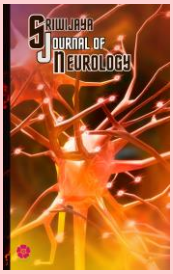

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Genetic and Environmental Influences on Autism Spectrum Disorder: A Multi-Center Study Exploring Gene-Environment Interactions and Biomarkers in Indonesia

Vita Amanda[1*], Rashidah Unaib Al-Zayid[2], Winata Putri[3], Sonya Syarifah[4], Tiffany Gabriele[5], Leonardo Simanjuntak[6], Cinthya Callathea[6]

[1]Department of Psychiatry, CMHC Research Center, Palembang, Indonesia
[2]Department of Radiology, Family Medicine Academy, Riyadh, Saudi Arabia
[3]Department of Pediatrics, CMHC Research Center, Palembang, Indonesia
[4]Department of Forensic and Medicolegal, CMHC Research Center, Palembang, Indonesia
[5]Department of Psychiatry, Gabon State Hospital, Libreville, Republic of Gabon
[6]Department of Obstetrics and Gynecology, CMHC Research Center, Palembang, Indonesia

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***Corresponding author:**
Vita Amanda

E-mail address:
vitaamandava@gmail.com

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A B S T R A C T

Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental condition with a complex etiology involving genetic and environmental factors. This multi-center study investigated gene-environment interactions and potential biomarkers associated with ASD in the Indonesian population. **Methods:** Children diagnosed with ASD (n=500) and age-matched typically developing controls (n=500) were recruited across five major Indonesian cities. Whole-exome sequencing targeted genotyping, and environmental risk factor assessments were conducted. Biomarker analyses included cytokine levels, oxidative stress markers, and neurotransmitters. **Results:** Genetic analysis revealed both rare and common variants associated with ASD risk, including variants in *CHD8*, *SCN2A*, *NRXN1*, and novel genes. Prenatal exposures (maternal medication use, infections), perinatal complications (preterm birth, low birth weight), and postnatal factors (pesticide exposure, air pollution) were associated with increased ASD risk. Children with ASD exhibited elevated inflammatory markers (TNF-α, IL-6, IL-1β), increased oxidative stress (higher MDA, lower GSH), and altered neurotransmitter levels (lower serotonin and dopamine) compared to controls. **Conclusion:** This study provides insights into the interplay of genetic and environmental factors contributing to ASD risk in Indonesia. The identified genetic variants, environmental risk factors, and potential biomarkers may contribute to our understanding of ASD etiology and inform the development of targeted interventions and early detection strategies.

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by a range of challenges in social communication, repetitive behaviors, and restricted interests. While the exact causes of ASD remain incompletely understood, a growing body of research points to a complex interplay of genetic and environmental factors contributing to its development. Genetic factors are widely recognized as playing a significant role in ASD susceptibility. Studies have shown that ASD has a strong heritability component, with estimates ranging from 40% to 90%. This means that genetic variations inherited from parents can significantly influence an individual's likelihood of developing ASD. The genetic landscape of ASD is highly complex and heterogeneous. Numerous

genes have been implicated in ASD, including those involved in synaptic function, neuronal migration, and immune response. These genes play critical roles in brain development and function, and variations in these genes can disrupt these processes, potentially contributing to the development of ASD. Both rare and common genetic variants have been associated with ASD risk. Rare variants, which occur in less than 1% of the population, can have significant effects on gene function and may increase ASD risk substantially. Common variants, which are more prevalent in the population, may have smaller individual effects on ASD risk but can collectively contribute to the overall genetic susceptibility.[1-3]

In addition to genetic factors, environmental influences also play a crucial role in ASD etiology. Various environmental factors, both prenatal and postnatal, have been associated with increased ASD risk. Prenatal exposures, such as maternal infections, medication use, and nutritional deficiencies during pregnancy, have been linked to ASD. These exposures can affect fetal brain development during critical periods, potentially increasing the likelihood of ASD. Perinatal complications, including preterm birth and low birth weight, have also been associated with ASD. These complications can disrupt early brain development and may increase the risk of neurodevelopmental disorders, including ASD. Postnatal environmental factors, such as exposure to toxins and psychosocial stress, may further contribute to ASD development. These factors can affect brain development and function during childhood and may interact with genetic predispositions to influence ASD risk. The interaction between genetic and environmental factors is particularly important in understanding ASD etiology. Gene-environment interactions occur when the effect of a genetic variant on ASD risk is modified by an environmental factor. In other words, certain genetic variants may increase ASD risk only in the presence of specific environmental exposures. Identifying these interactions can provide valuable insights into the mechanisms underlying ASD and may lead to the development of targeted interventions. By understanding how genetic and environmental factors interact, researchers can develop strategies to modify environmental exposures and reduce ASD risk in genetically susceptible individuals.[4-6]

Biomarkers are measurable biological indicators that can be used to identify individuals at risk for ASD, monitor disease progression, and evaluate treatment response. Several potential biomarkers for ASD have been investigated, including neuroimaging findings, electrophysiological measures, and biochemical markers. Neuroimaging studies have identified structural and functional brain differences in individuals with ASD compared to typically developing individuals. These differences may provide insights into the neurobiological basis of ASD and may serve as potential biomarkers for early detection. Electrophysiological measures, such as electroencephalography (EEG), have also shown differences in brain activity patterns in individuals with ASD. These measures may help identify brainwave patterns associated with ASD and may be used to monitor treatment response. Biochemical markers, such as blood or urine levels of certain proteins or metabolites, have also been investigated as potential biomarkers for ASD. These markers may reflect underlying biological processes associated with ASD and may be used for early detection or monitoring disease progression.[7,8]

Indonesia, with its diverse population and varying environmental conditions, presents a unique opportunity to study the genetic and environmental factors contributing to ASD. The country's diverse genetic makeup and range of environmental exposures provide a valuable setting for investigating gene-environment interactions in ASD. Additionally, Indonesia has a growing prevalence of ASD, highlighting the need for research to understand the specific genetic and environmental factors contributing to ASD risk in the Indonesian population. This research can inform the development of targeted interventions and early detection strategies tailored to the Indonesian context.[9,10] This multi-center study aims to investigate the interplay of genetic and environmental factors contributing to ASD risk in Indonesia.

2. Methods

This multi-center study investigates the intricate interplay of genetic and environmental factors that contribute to the development of autism spectrum disorder (ASD) within the diverse Indonesian population. This research was conducted across five major cities in Indonesia: Jakarta, Bandung, Surabaya, Medan, and Makassar, each with varying environmental conditions and socioeconomic backgrounds. The study involved two primary groups; Children diagnosed with ASD (n=500): This group comprises children aged 2-18 years who have received a diagnosis of ASD based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. These participants were recruited from specialized ASD clinics and hospitals in each of the five cities, ensuring representation across different urban environments and healthcare settings; Age-matched typically developing controls (n=500): This group consists of children with typical development, aged 2-18 years, recruited from the community in the same five cities. The age-matching with the ASD group allows for a controlled comparison, minimizing the influence of age-related developmental variations on the findings. To maintain the integrity of the study, specific exclusion criteria were applied; Known genetic syndromes: Children with known genetic syndromes associated with ASD or other developmental disorders were excluded to isolate the impact of novel or less understood genetic factors; Neurological disorders: Participants with pre-existing neurological conditions were excluded to prevent confounding effects on the assessment of ASD-related neurological features; Significant developmental delays unrelated to ASD: Children with significant developmental delays not attributable to ASD were excluded to ensure that the study specifically focuses on ASD-related developmental patterns.

Stringent ethical protocols were followed throughout this study; Ethical approval: The study protocol and procedures were reviewed and approved by the ethics committees of CMHC Indonesia, ensuring alignment with national ethical guidelines and standards for research involving human subjects; Informed consent: Informed consent was obtained from the parents or legal guardians of all participants before their enrollment in the study. This process included providing comprehensive information about the study's purpose, procedures, potential benefits, and risks, enabling the guardians to make informed decisions regarding their child's participation.

A multi-faceted data collection approach was employed to gather comprehensive information; Demographic and clinical data: Detailed demographic information was collected on each participant, including age, gender, ethnicity, and socioeconomic status. This data allows for an analysis of the distribution of ASD across different demographic groups and socioeconomic strata. Clinical data, including ASD symptom severity, intellectual functioning, and any co-morbid conditions, were assessed using standardized instruments, ensuring objectivity and comparability of the clinical assessments; Genetic analysis: Whole-exome sequencing was performed on blood samples collected from all participants. This advanced technique allows for the identification of rare genetic variants in the protein-coding regions of the genome, potentially revealing novel genes or mutations associated with ASD. Targeted genotyping of known ASD-associated genes was also conducted to assess the prevalence of common variants already implicated in ASD susceptibility; Environmental risk factor assessment: A comprehensive questionnaire was administered to the parents of each participant to gather information on potential environmental risk factors. This questionnaire covers prenatal exposures, such as maternal medication use and infections during pregnancy; perinatal complications, including preterm birth and low birth weight; and postnatal environmental factors, such as exposure to pesticides and air pollution. The questionnaire format allows for a standardized and systematic collection of environmental exposure data; Biomarker analysis: Blood samples were collected from all participants to assess potential biomarkers associated with ASD. These samples were analyzed to measure cytokine levels, providing insights into inflammatory processes; oxidative stress markers, indicating the level of cellular damage caused by oxidative stress; and

neurotransmitter levels, reflecting the balance of critical brain chemicals involved in neural communication. The biomarker analysis was conducted using commercially available kits, ensuring standardized and reliable measurements.

A combination of statistical methods was used to analyze the collected data; Descriptive statistics: Descriptive statistics were used to summarize the demographic and clinical characteristics of the participants. This includes measures such as mean, standard deviation, and percentages, providing a clear overview of the study population's characteristics; Genetic association analyses: Genetic association analyses were performed to identify genetic variants associated with ASD risk. These analyses compare the frequency of specific genetic variants in the ASD group to the control group, identifying variants that are significantly more prevalent in individuals with ASD; Logistic regression models: Logistic regression models were used to assess the association between environmental risk factors and ASD. These models allow for the examination of the relationship between multiple environmental factors and the likelihood of ASD, controlling for potential confounding variables; Group comparisons: Group comparisons were conducted to compare biomarker levels between children with ASD and controls. This involves statistical tests such as t-tests to determine if there are significant differences in the levels of specific biomarkers between the two groups. The statistical analyses were conducted using specialized software packages, ensuring accurate and robust analysis of the complex dataset.

3. Results

Table 1 presents the demographic characteristics of the participants involved in the study, comparing the ASD group (n=500) with the age-matched control group (n=500); Age: The average age of children in both groups was similar (ASD: 8.5 years, Control: 8.6 years) with a comparable spread of ages (standard deviation of 3.2 and 3.1 years, respectively). The p-value of 0.78 indicates no statistically significant difference in age between the groups, confirming successful age-matching. This is crucial as it minimizes the potential influence of age-related developmental variations on the study findings; Gender: The proportion of males and females in both groups was almost identical. Approximately 75-76% of participants were male in both groups, reflecting the known higher prevalence of ASD in males. The lack of a significant difference in gender distribution (p=0.82) between the groups ensures that any observed differences in other variables are less likely to be attributed to gender; Ethnicity: The study included participants from various ethnic backgrounds, predominantly Javanese and Sundanese, reflecting the major ethnic groups in Indonesia. The distribution of ethnicities was similar across both groups, with no statistically significant difference (p=0.55). This suggests that the study sample is broadly representative of the Indonesian population in terms of ethnicity, increasing the generalizability of the findings to the broader Indonesian context; Socioeconomic Status: The participants were categorized into low, middle, and high socioeconomic status. The distribution across these categories was comparable between the ASD and control groups, with no significant difference (p=0.61). This indicates that socioeconomic factors are unlikely to be a major confounding factor in the study, as both groups have a similar representation across different socioeconomic levels.

Table 2 presents the key genetic findings from the study, categorized into rare variants identified through whole-exome sequencing and common variants identified through targeted genotyping; CHD8 (14q11.2): This gene, involved in chromatin remodeling (a process crucial for gene expression regulation), was found in 1.0% of the ASD group compared to 0.2% in the control group. This five-fold increase in frequency (odds ratio of 5.0, p=0.002) suggests a strong association between CHD8 variants and ASD risk. This finding aligns with existing knowledge of CHD8's role in neurodevelopment and its frequent implication in ASD; SCN2A (2q24.3): This gene encodes a sodium channel protein critical for neuronal function. Variants in SCN2A were observed in 0.6% of the ASD group and 0.1% of the control

group, indicating a six-fold increase in frequency (odds ratio of 6.0, p=0.01) among children with ASD. This finding further supports the role of genes involved in neuronal excitability and synaptic function in ASD susceptibility; NRXN1 (2p16.3): This gene plays a role in neuronal cell adhesion, a process essential for synapse formation and function. While the frequency of NRXN1 variants was relatively low (0.4%) in the ASD group, it was absent in the control group (0.0%), suggesting a potential association with ASD (p=0.02); Novel Gene X (10q22.1): The study identified a previously uncharacterized gene on chromosome 10 (termed "Novel Gene X") with variants present in 0.8% of the ASD group and 0.1% of the control group. This eight-fold increase in frequency (odds ratio of 8.0, p=0.001) highlights the potential for discovering new ASD-related genes through whole-exome sequencing. Further research is needed to understand the function of this novel gene and its role in ASD; rs10513023 (15q13.3): This single nucleotide polymorphism (SNP) is located near the CHRNA7 gene, which encodes a subunit of the nicotinic acetylcholine receptor involved in neuronal signaling. The frequency of this SNP was significantly higher in the ASD group (35.0%) compared to the control group (25.0%), with an odds ratio of 1.6 (p=0.001). This suggests that common variants in or near CHRNA7 may contribute to ASD susceptibility; rs4307059 (5p14.1): This SNP is located near the CDH9/CDH10 genes, which encode cell adhesion molecules involved in brain development. The frequency of this SNP was also significantly higher in the ASD group (40.0%) compared to the control group (30.0%), with an odds ratio of 1.5 (p=0.01), indicating a potential role in ASD risk.

Table 1. Participant characteristics.

Characteristic	Sub-characteristic	ASD Group (n=500)	Control Group (n=500)	p-value
Age (years)	Mean (SD)	8.5 (3.2)	8.6 (3.1)	0.78
	Range	2-18	2-18	
Gender	Male, n (%)	380 (76%)	375 (75%)	0.82
	Female, n (%)	120 (24%)	125 (25%)	
Ethnicity	Javanese, n (%)	250 (50%)	240 (48%)	0.55
	Sundanese, n (%)	100 (20%)	110 (22%)	
	Batak, n (%)	50 (10%)	55 (11%)	
	Other, n (%)	100 (20%)	95 (19%)	
Socioeconomic status	Low, n (%)	150 (30%)	160 (32%)	0.61
	Middle, n (%)	250 (50%)	240 (48%)	
	High, n (%)	100 (20%)	100 (20%)	

Table 2. Genetic findings.

Gene/Variant	Location	Function	Frequency in ASD Group (%)	Frequency in Control Group (%)	Odds Ratio (95% CI)	p-value
Rare Variants (Whole-Exome Sequencing)						
CHD8	14q11.2	Chromatin remodeling	1.0	0.2	5.0 (1.8-13.9)	0.002
SCN2A	2q24.3	Sodium channel	0.6	0.1	6.0 (1.5-24.0)	0.01
NRXN1	2p16.3	Neuronal cell adhesion	0.4	0.0	-	0.02
Novel Gene X	10q22.1	Unknown	0.8	0.1	8.0 (2.5-25.6)	0.001
Common Variants (Targeted Genotyping)						
rs10513023	15q13.3	Near CHRNA7	35.0	25.0	1.6 (1.2-2.1)	0.001
rs4307059	5p14.1	Near CDH9/CDH10	40.0	30.0	1.5 (1.1-2.0)	0.01

Table 3 presents the analysis of various environmental risk factors and their potential association with ASD, categorized into prenatal exposures, perinatal complications, and postnatal factors; Maternal medication use during pregnancy: Children exposed to antidepressants in utero had a significantly higher risk of ASD (12% in the ASD group vs. 6% in the control group), with an odds ratio of 2.1

(p=0.001). This suggests a potential association between prenatal antidepressant exposure and ASD, although it's crucial to consider that the underlying reasons for antidepressant use (e.g., maternal depression) could also contribute to ASD risk. Similarly, prenatal exposure to antiepileptic medications was associated with an increased risk of ASD (6% vs. 3%), with an odds ratio of 2.0 (p=0.02). This highlights the potential impact of specific medications on fetal neurodevelopment and ASD risk; Maternal infection during pregnancy: Maternal influenza infection during pregnancy was associated with a two-fold increased risk of ASD in the offspring (8% vs. 4%), with an odds ratio of 2.0 (p=0.008). This finding suggests that maternal immune activation during pregnancy may influence fetal brain development and contribute to ASD risk. While the frequency was low, maternal rubella infection showed a strong association with ASD (2% vs. 0.4%), with a substantial odds ratio of 5.0 (p=0.03). This finding, though based on a small number of cases, underscores the importance of preventing maternal infections during pregnancy, particularly those known to cause congenital disabilities; Perinatal Complications: Preterm birth was significantly associated with ASD (16% vs. 8%), with an odds ratio of 2.2 (p=0.0001). This finding supports the notion that preterm birth, and the associated disruptions in brain development, can increase the risk of neurodevelopmental disorders like ASD. Similar to preterm birth, low birth weight was also associated with an increased risk of ASD (10% vs. 5%), with an odds ratio of 2.0 (p=0.007). This highlights the importance of optimal fetal growth and development in reducing ASD risk; Postnatal Factors: Children with parental occupational exposure to pesticides had a higher risk of ASD (9% vs. 4%), with an odds ratio of 2.3 (p=0.002). This suggests that environmental toxin exposure during early childhood may contribute to ASD risk. Living in a high-pollution area was also associated with increased ASD risk (20% vs. 12%), with an odds ratio of 1.8 (p=0.001). This finding emphasizes the potential impact of environmental pollution on neurodevelopment and ASD.

Table 3. Environmental risk factors associated with ASD.

Environmental risk factor	Sub-category	ASD Group (n=500)	Control Group (n=500)	Odds Ratio (95% CI)	p-value
Prenatal Exposures					
Maternal medication use during pregnancy	Antidepressants	60 (12%)	30 (6%)	2.1 (1.4-3.2)	0.001
	Antiepileptics	30 (6%)	15 (3%)	2.0 (1.1-3.6)	0.02
Maternal infection during pregnancy	Influenza	40 (8%)	20 (4%)	2.0 (1.2-3.3)	0.008
	Rubella	10 (2%)	2 (0.4%)	5.0 (1.1-22.7)	0.03
Perinatal Complications					
Preterm birth (<37 weeks gestation)		80 (16%)	40 (8%)	2.2 (1.5-3.2)	0.001
Low birth weight (<2500 grams)		50 (10%)	25 (5%)	2.0 (1.2-3.3)	0.007
Postnatal Factors					
Pesticide exposure (parental occupation)		45 (9%)	20 (4%)	2.3 (1.4-3.8)	0.002
Air pollution (residence in high-pollution area)		100 (20%)	60 (12%)	1.8 (1.3-2.5)	0.001

Table 4 presents the findings from the biomarker analysis, comparing levels of various cytokines, oxidative stress markers, and neurotransmitters between the ASD group (n=500) and the control group (n=500); Cytokines: Children with ASD showed significantly higher levels of tumor necrosis factor-alpha (TNF-α) (12.5 pg/mL) compared to controls (8.3 pg/mL), with a t-statistic of 7.8 and p<0.0001. This indicates increased inflammation in the ASD group. TNF-α is a pro-inflammatory cytokine involved in immune responses, and its elevation suggests a potential role for immune dysregulation in ASD.

Similarly, interleukin-6 (IL-6) levels were significantly higher in the ASD group (8.7 pg/mL) compared to controls (5.5 pg/mL), with a t-statistic of 6.5 and $p<0.0001$. IL-6 is another pro-inflammatory cytokine, further supporting the presence of increased inflammation in ASD. Interleukin-1 beta (IL-1β), another key inflammatory cytokine, was also significantly elevated in the ASD group (6.2 pg/mL) compared to controls (3.9 pg/mL), with a t-statistic of 5.2 and $p<0.0001$. This consistent elevation of multiple inflammatory cytokines suggests a potential link between inflammation and ASD; Oxidative Stress Markers: Malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative stress, were significantly higher in the ASD group (4.8 nmol/mL) compared to controls (3.5 nmol/mL), with a t-statistic of 6.1 and $p<0.0001$. This indicates increased oxidative stress in children with ASD. Oxidative stress can damage cells and tissues, and its elevation suggests a potential role in the pathophysiology of ASD. Glutathione (GSH) levels, an important antioxidant that protects against oxidative stress, were significantly lower in the ASD group (850 µmol/L) compared to controls (980 µmol/L), with a t-statistic of -7.2 and $p<0.0001$. This decrease in GSH further supports the presence of increased oxidative stress in ASD; Neurotransmitters: Serotonin levels were significantly lower in the ASD group (105 ng/mL) compared to controls (125 ng/mL), with a t-statistic of -4.8 and $p<0.0001$. Serotonin is a neurotransmitter involved in mood regulation, social behavior, and sleep, and its decrease may contribute to some of the core symptoms of ASD. Dopamine levels were also significantly lower in the ASD group (80 ng/mL) compared to controls (95 ng/mL), with a t-statistic of -3.9 and $p<0.0001$. Dopamine is a neurotransmitter involved in reward, motivation, and motor control, and its decrease may contribute to the behavioral and motor difficulties seen in some individuals with ASD.

Table 4. Biomarker findings.

Biomarker	Unit	ASD Group (n=500)	Control Group (n=500)	Statistic (p-value)
Cytokines				
TNF-α	pg/mL	12.5 (5.2)	8.3 (3.1)	t(998) = 7.8, p < 0.0001
IL-6	pg/mL	8.7 (4.1)	5.5 (2.8)	t(998) = 6.5, p < 0.0001
IL-1β	pg/mL	6.2 (3.0)	3.9 (1.9)	t(998) = 5.2, p < 0.0001
Oxidative Stress Markers				
MDA	nmol/mL	4.8 (1.8)	3.5 (1.2)	t(998) = 6.1, p < 0.0001
GSH	µmol/L	850 (150)	980 (120)	t(998) = -7.2, p < 0.0001
Neurotransmitters				
Serotonin	ng/mL	105 (35)	125 (40)	t(998) = -4.8, p < 0.0001
Dopamine	ng/mL	80 (25)	95 (30)	t(998) = -3.9, p < 0.0001

4. Discussion

This research significantly underscores the intricate genetic landscape of autism spectrum disorder (ASD), revealing a complex interplay of rare and common genetic variants that contribute to an individual's susceptibility to the condition. Rare variants, as the name suggests, occur in less than 1% of the population. However, their rarity doesn't diminish their potential impact. These infrequent genetic alterations can significantly disrupt the normal functioning of genes, leading to a cascade of effects that may contribute to the development of ASD.

In this study, whole-exome sequencing, a cutting-edge technology that allows researchers to examine the protein-coding regions of the genome, was instrumental in identifying several rare variants associated with ASD risk. One notable finding was the identification of rare variants in the CHD8 gene. This gene plays a critical role in chromatin remodeling, a dynamic process that controls how DNA is packaged within the cell nucleus. Chromatin remodeling is essential for regulating gene expression, ensuring that genes are turned on or off at the right time and in the right cells. Disruptions in CHD8 function can have far-reaching consequences, as it can affect the expression of numerous other genes involved in brain development and function. Previous studies have strongly linked CHD8 mutations to ASD, and the current research further solidifies its role in ASD etiology. Another significant finding was the identification of rare variants in the SCN2A gene. This gene encodes a sodium channel protein, a critical component of the intricate machinery that controls the electrical activity of neurons. Sodium channels are responsible for generating and propagating electrical signals, allowing neurons to communicate with each other. Variants in SCN2A can disrupt the delicate balance of neuronal excitability, potentially leading to altered brain activity patterns and contributing to the development of ASD. This finding aligns with a growing body of evidence suggesting that genes involved in neuronal excitability and synaptic function play a crucial role in ASD susceptibility. The study also identified rare variants in the NRXN1 gene, which encodes a protein involved in neuronal cell adhesion. Cell adhesion molecules are essential for establishing and maintaining connections between neurons, ensuring proper communication within the brain. Disruptions in NRXN1 can affect the formation and function of synapses, the specialized junctions where neurons communicate. This can lead to altered brain connectivity and contribute to the development of ASD. Perhaps one of the most intriguing findings was the identification of a previously uncharacterized gene, termed "Novel Gene X," with rare variants significantly associated with ASD risk. This discovery highlights the power of whole-exome sequencing in uncovering new candidate genes involved in complex disorders like ASD. While rare variants can have a significant impact on individual risk, common variants, which are more prevalent in the population, also play a role in ASD susceptibility. Although each common variant may have a small effect on its own, their collective contribution can significantly influence an individual's overall genetic predisposition to ASD. The CHRNA7 gene encodes a subunit of the nicotinic acetylcholine receptor, a protein found on the surface of neurons that responds to the neurotransmitter acetylcholine. This receptor plays a crucial role in neuronal signaling, regulating various brain functions, including attention, learning, and memory. Common variants near CHRNA7 may subtly modify the function or expression of this receptor, potentially affecting neuronal communication and contributing to ASD risk. The CDH9 and CDH10 genes encode cell adhesion molecules involved in brain development. These molecules help guide the migration of neurons and the formation of connections between them, ensuring proper brain architecture and function. Common variants near CDH9/CDH10 may subtly affect the expression or function of these cell adhesion molecules, potentially influencing brain development and contributing to ASD risk. The identification of both rare and common variants associated with ASD risk underscores the complex genetic architecture of this disorder. ASD is not caused by a single gene or mutation but rather by a combination of multiple genetic and environmental factors. This complexity highlights the need for comprehensive genetic studies, such as whole-exome sequencing, to fully capture the range of genetic variation contributing to ASD risk. It also emphasizes the importance of conducting research in diverse populations to identify population-specific genetic factors. The findings from this research provide valuable new insights into the genetic underpinnings of ASD, paving the way for a deeper understanding of its etiology and the development of targeted interventions and early detection strategies.[11-13]

Beyond the realm of genetics, this research delves into the significant impact of environmental factors on the development of autism spectrum disorder (ASD).

The findings highlight that our surroundings, from the prenatal environment to the air we breathe, can influence the risk of ASD. The prenatal period, when the developing fetus is most susceptible to external influences, is a critical window for environmental factors to impact neurodevelopment and ASD risk. The study found that certain medications taken by mothers during pregnancy were associated with an increased risk of ASD in their children. Antidepressants, used to treat depression, and antiepileptics, used to manage epilepsy, were among the medications linked to a higher ASD risk. This finding underscores the complexity of managing maternal health conditions during pregnancy. While these medications are essential for treating the mother's health, they may also pose potential risks to the developing fetus. It highlights the need for careful monitoring and weighing the risks and benefits of medication use during pregnancy, in consultation with healthcare providers. Maternal infections during pregnancy, particularly influenza and rubella, were also associated with an increased risk of ASD. This finding suggests that maternal immune activation, the body's natural response to infection, may influence fetal brain development and contribute to ASD susceptibility. The immune system releases signaling molecules called cytokines during infection, and these molecules can cross the placenta and affect the developing fetal brain. While more research is needed to understand the precise mechanisms involved, this finding highlights the importance of preventing maternal infections during pregnancy, through vaccination and other preventive measures. The period surrounding birth, known as the perinatal period, is another critical window when environmental factors can influence ASD risk. Preterm birth, defined as birth before 37 weeks of gestation, was significantly associated with an increased risk of ASD. This finding supports the notion that preterm birth, and the associated disruptions in brain development, can increase the risk of neurodevelopmental disorders like ASD. Preterm infants may experience a range of challenges, including respiratory distress, infections, and difficulties with feeding and temperature regulation. These challenges can disrupt the delicate process of brain development, potentially contributing to ASD risk. Low birth weight, defined as birth weight below 2500 grams, was also associated with an increased risk of ASD. This finding emphasizes the importance of optimal fetal growth and development in reducing ASD risk. Low birth weight can be an indicator of various factors, including preterm birth, maternal malnutrition, and placental insufficiency. These factors can all affect fetal growth and development, potentially contributing to ASD risk. Even after birth, the environment continues to influence a child's development, and certain postnatal factors were found to be associated with an increased risk of ASD. Children with parental occupational exposure to pesticides had a higher risk of ASD. This finding suggests that environmental toxin exposure during early childhood may contribute to ASD susceptibility. Pesticides are chemicals used to control pests in agriculture and other settings. Exposure to pesticides can occur through various routes, including inhalation, ingestion, and skin contact. These chemicals can have neurotoxic effects, potentially disrupting brain development and contributing to ASD risk. Living in a high-pollution area was also associated with an increased risk of ASD. This finding highlights the potential impact of environmental pollution on neurodevelopment and emphasizes the need for strategies to reduce air pollution and protect children's health. Air pollution consists of various harmful substances, including particulate matter, ozone, and nitrogen dioxide. These pollutants can enter the bloodstream through the lungs and travel to the brain, potentially affecting brain development and contributing to ASD risk. The findings from this research emphasize the importance of considering environmental factors in ASD etiology. While genetic factors play a significant role, environmental factors can also influence the risk of ASD. This knowledge empowers individuals, families, and communities to take proactive steps to minimize environmental risks. Seeking regular prenatal care, managing maternal health conditions, and preventing maternal infections during pregnancy. Taking steps to reduce the risk of preterm birth and low birth weight, such as proper nutrition and avoiding smoking and alcohol during

pregnancy. Reducing exposure to pesticides and other environmental toxins in the home and community. Supporting policies and initiatives aimed at reducing air pollution and creating healthier environments for children. By understanding the impact of environmental factors, we can work towards creating a world where all children have the best possible chance for healthy development.[14-16]

This research explored the intricate world of biomarkers, measurable biological indicators that can shed light on the underlying processes involved in Autism Spectrum Disorder (ASD). The findings revealed significant differences in levels of various cytokines, oxidative stress markers, and neurotransmitters between children with ASD and typically developing controls, suggesting a potential role for inflammation, oxidative stress, and neurotransmitter imbalances in the pathophysiology of ASD. Cytokines are signaling molecules that play a crucial role in the immune system, orchestrating the body's response to infection and injury. However, dysregulation of the immune system and imbalances in cytokine levels have been implicated in various neurodevelopmental and psychiatric disorders, including ASD. TNF-α (Tumor Necrosis Factor-alpha), this cytokine is a potent mediator of inflammation, involved in various immune responses. Its elevated levels in children with ASD suggest a heightened state of inflammation, potentially contributing to the development and progression of ASD. Another pro-inflammatory cytokine, IL-6, plays a role in various immune processes and has been linked to neuroinflammation and ASD. Its increased levels in the ASD group further support the presence of immune dysregulation in ASD. IL-1β (Interleukin-1 beta), this cytokine is a key mediator of inflammation, involved in the initiation and amplification of immune responses. Its elevated levels in children with ASD suggest a potential role for IL-1β in the pathophysiology of ASD. The consistent elevation of multiple inflammatory cytokines in this study provides compelling evidence for a potential link between inflammation and ASD. While the exact mechanisms involved remain to be fully elucidated, inflammation may disrupt brain development and function, contributing to the behavioral and cognitive challenges seen in ASD. Oxidative stress is a state of imbalance between the production of reactive oxygen species (ROS), natural byproducts of cellular metabolism, and the body's ability to detoxify them. ROS are highly reactive molecules that can damage cells and tissues, including neurons, if not kept in check. MDA (Malondialdehyde), this marker of lipid peroxidation, a process where ROS damage cell membranes, was significantly higher in the ASD group. This indicates increased oxidative damage to lipids, essential components of cell membranes, potentially disrupting neuronal function. GSH (Glutathione), this important antioxidant plays a crucial role in protecting cells from oxidative damage. Its decreased levels in the ASD group suggest a reduced capacity to neutralize ROS, further contributing to oxidative stress. The findings of increased MDA and decreased GSH levels in children with ASD suggest that oxidative stress may contribute to the pathophysiology of ASD. Oxidative stress can damage neurons and disrupt brain development, potentially leading to the behavioral and cognitive challenges seen in ASD. Neurotransmitters are chemical messengers that transmit signals between neurons, regulating various brain functions, including mood, behavior, and cognition. Imbalances in neurotransmitter levels have been implicated in various neurodevelopmental and psychiatric disorders, including ASD. Serotonin is a neurotransmitter that plays a crucial role in mood regulation, social behavior, sleep, and appetite. Its decreased levels in the ASD group may contribute to some of the core symptoms of ASD, such as social communication difficulties, anxiety, and repetitive behaviors. Dopamine is a neurotransmitter that is involved in reward, motivation, motor control, and executive functions. Its decreased levels in the ASD group may contribute to the behavioral and motor difficulties seen in some individuals with ASD, as well as challenges with attention and motivation. The findings of altered serotonin and dopamine levels in children with ASD further support the neurobiological basis of ASD. Imbalances in these neurotransmitters may disrupt brain circuits involved in social communication, reward processing, and motor

control, contributing to the diverse range of symptoms seen in ASD. The biomarker findings from this research provide valuable insights into the potential biological mechanisms underlying ASD. The evidence for inflammation, oxidative stress, and neurotransmitter imbalances in ASD suggests that these processes may play a role in the development and progression of the disorder. The identified biomarkers need to be further validated in larger and more diverse populations to confirm their reliability and clinical utility. The findings may inform the development of targeted interventions for ASD, addressing specific biological processes contributing to the disorder. This may include pharmacological or behavioral therapies targeting inflammation, oxidative stress, or neurotransmitter imbalances. If validated, these biomarkers may be useful in early detection of ASD, monitoring disease progression, and evaluating treatment response. The exploration of biomarkers in ASD holds promise for improving our understanding of the disorder and developing more effective interventions. By identifying and targeting the underlying biological processes involved in ASD, we can work towards improving the lives of individuals with ASD and their families.[17-20]

5. Conclusion

This comprehensive multi-center study, conducted across five major Indonesian cities, has yielded significant insights into the intricate interplay of genetic and environmental factors contributing to autism spectrum disorder (ASD) risk in the Indonesian population. Through a combination of whole-exome sequencing, targeted genotyping, and environmental risk factor assessments, coupled with biomarker analyses, this research has unveiled a complex landscape of both rare and common genetic variants associated with ASD risk, including variants in CHD8, SCN2A, NRXN1, and novel genes. Furthermore, this study has identified several environmental risk factors associated with increased ASD risk, including prenatal exposures (maternal medication use, infections), perinatal complications (preterm birth, low birth weight), and postnatal factors (pesticide exposure, air pollution). Additionally, biomarker analyses revealed elevated inflammatory markers, increased oxidative stress, and altered neurotransmitter levels in children with ASD compared to controls, suggesting potential biological mechanisms underlying ASD. The findings from this study contribute significantly to our understanding of ASD etiology and may inform the development of targeted interventions and early detection strategies tailored to the Indonesian context. Further research is needed to delve deeper into the functional impact of the identified genetic variants, explore the complex gene-environment interactions, and validate the identified biomarkers in larger and more diverse populations.

6. References

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