Review Article

Open Access Journal of
Biomedical Science
ISSN: 2690-487X

The Impact of Sequencing Human Genome on Idiopathic Diseases

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ABSTRACT

This abstract attempt to explore the clues of any idiopathic disease of uncertain or unknown origin arising spontaneously by exposure to metallic or wood dust or viral infection causing illnesses such as acute idiopathic polyneuritis, diffuse idiopathic skeletal hyperostosis, idiopathic pulmonary fibrosis, idiopathic scoliosis etc. With the completion of the Human Genome Project on April 3, 2003, our search for unknown diseases has come to an end. What it means is that we have the catalog of all genes in the Human Genome, we can search the entire genome and locate the spelling errors in the genetic script responsible for causing all diseases. Everything there is to know about human health and traits are written on our genes in nucleotide sequences. Our Genomes provides the catalog of all genes. When we compare the genome of a normal person (Reference Sequence) with the genome of a patient with idiopathic disease, we can identify the differences; or mutations, in genetic script responsible for causing the disease. Or when we compare their gene expression looking for a specific protein, from a specific gene and for a specific nucleotide sequence, we can identify a specific disease. With this knowledge in hands, a Physicians looks at the genetic profile of an idiopathic patient and say with certainty that I don't know what is wrong with you, but I do know where to find it. It is written in your Genome. Our Genome is a diagnostic road map of all our genes. By comparing the sequence of Chromosome-11, of idiopathic patient with the Reference Sequence, if the suspect XIAP gene is confirmed for coding a protein which triggers an inflammatory antigen by creating a cascade of reactions in affected tissue resulting in idiopathic disease, we could design drugs using similar rational like the one we described below for making AZQ to shut off XIAP gene to treat idiopathic diseases and bring them in line with other Western medicine.

KEYWORDS: Sequencing genome; Mutations; Genetic diversity; Genome diseases; Inbreeding

NOTES

a) A Note to my readers

The Impact of Sequencing Human Genomes is a series of lectures to be delivered to the scholars of the National Youth League Forum (NYLF) and the International Science Conferences. NYLF scholars are the very best and brightest students selected from all over the USA and the world brought to Washington by Envision, an outstanding organization that provides future leaders of the world. I am reproducing here part of the lecture which was delivered at the International Science Conference that was PCS 6the Annual Global Cancer Conference held on November 15-16, 2019, in Athens, Greece.

b) Special Notes

I am describing below the use of highly toxic lethal chemical weapons (Nitrogen Mustard) which was used during WWI and its more toxic analogs developed as more toxic weapons during WWII. I described the use of Nitrogen Mustard as anti-cancer agents in a semi-autographical way to accept the responsibility of its use. When we publish research papers, we share the glory with colleagues and use the pronoun "We" but only when we share the glory not the misery. In this article by adding the names of my coworkers, the animal handers, I will share only misery. The Safety Committee is interested to know who generated the highly lethal Chemical Waste,

Quick Response Code:

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Received: March 10, 2023 **Published:** April 06, 2023

How to cite this article: Hameed Khan A. The Impact of Sequencing Human Genome on Idiopathic Diseases. 2023- 5(2) OAJBS.ID.000554. **DOI:** 10.38125/OAJBS.000554

how much it was generated and how was it disposed. I accept the responsibility. The article below sounds semi-autobiographical, it is, because I am alone responsible for making these compounds of Nitrogen Mustard, Aziridines and Carbamate. To get a five-gram sample for animal screening, I must start with 80 grams of initial chemicals for a four-step synthesis. To avoid generating too much toxic chemical waste, instead of using one experiment with 80 grams, I conducted 80 experiments with one gram sample, isolating one crystal of the final product at a time. The tiny amount of waste generated at each experiment was burned and buried at a safe place according to safety committee rules.

INTRODUCTION

Any disease that is of uncertain or unknown origin may be termed idiopathic. An idiopathic orbital inflammatory disease is a "disease of itself," that is, one of uncertain origin, apparently arising spontaneously. Several diseases are referred to as idiopathic such as acute idiopathic polyneuritis, diffuse idiopathic skeletal hyperostosis, idiopathic pulmonary fibrosis, idiopathic scoliosis. Idiopathic means the cause is unknown. A well-known example is the Idiopathic pulmonary fibrosis (IPF) which is a disease of aging that has long had a mysterious etiology and pathogenesis, but new findings in the telomere biology field have begun to provide clues. IPF has been linked to exposure to certain antigenic substances such as certain types of dust, such as metal or wood dust. viral infections. Site of Inflammation's location is identified by CT or MRI scanner. Although we know its location, we still don't know the cause. Idiopathic is also a non-specific, non-infected inflammatory disease; it is a unilateral disease in adults and bilateral in children. Circulating antigen triggers an inflammatory cascade in affected tissues postulated as the cause of this disease resulting in edema, lid swelling, Lympho-proliferated lesions, thyroid ophthalmopathy, ophthalmic disease, causing pain due to inflammation. It is also responsible for causing fever, and reduced blood count. Treatments include radiotherapy, followed by treatment with corticosteroid, and immunosuppressive agents. If it fails to respond to either treatment, then we use the highly cytotoxic drugs such methotrexate which is used in chemotherapy of cancer, including neoplasm with benign condition. Using such extreme treatment could cause vision loss, headache, and nausea.

A RATIONAL APPROACH TO IDENTIFY AND TREAT IDIOPATHIC DISEASES

More than 70 years ago, it was established that all heritable diseases including idiopathic diseases characteristics of living organism are carried by a class of chemicals called the Deoxy ribonucleic acid (DNA). It is a long chain molecule basically made of four building blocks called nucleotide bases and they are Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C) or A-T, G-C.: they always come in pairs. (A) always linked to (T) and (G) is always linked to (C) and they are called nucleotide base pairs. These are the information molecules which code for life and their genetic code has been broken by Crick and Watson [1]. We found that out of four nucleotides bases, three code for an amino acid called codon. Different combination of four nucleotides, gives 64 codons which code for all 20 amino acids. Several codons are arranged together to code for a protein called a gene. A gene is a unit of inheritance. On a string of DNA nucleotides, a gene is recognized by a start and a stop codon. The start codon of a gene is UAG which codes for an amino acid called Methionine. After several hundred codons, a gene ends by one of the three stop codons and they are UAG, UGG, and UGA. Once the stop codon appears, DNA synthesis stops. In a growing fertilized egg, the genes produce proteins which act either to build the structure of the cell or to act as an enzyme to carry out the instruction for the growth of the cells.

In the Lab, we have learned not only to synthesize DNA, but also to cut, paste and copy DNA. We have isolated Restriction enzymes (molecular scissors) from bacteria to take the DNA apart. Using enzyme ligase, we put in DNA back together. We have also learned to combine synthetic DNA with natural DNA. We also learned to take DNA from one organism and put it in ten other organisms. They all work perfectly. If circumstances permit, the resulting organism manifests the newly transformed characteristics of a new synthesized gene. We learned to perform genetic engineering by cutting, pasting and copying a gene from the sequence of a DNA string. For example, we have taken a gene from the human pancreas which codes for a protein called Insulin and inserts it in plasmids. The transgenic plasmid is harvested in Yeast where it makes millions of copies of Insulin. By using restriction enzymes, we cut and release pure Insulin which is isolated from Yeast. Today, more than three hundred million diabetics patients around the world use Insulin to control their diabetes.

By reading the script of DNA letter by letter, words by words and sentence by sentence, (by sequencing), we identify five thousand spelling errors in the DNA scripts called mutation responsible for causing five thousand genetic diseases and disorders. One extreme example is Huntingdon disease which starts in its victims in their fifth decade of their lives. As the disease progresses, the patients' mental functions are lost. Physical function degrades, and over a period of several years, the person dies thoroughly miserable death. If a person is born with this mutation, his destiny is implicit in his genes. If you carry the gene, you will develop the disease. The most important question we ask ourselves is how many total genes are present in our genome. How many are good genes and how many are bad genes where are they located and what do, they do. We will know the answers to these questions only by reading the entire genome. We embark on the greatest biological experiment ever conceived by the human mind. To read our entire book of life under the title, The Human Genome Project (HGP); [2,3].

The HGP is the most important biological project which was completed 20 years ago. Its origin is based on medical concern and has consequently been focused on diseases, and disorders. Its goal is to create the first map of all the human genes which are collectively known as genome. By sequencing the human genome, we should be in a better position to identify and relate a specific piece of DNA to specific diseases and susceptibility. What are the consequences of this knowledge? First and most obviously will be diagnosis. If a disease is known to be genetic in the family and it is straight forward for those diseases whose gene locus is on DNA and has been identified to determine whether you carry that gene following diagnosis but not following close behind when we attempt prevention that is to intervene in some way or another to prevent the genetically programmed disorder from manifesting itself. Following would be the therapy which comes in two primary forms. They are gene therapy and drug therapy [4,5].

In 1990, US Congress authorized three billion dollars to our Labs at NIH to decipher the entire Human Genome under the title, "The Human Genome Project." We found that our genome contains six billion four hundred million nucleotides base pairs half comes from our father and another half comes from our mother. Less than two percent of our Genome contains genes which code for

proteins. The other 98 percent of our genome contains switches, promoters, terminators etc. The 46 Chromosomes present in each cell of our body are the greatest library of the Human Book of Life on planet Earth. The Chromosomes carry genes which are written in nucleotides base pairs. Before sequencing (determining the number and the order of the four nucleotides on a Chromosomes), it is essential to know how many genes are present on each Chromosome in our Genome. The Human Genome Project has identified not only the number of nucleotide base pairs on each Chromosome, but also the number of genes on each chromosome [6].

The following sequence of Human Genome provides the details composition of each Chromosome including the number of nucleotides and the number of genes on each Chromosome: We found that the Chromosome-1 is the largest Chromosome carrying 263 million A, T, G and C nucleotides bases and it has only 2,610 genes. Chromosome-2 contains 255 million nucleotides bases and has only 1,748 genes. Chromosome-3 contains 214 million nucleotide bases and carries 1,381 genes. Chromosome-4 contains 203 million nucleotide bases and carries 1,024 genes. Chromosome-5 contains 194 million nucleotide bases and carries 1,190 genes. Chromosome-6 contains 183 million nucleotide bases and carries 1,394 genes. Chromosome-7 contains 171 million nucleotide bases and carries 1,378 genes. Chromosome-8 contains 155 million nucleotide bases and carries 927 genes. Chromosome-9 contains 145 million nucleotide bases and carries 1,076 genes. Chromosome-10 contains 144 million nucleotide bases and carries 983 genes. Chromosome-11 contains 144 million nucleotide bases and carries 1,692 genes. Chromosome-12 contains 143 million nucleotide bases and carries 1,268 genes. Chromosome-13 contains 114 million nucleotide bases and carries 496 genes. Chromosome-14 contains 109 million nucleotide bases and carries 1,173 genes. Chromosome-15 contains 106 million nucleotide bases and carries 906 genes. Chromosome-16 contains 98 million nucleotide bases and carries 1,032 genes. Chromosome-17 contains 92 million nucleotide bases and carries 1,394 genes. Chromosome-18 contains 85 million nucleotide bases and carries 400 genes. The Chromosome-19 contains 67 million nucleotide bases and carries 1,592 genes. Chromosome-20 contains 72 million nucleotide bases and carries 710 genes. Chromosome-21 contains 50 million nucleotide bases and carries 337 genes. Chromosome-22 contains 56 million nucleotides and carries 701 genes.

The above 22-chromosomes are present in all somatic or body cells. Changes in the somatic cell that is the body cell are not heritable, only changes in the sex cells that is egg and sperm are passed down to the future generation. For example, damaged skin cells due to skin cancer are not passed down to the offspring. They are genetically not heritable, and their mutations are not passed down to the next generations. When a person with skin dies, the mutations dye with him. Diseases carried by the above twenty-two chromosomes are called autosomal traits. These traits are either dominant or recessive. In genetics, dominance is the phenomenon of one variant of a gene on a chromosome masking or overriding the effect of a different variant of the same gene on the other copy of the chromosome. The first variant is termed dominant and the second recessive.

Autosomal dominant traits pass from one parent onto their children. Autosomal recessive traits pass from both parents onto their children. Autosomal dominant inheritance is a way a genetic trait or condition can be passed down from parent to child. One copy of a mutated (changed) gene from one parent can cause the genetic condition. A child who has a parent with the autosomal mutated gene has a 50% chance of inheriting that disease. Examples of autosomal recessive disorders include cystic fibrosis, sickle cell anemia, and Tay-Sachs disease. Mutations in BRCA1 and BRCA2 genes-which have been associated with breast cancer-also are transmitted in this pattern. Some of the specific examples of the autosomal diseases are described below:

Sickle Cell Anemia

Autosomal traits which mean the gene that caused sickle cell anemia is caused in autosome that it is not sex cell It is a recessive trait that it skips generation that hybrid does not show the disease, common in sub-Saharan countries where malaria is common, and the hybrid are resistant to malaria. Normal is "A" and the diseases gene is small "a". If an individual inherits dominant and recessive so they are heterozygous, this condition makes them somewhat immune or mostly immune to malaria, If both parents are heterozygote, they both immune to malaria, but 25% of their offspring inherit the lethal combination of diseased mutation "a" and come down with malaria.

Tay-Sac Disease

It is another autosomal disease, it is also recessive, it causes a change in the gene produces an enzyme which allows to metabolize the Certain lipid it is used in brain tissues. Kids born with Tay-sac are unable to metabolize the lipid it builds up slowly in their brain and usually caused death within two years.

Huntington Disease

It is also caused by autosomal dominant mutations. The mutation produces excessive amount of glutamine in a protein that slowly damages the brain. It is a different mutation whose codon CAG which codes for amino acid Glutamine in a protein. Effected parents will pass down this mutation to half of their kids, because it is autosomal dominant, "H" passes down to their children. Sequencing of their genome has identified the mutation at the end of Chromosome-4. (Chromosome-4 is made of 203 million nucleotide base pairs which carry 1,024 genes). At the tail end of the Chromosome-4, we found the codon CAG repeats. Normal people have 26 CAG repeat they will not have the disease. but if you have more than 40 CAG repeats, you have overloaded the proteins with Glutamine and you are definitely going to be affected with mental retardations.

Down Syndrome

Down syndrome is a genetic disorder that causes abnormal cell division resulting in the addition of an extra chromosome-21. Down Syndrome is caused by a random error in cell division. This error is called "nondisjunction." During autosomal replication, each pair of chromosomes from the father and mother separate at conception, to provide one-half of the embryo's pairs of chromosomes. In Down syndrome babies, one of the pairs of 21st chromosome does not separate. As a result, the embryo has three 21st chromosomes instead of two, which are replicated throughout development. This is the most common cause of Down syndrome in both male and female also known as Trisome-21. About 6,000 children with Down syndrome are born each year in the USA. Finally, the last two chromosomes are called the sex chromosomes. All females carry (X) chromosome which is made of 164 million nucleotide bases and carries 1,141 genes. All males carry (Y) chromosome which is made of 59 million nucleotide bases and carries 255 genes. Inheritable diseases are usually caused by mutations in genes or the changes

in the sex cell chromosome. An example of sex-linked mutation is described below:

Color Blindness

It is a sex-linked mutation. A female carries a pair of XX chromosome while a male carries an XY chromosomes. The mutation in X-chromosome is responsible for causing color blindness. It is recessive mutation. It means that male cannot be a carrier. A male carries an X-chromosome and a Y-Chromosome. If male is carrying this mutation on X-Chromosome, they don't have another X-chromosome to cover up Because of the lack of protection, only male exhibits color blindness. On the other hand, a female carries two X-chromosomes. If this mutation is on one X-chromosome, they will be carrier, because they carry another X-chromosome to cover it up. If female carries these mutations, she will give this disease, the color blindness, to all her sons.

Germ-line gene therapy is forbidden because changes made in egg and sperm will pass on to future generations. Couples with a family history of genetic diseases should sequence their egg and sperm and compare with the Reference sequence to identify any genetic defect and discard mutated egg and sperm. They should use a healthy egg and sperm and conceived by *in vitro* fertilization. The advantage of *in vitro* fertilization is that they can sequence the embryo as well to make sure it is free from all genetic defects. In the olden days of Eugenics, authorities ordered for force sterilization of the genetically defected couples, either lock them up or kill them. In the New Eugenic it is not authority, but the parents decide if the child they are bringing to this world is an acceptable member of human society. If you add up all genes in the 23 pairs of Chromosomes in your genome, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes needed to keep us function normally because some genes don't code for any proteins. By sequencing the Human Genome, we have identified a total of 24,000 genes out of which 16,000 are good genes, 6,000 bad genes and 2,000 pseudo or neutral genes. Less than 2% of our genome codes for proteins. The remaining 98% of our genome carries pieces of DNA picked up from bacteria and viruses during evolution which serves as switches, promotors, enhancers etc. The greatest Darwinian transformation is controlled by switches. By switching on and off a gene, the body plan gene called the FOX gene can bring evolutionary changes in the body. Genes code for protein, it is the switches that turn genes on or off.

As I said above, a gene codes for a protein, not all 24,000 genes code for proteins. It is estimated that less than 19,000 genes code for protein. Because of the alternative splicing, each gene codes for more than one protein. Not all genes act simultaneously to make us function normally. Current studies show that a minimum of 2,000 genes are enough to keep human function normally; the remaining genes are backup support system, and they are used when needed. The non-functional genes are called Pseudogenes. For example, millions of years ago, humans and dogs shared some of the same ancestral genes; we both carry the same olfactory genes, only in dogs do they still function to search for food. Since humans don't use these genes to smell for searching food, these genes are broken and lose their functions, but we still carry them. We call them Pseudogenes. Recently, some Japanese scientists have activated the Pseudo genes, this work may create ethical problem in future as more and more Pseudo genes are activated. Nature has good reasons to shut off those Pseudogenes. All functional genes in our body make less than 50,000 proteins which interact in millions of different ways to give a single cell. Millions of cells interact to give a tissue, hundreds of tissues interact to give an organ and several organs interact to make a human.

On April 3, 2003, when we sequenced the entire Human Genome, we not only read the entire script of our genome, letter by letter, word by word, sentence by sentence, but also, we also read the number of letters and the order in which they are arranged (sequence) called under the title, "The Human Genome Project". We found that less than two percent of the Gene in our Genome codes for proteins and the rest is the non-coding regions which contains switches to turn the genes On or Off, pieces of DNA which act as promoters and enhancers of the genes. Using restriction enzymes (which act as molecular scissors), we can cut, paste, and copy genetic letters in the non-coding region which could serve as markers and which has no effect, but a slight change in the coding region makes a normal cell become abnormal or cancerous. Recent studies showed that mutations can also occur in switches, promoters and enhancers which are present in the non-coding regions and are also responsible for some unusual diseases. We need to go back and look at these regions more carefully. Our Genome provides the genetic road map of all our genes, past, present and future. For example, it can tell us how many good or bad genes we inherit from our parents and how many of those genes we are going to pass on to our children. If a family has too many bad genes, and have a family history of serious illnesses, we can break off the information flow and stop having children or stop donating mutated or defected eggs and sperms.

OUR SEARCH FOR UNKNOWN IDIOPATHIC DISEASES HAS COME TO A CLOSURE

There are two most powerful implications of human Genome Sequencing. One of them is that we have come to closure. What it means is that we have the catalog of all genes in the Human Genome, we can search the entire genome and locate the desired gene. We will not wonder in the wilderness anymore. Everything there is to know about human health and traits are written on these genes in nucleotide sequences. Our Genomes provides the catalog of all genes.

We can scan the whole genome (Reference Sequence) for its response to a given situation. When we look at a normal cell and compare it with an abnormal cell, we see the differences. Or when we compare their gene expression looking for a specific proteins, from a specific genes and for a specific nucleotide sequence, we can identify a specific disease. Now, we have completed the thousand genome project. To get precision and accuracy, we can compare a suspect gene with a thousand copies of the same gene sequence.

In the olden days, before scanners and the human genome, a physician would order the same tests for many different diseases such as cancers, cardiovascular diseases. Alzheimer, multiple sclerosis, osteoporosis, and AIDS. These tests were recommended which will allow them to check the functions of liver, kidneys and for monitoring our health to check the level of cholesterol, triglycerides, sugar, hormones, salt, potassium etc. After examining the data, the Physician would say to his patient, I don't know what is wrong with you, I will see if any of these tests show if my guess is right and if it is wrong, he will recommend few more tests to see if he could identify the illness. The guesswork and the trial-and-error days are over. Now, after sequencing the human genome, the physician would say I don't know what is wrong with you, but I do know where to find it. It is written in your Genome. Today, it would be easy for a Physician to scan the patient entire genome and compare against the Reference Sequence to identify the mutations responsible for

causing the disease. He will take a small blood sample of the patient, separate his WBC, extract DNA, sequence his Genome and compare with the Reference Sequence letter by letter, word by word by word and sentence by sentence, he can easily identify the mutations responsible for causing the disease. The sequencing result will provide the best diagnostic method to identify a disease. In other words, by comparing the sequence of Gene Profiles of normal genes with mutated genes, one can identify with precision and accuracy the exact location of mutated (altered or damaged) nucleotide sequence responsible for causing the diseases. Comparing Gene Profiles is an excellent diagnostic method which helps us design drugs to specifically shut off the mutated genes. Delivering drugs from injection site to the target site is the essential way of treating diseases. We cannot design novel drugs unless we find the abnormal mutations responsible for causing that disease.

Our Genome is not just a diagnostic road map of our genes, it also tells us how to clone the good genes and shut off the bad genes. Using good genes, it also tells us how to make its large-scale protein for worldwide use such as Insulin and Human growth hormone. On the other hand, identifying the bad genes, it tells us how to design drugs to shut off bad genes responsible for causing Cancers, Cardiovascular disease, and Alzheimer. We have already demonstrated that using genetic engineering techniques, we can cut, paste, copy, and sequence a good gene for industrial scale preparation such as Insulin to treat 300 millions of diabetic around the world. Similarly Human Growth Hormone, once available in minute quantities from the pituitary glands of humans' cadavers can now be produced in large amounts in the Labs using the same genetic engineering method. Many valuable medicines are being produced including Interleukin-II, for the treatment of the Kidney cancer, Factor-VIII for treating Hemophilia, Hepatitis B vaccine, Eleuthero protein for anemia, Whooping cough vaccine, and for Somatotropin for treating dwarfism.

Once the good and bad genes are identified, we can look up the genetic make-up of the most common illnesses such as coronary artery disease, stroke, respiratory tract infections, chronic obstructive pulmonary disease, tracheal, bronchial and lung cancer, diabetes, Alzheimer, and idiopathic diseases. We know that the good genes code for good proteins which keep us healthy, and the bad genes produce bad protein that make us sick. Genome sequencing of bad genes starts a new era of Genomic Medicine which is based on the genetic make-up of the individuals. The next step would be to design drugs to shut off the mutated genes. As I said above, Gene Therapy will work if the disease is caused by a single gene mutation. Drug Therapy will work if multiple genes are responsible for causing diseases such as Cancers, Cardiovascular diseases, and Alzheimer.

How can we protect you and your offspring from living a measurable life? By designing drugs to shut off the diseased gene. By eliminating diseased genes from your genome either by gene therapy or by drug therapy. Gene therapy will work on a single genetic defect. Drug therapy will work on multiple genetic defects. By designing drugs to shut off a diseased gene. In the following pages, I have described as a doctoral candidate, how I designed CB1954, a novel drug, to shut off a gene responsible for causing cancer in animal and in my post-doctoral studies how I translated animal work to human by designing AZQ for treating brain cancer in humans.

Throughout the past decade, there have been substantial advances in understanding the pathogenesis of idiopathic pulmonary fibrosis (IPF). Recently, several large genome-wide associations (GWAS) and linkage studies have identified common genetic variants in more than a dozen loci that appear to contribute to IPF risk. In addition, family-based studies have led to the identification of rare genetic variants in genes related to surfactant function and telomere biology. Mechanistic studies suggest pathophysiological derangements associated with rare genetic variants which are also found in sporadic cases of IPF. Current evidence suggests that rather than existing as distinct syndromes, sporadic and familial cases of IPF (familial interstitial pneumonia) probably reflect a continuum of genetic risk.

As we go forward with the development of novel treatments, we anticipate that advances in these genetic and genomic technologies will result in a re-organization of the way we define and classify interstitial lung disease based on molecular characterization. As we advance from a system of diagnosis based on histopathology to one based on a specific genetic/genomic signature reflecting the fundamental biology of the disease, there will be unique opportunities to develop and test therapies in specific patient populations based on the molecular profiles. Coupled with advances in detection of early disease, the coming decade offers an unprecedented opportunity to dramatically change the lives of patients with IPF. Deciphering the biological effects of common genetic variants identified by GWAS has proven challenging so far. It is possible that the relevant biological effect of most individual SNPs is subtle or manifest only in the context of unique additional genetic or environmental factors to confer disease risk. Despite challenges, future studies are needed to clarify the biological role of disease-associated with common genetic variants.

We anticipate that by understanding the biological mechanisms through which individual genetic variants contribute to disease pathogenesis, key pathways will be identified that will clarify the crucial molecular mediators of IPF pathogenesis. We anticipate that a role for molecular genetics in the classification of IIPs will emerge. The ultimate challenge that lies ahead is to develop an integrated understanding of the role of genetic variants (rare and common). As more and more genomes are sequenced, the emergent of new mutants will help us identify how these variants interact with each other and with environmental factors to produce idiopathic diseases and help us understand if they are caused by either epigenetic, transcriptomic, proteomic, or histopathologic. With increased understanding of the fundamental mechanisms of these diseases, the future is promising for development of new, targeted therapies to further improve treatment of IPF.

As I said above, a gene codes for a protein. We successfully sequenced the smallest gene in bacterial genome to the largest gene in Duchenne Muscular Dystrophy genome. To code for a protein, between start and stop codon, a gene has accumulated several hundred to several thousand codons. A single mutation (change or damage) in the coding region of a single codon will alter the gene function. Mutation is caused by either exposure to radiation, chemical or environmental pollution, genetic inheritance, viral infections or DNA deletion, insertion relocation or inversion. Mutation can be good, bad, or neutral. A good mutation can convert a single cell organism to a multicellular creature resulting in evolution. A bad mutation is responsible for coding for a wrong amino acid responsible for causing diseases. A neutral mutation can serve as a gene marker for identifying its presence close to a good or a bad gene. To understand the function of each gene, we must read the entire human genome.

In 2011, a genome-wide linkage study identified a locus on chromosome-11 that was significantly associated with IPF risk. Chromosome-11 is made of 144 million nucleotide base pairs and carries 1,692 genes. Resequencing of this region subsequently identified a common single nucleotide polymorphism (SNP) in the promoter region of the gene. On Chromosome-11, a single gene called XIAP is implicated for causing idiopathic disease. As I said above, a gene codes for protein. The XIAP gene provides instructions for making a protein that is found in many types of cells, including immune cells. Does it act as an antigen? An antigen is a foreign substance (usually harmful) that induces an immune response, thereby stimulating the production of antibodies. A substance that induces the immune system to form a corresponding antibody is called an immunogen. Does it trigger an inflammatory antigenic response which creates a cascade of reactions in affected tissue resulting in idiopathic disease? It may help protect these cells from self-destructing (undergoing apoptosis) by blocking (inhibiting) the action of certain enzymes called caspases, which are necessary for apoptosis. As I said above, a gene code for a protein. If you would identify a mutated nucleotide on a gene responsible for causing idiopathic disease, I would show how to design drugs to shut off the gene either by using an extremely reactive drug like Nitrogen Mustard or by using a non-toxic prodrug like Aziridines or Carbamates (as we have demonstrated below). If we succeed in shutting off that gene, Idiopathic diseases will not be diseases of unknown origin. We will bring them in line with other known western medicine.

For example, the XIAP gene codes for the XIAP protein that the body needs to regulate the immune system. People with this genetic change (mutation) either have problems with the XIAP protein, or they do not have the protein. Another name for this condition is XIAP deficiency. We have identified XIAP gene that regulates the immune cell. Once a mutation related to a disease is identified, our next challenge is to design drugs to shut off that mutated gene. Since the language of life of all living creatures is written in the same four genetic nucleotides, a drug design to treat genetic disease in animals could be tested in humans. If the association of idiopathic protein linkage to the XIAP gene on chromosome-11 is confirmed, then I could suggest how to design drugs to shut off the gene. This article describes how scientific rational was used to design drug like Aziridine, 2-4, Dinitrophenyl Benzamide (CB1954), to shut off a gene to treat a solid aggressive tumor like the Walker carcinoma 256 in Rats and how similar rational was used to design, (AZQ), Di-aziridine benzoquinone Carbamate (US Patent 4,233,215) to shut off a gene to treat solid aggressive brain tumor like Glioblastoma in humans.

Chemicals Weapons used to shut off genes by cross-linking DNA

Historical Background

Highly toxic chemicals were used as weapons during World War (WWI) and more toxic analogs were developed during World War (WWII). Among the worst chemicals was Bis-chloroethyl amino methane which smell like mustard seeds called the Nitrogen Mustard. When soldiers in trenches were exposed to Nitrogen Mustard, upon contact with skin, the bis-chloroethyl amino group was hydrolyzed to generate highly reactive carbonium ions which attack simultaneously cross-link both strands of DNA shutting off the genes resulting in sever burn causing death.

The supreme intellect for using chemical weapons to treat diseases is Ross, an Englishman, who is a Professor of Chemistry at London University, England. Professor WCJ Ross is also the Head of Chemistry Department at the Royal Cancer Hospital, Chester Beaty Research Institute, a post-graduate medical center of the London University. Ross was the first person to use chemical weapons for treating Cancers. He observed that Nitrogen Mustard can cross-link both strands of DNA that we inherit one strand from each parent. As I said above, Cross-linking agents such as Nitrogen mustard are extremely toxic and were used as chemical weapon during the First World War (WWI). More toxic derivatives were developed during the Second World War (WWII).

Using data for the toxic effect of Nitrogen Mustard on soldiers during the First World War, Ross observed that Soldiers exposed to Nitrogen Mustard showed a sharp decline of White Blood Cells (WBC) that is from 5,000 cell/CC to 500 cells/CC. He immediately realized that children suffering from Childhood Leukemia have a very high WBC count that is over 90,000 cells/CC. In sick children, most of the WBCs are premature, defective and unable to defend the body from microbial infections. Ross rationale was that cancer cells divide faster than the normal cell, by using Nitrogen Mustard in controlled doses to cross linking both strands of DNA, one can control and stop the abnormal WBC cell division in Leukemia patients. It was indeed found to be true. Professor Ross was the first person to synthesize hundreds of derivatives of Nitrogen Mustard over a decade. By using an analog of Nitrogen Mustard, called Chlorambucil [7,8] he was successful in treating Childhood Leukemia. In America, two Physicians named Goodman and Gilman from Yale University were the first to use Nitrogen Mustard to treat cancer in humans. Nitrogen Mustards and their analogs are highly toxic. Ross was a Chemist, over the years, he synthesized several hundred derivatives of Nitrogen Mustard derivatives to reduce the toxicity of Nitrogen Mustard [9].

Although analogs of Nitrogen Mustard are highly toxic, they are more toxic to cancer cells and more cancer cells are destroyed than normal cells. Toxicity is measured as the Chemotherapeutic Index (CI) which is a ratio between toxicity to Cancer cells versus the toxicity to Normal cells. Higher CI means that the drugs are more toxic to cancer cell. Most cross-linking Nitrogen Mustard have a CI of 10 that is they are ten times more toxic to cancer cells. Some of the Nitrogen Mustard analogs Ross made over the years are useful for treating cancers such as Chlorambucil for treating childhood leukemia (which brought down the WBC level down to 5,000/CC). Children with Childhood Leukemia treated with Professor Ross Chlorambucil showed no sign of Leukemia even after 20 to 25 years later. Chlorambucil made Ross one of the leaders of the scientific world. He also made Melphalan and Myrophine for treating Pharyngeal Carcinomas [10-13]. At the London University, I was trained as an Organic Chemist in the Laboratory of Professor WCJ Ross of the Royal Cancer Hospital, at the Chester Beaty Cancer Research Institute, a post-graduate medical center of the London University.

As I said above, Professor Ross was designing drugs to attack both strands of DNA simultaneously by cross-linking double stranded DNA using Nitrogen Mustard analogs, which are extremely toxic. A radiolabel study to understand the mechanism of action of Nitrogen Mustard showed that cross-linking of DNA occurred in two steps. The first step involved the formation of a three-member aziridine intermediate which attacks a single strand of DNA followed by the similar second step ending in cross-linking both strands of DNA. The aziridine intermediate could not be isolated. The intermediate Aziridine has great advantage over nitrogen mustard. It binds to only one strand of DNA, and it shuts off the gene. Aziridine is half as

toxic, instead cross linking both strands of DNA, it binds to only one strand of DNA. Aziridine acts as a pro-drug. The three, membered aziridine ring is stable in neutral and basic media. It is activated only in the presence of acid. Aziridine has specificity and selectivity. As tumor grows, it uses Glucose as a source of energy. Glucose is broken down to lactic acid. It is acid which opens the aziridine ring and its carbonium ion attacks N-7 Guanine of a single stand of DNA shutting off the gene. Only Since growing tumor produces acid. Only tumor cells are attacked. To attack a single strand of DNA, aziridine analogs are separately synthesized. As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking DNA strands, I am to design drugs to attack only one strand of DNA. This class of pro-drug is called Aziridines (Figure 1).

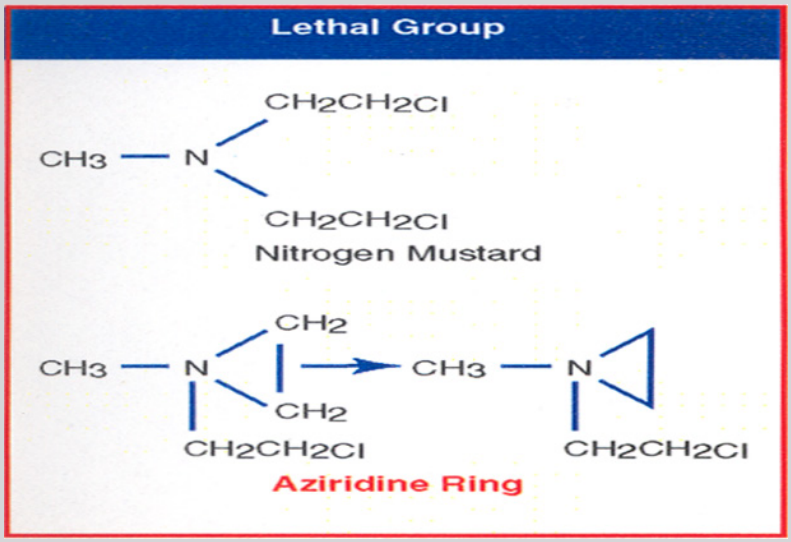

Figure 1: DNA binding aziridine group.

Mechanism of Action of Nitrogen Mustard

Nitrogen Mustard neither have selectivity nor specificity. They attacked all the divided cells including normal cells. As I stated above, during the study of the mechanism of action of radiolabeled Nitrogen Mustard on DNA, it was discovered that the two arms of Nitrogen Mustard do not bind to the double stranded DNA simultaneously. The first arm of the Nitrogen Mustard generates extremely reactive carbonium ion which attack to Nitrogen atom of the amino group forming a stable three-member aziridinium ion. We were unable to isolate the aziridinium ion intermediate as a growing tumor which produces acid which breaks down aziridinium ion.

The Carbonium ion generated by the second arm of the Nitrogen Mustard attacks the N-7 guanine of the DNA nucleotide. Within minutes, both strands of DNA are bound by both arms of Nitrogen Mustard. We were able to isolate cross-linking DNA products. This study showed that to attack a single strand of DNA, we must synthesize Aziridine in the Lab by using ethyl amino methyl sulphonate in sodium hydroxide. Pure Aziridine was distilled off.

Synthesis of Aziridine analogs will give two advantages over Nitrogen Mustard: first, instead of cross-linking, Aziridine binds to one strand of DNA, reducing its toxicity of the double stranded Nitrogen Mustard by half. Second, it gives selectivity, the Aziridine ring serves as prodrug opens only in the acidic medium. Once the active ingredient Aziridine was determined to attack DNA, the next question was what drug delivery method should be used to deliver Aziridine at the tumor site (Figure 2).

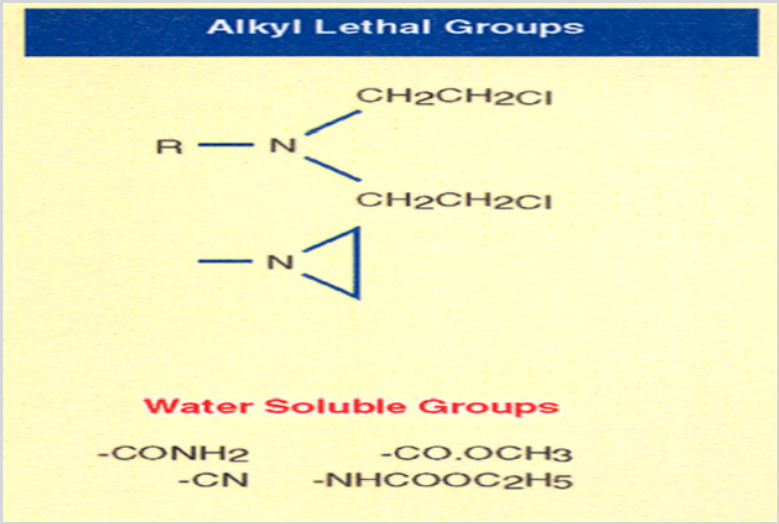

Figure 2: DNA binding lethal groups.

DESIGNING DRUGS TO BIND TO A SINGLE STRANDED DNA TO TREAT ANIMAL CANCERS

As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking both strands of DNA by Nitrogen Mustard, I am to design drugs to attack only one strand of DNA by making Aziridine analogues. We decided to use Aziridine moiety (as an intermediate of Nitrogen Mustard) that would be an excellent active component to shut off a gene by binding it to a single strand of DNA. To deliver Aziridine to the target site DNA, we decided to use Dinitrophenyl (DNP) moiety as a drug delivery agent. DNP is a dye which colors the tissues of the experimental animal tumor such as Walker Carcinoma 256 in Rats. It is well known that analogs of DNP such as Dinitrophenol disrupt the Oxidative Phosphorylation of the ATP (Adenosine Triphosphate) which provides energy to perform all our body functions. To provide energy to our body function, the high energy phosphate bond in ATP is broken down to ADP (Adenosine Diphosphate) which is further broken down to AMP (Adenosine Mono Phosphate), the enzyme Phosphokinase put the inorganic phosphate group back on the AMP giving back the ATP. This cyclic process of Oxidative Phosphorylation is prevented by Dinitrophenol. As a part of my doctoral thesis, I decided to use Dinitrophenol as a drug delivery method for the active ingredient aziridine. The analog of DNA such as Aziridine. Dinitrophenol could also serve as a dye which stains Walker Carcinoma 256, a solid and most aggressive tumor in Rat. The first molecule I made was by attaching the C-14 radiolabeled Aziridine to the DNP dye. The Dinitrophenyl Aziridine was synthesized using Dinitrochlorobenzene with C-14 radiolabeled Aziridine in the presence of Triethyl amine which removes the Hydrochloric Acid produced during the reaction. When the compound Dinitrophenyl Aziridine was tested against the implanted experimental animal tumor, the Walker Carcinoma 256 in Rats, showed a TI (Therapeutic Index) of ten. The TI of ten was like most of the analogs of Nitrogen Mustard. Since this Aziridine analog was not superior to Nitrogen Mustard, it was dismissed as unimportant.

STRUCTURE ACTIVITY RELATIONSHIP

On reexamination of the X-ray photographs of Dinitrophenyl Aziridine, it appeared that most of the radioactivity was concentrated at the injection site. Very little radioactivity was observed at the tumor site. It was obvious that we needed to make derivatives of Dinitrophenyl Aziridine to move the drug from the injection site to the tumor site. Because of the lack of fat/water solubility to be effective drug delivery method, Dinitrophenyl Aziridine stays at the injection site, a very small amount of radioactivity was found on the tumor site. I immediately realized that by altering structure, I could enhance biological activity by making water and fat-soluble analogs of Dinitrophenyl Aziridine. By attaching water soluble groups, I should be able to move the drug from the injection site to the tumor site (Figure 3). To deliver 2,4-Dinitrophenylaziridine form the injection site to tumor site, I could alter the structure of 2,4-Dinitrophenylaziridine by introducing the most water-soluble group such as ethyl ester to the least water-soluble group such as Cyano- group or to introduce an intermediate fat/water soluble such as Amido group.

Figure 3: Dinitrophenyl benzamide a novel drug delivery molecule for aziridine.

An additional substituent in the Dinitrophenyl Aziridine could give three isomers, Ortho, Meta, and Para substituent. Here confirmational chemistry plays an important role in drug delivery methods. Ortho substituent always give inactive drug. Model building showed that because of the steric hinderance, Aziridine could not bind to DNA shutting off the genes. On the other hand, Meta and Para substituents offer no steric hindrance and drug could be delivered to DNA. The following chart showed that I synthesized all nine C-14 radiolabeled analogs of 2,4-Dinitrophenyl aziridines and tested them against implanted Walker Carcinoma 256 in Rats (Figure 4).

The most water-soluble substituent

The first three compounds on top line of the above chart carry all three isomers of the most water-soluble Ethyl Ester group attached to 2,4-Dinitropehny aziridine. The compound *in vivo* is hydrolyzed ester to produce the most water-soluble carboxylic group. Within 24 hours of injection, the entire radioactive compound was extracted from the Rat's urine and washed down from the cages. Since the Ortho position was not available for DNA binding, it showed no biological activity, but the third compound in which Ortho position was free to bind to DNA showed some anti-tumor activity in Rats.

The least water-soluble substituent

On the other hand, when the least water-soluble Cyano-group was attached to all three isomers of the 2,4-Dinitrophenyl aziridine compound as shown in the second line of the above chart, most of the compound stayed at the injection site. Only the last Cyano-derivative attached to DNA showed some anti-tumor activity.

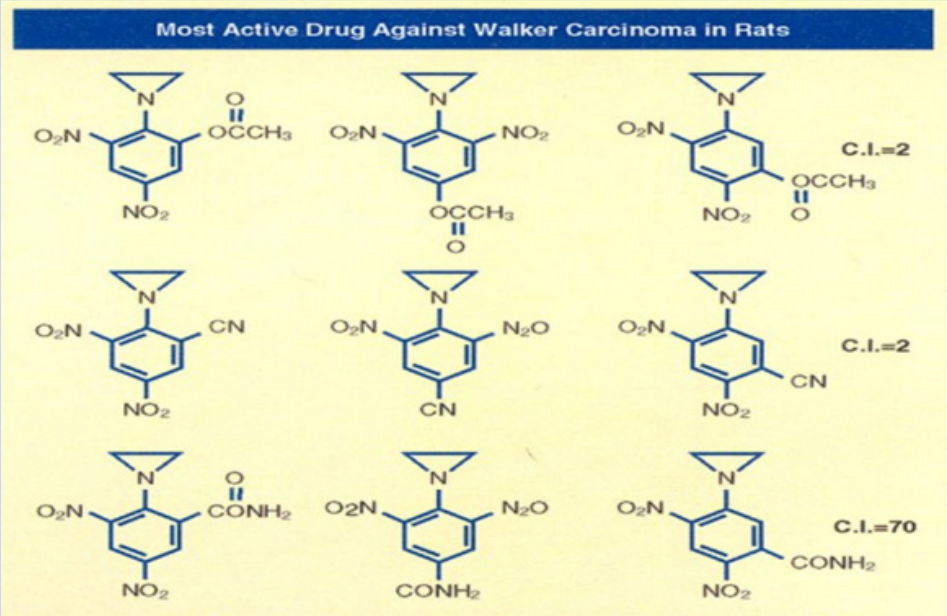

Figure 4: Derivatization of dinitro phenyl benzamide based on partition coefficient.

The moderately soluble amido-substituent

The last line of the above chart showed that the first two Amido groups were sterically hindered and did not bind to DNA and showed no biological activity, but the last compound presents the perfect drug delivery method. The entire drug was delivered from the injection site to the tumor site. The drug 1-Aziridine, 2,4-dinitro, 5-benzamide (CB1954) showed the highest anti-tumor activity. It has a CI of seventy; it is seventy times more toxic to cancer cells, the highest toxicity ever recorded against Walker Carcinoma 256 in Rats [14-16].

As I said above, Nitrogen Mustards are highly toxic because they have neither specificity nor selectivity. They attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as prodrugs and remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by growing cancer cells. Aziridine attacks DNA in acidic medium, particularly the N-7 Guanine. The dye Dinitro benzamide has great affinity for Walker Tumor. The Aziridine Dinitro benzamide (CB1954) has the highest toxicity to Walker Tumor cells ever recorded. As the tumor grows, it uses Glucose as a source of energy. Glucose is broken down by Lactic Acid. It is the acid which activates the Aziridine ring. The ring opens to generate a carbonium ion which attacks the most negatively charged N-7 Guanine of DNA (as shown below) shutting off the Walker Carcinoma gene in Rat. The following conjugate structure shows how CB1954 binds to a single stranded of DNA shutting off the gene (Figure 5).

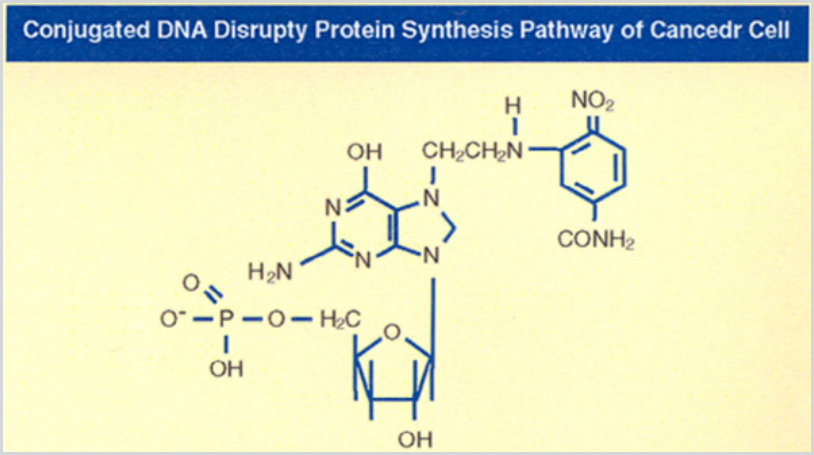

Figure 5: Conjugated DNA disrupting protein synthesis pathway of cancer cell.

For the discovery of CB1954, The University of London, honored with the Institute of Cancer Research (ICR) post-doctoral fellowship award to synthesize more analogs of CB1954. To improve drug delivery method, over the years, I made over a hundred additional analogs of Dinitro phenyl aziridines. To increase the toxicity of CB1954 to Walker Carcinoma, I made an additional 20 analogs as a postdoctoral fellow. When I attached one more Carbonium ion generating moiety, the Carbamate moiety to the Aziridine Dinitrobenzene, the compound Aziridine Dinitro benzamide Carbamate was so toxic that its Therapeutic Index could not be measured. We stopped work. Further work at London University was discontinued for safety reasons [17,18]; (Figure 6).

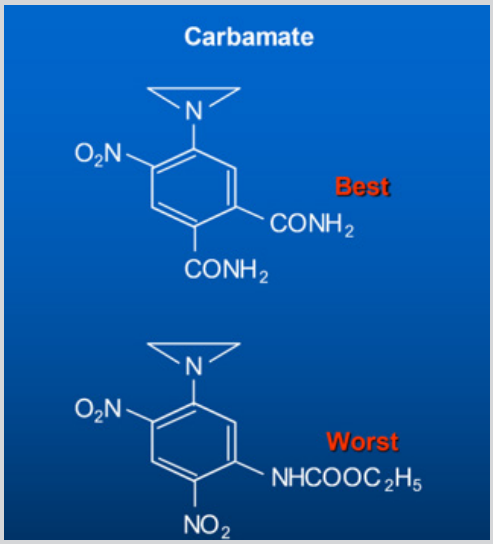

Figure 6: The best and the worst dinitro phenyl aziridine analogs.

As a part of the inter-government agreement between UK and USA, all novel drugs developed in England were sent to the National Cancer Institute (NCI) in America for further screening. To translate animal work to human, I was invited to continue my work on the highly toxic Aziridine/Carbamate combination in America when I was offered the Fogarty International Fellowship Award to continue my work at the National Cancer Institute (NCI) of the National Institutes of Health (NIH), USA. For making more Aziridine/Carbamates, I brought the idea from London University of attacking one strand of DNA using not only Aziridine, but also Carbamate without using the same dye Dinitro benzamide. My greatest challenge at NCI is to translate the animal work which I did in London University to humans.

Designing drugs to treat Glioblastoma, the human brain cancer

One day, I heard an afternoon lecture at the NIH in which the speaker described that radio labeled Methylated Quinone crosses the Blood Brain Barrier (BBB) in mice. When injected in mice, the X-ray photograph showed that the entire radioactivity was concentrated in the Mice's brain within 24 hours. I immediately realized that Glioblastoma multiforme, the brain tumor in humans, is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a novel drug delivery molecule to cross BBB delivering Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rationale to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone molecule to test against Glioblastomas in humans.

Glioblastoma (GBM) is a primary type of brain cancer which originates in the brain, rather than traveling to the brain from other parts of the body, such as the lungs or breasts. GBM is also called glioblastoma multiforme which is the most common type of primary brain cancer in adult humans. Attaching Nitrogen Mustard group to Quinone will produce highly toxic compound which will have neither specificity nor selectivity. Such a compound will attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as prodrugs that is they remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by cancer cells (Figure 7).

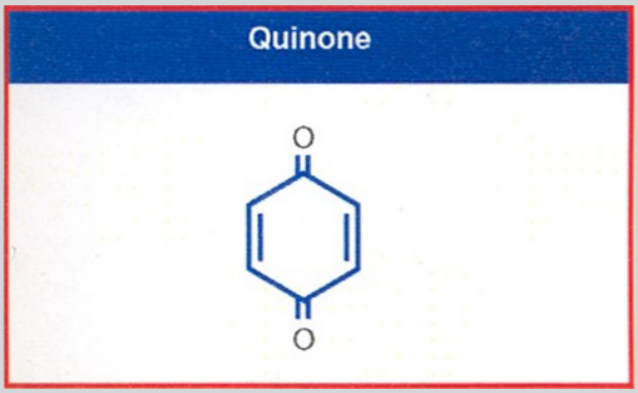

Figure 7: The structure of a non-toxic and non-addictive quinone used for crossing the Blood Brain Barrier (BBB).

DNA binding aziridines

I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rationale to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone to test against Glioblastomas in humans. Over the years, I made dozens of analogs of Aziridine Quinone. By attaching two Aziridines and two Carbamate moieties to Quinone, I synthesized the most useful

compound, Diaziridine Dicarbamate Quinone, I named this novel compound AZQ. Over a three-year period, I made 45 analogs of AZQ. They were all considered valuable enough to be patented by the US Government (US Patent 4,233,215). By treating brain cancer with AZQ, we observed that Glioblastoma tumor not only stops growing, but it also starts shrinking. I could take care of at least one form of deadliest old age cancers, Glioblastomas. Literature search showed that AZQ is extensively studied as a pure drug and in combination with other anti-cancer drugs [19].

As I said above, Glioblastomas, the brain cancers, is a solid and aggressive tumor and is caused by mutations on several chromosomal DNA. Deleterious genetic mutations are the result of damaging to DNA nucleotides by exposure to radiation, chemical and environmental pollution, viral infections, or genetic inheritance. The other factors responsible for causing DNA mutations are due to the fast rate of replication of DNA. For example, the bacteria E-coli grows so rapidly that within 24 hours, a single cell on a petri dish containing nutrients forms an entire colony of millions when incubated on the Agar Gel. Mistakes occur in DNA during rapidly replication such as Insertion of a piece of DNA, Deletion, Inversion, Trans location, Multiple Copying, Homologous Recombination etc. When an additional piece of nucleotide is attached to a DNA string, it is called Insertion, or a piece of DNA is removed from the DNA string; it is called Deletion or structural Inversion of DNA is also responsible for mutations. Since the gene in a DNA codes for Proteins, Insertion and Deletion on DNA have catastrophic effects on protein synthesis. With the Quinone ring as a carrier across BBB, I could introduce different combinations of Aziridine rings and Carbamate moieties to Quinine and could create havoc for Glioblastomas. My major concern was how toxic this compound would be to the human brain cells. Fortunately, brain cells do not divide, only cancer cells divide.

Attempting to find the site of mutations on Glioblastomas represents the greatest challenge. In Glioblastomas, three major changes occur on Chromosomes (C-7, C-9 & C-10) and two minor changes occur on Chromosomes (C-1 & C-19). These mutations are responsible for causing brain cancers in humans. Let us examine the effect on each chromosome. In a normal human cell, Chromosome-7 is made of 171 million nucleotide base pairs, and it carries 1,378 genes. When Insertion occurs on Chromosome-7. Ninety-seven percent of Glioblastoma patients are affected by this mutation. On the other hand, a different mutation occurs on Chromosome-9 which is made of 145 million nucleotide base pairs, and it carries 1,076 genes. A major Deletion of a piece of DNA occurs on Chromosome-9 which results in eighty- three percent patients who are affected by this mutation. A minor Deletion of DNA also occurs on Chromosome-10 which is made of 144 million base pairs, and it carries 923 genes. Although it is a minor deletion of a piece of DNA and yet it contributes to ninety-one percent patients with Glioblastoma. To a lesser extent, small mutation occurs on Chromosome-1 (the largest Chromosome in our Genome). It is made of 263 million nucleotide base pairs and carries 2,610 genes) and Chromosome-19 (it is made of 67 million base pairs and carries 1,592 genes) is also implicated in some forms of Glioblastomas.

All known Glioblastomas causing genes are located on five different chromosomes and carries a total of 9,579 genes. It appears impossible to design drugs to treat Glioblastomas since we don't know which nucleotide on which gene and on which chromosome is responsible for causing the disease. It becomes possible by using C-14 radiolabeled Aziridines, we can confirm the binding site of a nucleotide on a specific gene and on a specific chromosome. By comparing it with the mega sequencing project, we can further confirm the sites of mutations.

With the completion of the 1,000 Human Genome Project, it becomes easier. By simply comparing the patient's genome with the sequencing of one thousand genomes, letter by letter, word by word and sentence by sentence, we could identify the differences called the variants with precision and accuracy, the exact variants, or mutations responsible for causing the disease. Once the diagnosis is confirmed, the next step is how to treat the disease. As I explained above, by making CB 1054 to treat solid Walker Carcinoma in Rats, I established the structure activity relationship, and by making AZQ to treat human Glioblastoma, we have demonstrated that all bad genes can be shut off using Aziridine or Carbamate or both as attacking agents to shut off a gene. If you plan to develop drugs to treat other cancers, all we need to do is to identify carriers such as coloring dyes which stains a specific tumor. By attaching Aziridines and Carbamate moiety to carriers to the dyes, we could attack other tumors.

One of the greatest challenges of nanotechnology is to seek out the very first abnormal cell in the presence of billions of normal cells of our brain and shut off the genes before it spread. I worked on this assignment for about a quarter of a century; conducted over 500 experiments which resulted in 200 novel drugs. They were all tested against experimental animal tumors. Forty-five of them were considered valuable enough to be patented by the US Government (US Patent 4, 146, 622 & 4,233,215). One of them is AZQ which not only stops the growth of Glioblastoma, but also the tumor starts shrinking. For the discovery of AZQ, I was honored with, "The 2004 NIH Scientific Achievement Award." One of America's highest Award in Medicine. I was also honored with the India's National Medal of Honor, "Vidya Ratna" a Gold Medal (Exhibits 1,2,3,4).

Exhibit #1: 2004 NIH Scientific Achievement Award Presented to Dr. Hameed Khan by Dr. Elias Zerhouni, The Director of NIH

Dr. Khan is the Discoverer of AZQ (US Patent 4,146,622), a Novel Experimental Drug Specifically Designed to shut off a Gene that causes Brain Cancer for which he receives a 17-year Royalty for his invention (License Number L-0I9-0I/0). To this date, more than 300 research papers have been published on AZQ. The award ceremony was broadcast live worldwide by the Voice of America (VOA). Dr. Khan is the first Indian to receive one of America's highest awards in Medicine.

Exhibit #2

Exhibit #3: His Excellency, Dr. A.P.J. Abdul Kalam, the President of India, Greeting Dr. A. Hameed Khan. Discoverer of anti-cancer AZQ, after receiving 2004, Vaidya Ratna, The Gold Medal, One Of India's Highest Awards in Medicine at The Rashtrapathi Bhavan (Presidential Palace), in Delhi, India, during a Reception held on April 2, 2004. India's National Medal of Honor

Exhibit #4: Single Strand DNA Binding Aziridine and Carbamate

Exhibit #5: DNA Single Strand Binding Agents, Structure of AZQ for treating Glioblastoma

Exhibit #6: Gold Medal for Dr. Khan. Dr. A. Hameed Khan, a Scientist at the National Institutes of Health (NIH) USA, an American Scientist of Indian Origin was awarded on April 2, 2004. Vaidya Ratna; The gold Medal, one of India's Highest Awards in Medicine for his Discovery of AZQ (US Patent 4,146,622) which is now undergoing Clinical Trials for Treating Bran Cancer. For Discovering AZQ Dr. Khan was Awarded A Gold Medal.

Exhibit #7: Presented to by Dr. Duane Alexander, M.D. Director, NICHD, Dr. Robert Stretch, Director DSR and

Dr. Yvonne Maddox, Deputy Director, NICHD. In recognition of his superior commitment, dedication and accomplishment in the planning and executing of over 250 Peer Review Meetings for both Grants and Contracts. Dr. Khan was honored during the Director's Award Ceremony held on October 11, 2006.

Exhibit #8: Based on the Genetic make-up, what other cancers should be explored?

Exhibit # 1: During the NIH/APAO award ceremony held on December 3, 2004.

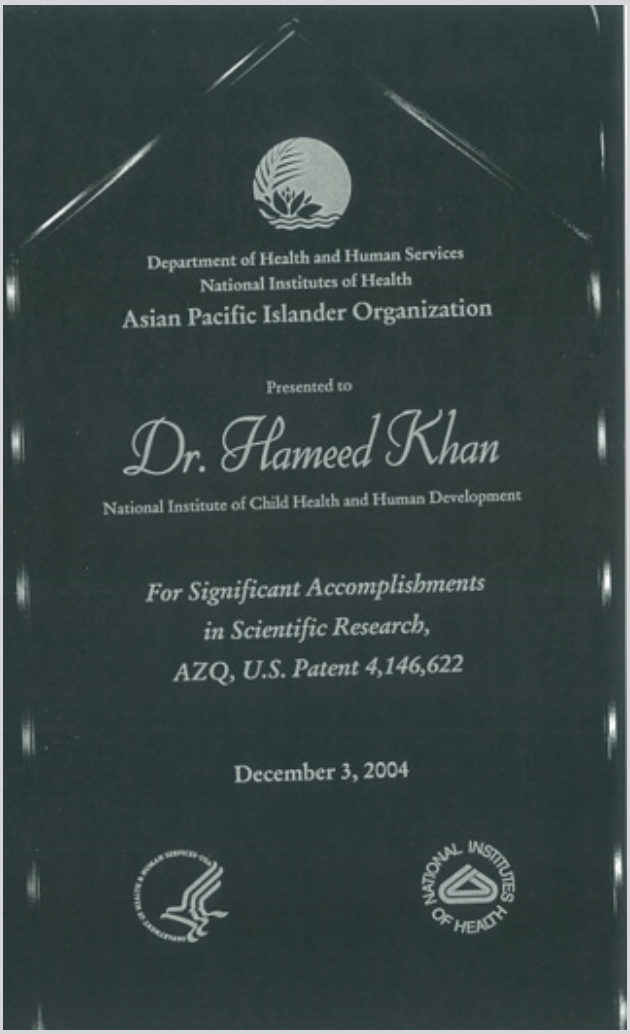

Exhibit # 2: 2004 NIH scientific achievement award.

Exhibit # 3: His Excellency, Dr. APJ Abdul Kalam, The President of India, Greeting, Dr. A. Hameed Khan.

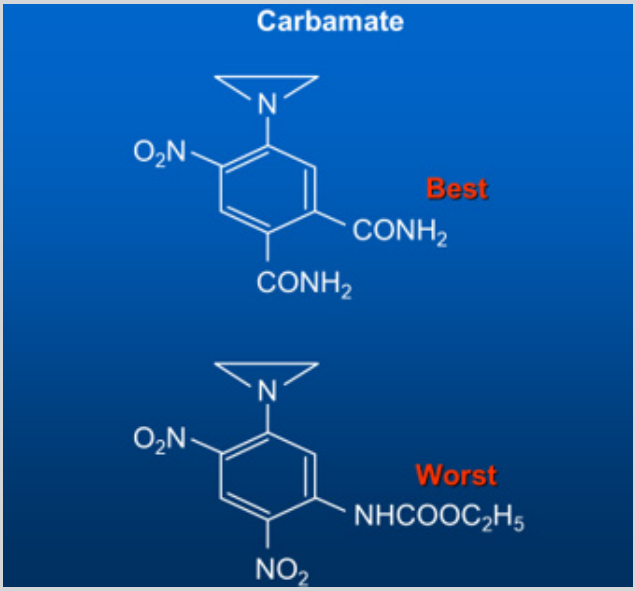

Exhibit # 4: Single strand DNA binding aziridine and carbamate.

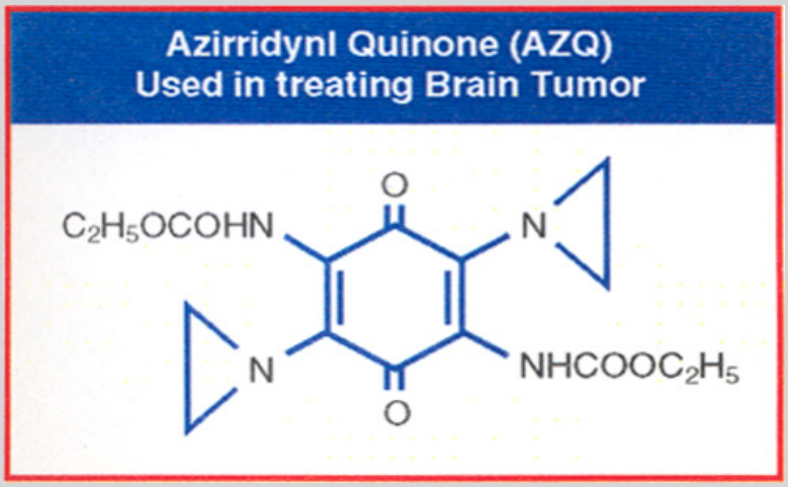

Exhibit # 5: DNA single strand binding agents.

Exhibit # 6: Gold Medal for Dr. Khan.

Exhibit # 7: Presented to by Dr. Duane Alexander, M.D. Director, NICHD, Dr. Robert Stretch, Director DSR and Dr. Yvonne Maddox, Deputy Director, NICHD.

Exhibit # 8: 2000 NIH speaker bureau award presented to Dr. Hameed Khan by Dr. Ruth Kirschstein, Acting Director of NIH & Dr. Vivian Pinn, Associate Director of NIH during the NIH/speaker bureau's award ceremony held on June 12, 2020.

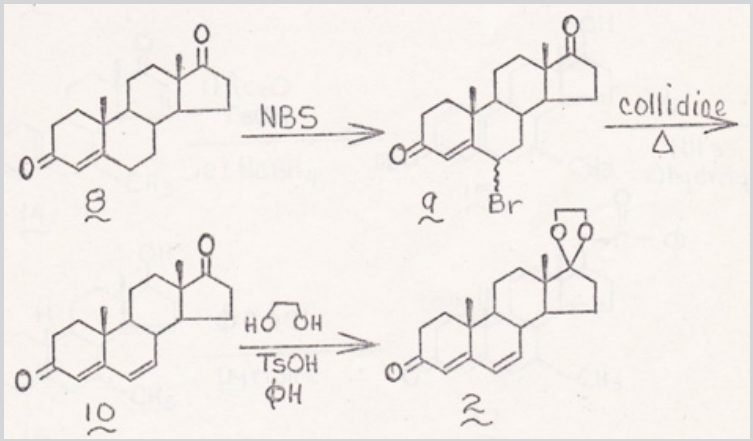

Exhibit # 9

Of all cancers, the largest killer of women is the Breast Cancer. Despite the use of highly advanced treatment methods such as Chemotherapy, Radiation therapy and Surgery, within three years, the tumor returns as metastatic cancer and kill the patients. On the rational basis, I propose the following approach to develop novel drug to treat Breast Cancer. Although mutations on BRCA1 gene responsible for causing Breast Cancer located on Chromosome-17 has been identified years ago, so few drugs were designed on rational grounds. Now, we have sequenced Chromosome-17. We found that it is made of 92 million nucleotide bases pairs carrying 1,394 genes. By comparing it with the Reference Sequence, we can easily identify which nucleotide on which gene of the Chromosome-17 is responsible for causing Breast Cancer. As I said above, Genomic medicine is a predictive medicine. By MRI (Magnetic Resonance Imaging which takes three-dimensional images) and gene sequencing, we should be able to predict if the abnormal changes in the cellular DNA will lead to Breast Cancer. Without this knowledge, it has been so difficult to design drugs on rational basis to treat Breast Cancer. By the time the Breast Cancer diagnosis is confirmed in a patient, the BRCA1 gene has accumulated more than three thousand mutations. Genotyping of the blood sample would also show the existence of many cells carrying mutated cells responsible for creating secondary deposits. It is also found in some cases when not detected earlier, by the time Breast Cancer is confirmed, metastatic cancer cells have already been spread from Liver Lung on their way to Brain.

As a Fogarty International Postdoctoral Fellow at the NCI, I was given the chance to work on any cancer, I was pleased. Since all other organs including Breast and Liver could be removed and replaced by organ transplant except Brain, I thought that protecting Brain is utmost important to save life. For years, I have worked on the development of AZQ. Once the AZQ was developed to protect the Brain Cancer, I could focus on Breast and Prostate Cancers. Recent, Radiolabeled studies in mice showed that male hormone Testosterone has great affinity for female organs like Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for male Prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could design novel drugs to attack both the Breast and the Prostate cancers. Now, I found that I could increase its toxicity several folds to abnormal cells by attaching more than four Aziridine and Carbamate moieties to both Male and Female Hormones.

In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 gene carries about three thousand mutations. These mutations are caused by exposure to radiation, chemical or environmental pollutants, viral infection, or genetic inheritance. To attack the mutated nucleotides among the three thousand mutations in BRCA1 gene, we could use male hormone, Testosterone, and bind multiple radio labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using three dimensional MRI, we could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone ring system, there are only three positions that is 1,3 and 17 are available for substitution on Testosterone ring system.

Carl Djerassi had demonstrated that we could activate additional positions for substitutions on hormone ring system such as the position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be de-brominated by Collidine to introduce a 9,10 double bond which we could be further brominated to produce 9,10 dibromo compound. These bromo ion could be replaced by additional Aziridines or Carbamate ions. We could increase or decrease the number of Aziridine and Carbamate ions to get maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties.

Similarly, we could use the female hormone Estrogen as a carrier and by attaching multiple Aziridine and Carbamate ions to attack Prostate tumor in Men. Since seventeen positions are also available on Estrogen ring as well; again, we could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit by using Djerassi' method as we did with Testosterone. The above methods are novel approach to designing drugs to treat Breast and Prostate cancers. Using genetic make-up of a patient to treat metastatic cancers, the future scientists (my students) will conduct these experiments.

Based on the Genetic make-up, could we design novel drugs to treat idiopathic diseases?

As I said above, our Genome is a diagnostic road map of all our genes including genes responsible for causing idiopathic diseases. By comparing the genome of idiopathic patient with the Reference Sequence, we identify XIAP gene that regulate the immune cell. Could this gene be responsible for causing idiopathic diseases? Once a mutated gene related to a disease is identified, our next challenge is to design drugs to shut off that mutated gene. Since the language of life of all living creatures are written in the same four genetic nucleotides (A-T and G-C), a drug design to treat genetic disease in

animals could be tested in humans. We described above how this scientific rational was used to design drug like CB1954 (Aziridine, 2-4, Dinitrophenyl Benzamide) to treat a solid aggressive tumor like the Walker carcinoma 256 in Rats and how similar rational was used to design, (AZQ), Di-aziridine benzoquinone Carbamate (US Patent 4,233,215) to treat solid aggressive brain tumor like Glioblastoma in humans. If XIAP gene is confirmed for coding a protein which triggers immunogenic response to cause idiopathic diseases, we could use similar rational like the one we used for making AZQ to design drugs to shut off the gene.

PREVENTION

After deciphering the entire genome more than twenty years ago, we embark on a more ambitious goal to complete the Personal Genome Project that is not only to sequence but also to inscribe the total genetic information of all eight billion people on a computer chip (DNA Profile Chip) at a cost of a hundred dollar per genome during the next ten years. It is only a matter of time to finish the work and provide a Genetic Profile Chip to every man, woman, and child on the face of the Earth. You will always carry your DNA chip with you. In case of medical emergency, the hospital staff will compare your genetic profiles with the Reference Sequence and would be able to provide instant medical help. Once we sequence everyone's genome, we will face some ethical problems. Do you want to know your genetic make-up? There might be bad news. Some will say yes, they would like to know what problems to expect in future, and others say no, they don't want to know. I would because I would prepare myself for future health problems. I can plan my future; what I should do, what I should not do. As a responsible person, you should prepare for financial planning for your dependence; medical planning for oneself and other essential family members. It is an individual choice.

The Human Genome Project has enlightened us in ways; we have never been enlightened before. Now we know the answers to questions like who are we? Where have we all come from? What was it that made us this way? We can answer with certainty that genetic studies confirm that you and I are the result of three and a half billion years of Darwinian evolution. We are the extension of the same single DNA molecule that was formed nearly four billion years ago at some remote corner of the Earth. The book of life in all of us is written in the same language of DNA. This is the evidence-based information which we should teach to our children.

The purpose of basic education is to provide an articulate framework of mind. A teacher is supposed to educate, inform, motivate, and inspire the next generation of students. The purpose of higher education in the 21st century is to produce in students a level of excellence. It should, help develop in students a secure sense of their own voice, enough mental toughness, and resourcefulness, and humanity to seek out the wisdom of others, and the ethical and emotional intelligence, required to live life reasonably and responsibly, in a diverse and complex world. At the end of all studies to get a doctoral degree, the knowledge gained will make them a good human being and a useful citizen ready to serve humanity.

Some advice to the future generations of scientists (My students) [20-27]. Once you finish your doctoral studies, you must proceed to the postdoctoral work. The period of Postdoctoral Fellowship is a holding tank. You just finished your doctoral studies and are waiting to get a new job to embark on a new career. The Postdoc fellowship provides you an opportunity to conduct any challenging experiments including the identification and confirmation of idiopathic diseases. Is there a gene which codes for a protein which is responsible for causing idiopathic diseases? To answer this question, you make a list of all suspected antigens and test them to see which one trigger immunogenic response by producing antibody. Isolate and sequence the antibody protein. The protein will point to the gene which codes for the antibody protein. Using C-14 radiolabeled DNA binding agents such as labeled Aziridine or Carbamate, we attack the mutated gene to identify the specific nucleotide responsible for causing idiopathic disease and bring them in line with other western medicine.

After identifying and cleaning up our genomes from defected genes, the quality control of the population will become a reality. When we finish the Personal Human Genome Project of all eight billion people, you will have access to the sequence data from Prisons, mental institutions, and Mental Asylums. The data will provide the goldmine of mutations. You will have the opportunity to compare these data with the Reference Sequence to identify defective mutations responsible for bringing individuals to these places. You will have unique opportunity not only to prevent these diseases, but also to design drugs to treat these diseases. A word about prevention: Newlywed couples can prevent passing on these genes with adverse mutations to the next generations. For example, in Klinefelter Syndrome, a germ line mutation, the male fetus inherits an extra X-chromosome from his mother. The child is born with XXY-chromosomes. Seventy percent of the XXY boys tend to have dyslexia and other learning disabilities and they have below average IQ, typically between 80 and 90. As these boys mature, they will have low level of male hormone, testosterone, enlarge breast, delayed puberty, infertility, reduced sex drive with little or no sperm. Since they cannot complete their sex act, they tend to be violent to their spouse and may be violent to the society. If there is a family history of Klinefelter on either side of the couple, it is best to have conception by *in vitro* fertilization. As the fertilized egg in the Petri Dish matures to 8-cell embryo, we can take a single cell out of the embryo and sequence its genome. We can easily identify the presence of an extra X-chromosome. It is very large and easily detectable. (X-chromosome is made of 164 million nucleotide base pairs and carries 1,144 genes.). The couple can easily discard the defected embryo and use the next healthy embryo for the conception that is to use an embryo which is free from any mutations. I want to make the future scientists (my students) clear. Society must take care of all those children who are already here whether they are gay or straight. They all have the same rights to life, liberty, and pursuit of happiness. They must have equal opportunity to work hard, study hard, obey the laws and follow the rules. Like all of us, they must have full opportunity to climb the ladder of success and get to the promised land and become Prime minister, President, or the leader of our society. What I am wondering about is the children who are not yet born. Do they have the same rights? Parents will look at the sequence of their egg, sperm, or embryo and will decide instead of terminating the pregnancy, they will conceive by vitro fertilization.

Over fifty years ago, a Scottish study showed that male violent inmates in a prison hospital showed seven out of 196 males at Carstairs, the Scottish State Hospital, had an additional Y-chromosome also known as 47, XYY. These studies of the inmates of similar institutions in England, Australia, Denmark, and the USA have confirmed these findings. Now, these studies are questioned because Gene sequencing data was not available to their parents. Today, if some parents refuse to look at the sequence data and decide to bring this child by natural conception, it is parental responsibility to pay all the medical expenses of their children for their entire life. They should know that parents of a severely disabled child can sue

his parents and his doctors who failed to inform them about fetal abnormalities that, if revealed, would have prompted the mother to have an abortion, the Iowa Supreme Court ruled.

CONCLUSION

Today, second and third generation Nanopore genome sequencers can sequence the entire genome cheaper, faster with precision and accuracy for about a hundred dollars. For an additional five dollars they can sequence X and Y Chromosomes or any other chromosomes. Parents must decide if mutated Retinoblastoma gene is identified in the embryo, is there a reason to bring the incurably blind child into this highly competitive world. This is the last generation of mutated children. We would have developed accurate genetic tests for all six thousand mutated genes sooner than you think. All those handicapped children who are here must be treated with utmost respect, utmost courtesy, and utmost friendship. They should be provided with all the medical help they need. Today, through cloning, we have the capability to replicate the entire human being down to the most microscopic detail of our anatomy. Through genetic engineering, we can grow crops that are resistant to insects and drought, but safe for human consumption. Should the quality control of the population be our primary goal? In New Eugenic, it is not the authority, but parents who decide if the child they are bringing to this world is an acceptable member of human society. One person cannot provide answers to all these ethical questions, we need debate and discussion and provide guidelines for society. What I want to do is to raise these questions in your mind. My aim will be fulfilled if I have made you think along these lines.

The opinions expressed in this article are mine and do not represent NIH policy.

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