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The Impact of Genomics on the Administration of Medicine

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ABSTRACT

The administration of medicine has improved enormously over the years, but the completion of the human genome sequence at the start of the 21st century is now rapidly ushering in a new era within medicine. With genome kits available online and holiday sales on human genome sequencing the ability to sequencing one's genome is rapidly changing the face of medicine and has led to the promise of 'personalized medicine'. This IQP attempts to look at the impact that the revolutionary field of genomics is having on the administration of medicine.

ACKNOWLEDGEMENT

To begin with, I want to appreciate the great hospitality of Duff for taking a chance on me as his first IQP student. I don't even know if he knew what he was getting himself into, but he gave me the chance to complete this project successfully. He was very helpful all the way: helping me to learn the skill of time management and also the basic understanding of the genomic project. I also want to thank him for guiding me through my academic career here at WPI. It was fun working with him.

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PROJECT OBJECTIVES

The objective of this IQP project was to begin to examine the impact of genomics on medicine. In today's society medical care is delivered almost exclusively by physicians and other health care professionals. The advent of genomics has led to companies providing direct to consumer (DTC) services involving medical information relevant to health care. The outcome of this project suggests that we are entering an era of "personalized" and "global" medicine, but also one that could remove an important component - physicians and other health care professionals, from the process.

CHAPTER 1: DNA – Discovery and Era of Recombinant DNA/Molecular Biology

DNA – STRUCTURE

The cell is known as the basic unit of life and the genetic instruction of cells in all living organisms and some viruses is Deoxyribonucleic acid, commonly known as DNA. This discovery and the unraveling of the structure of DNA were critical first steps in the development of the field of genomics (National Human Genome Research Institute, 2010).

At the molecular level, DNA gets its name from the sugar that it contains - deoxyribose. This five-carbon sugar (pentose) forms an essential component of the building blocks of DNA termed nucleotides. Nucleotides are made up of the sugar molecule (deoxyribose), a phosphate group, and nitrogenous bases (purines and pyrimidines). A nitrogenous base plus deoxyribose forms a nucleoside and the addition of the phosphate group generates the nucleotide. There exist four nucleotides: thymine, cytosine, adenine, and guanine. The nucleotides adenine and guanine are known as purines because they have a six-membered and a five-membered nitrogen-containing ring fused together. The nucleotides thymine and cytosine on the other hand are known as pyrimidines because they have only a six-membered nitrogen-containing ring fused together. A single strand of DNA is formed when these nucleotides are linked together through phosphodiester bonds formed between the 3' hydroxyl group on the sugar and 5' phosphate group.

Solution of the structure of DNA revealed that it has a very unique double helical structure with two antiparallel strands pairing through the nucleotides (National Human Genome Research Institute, 2010). Nucleotides of the DNA molecule are complementary--meaning the sequence of nucleotides on one DNA strand determines the sequence of nucleotides on the other strand. This is because under normal conditions nucleotides pair (via hydrogen bonds) with only

one other type of nucleotide. This specificity of nucleotide bonding between the two strands of DNA helps creates the well-known double helical structure of DNA as shown in Figure 1 below. The nucleotide pairing is in such a way that a purine is always bonded to a pyrimidine. In a normal DNA double helix, adenine pairs with thymine through two hydrogen bonds and guanine pairs with cytosine through three hydrogen bonds. Each nucleotide pair in the double helix is then commonly referred to as a base pair (for larger sequences of base pairs, one thousand base pairs equal one kilobase of DNA, while a million base pairs equal a megabase of DNA). Hydrogen bonds are relatively weak bonds, thus providing a key element of the ability of the two strands of DNA to be separated or unzipped. These unzipped strands can then be used as templates for the synthesis of additional copies of that DNA molecule; a process that underlies the field of DNA sequencing and genomics (Figure 2).

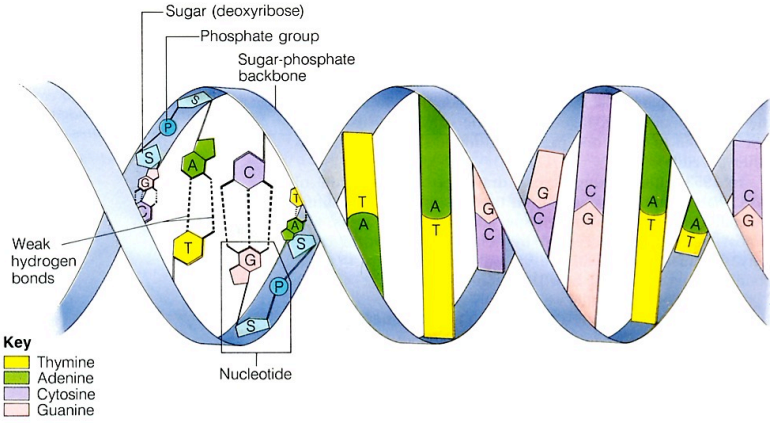

Figure 1: **DNA Structure.** (Taken from: Google Images: metal-domes.com)

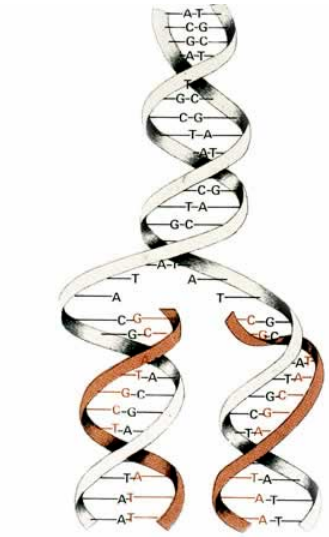

Figure 2: **DNA Replication.** Unzipping DNA provides two templates for the formation another Double Helix. *(Taken from: Google Images: biocorner.com)*

DNA - GENES AS FUNCTIONAL UNITS

To understand the role of DNA in controlling traits it is important to understand the central dogma of molecular biology, which states that information flows directionally from DNA to RNA to protein (Gerstein et…al, 2007; Figure 3). In its simplest form this information, a functional unit of DNA, is called a gene and can vary from hundreds to thousands of base pairs long. Through the process of transcription, genes are then transcribed (copied) into Ribonucleic acid (RNA), which is then read into a sequence of amino acids to form proteins through the process of translation. More recently however, the importance of RNA molecules and not proteins as the functional end for the flow of information from genes has become increasingly evident (Gerstein et…al, 2007).

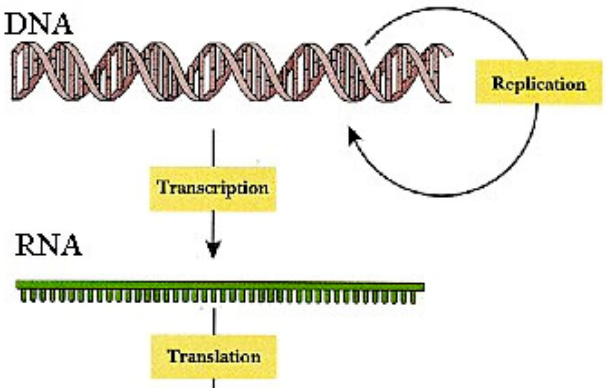

Figure 3: **Central Dogma of Biology**. DNA is transcribed into RNA which is then translated into protein. (Taken from: Google images: faculty.ksu.edu.sa)

At the molecular level, genes have two basic components, the transcription unit, or portion of DNA sequence that is physically copied into RNA, and the regulatory region that directs when and in what cells the gene will be transcribed (Gerstein et…al., 2007; Dillon et…al,. 2000). For genes encoding proteins, within the transcription unit lies the open reading frame or DNA sequence that is read in triplets (codons) to specify the correct sequence of amino acids in the protein (Dillon et…al,. 2000). At a higher level, genes are organized onto chromosomes, which allow for the proper segregation of genes from generation to generation. Through the proper segregation of chromosomes through a process called meiosis, genes can be passed on from parents to offspring.

Humans are diploid having two copies, one derived from each parent, each of twenty-three chromosomes, all of which contain many genes. Thus, we contain two homologous (similar) chromosome number ones, two homologous chromosome number twos and so on. Through the process of meiosis, each parent contributes only one set of their two sets of chromosomes to their offspring. Fertilization combines the set from each parent thereby creating the diploid zygote. It is important to note, however, that there can be considerable diversity in the chromosome set that an offspring inherits from a given parent due to a process known as recombination.

DNA – HISTORY

The quest to discover the genetic material of life began in ~1856 when Gregor Mendel, an Austrian monk, began experiments in genetics. His experiments with pea plants led him to formulate the basic laws of heredity (Orel, 1996). It also led to the growing interest of scientists in the study of genetics. Thomas Hunt Morgan followed this work in 1911 by demonstration that genes are located on chromosomes in a linear fashion, furthering the link between heredity and our molecular understanding of it (Ruben et…al., 2000).

In 1928, a British microbiologist named Frederick Griffith carried out experiments leading to the demonstration that DNA is the molecule of inheritance. He worked with the disease-causing bacteria Pneumococcus and laboratory mice (ThinkQuest, 2010). Two strains of the bacteria were used: a virulent and a non-virulent strain. One group of laboratory mice was injected with the virulent strain and the other group of laboratory mice was injected with the non-virulent strain. The virulent strain had a smooth coat and the non-virulent strain had a rough coat. Those injected with the virulent smooth coated strain died a few days later. Those injected

with the non-virulent rough coated strain continued to be healthy. He then killed the smooth coated virulent strain with heat and injected another group of mice with it. He observed that this group survived. He then thought that if he were to inject a group of mice with the rough-coated non-virulent and the heat-killed strains, the mice would survive. However when he did this experiment, he observed that the mice died. He thought that the heat-killed virulent bacteria had passed on a molecule to the non-virulent bacteria to make it virulent (new trait). He thought that this transforming factor was in the inheritance molecule or gene. He used the term transformation to describe the transfer of the inheritance molecule.

In 1948 Oswald Avery, Colin MacLeod, and Maclyn McCarty came into the picture of DNA research. They continued Griffith's experiment to see what the transforming factor was by destroying the various molecules present in the heat-killed virulent Pneumococcus extract. They destroyed lipids, ribonucleic acids, carbohydrates, and proteins, but still observed that transformation occurred. However, when they destroyed DNA, transformation did not occur. Together they had discovered the inheritance molecule, DNA, and confirmed the chemical nature of the gene (UDEL, 2004).

After this discovery, people began to come up with models that would aid in understanding how DNA works as an inheritance molecule. In 1940 a scientist by the name Erwin Chargaff noticed a pattern in the four bases: adenine, guanine, cytosine, and thymine (UDEL, 2004). From different cells, he took samples of DNA and realized that the amount of guanine was almost equal to cytosine and the amount of adenine was almost equal to the amount of thymine. This finding or discovery later became Chargaff's Rule.

The real key to unraveling DNA's role as the molecule of heredity arguably came from the work of four scientists, Rosalind Franklin, Maurice Wilkins, James Watson, and Francis

Crick. Franklin and Wilkins created crystal forms of DNA and used X-Ray diffraction to try and deduce the structure of DNA (UDEL 2004; ThinkQuest, 2010). In 1953 from this work, Watson and Crick were able to put together a model of DNA. When they set their eyes on Franklin and Wilkins' picture, they had enough information to make an accurate model. Their model has not been changed much since then. Their model described DNA as a double helix with little rungs connecting the two strands. These rungs they realized were the bases of a nucleotide. A problem set them back while they were staring at the model - the pairing of the bases, and also the sizes of the bases. If purines were to pair with purines and pyrimidines to pyrimidines then the model will look crooked, but if a purine bonded to a pyrimidine, then the model would be uniform. The pairing they came up with to resolve this problem was the following: Cytosine-Guanine and Adenine–Thymine. This base-pairing model was consistent with Chargaff's rule. Watson, Crick and Wilkins won a Nobel Prize a few years after the presentation of the DNA model. Rosalind Franklin died of cancer prior to the awarding of the Nobel and because they are not awarded posthumously she was not awarded a Nobel Prize. However, it should be noted that her work led to this great milestone in genetics and medicine.

Subsequent to the discovery of the structure of DNA, there have been enormous advances in our ability to manipulate DNA (Nature Review Genetics , 2007). Rather than present these in detail, key advances and discoveries over the past 50 years relevant to the field of genomics are highlighted in Tables 1 and 2 below.

Table 1: ***Timeline of discoveries key to the field of Genomics (1950s - 1980s)***

DATE	DISCOVERY	RELEVANCE TO GENOMICS
1952	Electrophoresis	Sorting of DNA and RNA molecules by size
1956	Discovery of DNA Polymerase	Enzyme that catalyzes the synthesis of DNA
1967	Discovery of DNA Ligase	Enzyme that catalyzes the joining of two DNA molecules
1969	Fluorescence in *situ* hybridization (FISH)	nucleic acids can be fluorescently tagged and hybridized to complementary sequences for mapping gene positions and gene expression
1970	Discovery of Restriction Enzymes	Enzymes that cut DNA at specific sequences
1970	Discovery of Reverse Transcriptase	Enzyme that converts RNA to DNA
1972	Creation of first recombinant DNA molecule	Ability to generate recombinant DNA
1975	Southern Blot	Characterize DNA structure/sequence
1977	DNA sequencing	Nucleic acid sequences could be determined
1977	First genome sequenced	Phage Φ-X174 5368bp
1980	Discovery of RFLPs (Restriction fragment length polymorphisms)	Sequence differences can be detected with restriction enzymes
1982	Whole genome shotgun (WGS) vs directed sequencing	WGS proposed as means for genome sequencing
1982	Transgenic Drosophila created	Modification of higher eukaryotic genomes
1983	RFLPs used to map Huntington Disease	Positional cloning of disease genes
1985	Discovery of Polymerase Chain Reaction (PCR)	Ability to amplify DNA
1985	DNA fingerprinting	Ability to distinguish DNA between individuals
1987	Development of yeast artificial chromosomes (YACs)	Ability to clone large DNA fragments (>100kb)
1987	Development of site directed mutagenesis of mouse genome	Ability to alter mouse genome, opens way for gene function studies
1988	Chromatin immunoprecipitation (ChIP)	Provided way to assess protein-DNA interactions

Table 2: ***Timeline of discoveries relevant to the field of Genomics (1990s -)***

DATE	DISCOVERY	RELEVANCE TO GENOMICS
1990	Creation of Human Genome Project	International project to sequence human genome
1990	Development of basic local alignment tool (BLAST)	Provides ability to compare DNA sequences, paves way for comparative genomics
1995	Development of Microarray Technology	Global analysis of genome expression and structure
1998	Discovery of RNA interference	Ability to inhibit gene function
1998	Development of pyrosequencing	Ability to sequence in a high throughput manner
2001	Publication of 1st human genome sequence	Advent of human genomics
2001	Launch of Genome Browsers (UCSC Genome Browser, Ensembl, NCBI map viewer)	Ability to browse genome sequence and annotation online
2003	Development of DNA assembly programs	Ability to reconstruct genome sequences from WGS approach
2004	Development of DNA annotation programs	Ability to annotate DNA sequences
2005	Creation of first Haplotype Map (HapMap)	Ability to carry out whole genome association studies for disease susceptibility variants
2006	Publication of genome-wide maps of DNA methylation	Increased understanding of epigenetic phenomena
2007	Publication of 1st diploid human genome sequence	Paves way for personalized genomics
2008	Publication of variation in eight human genomes	Increased understanding of genetic diversity in humans
2010	Publication of Neanderthal genome	Increased understanding of genetic ancestry of homo sapiens
2010	Commercially available genome sequencing	Individuals can have their genomes squenced

Chapter 2: Genomics – What is it and How is it done?

Genomics - what is it?

As described in Chapter One, Mendel's work in genetics led to the discovery that genes are the functional sections of chromosomes responsible for heredity. Studying and understanding the molecular structure and function of genes has been very valuable in providing insight to two of the biggest mysteries in biology: what makes a species what it is and what causes variation within species. Through many of the discoveries highlighted in Table 1 and Table 2 scientists have been able to gain a better understanding of genes and their roles in determining a species characteristics or traits, collectively called a phenotype, and the basis for variation of these traits within or between species. By understanding the relationship between these genes and the phenotype of an organism or species we can better understand how DNA sequences guide heredity. However, it is important to keep in mind that an organism's phenotype is not simply dictated by its genotype, as the environment is an important contributor to an organism's phenotype.

Historically, studies on the function of genes and their contributions to phenotype were predominantly done on a small scale, gene by gene. The development of sequencing and recombinant DNA technologies dramatically changed the ability to carry out these studies, allowing them to be performed on a much larger scale (Nature Review Genetics , 2007). This led to the development of genomics, a subfield in the field of genetics, whose goal is to understand the molecular organization and informational content of the entire DNA sequence, genome, of an organism (or species) and its role in directing the phenotype of that organism (or species).

Genomics – how is it done: Determining genome sequences

Determination of an organism's genome sequence is a key first step in genomics and was made possible by the discovery of DNA polymerase by Arthur Kornberg in 1956 and the development of methods for DNA sequencing, the determination of the sequence of nucleotides in a DNA strand, by two different groups in 1977 (U.S. National Library of Medicine, 2010). The first method, chain-termination, was developed by Frederick Sanger and involved "sequencing by synthesis" – using DNA polymerase to synthesize a strand of DNA complementary to the strand of interest (Frederick Sanger et...al., 1977). By incorporating the use of dideoxy-nucleotides, which lack a 3' hydroxyl and therefore prevent the further addition of nucleotides by DNA polymerase, the sequence of nucleotides in the template strand can be determined (Frederick Sanger et...al., 1977). The second method, developed by Allan Maxim and Walter Gilbert, was based on modification of the DNA and its subsequent cleavage at specific nucleotides. Since that time "sequencing by synthesis" has been the principal method for DNA sequencing (Halima et…al., 2008).

Sequencing of an individual genome requires four basic steps: (1) preparation of the DNA templates, (2) physical sequencing of these templates, (3) imaging/detection of the sequencing products, and (4) analysis of the sequence data (Kirkness et…al., 2010). Among the strategies for genome sequencing, whole genome shotgun (WGS) sequencing has becoming the standard. With respect to WGS sequencing template preparation (1) involves fragmenting the genomic DNA (chromosomes) into many small overlapping pieces. These small overlapping fragments are then subjected to sequencing (2) and the products of the sequencing reaction (3) detected (Kirkness et…al., 2010). Enormous improvements in these latter two steps (2&3) are occurring almost daily and diverse technologies exist so they will not be discussed in detail here. The last

step - analysis of the sequence data (4) from all of these fragments has become feasible and simplified in large part due to advances in computer science (Kirkness et…al., 2010). Using programs designed to detect overlapping sequences, the sequence of the genome is reconstructed *in silico*, or on the computer. Together advances in biology, chemistry, engineering, and computer science are revolutionizing our ability to rapidly and cheaply sequence genomes on a larger scale (Kirkness et…al., 2010). A future in which an individual's genome is sequenced as a standard part of their medical care is no longer the stuff of science fiction, but has now become a reality.

CHAPTER 3: Medicine - Macroscopic and Microscopic or Pre-Genomics

Arguably, the determination of the sequence of the human genome has been the most profound advance in medicine to date and a world where sequencing is a part of medical care is now here. As such, it is likely to dramatically alter the way we view our health and management thereof. To begin to understand the impact the human genome sequence is having and will continue to have on society and health care, this discovery is discussed within a historical context of important advances in medicine. Some of these advances in medicine are highlighted below. They lay out a logical progression of understanding the human body that moved from a ***macroscopic level*** in which medicine was focused on deciphering our physiology and anatomy to the current state of medicine, which, arguably, is increasingly focused on a ***molecular level*** scale to understanding the human body and its health.

Medicine: Transition from spiritual to macroscopic – anatomical & physiological based treatment

The Egyptians and Romans were the first to begin treating diseases physically. Prior to this time, people believed that diseases were caused by spirits or spiritual beings. As a result diseases were treated by so called "spiritual means" by medicine men and magicians. As time went on, the Egyptians and Romans began exploring the anatomy of the human body. Anatomical knowledge was attained through the dissecting of corpses and analyzing the various structures of the human body. Treatment of disease was therefore carried out on specific parts of the body as a result. So, for example, if one has pain in the hands, the hand becomes the focus of treatment. This led to the understanding that perhaps diseases were caused by a defect in some part of the human body and not just by spirits or spiritual beings. In other words, they were the

first to begin treating diseases based on a general understanding of human anatomy and physiology. Archeological digs have found evidence of people with the title of physicians as early as 1500 B.C. Irj was one of such physicians. Physicians like Imphotep, a physician to King Zoter, lived earlier in Ancient Egypt around 2600 B.C. Even after his death people worshipped him as the god of healing. As more knowledge about the anatomy of the human body was gained, the people gradually drifted away from so-called their "spiritual disease" myth. Despite the many myths and spiritual beliefs that had governed people of ancient times, ancient Egyptians and Romans and Greeks have provided modern historians with a great deal of knowledge and evidence about their attitude towards medicine and the medical knowledge they possess. However, documents provide evidence that when diseases could not be treated based on anatomical knowledge, patients were still referred to magicians and medicine men.

Medicine: Training Physicians – Development of Medical schools and Medical Education

Although it is very difficult to identify the origin of medical education, it is believed that medical knowledge began with the Greeks use of anatomical knowledge and rational inquiry to treat diseases (Garrison, 1921). This inquiry introduced the process of observation and reasoning regarding disease. This rational inquiry is believed to have led to teaching and thus the formation of medical schools such as that at Cos, which Hippocrates, the most famous of all ancient Greek doctors, founded in the 5th Century B.C. (Garrison, 1921). Though there were other physicians during his time, Hippocrates was the man leading the development of medicine in Greece and many see this man as the father of modern medicine even though he was born in Cos in 406 B.C. (Garrison, 1921).

Hippocrates believed in keeping the treatment of diseases away from spiritual beliefs and

in treating the body as a whole and not as separate parts as ancient knowledge of anatomy suggested. In other words, Hippocrates set the tone for understanding the physiology of the human body as a crucial aspect of medicine. He was also the author of some of the collection of sixty medical books that summed up ancient Greek medical knowledge (Garrison, 1921). This collection of books, the Hippocratic collection, was named after him even though evidence show that he didn't write a majority of these books, which covered a span of 150 years. The collection deals with anatomy, clinical subjects, diseases of women and children, prognosis, treatment, surgery and medical ethics. He was famous to the point that famous people like Aristotle and Plato wrote about him and his work made such a great impression on medicine that his name is still associated with medicine today. When medical students receive their medical degree they take the *Hippocratic Oath*, written by Hippocrates, before they are allowed to administer medicine (Garrison, 1921).

It is believed that Cos' medical institution was first to be ever built. Later, the Christian religion greatly supported the cause of not only treating disease but the establishment of institutions to produce physicians. During the middle ages, apprenticeship training in monastic infirmaries and hospitals dominated medical education. Medical schools like the current ones did not evolve until between the 9th and 11th centuries which saw the establishment of one in Salerno in Southern Italy. Even there, the teaching style was still by the apprentice system. The apprenticeship system was still in use because a clear-cut curriculum for medicine hadn't been established. After a while, an attempt was made at systemization of the medical knowledge of the time. Through this process, a series of health precepts was drawn up and approved for medical practice by the then Holy Roman Emperor Frederick II. During the same time, the Muslim world also saw similar developments. Medical institutions were established in cities like

Cairo, Baghdad and Cordoba (Garrison, 1921).

The development and establishment of Universities in Europe also had an impact on medicine. Medical studies led more often to familiarity with theories about disease than with actual sick people. However, the establishment of the Royal College of Physicians of London in 1518, through the energy of people like Thomas Linacre, produced a system that examined medical practitioners before they were authorized to practice medicine. The discovery of blood circulation by William Harvey also allowed for a scientific approach to the study of medicine. It also didn't support the tradition of studying the body through theories and doctrines (Garrison, 1921).

During the 17th and 18th centuries, this new practical approach to studying medicine, which preached the value of hospital experience, was reasserted (Rothstein, 1987). It also saw the training of student's sight, hearing and touch as the best way to apply increasing knowledge of natural science to the actual care of patients. There was also an encouragement of the systematic study of anatomy, chemistry, and botany, sciences that were considered to be the basis of medicine at that time (Rothstein, 1987). During these colonial times in England, three distinct groups practiced medicine: physicians -university trained, surgeons - apprenticed and hospital trained, and apothecaries - also apprenticed. In America, however, the distinction between physician and surgeon soon disappeared. The New Jersey Medical Society, chartered in 1766, represented the first group of medical professionals in America and paved the way for the development of training programs – medical schools (Rothstein, 1987). In the mid 1800s the American Medical Association (AMA) was formed and set educational standards for the practice of medicine (Rothstein, 1987).

These included:

1) liberal education in the arts and sciences,

2) certificate of training completion from preceptor over apprenticeship prior to entrance to Medical college,

3) an MD degree of 3yrs of study (2 six month lecture sessions, three months of dissection, at least 1 six month session of hospital training),

4) 16 week course in anatomy, medicine, surgery, midwifery, and chemistry, and

5) minimum 3yrs of study at least 2 under practitioner.

In America, the John Hopkins University School of Medicine in Baltimore became known as the first medical school. From there, the adequacy of medical schools was further improved by the Flexner report written by Abraham Flexner and published by the Carnegie Foundation for the Advancement of Teaching (Rothstein, 1987). In this report written in 1910, he outlined that medical education is actually a form of education rather than some apprenticeship. Also, this report clearly laid out what medical schools needed to be successful: laboratories, teaching rooms or lecture halls, libraries, large hospitals where the medicine can be practiced, an academic staff, etc. It was after this report that medical schools really advanced in the United States.

Medicine: Important Technical Advances in Treatment

Microscopes: The microscope is arguably one of the greatest inventions that man has ever made. In 1590, the compound microscope was invented by two spectacle makers, Hans Jannsen and his son (Hogg, 1854). In the 16th and 17th centuries, Italy and Holland were principal countries for the construction and use of microscopes. Galileo left a mark on this

invention by making several telescopes and microscopes that he called "ochialino" (Hogg, 1854). However, the word microscope was coined by an Italian, named James Faber, in 1625. Petrus Borellus in 1653 published a paper on the use of microscopes in medicine. In his paper, he described 100 medical observations and applications of microscopes including how to remove invisible in-growing eyelashes (Hogg, 1854). These observations opened doors for research into the cause of diseases using microscopes. Athanasius Kircher observed microscopic worms in plague victims, which he suspected killed millions of people in Europe during the 17th century. Most likely, he was looking at pus or cells in the blood because microscopes then could not see the *Bacillus pestis* that was causing plague (Hogg, 1854).

Brilliant observations made by Anton von Leeuwenhoek with his microscope excelled those of all microscopists at that time. Through his observations, he gave descriptions and illustrations of bacteria from the human mouth, spermatozoa, skeletal muscles, protozoa, and epithelial cells from warts. He is also known for his brilliant observation of red blood cells. Malpighi, another brilliant microscopist, histologist, and embryologist, was the first person to see anastomosis between arterial and venous capillaries. Every medical student knows his description of the Malpighian bodies of the kidney, the Malpighian corpuscles of the spleen and the Malpighian layer of the epidermis—among his great observations. Despite all this great observations and findings, clinical microscopy had a very slow beginning. It took more than two centuries before the value of microscopes began to be appreciated by clinical and laboratory scientists. As time went along electron microscopes were developed to even look at the smallest of all microbes. DNA was also first observed using this type of microscope (Rayment, 2010).

Anesthesia: Anesthesia is simply defined as the loss of feeling or sensation. Currently, this is a process used before all surgeries. This process was first used by an American

Physician, Crawford W. Long , in an operation in 1842 (Canton, 2007). However it was not recorded so official credit was given to William Morton in 1846 for demonstrating an operation involving anesthesia (Canton, 2007). It had a major impact because it allowed surgeons to do surgery without worrying about the pain that patients will go through during the process of surgery.

Prior to anesthesia, ancient methods were used to control pain during the process of surgery, including the use of drugs such as alcohol, hashish, and opium derivatives (Garrison, 1921). Also used were rudimentary physical methods of producing insensitivity to pain. These include packing limbs on ice, and an extreme method of inflicting a blow to the head. These methods performed the job of reducing pain but weren't safe. A safer method, anesthesia, began after the discovery of nitrous oxide by English Chemist Joseph Priestly in 1772. Ether, a similar gas, also became popular in the United States at that time. Attention returned to nitrous oxides when Horace Wells, a Connecticut dentist, learned about the effects of nitrous oxides by testing it on himself and his patients. He was happy with the results, so he shared this information about nitrous oxide with his friend William T. Morton, a student at Harvard medical school, who proceeded on a quest to find an even more potent agent. He began experimenting with sulfuric ether. Pleased with the outcome of the experimentation, he contacted a Harvard University colleague Dr. John C. Warren who would arrange for a public demonstration of surgery without pain using sulphuric ether. News of this spread worldwide and a new era of surgery was born. Oliver Wendell Holmes would later coin the term anesthesia to describe the condition brought on by ether (Canton, 2007).

Antibiotics: An antibiotic is a substance that kills bacteria or inhibits their growth. The term "antibiotic" was coined by Selman Waksman, in 1942, to describe any substance that is antagonistic to the growth of microorganisms. It came from the word "antibiosis", term coined by Louis Pasteur's pupil Paul Vuillemin in 1889, which means a process by which life could be used to destroy life. The original definition excluded naturally occurring substances that were not produced by microorganisms, but performed the same function of antagonism against bacteria. It also excluded synthetic antibacterial compounds such as sulfonamides. However as time went by, these exclusions became a part of the definition (ThinkQuest, 2010).

During ancient times, Egyptians, Chinese, and Indians of Central America all used molds to treat infected wounds without really understanding the antibacterial properties of it. However, the true search for antibiotics began in the 1800s with the growing acceptance of the *germ theory of disease* which linked microbes like bacteria to the causation of diseases. The search started when scientists worldwide began to figure out ways to take care of microbes that cause diseases. The quest began 1871 with surgeon Joseph Lister researching the phenomenon that mold-contaminated urine would interfere with the growth of bacteria. In 1890, the first antibiotic made up of microbes to be used in hospitals was made by German doctors, Rudolf Emmerich and Oscar Low. They named it pyocyanase. However, it didn't work as often as was expected (ThinkQuest, 2010).

In 1928 Sir Alexander Fleming demonstrated antibacterial properties by observing that the mold *Penicillium notatum* could destroy colonies of *Staphylococcus aureus*. This led to the biggest impact on medicine by antibiotics with the isolation and manufacturing of Penicillin by Howard Florey and Ernst Chain in 1942. Penicillin could now be sold as a drug. Fleming, Florey and Ernst were awarded the Nobel Prize in medicine in 1945 for their work on Penicillin. Penicillin

was the first working antibiotic. Currently it is still in use in hospitals to fight bacteria, though some bacteria have grown to be resistant to it. In 1935, Pronotosil, the first sulfa drug, was discovered by German Chemist Gerhard Domagk. In 1943, the antibiotic streptomycin, a drug made out of soil bacteria, was made by the American microbiologist Selman Waksman. Streptomycin would introduce a new class of drugs called aminoglycosides. Streptomycin could treat diseases but its side effects are often too severe. In 1955 Lloyd Conover patented Tetracycline, which would go on to become the most prescribed broad spectrum antibiotic in the United States. In 1957 Nystatin was patented and used to treat many disfiguring and disabling fungal infections. SmithKline would hit the market with Amoxicillin, a semisynthetic antibiotic, in the 1981 (ThinkQuest, 2010).

Antibiotics changed the way we looked at and approached the treatment and cure of many diseases. With the invention of microscopes and discovery of microbes, the concept of "germ theory", diseases caused by microbes, was developed. Such diseases could be treated by attacking the microbes that caused them through the use of antibiotics. That this concept is still important and impacting medicine is typified by the awarding of the Nobel prize in 2005 to Barry J. Marshall and J. Robin Warren for their discovery that gastritis and peptic ulcer disease was caused by the bacterium *Helicobacter pylori* (ThinkQuest, 2010).

In vitro fertilization: Infertility is the inability of a couple to become pregnant. Currently 10% - 6 million, of the general American population are affected by infertility (Bavister, 2002). To help treat this condition, Dr. Robert G. Edwards developed the process of *in vitro* fertilization. In *in vitro* fertilization egg cells are fertilized by sperm cells in a test tube - outside the body. Through this procedure, zygotes (fertilized egg) or embryos can then be

transferred into the uterus and result in pregnancy. It was successfully used for the first time in 1978 with the birth of the first test tube baby, Louise Brown (Bavister, 2002). Since then more than 250 babies have been born as a result of this wonderful technique. Dr. Brown was awarded the Nobel Prize in Physiology or Medicine for his work in developing this treatment in 2010.

Medicine: Transition to the Molecular Level

As medicine was transitioning to a cellular scale with the discovery of microbes and the development of techniques such as *in vitro* fertilization, our understanding of DNA and its role in human biology was becoming increasingly clear. As such, it was inevitable that medicine would begin to seek information on diseases at the molecular level. This was first done for sickle cell anemia, which in 1910 was shown to have defects at the cellular level – "peculiar elongated and sickle-shaped" cells by Ernest Irons and Dr. James Herrock. The disease showed evidence of inheritance, supporting a genetic basis. This was confirmed in 1949 by Linus Pauling with the demonstration that sickle cell anemia was due to an abnormal form of the protein hemoglobin (Pauling, 1959).

This discovery ushered in the future of medicine at the molecular level and led to the field of genetic counseling which was created to help patients better understand the impact of genetic information on their health. In 1971 the first genetic counselors graduated from Sarah Lawrence College (Coutts, 1994). Genetic testing for specific diseases has now become commonplace and in 1990 Ashanti DeSilva, a patient with severe combined immune deficiency (SCID), was the first person approved for gene therapy in the United States. In this technique, the disease is treated at the molecular level by providing a functional version of the affected gene to the genome of the diseased individual. In Ashanti's case, the nonfunctional form of adenoside

deaminase (ADA) in her genome was compensated for by inserting a functional form of the ADA gene into her cells. In Chapter 4, the impact of this transition to a molecular understanding of disease and of genomics on medicine is explored in greater detail.

Chapter 4: Medicine - The Impact of Genomics

Many advances in genetics have been made since the discovery of DNA about 70 years ago. All these advances have gotten the world closer to the ultimate goal of deciphering genomic information for a positive impact on society. However in 2001, the world saw a major breakthrough with the sequencing of the first human genome. If this had been predicted by anyone around the time of DNA's discovery, the world would have said it was impossible. The Human Genome Project began in 1990 and amounted to a total cost of ~$2.7 billion. Though very expensive by today's standards where genomes can be sequenced for $1000-$20,000, it is impossible to put a cost on the impact of the human genome project. It has moved the world into a new era and in a short time dramatically impacted our approach to medicine.

Prior to genomics, discoveries in biology provided incremental advances about genes—their regulation, expression, and function. With the advent of the human genome sequence, the world of science has been able to address gene function on a much larger scale. With respect to medicine, scientists have focused on a couple of questions: what does the sequence mean and how can genomic data be used to treat disease? These are complex questions and many of the answers lie in the future. However, among the simple answers we have learned is that as increasing numbers of individual genomes are sequenced, more insight is gained. This means that more people would need to agree to get their genomes sequenced so that research can be conducted over a large scale. However, cost and ethical and legal issues remain paramount concerns.

Since the Human Genome Project, which amounted to almost $3 billion, the cost of sequencing has decreased dramatically. This is due to the great advances in the sequencing technology, which has made the entire process quicker and more efficient. Currently with next

generation sequencing technology, a human genome can be sequenced for less than $10,000 and there is a bold prediction that this figure could decrease to $1000 per human genome in the future (Samani et al, 2010). Any economist would agree with the following statement: As the cost of a product decreases, there is always an increase in the amount of consumers for that product as opposed to more costly products that serve the same purpose. This will be no different for genome sequencing. For more people to be willing to sequence their genomes, the cost of sequencing needs to be reduced. Many private companies foresee opportunity in this technology and are aggressively working to reduce the cost, leading to the predictions of $100 genomes in the near future are not met with great skepticism any more.

More important than the cost of a genome sequence, is that society attempts to understand the benefits of this information and the ethical and legal issues that arise from having access to people's genome sequence. Despite the many issues, with private companies and members of the scientific community behind these efforts genomics has already impacted medicine in two very specific ways. First, the sequence of an individual's genome will provide direct information of relevance to his or her own health. This can lead to management of one's health in a highly individualized way, setting the stage for an era of "personalized medicine". Second, the ability to analyze genome sequences from large populations of individuals of similar and differing ethnic backgrounds, medical conditions etc. has begun to yield important information about the links between DNA sequence, human biology, and disease.

Genomics & Personalized Medicine

Increasing advances in genomic research have led to *personalized medicine*. This is an approach in which care is administered to a patient based on personal information about the

patient. Previously, personalized medicine had been administered with minimal to no information on a patient's genetic makeup. Given that our phenotype (and health) is dictated in large part by our genetic makeup, excluding this information from the diagnostic process seems destined to result in an incomplete and potentially inaccurate picture. With an individual's genome sequence in hand, doctors can now add this information to more standard obtain a more complete picture of the patient's health. This opens the door to developing treatments or health management plans based in part on the results of the patient's own genetic makeup.

Key to the wide spread use of personalized medicine will be commercially available, low-cost genome sequencing. With the belief in a large market demand in the near future, a number of companies have begun to offer genome-sequencing services. The services they offer do not require a physician or other health care professional and instead provide results directly to the consumer. Such services are known as Direct-to-Consumer (DTC) services. Currently, the majority of these services are offered online (Knoppers, 2010).

Recently, however, companies have embarked on efforts to bring these services onto the local the drugstore shelves. A notable example of this occurred in the summer of 2010 and involves Pathway Genomics. Pathway went into a contract with Walgreens, a nationally known Pharmacy to provide a genetic testing kit (Rugnetta, 2010). Under their agreement, Walgreens would sell Pathway's genetic test kit at 6000 Walgreens pharmacies nationwide (Rugnetta, 2010). However, the execution of the contract didn't happen because the United States Food and Drug Administration (FDA) stepped in. According to the FDA who sent out a letter to Pathway Genomics just when the product was about to hit the market, Pathway Genomics was selling what appeared to be a medical device under section 201(h) of the Federal Food Drug and Cosmetics act. This act defines a medical device as an instrument that is intended for use in the

diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease (Rugnetta, 2010). As part of the kit/service Pathway Genomics would sequence regions of the individual's genome and would scan their genes for a propensity for various diseases, such as breast cancer, diabetes, Alzheimer's, etc. (Rugnetta, 2010).

As of the end of 2010, the FDA is still investigating the legal ramifications of what Pathway Genomics offers and their product is pending approval. Curiously, while this off the shelf product is on hold, the online services are still being offered and results are mailed directly to consumers (or their doctors). Additional genome services provided online by companies include health risk tests, single nucleotide polymorphism (SNP) tests for the whole genome, and ancestry tests, among others. Table 3 below shows a list of some of these companies, what they offer, the cost of services, and how they distribute the results of their tests.

Table 3: *Companies providing DTC Genome Services*

Company	Services	Cost	Mechanism of Data Distribution
23and Me	Ancestry test Health risk tests for 119+ conditions Complete package of both	$499 for ancestry and health gene scan package N.B: 2010 Christmas sale for $99 Separate tests also offered: Ancestry tests alone = $399 Health edition (testing genetic risks for 119+ conditions = $425	Data sent to consumer and consumer logs onto website where there are tools that help them analyze the data
Pathway Genomics	Health Test Scan for 90+ conditions Ancestry Test (1000 maternal + 200 Paternal haplogroups)	Health Test Scan (tests for genetic risks for 90+ conditions = $299 Ancestry Test (gauged 1000 maternal + 200 paternal haplogroups) = $199 20% discount on all prices for OptumHealth Allies' members	Testing requires physician approval, data provided online to both consumer and physician
Navigenics	SNP-genotyping test for whole gene Genetic predisposition test to 10 common health conditions	SNP-genotyping =$999 Genetic predisposition data = $499	NA
DecodeMe	Full service providing genetic risk for 42 conditions Cancer Scan including genetic risk or 7 cancers Cardio Scan testing predisposition to cardiovascular events like heart attacks and atrial fibrillation	Full service scan =$985 Cancer scan= $225 Cardio Scan = $195	NA
New Hope Medical	Genetic testing for between 12-25 SNPs linked to certain conditions	between $475 and $900	NA
Gene Essence	Genetic testing using affymetrix 6.0 SNP array giving 1 million genetic variants (SNPs) and calculation of genetic susceptibility for common diseases and complex traits	= $1,195	NA

Table 3 (cont.): ***Companies providing DTC Genome Services***

Company	Services	Cost	Mechanism of Data Distribution
Knome	Full genome scans	$350,000	NA
Illumina	Whole Genome sequencing	$48,000	NA
Vugene	1.Basic Alzheimer's test 2.Alzheimers' for Women 3. OSA 4. Stroke Test 5. Weight Loss Success Test	1. $99.95 2. $109.95 (sale price 20% off) 3. 69.95 4. $49.95 5. $89.95	NA
Oxford Nanopore	Powerful and affordable whole genome sequencing		NA
Life Technologies	Combo of companies have come together to improve sequencing		NA
Pacific Biosciences	New technology for whole genome Sequencing		NA
Helicos Biosciences	True single-molecule sequencing TM Platform		NA
GnuBio	Whole genome sequencing	$45,000	NA

One may ask, so what does knowing information about ones genome does for them? What is the significance of such information? This question is better answered by answering the question: what are the applications of personalized genomic data? Though there are increasing numbers of DTC companies seeking to offer personalized genome sequencing services, our understanding of what this information means is still in its infancy. Even though predisposition services are offered by DTC companies the multifactorial nature of many diseases suggests that caution is necessary when interpreting the data.

Since the development of genome sequencing, there have been a handful of applications of personalized medicine. One of the major areas of application has been in cancer research. Cancer is triggered by a cascade of mutations resulting in an activation of oncogenes or inactivation of tumor suppressor genes. In August of 2010, the first example of genome-scale RNA and DNA sequencing to aid in clinical decision and therapeutic choice was published in the journal *Genome Biology* (Agency, 2010). The disease under research was a rare tumor on the tongue which had progressed to metastatic disease. Because of the rarity of this disease, no established treatment option for the disease existed. For the first time, scientists turned to the analysis of whole genome sequences – this allowed for the comprehensive discovery of genetic changes that had accumulated within the tumor. From the results of the analysis, a personalized drug regime was initiated to stabilize this aggressive cancer for several months (Agency, 2010).

This is a major advance and represents an important milestone in the use of genomics in medicine - treatment was tailored to an individual based on their genetic makeup. With the number of such instances increasing, it is safe to say the promise of genomics to personalize medicine is no longer a promise, but is now upon us. Although arguable, it is likely that therapeutic treatments will ever more rely on an individual's sequence as the primary source of

information.

Genomics & Global Medicine

Genomic research is also greatly impacting how we treat disease on a global scale. As a matter of fact the field of global medicine was the first to be impacted. Right after the sequencing of the entire human genome in 2003, it became clear that to make sense of genomic data comparisons among individuals had to be made. This led to projects like the *Personal Genome Project,* which aims to sequence the genomes of a group of individuals (on a volunteer basis) so such comparisons can be made.

Global healthcare deals with healthcare that is administered worldwide with considerations to the various regions around the world. There are ongoing efforts to improve global healthcare based on the different regions in the world. A case study conducted in a few regions around the globe revealed that DNA sequences slightly differ with respect to regions around the globe. As a result, medicine and treatments could be developed in such a way that target would be regionally or ethnically based.

Case studies were conducted in four countries in different regions around the world: Mexico, South Africa, India and Thailand, to assess the effect of genome information on medicine from a global perspective. In Mexico, the National Institute of Genomic Medicine (INMEGEN) was established in 2004 to carry out disease-related genomic studies (Singer et al, 2008). This organization aims to develop public health genomics in Mexico. The organization has begun studying genomic variations in sub-populations as a starting point. Mexico foresees the health and economic benefits that gaining genomic knowledge could bring. As a result, significant monetary investments have been made toward ensuring the success of INMEGEN.

In South Africa, a number of initiatives have already been made to study genetic variation within the local indigenous populations. South Africa is also beginning to apply genomics to address local health needs, including Human Immunodeficiency Virus (HIV) and Tuberculosis (TB) infections. South Africa even has an organization-- Africa Genome Education Institute, responsible for involving the public in genomic issues (Singer et al., 2008).

India, with the second largest population in the world, is also looking for high-tech science and technology that would improve its economic and health situations. Thus, India is also now working to develop genomics in the country through the Indian Genome Variation (IGV) Consortium, a government-funded collaborative network among seven local institutions (Singer et al., 2008). One of the goals of the Consortium is the identification of links between specific genome variants and disease, which would then be used to improve treatment options on a national scale.

Finally, Thailand has also been involved in efforts to use genomics to improve healthcare. Specifically, Thailand has launched two genotyping initiatives: the Thai SNP Discovery Project and the Thai Center for Excellence in Life Sciences Pharmacogenomics Project. Together these projects will help Thailand understand the genomic diversity of its population and how to predict drug response with this information. A big challenge is foreseen, however - Thailand will have to figure out a way to incorporate this knowledge into its young universal health-care system (Singer et al., 2008).

These case studies reveal the incredible impact that genomics is having on developing countries. It also reveals that genomics is having an impact globally on both poor and rich countries despite the economic challenges currently involved with genomic medicine. Countries have shown interest because increasingly genomics is positively impacting health and economic

situations.

One particular area in medicine that has taken a global initiative to use genomic data to treat diseases is in cancer research. Cancer is defined as the uncontrolled growth of cells caused by mutations in the human genome. Complications in cancer research stems from the fact that cancer is not caused by a single mutation, but rather a series of mutations. To make the situation even worse these mutations are not only point mutations, but are a combination of deletion, insertion, inversion, and repeat mutations. This makes identifying the major gene contributors to specific types of cancer very challenging.

To overcome this, a global effort to fight cancer with genomic information was first initiated in 2006 with studies of 35 colorectal cancers. Initially, this didn't reveal the gene primarily mutated in this type of cancer. Surprisingly, *IDH1I*, isocitrate dehydogenase - a lowly housekeeping enzyme involved in metabolism, was later proven to be among the many mutated genes in colorectal and other cancers. It was not until 300 more of such cancers were analyzed to confirm that *IDH1I* was in fact involved (Ledford, 2010). However there are many more genes yet to be discovered as components in causing cancer. In the past two years, labs around the world have teamed up to sequence thousands of genomes from tumor cells along with healthy cells from the same individuals (Ledford, 2010). The hope is that from the wealth of sequence information key mutations will appear again and again to help identify key pathways involved in cancer formation.

Along these lines, the International Cancer Genome Consortium (ICGC), formed in 2008, is coordinating efforts to sequence 500 tumors from each of fifty cancers—a project estimated to cost about $1 billion. Eleven countries have already signed on to cover twenty of these cancers. Table 4 shows the countries involved and the cancers they are working with. It is estimated that

it would cost $20 million to sequence each cancer type, but with help and funding from the countries and organizations like the European Union, the project is already under way (Ledford, 2010).

Table 4: ***Genomics and Global Cancer Research***

Country	**Number of Cancers Sequenced**	**Types of Cancers Being Sequenced**
United States	6+	Ovarian Cancer Brain Cancer Lung Cancer (squamous-cell carcinoma) lung cancer (adenocarcinoma) Acute Myeloid leukaemia colon cancer (adenocarcinoma)
Britain	3	Breast Cancer (ER-, PR-, HER-) Breast Cancer (Lobular) Breast Cancer (ER+, HER-)
France	3	Breast Cancer (HER2 Overexpressing) Liver Cancer (Alcohol-Associated) Renal-cell Carcinoma
Australia	2	Pancreatic Cancer (ductal adenocarcinoma)
Canada	1	Pancreatic Cancer (ductal adenocarcinoma)
Germany	1	Pediatric brain tumors
Spain	1	Chronic Lymphocytic Leukemia
India	1	Oral Cancer (gingivobuccal)
Italy	1	Rare Pancreatic Cancers (enteropancreatic endocrine, pancreatic exocrine)
Japan	1	Liver Cancer (Virus- Associated)
China	1	Gastric Cancer

One issue that the ICGC project doesn't address is the issue of functional link to cancer development. The appearance of specific mutations in certain cancers is strictly correlative. Therefore, to address the functional relationship issue, the US National Cancer Institute established two 2-year cancer projects in September of 2010 to look into high-tech methods for testing the cellular function of mutations identified from the project. Sites for this project are Dana Farber Cancer Center in Boston and Cold Spring Harbor Laboratory in NY. The ultimate goal of these two projects is to find a systematic way by which researchers can make sense of the mutations that are being identified in tumor cell genomes. Specifically, the Boston site is responsible for increasing and reducing the expression of correlated genes in cell cultures. In contrast, the Cold Spring Harbor site studies cancer-associated mutations using tumors transplanted into mouse. Together, these laboratories look to pave the way for us to understand and navigate through genomic data in cancer (Ledford, 2010).

Genomics & Global Medicine

Though genomics promises a bright future for healthcare, a few issues need to be resolved for the success achievement of this ultimate goal. Genomics is faced with both ethical and legal issues at the moment. The public is concerned about privacy. These concerns, beginning in the 1990s, were largely triggered by the Human Genome Project (HGP) and the establishment of population bio-banks in the following decade (Knoppers, 2010). Lawmakers were worried that genetic information could be used for discrimination purposes. This led to legislation both in the USA and Europe to protect the privacy of genetic information and prohibit genetic discrimination (Knoppers, 2010). Despite these protection laws, the public is still worried about how genetic information is distributed. Concerns were stirred up by popular online genetic

testing services that offer predictions to consumers of their health risks. Of paramount importance and concern to the public is ensuring the accuracy of information reported to consumers. In addition, the public is concerned about how this information could be distributed or used for research. Who gains access, the consumer, their doctors, and/or insurance companies? This is a big problem because if someone has a predisposition to some serious medical problem like cancer, an insurance company could pass on giving this person medical insurance. To resolve such issues, governments would have to set up legislation to prevent this from happening. This occurred in the U.S. in May of 2008 when President George Bush signed the Genetic Information Nondiscrimination Act into Law. This law prevents the improper use of genetic information to deny insurance and affect employment. This is an important first step in insuring consumers protection from the inappropriate use of their genetic information. However, a more complete understanding of all the legal and ethical issues surrounding genomics will require significant work in the future.

Chapter-5: Project Conclusions and Projections

Genomics has and continues to have a big impact on medicine. Looking at its impact personally and globally, it is fair to say that the future of medicine is dependent on our ability to identify, interpret, and possibly manipulate genomic information. When this in mind, doctors ability to make accurate diagnoses and carry out treatments will be greatly impacted. Patients on the other hand may feel more comfortable because treatments are being personalized based on our understanding of the human genome. As mentioned earlier, the goal of using genomics to improve medicine is far from being attained. Through genetic testing companies can provide predictive healthcare information, but to make solid, accurate, and factual conclusions that would impact medicine, the mystery behind the human genome needs to be unraveled. With all the focus on genomics, the mystery promises to be unraveled in our lifetimes.

REFERENCES

Agency, B. C. (2010). *Personalized Genome Sequencing In Cancer Treatment - A Major Breakthrough In Care*. Retrieved December 17, 2010, from Medical News Today: http://www.medicalnewstoday.com/articles/197516.php

Bavister, B. (2002). Early History of In Vitro Fertilization. *Reproduction* , 181-196 .

Biosciences, P. (2010). SMRT Biology Overview. *Pacific Biosciences*. <http://www.pacificbiosciences.com/smrt-biology/overview>

Canton, D. (2007). Anesthesia (The History). *Wiley Online Library* , 1-6.

Coutts, C.M (1994). Human Gene Therapy. <http://bioethics.georgetown.edu/publications/scopenotes/sn24.htm>

Donnelly, J. M. (2010). GnuBio launches as open-source genome sequencing startup. *American City Business Journal*.

Essence, G. (2008). Gene Essence. *SNPedia*. <http://www.snpedia.com/index.php/Gene_Essence>

Frederick Sanger et...al. (1977). DNA Sequencing with Chain -Terminating Inhibitors. *Proc. Nati. Acad. Sci. , 74* (12), 5463-5467.

Garrison, F. H. (1921). An Introduction to the history of medicine. In F. H. Garrison, *An Introduction to the history of medicine* (pp. 1-946). Philadelphia : W.B. Saunders Company.

Hogg, J. (1854). The microscope: its history, construction, and applications. In J. Hogg, *The microscope: its history, construction, and applications* (pp. 1-435).

Illumina. (2010). Every Genome Tells a Story. What's yours? *Illumina*. <http://www.everygenome.com/>

Institute, N. H. (2010, November 26). Deoxyribonucleic Acid (DNA). 1.

Kirkness, P. C. (2010). Whole Genome Sequencing. In P. C. Kirkness, *Genetic Variation, methods and Protocols* (pp. 215-226).

Knoppers, B. M. (2010). Consent to 'Personal' genomics and privacy. *European Molecular Biology Organization , 11* (6), 416-419.

Ledford, H. (2010). The Cancer Genome Challenge. *Nature , 464*, 972-974.

Mark B. Gerstein, C. B. (2007). What is a gene, post-ENCODE? History and updated definition.

Genome Research , 669-681.

Nature Review Genetics . (2007). Milestones in DNA Technologies. *Nature* , 1-20.

Niall Dillon, P. S. (2000). Functional gene expression domains: defining the functional unit of eukaryotic gene regulation. *BioEssays* , 657-665.

Oracle ThinkQuest (2010). DNA History.

<http://library.thinkquest.org/20830/Textbook/HistoryofDNAResearch.htm>

Oracle ThinkQuest (2010). History of Antibiotics.

<http://library.thinkquest.org/25462/history.html>

Orel, Vitezslav, (1996).The First Geneticist. Oxford & London: Oxford University Press.

Pauling, Linus (1959).Molecular Disease. *American Journal of Orthopsychiatry*, 29: 684–687.

Ray, T. (2009). Pathway's Deal with OptumHealth Yields Lowest-Priced Gene Scans in DTC Genomics Industry.

Rayment, W.J (2010). A History of the Microscope.
<http://www.indepthinfo.com/microscopes/history.htm>

Rothstein, W. G. (1987). American medical schools and the practice of medicine: a history. In W. G. Rothstein, *American medical schools and the practice of medicine: a history* (pp. 1-24). Oxford: Oxford University Press.

Rubin, G.M., Lewis, E.B. (2000): A Brief History of Drosophila's Contributions to Genome Research. *Science*, 287(5461):2216-8.

Rugnetta, M. (2010, May 13). FDA Intervention Shelves Plan for Drugstore Genome Tests.

Science Progress , p. 1.

Samani, N. J. (2010, May 1st). The personal genome--the future of Personalized medicine? *www.thelancet.com* , 1497-1498.

Singer, P. (2008). From Diversity to Delivery: the Case of the Indian Genome Variation Initiative. *Nature Reviews* , 9-14.

Singer, P. (2008). Genomics, Public Heaith and Developing Countries: the Case of the Mexican National Institute of Genomic Medicine . *Nature Reviews* , 55-59.

Singer, P. (2008). South Africa: from Species Cradle to Genomic Application. *Nature Reviews* , 19-24.

Singer, P. (2008). Universal Health Care, Genomic Medicine, and Thailand: Investing in Today and Tomorrow. *Nature Reviews* , 14-19.

Technologies, O. N. (2010). DNA Sequencing. *Oxford Nanopore*. <http://www.nanoporetech.com/sections/index/71>

University of Delaware (UDEL): The Race to Solve the Mystery: The Structure of DNA. November 2004. < http://www.ceoe.udel.edu/extreme2004/genomics/dnahistory.html>

U.S. National Library of Medicine. (2010). The Arthur Kornberg Papers. *U.S. National Library of Medicine* .

Vorhaus, D. (2011). 2011 Personal Genomics Preview: It's Deja Vu. <http://www.genomicslawreport.com/index.php/tag/pathway-genomics>

Y. Ben Halima, Y. J. (2008). Genetic Sequencing Research Approach for users' satisfaction in grid environments. In C. Branki, *Techniques and Applications for Mobile Commerce: Proceedings of Tamoco 2008* (pp. 132-133). Amsterdam: IOS Press.