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REVIEW ARTICLE

Systematic Review on Implication for DNA Assisted Technology into Molecular Medicine and the useful is the application of Genome Wide Studies

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ABSTRACT

Objective: The aim of this study is to explore the role of molecular DNA, DNA Phenotyping and Polymerase Chain Reaction in samples relevant to genetic investigation. The Deoxyribonucleic Acid (DNA) is a macromolecule which has propelled our capacity of understanding the function of an organism at the cellular level, how organism reproduce and replicate, and pass their subjective genetic information from one generation to the other. DNA is also referred to as the genetic "Blueprint" of an organism and found to possess all information pertaining to the specific being. Nuclear DNA is present within the nucleus and is significant in developing immunity for the cell and depends on the information incorporated within its framework. Mitochondrial DNA exists in varying locations in frequencies of two to ten copies within mitochondria. The most familiar body fluids come across in molecular medicine laboratories is blood, semen and saliva and further more are vaginal fluids, urine and sweat. Bones, teeth, soft tissues etc. are also essential biomarkers for DNA-phenotyping.

Methodology: The methods for research for this particular study is to demonstrate a review of relevant literature to examine the molecular genetics and the application of genome and DNA-amplification into molecular medicine investigations. To conduct a literature review a qualitative research design is the most suitable research design. It provides the rationale for assessing the human behaviour and assists to legalize and authenticate the data which is selectively collected from the secondary sources.

Results: Every individual's DNA consists of minor alterations a change in these restriction places would result in different profile of restriction fragments. Genome wide population substructure is large enough to determine ancestry with large number of Autosomal SNPs at the level of continental resolutions.

Conclusion: The advances in human genomics and molecular genetics have provided success and advances by determining the cellular origin and estimating the age of sample and disposition time. The Phenotypical characteristics like hair and eye color demonstration in criminal cases has yielded better inferences however, DNA phenotyping illustrates a limited approach wherein incorporated into biological material analysis. The accuracy of DNA technology is incredibly useful for professionals like lawyers, anthropologists, homicide detectives etc. in the field of molecular medicines and is expected to improve in years to come.

Key Words; Deoxyribonucleic Acid (DNA) , Polymerase Chain Reaction (PCR), Mitochondrial DNA (mtDNA) sequencing and Short Tardem Repeat (STR)

INTRODUCTION

This study aims to undertake a literature review on the available evidence relating to DNA technology in Molecular Genetics and human Molecular medicines.

DNA is an organic polymer found in each and every living organism. This polymer is comprised of three main components 1) A Central Phosphate Molecule 2) A Deoxyribose Sugar 3) a Nitrogenous Base. The phosphate and sugar component remains the same in all living organism but the nitrogenous base is the distinguishing factor in the polymer that differ from individual to individual (Hummel 2003).

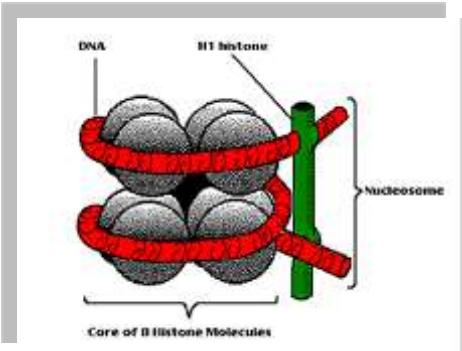

Figure 1: Structure of Nucleosome (Access Execellence at the National Health Museum, 2009).

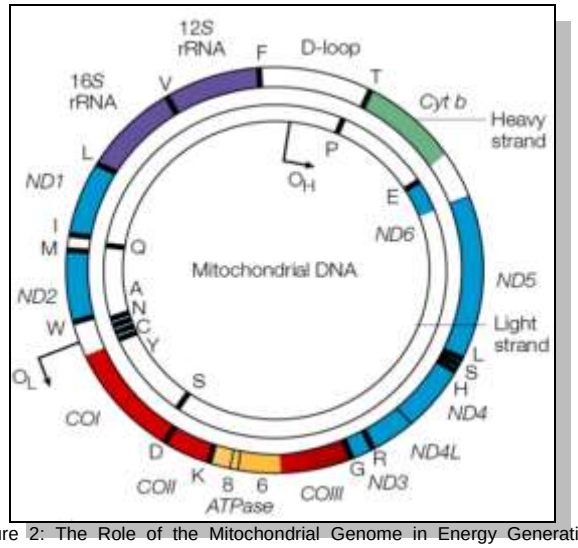

Figure 2: The Role of the Mitochondrial Genome in Energy Generation (Scitable, 2013)

Unlike nuclear DNA, the hereditary mode of transmission of mitochondrial DNA is purely maternal in nature (Hummel 2003). Therefore, all the maternally associated persons carry same mDNA in their genome and this depicts that the differentiating

powers of mDNA is not as precise and accurate as compared with the nDNA. However, mitochondrial DNA is useful in migratory studies for populations and is also used as an assisting mode in missing person cases since mtDNA is maternal related and can be utilized for a comparison to unknown individuals. Mitochondrial DNA is highly susceptible to mutation as it is not wrapped around by histones for protection. Mitochondrial DNA also lacks repair mechanisms to cater any mutation or damages (Budowle et al. 2003). Other factor that limits mitochondrial DNA's advantages is its maternal nature of inheritance as identification of individuals can only be made possible on familial level.

DNA-Based Methods for Human Identification: The use of DNA-based methods for analysis in criminal investigations highly depends on proper processing of biological samples collected from scenes. These available samples of biological materials can lead to criminal identification. However, during the process of collecting and processing the samples great caution needs to be taken to issues of proper handling, contamination and preservations because of minute amount of available DNA content. The chemical reactions that are the mode of Molecular medicine DNA analysis are specific to humans, but in few instances can show positive results for non-human primates e.g. Gorilla, Chimpanzee, Orangutan (Crouse et al. 1995).

Significance of the Study: This study is unique as it will review the previous and current studies related to Molecular Genetics and its molecular medicine attributions in finding solutions for complexed Phenotypical and DNA-extraction associated problems. This study will serve a handy assistance for the clinical researchers in the field of molecular medicine medicine in identifying the limitations for technology. This review will help the professionals in the field of genetics to identify the importance of biological tissue samples in retrieving better quantity and quality of DNA. The implications for PCR and Genome wide studies into molecular medicine investigations for recognizing unknown individuals can aid in better terms by demonstrating techniques to express phenotypes specific to the individual. These state-of-art techniques not only has provided assistance in solving molecular medicine queries but also has aided in solving investigations of ethnic origin of populations of remote past.

Aims and Objectives of the Study: To examine the general trends and challenges observed in the area of Molecular medicine DNA phenotyping and explore its ethical and legal issues.

Research Question: What is the implication for DNA assisted technology into Molecular medicine Medicine and How useful is the application of Genome wide studies, DNA Phenotyping and Polymerase Chain Reaction in samples relevant in molecular medicine investigation?

In clinical settings, the samples are collected and are preserved in excellent conditions, but however, due to ineffective handling of molecular medicine evidences like low amount of available template, degradation of material due to exposure from air, light or heat, and the age of specimen. Further, the samples can not be retested if problem arises after the first analysis and so the first attempt may be the only one. There are some physical traits that are determined in subjective terms with finite variabilities like eye colors. Are his eyes blue? Black? Are her skin color Mocha or chocolate etc. This problem can be representated in thee basic three terms; the testing can be considered useful so as to discern the differentiation of shades of color to predict approximation to the natural color determination; secondly, validity of testing methods need a reference value for the arbiter with the specific characteristics; thirdly, consensus among the professionals needed to be present and maintained so as to characterize a trait for the validated tests that can be translated by the people in the particular field, for example, police personnel can distinguish perpetrators with black or dark brown eye color.

Studies do suggest that, there is a growing body of evidence that hold promising results for DNA phenotyping as a pertinent component for molecular medicine investigations. The implication of molecular medicine chip is one of the firstly available tool that can analyze genetic information from genome and produce information for Biogeography ancestry (Brendan Keating et al. 2012). Using 3196 DNA samples of different quality in order to determine molecular medicine characteristics, the researchers found that nearly 95 % of the samples resulted in highest accuracy determination for sex and first to third degree relatives, 94 % illustrated accuracy regarding ancestry, 75 % correctness for specific eye color and 65 % for accurate prediction of hair color (Brendan Keating et al. 2012). Among all phenotypes age estimation studies demonstrated that the capacity to predict accurate age within 5 years margin is well established (Brendan Keating et al. 2012).

The morphology depicting the facial characteristics such as shape and size of nose, eyes, lips, and overall facial structure, can be accurately predicted by genetic variability (Levinia Paternoster et al. 2013). Future researches are expected to reveal indicators illustrating the probabilities for body height, baldness, and traits for hair morphology (Levinia Paternoster et al. 2013). Researchers are also aiming to solve the puzzle of genetic origin responsible for a variety of medical and psychological traits. Some of these phenotyping techniques are useful for molecular medicine investigations, for example, indicators for genetic diseases like Albinism, dwarfism, or traits for Sickle Cell Anemia, and left handedness (Levinia Paternoster et al. 2013).

The genetic probe predictors for Phenotypical traits like sexual disorientation, drug dependency, aggressiveness, all of which are directly related to molecular medicine and criminal justice. Behavioral characteristics constitute a variety of regulatory systems that are complex in execution as they are governed by other environmental influences. It seems in the short term time period that tools to interpret such information will be available for the law enforcement agencies for effective investigations. It is still expected that genetic information which is revealed today can not be understood or conceived but later can be successfully interpreted despite it initially holds low predictive powers.

Molecular medicine DNA Phenotyping and its Usefulness in Criminal Cases: In order to understand the application of Molecular medicine DNA Phenotyping into subject field of criminal investigations, the knowledge of scientifically assessing the biological materials to separately address unidentified molecular medicine samples and known individual samples. Unknown samples containing biological fluids or tissue materials retrieved from crime scenes can be belonging to a perpetrator, victim or a witness. Similarly, identified samples are collected from known subjects whether through voluntary or compulsory procedures. Testing of unknown samples help in identifying the perpetrators which requires precise determination of genetic testing to differentiate individuals from one another. These testing procedures for Phenotypical traits for distinguishing biological characteristics like Albinism or a stutter aid in isolating a suspect. This additional genetic testing apart from conventional methods for molecular medicine sciences prove to be helpful in resolving whether the suspects profile matched the profile that of the evidence. In cases when the known individuals' samples are associated with DNA phenotyping procedures, conventional DNA testing has an advantage over FDP.

Traditional methods illustrate more viable indicators of innocence and guilt for any offense which have genetic evidence as well. However, FDP application can be viewed as an aggravating factor for taking decisions about pre-trial release, sentencing, and in some instances preventative detentions. A study also demonstrated that by applying FDP into molecular medicine investigations at least 6 perpetrators have been arrested and charged with the offense (Tony Frudakis, 2007). Another prominent example can be taken into account from Louisiana where a serial killer linked to seven murders have been arrested successfully. Law enforcements had very limited evidential resources for this specific perpetrator; one information was of a white male in a white van. This perpetrator had no record in Law

enforcement data bases as he never got arrested previously (Tony Frudakis, 2007).

Investigators indulged molecular medicine profilers and psychologists and engaged every possible resources to catch this man but in vain. Molecular medicine experts contacted later a company that marketed a service allied a DNA Witness which test biological samples for ancestry (Tony Frudakis, 2007). FDP testing revealed that the unknown perpetrator's ancestry is expected to be 85 % African and 15 % American genotype. Further the studies demonstrated that, skin color for the subject is more likely to be averagely darker as compared to tones for African-American group of population (Tony Frudakis, 2007). Police focus their attentions to recent unsolved homicide cases in which assailants are identified as Black. Finally police identified a suspect from short list of perpetrators and identified the man as Derek Todd. A sample was tested for his phenotypical characteristics using FDP, and the results depicted as a positive match.

In October, 2004 he was apprehended and sentenced to death for one of the convicted murders. There are many examples that show the variability and reliability of DNA Phenotyping in solving difficult criminal cases but possess some limitations. The first limitation to its use is that with such a tool only perpetrators that are unknown can be successfully identified but a wide variety of crimes occur between people who are known to each other. Secondly, police test biological materials for identifying markers in molecular medicine DNA typing and then search databases for exact match to known person. In this way it is easier to link an assailant to his DNA profiling, but the use of Phenotyping techniques only allows the straightforward molecular medicine profile.

METHODOLOGY

The search of literature is defined as a systemic approach for retrieving, identifying and bibliographic material to manage the studies which are self governing. The main purpose of the study was to locate the information or knowledge on any particular topic for recognizing the study parts for future and forming the clinical practice guidelines. For establishing any search of literature, it is important to comprehend any research and its role in informing the clinical practice as well as questioning (Parahoo 2006).

Findings of this Review: The main findings are concerned to the morphology that depicts the facial characteristics can be accurately predicted by genetic variability. Almost all nations allow the application of Short Tandem Repeat Markers (STD-markers) in revealing non-coding regions for DNA profiling, regions that are not associated with non-visible traits. Testing of unknown samples help in identifying the perpetrators which requires precise determination of genetic testing to differentiate individuals from one another. Previous studies in molecular medicine sciences also reported that three SNPs are responsible to express 76 % of total variability in hair melanin and other phenotype characteristics.

DISCUSSION

With the completion of Human Genome project in the year 2003, researchers in the field of molecular genetics have focused on a set of research tools that include computerized databases containing reference Human Genome Sequences. These sequences contribute widely in assessing human genetic variations that in turn can easily and precisely analyze genome samples essential for pathological and molecular medicine investigations. Moxley et al. (2002) reported, the replications of Genotype-Phenotype association helpful in facilitating improvements in lowering thresholds for positive results, adjustments for population framework, and exploitation of linkage disproportions in candidate genes (Lueders et al. 2003).

In similar context, the technical method of Restriction Fragment Length Polymorphisms (RELP) in DNA sequestration, involves a restriction enzyme that bifurcates at a specific sequence is been used to break DNA into numerous fragments (Lueders et al. 2003). The relative size of the fragment provides a distinctive characteristics based on the location of sequences within DNA framework. Hence, a restriction profile for an individual's DNA can be formulated. Every individual's DNA consists of minor alterations a change in these restriction places would result in different profile of restriction fragments (Lueders et al. 2003). This technique has been the standard in Molecular medicine testing for a considerable time because it allowed an elevated degree of discrimination but however, it required a large amount of DNA material outside the human body which is often too difficult to obtain. In 1986, Kary Mullis developed a method called Polymerase-Chain Reaction (PCR) (Lueders et al. 2003).

The Methods for Preservation and Estimation of Age of DNA Samples: DNA is characterized on the basis of the sources it is collected from and also on the location on which it is situated. However, DNA samples can also be classified on the basis of age designated as Ancient or Modern. Ancient DNA (aDNA) refers to DNA molecules that are found in fossils or preserved biological materials (Brown 1994). On the other hand, Modern DNA is extracted from fresh materials and from samples which are less than 50 years old. In postmortem specimens, the speed of degradation of DNA is faster brought about by endogenous nucleases (Hofreiter et al. 2001b). These damaging nucleases can be inactivated in certain environmental conditions like extreme lower temperatures and by surrounding surfaces containing high salt concentrations (Hofreiter et al. 2001b,Sarwar B et al.2021).

Although modern DNA are more susceptible to damages from environmental causes, the degradation is minimal due to the presence of number of copies of DNA available. However, Ancient DNA samples have been exposed to different environmental conditions over a prolonged period of time nearly hundreds to thousands of years (O'Rourke 2000). These damaging processes have more time to accumulate and cause damages to DNA fragments typically those less than 300 base pairs in length (O'Rourke 2000). The processes of Hydrolysis and Oxidative Reactions are the prime methods that accelerated the DNA degradation process (O'Rourke 2000). Hydrolytic insult can cause degradation in two ways; by cleavaging Phosphodiester bonds found in Phosphate Sugar Backbone that resulted in stranded nicks, and the other is by cleavaging Glycosidic bonds found within Sugar backbone and Nitrous base creating fragmention of molecules (Paabo et al. 2004).

Oxidation takes place when water liberated Super Oxide radicals distort the helical structure and modify bases (O'Rourke 2000). These degradation processes keep on occurring in the living cells but are replaced by repair mechanisms found in the nucleus however, after death the repairing powers of the body cease and the degradation process continues. The analytical methods used by Hofreiter et al. (2001b) for depicting mitochondrial DNA fragments that contain aDNA from ancient sample has been illustrated. Following the first human analysis that was done by analyzing a 2400 year old mummy, *Alu fragment* was firstly determined shown to be essential in demonstrating better DNA detection (Paabo 1985). Moreover, these samples collected are available in limited amount and thus it is not possible to replicate the inferences to determine if the profile retrieved is the endogenous DNA. Similarly, the process of Inhibition can also effect the determination of DNA profiling at two stages; Extraction and Amplification.

During the process of extraction, Inhibitors can interfere with the chemical components of the processes by preventing the active agents from stabilizing proteins and liberating DNA (Muller et al. 1999). Polymerase Inhibitors interfere by inhibiting the amplification of DNA typically affecting the chemical reactibility of the molecule (Muller et al. 1999). Such an example can be of Mercury Inhibiting PK as these inhibitors are mostly encountered during a DNA analysis and can be Tannins, Fulvic acids, Humic acids all of these are soil derived degradation products (O'Rourkeet al. 2000,Khatoon F et al.2021)

CONCLUSION

The results of this study demonstrate that the efficacy of a molecular medicine technique used to analyze the Samples from crime scenes or for identification purposes rests on the type, quality, age and amount of DNA retrieved. The decontamination and sample purification methods employed before the process of extraction of DNA also impact the yield of DNA specially in removing the impurities and inhibitors. Further, the implementation of PCR into molecular genetics and the incorporation of Genome wide studies has not only boost the scientific technology that can be utilized into molecular medicine case analysis and for depicting better outcomes.

It is evident that molecular DNA techniques will evolve and advance along with the database technology for analyzing Molecular medicine evidences. The accuracy of DNA technology is incredibly useful for professionals like lawyers, anthropologists, homicide detectives etc. in the subsequent and is expected to improve in years to come. However, molecular technology possesses weaknesses and its limitations always need to be kept in mind. DNA can never be utilized in order to prove that an individual committed a particular crime but can only assess the presence of a person at the scene.

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