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SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

GENETIC ANALYSIS

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Article Received on 05/08/2018 **Article Revised on 26/08/2018** **Article Accepted on 17/09/2018**

The human body is composed of diploid cells that carry identical DNA and haploid gametes (egg and sperm) that carry sex chromosome either X or Y. The human genome is composed of over 3 billion base pairs of information organized into 23 chromosomes. Only 1.5% of the entire genome is called coding region and is responsible for encoding and regulating protein synthesis. Other 75% of the entire genome is called non coding region and is contain extragenic DNA, about 50% of these DNA formulated repetitive DNA, which has a particular use in forensic analysis.

Each gene has a specific place on the chromosome and this place called Locus. During mutation,genes can be altered in many forms called Alleles which occur at the same locus on homologous chromosomes. Therefore, genotype in diploid cells composed of two allelic forms of the homologous chromosomes. Thus, these loci used as Molecular Markers.

A DNA profile is a forensic technique and is represent a small set of DNA variations that is highly probably different in all unrelated individuals, thereby being unique to individuals as that of fingerprints and it is commonly used in parentage testing and criminal investigation, to identify a person at a crime scene.

DNA profile that taken at the site of crime scene will match with DNA profiles of suspected person, if a match occurs is called 'inclusion' result; otherwise, failure match called 'exclusion' result. Then a comparison of the DNA profile is made to a population database, which is a collection of DNA profiles obtained from unrelated individuals of a particular ethnic group.

There was a variety of workable sources of DNA evidence including blood, semen, vaginal fluid, nasal secretions, and hair with roots. While buccal swabs are used to obtain DNA samples for comparison, at which a sterile swab is scraped along the inside of the cheek to collect epithelial cells for DNA fingerprint production.

Many Characters should be present in a genetic markers to be effectively used like: highly Polymorphism, co-dominancy (absence of intra-locus interaction), multi-allelic, neutral (the substitution of alleles at the marker locus should not change the phenotype of an individual), Stability, not influences by the environment, Wide dispersion through the genome, Simplicity of observation, Low cost, Reproducibility, Portability between species.

The First Generation of Molecular Markers produces in 1980s which Based on DNA-DNA hybridizations, such as Restriction Fragment Length Polymorphism (RFLP). While the Second Generation of Molecular Markers produces in 1990s, One Based on polymerase chain reaction (PCR): Using random primers such as Random Amplified Polymorphic DNA (RAPD) and Using specific primers such as Simple Sequence Repeat Length Polymorphism(SSR) and Sequence Tagged Sites (STS). Others Based on PCR and restriction cutting such as Amplified Fragment Length Polymorphism (AFLP) and Cleaved Amplified Polymorphic Sequences (CAPs).

Recently the Third Generation of Molecular Markers Based on DNA point mutations Single Nucleotide Polymorphism (SNP) and can be detected by Single Strand Conformational Polymorphism (SSCP), DNA chip, sequencing etc.

MOST COMMON TYPES OF GENETIC ANALYSIS

1-RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS) ANALYSIS

Restriction Fragment Length Polymorphisms (RFLPs) was the first technique used to analyze variable lengths of DNA fragments that produced after in vitro DNA digestion by restriction enzyme. This procedure will produce different DNA fragments due to the varying location of digestion (cutting) of this restriction endonuclease enzyme.

By using gel electrophoresis, the resulted DNA fragments separated into many bands and then transferred to a membrane using the Southern Blot

technique at which labeled probe used to hybridize specific sequences of DNA fragments and identify them. Many reasons make RFLP technique no longer used in forensic analysis such as, this technique need large amounts of DNA, destroyed samples may not analyze with accuracy.

2- POLYMERASE CHAIN REACTION (PCR)

The process that involve replication of DNA regions by using an enzyme DNA polymerase in a test tube which permit DNA fragments amplification to about 10,000 base pairs length is called Polymerase Chain Reaction (PCR).

PCR reaction needs many requirements to proceed such as oligonucleotide primers, which are complementary to the DNA target to be amplified so that two primers needed, one primer binds to one side of the target DNA while the other primer binds to the other side of this target, and the DNA sequences between the two primers will be amplified. Fluorescent tags usually added to the primers to visualize amplified DNA products when isolated, after PCR, by electrophoresis.

PCR errors that respective to primers may occur either due to the miss priming with products that formed from non-target sites or due to the formation of excessive primer dimers, which are by-products of PCR formed when one primer is annealed to another leading to primer extension.

DNA polymerase enzyme is another requirement of PCR reaction permits the copying of the DNA strand by adding deoxy ribonucleotides to the 3' end of the primers. Taq polymerase is a thermo stable enzyme extracted from Thermus aquaticus organism, which is capable of surviving in temperatures over 70 ^{0}C .

However, this enzyme does not own the proof reading ability, resulting in errors in amplification. The higher the amplification, the more such errors will occur. Other requirement involve a reaction buffer with magnesium chloride (MgCl) to obtain an ideal conditions for the functioning of the DNA polymerase enzyme, deoxyribonucleotides for the construction of the DNA molecule, and template DNA.

The PCR cycle involved three primary steps: denaturation, annealing and extension and can be repeated as necessary until the original target sequence has been amplified. Each PCR cycle takes only 5 minutes and the process is usually conducted in a small, plastic centrifuge tube with the temperature carefully controlled using a thermal cycler.

In fact, PCR is not suitable for analysis of longer chains of DNA, and so cannot be used in RFLP. Many compounds can restrain PCR reactions like substances that associated with the stages of extracting and purifying of the DNA such as proteinase K (which degrades the polymerase enzyme), ionic detergents, and gel loading dyes. Other substances that present in blood can also restrain PCR, such as haemoglobin and heparin.

Multiplex Polymerase Chain Reaction includes the amplification of abundant DNA sequences in a single reaction by using primers that produce non-overlapping allele sizes, permitting abundant regions of a sample to be tested concurrently.

3-SINGLE NUCLEOTIDE POLYMORPHISMS ANALYSIS

The forensic DNA analysis depended on testing of many types of polymorphisms which involved variation in sequences, length and repetition such as single nucleotide polymorphisms, minisatellites (variable number tandem repeats), microsatellites (short tandem repeats) and mitochondrial DNA.

Single Nucleotide Polymorphisms (SNPs) are the simplest and most common type of genetic variation, composing about 90% of genetic variation in humans. There are about 10 million SNPs in the human genome, with one found at every 100-300 base pair.

Each human has a unique number and location of these polymorphisms and these properties produces a distinctive band pattern when analyzed, therefore it can be used as biological markers. SNP markers are used in gene mapping, detection of mutation at molecular level, detection of disease causing genes and positional cloning of a mutant locus.

Many factors may restrict the usage of SNP markers such as bialleleic and less informative properties of SNP than SSR markers, needed more than three times SNP in preparing genetic maps than SSR markers, some SNP assay are expensive.

Additionally, SNP markers needed DNA sequencing techniques which is not commonly used in forensic field except in the analysis of mitochondrial DNA, to recognize specific base present in the SNP.

Because SNPs have lower mutation rate therefore they are highly fixed in a population and could be population-specific. Ethnic origin can be evaluated depending on the presence of rare SNPs or STRs linked to particular population groups but people with mixed ancestry may cause problem.

Forensic DNA phenotyping (FDP) or phenotypic profiling is DNA analysis identified the genetic phenotypic characteristics of individual such as skin color, hair color and eye color.

Phenotype SNPs that are related to the pigmentation represent a number of pigmentation genes that are associated with various human hair, skin and eye color phenotypes such as SNP typing assay which is developed

by Forensic Science Service and is tested mutations in the human melanocortin 1 receptor gene (MC1R) that is related to red hair.

4-MINISATELLITES OR VARIABLE NUMBER TANDEM REPEATS (VNTRS) ANALYSIS

At the exon domain of DNA, there are highly polymorphic sequences 20-100 base pairs in length,repeated 4-40 times at various points along the chromosome, called Variable Number Tandem Repeats (VNTRs).

As VNTRs inherited from both parents, therefore no one will have the same VNTRs as either of their parents. Because of variable number of these tandem repeats therefore they gave different length of the DNA fragments after cutting with restriction enzyme and then they produced different position after electrophoresis.

Although Minisatellite was the first type of polymorphism used in a criminal investigation (British murderer and rapist Colin Pitchfork),but it was no longer used in forensic analysis for many reasons such as high cost, takes longer time as VNTRs having a greater length than STRs.

5- MICROSATELLITES OR SHORT TANDEM REPEAT (STR) OR SIMPLE SEQUENCE REPEAT (SSR) ANALYSIS

Microsatellites are regions of the genome consisted of approximately 1-5 bases and repeated up to 17 times. STR markers were either be simple (identical length repeats), compound (two or more adjacent repeats) or complex (several different length repeats).

They are observed on 22 autosomal chromosomes as well as on both X and Y chromosomes, though those on the Y chromosome less vary due to lack of recombination. STRs are likely to be highly polymorphic, highly abundant, and randomly dispersed throughout most genomes, co-dominant, and locus specific.

The Federal Bureau of Investigation (FBI) in USA has chosen 13 specific STR loci to serve as the standard for CODIS, while forensic DNA profiling in UK select10 specific STR loci. Both numbers of STR markers have high degree of polymorphism, suffer less environmental degradation, and provide a high degree of error free data, making them of particular important in forensic DNA analysis.

The aim of using a specific core set of STR loci is to ensure that all forensic laboratories can establish uniform DNA databases and share valuable forensic information. The variability in STR region, non-coding region, is came from inaccuracy of DNA polymerase in copying these region and high mutation rate, resulting in high variation between different people.

STR loci are ideal for use in forensic science for a number of reasons. They represent discrete alleles that are distinguishable from one another, they show a great power of discrimination, only a small amount of sample is required due to the short length of STRs, PCR amplification is robust and multiple PCR can be used, and there are low levels of artifact formation during amplification.

6- MITOCHONDRIAL DNA (MTDNA) ANALYSIS

Each cell inside the human body has about 100 - 1000 mitochondria, each one containing mitochondrial genome which has a circular shape DNA molecule with 16,569 base pairs. Mitochondrial DNA is only maternally inherited that mean it passed from mother to her son and daughter, but only passed on by daughter. Whereas son carry his mother's mitochondrial DNA without pass it on.

Therefore, a full DNA fingerprint of the individual cannot make, thus this technique is only useful if the DNA profiles of maternal relatives are available, such as the individual's mother, maternal grandmother, or siblings because they have identical mtDNA.

The sequence of mitochondrial DNA is entirely functional and highly conserved; therefore, there are very little differences between individuals. Moreover, there is a 1000 base pair non-coding D-loop called the control region, which have two hyper variable regions known as HV1 and HV2.

Single nucleotide polymorphisms (SNPs) are mostly the types of variations that occur within these regions (HV1 and HV2), which do not effect on the length of the mtDNA and it were the regions that directed by the forensic sciences.

Forensic laboratories can develop DNA profiles from Mitochondrial DNA when the biological evidences (blood and semen) are highly degraded and are inapplicable for RFLP or STR analysis as in disaster or accidents. Old remnants lacked nucleated cells like hair shafts, bones, and teeth that are unsuitable for STR and RFLP testing but are eligible for mtDNA testing.

Analysis of mitochondrial DNA firstly involve extraction of DNA from the mitochondria and then the HV1 and HV2 regions are amplified using PCR after that by using DNA sequencing the base pair sequence of these regions will determine.

The result will compare with the Cambridge Reference Sequence and the differences will note. Then other samples (individual relatives) will analyze and comparisons made to prove potential similarities. Mitochondrial DNA testing also supplies a haplo group that defines deep ancestry, such as European, African, Asian or Native American.

7- Y-CHROMOSOME ANALYSIS

The Y chromosome found only in males and transmitted directly from a father to all of his sons. It was a small chromosome and altered only through the rare occurrence of mutation.

Many genetic markers detected on the Y chromosome like amelogenin marker and specific STRs present on the Y chromosome that could be used to trace family relationships among males and in forensic analysis to target the male fraction of a biological evidence sample. By analyzing haplogroup on Y chromosome, the genealogists can define deep ancestry, such as European, African, Asian or Native American.

Generally, the differentiation by analyzing Y chromosome is relatively low, but it is mostly useful in cases of sexual assault and rape at which mixed DNA profiles may be meet.

8- X- CHROMOSOME ANALYSIS

The X-Chromosome is a part of the 23 chromosomes (autosomal chromosomes), it was inherited in a way that is vary from other chromosomes. The male inherited X-chromosome from his mother and Y- chromosome from his father while female inherited X- Chromosome from both of her parents. Therefore, these inheritance properties allow genetic analyzer to assess the results of X- Chromosome separately from the other autosomal Chromosome results.

REFERENCES

1. Bieber Frederick; et al. "Finding Criminals Through DNA of Their Relatives". Science, 2006; 312(5778): 1315–16. doi:10.1126/science.1122655. PMID 16690817.
2. Chambers, Geoffrey K.; Curtis, Caitlin; Millar, Craig D.; Huynen, Leon; Lambert, David M. "DNA fingerprinting in zoology: past, present, future". Investigative Genetics, 2014; 5: 3. doi:10.1186/2041-2223-5-3.
3. Christoffersen, John and Barakat, Matthew. "Other victims of East Coast Rapist suspect sought." Associated Press. Accessed May 25, 2011.
4. CODIS — National DNA Index System. Fbi.gov. Archived from the original on March 6, 2010. Retrieved 2010-04-03.
5. Diamond, Diane. "Searching the Family DNA Tree to Solve Crime". HuffPost Denver (Blog). The Huffington Post. Retrieved April 17, 2011.
6. Findlay, I. Taylor, A. Quirke, P. et al. DNA Fingerprinting from Single Cells. Nature, 1997; 389(6651): 555-556.
7. Helderman, Rosalind. "McDonnell Approves Familial DNA for VA Crime Fighting." The Washington Post accessed April 17, 2011.
8. Jeffreys, A J. Thein, S L. Wilson, V. Individual-Specific Fingerprints of Human DNA. Nature, 1985; 316(6023): 76-79.
9. Kaye, David H., (2013). "The Genealogy Detectives: A Constitutional Analysis of Familial Searching" American Criminal Law Review, 2013; 51(1): 109-163.
10. Miller, K.W.P.; Dawson, J.L.; Hagelberg, E. "A concordance of nucleotide substitutions in the first and second hypervariable segments of the human mtDNA control region". International Journal of Legal Medicine, 1996; 109: 107–113. doi:10.1007/bf01369668.
11. Murphy Erin Elizabeth."Relative Doubt: Familial Searches of DNA Databases" .Michigan Law Review, 2009; 109: 291–348.
12. Pankratz, Howard. "Denver Uses 'Familial DNA Evidence' to Solve Car Break-Ins." The Denver Post accessed April 17, 2011.
13. Samuels, J.E., E.H. Davies, and D.B. Pope. (2013). Collecting DNA at Arrest: Policies, Practices, and Implications, Final Technical Report. Washington, D.C.: Urban Institute, Justice Policy Center.
14. Staff. "Familial searches allows law enforcement to identify criminals through their family members". DNA Forensics. United Kingdom – A Pioneer in Familial Searches. Retrieved December 7, 2015.
15. Steinhaur, Jennifer. "'Grim Sleeper' Arrest Fans Debate on DNA Use." The New York Times accessed April 17, 2011.
16. Suter Sonia ."All in The Family: Privacy and DNA Familial Searching" . Harvard Journal of Law and Technology, 2010; 23: 328.
17. Tautz D."Hypervariability of simple sequences as a general source for polymorphic DNA markers". Nucleic Acids Research, 1989; 17: 6463–6471.doi:10.1093/nar/17.16.6463.