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Genome Wide Association Studies in Molecular Medicine

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Chapter 1: Introduction- The achievements in Human Genome Project and subsequent advancements observed in Genotyping techniques have led an influx of exciting new era of discovering human genome and genetics. These technologies have provided the scientists with a comprehensive data on human genomes and have introduced state-of-art methods of investigating the fundamentals of forensic medicine (Moore 2009). The Human genome is now capable to incite in depth and precise data information and allows access into processes that extract detailed sequences for genomics in order to analyze and answer not only theoretical questions but also identifies the practically feasible genetic characteristics (Moore 2009).

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I. CHAPTER 1: INTRODUCTION

The achievements in Human Genome Project and subsequent advancements observed in Genotyping techniques have led an influx of exciting new era of discovering human genome and genetics. These technologies have provided the scientists with a comprehensive data on human genomes and have introduced state-of-art methods of investigating the fundamentals of forensic medicine (Moore 2009). The Human genome is now capable to incite in depth and precise data information and allows access into processes that extract detailed sequences for genomics in order to analyze and answer not only theoretical questions but also identifies the practically feasible genetic characteristics (Moore 2009).

Genomic sequences aid in appreciating the origination and nature of the particular mutations in genetic data and thus, incorporate functional genetics, biological sciences and bioinformatics to extract the cause of a variation in genetic framework. In the evolutionary field of forensics, the combination of information and the understanding of genetic architecture lead to dissect the unidentified entities and translate for making connections in solving ongoing genotype and phenotype puzzles of unknown nature (Moore 2009).

The last five years in the field of genomic studies have been an era of ensurgement of discoveries for epidemiological studies associated with the genetic and genomic technological advancements. High-Throughput genotyping and sequencing centers are containing torrents of data and information related to surveying genetic variants across the genome for association of complex diseases, human phenotypical characteristics and quantitative traits (Park et al. 2010). The Genome Wide Association Studies has been the work force for these efforts and now is considered as a standard technique for disease gene mapping.

There are two fundamental and statistical challenges that Genome Wide studies currently faces, the first one is the majority of identified variants have minor impacts and so collectively only explains a small proportion of the entire genetic variances (Gibson, 2010; Park et al. 2010). Secondly, the total number of Single

Nucleotide Polymorphisms (SNPs) being analyzed are usually over half a million, and is often larger in magnitude as compared with the number of genetic observations recorded (Gibson, 2010). Referring to current situation, even a minute amount of error in the information about genetic data can have wide adverse effects that may greatly cost the power and credibility of Genome Wide Association Studies.

According to GWAS, the inductive spurious associations between a phenotype for the pathology and a variance or in case when there is no association between a true variant and the disease phenotype, subsequently assists in evaluating true relationships between genetic variables (Gibson, 2010). Therefore, it has been crucial for the success of genomic association studies to execute careful Quality Control (QC) and appropriate data cleaning before using the data for testing for genetic association purposes (Gibson, 2010).

a) *The Empirical Hallmarks for Misidentification in Forensic Genomic Sampling*

In recent times, Laurie et al (2010) and Anderson et al (2010) signified that the best guidelines for successful genomic association studies is the inclusion of per individual and per-SNP steps in sequencing. One essential error that appears during genomic association studies is the misidentification of the samples which result from pipetting errors in the laboratory during analyzing the samples, labeling mistakes and other data or information handling mistakes. These types of misidentification errors can lead to considerable loss of accuracy specifically in diseases with low prevalence (Edwards et al. 2005; Zheng and Tian, 2005).

Misidentification is an error that can be challenging to detect since it is a fundamental feature for many Genome Wide Association Studies that all the samples are unrelated to each other. Thus, the use of already known Mendelian relationships between pairs of individuals included in the study sample is for purpose to infer the tendency to make mistakes which is not exclusively possible to elicit successful outcomes. Instead, misidentification of samples can be detected by following comparisons between a). Sex characteristics associated with X and Y chromosomes data for genome with not established identity of sex b). The inferred data for constructing ancestry details based on principle component analysis with self proclaimed ancestry

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Genome Wide Association Studies for forensic determination?

2. What are the statistical methods followed in GWAS affecting the phenomenon of Epitasis that produces randomization in phenotype resulting in variances in allelic expression?

e) *Outline of the Study*

The Chapter 1 of this dissertation provides a general idea of this study from the perspective of the researcher will develop from, clearly introducing the question of this study and aim and objectives adopted for the research process.

The chapter 2 of this dissertation provides a brief overview of the topic concerned following a description over the aspects of Genome Wide Association Studies incorporated into the field of forensics and respective literature evidences.

The chapter 3 of the study is the methodology of the research used for describing the study adequately and how it is carried out together with the detailed procedure undertaken in relation to the critical review and the studies included in the study. The ethical considerations recognized essential for this dissertation will also be presented along with the justification demonstrating that this perspective is unbiased, by documenting the literature searching and critical appraisal process.

The chapter 4 of the study presents the analysis and discussion of the data determining the benefits of the studying this problem, assessing and comparing the studies related to our topic along with its discussion, and interventions.

The chapter 5 of the study opens up with the results shortened in the form of a conclusion to the dissertation along with the implications for research and practice.

II. CHAPTER 2: LITERATURE REVIEW

The section will open up with the review of literature of the published work related to the topic. It will also highlight the theories close to the subject of research along with the general review of the studies selected. The aim of this review is to study the application of Genome Wide Association Studies incorporated into Forensic Medicine.

a) *Ancestral Sampling in Genome Wide Association Studies*

The alternative approach used to amplify genetic causes of complex traits is population-based Genome studies. These studies focus on the correlation between genetic markers and relevant traits among unrelated population samples (Cardon et al. 2001). The reasons for ancestral mutation of genes that occurred hundreds of years ago and of which lineages of all descendants carry the casual variants, are thus

considered susceptible for polygenic mutations. If this casual variant is assayed into Genome Wide studies' perspectives, persons carrying the derived allele will disperse it in different phenotypes as compared to those who carry ancestral alleles (Cardon et al. 2001).

In human demographic history, the markers are in Linkage Disequilibrium with one another within the same population. The genotype segment can be in Linkage Disequilibrium with the casual variants efficiently tracking ancestral chromosomal mutations that have been long aroused but decayed due to recombination. This genotype marker will appear in association with the phenotype and allows the localization of genetic loci (Cardon et al. 2001). In accordance to this, several methods have been proposed for confounding effects of population substructure. Family based association tests (Chen & Abecasis, 2007), used to assess the transmission of alleles from the parents to the offspring and serve as useful tools for assessing ancestral characteristics within sub groups of population. The family-based test analysis is considered immune to potential genetic heterogeneity that lies between generations and among persons from similar families (Chen & Abecasis, 2007).

Global studies (Jakobsson et al. 2008) reveal that genome wide population structure on various levels can be assessed using statistical approaches. Individuals in different continents are differentiated in genetic framework through genome-wide SNP data but however, some level of over-lapping do exists between continental regions (Jakobsson, et al. 2008). This genetic overlapping increases with decreasing distances between continental regions. For example, there is a strong relationship between geographic correlation and genetics similarity of an individual who resides in Europe, but there is a considerable overlap of genetic similarity among neighboring European subpopulations which determines accurate determinations for genetic variability (Jakobsson, et al. 2008).

It is assumed that Genome Wide sets of Autosomal Single Nucleotide Polymorphisms (SNPs) that has become available through Microassays, helps in studying a wide range of geographical genetic variability. Furthermore, Genome wide population substructure is large enough to determine ancestry with large number of Autosomal SNPs at the level of continental resolutions (Mangold E. et al. 2010). Furthermore, the Y-chromosomal informative sets and mt DNA markers needed to be combined along with those from autosomes for precise DNA based inferences of biogeographic ancestry (Mangold E. et al. 2010). Genome Wide Association Studies provide researchers to identify relevant genes involved in creating a specific personality trait or genetic variations. This method scrutinizes the whole genome and

investigates the hundreds and thousands of SNPs, simultaneously.

Scientists can use data from this technique and search for genes that are responsible for predisposing a person at risk of developing disease or a trait important in terms of forensic interest (Lueders et al. 2003). Selective genotyping is an application used to identify different ethnic groups or a mix population and can be beneficial in identifying specific traits in a homogenous ancestry distribution. Similarly, by comparing relative allele frequencies between different phenotypic groups, Genome Wide Studies can detect SNPs that are associated specifically to a disease process or an ethnic trait. GWAS are preferred by many researchers because they provide more accurate localization of casual genes and also they provide unbiased detection of whole genome for genetic association (Lueders et al. 2003).

b) Genetic Mapping Scheme in Analyzing Familial Linkages

In order to demonstrate the heritable characteristics of an unidentified person and to detect mutations at the loci implies that Genetic Mapping Scheme is based on linkages in familial studies of randomly illustrated phenotypical characteristics. However, it is difficult to ascertain that as to how polymorphic loci are adhered to establish linkage relationship into practice. To address such problems, to adapt linkages to a polymorphic marker is better than the availability of no linkage (Lueders et al. 2003). For these purposes, tighter the linkage more accurate will be the predicted genotype of the individual and higher will be the degree of polymorphism. The construction of genetic linkage map requires lineages spanning multiple generations and can be selected from large pedigree. The application of restriction fragment fingerprinting that result from each probe, from each person can be recorded and analyzed for familial linkages (Lueders et al. 2003).

These can be made possible by distant translocations; however, can reveal unusual inheritance since the probe may be detecting such sequences that are genetically non-linking. The pedigree analysis can show linkage relationships between modifications of genes by depicting co dominant phenotype. The genes that are dominant or recessive or are structurally modified for a regulatory enzyme can bring about activity and expression. The application of such a system may affect genome detection with different probes already identified through mapping to reflect sequences imbedded in linkage groups. The mentioned characteristics can be distinguished by their dominancy state, rearrangements illustrating co dominance and modifying enzymes that can be dominant or recessive (Marsh et al. 2000).

The analysis for identifying the linkages in familial expressions allows the execution of frequency of

polymorphism among 71 proteins, and is studied in European populations (Osbon et al. 2000), and the inferences deduced state that 0.28 base changes per 1000 base pairs suggesting that DNA Sequence Polymorphism depends on the size of the average gene. The visible Electromorphs represents about 1/3 rd of Nucleic acid sequence changes and out these third base changes are not reflected in corresponding amino acids. This depicts that the DNA sequence polymorphism is about 0.001 for each of the base pairs among various protein coding sequences (Osbon et al. 2000). The repetitive DNA segments are exclusively found in humans and serves as a probe for specific loci susceptible to show polymorphism. This mobile sequence can be replicated, cloned and used to screen the genome library of humans. Forensic illustrations in genomic studies can be made possible by making possible the identification of unknown loci and polymorphism that is particular to a family and thus can assist in tracing the desired individual among entire set of population (Osbon et al. 2000).

c) Phenotypical Variance Incorporated into Genomic Studies and SNPs

There are numerous forensic cases in which DNA-based inferences of biogeographic ancestry information is vital to suggest police investigations to find unknown individuals or unidentified victims (Mangold E. et al. 2010). However, it is important to acknowledge that when and how biogeographic ancestry is applied in answering forensic queries and where DNA testing techniques can be introduced to determine the level of ethnic disparity using useful DNA marker sets in association studies. It is evident to keep in mind that the genetic diversity allows the involvements of various appearance traits likely to be error prone as no phenotype is restricted to a certain geographic region. In similar terms, appearances of the unidentified individuals can be known by utilizing markers from genes incorporated into data sets for genomic studies that are functionally active and strongly associated with person's appearances and skin color (Mangold E. et al. 2010).

The genetic effect of Single Nucleotide Polymorphisms (SNPs) on the phenotype of an individual depend on the number of contributing SNPs and non-genetic influences like environmental effects, in determining accurately an identity of a person. Phenotype studies illustrate that eye color is the most successfully predictable phenotype essential in accurately identifying basic physical factors in an individual (Mangold E. et al. 2010). The challenges undertaken in applying DNA prediction into categorizing appearance traits and eye color is its expected variability in conceptual understanding of trait information. For example, people assign same eye color to various color categories and therefore look different from others using

an eye color provided by DNA predictions. In order to minimize this problem, studies investigated about the genetic basis of variation in eye color utilizing SNP data analysis (Mangold E. et al. 2010).

Prediction for hair color can also be illustrated by using SNPs and more precisely for red hair (Mangold E. et al. 2010). A recent systemic study investigated 46 SNPs from 13 genes of physical characteristics of various hair colors and discovered models for differentiating between red and blond-red, and blond and dark blond. The lowest prediction accuracy for blond hair can be due to the fact that over the years this color undergoes under extensive changes and thus depicts high variations (Mangold E. et al. 2010). Previous studies in forensic sciences also reported that three SNPs are responsible to express 76 % of total variability in hair melanin (Mangold E. et al. 2010).

Two of the SNPs are believed to be highly specific to hair color while the third relates to reflect biogeographic ancestry rather than emphasizing on the hair color (Mangold E. et al. 2010). Another trait that expresses a physically visible characteristic can be useful in successfully predicting appearance of an individual specially age. Two DNA- based approaches for age predictions are based on mt DNA deletions and age-dependent telomere shrinkage have been suggested for forensic assistance but further research is needed to diversify their effects (Mangold E. et al. 2010). Genome wide studies, on age related features for analyzing DNA methylation pattern may provide extensive benefits for establishing suitable age-predictive biomarkers (Mangold E. et al. 2010). By targeting the Genome data that is relevant and informative can be obtained and is generally applicable. This is because of the fact that the specific information contained within the Genome is generally correlated with all the normal cells in the human body as all cells contain similar DNA sequences, same mutations and polymorphisms.

d) Genome Wide Association Studies Serves as a Tool to Understand the Complexity of Genomic Framework

With the embankment of the technologies in genetic sequencing and phenotyping, the execution of genome wide studies to evaluate relationships between phenotypical and genotypic variants can discover reliable inferences. Genome Wide Association Studies typically utilizes the high tech and thorough input platform to gather large quantity of valid data from millions of genetic variations (Manolio, 2008). The incorporation of Single Nucleotide Polymorphisms (SNPs) in genomic studies with larger DNA or RNA sequencing and variants from unknown individuals assist the scientists to interpret the genetic variability and polymorphism (Manolio, 2008). Genome Wide Association Studies have been quite helpful in

investigating the infliction of diseases, various types of lymphomas, carcinomas and tumors including chronic pathologies like Diabetes Mellitus and Insipidus (Gudmundsson, 2007).

The advancements of Genome Wide Studies in establishing etiologic, pathodiagnostic and physiochemical basis for the diseases has been diverse and its current use in forensic medicine is under study. Genome studies is not associated with objectives that are without any imperative challenges of which some are fatal in consequences as they are unable to replicate the findings of the initial studies and also are limited in availability of sample population (Chanock, 2007). This failure in replication has been referred to various reasons identified as higher potency of false positive results and lower power of true positive inferences which, causes poor replication in a follow up sampling (Chanock, 2007).

According to Tian (2008), the mixed population sampling without stratification yields the results for common ancestry but not the causation of the mutations. The problem of insufficient matching of replication samples with the initial population causes the depiction of risk variations that have not been found in the initial sampling. However, the false positive genetic variations in Genomic studies lead to significant disproportionate patterns, variability in allelic expressions and phenotypical differentiation between initial and replicated samples. The application of genomics into forensic investigations assists in finding genes unique to certain genotypic framework which was previously limited due to limitations in technology in genetic studies. Today, the Genome wide association studies help in useful data extraction from genetic information in predicting technical details like the heritability data found in complex traits (Monolio, 2009).

The complexities in the genetic architecture demand application of complex methods in analyzing data for explaining all causes of variations in complex ancestry (Monolio, 2009). The current advancements in GWAS are now increasingly formulating a tool that helps to elevate interest in understanding other types of structural variants that have not been considered for genome studies (Frazer, 2009). However, there has also been other reasons for which genomic studies has not yielded successes many thought they would; still a lot of effort is being put forward in finding better analytical methods in addressing problems associated with GWAS (Monolio, 2009).

e) Genetic Ancestry and Association Study Analysis

The aim and objectives for Genetic Ancestry approach was to adjust funding and utilized resources on designing a panel of Single Nucleotide Polymorphisms (SNPs) that magnifies the chances of observing common variations in a population sample (Need et al. 2009). The genomic studies has also been



carried within the European population for achieving better inferences in observing a common group of individuals for genomic variance in order to reduce the expenses and efforts for collecting samples and genotyping analysis (Rosenberg et al., 2010). Another reason for European population based studies was the availability of large and homogeneous sample for populations, such as in cohorts for Finland and Sardina derived population samples (Rosenberg et al., 2010). These cohorts are believed to contain extensive collaborations and the prior genetic and phenotypical information to help in analysis and interpretation of Genome Wide Association Studies (Rosenberg et al., 2010).

The genetic framework for the European derived genomes further helped in designing the first episode for GWA studies (Jakkula et. al. 2008). The comparative lower levels of sub structures within the population within European continent allowed the scientists to properly remodel the HapMap project for genomic determination (Conrad et. al. 2006). The implications for Single Nucleotide Polymorphisms (SNPs) into genomic association studies were initially designed only to observe the common variances among different populations within European territory using tag SNP approach (Gunderson et al., 2006). The relative higher level of similarity in genetic framework in European populations enable the tag SNPs approaches to be transferred to other regions within European geographical limits (Conrad et. al. 2006). The level of Linkage Disequilibrium (LD) has been extended over patterns in European populations, thus it would have been convenient to cover the genome by using fewer tag for Single Nucleotide Polymorphisms (SNPs) (Conrad et. al. 2006).

The implementation of filtering in Multifactor Dimensionality Reduction technique is still at a preliminary stage of evaluation, there is still more improvements need to be implied in MDR's utility to act as a filter for genome Wide Association Studies. Future aims to solve problems that have not been addressed in this study for reason attributable to the limitations of the research grounds and resources, for example lack of induction of significant testing of models before applying their unique characteristics into genomic studies. An analysis of more diversified approach is needed to better determine errors in classification for thresholds for more acceptable and precise effect sizes that can easily be tested in actual datasets.

f) Structured Association and Genetic markers in GWAS

The technique of Structured Association works on the principle of Hardy and Weinberg's Equilibrium within populations with linkages to genetic markers that are also responsible for establishing equilibrium to detect and correct stratification in a homogenous sub

group cluster based on genotypic characteristics. Test for detecting familial or hereditary pathological traits in the generations can be achieved by studying association of each genetic marker to disease expression phenotype (Alexander, 2009).

The current limitations of methods of optimization of power to detect associations at dual stages of analysis in which the selected models in the first stage can be further tested for main effects (Wang, 2006; Kwak, 2009). It can also be beneficial to incorporate biological information as a component of genomic strategy as it is predicted in current genomic association studies that the results from these analysis can be proved to be true for biological experimentations. As study by Moskvina, 2011 shows that the study of genetic association between subjects improves the chances of finding true associations among products with unrelated functionality.

Further, the same research revealed that filters like MDR also suffers from limitations from previous methodologies such as increased chances of false positive associations. It is therefore, essential to explore the in depth understanding of how unspecified genetic linkages are responsible for depicting interactions in genomics and phenotype analysis. In the future, the application of filter approach in real datasets will be needed to comprehend with important points to be considered. Firstly, in the simulation studies the correct number of SNPs needs to be identified whether mutations are a result of single locus effect or is due to interactions. It is desired for this study to serve as an initiating point for testing for single locus as well as interactional models.

In the coming years, the computational advancements will take place, high order interactions can be made feasible. Secondly, the implication of 'Post Hoc' analysis needs to be involved to study and understand the actual models. Post Hoc analysis can be applied to investigate the dual loci models through the involvement of MDR to understand the interactive effects. A higher rank of a locus does not necessarily signifies an interactive effect and the development of state of art techniques will help in the future to assist in detecting the underlying abnormalities of complicated genetic models as it can serve as an active research area in molecular genetics (Hu, 2010; Kooperberg, 2008).

The software named 'Structure' was formerly developed which works by considering structured approach to detect associations. Since, this structured process provides better correctional opportunities for removing stratifications, but however, the computational intensity for this technique allows the detection of partial cluster membership, hence limiting its usefulness for incorporating it into Genome Wide Association Studies. Another new package for software that determines the stratifications is 'Admixture' which is also based on

clustering cryptic relationship in family structures (Alexander, 2009).

g) The Use of Principal Component Analytical Method in Genome Association Studies

The method of Principal Components Analysis (PCA) has been in use to detect and control stratification in the population data and also been utilized in the form of multidimensional approaches to identify a consistent axis of deviation of data for genetic expression. In unknown, forensic samples, the genotype and phenotype are later adjusted by variables attributed to pedigree of ancestry along each of the base axis, forming sets for matched cases and their control samples (Price, 2006). The application of genomic association studies is then made possible by calculating the adjusted phenotypes and genotypes.

According to Price, (2006) 'Eigenstrat' is an another software package has been implemented in genomic studies to execute this method and is shown to be higher in power at detecting casual genetic markers, better at handling highly differing Single Nucleotide polymorphisms (SNPs) than is evident in genomic control (Price, 2006). This software induction also has enabled the effectiveness in tracing computational datasets as compare to Structure technique in handling large sample populations (Price, 2006).

In addition to the above mentioned advantages, Eigenstrat has been able to draw attention towards the impact of differential biasness which may illustrate quality control possible. It needs also be noted that the top two phenotype and genotype adjustment factors may not necessarily due to structure for population but also depends on familial relations and linkage disequilibrium (Tian, 2008). The application of Principal Components Analysis can detect familial structure effectively but on the other hand it is unable to identify correctional models that are individuals with defective phenotypes.

h) The Application of Mix Methodology Models in GWA Studies

These Mixed Methods can be implied within Genomic association studies and can model various stratification within the population, that is it studies the effects of structure of a sample population, structure of familial predisposition and cryptic relations between case and control sample size (Visscher, 2008). The primary principles on which these methods are based are the model phenotypes that uses the combination of fixed effects like candidate Single Nucleotide Polymorphisms, sex, biological age of the subject; and also requires input of data from random effects for example, phenotypical variants in matrices (Kang, 2010). When considering the implementation of these techniques into Genome Wide Association Studies, there are still queries as to whether structure for the

population sampling should be modeled as a fixed or random entity (Zhang, 2010).

While every of these mixed methods can sufficiently alter and detect stratifications in the genome, it has been assumed that stratification modeling as taken a random effect can leads to successful discovery of spurious associations specifically for unique SNPs (Price, 2010), as these all are newly introduced methods, more assessment studies need to be done on the best approach for modeling of stratification. When stratification modeling is termed as fixed effect it requires additional induction of previous Principal Components Analysis as a reservoir for accounting stratification of populations to investigate genetic ancestry of each person in the sample (Zhang, 2010).

III. CHAPTER 3: METHODOLOGY

The main objective of the literature review is to draw conclusions for Genomics and its relevance in applying techniques in forensic investigations. This chapter will highlight the definition of literature review and its significance as a method for this study. Then, it will outline the way to carry out this study including the searching strategy process, limitations, and the qualitative design employed in this study. Finally, it will conclude with ethical issues along with the inclusion and exclusion criteria.

a) Literature Review

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). This systemic process was defined by the researcher as utilizing the definite methods for cracking the problem and answering to the research question (Parahoo 2006). Parahoo suggest and argues that the findings of research were not the problem solutions however, the study was more likely offering the current information which assist in the decision making (Parahoo, 2006). Therefore, the eventual the purpose of the study is to expand, develop and refine the knowledge of the body (Parahoo 2006).

b) Qualitative Design

The main argument for this particular research study is to carry out a review to examine the molecular genetics and the application of genome and DNA-amplification into forensic investigations. To conduct a literature review a qualitative research design is the most suitable research design. The qualitative research design is the most appropriate research design to



examine the human behavior and it assists to legalize and authenticate the data which is selectively collected from the secondary sources. It helps in refining the research and additionally it adds a primary hand worth to it. For many of the researchers the secondary data is crucial in the health care and medicine field as it suggest the previous researches. Creswell (2009), stated that a qualitative research comprise of using exclusive steps of strategies and analysis of inquiry with researchers interpreting what they hear, see, and understand. The method used in this study is qualitative. As compared to quantitative research, Qualitative research is more subjective, and it is based on unlikely methods of gathering information. This research is more or less based on the literature review and the conclusions are drawn on the basis of actual resources (mainly primary researches).

c) Critical Analysis

The critical analysis is central process of any research study. It involves critical thinking which applies logical and rational thinking while deconstructing the text. It is a complex, intellectual activity involving analysis and critical comment on the material formerly gathered. Browne and Keeley (2001) defined the critical thinking as a set of awareness which interrelates with the research question under study which is expected to be critically analyzed. It is an ability to answer and ask the critical question at a specific time and it desires to use the critical questions actively). Consequently, it is not just a descriptive list of the set of accessible summary and material. Without a critical appraisal and systemic literature any academic study cannot be applied to a methodology or in any way put in to the knowledge (Hart, 2005). Critical appraisal of the literature also offers a room for the comparison of the results of diverse researches.

d) Search Strategy

For research evaluation on the molecular DNA and forensics, the inclusive review of most of the studies available was important. The databases used and accessed for this purpose included: ProQuest, PubMed, Cochrane Library, Science Direct and CINAHL. For the data bases searches following keywords were utilized including Molecular genetics, Genome-wide studies, Polymerase Chain Reaction, DNA Phenotyping, DNA-amplification and sources for DNA-extraction. The bibliographic references of studies selected for the literature review were also searched from these databases. All studies meeting up with the explicit inclusion & exclusion criteria for each literature review section were retained at the end of the search.

e) Inclusion criteria

This search strategy was based on evidence available within a range of past ten years and was basically primary researches. The search of literature

only found out the material published in English and for this reason it created no issues to reject any possible study for the reason of the translating complexities of the papers. This search of literature found researches including the literature reviews, scientific documents as well as the prospective studies fitting the remit of the search strategy.

f) Exclusion Criteria

Exclusion criteria included the articles not published as full manuscripts or not in the peer review literature. The studies including controversial ethical or legislative materials are not included. The studies which were conference papers or studies in progress, or government reports will be excluded. The searches will be restricted to retrieve the literature available in English.

g) Limitation

In this review there was a likelihood of publication bias. By rejecting those studies with negative outcomes and were unpublished it was probable to overrate the effects of techniques used, nevertheless, for the published literature the comprehensive search for potentially appropriate articles was undertaken by means of a strategy for systemic review for the purpose of avoiding bias. It was preceded by the contacts with the first and corresponding authors. As the nature of the study was provided (for instance it was difficult to fund, it was complex and expensive), as these diminutive negative trials were not likely and they were expected to manipulate the consequences.

h) Ethical Considerations

Most ethical issues in numerous research studies falls within the following criteria: protection from informed consent, honesty, harm, and right to privacy. The most thoughtful and prominent moral trouble that most authors cannot avoid is the utilization of knowledge that was produced by some other author while conducting a same inquiry. No falsification will be occurring while citing the literature. This study lies on primarily on the original and primary sources as it is known that the unethical, fraudulent and dishonest researchers can circumvent the scientific method. It is important to properly cite the person whose work is being used. This is because the person who has conducted an actual research may have served a lot of effort and time to extract the outcomes of the study and hence it is one's responsibility to give credit legally to the individual who has conducted that particular work.

IV. CHAPTER 4: ANALYSIS AND DISCUSSION

This section will include the thematic analysis and critique of the selected articles. This chapter will also discuss the selected researches in detail and their findings, based on the findings of the reviewed literature.

a) Critical Analysis

In 1999, Pritchard and Rosenberg proposed a technique which was based on the concept of differences in the genetic framework between cases and their controls that could be tested. The variations in the genetic details of cases and controls can be measured by utilizing markers which are unrelated with the mutations detected (Weir, 2006). This method has been successful in evaluating the specific structural characteristics in the population; however, it is unable to correct the detected abnormalities in the provided data information about genetic mutations. In the same year, Devlin and Roeder presented Genomic Control method which was also based on the concept of biological markers for genetic detections (Alexander, 2009). Genomic Control also detects significant stratification but effectively compensates for it to be corrected. Their technique was based on the assumption of population structure with respect to case and control data that can be alter by implying multiplicative factorization in proportion to structure of population in unidentified genetic markers.

The inflation factor can later be incorporated into analysis for detecting associations and for correcting stratifications. One benefit for this method is that it can easily be implemented for analyzing the various categories for DNA data information and sequestering. Further, it also allows the implication of more accuracy in results as the number of genetic markers used to calculate the inflation factor, which makes it an efficacious technique to be followed in Genome Wide Association Studies (Weir, 2006). But on the downhill, it cannot on consistent basis detect and correct for relations with the family structure when they are responsible for causing stratification and can lead to corresponding loss of power when markers are strictly differing across the ancestry of subsequent population framework (Weir, 2006).

The serious issue in Genomic Association studies is studying the influence of genomic ancestry on the design for Genome Wide Association Studies. The confounding effects of genetic ancestry in association studies play a significant effect in understanding the population based characteristics that differentiates one subset from the other regarding genotype and phenotype information (Need et al. 2009). Most of the Genome Wide Association Studies are been conducted on European derived populations (Need et al. 2009). In the initiation stages of GWAS, extensive researches were undertaken on Europeans mostly due to logistical and research design reasons. In logistical terms, the financial burden for the conduction of GWAS lead to many early studies to be conducted on same general populations with extensive variance in physical characteristics (Need et al. 2009).

b) Relevant Themes

Some of the common themes extracted from the studies above are described as follows:

Theme No. 1: Epitasis and its Relevance with Genome Wide Association Studies

The first definition of epitasis was provided by William Bateson in 1909, and was described in terms of deviation from Mendelian inheritance. Currently, epitasis is commonly defined as interaction among genes at various loci where the impact of allele at one locus is inhibited by an allele on the other locus (Moore, 2005). The effects of epitasis can be favorable in producing no random phenotype and can be harmful in various ways. In the current era, epitasis is now becoming widely accepted as a tool in determining the relations between phenotype and genotype (Moore, 2005). Biologists and forensic experts study gene to gene interactions by incorporating epitasis as physical and biochemical reactions which occur between genes and regulatory networks. Biological component of epitasis can be expressed in several ways; by interactions between factors responsible for transcriptions and by enzymes involved in metabolic pathways (Moore, 2005). For example, blood borne or metabolic abnormalities like Maple Syrup Urine Disease in forensic sampling can aid to separate the individual profile from the majority of the population, thus identity of the unknown can be made possible. Similarly, in Sickle Cell Disease there is a biomolecular interactions between globulin proteins, hence helps in differentiating sick from healthy individuals (Moore, 2005).

c) Epidemiology and Significance of Epitasis

Epitasis has been increasingly significant factor in understanding the genotype for complex abnormality (Shao, 2008). These inferences depict that epitasis can be more prevalent not only in failed replications for Genome Wide Studies but also have main effects in detecting heritability in samples with unique genetic characteristics (Greene, 2009). It has also been shown that simple Mendelian traits like identification of metabolic disorders in body secretions samples, Cystic Fibrosis in blood samples etc. can be made easy to detect when interactions between other genes and the mutant variations that serve to produce phenotypes for a particular genetic expression are assessed.

d) Challenges in Identifying Epitasis

The correct identification of a particular genotype in varying phenotypical expressions can be convoluted that can be one of the reasons epitasis can be challenging in giving success. This is mostly applicable on human subjects which limit the benefits of methods such as genetic manipulation on organisms that serves as models for humans, hence making the execution of techniques even more difficult. In order to overcome this problem, characterization of epitasis on

genome wide scale allows numerous biological, computational as well as statistical challenges not only in analyzing genomic data but also to prove functional validity of such inferences. This method presents even more challenges when human populations are assessed as researchers can not benefit from all tools that could be tested on model organisms (Lewontin, 2006).

The conventional methods for applying association theory comprised of using linear models, while these models work better in finding main effects, but use to fall short in finding interactive effects (Lewontin, 2006). The detailed and diverse data framework that needs to be analysed also presents analytical challenge and demand the development of better statistical techniques to cater non-handle components for these interactions which are influenced on by environmental factors. This technique requires the filtration and prioritization of biological relevance along with maintaining computational efficacy in evaluating genetic variance and their interactions with genetic environment. Several methods have been developed in order to study the epitasis with regards to Genome association studies, including logistic regression methods, penalized methods and data mining approaches such as Random Forests, Recursive Elimination of Feature-F, Grammatical Evolution Neural Networks, Multifactor Dimensionality Reduction and Combinational Partitioning (Motsinger-Reif, 2008; Wang, 2011, Moore, 2007).

e) *Logistic Regression Method in Determining Epitasis*

Regression based techniques have been deployed for analyzing epitasis interactions in genomic association studies. They use the statistical approaches to determine the relationships between linear values for predictor variables for example, genetic variants and the possible phenotypical expression. Logistic regression is also been in use when the outcome for the population is binary in nature that is either case or control. This method can also be applied for variable selections for example, in step wise regression, where in variables which elicit main effects are selected first followed by interactive factors that lie between them. The utilization of regression method, however, eliminates the possibility of finding interactions between variations with no profound effects. Logistic regression also has limitations when data are greatly multidimensional as is common when information is analyzed for association studies thus, can lead to higher rates for false positive results. The availability of software packages that help in performing statistical correlation for logistic regression on genomic population samples, one of such tools is named as Genome Analysis Tool (PLINK), which provides the option of logistic regression in association analysis (Purcell, 2007).

f) *Multifactor Dimensionality Reduction (MDR)*

It is a non-parametric estimation of model free genetic model to detect interactions in the absence of significant effects and has proved itself as an impressive technique to identify statistical differentiations in assessing interactions in GWAS (He, 2009). This technique comprised of incorporating the data into divisions according to total number of samples like 'N' across validity intervals for testing in order to avoid model over fitting such that the final result can predict all of the total data information. In the subsequent step of the method, the list of loci combinations is placed in tabular k-dimensions that contain all possible n-loci combinations (Velez, 2007). The ratio with the number of cases to control sampling for each genetic susceptibility is then calculated and compare with the existing baseline measurements. The entities below the threshold are termed as low risk and above are labeled as high risk. This process efficiently reduces the highly diverse information into two categories high and low risk factors.

The balance between unequal number of cases to control sampling and clarification accuracy needs to be calculated. This accuracy prediction gives a value for mutation risk in the testing sample. This method is repeated for all possible loci combinations and for every cross validation interval. There have been several amendments to Multifactor Dimensional Method including Log linear Based, Odd ratio Based, and Generalized Linear Model (Lou, 2007; Chung, 2007; Lee, 2007). All MDR sub-extensions have the ability to reduce highly diverse data input into high and low risk without illustrating any assumptions about the genetic framework. It serves as a powerful tool in identifying genotypic errors and data that has been gone missing (Richie, 2003). Conversely, MDR is limited in execution of heterogeneity as it losses its ability to detect best possible differences.

g) *Random Forests and Evaporative Cooling Methods in GWAS*

Random Forests and Evaporative Cooling Techniques are being used as learning machines that utilizes combinations of recursions, iterative logarithms to select subjects with maximum potentials for interactions. According to Breiman (2001), Random Forests Method allows the construction of classification tree to detect specific patterns in the data by using a bootstrap sample with variants at the tree nodes having being taken as randomly from the initial information. This tree is built in a fashion of node by node formation of genetic variations from the entire data and is sampled with replacement from the total sample size. The individuals are classified on the basis of size of forests of trees that have been included. Errors in predictability are later calculated from the persons that were not

initially selected for the building of the tree (Moore, 2007).

An approach that uses the calculation for finding the distance that lies between subjects based on the variability of similarity in genetic information despite the differences in phenotypes by applying Genome wide association studies. On the other hand, Evaporation Cooling technique is featured with the selection algorithm and combines them with thermodynamic principles incorporated in cooling mechanism of atoms. The main aim of this technique is to increase the density by evaporation of variants within the featured space, which contains the elements with highest potential of interaction (Breiman, 2001). Due to the fact that large quantity of variables are involved in genome wide studies and thus large amount of interactions need to be probed during the investigation of epitasis in order to find statistically essential interactions in filtering the inferences.

The concept of non-parametric and genetic data free approach, to detect gene to gene and gene to environment interactions with additional benefits of detecting higher interactions makes Multifactor approach successful for analysis of data. Further, this dissertation will analyze Genome Wide Association Studies' data and utilize it as a tool for detecting and exploring in depth explanations for problems associated with GWAS in accordance with epitasis (Ritchie, 2005). The minimum number of epitasis associations that can be investigated in Genomic Association Studies is tested for all possible paired interactions between genes and variants. For such types of studies, statistically essential information can be found that is not specific to simple interactions, then the implications for additional methods which can filter the inferences and select what is the most useful and helpful results.

MDR algorithm can also accomplish the testing of all interactive factors in a database and not only it selects single best outcome based on prediction accuracy but its unique framework also allows for retaining all the models for future evaluation since it goes for extensive search. MDR also follows the filtering technique that involves population stratification, a confounding source for Genome association studies which identifies this problem and to stratify the data into proper number of sub populations before analyzing it with Multifactor Dimensional Reduction technique (Breiman, 2001). The sub domains comprised of racial or ethnic lineages through which population clustering takes place. This technique resulted in separately analyzing each population and reduces the important sample size for the entire study.

h) Theme No. 2: Population Stratification with Respect to Genome Wide Association Studies

Population stratification can be defined as the differences in allele frequencies between controls and

cases that are related to commonalities in genetic ancestry but are unrelated to mutations in a heterogeneous population and serves as a confounder for Genome Wide Association Studies. These variations in allelic expressions between different populations like for example, geographical migratory patterns, genetic mutations, mating behaviors, drift and selections for sub-populations in the sample can be responsible for creating differences in the frequencies for the alleles over a period of time (Freedman, 2004).

Stratification acts like a tool for confounding subgroups for various populations within the sample that have different occurrences for a particular trait. These differences in the prevalence for the unique characteristics in a sub group of a population for most effected individuals can be studied coupled with a genetic variant of various allele frequencies that are over represented in association studies. When samples with diverse ethnic data and variations are analyzed for stratification, this can lead to depiction of false positive association. This population stratification can also yield results for samples that possess distant relations but similar ethnic backgrounds with no familial affiliations (Freedman, 2004). However, population stratification in some cases is the reason for failed attempts for replications of previous association studies as higher level of false positive inferences are found in these studies.

It is due to the fact that some of these association studies contains subjects from diverse heterogeneity in terms of genetic frameworks while the others contain information that are only true for a specific sub group of a entire population sample (Freedman, 2004). Therefore, population stratification may only minimizes the confounding effects of diversity in genetic determination in which casual genes differ in populations, along with elevated power for searching associations which differ among various population subgroups (Freedman, 2004). There have several been association studies which signify on characterizing the extend of stratification problems and also the impact that it can create on final association analysis (Helgason, 2005, Freedman, 2004).

Conversely, the genetic attribution can be the presence of occurrences within the population with genuine ancestry that contains differences in genetic encoding (Freedman, 2004). The problem of stratification is also enhanced with the increase in the sample size of the population (Marchini, 2004). The prevalence of Single Nucleotide Polymorphism (SNP) in a population, for example in European Americans, Afro-Americans or Asians is quantified and its impacts are studied in association tests. These results illustrate that the constant incidence of certain abnormalities or pathologies across populations can be made possible through stratifications analysis. The problem of not achieving proper stratification of population samples is



minimized when homogenous samples with small ratios for intermingled data are observed (Helgason, 2005). According to Helgason (2005) the mild concentrations of stratification can be a significant confounding factor in genomic association studies if not adequately measured and subsequently corrected.

One of the methods to remove errors from final results of Genome Wide Association Studies is the nature of study design adopted; the utilization of adequately synchronized control and case samples will eliminate variations in genetic backgrounds between the two groups under addressed. This prevention of stratification in Genome Wide Association Studies, works by assuming SNPs that are not directly part of the candidate genes there by in saves association signals that can be removed through the effect of stratification process. However, this matching criterion is not always successful in eliminating all forms of stratifications that are part of the initial data of the Genomic Association as several techniques have been developed to identify and correct the discrepancies in stratification of population; some of them are mentioned below.

i) *Transmission Disequilibrium Test (TDT) for Mendelian Law in GWA Studies*

The induction of designs for family relatedness like when relatives of the cases act as controls, have also been proposed as a means of minimizing the effects of stratification of population and this test is one of those effective methods to be employed. The parents of the affected individuals act as controls and this principle is based on Mendelian law that each of the parents transfers a single allele to its child. The knowledge of which of the alleles are transferred is afterwards used to form a placebo case control sample where the cases are defective alleles transferred from the parents to the offspring's; and untransmitted alleles are termed as the controls. This technique of case to control matching minimizes the impacts of stratifications by deleting bias from differences in allele frequency.

Whereas this method is able to eliminate the problem of population stratification, since the familial information is reduced in biasness the cost for this collection of data process is much higher as compared to other techniques and the design itself is not applicable specifically in cases when the mutation under study are late in expression. This method has been found to be less efficient as it needs a genotype of three persons to obtain to form a single case control match. This method also requires the parents of the subject to be heterozygous in order to know the information of allele that is passed from them to their offspring. The irrelevant data information that is not useful for the process of analysis that is some unconcerned genotypic information about distant relatives, can also result in loss of power for the reliability of the method.

j) *Theme No. 3: Genomic Association Data and its Discloser for Public Access, Barrier to Human Privacy and Protection*

Church et al. (2009) demonstrated that the given out of information pertaining to Genome Wide Genotypic Data from a person for the purpose of utilizing it for forensic sampling in Genome Wide Association Studies. This research further evaluates the actions taken by the authorities in USA to inhibit public access to Genome Wide Data dispersion but however, limitations have been observed to restrict sensitive information and also the use of genotype frequency data from each of the previous study which have been published using funding from public services agencies like National Institute of Health (NIH) and Wellcome Trust. Reactions to this implication in the public sector has been not so encouraging when authorities consider it too late and too little that has been done in maintaining privacy for individuals. However, the response from responsible persons in genetic field of experimentation models declared the breaching of public data as a high level beaurocratic response to a minimum risk which can unnecessarily inhibit the futuristic scientific research.

Other scientific concerns were also been highlighted concerning to situations when an individual's identity is accurately determined by applying Genome Wide Association Studies datasets. Several other researchers have also signified that misuse of an identification of an individual along with familial and medical history records and if genetics will not held these sensitive data information it will backfire to their own capabilities to carry further sequence of research network. Church et al. (2010) notified that appropriateness of measures in restricting access to information is likely to exclude the scientists who are specialist in handling sophisticated data. The active researchers in the field of genomic wide studies further argue that consent for complete disclosure for releasing genomic information should be taken from the respondents rather than making this procedure difficult to access for promises for anonymity.

In similar context, Martin and Bobrow compared and contrasted the related potential risks and advantages in execution, hence proposed four stepped representation; a). For current scenarios limited access to personal genetic information need to be implemented; b). Declaration of any activity that comes under the category of illegality need to be banned or be termed as malicious practice; c). Increase in the level of knowledge for the general public to identify the nature of their personal information that can be misused for purposes harmful for them in physical, social or financial frameworks; d). Encouragement for appropriate recognition need to be stimulated in order to understand the professional relationship between the genetic researchers and the study participants.

k) Population Genomics and Access to Genomic Datasets

Homer, Szelinger, Redman, Duggan, Tembe, et al. (2008) illustrated that Single Nucleotide Polymorphisms data from respondents inducted in Genome Association Studies can depict that individuals' genetic mixture consists of up to 1000 respondents whose DNA profiling have been disclosed for research proposes. As the mentioned case implies the basic statistical theory, the condition illustrated that this needs to elevate the frequency of subsequent discussions that can minimize the privacy concerns and trigger anticipating scientific possibilities (Human Genome Project Information, 2003).

Homer further entails that scientists who are continually using the sensitive data for research purposes, need to adhere to a code of conduct which is internationally known and accepted to provide the proof of research credibility as a bona fide researcher (Human Genome Project Information, 2003). A researcher then be awarded a permit and can be placed on a registry of users who are have refined access to genomic information in data bases (Braun et al. 2009). This can avoid the need to repeatedly applying to proof bona fide status to various regulatory bodies. Any misuse or misconduct by the researcher will be termed as denied for access to genomic data bases according to the ethical code of conduct. IN other terms, interim models should also be adopted which are consistent with the principles of permit mechanism (Braun et al. 2009).

The determinants of a bona fide researcher may be internet oriented but also require previous affiliation to an authentic institution (Braun et al. 2009). The permit also needs to link to a known and registered individual by using a universally accepted 'Researcher ID System' such as being promoted by GEN2-PHEN project (Human Genome Project Information, 2003). The individuals who are responsible for handling excessive secondary information need to be expected to follow the same standards and practices as primary data providers when disclosing information. To implement this work, global strategies like that of Burmuda Principles (Human Genome Project Information, 2003) is required to be followed. In order to get the researchers and their administrators sign a legal document, and if they do so it does not guarantee compliance. For example, during a forensic investigations access to high security data need psychosocial assessments of relatives, and personality evaluation that is highly personal and sensitive information. However, sometimes regulatory personnel possess some classified data outside the security zone.

Human genomics are observed to be extremely useful and is also considered to be capable of analyzing numerous numbers of angles. Another perspective has been illustrated in a Consortium (2003) over Celera in the map of human genome project (Wellcome Trust

Sanger Centre Press Releases, 2003). The individuals who have decided to display their genomic data and personal biological information to be freely accessible on the web have played a great part in introducing sharing of information with mutual consents. This approach of data sharing is termed as a norm in genomics research (Wellcome Trust Sanger Centre Press Releases, 2003) and is also a prerequisite for suitable funding for the project. However, genomic research is not limited to the research community.

There are now private institutions that are liable to collect sequence data and sensitive information (Jupp 2001). The real challenge for genomic studies is not the availability of the information within the realm of research community, the availability of the information about genomic sequence to people outside the research community, as these are the concerns safeguarding the rights, over sighting the confidentiality and evaluating the professional codes for conduct (Huang et al. 2009). There has been many factors identified that serve as risks for privacy for studying genomic sequence data but also breech to information can also takes place through availability of information on other resources (Huang et al. 2009).

The possibility of the datasets to get combined with relevant sources increases the risk for breeching of sensitive information (Greenbaum et al. 2008). For example, in a study by Gitschier it has been detailed that literary compare and contrast of subsequent surnames administered in genealogical data bases and information regarding Y chromosomes from HapMap project, genetic information about the concerned individuals can be detected by increased accuracy (Jupp 2001). Furthermore, Nyholt and colleagues depicted the difficulty of securing the sensitive genomic information by using Linkage Disequilibrium and data about polymorphisms to access information that has not been previously released (Nyholt et al. 2008). There information can be used by legal and health insurances in order to take decisions about the individuals (Jupp 2001).

The accessibility to web based approaches and face book utilization to extract birth records of individuals while these evolving data processing technologies allow effective collection of data records (Greenbaum et al. 2008). In order to raise these issues Greenbaum et al. also recently suggested that anonymity has already be a thing of the past due to the presence of infer data and the quantity, nature and variety of data that has been freely available (Greenbaum et al. 2008). The ethical consideration for the collected data has led to balance the privacy of the respondents using the principles of anonymity and informed consent. It is termed as unrealistic to promise respondents of complete confidentiality when genomic data is analyzed and assessed (Taylor 2008). The evident increment in the number of data sources that



are available in commercial and public sectors together with the ease in evaluating the genetic information suggests the content of data being released, has altered over couple of years (Taylor 2008).

If the researchers are not inclined to take better confidentiality measures towards the support of respondents and also to the public indulged into genomic research then it can have damaging consequences for futuristic scientific researches. In genuine terms ethical questions that arise from Genome Wide Association Studies or cohort design of research are actually derived mainly from funders or insurances to encourage far and wide sharing of basic research data and from the power of using internet tools for sharing of data (Greenbaum et al. 2008).

One contrasting likelihood, is the availability of liable genomic data to many intelligent minds which maximizes the chances of dispensing results of the researches to societal and community based groups but on the same time increases the breeching opportunities of privacy for research respondents. One such solution entails to the fact that to make only aggregated genetic data available publicly accessible on internet but with restrictions to accessing individuals' genotypic data (Greenbaum et al. 2008).

The presence of a person's DNA can be detected even if it is in minor concentrations in a mixture, and it is therefore possible that under very unique circumstances an irrelevant individual can deduce that particular individual is a part of a group of patients who has been suffering from a particular disease state, thus causing the data to be de-identified and the privacy of the person thus be invaded. If the researcher abandon large scale genomic studies and minimize the pressure for data sharing methods, it can be disproportionate response to the level of threat as is observed. The need to get consent from the respondents is a possible solution to the problem but the detailed, obfuscate and technical documents can only make respondents confused and can only be effective for shifting the legal burden that can be a part of the ethical consideration during the research process.

Braun et al. (2009), in his study depicted that is it possible for the individual with a genotype which is known and he belongs to a sample of respondents for whom only allele frequencies are identified? These allele frequencies can be identified through reference sampling and this query can be de phrased as; does the member of this group of individuals in a particular sample belong to a reference or test sample? Homer et al. discussed the wide subject of genomic wide association studies in a forensic context and stated that there has always an interest in acknowledging that a specific person belongs to a particular population and is a contributor to a mixed sample of genetic information like DNA from more than one individual (Braun et al. 2009). The implication of genome wide association

studies for determining allele frequencies but not for individual genotypes, are being made publicly accessible and available; also the interest of the individuals in knowing whether a specific person is a part of a genomic study should be restricted from public access (Braun et al. 2009).

V. CHAPTER 5: CONCLUSION

Over the span of last 10 years, the fields of genomics have under taken interesting and dramatic shifts in how to comprehend forensic detailing with respect to investigations about unknown pedigree, unidentified complex diseases and ethnogeographic ancestry of subjects (Scott, 2007). The wealth of information which can be extracted from immense map of human genome along with advancements in genotyping and related technologies has presented many opportunities for the scientists to test assumptions about the complex genomics for human. This has led a new era of genetic investigation like Genomic Association studies that incorporates to solve analytical questions about heritage and population based studies (Scott, 2007).

The genomic association approaches have enabled researchers to study in detail the effectiveness of candidate genes analysis which largely depends on previous information about the unique characteristics in a familial pedigree that have been based on inferences from previous assumptions (Scott, 2007). The Genome Wide Association Studies allows an effective scanning of the entire genomic data base at a single time, without the need of assumptions about genetic details for the missing encoding information. The implication of candidate analysis for gene expression has also added profound advantages of uncovering novel forensics.

In this study, the analysis for Genomics yield conclusive evidences for cases when common traits have been detected in genomic samples like detection of familial tendency of pathologies, mutation at fixed loci and phenotypical similarities (Fernando, 2008). For example, some of the phenotypic appearances are specific to an ethnic group, for example the hair color, skin color and morphological features. It has now been suggested that the prevalence of the minor allele frequencies have higher penetrance and may be responsible for familial diseases (Schork, 2009).

In 2005, Genome Wide Association Studies has evoked several other successful studies searching for answers for genetic variants related to complexed hereditary information (Scott, 2007; Hunter, 2007). However, other studies also illustrated that results cannot be replicated in other independent sampling and these scenarios demand deeper investigations in order to cater the replication failures. The popularity and the utilization of Genome Wide Association Studies have increased over the years, along with the challenges like

identification of stratifications in a population has been made feasible for forensic investigations.

Future implications for minimizing the failures in replicative studies pertaining to Genome Association Studies can lead to development of methods to define and evaluate the genomics and to prevent errors. The robust application of Genomics and associated studies can also have the confounding impacts as factors that can improve the reliability of the inferences extracted. On the other hand, the novelty of the technique has limitations to the amount of genetic variations detailed by the risk variants found to be related to the genetic factors under investigation.

The Genome Wide Association Studies has also suggested the limited approach of this method as it may not serve as a powerful tool to discover the complete genetic predisposition of the mutations unique to a familial trait, or even can predict a disease risk which has not been noted due to insufficient knowledge about genetic framework. This limitation can be attributed to the held assumption that the effects of main factors that act independently but chiefly contribute to the genotype for causation of mutations. However, this notion has now been challenged as more empirical studies have depicted that epistatic impact can occur despite of the presence of any main effect and thus, can essentially effect the phenotype, as has been proved solvable in Genome Wide Association Studies.

As concerning to epitasis in association studies, it sometimes becomes a burden on computational analysis for reasons because detecting and searching for epitasis will benefit the filtering techniques for prioritizing variants (Fernando, 2008). The utilization of next generation for sequencing data for genomic association studies also provides the challenge for implementing stratification techniques to the current datasets. Most of the Genome Wide Association Studies include a lot of rare variants as it signifies that the correction of the possessed stratification in the population samples are more important to be deleted (Fernando, 2008). There is an increased chance for the variants to be exclusive to ethnic, cultural and regional based variations, thus is responsible for causing an elevated number of stratifications.

It has also been a chance for these stratifications to deviate from the initial assumptions possessed by many currently in use methods for stratifications, as sequencing of the data is likely to be done on persons who have unique phenotype for the variants being studied, therefore depicting deviations from expected adaption of rare variants (Price, 2010). There are no available stratification techniques that can accept data for sequencing, hence if this has been possible then the genotyping from Single Nucleotide Polymorphisms associated with the rare variants as well that are scatter haphazardly across genome can be

adjusted for the confounding and stratification detection (Fernando, 2008).

Due to this realization about Genome Wide Association studies, 3 million or more variants are detected for interactive effects which presents as a computational challenge for GWAS analysis. These large number of variations examined for interactions also elevated the tendency for finding many essential combinations of risk variants, therefore genomic analysis methods need to be developed to filter and evaluate the most viable interactions. The influence of environmental factors on the variations in phenotype in the genetic analysis has been under much debate, for example the impact of toxins on human genome also accounts many contributions by GWAS (Thomas 2010).

The implications for statistical models for determining causal relationships between genetic variants and their biological interpretations can also limit success of functional validity in GWAS findings (Cordell, 2009). The predictions of genetics involving in complex phenotypical observations have limitations for appreciating single effects and thus wider possibility of gene to gene interaction (epitasis) is ignored. The use of technique of epitasis in genomic determination rests on the identification of variants that effect profoundly on final genetic expressions for alleles, gene to gene interactions and gene to environmental interactions, which are observed to significantly affect the nature of phenotype (Greene, 2009).

The genomic genotype sequencing based approach to discover variants in a sub group of a population require a defined location of a causal variant. This method subsequently allows the scientists and researchers to extract maximum advantage by reducing costs since the technique used for sequencing data require large sample size and thus are quiet expensive. New Genome processing and approaches are implemented into practice to study the interactive phenomenon between genes and environments in order to acknowledge the better understanding for biological mechanisms involve in unique characteristics for a sub population (Fernando, 2008).

Major study datasets have already contain genomic data that is available on the web and internet sources, these subsequent sites can be blocked to only gain access for the researchers on terms that the content of the data bases will be used appropriately and purposefully for research processing. If such obligations are made compulsory and are reinforced into genomic research field there would not be major obstacles for the utilization of the data and could be retained for indefinite time periods (Gill et al. 2009). Secondly, the declaration that the misuse of data to establish the identity of the individual is a legally punishable offense can also aid in maintaining confidentiality of respondents. To limit the deliberate invasion into data sets for breeching purposes is a fundamental principle for protection of

data but clear statements of punishments and the desire to reinforce these implications can be extremely helpful.

The induction of samples for population allele frequencies in genome wide association studies require the inductive process to be based on simulated and real data process to show original analysis. The susceptibility to Linkage Disequilibrium along with the markers for genetic determination rests on the identification of allele frequency between testing and referencing samples. With the elevating occurrence of the forensic DNA Profiling, that resulted from the determination of how the template amplifications and increasing number of samples for forensic investigations contain DNA from numerous contributors and the inferences drawn from such samples have progressed significantly (Gill et al. 2009).

As final point, Genome Wide Association Studies associated approaches are been incorporated into validation remains a standard, by which the acceptance of most of the hypothesis and techniques can be appraised. It will also be an important hallmark for the Genome Wide Association Studies to evaluate the same impacts in multiple populations for forensic determination in varying ancestries to extract a better outcome of population specific variants in order to depict effect on human identification in cases of mass disasters or for analyzing ancient samples for familial characteristic determination (Gill et al. 2009).

a) *The Road Ahead in Genomic Association Studies for Research and Practice*

Looking towards the future, it seems evident that the current enhancements in Genome Wide Association Studies is going to transform through changes for finding genetic susceptibilities that are responsible for causing characteristics unique to a familial trait (Bird, 2005). In 2010, Durbin provided a series of projects in the '1000 Genomic Project' that has provided an avenue for testing assumptions by utilizing sequencing techniques to identify rare variations in human genome. The use of sequential data for detecting rare genes for variant determination has also been observed possible through association analysis.

One of the barriers for effective execution of association studies in the samples containing higher amounts of heterogeneity that would be contaminated with variants in determining phenotype for each of the individual in the familial data (McClellan, 2010). Another feature for rare variants is their lower frequency of detections as the stratification techniques are not designed to consider this variation and is difficult to decide which stratification technique would be more beneficial. It is also thought that the implementation of new techniques is the need for current field of molecular genetics in order to identify rare variants for obtaining population based discreet data (Gill et al. 2009).

If individuals are inducted into distinct jurisdictions, then it can restrict the reach to their respective privacy by police, insurers, and state agencies and there would be a little risk of harm (Gill et al. 2009). This harm to a research participant is not the result of their participation into study process but from other activities, for example gain of access for data collection from hospital records and from private agencies keeping data sets for genomic studies, thus allows some genomic information which is related to identifying the basic phenotypical characteristics of the individual to become accessible to the data miner (Gill et al. 2009). Public discussion to alarm people and authorities to take legal actions can be made effective by placing genomic data on places that are secured rather than placing the information on potential places from where they are easily accessible. Further, individuals mostly do have confidential relationships with professionals like lawyers, bankers, doctors on whom they possess trust as they are bound by professional codes of conduct along with subsequent liable penalties (Fernando, 2008).

Researchers and the institutions that hire them to work for these agencies, probably require making their own statements in this regard as there need to be clear codes of conduct and punishments for breaching the records and information within their possession. Future clarifications for re identification of the individuals are mostly relevant in relation to the reference population, thus is hoped that future will entails greater sense of clarity and time to form a proportionate long term response (Gill et al. 2009).

According to Visscher and Hill, the need for further progress in genomic sensitivity for forensic genotyping means allelic drop is common and demand the utilization of sophisticated techniques to be induced in order to calculate the ratios. Secondly, during a forensic investigation, the scientists abandon the use of 13 to 20 microsatellite markers in favor of very large number of Single Nucleotide Polymorphisms (SNPs) as the sample under study contains a large data base for the offender profiling (Gill et al. 2009). In June 2009, there have been over more than million profiles available in US data bases in determining the forensic analyses for finding whether or not all the alleles are observed within the mixture of profiles and then calculating the ratios would be effective (Gill et al. 2009). The technology into genetics has been on the incline but at the same instance there lies a genuine fear of scientific abuse in medical and gene technology and a great potential of great harm to both research processes and health related implementations in identifying issues that have not been addressed sensitively (Gill et al. 2009). This has been termed essential to get the balance of anonymity protection and privacy restrictions, honest and uniform consent rights and implication for encouraging greater participations in the debate and

subjective education surrounding the issues (Gill et al. 2009).

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VII. PREFACE

The achievements in Human Genome Project and subsequent advancements observed in Genotyping techniques have led an influx of exciting new era of discovering human genome and genetics. These technologies have provided the scientists with a comprehensive data on human genomes as human genome is now capable to incite in depth and precise data information and allows access into processes that extract detailed sequences for genomics in order to analyze theoretical questions but also identifies the practically feasible genetic characteristics. According to GWAS, the inductive spurious associations between a

phenotype for the pathology and a variance subsequently assist in evaluating true relationships between genetic variables. Most of the Genome Wide Association Studies are designed in ways so as to include the single individual from the same population in order to minimize errors and biasness caused by population stratification. The phenotype traits which are useful tools for anthropometric measurements for forensic purposes include the color of eyes and hair, freckling, hair structure; elicit response to better tastes, urinary and creatinine excretion and metabolic rates. The discrepancies between sampling criteria and study populations and in matching the associations in identifying the individuals need to be kept under higher considerations while studying genomic association characteristics between populations.

VIII. METHODOLOGY

The methods for research for this particular study is to demonstrate a review of relevant literature to examine application of Genome Wide Association Studies in accordance with population based forensic investigations. To conduct a literature review a qualitative research design is the most suitable research design. It provides the rationale for assessing the human behavior and assists to legalize and authenticate the data which is selectively collected from the secondary sources. It is important to properly cite the person whose work is being used. This is because the person who has conducted an actual research may have served a lot of effort and time to extract the outcomes of the study and hence it is one's responsibility to give credit legally to the individual who has conducted that particular work. This search strategy was based on evidence available within a range of past ten years and was basically primary researches.

IX. ANALYSIS

The effects of epistasis can be favorable in producing no random phenotype and can be harmful in various ways. In the current era, epistasis is now becoming widely accepted as a tool in determining the relations between phenotype and genotype. It has also been shown that simple Mendelian traits like identification of metabolic disorders in body secretions samples can be made easy to detect when interactions between other genes and the mutant variations that serve to produce phenotypes for a particular genetic expression are assessed. The correct identification of a particular genotype in varying phenotypical expressions can be convoluted that can be one of the reasons epistasis can be challenging in giving success. Several methods have been developed in order to study the epistasis with regards to Genome association studies, including logistic regression methods, penalized methods and data mining approaches such as Random



Forests, Recursive Elimination of Feature-F, Grammatical Evolution Neural Networks, Multifactor Dimensionality Reduction and Combinational Partitioning. Stratification acts like a tool for confounding subgroups for various populations within the sample that have different occurrences for a particular trait. These differences in the prevalence for the unique characteristics in a sub group of a population for most effected individuals can be studied coupled with a genetic variant of various allele frequencies that are over represented in association studies. The accessibility to web based approaches and facebook utilization to extract birth records of individuals while these evolving data processing technologies allow effective collection of data records.

X. CONCLUSION

It has been a chance for the stratifications to deviate from the initial assumptions possessed by many currently in use methods for stratifications, as sequencing of the data is likely to be done on persons who have unique phenotype for the variants being studied, therefore depicting deviations from expected adaption of rare variants. The induction of samples for population allele frequencies in genome wide association studies require the inductive process to be based on simulated and real data process to show original analysis. Genome Wide Association Studies associated approaches are been incorporated into validation remains a standard, by which the acceptance of most of the hypothesis and techniques can be appraised.

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