Testing the Severity of the DeltaF508 Genotype in Patients With Cystic Fibrosis

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Abstract

The purpose of this study was to understand the various mutations of cystic fibrosis on chromosome seven, particularly the most commonly recognized mutation, ΔF508. Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in Caucasians, affecting about one in 2,500 live births, with a carrier frequency of one in 25. Through this research we demonstrate that ΔF508 genotype actually can predict disease severity in an ethnically diverse CF population, to an extent. Clinical and genetic data were collected for 214 Cystic Fibrosis patients and entered into a database. The diagnosis of Cystic Fibrosis is based on clinical symptoms, particularly respiratory and digestive complications, and a raise in the electrolytic ion concentration in sweat. Upon complete statistical analysis of the data, including haplotype verification, it can be determined that the ΔF508 genotype does suggests that patients with two ΔF508 allele demonstrate a greater degree of irregularity with the digestive system.
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LITERATURE REVIEW AND INTRODUCTION

A Clinical Look at Cystic Fibrosis

Cystic Fibrosis is the most common autosomal recessive disorder in Caucasians (Witt, 1991). It affects about one in 2,500 live births, and has a carrier frequency of one in 25. This inherited disease has its main effects on the digestive system and the lungs. It is usually diagnosed soon after birth, and symptoms occur throughout life. The name cystic fibrosis, or CF, originates from the changes in the pancreas that occur in patients diagnosed with this disease. The pancreas is a major organ in the body that manufactures digestive enzymes. The part of the pancreas that produces these enzymes is replaced by characteristic fibrous scar tissue with fluid filled spaces called cysts. In 1938 Dorothy H. Anderson from Columbia University gave this disease its name calling it "cystic fibrosis of the pancreas," based on these microscopic features she observed in the pancreas when performing autopsies on infants and children (Welsh, 1995). Later this disease simply became known as cystic fibrosis.

Much of what is known about the disease has been discovered in the last decade. Only until the beginning of this century did doctors recognize the disease in its own right. The various symptoms were merely seen as separate unrelated infections (Harris, 1987). In fact, it was not even recognized as a disease until the 1930's, when Dorothy Anderson documentated a correlation between symptoms and scars on the pancreas. Today much more is known about the disease, and not only the pancreas is affected. Manifestations relate to disruption of exocrine function of the pancreas and also to intestinal glands, biliary tree, bronchial glands, and
sweat glands. The presenting clinical symptoms of the disease are related mainly to chronic obstructive pulmonary involvement and malfunction of the exocrine glands, leading to pancreatic enzyme insufficiency and elevation of sodium and chloride ions in sweat (Witt, 1991).

The scientific community is becoming increasingly reluctant to view this disease as a fatal genetic disorder. The median age of survival has gone from less than five years in the late 1950's to about twenty-nine today (Silverstein, 1994). In patients with CF, a thick mucus is secreted into the lungs, which is unable to be removed by the normal ciliary action in the respiratory tree. As a result, a build up of mucus, which can have several consequences, arises in CF patients.

Emphysema can develop from the over-inflation of the lungs with the mucus; which can promote distended alveoli at the surface of the lung can rupture causing pneumothorax; air between surface of the lung and the chest wall, and even deformity. This thick mucus has a tendency to clog the upper and lower respirator tracts since they are not very easily cleared. Because this serves as a media for bacteria, bacterial infections and respiratory failure are often the cause of death in many patients. The most common strain of bacteria in these patients is initially *Staphylococcus aureus*, and later in the course of the disease, infection by *Pseudomonas* bacteria may occur. *Pseudomonas aeruginosa* only infects damaged tissue, and once it is acquired, it is seldom eradicated. However, lung transplantation is a treatment option in advanced pulmonary lung disease (Kerem, 1992).

Although most deaths are related to respiratory failure, another system that often exhibits complications is the digestive system. The major effects of cystic fibrosis are on the pancreas. Due to tissue damage caused by deposits
of dried up secretions, the pancreas ceases to function properly. As a result, patients with this disease often experience malabsorption (Food and nutrients are not adequately digested and cannot be absorbed normally in the intestine). A wide range of symptoms are observed including an abnormal excretion of fat in feces (steatorrhoea) and deficiency of fat soluble vitamins, proteins, carbohydrates, minerals, other vitamins, and water.

Another characteristic, and often the first indication of cystic fibrosis, is meconium ileus. This is a condition that occurs in ten to fifteen percent of babies with cystic fibrosis. Meconium (first stools of newborns) becomes impacted in the intestine and causes bilious vomiting and swelling of the abdomen. Surgery is generally necessary to remove the blockage, and a procedure called Bishop-Koop ileostomy is performed. The immediate prognosis improved remarkably after the introduction of this procedure in the late 1960's (Harris, 1987). Attacks of complete or impartial intestinal obstruction can occur later in life and are diagnosed as a meconium ileus equivalent.

Along with these respiratory and digestive complications of CF, many other problems arise. Patients can be afflicted with diabetes, cirrhosis of the liver, infertility, and a marked increase of electrolytes in their sweat. Increased electrolytes, measured from a sweat test in most circumstances, can confirm the diagnosis of cystic fibrosis. A normal child will contain sodium and chloride concentrations of between fifteen and thirty millimoles per liter. Concentrations of sodium and chloride of greater than seventy millimoles per liter are diagnostic of CF.
The Underlying Genetic Causes of CF

Many speculations and theories have arisen concerning the actual genetic and biochemical causes of CF. Some believed it was a gene affecting chloride ion channels; others proposed that it was a gene that produced dyskinesis of the cilia. Still others thought the defect was in an enzyme that puts fatty acids on glycoproteins. Although there were many more theories, all of which could explain some phenomenon in cystic fibrosis, a sufficient answer was not given and supported to account for all the symptoms of this genetic disorder until recently.

In order to truly understand this disease one must try to detect where and how a specific defect is upsetting an entire system. The cystic fibrosis literature is voluminous and filled with contradictory and confusing accounts with unreproducible results (Kolata, 1985). After surveying this literature, Efraim Racker of Cornell University remarked that "anyone who isn't thoroughly confused just doesn't understand the situation." However, great strides have been made recently to help clear up this confusion. The cystic fibrosis disorder is found to be located on chromosome 7. This finding was announced in 1985 by Lap-Chee Tsui of the Hospital for the Sick in Toronto, Bob Williamson in London and Ray White of the University of Utah. They established this by a demonstration of linkage to a set of polymorphic DNA markers in family studies. Once this was confirmed, the search for the actual gene began. This was done by a process of reverse genetics. In fact, cystic fibrosis represents the first genetic disorder elucidated by this process (Day, 1989). Later, this process of reverse genetics was renamed to positional cloning. It is a process performed on the basis of map location but without the availability of chromosomal rearrangements or deletions. The gene was
identified in 1989 by Lap-Chee Tsui and John Riordan of the Hospital for the Sick in Toronto, and Francis Collins of the University of Michigan, and their colleagues. The CF gene proves to be about 250,000 base pairs long and consists of 27 exons. The encoded polypeptide is made of 1480 amino acid residues, a number of deduced structural and functional domains, and two glycosalation sites.

With the identification of this gene it has become clearer as to how a defect of this sort can cause the disease. The encoded gene product was named cystic fibrosis transmembrane conductance regulator (CFTR). CFTR corresponds to a cAMP-regulated chloride channel found almost exclusively in the secretory epithelial cells. A major mutation that accounts for a single amino acid deletion (ΔF508) accounts for 70% of the disease alleles. In this prominent mutation a deletion occurs in an entire codon (3-base pairs) leaving out the code for phenylalanine, which is obviously an essential portion of the CFTR. Besides this common mutation, more than 550 additional mutant alleles of different forms have been detected, and another 5-10 are being uncovered each month (Zielenski & Tsui, 1995). Mutations in the CFTR gene fall into several different categories. There are missense, nonsense, frameshift, and RNA splicing mutations. Additionally there are several amino acid deletions, two large in-frame deletions, and a large, complex deletion spanning exons 4-7 and 11-18.

Knowledge of the functional properties of the CFTR gene was obtained through DNA transfection studies showing that CFTR cDNA could reverse the defect of chloride conductance of epithelial cell culture derived from CF patients. Also a reconstituted lipid bilayer study with purified CFTR gene product made in heterologous cells demonstrated that the protein could
function as a cAMP-regulated chloride channel. Now with an improved understanding of epithelial cell ion transport, we can construct a logical sequence for the pathogenesis of the lung insult and other disorders in patients with cystic fibrosis.

The CFTR gene codes for a multidomain polypeptide that shares similarity with many other transporter proteins (Zielenski & Tsui, 1995). In these proteins there are two nucleotide-binding folds that bind ATP (NBF1 and NBF2) and two hydrophobic transmembrane domains that each have six membrane-spanning segments. These membrane spanning segments are usually designated as transmembrane segments and numbered from one to twelve. They are labeled as merely the membrane spanning portion in Figure 1. These nucleotide binding folds (NBFs) contain sequence motifs called Walker A and Walker B. These are conserved among ATP-binding proteins. These NBFs are believed to bind and hydrolyze ATP following cAMP-mediated phosphorylation of the R-domain. The R-domain is a highly charged domain that links two halves of the polypeptide. It is believed that NBF1 is phosphorylated upon partial phosphorylation of the R-domain leading to the channel opening, whereas complete phosphorylation leads to phosphorylation of NBF2 causing the closing of the channel (Zielenski & Tsui, 1995). This protein constitutes a low-conductance (8-10pS) chloride channel in the apical membranes of epithelial cells.

The R-domain is a unique feature for CFTR. It contains a high portion of polar amino acids and several consensus phosphorylation sites believed to be important for regulation of the ion channel. Recent studies show that phosphorylation of different sites may have either a stimulatory or inhibitory effect on CFTR channel activity (Zielenski & Tsui, 1995). The
cAMP-activated phosphorylation of the R-domain is necessary for channel activity, but it is not sufficient for the CFTR channel opening. Presumably, phosphorylation of the R-domain by protein kinase A changes its conformation and regulates the gating of the CFTR chloride channel (Dulhanty, 1994). This conformation change may provide the ATP hydrolytic capacity of the NBFs that, in turn, controls the channel opening and closing.

The CFTR protein is believed to have two different functions. One part of this protein acts as an ion channel, and the other part acts as the control of the gate. This was unusual to see both the regulators of ion control and the ion channels in one protein (Silverstein, 1994). Therefore, as a result of malfunctioning of this protein, chloride ions are not excreted sufficiently and an increase in sodium ions are taken into the epithelial cell. As a result, the cell absorbs more water and allows for a more viscous mucous.

Figure 1. A. The Cystic Fibrosis transmembrane Conductance Regulator Gene
B. The Cystic Fibrosis transmembrane Conductance regulator Protein.
The relationship between the phenotype and the absence of this protein are not completely understood. Many of the problems associated with cystic fibrosis are inadequately explained with our current knowledge of this defect. For instance we still do not know how clubbing of the fingers or congenital absence of the vas deferens relates completely to the absence of this protein. However, we now understand how this ion trafficking disfunction can clog up many different systems. In one set of investigations, Paul M. Quinton found that the epithelial lining of the ducts of the sweat glands failed to take up chloride from the lumen, or cavity, of the glands. This explained why there is an abnormal amount of ions in the sweat. Sweat is produced at the base of the sweat glands; it then flows to the skin surface through a narrow duct. As it flows through the duct, ions should escape into the epithelium, leaving water behind. In patients with CF this intake of ions does not occur. As a result, diagnostic tests evaluating the amount of ions in the sweat was based on this naturally occurring phenomenon in these patients. Cystic fibrosis occurs when there is a disorder of the CFTR gene and the protein for which it encodes is either missing or altered in such a way that it cannot perform its specific function. These disorders of the gene result from mutations. It is possible to divide CFTR mutations into five general classes. The first class is where there are mutations affecting biosynthesis. In these patients there is absolutely no CFTR protein present in the apical epithelial cell. Another class is mutation affecting protein maturation. This is the class in which F508 falls. Here the protein is actually synthesized, but due to improper folding, it is mislocalized. As a result, it is soon degraded in a pre-Golgi compartment. Other classes include the mutation affecting chloride regulation, mutations affecting chloride conductance of channel gating, and
mutations causing reduced synthesis (Figure 2). In figure 2, we can see an example of how different types of mutations can result in the mislocalization or deformation of the CFTR protein. As mentioned earlier, a block in the processing of the CFTR is the cause of CF in patients with the genotype AF508.

**Figure 2.** Genetic problems that can arise in the CFTR protein in cystic fibrosis.

**Finding Trends in the ΔF508 Genotype**

Problems in the CFTR can arise due to a variety of malfunctions. Some examples of these are seen in figure 2. The one we have focused on in this research is the block in the processing of CFTR due to the ΔF508 deletion (Type II). Since this deletion is so prevalent (70%) among the cystic fibrosis
mutation. In order to do this, a complete data base was constructed from a population of CF patients. Everything from electrolytic sweat ion concentration to weight was analyzed and carefully sorted through in an attempt to either confirm or disclaim any trends or manifestations specifically identifiable with the ΔF508 genotype.
Materials and Methods

To determine the severity based on the mutation we had to first arrive at a consensus as to what determines severity. Realizing that this can be subjective, parameters were made, and specific symptoms of 214 patients were entered into a data base for further review and statistical analysis. The program that was used was SPSS 6.0 for Windows (SPSS Inc. in Chicago, Illinois). This is a comprehensive and flexible statistical analysis and data management program designed to take data from files and use it to obtain descriptive statistics, to perform complex statistical analyses, and to generate tabulated reports, charts, and plots of distributions and trends. Data was entered under the following headings:

(1) name
(2) gen1 (genotype)
(3) gen2
(4) twotype (ΔF508 summarization)
(5) haplotyp
(6) dob (date of birth)
(7) diag.dat (date of diagnosis)
(8) age
(9) deathage
(10) sex
(11) sweatcl (sweat chloride levels)
(12) sclwt (Sweat chloride weight)
(13) present (presentation of symptoms)
(14) p.cont (presentation continued)
(15) pneumoth
(16) hemoptys
(17) r.a.dis (reactive airway disease)
(18) atopy
(19) polyps
(20) oxygen
(21) giheight (gastrointestinal height)
(22) hper100 (percentile of height)
(23) giweight
(24) wper100
(25) pancreas
(26) r.prolap (rectal prolaps)
(27) enzyme
(28) diabetes
(29) vitamina (vitamin A levels)
(30) vitamine (vitamin E levels)
(31) igg (immunoglobulin G)
(32) ige (immunoglobulin E)
(33) iga (immunoglobulin A)
(34) fvc (forced vital capacity)
(35) fev (forced expiratory flow volume in 1 second expected volume)
(36) fef (forced expiratory flow)

1Due to the constraints of this program full complete labels could not be given to organize the data.
The previous headings for the data represents a complete collection of all the data obtainable for each patient. A few that need to be clarified are: (4) twotype, (13) present, (14) p. cont, (15) pneumoth, (16) hemopty, (18) atopy, (19) polyps, and (27) enzyme. The column labeled "twotype" was constructed to show the frequency of ΔF508 alleles. This column was added after the entry of the data. It represents whether or not the patients had either no, one, or both of the ΔF508 mutation in their diagnosis of cystic fibrosis. This became increasingly important in our study in finding a correlation between severity and this genotype. One factor that needs to be recognized is the small sample size of patients that did not have any ΔF508. Due to this small size, accurate hypothesis testing was impossible.

Present (presentation) and p. continued were columns that were constructed to show the presentational symptoms for each individual patient. Pneumoth (pneumothorax) is a condition where there is a partial or complete collapse of the lungs due to leakage of air that becomes trapped between the lung and the chest wall. Hemopty was an abbreviation for hemoptysis, which is merely the technical term for the coughing up of blood. Atopy describes a condition where a person is predisposed to a certain allergic response. Polyps are outgrowths of unnecessary tissue. The column labeled "enzyme" records whether or not the patient was currently taking digestive enzymes due to the deteriorating effects on the pancreas due to CF.

A column was made for each of the above headings, and the appropriate data was then entered into each one. If the data was not in a numerical form, a number was assigned and a label was then given to the number. This allowed us to run numerical tests on all the data. One example was with the genotypes. ΔF508 was entered into the data base as "one," and R117H was entered in as
"two." This allowed for quick and convenient entry, and also aided in the hypothesis testing.

This data was then analyzed to find any trends or differences that might be associated with the different genotypes. Then, the collected data was organized into two separate tables. In the first table (Table 1), binomial data, or data that could be answered as either yes or no was placed and analyzed. The number of people that were positive for a given symptom were tallied and recorded in one column. A percentage of the positive answers, which take into account the number of missing entries, was recorded for each group. In the other table (Table 2), data that involved numerical entries was compiled and the mean and standard deviation was computed with the aid of SPSS. Again, this data was collected from two hundred and fourteen cases. However, all these cases were not applicable for testing because of incomplete entries on the initial sheets. As a result, the number of missing cases were taken into account, and this was subtracted from the total number of cases to find the sample size (see Table 1).

The collected data from both tables was analyzed even further, and hypothesis testing was done at a 1% level of significance to see whether or not any correlation could be made. As recognized earlier, we had a very small sample size, particularly in the category of no AF508. Due to this factor contingency testing was not reliable. As a result, the data being tested for significance was only between the one and two AF508 categories. However, a list of data for the patients with no AF508 mutation was compiled, and we looked for trends or areas that might be tested in further studies. In the first table, a test of comparison between two proportions was used. The hypothesis being tested was whether the proportion of patients exhibiting a particular characteristic was significantly different (greater than or less than) for those in the two AF508 category compared to those in the one AF508 category.
<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Missing</th>
<th>% affected</th>
<th>No.</th>
<th>Missing</th>
<th>% affected</th>
<th>No.</th>
<th>Missing</th>
<th>% affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>120</td>
<td></td>
<td></td>
<td>74</td>
<td></td>
<td></td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>68</td>
<td>0</td>
<td>56.7%</td>
<td>29</td>
<td>0</td>
<td>39.2%</td>
<td>12</td>
<td>0</td>
<td>63.2%</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td>52</td>
<td>0</td>
<td>43.3%</td>
<td>45</td>
<td>0</td>
<td>60.8%</td>
<td>7</td>
<td>0</td>
<td>36.8%</td>
</tr>
<tr>
<td><strong>Atopey</strong></td>
<td>10</td>
<td>27</td>
<td>10.8%</td>
<td>6</td>
<td>22</td>
<td>11.2%</td>
<td>1</td>
<td>1</td>
<td>7.7%</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>6</td>
<td>8</td>
<td>5.4%</td>
<td>2</td>
<td>2</td>
<td>2.8%</td>
<td>0</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Enzyme oxygen</strong></td>
<td>113</td>
<td>6</td>
<td>99.1%</td>
<td>64</td>
<td>3</td>
<td>90.1%</td>
<td>17</td>
<td>0</td>
<td>89.5%</td>
</tr>
<tr>
<td><strong>Hemotops</strong></td>
<td>3</td>
<td>17</td>
<td>2.9%</td>
<td>10</td>
<td>10</td>
<td>9.4%</td>
<td>2</td>
<td>4</td>
<td>13.4%</td>
</tr>
<tr>
<td><strong>Pancreas insuf.</strong></td>
<td>111</td>
<td>8</td>
<td>99.1%</td>
<td>63</td>
<td>4</td>
<td>90%</td>
<td>17</td>
<td>0</td>
<td>89.5%</td>
</tr>
<tr>
<td><strong>rectal prolaps</strong></td>
<td>8</td>
<td>12</td>
<td>7.4%</td>
<td>5</td>
<td>7</td>
<td>7.5%</td>
<td>1</td>
<td>3</td>
<td>6.3%</td>
</tr>
<tr>
<td><strong>Polyps</strong></td>
<td>20</td>
<td>11</td>
<td>18.3%</td>
<td>14</td>
<td>7</td>
<td>20.9%</td>
<td>5</td>
<td>3</td>
<td>31.3%</td>
</tr>
<tr>
<td><strong>pnemoth</strong></td>
<td>2</td>
<td>13</td>
<td>1.9%</td>
<td>1</td>
<td>8</td>
<td>1.5%</td>
<td>0</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td><strong>r.a. dis</strong></td>
<td>28</td>
<td>20</td>
<td>28%</td>
<td>12</td>
<td>11</td>
<td>19%</td>
<td>6</td>
<td>2</td>
<td>35.3%</td>
</tr>
<tr>
<td><strong>M.ileus</strong></td>
<td>21</td>
<td>0</td>
<td>17.5%</td>
<td>18</td>
<td>0</td>
<td>24%</td>
<td>2</td>
<td>0</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Digestive</strong></td>
<td>43</td>
<td>0</td>
<td>36%</td>
<td>13</td>
<td>0</td>
<td>18%</td>
<td>4</td>
<td>0</td>
<td>21%</td>
</tr>
<tr>
<td><strong>Respiratory</strong></td>
<td>18</td>
<td>0</td>
<td>15%</td>
<td>17</td>
<td>0</td>
<td>23%</td>
<td>6</td>
<td>0</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Dig./Resp</strong></td>
<td>20</td>
<td>0</td>
<td>17%</td>
<td>19</td>
<td>0</td>
<td>26%</td>
<td>7</td>
<td>0</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td>17</td>
<td>0</td>
<td>14%</td>
<td>4</td>
<td>0</td>
<td>5%</td>
<td>1</td>
<td>0</td>
<td>5%</td>
</tr>
</tbody>
</table>

**Table 1.** Percentages were calculated by taking the No.(positively affected) and dividing by total number of people minus those that were missing.
<table>
<thead>
<tr>
<th></th>
<th>ΔF508/ΔF508 Alleles</th>
<th>ΔF508/other Alleles</th>
<th>Other/Other Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>St. Dev.</td>
<td>S.size</td>
</tr>
<tr>
<td>Age</td>
<td>11</td>
<td>8.3</td>
<td>110</td>
</tr>
<tr>
<td>Ige</td>
<td>219</td>
<td>445</td>
<td>63</td>
</tr>
<tr>
<td>Iga</td>
<td>181</td>
<td>131</td>
<td>60</td>
</tr>
<tr>
<td>IgG</td>
<td>1143</td>
<td>511</td>
<td>62</td>
</tr>
<tr>
<td>SweatCl</td>
<td>100.28</td>
<td>29</td>
<td>98</td>
</tr>
<tr>
<td>Vit. A</td>
<td>41</td>
<td>15.08</td>
<td>68</td>
</tr>
<tr>
<td>Vit. E</td>
<td>9</td>
<td>7.15</td>
<td>71</td>
</tr>
<tr>
<td>FVC</td>
<td>37</td>
<td>40.7</td>
<td>85</td>
</tr>
<tr>
<td>FEV</td>
<td>31</td>
<td>36</td>
<td>84</td>
</tr>
<tr>
<td>FEF</td>
<td>25</td>
<td>33</td>
<td>76</td>
</tr>
<tr>
<td>Weight/Height</td>
<td>1.2</td>
<td>1.24</td>
<td>105</td>
</tr>
</tbody>
</table>

**Table 2.** Calculated data.
Another area that was investigated, in hopes of clarifying this work and possibly showing some correlation, was the identification of the haplotype. Many of these had previously been done at the diagnosing hospital, however over one hundred were left undone. This was due largely to the fact that most of these patients were F508 homozygous. As a result most of these patients had the haplotype BB. The current literature suggests that 90% to 95% percent of the patients with F508 will be BB (Devoto, 1989).

In order to deduce that haplotype, samples of the DNA were obtained from Oakland. With these samples 5 ml of DNA were extracted and added with 15 ml of DNA primer to make a ratio of 1:4. Then 2 ml were taken from this solution in order to perform the amplification of the DNA. This was done with a polymerase chain reaction (PCR). First, a suitable solution was made to allow for the prime conditions. In this solution Taq1 DNA polymerase, DNA primers, water, and the DNA were mixed. Then the different markers were run separately for each DNA. These markers were KM1 and KM2 in one set and XV-2c in another. The PCR was run at 94 degrees for two minutes, and then for 30 cycles.

After the amplification a test gel was set up, and ethridium bromide was used to stain the DNA. The gel was run at 80 volts for ten to twenty minutes, and then we could see whether or not amplification had occurred under ultraviolet light (figure 3). When the bands were white we went to the next step, which was to digest the DNA. We used two restriction fragment length polymorphisms (RFLPs) that reveal strong linkage disequilibrium with cystic
fibrosis genotype. One is detected with an XV-2c probe and the restriction enzyme TaqI and the other with a probe designated KM-19 and the enzyme PstI.
Results and Discussion

In this study 120 (56.3 %) of the patients were homozygous for ΔF508, 74 (37.7%) were heterozygous, and the remaining 19 (8.9%) contained other mutations (Figure 3). For each category and characteristic, the number of missing entries is noted and each percent is based only on the entries that were present (The data collected from the two hospitals had some incomplete entries) (Table 1).

Figure 3. Distribution of the ΔF508 allele.
As was noted earlier, because of the minimal amount of data in the other/other category, which represented patients with no ΔF508 allele, most of the analyses had to be linked to a comparison of the characteristics between patients with two ΔF508, and those with one ΔF508 allele. Another point of interest that could not be shown, due to the minimal data, was those patients that died. In this study we only had two patients that died. Both were homozygous for ΔF508. One patient died at the age of eight and the other patient died at the age of twenty-eight. Further studies may show a stronger correlation between two F508 alleles and the severity of this disease, according to the age of death.

In the first table (page 15), the hypothesis testing failed to reject all categories except for the enzyme, pancreas, and digestive categories. These were strongly rejected with p values of 0.002, 0.002, and 0.003, respectively. This suggests that the result of these categories are not random, and that patients that are homozygous for F508 appear to have an increased risk of developing problems in the digestive system. These problems seem to arise in the pancreas, a major organ of digestive enzyme control. Over 99% of patients had pancreatic insufficiency, and thus needed supplementary enzymes, compared to only 90% of the patients that were heterozygous for this allele. This resulted in a higher percentage of patients with F508/ F508 developing problems with the digestive system.

Another interesting aspect that was investigated was the issue of age. It was noted that the mean age for the people with two ΔF508 alleles was 5.4 years less than the age of patients with only one ΔF508 allele. We speculated that this could be one determinant for testing severity. We presumed that as patients progressed through the disease they would unfortunately die. Measuring the current age of a random population of people with this disease
could therefore tell us, without a complete case study, the distribution of ages in these groups of cystic fibrosis patients. To see if these tests showed any significance a t-test for independent samples was performed. The test showed that the variances were unequal and that there is a significant difference between the two. The confidence interval or difference (95% confident) was between 2.159 to 8.695. The standard error of difference for age was 1.648. It was concluded that we can use this as one factor to show that the patients that are homozygous for ΔF508 mutation have a more severe case of CF. This was concluded because the 5.4 years difference in age is significantly less. This younger age of patients is most likely due to a younger age at which the homozygous ΔF508 died, leaving a younger living sample population. If this was not a random sample size, we could not conclude this.

As for the haplotype study, the results were entered into the data base, and hypothesis testing was performed. Although this data might become significant in another experiment, the results merely confirmed previous tests.² There were no trends or significant correlations that could be made from these results.

We are able to see some large differences between in some other categories, like immunoglobulin E and in the functional vital capacity. However, a large standard deviation causes a failure to reject the null hypothesis, and so no strong correlations could be made with our data to support any other trends or variances. However, as stated earlier, further testing may prove valuable.

² That 90-95% of delta F508 patients have the BB haplotype
Summary

Cystic fibrosis causes a wide range of symptoms and infects nearly all organ systems of the body. However, it appears that patients that are homozygous for the ΔF508 allele have an increased risk of developing problems with the pancreas. This increased risk of pancreatic malfunctioning has led to greater digestive problems and to a greater need for enzyme usage. One interesting point is that the leading cause of death in patients with cystic fibrosis is associated with infections of the lungs. Since we see a lower mean age in people with two ΔF508 alleles, we deduced that this is because the people at the end of the age bracket die earlier. One reason they may die earlier is because they may be more susceptible to bacterial infections. However, our data did not measure this, and further studies are needed to reach definitive conclusions. Taking this into account we do suggest that CF patients with homozygous ΔF508 have more severe symptoms than those that are heterozygous for the allele. Although all cases of CF are a cause for concern, our increased knowledge about the physiological and genetic aspects of this disease have greatly aided our understanding and ability to treat this disease. With our increased knowledge of this disease, a possible cure could be within reach. Currently the Cystic Fibrosis Foundation is involved in more than a dozen different clinical trials. Four major areas of treatment being tested are the thinning of the mucus, reducing inflammation of the lungs, reducing bacterial infections in the lungs, and treating body organs affected by CF.

Currently a number of new treatments are being developed. Ronald G. Crystal discovered that a drug called DNase helps reduce the thickness of lung

3 DNase is an enzyme that cleaves DNA into smaller fragments
secretions tenfold. In 1990, Michael Knowles and colleagues found that amiloride\(^4\), a blood pressure medicine, helped cells absorb sodium when breathed into the lungs. Dr. Knowles along with Dr. Boucher found that they could increase the amount of chloride secreted from the cells to a more normal level by using ATP\(^5\) and UTP\(^6\). Other drugs that are coming into the market are alpha-1 antitrypsin and ibuprofen, both of which prevent tissue damage from elastase. Researchers at the University of Washington also have shown that the antibiotic tobramycin is capable of killing the Pseudomonas bacteria and decreasing the amount of this organism from one hundred to ten thousand times.

Since we know the specific cause of the disease, the malformation or absence of the CFTR gene, a correction of this disorder could possibly lead to a cure for cystic fibrosis. Replacing defective genes is done through a technique called gene therapy. There are still barriers to this process. First of all, researchers want to make sure that they have the right vehicle for transferring the gene. This is usually done with the aid of viruses. It becomes necessary to make sure these viruses will be able to transfer the gene effectively and safely, and not affect any other tissues besides the one targeted. Although there are a significant number of obstacles that need to be overcome until there is a successful cure, researchers are optimistic that a cure is near, thus changing the outlook of cystic fibrosis patients from a painful and short-lived future, to a full life with unlimited options.

\(^4\) This compound helps stimulate the sodium potassium ATPase

\(^5\) Adenosine-triphosphate

\(^6\) Uridyl-triphosphate


Rosenstein, Beryl, and Flotte, Terence, "Cystic Fibrosis- Current topics," New Direction in Treatment, Wiley and Sons Ltd, 1993
