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Cystic Fibrosis-A Joujou for Pathogens

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Single nucleotide polymorphism (SNP) is a variation occurring in the single nucleotide of the genome. It is more precise to call it a point mutation. A single nucleotide polymorphism occurring in the CFTR gene causes cystic fibrosis. It includes the upset of Cl^- ion transport that in turn results in the absorption of Na^+ ions by the cells of the respiratory tree. This absorption later happens to be the initiating factor for various pathogens to breed in the respiratory tract with the sticky mucus as a supporting medium. The various pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas cepacia etc*. These pathogens communicate among themselves in a complicated way and organize into micro colonies so that they are able to pose an effective threat to the human defense mechanisms. This inter communication among pathogens is made possible by a complex and sensitive mechanism known as "Quorum Sensing" which involves micro molecules called "autoinducers". As a result of quorum sensing, the gene expressions within the pathogens are altered which enable the pathogen to diversify their virulence expression. The diversification of virulence factors leads to various hardships in the process of drug designing and targeting. Moreover several antimicrobial therapy and prophylaxis have been discovered in the past so as to safeguard the human race from the jaws of cystic fibrosis but all in vain. Present study focuses towards the proposal of an effective idea for this biological problem. This could be possible by making the concepts of chemistry join hands with bio-informatics so that a novel technique called "Chem-informatics" emerges. It is rather optimistic to trust on this emerging methodology so that something useful to the mankind may be contributed in the near future not only for cystic fibrosis but also for the rest of the diseases that swivel around the Homo sapiens.

Key Words: Single nucleotide polymorphism, CFTR gene, Cystic fibrosis, Quorum sensing, Autoinducers.

INTRODUCTION

The nucleus which is the structural and functional unit of the cell consists of numerous inheriting factors called genes[1]. These genes are constituted of nucleotide sequences that code for a protein. The arrangement of nucleotide bases (adenine [A], guanine [G], thymine [T] and cytosine [C]) in the nucleotide sequences are almost the same in the genome of that particular species. When there arises any alteration or variation in a single nucleotide of the genome, it results in single nucleotide polymorphism (SNP). Single nucleotide polymorphism's which are generally considered to be a form of point mutation, make up 90 % of all the genetic variations and they occur in every 100-300 bases along the human genome[1]. Variations in the DNA sequences can affect the human's ability to respond to diseases, bacteria, viruses, chemicals, drugs, *etc*.

Single nucleotide polymorphism's may fall in the intergenic regions or within the coding sequences. The former most probably doesn't cause any disease and so it serves as biological marker due to its proximity to the diseased region. It is also associated with absorbance and clearance of therapeutic agents. Eventually, it is used in drug development analysis and genealogical DNA testing processes. Single nucleotide polymorphism's within the coding sequences change the codon which in turn changes the amino acid in the protein sequence resulting in a diseased condition. One such diseased condition is cystic fibrosis (mucoviscidosis). It is an inherited disease occurring because of the single nucleotide polymorphism in the cystic fibrosis transmembrane conductance regulator gene (CFTR gene) which is essential for Cl^- ion transport across the membrane. This disease is characterised by abnormal fluid secretion. The alteration in the same CFTR gene may result in a different disorder of the mucous membrane known as Rhinosinusitis. Several methods and bio-informatics tools are available to analyze the impacts of single nucleotide polymorphism and its consequences[1,2].

Cystic fibrosis: An outlook: Cystic fibrosis is a fatal disease and it has served as a background for extensive research in the recent years. It is an inherited disease of the mucus and the sweat glands. The other areas of infection include the lungs, pancreas, liver, intestines, sinuses and sex organs.

Normally, the mucus secreted is thin and watery. It serves as a lining for certain organs thus keeping them moist. A single nucleotide polymorphism in the CFTR gene responsible for Cl^- ion conductance results in the thickening of mucus. This sticky and viscous mucus blocks the air passages, ducts of pancreas, intestines, *etc*., resulting in various complications. It may also lead to infertility in men. The minor symptoms of cystic fibrosis include frequent, coughing, bouts of pneumonia and bronchitis, salty-tasting skin, dehydration, abdominal discomfort and poor weight gain.

CFTR gene at a glance: The CFTR gene is located on the long arm "q" of human chromosome 7. Lap-Chee Tsui and John Riordan isolated the gene in 1989 and it was found that it is 25,000 base pairs long containing 27 exons. The CFTR protein is 1480 amino acids long and has a molecular weight of 1,68,173 Da. This polypeptide is a member of ATP binding cassette (ABC) transporter superfamily.

Detailed study of the CFTR protein reveals that it consists of five domains which include two membrane spanning domains (MSD) situated within the lipid bilayer that constitute the Cl^- ion channel, two nucleotide binding domains (NBD1 and NBD2) projecting into the cytoplasm that hydrolyse ATP and a regulatory domain which contains several serine residues. These serine residues can be phosphorylated by the protein kinase called PKA that is activated by the second messenger cyclic AMP (cAMP). The presence of regulatory domain is a unique feature of CFTR gene.

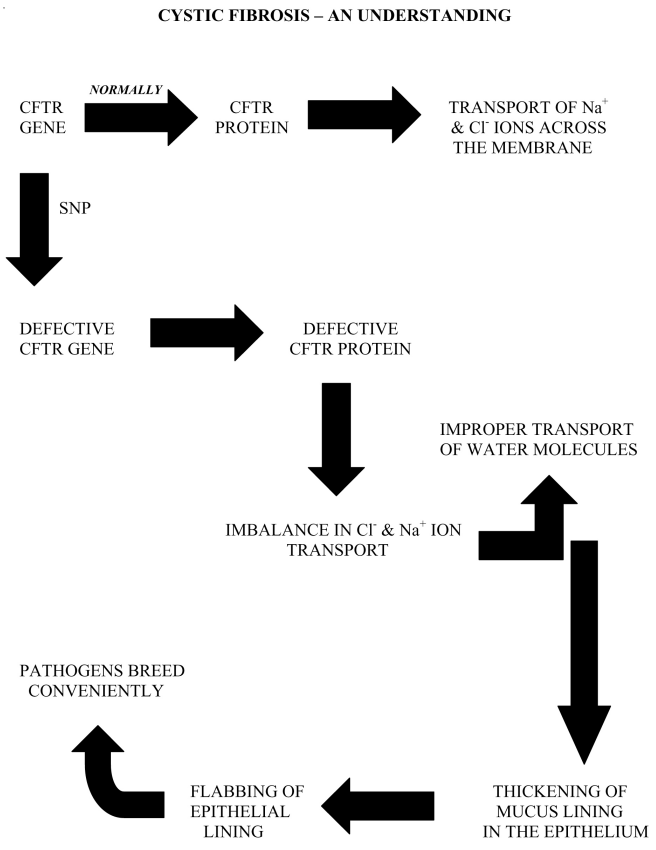
CFTR gene at work: CFTR gene acts as a channel and not as a transporter. This fact has been vividly brought to limelight after detailed study on the conclusions of various experiments conducted on purified CFTR protein. The CFTR protein plays a key role in Cl^- ion conductance.

Normally, within a membrane, the epithelial cells secrete a fluid that stimulates cAMP. The energy required by the secretary epithelia to release this fluid is provided by Na^+/K^+-ATPase which are located on their basolateral side. This fluid facilitates the maintenance of low Na^+ ion concentration inside the cell. The cells usually have a negative transmembrane potential as the interior of the cell possesses more negative charges. Thus the low Na^+ ion concentration coupled with the negative transmembrane potential paves way for the entry of Na^+ ion inside the cell. Meanwhile the entry of Cl^- ions into the cell is also observed and it is all by the action of $Na^+/K^+/2Cl^-$ co-transporter. The Na^+ and Cl^- ions keep on accumulating inside the cell and as the electrochemical gradient inside the cell shoots up beyond a certain limit, there is a need for the exit of Cl^- ions. It is at this juncture, CFTR protein plays a key role as Cl^- channel[3].

In a CFTR protein, opening and closing of the channel gate requires two independent events. (1) Phosphorylation of the regulatory domain. (2) Binding and hydrolysis of ATP by one of the NBDs.

Primarily, the phosphorylation of the R-domain takes place and it is followed by the binding and hydrolysis of ATP by the NBD1. This leads to the opening of the MSD thereby permitting the passage of Cl^- ions. Using this mechanism, Cl^- ions start to leave the cell and due to the attraction of like charges, Na^+ ions also leave the cell in a considerable amount. When the electrochemical gradient of the cell is restored, the binding and hydrolysis of ATP by NBD2 results in the closure of MSD. In the above process, the exit of water molecules too is observed due to the change in osmotic pressure inside the cell. These water molecules bring down the viscosity of secreted mucus so that the slimy nature of the outer membrane coverings could be maintained[4].

Single nucleotide polymorphism in CFTR gene: CFTR gene produces CFTR protein and it carried through the endoplasmic reticulum and then through the Golgi complex so that it can act as Cl^- ion channel at the required site. A single nucleotide polymorphism in the CFTR gene puts everything under trouble. Primarily, some defects arise in the formation of CFTR protein and later it is transported in an ineffective way to the required site. Therefore, there is an imbalance in the Cl^- ion transport at that particular site. Eventually, the transport of water molecules out of the cell is also obstructed. A reduction in the volume of surface liquid results in the increase in viscosity of the secreted mucus. Henceforth, cystic fibrosis comes into being as the thickened mucus couldn't moisturize that particular membrane lining[5].



Cystic fibrosis is caused by a recessive gene. Therefore, for a person to be affected by this disease, this gene must be inherited from both the parents. If a person has just one copy of the gene, he is denoted by the term "carrier". The child that is born to two cystic fibrosis carriers has 25 % probability for being free from cystic fibrosis (homozygous dominant) and the same 25 % probability for being affected by cystic fibrosis (homozygous recessive). Further more, there is 50 % probability for the child being a carrier of cystic fibrosis[5].

Pathogenesis of cystic fibrosis: Studying the pathogenesis of cystic fibrosis, we could come across a number of micro-organisms contributing to the disease. The nature of the existence of micro-organisms, their virulence, *etc*., has been discussed below.

Staphylococcus aureus

Background: *S. aureus* was the first organism recognized to cause chronic lung infections in young cystic fibrosis patients. Nearly 27 % of the lung infections occurring in cystic fibrosis patients are due to *S. aureus*.

Virulence: The virulence of *S. aureus* is dependant on 2 factors: (1) The ability to adhere to the respiratory epithelium. (2) The ability to evade immune clearance. The adherence to respiratory epithelium is in turn facilitated by two factors. (a) Teichoic acid. (b) Slime. Slime is a loosely cell-associated complex polysaccharide containing sugars, uronic acid and amino acids.

The evasion of immune clearance is made by leucocidins. It can lyse phagocytic cells, capsules and protein A.

Other virulence factors: Other virulence factors include hemolysins, hyaluronidase, catalase, coagulase and other exotoxins. All these factors produced by *S. aureus* collectively cause chronic lung infections which would prove fatal in later stages.

Pseudomonas aeruginosa

P. aeruginosa is usually isolated from the cystic fibrosis patients having prolonged chronic lung infections. This microorganism houses in the patient's respiratory secretions. Nearly six morphotypes of *P. aeruginosa* have been discovered so far. Among them, mucoid *P. aeruginosa* is the most common one. Studies reveal that approximately 80 % of the lung infections in cystic fibrosis patients arising due to *P. aeruginosa* are contributed by the mucoid morphotype.

Background: The mucoid morphotype is due to the production of large amount of polysaccharide that surrounds the cell. This material has been designated as "mucoid exopolysaccharide" (MEP). This MEP is a polymer composed of acetylated D-mannuronic acid, L-guluronic acid and it is commonly called alginate[6,7]. Moreover all strains of *P. aeruginosa* isolated from the cystic fibrosis patients produced MEP, even those classified as non-mucoid morphotypes.

Virulence: *P. aeruginosa* is found to produce numerous, diverse virulence factors including exotoxin A, exoenzyme S, elastase, alkaline protease and pyoverdin, two types of hemolysins, lipopolysaccharides, pili and mucoid exopolysaccharide.

Role of mucoid *P. aeruginosa*: Under some undefined environmental pressure, these organisms get converted into mucoid phenotype and it predominates during chronic lung infection. Mucoid *P. aeruginosa* grows as micro colonies in the lung and that micro colony formation plays an important role in the pathogenesis of this infection. Micro colony formation may be enhanced by proteases locally produced by *P. aeruginosa*. Pseudomonal proteases cause release of mucins from the respiratory epithelium. Mucins in the cystic fibrosis patients are highly sulfonated and when combined with mucoid exopolysaccharide in the presence of calcium ions form a highly viscous gel. Increased gel formation enhances micro colony formation, which may lead to even poorer ciliary clearance of these organisms.

Pseudomonas cepacia

Background: The pathogen *P. cepacia* is liable for detection in the cystic fibrosis patients only in the later stages of infection. Further more, isolation of *P. cepacia* is very difficult where galorious colonization of *P. aeruginosa* is found. Initially MacConkey agar was used as a culture medium to grow the organism. At present times, two agar mediums, PC and OFPBL have been developed to culture the same.

Pathogenic potential: The contradictory issue concerning *P. cepacia* is that, it could not be identified whether it is a true pathogen or a marker for severe lung disease. Patients affected by *P. cepacia* have poor pulmonary status and fulminant infection. In addition to this, the severity of *P. cepacia* infection may be dependant on tissue damage caused by other organisms.

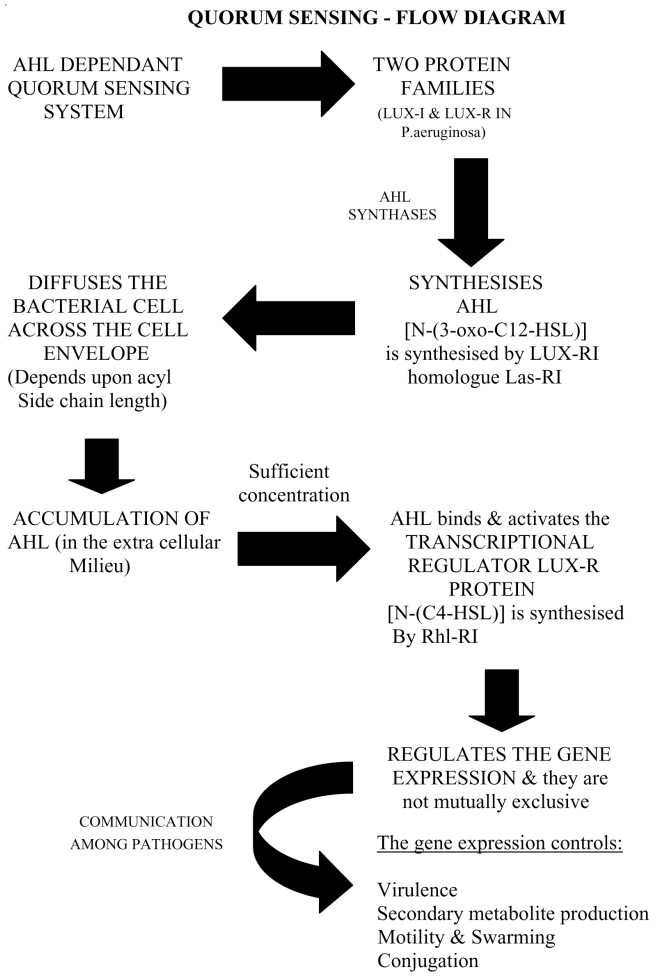
Other pathogenic agents: Apart from *S. aureus*, *P. aeruginosa* and *P. cepacia* other pathogens like *Haemophilus infuenzae*, *Streptococcus pneumoniae*, enterics, glucose non-fermenters, mycobacteria, fungal agents and certain viruses are found to contribute to the infections due to c.

But however, various hardships hinder the study on the above mentioned class of pathogens, the primary reason being their scarce colonization in the infected areas.

Quorum sensing: The pathogenic agents express their virulence in spite of the action of host defence mechanisms. This is possible for them only because of the existence communication system. Here the communication system happens to be a complex organization of signaling processes known as "Quorum Sensing". This sensing involves sensitive micro molecules called "Autoinducers". These autoinducer molecules usually contain peptides and they interact with specific repressor or activator sequence in DNA. The presence or absence of autoinducer molecules controls the production of mRNA and hence the protein. Signaling molecules usually belong to acylhomoserine lactone (AHL) family. All the above mentioned pathogens are found to produce acylhomoserine lactone.

P. aeruginosa for example is found to produce "Pseudomonas quinolone signal" molecule (PQS) and cyclic dipeptides apart from acylhomoserine lactone's. Acylhomoserine lactone dependant quorum sensing system consists of two protein families *viz*, LUX-I and LUX-R. LUX-I function as AHL synthases thus synthesizing acylhomoserine lactone. The synthesized acylhomoserine lactone either diffuses or is pumped into the bacterial cell across the cell envelope (dependant on the acyl side chain length). In the case of *P. aeruginosa* the LUX-I homologue Las-RI synthesises N-(3-oxododecanoyl homoserine lactone). Then this acylhomoserine lactone accumulates in the extra cellular milieu. On reaching sufficient concentration, the AHL binds and activates the transcriptional LUX-R protein. In *P. aeruginosa* the AHL N-(3-OXO-C12-HSL) activates the LUX-R homologue Rhl-RI which in turn synthesises N-butanoylhomoserine lactone. This regulates the gene expressions in the pathogens which account for virulence, secondary metabolite production, motility and swarming, conjugation, biofilm formation and growth inhibition. As a result the pathogens organize among themselves in an effective way so the activities of host defence mechanisms are at stake[8].

Now when a drug is targeted to break this complex and sensitive communication system, the orderly organisation methodology exhibited by the pathogens will be shattered. As a result the pathogens will not be able to withstand or oppose the action of host defense mechanisms. This would be possible by applying the concepts of chemistry in bio-informatics so that a brand new way of approach called "Chem-informatics" comes into being.



Conclusion

The single nucleotide polymorphism analysis clearly throws light upon the fact that cystic fibrosis is a result of variation occurring in the single nucleotide of the CFTR gene. Studies and research on cystic fibrosis needs a multidisciplinary approach. Apart from environmental factors and life-style factors, genetic factors too play a vital role in causing a disease[9]. The role of genetic factors could be understood from the topics discussed above. Being a fatal disease, it is necessary that a solution must be found at least in the near future.

In the recent years, numerous bio-informatics tools have been discovered[10-15]. Their efficiency depends upon their ability to sort out problematic issues to the core[16-23]. These tools should be exploited to the fullest extent so as to root the problem out. This approach towards the diseases with the aid of bio-informatics tools and concepts of chemistry is known as "chem-informatics". Moreover, the intercommunication among the pathogens has been understood and it has been found that it involves a sensitive mechanism by the name "Quorum Sensing". Using Chem-Informatics a drug could be targeted so that the intercommunication could be aborted. As a result the expression of virulence is averted. So we could hopefully expect that in the near future, this approach towards diseases would enable the researchers to discover novel solutions not only for cystic fibrosis but also for other deadly diseases.

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