



Abstract #182

# ASSESSING DISEASE LIABILITY OF MISSENSE CFTR MUTATIONS USING DIFFERING PERCENTAGES OF WILD-TYPE FUNCTION

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CFTR2 Team



**JOHNS HOPKINS**  
MEDICINE  
CYSTIC FIBROSIS CENTER

Funding for the CFTR2 project, including salary  
support provided by the US CF Foundation



| Adding *tomorrows* every day.

# ***Cystic Fibrosis***

## ***Mutation Database***

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

- 1820 distinct *CFTR* mutations
- Voluntary submissions by academic labs, commercial diagnostic labs
- Limited clinical information to assess the phenotypic consequences of these mutations
  
- Few of these mutations are clearly characterized as CF-causing *CFTR* mutations, or neutral variants

## CFTR2 Database

- 39,614 patients from US, Canada, Europe, Israel
- 1099 different CFTR mutations
- Clinical information on: Sweat chloride, pancreatic status, infection status, height, weight, lung function, age, age and reason for diagnosis
- MISSING: extent of genetic testing



## CFTR1 (CF Mutation Database)

- 1820 different *CFTR* mutations
- Limited clinical information

Novel *CFTR*  
Mutation

```
graph TD; A([Novel CFTR Mutation]) --> B[CF-Causing CFTR Mutation]; A --> C[Neutral Variant "polymorphism"]; A --> D["Variants of Uncertain Clinical Significance"]
```

CF-Causing *CFTR*  
Mutation

Neutral Variant  
“polymorphism”

“Variants of Uncertain  
Clinical Significance”

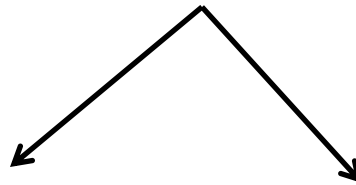
# CF-causing *CFTR* mutation

- Clinical criteria:
  - Patients carrying this mutation, and another CF-causing mutation should have characteristic phenotype associated with CF
  - Obstructive lung disease, bronchiectasis
  - Typical CF respiratory pathogens
  - Pancreatic disease
  - **Sweat Chloride concentration  $\geq 60\text{mEq/L}$**

# Sweat chloride is a useful filter to evaluate disease liability

159 mutations with allele frequency > 0.01% (95% of CF alleles)

## Average Sweat chloride



>=60mEq/l

147 mutations

- Average sweat 95.4
- FEV1% predicted 76.8
- 78% of patients PI

<60mEq/l

12 mutations

- Average sweat 48.2
- FEV1% predicted 90.3
- 39% of patients PI

# CF-causing *CFTR* Mutations

Clinical criteria alone are not enough:

- I148T, R117H as examples
- Functional criteria:
  - Predictions can be made based on the type of mutation (nonsense, frameshift, splice donor/acceptor site)
  - Functional assessment needed for missense mutations, ins/del that do not cause frameshift, other splice mutants

\* Also would like population confirmation that the mutation does not occur in asymptomatic carriers

# Functional Studies

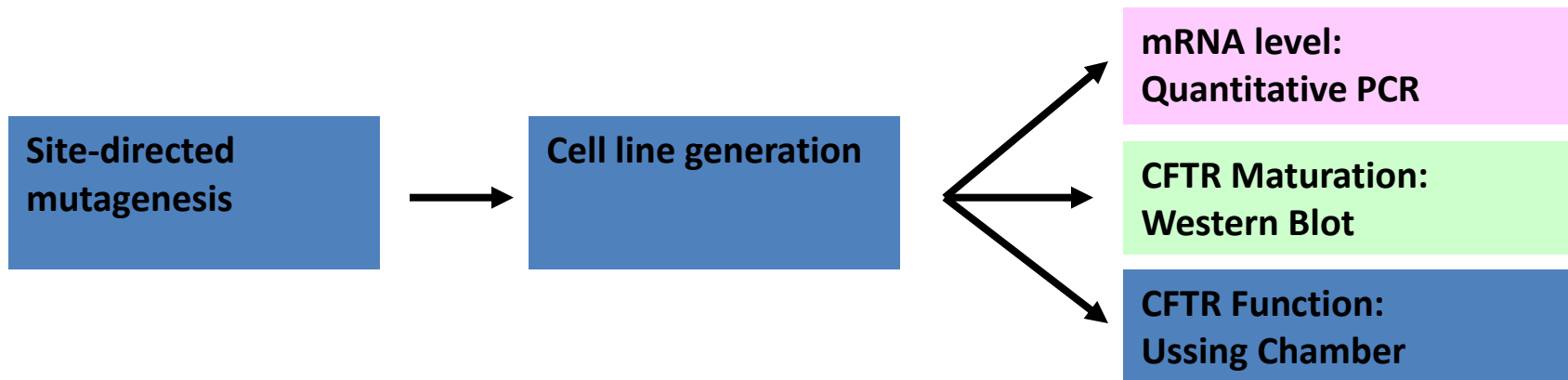
159 mutations are seen in 9 or more patients world-wide:

-47 of these were mutations and evaluated functionally (mostly missense)

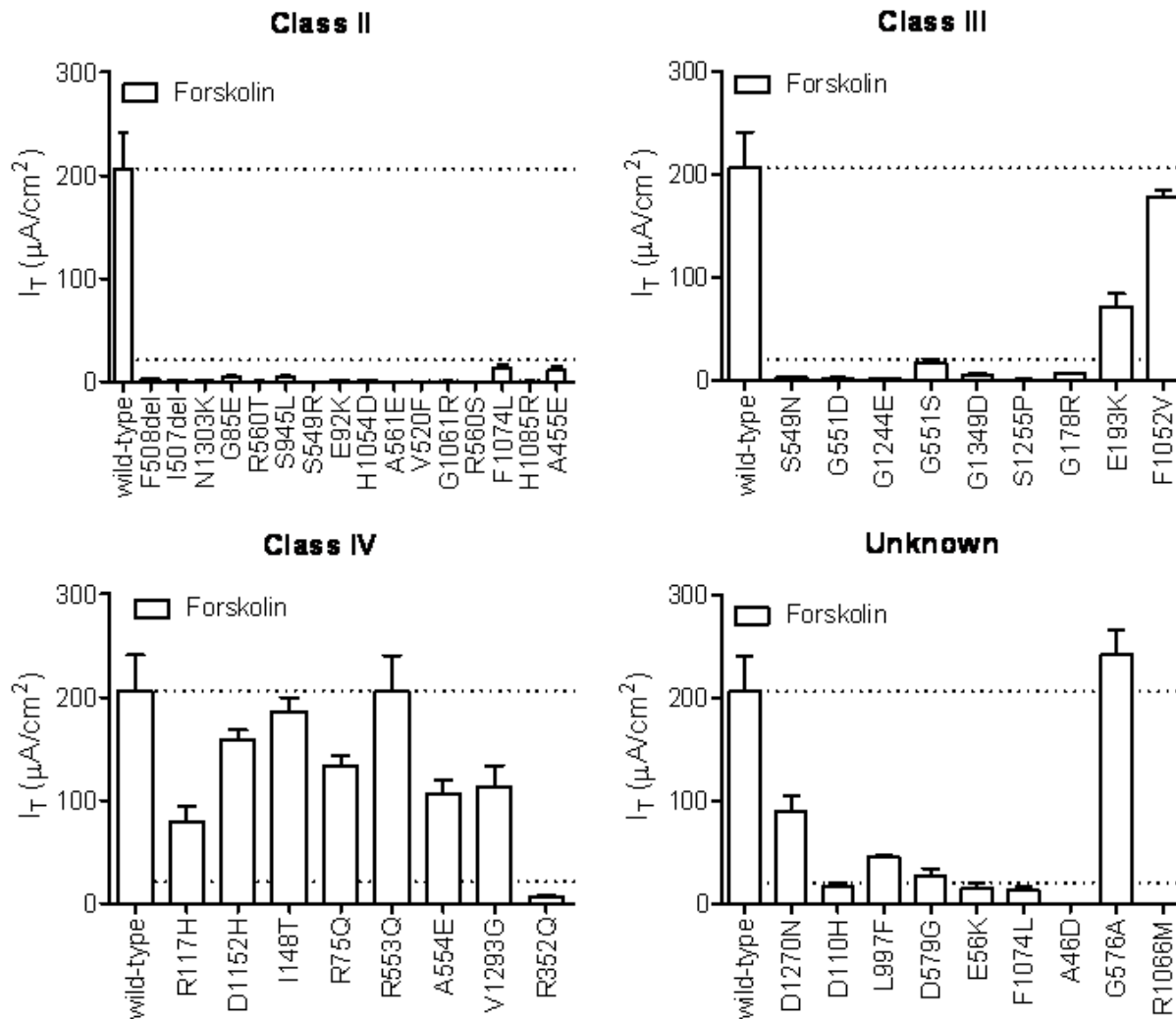
CFTR processing and function (Fred VanGoor, Haihui Yu)

Fisher Rat Thyroid (FRT) cells expressing CFTR from single cDNA integration

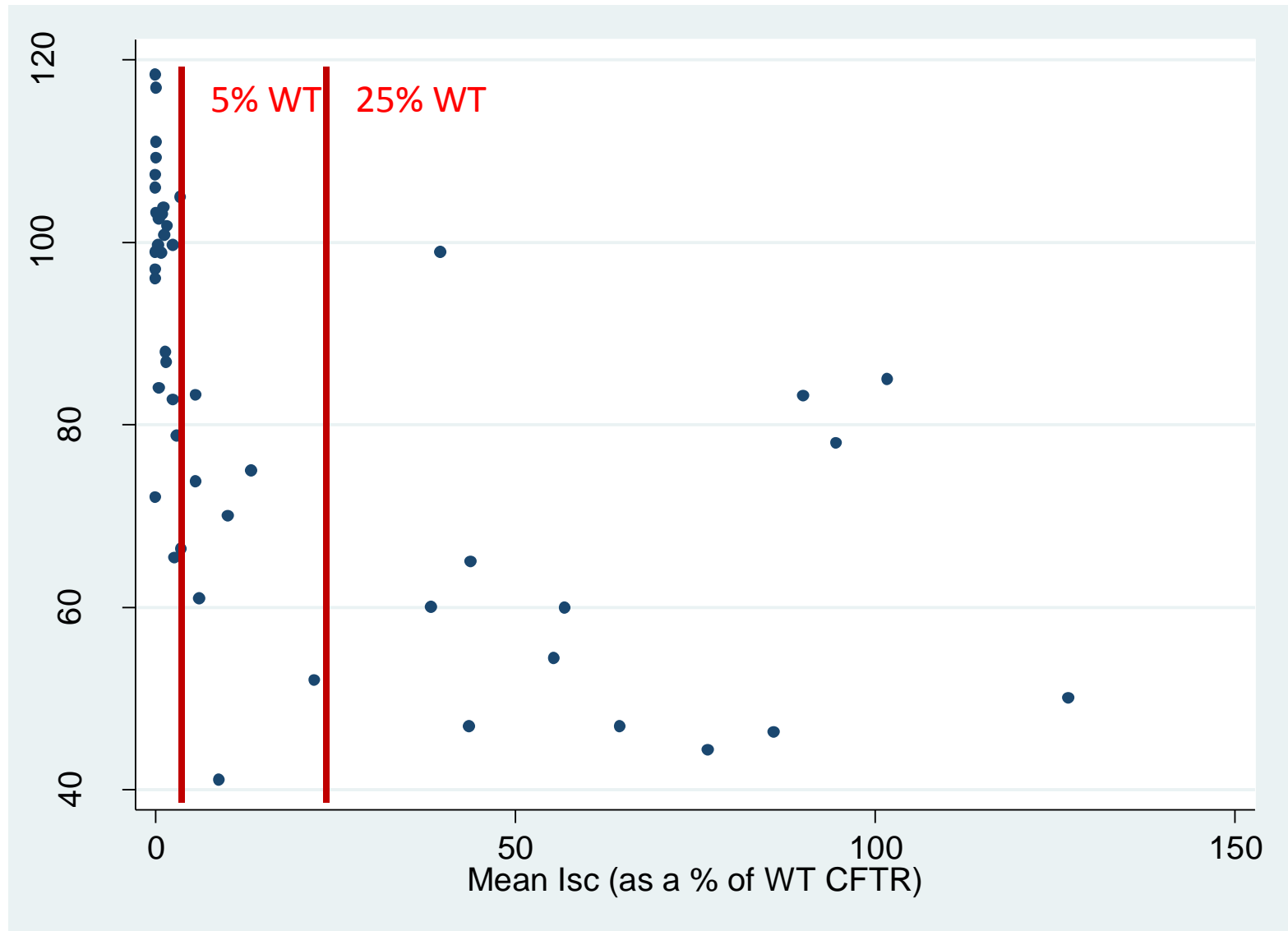
Characterize the processing and function of CFTR



# Forskolin-Stimulated $I_T$ in FRT Cells Expressing Various Mutant CFTR Forms

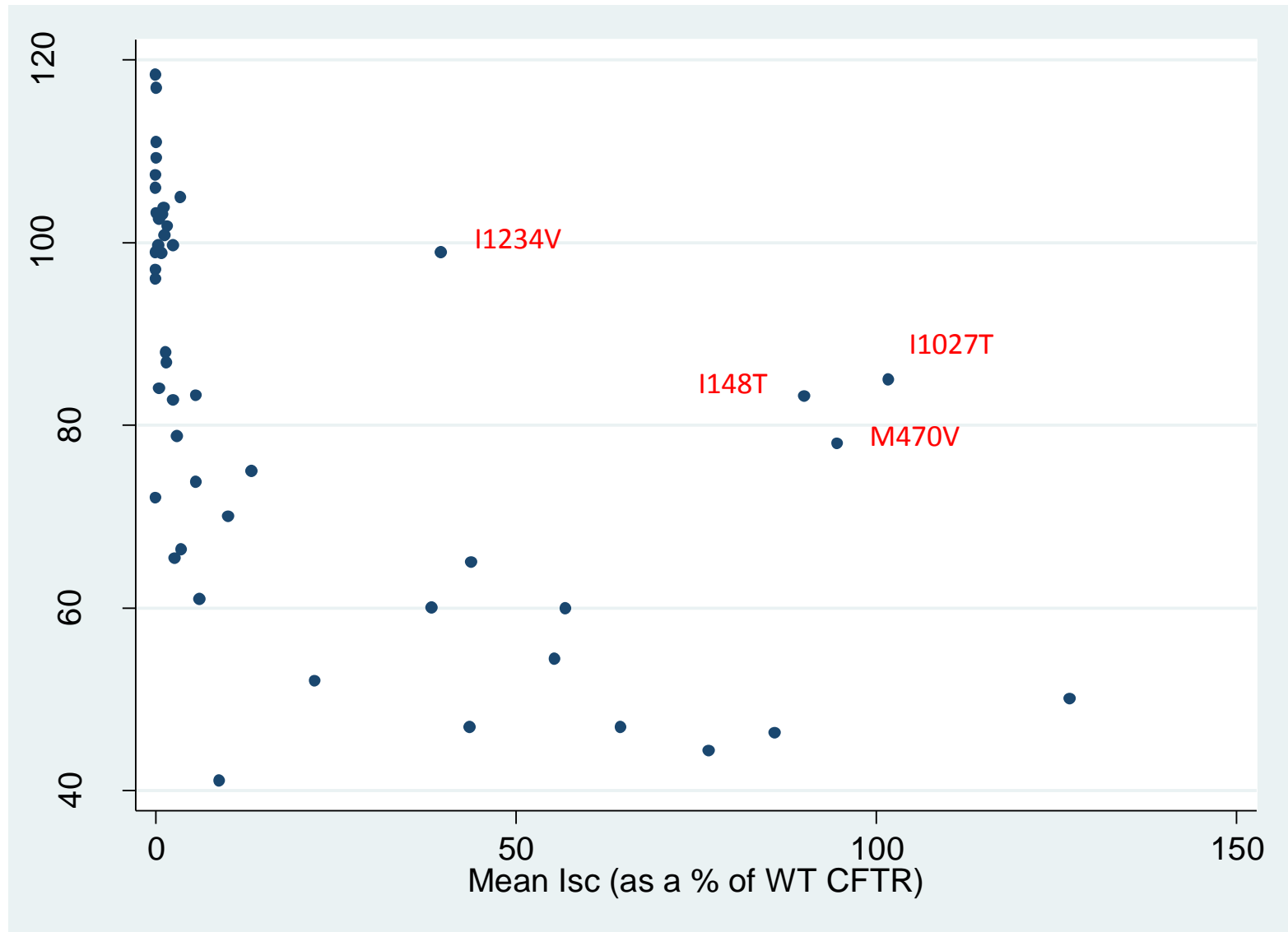


# In vitro Current vs. Average Sweat Chloride

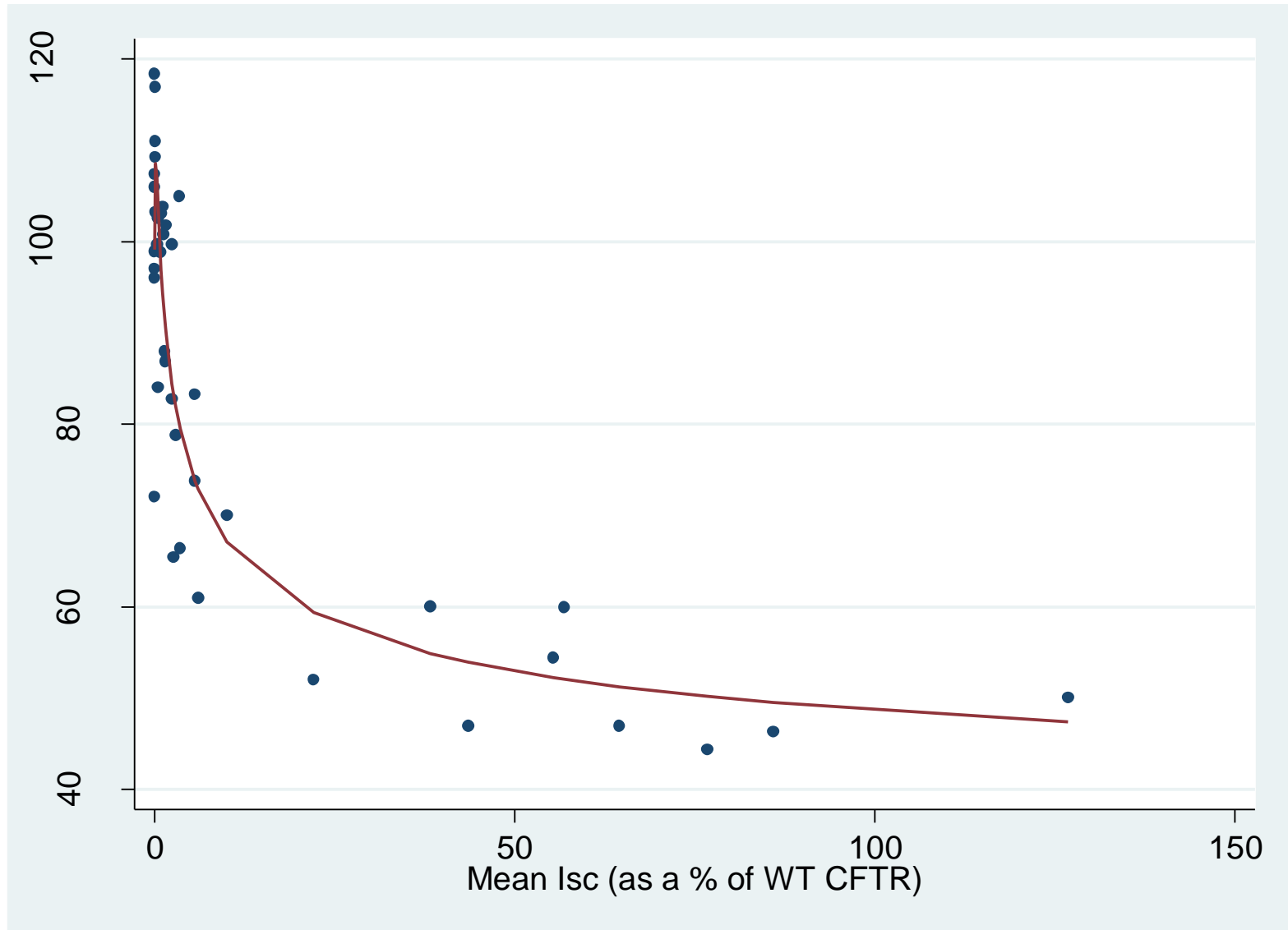


<b>Clinical Characteristic:</b>	<b><u>Mutations with Isc &lt;5% wild-type</u></b>	<b><u>Mutations with Isc 5-25% wild-type</u></b>	<b><u>Mutations with Isc &gt;25% wild-type</u></b>
# of mutations	25	7	13
Mutations Included	G551D, N1303K, G85E, R560T, R1066C, M1101K	A455E, L206W, P67L, R1070W, D110H, D579G, L997F	R117H, I1234V, R74W, D1270N, S1235R, R668C
Average sweat (std. dev.) chloride concentration (in trans with ACMG PI)	94.9 (13.2)	65.2 (14.7)	63.0 (17.8)
Average % of patients with PI (in trans with ACMG PI)	71.7 (29.4)	33.5 (21.3)	43.0 (22.3)
Average FEV1 %predicted (in trans with ACMG PI)	74.7 (7.7)	84.1 (7.9)	84.3 (6.6)

# Mutations with Short Circuit Current >25% wild-type do not fit in vitro/in vivo curve



# Mutations with Short Circuit Current >25% wild-type do not fit in vitro/in vivo curve



# Mutations with Short Circuit Current >25% wild-type

- Many of these mutations that lie on the curve relating in vivo sweat [Cl<sup>-</sup>] and in vitro Cl<sup>-</sup> current are mutations associated with variable expression:
  - D1152H
  - F1052V
  - R75Q
  - R668C
  - G576A

# Conclusions

- There is a non-linear (polynomial regression) correlation between in vitro mutation function analysis and in vivo sweat chloride concentration in patients that carry that mutation
- Mutations in which there is not a correlation are suspicious for complex alleles or intragenic modification

# Conclusions

- The cut-off to determine a mutation is CF-causing is arbitrary
- Mutations seen in CF patients with higher than 10% WT current in vitro are associated with incomplete penetrance of the CF phenotype; these mutations will be characterized as Mutations of Varying Clinical Consequence in CFTR2.

# CFTR2 Website: [www.cftr2.org](http://www.cftr2.org)



Languages:

scientific/medical view

## Quick Links

[Home](#) »»

[Search Database](#)

[Links](#)

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[CFTR<sub>2</sub> Contributors](#)

[CFTR<sub>2</sub> Team](#)

[How to use this site](#)

Welcome to the **C**linical and **F**unctional **T**Ranslation of CFTR (CFTR<sub>2</sub>) website. CFTR<sub>2</sub> is a website designed to provide information about specific cystic fibrosis mutations to patients, researchers, and to the general public.

### For patients:

#### WHO THIS SITE IS FOR:

This web site is intended for patients, family members and members of the general public who want to find out what we currently know about specific mutations related to cystic fibrosis.

This includes:

- Cystic fibrosis (CF) patients,
- Family members of CF patients,
- People who are carriers of a CF mutation, and
- Parents whose baby has just been diagnosed with CF through newborn screening.

#### WHO THIS SITE IS NOT INTENDED FOR:

- This website is not intended to help diagnose anyone with CF.
- This website is not intended to provide information about pancreatitis, diabetes mellitus, or other diseases associated with CF.
- This website provides information about specific CF mutations only.

### For health care providers/scientists:

This section provides scientific and medical descriptions, intended for CF researchers, health professionals, and members of the general public that are looking for more in depth, research-related information. Patients and their families are encouraged to visit the section "For patients" first.

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# CFTR2 Website




## Search Database Options

Mutation

← type mutation

Show All

 trouble finding a mutation

## Search results

MUTATION	NEW NOMENCLATURE(PROTEIN)	NEW NOMENCLATURE(NUCLEOTIDE)
G551D	p.Gly551Asp	c.1652G>A
R553X	p.Arg553X	c.1657C>T
A455E	p.Ala455Glu	c.1364C>A
V520F	p.Val520Phe	c.1558G>T
R352Q	p.Arg352Gln	c.1055G>A
A559T	p.Ala559Thr	c.1675G>A
Q552X	p.Gln552X	c.1654C>T
R851X	p.Arg851X	c.2551C>T
L558S	p.Leu558Ser	c.1673T>C
1548delG	p.Met472Metfs*55	c.1416delG
G551S	p.Gly551Ser	c.1651G>A

# CFTR2 Website



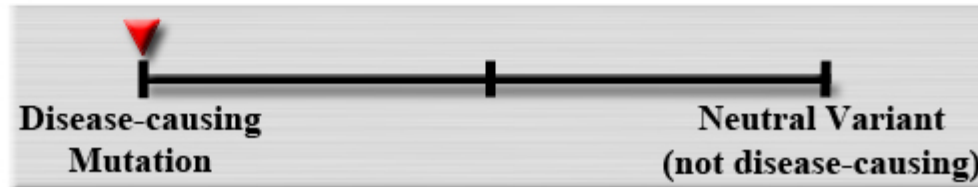
**This is the general user view. Click to switch to the scientific/medical view.**

**Summary:** This mutation is seen in our worldwide database. Based on the patients that carry **V520F**, and scientific experiments on the mutation, we could conclude that this mutation causes CF. That means that someone with one copy of this mutation, and no other CFTR mutations would be a CF carrier; and that someone with one copy of this mutation, and another CF-causing mutation would have CF. Patients with CF who have this mutation and a copy of F508del often have pancreatic insufficiency [↗](#)













# CFTR2 Website

This is the scientific/medical view. Click to switch to the general user view.

**Summary:** **V520F** is seen in 58 patients in our worldwide CF database. Based on the combination of clinical and functional evaluation, this is a mutation that **would cause CF**. Based on the patients we have reviewed we would expect this mutation would be associated with *pancreatic insufficient* CF.



The information displayed below shows how we came to this decision.

-   **Clinical Characteristics** 
-   **Mutation Characteristics** 
-   **Functional Testing** 
-   **Literature Review** 
-   **Carrier Screen** 
-   **Predictive Testing** 

## Quick Links

Home

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CFTR<sub>2</sub> Contributors


CFTR<sub>2</sub> Team


How to use this site


### Icons Legend:

 Evaluation of this mutation for this feature has been completed.




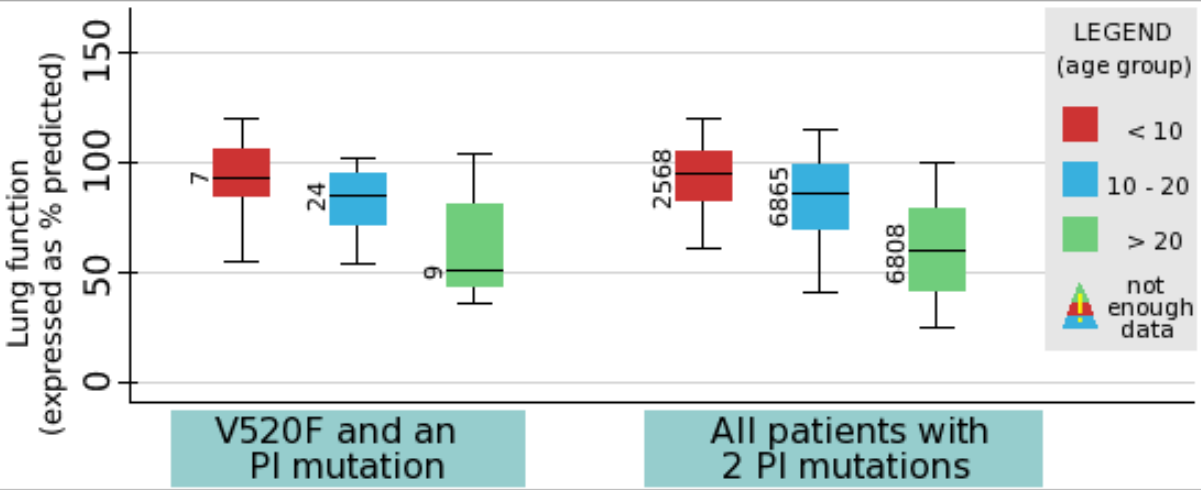


 Evaluation of this mutation for this feature has been completed. This mutation may not always cause CF.

 Evaluation of this mutation for this feature is being performed, but is not completed yet.

 Evaluation of this mutation for this feature will not need to be performed.

 Click for more information.

# CFTR2 Website

(RANGE IN INDIVIDUALS WITHOUT CF)	MUTATION V520F AND A PANCREATIC INSUFFICIENT MUTATION	PANCREATIC INSUFFICIENT MUTATIONS
<p>Sweat Chloride </p> <p><i>(non-CF is less than 40mEq/L in children and older, less than 30mEq/L in infants)</i></p>	110	102
<p>Lung Function  </p> <p>expressed as % predicted <i>(non-CF 80%-120% predicted)</i></p>	 <p>LEGEND (age group)</p> <ul style="list-style-type: none"> <li>&lt; 10</li> <li>10 - 20</li> <li>&gt; 20</li> <li>not enough data</li> </ul>	
<p>Pancreatic Insufficiency </p> <p><i>(0% of non-CF expected to be PI)</i></p>	100%	98%
<p>Pseudomonas </p> <p><i>(0% of non-CF expected to have Pseudomonas)</i></p>	41%	58%
Average Age	15	17

# **ACKNOWLEDGEMENTS:**

# **Clinical and Functional TRanslation of CFTR (CFTR2) Team**

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- Stuart Elborn, UK
- Phil Farrell, USA
- Michael Knowles, USA
- Isabelle Sermet, France

## Functional Evaluation

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- Gergely Lukacs, Canada
- David Sheppard, UK
- Phil Thomas, US

**Patient Privacy/Advocacy:** Michelle Lewis, Juliet Page, Barbara Karczeski, Suzanne Pattee, Bruce Marshall

# Contributors to CFTR2 (39,614 patients)

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<b>Patient registry of Mukoviszidose e.V., the GERMAN Cystic Fibrosis association</b>	Andreas Reimann, Martin Stern, Paul Wenzlaff	Martin Stern, MD, PhD, Director, CF Centre, Childrens' hospital, university hospital of Tuebingen, Germany Andreas Reimann, PhD, MBA, Chief Executive Officer, Mukoviszidose e.V. -German Cystic Fibrosis Association -, Bonn, Germany Paul Wenzlaff, MSc, Project Leader, Centre for Quality and Management in Medicine, Lower Saxony Chamber of Physicians, Hannover, Germany.
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<b>The NETHERLANDS CF Registry</b>		
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<b>NORTHERN GREECE CF Registry</b>	John Tsanakas Elpis Hatziagorou	
<b>UNITED STATES CF Patient Registry</b>	Bruce Marshall, Alexander Elbert, Ase Sewall	U.S. CF Foundation
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# Thank you

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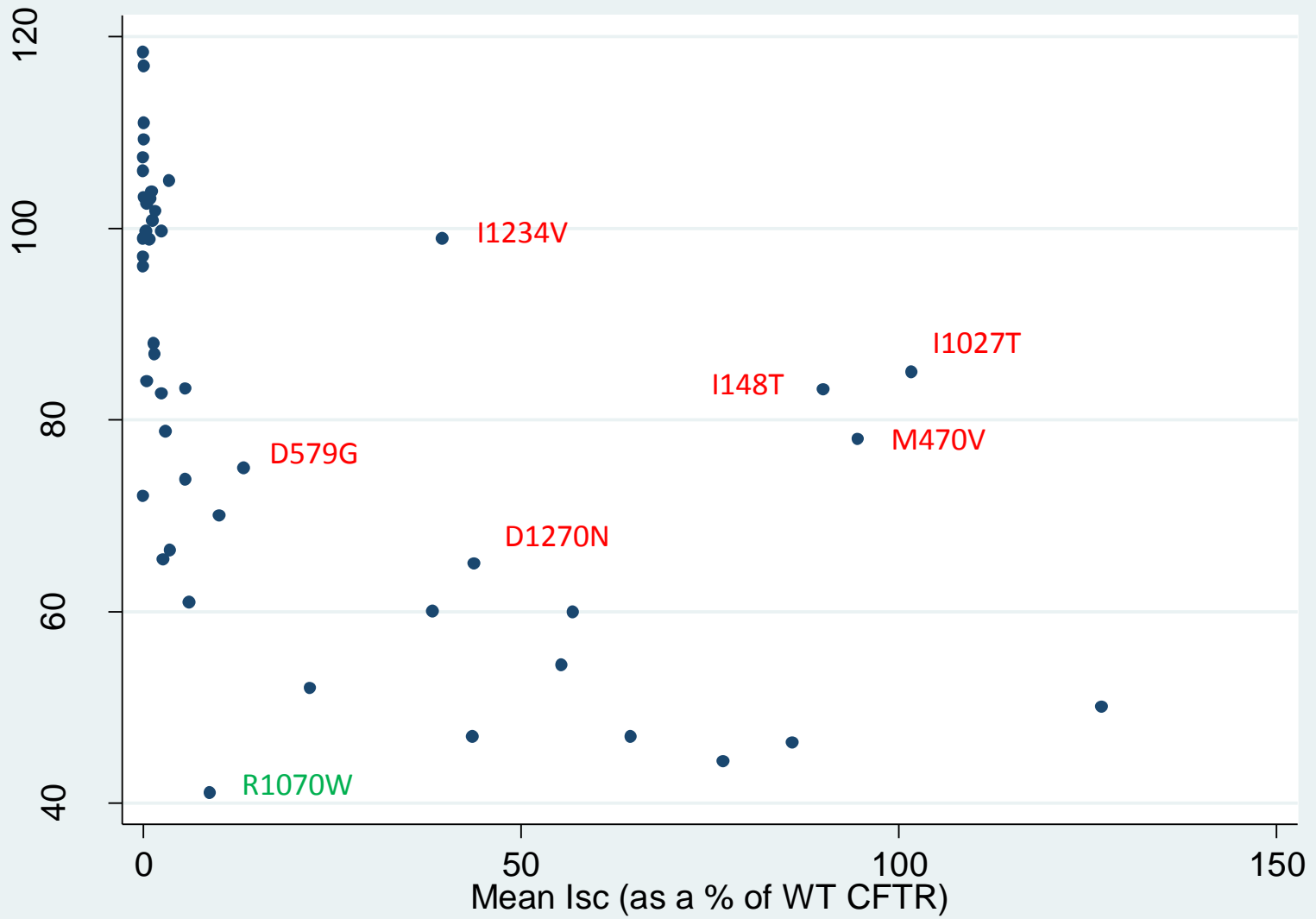
<http://robertmcclintock.com/>

	Isc <10% wild type CFTR (disease)	Isc >=10% wild type CFTR (neutral)
Sweat [Cl <sup>-</sup> ] > 60 (disease)	29 mutations: L1065P, L467P, A559T, L1077P, M1101K, R1066C, L927P, S549R, R560T, V520F, E92K, N1303K, G1244E, I336K, G551D, R334W, R1066H, T338I, S549N, S945L, G85E, R117C, R352Q, G178R, R347H, A455E, L206W, P67L, D110H	6 mutations: D579G, I1234V, D1270N, I148T, M470V, I1027T
Sweat [Cl] <=60 (neutral)	single mutation: R1070W	9 mutations: L997F, R117H, R74W, S1235R, R668C, R75Q, D1152H, F1052V, G576A

Mutation	# of patients	Mean Chloride conductance	Average sweat in trans with PI	Notes
G576A	40	127	51	Alternative splice
I1027T	41	87	101	Off curve (alternative splice)
M470V	39	94	70	Off curve (Complex allele/incomplete mutation analysis)
I148T	61	89	76	Off curve
F1052V	12	77	37	CBAVD
D1152H	185	76	46	
R75Q	20	65	55	
R668C	45	57	54	
S1235R	54	55	58	
D1270N	32	43	63	Average sweat 64
R74W	25	44	54	
I1234V	16	39	97	Off curve
R117H	739	38	60	Example of intragenic modification
L997F	27	22	59	
D579G	21	13.23	78	
D110H	30	10.0	69	

# Fractional Polynomial Fit

Graph uses the equation:  $y = \beta_0 + \beta_1((x + .1)/100)^{-2.0155} + \beta_2((x + .1)/100)^{.5} - \ln(x) + 4.641$



Mutations with Short  
Circuit Current < 5%  
wild-type

- 28 mutations
- Includes F508del, I507del, N1303K
- Average sweat [Cl<sup>-</sup>] of the patients that carry one of these mutations (in trans with CF-causing mutation) = 97 mEq/L
- 19/28 mutations are associated with pancreatic insufficiency

Mutations with Short  
Circuit Current 5-25%  
wild-type

- 7 mutations
- Includes A455E, D110H, L997F
- Average sweat [Cl<sup>-</sup>] of the patients that carry one of these mutations (in trans with CF-causing mutation) = 63 mEq/L
- 1/7 mutations are associated with pancreatic insufficiency

Mutations with Short  
Circuit Current >25%  
wild-type

- 13 mutations
- Includes R117H, R75Q, D1152H, M470V, I148T
- Average sweat [Cl<sup>-</sup>] of the patients that carry one of these mutations (in trans with CF-causing mutation) = 65 mEq/L
- 5/13 mutations are associated with pancreatic insufficiency

# Mutations with Short Circuit Current >25% wild-type

- Mutations known subject to intragenic variation: R117H
- Mutations known to be incorrectly identified as disease-causing allele: M470V, I148T
- Mutations suspected to cause disease through aberrant splicing: I1234V, I1027T (M. Amaral personal communication)

## Novel CFTR Mutation

```
graph TD; A([Novel CFTR Mutation]) --> B[CF-Causing CFTR Mutation]; A --> C[Neutral Variant "polymorphism"];
```

### CF-Causing CFTR Mutation

- Novel amino acid change not seen in CF carriers
- The variant is seen in unrelated individuals with *bona fide* CF
- Changes a highly conserved amino acid residue
- Creates a novel/cryptic splice site

### Neutral Variant "polymorphism"

- It is seen in trans with another disease causing mutation in an asymptomatic individual
- Silent exon variant, without splicing modification
- Intronic sequence variant that does not create a splice site
- Frequency in the general population is greater than 0.4%.