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Defining Allelic Heterogeneity among Clinically Important Mutations in CFTR

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Abstract

The allelic background upon which CFTR mutations occur has increasingly been viewed as a critical determinant of mRNA utilization, and protein folding. For example, the prevalent M470V polymorphism has recently been shown to modify F508del CFTR biogenesis (Bridges *et. al.*, *Pediatric Pulmonology*, 2013). Although virtually 100% of F508del alleles encode the M variant (likely due to a genetic founder effect and/or "identity descent"), earlier mechanistic and compound library screening efforts have often utilized a V470 background. For other common disease-associated mutations, very little is known regarding allelic context. In the present study, we evaluated more than 75,000 CFTR alleles for which full or partial sequence data is available using the Ambry database, including specific non-coding elements that mediate splice junction fidelity, poly T tracts, and other features. Our long-term plan is to apply available sequence data to help define allelic background for a diversity of CF-associated alleles. The information is vital to developing rational and effective drug discovery programs for patients with less-common CF defects, and will furnish new knowledge regarding intragenic modifiers of cystic fibrosis phenotype and disease severity. Here we provide our initial study of M470V polymorphism relevant to mechanistic studies and of cell line development.

Background

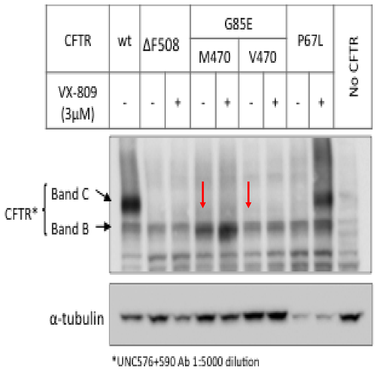
Linkage disequilibrium describing CFTR for a Caucasian ethnicity derived from HapMap (Cordovado et. al., *Mol Genet Metab.*, 2012). Two large haplotype blocks are noted, with genomic recombination evident in non-coding DNA between exons 22 and 23. Block structure of this sort is attributable to a limited number of ancestral founder alleles. DNA variants such as M470V are best explained by *de novo* mutation on a prominent ancestral haplotype. Among the Caucasian ethnicity, for example, the minor variant V470 occurs on the order of 30-40%, depending on the sub-ethnicity being examined.
Note: White and blue regions indicate no LD (LOD<2); shades of pink/red indicate regions of LD (LOD≥2). Intensity of red box color is proportional to the strength of the LD property of the marker pair (Cordovado et. al., *Mol Genet Metab.*, 2012).

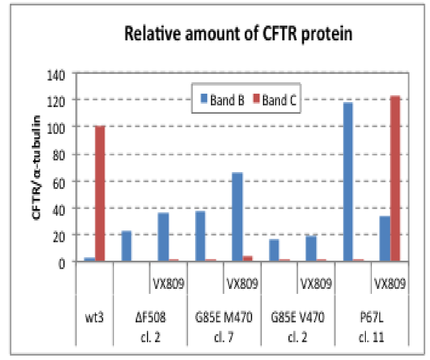
Results

	Legacy Name	nucleotide	domain	mutation class	prevalence (CFTR2)	Cl transport with Ivacaftor (% normal)	FRT flp4	M470V[1]	T854T[2]	comments
1.	wt					100% w/o VX770	yes	M470 or V470		
2.	R31C	91C>T	NT		13	ND		M or V		non CF causing mutation
3.	P67L	200C>T	NT	IV	76	43.4%	yes	M or V (rare)		likely pancreatic sufficient
4.	G85E	254G>A	TM1	II	301	NS	yes	V470	ACT	
5.	E92K	274G>A	TM1	II	12	2.1%	yes	-		
6.	R347H	1040G>A	TM6	IV	91	52%		M or V		pancreatic sufficient
7.	R347P	1040G>C	TM6	IV	224	NS		V470	ACT	Pancreatic insufficient?
8.	A455E	c.1364C>A	NBD1	V	219	17.8%	yes	M470	ACT	
9.	S492F	1475C>T	NBD1	II	16	NS	yes	M or V		pancreatic sufficient
10.	F508del	1521_1523delCTT	NBD1	II	32,834	5.9%	yes	M470	ACT	100% linked to c.869+11T>C (same as c.1001+11C>T)
11.	V520F	1558G>T	NBD1	III	73	NS		M or V		
12.	S549N	1646G>A	NBD1	III	83	99%		mostly M, some V		
13.	S549R	1645A>C or 1647T>G	NBD1	III	42	36%		Cannot be determined		A->C:1 T->G:10
14.	G542X	c.1624G>T	NBD1		1803	ND	yes	M470	ACT	
15.	G551D	c.1652G>A	NBD1	III	1392	55.3%		M470	ACG	
16.	R560T	1679G>C	NBD1	III	196	NS		M470	ACG	
17.	D614G	1841A>G	R		11	ND		M or V		under investigation
18.	L1077P	3230T>C	CL4	III	46	NS		M470		
19.	M1101K	3302T>A	CL4	IV	104	NS		V470		
20.	S1235R	3705T>G	NBD2		54	ND		M or V (rare)		non CF causing mutation
21.	W1282X	c.3846G>A	NBD2		939	NA		M470	ACG	
22.	N1303K	3909C>G	NBD2	II	1194	NS	yes	M470	ACT	

Preliminary analysis of the M470V polymorphism evaluated across a series of CFTR mutations, many of which cause severe clinical disease and are being pursued for personalized medicine- type interventions.

[1]Non-synonymous SNP M470V (A/G): CEU - G(0.513)/A(0.487), TSI – G(0.653)/A(0.347). NS: not significant
ND: not determined





Differential expression of G85E on the M470V background. The G85E processing mutation has been difficult to rescue by pharmacological or low temperature interventions based on results from a number of laboratories and the CFTR Folding Consortium. Because this clinically important mutation appears to occur exclusively on a V470 background , we tested influence of the polymorphism on CFTR expression levels and rescue. FRT "Flip in" stable cell lines encoding the two allels of G85E were generated in which CFTR was inserted into the same genomic locus and expressed similar levels of CFTR mRNA. The data indicates a strong enhancement of CFTR band B in the setting of the M variant, but no difference in susceptibility to correction by VX-809-based correction.

Number of times mutation was observed with other mutations listed														
nucleotide	Legacy Name	Homozygous	Delta508	W1282X	G542X	G551D	N1303K	Homozygous M or V	Observed Chromosomes	Total # of	#M	#V	%M	%V
91C>T	R31C		8		1	1		2	12	9	3	75%	25%	
200C>T	P67L	1	58			3	1		64	62	2	97%	3%	
254G>A	G85E	2	8		2			2	16	0	16	0%	100%	
274G>A	E92K	seen only once												
1040G>A	R347H		11		1			1	13	4	9	31%	69%	
1040G>C	R347P		10			2		3	15	0	15	0%	100%	
c.1364C>A	A455E		10	2			2	7	21	21	0	100%	0%	
1475C>T	S492F		6					5	11	5	6	45%	55%	
1521_1523delCTT	F508del	463		16	63	56	27		1088	1088	0	100%	0%	
1558G>T	V520F		3					3	5	4	1	80%	20%	
1646G>A	S549N		5					15	26	22	4	85%	15%	
1645A>C or 1647T>G	S549R	3	1						3	3	0	100%	0%	
c.1624G>T	G542X	1	72	4					82	82	0	100%	0%	
c.1652G>A	G551D		68	1	1				72	72	0	100%	0%	
1679G>C	R560T		4			1		1	16	16	0	100%	0%	
1841A>G	D614G		5		1				6	1	5	17%	83%	
3230T>C	L1077P	1	11		1		1	1	16	16	0	100%	0%	
3302T>A	M1101K	1	4					2	8	1	7	13%	88%	
3705T>G	S1235R	4	25	3					36	35	1	97%	3%	
c.3846G>A	W1282X	4	19		4	2	1		33	33	0	100%	0%	
3909C>G	N1303K		33	1	1	1			37	37	0	100%	0%	

Incidence of rare CFTR alleles across the Ambry database indicates allelic background polymorphism at position 470 for numerous CFTR mutations. Information of this sort may suggest numerous "hotspots" for CFTR SNP formation. If confirmed by other _____ CFTR sequence compendia, data of this sort should be incorporated into cell models being developed to evaluate rare CFTR defects.

Conclusions

1. Rare CFTR mutations occur on a multitude of allelic backgrounds that will require evaluation in order to properly develop new cystic fibrosis cell lines for these type of analyses.
2. The M470V variant has important impact on CFTR biogenesis for at least 2 clinical contexts (F508del (Bridges *et. al.*, *Pediatric Pulmonology*, 2013) and G85E). Other CF mutations which occur on more than one background will require characterization as shown here.
3. CFTR polymorphisms in addition to M470V may provide new insight regarding both disease mechanisms and therapeutic development, and planned for future study.