THE ROLE OF *CFTR* MUTATIONS IN CAUSING CYSTIC FIBROSIS (CF)

Program objectives

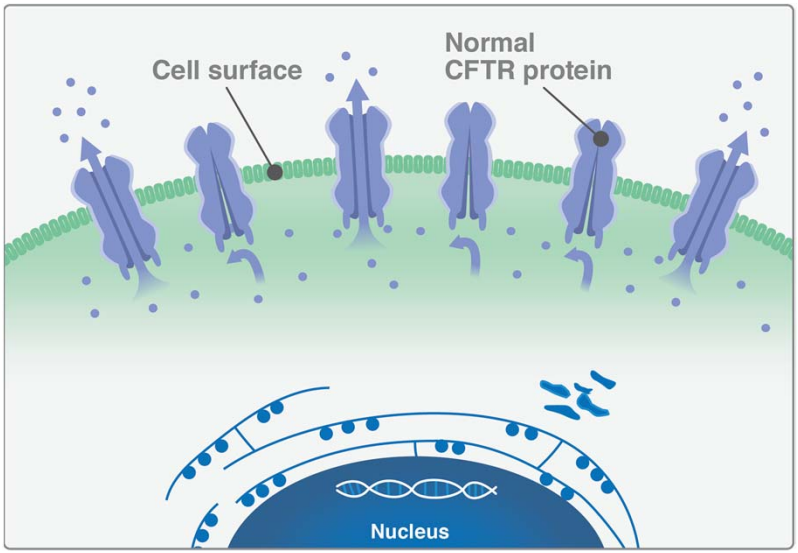
Increase your knowledge of:

- The importance of CFTR protein regulation in the body
- How *CFTR* mutations are inherited, and how they affect CFTR protein quantity and/or function and total CFTR activity
- The links between *CFTR* mutations, CFTR activity, and CF disease development

CFTR, cystic fibrosis transmembrane conductance regulator.

CFTR PROTEINS IN NORMAL CELLS

CFTR proteins: An important regulator of fluid and ion balance in organs throughout the body



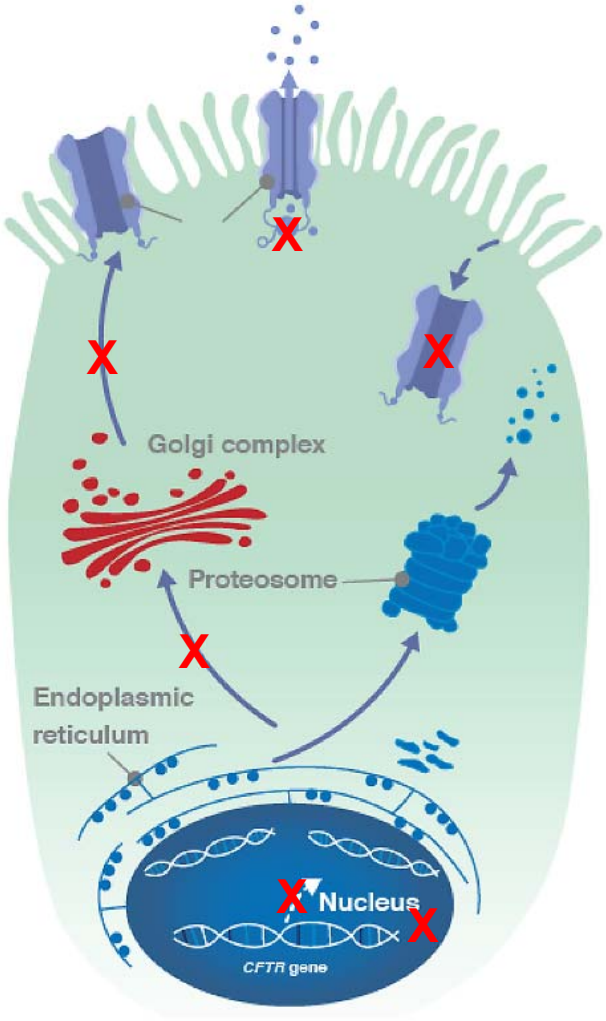
- CFTR proteins are found on epithelial cell surfaces in organs throughout the body[1-4]
- Normally, CFTR protein channels transport ions, such as chloride and bicarbonate, through the epithelial cell surface in these organs[1-4]
- Maintaining water and salt balance at the epithelial cell surface requires an adequate quantity and function of CFTR proteins[5]

- **Total CFTR activity** can be defined as total ion transport mediated by CFTR protein channels at the cell surface. It is dependent on CFTR protein **quantity** and **function**[6,7]

CFTR quantity X **CFTR function** (**Channel-open probability** X **Conductance**) = **Total CFTR activity**

References: 1. Zielenski J. *Respiration*. 2000;67(2):117-133. **2.** O'Sullivan BP, Freedman SD. *Lancet*. 2009;373(9678):1891-1904. **3.** MacDonald KD et al. *Pediatr Drugs*. 2007;9(1):1-10. **4.** Derichs N. *Eur Respir Rev*. 2013;22(127):58-65. **5.** Elborn JS. *Lancet*. 2016;388(10059):2519-2531. **6.** Derichs N. *Eur Respir Rev*. 2013;22(127):58-65. **7.** Boyle MP, De Boeck K. *Lancet Rep Med*. 2013;1:158-163.

CFTR proteins reach the membrane through a multi-step process



CFTR protein function and breakdown

CFTR protein trafficking (delivery to the cell surface)

CFTR protein synthesis

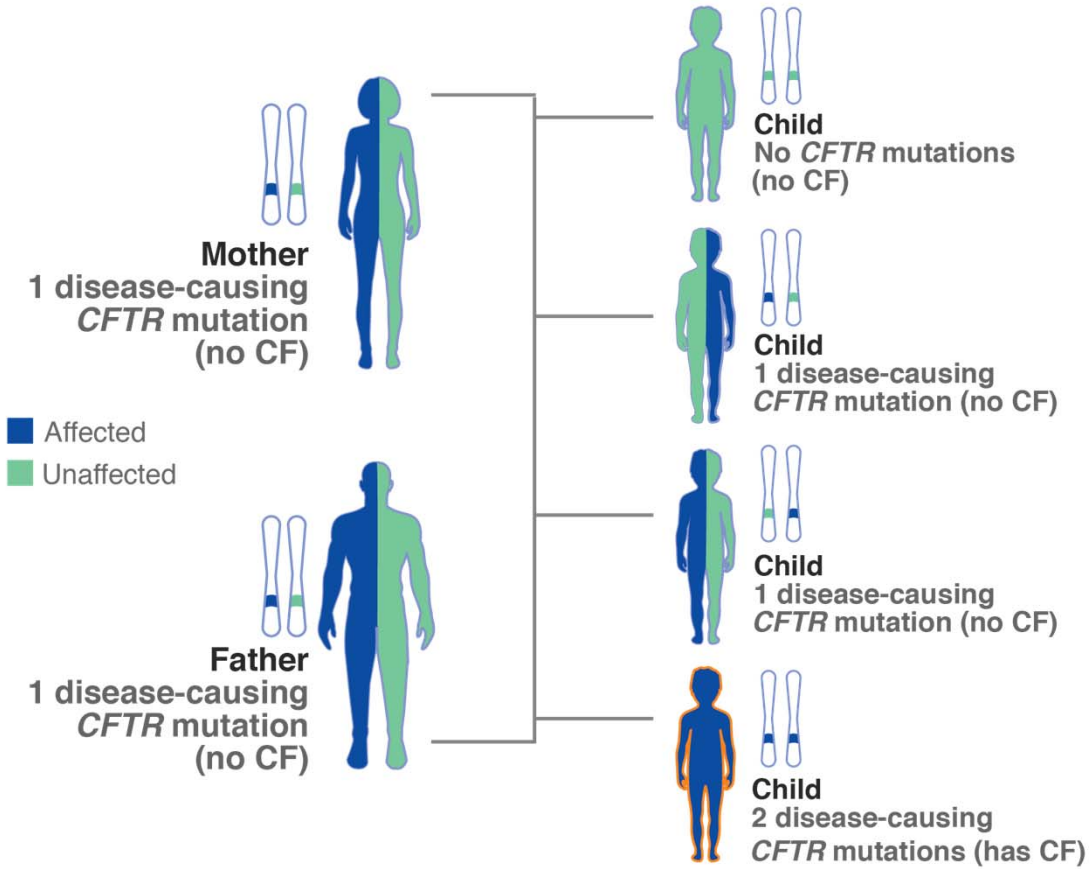
***CFTR* gene expression**

CFTR *mutations may affect different steps of this process to reduce CFTR quantity and/or function and reduce total CFTR protein activity*[1,2]

References: 1. Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **2.** Derichs N. *Eur Respir Rev.* 2013;22(127):58-65.

CFTR MUTATIONS AND THEIR EFFECTS ON CFTR PROTEIN ACTIVITY

CFTR mutations are inherited genetically

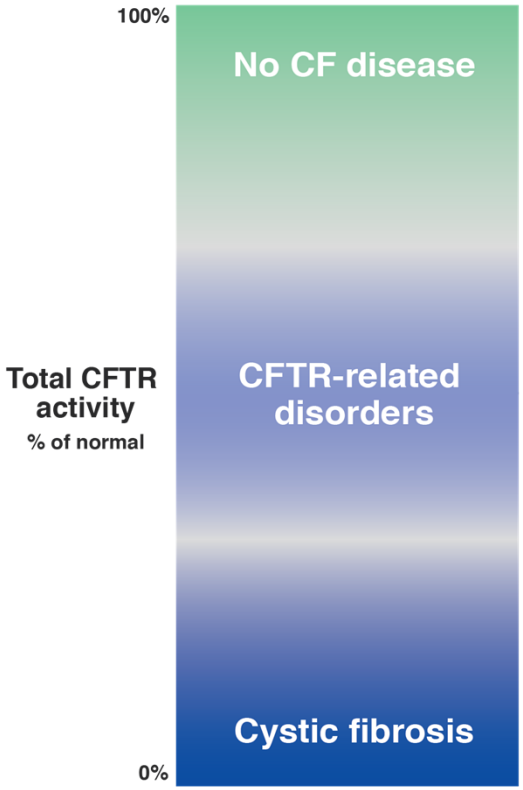


- If a person inherits 2 copies of a disease-causing *CFTR* mutation (one from each parent), CFTR protein dysfunction can occur[1]
  - CFTR protein dysfunction impairs cellular chloride transport
  - CF disease can be the result

Reference: 1. Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163.

CFTR mutations may affect CFTR quantity and/or function, reducing total CFTR activity

Spectrum of phenotypes associated with total CFTR activity[1]



- People with 2 *CFTR* mutations resulting in loss of CFTR activity generally have a CF phenotype, which may include[1,2]
  - Elevated sweat chloride (>60 mmol/L)
  - Pancreatic insufficiency
  - CBAVD
  - Lung function decline over time
  - *Pseudomonas aeruginosa* colonization

Certain *CFTR* mutations result in some CFTR activity[1,2]

Other *CFTR* mutations result in little to no CFTR activity[1,2]

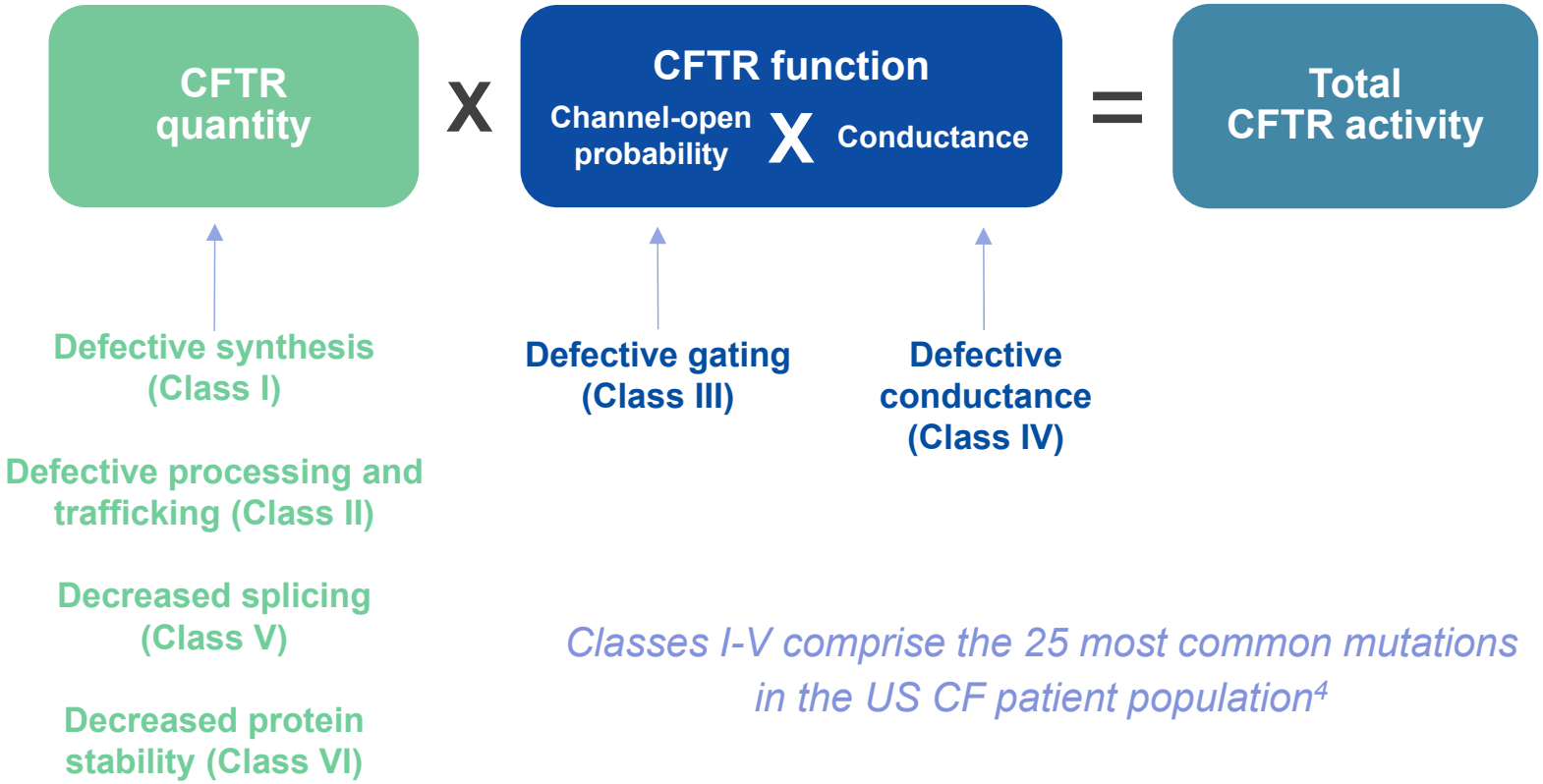
Adapted with permission from Castellani and Assael.

CBAVD, congenital bilateral absence of vas deferens.

References: 1. Castellani C, Assael BM. *Cell Mol Life Sci.* 2017;74:129-140. **2.** Derichs N. *Eur Respir Rev.* 2013;22(127):58-65.

Different *CFTR* mutations produce different effects on CFTR protein quantity and function

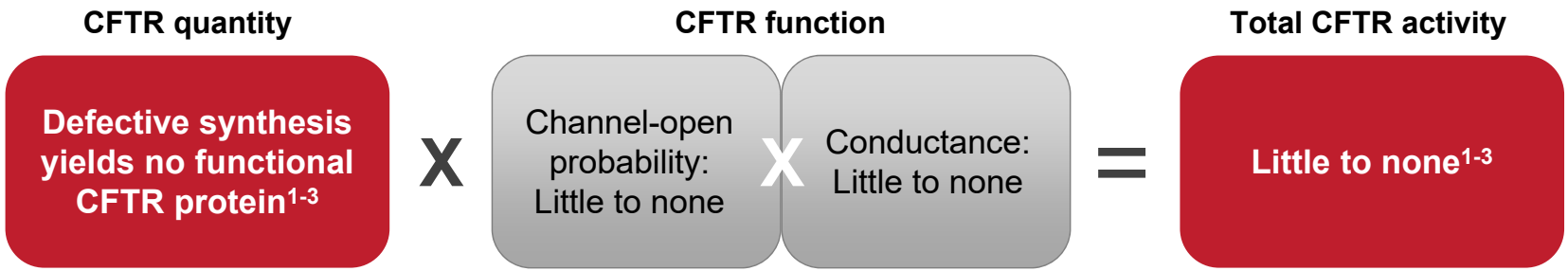
CFTR mutations are grouped into classes according to their effects on CFTR protein synthesis, trafficking, or function[1-3]



Classes I-V comprise the 25 most common mutations in the US CF patient population[4]

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **3.** Zielenski J. *Respiration*. 2000;67(2):117-133. **4.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

Examples of *CFTR* mutations that result in defective biosynthesis of the CFTR protein



An absence of CFTR protein…

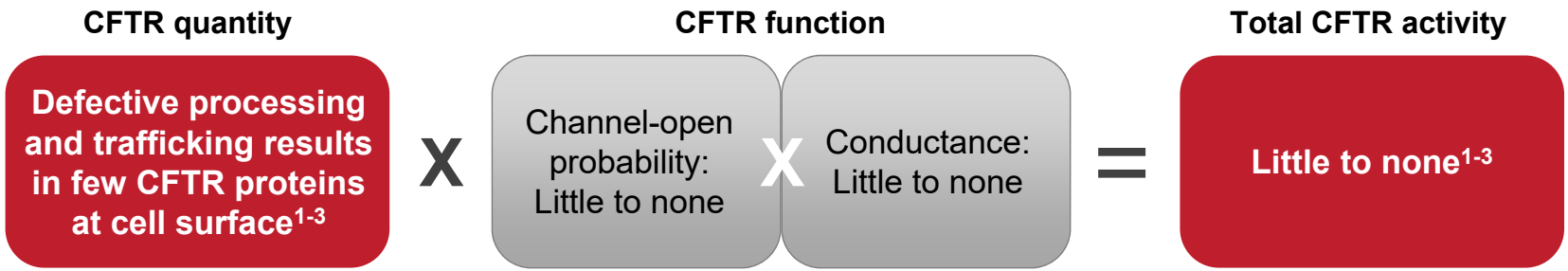
…regardless of function since few to no CFTR proteins reach the surface...

…results in little to no total CFTR activity

Examples (Class I)	Type[4]	US prevalence (%)[5]
G542X	Nonsense	4.7
1717-1G→A	Splice defect	1.6
3659delC	Frameshift	0.7

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **3.** Zielenski J. *Respiration.* 2000;67(2):117-133. **4.** Ogino S et al. *J Mol Diagn.* 2007;9(1):1-6. **5.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

Examples of *CFTR* mutations that result in defective processing and trafficking of the CFTR protein



Significantly reduced CFTR protein…

…regardless of function since few to no CFTR proteins reach the surface...

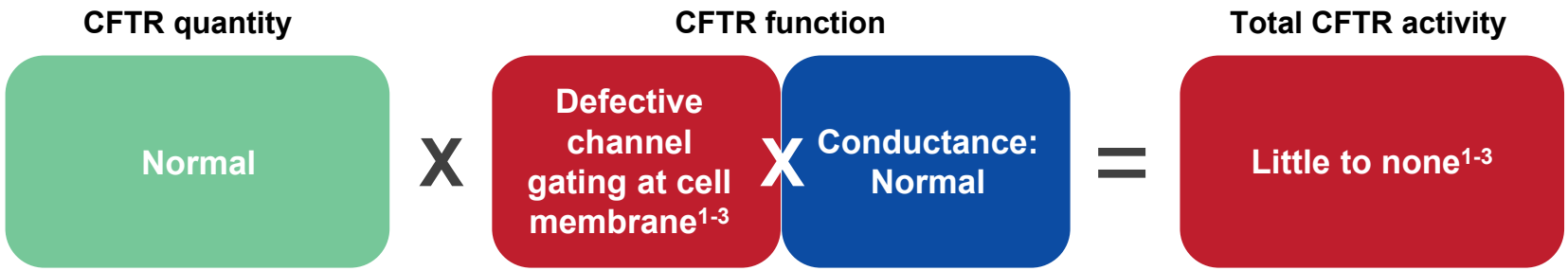
…results in little to no total CFTR activity

Examples (Class II)	Type[4]	US prevalence (%)[5]
F508del	Amino acid deletion	86.4[a]
N1303K	Missense	2.4

[a]Homozygous and heterozygous.

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **2.** Zielenski J. *Respiration.* 2000;67(2):117-133. **4.** Ogino S et al. *J Mol Diagn.* 2007;9(1):1-6. **5.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

Example of *CFTR* mutations that reduce channel-open probability in the CFTR protein



Although CFTR protein quantity may be normal…

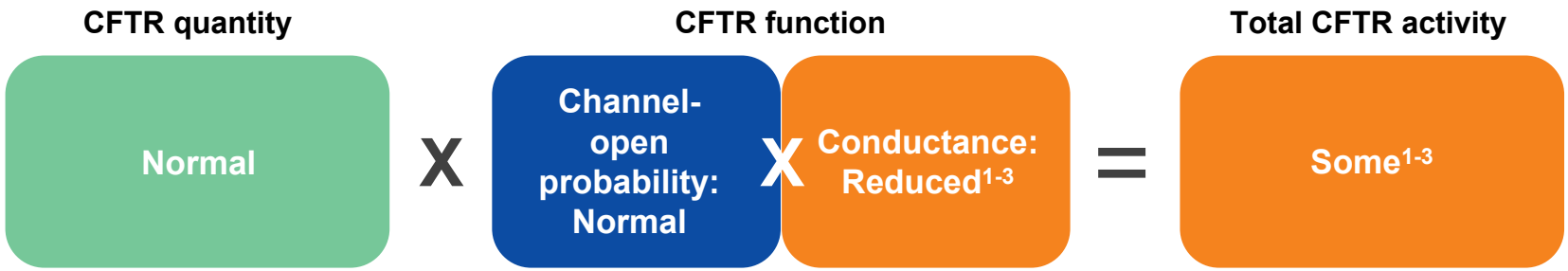
…function is severely reduced due to decreased channel-open probability...

…and results in little to no total CFTR activity

Examples (Class III)	Type[4]	US prevalence (%)[5]
G551D	Missense	4.4

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **3.** Zielenski J. *Respiration.* 2000;67(2):117-133. **4.** Ogino S et al. *J Mol Diagn.* 2007;9(1):1-6. **5.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

Example of *CFTR* mutations that result in a CFTR protein with defective conductance



Although CFTR protein quantity may be normal…

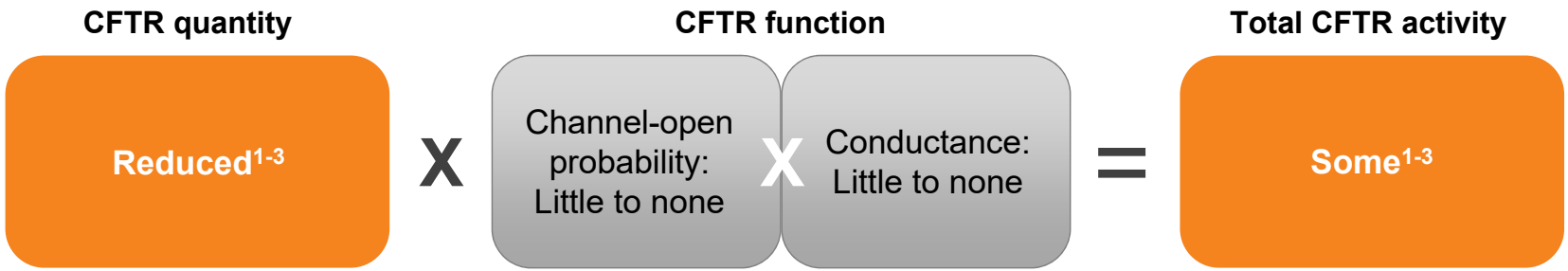
…function is reduced due to decreased conductance...

…and results in some total CFTR activity

Examples (Class IV)	Type[4]	US prevalence (%)[5]
R117H	Missense	3.0

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **3.** Zielenski J. *Respiration.* 2000;67(2):117-133. **4.** Ogino S et al. *J Mol Diagn.* 2007;9(1):1-6. **5.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

Examples of *CFTR* mutations that result in some functional CFTR at the cell surface



CFTR protein quantity is reduced…

…even though CFTR function is not impaired...

…and results in some total CFTR activity

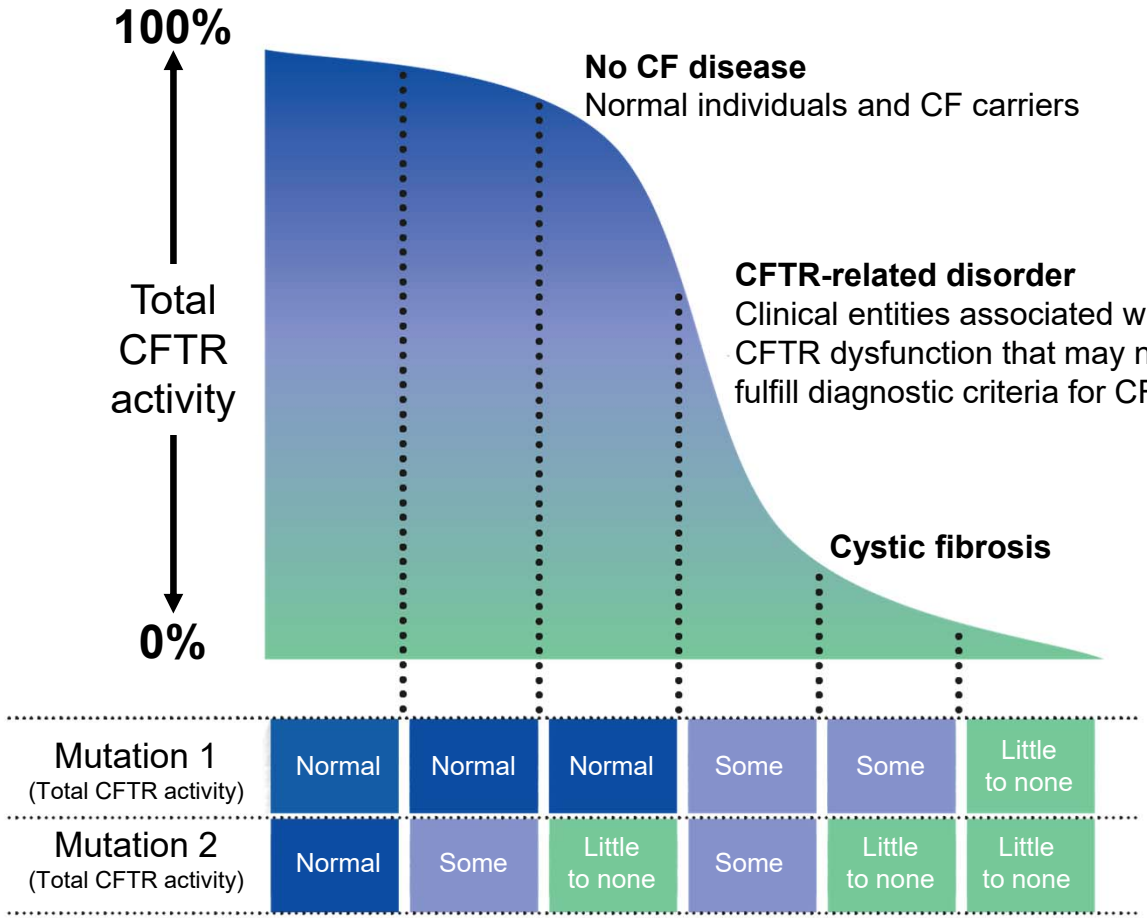
Examples (Class V)	Type[4]	US prevalence (%)[5]
3849+10kbC→T	Splice defect	3.0
A455E	Missense	0.6

References: 1. Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **2.** Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **3.** Zielenski J. *Respiration.* 2000;67(2):117-133. **4.** Ogino S et al. *J Mol Diagn.* 2007;9(1):1-6. **5.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation.

THE IMPACT OF REDUCED CFTR ACTIVITY

Both *CFTR* mutations play a role in determining phenotype and disease severity

As CFTR activity declines, CF development becomes more likely[1]



Adapted with permission from Zielenski.

CFTR genotype affects sweat chloride concentration, an indicator of underlying CFTR activity and a principle diagnostic marker for CF[1]

Normal: No CF-causing mutation.
Some: Classes IV-VI.
Little to none: Classes I-III.

Reference: 1. Zielenski J. *Respiration.* 2000;67(2):117-133.

Summary

- CFTR protein is an important cellular regulator in organs throughout the body[1,2]
  - *CFTR* mutations, which are acquired genetically, decrease CFTR protein function and/or quantity to reduce total CFTR activity
  - Loss of CFTR protein activity is the underlying cause of CF
- Different types of *CFTR* mutations vary in their effects on CFTR protein quantity and/or function, because they affect CFTR protein formation in different ways[2,3]
- Both *CFTR* mutations play a role in determining phenotype or disease severity[4]

References: 1. Boyle MP, De Boeck K. *Lancet Rep Med.* 2013;1:158-163. **2.** Derichs N. *Eur Respir Rev.* 2013;22(127):58-65. **3.** Cystic Fibrosis Foundation. *Patient Registry Annual Data Report 2016*. Bethesda, MD. © 2017 Cystic Fibrosis Foundation. **4.** Zielenski J. *Respiration*. 2000;67(2):117-133.