Contents

Abstract

Cystic Fibrosis (CF) is a genetic disease which is caused by mutations in the CFTR[1] gene leading to incorrect protein formation. It can be severe or mild its presentation affecting mainly the lungs and digestive system but also other organs as well. To inherit the disease both parents must carry this defective gene as well as pass it on (recessive genetic disease). Previously, traditional pathology tests were performed to diagnose CF, but with the advancement of molecular science, simple genetic tests targeting the entire gene or areas known to contain mutations are conducted regularly in susceptible people, both pre and post natal. The primary mutation is known as F508del, which is the deletion of three codes leading to a missing amino acid at 508 in the CFTR sequence. Primarily these tests involve targeting areas of the CFTR gene and looking for missing or mutated codes. Non-genetic tests are still carried out but almost always in conjunction with genetic testing.

Cystic Fibrosis

[1] cystic fibrosis trans-membrane conductance regulator

CF is a recessive genetic disease of the sweat and mucus glands that affects the liver, lungs, pancreas, sinuses and reproductive organs. It causes growth problems, infertility, liver pancreas and lung and airway complications. The most serious problem can be difficulty breathing and digestive problems, which can become life threatening.

The CFTR gene

CF is caused by a mutation in the CFTR gene (fig 1) resulting in a mutation of the protein *cystic fibrosis trans-membrane conductance regulator (fig 2).* The CFTR family is located on chromosome 7, containing 250,000 DNA nucleotides, in turn 27 mRNA sequences, encoding 1,480 amino acids.

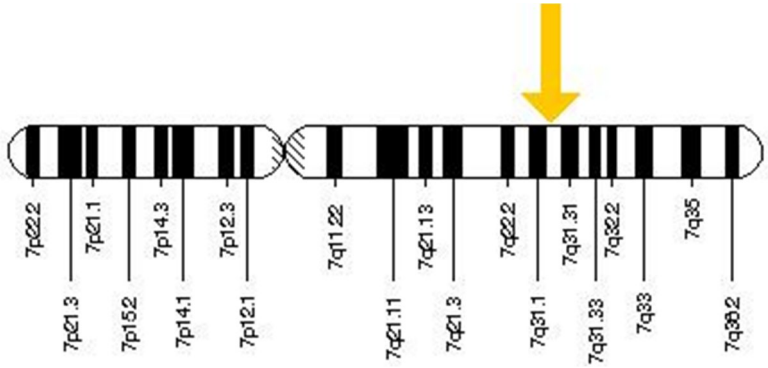

Figure 1 Position CFTR gene on chromosome 7 (http://ghr.nlm.nih.gov/gene/CFTR)

This protein is responsible for movement of chloride ions, in and out of cells that produce mucus, saliva, sweat and digestive enzymes. This movement is responsible for the creation and regulation of mucus which is needed as lubricant throughout the body. This abnormality is responsible for many of the respiratory and organ issues associated with this disease.

Figure 2 the resultant cystic fibrosis trans-membrane conductance regulator protein (http://commons.wikimedia.org/wiki/File:Protein_CFTR_PDB_1xmi.png)

Pathogenesis

There are more than 1,200 mutations of the CFTR gene, known to cause CF, although in 70% of cases it is the deletion of the 508th (fig 3) amino acid in the resultant protein, known as ΔF508. This is due to 3 missing nucleotides in the encoding DNA.

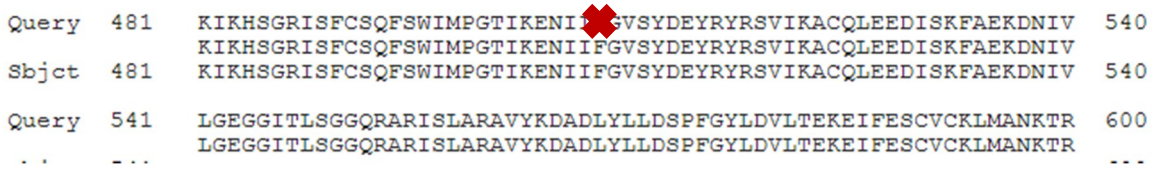

Figure 3 ΔF508 - deletion of the 508th (phenylalanine) amino acid
http://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_90421313

This deletion causes the amino acid sequence to fold incorrectly into its final structure which causes it to breakdown shortly after folding is complete. This means it never reaches the cell membrane and thus cannot complete its job of chloride ion transfer. Other mutations include, shortening of the CFTR protein due to premature folding completion and incorrect amino acid placement due to incorrect DNA coding. See fig 4 for a detailed list of mutations causing Cystic Fibrosis. It is believed there are many more mutations of which the phenotype is unknown. Whatever the mutation, CF is caused by improper formation of the CFTR protein which affects the ability to transfer chloride ions into and out of cells.

Class	Effect on CFTR protein	Example of mutation	% CF patients (Europe)
I	Shortened protein	W1282X Instead of inserting the amino acid tryptophan (W), the protein sequence is prematurely stopped (indicated by an X).	7
II	**Protein fails to reach cell membrane**	**ΔF508 A phenylalanine amino acid (F) is deleted**	**85**
III	Channel cannot be regulated properly	G551D A "missense" mutation: instead of a glycine amino acid (G), aspartate (D) is added	<3
IV	Reduced chloride conductance	R117H Missense	<3
V	Reduced due to incorrect splicing of gene	3120+1G>A Splice-site mutation in gene intron 16	<3

Table 1 Mutations grouped into classes according to their effect on the CFTR protein. Different mutations can have different effects on the disease. The most common is the deletion of amino acid 508 as seen above
http://www.nchpeg.org/nutrition/index.php?option=com_content&view=article&id=462..&limitstart=4

Inheritance

As CF is an autosomal recessive disease, inheritance occurs only when a child gets a mutated copy of CFTR gene from each parent. If only one parents hands down a defective gene the child will express enough normal genes to avoid CF complications. People who have only one affected gene are known as carriers and would not know unless genetic screening was performed. To avoid having a child with CF parents can undergo genetic tests prior to conception. The chance of having a child with CF when both parents are carriers is 25%.[2]

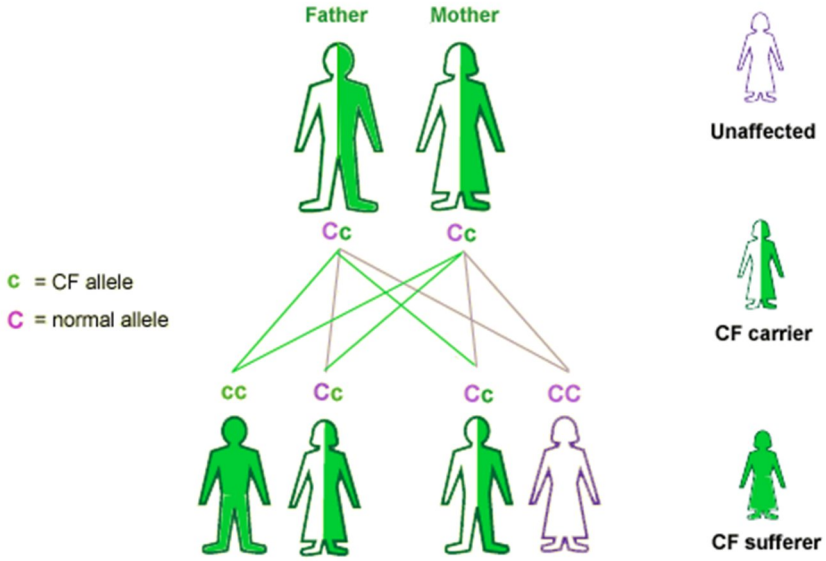

Figure 4 Inheritance of CF http://www.bbc.co.uk/schools/gcsebitesize/science/21c/genes/genetic_diseasesrev2.shtml

Pathophysiology

Depending on the mutation the effects of CF can be:

- Idiopathic pancreatitis – inflamed pancreas
- Rhinosinusitis – chronic inflammation of the sinus
- Bronchiectasis - damage to airways due to widening
- Allergic bronchopulmonary aspergillosis – over response of the immune system
- congenital bilateral absence of the vas deferens – defective male sex organs leading to infertility
- Children born with CF are generally underweight.

[2] http://cysticfibrosis.about.com/od/cysticfibrosis101/f/CFcarrier.htm

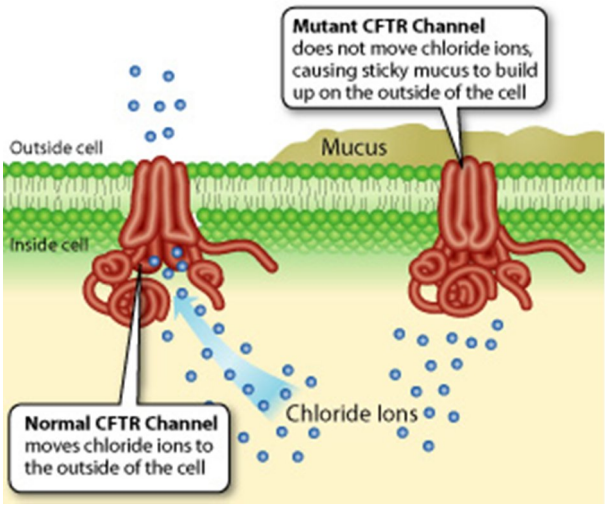

Figure 5 mutant formation vs. normal formation and its effects on the cell

http://learn.genetics.utah.edu/content/disorders/whataregd/cf/

Testing and diagnosis

Non-genetic tests

- **Sweat test**: Test to find the level of chloride in sweat. The higher it is, the more likely hood the subject has CF.
- **Phenotypical features**: *see pathophysiology section*
- **Prenatal ultrasonic findings**: hyperechoic bowel pattern can be a prenatal marker for CF
- **Meconium (early foetus faeces) test**: test for abnormally high protein concentration
- **Observable meconium abnormalities**:
    - Delayed passage of Meconium: delay in first antenatal stool
    - Meconium ileus or Meconium plug syndrome: blockage caused by thickening of the stool due to thick mucus associated with CF.

Broader genetic methods and gene sequencing

Below are the different techniques to look for missing or mutated genes. The aim in all cases to seek out areas of interest and check DNA is coding, properly, for all areas of the CFTR protein.

- **PCR**: Creating copies of DNA. In the case of CF primers[3] can be used to copy the offending area and analyse it. For example, as a CF gene has 3 missing base pairs the resultant copy will be 151bp as opposed to the normal 154bp.

[3] Short nucleic acid sequences that are designed to replicate a certain region of DNA

- **Micro-arrays**: to test gene expression of cells known to effect CF
- **FISH**: using a florescent copy DNA or RNA copy of the area (sequence) to find the area (CFTR) so it can be analysed.

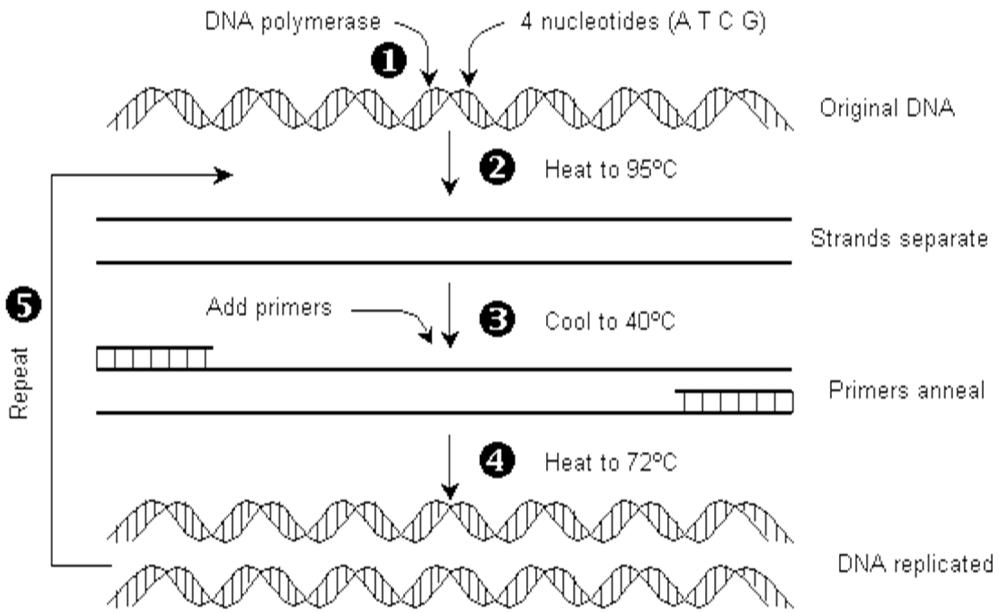

Figure 6 an example of PCR. In this example primers would be designed based on the target area of the gene

http://www.biologymad.com/master.html?http://www.biologymad.com/geneticengineering/geneticengineering.htm

Specific tests

Below are the tests a doctor might order if CF is suspected. Appropriate samples can be taken from blood or blood spots, tissue or amniotic fluid.

- **Poly-T analysis:** Differences in length of the polythymidine (Poly-T) tract in intron 8 can alter the disease state. The poly-T tract, which is present in all copies of CFTR, occurs in one of three forms: 9T, 7T or 5T. Which one is present will indicate the severity and presentation of the disease. *See table 2*
- **ACMG/ACOG Mutation Panel**: using primers designed to seek out known mutations, this test targets 23 mutations as recommended by the ACMG[4] and the ACOG[5] that is shown to reveal 98% of CF cases. *See PCR above*
- **Full CF Gene sequencing**, usually done by shotgun sequencing: This is analysing the entire gene to find out which codes are or are not present. As it is not targeting a specific area, but

[4] American College of Medical Genetic
[5] American College of Obstetricians and Gynecologists

looking at the entire gene, it is expensive and time consuming, but very successful and will find around 98% of all mutations.[6]

Second mutation (R117H + x)	Predicted outcome
5T	Will likely cause CF disease
7T	Will unlikely cause CF disease
9T	Will highly unlikely cause CF disease

Table 2 Predicted outcomes based on which Poly-T form is present in combination with CF causing mutation F508del

Target patients

- Prenatal where one or both parents are known to carry a mutation of the CFTR
- Carrier[7] testing via to conception
- Patients with positive sweat test results or presenting signs of CF

Conclusion

Cystic Fibrosis is one of the most common chronic, genetic illnesses in the world. Approximately 1 in 25[8] people are carriers but few would even know they have it. Until recently non-genetic tests were done based on presenting symptoms and it was rarely detected pre-birth, let alone predicted before conception.

Target testing of small area of a gene have vastly improved in recent years enabling "needle in a haystack" type precision when it comes to locating and analysing desired known areas. This coupled with a massive improvement in the knowledge of the CFTR gene, and it's mutations, has enabled scientists to develop cheap, quick, accurate and non-invasive tests to detect CF at all stages of the disease. As CF is common many tests have been developed by many different groups to meet the high demand. For very rare genetic diseases this is not always the case, however the full human genome sequence has gone a long way to providing such diseases with appropriate tests.

Map TODO import

http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?taxid=9606&build=current&chr=1&MAPS=ugHs,genes,rnaHs-r&cmd=focus&fill=40&query=uid(-2099664007)&QSTR=7q31%2E2

[6] http://cysticfibrosis.about.com/od/diagnosis/a/adv_DNA_test.htm
[7] Subjects with only one copy of a mutated gene
[8] https://www.cysticfibrosis.org.au/

Appendix

A. CFTR nucleotide sequence

```
   1 atggatttgg atgctgtgga gaaaatattc agggtgagac aaagcaactc cagaattaat
  61 gctagagtat ttttaaatct ttttttttct tttttaaatt tgcttagatg gacaaaccca
 121 attttgaaaa agggttacag acgacggttg gagctctcag atatttatca aatcccttct
 181 gcagactctg ctgataatct ttctgaaaaa ctggaaagag aatgggacag agagctagca
 241 acttcagaga agaaacccaa actcatcaat gctctgcgac gatgcttttt ctggaaattt
 301 atgttctatg gaataatttt atatttagcg gaagtcacca aagctgtgca gcccccttctg
 361 ctggggagaa taatagcttc ctatgaccca gacaactctg atgaaagatc catagcctac
 421 tacctgggca ttggcttgtg cctcctcttt gttgtgagaa cactgcttat ccaccctgct
 481 atattcggtc ttcatcacat cggaatgcaa atgaggatag ctatgtttag cttgatttat
 541 aagaagatcc taaagctgtc aagcagagtt ctagataaaa taagtactgg acaattggtc
 601 agtcttcttt ccaacaacct gaacaagttt gatgagggtc ttgctctggc tcattttgta
 661 tggattgcac cattacaagt ggcactgctg atgggattgc tctgggatat gttagaagct
 721 tctgcatttt ctggacttgc ttttctaata gtcatggtct tattccaagc ctggctggga
 781 caaatgatga tgaaatacag gaataagaga gcaggaaaga tcaatgagag acttgtcatc
 841 acttcagaaa taattgaaaa tatacagtca gttaaagcct attgttggga agatgcaatg
 901 gaaaaaatga ttgaaaacat ccgtgaaact gaactgaagc ttacccgaaa agctgcttat
 961 gtgagatatt tcaacagctc agccttcttc ttctcaggct tttttgtggt gttcttggct
1021 gtgcttccgt atgctgtgat taaaggaatt actctcagaa aaatattcac caccatttcc
1081 ttctgcattg ttcttcgaat gacagtgacc aggcagtttc ccggctctgt gcagacctgg
1141 tatgactcta ttggagcaat aaacaaaata caggatttct tgctgaaaaa agaatataaa
1201 gctctggagt ataatctaac aaccactggg gttgagctgg acaaagtaac agctttttgg
1261 gatgagggaa ttggagagct atttgtaaaa gcaaaccagg aaaacaacaa tagcaaagct
1321 cctagcactg acagaaatct cttcttcaat aattttcccc ttcatgcctc tcctgttctt
1381 caggacataa atttcaagat tgagaagggg cagctattgg ctgtttctgg atctactgga
1441 gccggcaaga cttctcttct gatgttgata atgggagaat tggagccttc acagggtaag
1501 attaagcaca gtggaaggat atccttttca cctcaagtct cctggatcat gcctggaaca
1561 ataaaggaga acataatttt tggtgtatcc tatgatgaat accggtacag gagtgtcatc
1621 aaagcctgcc aactagagga ggatatttcc aagttcccag aaaaagatta tactctgctg
1681 ggtgaaggtg gaatcattct gagtggaggt caacgagcac ggatctcact ggcaagggca
1741 gtgtacaaag atgctgattt gtatctcctg gattctcctt ttggacactt agacattttt
1801 acagaaaaag agatatttga aagctgtgta tgcaagttga tggcgaataa aacaaggatc
1861 ttggttactt caaaattgga acacttaaag attgctgaca aaatattaat cttacatgaa
1921 gggagctgct atttctatgg aacattttct gaacttcagg gtcaacggcc agacttcagc
1981 tcggagctga tgggatttga ctcttttgat cagttcagtg cagagagaag aaattcaatc
2041 cttactgaga ctctccgacg attttccatt gaaggagaag gcgtgggatc gcgcaatgaa
2101 ataaagaagc aatcttttaa acaaaaatca gatttgaacg acaagaggag gaactcagtg
2161 attattaacc cccttaacgc aaataggaag ttctcagtgg tgcagaagaa cggcatgcag
2221 gtcaatggga tagaggatgg gcacagtgac ccgctggaga ggagggtctc gctggtgccc
2281 gacgtggagc agggtgatgt ggctctgctg aggagtggga tgctcaagcc tgaccatctg
2341 ctgcagggcc acaggaggca gtcggtgctc aacctcatga cgagcacctc tgtcagctac
2401 ggaccaaact tccccaagaa gggaagtaca acctttcgga aaatgtccat ggtgcctcag
2461 acaaacctgt ctgctgagat ggatatctac accaggcggg tgtcacgaga cagcgtcctg
2521 gacataactg atgagatcaa tgaagaggat ctgaaggagt gcttcagtga tgatgctgaa
2581 agcatgggta ctgttactac atggaatacc tatttcagat acgttactat ccacaaaaac
2641 ttgatttttg ttctgatttt gtgtgtgatt gtcttcttag ctgaggtagc tgcttctctt
2701 gctggggtat ggtttcttca aaagacagct ttgaaggcaa attcaaccca gtcagaaaac
2761 agcacctctg acaaacctcc tgtgatagtc actgacacta gcagctacta catcgtttac
2821 atctatgtgg gtgtggcaga tactctgctt gccatgggaa tcttcagagg tttgcccctt
2881 gtgcacacac ttataacagt gtctaaaact cttcatcaga agatggtgca tgcagttctt
2941 catgcaccta tgtcaacctt caactcttgg aaagcaggtg gtatgcttaa cagattctca
```

```
3001 aaagatacgg ccatactgga tgacatactt ccacttacag tatttgactt cattcagtta
3061 atactgattg tgattggcgc tataacagta gtctcaatat tagaacccta catcttcctg
3121 gcatcagtgc ctgtgatagc agcctttatt ctgttaaggg catacttcct ccacacttct
3181 cagcagctga aacaactgga atctgaagct cggagtccaa ttttcaccca ccttgttaca
3241 agcttaaaag ggctgtggac actgcgagct tttgggcggc agccgtattt tgagaccttg
3301 tttcacaaag ctctgaacct ccacacagca aactggttcc tctacctgtc aacactgcgc
3361 tggttccaga tgaggataga aatcattttt gttgtctttt ttgttgctgt ggcatttatc
3421 tctatcataa ctacaggtga aggaccaggt acagctggaa ttattcttac tttagctatg
3481 aacatcatgg gaaccttgca gtgggcagta aactcaagca tagatgttga tagtttgatg
3541 cggtctgtcg gacgaatatt taaattcatt gacatgccaa cagaagagac agaaagcatt
3601 aagccacaga agaataatca gttttcagat gcccttgtaa ttgaaaacag gcatgtgaag
3661 gaagagaaga actggccatc tgggggccaa atgactgtga aagaccttac agccaaatat
3721 ggtgaaggtg gacctgctgt actagaaaac atttcttttt caataagttc agggcagagg
3781 gtgggccttt taggaagaac aggttcagga aaaagcactt tacttttgc atttttaaga
3841 ctgctgaata cagaagggga tatccagatt gatggagtct cctggaacac agtcagcgtg
3901 cagcaatgga gaaaagcatt tggagtgatt cctcagaaag tgttcatatt ttctgggagt
3961 tttaggatga atttagatcc ttatgggcag tggaatgatg aagaaatatg gaaagttgca
4021 gaggaggttg ggctgaagtc tgtaatagag caatttccag gcaagcttga ttttgttctt
4081 gtggatggag gctgtgtcct cagccatggc cacaagcagc tgatgtgcct tgcccggtca
4141 gttctcagca aagctaaaat cctgctgctt gatgaaccaa gtgctcacct ggaccctgta
4201 acttcccaag tgatcaggaa gaccctgaag cacgcatttg ccaactgcac agtgatactc
4261 tccgagcaca gactggaggc aatgctggaa tgccagagat ttttggtgat tgaggacaat
4321 aagctgaggc agtacgaatc ccttcagaag ctgctgaatg agaagagctc cttcaggcag
4381 gccatcagtc acgctgagcg ccttaagctg ctgccagcac accacagaaa ctccagcaaa
4441 cgtaaacccc gaccccaaat cactgccttg caggaggaga cagaggaaga agtgcaggag
4501 acaagactgt ga
```

B. CFTR resultant protein sequence

Amino acid sequence which will fold into the CFTR protein as coded by the CFTR gene

MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLEREWDRELASKKNPKLI
NALRRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEERSIAIYLGIGLCLLFIVRTLLLHP
AIFGLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFVWIAPLQVAL
LMGLIWELLQASAFCGLGFLIVLALFQAGLGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEEA
MEKMIENLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVFLSVLPYALIKGIILRKIFTTISFCIVLRMAV
TRQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGFGELFEKAKQNNNNRK
TSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGKTSLLMVIMGELEPSEGKIKHSGRISF
CSQFSWIMPGTIKENIIFGVSYDEYRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLAR
AVYKDADLYLLDSPFGYLDVLTEKEIFESCVCKLMANKTRILVTSKMEHLKKADKILILHEGSSYFYGTF
SELQNLQPDFSSKLMGCDSFDQFSAERRNSILTETLHRFSLEGDAPVSWTETKKQSFKQTGEFGEKRKNS
ILNPINSIRKFSIVQKTPLQMNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVL
NLMTHSVNQGQNIHRKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEINEEDLKECFFDDMESI
PAVTTWNTYLRYITVHKSLIFVLIWCLVIFLAEVAASLVVLWLLGNTPLQDKGNSTHSRNNSYAVIITST
SSYYVFYIYVGVADTLLAMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSKDI
AILDDLLPLTIFDFIQLLLIVIGAIAVVAVLQPYIFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSP
IFTHLVTSLKGLWTLRAFGRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISIL
TTGEGEGRVGIILTLAMNIMSTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEGKPTKSTKPYKNGQLSKV
MIIENSHVKKDDIWPSGGQMTVKDLTAKYTEGGNAILENISFSISPGQRVGLLGRTGSGKSTLLSAFLRL
LNTEGEIQIDGVSWDSITLQQWRKAFGVIPQKVFIFSGTFRKNLDPYEQWSDQEIWKVADEVGLRSVIEQ
FPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLKQAFADCTVILC
EHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAISPSDRVKLFPHRNSSKCKSKPQIAALKEE
TEEEVQDTRL

Works Cited

1. Orenstein DM, Rosenstein A, Stern D, Cystic Fibrosis, Lippincott Williams & Wilkins; 2000. p. 21-37.
2. Genetics Home Reference, your guide to understanding genetic conditions, CFTR, Available from http://ghr.nlm.nih.gov/gene/CFTR
3. Winikates, Kristina, "Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene". *Embryo Project Encyclopedia* (2012-01-01). ISSN: 1940-5030 http://embryo.asu.edu/pages/cystic-fibrosis-transmembrane-conductance-regulator-cftr-gene

http://ghr.nlm.nih.gov/gene/CFTR

http://embryo.asu.edu/pages/cystic-fibrosis-transmembrane-conductance-regulator-cftr-gene

http://www.nchpeg.org/nutrition/index.php?option=com_content&view=article&id=462..&limitstart=4

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3313698/

http://www.cftr2.org/r117h.php