Bioinformatics analysis of homologies between pathogen antigens, autoantigens and the CFTR cystic fibrosis protein: A role for immunoadsorption therapy?

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Abstract

The cystic fibrosis CFTR chloride channel is involved in pathogen entry into epithelial cells, and provides the glutathione and hypochlorous acid necessary for bactericidal and viricidal actions. CFTR mutations block these effects, diminishing pathogen defence and allowing pathogen accumulation in the extracellular space, where antibody encounter is likely. The pathogen antigens observed in cystic fibrosis (including P. Aeruginosa, S.Aureus and S.Maltophilia proteins) are homologous to the autoantigens reported in cystic fibrosis and all are homologous to the CFTR protein itself. Antibodies to pathogens and autoantigens may also target the CFTR protein, acting as antagonists, further compromising its function. The tripartite relationship between pathogen antigens, autoantigens and the CFTR protein creates a feed forward cycle, diminishing the function of the CFTR protein and increasing the probability of pathogen accumulation and further antibody encounters at every turn. Kegg pathway analysis of the CFTR/autoantigen interactome indicates that the CFTR protein is also involved in pathogen entry pathways, diabetes and pancreatic and gastric acid secretion pathways, in pathways related to cardiac myopathy, and in the gonadotrophin signalling network, all which are relevant to cystic fibrosis. Interruption of this cycle by antigen and antibody adsorption, and possible by immunosuppressant therapy may perhaps be of clinical benefit in cystic fibrosis.

Introduction.

Cystic fibrosis is a devastating condition caused by mutations in the cystic fibrosis transmembrane conductance regulator CFTR chloride channel. The disease affects many organs resulting in general debilitation but especially targets the respiratory system leading to difficulty in breathing . There is no apparent cure or preventive strategy. The disease appears to have an immune and autoimmune component as antibodies to Saccharomyces cerevisiae and Stenotrophomonas Maltophilia{ and to neutrophil cytoplasmic antigens and bactericidal/permeability-increasing protein (BPI) and many other proteins (the adrenoreceptor ADRB2, Calgranulin, heat shock proteins, mucins, myeloperoxidase, rheumatoid factor and tumour necrosis factor, inter alia are observed in many patients Bae, Choi, et al. 2010 135301 /id}. The disease is also influenced by infection. For example Burkholderia infection causes severe respiratory infections in cystic fibrosis patients and is often associated with this condition (LiPuma, 1998, LiPuma, 1998, Coutinho, 2007). Stenotrophomonas maltophilia infection has also been reported to worsen pulmonary symptoms while infection with S.Aureus or P.Aeruginosa are known to decrease the lifespan of cystic fibrosis patients .

Many bacteria and viruses cause problems by molecular mimicry of human proteins. When homologous to receptors, they may act as decoys, or when homologous to peptide ligands that may act as dummy ligands or decoy substrates. For example the measles virus V protein is a decoy substrate for IkappaB kinase (Pfaller & Conzelmann, 2008). They may also use the host's cognate receptors to gain entry, as is the case with the AIDS virus and the CCR5 or CXCR4 chemokine receptors . When such mimics are antigenic and homologous to host proteins they may cause problems related to autoimmunity. Such mimicry is extensive (Elde & Malik, 2009)and has been observed between Herpes simplex, a risk factor in Alzheimer's disease, and Alzheimer's disease susceptibility gene products (Carter, 2010b), or between the proteins of the Epstein Barr virus or of gut bacterial flora and multiple sclerosis autoantigens (Westall, 2006, Toussirot & Roudier, 2008) .

As reported below, proteins from pathogens implicated in cystic fibrosis, and many others (bacteria, fungi and viruses) are homologous to diverse CFTR mutants. Many of these homologous regions are immunogenic, suggesting an important autoimmune component to cystic fibrosis that may be amenable to therapy.

Methods

Mutant CFTR proteins were identified from the Cystic fibrosis mutation database http://www.genet.sickkids.on.ca/app

A "polymutant" protein was constructed (Fig 1) that included 19 point mutations, and was used for bioinformatics analysis. The sequence of this protein as well as the common DeltaF508 deletion mutation was compared with viral, bacterial and fungal proteins using the NCBI BLAST server . Heptapeptides centred on the point mutation were also screened against viral and bacterial proteomes. Pathogen antigen and autoantigens described in cystic fibrosis were aligned with the delta508 mutant using the Uniprot CLUSTAL alignment server http://www.uniprot.org/. Antigenicity was predicted using the immune epitope database server http://tools.immuneepitope.org/main/index.html. Antigenicity predictions from these

programmes are calculated on the basis of charge, hydrophobicity and surface localisation. B cell antigenicity was determined using the BepiPred linear epitope prediction method {Larsen, Lund, et al. 2006 1725 / (See Table 1 for the predicted antigenicity of individual amino acids) and T cell antigenicity using the Average Relative Binding matrix methods that predicts IC_{50} values for the binding of epitopes to major histocompatibility complex (MHC) molecules . The B cell antigenicity of the native and mutant proteins can be directly compared, as the algorithm defines antigenicity, amino acid by amino acid, along the length of the protein. In contrast, T cell epitopes are referenced as 9 amino acid strings, and each mutation generates a series of epitopes that are distinct from those in the native protein. There are numerous T cell epitopes across multiple HLA-antigens and the native/mutant comparisons were restricted to HLA DRB1*0301, one of the most common alleles . The CFTR interactome was downloaded from the Protein, Signalling, Transcriptional Interactions & Inflammation Networks Gateway (pSTIING) database http://pstiing.licr.org/ and pathway analysis performed at KEGG pathways . http://www.genome.jp/kegg/tool/color_pathway.html . Host proteins interacting with viruses were obtained from the VirusMint database http://mint.bio.uniroma2.it/virusmint/ and from the Herpes/host viral interaction database (Carter, 2010c) http://www.polygenicpathways.co.uk/herpeshost.html The BLAST analyses return a large number of hits to multiple proteins from hundreds of bacterial, viral and fungal species. The CFTR protein is homologous to several proteins from the same species, resulting in a certain number of overall hits per species. These were semi-quantitatively analysed using a tag cloud server at http://www.tagcloud-generator.com/generator.php#anker which generates tags, sized according to the number of hits per species. The tag size was set to a font size of 4 to 30. Because of the large volume of data generated by the BLAST analyses, the original saved BLAST searches and the maps of the KEGG pathway analysis are stocked in an online database at http://www.polygenicpathways.co.uk/cysfib.htm

Results

The immunity spectrum of the CFTR mutants.

The localisation of the mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) that were examined is depicted in Fig 1.

Several of these mutations are in regions of high predicted B-cell antigenicity (R171H, G480C, G551D, S895N, K1250A, N1303K) while others are less so (Fig 2).

The CFTR F508 deletion or point mutations can dramatically change the antigenicity, not only of the amino acid concerned, but also of the surrounding peptide as shown in Fig 2. For example the F508Del mutant markedly increases the predicted B-cell antigenicity over a long stretch of amino acids, not only confined to the deleted amino acid and also generates two T cell epitopes that have a higher affinity (1.5 - 3-fold) than those of the native protein (Fig 3). For the 19 other mutants, B cell antigenicity can be increased, decreased or little changed by the point mutation (Fig 2).

The T cell epitope landscape is dramatically changed by the 19 point mutations as shown in Fig 2. All of these sequences are T cell epitopes and will bind to MHC molecules, although with different affinities. High concentrations of antigens, a likely consequence of the hyper colonisation by pathogens containing these epitopes

(see below), might be expected to saturate all MHC binding sites. 57 T cell epitopes were generated by the polymutant protein, compared to 50 for the native protein. The following epitope changes resulted in large increases in T cell epitope affinity (LSHGHKQLM > LSHDHKQLM (127 fold); ITLSGGQRA > ITLSGDQRA (63 fold); CVLSHGHKQ > CVLSH**D**HKQ (63 fold); FDDMESIPA > FDDMESIRA (6 fold); IAIYLGIGL > IAIYLCIGL (3.9 fold); DMESIPAVT > DMESIRAVT (5.2 fold). Certain epitopes in the native protein are lost due to the mutation (N=27), while others are gained (N=34), many of which were of intermediate T cell affinity (Fig 2).

The ten T cell epitopes (mutant Delta F508 protein) with the highest affinity are homologous to proteins expressed by a number of bacterial species, including S.Aureus. Other noteworthy species containing CFTR epitope homologues included Clostridial species, and Klebsiellae (Table 2, which are known to colonise CF patients (see Table 3). This type of epitope mapping may be of use in identifying novel pathogen suspects that may pose a problem in cystic fibrosis. For example, proteins from B.cereus and Brachyspira species were well represented as CFTR epitope matches (See Table 2).

F508del CFTR homology with autoantigens and pathogen proteins

The F508del mutant protein is homologous to ten autoantigens and four P.Aeruginosa and S.Maltophilia antigens reported in cystic fibrosis. The autoantigens are in turn homologous to proteins from three major pathogens implicated in cystic fibrosis (S.Aureus, P.Aeruginosa and S.Maltophilia) (Table 4), suggesting that the autoantigens are likely to have been created by antibodies that initially targeted the pathogen proteins. The Blast results for this exercise are available at http://www.polygenicpathways.co.uk/cftrpathant.htm

CFTR/pathogen protein homologies

Several viral or bacterial pathogens colonise cystic fibrosis patients to a much greater extent than observed with the normal population . Many of these pathogens have been reported to worsen symptomatology, for example S.Maltophilia and even to decrease the lifespan of infected patients (S.Aureus or P.Aeruginosa) . These effects are summarised in Table 3.

The heptapeptides surrounding the 19 point mutations, or the octapeptide surrounding the F508del mutation, are all homologous to proteins expressed by S.Aureus, P.Aeruginosa or S.Maltophilia, (Table 5) as well as to many other strains (not shown: see website BLASTs) .

The delta508 mutant or the entire polymutant is also homologous to proteins expressed by multiple viral, bacterial and fungal strains, many of which, in particular P.Aeruginosa, S.Aureus and S.Maltophilia, are known to hypercolonise cystic fibrosis patients or to be associated with symptom exacerbation (Table 6). This survey also identified many other pathogens expressing proteins with CFTR homology, which might perhaps be considered as potential antibiotic targets. These included B.Cereus, Gordonia bronchialis and several clostridial species (Table 6).

The CFTR protein, mutated or not, contains a large number of T cell epitopes (82,376: vs. various MHC alleles) of which 1303 were of high affinity (< 100nM). The pathogen protein homology with the 10 highest affinity epitopes is shown in Table 7. Numerous pathogen species are represented, with P.Aeruginosa. S.Aureus and S.Maltophilia figuring highly as pathogens expressing proteins with homology to theses CFTR epitopes.

P.Aeruginosa and S.Aureus vatches in the mutant CFTR protein

Vatches (Viral mATCHES are short contiguous amino acid stretches covering the entire human proteome that are identical in human, and viral proteins and also in the proteins of other pathogens (see http://www.polygenicpathways.co.uk/blasts.htm . They are a probable legacy of our evolutionary decent from microorganisms, and of pathogen mimicry of human proteins: Despite chromosomal shuffling over millions of years, the current human DNA can still encode for quite large peptide stretches that are identical to those expressed by pathogen proteins (Carter, 2010d, Carter, 2010a, Carter, 2010b). The S.Aureus and P.Aeruginosa vatches within the CFTR polymutant are shown in Fig 4. The CFTR polymutant displays extensive homology with proteins expressed by these two pathogens. The homologous regions are often within highly immunogenic regions of the CFTR and pathogen proteins, and also cover the CFTR point mutations.

Homology with the native CFTR protein

As the mutations in cystic fibrosis are point mutations, the native protein too is evidently homologous to these same pathogen proteins. However, the pathogen riddance pathways are intact in these cases, and the immune system is not compromised by CFTR mutations. There is no reason to suppose that high levels of pathogen proteins could be attained, or that the host could not appropriately deal with the pathogens . Whether the CFTR mutations increase or decrease homology to pathogens is also perhaps irrelevant, as the hyper colonisation by pathogens would be an expected consequence of any functional mutation (see discussion); an outcome that would favour antibody production that could target any CFTR matching epitopes. As antibodies are able to enter cells , such targeting could be relevant to domains in both the intracellular and extracellular portions of the CFTR protein.

Pathway analysis of the CFTR interactome (Fig 5)

Pathway analysis of protein interaction networks is a powerful tool for divining the functions of particular proteins. Those proteins shown to interact with the CFTR protein, from pSTIING, are shown in Table 8.

Pathway analysis of the CFTR interactome (Table 9) also included the autoantigens reported in cystic fibrosis, as their function is also likely to be compromised by their respective autoantibodies. This pathway analysis clearly demonstrates an important role for the CFTR protein in the immune system and in pathogen invasion (Table 9:Fig 5 See http://www.polygenicpathways.co.uk/cysfib.htm for coloured KEGG pathways). For example, a number of CFTR binding partners are involved in antigen processing or

chemokine signalling and in lysosomal function which is also related to antigen processing and pathogen destruction ,as well as in chemokine signalling, while others are involved in bacterial invasion and Vibrio infection or pathogen destruction (endocytosis, junctions, phagosomes and lysosomes).These pathways are illustrated in Fig 5.

Interaction with viruses in the CFTR interactome.

The virusMINT and HSV-1 interactions showed that a number of the CFTR interacting proteins also interact with viral proteins from the adenovirus and papillomavirus as well as the Epstein-Barr, Herpes simplex, Hepatitis B and C and HIV-1 viruses (Table 8) , all of which also express proteins with homology to the CFTR protein (Table 7) . In other words, certain viral proteins with homology to CFTR may bind to the same targets as the CFTR protein and, when present, could form an integral part of the CFTR interactome. With the exception of a replete HIV-1 interaction database, viral/human protein networks are not extensively referenced in online databases, and more interactions are likely to exist.

Certain of the CFTR interactome pathways trace out a route that is used by the Herpes simplex virus, and probably other related viruses, during its life cycle. This involves entry and endocytosis, entry and exit to and from lysosomes , phagosomes and nuclei, and interference with protein processing pathways (see http://www.polygenicpathways.co.uk/herpeshost.html for a detailed view). These pathways suggest that the CFTR protein is involved in both bacterial and viral defence (Fig 5).

The pancreas, cardiac myopathy and the vas deferens in cystic fibrosis

Pancreatic insufficiency and diabetes are common features of cystic fibrosis as are cardiac myopathy and related cardiovascular problems (Moss, 1982). Bilateral loss of the vas deferens in men, or of the uterus and vagina in women are also commonly associated with cystic fibrosis . The CFTR/autoantigen pathway analysis indicates that the CFTR protein is involved in pancreatic and gastric acid secretion pathways, in several pathways related to cardiac myopathy, and in the gonadotrophin signalling network, which latter controls the development of the sexual organs. The autoantigens implicated in cystic fibrosis are also members of a signalling network related to diabetes (Table 8; Fig 5). These pathways relate to all of the coexisting conditions described above. The involvement of the CFTR protein in these signalling networks indicates that these associated conditions are a direct result of defects in CFTR signalling.

Immune related genes that modify cystic fibrosis symptomatology or pathogen colonisation

Many genes that modify the progression or severity of the cystic fibrosis are related to immune function. These include inflammation related genes (interleukins IL1B, IL8 and IL10, transforming growth factor-beta1, tumour necrosis factor-alpha and its receptor TNFR) antioxidant related genes (glutathione-S-transferase),

prostaglandin-endoperoxide synthase genes (COX1 and COX2) as well as CD95, Toll receptor TLR9, T cell receptor beta and HLA antigens .Immune activation and inflammation also play a key role in the airways in cystic fibrosis (Machen, 2006b) There are a large number of MHC molecules, each of which has differing affinity for distinct epitopes. HLA-DR2, (which recognises HLA-DRB1*15 and HLA-DRB1*16 alleles) , as well as HLA-DQB1*0201, HLA-DRB1*0301, and DR7/ DQA*0201 and HLA-B-18 have all been associated with cystic fibrosis symptomatology or pathogen colonisation .

Discussion

Nearly 2,000 mutations/polymorphisms have been described in cystic fibrosis patients. The most common is the DeltaF508 deletion which is expressed in almost 70% of patients and the G551D, G542X, and R553X mutations are also relatively common . 20 different mutations were covered by this survey. Several mutations, particularly truncations, result in non-expression of the CFTR protein or compromised delivery to the cell surface (Davidson & Porteous, 1998). The bacterial and viral homology is of less direct relevance to these mutants, although defects in the immune and microbial related functions of the CFTR protein would also favour pathogen colonisation and immune dysfunction. These and other mutant proteins result in malfunction of the chloride channel encoded by the CFTR protein, with the resultant pulmonary pathology associated with cystic fibrosis.

In addition to its actions as a chloride channel, CFTR has a number of other properties that are highly relevant to immunity and microbiology. For example it controls the efflux of glutathione which exerts viricidal and bactericidal properties, including the S.Aureus and P.Aeruginosa targets . Glutathione levels are reduced in cystic fibrosis and glutathione aerosols have been reported to ameliorate lung epithelia oxidative stress in cystic fibrosis patients . Clinical trials with glutathione or its prodrugs are ongoing . CFTR is also important in pathogen defence, providing the chloride for the generation of hypochlorous acid by myeloperoxidase in neutrophil phagosomes. This bactericidal mechanism is defective in cystic fibrosis , likely rendered the more so by the presence of myeloperoxidase autoantibodies in cystic fibrosis .

The CFTR protein is also expressed in lymphocytes and negatively regulates the nuclear factor kappa beta (NFKB) and toll receptor (TLR4) mediated innate immune response . The delta F508 mutation has also been shown to inhibit the antigen presentation pathway (Hampton & Stanton, 2010), and autoantigens and other antigens in cystic fibrosis would therefore not be properly processed. CFTR mutations also increase immune activation in mice .

In addition to these effects, CFTR is a pattern recognition receptor that recognises P.Aeruginosa . The CFTR protein appears to be involved in P.Aeruginosa ingestion and destruction, as the delta508 mutation in infected transgenic mice increases the pulmonary P.Aeruginosa burden and decreases its clearance. This mutation-related reduced uptake of the pathogen into epithelial cells favours multiplication of P.Aeruginosa within the lungs , The CFTR protein is also an entry portal for Chlamydia Trachomatis, and Salmonella Typhi, but not the closely related murine S. typhimurium and the delta508 mutation also reduces pathogen entry into epithelial cells . C.Trachomatis binding to CFTR also reduces its chloride channel activity . Not all bacteria use the CFTR protein which may itself thus determine which bacteria are

most likely to be present in large quantities. The Kegg pathway analysis of the CFTR binding proteins also revealed a key role in immunity and in pathogen entry and/or elimination.

Thus the CFTR mutations might be expected to compromise not only the chloride channel, but also the ability to kill pathogens via glutathione, or hypochlorous acid. Mutations might also be expected to alter the ability to process antigens to pathogens, or to self. CFTR mutations also activate the immune system.

Many of the mutations in the CFTR protein lie within regions that are highly immunogenic, and such high immunogenicity would be shared by the viral, bacterial and fungal homologues of the protein, of which there are several thousand. The autoantigens reported in cystic fibrosis, as well as P.Aeruginosa antigens are also homologous to the Delta508 mutant protein, again within regions that are highly immunogenic. Given the vast number of pathogen proteins that show homology with various regions of the CFTR protein, and the fact that such species are more abundant in cystic fibrosis patients, cross-reactivity with the CFTR protein would seem inevitable, although to date no antibodies to CFTR have been reported or apparently assessed. Although many of the CFTR mutations are intracellular, antibodies do enter cells , and even if not mounting an intracellular immune response would be expected to bind to the immunogenic regions of the CFTR protein, in effect producing protein knockdown, equivalent to the effects of the truncated mutants that fail to reach the cell surface. It is also clear that the viral homologues of the CFTR protein are capable of binding to CFTR binding partners, potentially modifying the function of CFTR by interactome interference.

Infliximab

Infliximab is a tumour necrosis factor -alpha (TNF) monoclonal antibody used to treat autoimmune disorders. TNF antagonism prevents the activation of other inflammatory cytokines and leukocyte activation and this approach is a target in many autoimmune and inflammatory conditions (Hoffman, 2009). A recent case study has reported 2 year remission in a cystic fibrosis patient treated with infliximab . Apart from the use of immunosuppressants in cystic fibrosis lung transplant patients, and limited studies with cyclosporine, the therapeutic potential of this class of drug does not appear to have been widely studied . TNF is one of the autoantigens reported in cystic fibrosis , and shares sequence similarities with the CFTR protein (Table 2). Although certain TNF antibodies would be expected to cross-react with the CFTR protein, such effects would depend upon the epitopes targeted by the antibody, and these details are not available.

A possible scenario for cystic fibrosis (Fig 6)

Irrespective of any homology to pathogens, CFTR mutations lead to defects in chloride channel function, but also to a reduction in glutathione levels and defects in hypochlorous acid production, that would compromise viral and bacterial destruction. The channel itself is involved in bacterial entry, and impaired CFTR function reduces bacterial entry into epithelial cells, resulting in increased colonisation of the extracellular milieu. In this space, the likelihood of encountering immunocompetent cells is increased, favouring the production of anti-pathogen antibodies. Pathogen binding to the CFTR channel also impairs its function. Such mutations may also compromise the immune system, rendering it less able to process antigens, but more

susceptible to activation. Polymorphisms in immune, inflammation and glutathione related genes fine tune this network, modifying its function, for better or worse.

Upon infection, the surfeit of pathogens triggers an immune response that generates antibodies to the pathogen that also target human proteins that are homologous to the antigenic pathogen proteins, generating the autoantigens observed in cystic fibrosis. As judged by epitope homology, antibodies to pathogen proteins and to autoantigens may also tag the CFTR protein, rendering it incapable of assuming its normal functions. The constant presence of the pathogens and of the autoantigens sustains this immune response. Viral infections, in particular, would also be expected to modify CFTR function via the theft of interactome partners. Thus, antibody knockdown would have the same effect on CFTR function as the mutations that prevent CFTR expression, or its delivery to the cell surface. In these cases, the antibodies are acting as antagonists, rather than as immune activators. In extreme cases, an autoimmune response to the CFTR protein might be expected to damage, or kill the cells in which the protein resides. The bioinformatics analysis suggests that antibodies to the CFTR protein should be detectable in cystic fibrosis. This does not appear to have been assessed, judging from the absence of any mention of CFTR autoantibodies in the literature. However, the high titre of pathogen antibodies, whose antigen targets are homologous to the CFTR protein, suggests that even low affinity T cell epitope binding sites would be saturated.

Taken together, although clearly a genetic disorder, these data suggest that cystic fibrosis has a crucial autoimmune component, triggered by pathogens with homology to the mutant and related proteins.

Antibacterial agents are already used in cystic fibrosis (Wat, 2003). There are no phage or bacterial vaccines as yet, and antiviral agents and vaccination strategies could also perhaps be useful. Unfortunately, the repertoire of pathogens colonising cystic fibrosis patients is so vast that polypharmacy, with its attendant risks, might seem the only plausible option. Clearly the potential benefits of glutathione supplementation appear to be promising . Other methods of enhancing pathogen defence require further research.

It is possible that immunosuppression might be of benefit in cystic fibrosis. This is extremely counter-intuitive, given the problems of multiple infections in these patients, but a carefully controlled and supervised clinical trial may well be warranted. Indeed, the reported benefits of Infliximab (see above) , although only so far reported in a cse study suggest that such approaches may be of more general clinical use.

If the problems in cystic fibrosis stem even partly from autoantigens and autoantibodies, then their riddance can only be beneficial. Immunoadsorption/plasma exchange has been reported to be of benefit in the autoimmune disorder, myasthenia gravis and this type of therapy may be applicable to cystic fibrosis, using targeted antigen and antibody columns to remove the circulating antibodies and antigens. Tryptophan or phenylalanine columns have also been reported to be of use in antibody adsorption .

In summary, CFTR mutations are themselves responsible for bacterial hypercolonisation, and for reduced bactericidal and viricidal effects, creating a situation where antibody generation to a plethora of pathogens in inevitable. These antibodies target other antigens that are homologous to the pathogens' proteins, and these include the various autoantigens that have been recorded in cystic fibrosis. The pathogen antigens and autoantigens are both homologous to the CFTR protein itself, and antibody related CFTR antagonism is a likely consequence of these effects. Interruption of this feed forward cycle may be of clinical benefit in cystic fibrosis.

Table 1: The antigenicity index (B-cell epitope) for single amino acids defined by the BepiPred server . The top 6 scoring amino acids are marked in red in other tables

Symbol	Amino acid	B-epitope antigenicity
P	Proline	0.145
G	Glycine	0.035
D	Aspartate	0.018
E	Glutamate	0.003
S	Serine	-0.008
T	Threonine	-0.011
Q	Glutamine	-0.012
N	Asparagine	-0.013
A	Alanine	-0.024
W	Tryptophan	-0.025
K	Lysine	-0.031
R	Arginine	-0.062
H	Histidine	-0.071
V	Valine	-0.112
F	Phenylalanine	-0.138
I	Isoleucine	-0.138
M	Methionine	-0.138
C	Cysteine	-0.175

Table 2:
T cell epitopes of the F508del mutant and their homologies in relation to bacterial and viral proteins. Genera or individual species known to colonise the airways in cystic fibrosis are highlighted in bold.

Allele	Epitope	IC50 nM	Equivalent Pathogen sequence and pathogens
HLA A*0250	TIKENIIGV	3.5	• TIKENIIG: Anaerococcus prevotii: Chryseobacterium gleum;**Prevotella** copri • TIKEFIIGV: Bacillus Cereus • TIKENIFIG: **Staphylococcus** lentus
HLA A*0211		28.4	
HLA A*0203		57.9	
HLA A*0212		98.8	
HLA A*0250	IKENIIGVS	8.8	• IKENII-VS: **Bacteroides** ovatus • IKEVNIIGV: Filifactor alocis • KENIIGIVS: Brachyspira pilosicoli • KEQNIIGVS: Clostridium perfringens
HLA A*0250	IGVSYDEYRI	44.4	• GISYDEYR: Brachyspira pilosicoli • IGVSY-EYR: **Prevotella** marshii • IGDSYDEYR: Acinetobacter calcoaceticus • IIGVSIYDE: Coraliomargarita akajimensis • IIGVSYMDE Brevibacillus brevis • IIGVSCYDE Xanthomonas campestris • IIGVSYTDE: Burkholderia phage
HLA A*6801		61.7	
HLA B*1503	KENIIGVSY	45.9	• KENIIPVSY: Shewanella frigidimarina • KENIIGIS :Clostridium botulinum • KENDIIGVS: **Clostridium difficile** • KEQNIIGVS Clostridium Perfringens • KENIIGIVS:Brachyspira pilosicoli • NIIGVS: **Staphylococcus aureus** • KENIIG: **Staphylococcus aureus**
HLA A*3201		63.5	
HLA A*0250	NIIGVSYDE	62.6	• IIGVSYD: Pantoea sp AND Cellulomonas flavigena and Klebsiella sp.AND Cronobacter turicensis and Sorangium

			cellulosum AND Enterobacter sakazakii AND Buchnera aphidicola AND Pelobacter propionicus • NIIGVSY: Clostridium acetobutylicum
HLA B*1503		76.6	• GVSYDEY: Sulfurimonas autotrophica AND Eubacterium cylindroide • IIGVSYD: Pantoea sp AND Cellulomonas flavigena and Klebsiella sp.AND Cronobacter turicensis and Sorangium cellulosum AND Enterobacter sakazakii AND Buchnera aphidicola AND Pelobacter propionicus

Table 3 A summary of some of the pathogen species isolated from cystic fibrosis patients and their effects on disease.

Bacteria	Colonisation and effects on symptoms
Achromobacter. xylosoxidans	Prevalent in CF patients
Acinetobacter baumanii	Isolated from Russian children with CF
Burkholderia Cepacia	Associated with cystic fibrosis
Chlamydia pneumoniae	Associated with exacerbation of symptoms
Clostridium difficile	Increased in CF patients
Corynebacterium pseudodiphtheriticum	Isolated from CF children's sputum
Haemophilus influenzae	Often recorded in CF sputum
Helicobacter pylori	Increased in patients with pancreatic sufficiency : Certain Mutations protect against H.Pylori infection in patients with pancreatic insufficiency
Klebsiella species	Increase in CF patients
Multiple strains of Mycobacteria	UK case report (Brown, 2010)
Pneumocystis jirovecii	Isolated from French children with CF
Prevotella species	Isolated from the airways of CF patients
Pseudomonas Aeruginosa	Infections decrease the life expectancy of CF patients
Staphylococcus Aureus	
Stenotrophomonas maltophilia	Associated with worsened clinical status
Streptococcus Millerii	Isolated from CF airways
Pseudomonadaceae, Xanthomonadaceae, Moraxellaceae and Enterobacteriaceae	These species are prevalent in the airways of cystic fibrosis patients
Others	Over 60 bacterial genera, not typically associated with cystic fibrosis were isolated from the sputum of CF patients including species of :- Actinobacillus, Aggregatibacter, Chryseomonas, Flavimonas, Haemophilus, Pseudomonas, Strenotrophomonas, Vibrio, Acidovorax, Azonexus, Comomonas, Delftia, Eikenella, Kingella, Neisseria, Brevundimonas, Spingobium, Sphingopyxis, Xanthobacter, Abiotrophia, Enterococcus, Gemella, Granulicatella, Lactobacillus, Lactococcus, Leuconostoc, Staphylococcus, Streptococcus, Butyrovibrio, Catonella, Dialister, Megasphaera, Moryella, Oribavterium, Peptoniphilus, Peptostyreptococcus, Selenomonas, Veillonella, Bulleida, Fusobacterium, Leptotrichia, Actinomyces, Arthrobacter, Atopobium, Corynebacterium, Micrococcus, Propionibacterium, Rhodococcus, Rothia, Scardovia, Tessaracoccus, Bacteroides, Porphyromonas, Prevotella, Bergeyella, Capnocytophage, Mycoplasma, treponema

Viruses	
Epstein-Barr	Infection can exacerbate respiratory symptoms (Winnie & Cowan, 1992)
Herpes simplex HSV-1	Association has been observed but appears to be rare
Cytomegalovirus (Herpesvirus 5)	Infection is a consistent problem in lung transplant CF patients
Hepatitis B	Occasionally observed in CF patients
Hepatitis C	Increased in CF patients
Influenza	Infection worsens symptoms (Dharmaraj & Smyth, 2009)
Respiratory syncytial virus	Increased in CF children
Rhinovirus	Rhinoviruses (common cold virus) exacerbate CF symptoms (Brownlee & Turner, 2008)
Fungi	
Candida and Aspergillus species; Scedosporium apiospermum and Exophiala dermatitidis	Isolated from the respiratory tract of CF patients (Muller & Seidler, 2010)

Table 4 Clustal alignment of the autoantigens or of the antigens to P.Aeruginosa and S.Maltophilia recorded in cystic fibrosis, with the Delta505F mutant. The top 6 high scoring immunogenic amino acids (B cell epitope) are marked in red. * = identical : = conserved . = semi-conserved: The autoantigen sequences were subsequently compared with S.Aureus (S.Aur) , S.Maltophilia (S.Malt) and P.Aeruginosa (P.Aer) proteins, as shown below the Clustal alignments. These alignments are shown bythe boxed regions or by double underlined regions in the autoantigen sequences. Original Lineups are at http://www.polygenicpathways.co.uk/cftrpathant.htm

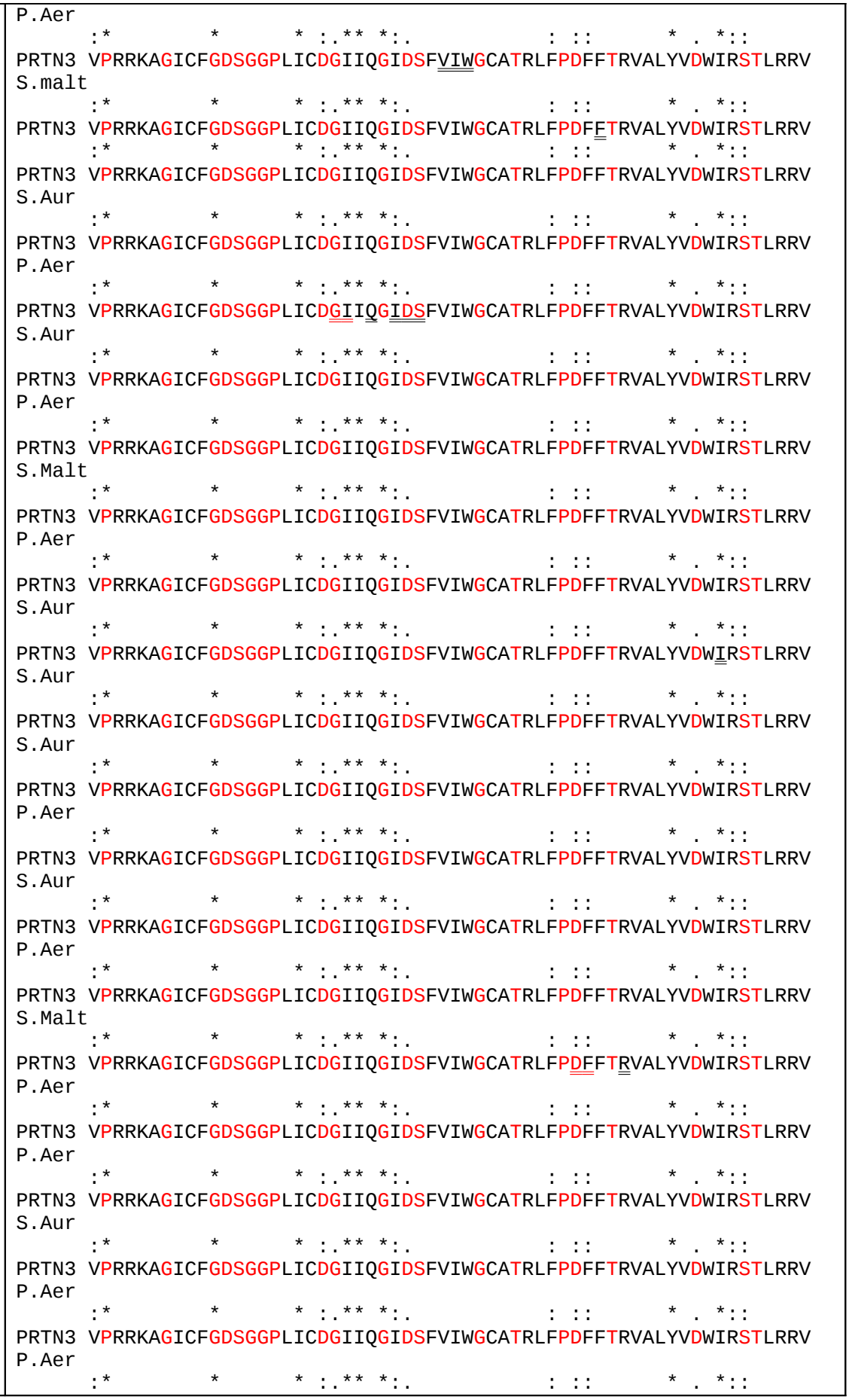
Antigen	CFTR Delta508F/antigen/pathogen alignment
Adrenergic beta receptor 2 (Fraser & Venter, 1982)	CFTR MPGTIKEN-IIGVS-Y----DEYRYRSVIKA- 25 ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 P.Aer :..:*: :*.*. * . ::*:*::. ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 S.Aur :..:*: :*.*. * . ::*:*::. ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 S.Aur :..:*: :*.*. * . ::*:*::. ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 S.Aur :..:*: :*.*. * . ::*:*::. ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 S.Malt :..:*: :*.*. * . ::*:*::. ADRB2 VTASIETLCVIAVDRYFAITSPFKYQSLLTKN 32 S.Aur :..:*: :*.*. * . ::*:*::.
Bactericidal/permeability-increasing protein BPI	CFTR MPG-TIKENIIGVSYD----------EYR------YRSVIKA 25 BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 S.Aur ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 S.Malt ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 S.Aur ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 P.Malt ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 P.Malt ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 P.Aer ** .:: . *:.* * : * . :* BPI NPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKI 42 S.Aur ** .:: . *:.* * : * . :*
Calgranulin B (S100A9)	CFTR MP---GTIKENIIGVS--YDEY------------RYRSVIKA 25 S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 P.Aer *. . ::.** : :.:* .::.::: S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur *. . ::.** : :.:* .::.::: S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 P.Aer *. . ::.** : :.:* .::.::: S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur *. . ::.** : :.:* .::.::: S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur *. . ::.** : :.:* .::.::: S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur *. . ::.** : :.:* .::.:::

	S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Malt S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 P.Aer S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 P.Aer S100A9 MTCKMSQLERNIETIINTFHQYSVKLGHPDTLNQGEFKELVRK 43 S.Aur
Glutamate decarboxylase GAD2 (GAD65)	CFTR MP---G-----TIKENIIGV-------SYD------EYRYRSVIKA 25 GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Malt GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 P.Aer GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 P.Aer GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 P.Aer GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 P.Aer GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur Bix GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.malt GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.malt GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Malt GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 P.Aer GAD2 LPACDGERPTLAFLQDVMNILLQYVVKSFDRSTKVIDFHYPNELLQ 46 S.Aur
Heat shock protein 60	CFTR MP----------G-TIKENIIG----VSYDEYR--YRSVIKA 25 HSPD1 IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aur HSPD1 IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer HSPD1 IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer HSPD1 IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aur HSPD1 IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aur

	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aur
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Malt
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Malt
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Malt
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aur
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Malt
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 P.Aer
		:* * * *:*: *.** : .:::
	HSPD1	IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEK 43 S.Aer
		:* * * *:*: *.** : .:::
Mucin 1 (tracheal)	CFTR	MPG--------TIKENIIGVS-----YDEY------RYRSVIKA 25
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Malt
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Malt
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Malt
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Malt
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer
		** :::*. *.* :::* **. .*.
	MUC1	RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 P.Aer

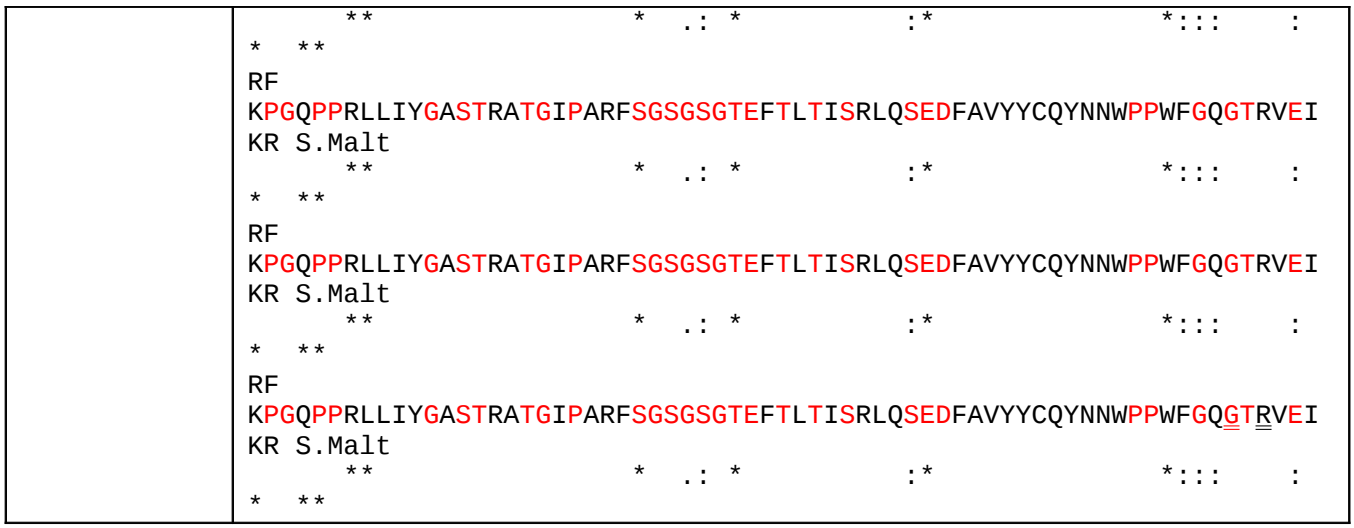
	** :::*. *.* :::* **. .*. MUC1 RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur ** :::*. *.* :::* **. .*. MUC1 RPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISD 44 S.Aur ** :::*. *.* :::* **. .*.
Myeloperoxidase MPO	CFTR MP------GTIKENIIGVSYDEYRY-RSVIKA 25 MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRS**YNDS**VDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Aur :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 P.Aer

	:* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:. MPO LPTYRSYNDSVDPRIANVFTNAFRYGHTLIQP 32 S.Malt :* .::. .* .* : :** :::*:.
Proteinase 3	CFTR MP--------GT-----IKENII-GVS----------YDEYR-----YRSVIKA----- 25 PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Malt :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Malt :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Malt :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Aur :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Aur :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Malt :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Malt :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV P.Aer :* * * :.** *:. : :: * . *:: PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV



	PRTN3 VPRRKAGICFGDSGGPLICDGIIQGIDSFVIWGCATRLFPDFFTRVALYVDWIRSTLRRV S.Aur :* * * :.** *:. : :: * . *::
Rheumatoid factor	CFTR MPG---------------TIKENIIG----------VS--------------YDEYR--- Y---RSVIKA RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Malt ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Malt ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDF AVYYCQYNNWPPWFGQGTRVEIKR S.Aur ** * .: * :* *::: : * ** RF

	KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Malt ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Malt ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR S.Aur ** * .: * :* *::: : * ** RF KPGQPPRLLIYGASTRATGIPARFSGSGSGTEFTLTISRLQSEDFAVYYCQYNNWPPWFGQGTRVEI KR P.Aer



1
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4

Table 5 : Proteins from S.Aureus, P.Aeruginosa or S.Maltophiulia, that contain regions homologous to the regions surrounding vartious CFTR mutants. The position of the mutant amino acid is shown in red within the sequences used for BLAST analysis.

Mutant	Pathogen protein homologue
F508Del ENII~~F~~GVSY Del = ENIIGVSY	• >gb|ADI98793.1| probable regulatory protein DeoR family [Staphylococcus aureus subsp. aureus ED133]8 ENII +SY • GENE ID: 3237744 SACOL0921 | CBS domain-containing protein [Staphylococcus aureus subsp. aureus COL] +NIIGV • GENE ID: 6476997 Smal_2508 | hypothetical protein Stenotrophomonas maltophilia R551-3] +IIGV Y • >ref|ZP_01368311.1| hypothetical protein PaerPA_01005469 [Pseudomonas aeruginosa PACS2] EN+IGV
R74W NALWRCF	• >ref|ZP_06881604.1| adenylate cyclase [Pseudomonas aeruginosa PAb1] NALWR • GENE ID: 6477391 Smal_2892 | tRNA(Ile)-lysidine synthetase [Stenotrophomonas maltophilia R551-3] LWRC • GENE ID: 3793024 SAB1831c | hypothetical protein [Staphylococcus aureus RF122] NA WRC
R117H KEEHSIA	• >gb|ACD39272.1| hypothetical protein PACL_0484 [Pseudomonas aeruginosa] EEH IA • gb|EFM07570.1| staphylococcal accessory regulator U [Staphylococcus aureus subsp. aureus ATCC BAA-39] K EHSI • GENE ID: 5759828 pEDINA_p19 | hypothetical protein [Staphylococcus aureus] KEEH • GENE ID: 6476459 Smal_3331 | threonine dehydratase [Stenotrophomonas maltophilia R551-3] EEH IA

G124C IYLCIGL	• 0891 | RND efflux transporter [Pseudomonas aeruginosa PA7] IYLC G • GENE ID: 5356552 PSPA7_0172 | 3-oxoacyl-(acyl carrier protein) synthase [Pseudomonas aeruginosa PA7] LCIGL • >gb|ADI96785.1| hypothetical protein SAOV_0248 [Staphylococcus aureus subsp. aureus ED133] LCIGL • GENE ID: 6393293 Smlt3043 | putative ISXac3 like transposase [Stenotrophomonas maltophilia K279a] YLCI
V201M AHFMWIA	• GENE ID: 3913891 SAUSA300_0980 | hypothetical protein [Staphylococcus aureus subsp. aureus USA300_FPR3757] HFMWI • >ref|ZP_06876458.1| putative acyltransferase [Pseudomonas aeruginosa PAb1] MWIA • GENE ID: 6477949 Smal_0246 | hypothetical protein[Stenotrophomonas maltophilia R551-3] MWIA
N287K MIEKLRQ	• >gb|ADI98383.1| hypothetical protein SAOV_1921c [Staphylococcus aureus subsp. aureus ED133] MIEKL Q • GENE ID: 6477998 Smal_0813 | hypothetical protein [Stenotrophomonas maltophilia R551-3] MIEKLR • [Pseudomonas aeruginosa LESB58] M+EKLR
R344W IILWKIF	• >dbj|BAA88419.1| hydrophobic transmembrane protein [Staphylococcus aureus] IILW IF • GENE ID: 5355417 PSPA7_0951 | hypothetical protein [Pseudomonas aeruginosa PA7] IL WKIF • GENE ID: 6476673 Smal_2260 | hypothetical protein [Stenotrophomonas maltophilia R551-3] WKIF
R352E AVTEQFP	• emb|CAW29475.1| Gene info linked to CAW29475.1 probable major facilitator superfamily (MFS) transporter [Pseudomonas

	aeruginosa LESB58] AV EQFP • >gb|ADL22468.1| Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrD [Staphylococcus aureus subsp. aureus JKD6159] VTEQF Sbjct 490 VTEQF 494 • GENE ID: 6476745 Smal_3466 | RND efflux system, outer membrane lipoprotein, NodT family [Stenotrophomonas maltophilia R551-3] VTEQF
K464A GAGATSL	• >ref|ZP_06878929.1| fimbrial subunit CupA4 [Pseudomonas aeruginosa PAb1] Length=402 GAGAT L • GENE ID: 6395375 Smlt1512 | putative exported fimbriae-related chaperone [Stenotrophomonas maltophilia K279a] AGATSL • gb|EFM07900.1| molybdate ABC superfamily ATP binding cassette transporter, binding protein [Staphylococcus aureus subsp. aureus ATCC BAA-39] AGATS
M469I SLLIVIM	• >gb|ADL66280.1| Sec f amily Type I general secretory pathway preprotein translocase SecY_1 [Staphylococcus aureus subsp. aureus str. JKD6008] SLLIVI • GENE ID: 6478455 Smal_1028 | hypothetical protein [Stenotrophomonas maltophilia R551-3] LLIV+M
G480C PSECKIK	• gb|EES98134.1| conserved hypothetical protein [Staphylococcus aureus subsp. aureus TCH130] SECKI • GENE ID: 6394958 sucD | succinyl-CoA synthetase subunit alpha [Stenotrophomonas maltophilia K279a] P ECKI • >gb|AAD21623.1| succinyl-CoA synthetase alpha subunit [Pseudomonas aeruginosa PAO1] P ECKI Sbjct 130 PGECKI 135
V510D IFGDSYD	• GENE ID: 6477620 Smal_0071 | beta-lactamase [Stenotrophomonas maltophilia R551-3]

	FGDSYD • >gb|ADI96775.1| conserved hypothetical protein [Staphylococcus aureus subsp. aureus ED133] IF GDSYD • >ref|ZP_06881234.1| hypothetical protein PaerPAb_26559 [Pseudomonas aeruginosa PAb1] FGDSY
G551D SGDQRA	• >gb|ADL23188.1| oligopeptide ABC superfamily ATP binding cassette transporter, membrane protein [Staphylococcus aureus subsp. aureus JKD6159] SGDQRA • >ref|ZP_06881833.1| DNA polymerase I [Pseudomonas aeruginosa PAb1] SGDQR • gb|ACF51726.1| Gene info linked to ACF51726.1 efflux transporter, RND family, MFP subunit [Stenotrophomonas maltophilia R551-3] GDQRA Sbjct 46 GDQRA 50
A561E LAR EVYK	• A37 thiotransferase enzyme MiaB [Staphylococcus aureus ST398] LARE YK • GENE ID: 6474448 | AAA ATPase [Stenotrophomonas maltophilia R551-3] AREVY • GENE ID: 5354737 | hypothetical protein [Pseudomonas aeruginosa PA7] LARE YK > gb|AAK50437.1| unknown [Pseudomonas aeruginosa] AREVY
P841R ESIRAVT	• GENE ID: 6391398 Smlt0713 | hypothetical protein [Stenotrophomonas maltophilia K279a] ESIRAV • >ref|ZP_06879943.1| succinyl-diaminopimelate desuccinylase [Pseudomonas aeruginosa PAb1] SIRAVT • >gb|ADL23193.1| phosphate ABC superfamily ATP binding cassette transporter, membrane protein [Staphylococcus aureus subsp. aureus JKD6159] E IRAV Sbjct 180 EAIRAV 185
S895N KGNNTHS	• >pdb|3ITP|A Structure related to 3ITP_A Chain A, Crystal Structure Of Staphylococcal Nuclease Variant

	Delta+phs F34k At Cryogenic Temperature KGNNTH • >ref|ZP_04933853.1| hypothetical protein PA2G_01187 [Pseudomonas aeruginosa 2192] KGNN H • GENE ID: 6474583 Smal_3703 | hypothetical protein [Stenotrophomonas maltophilia R551-3] KG NTHS
D993R TIFRFIQ	• >gb|ADL24479.1| intercellular adhesion protein IcaC [Staphylococcus aureus subsp. aureus JKD6159] IFRFI • GENE ID: 6478446 Smal_1019 | alpha-glucosidase [Stenotrophomonas maltophilia R551-3] FRFI+ • GENE ID: 7179795 PLES_56671 | hypothetical protein [Pseudomonas aeruginosa LESB58] +RFIQ
K1250A GSGASTL	• GENE ID: 6477662 Smal_0113 | filamentous haemagglutinin family outer membrane protein [Stenotrophomonas maltophilia R551-3] GSG STL & GSGA T & ASTL • >ref|ZP_06881106.1| Putative methyltransferase [Pseudomonas aeruginosa PAb1] G GASTL
N1303K FRKKLDP	• GENE ID: 6391536 O | presumed capsid scaffolding protein (gpo) [Stenotrophomonas maltophilia K279a] FRKKLD • >gb|ACD38843.1| proline racemase family protein [Pseudomonas aeruginosa] FRK LD
G1349D LSHDHKQ	• >ref|ZP_06879005.1| hypothetical protein PaerPAb_15331 [Pseudomonas aeruginosa PAb1] LSHD KQ • GENE ID: 6395416 Smlt1637 | putative transmembrane protein [Stenotrophomonas maltophilia K279a] LSHDH

Table 6 : Tag clouds of the bacterial, viral and fungal species with homology to the CFTR polymutant or to the Delta508F CFTR mutant (an octapeptide surrounding the deletion point): Tag sizes range from 4 to 30 and are correlated with the number of CFTR homologies per pathogen species. The pathogens in red (genera or species) have been recorded as overpopulating cystic fibrosis patients (from Table 3). See http://www.polygenicpathways.co.uk/cysfib.htm for raw BLAST data.

Polymutant vs Bacteria	anthomonas campestris Staphylococcus epidermidis Thermoanaerobacter italicus Clostridium sporogenes Pseudomonas Aeruginosa Streptococcus mutans Caldicellulosiruptor saccharolyticus Brucella Roseburia inulinivorans Geobacillus thermodenitrificans Gardnerella vaginalis Clostridium bartlettii Staphylococcus Aureus Corynebacterium pseudotuberculosis Clostridium butyricum Mycobacterium smegmatis Carydibrachium pacificum Staphylococcus carnosus BdelloVibrio bacteriovorus Clostridium botulinum Clostridium kluyveri Xanthomonas oryzae Lysinibacillus sphaericus Caldicelulosiruptor becscii Geobacillus kaustophilus Thermotoga lettingae Lactobacillus johnsonii Coprococcus comes Orientia tsutsugamushi Pneumocystis jirovecii Clostridium caridivorans Thermoanaerobacter mathranii Burkholderia Cepacia Chlamydophila pneumoniae Blautia hansenii Ruminococcus torques Streptococcus pneumoniae Stenotrophomonas maltophilia Orientia tsutsugamushi Clostridium hiranonis Listeria seeligeri Clostridium scindens Verrucomicrobiae bacterium Turicibacter sp ButyriVibrio proteoclasticus Thermocrinis albus Haemophilus influenzae Lactobacillus jensenii Porphyromonas gingivalis Corynebacterium efficiens Bulleidia extructa Subdoligranulum variabile Clostridium spiroforme Shigella Streptococcus sanguinis Streptococcus agalactiae Paenibacillus Bacteroides capillosus Bacteroides pectinophilus Haemophilus parainfluenzae Alkaliphilus metalliredigens Listeria monocytogenes Escherichia coli Bacillus cereus Listeria grayi Streptococcus gallolyticus Symbiobacterium thermophilum Brevibacillus brevis Clostridium hatheway Clostridium thermocellum Ruminococcus

	obeum Eubacterium eligens Bacillus pumilus Opitutus terrae marine gamma proteobacterium Exiguobacterium sibiricum Erwinia amylovora Bifidobacterium catenulatum Xylanimonas cellulosilytica Nostoc sp Microcystis aeruginosa Bacillus cellulosilyticus Prevotella Streptococcus suis Bacillus pseudofirmus Cytophaga hutchinsonii Gemella haemolysans Geobacillus sp Enterobacter cloacae Clostridium lentocellum Lactobacillus delbrueckii Dethiobacter alkaliphilus Streptococcus Millerii Lactobacillus sakei Bacillus thuringiensis Aeromonas hydrophila Parvimonas micra Listeria welshimeri Bacillus anthracis Aeromonas salmonicida Lysinibacillus fusiformis Aggregatibacter aphrophilus Vibrio Desulfitobacterium hafniense Bacillus mycoides Lactobacillus crispatus Streptococcus gordonii Streptococcus infantarius Dehalococcoides ethenogenes Scardovia inopinata Streptococcus uberis Granulicatella elegans Prochlorococcus marinus Bacillus subtilis
Polymutant Viruses	Measles Xestia c-nigrum granulovirus Foot-and-mouth disease virus Influenza A virus West Nile virus Newcastle disease virus Synechococcus phage Neodiprion sertifer NPV Theiler's encephalomyelitis virus Lettuce mosaic virus Streptococcus phage Leuconostoc phage Aeromonas phage Hyphantria cunea nucleopolyhedrovirus Hepatitis B virus Gordonia terrae phage Dengue virus Human adenovirus Cryptophlebia leucotreta granulovirus Cucumber Bulgarian latent virus Regina ranavirus Enterobacteria phage Natrialba phage Human papillomavirus Human herpesvirus 1 Synechococcus phage lumpy skin disease virus Breda virus Acanthocystis turfacea Chlorella virus Norwalk-like virus Acidianus rod-shaped virus Turkey coronavirus Clostridium phage Jembrana disease virus Human astrovirus Human Rhinovirus Pseudomonas phage Staphylococcus phage Mamestra configurata NPV-A Staphylococcus phage Black queen cell virus Hepatitis E virus Human immunodeficiency virus 1 Batai virus Human herpesvirus 5 Salmonella phage Cereal yellow dwarf virus Streptomyces phage Mammalian orthoreovirus Murid herpesvirus

	1 Border disease virus Anticarsia gemmatalis nucleopolyhedrovirus Human parvovirus Chuzan virus Mycobacterium phage Infectious bronchitis virus Rotavirus GB virus C Escherichia phage Bacillus phage Equine arteritis virus Listeria phage Human immunodeficiency virus 2 Invertebrate iridescent virus Human herpesvirus 2 Hosta virus X Human Respiratory syncytial virus Human herpesvirus 8 Epstein-Barr Stretch Lagoon orbivirus Human Herpesvirus 3 Acinetobacter phage Human bocavirus Tyuleniy virus Feldmannia species virus Vaccinia virus Rubella Human herpesvirus 7 Escherichia phage Siberian sturgeon herpesvirus Maguari virus Mycoplasma fermentans Human calicivirus Mokola virus Epizootic hemorrhagic disease virus Brochothrix phage Hepatitis C virus Lactobacillus phage Human T-lymphotropic virus Human enterovirus Choristoneura fumiferana Human endogenous retrovirus K Sinorhizobium phage Japanese encephalitis virus Amsacta moorei entomopoxvirus Burkholderia phage Mumps Lactate dehydrogenase-elevating virus Norovirus
Polymutant Fungi	Candida albicans Candida glabrata Zygosaccharomyces rouxii Debaryomyces hansenii Laccaria bicolor Ustilago maydis Vanderwaltozyma polyspora Ashbya gossypii Moniliophthora perniciosa Penicillium marneffei Lachancea thermotolerans Kluyveromyces lactis Sordaria macrospora Aspergillus fumigatus Coprinopsis cinerea Emericella nidulans Podospora anserina Scheffersomyces stipitis Nectria haematococca Schizosaccharomyces pombe] Tuber melanosporum Malassezia globosa Aspergillus flavus Saccharomyces cerevisiae Cryptococcus neoformans Phaeosphaeria nodorum Schizosaccharomyces pombe Aspergillus terreus Aspergillus clavatus Ajellomyces capsulatus Aspergillus niger Pichia pastoris Lodderomyces elongisporus Schizophyllum commune Yarrowia lipolytica Gibberella moniliformis Trichophyton verrucosum Aureobasidium pullulans Penicillium chrysogenum Phanerochaete chrysosporium Glomus intraradices Alternaria brassicicola Schizosaccharomyces japonicus Aspergillus oryzae Talaromyces

	stipitatus Phaeosphaeria nodorum Neurospora crassa Debaryomyces hansenii Nectria haematococca Gibberella zeae Ajellomyces dermatitidis Chaetomium globosum Postia placenta Verticillium albo-atrum Kluyveromyces lactis Candida dubliniensis Aspergillus nidulans Sclerotinia sclerotiorum Arthroderma benhamiae Schizosaccharomyces japonicus Magnaporthe oryzae Coccidioides posadasii Candida tropicalis Uncinocarpus reesii Candida tropicalis Neosartorya fischeri Pichia guilliermondii Fusarium oxysporum Coccidioides immitis Meyerozyma guilliermondii Botryotinia fuckeliana Pyrenophora tritici-repentis Chaetomium globosum Pichia guilliermondii Magnaporthe oryzae Clavispora lusitaniae Paracoccidioides brasiliensis Lodderomyces elongisporus Vanderwaltozyma polyspora
DeltaF508 Bacteria	Stenotrophomonas maltophilia Gordonia bronchialis Catenibacterium mitsuokai Lactobacillus ultunensis Aggregatibacter actinomycetemcomitans Porphyromonas gingivalis Burkholderia graminis Flavobacteriales bacterium Cellulomonas sp Eubacterium biforme Neisseria gonorrhoeae Orientia tsutsugamushi Pseudomonas syringae Clostridium nexile Waddlia chondrophila Fusobacterium varium Streptomyces scabiei Bacillus thuringiensis Vibrio harveyi Anaerococcus vaginalis Actinomyces odontolyticus Pedobacter Selenomonas Bacteroides vulgatus Streptococcus Millerii Bacillus cereus Sebaldella termitidis Idiomarina loihiensis DesulfoVibrio Clostridium spiroforme Treponema denticola Francisella tularensis Catenulispora acidiphila Rhizobium leguminosarum Bacteroides thetaiotaomicron Clostridium cellulolyticum Clostridium perfringens Frankia Photobacterium damselae Bacteroides pectinophilus Rhodococcus Helicobacter pylori Clostridium papyrosolvens Peptoniphilus

	duerdenii Lactobacillus casei Pediococcus acidilactici Oribacterium Enterococcus faecalis Burkholderia cenocepacia Burkholderia glumae Bacteroides Hahella chejuensis Francisella philomiragia Rickettsia Streptococcus pneumoniae Ethanoligenens harbinense Staphylococcus Aureus Azotobacter vinelandii Salmonella enterica Novosphingobium aromaticivorans Burkholderia Cepacia Clostridium ramosum Lactococcus lactis ButyriVibrio fibrisolvens Pseudomonas Aeruginosa Ruminococcus flavefaciens Clostridium phytofermentans Streptococcus pyogenes Veillonella Proteus mirabilis Croceibacter atlanticus Paenibacillus Haemophilus influenzae Citrobacter rodentium Candidatus Carsonella Symbiobacterium thermophilum Xanthomonas Clostridium difficile Actinobacillus pleuropneumonia Alistipes putredinis Pneumocystis jirovecii Shewanella violacea Mycobacterium tuberculosis Corynebacterium pseudodiphtheriticum Macrococcus caseolyticus Pelobacter propionicus Trichodesmium erythraeum Lactobacillus salivarius Streptococcus infantarius Psychroflexus torquis Campylobacter jejuni Chlamydophila pneumoniae Campylobacterales bacterium Candidatus Pelagibacter Photorhabdus luminescens Mycobacterium gilvum Clostridium botulinum Mitsuokella multacida Saccharophagus degradans Legionella pneumophila ParaBacteroides distasonis Sphingomonas Anabaena variabilis Corynebacterium diphtheriae Chlamydia pneumoniae Desulfohalobium retbaense ButyriVibrio crossotus Pseudomonas putida Eubacterium dolichum Streptococcus mitis Legionella drancourtii Opitutaceae bacterium Prevotella melaninogenica Catenulispora acidiphila Leptotrichia goodfellowii
DeltaF508 Viruses	Trichoplusia ni Equine infectious anemia virus Hepatitis C virus Escherichia phage Human immunodeficiency virus Mycobacterium phage Tamiami virus Mushroom bacilliform virus Staphylococcus phage Geobacillus virus Molluscum

	contagiosum virus Cyanophage Lumpy skin disease virus Feline coronavirus Dengue virus Human herpesvirus 3 Norovirus Lactobacillus phage Human herpesvirus 7 Influenza A virus Human papillomavirus Grapevine virus Antheraea mylitta cypovirus Enterobacteria phage Marseillevirus Human Herpesvirus 1 Mycobacterium phage Main Drain virus Measles Acanthamoeba polyphaga mimivirus Chronic bee paralysis virus Japanese encephalitis virus Capsicum chlorosis virus Infectious hypodermal and hematopoietic necrosis virus oatpox virus Pellor Fowlpox virus Broad bean true mosaic virus Enterobacteria phage Synechococcus phage haemorrhagic kidney syndrome virus Northway virus Amsacta moorei entomopoxvirus Flavobacterium phage Hepatitis B virus Adoxophyes orana nucleopolyhedrovirus Highlands J virus Western equine encephalomyelitis virus Bacillus phage Campylobacter phage Human poliovirus Polyomavirus Sclerophthora macrospora virus Pseudomonas phage Musca domestica salivary gland hypertrophy virus Deerpox virus Liao ning virus Murine cytomegalovirus Toscana virus Soybean chlorotic mottle virus Acinetobacter phage Human herpesvirus 6 Epstein-Barr Turdivirus Gloxinia tospovirus Muromegalovirus Campylobacter phage Human enterovirus 71 SARS coronavirus Neodiprion sertifer NPV Emiliania huxleyi virus Adoxophyes honmai NPV Klebsiella phage Canna streak virus Infectious bronchitis virus Acanthocystis turfacea Chlorella virus Human coxsackievirus Human bocavirus Epizootic hemorrhagic disease virus Acidianus filamentous virus Foot-and-mouth disease virus Gremmeniella abietina Cotesia plutellae polydnavirus Leptospira biflexa temperate bacteriophage Paramecium bursaria Chlorella virus Simian-Human immunodeficiency virus Erwinia phage Mumps African swine fever virus Bidens mottle virus Peanut mottle virus Aeromonas phage Human herpesvirus 8 Brochothrix phage Streptococcus phage Clostridium phage Human endogenous retrovirus K Vaccinia virus Human Respiratory syncytial virus Neisseria meningitidis phage Human Rhinovirus wheat yellow mosaic virus Elephant endotheliotropic herpesvirus 2 Human endogenous retrovirus Massilia virus
DeltaF508 Fungi	Ashbya gossypii Lachancea thermotolerans Arthroderma otae Puccinia sorghi Hypocrea jecorina Moniliophthora perniciosa Podospora anserina Trichoderma hamatum Aspergillus

oryzae Aspergillus terreus Cochliobolus heterostrophus Penicillium marneffei Nectria haematococca Lentinula edodes Sclerotinia sclerotiorum Puccinia graminis Vanderwaltozyma polyspora Schizosaccharomyces pombe Fusarium oxysporum Puccinia recondita Paracoccidioides brasiliensis Debaryomyces hansenii Zygosaccharomyces rouxii Cadophora gregata Schizosaccharomyces japonicus Enterocytozoon bieneusi Dekkera bruxellensis Emericella nidulans Ajellomyces capsulatus Coccidioides posadasii Coccidioides immitis Verticillium albo-atrum Clavispora lusitaniae Botryotinia fuckeliana Talaromyces stipitatus Ajellomyces dermatitidis Uncinocarpus reesii Chaetomium globosum Debaryomyces hansenii Postia placenta Trichoderma asperellum Aspergillus niger Brettanomyces custersianus Coprinopsis cinerea Sordaria macrospora Gibberella zeae Saccharomyces cerevisiae Candida tropicalis Neurospora crassa Penicillium chrysogenum Phaeosphaeria nodorum Neosartorya fischeri Aspergillus fumigatus Scheffersomyces stipitis Laccaria bicolo Puccinia triticina Lodderomyces elongisporus Pichia guilliermondii Puccinia horiana Puccinia striiformis Aspergillus clavatus Tuber melanosporum Candida albicans Trichophyton verrucosum Pichia stipitis Arthroderma benhamiae Podospora anserina Aspergillus nidulans Yarrowia lipolytica Ajellomyces dermatitidis Aspergillus flavus Chaetomium globosum Arthroderma otae Lodderomyces elongisporus Pichia pastoris Arthroderma benhamiae Aspergillus nigere Magnaporthe oryzae Candida dubliniensis Kluyveromyces lactis Cryptococcus neoformans Penicillium marneffei Schizophyllum commune Malassezia globosa Coprinopsis cine Opegrapha varia Pyrenophora tritici-repentis Encephalitozoon intestinalis Candida glabrata Clavispora lusitaniae Blastocladiella emersonii

1Table 7: Bacterial and viral homologues of the ten highest affinity CFTR T cell
2epitopes ; Genera or species known to colonise CF patients are shown in bold.
3

Allele	CFTR Position	Epitope	Ic50 nM	Pathogen homologue
HLA A*0211	1:263-271	EMIENIQSV	2.1	EMIENIQ Flavobacterium johnsoniae: Paenibacillus curdlanolyticus **Xanthomonas** fuscans: **Xanthomonas** oryzae IENIQSV **Vibrio** fischeri EMIENTQ **Klebsiella** pneumoniae
HLA A*0250	1:263-271	EMIENIQSV	2.3	
HLA A*0211	1:869-877	FLAEVAASL	1.9	LAEVAASL Slackia heliotrinireducens: Actinosynnema mirum: Brevibacillus brevis; Dinoroseobacter shibae FLAEVADSL **Pseudomonas** fluorescens FLAQEVAASL **Burkholderia cenocepacia** FLAEVA **Stenotrophomonas maltophilia** FLAEVAA **Ruminococcus sp:** Pseudomonas mendocina
HLA A*0250	1:869-877	FLAEVAASL	2.1	
HLA A*0202	1:869-877	FLAEVAASL	2.3	
HLA A*0211	1:199-207	FMWIAPLQV	1.6	MWIAPL Lactobacillus delbrueckii WIAPLQ Comamonas testosterone: Ferrimonas balearica: Starkeya novella: Rhodobacter capsulatus Vibrio parahaemolyticus: Xanthomonas campestris WIAPLTV **Staphylococcus aureus** WIRPLQV **Pseudomonas aeruginosa** FMWGAPL **Stenotrophomonas maltophilia** WLAPLQV Borrelia recurrentis FMWIA Prevotella oris: Clostridium carboxidivorans
HLA A*0250	1:199-207	FMWIAPLQV V201M	2.2	
HLA A*0250	1:1138-1146	IMSTLQWAV	2.4	MSTSALQWAV Streptomyces lividans IMGTLQW
HLA A*0211	1:1138-	IMSTLQWAV	2.4	

	1146			Bacillus thuringiensis: Bacillus cereus MSTLQW Xanthomonas campestris MSLLQWAV Novosphingobium aromaticivorans ISSTLQWA **Leuconostoc** gasicomitatum ILSTLQW Corynebacterium ammoniagenes IMSTLQQW Serratia proteamaculans TLQWAV Micrococcus luteus TLQWAV Spiroplasma citri TLQWA **Pseudomonas aeruginosa**
HLA A*0250	1:136-144	LLHPAIFGL	2.1	LHPAIFGL Gluconobacter oxydans LHPAIFG Cytophaga hutchinsonii LLQPAIFG Photorhabdus asymbiotica LNPAIFGL Brachyspira pilosicoli LHPALFGL Brevundimonas sp LLHPDIFG Sinorhizobium medicae LLHP-VFGL **Stenotrophomonas maltophilia** LLHIPAIF **Pseudomonas aeruginosa**
HLA A*0211	1:209-217	LLMGLIWEL	1.9	LLMSLIWE Atopobium vaginae LLMGLIW Marinomonas sp: Colwellia psychrerythraea LLMGLFWQL **Pseudomonas** entomophila MGLIWDL Stigmatella aurantiaca MGLIWE Bacteroidetes oral taxon LLMGLVW **Helicobacter pylori** LMGLIRDL **Staphylococcus aureus** LLMMLFWEL **Pseudomonas aeruginosa** LLMGLAW **Stenotrophomonas maltophilia**
HLA A*0250	1:209-217	LLMGLIWEL	1.9	
HLA A*0219	1:209-217	LLMGLIWEL	2.3	
HLA A*0202	1:209-217	LLMGLIWEL	2.4	
HLA A*0250	1:1154-1162	SLMRSVSRV	1.9	SLVDMRSVSRV

HLA A*0211	1:1154-1162	SLMRSVSRV	2.3	Citromicrobium bathyomarinum LMRSVSR Pseudocowpox virus LMRNVSRV Clostridium asparagiforme MRSVSRV Erythrobacter litoralis SLMR-VSR **Stenotrophomonas maltophilia** **LMR**QA**RSVSR** **Pseudomonas aeruginosa**
HLA A*0250	1:768-776	VLNLMTHSV	2.1	VLNLMTH Ralstonia sp: Leptothrix cholodnii: Rhodoferax ferrireducens LNLMTH **Lactobacillus** jensenii +LMTHSV Streptomyces clavuligerus LNLMT S **Human herpesvirus 5 (cytomegalovirus)** NLMTH **Staphylococcus aureus**
HLA A*0250	1:121-129	YLCIGLCLL G124C	2.2	LCIGLCL **Bacteroides sp**: Bacillus cereus: Bacillus thuringiensis : Chlorobaculum parvum IGLCLL **Stenotrophomonas maltophilia**
HLA A*0211	1:88-96	YLGEVTKAV	1.7	YLGE-VTKAV **Streptococcus** oralis **Streptococcus** pneumoniae and other strep species: Eubacterium cylindroide: Lysinibacillus fusiformis
HLA A*0250	1:88-96	YLGEVTKAV	2.1	

1Table 8: The binding partners of the CFTR protein as defined by pSTIING. The viral
2binding partners of these proteins are also noted.
3

Gene symbol	**Name**	**Viral binding**
ADCY8	Adenylate cyclase type 8 activated adenylyl cyclase)	-
AHSA1	Activator of 90 kDa heat shock protein ATPase homolog 1	-
AIFM1	Apoptosis-inducing factor 1, mitochondrial precursor	-
AP1B1	AP-1 complex subunit beta-1	HIV-1
APOA2	Apolipoprotein A-II precursor	Hepatitis C, HSV-1 (Carter, 2010c)
ATAD3A	ATPase family AAA domain-containing protein 3A	-
ATP2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 2) ATPase)	-
ATP2A3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3 3)	-
ATXN2L	Ataxin-2-like protein	-
BCR	Breakpoint cluster region protein	-
C6orf48	Protein G8	-
C8orf55	C8orf55 protein	
CALU	Calumenin precursor	-
CANX	Calnexin precursor	HIV1, HSV-1
CAPNS1	Calpain small subunit 1	-
CD59	CD59 glycoprotein precursor	HIV-1, HSV-1 (Carter, 2010c)
CDH1	Epithelial-cadherin precursor	-
CLCA1	Chloride channel, calcium activated, family member 1	-
CLINT1	Clathrin interactor 1	-
CLTA	Clathrin light chain A	-
CLTCL1	Clathrin heavy chain 2	-
COPB1	Coatomer subunit beta	HIV-1
CSE1L	Exportin-2	Epstein-Barr,
CSTB	Cystatin-B	-
DAB2	Disabled homolog 2	-
DERL1	Derlin-1	-
DNAJA1	DnaJ homolog subfamily A member 1	Moloney murine leukemia virus
DNAJA2	DnaJ homolog subfamily A member 2	-
DNAJB1	DnaJ homolog subfamily B member 1	HSV-1 (Carter, 2010c)
DNAJC5	DnaJ homolog subfamily C member 5	-
EDG4	Lysophosphatidic acid receptor Edg-4	-
EMD	Emerin	HSV-1 (Carter, 2010c)
EPS8	Epidermal growth factor receptor kinase substrate 8	-
EXO1	Exonuclease 1	-

FAM120A	UPF0318 protein FAM120A	-
FAT	Cadherin-related tumor suppressor homolog precursor	-
FLOT2	Flotillin-2	-
GNA11	Guanine nucleotide-binding protein subunit alpha-11 subunit alpha)	-
GNAI2	Guanine nucleotide-binding protein G(i), alpha-2 subunit	-
GNB2L1	Guanine nucleotide-binding protein subunit beta 2-like 1	Epstein-Barr , Human adenovirus 2 and 5, HIV-1,
GOPC	Golgi-associated PDZ and coiled-coil motif-containing protein	Human papillomavirus type 18
GPIAP1	GPI-anchored membrane protein 1	Vaccinia Virus
GRN	Granulins precursor	HIV-1
HAX1	HS1-associating protein X-1	Epstein-Barr, HIV-1
HCLS1	Hematopoietic lineage cell-specific protein	-
HSP90AB1	Heat shock protein HSP 90-beta	HSV-1 (Carter, 2010c)
HSPA1A	Heat shock 70 kDa protein 1	Epstein-Barr HSV-1 (Carter, 2010c)
HSPA1L	Heat shock 70 kDa protein 1L	Simian virus 40
HSPA2	Heat shock-related 70 kDa protein 2	-
HSPA5	78 kDa glucose-regulated protein precursor protein grp78)	Epstein-Barr, HSV-1 (Carter, 2010c)
HSPA7	Heat shock 70 kDa protein 7	-
HSPA9B	Stress-70 protein, mitochondrial precursor	-
HSPB1	Heat-shock protein beta-1	Epstein-Barr,
HSPD1	60 kDa heat shock protein, mitochondrial precursor	Epstein-Barr, HIV-1
IL1RAPL1	X-linked interleukin-1 receptor accessory protein-like 1 precursor	-
IPO7	Importin-7	-
Kab	KARP-1-binding protein 1	-
KPNB1	Importin beta-1 subunit	HIV-1, HSV-1, Simian virus 40, Papillomavirus, HSV-1 (Carter, 2010c)
LGALS3	Galectin-3	-
LGALS4	Galectin-4	-
LIMA1	LIM domain and actin-binding protein 1	-
LIN7C	Lin-7 homolog C	-
LMNA	Lamin-A/C	Adenovirus, HIV-1, HSV-1, Papillomavirus, HSV-1 (Carter, 2010c)
LMO7	LIM domain only protein 7	-
LRRFIP2	Leucine-rich repeat flightless-interacting protein 2	-
MLP	Mucin-like protein	-

MS4A5	Membrane-spanning 4-domains subfamily A member 5	-
MUC13	Mucin-13 precursor	-
PCMT1	Protein-L-isoaspartate(D-aspartate) O-methyltransferase	-
PDCD6	Programmed cell death protein 6	HSV-1 (Carter, 2010c)
PDZK1	PDZ domain-containing protein 1 exchanger regulatory factor 3)	-
PLD2	Phospholipase D2	-
PLEKHA6	Pleckstrin homology domain-containing family A member 6	-
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	HIV-1, Papillomavirus, Simian virus 40
PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	Adenovirus, Hepatitis B, HIV-1
PRKDC	DNA-dependent protein kinase catalytic subunit	Adenovirus, HIV-1, HSV-1,
PSAP	Proactivator polypeptide precursor	-
PSMD2	26S proteasome non-ATPase regulatory subunit 2	Epstein-Barr
PSME2	Proteasome activator complex subunit 2	-
RCN1	Reticulocalbin-1 precursor	Epstein-Barr
RCN2	Reticulocalbin-2 precursor	Epstein-Barr, Papillomavirus,
REPS1	RalBP1-associated Eps domain-containing protein 1	-
RNF5	E3 ubiquitin-protein ligase RNF5	-
RPS27A	Ubiquitin	HIV-1
RYK	Tyrosine-protein kinase RYK precursor	-
RYR2	Ryanodine receptor 2	-
S100A7	Protein S100-A7	-
S100A9	Protein S100-A9	-
SEC61A1	Protein transport protein Sec61 subunit alpha isoform 1	-
SEC61A2	Protein transport protein Sec61 subunit alpha isoform 2	-
SFXN3	Sideroflexin-3	-
SH3BGRL2	SH3 domain-binding glutamic acid-rich-like protein 2	-
SLC9A2	Sodium/hydrogen exchanger 2 exchanger 2)	-
SLC9A3R1	Ezrin-radixin-moesin-binding phosphoprotein 50 exchange regulatory cofactor NHE-RF) exchanger)	-
SLC9A3R2	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	-
SNX4	Sorting nexin-4	-
SNX9	Sorting nexin-9	-

SORL1	Sortilin-related receptor precursor	-
SPTLC1	Serine palmitoyltransferase 1	-
SQRDL	Sulfide:quinone oxidoreductase, mitochondrial precursor	-
STX1A	Syntaxin-1A	-
SVIL	Supervillin	-
TACSTD1	Tumor-associated calcium signal transducer 1 precursor	-
TCEB1	Transcription elongation factor B polypeptide 1	HIV-1
TCEB2	Transcription elongation factor B polypeptide 2	HIV-1
TFG	Protein TFG	-
TIAM1	T-lymphoma invasion and metastasis-inducing protein 1	-
TJP1	Tight junction protein ZO-1	-
TJP3	Tight junction protein ZO-3	-
TMEM43	Transmembrane protein 43	-
TMOD3	Tropomodulin-3	-
TPM3	Tropomyosin alpha-3 chain	Ectromelia virus strain Moscow
TRIP12	Thyroid receptor-interacting protein 12	-
UBE2J1	Ubiquitin-conjugating enzyme E2 J1	-
UBE3A	Ubiquitin-protein ligase E3A	Papillomavirus
UNQ1922	Galactosyltransferase	-
VCP	Transitional endoplasmic reticulum ATPase ATPase p97 subunit)	-
VPS4A	Vacuolar protein sorting-associating protein 4A	-
WFS1	Wolframin	-
WSB1	WD repeat and SOCS -containing protein 1	-
XPNPEP3	Putative Xaa-Pro aminopeptidase 3	-
XPO1	Exportin-1	HIV-1

1Table 9: Kegg pathway analysis of the binding partners of the CFTR protein

Pathway and number of proteins	**Gene symbols**	**Comments**
Protein processing in endoplasmic reticulum (18)	CANX, DERL1, DNAJA1, DNAJB1,DNAJC5, HSP90AB1, HSPA1A, HSPA1L, HSPA2, HSPA5,BIP, RNF5, SEC61A1, SEC61A2, UBE2J1,VCP, WFS1+ CALU	Endoplasmic reticulum stress is a feature of cystic fibrosis
Ubiquitin mediated proteolysis (5)	TCEB1, TCEB2,TRIP12, UBE2J1, UBE3A	Protein degradation via this pathway is impaired in CF patients (Paul, 2008)
Protein export (3)	HSPA5, SEC61A1, SEC61A2	
Antigen processing and presentation (8)	CANX, HSPA1A, HSPA1L, HSPA2, HSPA5, HSP90AB1, PSME2 + Autoantigen TNF	The delta F508 mutation has also been shown to inhibit the antigen presentation pathway (Hampton & Stanton, 2010),
Chemokine signalling pathway (5)	ADCY8, GNAI2, TIAM1 + IL1RAPL1, + autoantigen TNF	CFTR controls the NFKB mediated chemokine inflammatory response
Endocytosis (10)	CLTA, CLTLC1, DAB2, HSPA1A, HSPA1L HSPA2, PLD2, VPS4A + , CLINT1, COPB1,	CFTR is a pattern recognition receptor that allows entry of P.Aeruginosa by endocytosis
Vibrio cholerae infection (4)	CFTR, SEC61A1, SEC61A2, TJP1	Inflammation of airway epithelial cells due to bacterial colonisation is a characteristic feature of cystic fibrosis (Machen, 2006a)
Bacterial invasion of epithelial cells (4)	CDH1, CLTA, CLTCL1, HCLS1.	
Chagas disease (3)	GNA11, GNAI2, PPP2R1A	
Toxoplasmosis (5)	GNAI2,HSPA1A, HSPA1L, HSPA2 + autoantigen TNF	-
Lysosome (5)	AP1B1, CLTA, CLTCL1, PSAP + CSTB	The CFTR protein is involved in lysosomal acidification in alveolar macrophages and these cells are less able to kill bacteria in CFTR knockout mice
Phagosome (3)	CANX, SEC61A1, SEC61A2	CFTR provides the

		chloride for the generation of hypochlorous acid by myeloperoxidase in neutrophil phagosomes. This bactericidal mechanism is defective in cystic fibrosis
Dilated cardiomyopathy (7)	ADCY8,RYR2,TPM3,ATP2A2, EMD, LMNA + Autoantigen TNF	Cardiomyopathy is a complication of cystic fibrosis
Hypertrophic cardiomyopathy (HCM) (6)	ATP2A2, EMD, LMNA, RYR2, TPM3 + Autoantigen TNF	
Arrhythmogenic right ventricular cardiomyopathy (ARVC) (4)	ATP2A2, EMD, LMNA, RYR2 + TMEM43	
Cardiac muscle contraction (3)	ATP2A2, RYR2, TPM3,	
Pathways in cancer (9)	BCR,CDH1, FAT, HSP90AB1, TCEB1, TCEB2, TFG, TPM3 + WSB1	Kidney, thyroid, endocrine, lymphoma and nonmelanoma skin cancer risk is increased in cystic fibrosis .
Thyroid cancer (3)	CDH1, TFG, TPM3.	
Spliceosome (3)	HSPA1A, HSPA1L, HSPA2	-
Pancreatic secretion (6)	ADCY8, ATP2A2, ATP2A3, CFTR, CLCA1, RYR2	Pancreatic function is impaired in CF patients
Type 1 Diabetes	GAD2 , HSPD1 and and TNF autoantigens	Diabetes is a frequent complication in cystic fibrosis
Tight junction (5)	GNAI2, HCLS1, PPP2R1A, TJP1, TJP3	Gap junction function is impaired in cystic fibrosis pancreatic duct cells Airway epithelial tight junction function is compromised in cystic fibrosis (Godfrey, 1997)
Gap junction (4)	ADCY8, GNA11, GNAI2, TJP1,	
Adherens junction (3)	CDH1, LMO7, TJP1	
Apoptosis (6)	AIFM1, CAPNS1, LGALS3 PRKAR2A, PDCD6, TNF	The calcium, calpain caspase apoptosis cascade is modified F508del expressing cells
Calcium signalling pathway (5)	ADCY8, ATP2A2, ATP2A3, GNA11, RYR2. Added RCN1, RCN2	
MAPK signalling pathway (4)	HSPA1A, HSPA1L, HSPA2, HSPB1 + autoantigen TNF	P.Aeruginosa inhibits airway epithelial sodium transport via

		activatioin of MAPK signalling
Gonadotrophin releasing hormone signalling pathway (3)	ADCY8, GNA11, PLD2;	CFTR is expressed in the hypothalamus, in Gnrh containing cells, and controls the reproductive endocrine axis
Progesterone-mediated oocyte maturation (3)	ADCY8, GNAI2, HSP90AB1	Respiratory epithelial ion transport is regulated by progesterone and oestrogen
Gastric acid secretion (3)	ADCY8, CFTR, GNAI2	The CFTR chloride channel modulates gastric acid secretion (Schubert, 2010)
Long-term depression (3)	GNA11, GNAI2, PPP2R1A	-

Fig 1

The position and nature of the CFTR mutations studied (highlighted in red). Del = deletion; X = stop codon termination.

A polymutant protein containing all of the point mutations was constructed for bioinformatics analysis

NATIVE

```
MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLEREWDRELASKKNPKLI 70
NALRRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEERSIAIYLGIGLCLLFIVRTLLLHP 140  R>W R>H G>C
AIFGLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFVWIAPLQVAL 210  V>M
LMGLIWELLQASAFCGLGFLIVLALFQAGLGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEEA 280
MEKMIENLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVFLSVLPYALIKGIILRKIFTTISFCIVLRMAV 350  N>K R>W
TRQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGFGELFEKAKQNNNNRK 420  R>K/A/E
TSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGKTSLLMVIMGELEPSEGKIKHSGRISF 490  K>A M>I G>C
CSQFSWIMPGTIKENIIFGVSYDEYRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLAR 560  F>Del V>D G>D
AVYKDADLYLLDSPFGYLDVLTEKEIFESCVCKLMANKTRILVTSKMEHLKKADKILILHEGSSYFYGTF 630  A>E
SELQNLQPDFSSKLMGCDSFDQFSAERRNSILTETLHRFSLEGDAPVSWTETKKQSFKQTGEFGEKRKNS 700
ILNPINSIRKFSIVQKTPLQMNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVL 770
NLMTHSVNQGQNIHRKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEINEEDLKECFFDDMESI 840
PAVTTWNTYLRYITVHKSLIFVLIWCLVIFLAEVAASLVVLWLLGNTPLQDKGNSTHSRNNSYAVIITST 910  P>R S>N
SSYYVFYIYVGVADTLLAMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSKDI 980
AILDDLLPLTIFDFIQLLLIVIGAIAVVAVLQPYIFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSP 1050 D>R
IFTHLVTSLKGLWTLRAFGRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISIL 1120
TTGEGEGRVGIILTLAMNIMSTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEGKPTKSTKPYKNGQLSKV 1190
MIIENSHVKKDDIWPSGGQMTVKDLTAKYTEGGNAILENISFSISPGQRVGLLGRTGSGKSTLLSAFLRL 1260 K>A
LNTEGEIQIDGVSWDSITLQQWRKAFGVIPQKVFIFSGTFRKNLDPYEQWSDQEIWKVADEVGLRSVIEQ 1330 D>N W>X N>K
FPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLKQAFADCTVILC 1400 G>D
EHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAISPSDRVKLFPHRNSSKCKSKPQIAALKEE 1470
TEEEVQDTRL
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Polymutant

```
MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLEREWDRELASKKNPLINA
LWRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEEHSIAIYLCIGLCLLFIVRTLLLHPAIF
GLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFMWIAPLQVALLMGL
IWELLQASAFCGLGFLIVLALFQAGLGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEEAMEKMI
EKLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVFLSVLPYALIKGIILWKIFTTISFCIVLRMAVTEQFPW
AVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGFGELFEKAKQNNNNRKTSNGDDS
LFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGATSLLIVIMGELEPSECKIKHSGRISFCSQFSWIM
PGTIKENIIFGDSYDEYRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGDQRARISLAREVYKDADLY
LLDSPFGYLDVLTEKEIFESCVCKLMANKTRILVTSKMEHLKKADKILILHEGSSYFYGTFSELQNLQPDF
SSKLMGCDSFDQFSAERRNSILTETLHRFSLEGDAPVSWTETKKQSFKQTGEFGEKRKNSILNPINSIRKF
SIVQKTPLQMNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVLNLMTHSVNQGQN
IHRKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEINEEDLKECFFDDMESIRAVTTWNTYLRYI
TVHKSLIFVLIWCLVIFLAEVAASLVVLWLLGNTPLQDKGNNTHSRNNSYAVIITSTSSYYVFYIYVGVAD
TLLAMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSKDIAILDDLLPLTIFRFI
QLLLIVIGAIAVVAVLQPYIFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSPIFTHLVTSLKGLWTLR
AFGRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISILTTGEGEGRVGIILTLAM
NIMSTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEGKPTKSTKPYKNGQLSKVMIIENSHVKKDDIWPSGG
QMTVKDLTAKYTEGGNAILENISFSISPGQRVGLLGRTGSGASTLLSAFLRLLNTEGEIQIDGVSWDSITL
QQWRKAFGVIPQKVFIFSGTFRKKLDPYEQWSDQEIWKVADEVGLRSVIEQFPGKLDFVLVDGGCVLSHDH
KQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLKQAFADCTVILCEHRIEAMLECQQFLVIEENKV
RQYDSIQKLLNERSLFRQAISPSDRVKLFPHRNSSKCKSKPQIAALKEETEEEVQDTRL
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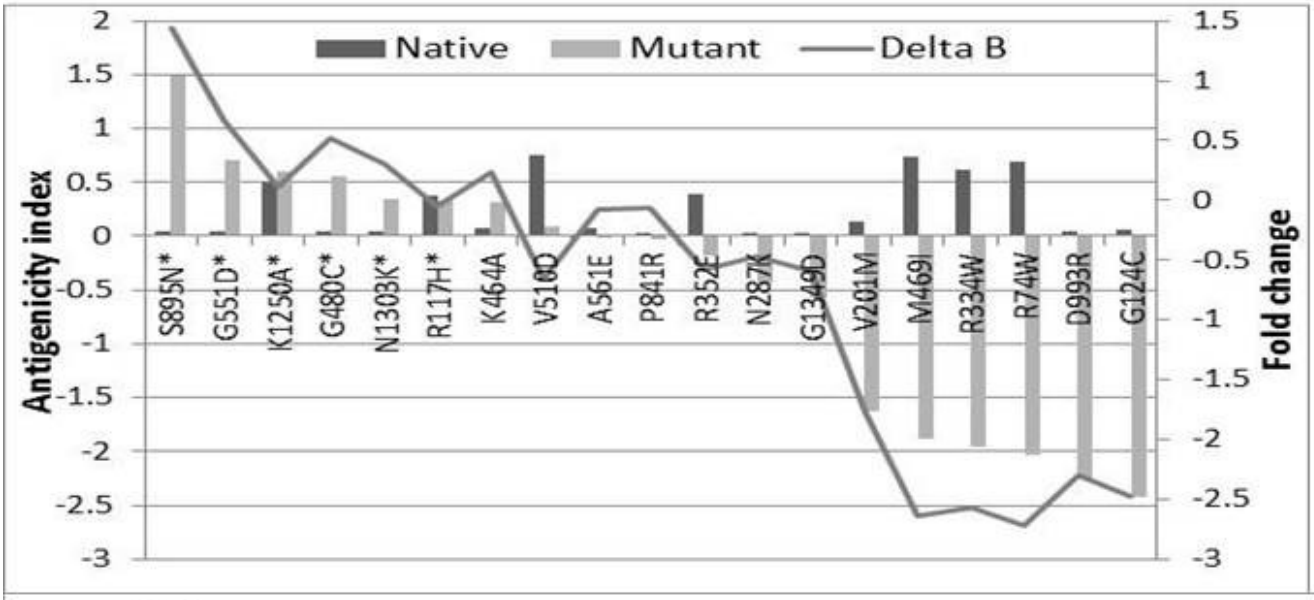
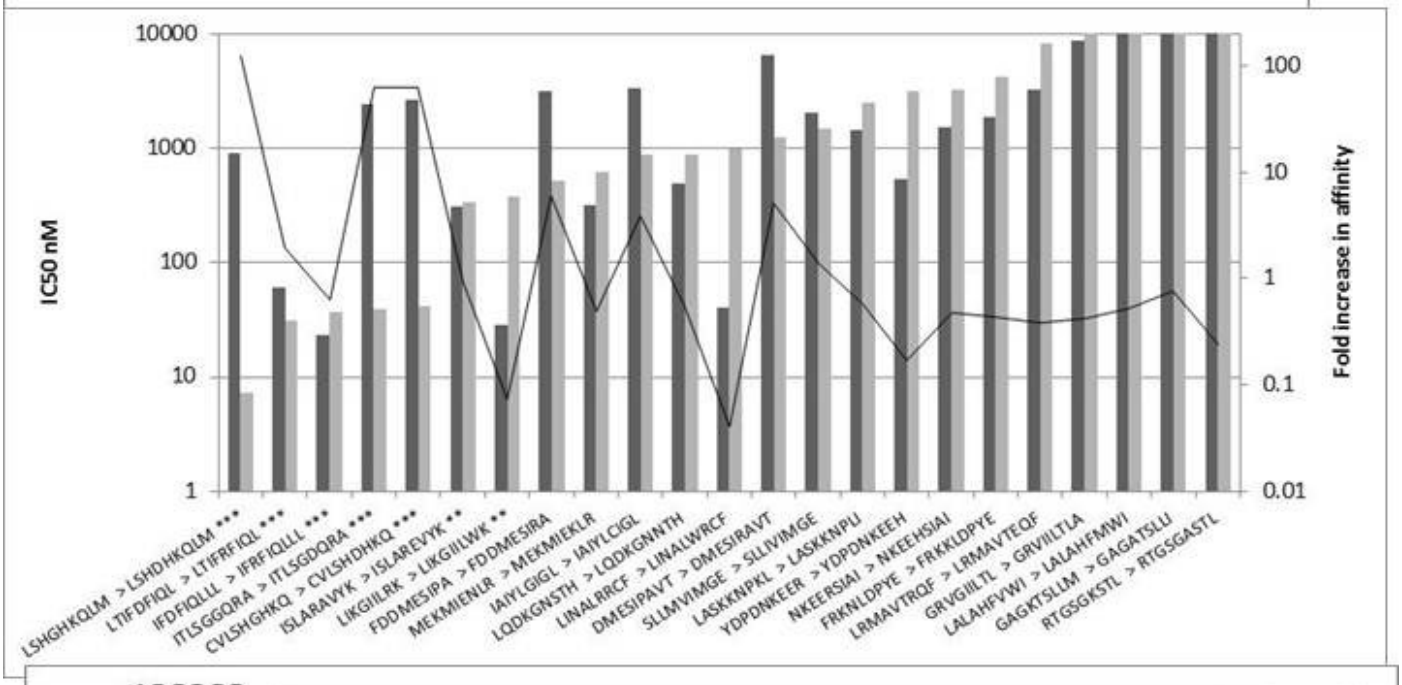
1Fig 2 The B-cell and T-cell immunogenic profile of the CFTR mutations. The antigenicity is based on a scan
2of the whole protein and not simply of the amino acid concerned.





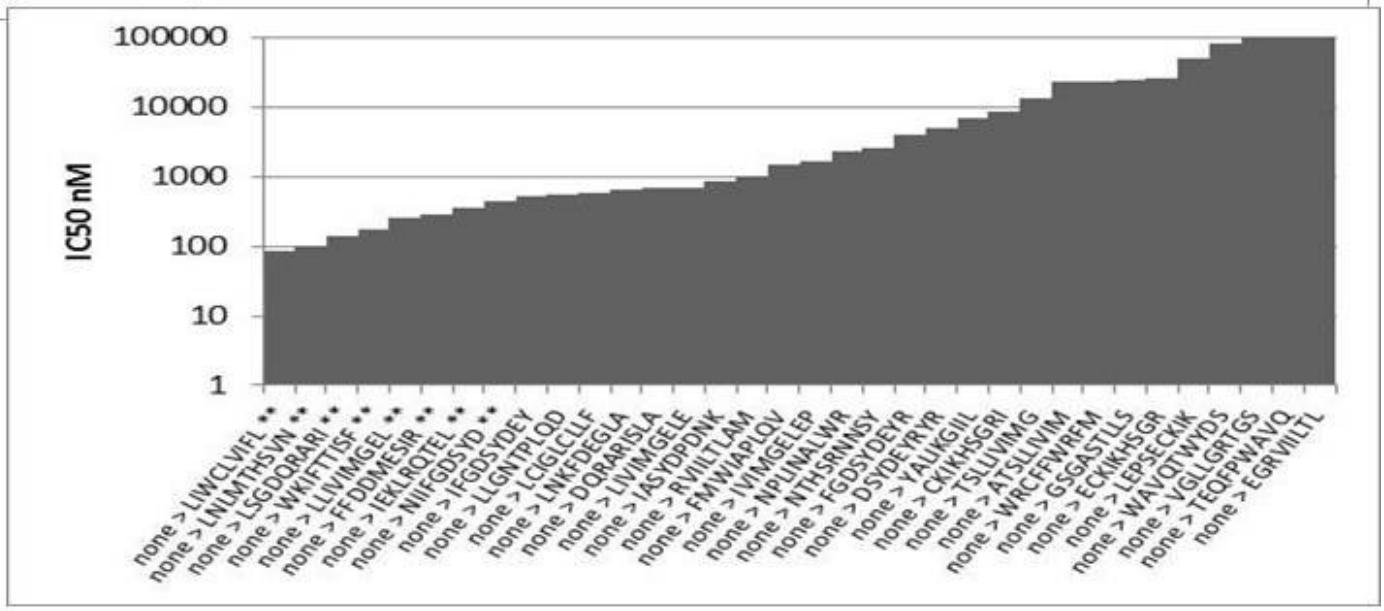


Fig 3

The effects of CFTR mutations on B-cell and T-cell immunogenicity. The plots are based on scans of the entire CFTR protein. All mutants were used to constitute a polymutant protein. The epitope prediction servers generate a table of antigenicity values for each amino acid along the entire length of the protein. The delta values reflect subtraction of the native from the CFTR values.

As a rough guideline, peptides with IC_{50}values <50 nM are considered high affinity, <500 nM intermediate affinity and <5000 nM low affinity

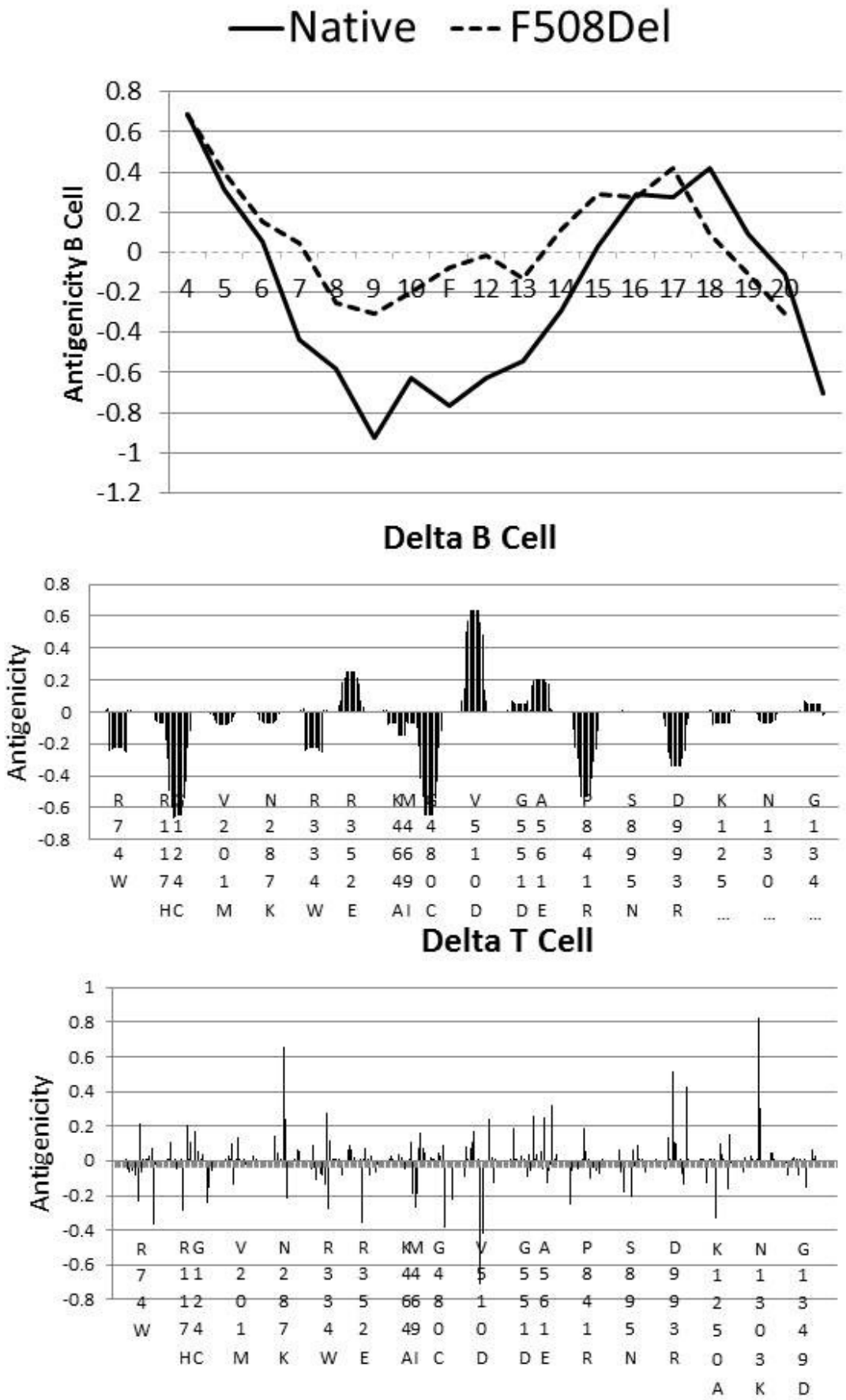


Fig 4: Examples of P.Aeruginosa or S.Aureus vatches within the polymutant CFTR protein. Highly immunogenic regions are in larger font (B cell epitope prediction > 0.5: The Bepipred server sets the epitope prediction threshold at 0.35) The alignment regions are boxed and the identical amino acids shaded in grey. Red amino acids are the point mutations within the CFTR protein.

P.Aeruginosa

>gi|90421313|ref|NP_000483.3| cystic fibrosis transmembrane conductance regulator [Homo sapiens]B cell epitopes > 0.5 P.Aeruginosa

MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLERERWDRELASKKN

PLINALWRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEEHSIAIYLCIGLCLLFIVRTLLLHPAIFGLHHIGM

QMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFMWIAPLQVALLMGLIWELLQASAFCGLGFLIVLALFQAG

LGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEEAMEKMIEKLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVFLSV

LPYALIKGIILWKIFTTISFCIVLRMAVTEQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGF

GELFEKAKQNNNNRKTSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGATSLLIVIM

GELEPSECKIKHSGRISF

CSQFSWIMPGTIKENIIFGDSYDEYRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGDQRARISLAR

EVYKDADLYLLDSPFGYLDVLTEKEIFESCVCKLMANKTRILVTSKMEHLKKADKILILHEGSSYFYGTFSELQNLQPDFSSKLMGC

DSFDQFSAERRNSILTETLHRFSLEGDAPVSWTETKKQSFKQTGEFGEKRKNSILNPINSIR

KFSIVQKTPLQMNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVLNLMTHSV

NQGQNIHRKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEINEEDLKECFFDDMESIRAVTTW

NTYLRYITVHKSLIFVLIWCLVIFLAEVAASLVVLWLLGNTPLQDKGNNTHSRNNSYAVIITSTSSYYVFYIYVGV

ADTLLAMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSKDIAILDDLLPLTIFRFIQLLLIVIGAIAVVAVLQPY

IFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSPIFTHLVTSLKGLWTLRAFGRQPYFETLFHKALNLHTANWFLYLSTLRW

FQMRIEMIFVIFFIAVTFISILTTGEGEGRVGIILTLAMNIMSTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEGKPT

KSTKPYKNGQLSKVMIIENSHVKKDDIWPSGGQMTVKDLTAKYTEGGNAILENISFSIS

PGQRVGLLGRTGSGASTLLSAFLRLLNTEGEIQIDGVSWDSITLQQWRKAFGVIPQKVFIFSGTFRKKLDPYEQ

WSDQEIWKVADEVGLRSVIEQFPGKLDFVLVDGGCVLSHDHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLK

QAFADCTVILCEHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAISPSDRVKLFPHRNSSKCKS

KPQIAALKEETEEEVQDTRL

S.Aureus

>gi|90421313|ref|NP_000483.3| cystic fibrosis transmembrane conductance regulator [Homo sapiens]B cell epitopes > 0.5

MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLERERWDRELASKKN

PKLINALWRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEEHSIAIYLCIGLCLLFIVRTLLLHPAIFGLHHI

GMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFMWIAPLQVALLMGLIWELLQASAFCGLGFLIVLALFQ

AGLGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEEAMEKMIEKLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVF

LSVLPYALIKGIILWKIFTTISFCIVLRMAVTEQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWE

EGFGELFEKAKQNNNNRKTSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGATSLL

IVIMGELEPSECKIKHSGRISFCSQFSWIMPGTIKENIIFGDSYDEYRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLS

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3

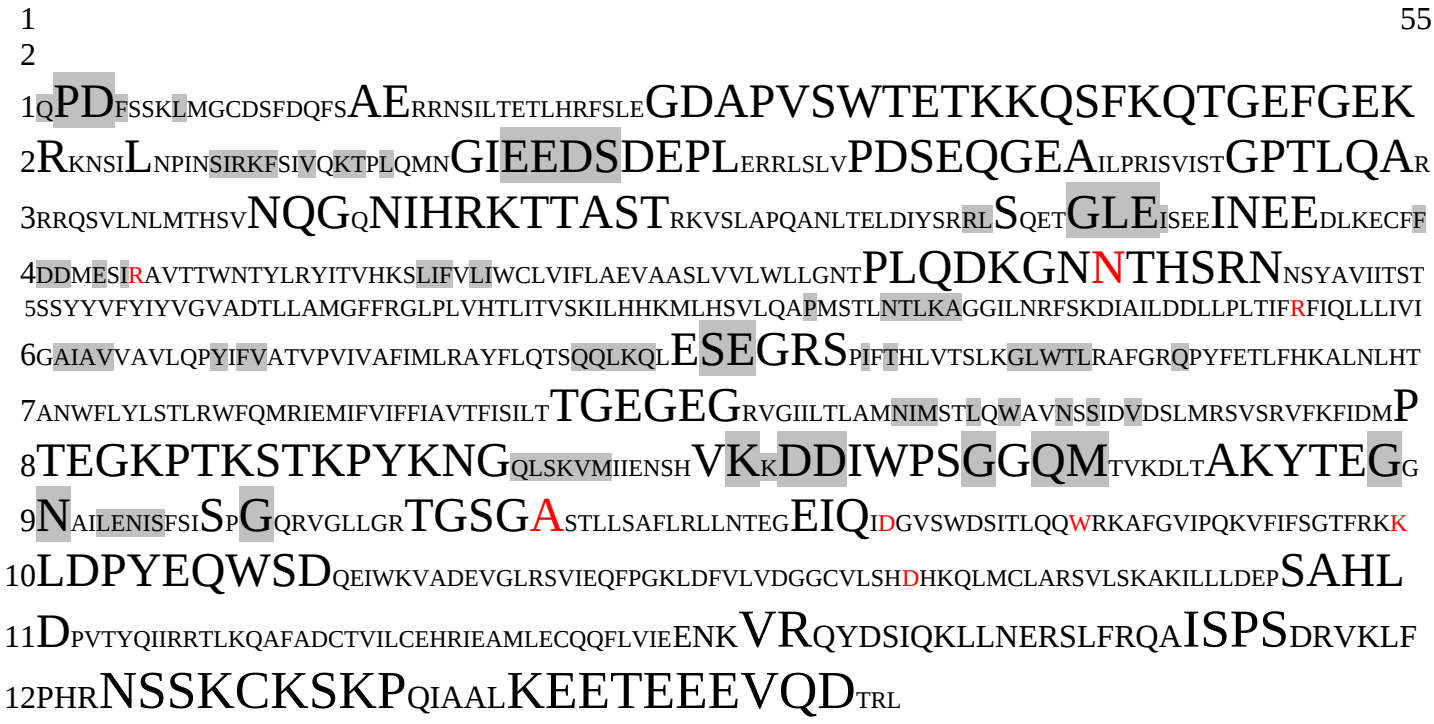


Fig 5
Results of the Kegg pathway analysis of the CFTR interactome, including the autoantigens observed in cystic fibrosis. The spokes radiating from the CFTR protein contact with proteins within the CFTR interactome. Proteins known to bind to viral proteins are highlighted in white.The pathways on the right (entry/endocytosis/intercellular spread/protein removal) are those used by Herpes simplex and manyt other viruses during their sojourn in the host cell. The pathways relating to diseases are shown on the left.

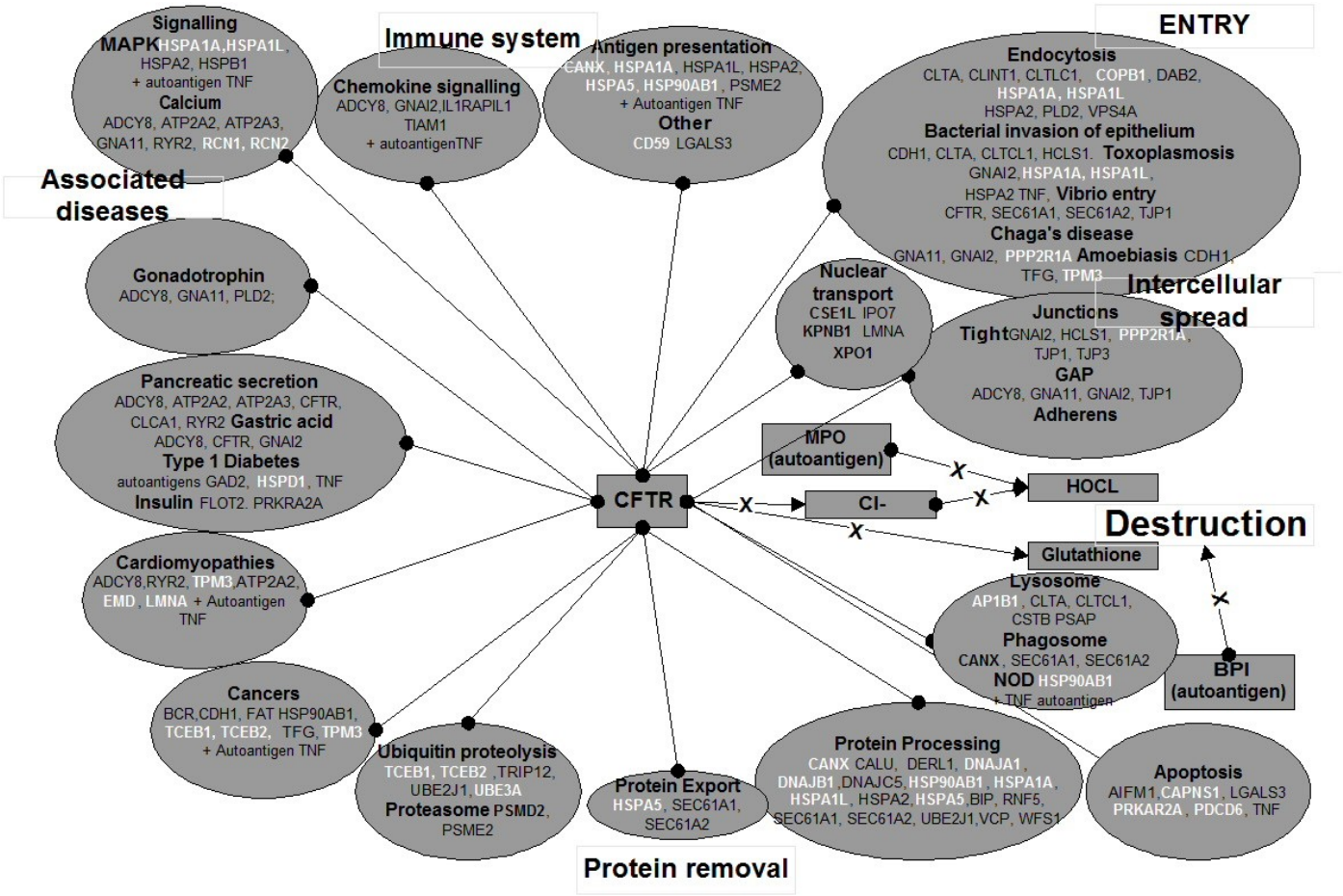
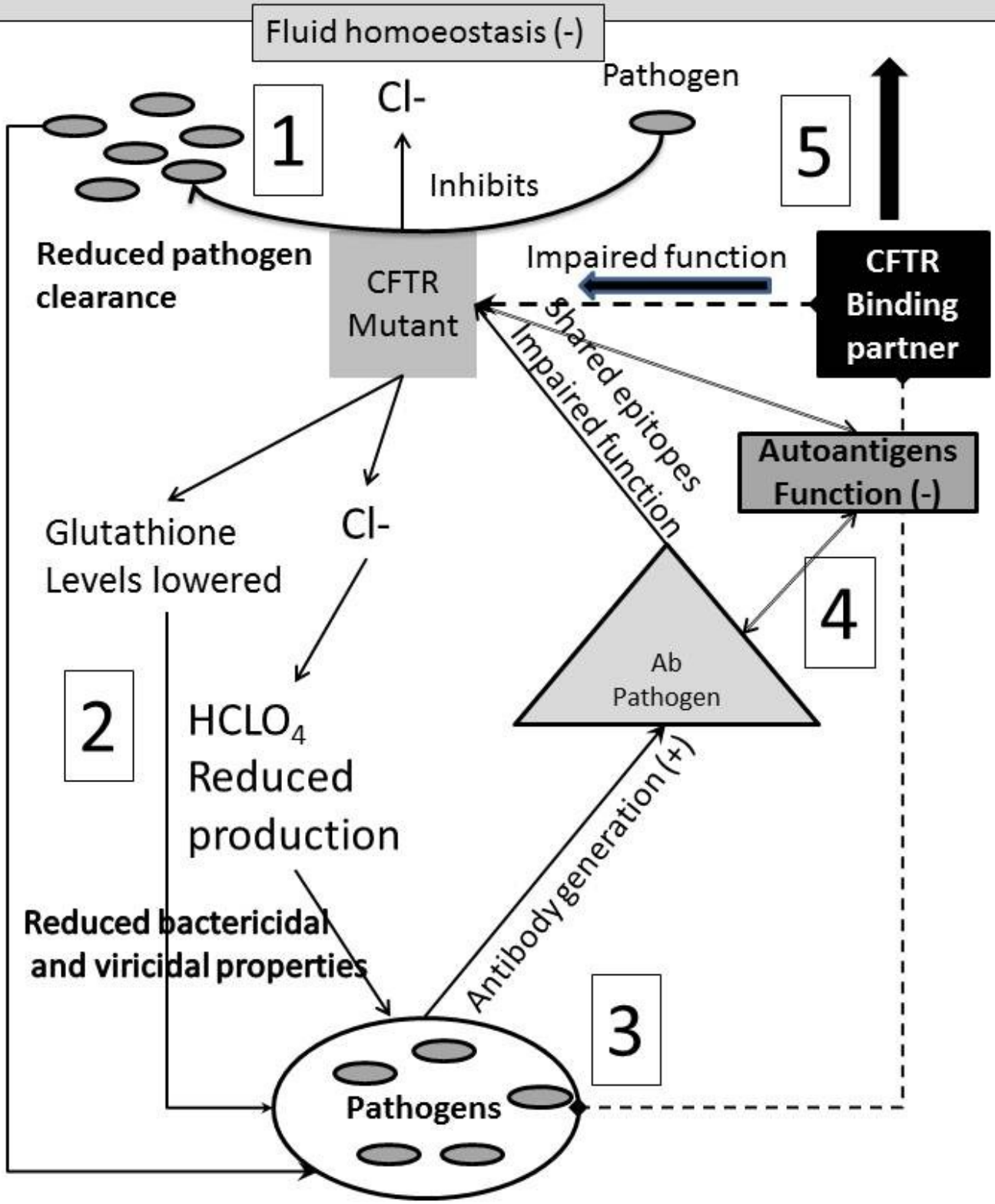


Fig 6.

A pathogenic feed forward cycle in cystic fibrosis

1: CFTR mutations result in chloride channel deficiency with associated problems in fluid homoeostasis/ They also favour pathogen accumulation in the extracellular mileu. 2 : CFTR mutations also compromise glutathione and hypochlorous acid availability, reducing bactericidal and viricidal effects. 3: Hypercolonistaion by diverse pathogtens results in antibody production. 4: Because of pathogen mimicry these antibodies also target autoantigens, and possible the CFTR protein itself. Epitope sharing between pathogen/autoantigen and the CFTR protein favours the maintenance of antibody production. 5: Reductions in CFTR and autoantigen function, compromises CFTR related pathways, which include those related to the associated pathologies of cystic fibrosis. Repeated reductions in CFTR function continue the cycle, resulting in further pathogen colonisation etc…….

Associated diseases Pulmonary , cardiomyopathy, pancreas, gastric secretion, diabetes
Disrupted Pathways: Chemokines; ER stress; Protein processing; Lysosome; Phagosome; Junctions , etc



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