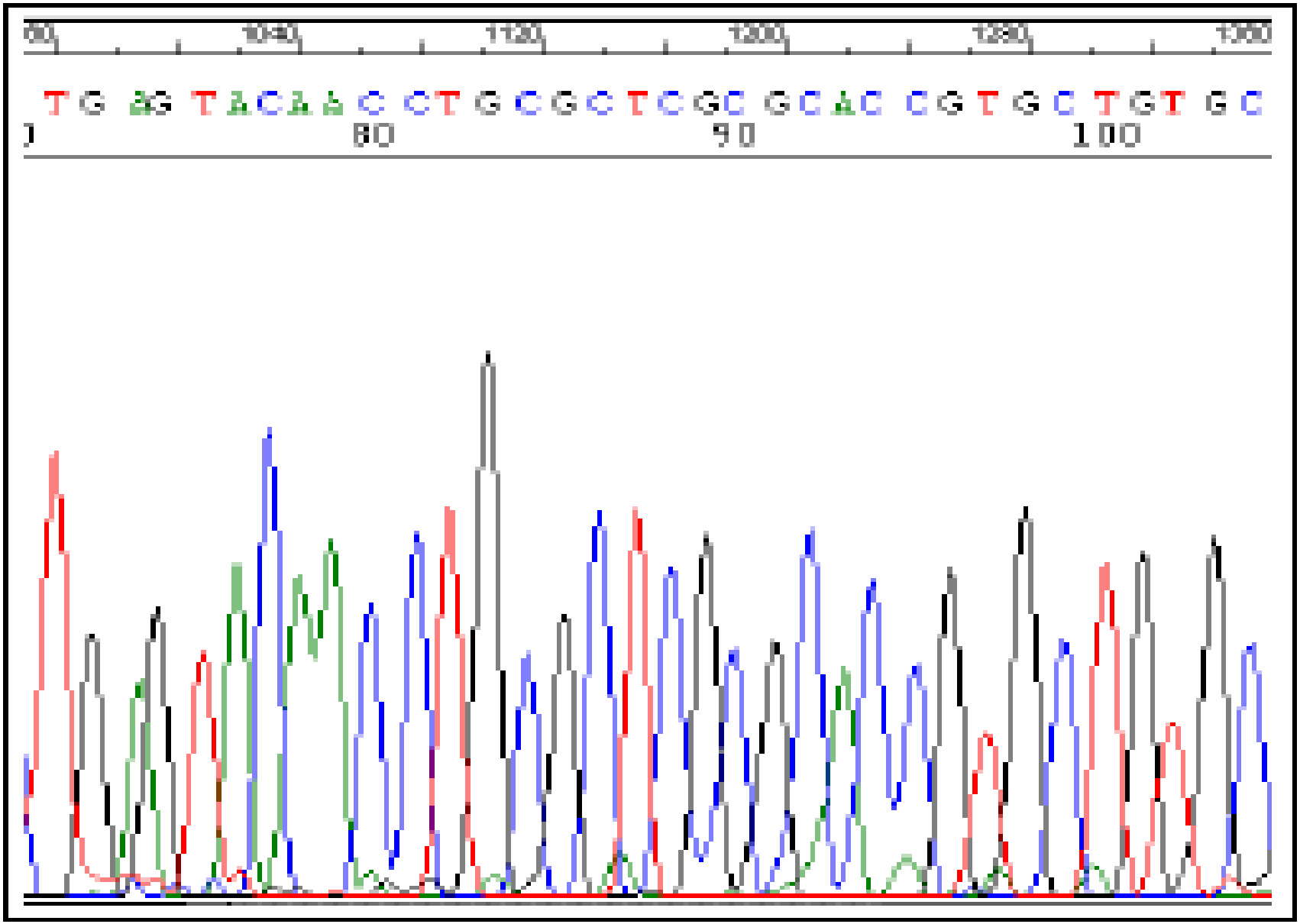


Recherche de mutation par RT PCR et sonde spécifique d'allèle : méthode Taqman

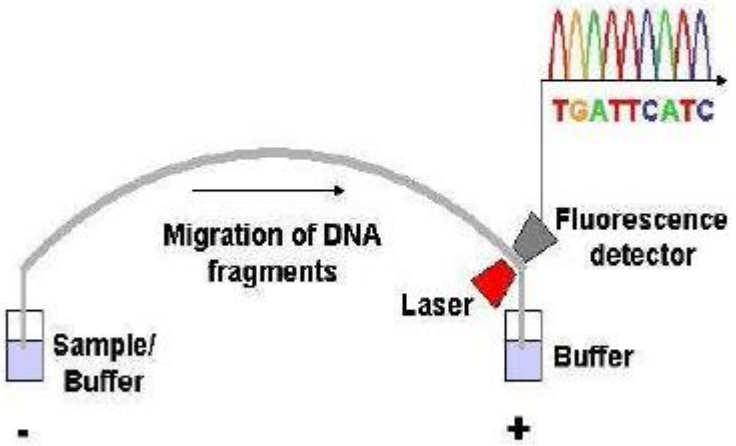
LE SEQUENÇAGE DE L'ADN

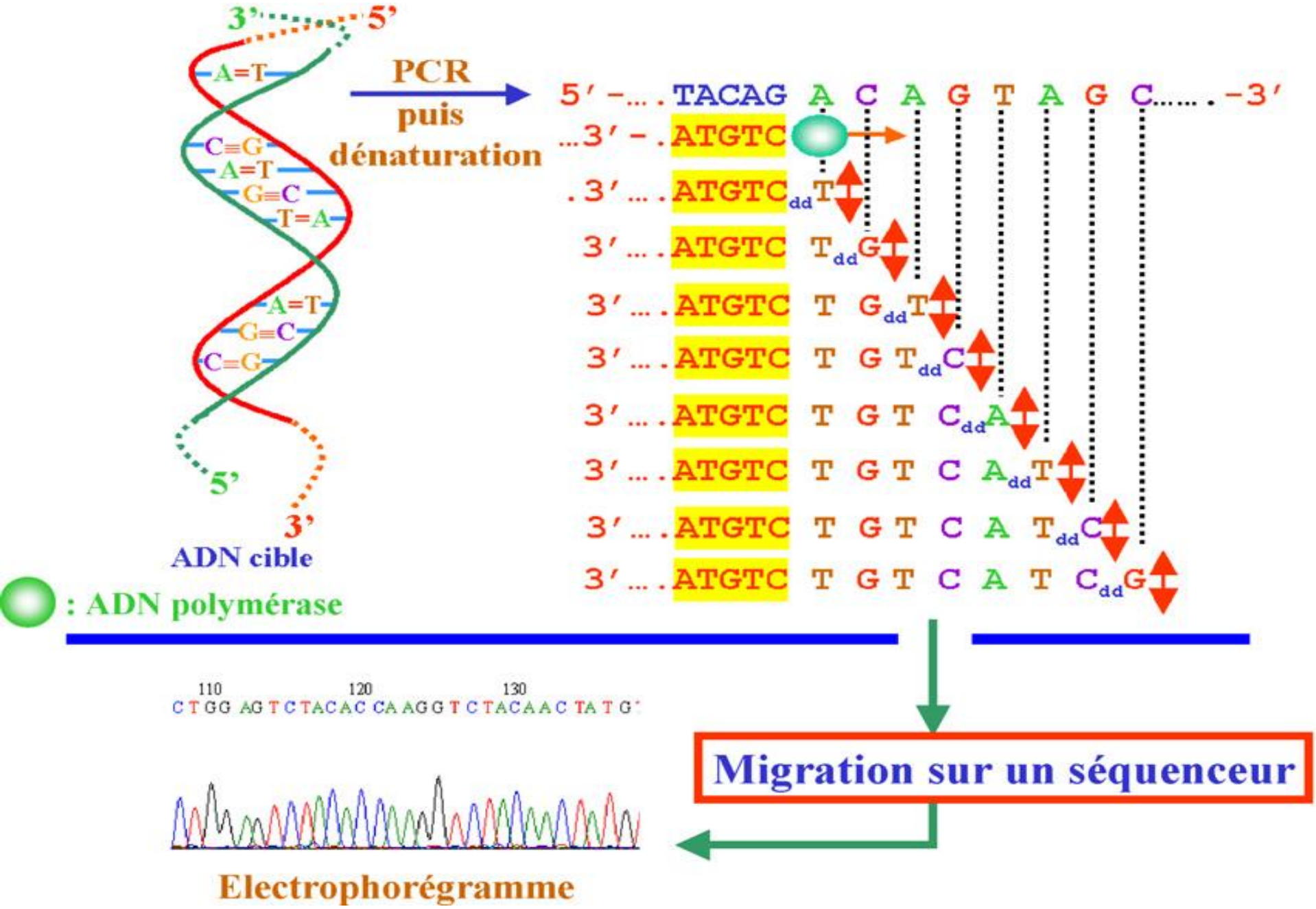


LE SEQUENÇAGE DE L'ADN



2ème génération de séquençage d'ADN: séquençage capillaire




3'
5'
A=T
C≡G
A=T
G≡C
T=A
A=T
G≡C
C=G
5'
3'
ADN cible
PCR
puis
dénaturation
5'-....TACAG A C A G T A G C.....-3'
...3'-.ATGTC
.3'....ATGTC Tdd
3'....ATGTC Tdd G
3'....ATGTC T Gdd T
3'....ATGTC T G Tdd C
3'....ATGTC T G T Cdd A
3'....ATGTC T G T C Add T
3'....ATGTC T G T C A Tdd C
3'....ATGTC T G T C A T Cdd G
: ADN polymérase
110
120
130
CTGG AG T C TACAC CA AGG T C TACAA C TA T G
Migration sur un séquenceur
Electrophorégramme

Sang total
(tube sur EDTA)

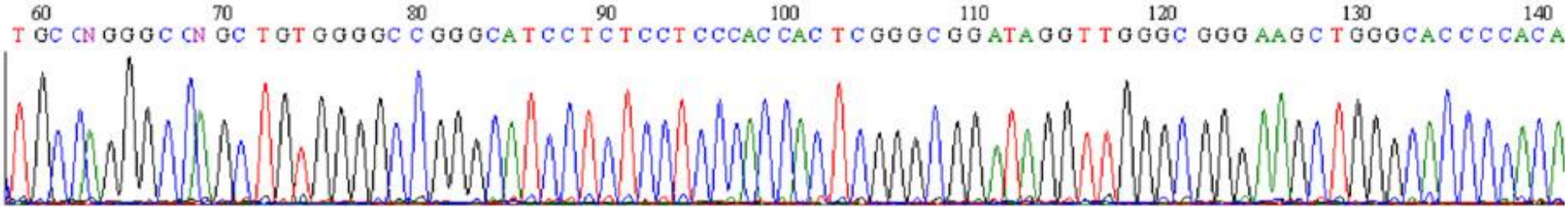
Extraction de l'ADN 1

Amplification de l'ADN (cf.PCR)
(taille moyenne: 250 à 500 pb) 2

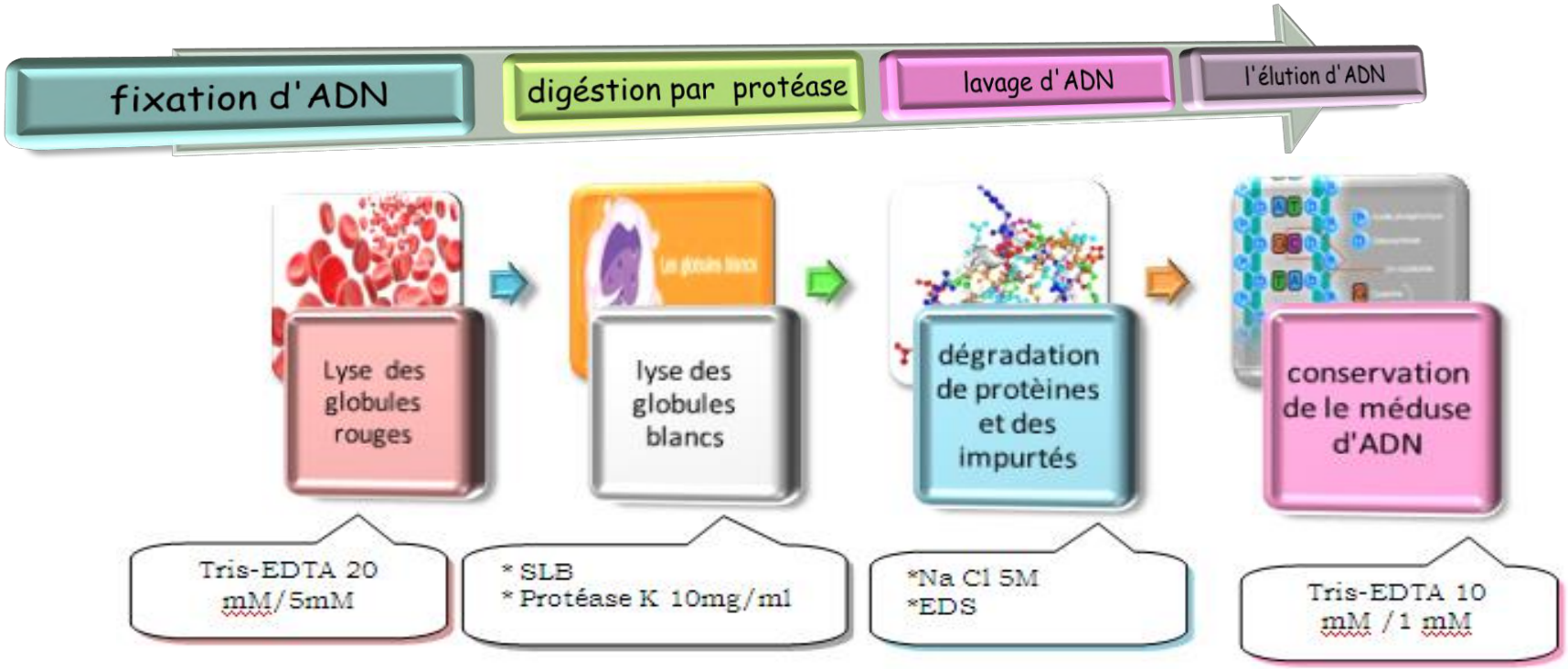
Séquençage de l'ADN
(technique de Sanger) 3

Migration sur un séquenceur
(cf electrophorèse capillaire) 4

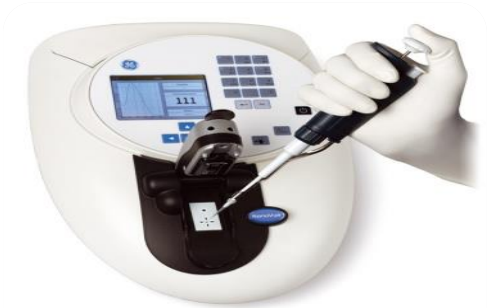
Lecture des séquences 5



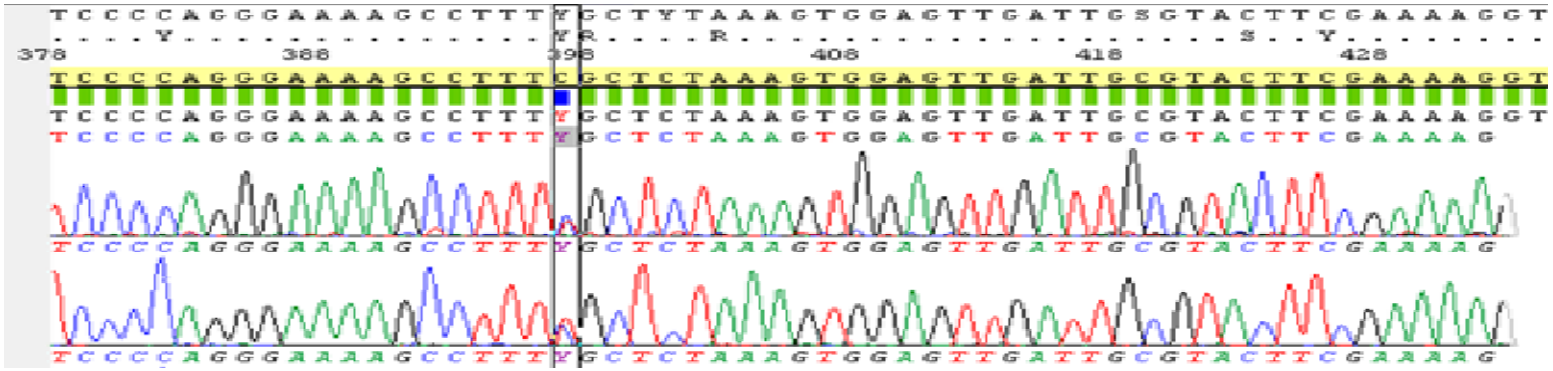
Extraction d'ADN par SEL se base sur 4 étapes essentielles



Dosage d'ADN extrait



Dosage d'ADN par NanoDro



```
 Score =  660 bits (357),  Expect = 0.0
 Identities = 359/360 (99%), Gaps = 0/360 (0%)
 Strand=Plus/Plus

Query  1    TCCCCAGGGAAAAGCCTTTYGCTCTAAAGTGGAGTTGATTGCGTACTTCGAAAAGGTAGG  60
            ||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  604  TCCCCAGGGAAAAGCCTTTCGCTCTAAAGTGGAGTTGATTGCGTACTTCGAAAAGGTAGG  663

Query  61   CGACACATCCCTGGACCCTAATGATTTTGACTTCACGGTAACTGGGAGAGGGAGCCCCTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  664  CGACACATCCCTGGACCCTAATGATTTTGACTTCACGGTAACTGGGAGAGGGAGCCCCTC  723

Query  121  CCGGCGAGAGCAGAAACCACCTAAGAAGCCCAAATCTCCCAAAGCTCCAGGAACTGGCAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  724  CCGGCGAGAGCAGAAACCACCTAAGAAGCCCAAATCTCCCAAAGCTCCAGGAACTGGCAG  783

Query  181  AGGCCGGGGACGCCCCAAAGGGAGCGGCACCACGAGACCCAAGGCGGCCACGTCAGAGGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  784  AGGCCGGGGACGCCCCAAAGGGAGCGGCACCACGAGACCCAAGGCGGCCACGTCAGAGGG  843

Query  241  TGTGCAGGTGAAAAGGGTCCTGGAGAAAAGTCCTGGGAAGCTCCTTGTCAAGATGCCTTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  844  TGTGCAGGTGAAAAGGGTCCTGGAGAAAAGTCCTGGGAAGCTCCTTGTCAAGATGCCTTT  903

Query  301  TCAAACTTCGCCAGGGGGCAAGGCTGAGGGGGGTGGGGCCACCACATCCACCCAGGTCAT  360
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Sbjct  904  TCAAACTTCGCCAGGGGGCAAGGCTGAGGGGGGTGGGGCCACCACATCCACCCAGGTCAT  963
```
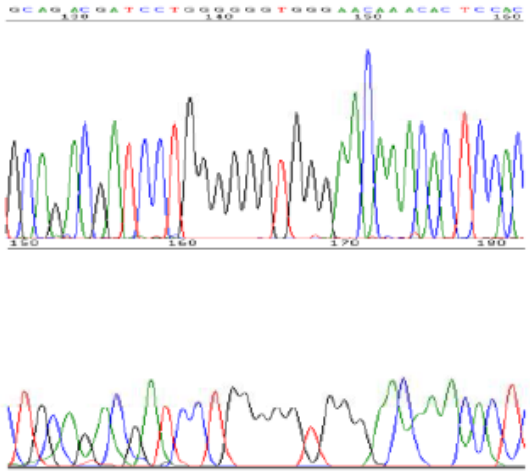

Analyse de la séquence de l'exon 4 de gène MECP2 par l'amorce 4A (F,R) de la patiente 4 .

Les mutations décalant le cadre de lecture (Frame-Shift)

Exemple: La mutation **c.35delG** du gène *GJB2* est une mutation fréquente dans les surdités non syndromiques autosomiques récessives.

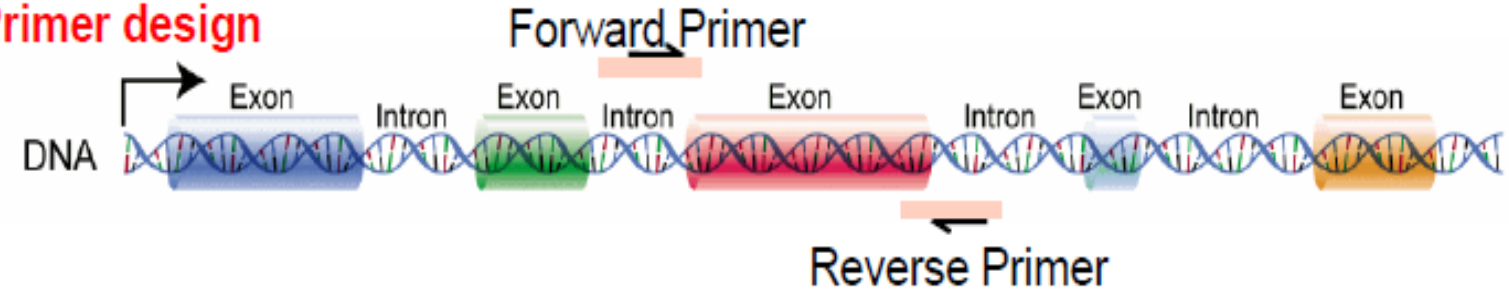


Séquence normale TCCT**GGGGGG**TGGG

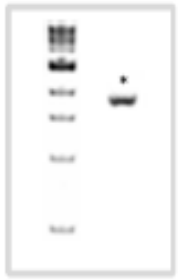
Séquence mutée TCCT**GGGGG**TGGG

Séquençage par la méthode de Sanger

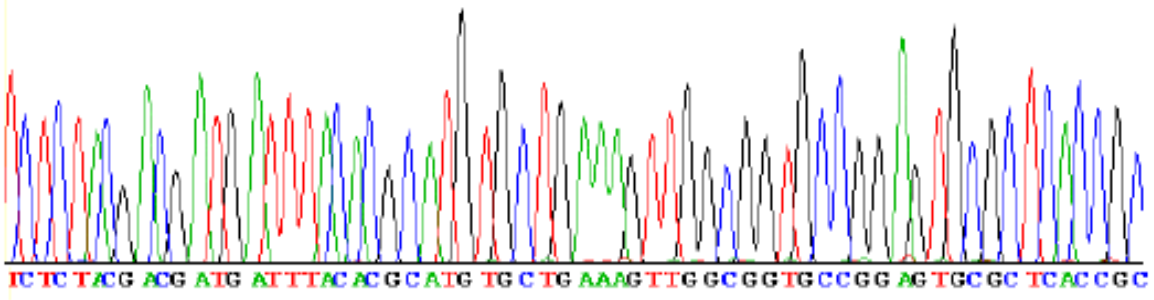
1. Primer design



2. PCR



3. Sanger Sequencing (based on terminator di-deoxy nucleotides)



Generate sequence

4. Mutation detection

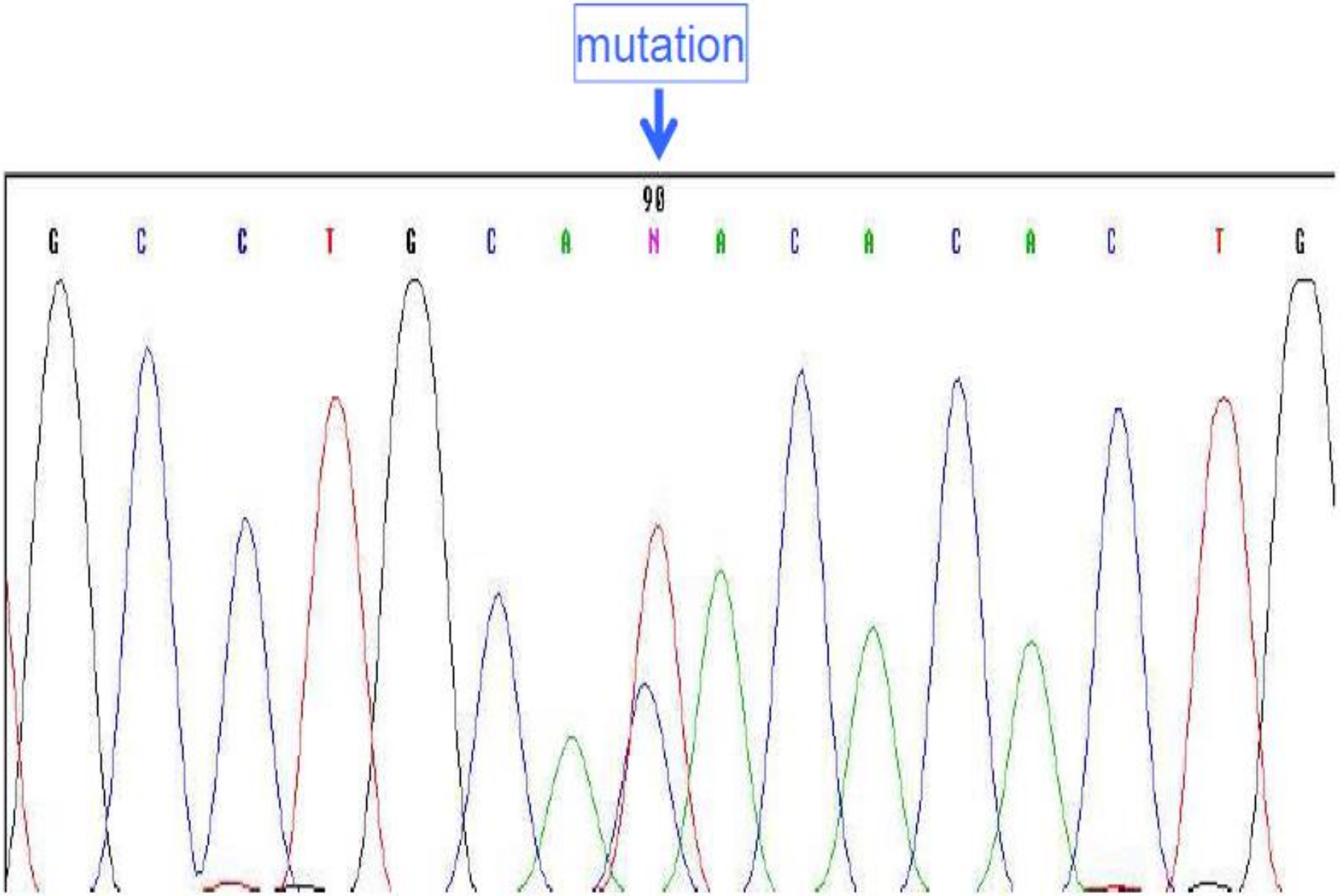
....TCTCTACGACGATGATTTACACGCATGTGCTGTAAGTTGGCGGTGCCGGAGTGCGCT
CACCGC...

Compare to reference genome

....TCTCTACGACGATGATTTACACGCATGTGCTGAAAGTTGGCGGTGCCGGAGTGCGCTCACCGC...
....TCTCTACGACGATGATTTACACGCATGTGCTGTAAGTTGGCGGTGCCGGAGTGCGCTCACCGC...

Lysine → Stop!

Maladies monogéniques et mutations à impact considérable



Variation d'un seul gène avec effet majeur

.....ATGAGCCAGTACCTGTTTAAGGTTCTCATCGTGGGTGAC
ACCGGCGTGGGCAAATCCTCCCTGATGATGCGTTTCACGGAGAAC
AAATTCCTCGAGAACTACGTGTGCACGGTGAGCATGGATATCAGG
GCGAGCTACGTGGAGCTGCTCGAGGGCAAGATGATGCTGGAGGT
CTGGGACACCACCGGCGACGAGCGCTTGAAGTCGGCGATGCCGT
CCTTTTATCGTGGTGCCCATGGCGTACTGCTCGTTTACGACACAAC
GTCGTCCAAAAGTTTCGAAAACATCGGTGGCTGGCTGAAGGAGAT
CATGCGCATGTGTCCGGATAAGCTGAACGTCGTGCTGGTGGGGA
ACAAGTGTGATGATCTGGACCATCGCCAGGTGGACCCTGAGCAG
GCCCTCCAATATGCCCGTCGTCGGGGATTCCACTCTGATGTGGTT
TCCGCCAAGAGTGGCAAGAATGTATATAACTTATTCCGTTCGTTGA
CATTTGACATGCACGATCGTATTGTGCGTCACGGGAGATTCGAGG
ACATTAGAGAGCTACCGGATGAACCAATTAATCCAGCTGACACAGA
TCGCCAGGGGGGCAATGACCCCAATACCTGCTGTGCGGTGGACG
TAGCTTCTACACATACGCAGGAACAGCTATGACCATCTCGAGCAG
CTGAAGCTCCAATGTGGTGGAATTCTACTTATTAAACCGAACCGAA
TTGACGATGAGCAACTTCCAGTACCTGTTTAAGGTTCTCATCGTGG
GTGACACCGGCGTGGGCAAATCCTCCCTGATGATGCGTTTCACGG
AGAACAAATTCCTCGAGAACTACATG/AAGCACGGTGAGCTTGGAT
ATCAGGGCGAGCTACGTGGAGCTGCTCGAGGGTAAGATGATGCT
GGAGGTCTGGGACACCACCGGCGACGAGCGCTTGAAGTCGGCGA
TGCCGGCCTTTTATCGTGGTGCCCATGGCGTACTGCTCGTTTACG
ACATAACGTCGTCCAAAAGTTTCGAAAACATCGGTGGCTGGCTGA
AGGAGATCATGCGCATGTGTCCGGATAAGCTGAACGTCGTGCTGG
TGGGGAACAAGTGTGATGATCTGGACCATCGCCAGGTGGACCCT
GAGCAGGCCCTCCAATATGCCCGTCGTCGGGGATTCCACTCTGAT
GTGGTTTCCGCCAAGAGTGGCGAGAATGTATATAACTTATTCCGTT
CGTTGACATTTGACATGCACGATCGTATTGTGCGTCACGGGAGGT
TCGAGGACATTAGAGAGCTACCGGATGAACCAATTAATCCAGCTG
ACACAGATCGCCAGGTGACCACAGATGACCCCAATACCTGCTGTT
AAGCACCGCGACTTCATTTGAATATATGATATCATAATACATTTTAT
AATTTCTATTACACAATAGCACAACAAACTCCGTAAACTTCTTGCCT
AATGAAAAATCAAATATTTGTAATGTGAAACGGGAAGTTTGCGAAA
ATGAACATAAATAAAGTTTCCCAGTTGATGCAGTGAAAGCGCATTT
TGTTACAATTCACGATATATGATGATGTCACTTTGCCCAGTATTCCA
TCCCTCGAAAATACTGATTATTATACCAATGACAAATCATGCGCAT
GTGTAACGTCGTGCTGGTGGGGAACAAGTGTGATGATCTGGACCA
TCGCCAGGTGGACCCTGAGCAGGCCCTCCAATATGCCCGTCGTC
.....

G G G G G

A

Une erreur sur les 3 milliards de perles de notre génome

Limites des techniques "traditionelles" de séquençage d' ADN

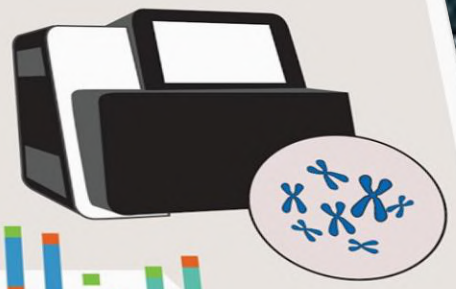
- L'analyse d'un gène entier est souvent laborieuse
- Plusieurs gènes sont souvent impliqués dans une maladie:
  - Cancer du côlon héréditaire (8 gènes)
  - Ataxies (>80 gènes)
  - Vision/cécité (>100 gènes)
  - Retard mental (~500 gènes)
- Problèmes de détection des variations structurelles (de grande taille)
- Bases génétiques des maladies complexes peu connues

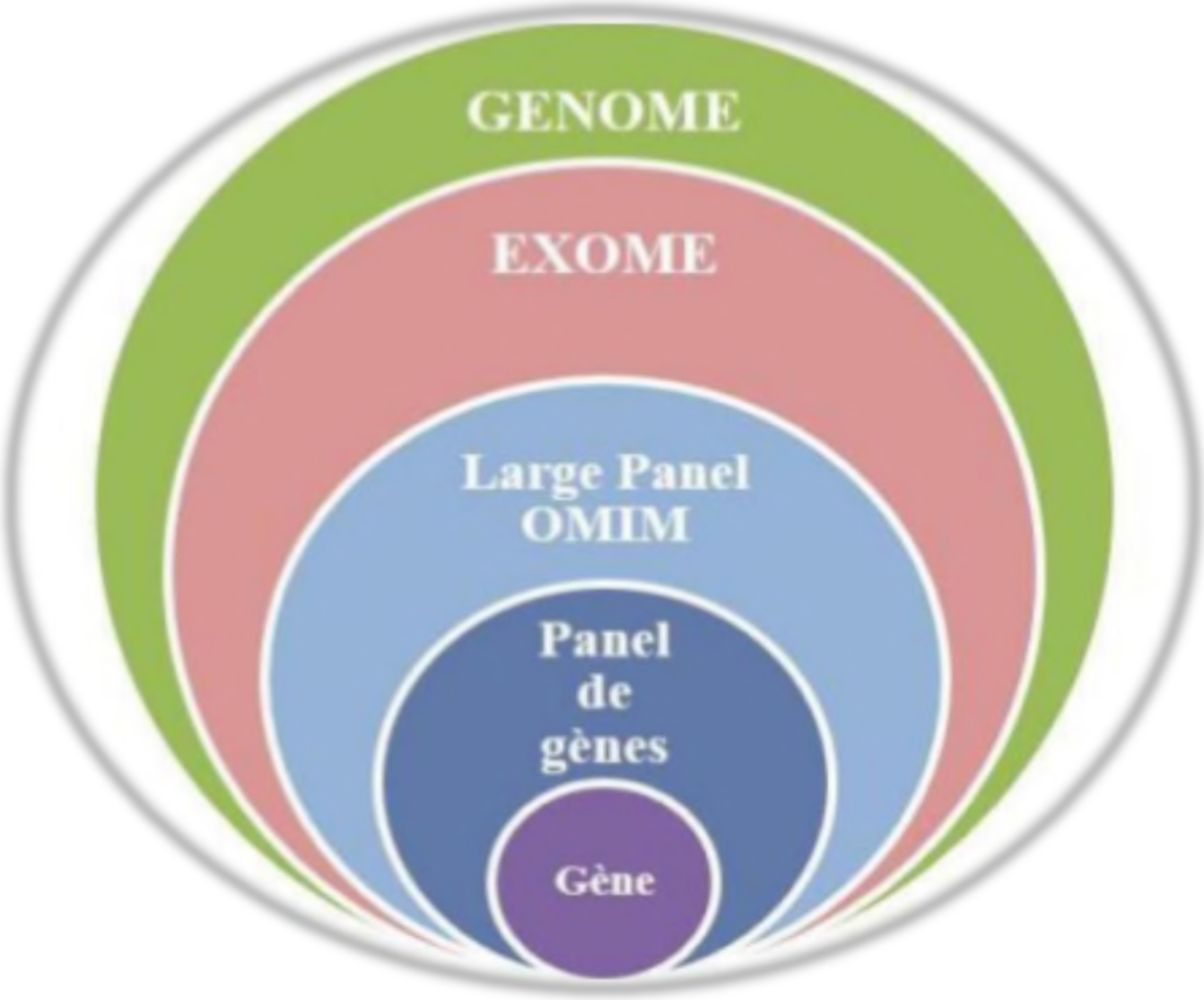
→ **pas de test pour beaucoup de maladies monogéniques**

→ **pas de test pour les maladies complexes**

NEXT GENERATION SEQUENCING

AN INTRODUCTION

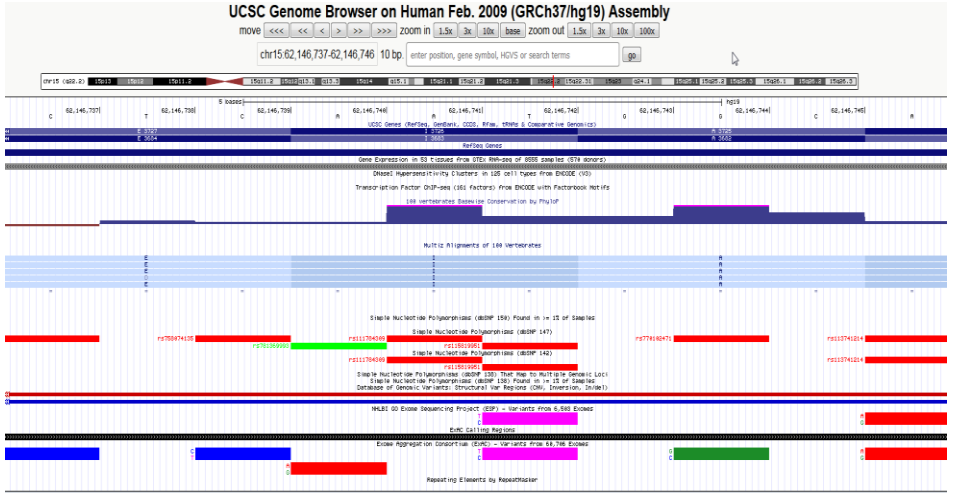




Les différents niveaux d'approche du séquençage

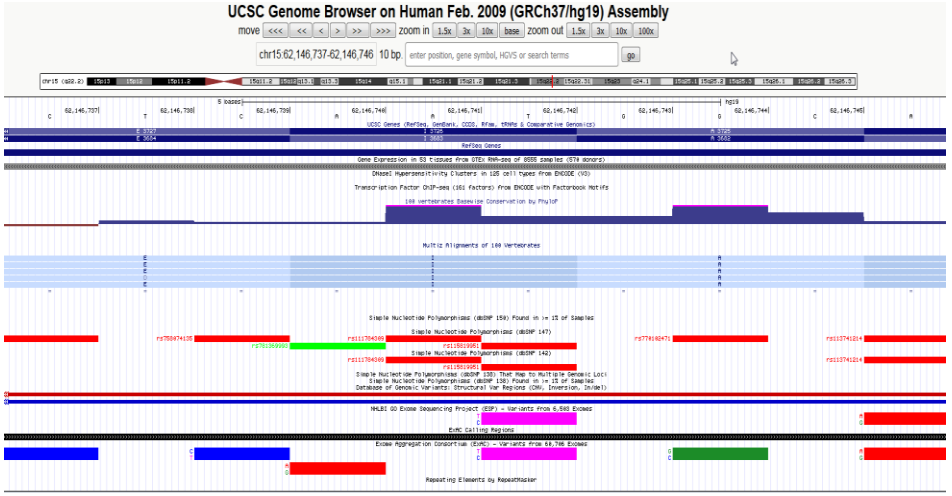
Human Genome Reference

Sequencing human genome

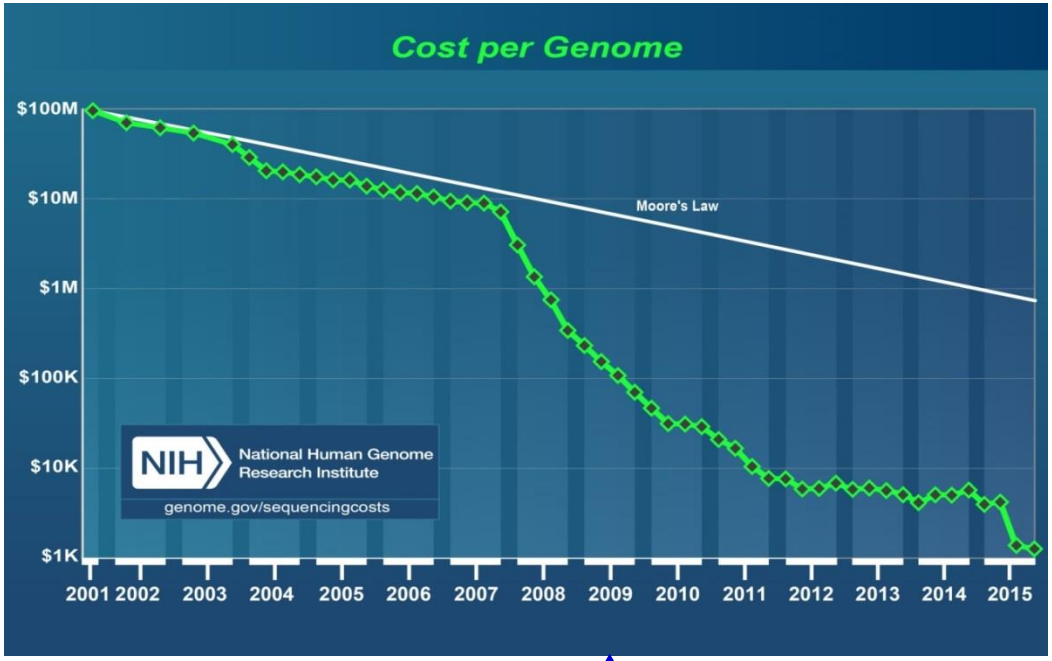


Human Genome Reference

Sequencing of 3 billions bp



NextGen : The revolution



Clinic

3 billions $/Genome

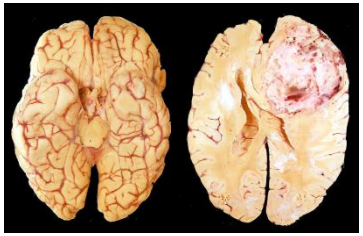
<1000 $/Genome

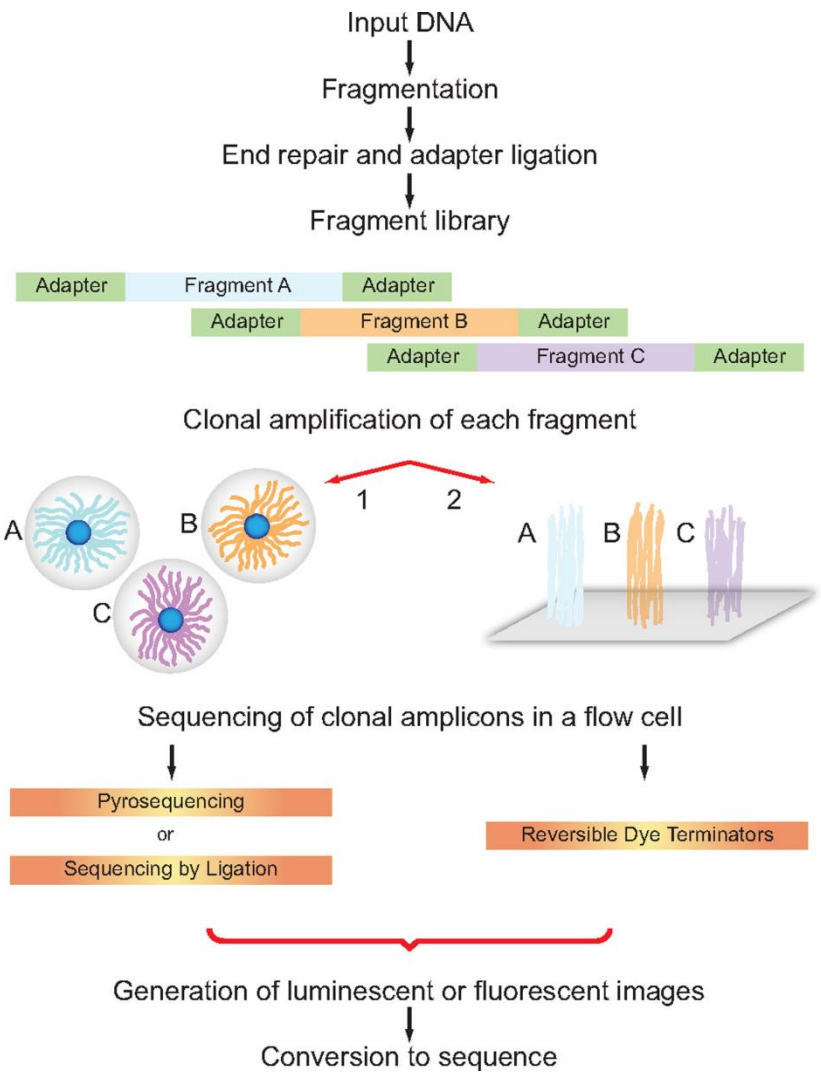
13 years = 4745 days

2 days

Next generation sequencing

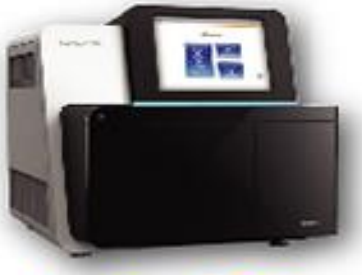
- **DNA :**
  - **Blood**
  - **Saliva**
  - **Tissues :**
    - **Normal**
    - **Tumor**
  - **Fresh-frozen or FFPE tissues**





Platforms features

MiSeq®



NextSeq® 500

HiSeq® 2500

HiSeq® 3000

Next Generation Sequencing
platforms from trusted names

Ion Torrent™

PacBio RS II System

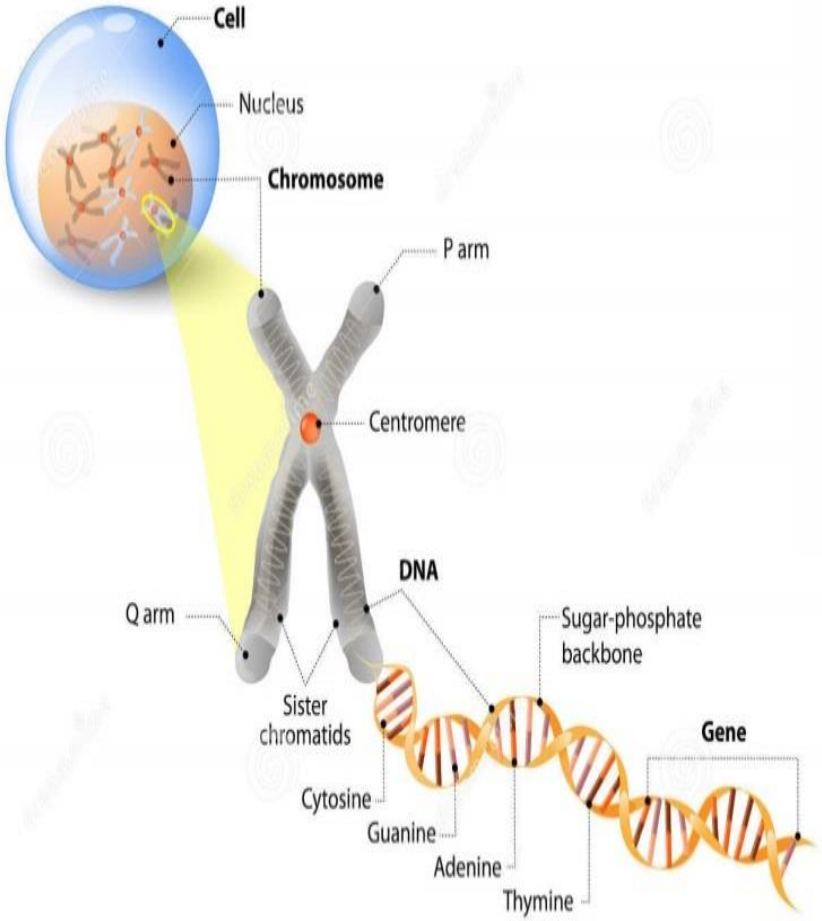
HiSeq® 4000

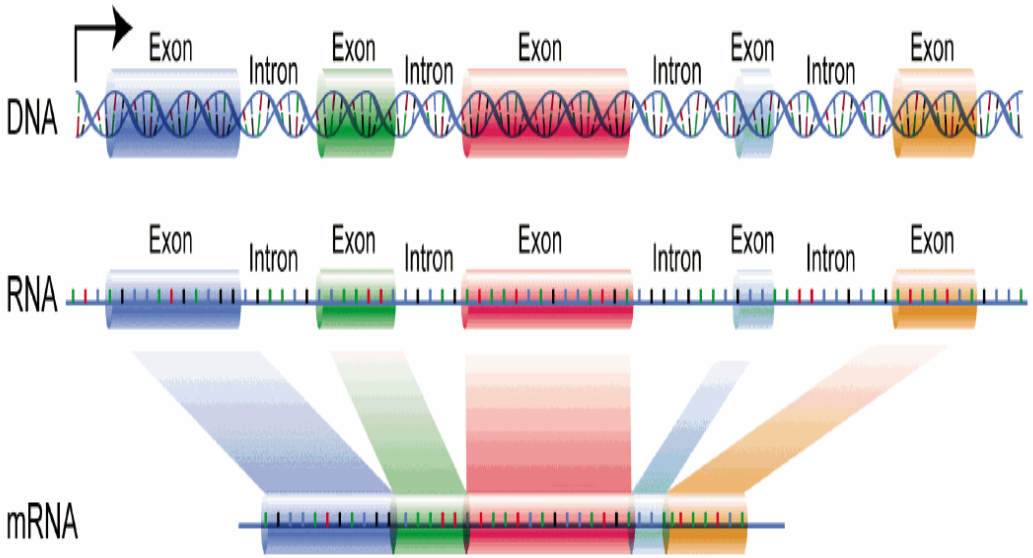
Sequencing data : the flood

- One Human Genome sequence at 30X (deep) = 100-150 GigaB
- Computational challenges :
  - Acquisition
  - Storage
  - Distribution
  - Analysis
  - Privacy

Whole exome sequencing

- Sequencing 1% of the whole genome = **Sequencing EXOME**
- Interrogating coding regions of **20 000 genes = 180 000 exons**



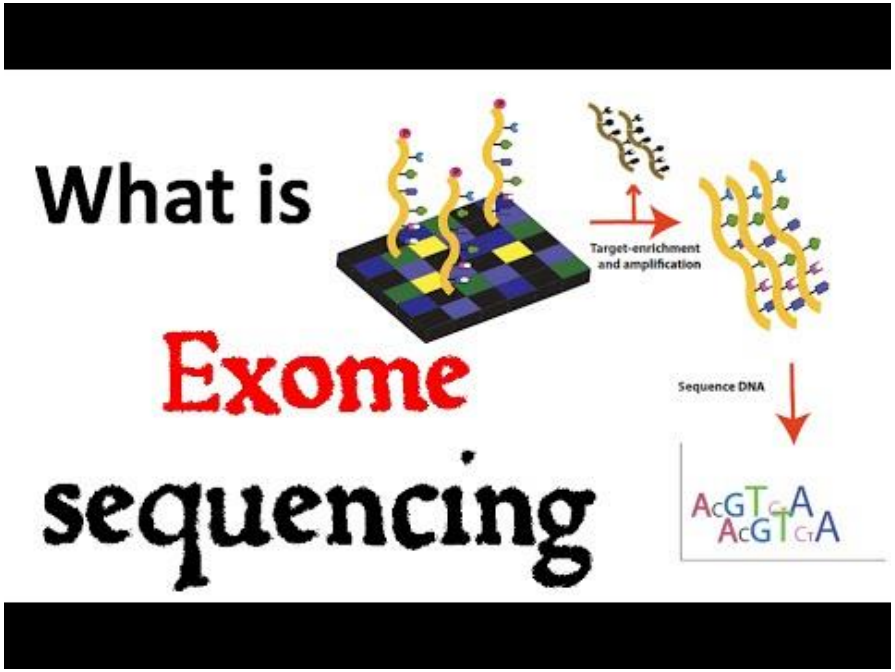


Coding DNA = Exome ~ 1%

85% of genetic diseases

Whole exome sequencing WES

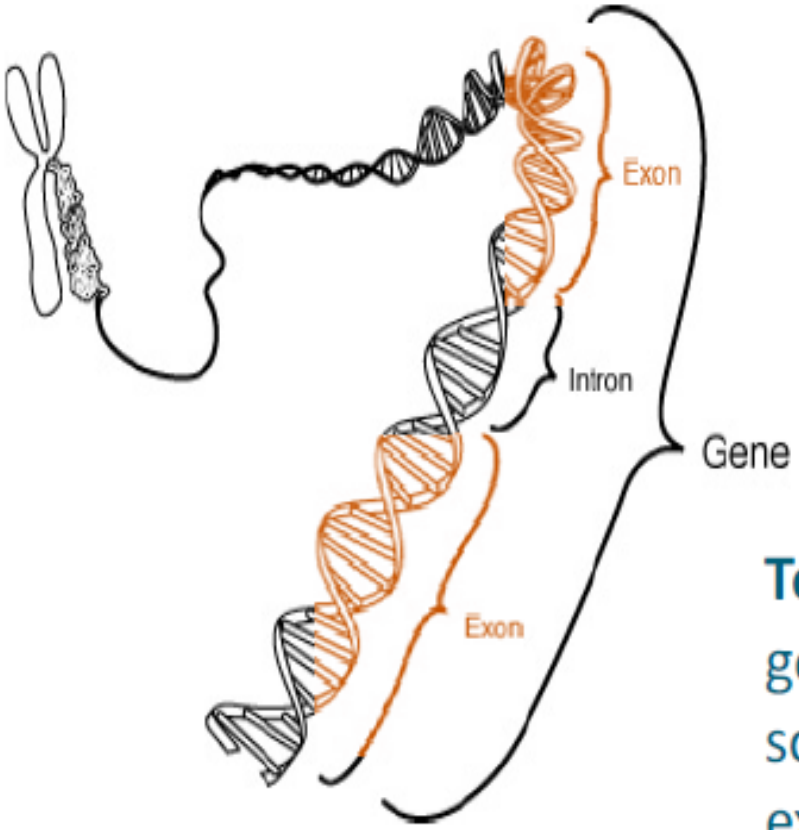
- **Specificity** : false-positive rate [FPR]
  - not a major issue in NGS, confirmation by Sanger sequencing (99,9%)
- **Sensitivity** : false-negative rate [FNR]
  - Critical outcome parameter
    - GC rich regions
    - Repeat regions
- WES provides coverage for more than 95% of human exons to investigate the protein-coding regions (CDS)



séquençage d'exomes

'Exome' = ensemble des exons d'un génome

~1.5 % du génome humain



Toutes les parties codantes d'un génome humain (>180,000 exons), sont séquencées lors d'une seule expérience

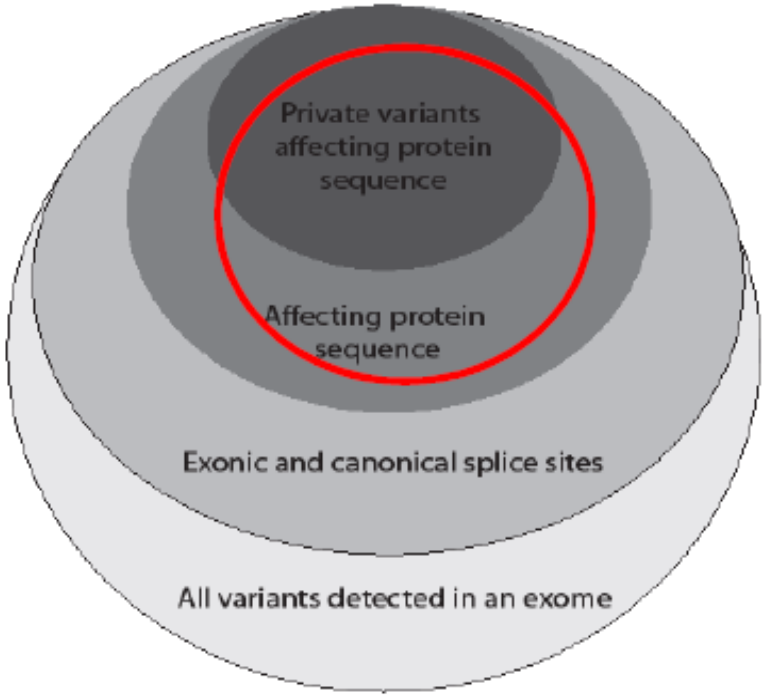
Séquençage d'exomes– nombre de variants identifiés

- Nombre total de variants codants:

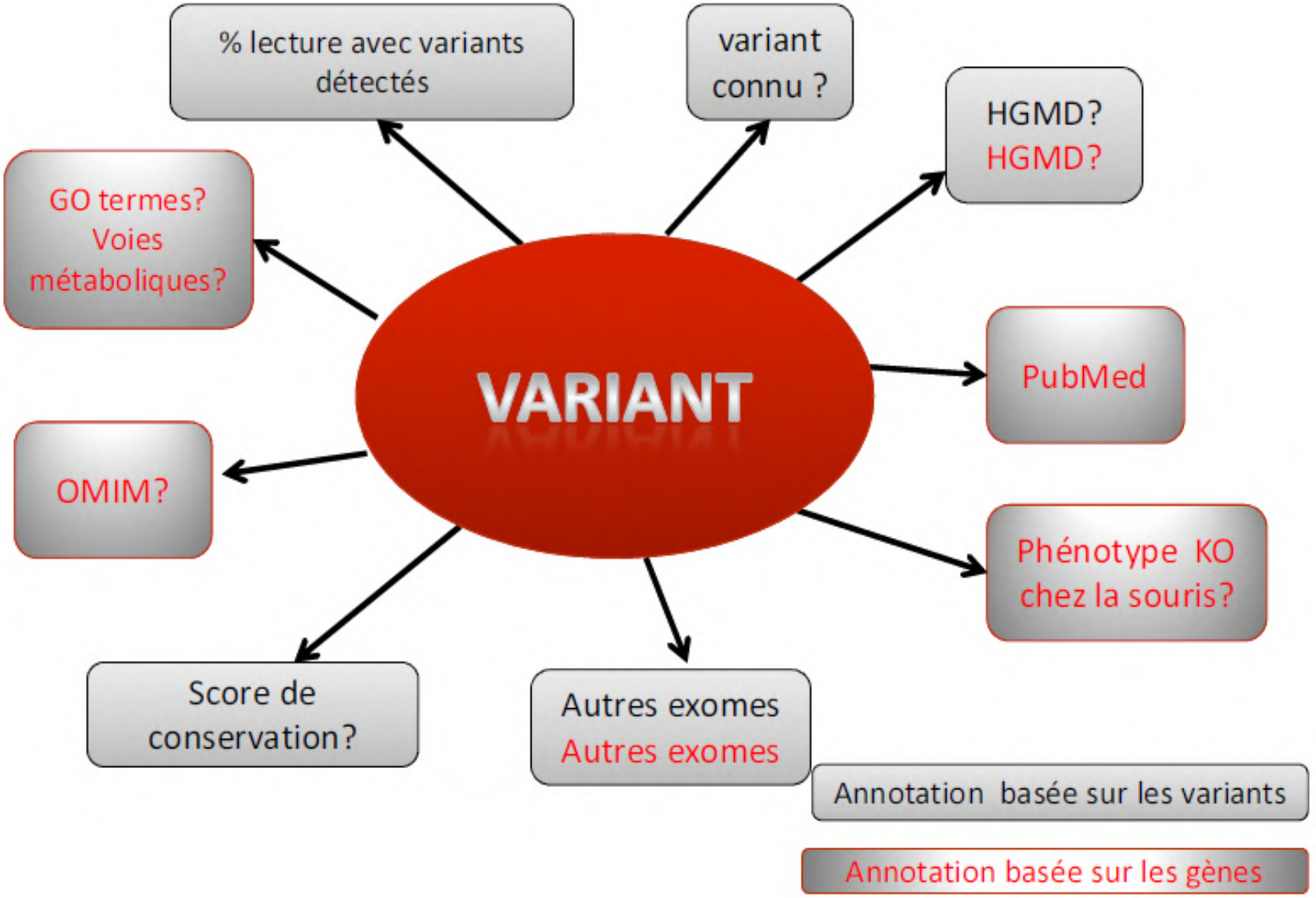
  ~ 12,000

- **Variants privés* (non-synonymes):**

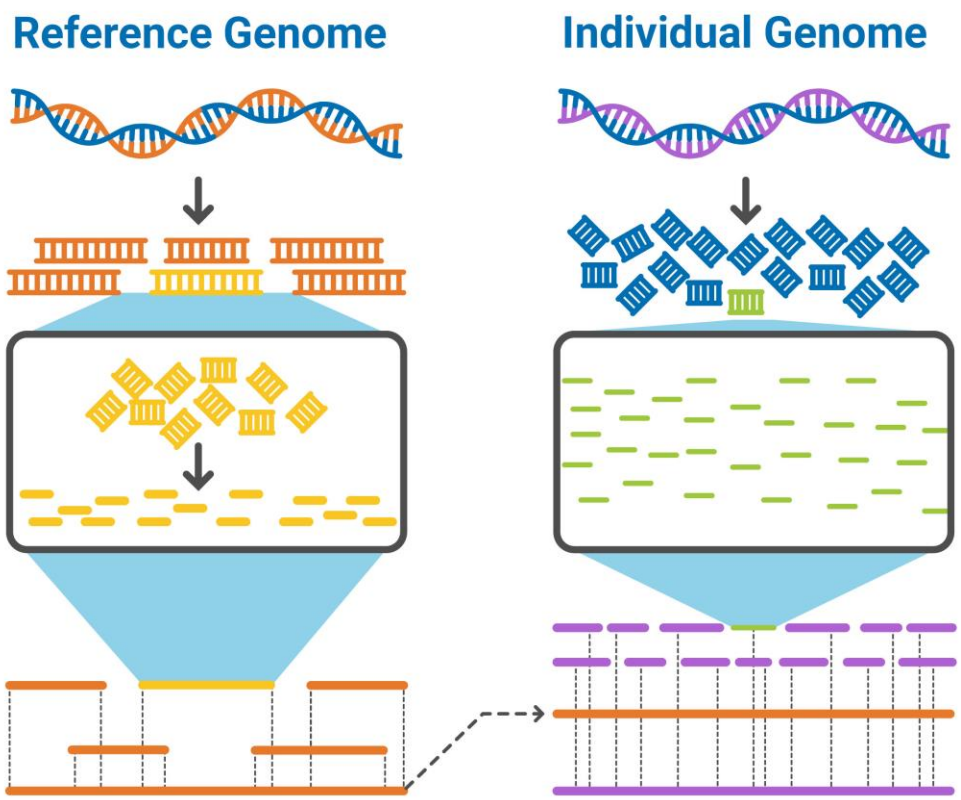
  ~ 150-200



*: *jamais identifés localement*, absents de la database de SNP


% lecture avec variants détectés
variant connu ?
HGMD?
HGMD?
GO termes?
Voies métaboliques?
VARIANT
PubMed
OMIM?
Phénotype KO chez la souris?
Score de conservation?
Autres exomes
Autres exomes
Annotation basée sur les variants
Annotation basée sur les gènes

WHOLE GENOME SEQUENCING



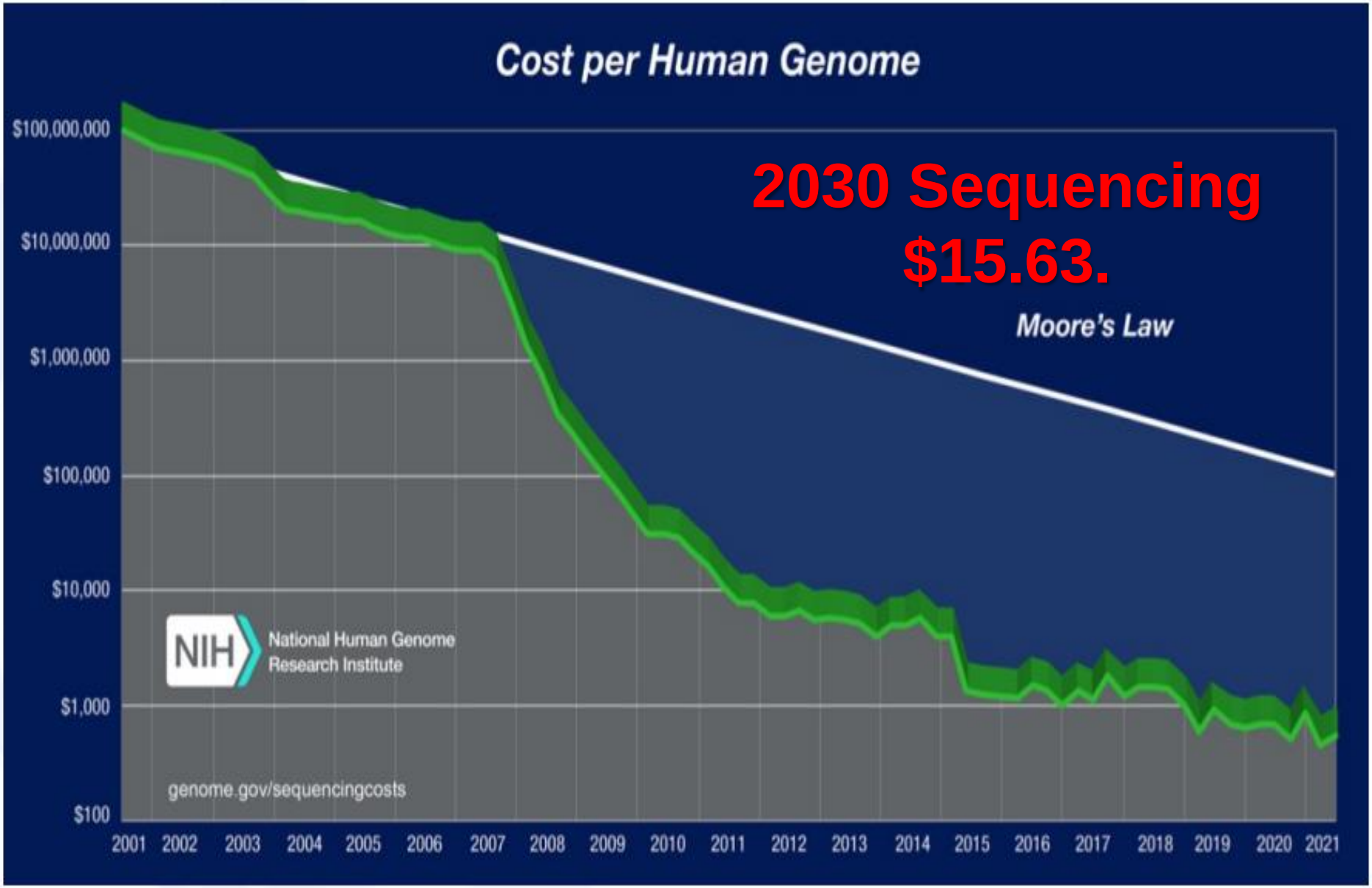
1. Break genome into large fragments and clone
2. Break individual clone into small fragments
3. Generate thousands of sequence reads
4. Assemble sequence reads for each clone

Reference genome

1. Break genome into small fragments
2. Generate millions of sequence reads
3. Align sequence reads into a reference genome

Individual genome

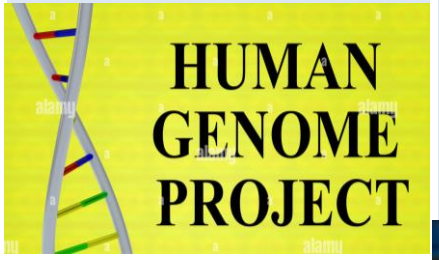
Coût du séquençage nouvelle génération (**NGS**) Génome Humain



Human genomic data past 30 years

1990–2000

Launch of the « Human Genome Project and related endeavors ».



2000–2010

- Law
- Ethics
- Research infrastructures (biobanks)
- Citizenship and ‘public goods’

2010–2020

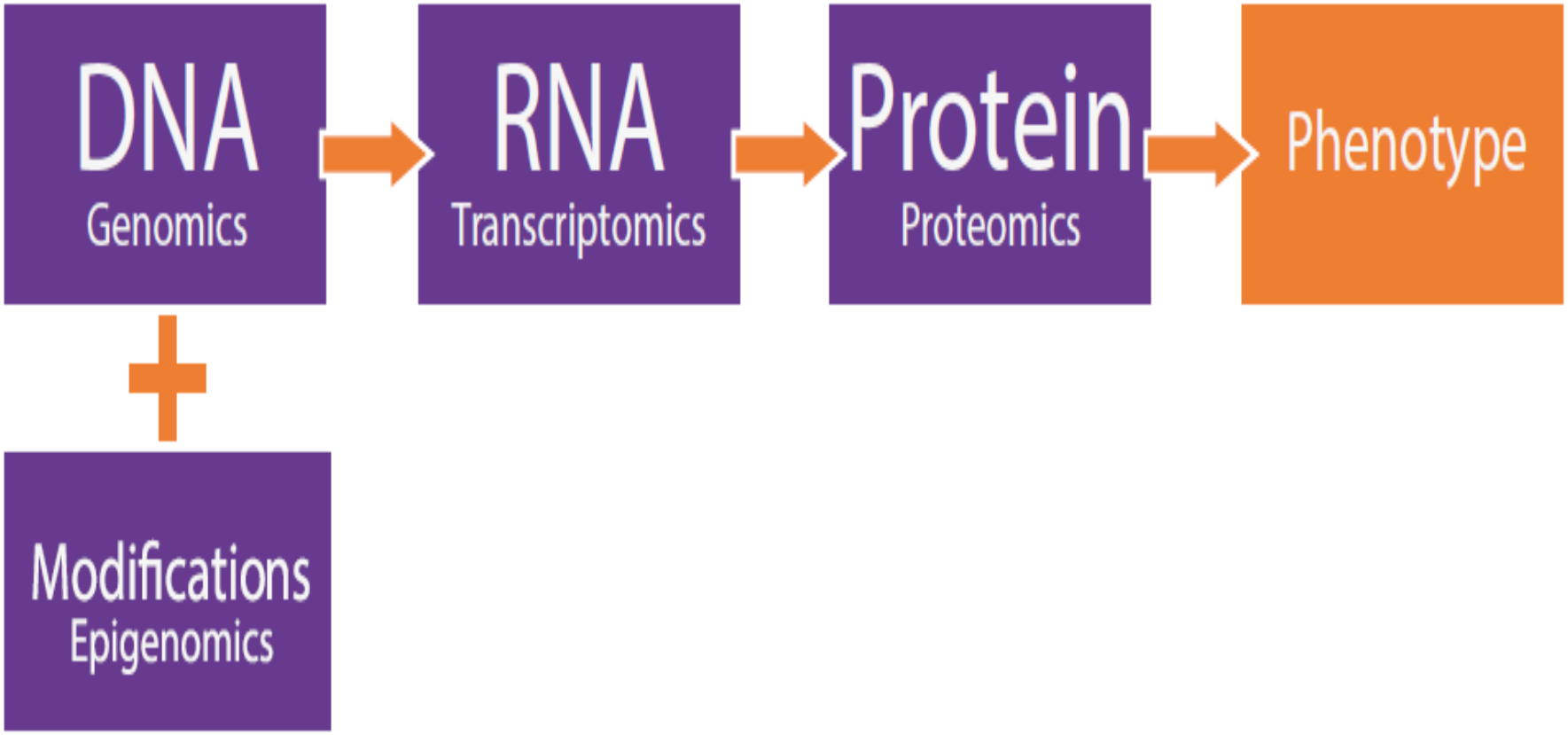
Genetic privacy in response of large international research consortia and big data.

2020……2050….2100….

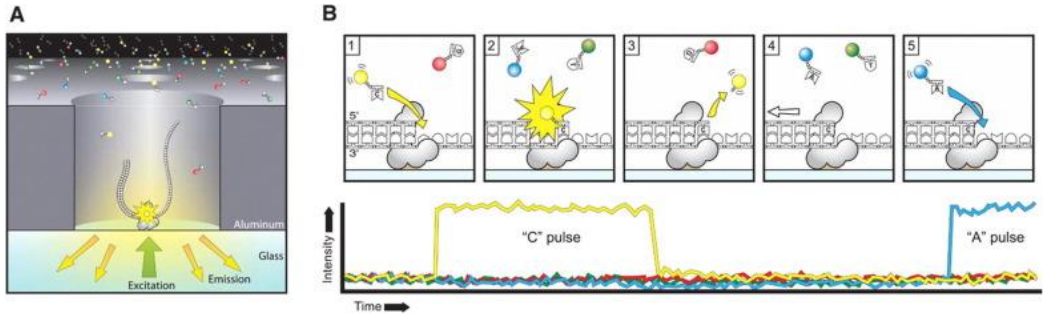
- **Big Data**
- **Artificial intelligence (AI)**
- **Gene and cell therapies**
- **Nanotechnology**

Bartha Maria Knoppers , Michael J S Beauvais Three decades of genetic privacy: a metaphoric journey Hum Mol Genet2021 Oct 1;30(R2):R156-R160. doi: 10.1093/hmg/ddab164.

The landscape of genomic technologies in healthcare and biomedical research



PacBio Sequencing



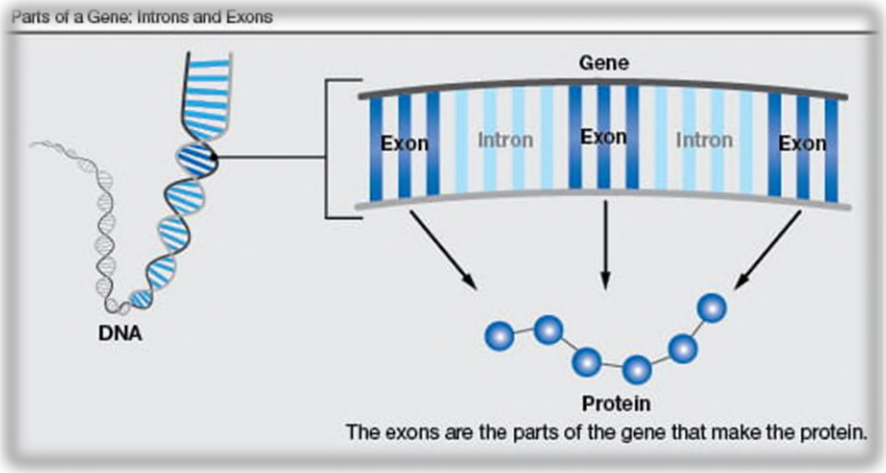
华大基因
BGI

Nanopore sequencing

Genomics and sequencing of DNA

Whole genome sequencing (WGS)

Whole exome sequencing (WES)



	Where we are today	Where we will be in 2030
Clinical applications		
Genomics for disease	Primarily limited to rare disease and select cancers.	Genomics is routine. Genetic causes and targeted therapies are discovered for many "common" diseases. Microbiome measures are routinely included.
Pharmacogenomics (PGx)	Common in cancer and within select applications of older medications at select sites.	Genome-aware EHRs make PGx easy and automatically update rules from central guidelines. New PGx associations discovered from clinical data.
Genomics for healthy individuals	In research, whole-genome sequencing and search for mutations in one of the ACMG59 genes, present in about 3% of people. Variant interpretation is hard.	ACMG59 grows to > 200, variant interpretation improved by huge, diverse sequenced populations. Cell-free DNA becomes a mainstay of cancer screening
EHRs	Episodic capture from healthcare without robust genomics support. EHR data is essentially not portable.	Genome- and device- enabled. Data can be easily moved between EHRs and to participant apps.
Environmental influences on health	Patient-reported habits and exposures	Geocode-based exposure linkage Real time monitoring of multiple environmental exposures Precision nutrition
Wearable sensors	Ad hoc use of activity monitors	Continuous monitoring of physical activity, sleep, metabolic parameters

Routine clinical genomics to guide prevention, diagnosis, and therapy

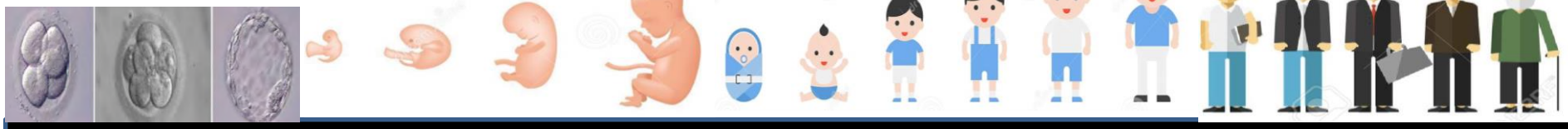
Sequencing $15.63.

American College of Medical Genetics and Genomics (ACMG)

Electronic health record (EHR)

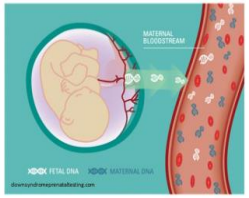
Joshua C Denny 1, Francis S Collins 2 Precision medicine in 2030-seven ways to transform healthcare 2021 Mar 18;184(6):1415-1419.

« Personalized Medicine & Medical Genetics »



Preimplantation genetic diagnosis (PGD)

Noninvasive prenatal testing (NIPT)



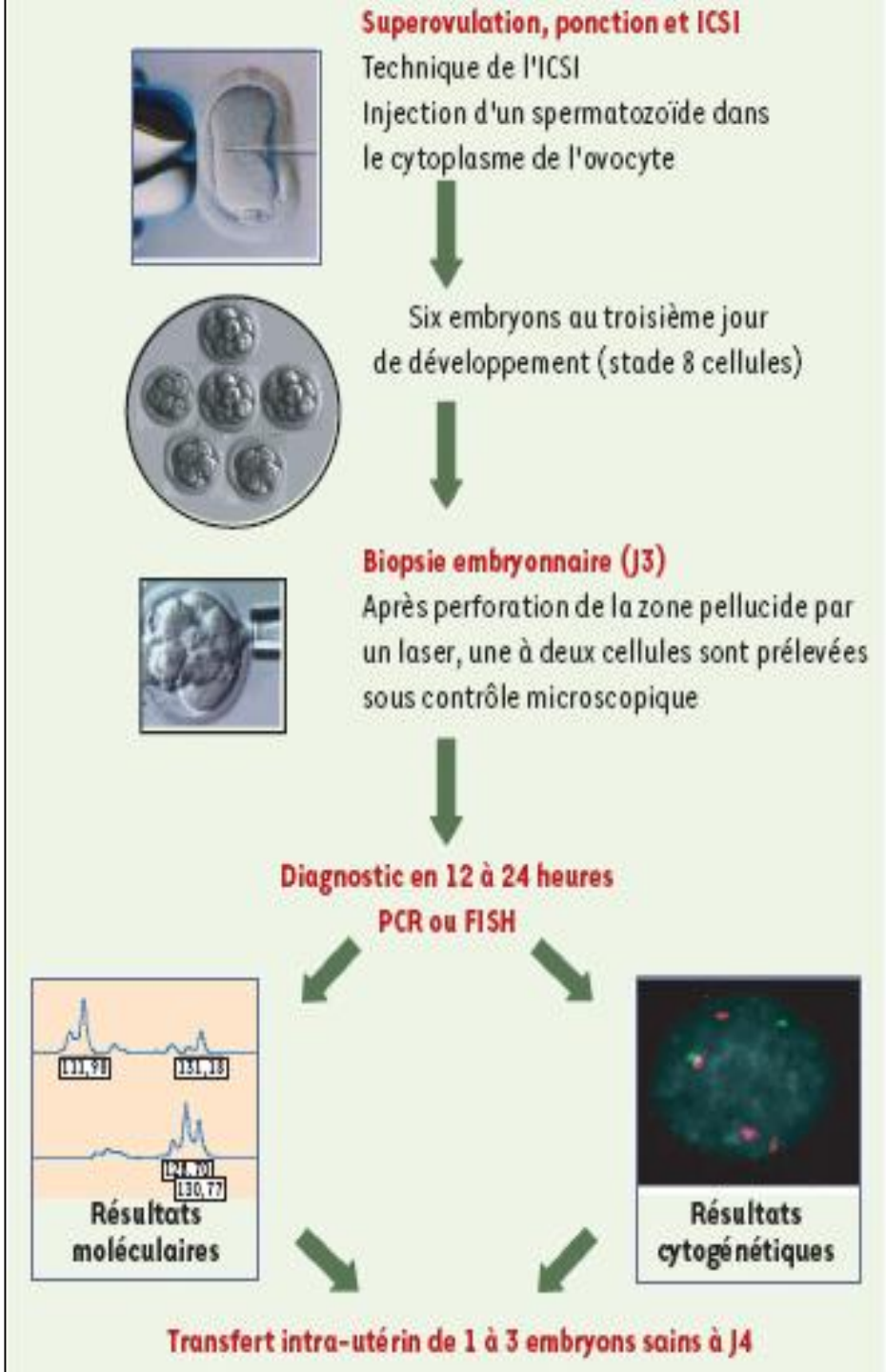
Fetal medicine

Newborn screening (NBS)

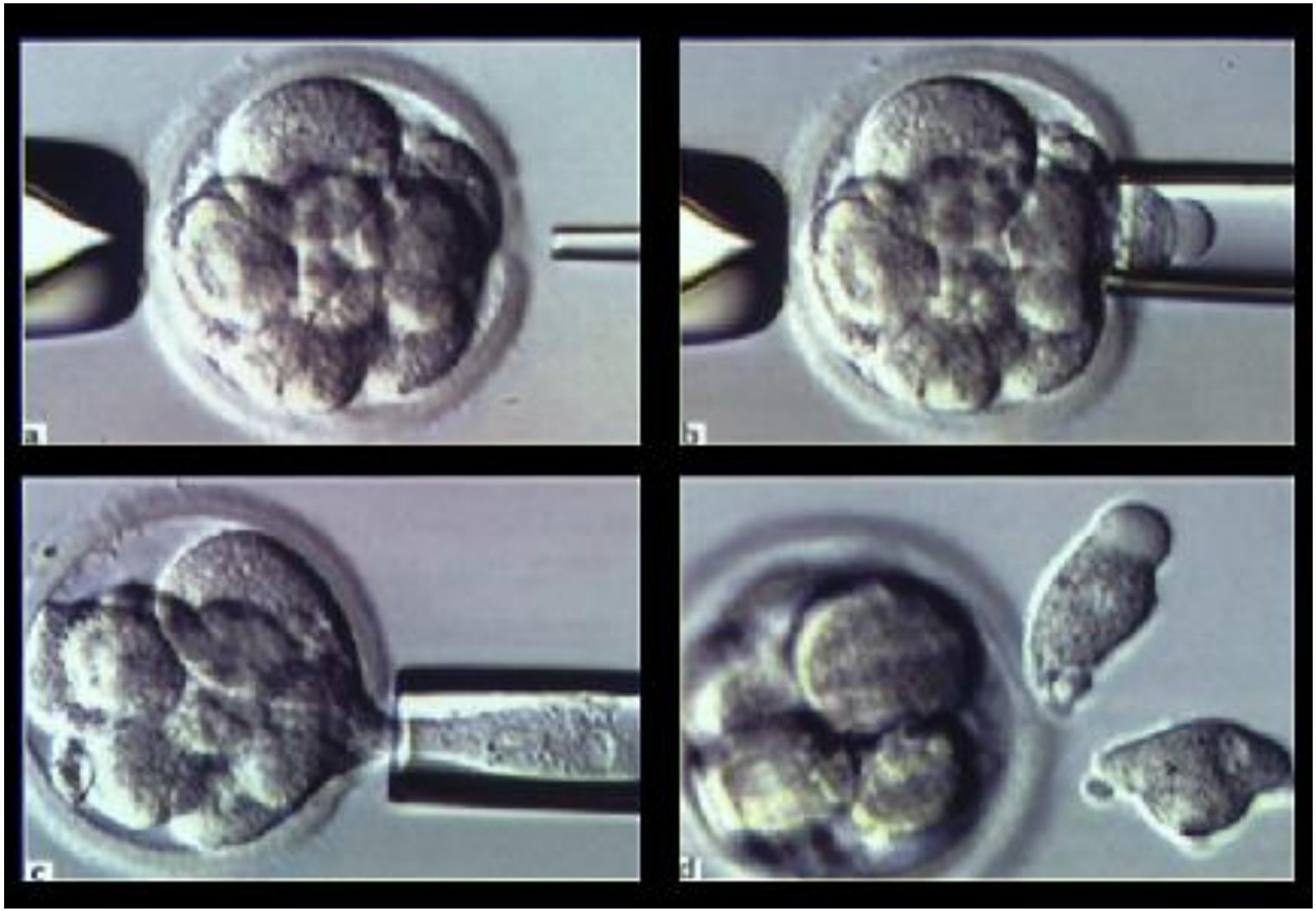
Precision medicine

Diagnostic préimplantatoire

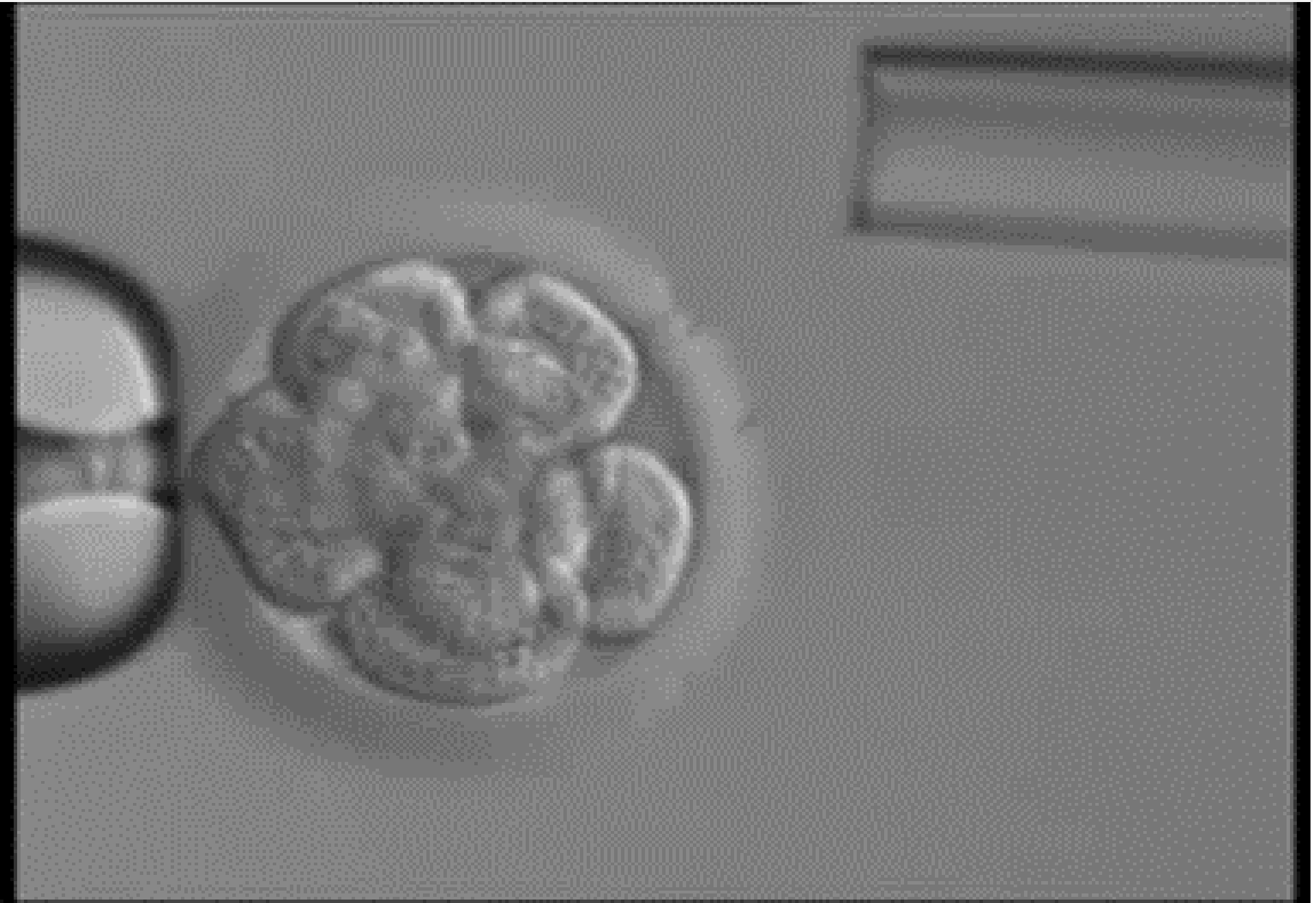
- Fécondation in vitro
- Prélèvement d'un blastomère au stade morula
- Test génétique sur 1 cellule
- ADN



Blastomère



Preimplantation Genetic Diagnosis (PGD)



Preimplantation genetic (PG) testing

Who benefits from PG testing?

PG testing has two broad categories

PG diagnosis

Tests embryos for specific genetic abnormalities that have been shown to exist in one or both parents

Purpose is to prevent the birth of affected children from parents with a known genetic abnormality

Widely acknowledged as acceptable for routine clinical application

PG screening

Tests for aneuploidy in embryos; parents have no diagnosed genetic abnormality

Purpose is to identify optimal embryos for uterine transfer in an IVF cycle and, in so doing, improve pregnancy success in certain patient populations

Its routine clinical application remains controversial

Brezina PR Brezina DS, Kearns WG Preimplantation genetic testing. BMJ. 2012 Sep 18;345

Preimplantation genetic (PG) testing

What is PG diagnosis?

PG diagnosis is the testing of embryos for **specific genetic abnormalities known** to exist **in one or both parents**

Diagnosis is appropriate for:

- Autosomal recessive diseases in which both parents are known genetic carriers, such as cystic fibrosis or sickle cell disease
- Autosomal dominant diseases in one or both parents, such as Huntington's disease
- X linked diseases (such as haemophilia)
- Any parent who harbours certain balanced chromosomal translocations or inversions

Diagnosis is not appropriate for:

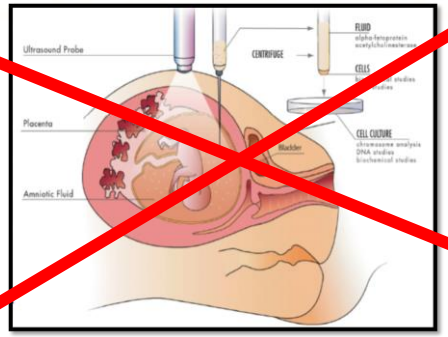
- Medical conditions in parents in whom a definitive genetic cause has not been identified
- Testing for non-medical phenotypic traits, such as eye or hair colour

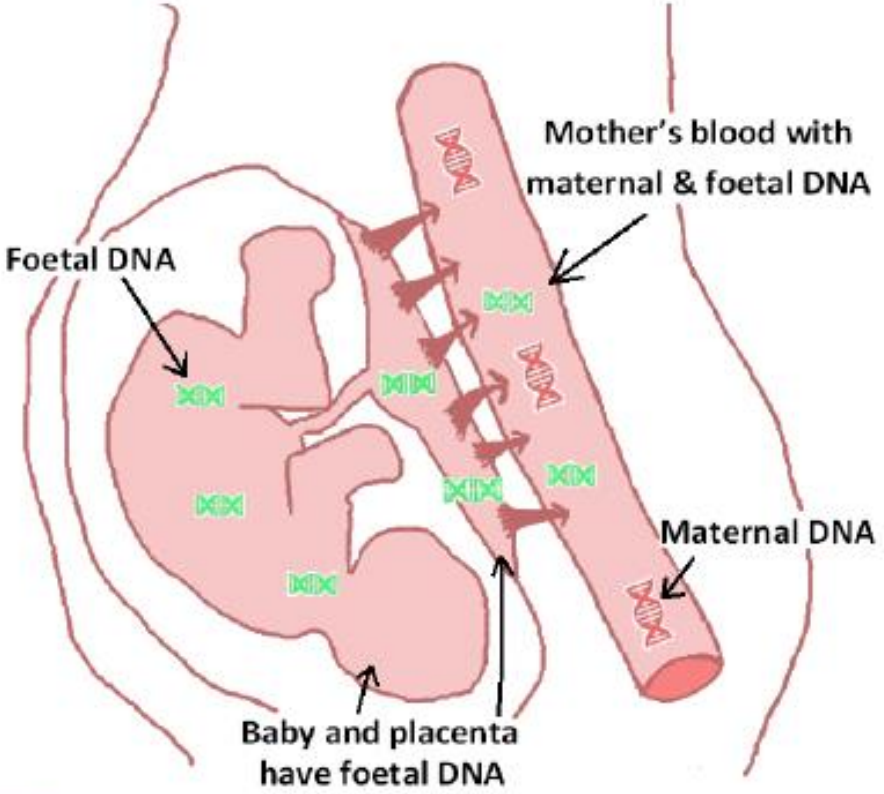
Diagnosis is controversial for:

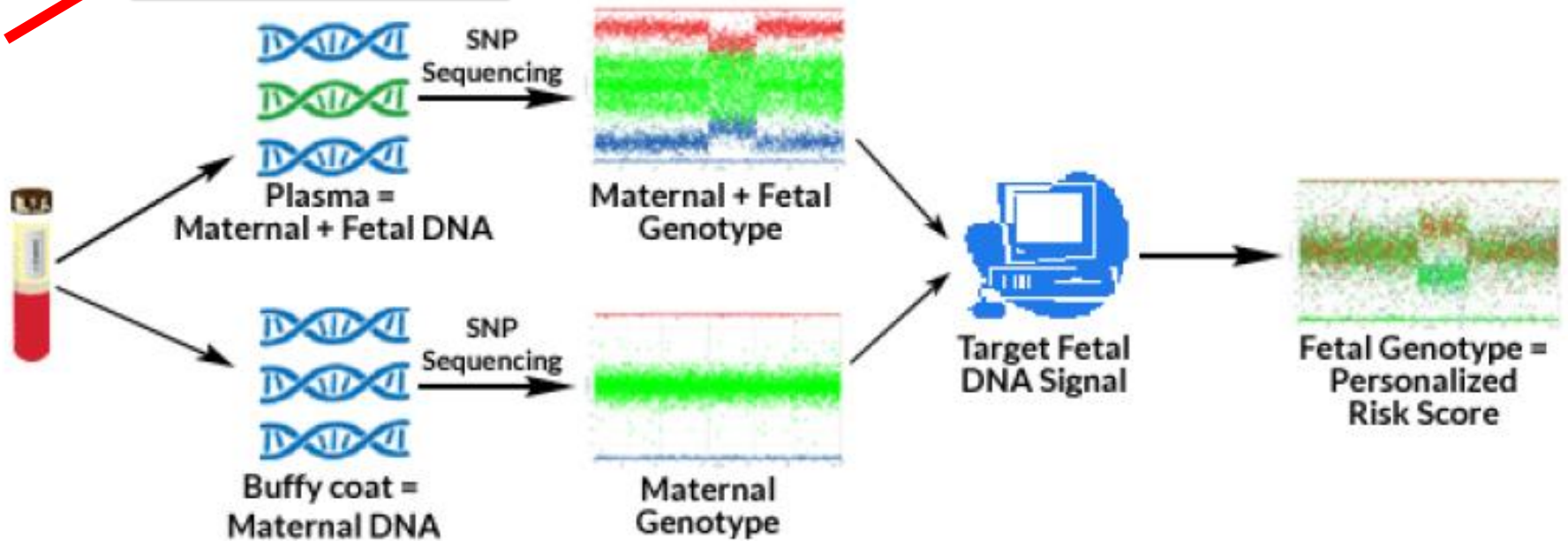
- Sex selection for the purposes of family balancing
- HLA matching for the purposes of creating a tissue donor for an existing diseased sibling

Brezina PR Brezina DS, Kearns WG Preimplantation genetic testing. BMJ. 2012 Sep 18;345

NIPT: non-invasive prenatal test







Diagnostic prénatal non invasif

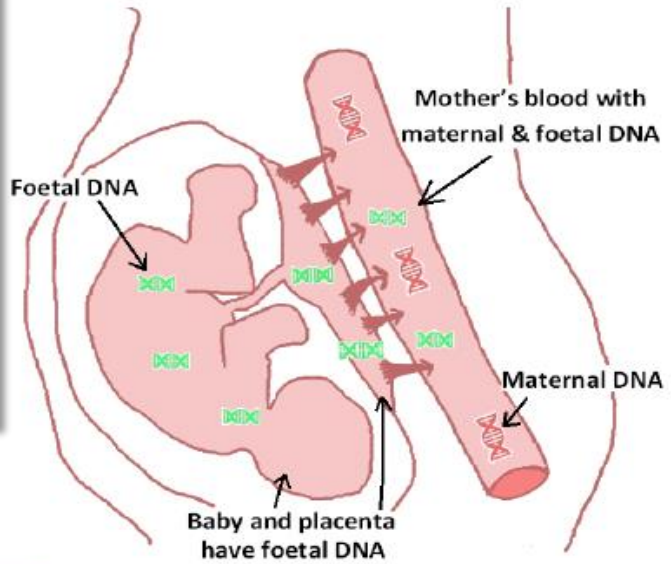
Clinical information: normal pregnancy, gestational age: 12 weeks, maternal weight: 66kg, maternal height: 163cm.

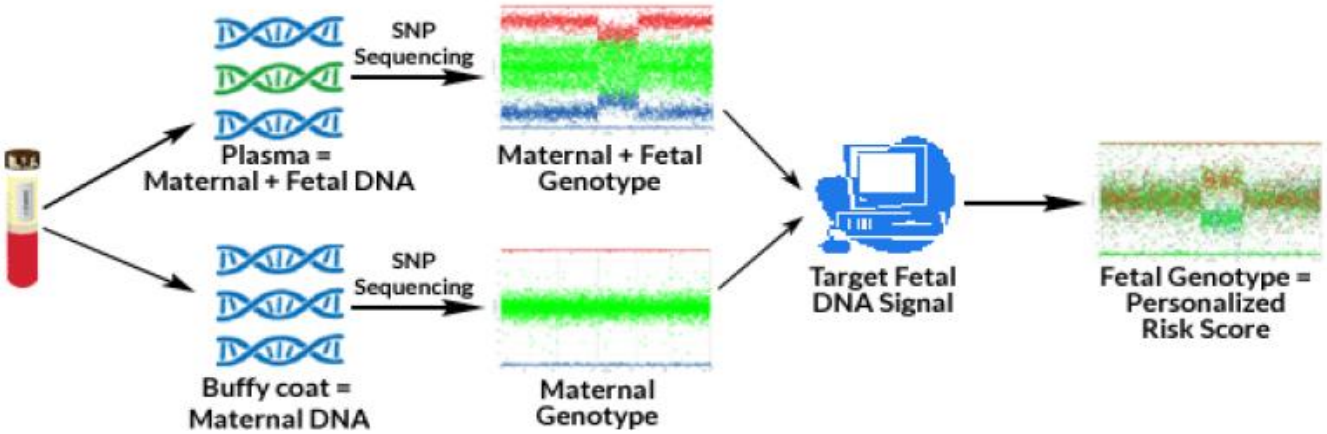
Results:

	Aneuploidy detected (Y/N)
Chromosome 21	No
Chromosome 18	No
Chromosome 13	No
Gonosomal chromosomes	No
Fetal Fraction (xx %):	7%
Fetal gender	male

The analysis did not indicate a trisomy of chromosome 13, 18, or 21 or gonosomal abnormalities.

However, the test cannot entirely exclude this due to the possibility of fetoplacental mosaicism. If the fetus shows abnormalities on ultrasound investigation, or if a family history of fetal abnormalities or other genetic disorders exists, invasive testing and subsequent analysis by karyotyping or additional genetic analysis should be considered.





Male infertility…… Genetic factors

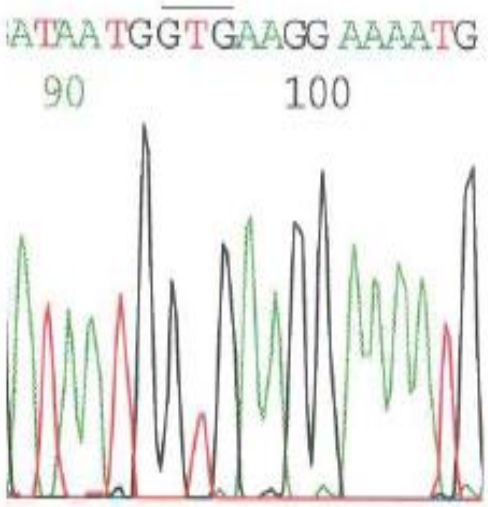
The common Cresponsible for male infertility are :

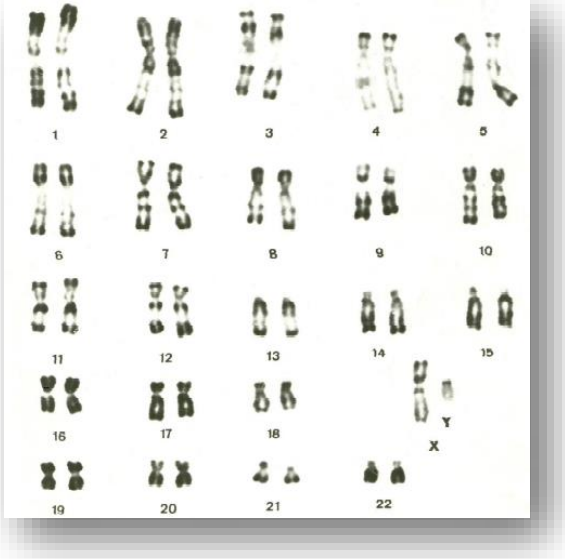
- **Chromosomal abnormalities**
- **Yq microdeletion**
- **Cystic fibrosis.**

30 % **percent cases of male infertility.**

- **About 40 % cases of male infertility are categorized as idiopathic :**

Genetic and genomic abnormalities.



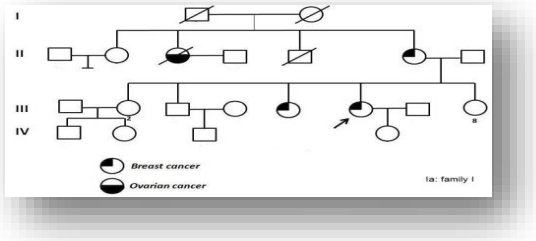


Halder A, Kumar P, Jain M, Kalsi AK. Genomics: Tool to predict and prevent male infertility. Front Biosci (Schol Ed). 2017 Jun 1;9:448-508.

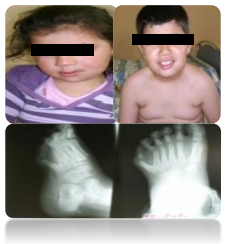
Couples with ..... Male infertility

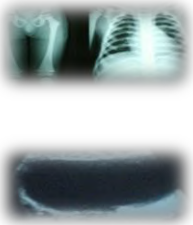
General considerations on genetic testing

Clinical Genetics



Genetic consuling





Clinical Diagnosis

Genetic counseling

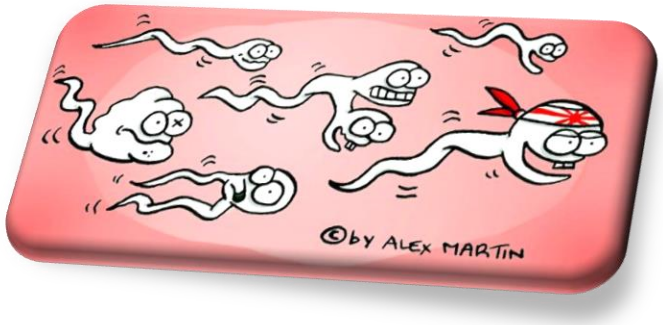
Genetic testing

Familial history

Clinical-genetic examinations

Genetic counseling, focusing on an extensive evaluation of the familial history and, if necessary, clinical-genetic examinations, is required in order to decide whether and which further genetic testing is appropriate for the couple.

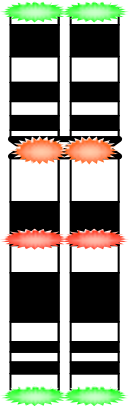
Male infertility..... Medical Genetics

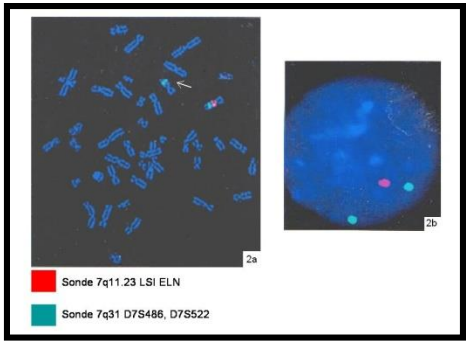


- Chromosomal abnormal
- Yq microdeletion
- Copy number variations (CNVs)
- Monogenic
- Multifactorial
- Mitochondrial
- Epigenetic abnormalities.

Chromosome analysis

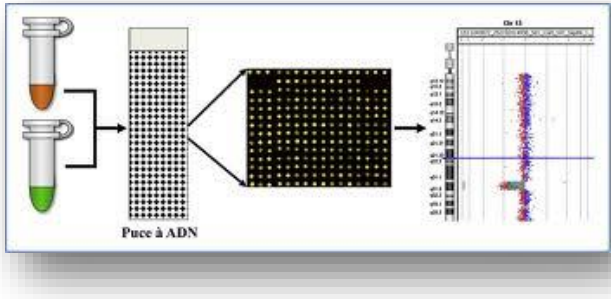
FISH





CGH arrays ACPA

Copy Number Variations (CNVs),



DNA sequencing

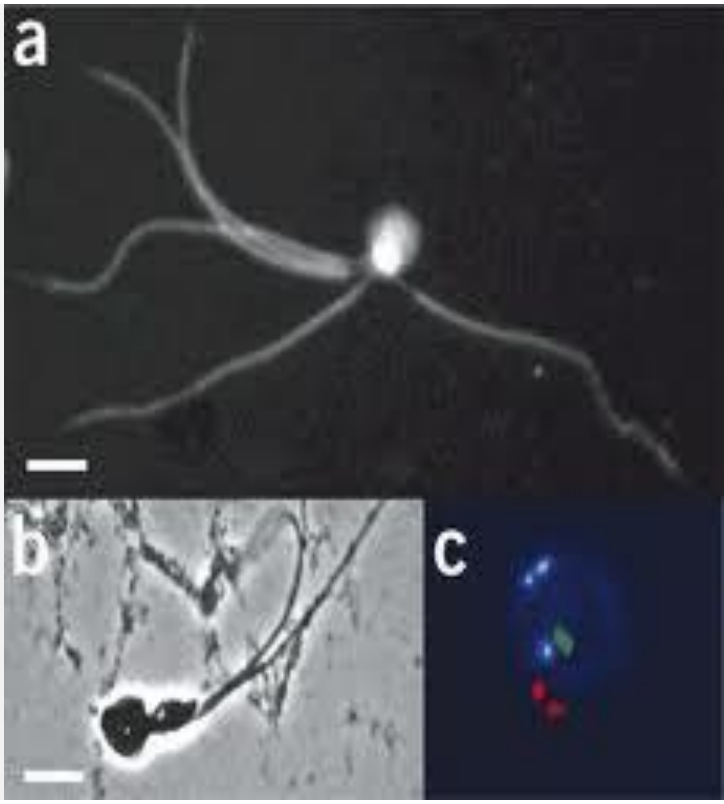
ACTGACTGACTG

GENETIC CAUSES OF MALE INFERTILITY

Testicular failure

Mutations AURKC gene

- **Infertility in men from the Maghrebian region :**
  - Morocco,
  - Tunisia
  - Algeria
- **Homozygous c.144delC AURKC**
- **Sperm with :**
  - 4N chromosomal complement,
  - large heads
  - often multiple tails.



Homozygous mutation of AURKC

Dieterich K, Soto Rifo R, Faure AK, Hennebicq S, Ben Amar B, Zahi M, Perrin J, Martinez D, Sèle B, Jouk PS, Ohlmann T, Rousseaux S, Lunardi J, Ray PF. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. Nat Genet. 2007 May;39(5):661-5. Epub 2007 Apr 15.

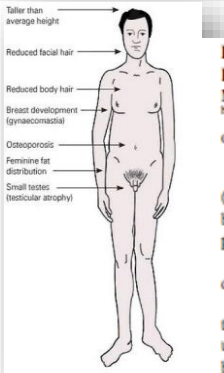
GENETIC CAUSES OF MALE INFERTILITY

Testicular failure

Aneuploidy

Klinefelter syndrome (47,XXY)

- Clinical and cytogenetic heterogeneity :
  - 47,XXY
  - mos 47,XXY/46,XY ….



- Progeny of XXY men by ICSI (intracytoplasmic sperm injection) after TESE (testicular sperm extraction) have been described.
- They have an increased risk for gonosomal aberrations.

47,XXY

HEREDITARY RISK IN ICSI WITH SPERM FROM NON-MOSAIC KLINEFELTER SYNDROME PATIENTS. T. Miki,[a] A. Tanaka,[a] M. Nagayoshi,[a] S. Watanabe.[b] [a]Saint Mother Hospital, Kitakyusyu, Japan; [b]Anatomical Science, Hirosaki University Graduate school of Medicine, Hirosaki, Japan.

OBJECTIVE: To verify the actual risk of hereditary Klinefelter Syndrome (KS) or incidental aneuploidy in ICSI treatment of KS patien… which has been warned in previous cytogenetic studies using testic… om KS patients.

DESIGN: Cytogenetic analysis in KS patien… nd delivered babies.

MATERIALS AND METHODS: 1) … treatment of KS patients in our ho… using amniocentesis or periph… bridization (FISH) analy… out in morphological… identified and is… tients, respe… ents an… de…

O-160 Tuesday, October 18, 2016 12:00 PM

HEREDITARY RISK IN ICSI WITH SPERM FROM NON-MOSAIC KLINEFELTER SYNDROME PATIENTS. T. Miki,[a] A. Tanaka,[a] M. Nagayoshi,[a] S. Watanabe.[b] [a]Saint Mother Hospital, Kitakyusyu, Japan; [b]Anatomical Science, Hirosaki University Graduate school of Medicine, Hirosaki, Japan.

…were … KS patients, and their parents … chromosome was de… s, according to the manufacturer's … extracted from blood sample… sent. Multiplexed PCR amplification … ducted using an Investigator Argus … resis was run on an ABI PRISM 3100 … products. The data obtained was analyzed … ware.

… chromosomal abnormality was found in 45 KS patients examined. 2) In the most of 5 KS patient's testes examined, sp… osaki, Japan … showed XY and XXY mosaicism. However, the sex chromosome constitution of all primary spermatocytes and spermatids was normal, suggesting the possibility that XXY spermatogonia can not enter meiosis. In one case, there were no XXY spermatogonia. 3) X-chromosomal STR DNA profiles were compared among KS patient and their parents. In 3 of the 4 KS patients, both two X chromosomes were maternal origin, showing that an extra X chromosome was left in an oocyte as a result of chromosomal non-disjunction at the 1st or 2nd meiotic division. In one patient, X-chromosomes were inherited from parents, suggesting that fertilization of XY-sperm is the cause of KS. In addition, it was surmised that 12 cases in 18 patients showed maternal origin and 6 in 18 patients were paternally. Although the sample number applied for X-chromosomal STR DNA profiling is not enough, the present data may indicate that contribution of XX oocyte to the production of XXY embryos is greater than XY sperm. Namely, a XX oocyte penetration by a Y sperm is the main cause of KS.

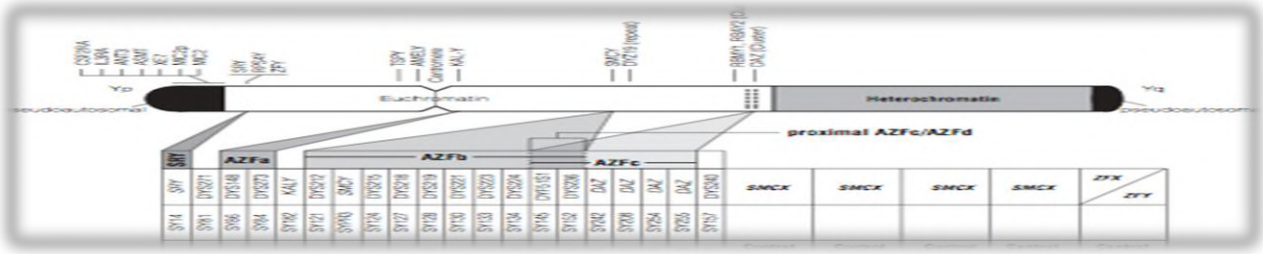
CONCLUSIONS: All data indicates that the risk of KS baby resulting from ICSI treatment of KS patient couples may be a lot lower than expected from the previous studies. Cytogenetic analysis with smear of testicular cell mixture that was used in the studies may overestimate chromosomal abnormality. This finding encourages the opinion that we should strongly recommend vigorous treatment with ART for KS patients.

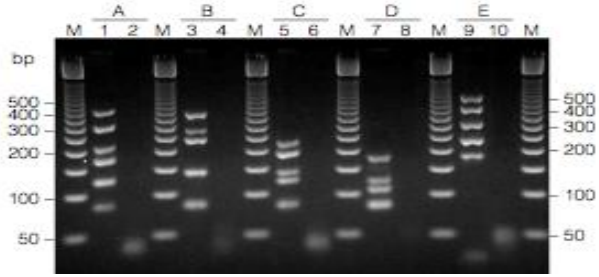
Couples with infertility

2. Genetic testing in male infertility

Molecular genetic testing

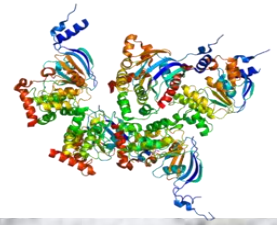
Azoospermia factor





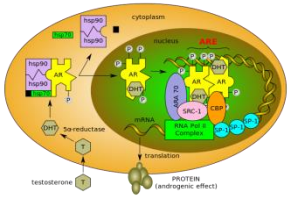
Cystic fibrosis gene mutations

Homozygous or compound heterozygous for CFTR mutations.



Further mutations and syndromes

Several hundred mutations of AR have been described with resultant phenotypes ranging from testicular feminization to partial androgen insensitivity syndrome to male infertility

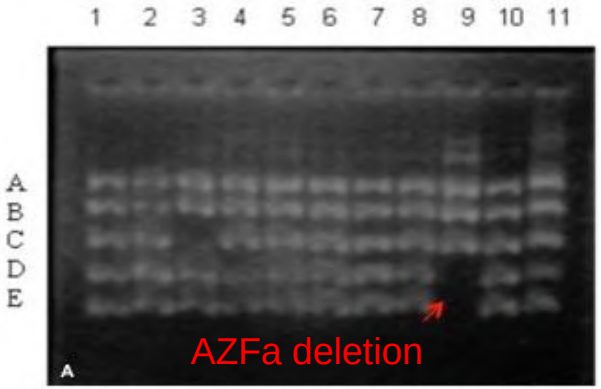


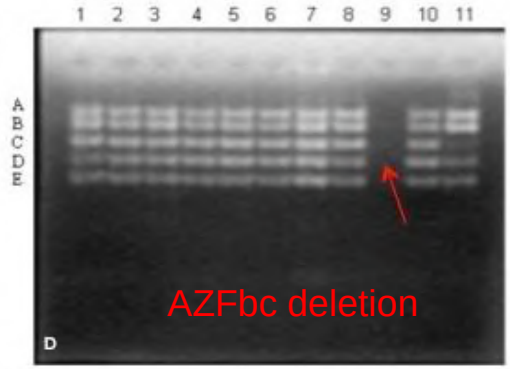
GENETIC CAUSES OF MALE INFERTILITY

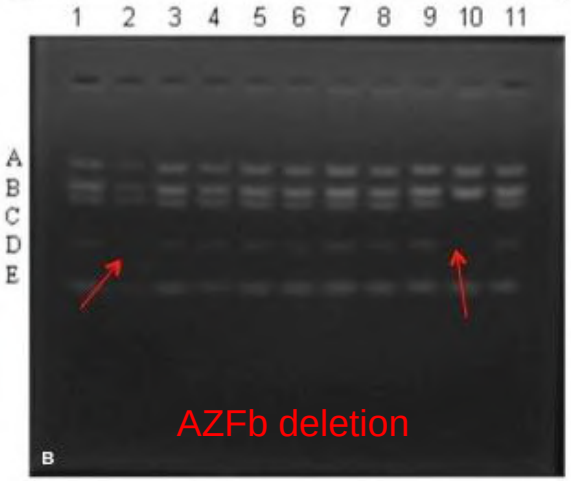
Testicular failure

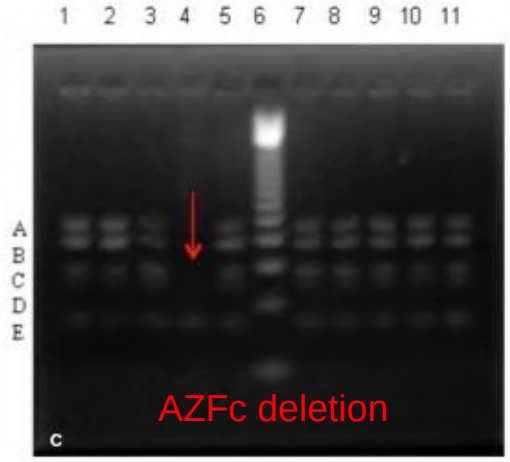
Azoospermia factor

Microdeletions of the long arm of the Y chromosome are now recognized as a relatively common cause of primary testicular failure (severe oligospermia and azoospermia), affecting up to 20% of men with infertility









A summary of potential gene biomarkers involved in male infertility.

Gene	Name	Location
AZF	Azoospermia factor	Yq11
CFTR	Cystic fibrosis transmembrane regulator	7q31.2
SHOX	Short stature homeobox	Xp22.33; Yp11.3
USP8	Ubiquitin-specific peptidase 8	15q21.2
UBD	Ubiquitin D	6p21.3
EPSTI1	Epithelial-Stromal interaction 1	13q13.3
LRRC32	Leucine-rich repeat containing 32	11q13.5–q14
PDE3A	Phosphodiesterase 3A	12p12
EFCAB4B	EF-hand calcium-binding domain 4B	12p13.32
COBL	Cordon-bleu WH2 repeat protein	7p12.1
ATP8A1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	4p13
MASP1	Mannan-binding lectin serine peptidase 1	3q27–28
PROK2	Prokineticin 2	3p13
AHRR	Aryl-hydrocarbon receptor repressor	5p15.3
MTHFR	Methylenetetrahydrofolate reductase	1p36.3
UBE2B	Ubiquitin-conjugating enzyme E2B	5q31.1
CREM	cAMP responsive element modulator	10p11.21
TSPY1	Testis-specific protein, Y-linked 1	Yp11.2
CLU	Clusterin	8p21–p12
PRM2	Protamine 2	16p13.2
PSG1	Pregnancy-specific beta-1-glycoprotein 1	19q13.2
HLA-E	Major histocompatibility complex, class I, E	6p21.3
PLCD1	Phospholipase C, delta 1	3p22–p21.3
ADD1	Adducin 1 (alpha)	4p16.3
ACVRL1	Activin a receptor type II-like 1	12q13.13
AR	Androgen receptor	Xq12
ARNT	Aryl hydrocarbon receptor nuclear translocator	1q21
hCAP18	cAMP cathelicidin antimicrobial peptide	3p21.3
SPIN1	Spindlin 1	9q22.1
TEX101	Testis expressed 101	19q13.31
PGK2	Phosphoglycerate kinase 2	6p12.3
HIST1H2BA	Histone cluster 1, H2ba	6p22.2
SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14	12p13.31
SPACA3	Sperm acrosome associated 3	17q11.2
GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	19q13.12
AKAP4	A kinase (PRKA) anchor protein 4	Xp11.2
SPAG11B	Sperm-associated antigen 11B	8p23.1
SAMP32/SPACA1	Sperm acrosome associated 1	6q15

Kovac. Biomarkers of male factor infertility. Fertil Steril 2013.

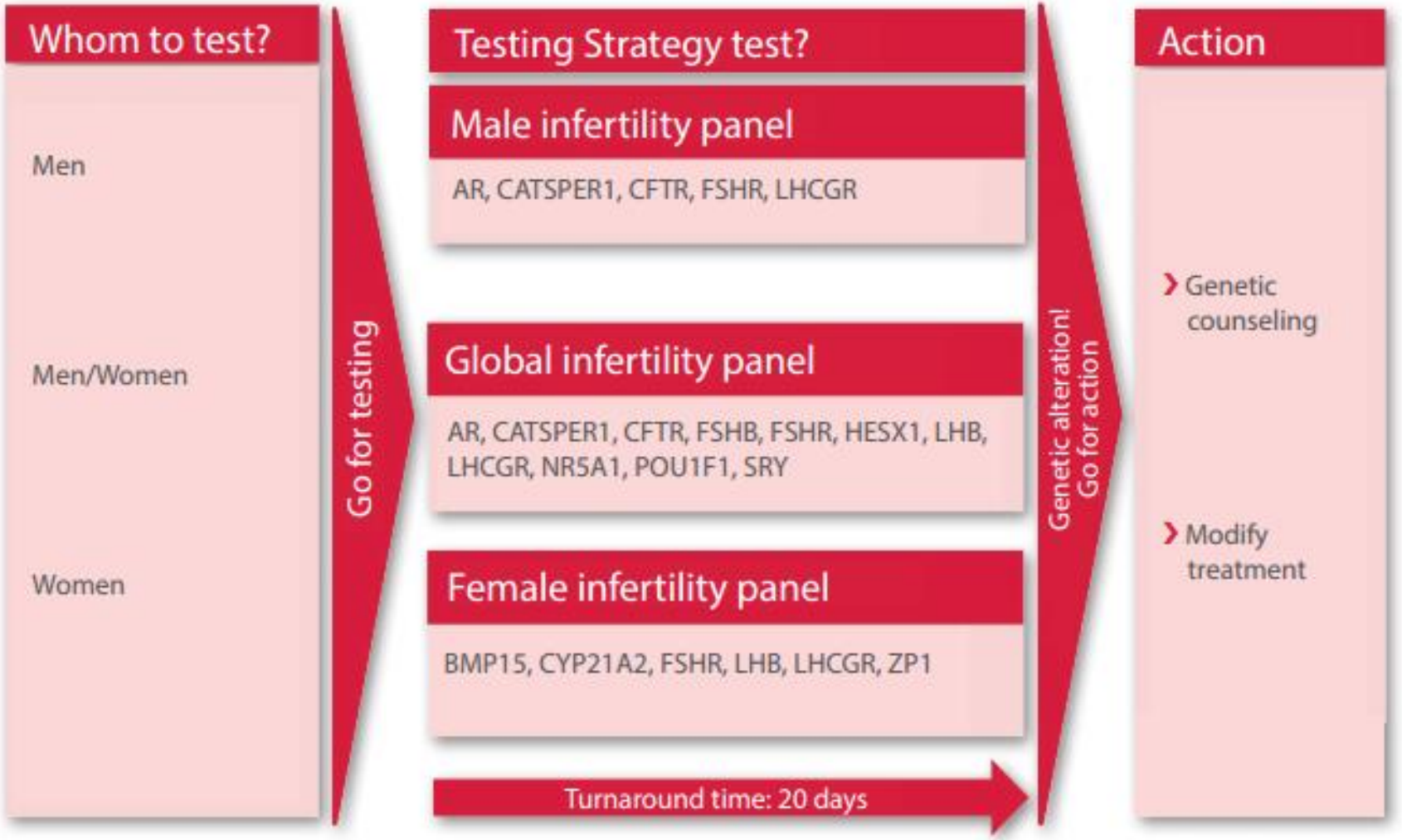
Séquençage
nouvelle génération
Haut débit
NGS

Diagnostic strategy

Postnatal karyotype

FISH postnatal (CEP X, LSI SRY,, …)

Comparative Genomic Hybridisation arrays : CGHarrays



Dépistage néonatal de la surdité
Stratégie « Génétique Médicale »

Surdité non syndromique

Surdité syndromique

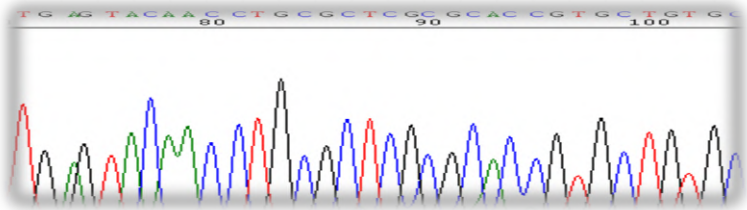
Consultation de Génétique

Analyse ADN ciblée

35delG of the gene of the connexin 26

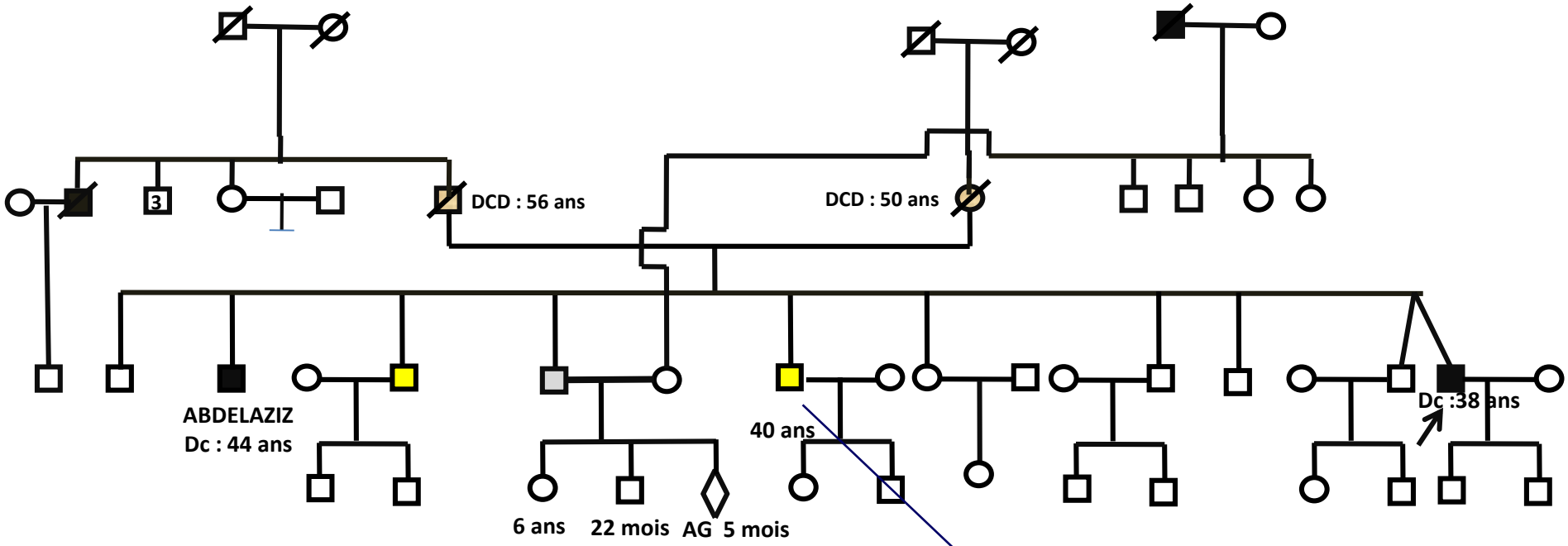
Gènes GJB2 / GJB6 / GJB3

Non-Syndromic Hearing Loss Panel



Analyse « Panel de gènes » 93 GENES

Famille HNPCC



- CCR
- Cancer hépatique (sur foi sain?)
- Colite interstitielle sans caractère spécifique ou tumoral
- Symptomatologie digestive

Dc presymptomatique en cours

Résultats

MYH (-) **MSI** ***Mutation MLH1***

Oncol Lett. 2011 Mar;2(2):389-393. doi: 10.3892/ol.2011.248. Epub 2011 Jan 21.

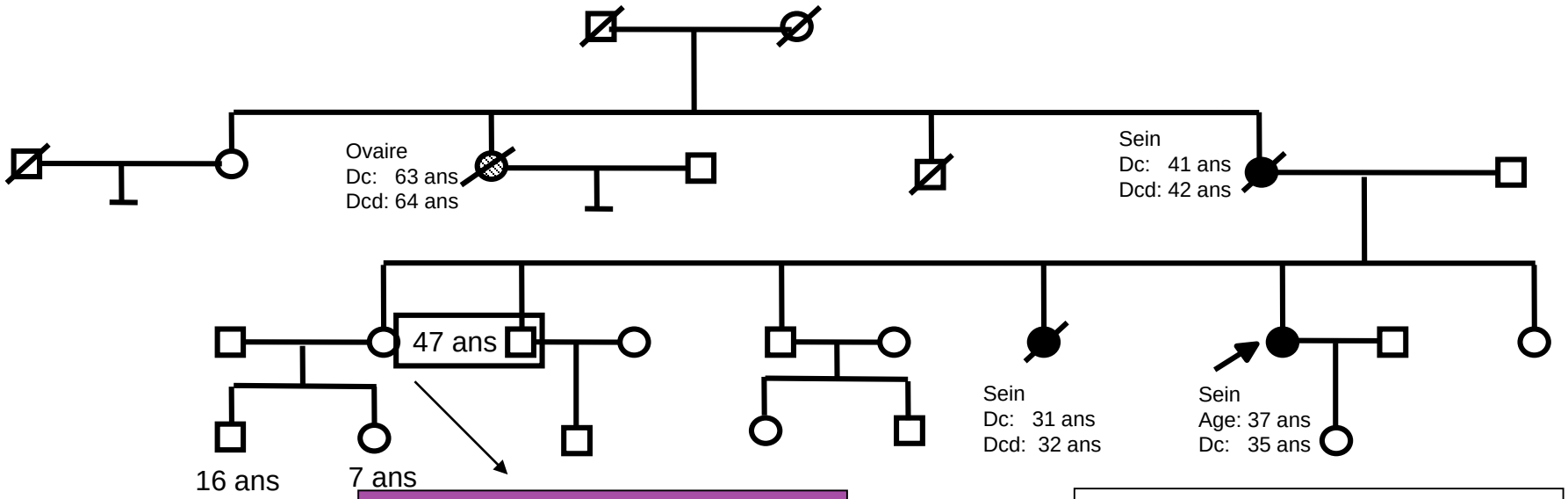
Genetic testing and first presymptomatic diagnosis in Moroccan families at high risk for breast/ovarian cancer

Fatima Zahra Laarabi [1], Imane Cherkaoui Jaouad, Karim Ouldim, Nisrine Aboussair, Abdelouahed Jalil, Brahim El Khalil El Gueddari, Noureddine Benjaafar, Abdelaziz Sefiani

Affiliations + expand

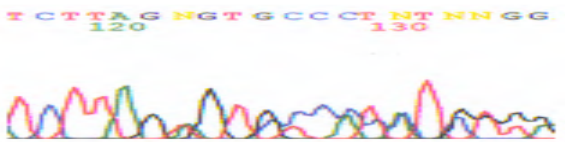
PMID: 22866093 PMCID: PMC3410606 DOI: 10.3892/ol.2011.248

Free PMC article



Diagnostic presymptomatique: Présence de la mutation 185delG

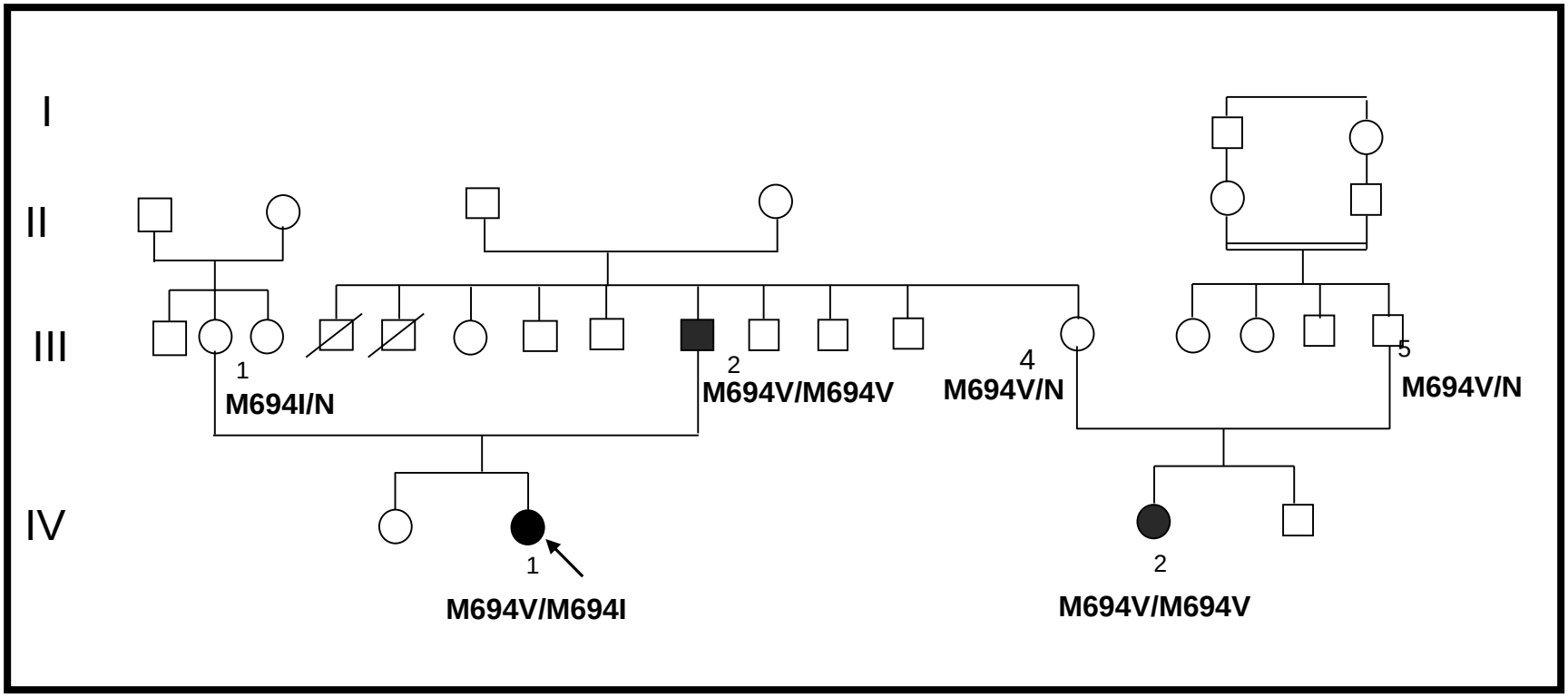
Mutation BRCA1 : 185delAG



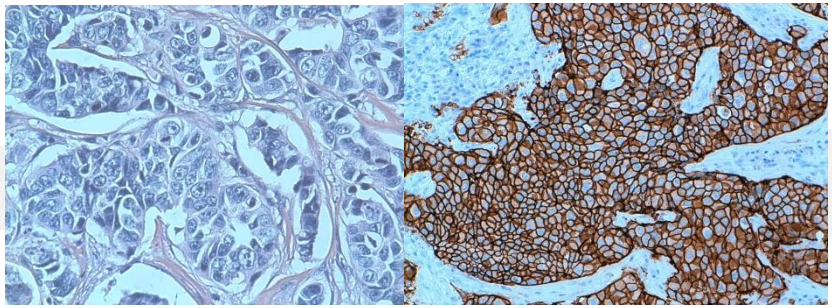
Premier diagnostic presymptomatique au Maroc

P.e.c : Première expérience marocaine

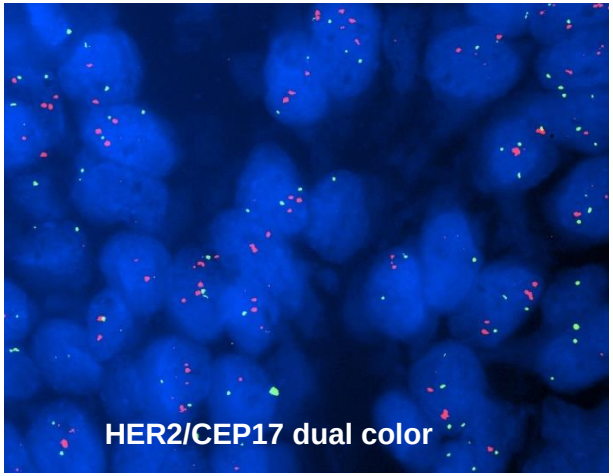
Tum utilisable sur biopuce



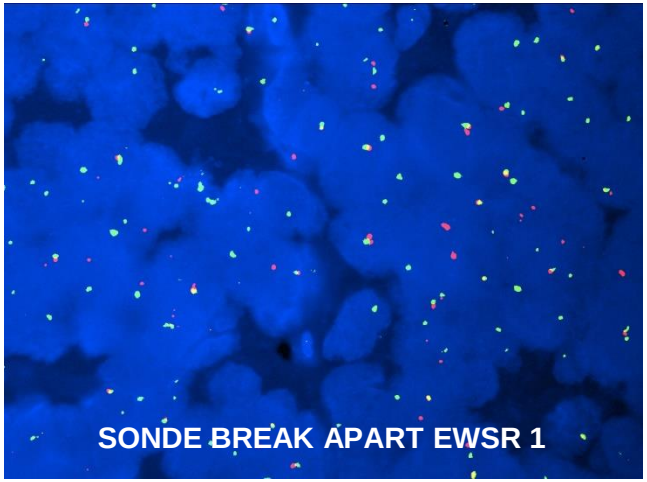
Fièvre méditerranéenne familiale (FMF)

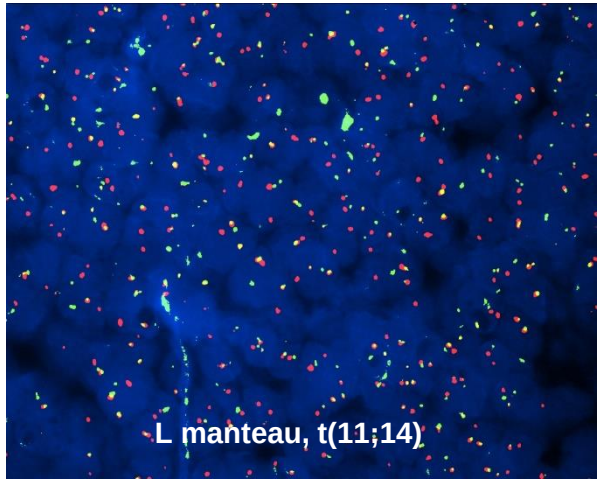


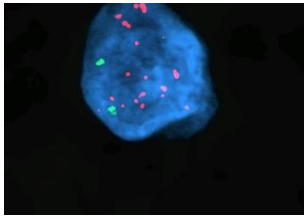
Cytogénétique moléculaire (FISH)



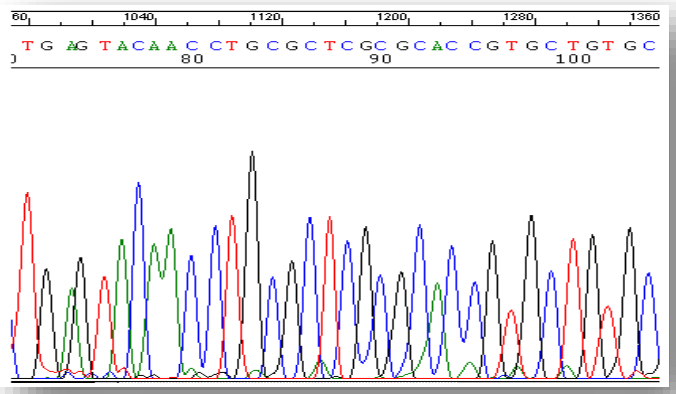
Cancer du sein







HER2 amplifié



Molecular biology

DNA sequencing **ACTGACTGACTG**

Oncohématologie

Moelle osseuse et sang, ganglions..

Oncogénétique tumorale:

Mutation somatique

Tumeurs

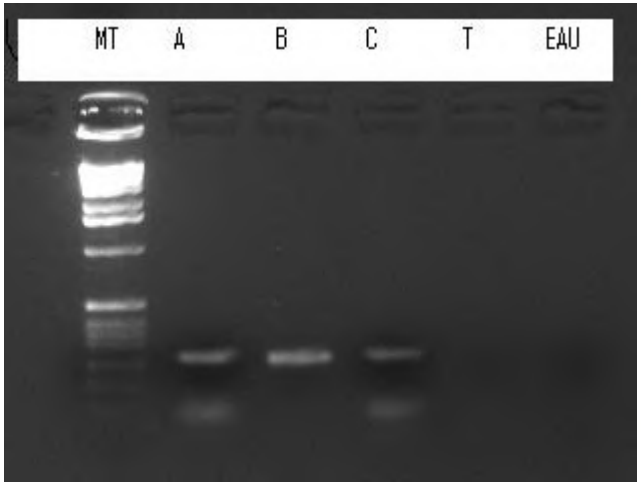
Oncogénétique constitutionelle :

Mutation constitionnelle

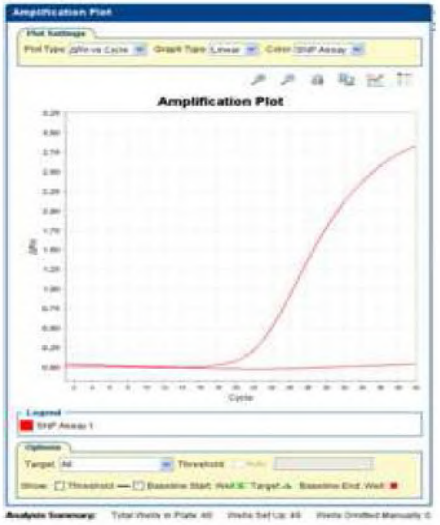
Sang

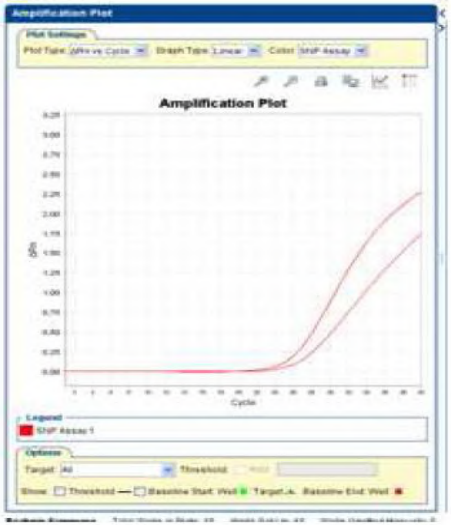
Oncogénétique
Biologie moléculaire

PCR conventionnelle



PCR en temps réel



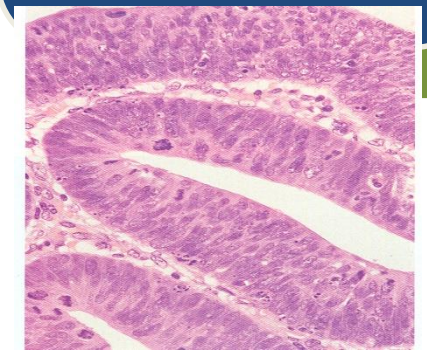


Pathologie?

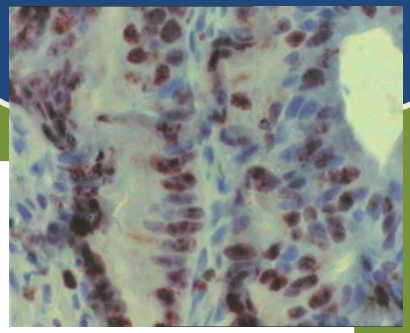
Morphologie

HE

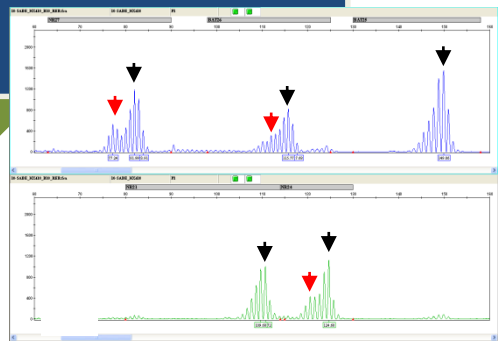
Colo spé



IHC



Anomalies génétiques des tumeurs



Parmacogénétique en cancérologie

Pharmacogénétique:
Mutation constitionnel
Sang

Table 2 | Selected germline pharmacogenomic markers.

Pharmacogenomic marker	Drug (s)	Genome	Outcome	Multi-tumor marker*
BIM	Imatinib	Germline	Efficacy	No
CYP2B6	Cyclophosphamide	Germline	Toxicity	No
CYP2D6	Tamoxifen	Germline	Efficacy	No
DPYD	Capecitabine, fluorouracil	Germline	Toxicity	No
G6PD	Rasburicase	Germline	Toxicity	No
MLH1, MSH2, MSH6, PMS2	Fluorouracil	Germline	Efficacy	Yes
SLCO1B1	Methotrexate	Germline	Toxicity	No
SLC28A3	Anthracyclines	Germline	Toxicity	No
TCL1A	Aromatase inhibitors	Germline	Toxicity	No
TPMT	Mercaptopurine, thioguanine, cisplatin	Germline	Toxicity	No
UGT1A1	Irinotecan	Germline	Toxicity	No

*Commercially available multi-marker tumor panels.

Arup Laboratories (http://ltd.aruplab.com/Tests/Pub/2007991).

AsuraGen (http://asuragen.com/products-and-services/genomic-services/next-generation-sequencing-services/).

Foundation Medicine (http://www.foundationone.com/).

Parmacogénétique en cancérologie

Pharmacogénétique: Mutation Somatique Tumeurs

Table 1 | Selected somatic pharmacogenomic markers.

Pharmacogenomic marker	Drug (s)	Genome	Outcome	Multi-tumor marker*
ABL	Bosutinib, dasatinib, imatinib, nilotinib, ponatinib	Somatic	Efficacy	Yes
ALK	Crizotinib	Somatic	Efficacy	Yes
BRAF	Vemurafenib	Somatic	Efficacy	Yes
EGFR	Afatinib, cetuximab, erlotinib, panitumumab, vandetinib	Somatic	Efficacy	Yes
FcγR	Cetuximab, rituximab, trastuzumab	Somatic	Efficacy	No
HER2	Lapatinib, pertuzumab, trastuzumab, trastuzumab emtansine	Somatic	Efficacy	No
KRAS	Cetuximab, panitumumab	Somatic	Efficacy	Yes
KIT	Imatinib	Somatic	Efficacy	Yes
MET	Trametinib	Somatic	Efficacy	Yes

*Commercially available multi-marker tumor panels.

Arup Laboratories (http://ltd.aruplab.com/Tests/Pub/2007991).

AsuraGen (http://asuragen.com/products-and-services/genomic-services/next-generation-sequencing-services/).

Foundation Medicine (http://www.foundationone.com/).

Somatic mutations in cancer pharmacogenomics

Drug	Drug target	Cancer type (or types)	Somatic markers
Cetuximab	EGFR	Colorectal, head and neck	*EGFR* and *KRAS*
Erlotinib	EGFR	Lung, pancreatic	*EGFR*
Exemestane	Aromatase	Breast	*ESR1*, *ESR2* and *PGR*
Gefitinib	EGFR	Lung	*EGFR*
Imatinib	BCR–ABL, KIT and PDGFRα tyrosine kinases	Chronic myeloid leukaemia, gastrointestinal	Philadelphia chromosome, *KIT* and *PDGFRA*
Lapatinib	ERBB2 receptor	Breast	*ERBB2*
Letrozole	Aromatase	Breast	*ESR1*, *ESR2* and *PGR*
Panitumumab	EGFR	Colorectal	*EGFR* and *KRAS*
Tamoxifen	Oestrogen receptor	Breast	*ESR1*, *ESR2* and *PGR*
Trastuzumab	ERBB2 receptor	Breast, stomach	*ERBB2*

HEMATOLOGIE

Syndromes Myéloprolifératifs

Leucémie myéloïde chronique (LMC)

t(9;22)(q34.1 ;q11.2)

Definition of Hematologic, Cytogenetic and Molecular Response

Hematologic	Complete (CHR)	WBC < 10 x 10^9/L
		Basophils < 5%
		No myelocytes, promyelocytes, myeloblasts in the differential
		Platelet count < 450 x 10^9/L
		Spleen non palpable
Cytogenetic	Complete (CCgR)	No Ph+ metaphases
	Partial (PCgR)	1- 35% Ph+ metaphases
	Minor (mCgR)	36-65% Ph+ metaphases
	Minimal (minCgR)	66-95% Ph+ metaphases
	None (noCgR)	> 95% Ph+ metaphases
Molecular	Complete (CMolR)	Undetectable BCR-ABL mRNA transcripts by real time quantitative and/or nested PCR in two consecutive blood samples of adequate quality (sensitivity > 10^4)
	Major (MMolR)	Ratio of BCR-ABL to ABL (or other housekeeping genes) ≤ 0.1% on the international scale

Baccarani et. al., JCO 2009; 10.1200/JCO.2009.25.0779

US FDA-approved oncology drugs with package inserts containing pharmacogenetics and pharmacogenomics information

Drug	Pharmacogenomic biomarker(s)
Ado-trastuzumab emtansine	ERBB2
Afatinib	EGFR
Anastrozole	ESR1, PGR
Arsenic trioxide	PML/RARA
Bosutinib	BCR/ABL1
Brentuximab vedotin	TNFRSF8
Busulfan	Ph chromosome
Capecitabine	DPYD
Cetuximab	EGFR, KRAS
Cisplatin	TPMT
Crizotinib	ALK
Dabrafenib	BRAF, G6PD
Dasatinib	BCR/ABL1
Denileukin diftitox	IL2RA
Erlotinib	EGFR
Everolimus	ERBB2, ESR1
Exemestane	ESR1

Edward D Esplin‡,1, Ling Oei‡,1 & Michael P Snyder*,1 Pharmacogenomics (2014) 15(14), 1771–1790

US FDA-approved oncology drugs with package inserts containing pharmacogenetics and pharmacogenomics information.

Everolimus	ERBB2, ESR1
Exemestane	ESR1
Fluorouracil	DPYD
Fulvestrant	ESR1
Ibritumomab tiuxetan	MS4A1
Imatinib	KIT, BCR/ABL1, PDGFRB, FIP1L1/PDGFRA
Irinotecan	UGT1A1
Lapatinib	ERBB2
Letrozole	ESR1, PGR
Mercaptopurine	TPMT
Nilotinib	BCR/ABL1, UGT1A1
Obinutuzumab	MS4A1
Ofatumumab	MS4A1
Omacetaxine	BCR/ABL1
Panitumumab	EGFR, KRAS
Pazopanib	UGT1A1
Pertuzumab	ERBB2
Ponatinib	BCR-ABL T315I
Rasburicase	G6PD
Rituximab	MS4A1
Tamoxifen	ESR1, PGR, F5, F2
Thioguanine	TPMT
Tositumomab	MS4A1
Trametinib	BRAF

F2: Prothrombin; F5: Factor V Leiden; Ph: Philadelphia.
Data taken from [32].

Edward D Esplin‡,1, Ling Oei‡,1 & Michael P Snyder*,1 Pharmacogenomics (2014) 15(14), 1771–1790

US FDA-approved oncology drugs with package inserts containing pharmacogenetics and pharmacogenomics information.

Everolimus	ERBB2, ESR1
Exemestane	ESR1
Fluorouracil	DPYD
Fulvestrant	ESR1
Ibritumomab tiuxetan	MS4A1
Imatinib	KIT, BCR/ABL1, PDGFRB, FIP1L1/PDGFRA
Irinotecan	UGT1A1
Lapatinib	ERBB2
Letrozole	ESR1, PGR
Mercaptopurine	TPMT
Nilotinib	BCR/ABL1, UGT1A1
Obinutuzumab	MS4A1
Ofatumumab	MS4A1
Omacetaxine	BCR/ABL1
Panitumumab	EGFR, KRAS
Pazopanib	UGT1A1
Pertuzumab	ERBB2
Ponatinib	BCR-ABL T315I
Rasburicase	G6PD
Rituximab	MS4A1
Tamoxifen	ESR1, PGR, F5, F2
Thioguanine	TPMT
Tositumomab	MS4A1
Trametinib	BRAF

F2: Prothrombin; F5: Factor V Leiden; Ph: Philadelphia.
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Edward D Esplin‡,1, Ling Oei‡,1 & Michael P Snyder*,1 Pharmacogenomics (2014) 15(14), 1771–1790

US FDA-approved oncology drugs with package inserts containing pharmacogenetics and pharmacogenomics information.

Trastuzumab	ERBB2
Tretinoin	PML/RARA
Vemurafenib	BRAF

F2: Prothrombin; F5: Factor V Leiden; Ph: Philadelphia.
Data taken from [32].

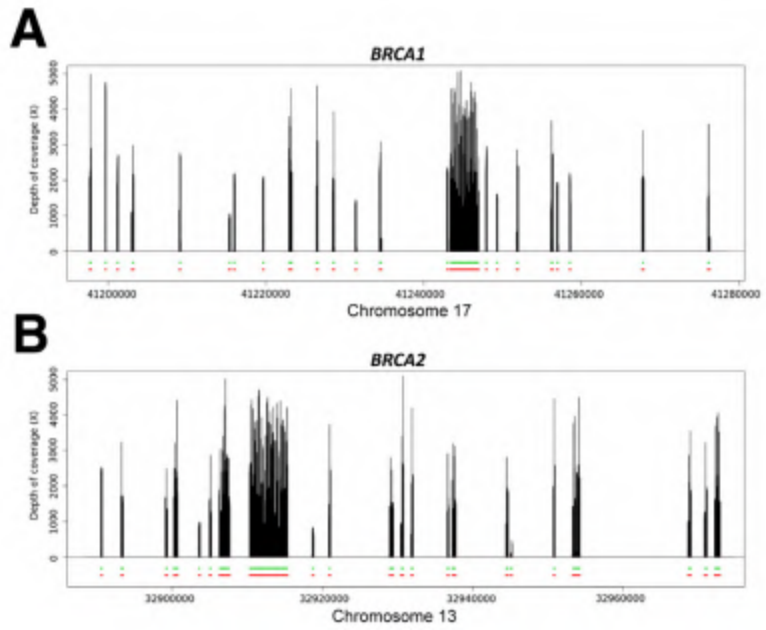
Edward D Esplin‡,1, Ling Oei‡,1 & Michael P Snyder*,1 Pharmacogenomics (2014) 15(14), 1771–1790

Breast ovarian cancer panel

CDH1, PTEN, STK11, TP53

Breast ovarian cancer panel PLUS

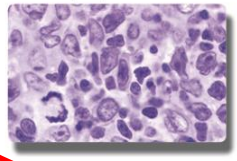
ATM, BARD1, BRIP1, CHEK2, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, XRCC2



Gene*	Location	cDNA change	Protein change	dbSNP138	HGMD	Variant type	Validation cohort	Discovery cohort	Homozygous/ heterozygous of total
BRCA1	Ex. 07	c.503A>C	p.(K168T)	rs273901743	—	Uncertain	1	0	0/1 of 210
BRCA1	Ex. 07	c.536A>G	p.(Y179C)	rs56187033	CM030786	Uncertain	1	4	0/5 of 210
BRCA1	Ex. 10	c.1456T>C	p.(F486L)	rs55906931	—	Uncertain	1	4	0/5 of 210
BRCA1	Ex. 10	c.1648A>C	p.(N550H)	rs56012641	CM025218	Uncertain	1	4	0/5 of 210
BRCA1	Ex. 10	c.2071del	p.(R691fs)	rs80357688	CD982486	Definitely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 10	c.3569C>T	p.(P1190L)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 12	c.4236del	p.(A1412fs)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 15	c.4535G>T	p.(S1512I)	rs1800744	CM960183	Uncertain	1	0	0/1 of 210
BRCA1	Ex. 16	c.4787C>A	p.(S1596*)	—	—	Likely pathogenic	2	3	0/5 of 210
BRCA1	Int. 16	c.4986+2T>A	p.(?)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 17	c.5062G>T	p.(V1688F)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 18	c.5096G>A	p.(R1699Q)	rs41293459	CM034007	Likely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 19	c.5177_5180del	p.(1726_1727del)	rs80357975	CD972067	Definitely pathogenic	1	0	0/1 of 210
BRCA1	Ex. 20	c.5266dup	p.(Q1756fs)	rs80357906	CI941841	Definitely pathogenic	1	2	0/3 of 210
BRCA1	Int. 23	c.5468-10C>A	p.(?)	rs8176316	CS086718	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 03	c.122C>T	p.(P41L)	—	—	Likely pathogenic	1	1	0/2 of 210
BRCA2	Ex. 05	c.467_468insT	p.(D156fs)	—	CI020251	Definitely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 10	c.965_968del	p.(322_323del)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 10	c.1151C>T	p.(S384F)	rs41293475	CM065036	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 10	c.1550A>G	p.(N517S)	rs80358439	—	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 10	c.1792A>G	p.(T598A)	rs28897710	CM035689	Uncertain	1	1	0/2 of 210
BRCA2	Ex. 10	c.1813dup	p.(I605fs)	rs80359308	CI972557	Definitely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 11	c.2803G>A	p.(D935N)	rs28897716	CM994285	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 11	c.3318C>G	p.(S1106R)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 11	c.3503T>C	p.(M1168T)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 11	c.4258G>T	p.(D1420Y)	rs28897727	CM003133	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 12	c.6935A>T	p.(D2312V)	rs80358916	CS119639	Likely pathogenic	1	0	0/1 of 210
BRCA2	Int. 13	c.7008-62A>G	p.(?)	rs76584943	CS014426	Uncertain	1	1	0/2 of 210
BRCA2	Ex. 14	c.7068_7069del	p.(2356_2357del)	—	—	Likely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 15	c.7544C>T	p.(T2515I)	rs28897744	CM994287	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 18	c.8187G>T	p.(K2729N)	rs80359065	CM021957	Uncertain	1	0	0/1 of 210
BRCA2	Ex. 22	c.8851G>A	p.(A2951T)	rs11571769	CM970186	Uncertain	1	1	0/2 of 210
BRCA2	Int. 22	c.8954-3C>G	p.(?)	rs81002844	CS124767	Likely pathogenic	1	0	0/1 of 210
BRCA2	Ex. 23	c.9097_9098insT	p.(T3033fs)	—	—	Likely pathogenic	1	0	0/1 of 210

Oncogenetics

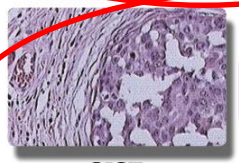
Lymphoma



t(11 ;14) lgH-CCND1
t(14 ;18)lgH-BCL2
C MYC rearrangement

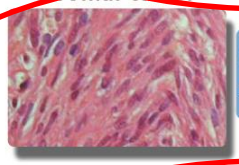
Chromosome breakage analysis

breast cancer



HER2 amplification
TOP 2A

GIST
Gastrointestinal Stromal tumor

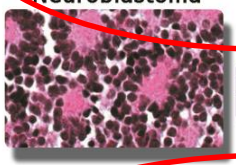


C KIT mutations
PDGFR x mutations

Colorectal cancer

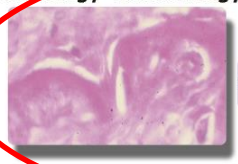
KRAS mutations
MSI (microsatellite instability)*

Neuroblastoma



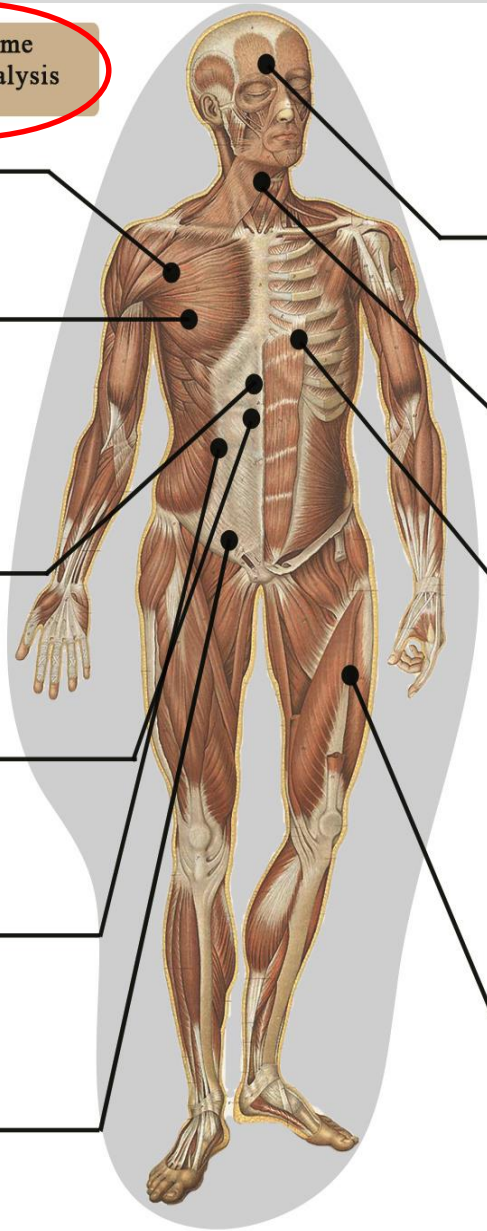
N-MYC amplification

oncology-hematology



BCR-ABL

FISH Postnatal

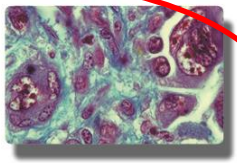


Clinical Genetics
(Genetic consuling ,oncogenetic consultation)

Postnatal karyotype

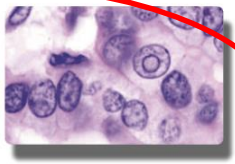
Brain cancer

GLIOMA : 1p36



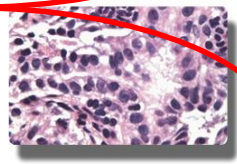
Thyroid cancer

RAS and BRAF mutations
MEN2 (RET gen)



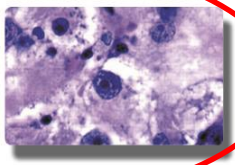
Lung cancer

EGFR mutations
EGFR amplification



Soft tissue tumors

Ewing sarcoma
EWSR1 Break Apart rearrangement



COLOUR CODE

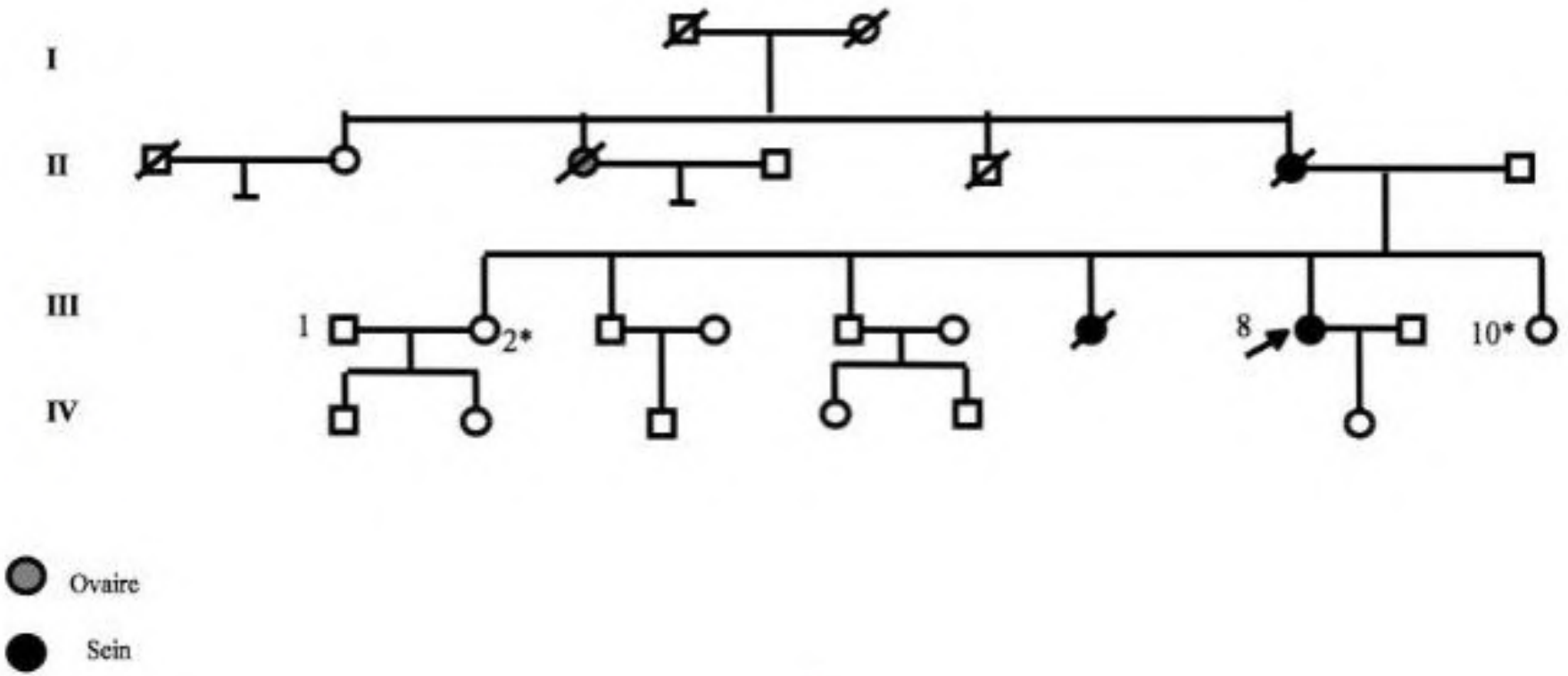
FISH analysis | MOLECULAR BIOLOGY analysis

oncogenetics

Cancer héréditaire

- ✓Age précoce du cancer
- ✓Plus d'un membre de la famille atteint d'un cancer
- ✓Association de deux cancers ou plus chez le même individu (Cancer du colon / Cancer de l'endomètre, Cancer du Sein/ Cancer de l'ovaire, Mélanome / Cancer du pancréas…)
- ✓Plusieurs générations atteintes d'un cancer
- ✓Cancer bilatéral (cancer du sein bilatéral…)
- ✓Cancers rares
- ✓Lésions précancéreuses
- ✓Associations avec d'autres manifestations (dysmorphie, taches café-au-lait, hamartomes, macrocéphalie…)
- ✓Cancer du sein chez l'homme à tout âge
- ✓Cancer médullaire de la thyroïde à tout âge
- ✓Polypes adénomateux du côlon (10 ou plus) surtout si la découverte des premiers polypes avant l'âge de 50 ans
- ✓L'origine ethno-géographique

Famille 1

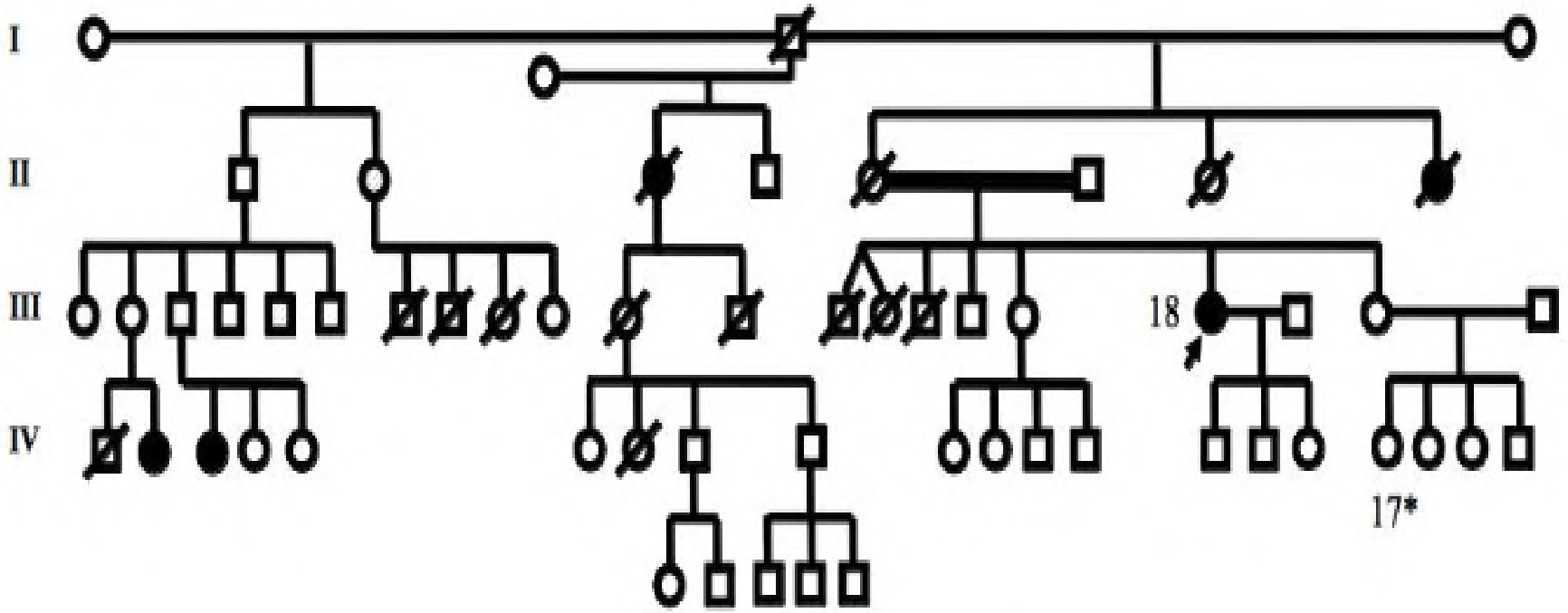


BRCA1- 185delAG (c.68_69delAG)

III-2: 48 ans, Cancer du sein

III-10: 42 ans, Tumeur de l'ovaire T2N0M0

Famille 2



BRCA2 - c.5073dupA; p.Trp1692MetfsX3

Suivi:

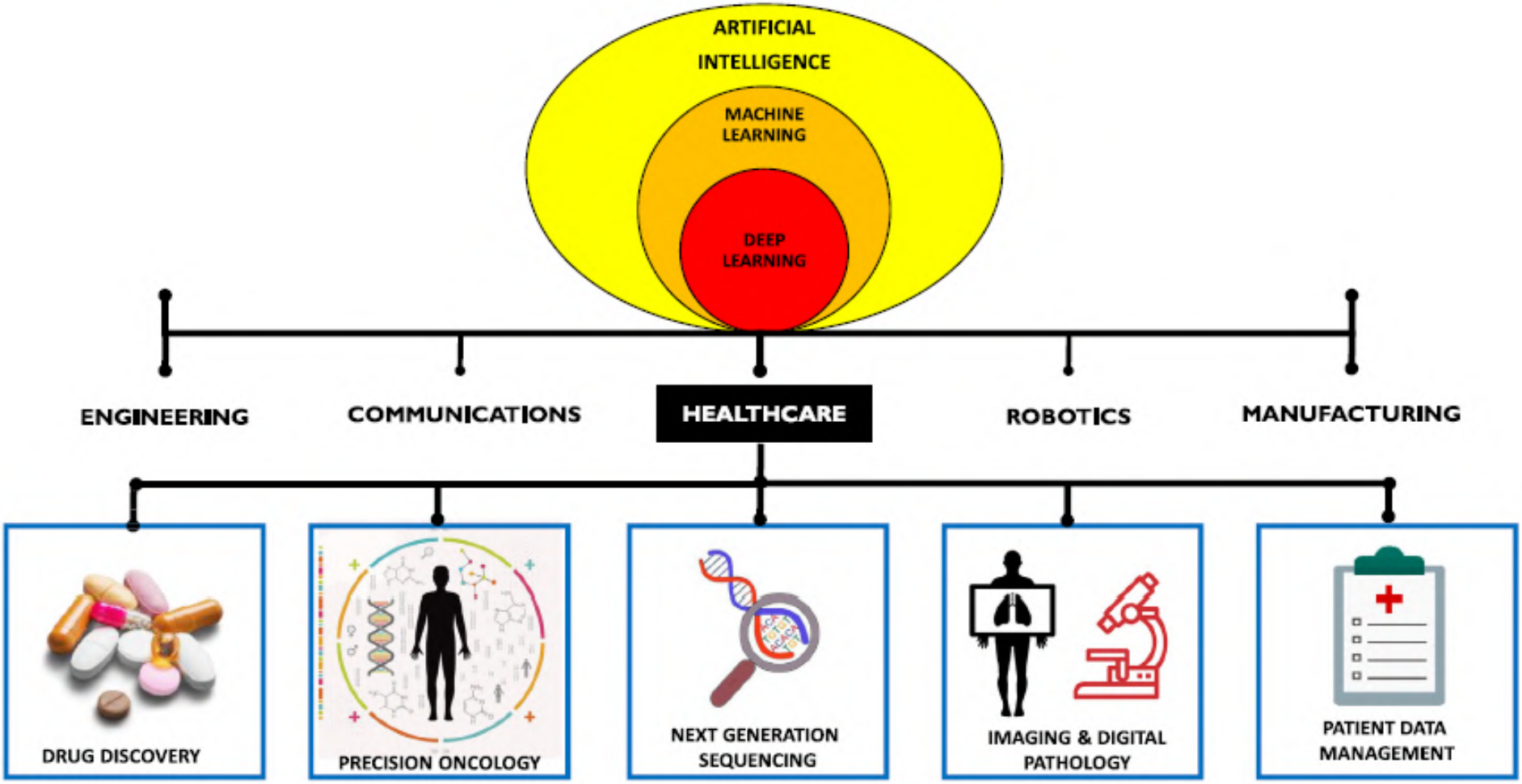
- ❑Examen clinique semestriel
- ❑Mammographie et IRM mammaire annuelles
- ❑Echographie transvaginale

Liste des tests et panels

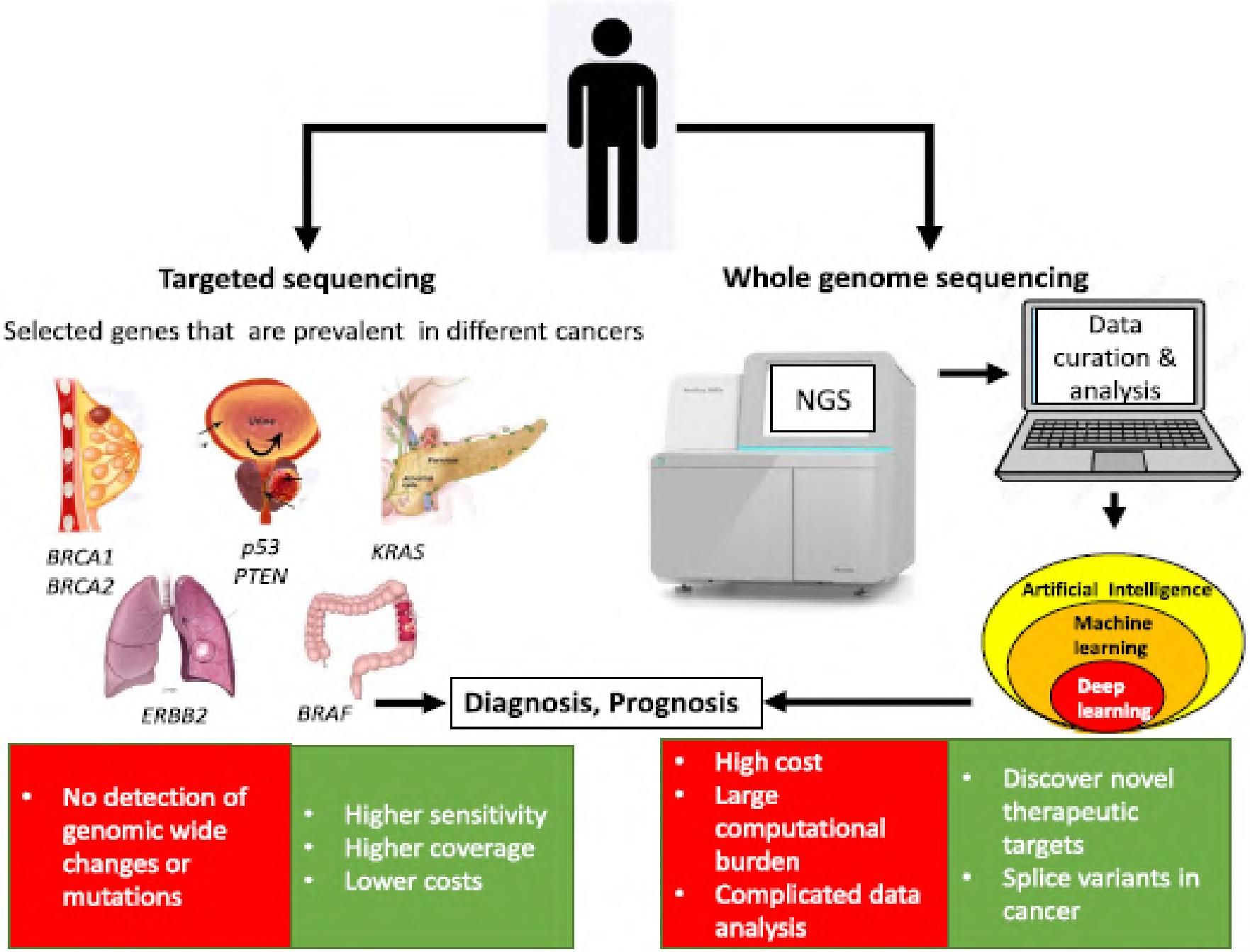
Panels « séquençage à haut débit NGS Panel

- **Breast ovarian cancer panel (NGS Panel; ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MSH6, NBN, PALB2, PTEN, RAD51, RAD51C, STK11, TP53)**
- **Colon cancer and polyposis syndrome panel (NGS Panel; APC, BMPR1A, ENG, EPCAM, FLCN, MLH1, MSH2, MSH3, MSH6, MUTYH, PMS1, PMS2, PTEN, SMAD4, STK11)**
- **Fanconi anemia panel (NGS Panel; BRCA2, BRIP1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, PALB2, SLX4, XRCC2)**
- **Neurofibromatosis panel (NGS Panel; NF1, NF2, SPRED1)**
- **Pheochromocytoma panel (NGS Panel; MAX, PRKAR1A, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, VHL)**

D'autres panels spécifiques aux patients marocains peuvent être développés



Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311

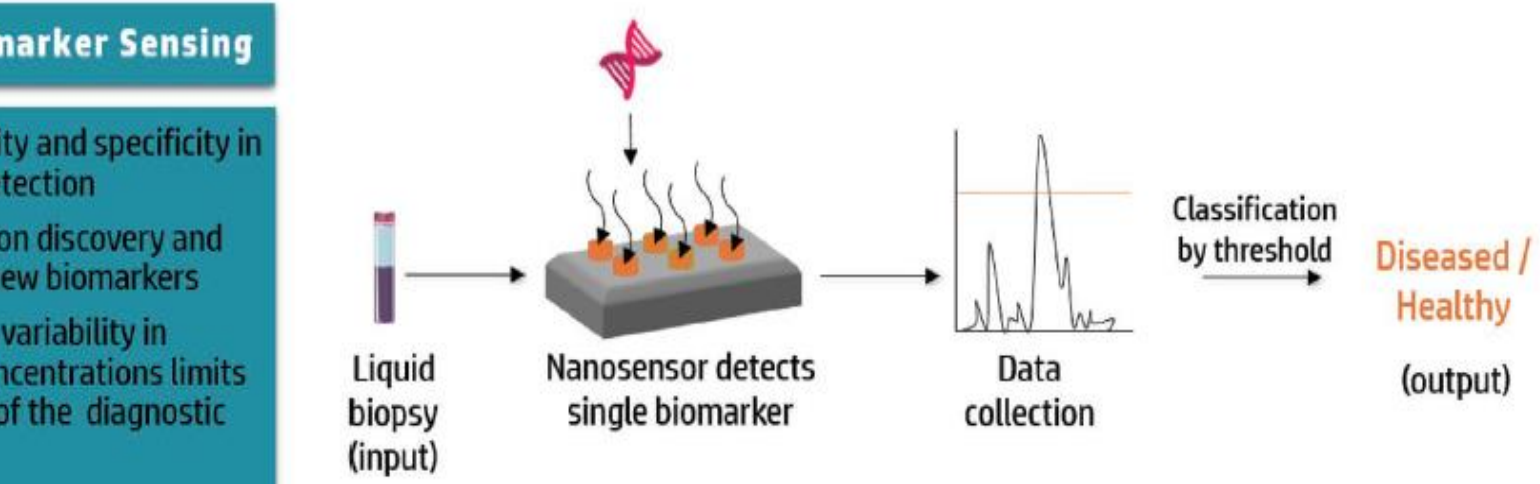


Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311

Integrating Artificial Intelligence and Nanotechnology for Precision Cancer Medicine

Single Biomarker Sensing

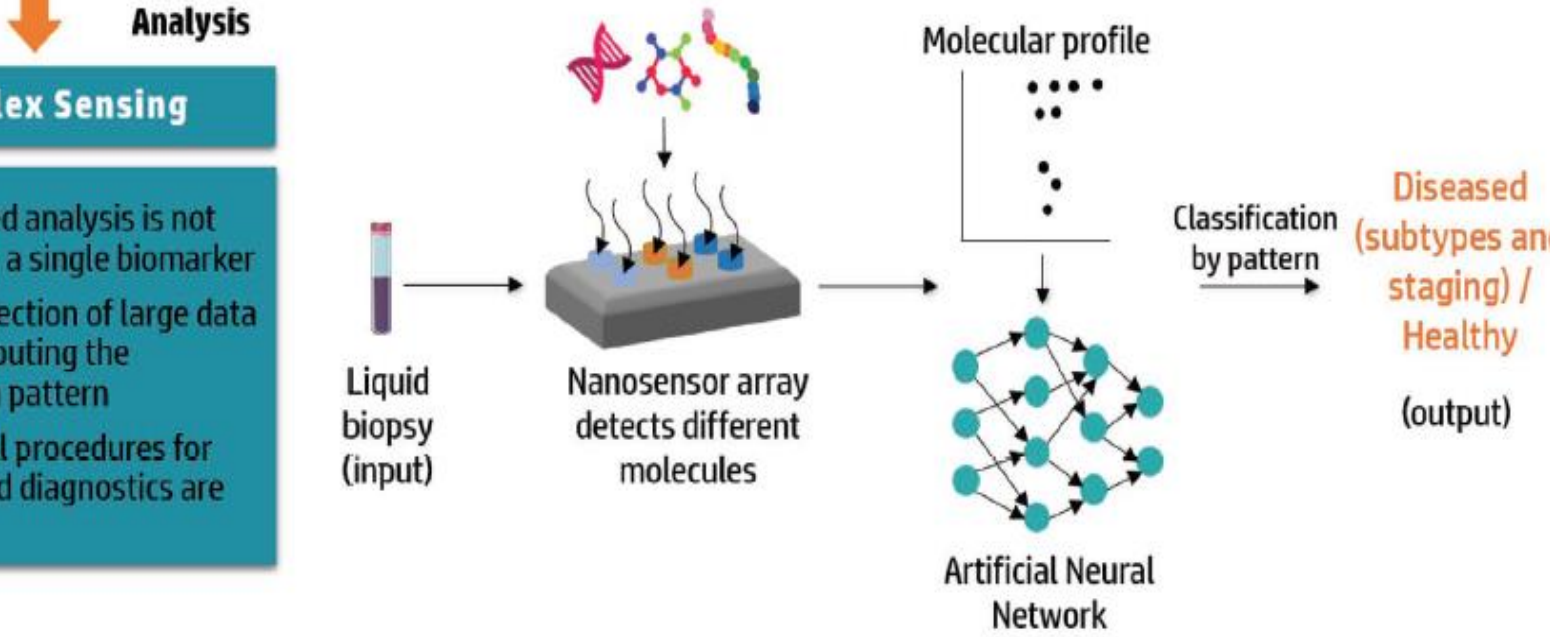
- High sensitivity and specificity in biomarker detection
- Dependence on discovery and approval of new biomarkers
- Inter-patient variability in biomarker concentrations limits the accuracy of the diagnostic prediction



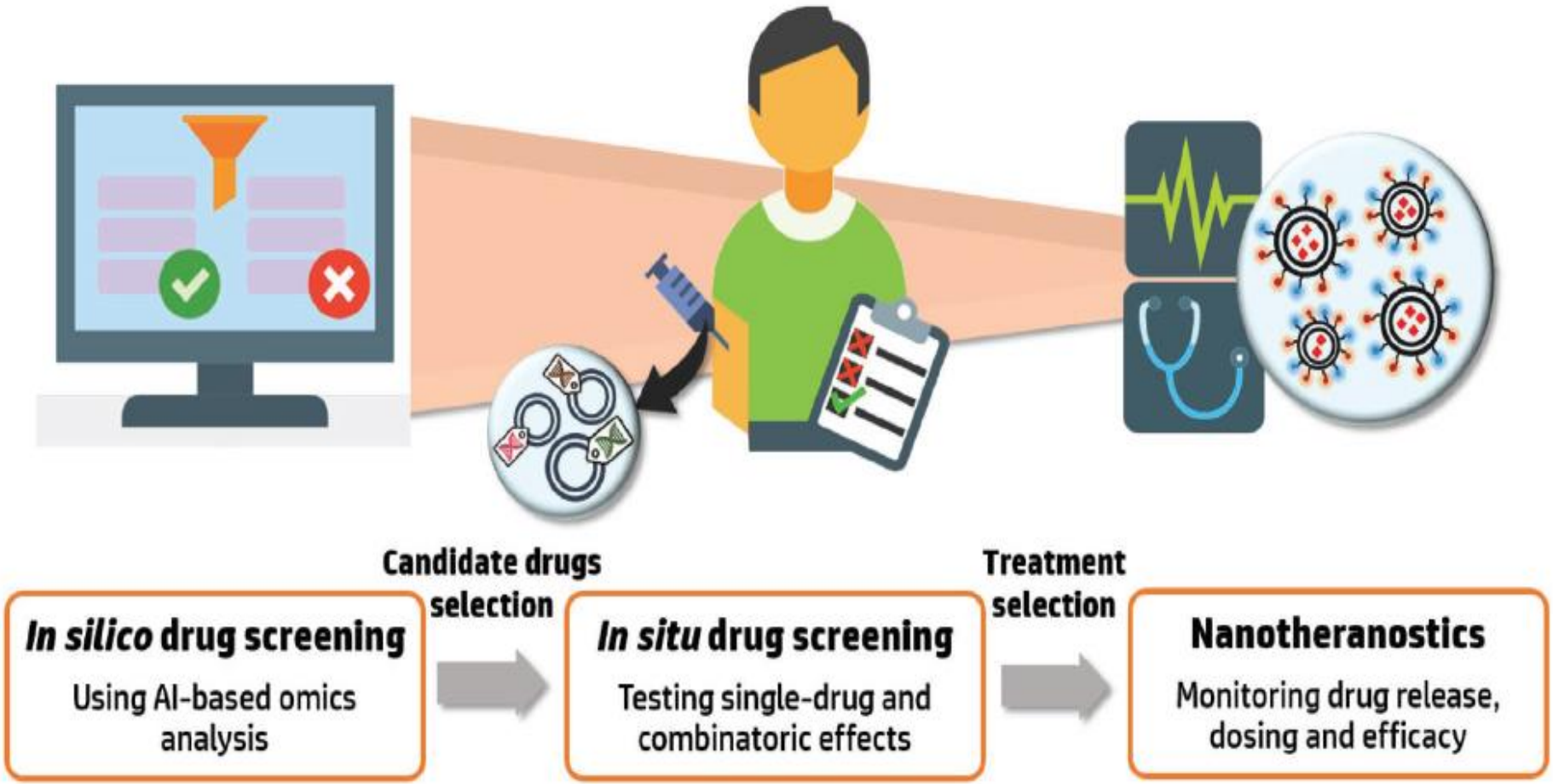
Progress Towards Big Data Analysis

Multiplex Sensing

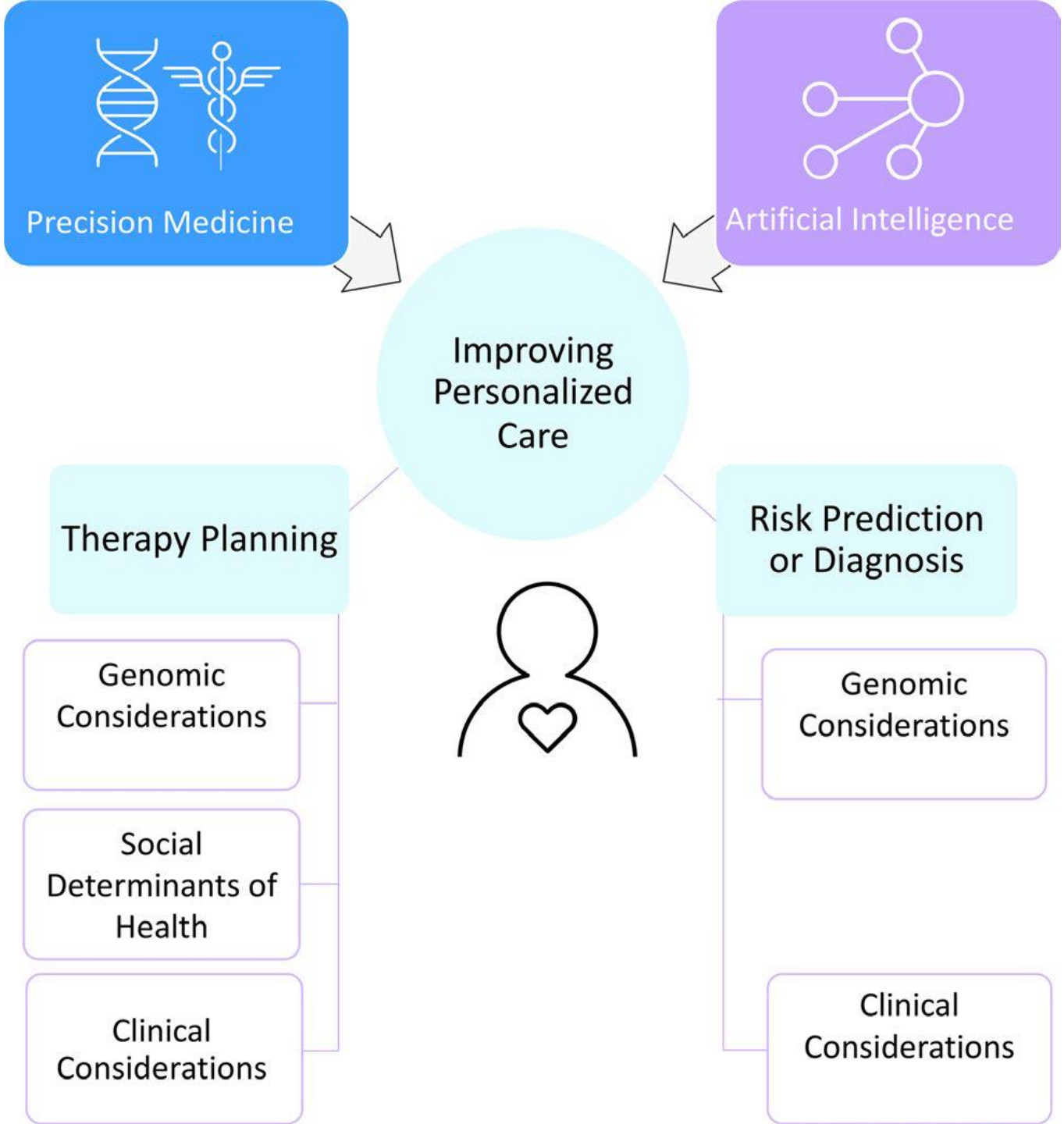
- Pattern-based analysis is not depended on a single biomarker
- Requires collection of large data sets for computing the classification pattern
- New approval procedures for pattern based diagnostics are required



Omer Adir et al Integrating Artificial Intelligence and Nanotechnology for Precision Cancer Medicine adma.2. Epub 2019 Jul 9.

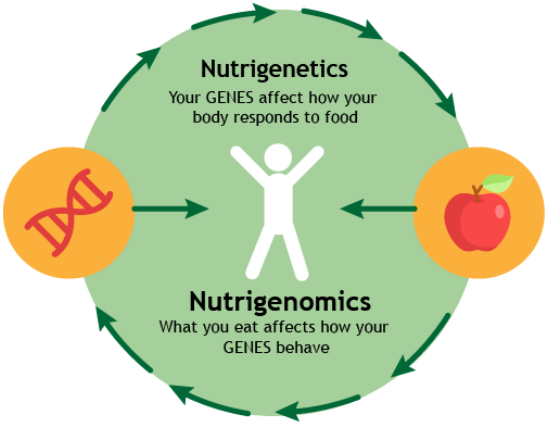


Omer Adir et al Integrating Artificial Intelligence and Nanotechnology for Precision Cancer Medicine adma.2. Epub 2019 Jul 9.

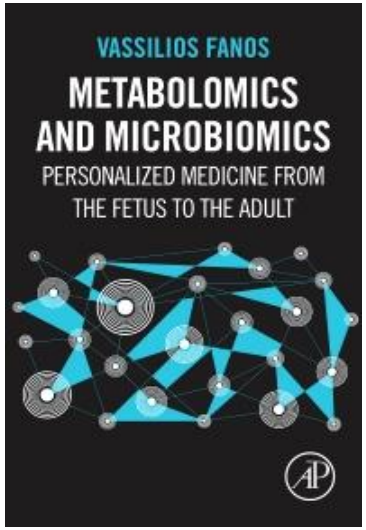

Precision Medicine
Artificial Intelligence
Improving Personalized Care
Therapy Planning
Genomic Considerations
Social Determinants of Health
Clinical Considerations
Risk Prediction or Diagnosis
Genomic Considerations
Clinical Considerations

The landscape of genomic technologies in healthcare and biomedical research

Pharmacogenomics (PGx)



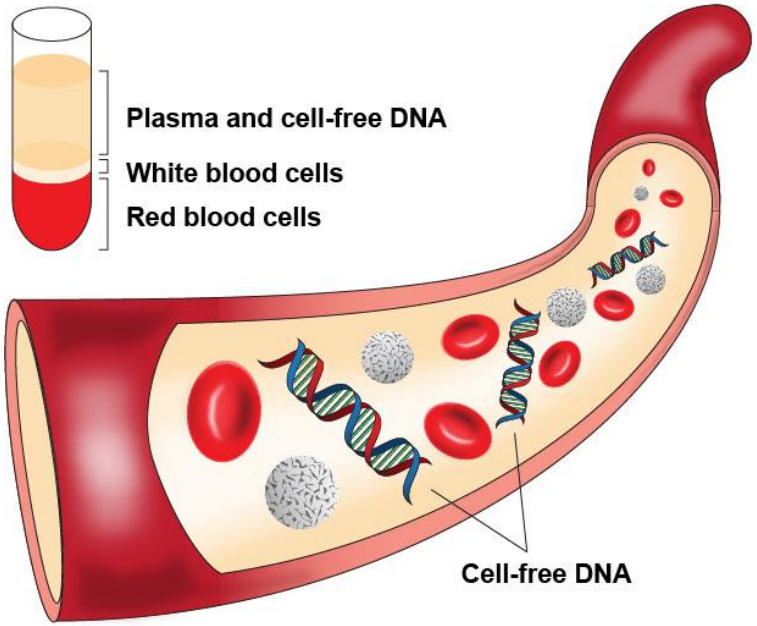
Nutrigenomics (or nutritional genomics)

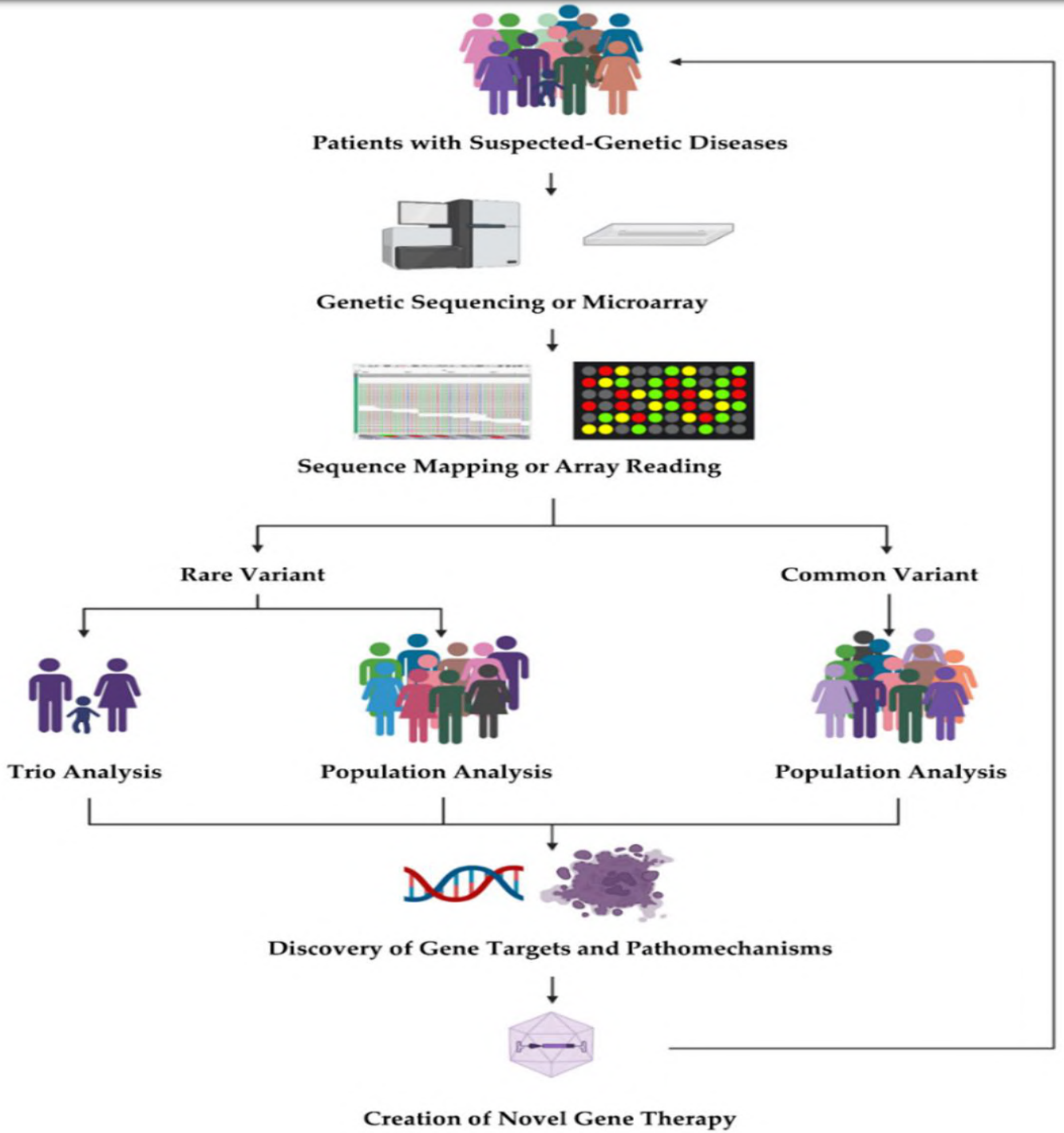


Microbiome analysis (microbiomics)

The landscape of genomic technologies in healthcare and biomedical research

- Cell-free DNA (cfDNA): germline DNA, fetal DNA, cancer DNA, or potentially pathogen DNA,ease.
- Single cell sequencing (scSeq)
- Epigenomics
- Transcriptomics
- Proteomics
- OMICS




Patients with Suspected-Genetic Diseases
Genetic Sequencing or Microarray
Sequence Mapping or Array Reading
Rare Variant
Common Variant
Trio Analysis
Population Analysis
Population Analysis
Discovery of Gene Targets and Pathomechanisms
Creation of Novel Gene Therapy

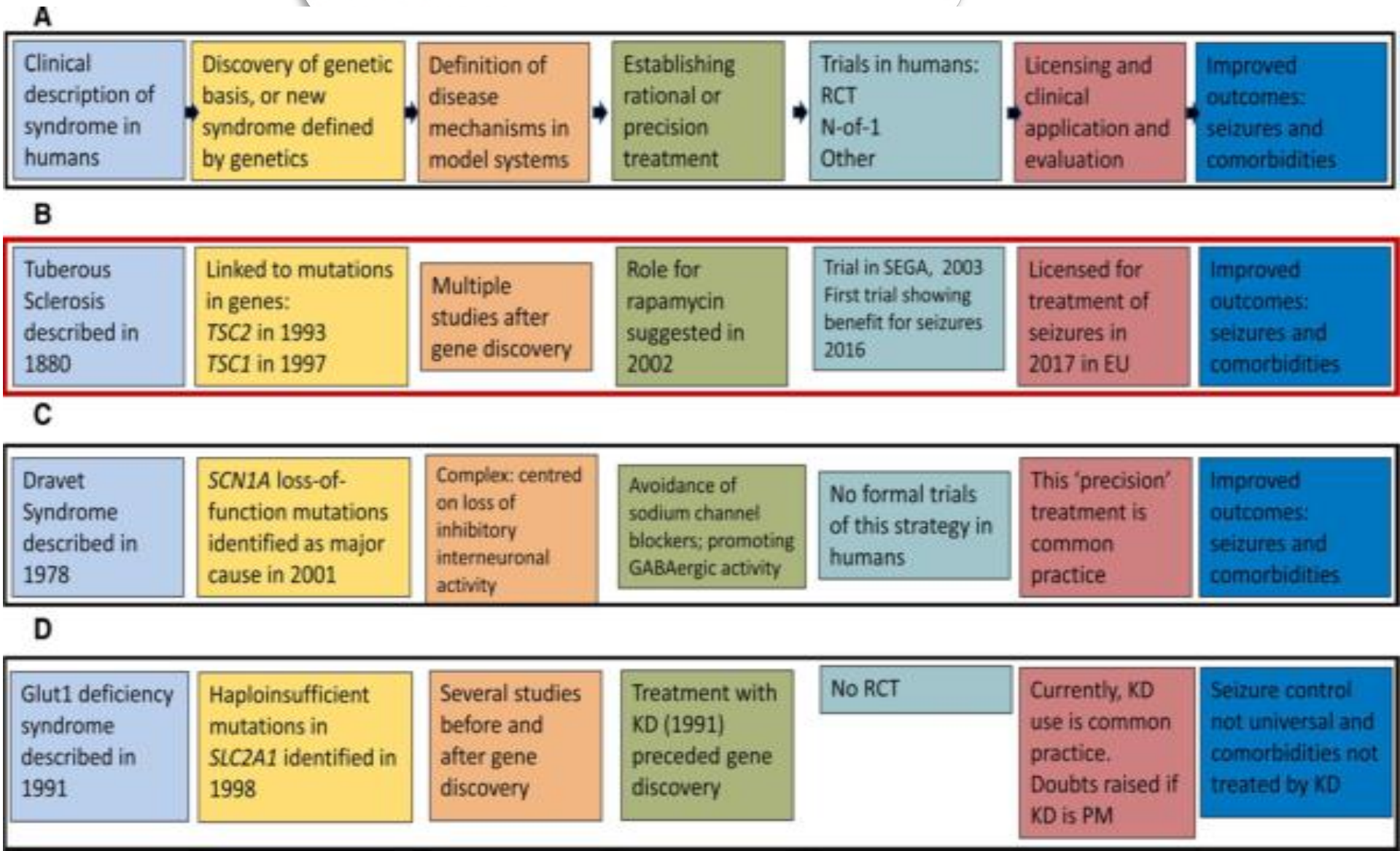
Received: 13 February 2020 | Revised: 20 April 2020 | Accepted: 21 April 2020
DOI: 10.1111/epi.16539

SUPPLEMENT ARTICLE

Epilepsia

Precision medicine and therapies of the future

Sanjay M. Sisodiya[1,2]

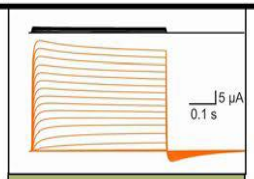
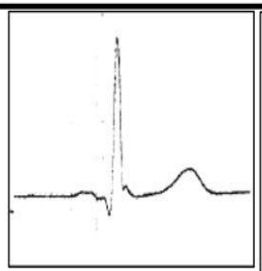


Real-life examples of more complex PM scenarios.

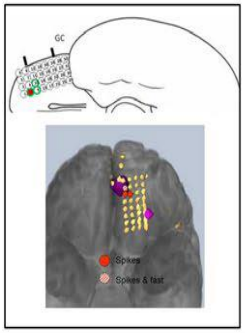
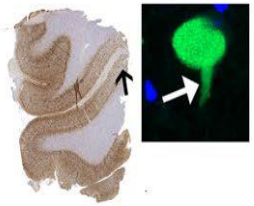
A

Clinical description of syndrome in humans	Discovery of genetic basis, or new syndrome defined by genetics	Definition of disease mechanisms in model systems	Establishing rational or precision treatment	Trials in humans: RCT N-of-1 Other	Licensing and clinical application and evaluation	Improved outcomes: seizures and comorbidities

B

No previous syndromic description	*KCNA2* de novo mutation-related DEE GoF in mutant protein	 Reversed in vitro by 4-AP	N-of-1 trials: seizure frequency increased in one patient with L298F; seizure control in another	3 other unique inherited variants on WES reanalysis: *CACNA1C* LoF*		Patient intolerant of prolonged ECG/EEG, ajmaline not possible. Relevance of *variant? Cause of increased seizures?

C

Focal-onset seizures to bilateral convulsions; no ID; family history	Treatment-resistant Normal high-resolution MRI Life-threatening and life-limiting seizures		Surgical resection as PM 	No benefit from surgery WGS reveals inherited *DEPDC5* stop gain variant	Trial of everolimus?	No trial data in *DEPDC5* epilepsy; young patient unwilling to try; regulatory hurdles

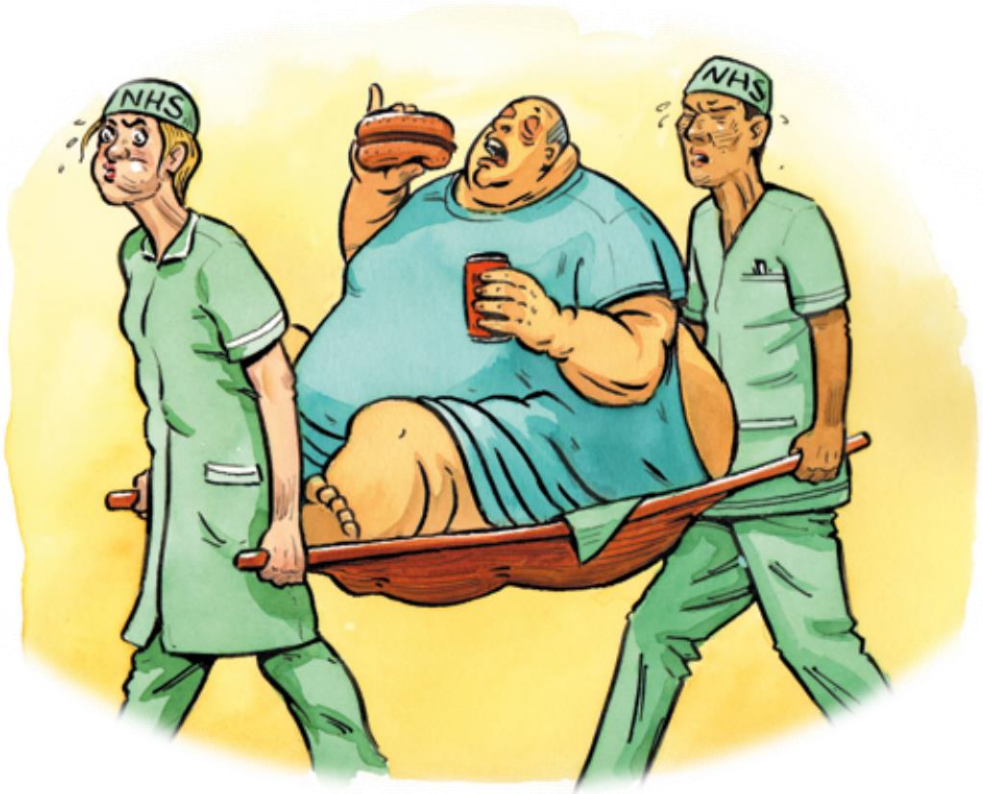
D

Brothers with severe epilepsy & ID; symptom onset age 7 years	Homozygous mutation identified in *GAMT* at age 26 years. First described 1996	Cerebral creatine metabolism disorder	Creatine supplementation	Seizures stopped, ASD withdrawn No RCT for this PM	Significant behavioural decline necessitates ASD reintroduction. Developmental component irreversible. PM treats seizures only.

Published in final edited form as:

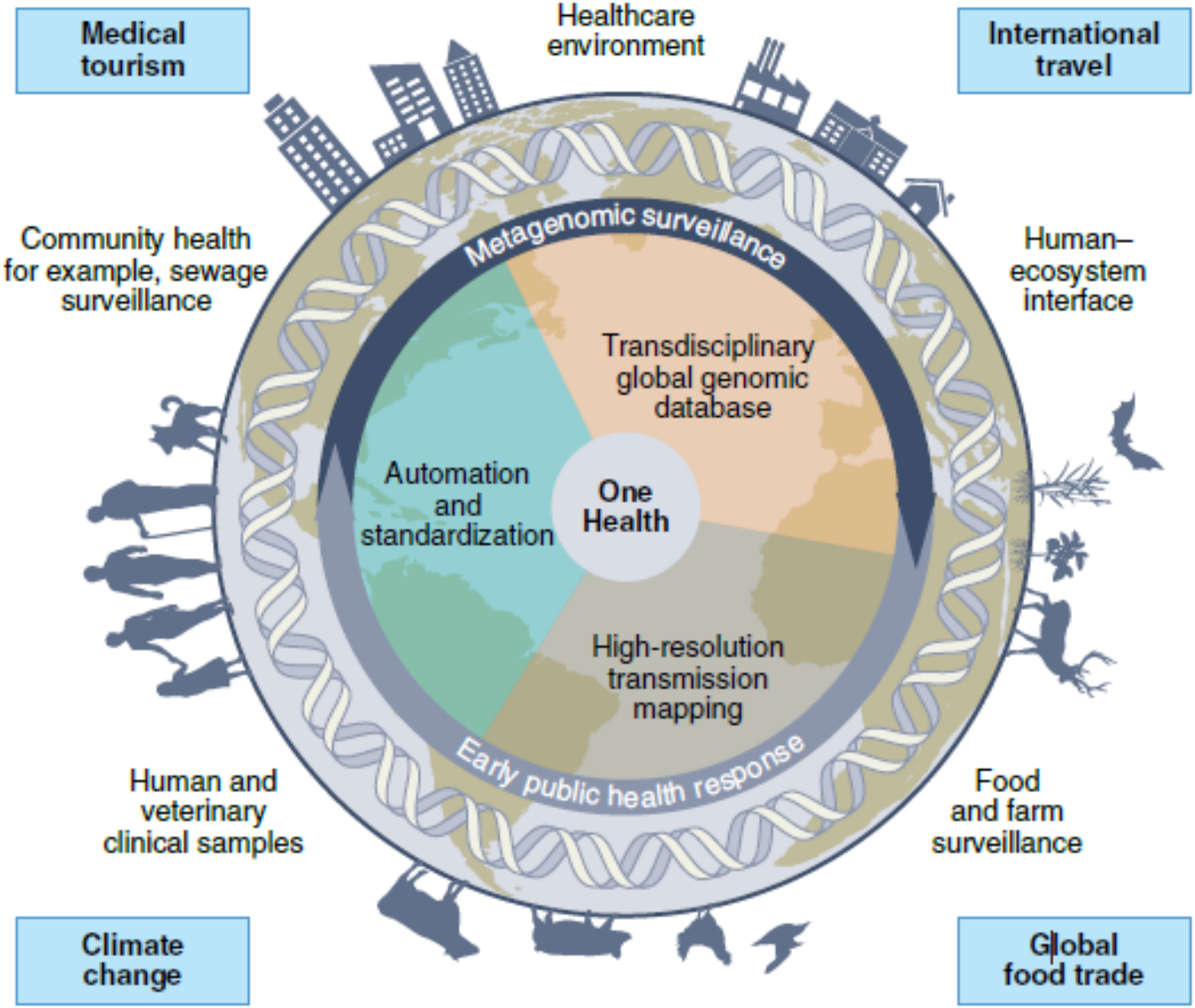
Gastroenterol Clin North Am. 2021 March ; 50(1): 127–139. doi:10.1016/j.gtc.2020.10.005.

Precision Medicine and Obesity

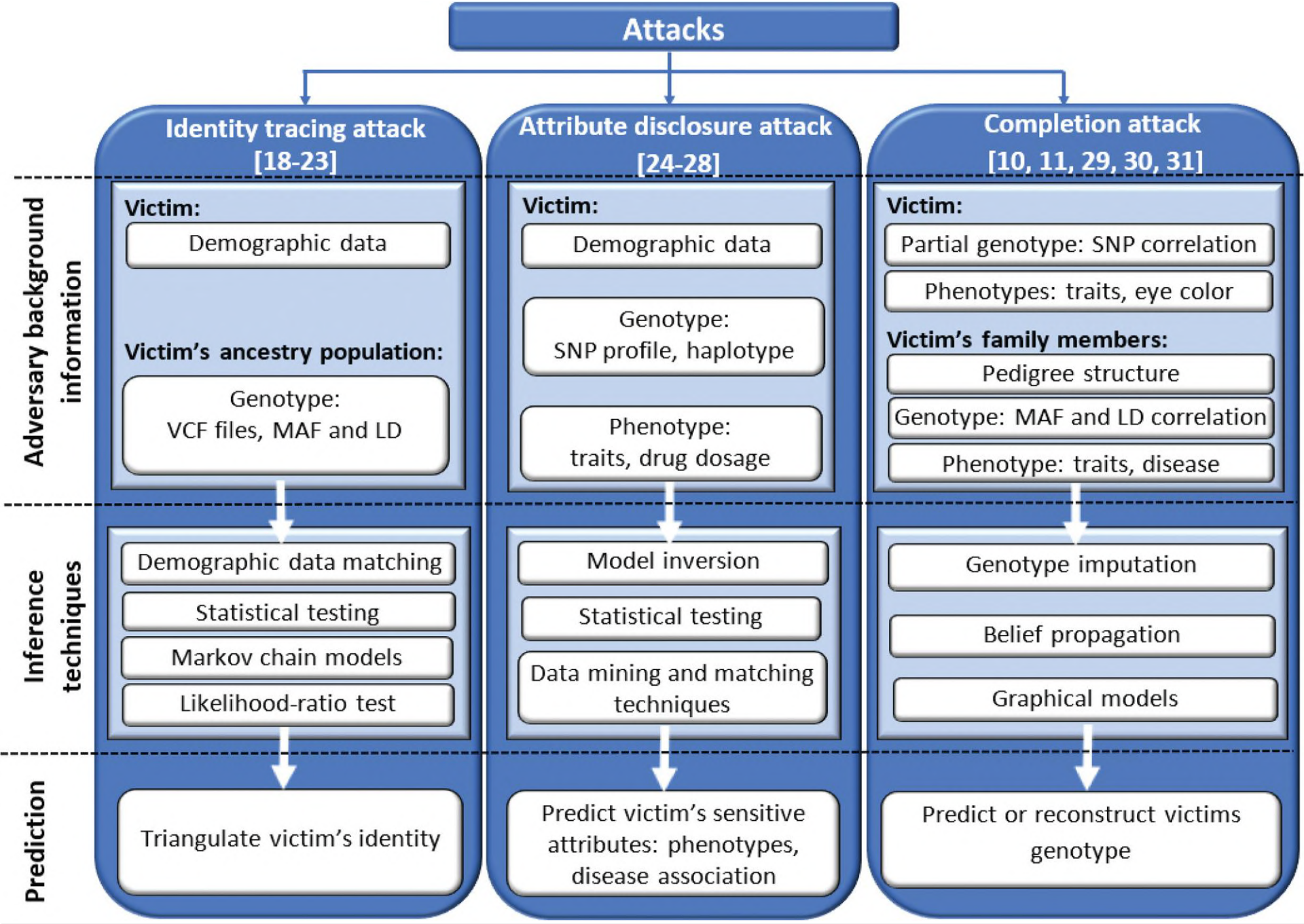


SNPs [12]		
Gene	**Phenotype**	
FTO	BMI, waist circumference, fat percentage, extreme obesity	
MC4R	BMI, waist circumference, extreme obesity	
MC3R, SLC6A14	Obesity	
BDNF, TMEM18	BMI, extreme obesity	
POMC, NEGR1, PCSK1, GNPDA2, MAP2K5, SEC16B	BMI	
Epigenetically modified genes [26]		
Gene	**Phenotype**	
POMC, NPY, SLC6A4, MCHR1	Overall obesity	
FTO, LPL, IRS 1, TMEM18	Fat distribution	
PPARG	Percentage body fat	
LEP	Overall obesity, fat distribution, BMI	
SNPs-diet interactions [43]		
Gene	**Diet Interaction**	**Putative disease risk**
FTO	High Fat and High carbohydrate	Obesity
LCT	Dairy products	
PPARG, GIPR	High fat	
TXN	Low vitamin E	Abdominal obesity
MC4R	Western dietary pattern and high saturated fatty acids	Metabolic syndrome
APOB	High fat	
TCF7L2	High saturated fatty acids	
APOC3, APOA1	Western dietary pattern	
Deregulated metabolic signatures [64]		
Metabolic pathway	**Phenotype**	
Branched-chain amino-acid metabolism	Obesity and insulin resistance	
Androgen synthesis	Childhood obesity	

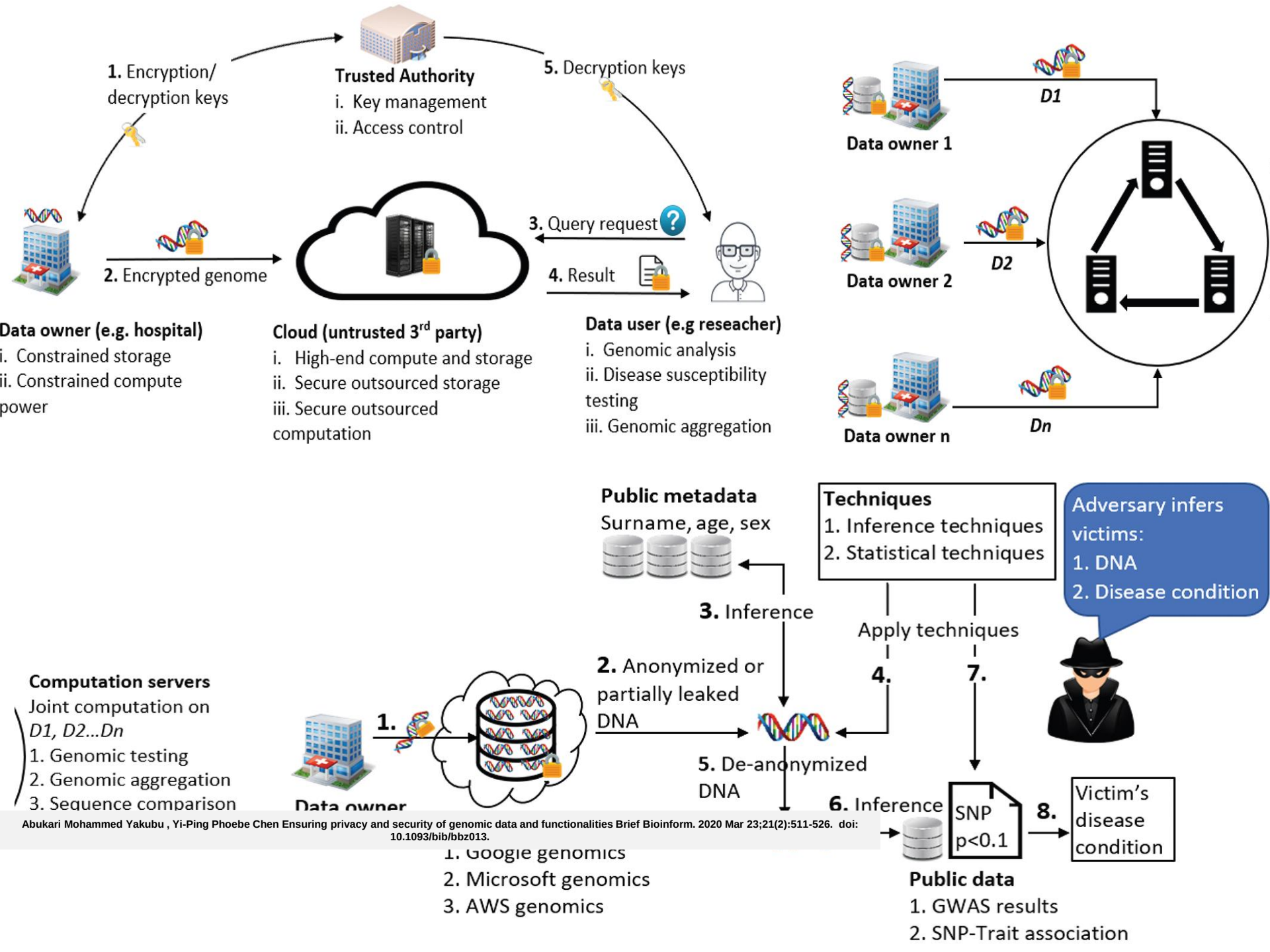
Common SNPs, Epigenetically Modified Genes, SNPs-diet Interactions and Metabolic Pathways Associated with Obesity and Obesity Traits.



Harmonized pathogen surveillance using metagenomics.



Abukari Mohammed Yakubu , Yi-Ping Phoebe Chen Ensuring privacy and security of genomic data and functionalities Brief Bioinform. 2020 Mar 23;21(2):511-526. doi: 10.1093/bib/bbz013.



Abukari Mohammed Yakubu , Yi-Ping Phoebe Chen Ensuring privacy and security of genomic data and functionalities Brief Bioinform. 2020 Mar 23;21(2):511-526. doi: 10.1093/bib/bbz013.

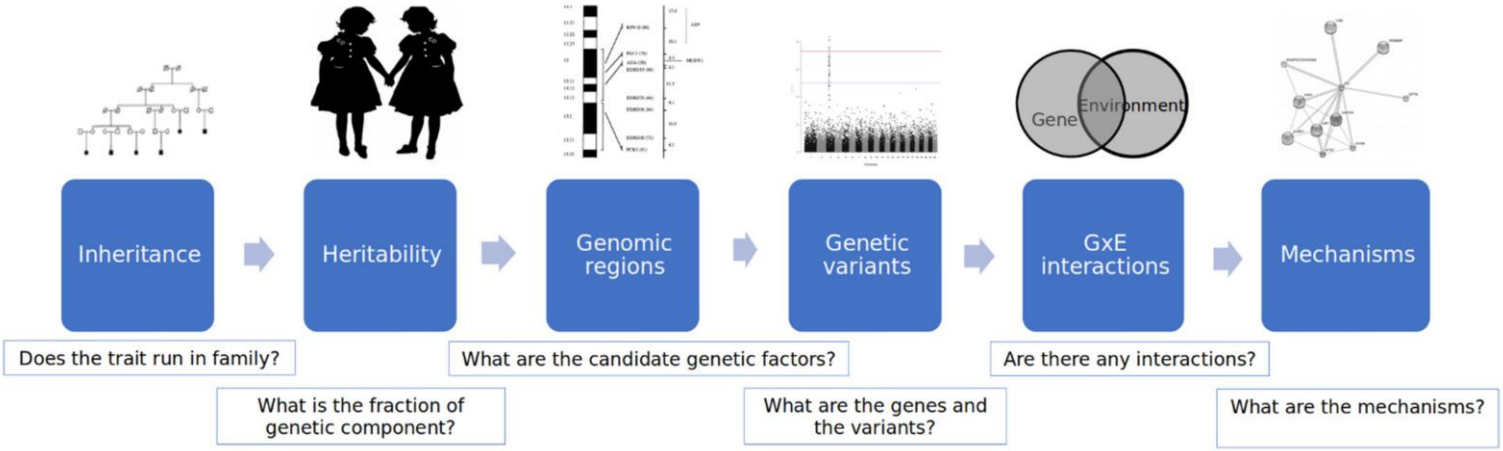
behavioral sciences

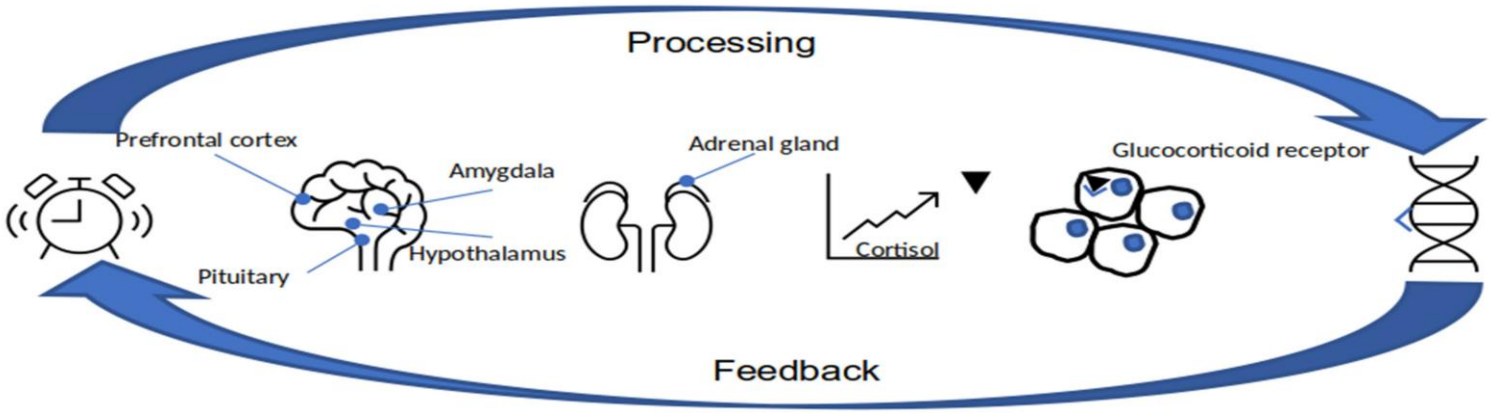
MDPI

Review

CyberGenomics: Application of Behavioral Genetics in Cybersecurity

Ingrida Domarkienė [1,*], Laima Ambrozaitytė [1], Linas Bukauskas [2], Tautvydas Rančelis [1], Stefan Sütterlin [3,4], Benjamin James Knox [3,4,5], Kaie Maennel [4], Olaf Maennel [4], Karen Parish [5], Ricardo Gregorio Lugo [3,6] and Agnė Brilingaitė [2]

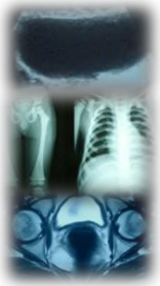


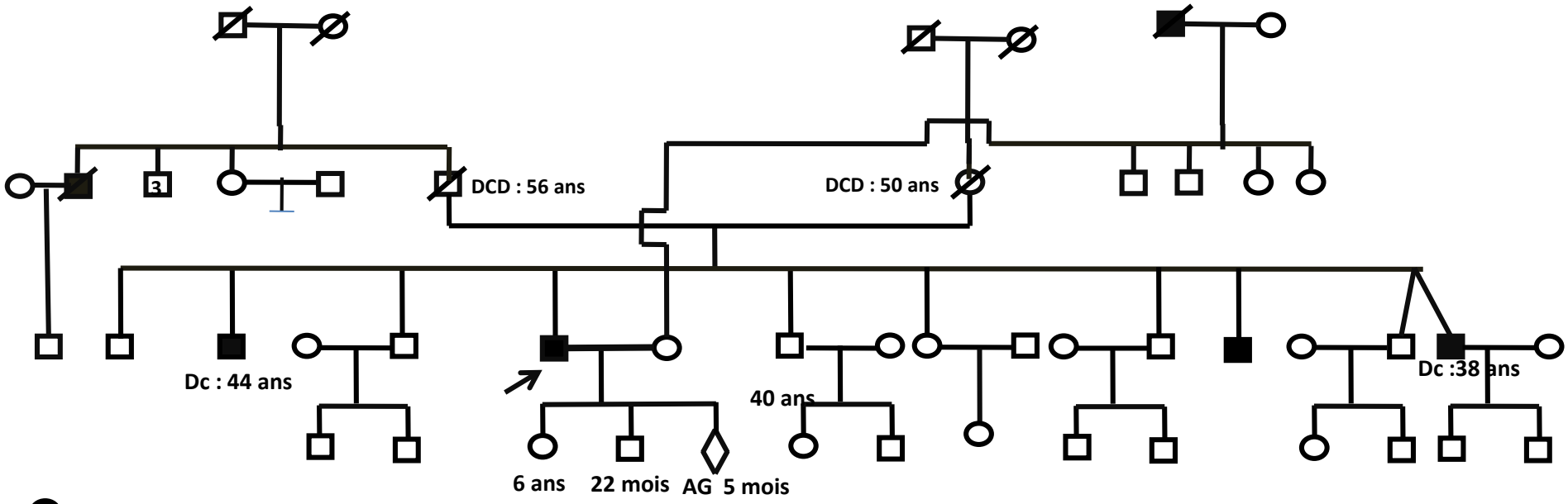


Oncogénétique clinique : consultation et conseil génétique

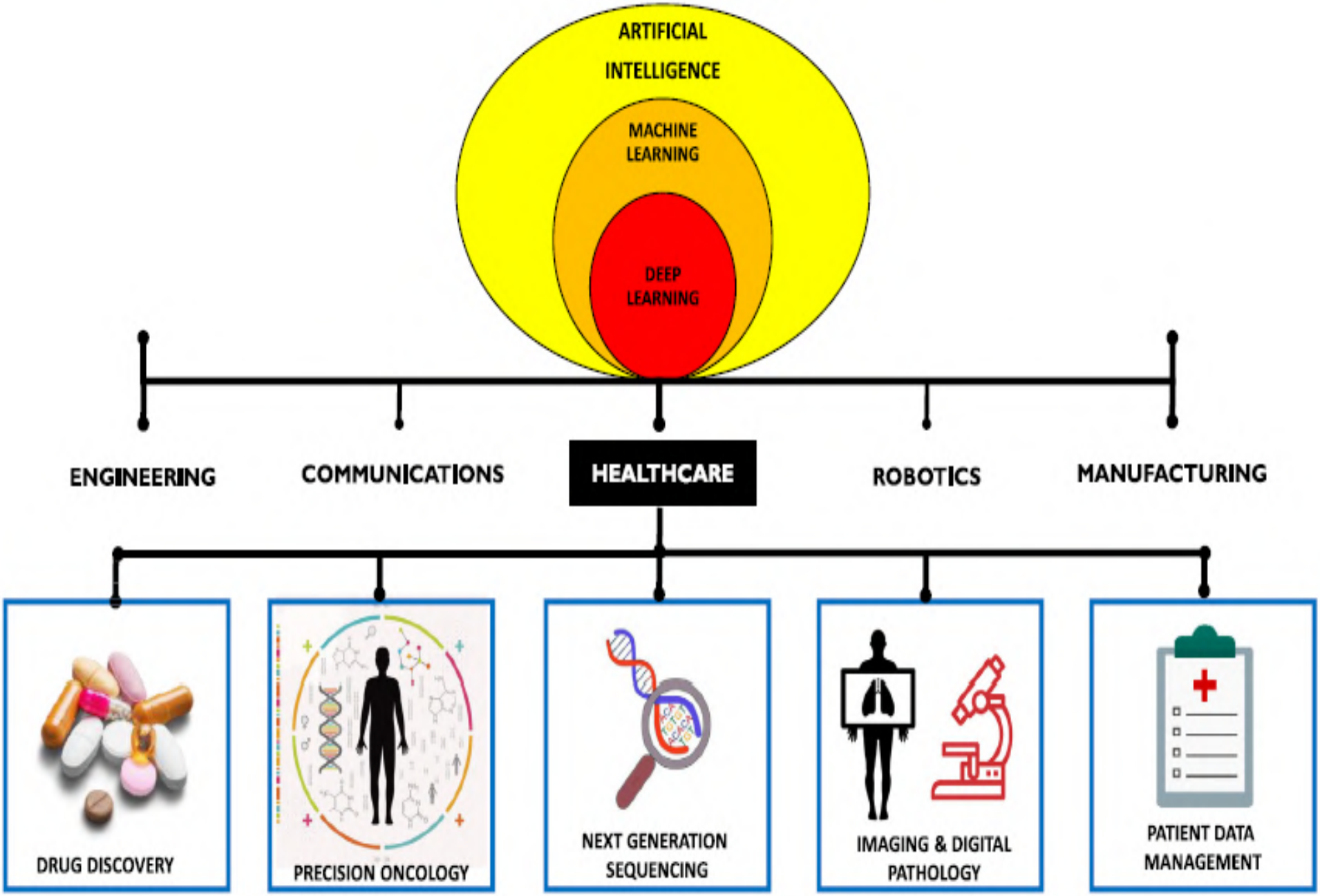
Prédispositions héréditaires au cancer

- Cancer du sein
- Cancer de l'ovaire
- Cancer du colon
- Cancer de la thyroïde…





Famille marocaine : Cancer du colon héréditaire (mutation du gène *hMLH1)*

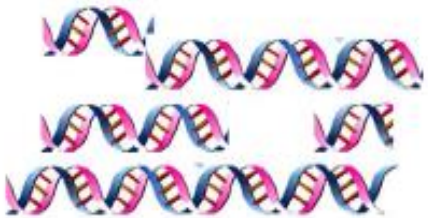


Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311

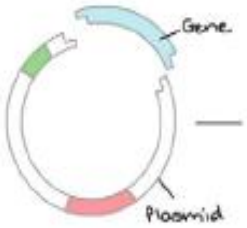
NGS and molecular profiling

1st Generation

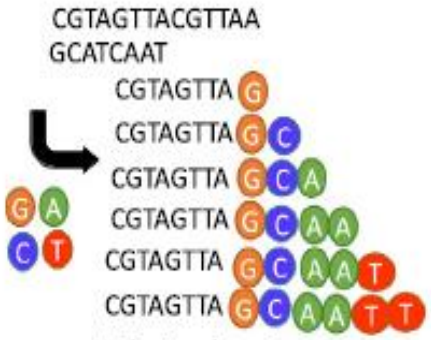
1. DNA fragmentation



2. Cloning



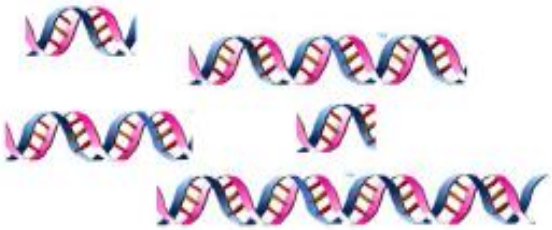
3. Cycle sequencing



4. Electrophoresis

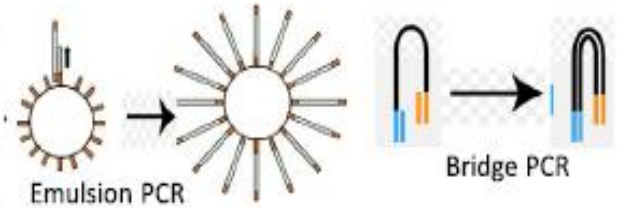
2nd Generation

1. Library preparation - DNA fragmentation



2. In vitro adapter ligation

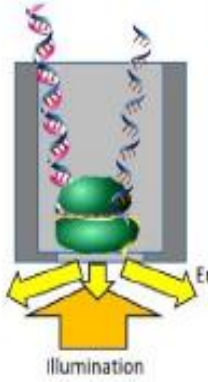
3. Clonal amplification



4. Cyclic array amplification

Pyrosequencing (454 sequencing)
Sequencing by ligation (SOLID platform)
Sequencing by synthesis (Solexa technology)
Reversible dye terminator (Illumina)

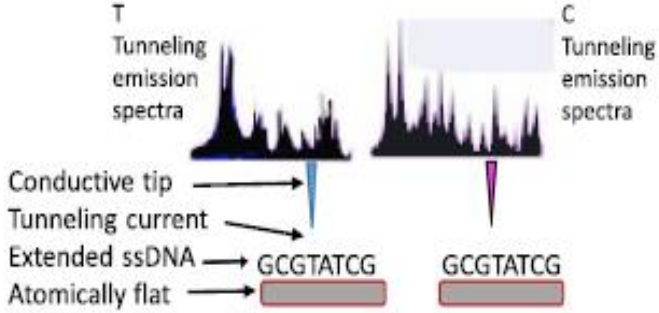
3rd Generation



1. Pacific biosciences

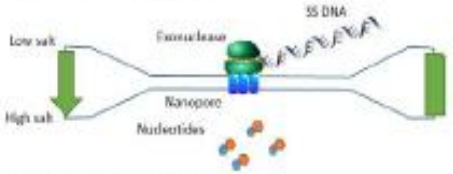
Fluorescence detection of gamma-labelled phosphonucleotides

2. Direct inspection



Oxford Nanopore

Translocation of nucleotides across a pore driven by ion concentration

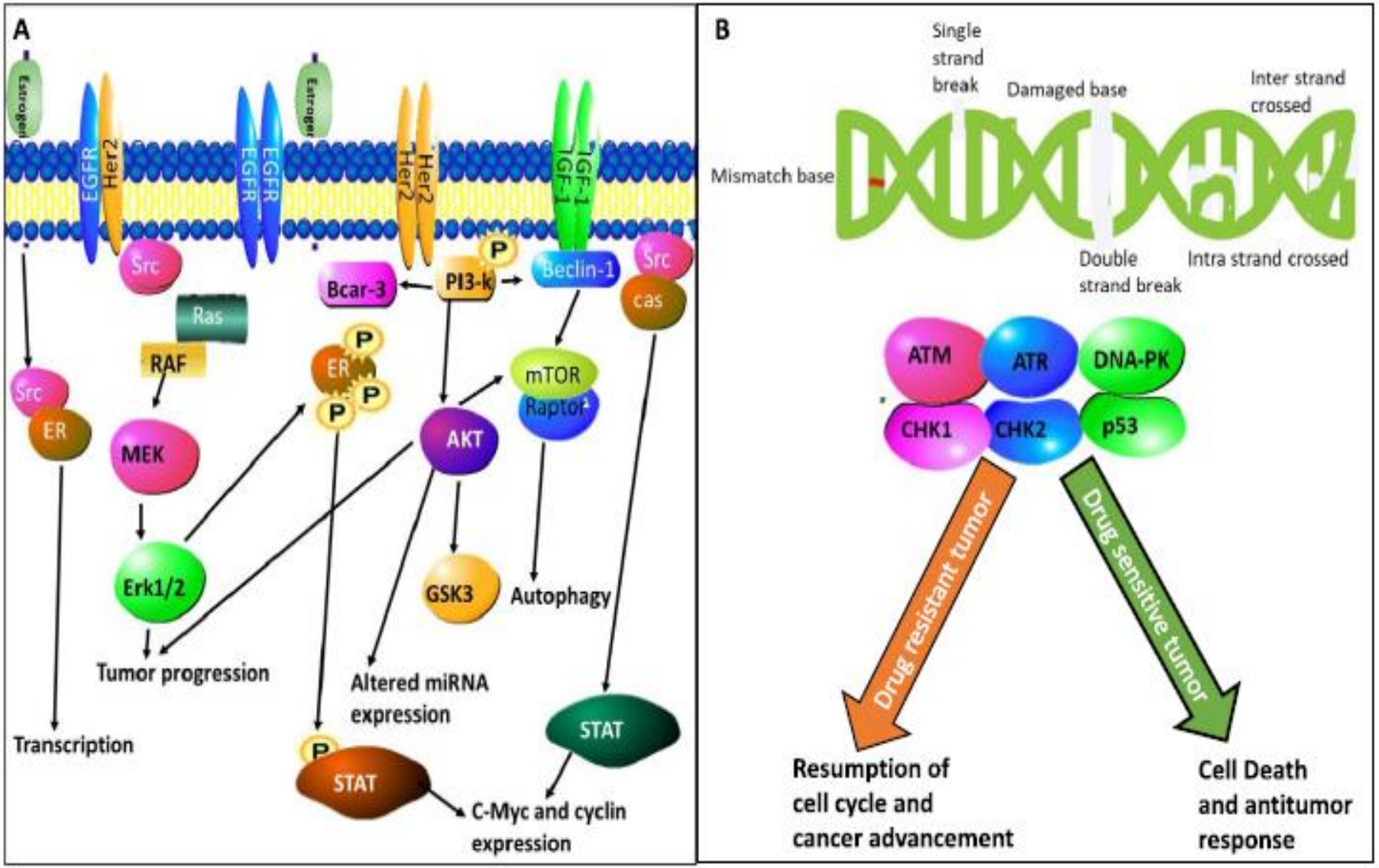


IBM DNA Transistor

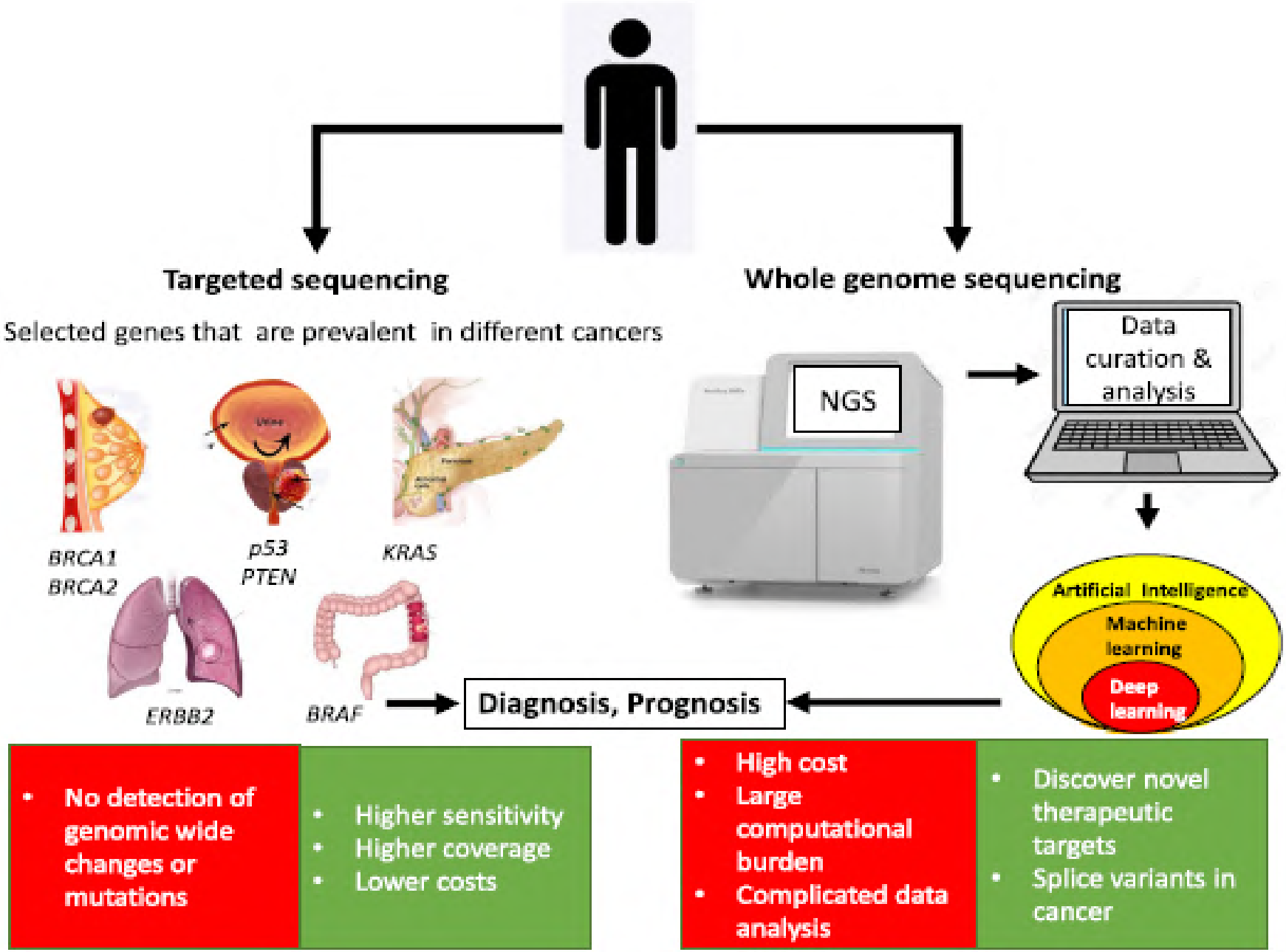
Individual bases are read as the ssDNA passes through the aperture based on the molecules unique electronic signature

Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311

Artificial intelligence : Cancer therapy



Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311



Z. Dlamini et al. Artificial intelligence (AI) and big data in cancer and precision oncology, Computational and Structural Biotechnology Journal 18 (2020) 2300–2311

Integrating Artificial Intelligence and Nanotechnology for Precision Cancer Medicine

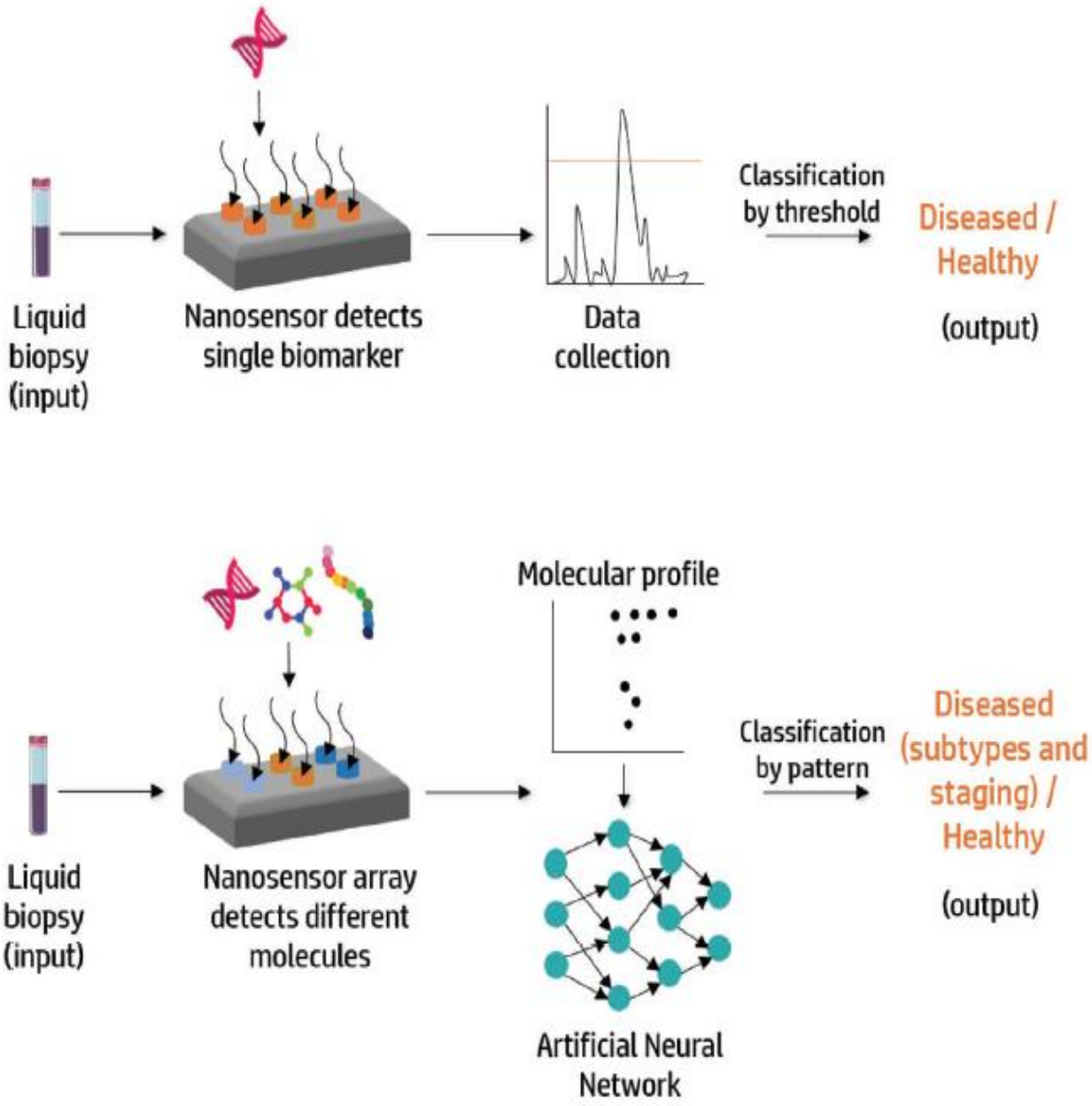
Single Biomarker Sensing

- High sensitivity and specificity in biomarker detection
- Dependence on discovery and approval of new biomarkers
- Inter-patient variability in biomarker concentrations limits the accuracy of the diagnostic prediction

Progress Towards Big Data Analysis

Multiplex Sensing

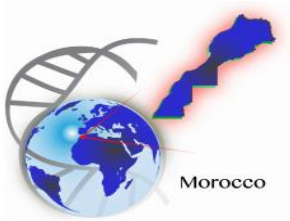
- Pattern-based analysis is not depended on a single biomarker
- Requires collection of large data sets for computing the classification pattern
- New approval procedures for pattern based diagnostics are required



Omer Adir et al Integrating Artificial Intelligence and Nanotechnology for Precision Cancer Medicine adma.2. Epub 2019 Jul 9.

Diagnostic test	Advantages	Disadvantages
Direct PCR	Simple Rapid Inexpensive Potential for quantitative PCR	Depends on hypothesis Requires primers that may not always work Limited to a very small portion of genome
Multiplex PCR	Rapid Able to detect multiple organisms	Low specificity and false positives for many organisms due to difficulty in quantitation Often requires more than one amplification Limited to a small portion of genome Requires primers that may not always work
Targeted universal multiplex PCR (e.g., 16S, ITS) for Sanger sequencing	Can differentiate multiple species within one pathogen type	Requires primers that may not always work Limited to a very small portion of genome
Targeted universal multiplex PCR (e.g., 16S, ITS) for NGS	Can differentiate multiple species within one pathogen type Multiplexing capability Potential for quantitation	Requires primers that may not always work Expensive and time consuming Often requires more than one amplification Limited to a very small portion of genome
Targeted NGS	Sensitive detection for selected organism types Potential for quantitation Potential to be combined with 16S NGS (see above)	Sequencing library preparation more complex, typically with more than one amplification Limited to a small portion of genome Expensive and time consuming Prone to contamination with environmental species
Metagenomic NGS	Hypothesis-free, or unbiased, testing Discovery of new or unexpected organisms Potential for quantitation Ability to detect any portion of genome	Must also sequence human host background Expensive Time consuming Not all genomes are available Prone to contamination with environmental species
Serology	Potential for diagnosis after acute infection Inexpensive	May be negative during early infection False-negatives in humoral immune deficiencies False-positives
Microscopy and staining (e.g., Gram stain, auramine–rhodamine, calcofluor-white)	Rapid Inexpensive	Low sensitivity unless there is a high burden of disease Low specificity
Culture	Able to accommodate large sample volumes Inexpensive Well studied	Sensitivity limited by use of antibiotics and antifungals Sensitivity limited for fastidious organisms Limited use in viral testing Long time to result, especially in acid-fast and fungal cultures
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	High specificity Rapid after culture	Requires culture-positive isolate

Abbreviations: ITS, internal transcribed spacer; NGS, next-generation sequencing; PCR, polymerase chain reaction.



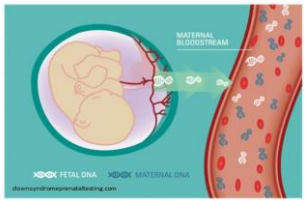
Génétique : 1 cellule ……Vieillissement

1000 Jours

Diagnostic préimplantatoire

Prévention préconception

Diagnostic prénatal DPN Invasif et non invasif



Naissance

DNN programme National

Pédiatrie

Médecine Adulte

Prevention

Diagnostic

Prise en charge et suivi

Pharamcogénomique

Oncogenetique

Microbiologie microbieome

k.Ouldim 2023

Registry **Biobank** **Telemedicine** **National Network** **Bioinformatics**

Data **drug** **Clinical trials**

Omics

Genomics

DNA

Transcriptomics

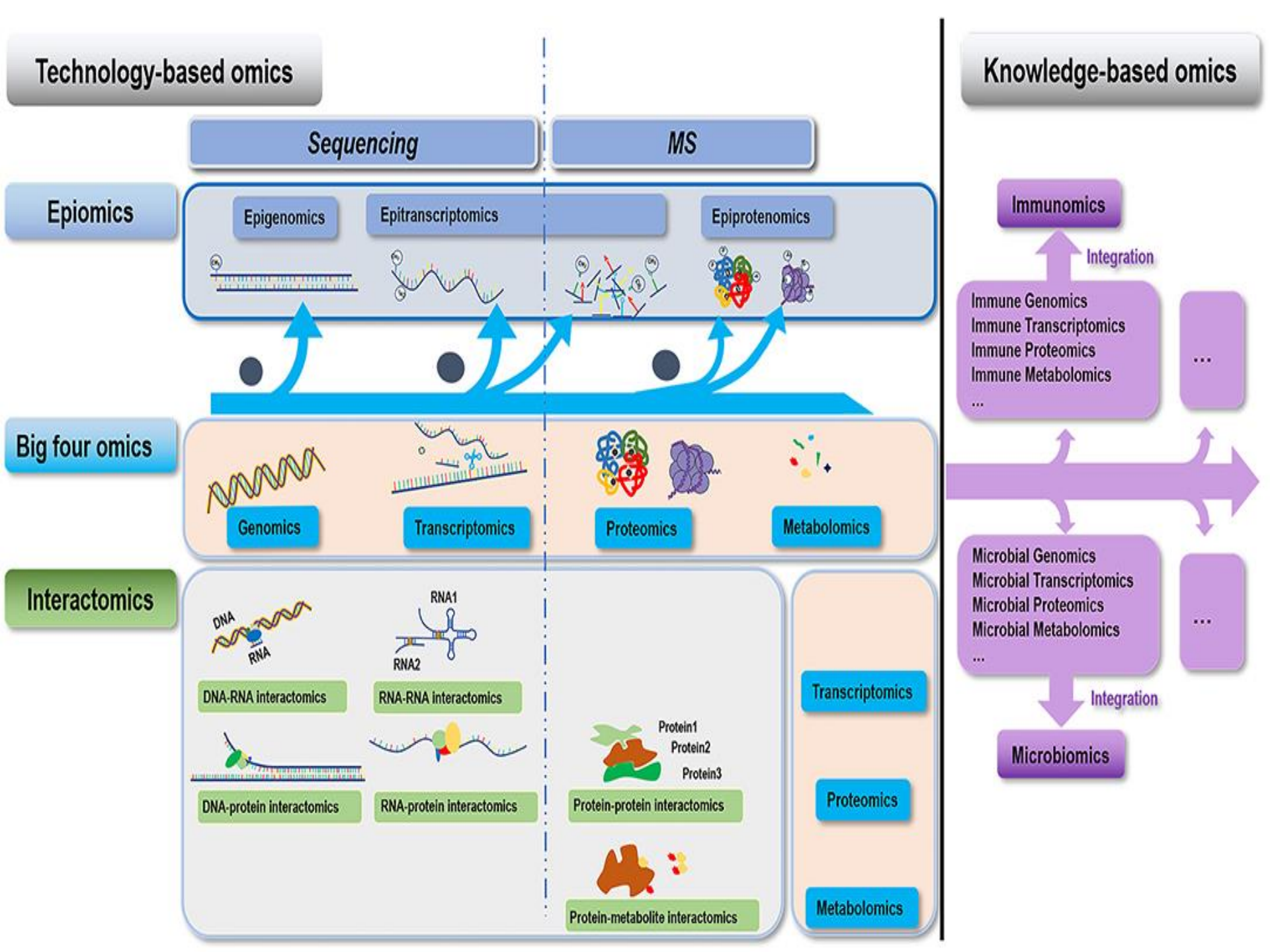
RNA

Proteomics

Proteins

Metabolomics

Metabolites


Technology-based omics
Knowledge-based omics
Sequencing
MS
Epiomics
Epigenomics
Epitranscriptomics
Epiprotenomics
Big four omics
Genomics
Transcriptomics
Proteomics
Metabolomics
Interactomics
DNA
RNA
DNA-RNA interactomics
RNA1
RNA2
RNA-RNA interactomics
DNA-protein interactomics
RNA-protein interactomics
Protein1
Protein2
Protein3
Protein-protein interactomics
Protein-metabolite interactomics
Transcriptomics
Proteomics
Metabolomics
Immunomics
Integration
Immune Genomics
Immune Transcriptomics
Immune Proteomics
Immune Metabolomics
...
...
Microbial Genomics
Microbial Transcriptomics
Microbial Proteomics
Microbial Metabolomics
...
...
Integration
Microbiomics

Analyse génomique

- ***Sujets normaux*** (médecine préventive): Génomique à la demande
- **Génome blastomére, fœtus, ADN fœtal circulant** chez la mère: DPI, DPN
- **Génome des tumeurs :** diagnostic, un pronostic et d'optimiser les cibles thérapeutiques
- ***Diagnostic des maladies héréditaires***
- **Adaptation thérapeutique** en fonction du fond génomique: pharmacogénomique (oncologie +++++).

Big Data, Artificial Intelligence and Health

Precision medicine

1000 Jours

Diagnostic préimplantatoir e

Prévention préconception

Diagnostic prénatal DPN Invasif et non invasif

Naissance

DNN programme National

Pédiatrie

Médecine Adulte

Diagnostic
Prise en charge et suivi

Registry | Biobank Data | Telemedicine Orphan drug | National Network Clinical trials | Bioinformatics

k.Ouldim 2022

Cours de Génétique Médicale

1ère année médecin 2023 / 2024

Faculté de médecine et de Pharmacie d'Errachidia

1. Les acides nucléiques et Génome Humain
2. Réplication et systèmes de réparation de l'ADN
3. Transcription
4. Traduction
5. Contrôle de l'expression génique
6. Cytogénétique classique et moléculaire
7. Types et mécanismes des anomalies chromosomiques
8. Techniques d'analyse de l'ADN
9. Mutations et leurs conséquences en pathologie humaine
10. Mode de transmission des Maladies héréditaires