FIBROSI CISTICA

Fibrosi Cistica:
" La malattia del bacio salato"

Già le mamme romane, 2000 anni fa, riconoscevano il bambino malato per il sapore di mare nel baciargli la fronte. In tal modo, la saggezza popolare aveva già anticipato quanto l'osservazione medica avrebbe poi scoperto negli anni '50 e la ricerca scientifica non ancora completamente chiarito indicando nella concentrazione di sali nel sudore, il **metodo di diagnosi** per la Fibrosi Cistica. È proprio questa caratteristica del sudore particolarmente salato, avvertito dalle madri quando baciavano i bambini affetti, che farà chiamare la Fibrosi Cistica **"la malattia del bacio salato"**

La Fibrosi Cistica

- **Più comune MALATTIA GENETICA potenzialmente LETALE della razza Caucasica**
- **Trasmissione AUTOSOMICA RECESSIVA**
- **Incidenza in ITALIA di circa 1/3.000 nascite (nati/anno oltre 200). Attualmente si registrano in Italia 6000 pazienti in vita**
- **ETA' mediana alla MORTE di 37.5 anni**
- **Frequenza di PORTATORI di circa 1/29 (oltre 2.200.000)**
- **Determinata dal MALFUNZIONAMENTO della proteina CFTR (*Cistic Fibrosis Transmembrane Regulator* - canale del cloro) per la presenza di DUE MUTAZIONI nel gene**

FIBROSI CISTICA IN 3 MINUTI

- *Malattia **grave***
- *La più **frequente** delle genetiche gravi*
- ***Poco** o niente affatto **visibile***
- *Accorcia sensibilmente la vita*
- *Necessità di **cure quotidiane***
- *Cure per allungare la vita, **non per guarire***
- ***Qualità di vita discreta***
- *Fase **terminale** molto **critica***

FIBROSI CISTICA CLASSICA – Fenotipo

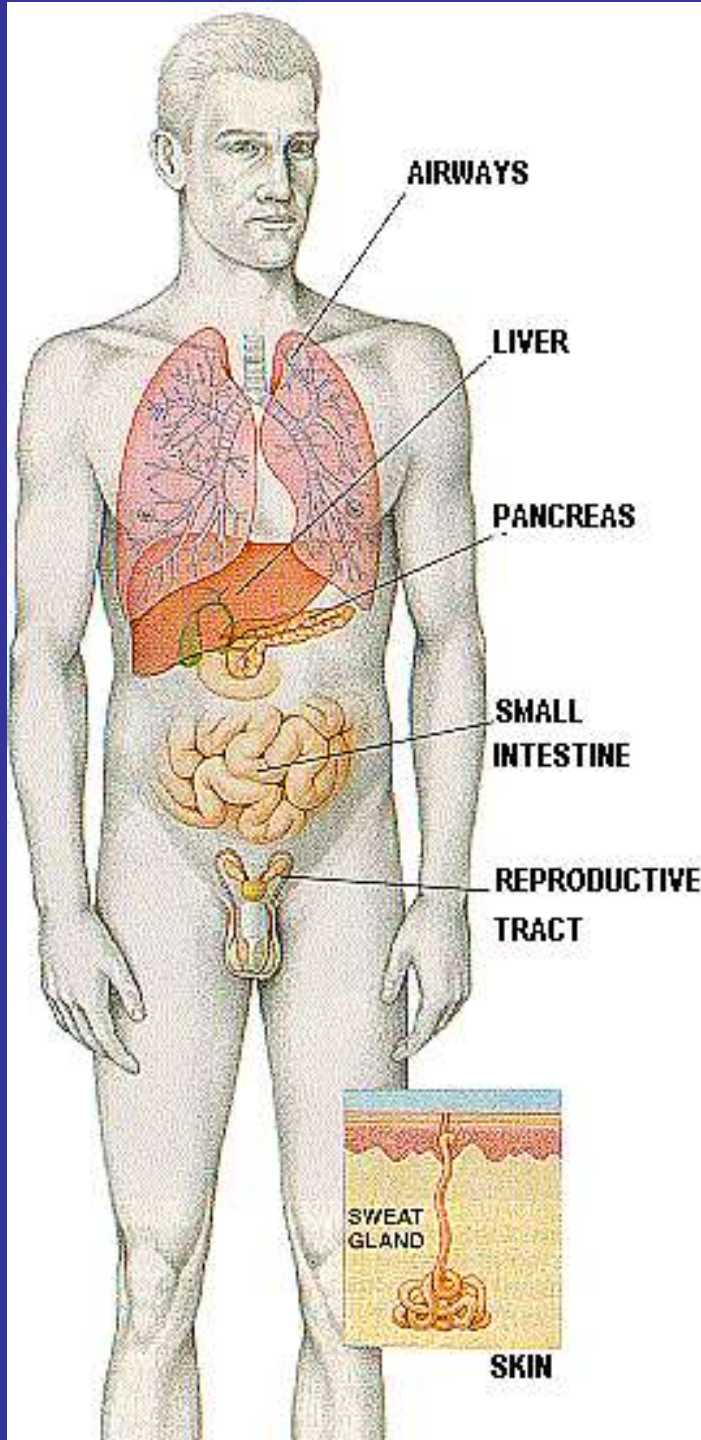
1. Vie aeree: ostruzione e infezione, causa della maggior parte dei decessi

2. Pancreas: occlusione dotti ed enzimi digestivi insufficienti nell'85% dei pazienti

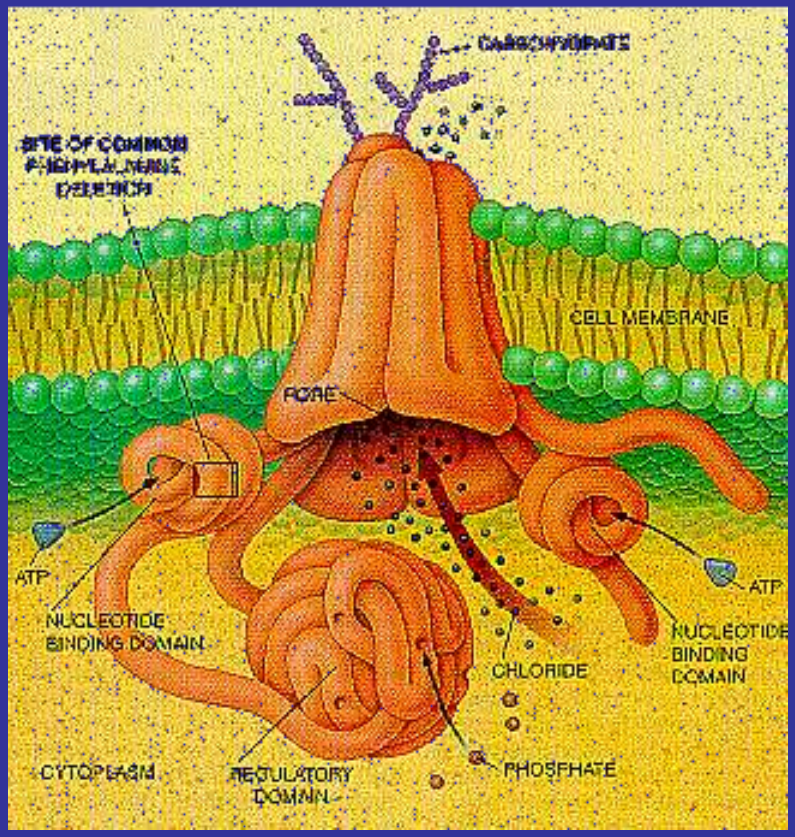
3. Intestino non riceve abbastanza enzimi digestivi specifici per i grassi provocando denutrizione ed eccesso di grassi nelle feci (steatorrea).
Occlusione fecale nel 10% dei neonati (ileo da meconio)

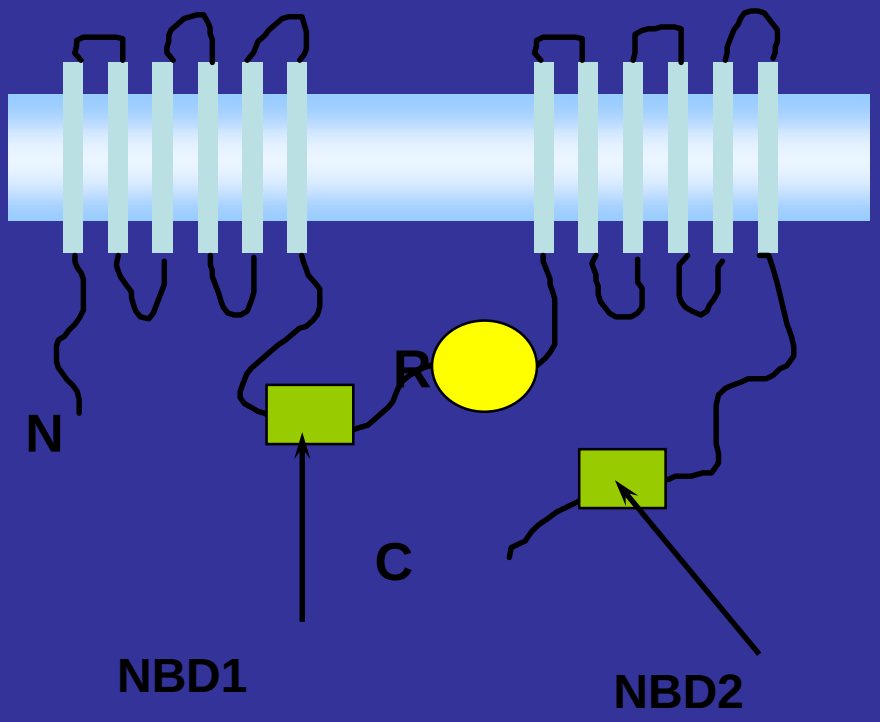
4. Apparato riproduttivo: nel 95% dei maschi assenza congenita dei dotti deferenti.
Nelle donne possibile tappo mucoso uterino

5. Ghiandola sudoripara:eccesso di cloro nel sudore,test diagnostico fondamentale



Regolazione di CFTR

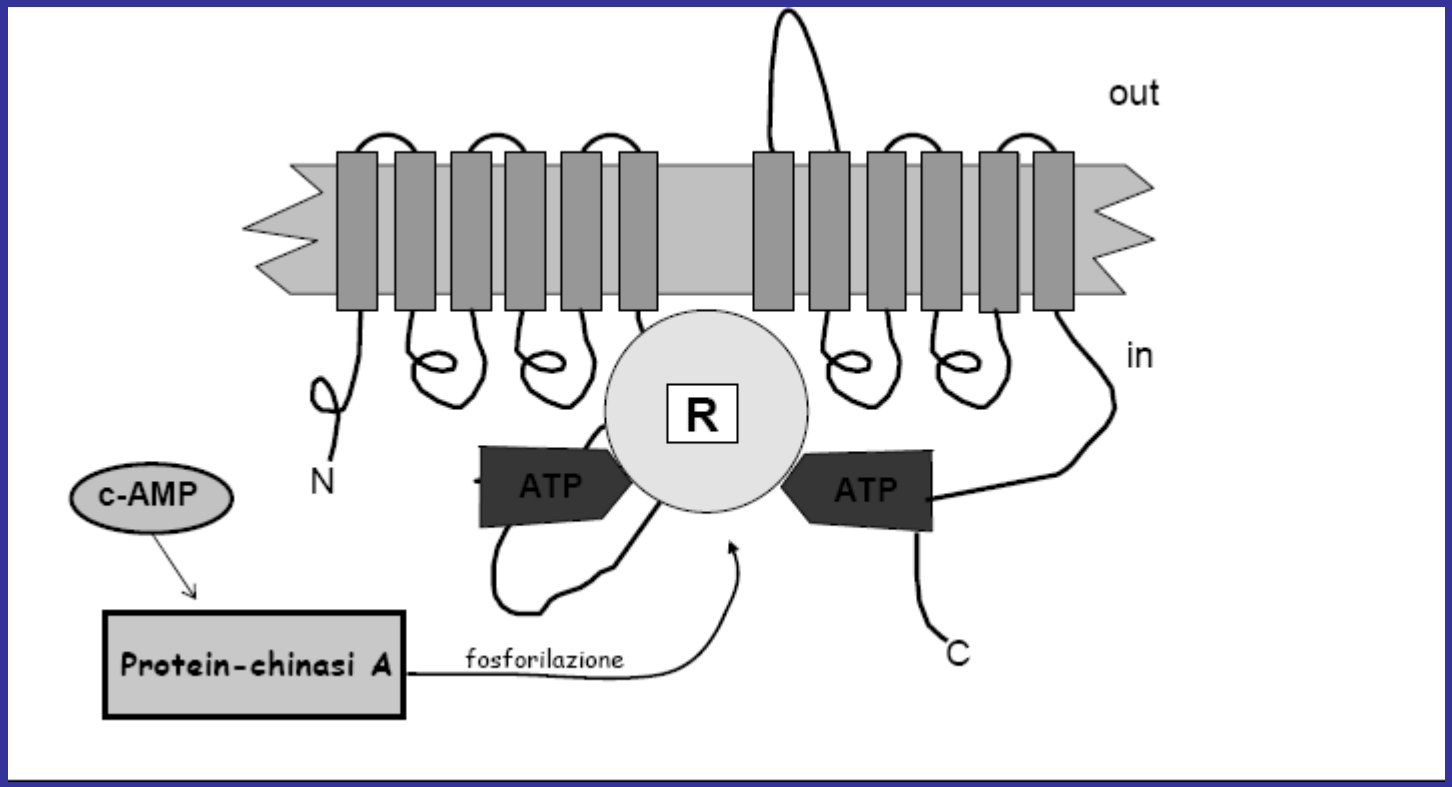




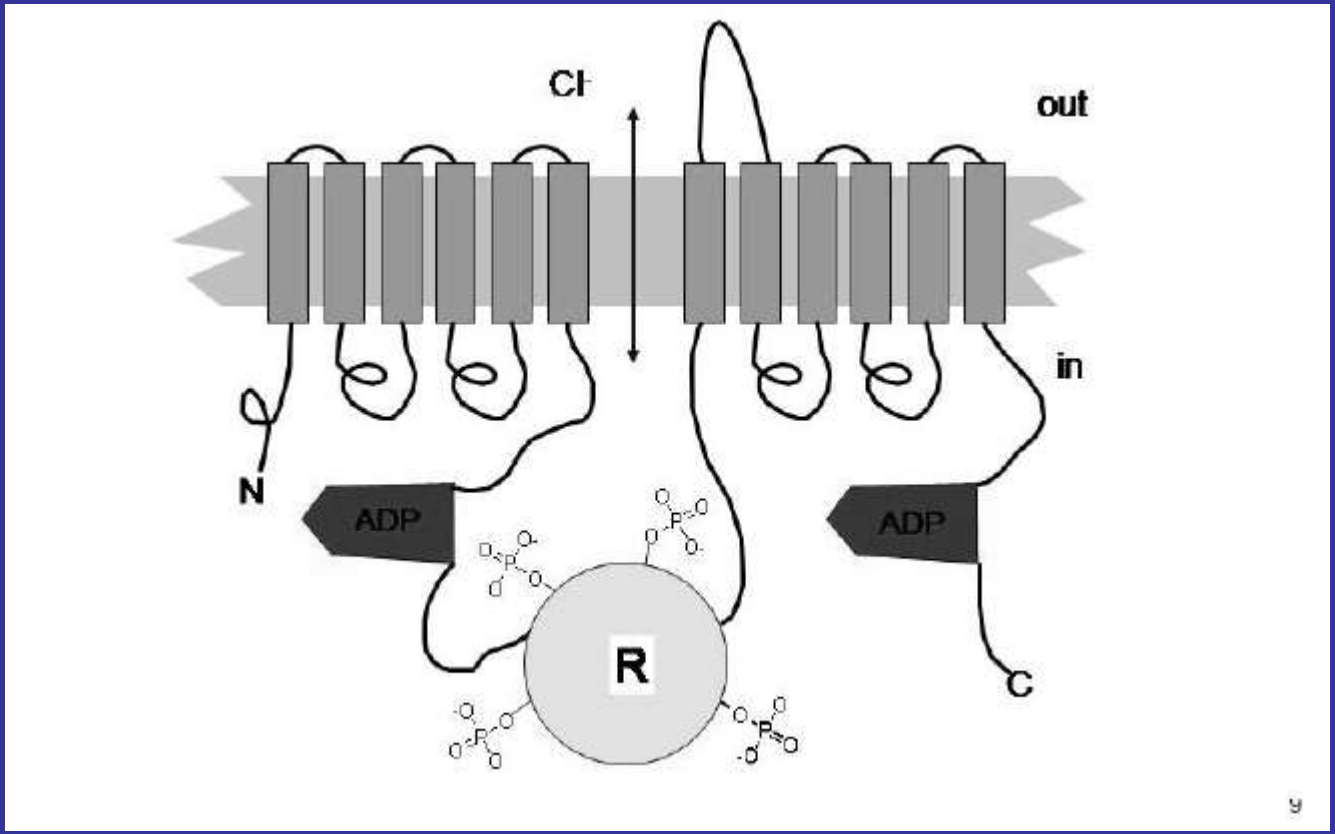
- **CFTR si apre se:**

È fosforilato nel dominio R da PKA, PKC, PKG

Si lega ATP *(con e senza* idrolisi) a NBD1 & NBD2



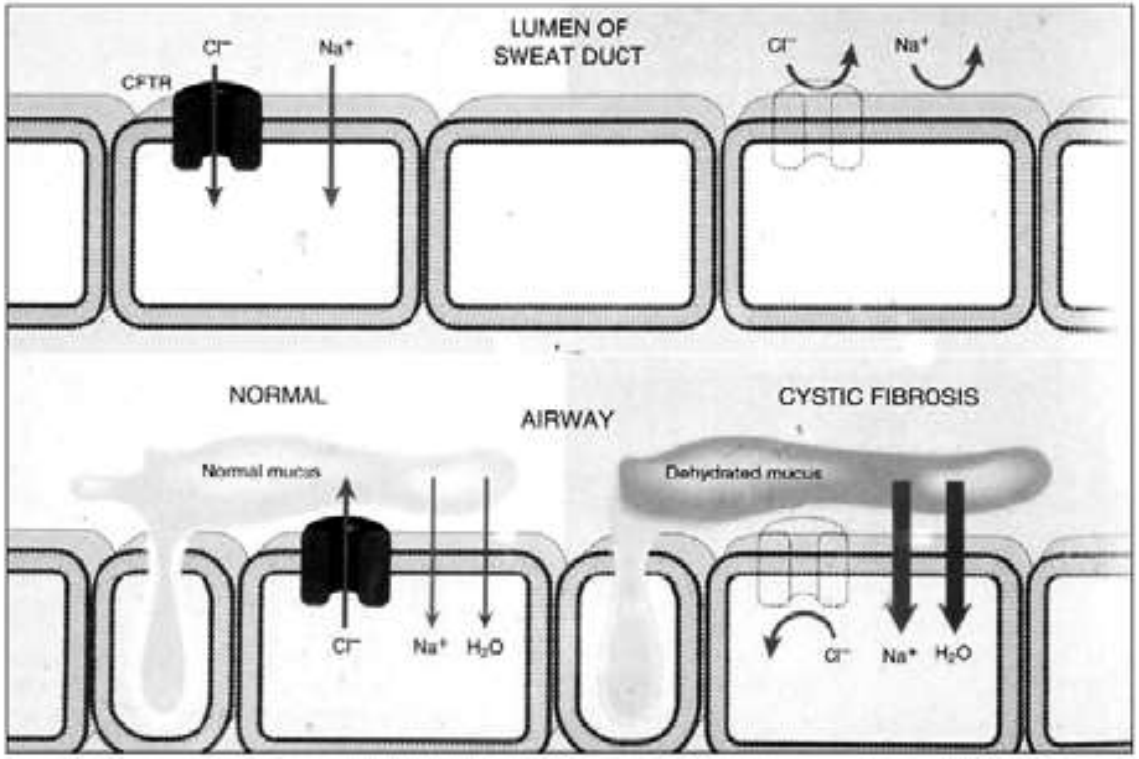
1. In assenza di fosforilazione del dominio R, il canale è chiuso
2. PKA (attivata da c-AMP) determina la fosforilazione del dominio R
3. Il dominio R fosforilato aumenta l’affinità dei domini NBD per l’ATP



4. L’ATP viene idrolizzato ad opera di fosfatasi
5.Cambio conformazionale che determina l’apertura del canale
6.Il passaggio attraverso il dominio transmembrana è dipendente dal gradiente elettrochimico

Funzione della proteina

La principale funzione è il trasporto di Cl^-. La direzione del trasporto dipende dalla specifica funzione del tessuto.



Ghiandole sudoripare

N: Cl^- va dal lume al citoplasma

FC: difetto assorbimento Cl^- e Na^+. Aumentata concentrazione di sale nel sudore.

Epitelio respiratorio

N: Cl^- va dall'interno all'esterno della cellula

FC: difetto secrezione Cl^- ed eccesso assorbimento Na^+ e di H_2O. Disidratazione ed ispessimento del muco sulla superficie dell'epitelio.

TEST DEL SUDORE

Test normale
< 30 mEq/L cloro

Test borderline
> 30 mEq/L cloro

Test patologico
>60 mEq/L cloro

STIMOLAZIONE con **PILOCARPINA**

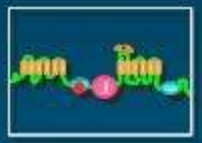
RACCOLTA del SUDORE

DOSAGGIO del **CLORO:**
Clorurimetro

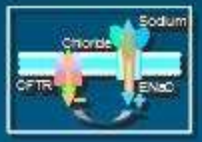
The CF Pathogenesis Cascade

Defective CF Gene

Deficient CFTR Protein



Abnormal Chloride Permeability
Altered Ionic Transport



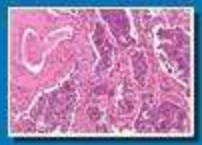
Act here to rescue the basic CF defect and block the cascade!

Decreased Water in ASL
Abnormal Mucus Composition

Bronchial Obstruction

Bacterial Infections

Inflammation



Bronchiectasis + Lung Insufficiency

Most current therapies in CF!

Amaral & Kunzelmann (2007) *Trends Pharmacol Sci* **28**, 334-341

Correlazione genotipo-fenotipo

(malattia pancreatica)

- Buona correlazione genotipo e funzionalità pancreatica
- Mutazioni classificate in "severe" e "lievi" in riferimento alla funzionalità pancreatica
- Le mutazioni associate ad un pancteas funzionante (lievi) sono dominanti fenotipicamente su quelle associate ad insufficienza pancreatica.

Funzione	Tipo di mutazione	Esempi
Insufficienza	severa/severa	ΔF508/ΔF508 ΔF508/R1162X
Sufficienza	severa/lieve lieve/lieve	ΔF508/R117H T3381I/R334W

Correlazione genotipo-fenotipo

(malattia respiratoria)

- Non è possibile correlare il genotipo alle manifestazioni respiratorie.
- Pazienti con lo stesso genotipo (es. ΔF508/ΔF508) hanno gravità dei sintomi polmonari molto variabile.
- Mutazioni non classificabili in "severe" e "lievi" per quanto riguarda la funzionalità respiratoria.
- Tessuti bronchiali esposti a fattori ambientali, accessibili a virus, batteri, interventi terapeutici.

CRITERI DIAGNOSTICI

THE DIAGNOSIS OF CYSTIC FIBROSIS: CONSENSUS STATEMENT

one or more characteristic phenotypic features
and/or
history of CF in a sibling
and/or
positive newborn screening test

+

elevated sweat chloride concentrations
and/or
identification of two CF-causing mutations
and/or
demonstration of characteristic abnormalities in ion transport across the nasal epithelium

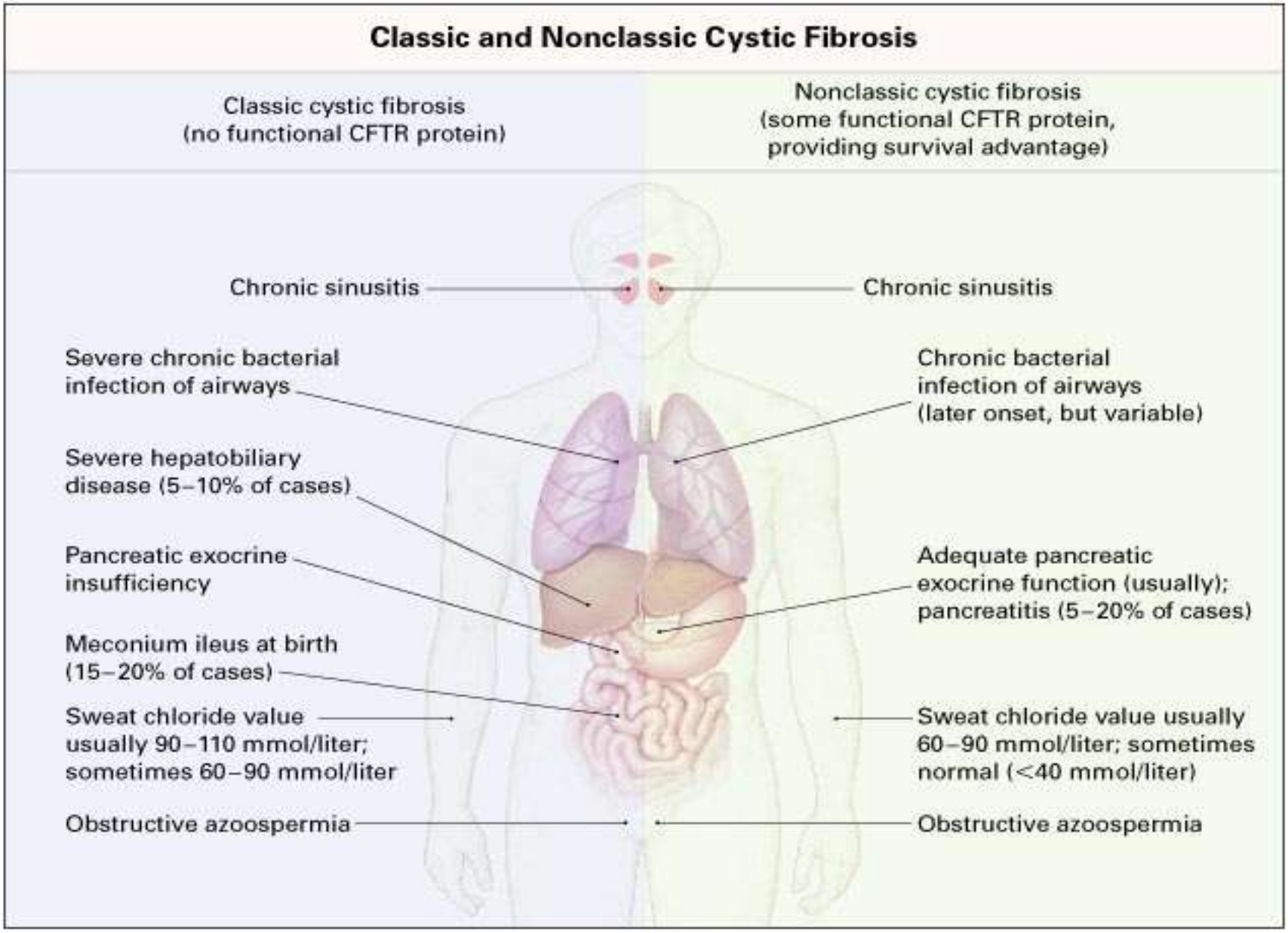
=

CF

Rosenstein BJ et al. J. Pediatr 1998; 132: 589-95


Calshake
Volumatic
Space Device

Classic and Nonclassic Cystic Fibrosis



Spectrum of Cystic Fibrosis

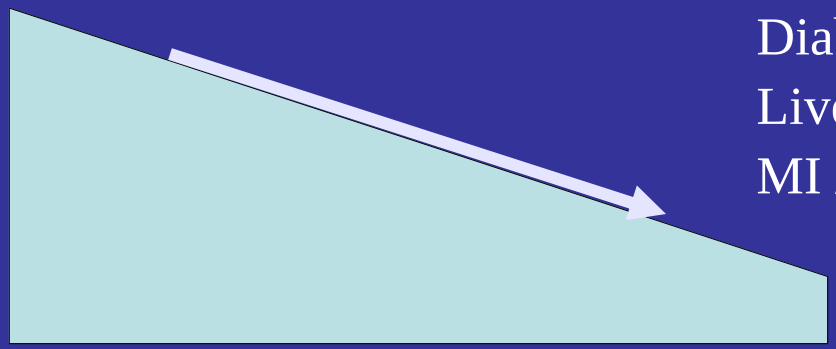
CBAVD: **C**ongenital **B**ilateral **A**bsence of the **V**as **D**eferens

CFSPID: **CF S**creening **P**ositive **I**nconclusive **D**iagnosis

CRMS: **C**FTR **R**elated **M**etabolic **S**yndrome

CFTR-RD: **CFTR**-**R**elated **D**isorder

PS
Less severe lung disease
Male infertility
Pancreatitis

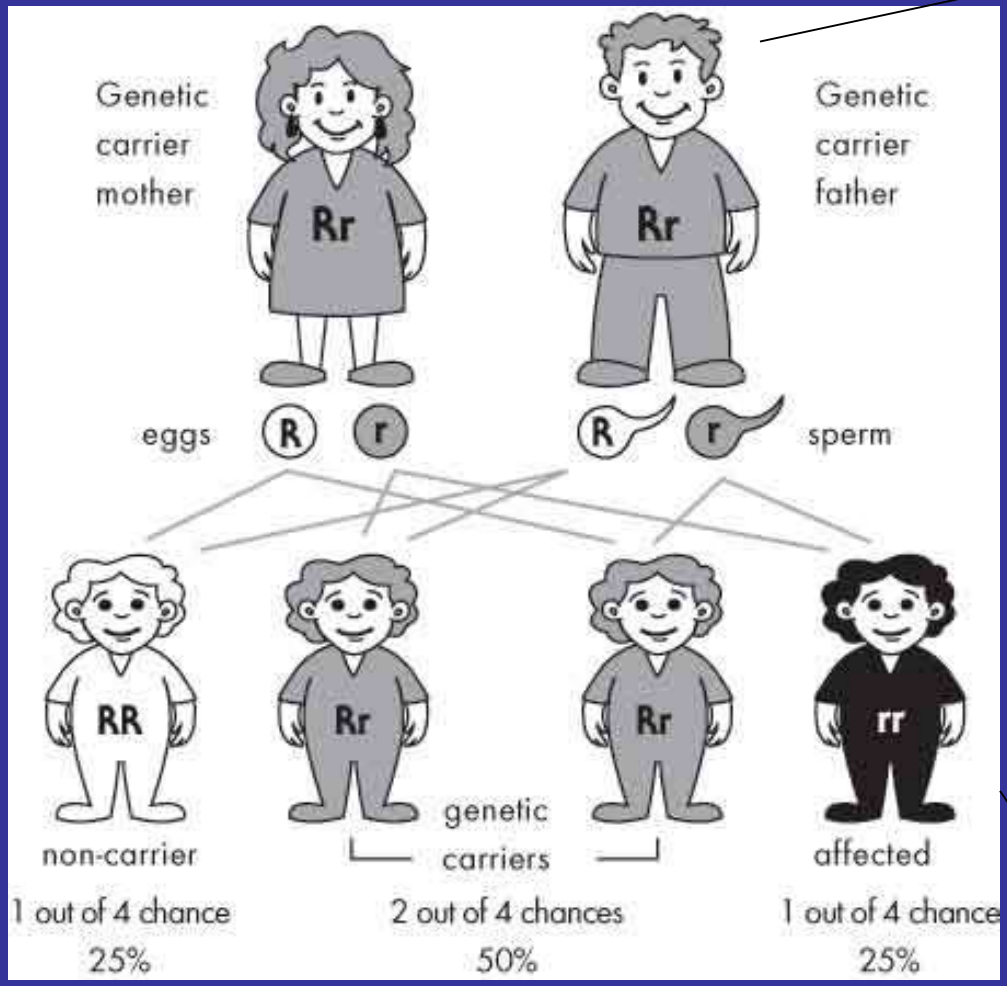


PI
Severe lung disease
Diabetes
Liver disease
MI / DIOS

CFTR Mutations and the Phenotypic Continuum

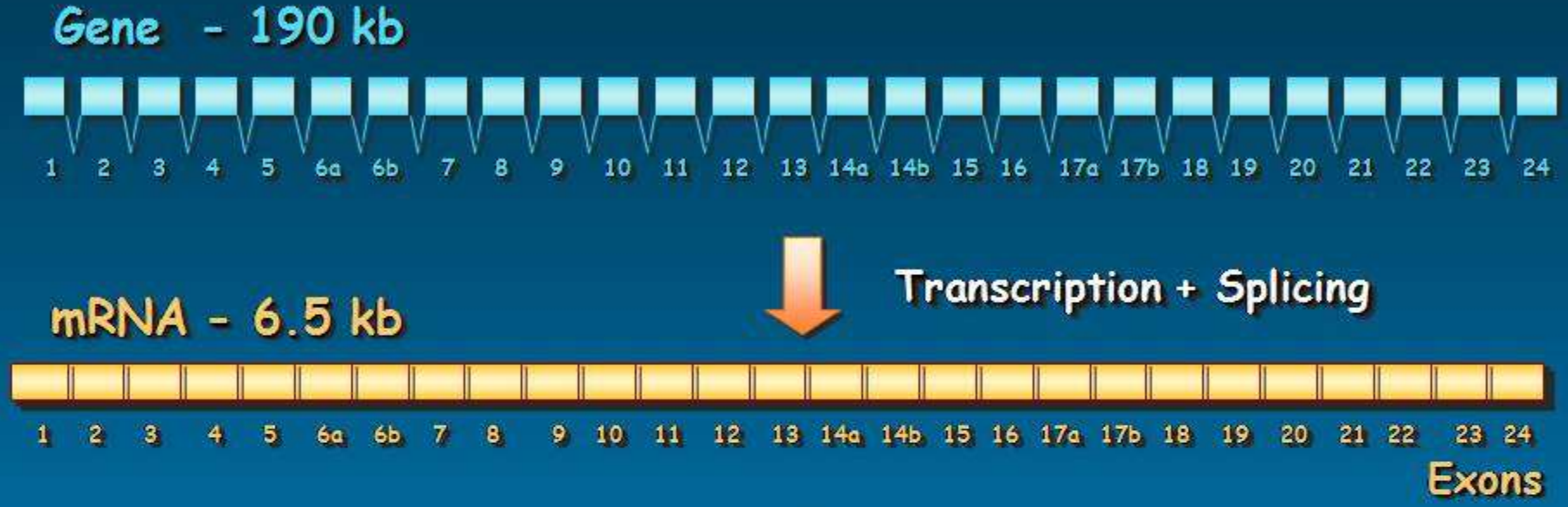
Cystic Fibrosis	CFTR-related disorders

1/25-30

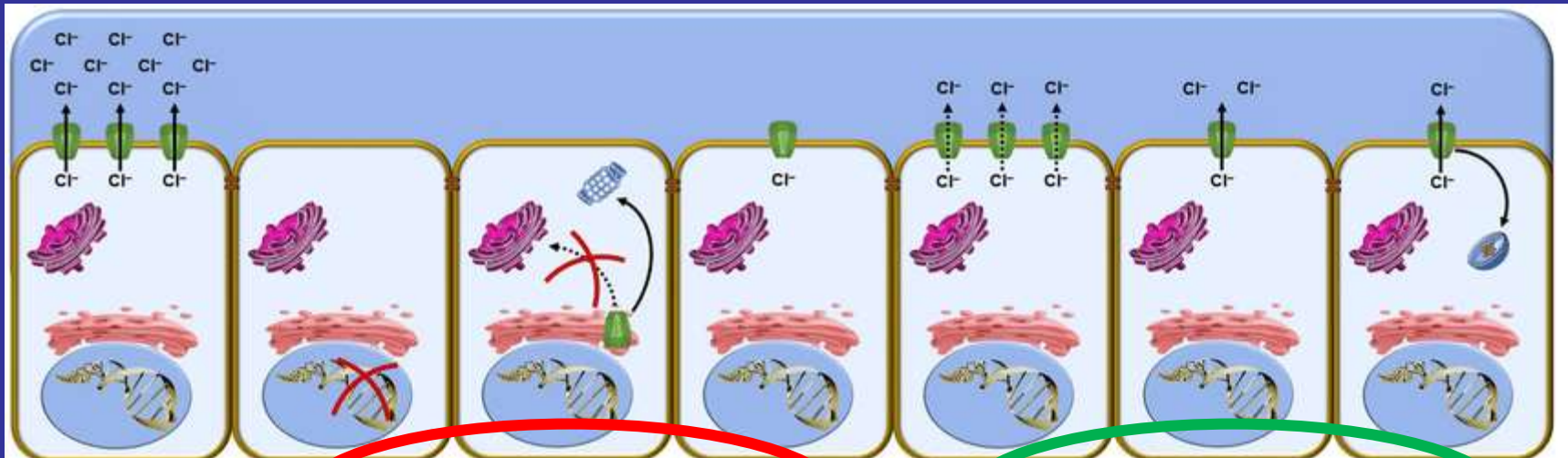


1/3000-3500

CFTR: from Gene to Protein

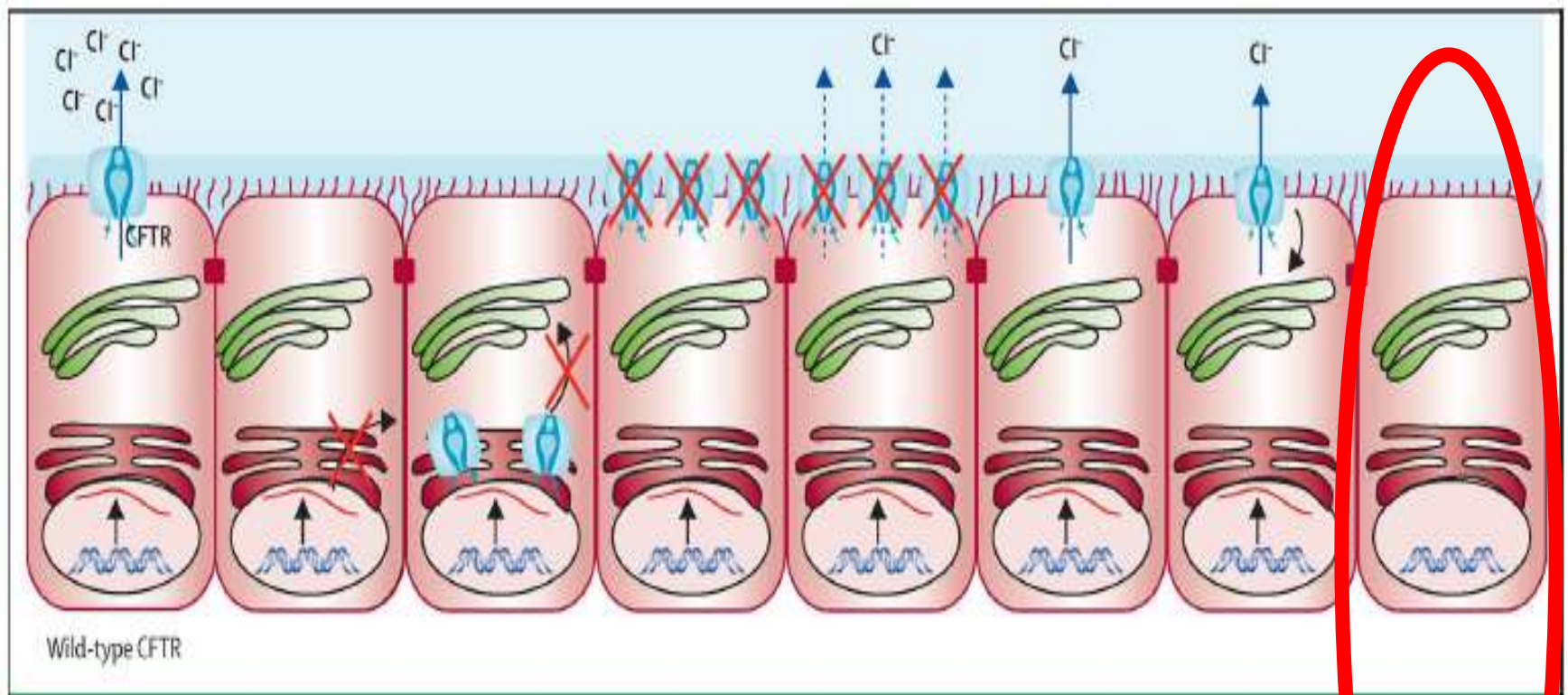


Più di 2000 mutazioni note: 6 classi funzionali



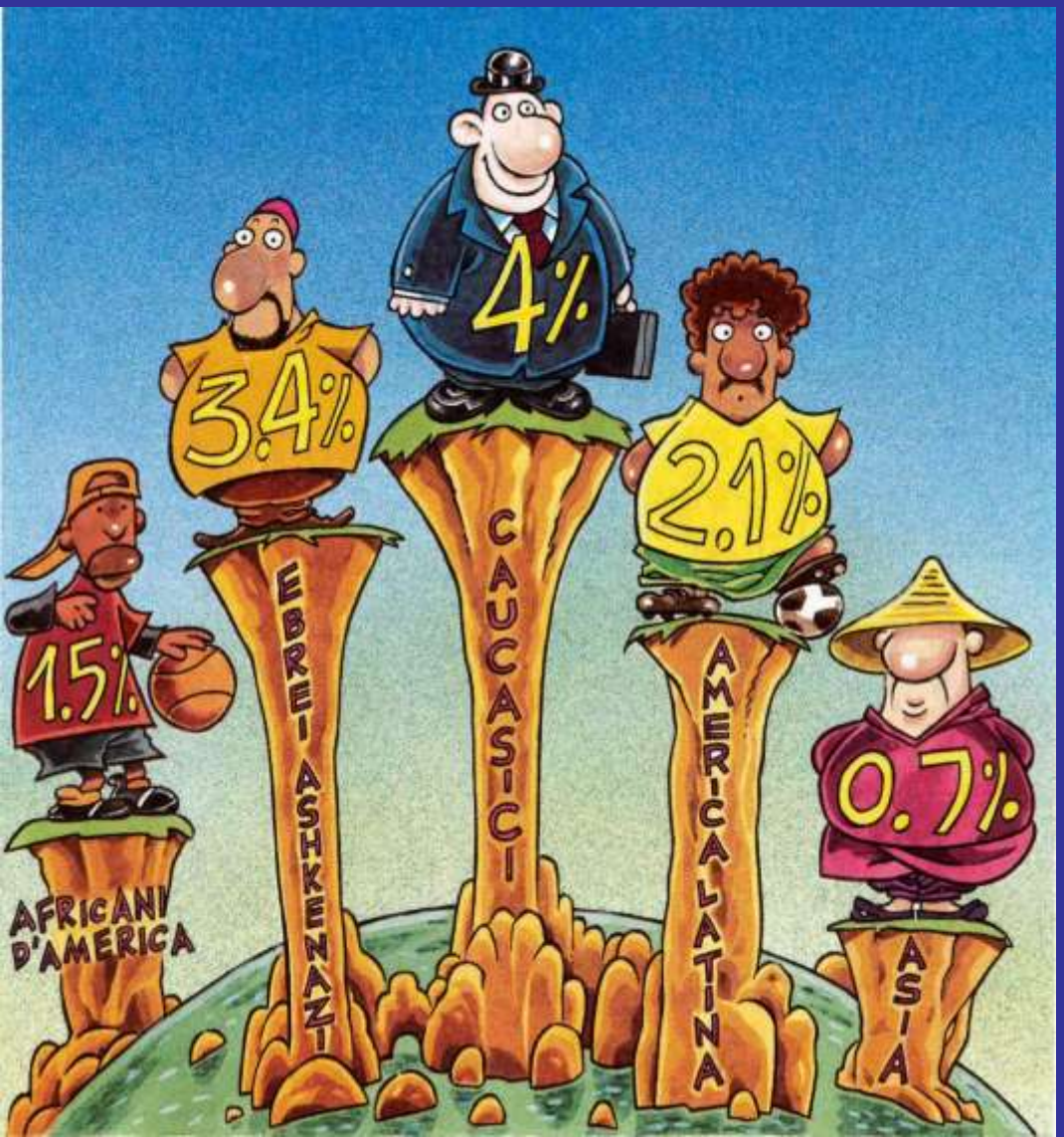
	wt-CFTR	I	II	III	IV	V	VI
Defect types		**No protein**	**No traffic**	**No function**	**Less function**	**Less protein**	**Less stable**
Mutation examples		G542X R553X W1282X	G85E ΔI507 **ΔF508** N1303K	V520F S549R G551D	R117H R334W S1235R	A455E 1680-886A>G 2657+5G>A	Q1412X
Required approaches		Rescue protein synthesis	Correct protein folding	Restore channel conductance	Restore channel conductance	Maturation / Correct misplicing	Promote protein stability

FENOTIPO	S	S	S/M	M	S/M/Atip	M



	Class I	Class II	Class III	Class IV	Class V	Class VI	Class VII
CFTR defect	No protein	No traffic	Impaired gating	Decreased conductance	Less protein	Less stable	No mRNA
Mutation examples	Gly542X, Trp1282X	Phe508del, Asn1303Lys, Ala561Glu	Gly551Asp, Ser549Arg, Gly1349Asp	Arg117His, Arg334Trp, Ala455Glu	Ala455Glu, 3272-26A→G, 3849+10 kg C→T	c.120del23, rPhe508del	dele2,3(21 kb), 1717-1G→A

QUANTI SONO I PORTATORI SANI DEL GENE DELLA FIBROSI CISTICA NEL MONDO



VENETO

Mutazione	%
DF508	44.84
R1162X	9.00
2183AA->G	7.96
1717-1G->A	3.83
N1303K	3.54
711+5G->A	2.65
2789+5G->A	2.51
G542X	2.36
R553X	1.77
G85E	1.62
Q552X	1.47
DI507	1.18
3132delTG	0.59
3849+10kbC->T	0.44
621+1G->T	0.44
Altro*	2.36
UN	**13.40**
Totale	100.00

SARDEGNA

Mutazione	%
DF508	53.10
T338I	13.10
G542X	5.63
2183AA->G	3.75
N1303K	2.50
3849+10kbC->T	1.88
Altro*	8.75
UN	**11.30**
Totale	100.00

≈2000 MUTAZIONI

CFTR variants in Chinese population

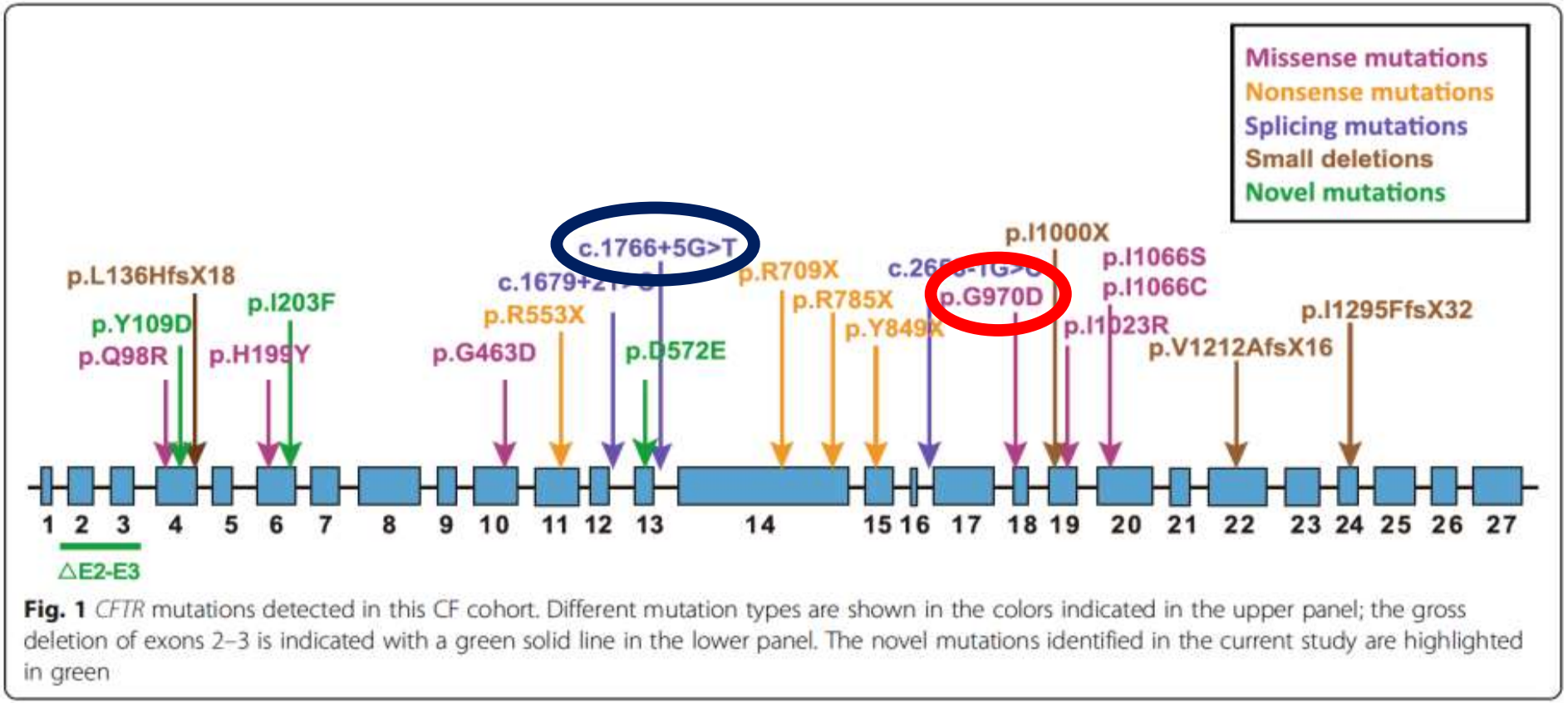


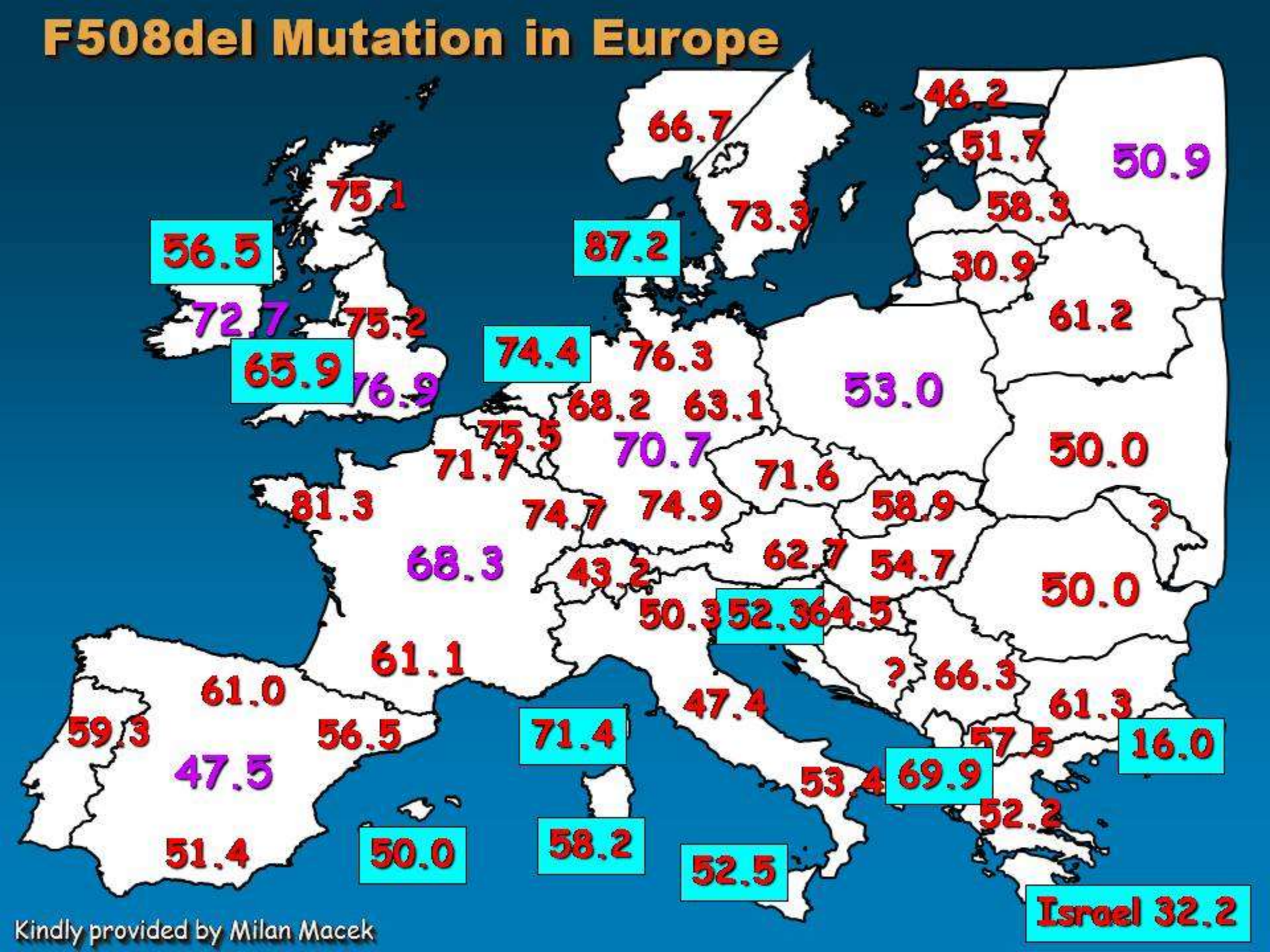
Fig. 1 *CFTR* mutations detected in this CF cohort. Different mutation types are shown in the colors indicated in the upper panel; the gross deletion of exons 2–3 is indicated with a green solid line in the lower panel. The novel mutations identified in the current study are highlighted in green

p.Phe508del assente o rara

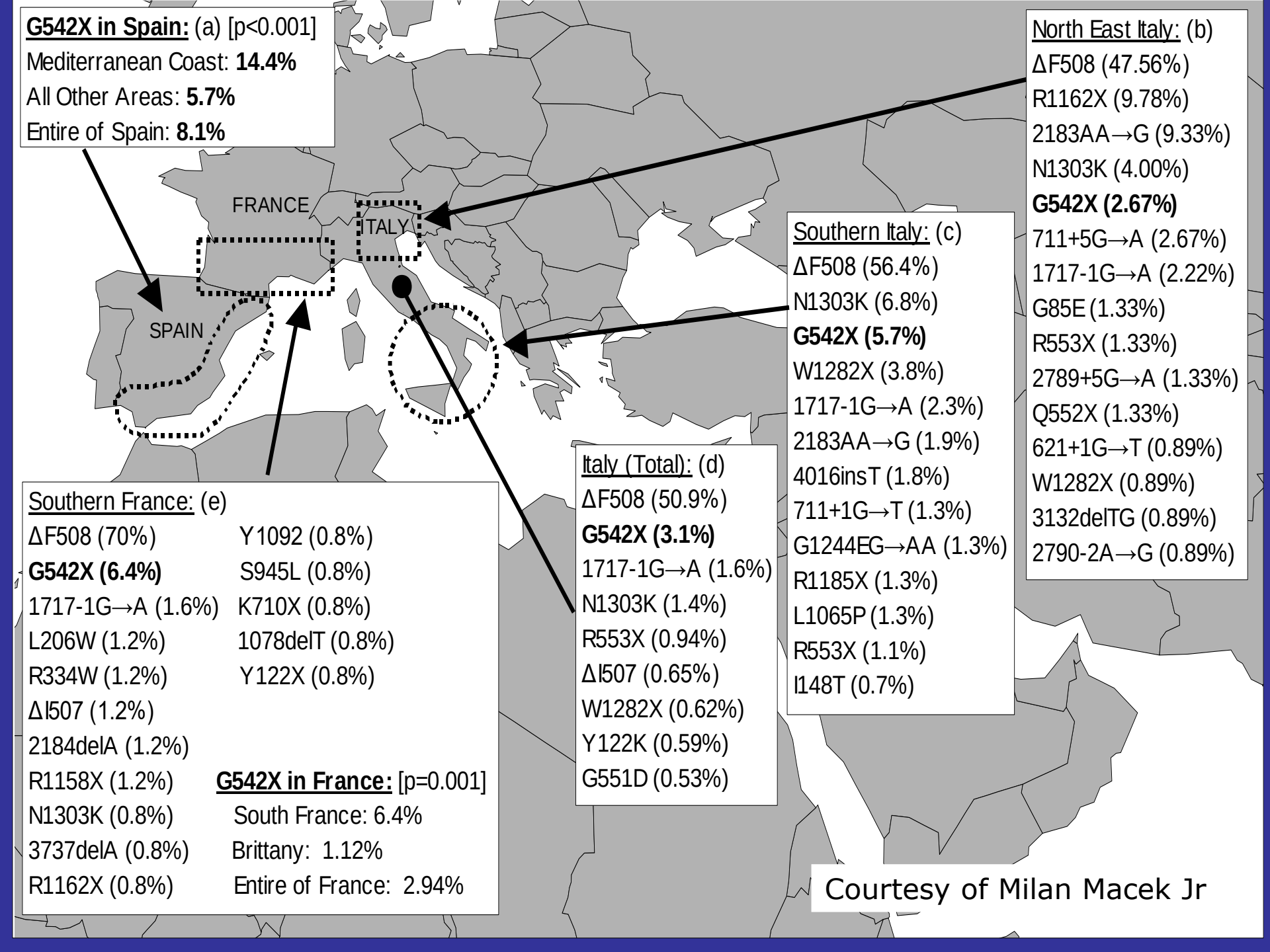
p.Gly970Asp mutazione più frequente in China (assente in CFTR2)

c.1766+5G>T non identificata nella popolazione Caucasica (presente in CFTR2 come CF-causing)

F508del Mutation in Europe



Kindly provided by Milan Macek


G542X in Spain: (a) [p<0.001]
Mediterranean Coast: **14.4%**
All Other Areas: **5.7%**
Entire of Spain: **8.1%**
FRANCE
ITALY
SPAIN
North East Italy: (b)
ΔF508 (47.56%)
R1162X (9.78%)
2183AA→G (9.33%)
N1303K (4.00%)
G542X (2.67%)
711+5G→A (2.67%)
1717-1G→A (2.22%)
G85E (1.33%)
R553X (1.33%)
2789+5G→A (1.33%)
Q552X (1.33%)
621+1G→T (0.89%)
W1282X (0.89%)
3132delTG (0.89%)
2790-2A→G (0.89%)
Southern Italy: (c)
ΔF508 (56.4%)
N1303K (6.8%)
G542X (5.7%)
W1282X (3.8%)
1717-1G→A (2.3%)
2183AA→G (1.9%)
4016insT (1.8%)
711+1G→T (1.3%)
G1244EG→AA (1.3%)
R1185X (1.3%)
L1065P (1.3%)
R553X (1.1%)
I148T (0.7%)
Italy (Total): (d)
ΔF508 (50.9%)
G542X (3.1%)
1717-1G→A (1.6%)
N1303K (1.4%)
R553X (0.94%)
ΔI507 (0.65%)
W1282X (0.62%)
Y122K (0.59%)
G551D (0.53%)
Southern France: (e)
ΔF508 (70%)
G542X (6.4%)
1717-1G→A (1.6%)
L206W (1.2%)
R334W (1.2%)
ΔI507 (1.2%)
2184delA (1.2%)
R1158X (1.2%)
N1303K (0.8%)
3737delA (0.8%)
R1162X (0.8%)
Y1092 (0.8%)
S945L (0.8%)
K710X (0.8%)
1078delT (0.8%)
Y122X (0.8%)
G542X in France: [p=0.001]
South France: 6.4%
Brittany: 1.12%
Entire of France: 2.94%
Courtesy of Milan Macek Jr

Cystic Fibrosis Mutation Database

F508del occurs in 70% of CF chromosomes worldwide

CFMDB Statistics

There are currently **2119** mutations listed in this CFTR mutation database.

Statistics by mutation type:

Mutation Type	Count	Frequency %
Missense	816	38.51
Frameshift	343	16.19
Splicing	231	10.90
Nonsense	178	8.40
In frame in/del	43	2.03
Large in/del	59	2.78
Promoter	17	0.80
Sequence variation	269	12.69
Unknown	163	7.69

In collaboration with:

CFTR2 Clinical and Functional Translation of CFTR

CFTR2 was last updated on April 7, 2023

CYSTIC FIBROSIS FOUNDATION ADDING TOMORROWS

JOHNS HOPKINS MEDICINE

sequenom Laboratories

Welcome to the CFTR2 website

Our Purpose:

CFTR2 is a website that provides information for patients, researchers, and the general public about specific variants in what is commonly referred to as the cystic fibrosis (CF) gene.

For each variant or variant combination included in the database, the website will provide information about:

1. Whether the variant or variant combination is CF-causing, and

2. Information about sweat chloride, lung function, pancreatic status, and Pseudomonas infection rate in patients in the CFTR2 database with this variant or variant combination.

Information on the CFTR2 website is being updated as further analysis is completed. The most up-to-date clinical information and results of functional testing are available on individual variant pages. For a complete list of CFTR2 variants and their characterizations, please visit CFTR2 Variant List History.

What this site is intended to do:

- This website provides information for members of the general public, including cystic fibrosis patients and their family members, about what is currently known about specific genetic variants related to cystic fibrosis.
- Patients and their family members are encouraged to visit the section, "For patients and family members" first.
- This website also provides more in-depth research-related information for health care professionals and researchers.

What this site is NOT intended to do:

This website is not intended to help diagnose anyone with CF.

- The information about groups of patients contained on this website should not be used to predict the clinical course of individual patients.
- This website is not intended to provide medical advice to individual patients.
- This website is not intended to provide information about pancreatitis, diabetes mellitus, or other diseases associated with CF.

For more information about CF, click here.

Note: If you have questions about any of the information contained in this website, please consult your doctor.

In collaboration with:

CFTR2 Clinical and Functional Translation of CFTR

CFTR2 was last updated on April 7, 2023

CYSTIC FIBROSIS FOUNDATION ADDING TOMORROWS

JOHNS HOPKINS MEDICINE

seq

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Informa analysis and res variant charact

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y of the please

The information about groups of patients contained on this website should **not** be **used to predict** the **clinical course** of individual patients.

Mutation analysis is not the answer to every diagnostic dilemma:its limitations and role must be understood by the **clinician**,who **has to interpret and use it in the context of the clinical setting**

G551D

I148T

D1152H

http://www.genet.sickkids.on.ca/cftr/Home.html

↓

2119 variazioni di sequenza

CF-causing: 804 (38%)
Variants of varying clinical consequence (*VCC*): 49 (2.3%)
Non CF-causing: 25 (1.1%)
Variant of Unknow Significance (*VUS*): 11 (0.5%)

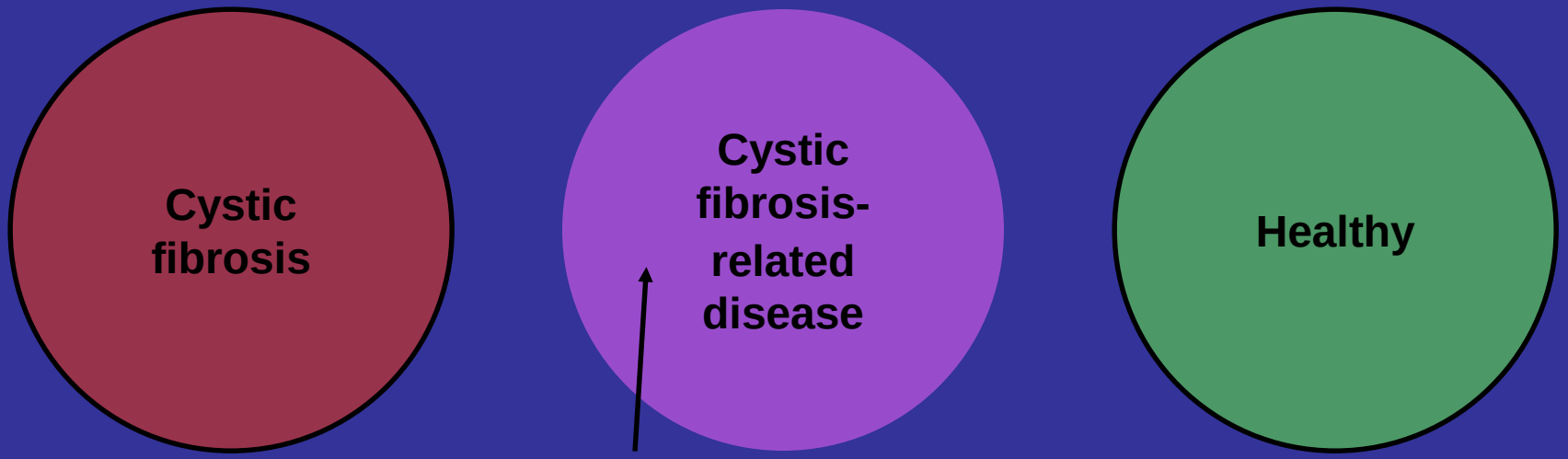
The list of mutations that is acceptable as diagnostic evidence will need to be expanded.

The Clinical and Functional TRanslation of CFTR (CFTR2) Project

Raccoglie dati da registri di Europa e Nord-America

www.cftr2.org

Predicting a spectrum of disease is difficult



For some mutations, it isn't entirely clear if they cause CF or a CF-related disease.

Table 4 Classification of *CFTR* mutations with regard to their potential for causing disease

Mutation group	*Examples*
CF-causing	F508del Mainly nonsense, frameshift, splicing (invariant dinucleotide): G542X, R553X, W1282X, 2183AA>G, 3659delC, 1717-1G>A, 3120+1G>A Missense that severely affects CFTR synthesis or function: G551D, N1303K, R347P 2789+5G>A, 3849+10kbC>T, 3272-26A>G, L206W[a], D1152H[a], (TG)13(T)5[a]
CFTR-related disorders associated	L206W[a], D1152H[a], (TG)13(T)5[a] [R117H;(T)7], (TG)12(T)5, L997F, V562I, [R668C;G576A;D443Y], [R74W;D1270N] (TG)11(T)5[b], S1235R[b]
No clinical consequences	875+40A>G, M470V (1540A>G), I506V (1648A>G), F508C (1655T>G), 1716G>A, 2694T>G, 4002A>G, 2752-15G>C (TG)11(T)5[b], S1235R[b]
Unproven or uncertain clinical relevance	Mainly missense mutations G622D, R170H, V938G, I125T Putative splice mutations: 406-6T>C, 2752-26A>G, 3601-17T>C

Only a fraction of mutations and patients have been characterized in detail and, with the exception of frequent mutations, only small numbers of patients have been available for the study of most mutations. Data shown here have to be interpreted with caution.
[a]Mutations that are associated with a wide phenotypic spectrum and which may belong either to group A or to group B.
[b]Mutations that may belong either to group B or to group C.

RISCHIO RESIDUO

RISCHIO RESIDUO

RISCHIO RESIDUO

III° Livello

Ricerca dei riarrangiamenti nel gene
Tasso di identificazione 98%

II° Livello

Scanning del gene
Tasso di identificazione 97%

I° Livello

Pannello di mutazioni
Tasso di identificazione 84.1%

Analisi di **II livello**: *scanning* di tutti gli esoni e delle regioni limitrofe, riconoscimento di variazioni di sequenza, sequenziamento della specifica regione del gene.

I test di II livello permettono un tasso di individuazione (*detection rate)* migliore, ma il significato fenotipico sultato molecolare può essere ile etazione.

- RDB copre circa :

93% delle mutazioni in Inghilterra

97% delle mutazioni in Ashenazi Jews

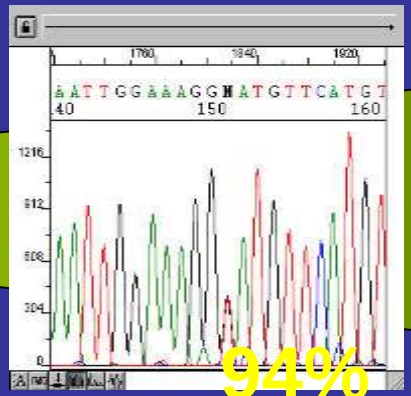
85% delle mutazioni in Italia

60% delle mutazioni in Portogallo

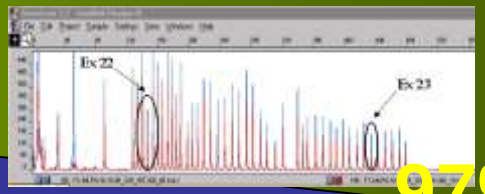
<30% delle mutazioni in Pakistan

Livelli di analisi molecolare

I° Livello	II° Livello	III° Livello
Kit commerciali che includono l'analisi delle **mutazioni più frequenti** nelle regioni di riferimento: OLA, RDB	**Scanning di tutti gli esoni** del gene CFTR e delle regioni limitrofe, riconoscimento di variazioni di sequenza: DGGE, DHPLC, SEQUENZIAMENTO	Ricerca di **grosse delezioni/inserzioni**: MLPA, MULTIPLEX-PCR, analisi dell'mRNA



etection Rate



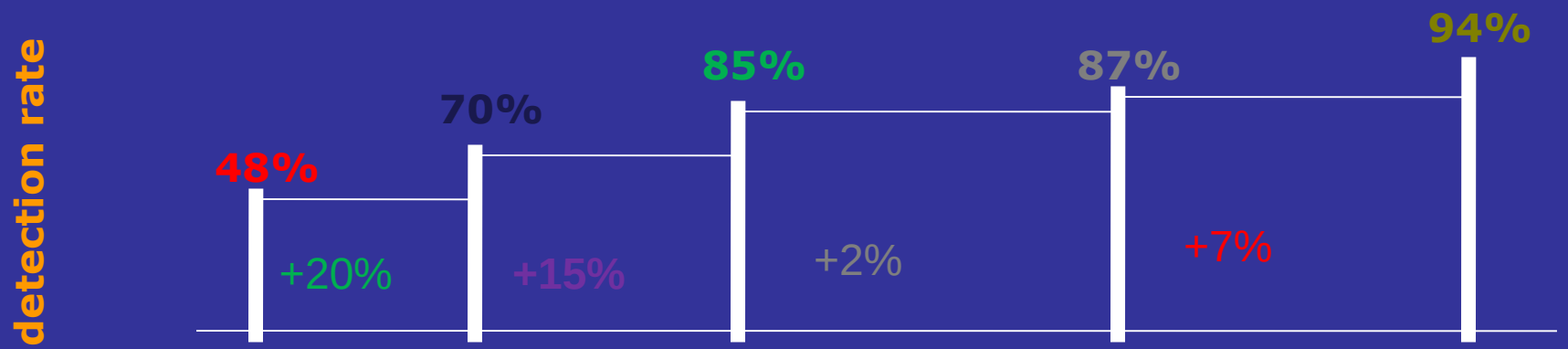
84,1%

94%

97%

How should we continue our ascent?

View to the West from Mt. Everest S. summit at dawn



F508del

R1162X
2183AA>G
N1303K

+3 M

F508del
G551D
G542X
DI507
R553X
N1303K
W1282X
1717-1G>A
V520F
R560T
S549R
3849+10kbC>T
R1162X
3659delC
3905insT
G85E
621+1G>T
R117H
711+1G>T
1078delT
R347P
R347H
R334W
A455E
1898+1G>A
2183AA>G
2789+5G>A
3876delA

+25 M

F508del
G542X
R553X
W1282X
CFTRdel2.3(21Kb)
3199del6
S1251N
Q552X
2184delA
R560T,
R1162X
3905insT
621+1G>T
711+1G>T
R347P
A455E
2183AA>G
2143delT
4016insT
852del22Bp
G1244E
I502T
R1158X
991del5
1898+3
R10
H

G551D
DI507
N1303K
1717-1G>A
3272-26A>G
3120+1G>A
394delTT
711+5GA.
3849+10KbC>T
3659delC
G85E
R334W
1898+1G>A
2789+5G>A
1259insA
4382delA
D579G
G1349D
L1065P
T338I
D1152H
R1070Q
621+3A>G
R334Q
R347P

+26 M

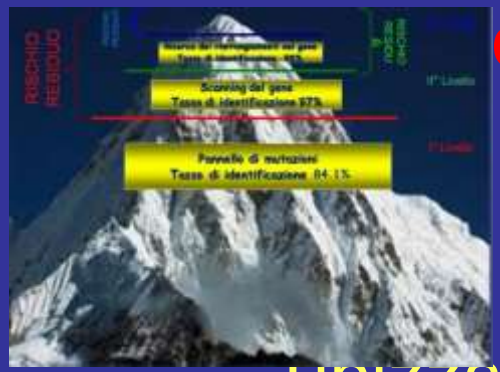
all exons scanning

>2000 M

VENETO

Mutazione	%
DF508	44.84
R1162X	9.00
2183AA->G	7.96
1717-1G->A	3.83
N1303K	3.54
711+5G->A	2.65
2789+5G->A	2.51
G542X	2.36
R553X	1.77
G85E	1.62
Q552X	1.47
DI507	1.18
3132delTG	0.59
3849+10kbC->T	0.44
621+1G->T	0.44
Altro*	2.36
UN	**13.40**
Totale	100.00

INDICAZIONI AL TEST



e dello stato di portatore nei familiari

molecolare nei partner dei portatori sani/affetti — I° livello / II° livello

Consanguineità — I° livello

Tipizzazione molecolare in pazienti affetti — II° livello

Diagnosi prenatale in coppie a rischio — Mirata

Coppie in gravidanza con quadri ecografici di anse intestinali iperecogene — I° livello / II° livello

Screening neonatale — I° livello

Tipizzazione genetica in pazienti con quadri clinici CF-Like — II° livello

Analisi molecolare in pazienti che accedono alla fecondazione medicalmente assistita

I° livello / II° livello

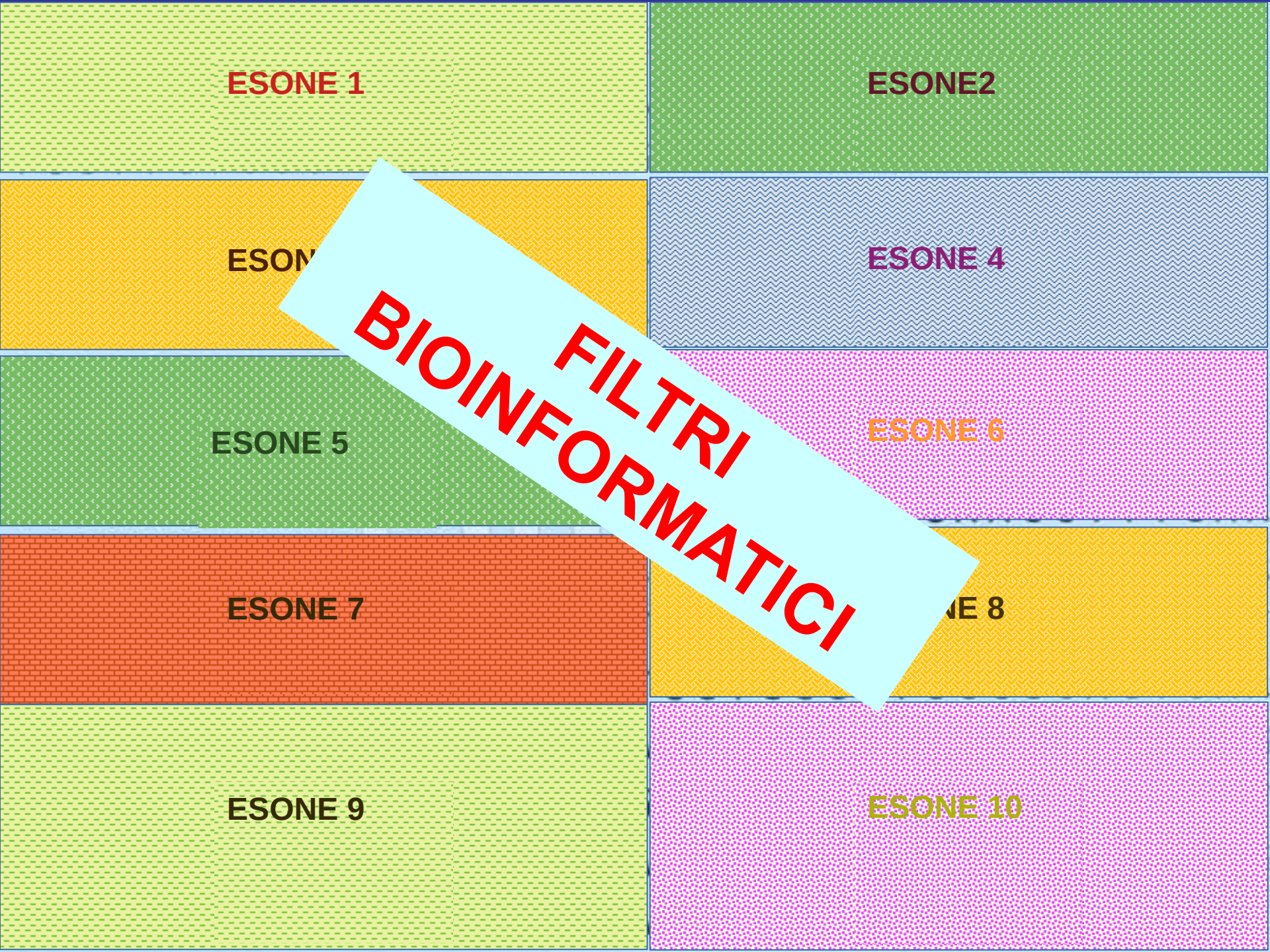
IL TEST GENETICO

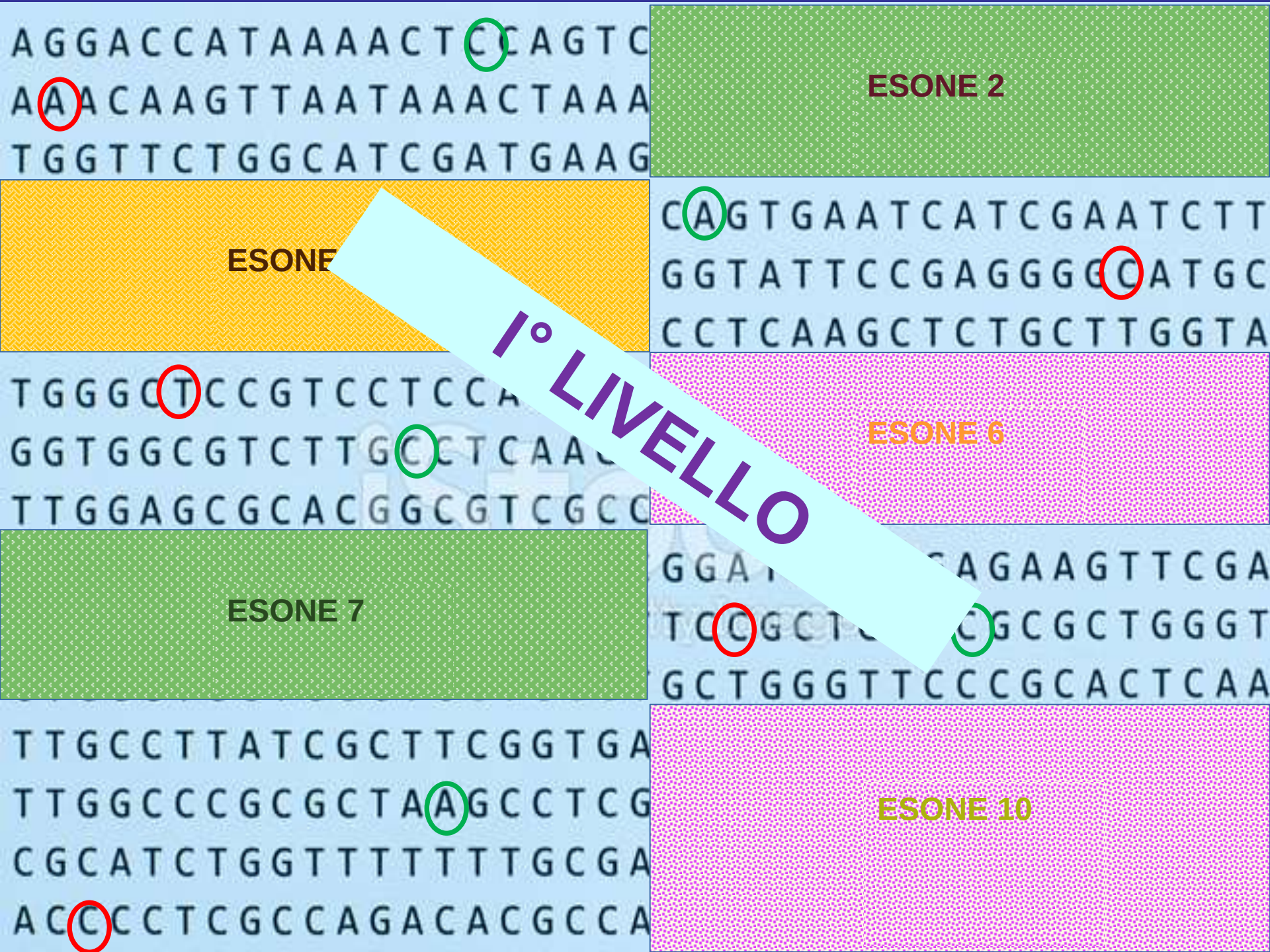
TEST MIRATI

Ricerca di un **numero ristretto** di *varianti patogenetiche*

NEXT GENERATION SEQUENCING (NGS)

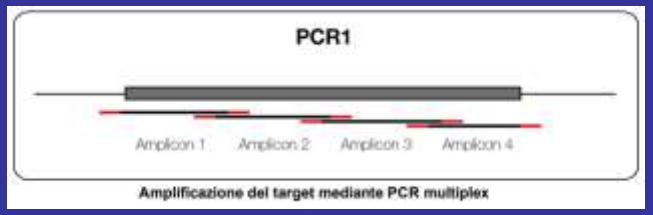
Estesi pannelli mutazionali (I° livello)
Sequenziamento completo (II° - III° livello)


ESONE 1
ESONE2
ESON
ESONE 4
ESONE 5
ESONE 6
ESONE 7
NE 8
ESONE 9
ESONE 10
FILTRI
BIOINFORMATICI

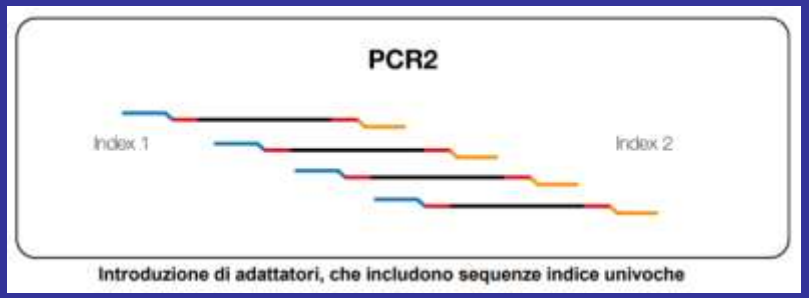

ESONE 2
ESONE
I° LIVELLO
ESONE 6
ESONE 7
ESONE 10


II° LIVELLO

STEP - NGS

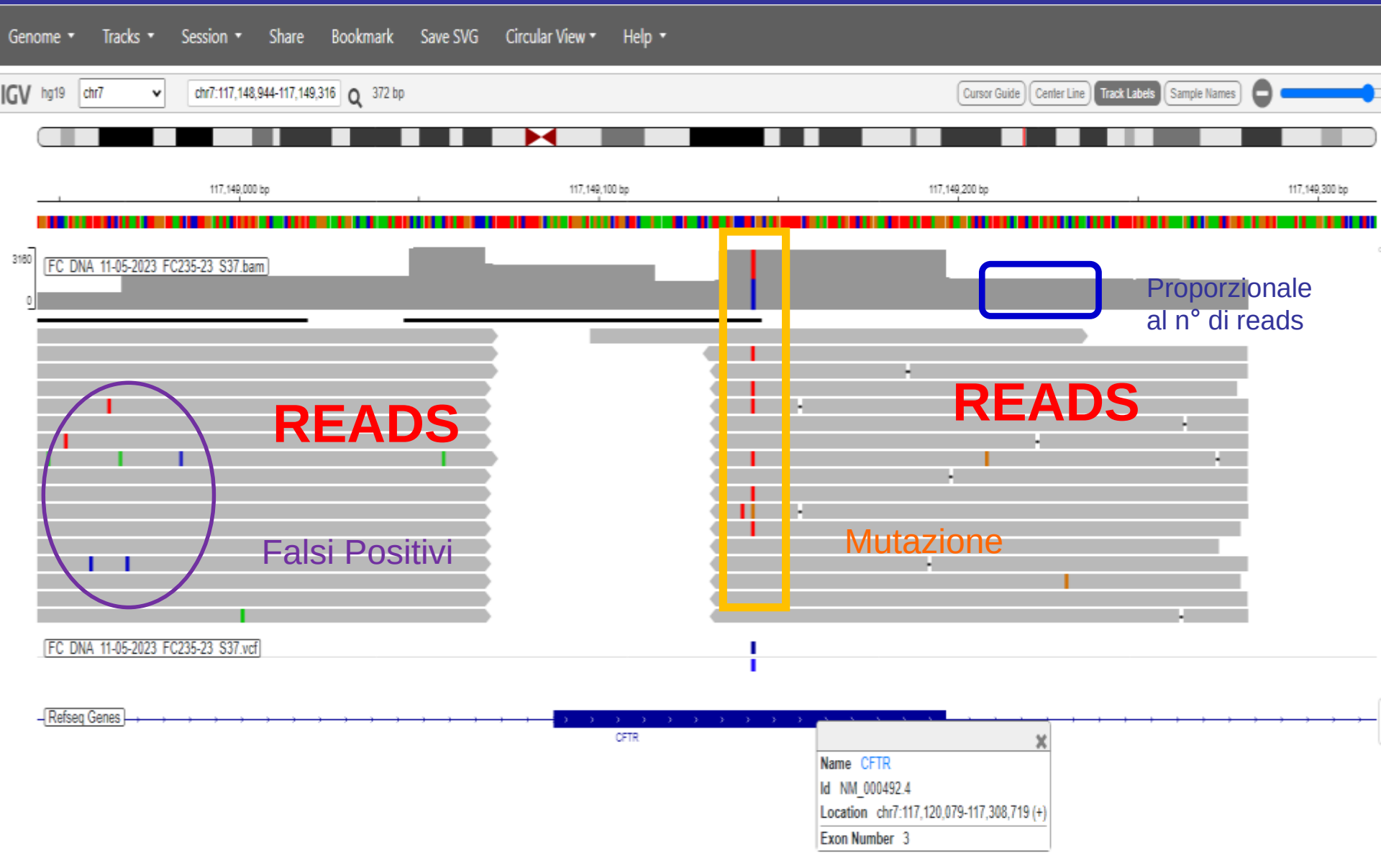


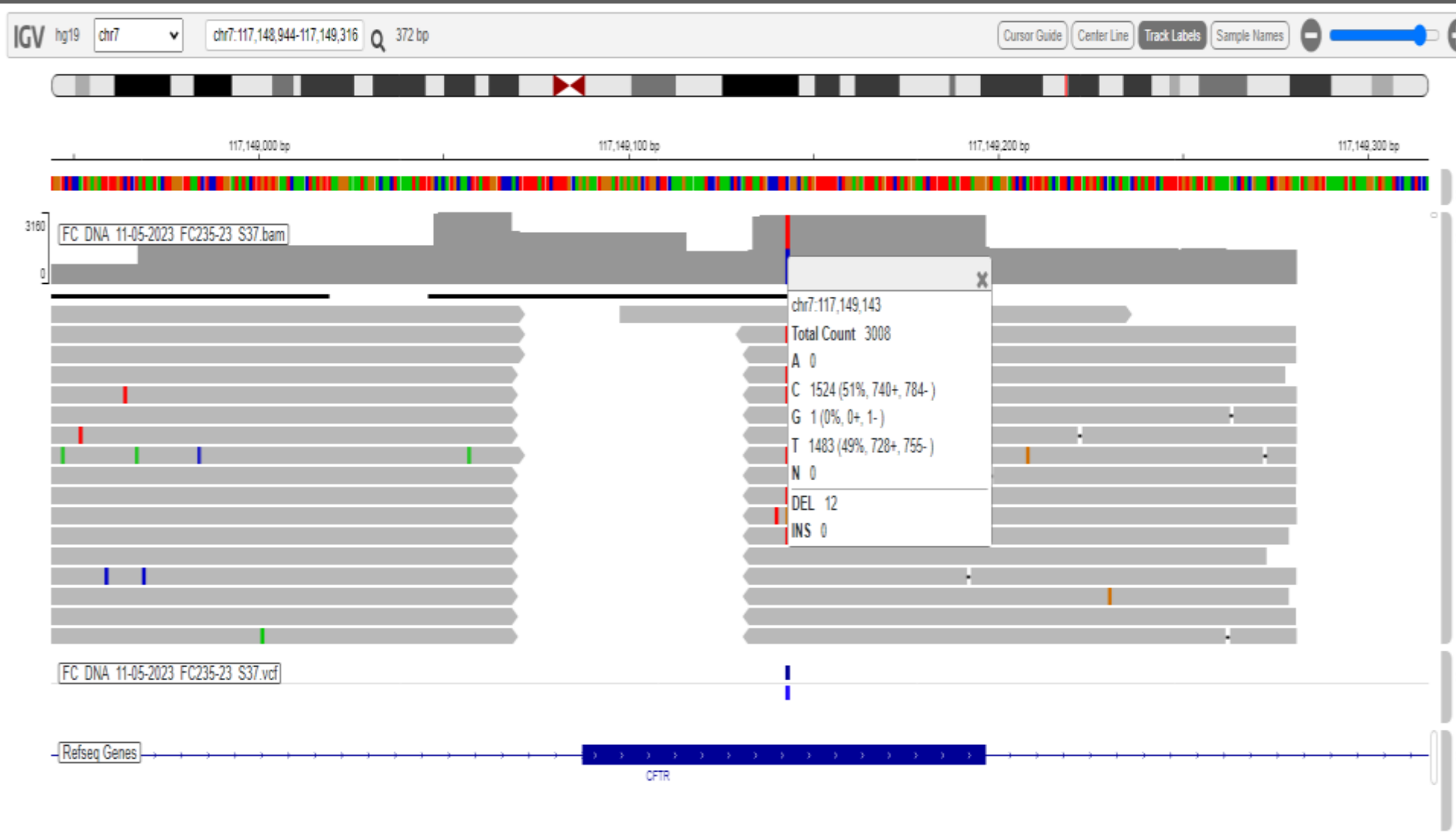
Amplificazione del target mediante PCR multiplex



Introduzione di adattatori, che includono sequenze indice univoche

Allineamento prodotti di sequenza (IGV): file fastq, .bam, .bai




IGV
hg19
chr7
chr7:117,148,944-117,149,316
372 bp
Cursor Guide
Center Line
Track Labels
Sample Names
117,149,000 bp
117,149,100 bp
117,149,200 bp
117,149,300 bp
3160
0
FC_DNA_11-05-2023_FC235-23_S37.bam
chr7:117,149,143
Total Count 3008
A 0
C 1524 (51%, 740+, 784-)
G 1 (0%, 0+, 1-)
T 1483 (49%, 728+, 755-)
N 0
DEL 12
INS 0
FC_DNA_11-05-2023_FC235-23_S37.vcf
Refseq Genes
CFTR

SCREENING | GENES | SNPs-INDELs | CNVs | WARNINGS

CFTR

HotSpots												Variants						
id	type	name	status	chr	gene	transcript	reads	start	end	exo...	codon	//	VF%	depth	Protein	c.DNA	alt	ref	IGV
c4	cdna	394delTT	undetected	7	CFTR	NM_000492	476	117149185	117149186										IGV
c5	cdna	406-1G>C	undetected	7	CFTR	NM_000492	499	117170952	117170952										IGV
c6	cdna	457TAT>G	undetected	7	CFTR	NM_000492	499	117171004	117171006										IGV
c7	cdna	541delC	undetected	7	CFTR	NM_000492	499	117171088	117171088										IGV
c8	cdna	541del4	undetected	7	CFTR	NM_000492	499	117171088	117171091										IGV
c9	cdna	546insCTA	undetected	7	CFTR	NM_000492	499	117171094	117171095										IGV
c10	cdna	574delA	undetected	7	CFTR	NM_000492	499	117171121	117171121										IGV
c11	cdna	621+1G->T	undetected	7	CFTR	NM_000492	499	117171169	117171169										IGV
c12	cdna	663insT	undetected	7	CFTR	NM_000492	355	117174371	117174371										IGV
c13	cdna	711+1G->T	undetected	7	CFTR	NM_000492	355	117174420	117174420										IGV
c14	cdna	711+3A->G	undetected	7	CFTR	NM_000492	355	117174422	117174422										IGV
c15	cdna	711+5G->A	undetected	7	CFTR	NM_000492	355	117174424	117174424										IGV
c16	cdna	852del22	undetected	7	CFTR	NM_000492	425	117175445	117175466										IGV
c17	cdna	875+1G>A	undetected	7	CFTR	NM_000492	425	117175466	117175466										IGV

SCREENING | GENES | SNPs-INDELs | CNVs | WARNINGS

CFTR

HotSpots												Variants						
id	type	name	status	chr	gene	transcript	reads	start	end	exo...	codon	//	VF%	depth	Protein	c.DNA	alt	ref	IGV
p49	codon	R553X	present	7	CFTR	NM_000492	355	117227865	117227867	12	553		99.72	355	p.Arg553*	c.1657C>T	T	C	IGV
c1	cdna	175insT	undetect...	7	CFTR	NM_000492	464	117120198	117120199										IGV
c2	cdna	186-13C>G	undetect...	7	CFTR	NM_000492	325	117144294	117144294										IGV
c3	cdna	296+2T>G	undetect...	7	CFTR	NM_000492	325	117144419	117144419										IGV
c4	cdna	394delTT	undetect...	7	CFTR	NM_000492	608	117149185	117149186										IGV
c5	cdna	406-1G>C	undetect...	7	CFTR	NM_000492	805	117170952	117170952										IGV
c6	cdna	457TAT>G	undetect...	7	CFTR	NM_000492	805	117171004	117171006										IGV
c7	cdna	541delC	undetect...	7	CFTR	NM_000492	805	117171088	117171088										IGV
c8	cdna	541del4	undetect...	7	CFTR	NM_000492	805	117171088	117171091										IGV
c9	cdna	546insCTA	undetect...	7	CFTR	NM_000492	805	117171094	117171095										IGV
c10	cdna	574delA	undetect...	7	CFTR	NM_000492	805	117171121	117171121										IGV
c11	cdna	621+1G->T	undetect...	7	CFTR	NM_000492	805	117171169	117171169										IGV
c12	cdna	663insT	undetect...	7	CFTR	NM_000492	632	117174371	117174371										IGV
c13	cdna	711+1G->T	undetect...	7	CFTR	NM_000492	632	117174420	117174420										IGV
c14	cdna	711+3A->G	undetect...	7	CFTR	NM_000492	632	117174422	117174422										IGV
c15	cdna	711+5G->A	undetect...	7	CFTR	NM_000492	632	117174424	117174424										IGV
c16	cdna	852del22	undetect...	7	CFTR	NM_000492	474	117175445	117175466										IGV

OVERVIEW | SCREENING | GENES | SNPs-INDELs | CNVs | WARNINGS

Static Filters

Retained 9

A: Most Likely Patho... 1 — 1

B: Potentially Pathoge... — 0

C: VUS (Unknown Sign... — 1

D: Likely Benign 4 — 7

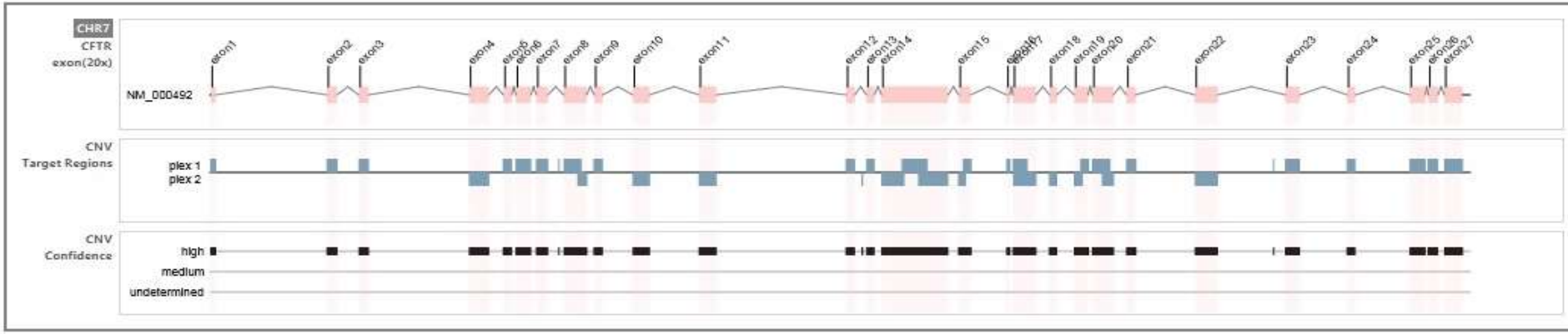
Low Confidence

Variant List - sorted by: PRED_CAT > PATHOGENICITY_CLASS > GENE

Pred.	Pat.	id	type	cod. cons.	gene	refSeqId	c.DNA	Protein	VF%
A		21	IVS8_polyT	splice_acceptor_indel	CFTR	NM_000492	c.1210-34TG[10];[=]		
C	1	15	INDEL	intronic	CFTR	NM_000492	c.2619+86_2619+87delTA		54.75
C	1	13	SNP	intronic	CFTR	NM_000492	c.1680-870T>A		46.61
C	1	17	SNP	intronic	CFTR	NM_000492	c.2909-92G>A		51.25
C	1	19	SNP	intronic	CFTR	NM_000492	c.4137-139G>A		47.43
C		11	SNP	intronic	CFTR	NM_000492	c.1585-9218G>A		48.05
D	1	3	SNP	synonymous	CFTR	NM_000492	c.4389G>A	p.= (p.Gln1463Gln)	51.78
D	1	2	SNP	synonymous	CFTR	NM_000492	c.2562T>G	p.= (p.Thr854Thr)	50.11
D	1	1	SNP	missense	CFTR	NM_000492	c.1408G>A	p.Val470Met	52.32

Global CNV Analysis — normal | insertion | deletion | failed

Patient	Gene	Noise	CNV	Amplicons (Copy Number)
33205-184...	CFTR	medium		2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2



Analisi di laboratorio:

Raccolta del campione

Analisi di laboratorio

Elaborazione del risultato

Referto

Test genetico:

Preparazione, Informazione, Consenso informato

(Consulenza pre-test)

Raccolta del campione

Analisi di laboratorio

Elaborazione del risultato

Interpretazione, Referto,
Consulenza post-test

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO

Laboratorio di Genetica Medica
Settore di Genetica Molecolare
via Commenda 12, 20122 - Milano
tel: 02 55032432-2433

INDAGINI MOLECOLARI PER LA FIBROSI CISTICA

Milano, **16/02/2012**

Data prelievo **09/02/2012**

Nome paziente: **Cod.** FC95/12

D.nascita **Sesso**

Reparto/Centro:

Motivo della richiesta: *Accertamenti pre-fecondazione assistita*

Materiale pervenuto: Sangue intero **Materiale utilizzato:** DNA estratto da leucociti del sangue periferico

Risultati **Presenza in eterozigosi della mutazione : c.3909C>G, p.Asn1303Lys (N1303K in eterozigosi)**

Interpretazione Il soggetto in esame è risultato portatore di una mutazione che causa Fibrosi Cistica ed è pertanto da considerarsi eterozigote per fibrosi cistica.
E' opportuno estendere l'indagine molecolare per la fibrosi cistica al partner ed ai familiari.

Si consiglia consulenza genetica

Il Biologo
Dott. Luigi Porcaro

Il responsabile del Laboratorio di Genetica Molecolare
Dott.ssa Manuela Seia

Mutazioni studiate [delta]F508,G551D,G542X,[delta]I507,R553X,N1303K,W1282X,1717-1G->A,CFTRdel2.3(21Kb), 3199del6,3272-26A>G,S1251N,Q552X,R560T,3905insT,3120+1G>A,711+1G->T, 1898+1G->A,394delTT,2184delA,711+5GA,3849+10kbC->T,R1162X,3659delC,G85E, 621+1G->T,R117H,1078delT,R347P,R334W,A455E,2183AA->G,2789+5G->A,2143delT, E60X,1259insA,4016insT,4382delA,852del22Bp,D579G,G1244E,G1349D,I502T,L1065P, R1158X,T338I,S549R(A>C),991del5,D1152H,1898+3A->G,R1070Q,R1066H,621+3A->G, E217G,R334Q,R347H,L1077P,1677delTA,1706del17, E585X, R1066C, S912X.
Screening delezioni es.1, es.2 (ins.182bp), es.2, es.2-3, es. 14b-17b, es. 22-23, es. 22-24, es. 17a-18 (3120+1Kb del. 8.6Kb).
Le mutazioni ricercate coprono circa l'85% dei difetti che causano Fibrosi Cistica (frequenze analizzate su 1200 alleli CF – dati casistica interna).

Polimorfismi osservati Poli T, Intr. 8 = 7/9

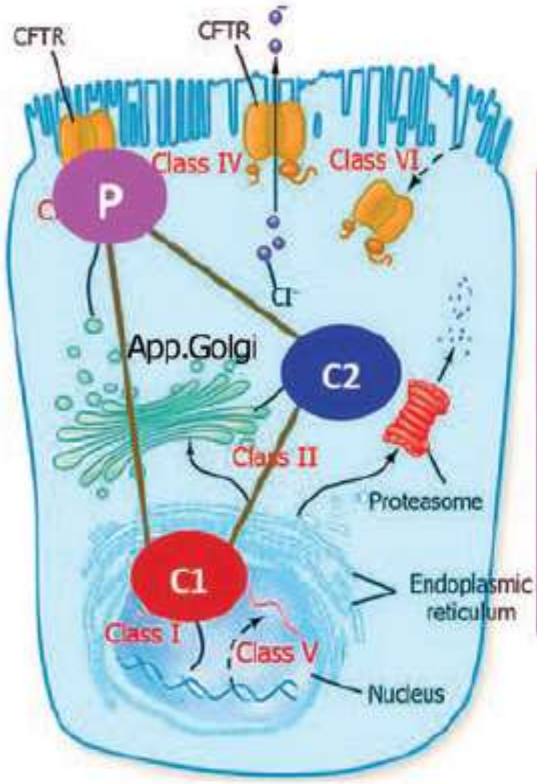
Con tecnica di sequenziamento sono stati inoltre indagati per la presenza di mutazioni i seguenti esoni del gene CFTR:
13

Metodiche utilizzate INNO-LIPA CFTR19, CFTR17+TN Update,CFTR Italian Regional; CFTR Deletions +6 (reverse dot blot)

Riferimento numerazione sequenza NM_000492.3. Nomenclatura secondo HGVS V 2.0 (in parentesi nomenclatura tradizionale: Cystic Fibrosis Mutation Database: www.genet.sickkids.on.ca)

Il laboratorio di Genetica Molecolare partecipa al controllo di qualità Europeo organizzato dal Cystic Fibrosis European Network.

MODULATORI - *Tipologia*



Potenziatori

	fase
• Kalydeco (in commercio)	
• GLPG1837	(2)
• ABBV/GLPG2451	(1)
• ABBV/GLPG3067	(1)
• QBW-251	(2)
• CTP-656	(2)
• FDL-176	(1)
• PTI-808	(1)

Correttori C1

	fase
• Lumacaftor+Kalydeco -Orkambi (in commercio)	
• Tezacaftor+Kalideko -Symdeko (in commercio USA)	
• ABBV/GLPG2222	(2)
• ABBV/GLPG2851	(2)
• FDL-169	(1/2)
• PTI-801	(1/2)
• FFC/ARN23765	pred.

Correttori C2

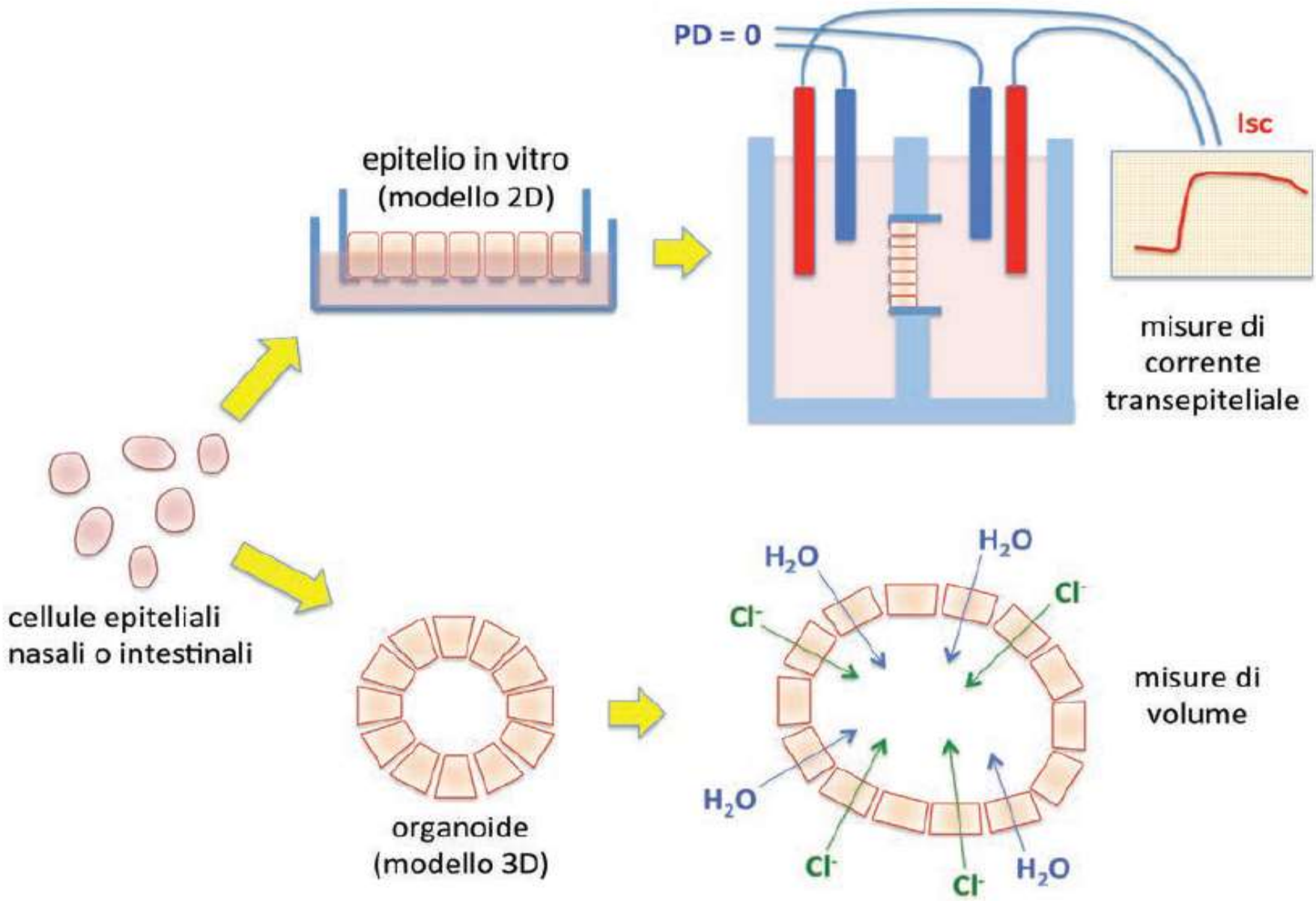
	fase
• VX-152	(2/3)
• VX-440	(2)
• VX-659	(2/3)
• VX-445	(1/2)
• ABBV/GLPG2737	(1)
• ABBV/GLPG3221	(1)
• FD2052160	pred.

Amplificatori: PTI-428 (fase 1)

Modulatori della proteina CFTR mutata per F508del e altre mutazioni.

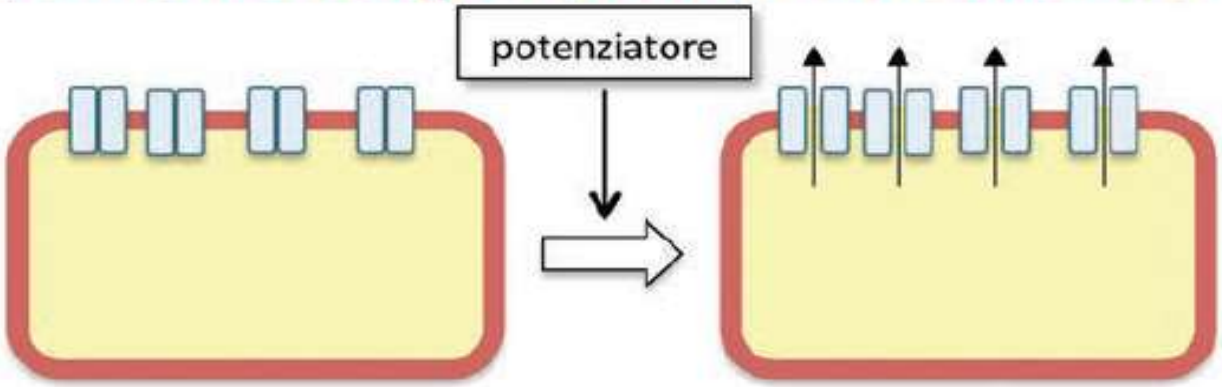
*I modulatori di CFTR mutata sono distinti in **Potenziatori (P)**, che intervengono su CFTR quando è sulla membrana della cellula, per potenziare la sua funzione di canale per il cloro, e in **Correttori (C1 e C2)** che intervengono nella maturazione di CFTR e nel suo percorso verso la membrana cellulare. Si ritiene che il meccanismo d'azione dei **Correttori C1 (Correttori di prima generazione)** sia rivolto direttamente a CFTR nelle prime fasi di maturazione fuori dal nucleo; quello dei **Correttori C2 (Correttori di seconda generazione)** sia rivolto alle proteine contenute nel citoplasma della cellula, che indirizzano CFTR sulla membrana o l'avviano alla degradazione. Una nuova categoria di modulatori, chiamati **Amplificatori** è in grado di aumentare la produzione della proteina CFTR, indipendentemente dal tipo di mutazione, e incrementa quindi l'azione di Potenziatori e Correttori.*

MODULATORI – "*In Vitro*"

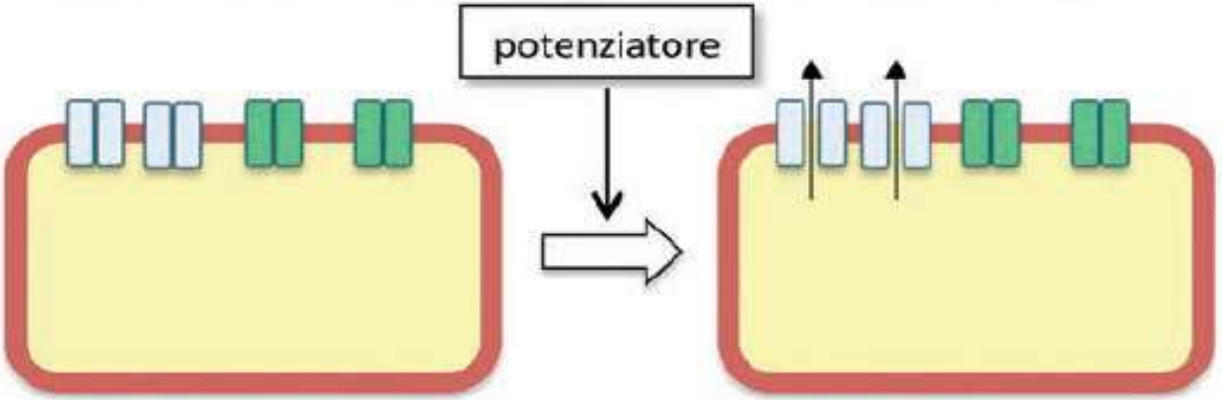


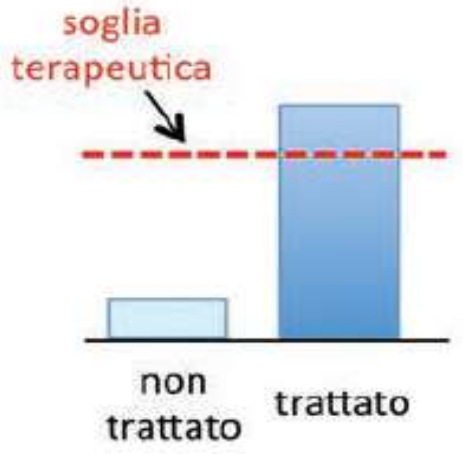
MODULATORI - *Effetti*

paziente omozigote per mutazione di classe 3

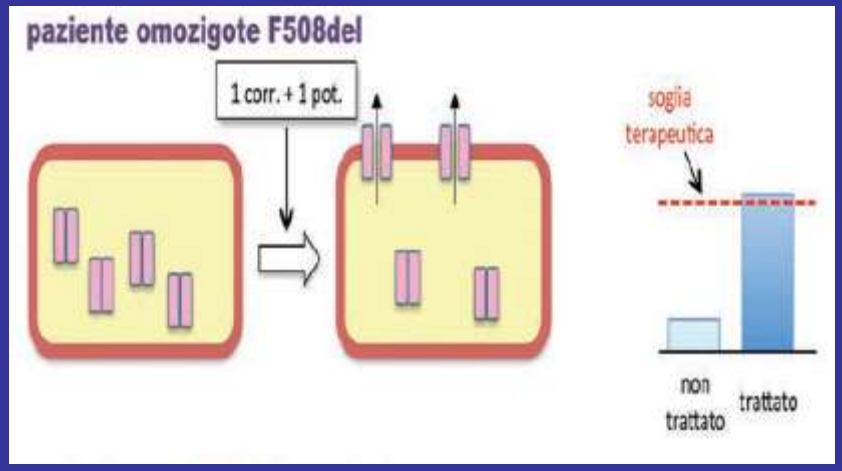


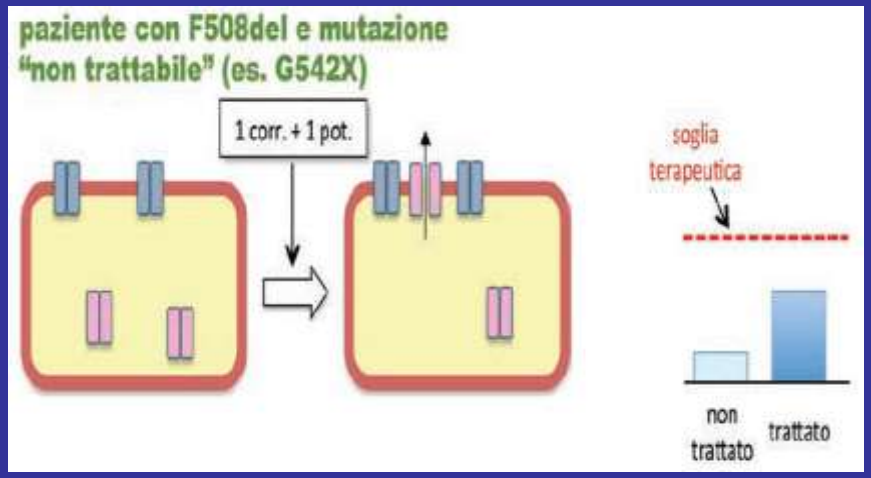
paziente con mutazione di classe 3 e mutazione "non trattabile" (es. G542X)

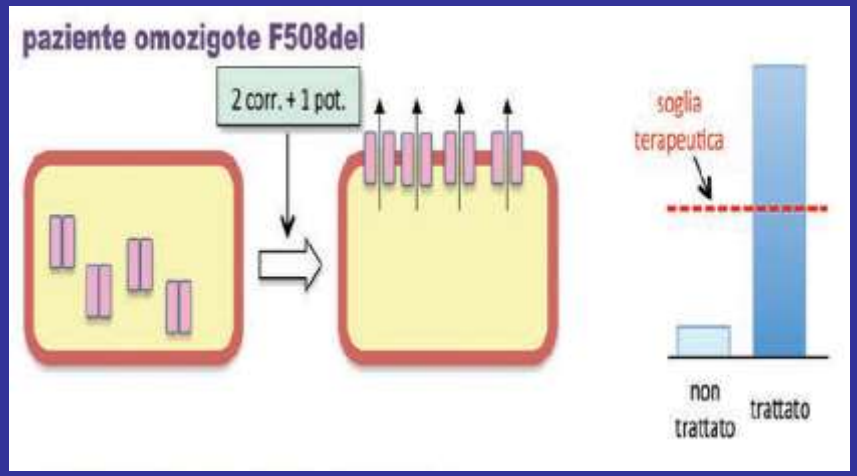


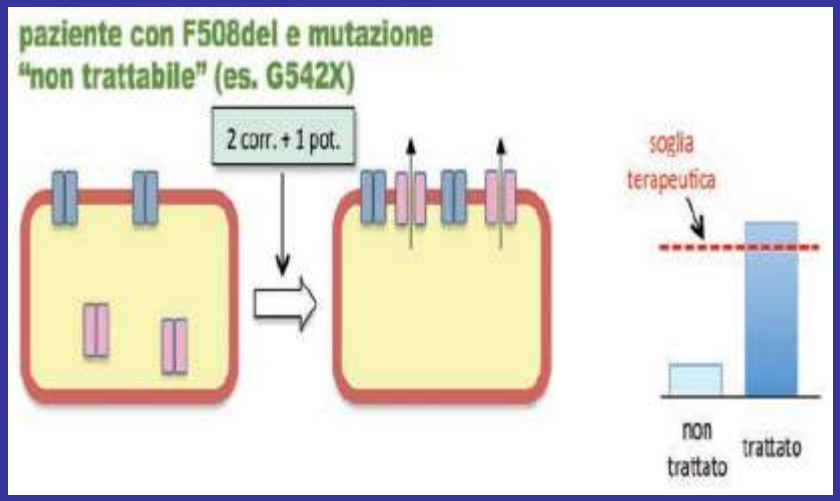


MODULATORI - *Combinazioni*

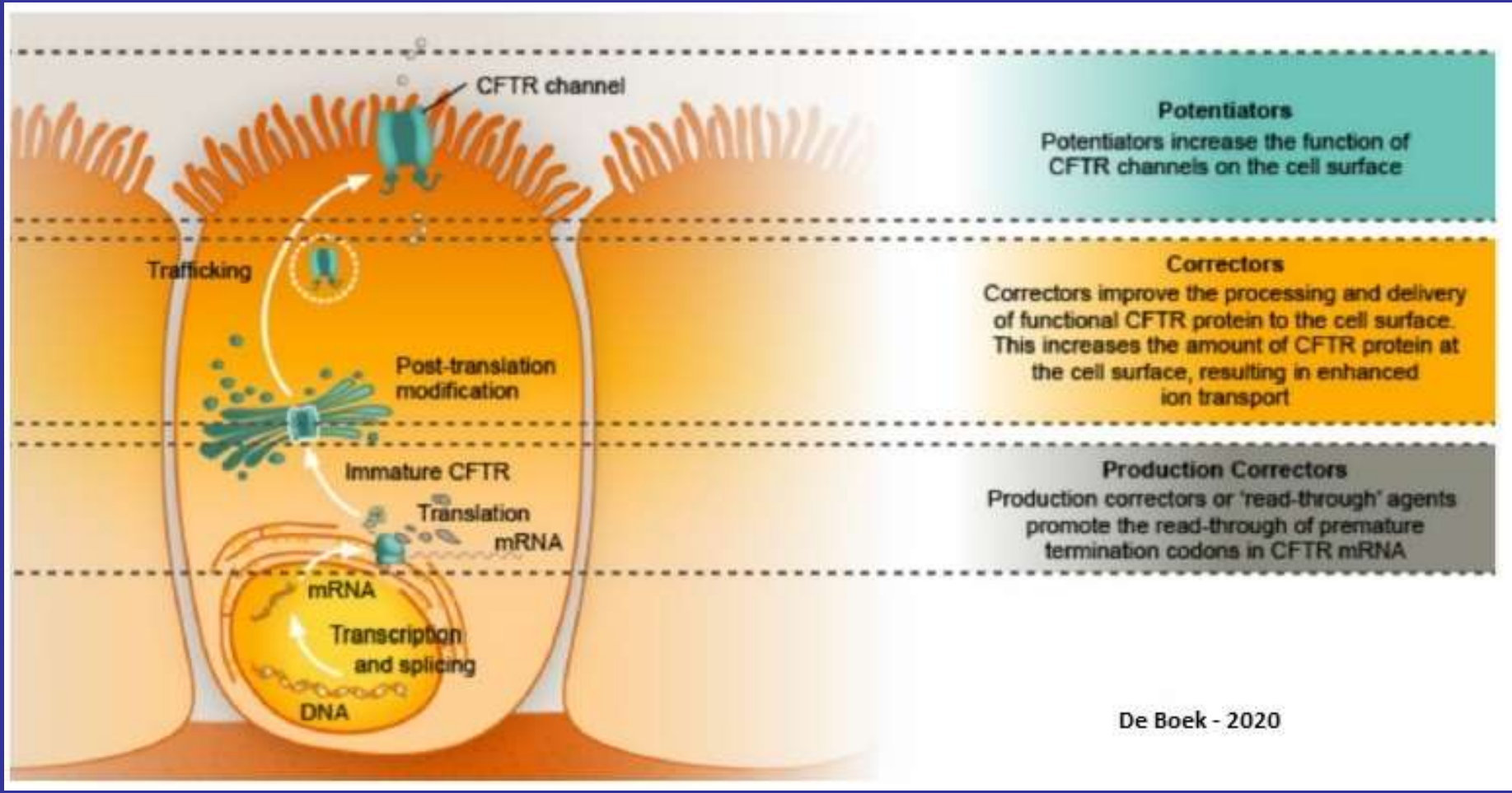


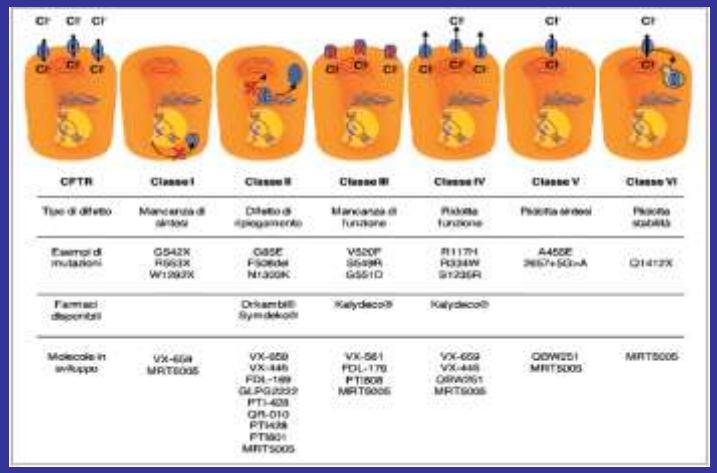






MODULATORI - *Riassunto*





CFTR modulator	Mutations	From age of
Kalydeco (ivacaftor) **Potenziatore**	9 class III mutations: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N and S549R	12 mo
	R117H	18 y
Orkambi (lumacaftor + ivacaftor)	F508del homozygous	2 y
Symkevi, Symdeko (tezacaftor + ivacaftor) **Correttore + Potenziatore**	F508del homozygous	12 y
	Patients heterozygous for F508del and one of the following mutations: P67L, R117C, L206W, R352Q, A455E, D579G, 711 + 3A>G, S945L, S977F, R1070W, D1152H, 2789 + 5G>A, 3272 26A > G, OR 3849 + 10kbC>T	12 y

Sources: https://www.ema.europa.eu/en/medicines/human/EPAR/kalydeco, https://www.ema.europa.eu/en/medicines/human/EPAR/orkambi, https://www.ema.europa.eu/en/medicines/human/EPAR/symkevi.