Personalised medicine of Cystic fibrosis

Biochemistry and Molecular biology
Pharmacology and Therapeutics
Pharmaceutical technology

Antoni Cabré Juan
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Index

1. Abstract / Resum .................................................................................................................. 1
2. Integration of three knowledge areas ................................................................................. 2
3. Introduction .......................................................................................................................... 3
4. Personalised medicine of cystic fibrosis ............................................................................ 11
   4.1 Ivacaftor .......................................................................................................................... 16
5. Aims ....................................................................................................................................... 20
6. Materials and methods ....................................................................................................... 20
7. Results and discussion. ....................................................................................................... 21
   7.1 Structure, functions and mutations of CFTR .............................................................. 21
   7.2 Microarray analysis and network construction .......................................................... 25
   7.3 Impact of G551D mutation .......................................................................................... 25
   7.4 Pharmacogenetic chip for Ivacaftor eligibility ............................................................ 28
8. Conclusions ......................................................................................................................... 30
9. Bibliography ........................................................................................................................ 31
10. Annex 1 .............................................................................................................................. 36
1. Abstract

The European commission defines personalised medicine as a medical approach that uses molecular insights into health and disease brought on by the sequencing of the genome to guide decision-making with regard to the prediction, prevention, diagnosis and treatment of illnesses. Its main aim is generally perceived to be “the right treatment for the right person at the right time”.

This new approach to medicine attracted worldwide attention in 2012 when president Obama launched a research project with the objective to implement personalised medicine principles in America. On his speech, he used Ivacaftor as an example of the potential of personalised medicine. The importance of Ivacaftor development lays on the fact that it’s the first drug capable of treating cystic fibrosis aetiology rather than its symptoms in a specific subset of mutations.

Given the impact Ivacaftor had in the media and that cystic fibrosis is not sufficiently known in our society, the objective of this project is to try to understand the underlying mechanisms of cystic fibrosis disease and shed light on what makes Ivacaftor a remarkable advancement in cystic fibrosis therapy and personalised medicine.

1. Resum

La Comissió Europea defineix la medicina personalitzada com a un enfocament mèdic que utilitza els coneixements moleculars de salut i malaltia obtinguts a través de la seqüenciació del genoma per guiar la presa de decisions pel que fa a la predicció, prevenció, diagnòstic i tractament de malalties. El seu objectiu principal generalment es defineix com “el tractament adequat per a la persona adequada en el moment adequat”.

Aquest nou enfocament de la medicina va atreure l’atenció mundial al 2012 quan el president Obama va llançar una campanya d’investigació amb l’objectiu d’implementar els principis de la medicina personalitzada a Amèrica. En el seu discurs, va utilitzar l’Ivacaftor per exemplificar el potencial de la medicina personalitzada. El desenvolupament d’Ivacaftor és molt important ja que és el primer fàrmac que tracta l’etiologia de la fibrosi quística enlloc dels seus símptomes en un conjunt de mutacions.

Atès al gran ressò mediàtic que l’Ivacaftor va assolir i la gran desconeixença de la societat sobre la fibrosis quística, l’objectiu d’aquest treball ha estat conèixer la fisiopatologia d’aquesta malaltia i entendre perquè aquest fàrmac suposa un gran avanç en medicina personalitzada i en la terapèutica de la fibrosis quística.
2. Integration of three knowledge areas

The objective of this project is applying and linking the different knowledge acquired throughout the degree with different pharmaceutical knowledge areas. This project has been supervised by the Biochemistry and Molecular Biology unit, which mainly focuses on the study of the structure and function of naturally occurring macromolecules in living organisms. Nevertheless, other areas such as Therapeutical Pharmacology and Pharmaceutical Technology will also be considered.

As mentioned above, the field of Biochemistry and Molecular Biology is the backbone of this project. Consequently, the project will explore, from a biomolecular perspective, cystic fibrosis patophysiology and diagnosis as an example of applied personalised medicine in modern healthcare.

In the secondary area, therapeutical pharmacology, the project will study the therapeutics of Ivacaftor, a novel drug used to treat certain cystic fibrosis variants with specific mutations in the cystic fibrosis transmembrane regulator (CFTR) gene.

Lastly but not leastly, the birth of personalised medicine cannot be fully understood without the recent technological advancements in the field of genomics and bioinformatics. For this reason, I will keep track of these recent technological leaps that have helped shape nowadays personalised medicine approach.

In summary, the effort done in integrating the above mentioned areas of knowledge will help make possible the achievement of the main objective of the project, which was gaining an insight into the potential benefits of personalised medicine, not only from a genomic understanding, but also in the fields of drug therapy and the pharmaceutical technology, through the study of Ivacaftor for cystic fibrosis, a revolutionary drug where personalised medicine principles were recently applied and remarkable success was achieved.
3. Introduction

According to the European Commission, personalised medicine refers to a medical approach that uses molecular insights into health and disease brought on by the sequencing of the genome to guide decision-making with regard to the prediction, prevention, diagnosis and treatment of illnesses. It’s main aim is generally perceived to be “the right treatment for the right person at the right time”(1). This translates in offering care based both on current evidence and patients unique background characteristics to enable proper predictions of their clinical outcome.

Everyone is interested in the idea that their medicine should be personalized. But in fact, the idea that we should take care of patients in a highly-individualized way is not something new. Back in ancient Greece, Hippocrates is quoted for saying “It is more important to know what sort of person has a disease than to know what sort of a disease a person has”(2). In other words, it's more important understanding patient’s background before trying to understand his disease. More recently, Sir William Osler, considered by many the founder of modern medicine, is widely quoted for another remarkable statement “The good physician treats the disease. The great physician treats the patient who has the disease.” Again, great physicians understand what it is about their patient that's special, that's individual, that's unique and try to tailor therapy for them.

In essence, physicians have known for millennia that occasional patients, for unknown reasons, seem to differ from the average somehow. How susceptible or resistant they are to certain diseases, how well they respond to certain treatments or how badly they tolerate certain drugs has always been a topic of interest in medicine.

The excitement in personalised medicine is that now we have the technological capability to study and understand what was unknown before. With the new tools that our ever-increasing understanding of genetics and molecular biology provide, we have now the potential to explore and determine the causes of this variability in relation to drug response.

The recent advancements in our disease understanding, inevitably results in the identification of differences across the same disease. In consequence, stratification into smaller subtypes of said disease usually takes place. However, classic drug development has always been about the performance of drugs in very large population samples. This approach, the so called the “one drugs fits all”, dismisses the inherent individual variability among individuals while targeting a common condition that hasn’t been properly stratified yet. Consequently, it’s rare for classic drugs to be both safe and effective to everyone, and in most cases several irresponsive patients appear.
Luckily, we are now moving away from this era of blockbuster drugs designed to treat huge amounts of patients with a common complex disease. For this and other reasons, modern healthcare and the pharmaceutical industry is undergoing a radical shift towards a more specific, tailored and patient driven drug development. In this new model, regulatory agencies no longer focus drug evaluation solely in drug’s average efficacy and safety in a given population. Instead, they also search for resistant subgroups of patients whose treatment failed and demand further investigation to explore these phenomena.

However, traditional clinical medicine approach for treating these irresponsive groups of patients is following a flowchart of treatment options, whereby trial and error is the way to reach the ‘best’ treatment for an irresponsive patient(3). This methodology, frequently used while treating common multifactorial diseases such as diabetes, hypertension or depression, often leads to several inappropriate and ineffective prescriptions, causing inadequacies and several adverse drug events. Unfortunately, this keeps going on until the most tolerated, but not necessarily best, combination of drugs to treat a “resistant” individual is prescribed.

The acknowledgment of the magnitude of adverse drug events impact, which accounts in the USA for an annual sum of 120.000 deaths and its economic impact of 21M$ per 100.000 habitants(4), can give us a good idea of the importance of preventing such events both for patients well-being and for healthcare providers budget.

There are several confounding factors that can determine whether a patient will be responsive to a drug or suffer an adverse drug event. Just to name a phew: miss-dosing, genetic background, drug interactions and allergies among others. Although this may be true, a patient’s individual genetic predisposition for inappropriate drug response remains the least studied factor(5). This is surprising because provided that it’s been well-documented, from as early as the 90s, that in some cases, polymorphisms in drug transporters or key drug metabolizing enzymes can increase toxicity, cause adverse drug events or end up with therapeutic failure(6).

With the promise of the ability to properly estimate patient’s likelihood to respond to a given drug and identifying those at most risk to suffer an adverse drug event, predicting its risk and intervening accordingly, a new emerging approach is gaining recognition: personalised medicine.

Notable areas of study where personalised medicine can shed some light and advance clinical practice are the general lack of efficacy of many pharmacological treatments, shown in figure 1, or explore and account for the diverse and complex variability of drug rates of metabolism among patients, displayed in figure 2.
One of these new tools that can help boost our understanding of these differences in patient’s response is a much deeper understanding of genomics. From 1953 Watson and Crick’s outstanding discovery of DNA structure(9), we are now capable of sequencing a whole human genome in 24 hours for 1500$\text{)(10)}$. The recent advancement in next-generation sequencing (NGS) methods provide a cheap and reliable large-scale sequencing technology. They are used extensively for sequencing, disease mapping, SNP tagging, to quantify expression levels through RNA sequencing and also in population genetic studies such as genome wide association studies (GWAS) that help scientists study and determine genes role in relation to disease(11).

As demonstrated in figure 3, these technologies are in continuous improvement. In fact, the cost per Mb of data has decreased tremendously, from nearly 3000$\text{)/Mb in the first platforms towards a mere amount of 0.07$\text{)/Mb in the platform Illumina Hi-seq 2000\text{)(12)}$. It is theorized that this rate of acceleration in sequencing output is clearly just beginning. Recent NGS platforms just introduced to the market will have a continuing and lasting impact on biomedical research for years to come. As technology advances, current instruments will continue to evolve, at a rate even faster than the rate of advancement in computer throughput pedicted by Moore’s law (10). In other words, the time it takes for DNA sequencing output to double is less than one year. Correspondingly, NGS technologies have room for improvement for many years(13).

But it’s important to understand that without the implosion of modern bioinformatics, among many other disciplines, we would not be able to gather and analyse those huge DNA sequences and understand how they should be applied to individual patients. Whereas in the past, in early Sanger’s sequencing times, when sequencing was extremely slow, sequence output was the true rate limiting in advancing biological understanding, nowadays, with the current platforms, it’s data interpretation, bioinformatics, that limits the rate of advancement in biological understanding(13).
To clarify, the revolution in personalised medicine is mainly taking advantage of two developments, one in the science of genomics, with advancements in genetic technologies and techniques such as genotyping or microarrays, and the other, in bioinformatics, with advancements in algorithms, huge data processing and even deep machine learning. Both have been pillars and sources of information to effectively apply personalised medicine. An important distinction to draw is that by huge data sets we are not just limited to molecular DNA sequencing data, but also many other types of molecular data, ranging from epigenetics, mRNA expression, metabolomics or proteomics. Another equally important type of data is none molecular data, such as the information contained in patient record files or from patient’s blood analysis.

All these sorts of data are what’s shaping modern bioinformatics and what makes feasible the current personalised medicine approach. In the future, we will learn more about disease susceptibility and drug response by looking at molecular data such as DNA patterns or mRNA expression levels combined with none molecular data such as patterns in electronic medical records or blood samples and understand what it is that makes an individual or a disease special. In this setting, bioinformatics purpose is to investigate those tremendously large data sets, analyse their interrelationships and extract clinically valuable information for both scientists and physicians.

Therefore, personalised medicine seeks to move away from the classic heavily symptom-based diagnostics towards a model where molecular characterisation is the main indicator of disease diagnosis and prognosis. This approach, alternatively known as biomarkers based medicine, is what ultimately will guide and improve physician’s decision making and enable a true individualisation of patients.

Figure 3: Changes in NGS instrument capability over the past decade and the timing of major sequencing projects. At the top, increasing scale of data output per run plotted on a logarithmic scale. In the middle, a timeline representing major milestones in massively parallel sequencing platforms. Bottom, the timing of important projects and milestones in the field of genomics (13).
Although personalised medicine is often considered to be mostly based on genomics and bioinformatics, it’s the result of a far more complex network from the combination of science, technology, engineering and medicine fields (STEM) as shown in figure 4.

In addition to the genes, several other factors that vary among individuals can influence drug response or disease course, and thus, must also be studied and properly addressed. For instance, the characterization of the intestine flora, the microbiome, which has been demonstrated to interfere in drug metabolism and to play a central role in human health, has the potential to help determine current patient immune status and help predict patients susceptibility to certain diseases such as Crohn’s disease, Inflammatory bowel disease or obesity(15).

Another example of a factor to consider in personalised medicine is circadian rhythms, which through various endogenous physiological and metabolic processes determine the daily fluctuations of parameters such as the heart rate, renal activity or endocrine secretion. These fluctuations, which broadly differ in relation to gender, age and health state, can directly affect drugs half-life. Recently, chronoparmacokineticists demonstrated that 56 out of the 100 best selling drugs in the US target products of genes in clinically relevant organs whose expression cycles significantly oscillate according to circadian rhythms, and thus, their efficacy fluctuates depending on the time of administration(16). Therefore, we must transition from the general three daily standard recommendation to virtually everyone and instead, if necessary, establish the optimal hours of administration according to each patient’s unique characteristics.

But besides the difficulties of the challenge of individualising healthcare, the implementation of this new approach faces many other problems of different nature. What many healthcare professionals encounter in their daily practice is that patients level of excitement around the concepts of personalised medicine vary broadly. This happens for many reasons unrelated to their biological health, but rather with their environment, the way they were brought up, their educational level and their religious beliefs. Popular examples include the Amish denial of modern medicine, Church of Scientology stance on any sort of psychiatry or Jehovah witnesses refusal of any blood transfusion. By and large, the way patients were brought up can make a big difference in their attitude towards personalised medicine.

Figure 4: A schematic diagram intending to display the complex relationships established between different STEM fields that currently shape personalised medicine(14).

April 2017
Given these points, a more updated and mandatory health education should be encouraged(3). We should promote early educational campaigns designed to facilitate the communication, in layman terms, of basic key health concepts of personalised medicine so they are aware of this new paradigm shift. Understanding terms such as what a mutation is or what is the purpose of sequencing the genome, should become common knowledge to virtually everyone.

Equally important is promoting its implementation in healthcare services. Despite the efforts done in this direction, a survey conducted to 2000 Canadian physicians in 2015 outlined that although the majority of practitioners recognised the positive potential of genetic testing in disease management (71%); problems such as lack of clinical evidence (53%), the prohibitive cost of tests (48%) or lack of adequate guidelines (60%) are still fundamental barriers for adoption of use(17).

Correspondingly, to allow widespread personalised medicine implementation, regulatory bodies across the world are developing a range of different initiatives. Notable examples in Europe include EMA’s development of Supplementary Information S1, a regulatory framework aiming to harmonize, among other things, current biospecimen sampling and guarantee proper clinical validity of new biomarkers or the programme Horizon 2020, a 70€ billion investment by the European Commission, to fight current lack of funding in collaborative health research and boost our disease understanding are examples of such initiatives(5).

On the other side of the Atlantic, FDA’s 2011 report titled “Paving the way for personalised medicine” outlined the steps the agency would take to integrate genetic and biomarker information, to develop a better regulatory science and to help advance drug development (18).

Moreover, back in 2014, Obama’s administration launched the Precision Medicine Initiative, shown in figure 5, to accelerate biomedical research and provide clinicians with new tools to select therapies that work best in individual patients with specific diseases(19).
Semantically, both personalised medicine and precision medicine are often used interchangeably. However, there is a concern that the word "personalized" could be misinterpreted to imply that treatments and preventions are being developed uniquely and solely for each individual patient, although this could be true to some extent, the precision medicine term focuses mainly on identifying which approaches will be effective for patients based on genetic, environmental and lifestyle factors(20). This distinction, coupled with the fact that precision medicine current definition among experts is generally understood as an approach to understand disease in a deeper level in order to develop a more targeted therapy, makes the precision medicine term preferable.

During Obama’s presentation speech of the Precision Medicine Initiative, he utilised as an example of the potential benefits of applying precision medicine towards disease advancement the case of cystic fibrosis and the recent development of Ivacaftor, a novel drug used for a specific subset of cystic fibrosis variants.

Cystic fibrosis (CF) is a rare, life threatening, autosomal-recessive genetic disorder that affects many systems, including the gastrointestinal, reproductive and specially, the respiratory system. It is the most common fatal genetic disorder, most prevalent in caucasian descent population, as shown in figure 6, and has a highly variable clinical presentation and course depending on specific mutations, several genetic modifiers and environmental factors. Until recently, no treatments were available that targeted the underlying cause of cystic fibrosis and consequently, all available treatment, consisting of preventive antibiotics, bronchodilators, mucus thinners, steroids and enzymes, was merely symptomatic, focused on treating its manifestations and secondary diseases.

![Figure 6: Estimated prevalence of cystic fibrosis per 100,000 habitants(21).](image)

Luckily, this all changed with the market launch in 2012 of Ivacaftor, a novel first-in-class drug that specifically targets cystic fibrosis aetiology rather than symptoms. It works by potentiating the function of the CFTR channel (the dysfunction of which causes cystic fibrosis) in a subgroup of patients carrying specific mutations.
The drug was discovered after decades of intense research after application of genomics in different stages of the drug development. After several collaborative projects between patients, scientists and the drug manufacturer, researchers investigating CF finally understood, at a molecular level, the reasons why CFTR fails to function. Based on this knowledge, the drug manufacturer designed successful cystic fibrosis *in vitro* models and via high-throughput screening identified a molecule capable of reverting this malfunction. Then, through iterative medicinal chemistry techniques, the manufacturer developed a drug capable of improving CFTR activity in a concrete subset of specific mutations(22). Shortly after, the developer of Ivacaftor designed a genetic test to determine patient’s eligibility for treatment. Without delay, the drug was studied in clinical trials with patients possessing these mutations. It’s success lead to the EMA’s and FDA’s approval of the first CFTR potentiator. Both drug applications were granted the orphan drug designation and the priority review status. In FDA’s case, it set an historical all time low record time of approval of 3 months(18).

Another remarkable aspect in the drug development of Ivacaftor that sets it as the perfect example for future drug development is the path that ultimately led to the discovery of Ivacaftor was remarkably patient driven. The drug itself came out of a collaboration between the drug’s manufacturer and the Cystic Fibrosis Foundation (CFF)(23). CFF has fought cystic fibrosis for many years, organizing and coordinating the patient community and helping fund the research that led to the discovery of CFTR sequence back in 1989(24). CFF has also helped in the establishment of a large public CF patient registry mutation databank (Cystic Fibrosis Mutation Database) accessible for free for researchers all over the world(25) and has helped establish the clinical trial network for investigating and determining the genetics of the disease while recruiting participants for testing candidate drugs aswell(18). The foundation itself helped fund Ivacaftor discovery and development with a total amount of 75M$(26).

Overall, the drug itself was such a breakthrough in both drug development and in cystic fibrosis clinical management, that it was described as a wonder drug among the medical community and labelled as “The most important drug of 2012” by Forbes magazine(27).

All things considered, the special nature of cystic fibrosis disease, the amount of research conducted over several decades and the singular characteristics of Ivacaftor development make it a great example of the revolutionary potential of personalised medicine. With all this in mind, I decided to investigate further cystic fibrosis and Ivacaftor throughout the course of this project.
4. Personalised medicine of cystic fibrosis

Cystic fibrosis (CF) is a classic Mendelian disorder caused by mutations in the gene that encodes an epithelial ion channel known as the cystic fibrosis transmembrane conductance regulator (CFTR). The disease is transmitted in an autosomal recessive manner and is the most common lethal inherited genetic disorder, affecting over 70,000 individuals worldwide.(19). It is most prevalent in caucasians with an estimated incidence of 1:2500 in this particular population as shown in figure 6.

CF pathophysiology is caused by CFTR protein malfunction, which is expressed across many cell types and is involved in the production of sweat, digestive fluids and mucus in several organs. Its impairment affects airways, sinuses, the intestinal tract, the genitourinary system, the pancreas and the biliary system. This leads to a wide and variable array of clinical manifestations and complications, shown in figure 7, the severity of which depend not only on the specific CFTR genotype but also on environmental factors and genetic modifiers, regions of the genome outside the CFTR gene that influence cystic fibrosis course.

Although it has been theorized that cystic fibrosis has been around from as early as 3000BC, it was first described on medical literature by Dr. Hansine in 1936(28), who first linked cystic fibrosis pancreatic problems with abnormal pulmonary and intestinal malfunction. She was the first physician to use pancreatic enzyme replacement therapy to treat affected children and established the first cystic fibrosis diagnosis test. When CF was first described, patient’s life was painful and short as seen in figure 8. However, as pancreatic supplementation became widely available and preventive antibiotics began to be used, coupled with an improved patient care and nutrition, the life expectancy and quality of life for cystic fibrosis patients increased dramatically.

Figure 7: The most common cystic fibrosis traits and their relative relationship with the CFTR genotype. As displayed in the image, CFTR genotype alone doesn’t account for all CF phenotypes. However, for some traits, such as pancreas exocrine insufficiency, CFTR genotype is the primary determinant. On the other hand, for other traits such as cystic fibrosis-related diabetes, the main determinant of the phenotype are the genetic modifiers(30).

Figure 8: Growth in CF patient’s life expectancy in the last century. Reaching a total of 32 life years in 2000, it’s expected to surpass the 50 life years mark in new borns after 2000(29). (Data source: Cystic Fibrosis Foundation(26)).
One of the most challenging problems in the management of cystic fibrosis and the major determinant of patient’s quality of life and life span is pulmonary disease(31). Due to CFTR malfunction, as shown in figure 9, the lack of transport of chloride and excessive sodium reabsorption cause absorption of water via osmosis across all respiratory tract leading to a dehydrated airway surface fluid and impairing mucociliary clearance. Ultimately, this excessive viscid mucus drastically increases the incidence of infections by the entrapment of bacteria in airway secretions and causes local inflammation in response(32). As a result, chronic lung infection incidence in cystic fibrosis patients is high and steadily increases with age due to different mechanisms.

Firstly, bacterial adaptation to the airway environment ensues with a shift from planktonic to a biofilm mode of growth by pressure selection of mutant bacteria with abundant exopolysaccharide production capable of eluding physiological phagocytosis. Secondly, the nature of the infection causal agents varies a lot and shifts with age (figure 10). Finally, and more importantly, is the appearance of multiresistant bacteria promoted by the persistent prophylactic antibiotic use. Therefore, failure in antibiotic therapy is common, explaining the difficulty of CF pulmonary disease management.

As bacteria chronicification advances, the tissue promotes a much more intense inflammation causing severe obstruction of airway flow, destruction of airway walls, fibrosis and characteristic cysts(33), clearly seen in figure 11, hence the disease name. The sum of all these physical changes in the respiratory tissue lead to a life-threatening decreased lung function to the point that some CF patients must undergo pulmonary transplant to carry on living if they don’t die first from this condition(29).

Figure 9: Cartoon displaying the mechanism underlying the surge in infections in CF patients. The deficient CFTR ion transport cannot properly hydrate airway mucus by causing bacteria entrapment(34).

Figure 10: Evolution of the causal agents and incidence of infection in relation to age. The colonization occurs typically in early ages with S. aureus and shifts towards P. aeruginosa in adulthood(26). (CFF, 2010).

Figure 11: Comparison of two X-ray chest radiographs. On the left, a healthy pair of adult lungs. On the right, the lungs of an adult with advanced cystic fibrosis. Quoting the original pneumologist description: In the CF lungs we can observe a severe bronchiectasis with numerous mucoid impactions, a retraction of both hilar regions due to tissue scarring and a marked hyperexpansion. (Source of images: Carol Black, MD, UpToDate, 2017).
Interestingly, this exacerbated local inflammation does not only occur in response to the presence of microbial signalling. CFTR mutations alone have been associated with constitutive pro-inflammatory signalling, increased oxidative stress and exaggerated response towards bacteria. Moreover, CFTR is also expressed in lymphocytes and some mutations have been shown to alter host-pathogen interactions, interfering in innate immune lung cell function and causing a pulmonary mucosal immunodeficiency(32).

In the pancreas, the build-up of mucus prevents the release of digestive enzymes that help break down food and absorb nutrients. Consequently, people with CF often have a marked malnutrition and poor growth. To tackle this problem oral pancreatic enzyme supplementation is strongly recommended as 85% of CF patients have pancreatic insufficiency(21). Those supplements are directly obtained from pig pancreatic tissue lysis and posterior protein purification. They consist of a combined form of lipase, protease and pancreatic amylase enzymes mixture and are dose-adjusted according to patient exocrine degree of insufficiency. Furthermore, thick mucus can also block the bile duct in the liver and, in some cases, cause liver diseases.

Lastly, male infertility rates in CF are extremely high, 97%(35). Male new borns often have a congenital bilateral absence of vas deferens, the tube that carries sperm from the testicles to the penis, resulting in an obstructive azoospermia that cannot be surgically corrected. However, modern fertility techniques that combine testicular sperm extraction and intracytoplasmic sperm injections can help assist some cases.

However, contrary to the popular belief, cystic fibrosis does not cause mental retardation or learning problems. Neither affects mobility or mean a person will have to use a wheelchair. In fact, children born today with CF are expected to have normal schooling and reach average academic achievement(36).

Cystic fibrosis diagnosis usually occurs in early ages (figure 12) but used to be troubling way back when genotyping of CFTR or new born screening (NBS) weren’t a routine medical procedure. Physicians had to rely on diagnosis based on new born signs and symptom such as delayed growth, failure to obtain normal weight, lack of bowel movements or salty-tasting skin. As we gained more knowledge of CF and sequencing technology advanced, besides a much thorough and broader study of symptoms, we rely now on immunoreactive trypsinogen test (NBS), genetic testing and sweat chloride test to diagnose CF. Other tests include secretin stimulation tests, X-rays of chest, upper GI and small bowel, lung function test or the analysis of meconium fat.

![Figure 12: Snapshot of 2007 displaying the age of diagnosis for CF in the USA. As we can see in the graph, 66% of patients with CF were diagnosed in their first life year and only 2% of CF patients are diagnosed with CF before being born. In 2013, 60% of new diagnoses of CF were detected by NBS. (Source: Cystic fibrosis foundation(26)).](image-url)
The complete NBS for CF is increasingly being implemented across the globe because early detection permits sooner access to specialized medical care which significantly improves patient’s outcomes. Nowadays, NBS in Europe and the USA is universal(37), with variations of the exact screening panel between countries.

Although the sweat chloride test remains the gold standard for CF diagnosis, it does not always give a clear and definitive diagnosis as shown in figure 13. Instead, modern guidelines for diagnosing CF recommend that an individual should meet all four items of the following criteria(37): In the first place, patient has to present evident clinical manifestations of CF; secondly, a demonstrated CFTR dysfunction as measured by sweat chloride test; thirdly, positive testing in NBS; finally, found carrier of a CFTR pathogenic variant in a gene analysis. While CF diagnosis is evident when subjects are carriers of CF-causing variants on both alleles, further extended CFTR testing is recommended if sweat chloride test results are inconclusive and only a single CF mutated allele with a suspected but not confirmed to cause CF mutation is found(38).

In an attempt to prevent CF, genetic testing on parents with CF family history prior to pregnancy is often recommended(37) because development of CF in the fetus requires each parent to pass on a mutated copy of the CFTR gene. Since CF genotyping is expensive, testing is often performed initially on one parent. If results indicate that a parent is a CFTR gene mutation carrier, the other parent is tested and the risk of their child to develop CF is calculated to guide parenthood decision making. However, in 2016, only the most common mutations, such as ΔF508, are tested(29,40).

Recently, advancements in genomics, especially in genome wide association studies (GWAS), have made possible the discovery that CFTR genotype alone accounts for a modest portion of patient’s cystic fibrosis phenotype as shown in figure 5. These numerous none-CFTR genetic modifiers, regions of the genome with demonstrated linkage with the likelihood to suffer severe cystic fibrosis secondary diseases, play a role in the development of several clinical features such as obstructive lung disease, intestinal obstruction, diabetes or susceptibly versus P. aeruginosa infection(41,30).
This raises an obvious question, if modern technology can make feasible the determination of cystic fibrosis patient’s predisposition to suffer certain complications or secondary diseases, in a certain degree, such as cystic fibrosis-related diabetes, by determining their full genotype and intervening accordingly, shouldn’t healthcare services recommend whole genome sequencing (WGS) for cystic fibrosis patients? As of today, 5 genome wide association studies (GWAS) have been published in the GWAS Catalogue Registry that helped establish 20 genes/locus in the genome now classified as genetic modifiers for cystic fibrosis disease(41). These findings provide additional genetic targets and might enable individualized treatment of CF. With the rate of on-going research, this number will only increase in the future. Should patients wait?

We have seen that sequencing the whole genome costs today 1000$ and this price is projected to halt in a near future. Under this premise, the potential clinical benefits of WGS are obvious. CF patients are already more prone to develop severe, and possibly life-threatening, diseases and these tools can effectively help physicians tailor treatment, offering personalised preventive action and help guide decision making regarding patients follow up.

From an economic standpoint, the mean annual cost of CF treatment per patient is 15500$(42), taking into account the basic healthcare principle “it’s always cheaper to prevent rather than threat disease”, there is little doubt that implementing this approach, in a personalised medicine fashion, would certainly be cost-effective. As a mode of example, if a certain patient has its genome sequenced and its susceptibility to suffer P. aeruginosa determined, physicians could prescribe a more adequate antibiotic scheme and design a more tailored follow up. Conversely, if a patient’s degree of predisposition to suffer cystic fibrosis-related diabetes is previously determined, perhaps an early introduction of preventive hygienic-diet actions could stop the disease development. In all these hypothetical scenarios, patients could benefit greatly from whole genome sequencing.
4.1 Ivacaftor

Ivacaftor is a cystic fibrosis transmembrane conductance potentiator (CFTR) indicated for the treatment of CF in patients 2 years of age and older who have at least one allele with the following mutations in the CFTR gene: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R or R117H(43–57). Ion channel modulators aim to correct the underlying cellular defect (chloride ion transport) responsible for CF instead of the severity of symptoms. Despite the classic agonist/antagonist denomination often used for receptor modulators, Ivacaftor was catalogued as a CFTR potentiator because it doesn’t open the channel on its own, instead, it works by increasing the likelihood of CFTR channel opening(46).

The exact mechanism of action of Ivacaftor has not been fully elucidated. However, recent research suggests that it interacts with CFTR in an ATP independent manner. In addition of channel gating promotion in both G551D-CFTR and wild type-CFTR channels, Ivacaftor demonstrated to also regulate other CFTR downstream airway functions, such as airway surface liquid depth and ciliary activity(22).

In preclinical studies assessing Ivacaftor’s efficacy, the change in G551D-CFTR activity was first studied in Fischer rat thyroid cells, where it was shown to increase both chlorine transport and open probability. To escalate experimenting towards humans further, a model with greater similarity with CF lung pathogenesis was used, which consisted in an in vitro model of human bronchial epithelial cells directly obtained from CF patients. In this setting, ivacaftor treatment displayed increased G551D-CFTR activity with sufficient efficacy to restore salt and fluid balance(46).

Ivacaftor’s selectivity for CFTR was excellent on an activity screening against a panel of enzymes and receptors, which included 11 other ion channels. Moreover, in safety pharmacology studies, no adverse effects were observed in in vivo dog cardiovascular safety evaluation, neither in neurological and respiratory functions. However, gastrointestinal studies indicated a remarked decrease in motility up to 49% and in hERG assay in HEK239 cells Ivacaftor displayed a 35% inhibition in maximal concentrations(47).

In pharmacokinetic studies, Ivacaftor metabolism after oral administration, occurs mainly through CYP3A4 resulting in two major metabolites M1 and M6, with 1/6 and 1/50 of ivacaftor potency respectively(43). Consequently, Ivacaftor interacts with all drugs heavily metabolized by CYP3A and thus, the coadministration of these drugs with Ivacaftor must be carefully followed(48).
In clinical trials, in patients carrying in at least one allele G551D mutation, ivacaftor therapy was associated with improved forced expiratory volume in 1 second (FEV1), decreased risk of pulmonary exacerbation, increased quality of life, improved nutrition and a large correction in the typical CF elevated sweat chloride concentrations(49). These ivacaftor clinical trials findings were first published back in 2011 in NEJM under the title “A CFTR potentiator in patients with cystic fibrosis and the G551D mutation”(49), the clinical results of which are synthetized in figure 17.

**Figure 17:** Changes from baseline of different clinical parameters after 48 weeks of treatment with Ivacaftor or placebo. This clinical trial had 213 participants and Ivacaftor was studied against placebo in cystic fibrosis patients with at least one allele for G551D-CFTR mutation. Panel A shows the mean improvement in forced expiratory volume (FEV1), which ranged from 10.6% to 12.5%. Panel B shows the time to the first pulmonary exacerbation, in those treated with Ivacaftor the risk of pulmonary exacerbations was 55% lower. Panel C displays the effect that Ivacaftor had on pulmonary function, measured with the standard Cystic Fibrosis Questionnaire (a 100-point scale where higher numbers indicate a lower effect of symptoms on the patient’s quality of life), where Ivacaftor receiving group scored 8.6 points higher. Panel D shows the change in weight which averaged a 3.1kg gain with Ivacaftor. Additionally, in panels E&F, the change in sweat chloride, which reached values below what qualifies for CF diagnosis 60mMol, ranging from a -48 to -54 mmol/L improvement in the group treated with Ivacaftor(49).
The clinical benefits of Ivacaftor were truly remarkable in clinical trials. Moreover, in the extension studies, patients previously treated with Ivacaftor displayed a sustained improvement in FEV1, weight and rate of pulmonary exacerbations for up to 144 weeks of treatment[50]. However, there was a lack of long-term data regarding critical parameters such as mean survival improvement or the decline in pulmonary transplantation rates. At the same time, authorities noted that the inclusion criteria for this clinical trial left out patients with predicted <40% FEV1, those with most severe pulmonary affection and that extrapulmonary variables such as the improvement in pancreatic insufficiency were not properly addressed[44,51].

Regardless of the positive clinical impact Ivacaftor showed, it is an extremely expensive treatment so healthcare providers heavily discussed its inclusion in their services. In the USA, the cost of the medicine per year of treatment is 294.000$[27] although the manufacturer offers both a co-pay assistance and free medicine program for those in most need who can’t afford the treatment[23]. In the UK, the cost for Ivacaftor is 182.625£ per year. To assess its cost-effectiveness, the NHS designated a clinical commission to evaluate the drug and calculated the incremental cost effectiveness ratio (ICER) for Ivacaftor in comparison to current treatments which ranged from 335.000£ per QALY (quality-adjusted life year) in the most optimistic case to 1.274M£ in a more conservative scenario. Although the cost of Ivacaftor fell pretty far outside from the 20-30.000£ per QALY threshold typically used to determine cost-effectiveness in the NHS, it was noted that the ICER for the optimistic scenario was within the range of other ultra-orphan medicines and thus would include the drug in its services and recommend its use for patients with eligible mutations[29].

Back home, in Spain, each Ivacaftor package containing 58 tablets costs 18.720€, assuming the normal posology of 1 tablet every 12 hours, the treatment costs a total amount of 244.028€ per year. To illustrate just how expensive this treatment is in comparison to current CF treatments that also reduce FEV1, figure 18 was elaborated.

<table>
<thead>
<tr>
<th>Name</th>
<th>Ivacaftor</th>
<th>Hypertonic serum</th>
<th>Dornase alfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>Kalydeco® tablets</td>
<td>Hyaneb® NaCl 7% + Hialuronic 0,1% Inh. Sol. 30 bottles</td>
<td>Pulmozyme® 2500 U/2,5 ml Inh. Sol. 30 bottles</td>
</tr>
<tr>
<td>Posology</td>
<td>150 mg/12 h</td>
<td>5 ml/12 h</td>
<td>2500 U/24 h</td>
</tr>
<tr>
<td>Change in FEV1 (%)</td>
<td>10,5% (week 24)</td>
<td>3,2% (week 48)</td>
<td>5,8% (week 24)</td>
</tr>
<tr>
<td>Exacerbations reduction (%)</td>
<td>60% (week 24)</td>
<td>66% (week 48)</td>
<td>22% (week 24)</td>
</tr>
<tr>
<td>Annual treatment cost (£)</td>
<td>244.028</td>
<td>2.175</td>
<td>7.227</td>
</tr>
</tbody>
</table>

Figure 18: Table comparing current treatments for cystic fibrosis patients that influence FEV1 and pulmonary exacerbations. Although Ivacaftor has the most clinical benefit, the cost is 100x/35x times more expensive than other alternatives. To elaborate this table I consulted Genesis-SEFH study from Andalucia Pharmacotherapy Group[52].
Despite the prohibitive cost of Ivacaftor, in Catalonia, the CatSalut commission “Consell Assessor de Tractaments Farmacològics d’Alta Complexitat (CATFAC)” recommended the drug as another form of treatment for CF if patients are carriers of one of the eligible mutations and meet all this specific criteria:\(^\text{(51)}\):

1. Patient’s sweat chlorine concentration is higher than 60≥ mmol/L or is a demonstrated carrier of 2 CF-causing mutations.

2. Phenotypic presence of sinopulmonary disease (with or without other manifestations characteristic of CF such as pancreatic insufficiency, sputum cultures positive for pathogens associated with CF or salt-wasting syndrome).

3. Patient’s predicted FEV1 is ≥30%. This is because the scientific evidence in these patients with predicted FEV1 <40% is currently lacking.

Furthermore, all eligible patients for Ivacaftor treatment require an individualised doctor request authorisation, approved by the hospital medical director, and further evaluation and authorisation, on a case by case basis, by CATFAC. If the request is approved, the treatment will require a specific follow up consistent in a periodic treatment efficacy evaluation, with FEV1 as the main clinical indicator, in months 3, 6, 12, 24... to properly assess patient response to treatment\(^\text{(53)}\). The current doctor request form file to request Ivacaftor is attached in Annex 1.

To determine a patient Ivacaftor eligibility, genetic testing of CFTR is mandatory. The current scheme recommended from Clinical Consortium of Pharmacogenetics for Ivacaftor treatment eligibility is presented in figure 19. To do this genetic determination, there are currently 84 different laboratories offering 186 different clinical genetic tests registered in NCBI’s Genetic Testing Registry database. From those, 100 are designed to sequence the entire coding region of CFTR gene and 74 are tests targeted for specific variants. In the same fashion, I’ll try to design my own genetic test targeted for all current Ivacaftor eligible CFTR mutations.

Figure 19: Current 2017 recommended scheme for Ivacaftor eligibility. If a patient is proven to be homozygous for Δ508del, Ivacaftor monotherapy is not recommended. However, if he is heterozygous for Δ508del and/or homozygous/heterozygous for any of the other mentioned SNP’s in the picture, Ivacaftor use is recommended. It is important to note that the drug costs over 300.000$ per year of treatment and currently under a strict case on case surveillance, so patient administration of the drug should only be administered if eligibility criteria is met. (Source: PharmGKB\(^\text{(54)}\))
5. Aims

My first objective in this project is to make a bibliographic research about the current situation of personalised medicine to understand it’s impact in our future disease management.

Secondly, I want to learn more about cystic fibrosis and Ivacaftor, understand the underlying mechanisms of cystic fibrosis disease and try to shed light on what makes Ivacaftor such a remarkable advancement in cystic fibrosis therapy.

Finally, I want to widen my practical knowledge in genomic tools available to the general public to improve my understanding of genomics. In order to achieve this objective, I will study CFTR protein structure and design a genetic chip to determine patient’s eligibility for Ivacaftor.

6. Materials and methods

I first researched about personalised medicine in university while completing an assignment of the subject Research in pharmaceutical biotechnology. I found the subject to be particularly fascinating and consequently decided that the topic of my TFG would revolve around this subject. To achieve this, I will conduct a much deeper investigation by navigating through different internet databases.

The methodology of this project has been based in a thorough bibliographic research and the subsequent synthesis of the articles found. To do so I’ll use scientific databases as my main source of information, searching for terms such as “precision medicine”, “cystic fibrosis” or “CFTR structure”, which mainly will be PubMed and Web Of Science, available at: www.ncbi.nlm.nih.gov/pubmed and http://webofknowledge.com/.

As for the practical experimental part of this project at the Bioinformatic level in silico, I utilised a broader range of resources besides bibliographic research. To study CFTR structure I will use the data and tools found in Uniprot, Protein Data Bank (PDB), Phyre2 and the protein visualisation software Pymol. To design the genetic chip to test Ivacaftor eligibility I will rely on PharmGKB, NCBI’s dbSNP and SNPedia databases.
7. Results and discussion.

7.1 Structure, functions and mutations of CFTR

The cystic fibrosis transmembrane conductance regulator (CFTR), first characterized in 1989, was the first epithelial cell ion channel to have its primary sequence structure determined. Functionally classified as an intracellular ATPase-gated chloride channel, CFTR consists of 1480 amino acids with a total mass of 170,000 Da(25). Structurally, it is a member of the ATP binding cassette (ABC) transporters superfamily with the distinct functional difference of performing as an ion channel(55). CFTR structure can be divided into five functionally different domains, shown in figures 20, 21 & 22:

- Two membrane-spanning domains (MSD1 and MSD2), each composed of six transmembrane segments that anchor the protein to the plasmatic membrane.
- Two nucleotide-binding domains (NBD1 and NBD2) which together form a heterodimer complex that interacts with nucleotides, mainly ATP, to regulate chloride channel activity by regulating the opening and closing of the MSD’s.
- The regulatory domain (R), which is unique across ABC family, that quantitatively regulates channel activity while activated.

![Diagram of CFTR structure](image)

Figure 20, 21 & 22: Comparison of CFTR structure depicted in a cartoon image(21) and two far more realistic images of CFTR structure done by myself. To obtain these images I used Pymol software and the available data of dephosphorylated, ATP-free CFTR crystal structure obtained in a 2017, not yet published, study (PDB 5UAK(63)). Interestingly, in this protein conformation, we can fully appreciate both MSD 6 α-helices and locate the R domain. To identify all domains I used the aminoacidic sequences available in Uniprot (P13569(64)). The exact domain sequences are: MSD1: 81-103+118-138+195-215+221-241+308-328+331-350; NBD1: 433-584; R: 590-831; MSD2: 860-880+912-932+991-1011+1014-1034+1103-1123+1129-1149 & NBD2: 1219-1382.
The discrepancies between the images I obtained from the crystal structure of CFTR and the cartoon are obvious. The cartoon, which is an artistic impression, represents the conformation of CFTR while inserted into the cytoplasmic membrane. On the other hand, what is seen in the other two images is the native dephosphorylated, ATP-free CFTR conformation, where NBD are not fully dimerised. Nevertheless, the 5 regions of CFTR are easily identifiable on all three images.

As seen in the cartoon representation and in the sequences annotated in the figure’s description, following the direction of the protein primary structure, the transmembrane domains MSD1 and MSD2 consist of six regions arranged in line, separated by NBD1. Next, comes the regulatory domain, located between 2 β-strands of NBD1. Finally, NBD2 is located at the terminal carboxyl portion of the protein.

The cellular process that produces mature CFTR is complicated. Once CFTR is encoded, it is subsequently integrated into the endoplasmic reticulum membrane where it is N-glycosylated by the addition of two glycosylated groups, increasing its weight from 130 to 150 kDa(58). With the help of chaperone molecules, such as Calnexin and Hsp70, the protein is folded, acquiring resistance to the cellular proteases and transported to the Golgi apparatus. This critical step is apparently very inefficient since only 25% of the folded protein acquires effective resistance towards proteases, making the transport to Golgi apparatus difficult. At this site, the glycosylated groups are modified resulting in a mature 170 kDa protein which will be transported to the apical membrane. Upon reaching the cell membrane, the protein enters a cycle of endocytosis through vesicles and is recycled back to the cell membrane. Finally, CFTR has a half-life of 16 hours and is degraded in the cellular cytoplasm by lysosomes(59).

Different studies have demonstrated that CFTR transport across the cell membrane is regulated by phosphorylation of Protein Kinase A (PKA) dependent of AMPc. Mechanistically, to activate CFTR channel a previous preparation is required, which consists in the phosphorylation of the R domain and subsequent interaction with the NBD domains and with other parts of the protein. NBD1 activation is necessary for channel opening and is the determinant of the closure time. Similarly, NBD2 regulates the opening time but this domain is not required to initiate channel opening(60).

Although CFTR was initially defined as a chlorine channel, the presence of phosphorylation points, as shown in figure 20, coupled with the fact CFTR can hydrolyse ATP, indicate that the protein requires energy to perform its function. Therefore, some scientists reclassified CFTR as a chlorine active transporter instead.

In addition to its function as a chlorine channel, CFTR participates in other cellular processes such as regulation of other ion channels, traffic of other transmembrane proteins through the cell or the control of intracellular pH(61).
To date, over 2000 CFTR mutations have been identified(25,26,41), the nature of which is very diverse as figure 23 demonstrates. These mutations can affect CFTR protein in several ways. Therefore, in order to seek better approaches to treat CF patients and maximize therapeutic effects, CFTR mutants have been stratified into six groups according to their functional defect(55,57,61). In figure 22 we can observe in which cellular step CFTR dysfunction occurs in relation to each mutational class.

- **Class I** mutations include nonsense mutations, frame shift mutations and splice site mutations that produce premature termination signals. Consequently, unstable transcripts and/or aberrant proteins containing deleted amino acid sequences are formed. Such proteins are expected to be degraded rapidly, producing a net effect of low amount of CFTR on cell surface.

- **Class II** mutations display a trafficking problem caused by a misfolded or improperly processed CFTR protein. Under these circumstances, upon entrance into the endoplasmatic reticulum, most protein is degraded by cellular quality control. A classic example of this group is the Δ508del mutation, a sequence deletion of an entire phenylalanine located at position 508 in CFTR protein. Δ508del is the most prevalent mutation, present, in at least in one allele, in 86% of patients with cystic fibrosis(30).

- **Class III** are mutations that result in a full-length CFTR protein that has difficulties with the activating/gating of the channel. Most of these mutations are found in NBD and interfere with the binding of ATP to these domains or with the stimulation of the channel by ATP, resulting in a decreased net chloride transport activity of the channel. The most notable mutation of this class is the G551D mutation, the mutation for which Ivacaftor was first approved.
• Class IV mutations have affected amino acids in MSD1, located in the pore of the channel. Consequently, although the ATP binding and the quantity of CFTR on surface is normal, the proteins displays a defective conductance because the movement of chloride through the pore is compromised.

• Class V mutations display a decreased transcription of CFTR gene, often caused by a splicing defect or a missense mutation in CFTR promoter. The net result is a very low production of CFTR and thus, these mutations cause severe forms of CF.

• Class VI mutations produce unstable protein with low residence time due to premature degradation.

Although the heterogeneity of CFTR mutations is extremely complex and daunting, as demonstrated in figure 24, where the location distribution of CFTR mutations across CFTR gene is displayed, a few select mutations account for most CF clinical cases(55). More importantly, not all these mutations cause cystic fibrosis and from those that do cause CF, not all of them do so in the same degree of severity. As seen in figure 25, the final phenotype of the patient is the result of the combination of both CFTR gene alleles. Depending on the exact mutation on each of these alleles patients will display a more or less aggressive form of cystic fibrosis.

However, the genetic classification discussed above is impractical in a clinical setting scenario. Instead, a simpler classification is used to improve CF patient communication. Shown in figure 25, CFTR mutations variants are classified into 3 groups (normal, residual activity or little to none activity). The CFTR activity value for each allele is proportional to the total number of CFTR channels in the apical membrane weighted for their functionality as measured by their conductance and probability to open. Then, the total CFTR activity of a genotype is the sum of both alleles intrinsic activity.
7.2 Microarray analysis and network construction.

Using the genomic data from differentially expressed genes between normal and CF human cells from a recent gene expression study where researchers compared the transcriptome of colon epithelial cells between healthy participants and cystic fibrosis patients I generated figure 26. In this network of genes, each sphere represents a single gene with its size correlated with the magnitude of differential expression between the two groups and each connection between genes (nodes) accounts for a predicted protein-protein interaction. The quantity of nodes and the degree of clustering in these networks give scientists insight of the nature and severity of alteration that CF causes in gene expression. In this particular network, we can appreciate 2 clusters of proteins connected via several nodes. As demonstrated in the original study, the protein expressed by ACTB gene (β-actin), highlighted in red, was identified as a CF genetic modifiers and proven to interfere in several biomolecular processes, indicating it might be an interesting target for future drug development.

7.3 Impact of G551D mutation

G551D, displayed in figure 27, is a single nucleotide polymorphism (SNP) consistent in a change of a guanine to adenine (G>A) nucleotide change in the position 1784 of exon 11 of CFTR gene which effectively changes a glycine towards an aspartate aminoacid in position 551 of CFTR protein during translation(34,55). It is a class III mutation, a missense, that causes a severe reduction in channel-open probability of CFTR(54) and is associated with a severe CF phenotype characterized for a marked pulmonary dysfunction and pancreatic insufficiency(64). Of the approximately 70,000 cases of cystic fibrosis worldwide, 3-4% are carriers of the G551D mutation, making it the third most prevalent CF mutation(23,65). The allele frequency of G551D in the whole world population is very low and highly varies with ethnicity/race, with values between 0.02069% among caucasians and 0.0013% in middle eastern populations(54).
To understand the changes that G551D causes in CFTR structure, we must first acknowledge the fact that when both NBD domains dimerise, in a head to tail manner, they form two ATP binding pockets (ATBP1 and ATBP2), with the ATP molecules sandwiched at the interface, as seen before in figure 20. The aminoacids that conform both pockets are key signature sequence (regions of a protein very conserved among different species) of CFTR. Experiments have demonstrated that the proper formation of both ATBP pockets in this interaction is critical for the ATP-dependent opening of the CFTR channel(66,67). Moreover, changes in the aminoacids of these sequences have a much lower CFTR ATP-mediated activity and are associated with CF(64).

The exact aminoacids that conform ATBP2 is known for both NBD1 and NBD2 signature sequences, and Glycine551, located on NBD1’s ATBP2 pocket, is one of them. Mechanistically, the G551D mutation difficults the ability of ATP to bind into ATBP2, completely eliminating the ability of ATP to increase the opening rate of CFTR(67). To be precise, scientists have estimated that that mutant G551D-CFTR has a 120 fold decreased conductance in comparison to wild type CFTR(64).

Figure 27: Above we can see the exact location of this mutation using NCBI’s Geneview navigation tool. On the right side of the image, in pink colors, the exon 11 exact sequence is displayed with the Gly551 aminoacid circled in green.

To study the impact of G551D mutation on CFTR structure, Phyre2 protein modelling portal was consulted. Introducing G551D-CFTR FASTA sequence the website predicted that this mutation would not display any major significant changes in the whole CFTR structure stability, in line with current research(67). Then, using Pymol software, and the experimental 5UAK crystal structure data, I proceeded to locate and highlight where Gly551 is located, shown in figure 28, demonstrating its location is in the outer surface of the region of NBD1 domain that dimerises with NBD2. This observation, taking into account that Gly551 participates in the formation of ATBP2, the change of this glycine for an aspartic acid, a much more acidic and bigger in volume residue, could easily difficult the formation of ATBP2 and the ATP interaction, explaining the dramatic change in the opening rate of G551D-CFTR seen on experiments.
Restoring the mutant CFTR dysfunction of G551D was thought to be possible by identifying and correcting the specific cause that changes total CFTR activity, which as explained before, depends on the total amount of CFTR available at the plasma membrane and their residence time once there; the gating, the probability of the channel to remain open in the presence of activation stimuli; the conductance of the channel and finally, the ability of ions to permeate open CFTR channel.

However, as easy as it sounds, strategies aiming to restore function to mutant CFTR date back to the 90s. Both pharmacological and nonpharmacological strategies have been designed. Notable examples include treatment of cells with co-chaperone molecules, suppression of premature termination codons with aminoglycosides and drug based treatments with Curcumin, Misglustat or Ataluren(22). The latter, is currently in phase III clinical trials(26).

Until Ivacaftor, first approved only for G551D mutation on 2012, there wasn’t commercialised any agent capable of reverting mutant CFTR dysfunction. Therefore, Ivacaftor was a huge breakthrough in CF therapy and a ray of hope for cystic fibrosis patients as this first-in-class drug, aimed only for 4% of CF patients, could lead to future treatments with greater scope of mutations. This was the case because in 2015, the launch of Orkambi (Ivacaftor/Lumacaftor), is able to treat CF patients homozygotic for Δ508del. The development of this drug wouldn’t have been possible without the previous CF knowledge acquired throughout the development of Ivacaftor.

In the near future, inhaled gene therapy has been proposed as the final cure of cystic fibrosis as it could completely revert disease. To date, over 25 clinical trials have been conducted with both viral and non-viral gene vectors trying to insert in the genome WT-CFTR and all of them failed to show sustained clinical benefits(68). However, with the recent developments in CRISPR/Cas9 technology, the possibility to cure any type of cystic fibrosis mutation is gaining strength. With this technique, the scenario where embryos diagnosed with CF are routinely genetically corrected to prevent the development of CF might not be science-fiction anymore.
7.4 Pharmacogenetic chip for Ivacaftor eligibility

In order to be able to design the probes to test Ivacaftor eligibility I consulted the current EMA approved eligible mutations(48). The methodology I used, after knowing which mutations the test should account for, consisted of a PharmGKB research of said mutations to obtain their respective reference sequence. After that, I retrieved from dbSNP NCBI’s database the fragments of the open reading frame consensus sequence of CFTR gene for each mutation. Then, the next step was the identification of the exact SNP base variation(s) from the consensus sequence, marked in red in figure 29. Lastly, I proceeded to obtain the complementary sequences of these consensus sequences to finally obtain the final DNA molecule that will be used as probes and, if desired, implemented in an Amplichip test to easily assess cystic fibrosis genotype and use Ivacaftor treatment when necessary.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence in CF (34)</th>
<th>SNP Ref. Seq.</th>
<th>Probe sequence to introduce to Amplichip</th>
<th>If probe hybridizes,</th>
</tr>
</thead>
<tbody>
<tr>
<td>G178R</td>
<td>0.059%</td>
<td>rs80282562</td>
<td>TTCCTCTGATTGTTCAACAGTTATGAAATAAATAGTCTT</td>
<td>G178R present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TTCCTCTGATTGTTCAACAGTTATGAAATAAATAGTCTT</td>
<td>G178R not present</td>
</tr>
<tr>
<td>S549N</td>
<td>0.144%</td>
<td>rs121908755</td>
<td>TTCCTCCTTGTGAGCTCACTCCTGATGTTCTTCTTA</td>
<td>G549N present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TTCCTCCTTGTGAGCTCACTCCTGATGTTCTTCTTA</td>
<td>G549N not present</td>
</tr>
<tr>
<td>S549R</td>
<td>0.050%</td>
<td>rs121909005</td>
<td>CTCTCACTCCTGATGTTCTCCTGAGTACCTCTTCTTA</td>
<td>G549R not present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTCTCACTCCTGATGTTCTCCTGAGTACCTCTTCTTA</td>
<td>G549R not present</td>
</tr>
<tr>
<td>G551D</td>
<td>2.111%</td>
<td>rs75527207</td>
<td>CACCTTATGTGACTCATCTACTAGTGTCTGTCTTAAGA</td>
<td>G551D present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CACCTTATGTGACTCATCTACTAGTGTCTGTCTTAAGA</td>
<td>G551D present</td>
</tr>
<tr>
<td>G551S</td>
<td>0.013%</td>
<td>rs121909013</td>
<td>CACCTTATGTGACTCATCTACTAGTGTCTGTCTTAAGA</td>
<td>G551S present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CACCTTATGTGACTCATCTACTAGTGTCTGTCTTAAGA</td>
<td>G551S not present</td>
</tr>
<tr>
<td>G1244E</td>
<td>0.075%</td>
<td>rs267606723</td>
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<td></td>
<td>GAATACCCCACTGAGGAACCTTCTGACTCTTCTTAAGA</td>
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<tr>
<td>G1349D</td>
<td>0.016%</td>
<td>rs193922525</td>
<td>CCGGAGACACGATGCGTCACTGAGTGTGTTCTGCACTACAGA</td>
<td>G1349D present</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CCGGAGACACGATGCGTCACTGAGTGTGTTCTGCACTACAGA</td>
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<tr>
<td>S1251N</td>
<td>0.085%</td>
<td>rs74503330</td>
<td>TTCTCTGACCTGACTCTACTGACTCTGACTCTTCTTAAGA</td>
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<td>TTCTCTGACCTGACTCTACTGACTCTGACTCTTCTTAAGA</td>
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<td>0.007%</td>
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</tr>
<tr>
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<td></td>
<td>GCTCTCTTCTGACCTGACTCTACTGACTCTGAGTAC</td>
<td>S1255P not present</td>
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<td>R117H</td>
<td>1.310%</td>
<td>rs78655421</td>
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<td>GGGGCTATTGTCTCTCGTGAGATAGCGCCTAAATAGTCTA</td>
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<td></td>
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<td></td>
<td>GGGGCTATTGTCTCTCGTGAGATAGCGCCTAAATAGTCTA</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GGGGCTATTGTCTCTCGTGAGATAGCGCCTAAATAGTCTA</td>
<td>R117H present</td>
</tr>
<tr>
<td>ΔA5O8del</td>
<td>69.856%</td>
<td>rs113993960</td>
<td>TGTTAAATCTTCTTATAGTAACACAAAAAGTACTACTA</td>
<td>ΔA508del present</td>
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<td>TGTTAAATCTTCTTATAGTAACACAAAAAGTACTACTA</td>
<td>ΔA508del present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TGTTAAATCTTCTTATAGTAACACAAAAAGTACTACTA</td>
<td>ΔA508del present</td>
</tr>
</tbody>
</table>

Figure 29: To determine if a patient is homozygous for a certain variant, say [G551D/G551D], hybridization must only occur with the G551D pathogenic probe (light reddish). On the other hand, a heterozygous genotype such as [ΔA508/S1255P] would display in the assay with hybridization occurring in both pathogenic probes. If no pathogenic probe hybridizes in the test, patient might still be carrier of other mutations CF-causing mutations. The predicted prevalence in the table is calculated from the frequency seen in CFTR2 registry database which consists of a total amount of 88.664 registered cystic fibrosis patients(34).
A minimum of 16 pathogenic probes, highlighted in red, are needed to determine the 13 mutations eligible for Ivacaftor treatment. On the other hand, for the not pathogenic ones, highlighted in green, a minimum of 9 probes are required because S549N, S549R, G551D & G551S and S1251N & S1255P could share the same none pathogenic probes. In total, 25 unique probes should be inserted in the Amplichip test.

The Amplichip P450 test is a patented clinical microarray-based genetic test developed by Roche and approved for use by FDA in 2004 that tests for 34 CYP2D6 variants(69). The methodology to do the test is quite simple, firstly, starting from a blood or saliva sample, the patient’s DNA is extracted. Then, the CFTR gene is amplified by PCR techniques, fragmented and labelled. The next step is a hybridization of the PCR products on to the Amplichip microarray that contains all the predefined probes. Finally, the chip is scanned and the data is analysed to determine patients genotype.

With all the information given in figure 29 we could design our test and implement it in an Amplichip test. With it we could accurately diagnose 11 CFTR mutations and easily determine patient’s eligibility for Ivacaftor treatment.

However, in practice, patients first should test for Δ508del and only proceed further with my pharmacogenetic chip if not found Δ508del/Δ508del or Δ508del/WT because Amplichip tests are quite expensive, with a price between 600-1200€. This preliminary mutation test is available all over the world and easily identifies CF patients with genotypes Δ508del/Δ508del or WT/Δ508del, which are not candidate for Ivacaftor. Particularly, in Spain, as seen in figure 30, Imegen, a genetics laboratory located at Valencia, commercialises this test for 104€ with an estimated delivery of results of 20 days. Interestingly, the very same laboratory also offers a CFTR genetic tool kit aimed at researchers and clinicians to carry on a molecular genetic analysis of the full CFTR gene via PCR and posterior Sanger sequencing.
8. Conclusions

• With the current advancements in bioinformatics and genomic technologies, personalised medicine has the potential to explore the causes of the variability in drug response, improving efficacy of treatments and preventing adverse drug events. Consequently, regulatory bodies all over the world are developing several policies to promote its implementation in modern healthcare systems.

• Despite having been studied for many decades and being a monogenic disease, cystic fibrosis is a very complex, chronic, life-threatening disorder the clinical management of which still has room for improvement. In the future, it will be important to develop drugs that affect whole functional classes of CFTR mutations rather than specific alleles to expand treatment to all patients with cystic fibrosis.

• Even though cystic fibrosis is the most common and widespread genetic disease, the exact reason to explain its prevalence has not been fully elucidated yet.

• The enormous variability in cystic fibrosis phenotypes is caused by several confounding factors. Firstly, the variety in genotypes is immense because there are described over 2000 different mutations all of them causing different alterations in CFTR function; secondly, CFTR is heavily pleiotropic, it is expressed in many tissues and can alter several systems in various degrees all at once; finally, there are described several genetic modifiers locus in the genome capable of modifying the disease course. Therefore, individualising patient care, personalising CF medicine, can have a huge impact in the management of cystic fibrosis.

• The total CFTR activity is determined by CFTR genotype which results from the specific combination of variants present on both alleles. Each mutation has an intrinsic CFTR activity value proportional to the total number of CFTR channels in the membrane weighted for their functionality as measured by their conductance and probability to open. The sum of both allele’s value give us a total CFTR activity.

• The G551D mutation takes places in NBD1 and this single aminoacid change affects the formation of ATBP2 when both NBD dimerise. As a result, the ATP-mediated conductance of CFTR decreases heavily.

• Ivacaftor works by increasing the probability of CFTR channel to remain activated once opened. Although it was first commercialised for G551D mutation, today it is approved for another 9 mutations.

• To design an Amplichip to assess Ivacaftor treatment eligibility, 25 unique DNA probes are necessary to properly asses all candidate mutations.
9. Bibliography


51. Consell Assessor de Tractaments Farmacològics d’Alta Complexitat (CATFAC) del Catsalut. “Dictamen del CATFAC sobre l’ús d’ivacaftor (Kalydeco*) per al tractament de la fibrosi quistica”. Barcelona: Departament de salut, Generalitat de Catalunya; Available from: http://catsalut.gencat.cat/web/content/minisite/catsalut/proveidors


67. Bompadre SG, Li M, Hwang TC. Mechanism of G551D-CFTR (cystic fibrosis


Annex 1

The current official doctor request form file to request Ivacaftor treatment in Catalonia

### Annex 1

**Sol·licitud d’autorització de tractament farmacològic amb ivacaftor per a fibrosi quística**

<table>
<thead>
<tr>
<th>Dades identificatives del/de la pacient</th>
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<table>
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<th>Dades del tractament farmacològic sol·licitat</th>
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<td>Ivacaftor</td>
</tr>
<tr>
<td>Indicació terapèutica</td>
<td>Fibrosi quística</td>
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<tr>
<td>Data del diagnòstic</td>
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<tr>
<td>Nom i cognoms del metge/essa responsable del tractament</td>
<td>Núm. de col·legiat/ada</td>
</tr>
<tr>
<td>Adreça electrònica del metge/essa</td>
<td>Telèfon de contacte del metge/essa</td>
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<tr>
<th>Dades del director/a mèdic/a sol·licitant</th>
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<td>Nom</td>
<td>1r cognom</td>
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<tr>
<td></td>
<td>2n cognom</td>
</tr>
</tbody>
</table>

**Sol·licito** que s’avaluï la idoneïtat d’aquest tractament farmacològic per al pacient esmentat.

Signatura del director/a mèdic/a (només en inicia tractament)

Data
## Dades del malalt

**Edat:**

**Mutació (classe III) identificada:**

Presenta 2 mutacions identificades que causen FQ (una d’elles classe III): Sí ☐  No ☐

Presència de malaltia crònica sinopulmonar: Sí ☐  No ☐

Presència anormalitat gastrointestinal / nutricional: Sí ☐  No ☐

## Inici o seguiment de tractament amb ivacaftor per FQ

Notificació d’inici de tractament ☐

Sol·licitud de continuació de tractament ☐

## Dades cliniques del malalt

**Pès (kg):**

**Alçada (cm):**

Concentració de clorurs a suor (mmol/L):

FEV1: percentatge del predit (%)

FEV1: volum absolut (ml):

Nombre d’exacerbacions pulmonars en l’any previ:

(nombre en inici de tractament)

Nombre d’exacerbacions pulmonars des del darrer seguiment:

(nombre en continuació de tractament)

Tractament concomitant per la fibrosi quística:

(Descriure)
Tractament concomitant amb inhibidors i/o inductors del CYP3A:
(Descríure-ho)

Qüestionari FQ - Valors del CFQ-R Domini Respiratori:
Judici clinic del malalt:

Documents necessaris a adjuntar
Informe clinic