Genome Editing and Engineering
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Module - 11
Personalized Therapy
Lecture - 44
History and Basics - Part A

Welcome to my course on Genome Editing and Engineering. Today, we will be discussing about Personalized Therapy. And in this lecture, we are going to discuss about the History and Basics of Personalized Therapy.

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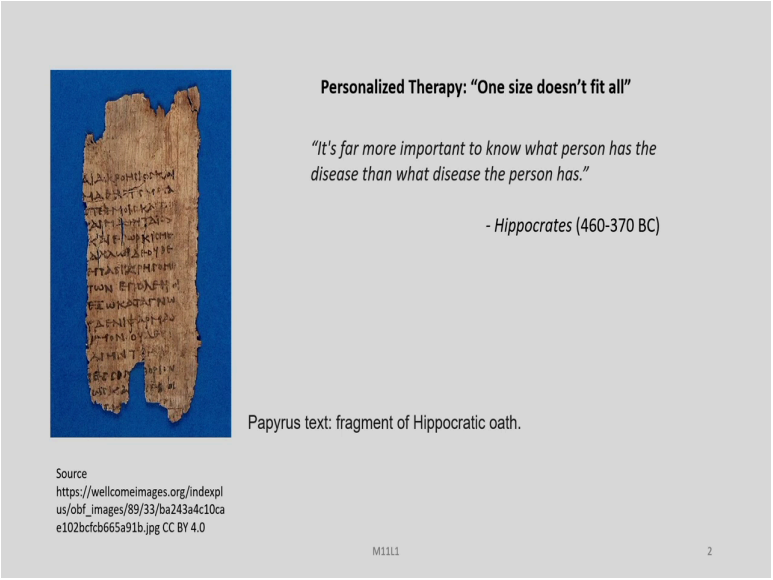

So, this is a very famous museum exhibit which shows a fragment of the Hippocratic oath. And the very same Hippocrates also spoke something very very interesting about therapy, where he told that it is far more important to know what person has the disease then what disease the person has. So, as long as 370 BC or more, the idea of personalized therapy existed in some way. And we have to know that therapy may be very very specific to individuals and as other things in this world, one size does not fit all.

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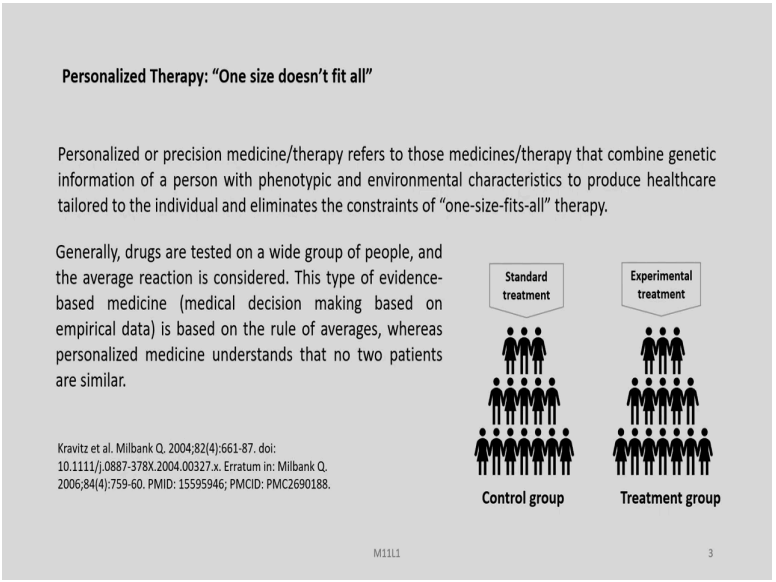

Although we have medicines made to be prescribed for certain diseases. As we progress through this lecture we will come to know that certain medicines prescribe to certain patients for certain disease may in fact, cause them harm rather than cure them.

So, in continuation of these paradigm, one size doesn't fit all, personalized or precision medicine or therapy refers to those medicines and therapy that combine genetic information of a person with phenotypic and environmental characteristics to produce healthcare tailored to the individual and eliminates the constraints of "one-size-fits-all" therapy.

In simple terms, our genotype determines our phenotype and our disease conditions also are governed by the genotypes to a large extent in conjunction with the environment. So, the therapies also has to be tailored to fit into such type and landscape. Generally, drugs are tested on a wide group of people, and the average reaction is considered in the drug development process.

This type of evidence-based medicine where medical decision making is done based on empirical data is based on the rule of averages, whereas personalized medicine understands that no two patients are similar.

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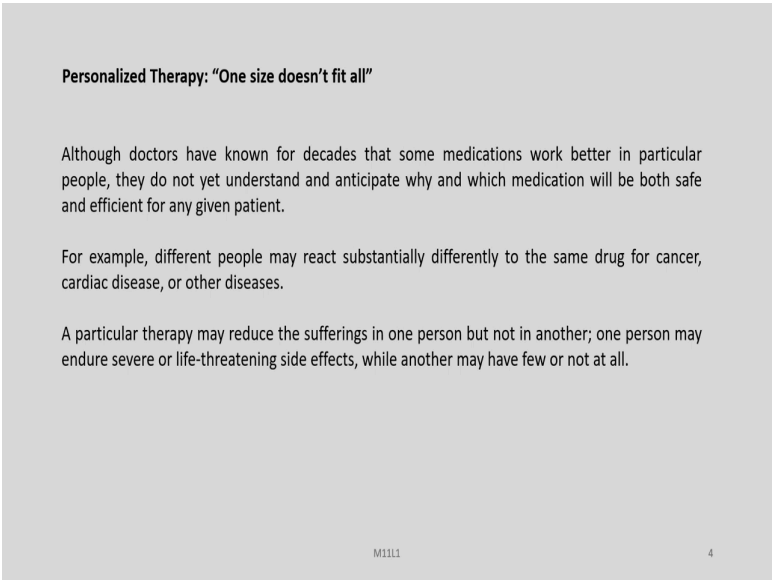

Although doctors have known for decades that some medications work better in particular people, they do not yet understand and anticipate why and which medication will be both safe and efficient for any given patient. For example, different people may react substantially differently to the same drug for cancer, cardiac disease or other diseases. A particular therapy may reduce the sufferings in one person but not in another, one person may endure severe or life-threatening side effects, while another may have few or no reactions at all.

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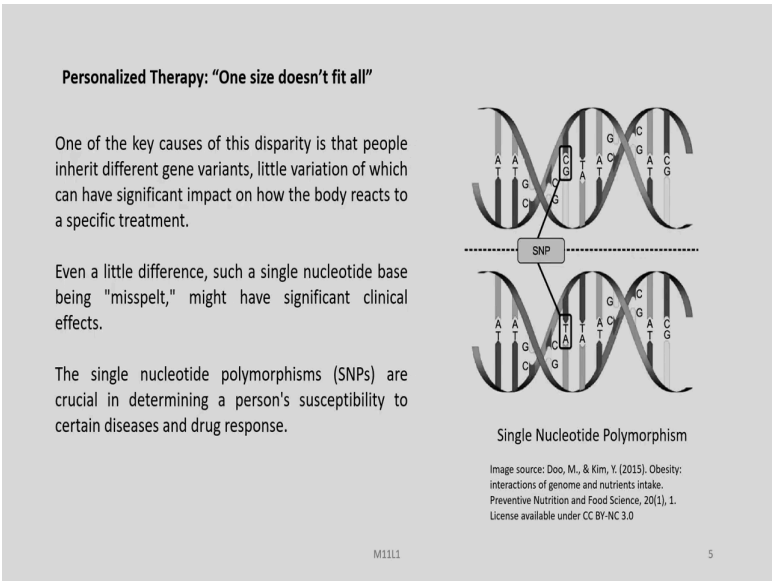

One of the key causes of this disparity is that people inherit different gene variants or alleles, a little variation of which can cause a significant impact on how the body reacts to a specific treatment. Even a little difference, such as a single nucleotide base being "misspelt," might have a significant clinical effect. The single nucleotide polymorphisms are very crucial in determining a person's susceptibility to certain diseases as well as drug responses.

Let us have a small peep into the history of personalized medicine. As I already told you about a Hippocrates, the idea of personalized medicine is actually not very new.

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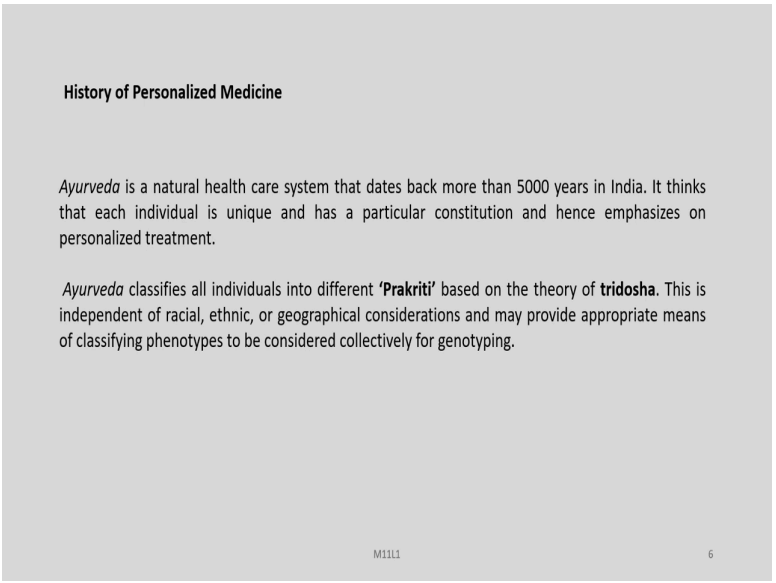

Ayurveda is a natural healthcare system that date backs to more than 5000 years in India. It thinks that each individual is unique and has a particular constitution and hence emphasizes on personalized treatment. Ayurvedic classifies all individuals into different 'Prakriti' based on the theory of tridosha. We have to understand that Ayurveda is not based on the genetic concepts on which personalized medicine is today based on. But nevertheless, it had some idea that every individual is very very unique or and at least they fall into certain classes.

This is independent of the racial, ethnical, or geographical considerations and may provide appropriate means of classifying phenotypes to be considered collectively for genotyping.

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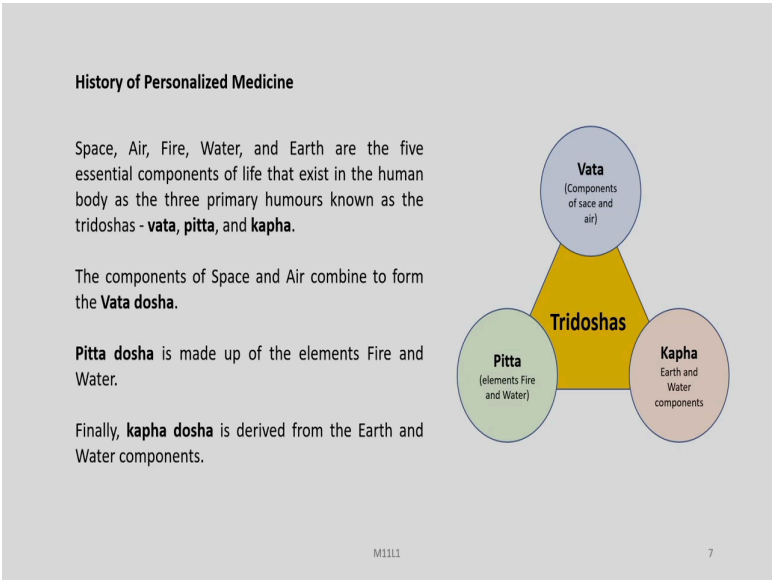

Space, Air, Fire, Water, and Earth are the 5 essential components or elements of life as laid out in Ayurveda that exists in the human body as the 3 primary humors known as the tridoshas, vata, pitta and kapha you can see. And there is a interaction between the 3. The components of these Space and Air combined form the vata dosha. The pitta dosha is made up of the elements Fire and Water. Finally, kapha dosha is derived from Earth and Water components.

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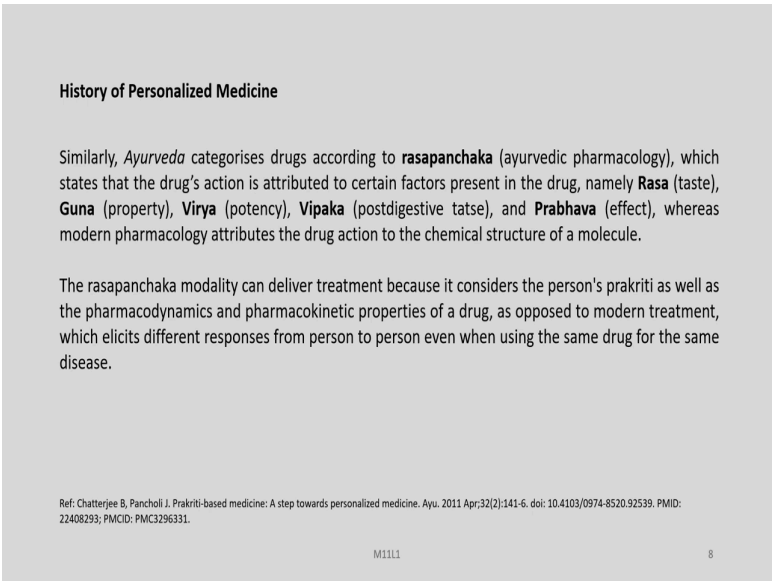

So, this is just to give an idea about the science of Ayurveda, how it treats the various phenotypes into different classes. Similarly, Ayurveda categorises drugs as in the way it categorises patients or individuals to rasapanchaka or ayurvedic pharmacology which states that the drug's action is attributed to certain factors present in the drug. Namely, Rasa, which is taste, Guna which is property, Virya which is potency, Vipaka which is post-digestive taste and Prabhava the effect.

Whereas, modern pharmacologic attributes the drug action to the chemical structure of a molecule. The rasapanchaka modality can deliver treatment because it considers the person's prakriti, the nature of the person, as well as the pharmacodynamics and pharmacokinetic properties of a drug, as opposed to modern treatment, which elicits different responses from person to person even when using the same drug from the same diseases.

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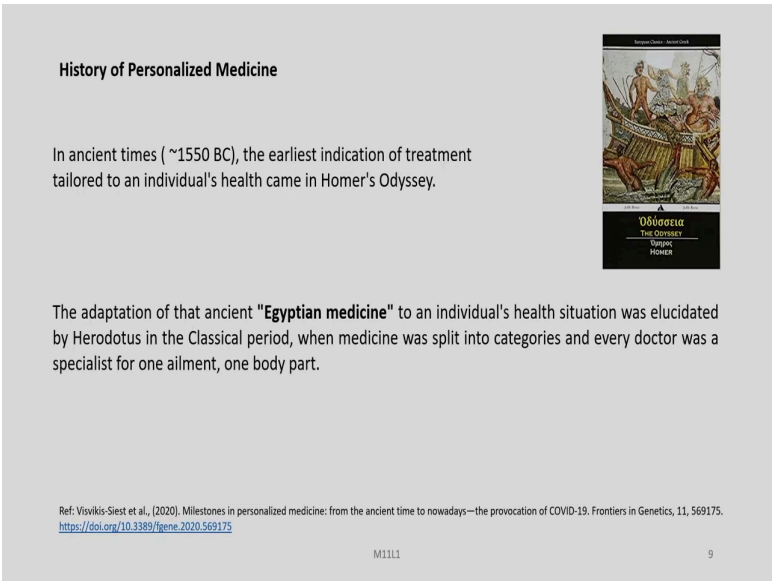

In ancient times around 1550 BC, the earliest indication of treatment tailored to an individual's health came in Homer's Odyssey. The adaptation of that ancient "Egyptian medicine" to an individual's health situation was elucidated by Herodotus in the Classical period, when medicine was split into categories and every doctor was a specialist for one ailment, and one body part.

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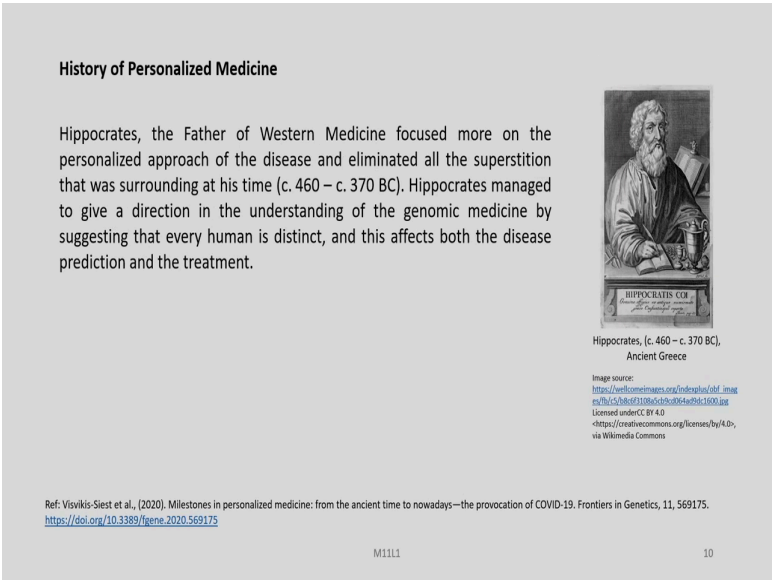

Hippocrates is the father of Western medicine about whom we have already discussed in the beginning. Focused more on the personalized approach of the disease and eliminated all the superstition that was surrounding at his time. Hippocrates managed to give a direction in the understanding of the genomic medicine by suggesting that every human is distinct and this affects both the disease prediction and the pre-treatment and the treatment.

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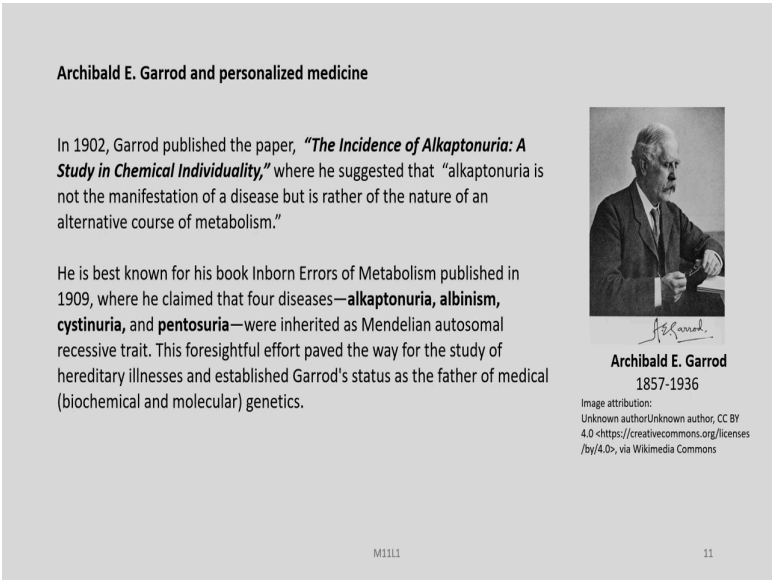

In 1902, Archibald E. Garrod published the paper, "The Incidence of Alkaptonuria: A Study in Chemical Individuality," where he suggested that "alkaptonuria is not the manifestation of

a disease, but is rather of the nature of a alternative course of metabolism." He is best known for his book, Inborn Errors of Metabolism published in 1909, where he claimed that four diseases alkaptonuria, albinism, cystinuria, and pentosuria were inherited as Mendelian autosomal recessive trait.

This foresightful effort paved the way for the study of hereditary illnesses and established Garrod's status as the father of medical, biochemical and molecular genetics.

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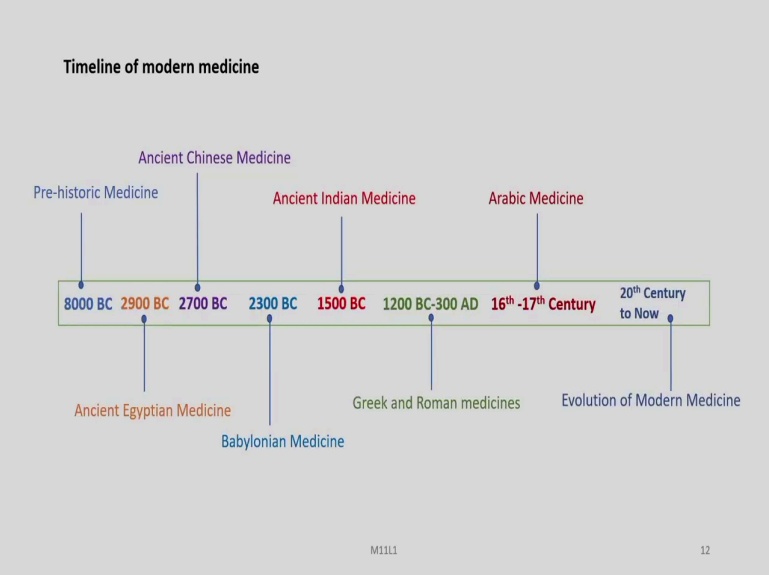

If you look into the timeline of modern medicine, it goes back to the pre-historic era which around 8000 BC. Then, you have the ancient Egyptian medicine, and then ancient Chinese medicine and then ancient Indian medicine and Babylonian medicine, Greek and Roman medicines, Arabic medicines and Evolution of Modern Medicines. Many of these systems in various instances has laid emphasis on the personalized nature of diseases and therapies.

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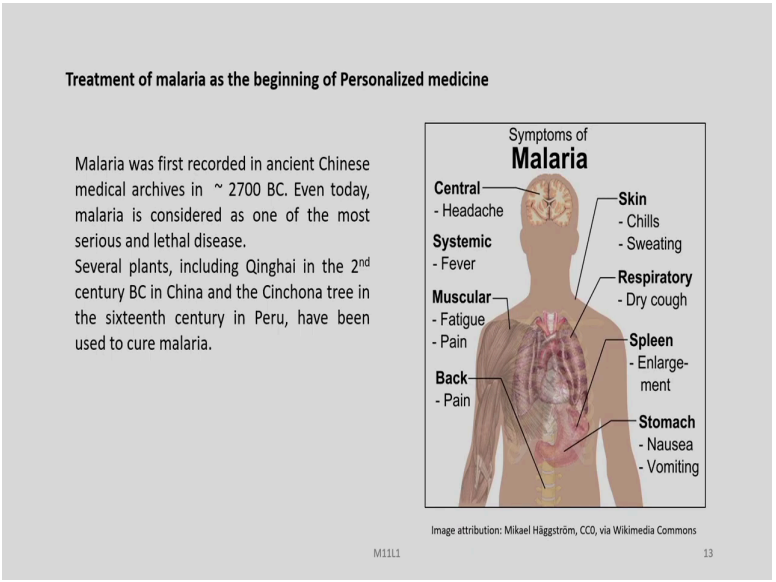

However, if we want to have some glaring example of personalized medicine, we need to discuss about malaria which was first recorded in ancient Chinese medical archives in around 2700 BC. And even today it is considered as one of the most serious and lethal diseases. Several plants, including Qinghai in the 2nd century BC in China and the Cinchona tree in the 16th century in Peru, have been used to cure malaria.

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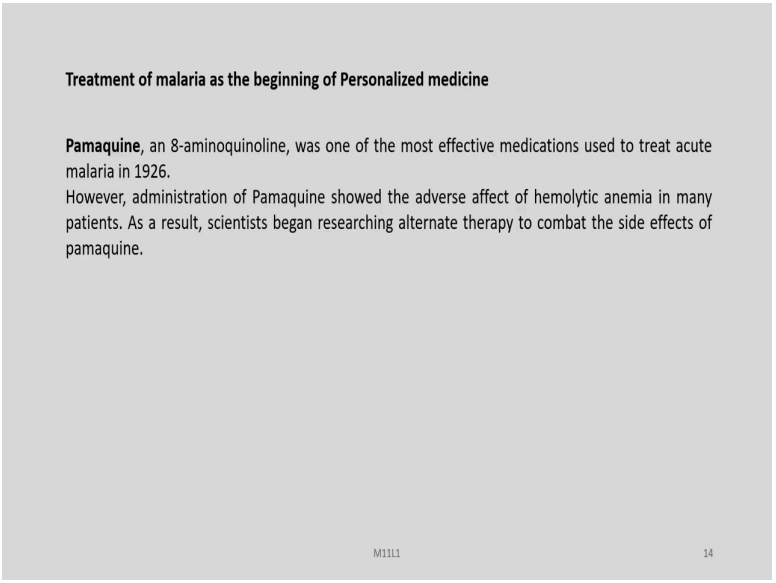

Pamaquine, an 8-aminoquinoline was one of the most effective medications used to treat acute malaria in 1926. However, administration of these drugs showed the adverse effect of

hemolytic anemia in many patients. As a result, scientists began researching alternate therapy to combat the side effects of pamaquine.

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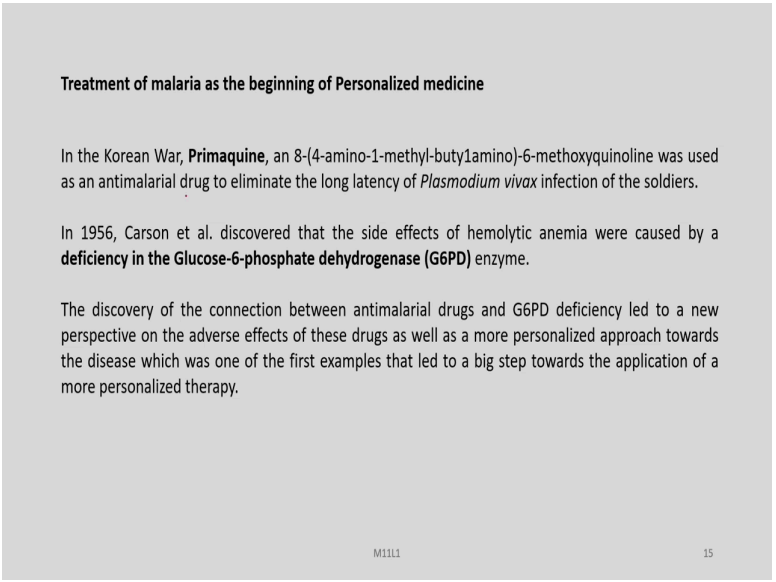

In the Korean War, Primaquine, an 8-4-amino-1-methyl-butylamino-6-methoxyquinoline was used as an anti-malarial drug to eliminate the long latency of Plasmodium vivax infection of the soldiers. In 56, Carson et al, discovered that the side effect of the hemolytic anemia were caused by a deficiency in the Glucose-6-phosphate dehydrogenase enzyme.

The discovery of the connection between antimalarial drugs and glucose-6-phosphate dehydrogenase deficiency led to a new perspective on the adverse effects of these drugs as well as a more personalized approach towards the disease which was one of the first examples that led to a big step towards the application of a more personalized therapy.

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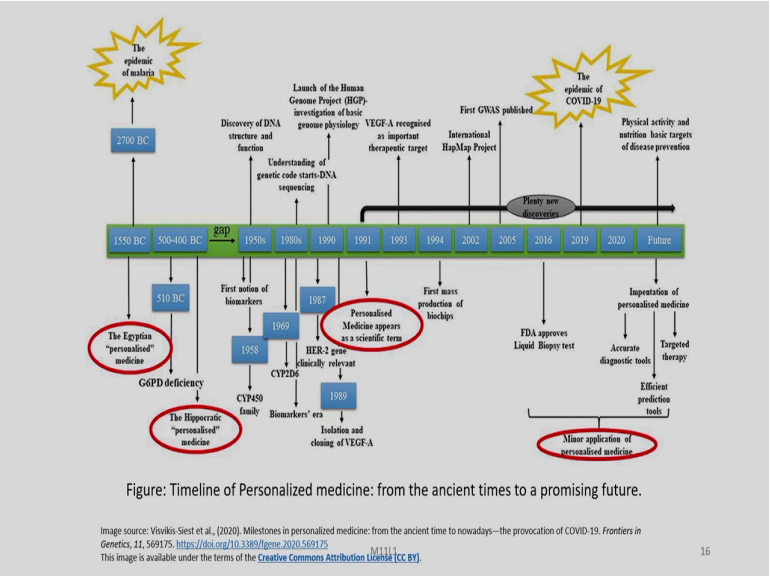

Figure: Timeline of Personalized medicine: from the ancient times to a promising future.

So, as I have already given some indications that if you look into the history of medicine, the history of a personalized medicine goes along with it. And each and every system of reported medicine or therapy has some kind of record to some extent regarding personalized medicine. And you can see as long as 1500 BC the Egyptian personalized medicine existed. And we have discussed about the Hippocratic Hippocrates approach towards the personalized medicine.

Then, various other landmarks are important here in understanding the progress of personalized medicine like the discovery of DNA or even the development of the first notion of biomarkers. As we have discussed about the glucose-6-phosphate deficiency with regards to malaria treatment earlier. So, presence, in absence of certain markers are very important in the context of personalized medicine. So, each and every progress in the track of genomics is in infantile landmark itself in the progress of personalized medicine.

However, the personalized medicine first appeared as a term scientific term roughly around 1901. Then there is a other important developments in the way of the glucoses phosphate with regards to malaria. We have hard to gene which is clinically relevant in the case of breast cancer and so on and so forth. And today, we have the international HapMap project which was completed around 2002. And since then, we live in age of modern genomics and also omics. And many of these omics technologies today play a huge role in the development of personalized medicine.

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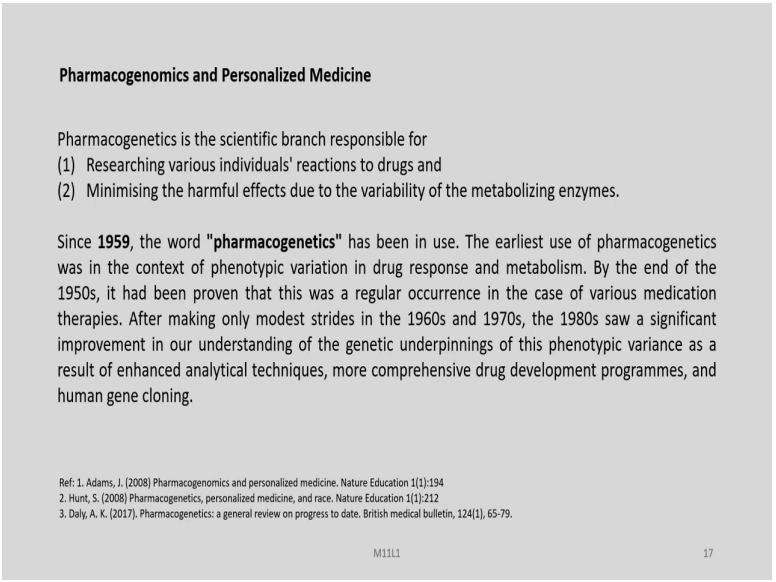

Pharmacogenomics and personalized medicine. Pharmacogenetics is the scientific branch responsible for researching various individual's reactions to drugs and minimizing the harmful effect due to due to the variability of the metabolizing enzymes. Since, 1959, the word "pharmacogenetics" has been in use. The earliest use of pharmacogenetics was in the context of phenotypic variation in drug response and metabolism. By the end of the 1950s, it had been proven that this was a regular occurrence in the case of various medication therapies.

After making only modest strides in 1960s and 70s, the 1980s saw a significant improvement in our understanding of the genetic underpinnings of this phenotypic variants. As a result of enhanced analytical techniques, more comprehensive drug development programs, and human gene cloning became possible.

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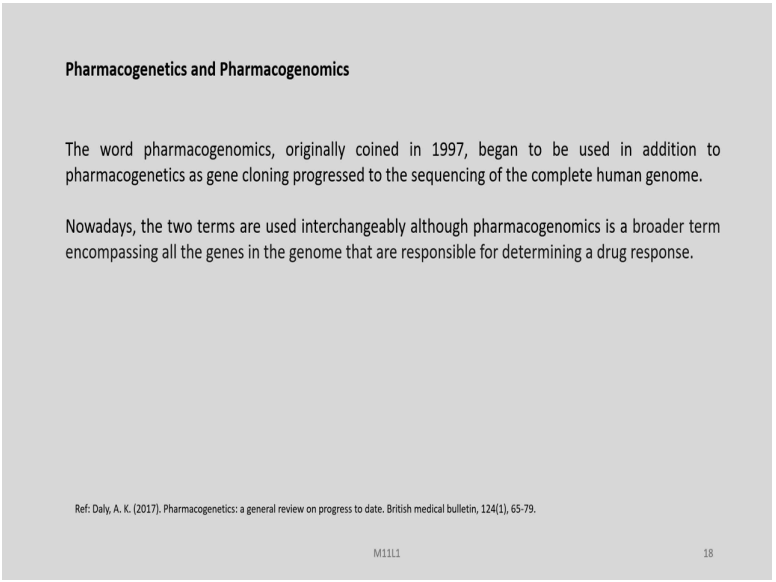

The word pharmacogenomics was originally coined in 1997, and it began to be used in addition to pharmacogenetics as gene cloning progressed to the sequencing of the complete human genome. Nowadays, the two terms are used interchangeably although pharmacogenomics is a broader term encompassing all the genes in the genome that are responsible for determining a drug response.

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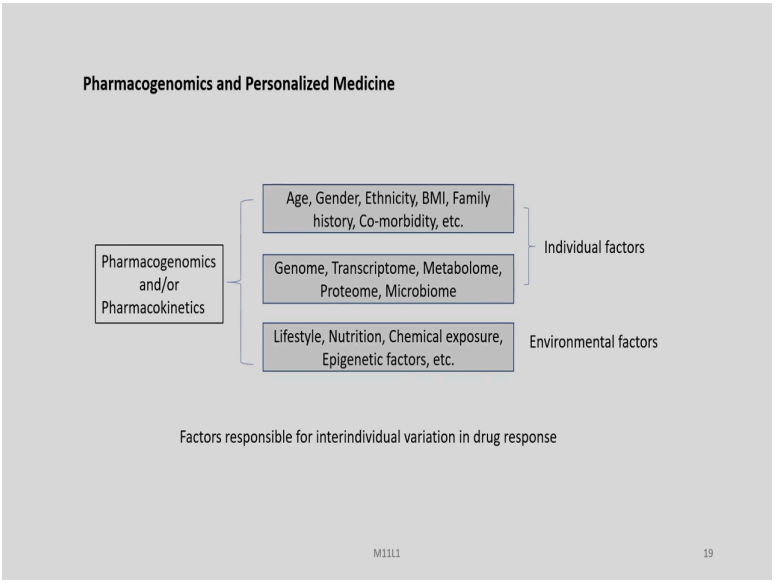

The various factors which are responsible for inter individual variation in drug response and which range from age, gender, ethnicity, BMI, family history, co-morbidity or genome,

transcriptome, metabolome, proteome, and microbiome which are basically considered as individual factors. And the lifestyle, the nutrition, the chemical exposure, epigenetic factors, and even the place of residence play an important role. And these are part of the environmental effectors.

Together these individual factors and environmental factors influence the pharmacogenomics and or the pharmacokinetics with respect to disease as well as personalized medicine.

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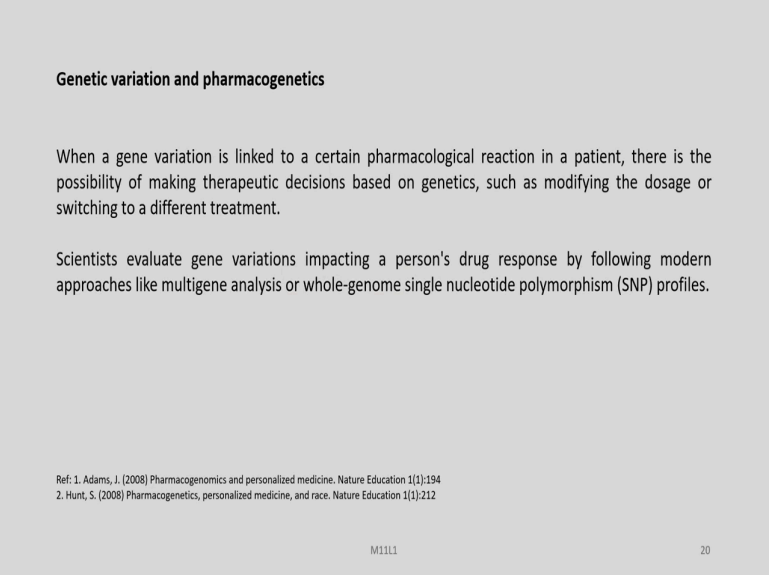

When a gene variation is linked to a certain pharmacological reaction in a patient, there is a possibility of making therapeutic decisions based genetics, such as modifying the dosage or switching to a different treatment. Scientists evaluate gene variations impacting a person’s drug response by following modern approaches like multigene analysis or whole-genome single nucleotide polymorphism profiles.

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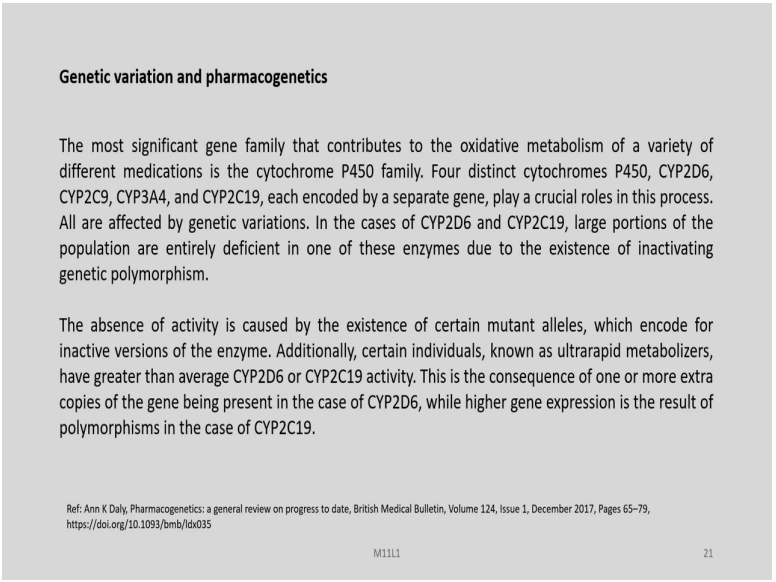

Genetic variation and pharmacogenetics: The most significant gene family that contributes to the oxidative metabolism of a variety of different medications is the cytochrome P cytochrome P450 family. Four distinct cytochromes P450, CYP2D6, CYP2C9, CYP3A4 and CYP2C19, each encoded by a separate gene, play a crucial role in this process.

All are affected by genetic variations. In the cases of CYP2D6 and CYP2C19, in large portions of the population are entirely deficient in one of these enzymes due to the existence of inactivating generic polymorphisms.

The absence of activity is caused by the existence of certain mutant alleles, which encode for inactive versions of the enzyme. Additionally, certain individuals known as ultrarapid metabolizers, have greater than average CY CYP2D6 or CYP2C19 activity. This is the consequence of one or more extra copies of the gene being present in the case of CYP2D6, while higher gene expression is a result of polymorphisms in the case of a CYP2C19.

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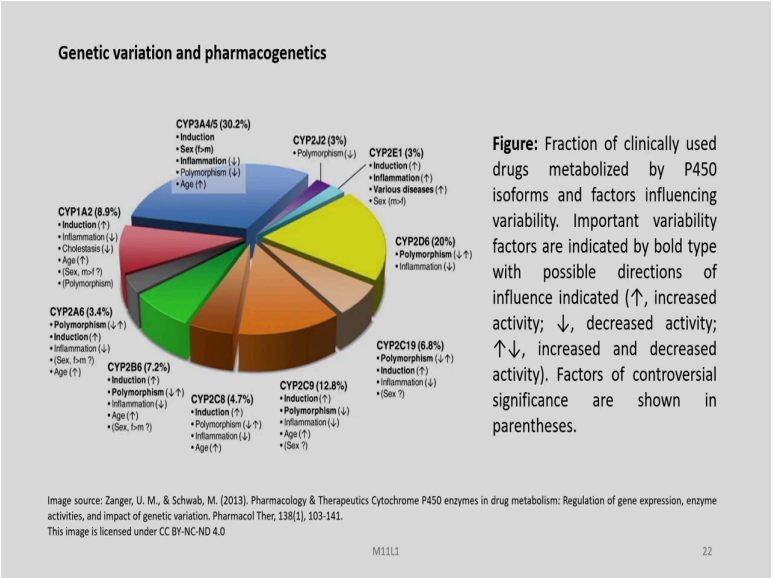

And here in this figure, you can see some of the genes or some of the symptoms getting much more worse and some of them are less. For example, if we consider the CYP3A4 4 by 5, here the inflammation goes down and there is less polymorphism and it progresses with age. So, the fraction of clinically used drugs metabolized by P450 isoforms and factors influence variability. Important variability factors are indicated by the bold types as you can see here, with possible directions of the influences mediated, either it is increased or which goes up or decreased which goes down.

Factors of controversial significance are shown in parentheses. For example, in this case the gender of the person, it is not very very clear. And similarly, here the polymorphism is not a very very clear concept or clear phenomena with regards to CYP1A2.

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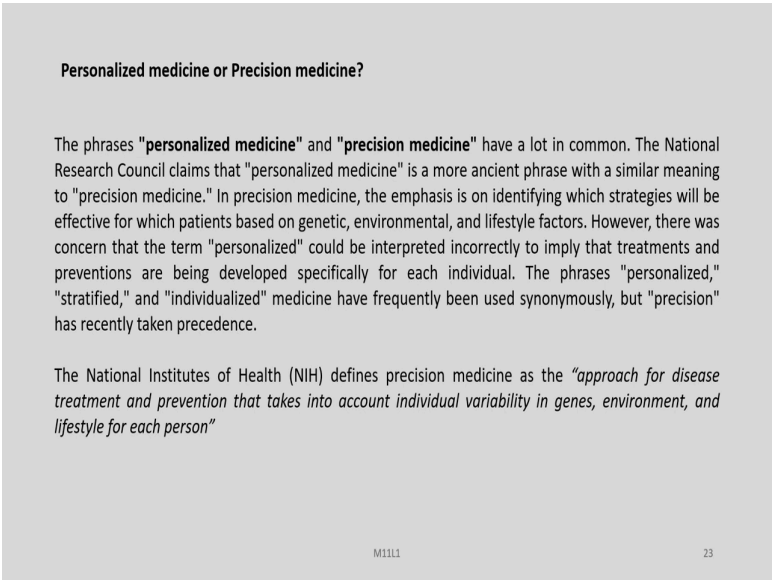

Personalized medicine or precision medicine? This is one important topic we need to discuss before we proceed to the next part of the lecture. These phrases “a personalized medicine” and “a precision medicine” has in fact a lot in a common. Although they have been used under various contexts, in various cases, the national resource council claims that personalized medicine is a more ancient phrase with a similar meaning to precision medicine. So, they are not different, they are same. But personalized medicine is a more primitive term.

In precision medicine, the emphasis is on identifying which strategies will be effective for which patients based on genetic, environmental and lifestyle factors. However, there was concern that the term “personalized” could be interpreted incorrectly to imply that treatments and preventions are being developed specifically for each individual. The phrases “personalized,” “stratified,” and “individualized” medicine have frequently been used synonymously, but “precision” has recently taken a precedence over all these.

The National Institutes of Health defines precision medicine as the “approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each person.”

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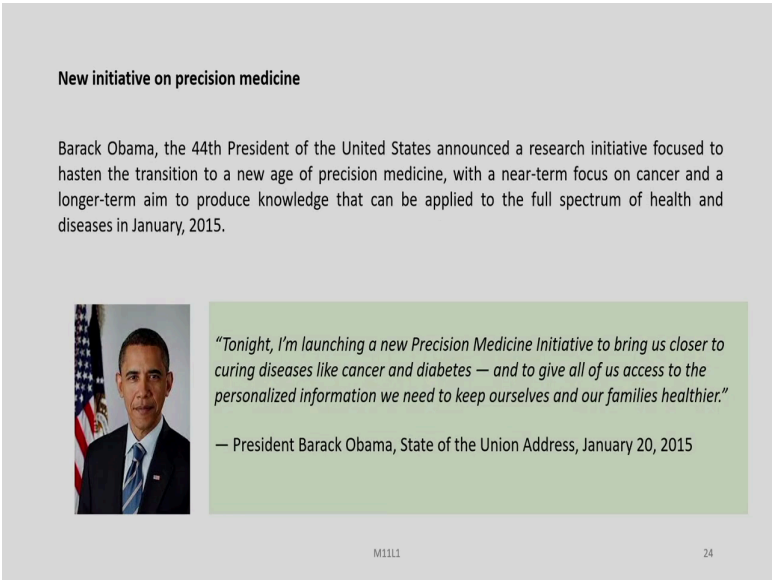

Barack Obama, the 44th president of the United States announced a research initiative focused on hastening the transition to a new age of precision medicine, with a near-term focus on cancer and a longer-term aim to produce knowledge that can be applied to the full spectrum of health and diseases in January, 20 2015.

So, on this particular date, Obama came out with this famous declaration, "Tonight, I am launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes and to give us give all of us access to the personalized information we need to keep ourselves and our families healthier."

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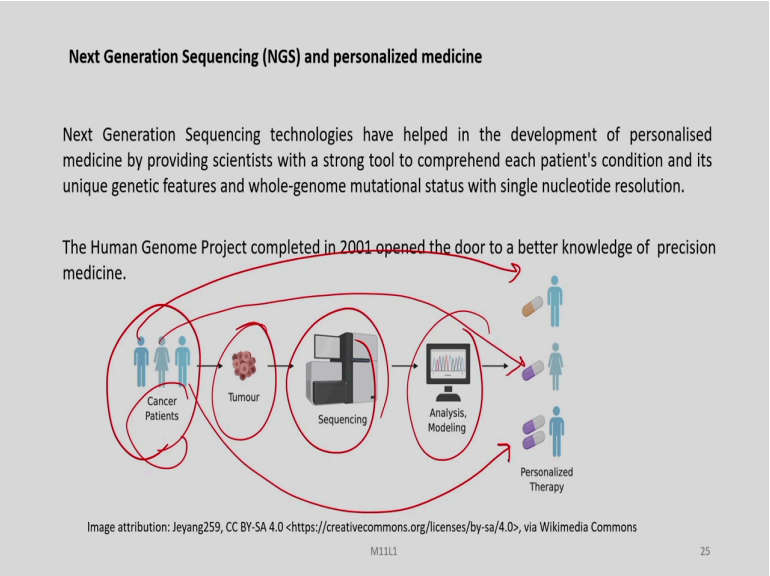

One of the important technologies that is playing a crucial role in the domain of personalized and precision medicine is the Next Generation Sequencing. So, let us briefly have some idea about this NGS technologies which has helped in the development of personalized medicine by providing scientists with a strong tool to comprehend each patient's condition and its unique genetic features and whole-genome mutational status with single nucleotide resolution.

The human genome product was completed in 2001 and it opened the door to a better knowledge of precision medicines. So, if we have for example, a particular person with a particular disease for instance cancer, we take out the particular tissue. Yeah, in this case is the tumour. And then we subject it to a sequencing and then to do the analysis and the modeling and depending on the different persons we may have the option of prescribing different cancer drugs. So, this is based exemplified by drugs like Herceptin.

So, for a better understanding of these, there is a very famous movie called Leaving Proof. It is worth watching this movie to understand how personalized medicine plays important role in cancer therapeutics and that is also is true for other diseases.

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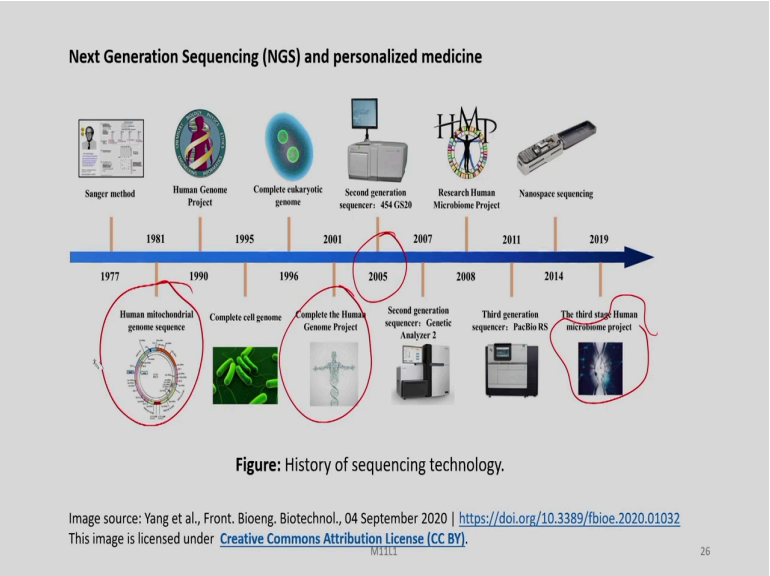

What is the connection of next generation sequencing and personalized a medicine? So, we have told that the history of medicine overall and the history of personalized medicine have been entry greatly linked to it one another since the beginning. And so also, the development of various genomics technologies and platforms which are being used to understand our genetic composition or genetic sequences.

And here in the linkage of personalized medicine to sequence in technologies is very very in intimate. I will not go into details of the various methods for sequencing like Sanger which was developed as old as 1977 in various milestones, where the human mitochondrial genome sequence in 81. Then, the second generation sequences came after the complete human genome was elucidated and so on and so forth.

Today, we have nano sequences. And we are going little bit farther into understanding the human microbiome which is also considered as one of the important component of the human health.

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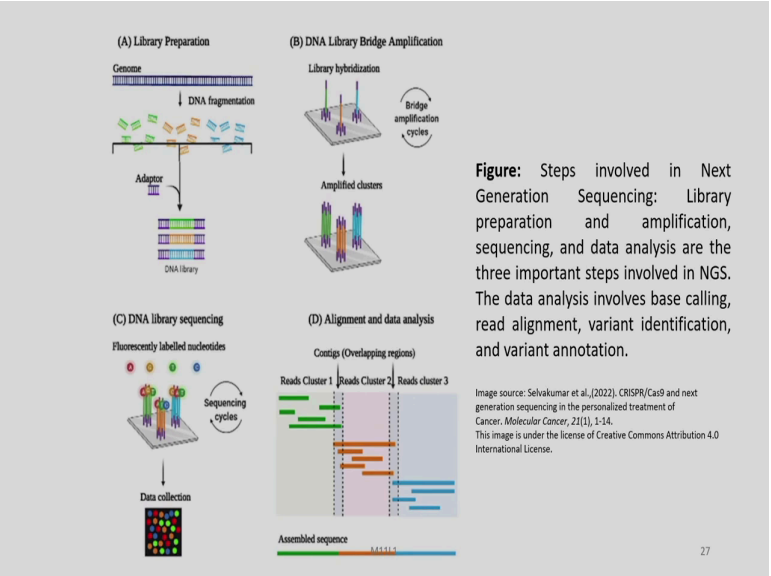

Figure: Steps involved in Next Generation Sequencing: Library preparation and amplification, sequencing, and data analysis are the three important steps involved in NGS. The data analysis involves base calling, read alignment, variant identification, and variant annotation.

Image source: Selvakumar et al.,(2022). CRISPR/Cas9 and next generation sequencing in the personalized treatment of Cancer. *Molecular Cancer*, *21*(1), 1-14.
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So, here are the various steps involved in the next generation sequencing. For example, it starts with library preparation, then the DNA library bridge amplification is done and finally, the DNA library sequencing is done and once that the raw data is obtained, the alignment and data analysis is completed. The key concerns of medical diagnosis is to identify the genes and mutations responsible for human disorders.

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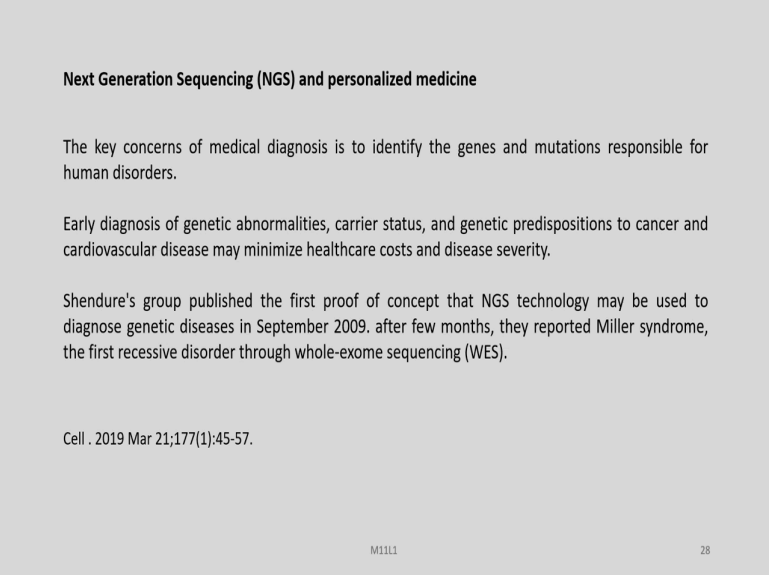

And in this context, next generation sequence is sequencing plays very important role. Early diagnosis of genetic abnormalities, carrier status, the genetic predispositions to cancer and

cardiovascular diseases may minimize healthcare cost and this is severity. Shendure's group published the first proof of concept that NGS technology may be used to diagnose a genetic disease in September 2009. After few months, they reported Miller syndrome, the first recessive disorder through whole genome whole-exome sequencing.

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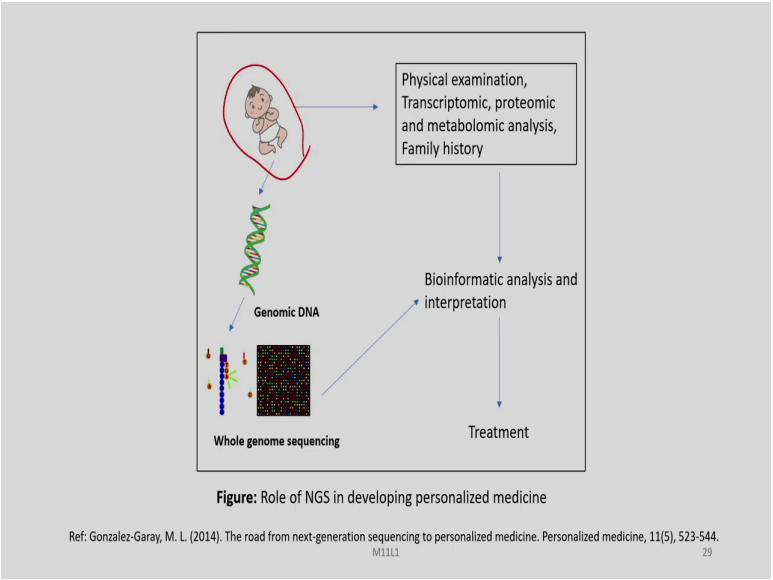

Figure: Role of NGS in developing personalized medicine

Ref: Gonzalez-Garay, M. L. (2014). The road from next-generation sequencing to personalized medicine. Personalized medicine, 11(5), 523-544.

So, here you can see a an individual, who may be normal or deceased. He is subject to physical examination and transcriptomic, proteomic, and metabolomic analysis and the family history is also recorded. And here his DNA is, genomic DNA is extracted and the whole genome sequencing is done.

So, along with these records of physical examination, transcriptomic, proteomic and metabolomic analysis, families history and the genomic information, by an informatic analysis and interpolation is done, to derive at the correct diagnostics with the help of genetic information.

And based on that, the treatment is decided for the particular individual. So, this is the role of NGS in developing personalized medicine as you can visualize from this particular diagram.

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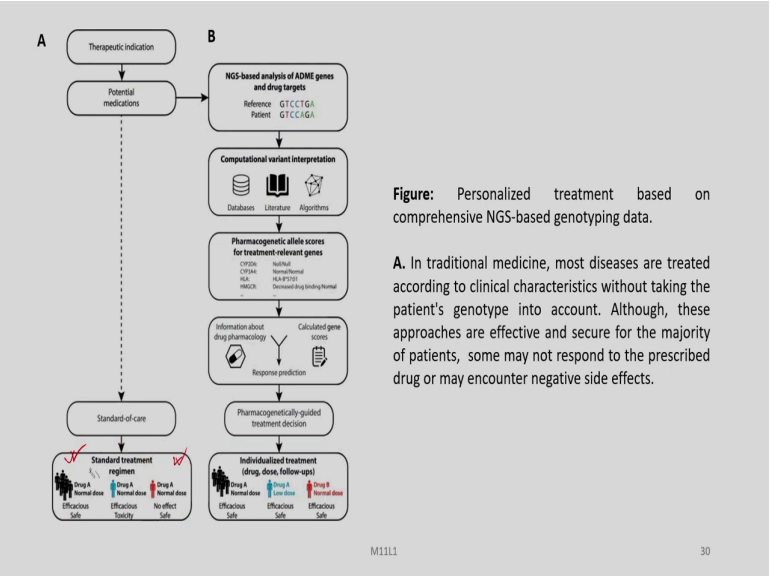

Personalized treatment based on comprehensive NGS-based genotyping data. In A, you can see in traditional medicine, most diseases are treated according to clinical characteristics. There is a therapeutic indication and their potential medications and then standard of care, the standard treatment regimen will be given drug, a normal dose drug a normal dose to a group and to different individuals. Although, these process are effective and secure for the majority of patients, some may not respond to the prescribed drug or may encounter negative side effects.

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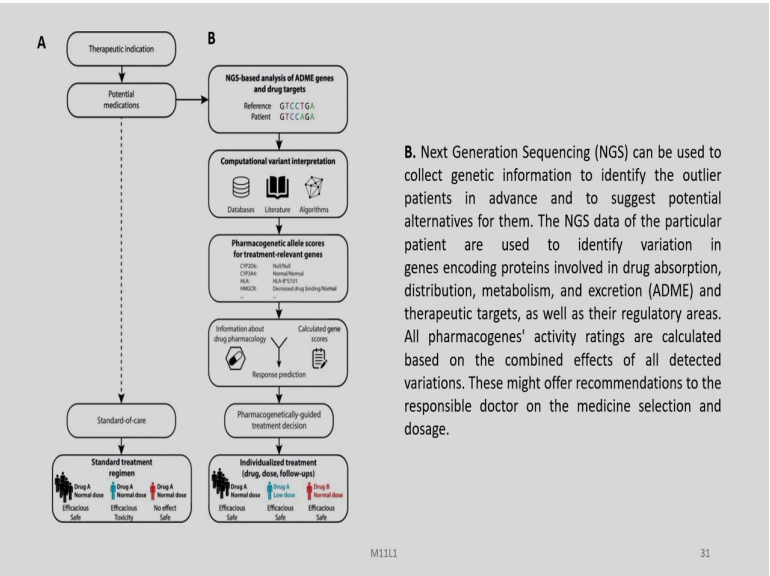

In the next generation sequencing based treatment approach, NGS can be used to collect genetic information to identify the outlier patients in advance and to suggest potential alternatives for them. The NGS data of the particular patient are used to identify variation in genes encoding proteins involved in drug absorption, distribution, metabolism and excretion and therapeutic targets, as well as their regulatory areas. All pharmacogenes' activity ratings are calculated based on the combined effects of all the detected variations.

These might offer recommendations to the responsible doctor on the medicine selection and dosage. So, we see a huge paradigm shift in the therapeutic approaches in the pre and post NGS era.

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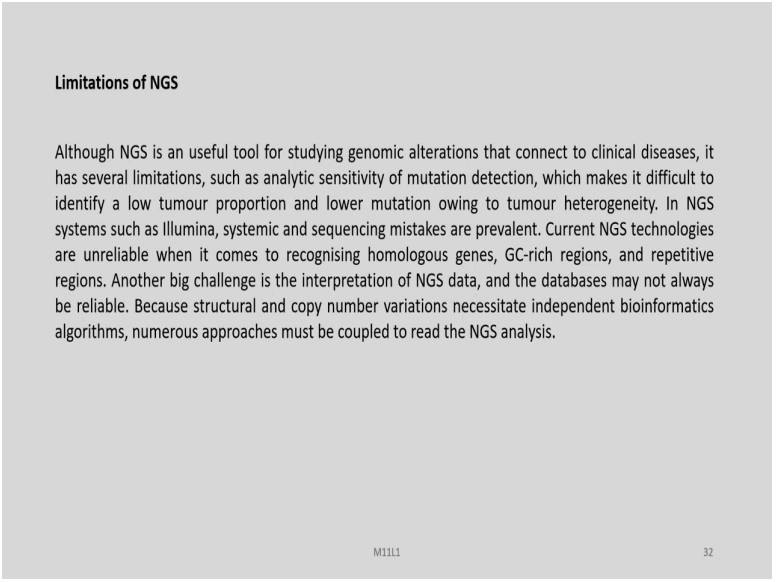

However, there are certain limitations of NGS, although, it is a very useful tool for studying genomic alterations that connect to clinical diseases. It has several limitations, such as analytical sensitivity of mutation detection, which makes it difficult to identify a low tumour proportion and lower mutation owing to tumour heterogeneity. In NGS systems such as Illumina, systemic and sequencing mistakes are prevalent. Current NGS technologies are unreliable when it comes to recognizing homologous genes, GC-rich regions, and repetitive regions.

Another big challenge is the interpretation of NGS data and the databases may not always be reliable. Because structural and copy number of variations necessitates the inter depend

independent bioinformatics algorithms, numerous approaches must be coupled to read the NGS analysis.

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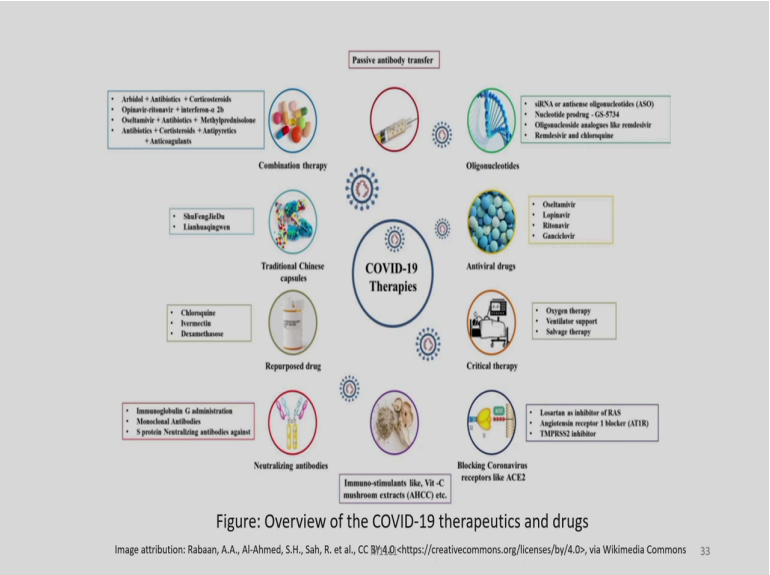

Figure: Overview of the COVID-19 therapeutics and drugs

So, this is the overview of COVID-19 therapeutics in drug. The scenario is still emerging. It is one of the biggest challenge of modern epidemiology. And we are still trying to cope up with it. So, various combination therapy, then even traditional medicines or repurpose drug and then neutralizing antibodies and even using a passive antibody transfer, oligonucleotides, antiviral drugs and other critical therapies. Then blocking coronavirus receptors like ACE2 or giving immuno-stimulants like vitamin-C, mushroom extracts etcetera has been tried.

Some of these work for certain type of patients, while some does not work for any others. And in certain cases none of these works for any of the individuals. So, it has make a very complex situation and current healthcare workers and as well as scientists are actually puzzled regarding the complexity of corona virus.

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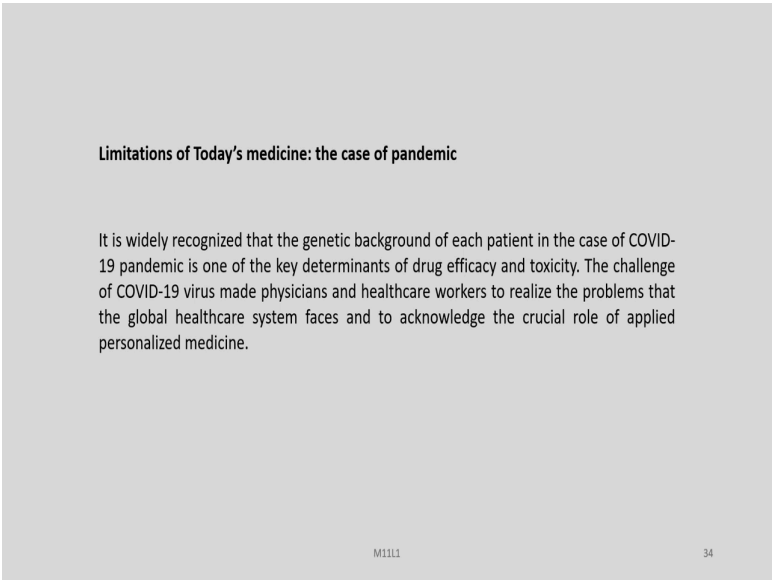

So, there are certain limitations as we understand from these big pandemic we have. It is widely recognized that the genetic background of each patient in the case of COVID-19 pandemics maybe one of the key determinants of drug efficacy and toxicity. And that is the reason why they some of them respond to certain treatment regimes probably. And these are all guesses made. But in the context of personalized medicine, the coronavirus can be a very important area of research in the future.

The challenge of COVID-19 virus made physicians and healthcare workers to realize that problems that the global healthcare system faces and to acknowledge the crucial role of applied personalized medicine. With this, we come to end of this part.

Thank you for your patient hearing. We will be continuing this lecture in part b.