

Les bases du diagnostic en Génétique moléculaire

Marie Legendre, PharmD PhD

U.F. de Génétique moléculaire

Sorbonne Université, Assistance Publique Hôpitaux de Paris

UMR_S933 « Maladies génétiques d'expression pédiatrique »

Sorbonne Université, Inserm – Prof. Serge Amselem

Hôpital Armand Trousseau, Paris, France

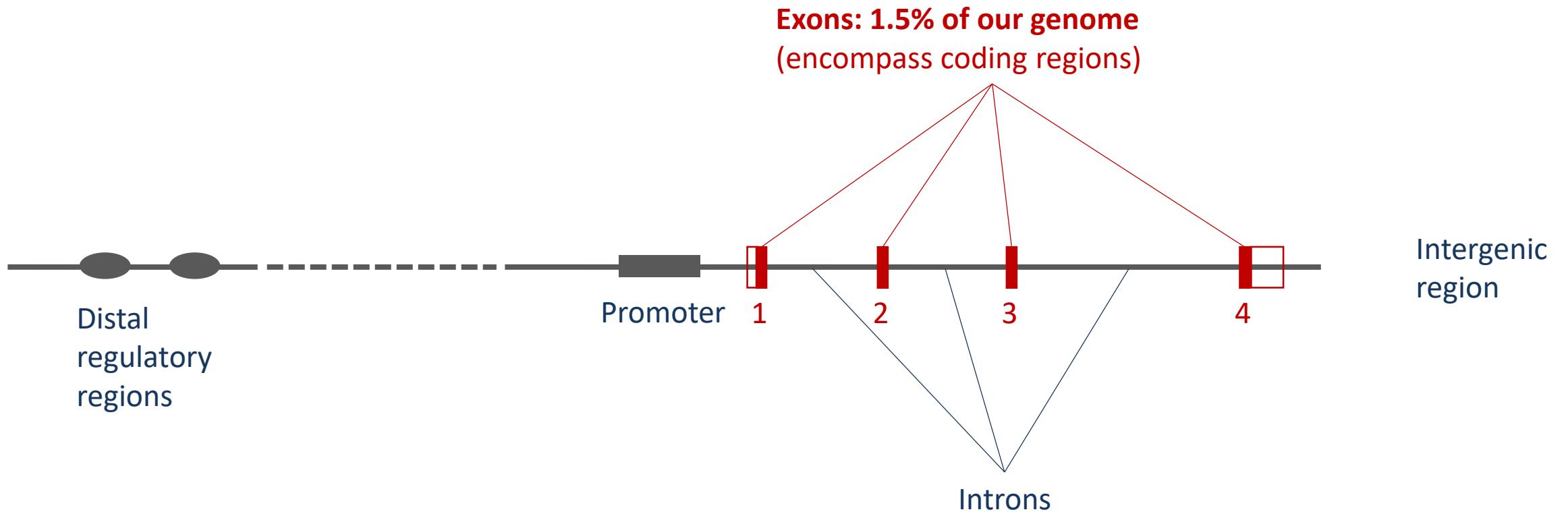
marie.legendre@aphp.fr



1. Diversity of our genomes & interpretation challenges
2. Genetic counseling and legal issues
3. Severity range of genotypes (gene, variation)
4. Tips for molecular genetics reports
5. Main diagnostic tests & their limitations

Basics

Structure of a gene



Next-Generation Sequencing (**NGS**)

Diagnostics
Research

Targeted gene panels

**Exons (~ coding regions)
of selected genes**
implicated in a disease

Whole Exome Sequencing
(WES)

**Exons of all the
known genes**

Whole Genome Sequencing
(WGS)

**Exons, introns,
regulatory regions,
intergenic regions**

Main techniques and their applications

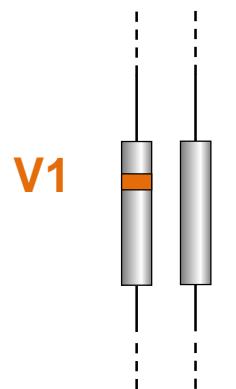
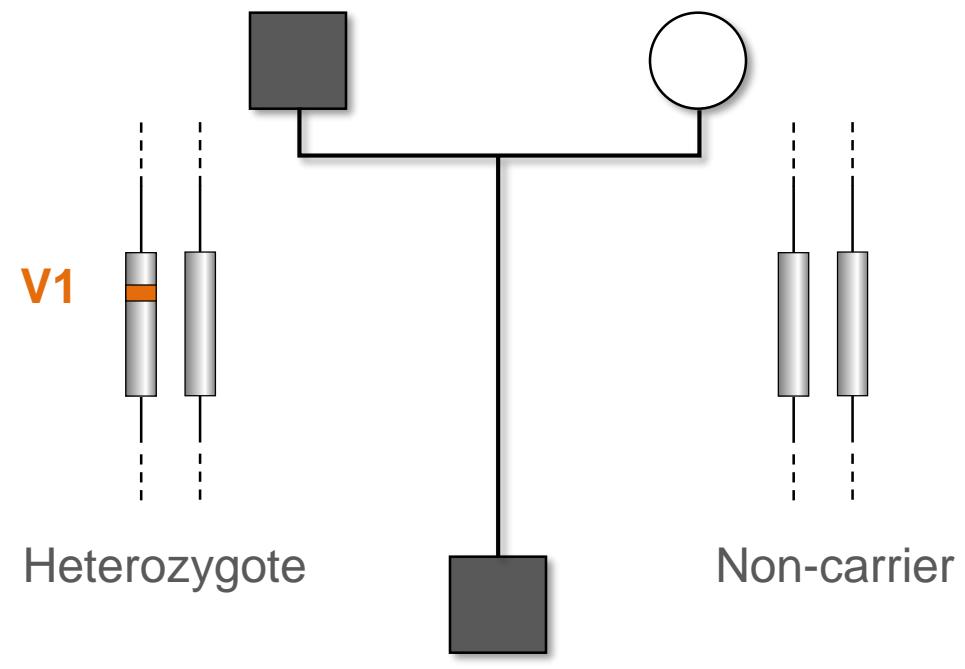
	Point variations and small insertions/deletions in coding regions	Large heterozygous del/dup (≥ 1 exon)	Large homozygous del/dup (≥ 1 exon)	Deep-intronic or intergenic mutations
Sanger	+	-	≈	-
Targeted NGS (gene panel)	+	≈	≈	-
Whole Exome Sequencing (WES)	+	≈	≈	-
Whole Genome Sequencing (WGS)	+	≈	≈	+

⊕ detected

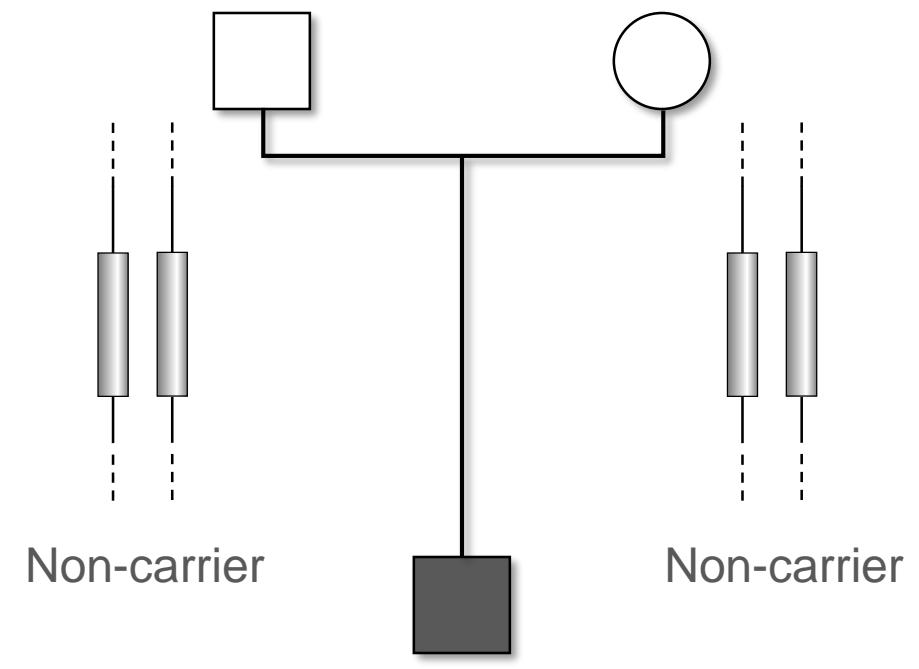
≈ variable detection

- no detection

Dominant disease



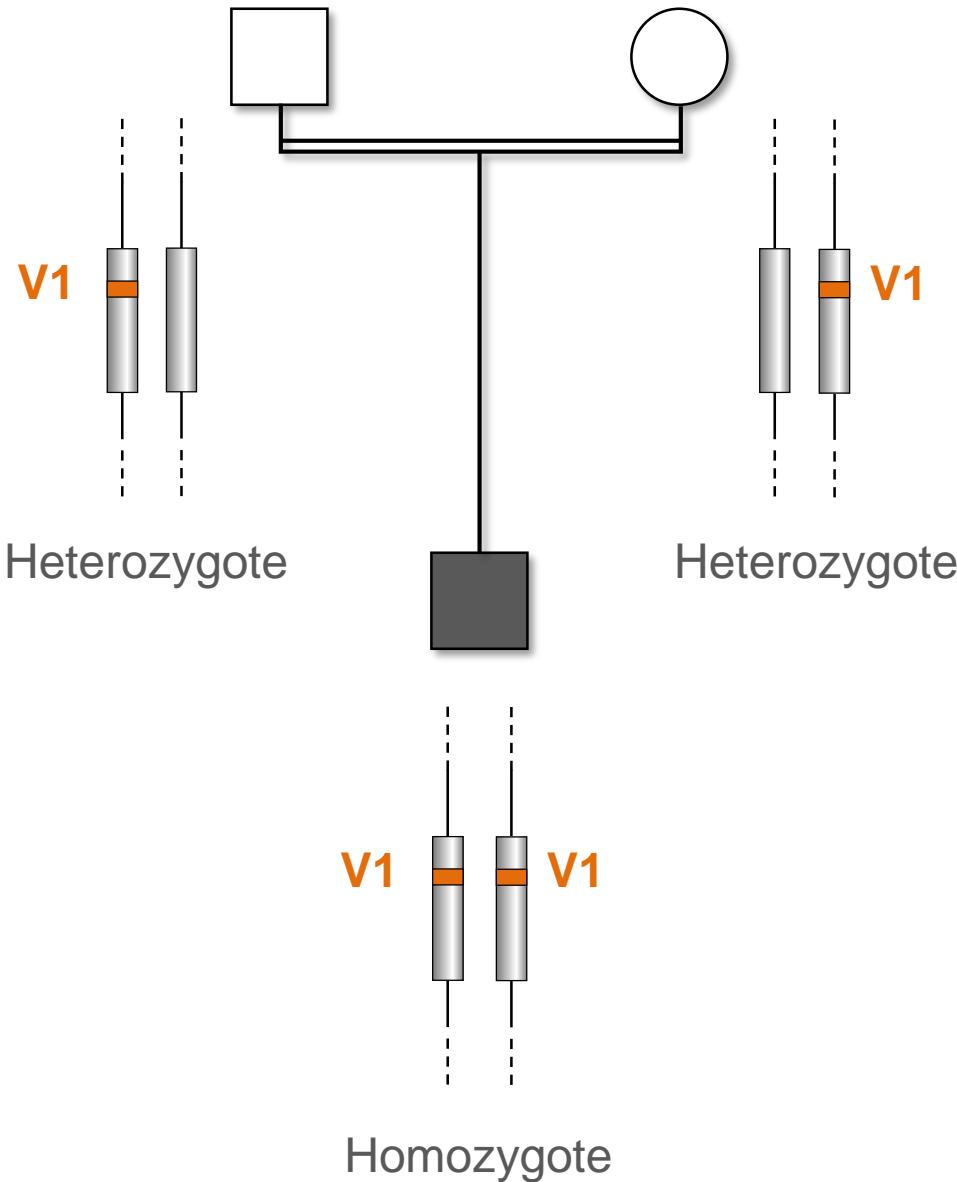
Heterozygote



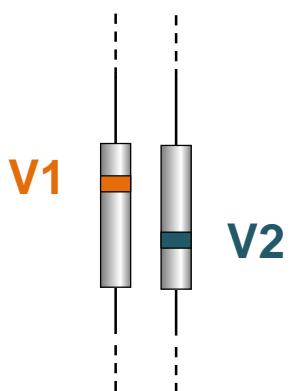
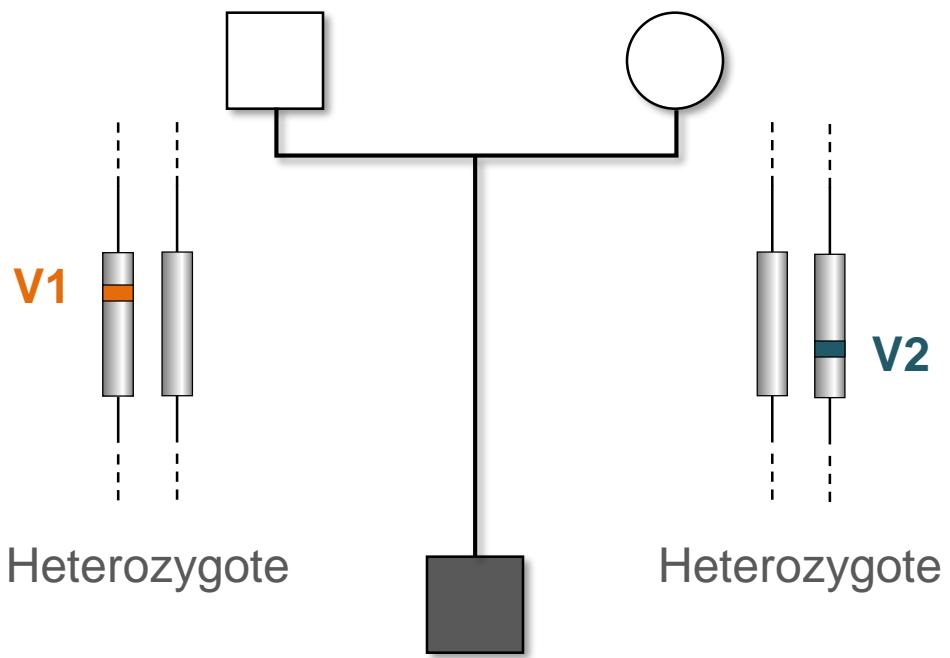
Heterozygote

V1
de novo

Recessive disease

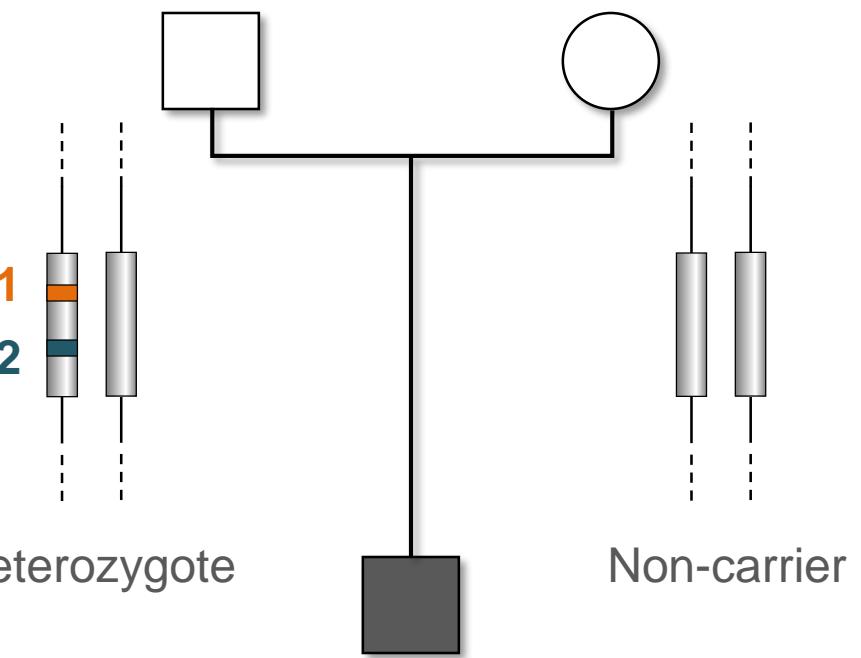


Recessive disease



Compound heterozygote

or



Heterozygote

Detailed phenotype / pedigree

Diagnostic des dyskinésies ciliaires primitives (DCP)
Syndrome de Kartagener

Formulaire de renseignements cliniques - Remplir un formulaire par individu **1/2**

Le diagnostic de DCP doit être au préalable établi sur les résultats des études ciliaires.
Le phénotype ultrastructural est indispensable pour guider le choix des gènes à étudier.

Identité du patient :	Date : Nom du médecin senior : Code RPPS : Service : Hôpital : email : N° de téléphone :
NOM : Prénom : <i>ou étiquette</i> Nom de jeune fille : <i>patient</i> Date de naissance : Sexe :	Origines géographiques : du père : de la mère :
Consanguinité parentale : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>si oui préciser :</i>	Atteinte uro-génitale : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Polykystose rénale oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Hypo fertilité/stérilité oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>si oui préciser :</i>
Présentation clinique du patient :	Atteinte sensorielle : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i>
Age au début des manifestations respiratoires :	Rétinite pigmentaire oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Hypoacusie/surdité oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i> de transmission <input type="checkbox"/> centrale <input type="checkbox"/> mixte <input type="checkbox"/> ND <input type="checkbox"/>
Détresse respiratoire néonatale : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>	Autre(s) : préciser :
Manifestations bronchopulmonaires :	Autres manifestations : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i>
Encombrement bronchique oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Syndrome du lobe moyen oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> BPCO* oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Pneumopathies oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Asthme oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>	Retard psychomoteur oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Hydrocéphalie oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Polydactylie oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Obésité oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
DDB* oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i> localisée <input type="checkbox"/> diffuse <input type="checkbox"/> ND <input type="checkbox"/>	Autre(s) pathologie(s) et remarque(s) éventuelle(s) :
Manifestations ORL :	
Rhinosinusite oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Polypose nasosinusiennne oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Otites séromuqueuses oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Otites moyennes aigues oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>	
Autre(s) : préciser :	
Malposition viscérale : oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i>	Mesure NO nasal : <input type="checkbox"/> nL/min <input type="checkbox"/> ppb
Situs inversus complet oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> Situs inversus incomplet oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/> <i>préciser :</i> thoracique <input type="checkbox"/> abdominal <input type="checkbox"/> ND <input type="checkbox"/> Dextrocardie oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>	Biopsie pour analyse en microscopie électronique : Date(s) : Muqueuse nasale <input type="checkbox"/> bronchique <input type="checkbox"/> Résultat(s) :
Autre(s) : préciser :	

Identité du patient :

NOM :
Prénom :

Etude de la famille :

Antécédents familiaux :
(préciser sur l'arbre)

Maladie respiratoire
si oui préciser :

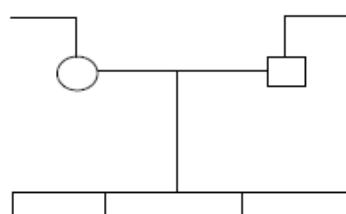
DCP	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
BPCO*	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
DDB*	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Rhinosinusite	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Polypose nasosinusiennne	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Autre(s) : <i>préciser :</i>	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>

Malposition viscérale	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Rétinite pigmentaire	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Surdité	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Polykystose rénale	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Stérilité ou hypofertilité	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>
Retard psychomoteur	oui <input type="checkbox"/> non <input type="checkbox"/> ND <input type="checkbox"/>

Autre(s) : préciser :

Arbre généalogique à compléter :

Signaler dans l'arbre généalogique :
- par un symbole plein (noir) : le(s) patient(s)
- par un symbole clair (blanc) : le(s) sujet(s) sain(s)
- par une flèche : le(s) sujet(s) prélevé(s)



○ Sujet féminin
□ Sujet masculin

Legal issues

- **Consentement éclairé :**
 - adultes, mineurs
 - RGPD
 - Données incidentes / actionnables
 - Prescripteur(s): en charge du conseil génétique ou de son exécution
- **Compte-rendu de moléculaire :**
 - Labo → prescripteur seulement
 - Orbis
- **Mineurs asymptomatiques :**

pas d'étude sauf si bénéfice pour elle/lui ou un apparenté

Legal issues

- DPN : contact avec le labo expert **et** CPDPN
- DPI : contact avec le labo de DPI régional
- **Patient décédé / ne pouvant exprimer sa volonté : étude possible si**
 - suspicion d'une cause génétique à une maladie grave (risque décès prématuré, handicap sévère),
 - intérêt pour les apparentés,
 - demande d'un apparenté,
 - le patient ne s'y est pas opposé précédemment.

Arrêté du 11 septembre 2023 fixant les critères déterminant les situations médicales justifiant, chez une personne hors d'état d'exprimer sa volonté ou décédée, la réalisation d'un examen de ses caractéristiques génétiques à des fins médicales dans l'intérêt des membres de sa famille potentiellement concernés, en application de l'article L. 1130-6 du code de la santé publique

Our genomes are diverse

Variation classification

Class 5

Pathogenic

Class 4

Probably pathogenic

Class 3

Uncertain Significance (VUS)

Class 2

Probably benign

Class 1

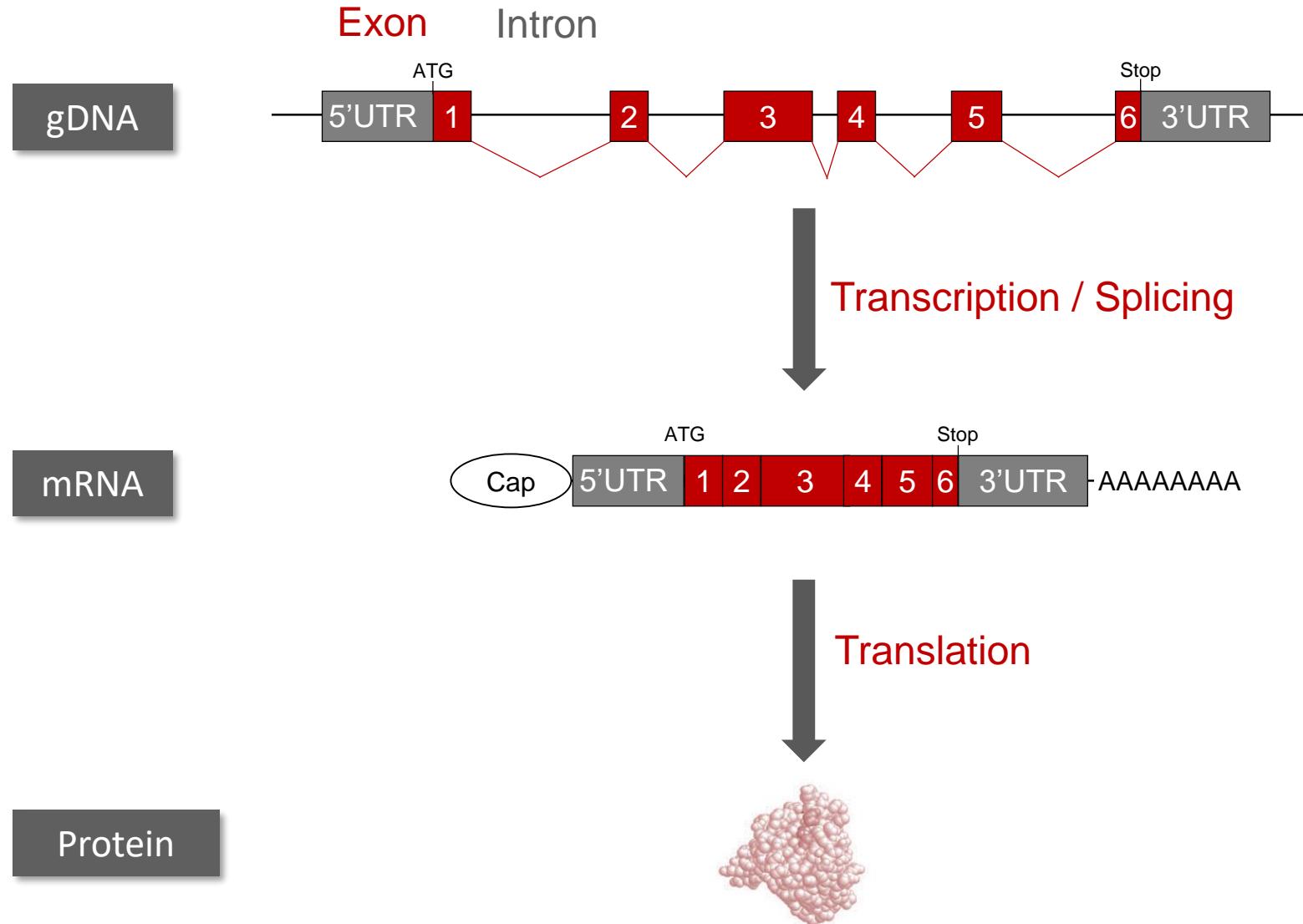
Benign

Genetic counseling

No genetic counseling

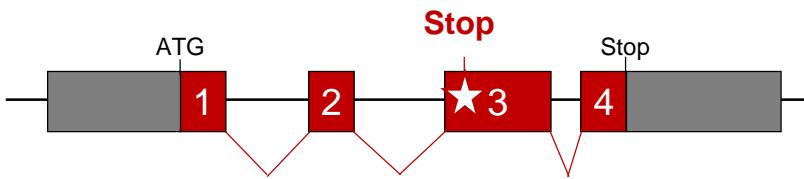
See Richards et al. *Genet Med* 2015

From gene to protein

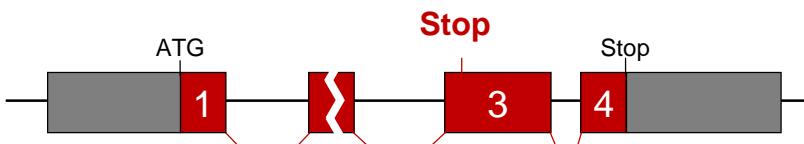


Types of pathogenic variations

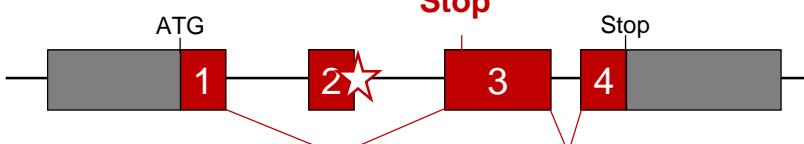
Nonsense



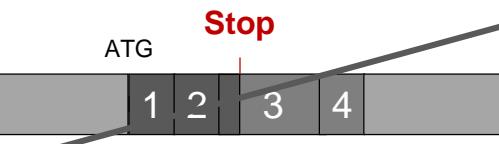
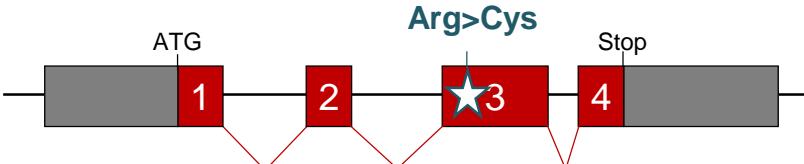
Frameshift



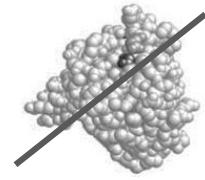
Splice



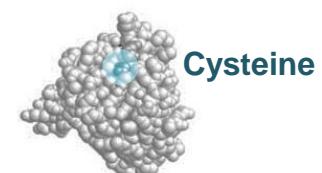
Missense



Nonsense-mediated
mRNA decay



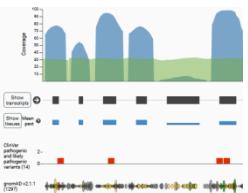
No protein



Protein with an
amino-acid change

To assess the pathogenicity of a missense variation

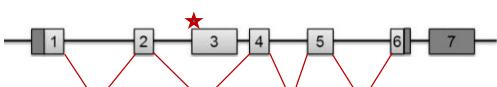
Frequency in ‘control subjects’ databases?



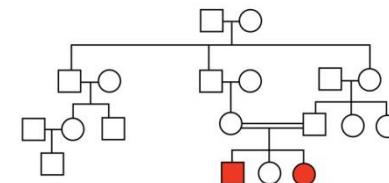
Conservation?

Homo_sapiens	ITILVALRRLHCPRNYVHTQLF
Canis_lupus	IAILVALRRLHCPRNYIHTQLF
Mus_musculus	IAILVALRRLHCPRNYIHTQLF
Rattus_norvegicus	IAILVALRRLHCPRNYIHTQLF
Gallus_gallus	VTIVLMAFRLRCPRNYIHSQLF
Danio rerio	VLILLLFRLRHTCRNYIHMQLF
:	: * : ; *** ; * , *** ; * ***

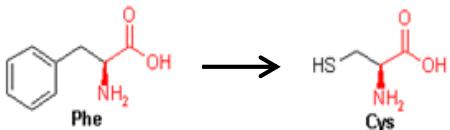
Splicing?



Segregation?

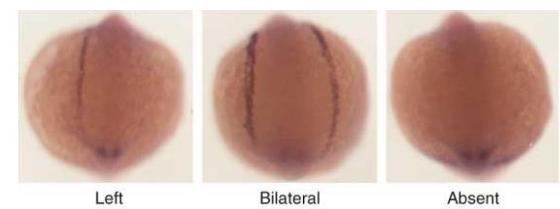
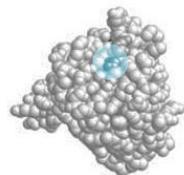


Type?



Functional studies (research)

Location?



Merveille et al. *Nat Genet* 2011

With **exome sequencing**, how many variations are detected in a single subject?

Vote

1. 30
2. 300
3. 30,000
4. 3 million
5. I am not quite sure

With **exome sequencing**, how many variations are detected in a single subject?

1. 30
2. 300
- 3. 30,000**
4. 3 million
5. I am not quite sure

Exome sequencing = all the exons from a subject

1 subject

Exome
analysis

30,000 variations / reference genome

**3,000 rare or not reported
in general population databases**

300 potentially pathogenic

100 synonymous

300 missense

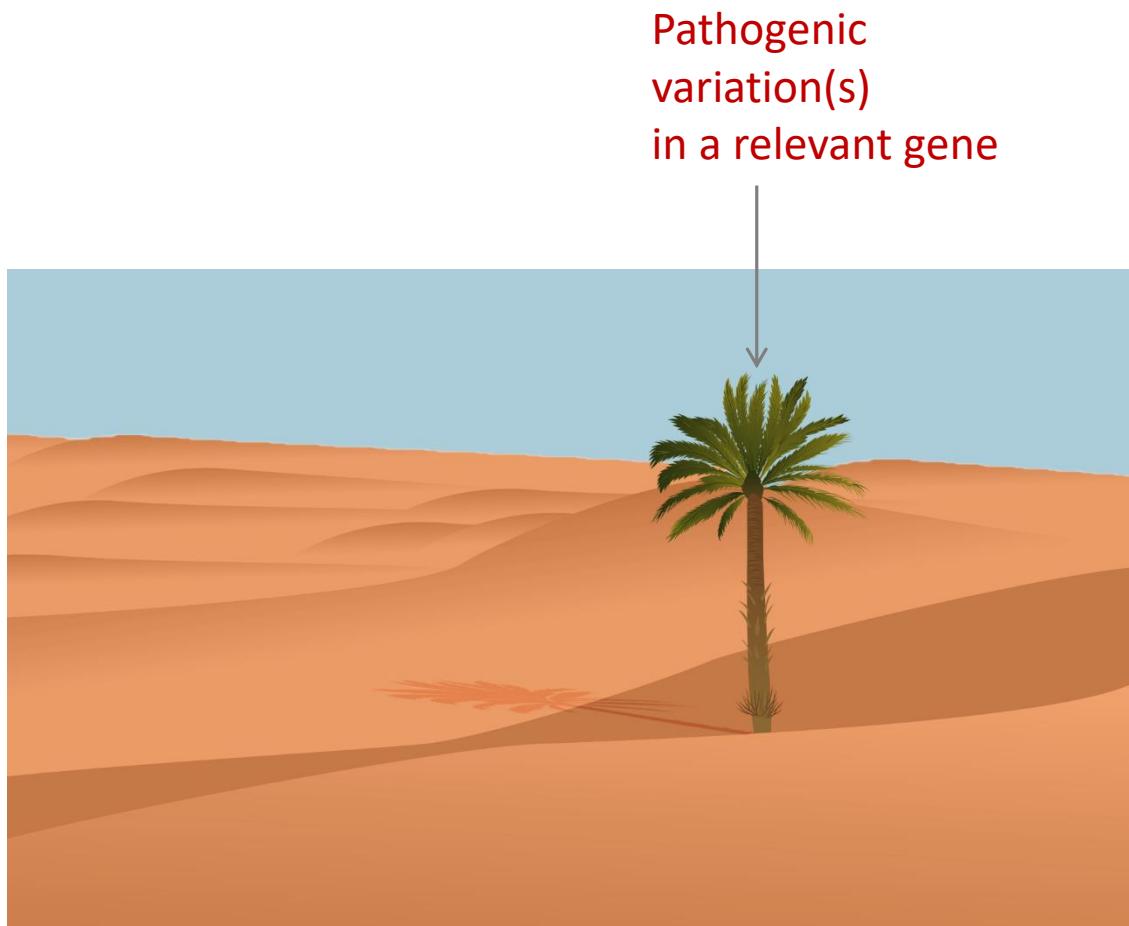
7 nonsense

5 splice

1 frameshift

10-100 = pathogenic variations

Finding the relevant pathogenic mutation(s) among hundreds of variations



Pathogenic variation(s) in a relevant gene

One heterozygous probably pathogenic variation in a relevant gene

One heterozygous VUS in a relevant gene

One heterozygous pathogenic variation in another relevant gene

2 VUS in a good candidate gene

One heterozygous pathogenic variation in a gene of unknown function

2 VUS in another airway disease gene

One heterozygous pathogenic mutation in a disputed gene

One heterozygous potentially pathogenic variation in a good candidate gene

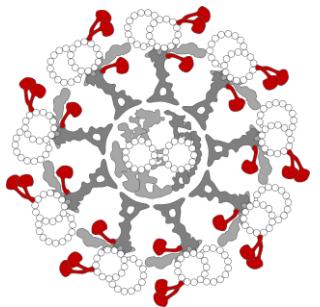
Variable pathogenicity

Phenotype/genotype correlation: gene level

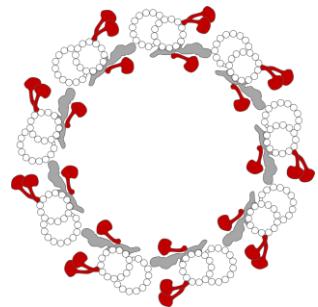
Primary ciliary dyskinesia

Dynein arm genes

Airway cilia

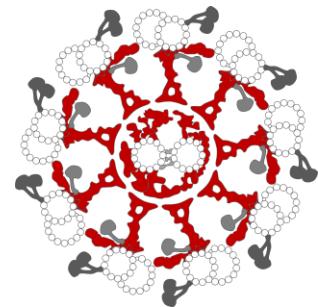


Nodal cilia

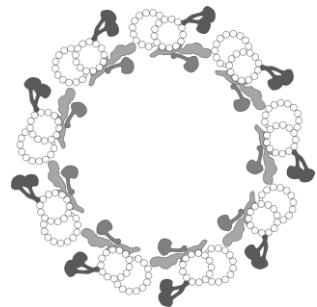


Central complex / radial spokes / DRC genes

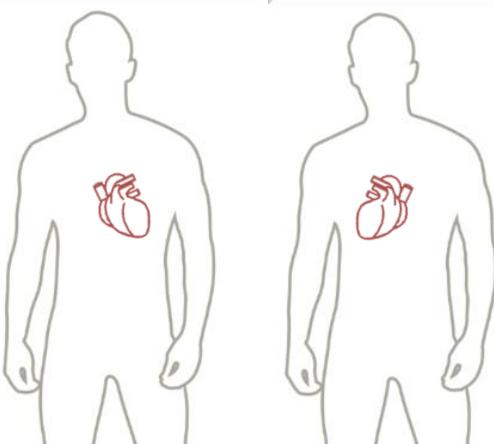
Airway cilia



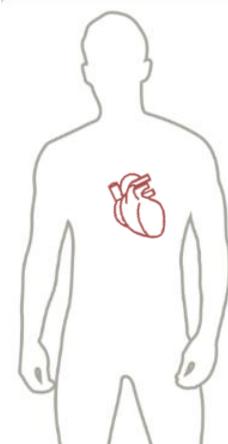
Nodal cilia



Situs inversus: 50%



No *situs* anomaly



Phenotype/genotype correlation: variation level

Surfactant diseases: ABCA3 (phospholipid transport)

Neonatal respiratory failure
Death at 1 month



Pediatric ILD
Survival > 16 year of age

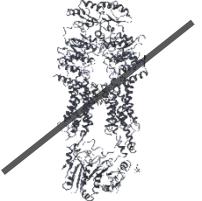


p.(Trp165*)
Nonsense



No protein

p.(Leu1414Cysfs*16)
Frameshift



No protein

c.3704-1G>T
Splice



No protein

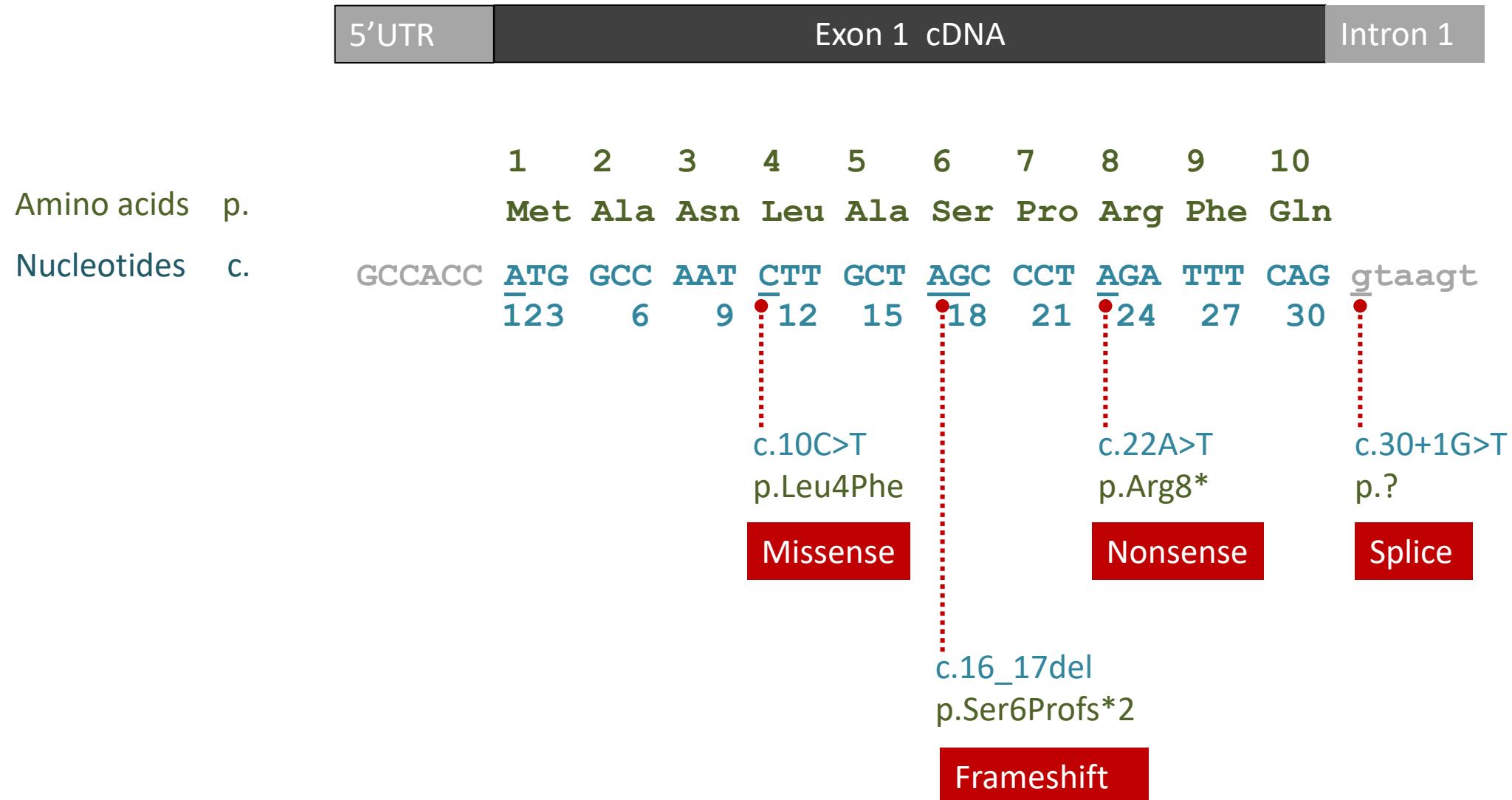
p.(Glu292Val)
Missense



↓ phospholipid
transport

Tips for genetic reports

Naming a variation



Genetic testing report of a proband

Reason for testing: primary ciliary dyskinesia

Gene (isoform)	Inheritance	Mutation	Zygosity	Classification
<i>DNAH11</i> (NM_001277115)	Autosomal recessive	c.9313A>T p.Lys3105*	Heterozygous	Likely pathogenic
<i>DNAH11</i> (NM_001277115)	Autosomal recessive	c.5461G>A p. Val1821Ile	Heterozygous	VUS (uncertain significance)

- *DNAH11* implication is not certain as p.Val1281Ile is a VUS
- Parental DNAs should be analysed to confirm compound heterozygosity

Diagnostic tests and their limitations

Next-Generation Sequencing (NGS)

Diagnostics

Research

Targeted gene panels

Number of variations
per subject:

Several 100s

Limitations:

Whole Exome Sequencing
(WES)

30,000

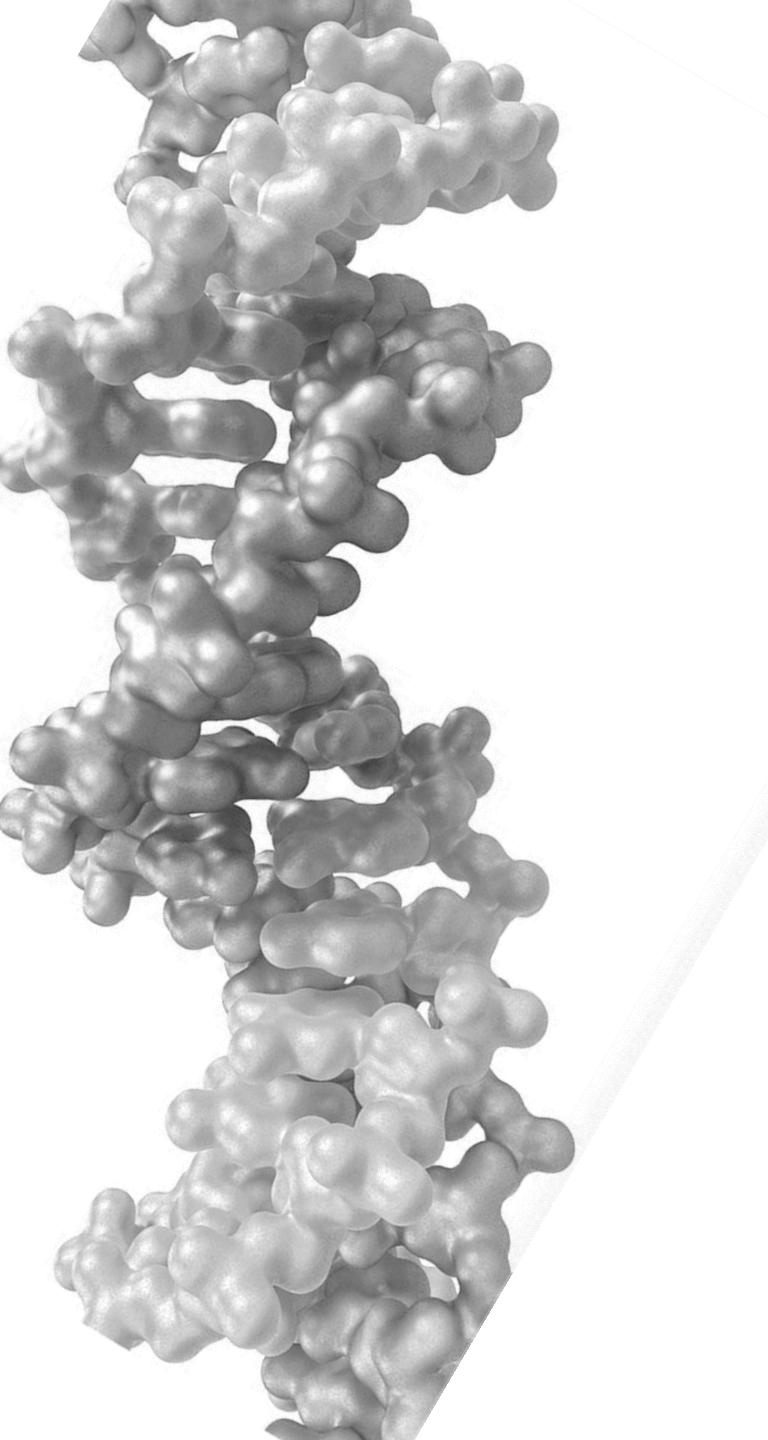
Only coding regions
of selected genes
Constant updating

Whole Genome Sequencing
(WGS)

5,000,000

Poor coverage
of GC-rich regions

Lower sequencing depth
→ artefacts
Interpretation...



Take home messages

- 30,000 variations in an exome
- We all carry pathogenic variations (~10-100)
- Phenotype and prognosis depend on the gene and on the variation(s)

Hôpital Trousseau, Paris:

UMR_S933, Serge Amselem
Inserm & Sorbonne Université
PCD & ILD research teams

Tifenn Desroziers
Estelle Escudier
Camille Louvier
Nadia Nathan
Lucie Thomas

U.F. Génétique moléculaire
Marie Legendre, AP-HP
PCD & ILD ref. diagnostic lab

Florence Dastot
Julie Galimand
Guy Montantin
Valérie Nau
William Piterboth
Sylvie Tissier

Lucie Thomas

Estelle Escudier

Tifenn Desroziers

Sylvie Tissier

Serge Amselem

Camille Louvier

Guy Montantin

Nadia Nathan



<https://geneticdiseases-lab.fr>

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CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs.
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Genotype-phenotype correlations for infants and children with ABCA3 deficiency.
Am J Respir Crit Care Med. 2014 Jun 15;189(12):1538-43