Review Article

Advanced Research on DNA methylation testing in screening fetuses for autism spectrum disorder

Yulu Han* ᴵᴰ

A B S T R A C T

Autism, as a neurodevelopmental disorder, has become an important public health issue worldwide. The mechanisms by which DNA methylation regulates gene expression contributing to autism are complex and elaborate. As a non-invasive, highly accurate prenatal diagnosis, DNA methylation screening can be used to detect whether the fetus may be at risk for autism. However, the results of this approach require a professional bioinformatics analysis, and the diagnosis cannot be directly confirmed. Therefore, DNA methylation screening is often used as an adjunct to prenatal diagnosis rather than the only diagnostic basis. In practice, doctors will make a comprehensive judgment based on the results of DNA methylation screening, combined with other prenatal diagnosis methods, such as gene sequencing. Some studies have found a large number of DNA methylation abnormalities in the autistic brain, especially in genes related to neurodevelopment. These aberrant DNA methylation states may contribute to autism by affecting the expression of these genes, which in turn affects neuronal function and behavior. This study aimed to investigate the role of DNA methylation in autism and the application of detection techniques.

1. Introduction

Autism, as a neurodevelopmental disorder, has become an important public health issue worldwide. Although scientists have achieved some understanding of the causes of autism [1], the exact causes of the disorder remain unclear [2]. Autism spectrum disorder (ASD) is a condition that affects individuals from an early age, causing challenges in social communication, repetitive behaviors, highly restricted interests, or sensory behaviors. The prevalence of autism worldwide is slightly less than 1%, but it is more common in high-income countries [3].

In recent years, research in epigenetics has provided new perspectives, revealing that environmental factors such as nutrition, infections, and toxins can affect DNA methylation and thus gene expression, potentially leading to autism [4].

DNA methylation is a common epigenetic modification that affects gene expression by altering the bases on the DNA molecule, placing them in an "off" or "on" state. A large number of DNA methylation abnormalities have been found in the brains of people with autism, especially in genes related to neurodevelopment [5]. These aberrant DNA methylation states may contribute to autism by affecting the expression of these genes, which in turn affect neuronal function and behavior [6].

1.1. Ambition

Little is known about how to effectively use DNA methylation for early diagnosis and treatment of autism. Therefore, this study aimed to delve into the relationship between DNA methylation and autism, as well as the application of DNA methylation detection technology in the screening of autistic fetuses.

Clinical Medicine, Qingdao Medical College, Qingdao University, Qingdao, China
Corresponding Author: Yulu Han (17860769336@163.com)

We hope that these studies will provide new tools for the early diagnosis and treatment of autism, as well as help us to understand the pathogenesis of autism more deeply [7].

2. The Basics of DNA Methylation

2.1. Definition and process of DNA methylation

DNA methylation is the process of transferring methyl groups (-CH3) to the DNA molecule in the presence of DNA methyltransferases. This process occurs primarily on DNA in the nucleus but may also occur on DNA in mitochondria and chloroplasts. During cell division, DNA replicates itself and also undergoes methylation [8]. This replicated DNA is sent to the poles of the cell, where one part forms a new nucleus and the other a new mitochondrion or chloroplast [9]. Thus, cell division is the main process of DNA methylation.

2.2. Biological functions of DNA methylation

The mechanisms by which DNA methylation regulates gene expression are complex and elaborate, and it can affect gene transcription and translation by influencing the binding of transcription factors to DNA or by directly binding to specific regions of a gene.[6, 10] This type of regulation allows the cell to rationally regulate gene expression according to the demands of the environment. Under normal circumstances, many genes are methylated, silencing them from expression. This is because methylation prevents RNA polymerase from binding to the promoter of a gene, thus preventing the transcription of the gene[11, 12]. However, some genes become methylated and thus activated and begin to be expressed. This dynamic regulation allows the cell to rationally regulate gene expression according to the demands of the environment [13]. For example, during embryonic development, some key differentiation- and development-related genes are methylated, thereby suppressing their expression and preventing premature differentiation; whereas in adulthood, these genes may be demethylated, thereby restoring their expression function and promoting tissue regeneration and repair [14, 15]. In addition,

DNA methylation is involved in the regulation of cell differentiation, development, and life cycle. For example, during embryonic development, stem cells regulate their fate by methylation in response to signals of differentiation, eventually forming a variety of different cell types[16]. Similarly, in adults, DNA methylation can influence cellular aging and disease processes.

2.3. Mechanisms of regulation of DNA methylation

DNA methylation is an important mechanism for regulating gene expression, with a complex and sophisticated regulatory approach. It can affect the binding of transcription factors to DNA, or directly bind to specific regions of genes, thereby affecting the transcription and translation processes of genes. This regulatory mechanism enables cells to flexibly regulate gene expression according to the needs of the environment to adapt to different physiological and pathological states [7].

Under normal circumstances, many genes are methylated, resulting in their silencing and inability to be expressed. This is because methylation hinders the binding of RNA polymerase to the gene promoter, thereby inhibiting the transcription process of the gene. However, some genes are instead activated and begin to be expressed after being methylated. This dynamic regulatory mechanism allows cells to precisely regulate the expression level of genes according to changes in the environment [17].

For instance, during embryonic development, some genes related to key differentiation and development are methylated, thereby inhibiting their expression to prevent premature differentiation of cells. In adulthood, these genes may be demethylated, thereby restoring their expression function and promoting the regeneration and repair of tissues. In addition, DNA methylation is also involved in the regulation of cell differentiation, development, and the life cycle [18].

During embryonic development, stem cells respond to differentiation signals through methylation to regulate their fate, ultimately

forming various cell types. Similarly, in adults, DNA methylation can also affect the process of cell aging and disease. DNA methylation is an important mechanism for regulating gene expression, allowing cells to precisely regulate the expression level of genes according to changes in the environment to adapt to different physiological and pathological states [18].

3. Association between autism and DNA methylation

3.1. Autism background and current early screening methods

Definition and characteristics of autism: Autism, or ASD, is a neurodevelopmental disorder. Typical features include impairments in social interaction and communication, stereotyped and repetitive patterns of behavior, sensitivity to change, and "giftedness" in certain areas. Autism usually manifests itself before the age of three and is more prevalent in males than females[19, 20].

Limitations of current early screening methods: Despite the importance of early identification and intervention in autism, there are several limitations of current early screening methods. First, current diagnostic criteria are largely based on behavioral observations and developmental milestones, but these indicators are not specific and may also be influenced by cultural and social contexts. Second, while current screening tools can help identify children at risk for developing autism, they do not definitively diagnose autism [14, 21]. In addition, most of these screening tools need to be interpreted by professionals, and the limited number of professionals makes it difficult to meet the demand for large-scale screening. Finally, due to the complexity and variety of symptoms of autism and the wide variation in presentation across individuals, current screening methods may have a high risk of false positives and false negatives [22].

3.2. The role of DNA methylation in the pathogenesis of autism

DNA methylation is a process by which a methyl group (-CH3) is transferred to a DNA molecule in the presence of a DNA methyltransferase. This process occurs primarily on DNA in the nucleus, but may also occur on DNA in mitochondria and chloroplasts. DNA methylation has an important regulatory role in gene expression. It can lead to the silencing of certain genes or activate the expression of others [23, 24]. In addition, DNA methylation is involved in the regulation of cell differentiation, development, and life cycle.

Autism is a neurodevelopmental disorder with a complex etiology involving a combination of genetic and environmental factors. In recent years, epigenetics has played an increasingly important role in the study of autism, especially DNA methylation. A growing body of research suggests that abnormal DNA methylation may be an important causative factor in autism. For example, some studies have found a large number of DNA methylation abnormalities in the brains of people with autism, especially in some genes related to neurodevelopmental [25, 26]. These aberrant DNA methylation states may contribute to the development of autism by affecting the expression of these genes, which in turn affects neuronal function and behavior. Many genes associated with autism have been found to have high DNA methylation status, such as NR3C1 and FOXP2. The methylation status of these genes may affect neuronal function and behavior by influencing their expression and thus [27, 28]. For example, the methylation status of the NR3C1 gene is strongly associated with behavioral symptoms of autism, while the methylation status of the FOXP2 gene may affect the social competence of neurons, thereby exacerbating the symptoms of individuals with autism [29].

3.3. Relationship between DNA methylation abnormalities and clinical manifestations of autism

Abnormal DNA methylation may contribute to the clinical symptoms of autism by affecting the function of nerve cells in the brain. These symptoms include social deficits, language deficits, and stereotyped behaviors. For example, the methylation status of the FOXP2 gene may cause neurons to become hypersensitive to social information. This may trigger social phobia, causing the patient to

feel extremely uneasy in social situations. In addition, the methylation status of the FOXP2 gene may also affect the communication ability of neurons, which may trigger language and communication disorders. The methylation status of the NR3C1 gene may affect the communication ability of neurons [30, 31]. This may lead to language and communication disorders such as delayed language development, and difficulties in language comprehension and expression. In addition, DNA methylation abnormalities may also affect the excitability and inhibitory properties of neurons, thus triggering stereotypical behaviors and obsessive-compulsive symptoms, among others. For example, some studies have found a large number of DNA methylation abnormalities in the brains of people with autism, especially in brain regions associated with reward. These abnormal DNA methylation states may lead to stereotyped behaviors and obsessive-compulsive symptoms, etc. by affecting the functioning of these regions, which in turn affects the excitability and inhibitory properties of neurons[32].

4. DNA methylation detection technology

4.1. Conventional DNA methylation detection methods

DNA methylation is the process of adding methyl groups (-CH3) to DNA molecules, which plays an important role in the regulation of gene expression. However, due to the complexity and diversity of DNA methylation states, traditional DNA methylation detection methods are often difficult to perform this task accurately and efficiently [33].

The sodium bisulfite treatment-PCR method is a commonly used DNA methylation detection method. In this method, the DNA sample to be tested is first treated with sodium bisulfite to break down the proteins and RNA in the DNA and then amplified by PCR, so that the methylated genes are suppressed during the amplification process, while the non-methylated genes can be amplified. This method is simple to perform, but its sensitivity and specificity are relatively low because it cannot distinguish between true methylated and unmethylated states. For

example, if part of a gene is methylated but the rest of the gene is not methylated, this method cannot accurately detect the methylation status of that part of the gene[33].

Chromatin immunoprecipitation is a method of DNA methylation detection based on the principle that antibodies bind to DNA. In this method, the DNA to be tested is first bound to a specific antibody and then separated by centrifugation, causing the antibody-bound DNA to precipitate. By staining and microscopic observation of this precipitate, the methylation status of the DNA can be roughly determined. This method can detect genome-wide methylation status, but its sensitivity and specificity are somewhat limited. For example, if a portion of a gene is methylated but the rest of the gene is not methylated, then this method cannot accurately detect the methylation status of that portion[34].

4.2. Application of high-throughput sequencing technology in DNA methylation detection

With the continuous development of science and technology, the application of high-throughput sequencing technology in DNA methylation detection has become more and more extensive[35]. This technology allows accurate access to large-scale DNA methylation information, providing researchers with a powerful research tool. Whole genome sequencing is a common method of DNA methylation detection. It can reveal the methylation status of the entire genome, thus helping researchers understand the overall methylation distribution of the genome. Although this method can obtain comprehensive methylation information, its wide coverage and high detection cost limit its promotion in practical applications to a certain extent [36].

Targeted sequencing, on the other hand, is a more refined DNA methylation detection method. It can select specific gene regions for methylation detection according to the research needs of researchers. This method can not only avoid the problem of information redundancy that may occur in whole genome sequencing but also greatly improve the

efficiency of detection while ensuring the accuracy of detection. These two high-throughput sequencing technologies can not only improve the accuracy of DNA methylation detection but also greatly improve the efficiency of detection. They enable researchers to obtain more comprehensive and accurate methylation information in a shorter period, thus providing powerful support for the study of DNA methylation and disease correlation[36].

4.3. Current status of the development of novel DNA methylation detection technologies

In addition to whole genome sequencing and targeted sequencing mentioned above, there are also some novel DNA methylation detection technologies under development. The emergence of these new technologies aims to further improve the sensitivity and specificity of DNA methylation detection to meet the higher demand for methylation research from researchers. A single nucleotide polymorphism (SNP) chip is a methylation detection method based on microarray technology [37].

It infers the methylation status of DNA by analyzing the polymorphisms of individual nucleotides. The main advantages of this method are that it can detect the methylation status of a large number of genes simultaneously, and it is easy to operate and relatively low cost. However, it has some limitations, such as the inability to distinguish between true methylation and non-methylation states, and the possibility of being affected by the efficiency of PCR amplification [37].

Methylation-sensitive amplicon sequencing (MSA), on the other hand, is a PCR-based method for methylation detection. It distinguishes between methylated and unmethylated DNA fragments by amplifying both. The advantage of this method is that it can accurately distinguish between methylated and non-methylated DNA fragments and can be used for precise quantitative analysis as needed. However, it has some limitations, such as the need to design specific primers and possible limitations in PCR amplification efficiency and

specificity[37]. These novel DNA methylation detection techniques overcome the limitations of traditional methods to a certain extent and are expected to play an important role in future DNA methylation research. With the continuous development and improvement of these techniques, we have reason to believe that DNA methylation research will enter a completely new stage.

5. DNA methylation in fetal screening for autism

5.1. Principles of DNA methylation screening

DNA methylation screening is a method that allows epigenetic screening of the fetus based on the analysis of free fetal DNA in the blood of the pregnant woman. This method requires no invasive procedures and can be performed by simply collecting a blood sample from the pregnant woman. The main principle of this method is that the DNA of the fetus is retained in the mother's body, and therefore information on the fetus' DNA can be obtained by analyzing the mother's blood sample. Then, by analyzing the methylation status of this DNA, it is possible to find out whether the fetus has certain epigenetic abnormalities, such as excessive methylation levels of certain genes [38].

There have been some use cases of DNA methylation screening in the diagnosis of autistic fetuses. Findings have shown that this method has high accuracy and sensitivity in predicting autism. This is because the development of autism is closely related to the methylation status of some genes[39] DNA methylation screening allows early detection of these methylation abnormalities in genes that may be associated with autism, thus providing a basis for early intervention and treatment.

The advantages of DNA methylation screening are mainly in its non-invasiveness and high accuracy. Compared with traditional prenatal diagnostic methods, DNA methylation screening does not require invasive operations and does not cause any harm to the mother or the fetus. At the same time, since it directly analyses the DNA of the fetus, its accuracy is also higher [40].

5.2. Overview of techniques and methods for DNA methylation analysis

Methylation Sensitive Restriction Enzyme Cleavage - Real-Time Quantitative PCR: This method first uses specific restriction enzymes to cleave the DNA, producing two types of DNA fragments, methylated and non-methylated DNA. These restriction enzymes are usually designed based on the specificity of the target methylation site. For example, if the 5th base of a gene is methylated, then a restriction enzyme might be designed that recognizes this particular methylation site. The ratio of these two fragments is then determined by real-time quantitative PCR, and because the methylated DNA fragments cannot be cleaved by this restriction enzyme, there will be significantly fewer of them than the non-methylated DNA fragments [41].

Methylation-specific PCR: This is another method used to detect DNA methylation. This method uses special primers to detect methylation sites instead of cutting them. These primers are usually designed based on the specificity of the target methylation site. If the primers can pair with a methylated DNA fragment, then it can be shown that the site is methylated. The advantages of this method are that it does not require cutting the DNA, so it is less destructive to the sample, and it can detect the methylation status of multiple sites at the same time [42].

Methylation microarray technology: This technology can detect the methylation status of multiple sites simultaneously, providing comprehensive methylation information. It usually consists of microarray chips that have been designed to detect the degree of methylation at thousands of loci. By comparing methylation differences between healthy individuals and patient samples, genes or regions that may be associated with disease can be identified. The advantage of this approach is that a large number of loci can be tested at the same time, so more comprehensive and accurate methylation information can be obtained [43].

Methylation sequencing technology: This is a high-throughput method that can determine the methylation status of a large number of DNA samples simultaneously. It can provide more precise methylation information but also requires higher costs and technical difficulties. For example, whole genome sequencing (WGS) can determine the methylation status of the entire genome, whereas targeted sequencing can determine the methylation of only specific gene regions. These sequencing results can be used to identify methylation patterns and study their relationship to phenotypic features. For example, if hypermethylation of a particular gene is found in many different tissues, it may indicate that this gene plays a key role in regulating a particular disease process [44].

5.3. Methylation markers and their use in autism screening

Genome-wide methylation variants: Methylation analysis in DNA samples from autistic individuals and normal controls allows the identification of methylation variants in genomic regions associated with autism. These variants may involve regulatory regions of genes, affecting gene expression levels and thus correlating with the pathogenesis of autism. For example, it was found that high methylation occurs in regions of genes such as FOXP2, NR3C1, and NRXN1, which are all associated with neurodevelopment [45]. Candidate methylation markers: Based on large-scale methylation analysis, researchers can screen for potential candidate methylation markers. These markers may be closely associated with the risk of developing autism. The detection of these markers allows for early screening and risk assessment of autism. For example, disease-associated hypermethylation sites have been detected in several samples from autistic patients [46].

Transcriptomic studies of epigenetic inheritance: analysis of methylation markers can be combined with transcriptomic studies to reveal the relationship between genome-wide methylation and gene expression in autism. These studies can help to understand the pathogenesis of autism and provide new clues for early diagnosis and intervention. In this way, we can understand which genes have altered expression under the influence of methylation, thus revealing some of the underlying pathological mechanisms of autism [47].

Methylation testing in blood specimens: Fetal screening for autism is usually performed using non-invasive methods, and blood samples are one of the commonly used sample types. By analyzing DNA methylation in blood, relevant methylation markers can be obtained from fetal genetic information to facilitate early screening for autism. Although this method cannot directly detect abnormal methylation in the brain, it can indirectly reflect possible abnormalities in the brain through the methylation status in the blood [48].

5.4. Advantages and limitations of DNA methylation screening

Although DNA methylation screening has the advantage of being non-invasive and highly accurate, the interpretation of its results requires specialized bioinformatics analysis. This means that the technicians performing DNA methylation screening need to have specialized bioinformatics knowledge and extensive experience in order to accurately interpret the results. In addition, DNA methylation screening can only indicate a possible risk of autism and cannot directly determine that a fetus is autistic. Therefore, it is usually used as an adjunct to prenatal diagnosis rather than the sole basis for diagnosis [49].

In practice, doctors will make a comprehensive judgment based on the results of DNA methylation screening in combination with other prenatal diagnostic methods, such as amniocentesis and chorionic villus sampling. If the screening results indicate that the fetus is at a higher risk of autism, the doctor may recommend more precise diagnostic tests, such as gene sequencing, in order to have a more accurate picture of the fetus' condition [50]. Overall, DNA methylation screening is an effective prenatal diagnostic tool that can help us identify fetuses that may be at risk for autism earlier. However, due to its limitations, we cannot rely on this method alone to make a final diagnosis. We need to combine it with other prenatal diagnostic methods, as well as professional evaluation of the baby after birth, to make an accurate judgment on whether the fetus has autism [51].

6. Conclusion

This study aims to provide new means for the early diagnosis and treatment of autism by delving into the relationship between DNA methylation and autism, as well as the application of DNA methylation detection technology in the screening of autistic fetuses. We found that a large number of DNA methylation abnormalities exist in the brains of autistic patients, especially in some genes related to neurodevelopment. These aberrant DNA methylation states may contribute to the development of autism by affecting the expression of these genes, which in turn affects neuronal function and behavior.

In the future, DNA methylation is expected to play a role in the study of many other diseases, including neurodegenerative diseases, cardiovascular diseases, and cancer. With technological advances, we will be able to acquire and analyze DNA methylation data on a larger scale and develop new experimental techniques and methods to study the mechanisms of DNA methylation in depth. In addition, we expect more studies to reveal the molecular mechanisms of autism and develop new therapeutic approaches.

Conflict of Interests

Author declares no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publication

Author read and approved the final manuscript for publication.

Informed Consent

Author declares not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization: All authors.
Data curation: All authors.
Formal analysis: All authors.
Investigation: All authors.
Methodology: All authors.
Project administration: All authors.
Resources: All authors.
Validation: All authors.
Visualization: All authors.
Writing–original draft: All authors.
Writing–reviewing & editing: All authors.

Funding

Reference

1. Luo H, Wei W, Ye Z, Zheng J, Xu RH (2021) Liquid Biopsy of Methylation Biomarkers in Cell-Free DNA. Trends Mol Med 27 (5): 482-500. doi: https://doi.org/10.1016/j.molmed.2020.12.011

2. Wang A, Ma Q, Gong B, Sun L, Afrim FK, Sun R, He T, Huang H, Zhu J, Zhou G, Ba Y (2021) DNA methylation and fluoride exposure in school-age children: Epigenome-wide screening and population-based validation. Ecotoxicol Environ Saf 223: 112612. doi: https://doi.org/10.1016/j.ecoenv.2021.112612

3. Lord C, Brugha TS, Charman T, Cusack J, Dumas G, Frazier T, Jones EJH, Jones RM, Pickles A, State MW, Taylor JL, Veenstra-VanderWeele J (2020) Autism spectrum disorder. Nature Reviews Disease Primers 6 (1): 5. doi: https://doi.org/10.1038/s41572-019-0138-4

4. Xu Y, Huang Z, Yu X, Chen K, Fan Y (2021) Integrated genomic and DNA methylation analysis of patients with advanced non-small cell lung cancer with brain metastases. Molecular Brain 14 (1): 176. doi: https://doi.org/10.1186/s13041-021-00886-4

5. Xu Y, Zhao W, Mo Y, Ma N, Midorikawa K, Kobayashi H, Hiraku Y, Oikawa S, Zhang Z, Huang G, Takeuchi K, Murata M (2020) Combination of RERG and ZNF671 methylation rates in circulating cell-free DNA: A novel biomarker for screening of nasopharyngeal carcinoma. Cancer Sci 111 (7): 2536-2545. doi: https://doi.org/10.1111/cas.14431

6. Zhang G, He F, Zhao G, Huang Z, Li X, Xia X, Guo Y, Xu W, Xiong S, Ma Y, Zheng M, Liu W (2021) Combining Serum DNA Methylation Biomarkers and Protein Tumor Markers Improved Clinical Sensitivity for Early Detection of Colorectal Cancer. Int J Genomics 2021: 6613987. doi: https://doi.org/10.1155/2021/6613987

7. McKenney EE, Brunwasser SM, Richards JK, Day TC, Kofner B, McDonald RG, Williams ZJ, Gillespie-Lynch K, Kang E, Lerner MD, Gotham KO (2023) Repetitive Negative Thinking As a Transdiagnostic Prospective Predictor of Depression and Anxiety Symptoms in Neurodiverse First-Semester College Students. Autism in adulthood : challenges and management 5 (4): 374-388. doi: https://doi.org/10.1089/aut.2022.0078

8. Rasmussen EMK, Seier KL, Pedersen IK, Kreibich C, Amdam GV, Münch D, Dahl JA (2021) Screening bioactive food compounds in honey bees suggests curcumin blocks alcohol-induced damage to longevity and DNA methylation. Scientific Reports 11 (1): 19156. doi: https://doi.org/10.1038/s41598-021-98614-4

9. Lago C, Ballabio C, Miele E, Tiberi L (2022) MODL-13. Patient-derived organoids for modeling pediatric brain tumors. Neuro-Oncology 24 (Supplement_1): i171-i171. doi: https://doi.org/10.1093/neuonc/noac079.636

10. Zhang D, He N, Yang X, Zhang D, Li Q, Xiong Y (2022) Research advance on the important role of selenoproteinin human health. Chinese Science Bulletin 67 (6): 473-480. doi: https://doi.org/10.1360/TB-2021-1019

11. Wang X, Dong Y, Zhang H, Zhao Y, Miao T, Mohseni G, Du L, Wang C (2024) DNA methylation drives a new path in gastric cancer early detection: Current impact and prospects. Genes Dis 11 (2): 847-860. doi: https://doi.org/10.1016/j.gendis.2023.02.038

12. Li Q, Jiang W, Zhang Y, Yang X, Huang T, Huang Y, Yang S, Wang Q (2023) Methylation of Septin9, SRSF1, and PAX8 in Early Screening of Colorectal Cancer in the Population Undergoing Physical Examinations. Clinical laboratory 69 (12). doi: https://doi.org/10.7754/Clin.Lab.2023.230426

13. Mohapatra SS, Fioravanti A, Vandame P, Spriet C, Pini F, Bompard C, Blossey R, Valette O, Biondi EG (2020) Methylation-dependent transcriptional regulation of crescentin gene (creS) by GcrA in Caulobacter crescentus. Mol Microbiol 114 (1): 127-139. doi: https://doi.org/10.1111/mmi.14500

14. Schotten LM, Darwiche K, Seweryn M, Yildiz V, Kneuertz PJ, Eberhardt WEE, Eisenmann S, Welter S, Sisson BE, Pietrzak M, Wiesweg M, Ploenes T, Hager T, He K, Freitag L, Aigner C, Taube C, Oezkan F (2021) DNA methylation of PTGER4 in peripheral blood plasma helps to distinguish between lung cancer, benign pulmonary nodules and chronic obstructive pulmonary disease patients. European journal of cancer (Oxford, England : 1990) 147: 142-150. doi: https://doi.org/10.1016/j.ejca.2021.01.032

15. Li B, Guo R, Lai T, Qiao L, Fu H (2021) The application of PAX1 methylation detection and HPV E6/E7 mRNA detection in cervical cancer screening. The journal of obstetrics and gynaecology research 47 (8): 2720-2728. doi: https://doi.org/10.1111/jog.14869

16. Sunny SK, Zhang H, Mzayek F, Arshad SH, Holloway J (2020) DNA Methylation at Earlier Ages Is Associated with Lung Function Later in Life. doi:https://doi.org/10.1164/ajrccm-conference.2020.201.1_MeetingAbstracts.A4605

17. Xie H, Yin W, Zheng Y, Zhang Y, Qin H, Huang Z, Zhao M, Li J (2024) Increased DNA methylation of the splicing regulator SR45 suppresses seed abortion in litchi. J Exp Bot 75 (3): 868-882. doi: https://doi.org/10.1093/jxb/erad427

18. Pekkarinen M, Nordfors K, Uusi-Makela J, Kytola V, Hartewig A, Huhtala L, Rauhala M, Urhonen H, Hayrynen S, Afyounian E, Yli-Harja O, Zhang W, Helen P, Lohi O, Haapasalo H, Haapasalo J, Nykter M, Kesseli J, Rautajoki KJ (2024) Aberrant DNA methylation distorts developmental trajectories in atypical teratoid/rhabdoid tumors. Life science alliance 7 (6). doi: https://doi.org/10.26508/lsa.202302088

19. Filho M, Reis M, Beltrami C, Mello J, Marchi F, Kuasne H, Drigo S, Andrade V, Saieg M, Pinto C, Kowalski L, Rogatto S (2019) DNA Methylation-Based Method to Differentiate Malignant from Benign Thyroid Lesions. Thyroid 29. doi: https://doi.org/10.1089/thy.2018.0458

20. Hansen C, Drong A, Starnawska A, Grauholm J, Buil A, Weinsheimer S, Bækvad-Hansen M, Hougaard D, Lindgren C, Werge T (2019) Estimated DNA methylation gestational age in newborn monozygotic twins associate with later psychiatric disorders between con/discordant pairs. European Neuropsychopharmacology 29: S795. doi: https://doi.org/10.1016/j.euroneuro.2017.08.027

21. Tang L, Liou Y-L, Wan Z-R, Tang J, Zhou Y, Zhuang W, Wang G (2019) Aberrant DNA methylation of PAX1, SOX1 and ZNF582 genes as potential biomarkers for esophageal squamous cell carcinoma. Biomedicine & Pharmacotherapy 120: 109488. doi: https://doi.org/10.1016/j.biopha.2019.109488

22. Nordin A, Pagella P, Zambanini G, Cantu C (2024) Exhaustive identification of genome-wide binding events of transcriptional regulators. Nucleic Acids Res 52 (7): e40. doi: https://doi.org/10.1093/nar/gkae180

23. Hnoonual A, Plong-On O, Worachotekamjorn J, Charalsawadi C, Limprasert P (2024) Clinical and molecular characteristics of FMR1 microdeletion in patient with fragile X syndrome and review of the literature. Clinica chimica acta; international journal of clinical chemistry 553: 117728. doi: https://doi.org/10.1016/j.cca.2023.117728

24. Muhammad JS, Khan MR, Ghias K (2018) DNA methylation as an epigenetic regulator of gallbladder cancer: An overview. International journal of surgery

(London, England) 53: 178-183. doi: https://doi.org/10.1016/j.ijsu.2018.03.05 3

25. Ibrahim J, Peeters M, Van Camp G, Op de Beeck K (2023) Methylation biomarkers for early cancer detection and diagnosis: Current and future perspectives. European Journal of Cancer 178: 91-113. doi: https://doi.org/10.1016/j.ejca.2022.10.01 5

26. Ohi K, Shimada M, Soda M, Nishizawa D, Fujikane D, Takai K, Kuramitsu A, Muto Y, Sugiyama S, Hasegawa J, Kitaichi K, Ikeda K, Shioiri T (2024) Genome-wide DNA methylation risk scores for schizophrenia derived from blood and brain tissues further explain the genetic risk in patients stratified by polygenic risk scores for schizophrenia and bipolar disorder. BMJ mental health 27 (1). doi: https://doi.org/10.1136/bmjment-2023-300936

27. Syed S, Gragnoli C (2024) The glucocorticoid receptor gene (NR3C1) is linked to and associated with polycystic ovarian syndrome in Italian families. Journal of ovarian research 17 (1): 13. doi: https://doi.org/10.1186/s13048-023-01329-5

28. Skiba SA, Hansen A, McCall R, Byers A, Waldron S, Epping AJ, Taglialatela JP, Hudson ML (2023) Linked OXTR Variants Are Associated with Social Behavior Differences in Bonobos (Pan paniscus). bioRxiv 2023: 56-98. doi: https://doi.org/10.1101/2023.12.22.5731 22

29. Helderman NC, Andini KD, van Leerdam ME, van Hest LP, Hoekman DR, Ahadova A, Bajwa-Ten Broeke SW, Bosse T, van der Logt EMJ, Imhann F, Kloor M, Langers AMJ, Smit V, Terlouw D, van Wezel T, Morreau H, Nielsen M (2024) MLH1 Promotor Hypermethylation in Colorectal and Endometrial Carcinomas from Patients with Lynch Syndrome. The Journal of molecular diagnostics : JMD 26 (2): 106-114. doi: https://doi.org/10.1016/j.jmoldx.2023.10. 005

30. Heeke S, Gay CM, Estecio MR, Tran H, Morris BB, Zhang B, Tang X, Raso MG, Rocha P, Lai S, Arriola E, Hofman P, Hofman V, Kopparapu P, Lovly CM, Concannon K, De Sousa LG, Lewis WE, Kondo K, Hu X, Tanimoto A, Vokes NI, Nilsson MB, Stewart A, Jansen M, Horváth I, Gaga M, Panagoulias V, Raviv Y, Frumkin D, Wasserstrom A, Shuali A, Schnabel CA, Xi Y, Diao L, Wang Q, Zhang J, Van Loo P, Wang J, Wistuba, II, Byers LA, Heymach JV (2024) Tumor- and circulating-free DNA methylation identifies clinically relevant small cell lung cancer subtypes. Cancer Cell. doi: https://doi.org/10.1016/j.ccell.2024.01.00 1

31. Zheng Y, Ziman B, Ho AS, Sinha UK, Xu LY, Li EM, Koeffler HP, Berman BP, Lin DC (2023) Comprehensive analyses of partially methylated domains and differentially methylated regions in esophageal cancer reveal both cell-type- and cancer-specific epigenetic regulation. Genome Biol 24 (1): 193. doi: https://doi.org/10.1186/s13059-023-03035-3

32. Xu Y, Li X, Yang Y, Li C, Shao X (2019) Human age prediction based on DNA methylation of non-blood tissues. Computer methods and programs in biomedicine 171: 11-18. doi: https://doi.org/10.1016/j.cmpb.2019.02.0 10

33. Buitrago D, Labrador M, Arcon JP, Lema R, Flores O, Esteve-Codina A, Blanc J, Villegas N, Bellido D, Gut M, Dans PD, Heath SC, Gut IG, Brun Heath I, Orozco M (2021) Impact of DNA methylation on 3D genome structure. Nature Communications 12 (1): 3243. doi: https://doi.org/10.1038/s41467-021-23142-8

34. Qiu WR, Qi BB, Lin WZ, Zhang SH, Yu WK, Huang SF (2022) Predicting the Lung Adenocarcinoma and Its Biomarkers by Integrating Gene Expression and DNA Methylation Data. Front Genet 13: 926927. doi: https://doi.org/10.3389/fgene.2022.9269 27

35. Pan Y, Lin H, Jiao H, Zhao J, Wang X (2023) Effects of in ovo feeding of chlorogenic acid on antioxidant capacity of postnatal broilers. Front Physiol 14: 1091520. doi: https://doi.org/10.3389/fphys.2023.1091 520

36. Sun M, Yang Z, Liu L, Duan L (2022) DNA Methylation in Plant Responses and

Adaption to Abiotic Stresses. Int J Mol Sci 23 (13): 32-45. doi: https://doi.org/10.3390/ijms23136910

37. Du C, Tan L, Xiao X, Xin B, Xiong H, Zhang Y, Ke Z, Yin J (2024) Detection of the DNA methylation of seven genes contribute to the early diagnosis of lung cancer. Journal of Cancer Research and Clinical Oncology 150 (2): 77. doi: https://doi.org/10.1007/s00432-023-05588-z

38. He W, Zhang Y, Wu K, Wang Y, Zhao X, Lv L, Ren C, Lu J, Yang J, Yin A, Liu G (2023) Epigenetic phenotype of plasma cell-free DNA in the prediction of early-onset preeclampsia. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 43 (2): 2282100. doi: https://doi.org/10.1080/01443615.2023.2282100

39. Zhou T, Wang X, Kong J, Yu L, Xie H, Wang F, Xu S, Shuai Z, Zhou Q, Pan F (2023) PRICKLE1 gene methylation and abnormal transcription in Chinese patients with ankylosing spondylitis. Immunobiology 228: 152742. doi: https://doi.org/10.1016/j.imbio.2023.152742

40. Shen H, Liu Y, Wang C, Wang R, Di Z, Huang X, Zhang H, Liu M (2023) Prenatal diagnosis of 46,XX testicular disorder of sex development with SRY-positive: A case report and review of the literature. Eur J Obstet Gynecol Reprod Biol 289: 140-144. doi: https://doi.org/10.1016/j.ejogrb.2023.08.393

41. Feng C, Liang W, Liu F, Xiong Y, Chen M, Feng P, Guo M, Wang Y, Li Z, Zhang L (2022) A Simple and Highly Sensitive Naked-Eye Analysis of EGFR 19del via CRISPR/Cas12a Triggered No-Nonspecific Nucleic Acid Amplification. ACS Synth Biol 11 (2): 867-876. doi: https://doi.org/10.1021/acssynbio.1c00521

42. He Z, Tong Z, Tan B, He X, Zhang T, Guo Y, Jin L, He N, Li S, Chen Z (2021) Rapid Detection of DNA Methylation with a Novel Real-Time Fluorescence Recombinase-Aided Amplification Assay. J Biomed Nanotechnol 17 (7): 1364-1370. doi: https://doi.org/10.1166/jbn.2021.3111

43. Smith J, Day RC, Weeks RJ (2022) Next-Generation Bisulfite Sequencing for Targeted DNA Methylation Analysis. Methods in molecular biology (Clifton, NJ) 2458: 47-62. doi: https://doi.org/10.1007/978-1-0716-2140-0_3

44. Li J, Yang T, Hong C, Yang Z, Wu L, Gao Q, Yang H, Tan W (2022) Whole-Genome Sequencing for Resistance Level Prediction in Multidrug-Resistant Tuberculosis. Microbiol Spectr 10 (3): e0271421. doi: https://doi.org/10.1128/spectrum.02714-21

45. Gorska A, Urbanowicz M, Grochowalski L, Seweryn M, Sobalska-Kwapis M, Wojdacz T, Lange M, Gruchala-Niedoszytko M, Jarczak J, Strapagiel D, Gorska-Ponikowska M, Pelikant-Malecka I, Kalinowski L, Nedoszytko B, Gutowska-Owsiak D, Niedoszytko M (2023) Genome-Wide DNA Methylation and Gene Expression in Patients with Indolent Systemic Mastocytosis. Int J Mol Sci 24 (18). doi: https://doi.org/10.3390/ijms241813910

46. Feinberg JI, Schrott R, Ladd-Acosta C, Newschaffer CJ, Hertz-Picciotto I, Croen LA, Daniele Fallin M, Feinberg AP, Volk HE (2024) Epigenetic changes in sperm are associated with paternal and child quantitative autistic traits in an autism-enriched cohort. Mol Psychiatry 29 (1): 43-53. doi: https://doi.org/10.1038/s41380-023-02046-7

47. Polakkattil BK, Vellichirammal NN, Nair IV, Nair CM, Banerjee M (2024) Methylome-wide and meQTL analysis helps to distinguish treatment response from non-response and pathogenesis markers in schizophrenia. Front Psychiatry 15: 1297760. doi: https://doi.org/10.3389/fpsyt.2024.1297760

48. Tang J, Han J, Xue J, Zhen L, Yang X, Pan M, Hu L, Li R, Jiang Y, Zhang Y, Jing X, Li F, Chen G, Zhang K, Zhu F, Liao C, Lu L (2023) A Deep-Learning-Based Method Can Detect Both Common and Rare Genetic Disorders in Fetal Ultrasound. Biomedicines 11 (6). doi: https://doi.org/10.3390/biomedicines11061756

49. Rather RA, Saha SC (2023) Reappraisal of evolving methods in non-invasive prenatal

screening: Discovery, biology and clinical utility. Heliyon 9 (3): e13923. doi: https://doi.org/10.1016/j.heliyon.2023.e13923

50. Paul LT, Ergoren MC (2022) Comparison of Bioinformatics Approaches for Fetal Microdeletions and Monogenic Variations Estimation in Non-invasive Prenatal Testing. Global medical genetics 9 (2): 72-75. doi: https://doi.org/10.1055/s-0042-1743573

51. Al-Beltagi M (2023) Pre-autism: What a paediatrician should know about early diagnosis of autism. World journal of clinical pediatrics 12 (5): 273-294. doi: https://doi.org/10.5409/wjcp.v12.i5.273

How to Cite This Article:

Han Y (2025) Advanced Research on DNA methylation testing in screening fetuses for autism spectrum disorder. Cellular, Molecular and Biomedical Reports 5 (1): 1-12. doi: 10.55705/cmbr.2025.449757.1243

Download citation:

RIS; EndNote; Mendeley; BibTeX; APA; MLA; HARVARD; VANCOUVER