Volume 9, Issue 7, July – 2024 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

A Review on Epigenetics of Human Inherited Diseases: Molecular Diagnosis

Bibi Shazia Sabir
University of Bahrain

Abstract:- Epigenetics are the changes due to DNA methylation, histone modifications, and noncoding RNA regulation, which play a crucial part in the expression of genes. Although these changes do not alter the sequence of the DNA, they are significant in determining the phenotype and inheritance of the genes. The impact of epigenetics on inherited diseases like Fragile X syndrome, Angelman syndrome, and Prader-Willi syndrome emphasizes the significance of studying epigenetic dysregulation in disease pathology. The current review aims to investigate the role of epigenetic mechanisms in inherited diseases and to evaluate the utility of molecular diagnostic tools for epigenetic analysis in disease diagnosis. Case studies of diseases such as Duchenne muscular dystrophy and Prader-Willi syndrome highlight the clinical relevance of epigenetic analysis in disease diagnosis and management. This research is conducted through an extensive analysis of recent literature to explore the epigenetic mechanisms underlying inherited diseases. Additionally, advanced molecular diagnostic techniques like droplet digital PCR (ddPCR), transposase-based bisulfite tagging, and data mining coupled with conventional experimental procedures. However, the intricate relationship between genetic and epigenetic factors and challenges related to inclined data need to be addressed. Future research endeavors to elucidate the role of epigenetic modifications in disease pathogenesis and develop strategies for personalized medicinal treatment and therapeutic intervention.

I. INTRODUCTION

The term epigenetics was introduced by Conrad H. Waddington, a British embryologist, in 1942. DNA methylation and demethylation, histone protein modifications, such as methylation, acetylation, phosphorylation, ubiquitination, or incorporation of histone variants gene regulation by noncoding RNAs (ncRNAs) are essential epigenetic signals. These epigenetic changes predominate spontaneously under normal development, health, aging, and disease (Rugowska et al., 2021). DNA sequence is an essential component that acts as a blueprint for the human genome. It is responsible for encoding all the genetic information that determines phenotype and genotype of an organism. However, the functionality of the genome is not only limited to its sequence, but also to over twenty identified epigenetic mechanisms. The epigenome network is a complex system of chemical modifications of the genome and its associated proteins that work together to regulate gene expression and ultimately control an organism's cellular processes. However, these modifications don't change the sequence of DNA but can transfer to the next generation. Furthermore, nutrition, inflammation, stress, metabolic effects, physical activity, and drugs have all been found to play a significant role in this complex biological process (Garcia-Gimenez, 2015).

Menezo et al., 2022 explain that environmental changes could compromise long-term health concerns in future generations by creating epigenetic aberrations. Both epigenetics and imprinting play a crucial role in transcriptional silencing and the regulation of imprinted genes. However, the effect of methylation greatly differs among male and female genomes. Furthermore, the maternal oviduct endures significant changes in epigenetic reprogramming upon fertilization. Flag of epigenetics in sperm cells occurs at later stages of embryogenesis and contributes to neurovegetative anomalies due to aberrant methylation. Another study conducted by Janssens et al., 2019 describes that histone methylation guides DNA methylation while DNA methylation serves as a template for histone modifications, contributing to the complexity of epigenetics. DNA methylation is a crucial process that is indispensable for maintaining genomic imprinting, ensuring genome stability, and repressing retrotransposons. It is worth noting that DNA methylation is a stable process, and demethylation, which is essential for gene reprogramming, occurs exclusively through passive dilution or an active hydroxy methylation process. Genes activation is due to the unfolding of chromatin that in turn is due to histone Acetylation. In contrast, histone methylation engages proteins that can either activate or repress genes. To reverse the effects of acetylation, deacetylases and histone demethylases play a crucial role. Notably, there are 18 different HDACs, which are classified into four classes that are involved in this process. Interestingly it is also observed that ncRNAs particularly miRNAs (19-24 nucleotides long) add another level of regulation to the epigenetic machinery by targeting mRNA and other ncRNAs to regulate gene expression.

Modern efficient molecular assays together with exceptional bioinformatics tools give insight into understanding complex hereditary diseases by using DNA methylation, histone modifications, and epitranscriptomes as biomarkers. Several diagnostic tools have been developed using chromatin immunoprecipitation, bisulfite treatment, methylated DNA immunoprecipitation (MeDIP), methylation-sensitive restriction enzyme digestion (MRE),

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

and RNA profiling. Dynamics of epigenomic marks allow a data-driven approach to understand Enhancers activity patterns across tissues for common gene functions and human phenotypes (Kundaje et al., 2015).

II. EPIGENETIC MECHANISMS IN INHERITED DISEASES

During sexual reproduction, meiosis causes some traits to be lost, while Histone and DNA modifications tend to be stable, regulating gene expression across many cell divisions (Harvey et al., 2018). In females, gene expression from only one X chromosome is assured by X chromosome inactivation. Moreover, the inclusion of macro histones like MacroH2A, DNA methylation and repressive chromatin modifications such as H3K27me3 leads to heterochromatin formation. It is believed that inactive X chromosome is involved in transcription silencing by a large cis-acting non-coding RNA. Alternatively, DNA methylation leads to the suppression of Xist gene on the active X chromosome by antisense transcript Tsipx.

Certain diseases, like Fragile X syndrome, Angelman syndrome, and Prader-Willi syndrome, are linked to epigenetic abnormalities affecting gene expression and genetic integrity. In the case of Fragile X syndrome, hypermethylation of CGG trinucleotide repeat in the 5' untranslated region results in silencing of FMR1. In Angelman syndrome, a deletion of the 15q11-q13 locus leads to the loss of maternally expressed ubiquitin-protein ligase E3A (UBE3A). On the other hand, Prader-Willie syndrome, a condition of uniparental disomy of maternal chromosome or due to microdeletions of the 3' end of imprinting control regions at 15q11-q13 locus at parental chromosome consisting of SNURF gene that codes for SNRPN upstream reading frame protein.

The mechanism responsible for encoding epigenetic markers plays a crucial role in certain diseases, like in the case of Rett syndrome. Mutation in methyl-CpG-binding protein 2 gene (MECP2) and DNA hypomethylation in T cells due to reduced levels of de novo methyltransferases 1 (DNMT1) leads to Systemic lupus erythematosus. Epigenetic changes also play a role in complex disorders such as schizophrenia and bipolar disorder, which exhibit different symptoms with varying severity (Thiagalingam, 2020).

It has been observed that Epigenetic disturbs biological pathways in some inherited diseases, like in the case of Duchenne Muscular Dystrophy (DMD). Inadequate production of miR-133/1 and miR-29 cause the sinhibition of muscle differentiation of muscle genes. Conversely, the higher level of miR-206 leads to an imbalance between proliferative and differentiated states. Moreover, miR-144 and miR-223 results in increased inflammation and muscle degeneration. (Rugowska et al., 2021).

Epigenetic dysregulation of repetitive sequences plays a crucial role in human disease. The human reference genome GRCh38 is linked to difficult-to-probe repeat sequences that lead to facioscapulohumeral muscular dystrophy (FSHD), schizophrenia, neuroblastoma, lung cancer, pancreatic ductal adenocarcinomas, immunodeficiency, centromeric region instability, and facial anomalies syndrome (ICF). Moreover, epigenetics silences evolutionarily older paralogs and activates newer copies through the regulation of gene paralogs. (Gershman et al., 2022).

- *Epigenetic Inheritance*

Epigenetic inheritance is a process that does not involve changes to chromosomes, instead, heritable changes evolved because of protein-based epigenetic elements known as Prions. Many are transcription factors and RNA-binding proteins that serve key roles in regulating information flow. Prions are not physically connected to chromosomes, which enables them to transmit their traits to all offspring through cytoplasmic inheritance in a robust manner (Harvey et al., 2018). Although epigenetic inheritance mechanisms were discovered in the 1900s, the idea of acquired traits remained hot research for scientists for more than 200 years. Moreover, the influence of environmental factors on the genetic material of mature sperm can extend to future generations, which may have an impact on their offspring (Casas & Vavouri, 2020).

- *Molecular Diagnostic Tools for epigenetic Analysis*

Recent research and extensive knowledge of the epigenetic impact on inherited diseases have paved the way for the tremendous evolution of diagnostic techniques. It is a well-established fact that circulating DNA, histones, and certain types of RNA can be interestingly traced in biological fluids like blood, serum, and plasma. Furthermore, DNA methylation, histone protein modification, and ncRNA are quite stable mutations and serve as potential clinical biomarkers in tissue damage, inflammation, and cellular apoptosis (Garcia-Gimenez, 2015). This review describes a few of the advanced techniques that promise significant diagnosis of inherited diseases with the understanding of epigenetic mechanisms.

- *Transposase-based Whole Genome Bisulfite Sequencing*

Cytosine methylation in DNA can be detected through Whole-genome bisulfite sequencing, where cytosine is converted to uracils through deamination by sodium bisulfite. Methylated cytosine refrains from conversion and is read as cytosine while unmethylated cytosines are converted to thymidines. Genomic DNA from IMR-90 and GM12878 cells were used, and libraries were optimized using transposase-based bisulfite tagging. The libraries were purified using Agencourt AMPure XP beads after PCR amplification. Massive parallel sequencing was done by the Illumina HiSeq X system and was aligned to the Homo sapiens reference genome after the removal of the adaptor. After segmentation, methylated cytosines were used in end-repair to avoid artifact that happens because of the unexpected insertion of methylated cytosines deeper into the duplex fragment. Furthermore, the artifact was corrected by a special Perl script that removes four consecutive methylated cytosines after the first nine bases (Suzuki et al., 2018).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

- *MspJI-assisted Hemi-Methylation Sequencing (Mhemi-seq)*

DNA methylation is one of the most explored mechanisms that primarily occurs at cytosines on both strands within CpG dinucleotides, playing a crucial role in regulating gene expression. However, during DNA replication, demethylation, or de novo methylation, hemimethylated CpG sites are generated in the genome. Moreover, hemi-methylation is more frequent and potent with high stability to be inherited across cell divisions, serving as potential epigenetic marks during embryonic development. Recently developed Mhemi-seq is a bisulfite-free method to identify allele-specific hemimethylated cytosine dyads. However, the traditional method of identifying hemi-methylation is harsh and leads to DNA degradation and loss of methylation information. Mhemi-seq provides a faster and more reliable method for identifying DNA hemi-methylation. In this method, MspJI is used to cut DNA at 9/13 bp downstream of methylated CNNR sites. NGS is required after ligation is done by sequencing adapters. Furthermore, Mhemi-seq is a useful technique in moderate to highly methylated genomes and in rare biopsies when the sample size is small. Moreover, another rare gene regulation cytosine modification, 5hmC can also be identified by MspJI (Xiong et al., 2024).

- *Data Mining Coupled with conventional Experimental Procedure*

The RNA modifications also described as epitranscriptome such as methylation of adenosine (A) and 5-methylcytosine (m5C), play key roles in certain types of cancers. Therefore, it serves as a biomarker in the detection of different types of cancer, particularly liver cells. Furthermore, altered expression of 5-methylcytosine RNA methyltransferases, an important enzyme in MYC signaling, causes destabilization of the CCDC9B mRNA and upregulation of its partner IVNS1ABP in liver cancer. In this method, the HumanMethylation450 Beadchip is used to check the level of Genome-wide promoter methylation following DNA lysis with buffer and sodium salt. A Nanodrop spectrophotometer is used to measure the concentration of the DNA, while an EZ DNA Methylation-Gold kit is used for bisulfite conversion. Bisulfite sequencing PCR is used for the analysis of the methylation status of the 5'-end promoter-associated CpG island of candidate genes. Bioinformatic tool Methyl Primer Express v1.0 is used to find CpG islands and Promoter regions were retrieved from the UCSC Genome Browser. BioEdit software is used to align reads, however, methylation of the promoter-associated island region was analysed through BSMap software. Moreover, expression of RNA is achieved by real-time qRT-PCR, and Primer3web ((Aartsma-Rus et al., 2019, p. 14)) is used for PCR primers for the sequences of interest, containing the potentially methylated cytosines. Small sizes of amplicon were used to reduce the amplification of uncovered cytosines. The total RNA was extracted from cell pellets using the SimplyRNA kit (Promega) on a Maxwell RSC device (Promega). Then, proteins were resolved on SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and subsequently blotted against specific antibodies. To study the effect of NSUN7 overexpression cloning of the cDNA sequence of NSUN7 is done into expression vectors and cellular overexpression was retrieved through lentiviruses. While The NSUN7-silenced SNU-423 cell line was lentiviral-transduced with GFP or Tomato-FACS. Furthermore, CRISPR was used to knock-out NSUN7 expression in hepatic cancer cells to observe the effects of NSUN7 depletion on cell proliferation. Expression of NSUN7-RNA is observed in unmethylated liver cancer cell lines, hypermethylated cells showed absence or minimal expression of the transcript (Ortiz-Barahona et al., 2023).

- *Droplet Digital PCR (ddPCR)*

Cells that undergo apoptosis or necrosis release various circulating cell-free DNA (ccDNA), which reflects the genome or epigenome of various tumors. Highly sensitive assays can accurately detect the quantity of ctDNA for diagnosis of certain cancer. Dickkopf-related protein 3 (DKK3) is a member of the human DKK-related gene family. Epigenetic silencing of this gene by methylation at its promoter region leads to recurrent in gastric and breast cancer. Droplet digital PCR (ddPCR) is a recent molecular technique that detects traces of circulating cell-free DNA (ccfDNA) in the serum of mesothelioma patients. In this method, ccfDNA is extracted from serum or plasma samples using QIAamp MinElute ccfDNA Mini Kit, Bisulfite conversion of ccfDNA as a template for droplet digital methylation-specific PCR (ddMSP) using the QX200 system. UCSC genome browser (http://genome.ucsc.edu/) was used to get the sequence of DKK3 CpG island and analyzed through using MethPrimer 2.0 (http://www.urogene.org/methprimer2/ tester-invitation.html). Thermo Fisher Scientific synthesized primers and Taqman-MGB probes were used. DKK3_island_M assay is used to amplify methylated DKK3-derived sequences, while Unmethylated DKK3-derived sequences were amplified with the DKK3_island_U assay. Bisulfite converted EpiScope-Methylated HCT116 gDNA and unmethylated HCT116 DKO gDNA were used as positive and negative controls for methylation-specific PCR. 10 µL of ddPCR Supermix for Probes used used in 20 µL of the total volume of the PCR mixture in the assay. C1000 Touch Thermal Cycler equipped with 96 Deep well reaction modules were used for methylation-specific PCR and non-methylation-specific PCR. The PCR products were subsequently read and analyzed using the QX-200 droplet reader and QuantaSoft analysis software.

Normal human serum and normal human plasma samples were used as normal controls while Peripheral blood samples from patients with malignant mesothelioma were collected. Patients' serum was pooled, and ccfDNA was purified from 4 mL. Cultured assays were used for both normal and mesothioma cells to further validate the results of ddMSP. No copies of the methylated genome were detected in the assay or ccfDNA derived from normal serum and normal plasma suggesting that these MSP regions were specific to the tumor tissue. 30-40 copies of cell-free DNA per 4mL is considered as detectable amount of ctDNA (Araki et al., 2022).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

➢ *T2T-CHM13 Profiling*

Epigenetic profiling like T2T-CHM13 provides unparalleled accuracy in complete genome assemblies and long-read epigenetics. Long read nanopore sequencing technology demonstrate CpG sites, leading to a more comprehensive understanding of DNA methylation patterns in different cell types, such as the trophoblastic cell line CHM13 and the terminally differentiated lymphoblast cell line HG002. The observation of particularly histone marks at the HLA locus strongly indicates the possibility of epigenetic dysregulation in prostate cancer.

To achieve this, primary alignments using SAMtools was followed by alignment of nanopore reads to the CHM13 genome using Winnowmap version 2.0. CpG methylation in nanopore data were measured by using Nanopolish and Megalodon. Overlapping at annotated promoter of a protein-coding gene is determined by Methylation clustring. Marker-assisted mapping of CUT&RUN was performed to sample-specific reference. Several ChIP-seq datasets generated as part of the ENCODE project,to achieve unique k-mers. Such epigenetic annotation plays crucial role in determining evolutionary paralog-specific function. For example, it was challenging to sequence the crucial SMN1/2 gene of spinal muscular atrophy (SMA). However, peaks of the activating H3K4me3 mark at the SMN2 gene promote were identified by ENCODE cell lines. It suggests high transcriptional activity among different tissues (Gershman et al., 2022).

➢ *miRNA as Biomarker*

MicroRNAs (miRNAs) are small non-coding RNAs, which is approximately 22 nucleotides in length. Many studies have implied that miRNAs are released into extracellular fluids. MicroRNAs exhibit hormone-like activities because extracellular miRNAs may be delivered by their target cells and they may act as autocrine, paracrine, and/or endocrine regulators to modulate cellular activities.

In 2019, Al-Hayali et al. explored that microRNA-1 (miR-1) and microRNA-21 (miR-21) are hugely optimistic diagnostic markers for heart failure (HF) and silent coronary artery disease (SCAD) in asymptomatic type 2 diabetes mellitus (T2DM) patients. They demonstrated a strong correlation between miR-1 and miR-21 expression levels and diabetes mellitus (DM). Patients of DM showed low levels of miR-1 and higher expression of miR-21 as compared to controls. The study also disclosed noteworthy differences in miR-1 and miR-21 levels among DM, coronary artery disease (CAD) + DM, and heart failure (HF) + DM groups, showing miR-21 as an extraordinary potential biomarker for distinguishing HF + DM. Altered miR-1 and miR-21 expression can be used in the diagnosis of cardiovascular risk factors and suggests that miR-21 could serve as a highly accurate early predictor of heart failure in asymptomatic patients with type 2 diabetes mellitus (T2DM).

III. CASE STUDIES

Epigenetic mechanisms are important as a link to diagnose certain inherited diseases. methylation modification is the most explored modification among more than 17 types of modification in DNA and 160 posttranscriptional modifications. Modern technologies of sequencing together with differential assay have facilitated the identification of epigenetic inherited diseases (Zhang et al., 2021).

➢ *Duchenne Muscular Dystrophy*

DMD is a rare neuromuscular disorder due to mutations in the DMD gene. It causes muscle weakness and motor function decline because of nonfunctional dystrophin protein affecting 1 in 3,600-6,000 male newborns (Alghamdi et al., 2021). They presented 4 case studies on diagnosing Duchenne muscular dystrophy (DMD). A neuromuscular specialist should be consulted if signs of DMD or an increase in serum CK levels are observed. Screening techniques like MLPA or array CGH are used to identify deletions or duplications in the DMD gene. Genetic sequencing is done if no mutations are detected. A muscle biopsy is performed if the diagnosis remains unclear.

Hypotonia and motor milestones delay was observed in a 10-month-old child in Kuwait, who had a family history of DMD. MLPA analysis confirmed the diagnosis of DMD, upon observation of elevated level of Ck. Results showed deletion of exons 46-49 in the DMD gene when the patient was 3 years and 4 months old. The clinical geneticist communicated the diagnosis to the family, who were referred to the local hospital for genetic counseling. Similarly, MLPA testing in twin brothers from Saudia Arabia revealed a hemizygous duplication of exon 12 of the DMD gene.

Females with DMD gene mutation are usually asymptomatic carriers, very rare, translocation can cause DMD. One such case was observed in Syria, where a 5-year-old female with no family history of DMD showed an elevated level of CK of 8,000 U/L after the presentation of frequent falls and difficulty in walking. The parents were identified as carriers of these variants, upon Gene sequencing and MLPA analysis of 123 neuromuscular genes. However, an array comparative genomic hybridization analysis did not reveal any mutations. When the patient was around 9 years old, a whole-genome sequencing showed a negative test result. However, a second muscle biopsy was conducted when the patient was almost 10 years old, and the results confirmed the absence of dystrophin protein through multiplex immunoblot analysis. A karyotype analysis revealed the presence of a new X-chromosome translocation near the DMD gene site. The patient was subsequently treated with physiotherapy, occupational therapy, and vitamin supplements.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

➢ *Prader–Willi Syndrome in Ethiopia*

Prader-Willi syndrome (PWS) is a rare genetic disorder that occurs due to the most prevalent inactivation of paternally expressed genes in the human chromosome region 15q11–q13 among other three classes of chromosomal mutation. According to recent studies, its occurrence is 1 in 25,000 births and prevalent among the population with a rate of 1 in 50,000. It is characterized by short stature, small hands or feet, and characteristic facial appearance. In late childhood and adolescence, behavioral issues, hypogonadism or hypogenitalism, delayed or incomplete secondary sexual characteristics, and worsening weight are commonly observed. Common clinical manifestations including hypotonia, a poor suck, or failure to thrive during infancy can be diagnosed by A DNA methylation analysis.

In the present study, A 14-year-old Ethiopian male patient with increased weight gain since childhood, gaining 8 kg at 6 weeks was brought to the hospital. During his childhood, genetic testing was not prescribed due to the poor prognosis during his early childhood. Additionally, a history of diabetes mellitus, hypertension, or chronic illnesses was not present in his family. His vital signs were normal, however, his BMI was 65kg/m^2 classifying him in class 3 obesity. According to consensus clinical diagnostic criteria for PWS 1993, patients exhibit six of eight major criteria of excessive weight gain, facial features, hypogonadism, recent learning difficulties, and hyperphagia. Furthermore, five of eleven criteria like characteristic behavior problems, short stature, small hands, eye refractory error, and speech articulation. Altogether a score of 8.5 was achieved for the patient is significant enough for the diagnosis of PWS. All basic laboratory tests, including complete blood count, renal function test, liver enzymes, serum bilirubin, serum albumin, and serum electrolytes, were in the normal range. Refractive error was diagnosed upon ophthalmological evaluation; however, the retinal exam was normal. The abdominal ultrasound revealed no abnormalities, except an 18-cm non-specific hepatomegaly that had a smooth contour and homogenous echo pattern. Moreover, a half-empty sella was the only noteworthy finding on the brain magnetic resonance imaging (MRI). Genetic testing using SALSA® MLPA® (Multiplex ligation-dependent probe amplification) was performed. Results confirmed an irregular methylation pattern at region 15a11.

The patient is advised for strict lifestyle adaptation, parenteral testosterone treatment, vitamin D, and spectacles. In addition to these therapies, the patient is being monitored by various professionals. Despite improvement in overall health and normalization of serum vitamin D and glycemia, no weight loss has been achieved so far. Furthermore, medical and surgical intervention options are being considered to address potential comorbidities (Belay et al., 2023).

➢ *Fragile X Syndrome*

Fragile X syndrome is the most prevalent hereditary neurodevelopmental disorder, due to the excessive abundance of CGG repeats located on the Xq27.3 chromosome at 5UTR of the FMR1 gene. Consequently, hypermethylation at this region leads to silencing of this gene resulting in inadequate production of the subsequent protein FMRP, which plays an important role in brain development. However, associated characteristics of the intellectual disorder, puffiness around the eyes, soft skin, hypotelorism, prominent ears, long palpebral fissures, broad philtrum, facial hypotonia, macroorchidism, hyper-flexible joints, and autism-like behavior are observed upon puberty.

The case presentation describes a 37-year-old female with intellectual disability and fertility issues, from mild MR consanguineous ancestors.

The proband and her entire family were tested for FXS using triplet-primed polymerase chain reaction (TP-PCR). DNA extraction was done from peripheral blood samples using the Roche DNA Isolation Kit for Cells and Tissues. Alleles with more than 200 repeats were detected by the Epoch Nanodrop UV-VIS Spectrophotometer (BioTek Instruments). Fragment inspection through the TP-PCR method and FastFraX™ FMR1 Sizing Kit was carried out on proband's sister's paraffin-embedded tissue sample to detect female zygosity. Furthermore, G-banding karyotype tests conducted for both the husband and the proband indicate the presence of fragile X at locus q27.3 in the proband, while the husband shows a normal karyotype. This confirms the genetic link to Fragile X Syndrome within the family.

IV. FUTURE PERSPECTIVES AND CHALLENGES

Although the epigenetic mechanism is crucial in determining the phenotype of an organism, still there are concerns about whether epigenetic variation itself is controlled by genetic variation (Husby, 2022). Despite many advances in technology and science, linking epigenetic changes to its outcome is challenging. Additionally, sufficient sample numbers are required to observe small changes made by epigenetic factors. (Fallet et al., 2023). DNA methylation is a widely explored mechanism in known cases and there is a need to develop strategies to investigate another mechanism as well. For instance, the role of histone modifiers requires the identification of non-histone substrates to understand whether signaling pathways are more critical in certain cancers (Zhao & Shilatifard, 2019). Similarly, revealing the full spectrum of human genetic variation through advancements in long-read sequencing technologies will undoubtedly discover the epigenetics of many diseases. Short-read sequencing is also challenging in terms of highly repetitive undiscovered regions. Although MspJI can identify the rare 5hmC cytosine mutation, there is still a need to explore this mutation by extensive experimentation (Gershman et al., 2022). Moreover, analyses and interpretation of epigenetic data are challenging because of lacking their functionality information.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

V. CONCLUSION

To conclude, epigenetics is a rapidly growing field that has broadened the concept of inherited diseases. It is a widely recognized fact that the epigenetic mechanism is remarkably influenced by environmental factors like diet and exercise. Nonetheless, a collaborative effort of precise experimental and computational methods is crucial to ultimately delineate the importance of epigenetics. Overall, the field of epigenetics is exceptionally complex and continuously progressing, offering tremendous potential for enhancing our understanding of gene regulation in hereditary diseases.

REFERENCES

[1]. Alghamdi, F., Al-Tawari, A., Alrohaif, H., Alshuaibi, W., Mansour, H., Aartsma-Rus, A., & Mégarbané, A. (2021). Case Report: The Genetic Diagnosis of Duchenne Muscular Dystrophy in the Middle East. *Frontiers in Pediatrics*, *9*, 716424. https://doi.org/10.3389/fped.2021.716424

[2]. Al-Hayali, M. A., Sozer, V., Durmus, S., Erdenen, F., Altunoglu, E., Gelisgen, R., Atukeren, P., Atak, P. G., & Uzun, H. (2019). Clinical Value of Circulating Microribonucleic Acids miR-1 and miR-21 in Evaluating the Diagnosis of Acute Heart Failure in Asymptomatic Type 2 Diabetic Patients. *Biomolecules*, *9*(5), 193. https://doi.org/10.3390/biom9050193

[3]. Araki, K., Kurosawa, A., & Kumon, H. (2022). Development of a quantitative methylation-specific droplet digital PCR assay for detecting Dickkopf-related protein 3. *BMC Research Notes*, *15*(1), 169. https://doi.org/10.1186/s13104-022-06056-6

[4]. Belay, K. E., Ayalew, B. L., Amogne, M. T., Alemneh, T. A., & Geletew, T. K. (2023). A 14-year-old male patient with diagnosis of Prader–Willi syndrome in Ethiopia: A case report. *Journal of Medical Case Reports*, *17*(1), 530. https://doi.org/10.1186/s13256-023-04282-5

[5]. Casas, E., & Vavouri, T. (2020). Mechanisms of epigenetic inheritance of variable traits through the germline. *Reproduction*, *159*(6), R251–R263. https://doi.org/10.1530/REP-19-0340

[6]. Fallet, M., Blanc, M., Di Criscio, M., Antczak, P., Engwall, M., Guerrero Bosagna, C., Rüegg, J., & Keiter, S. H. (2023). Present and future challenges for the investigation of transgenerational epigenetic inheritance. *Environment International*, *172*, 107776. https://doi.org/10.1016/j.envint.2023.107776

[7]. Garcia-Gimenez, J. L. (2015). *Epigenetic Biomarkers and Diagnostics*. Academic Press

[8]. Gershman, A., Sauria, M. E. G., Guitart, X., Vollger, M. R., Hook, P. W., Hoyt, S. J., Jain, M., Shumate, A., Razaghi, R., Koren, S., Altemose, N., Caldas, G. V., Logsdon, G. A., Rhie, A., Eichler, E. E., Schatz, M. C., O'Neill, R. J., Phillippy, A. M., Miga, K. H., & Timp, W. (2022). Epigenetic patterns in a complete human genome. *Science*, *376*(6588), eabj5089. https://doi.org/10.1126/science.abj5089

[9]. Griñán-Ferré, C., Corpas, R., Puigoriol-Illamola, D., Palomera-Ávalos, V., Sanfeliu, C., & Pallàs, M. (2018). Understanding Epigenetics in the Neurodegeneration of Alzheimer's Disease: SAMP8 Mouse Model. *Journal of Alzheimer's Disease*, *62*(3), 943–963. https://doi.org/10.3233/JAD-170664

[10]. Harvey, Z. H., Chen, Y., & Jarosz, D. F. (2018). Protein-Based Inheritance: Epigenetics beyond the Chromosome. *Molecular Cell*, *69*(2), 195–202. https://doi.org/10.1016/j.molcel.2017.10.030

[11]. Husby, A. (2022). Wild epigenetics: Insights from epigenetic studies on natural populations. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1968), 20211633. https://doi.org/10.1098/rspb.2021.1633

[12]. Janssens, Y., Wynendaele, E., Vanden Berghe, W., & De Spiegeleer, B. (2019). Peptides as epigenetic modulators: Therapeutic implications. *Clinical Epigenetics*, *11*(1), 101. https://doi.org/10.1186/s13148-019-0700-7

[13]. Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., Kheradpour, P., Zhang, Z., Wang, J., Ziller, M. J., Amin, V., Whitaker, J. W., Schultz, M. D., Ward, L. D., Sarkar, A., Quon, G., Sandstrom, R. S., Eaton, M. L., Wu, Y.-C., … Kellis, M. (2015). Integrative analysis of 111 reference human epigenomes. *Nature*, *518*(7539), 317–330. https://doi.org/10.1038/nature14248

[14]. Menezo, Y., Elder, K., Clement, P., Clement, A., & Patrizio, P. (2022). Biochemical Hazards during Three Phases of Assisted Reproductive Technology: Repercussions Associated with and Imprinting. *International Journal of Molecular Sciences*, *23*(16), Article 16. https://doi.org/10.3390/ijms23168916

[15]. Nilsson, E. E., Sadler-Riggleman, I., & Skinner, M. K. (2018). Environmentally induced epigenetic transgenerational inheritance of disease. *Environmental Epigenetics*, *4*(2), dvy016. https://doi.org/10.1093/eep/dvy016

[16]. Ortiz-Barahona, V., Soler, M., Davalos, V., García-Prieto, C. A., Janin, M., Setien, F., Fernández-Rebollo, I., Bech-Serra, J. J., De La Torre, C., Guil, S., Villanueva, A., Zhang, P.-H., Yang, L., Guarnacci, M., Schumann, U., Preiss, T., Balaseviciute, U., Montal, R., Llovet, J. M., & Esteller, M. (2023). Epigenetic inactivation of the 5-methylcytosine RNA methyltransferase NSUN7 is associated with clinical outcome and therapeutic vulnerability in liver cancer. *Molecular Cancer*, *22*(1), 83. https://doi.org/10.1186/s12943-023-01785-z

[17]. Rugowska, A., Starosta, A., & Konieczny, P. (2021). Epigenetic modifications in muscle regeneration and progression of Duchenne muscular dystrophy. *Clinical Epigenetics*, *13*(1), 13. https://doi.org/10.1186/s13148-021-01001-z

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL447

[18]. Suzuki, M., Liao, W., Wos, F., Johnston, A. D., DeGrazia, J., Ishii, J., Bloom, T., Zody, M. C., Germer, S., & Greally, J. M. (2018). Whole-genome bisulfite sequencing with improved accuracy and cost. *Genome Research*, *28*(9), 1364–1371. https://doi.org/10.1101/gr.232587.117

[19]. Thiagalingam, S. (2020). Epigenetic memory in development and disease: Unraveling the mechanism. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1873*(2), 188349. https://doi.org/10.1016/j.bbcan.2020.188349

[20]. Xiong, X., Chen, H., Zhang, Q., Liu, Y., & Xu, C. (2024). Uncovering the roles of DNA hemi-methylation in transcriptional regulation using MspJI-assisted hemi-methylation sequencing. *Nucleic Acids Research*, gkae023. https://doi.org/10.1093/nar/gkae023

[21]. Zhang, J., Xie, S., & Wan, S. (2021). Cancer Biomarkers Discovery of Methylation Modification With Direct High-Throughput Nanopore Sequencing. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.672804

[22]. Zhao, Z., & Shilatifard, A. (2019). Epigenetic modifications of histones in cancer. *Genome Biology*, *20*(1), 245. https://doi.org/10.1186/s13059-019-1870-5