Original Article

Role of Autoimmune Regulator Protein (AIRE-P) in the Pathophysiology of Autism Spectrum Disorder (ASD)in Saudi Children

Hayfa Ali Al-Ghabban[1*], Laila Yusuf Al-Ayadhi[2]

[1]Department of Physiology, Faculty of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia. [2]Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia & Autism Research and Treatment Center, Riyadh, Saudi Arabia.

Abstract

Numerous studies show the significance of immunological deficits, abnormal cytokine and immune cell production, and autism. Through the use of the childhood autism rating scale (CARS), the social responsiveness scale (SRS), and the computerized Cambridge neuropsychological test automated battery (CANTAB), the current study aimed to investigate the potential role of the autoimmune regulator protein AIRE-p as an immune biomarker in the pathophysiology of autism in Saudi children.

According to the study's findings, plasma levels of AIRE-p in 37 autistic children (n=37) were considerably (p=0.003) lower than those in 37 healthy controls (n=37) at 0.629 (0.776) pg/ml [median (IQR)]. Based on CARS ratings, there was no difference between AIRE-p levels in children with mild to moderate autism (median, 0.661 (0.666) pg/ml; interquartile range, 0.365 (1.114) pg/ml; p = 0.365) and children with severe autism (median, 0.365 (1.114) pg/ml; interquartile range, 0.365). On the basis of SRS, a comparable pattern between mild to moderate and severe autism was also seen. AIRE-p may be involved in the physiology of autism as shown by the reduced AIRE-p plasma levels in patients with ASD. However, unless more studies are carried out using bigger sample sizes to ascertain if the drop in AIRE-p plasma level is only a side effect of autism or whether it plays a pathogenic role in the condition, these results should be viewed with care. If AIRE-p levels may be employed as a biomarker for ASD, further research including larger patient and control cohorts would be required.

Keywords: Autism spectrum disorder, Autoimmune regulator protein, Autoimmunity, Childhood autism rating scale, Cambridge neuropsychological test automated battery, Childhood neurodevelopmental disorder

Introduction

Early childhood neurodevelopmental disorder known as autism spectrum disorder (ASD) is characterized by a shared spectrum of social interaction, qualitative impairments, and communication deficits in varying degrees. It is also characterized by noticeably restricted interests and repetitive behaviors [1]. The disease starts developing about the time a child turns three and persists throughout life. There is growing evidence and concern that abnormal immune responses contribute significantly to the pathophysiology and pathogenesis of ASD [2]. The pathogenesis of ASD is still unknown, and there are no definitive biochemical or clinical indicators for identifying ASD, despite significant research efforts over the last several years. Numerous studies have examined proteome patterns in the peripheral blood of ASD patients in search of possible biomarkers [3]. Numerous factors are thought to be involved in the development of ASD, including genetic, infectious, neurological, metabolic, environmental, and immunological factors [4]. The neurological cause of ASD is still unknown, and further research is needed to understand the significance of immunological abnormalities and autoimmune diseases.

The relationships between the immunological and nervous systems are well understood and have been the subject of much investigation. Numerous studies have shown that autism suffers from immune system abnormalities, including irregular synthesis of cytokines, immune cells, and antibodies [5]. Numerous neuroactive substances have also been shown to have potent immunomodulatory effects and to be crucial in the development of illness [6, 7].

Address for correspondence: Hayfa Ali Al-Ghabban, Department of Physiology, Faculty of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia. ghabbana@yahoo.com

How to cite this article: Al-Ghabban HA, Al-Ayadhi LY. Role of Autoimmune Regulator Protein (AIRE-P) in the Pathophysiology of Autism Spectrum Disorder (ASD)in Saudi Children. Arch Pharm Pract. 2023;14(3):83-90. https://doi.org/10.51847/GUzYRK8poy

By detecting markers suggesting autoimmune, as well as cytokines and other biomarkers that signal pro- and inflammatory states, many studies have connected autism to autoimmunity. Additionally, they found a correlation between illness severity and blood levels of autoantibodies and pro-inflammatory indicators in certain Saudi autistic individuals [8, 9].

AIRE-p, a transcription factor found on chromosome 21, promotes autoimmunity by controlling the expression of promiscuous genes (pGE) [10]. By aiding the negative selection of T cells in the thymus, creating the thymic microarchitecture, and generating a particular fraction of regulatory T cells, AIRE-p plays a crucial part in modeling central immunological tolerance [11]. Additionally, AIRE-p inhibits autoimmunity by encouraging the development of self-antigens in medullary thymic epithelial cells, causing developing T cells that identify these self-antigens to undergo clonal deletion in the thymus [12]. However, since the AIRE-p gene causes severe organ-specific autoimmune diseases [13], it is one of the finest choices to explain the complicated picture of autoimmunity [14].

AIRE-p has a significant impact in the expression of a number of tissue-restricted Ags (TRAs) in medullary thymic epithelial cells (mTEC), according to a research using a mouse model [15]. The negative selection process, in which self-reactive thymocytes with enhanced affinity for TRAs are eliminated before they enter peripheral blood circulation, depends on the expression of TRAs. Therefore, any alteration in AIRE-p levels may have complicated repercussions on thymic selection. It could result in a rise in the prevalence of autoimmune symptoms [16].

Previous research suggested that other elements interact with AIRE-p and support its activity. Abramson *et al*.'s investigation discovered two AIRE-p complexes that controlled the pre-mRNA processing of tissue-restricted antigens (TRAs) and one complex that was based on DNA-dependent PDNA-dependent protein Kinase (DNAPK), which seemed to increase TRA transcription [17, 18]. Another work revealed a cascade regulatory mechanism for these proteins by demonstrating how AIRE-p functions via the gene node Gucy2d, which is connected to the TRAs in medullary thymic epithelial cells (mTECs) [19]. In contrast, a research found that the transactivation of AIRE-p is inhibited by the interaction between AIRE-p and the DAXX protein [20]. As a result, interactions with other variables may be necessary for AIRE-p to transactivate.

Numerous autoimmune disorders and neurological illnesses have been said to be primarily caused by AIRE gene mutations and protein abnormalities [21]. In order to prevent autoimmunity, testosterone upregulates AIRE-p p-mediated thymic tolerance, according to Zhu *et al*. [22]. They discovered that men express more thymic AIRE-p than females, which suggests that the administration of androgens and male gender prevent autoimmunity in a multiple sclerosis mouse model in a way that is AIRE-dependent.

Additionally, it was thought that AIRE-p dysregulation, which reduces promiscuous gene expression (pGE), promotes autoimmunity in Down syndrome (DS) [23]. In thymus samples from DS patients and controls, the expression of AIRE-p and numerous peripheral tissue-specific Ag genes was evaluated. When DS individuals were compared to controls, it was shown that AIRE-p expression was drastically decreased by two times.

This study's goal was to investigate AIRE-p's potential function in ASD patients. Never before has the function of AIRE-p in the pathophysiology of autism been discussed. There hasn't yet been a single research that evaluated the AIRE-p levels in autistic people. We hypothesized that AIRE-p could play a pathogenic role in inflammation and autoimmune disorders given that autoimmunity has been shown to be one of the pathophysiological causes of autism and given its role in the transcription of a wide variety of self-antigens to start the negative selection process in the thymus. These details have motivated us to look for AIRE-p as a possible biomarker that might help identify ASD sooner.

Materials and Methods

Participants

In the current study, 37 ASD patients with ages ranging from 2 to 12 (mean SD = 5.47 2.52 years) from the King Saud University in Riyadh, Saudi Arabia, as well as 37 age- and sex-matched healthy children from the pediatric clinic at the King Saud Medical City in Riyadh, Saudi Arabia, made up the control group. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) was used to make the diagnosis of ASD [24]. The research excluded participants who had epileptic seizures, obsessive-compulsive disorder, affective disorders, fragile X syndrome, or any other mental or neurological conditions. The current research was authorized by the IBR Committee of the King Khalid Hospital at King Saud University in Riyadh, Saudi Arabia. All registered individuals had their parents or legal guardians sign informed written permission on their behalf.

CARS, or the Childhood Autism Rating Scale

Autism severity was evaluated using CARS [25]. It assigns scores for 15 different symptoms or dimensions (such as activity level, object use, body use, imitation, verbal and nonverbal communication, emotional response, relation to others, response to listening, nervousness or fear, reliability and intellectual response level, adaptation to changes, visual responses, responses to touch, smell, and taste, and general impressions) to children ranging in age from one to four. Scores below 30 on this measure strongly suggest the presence of autism. Children with scores between 30 and 36.5

had mild to moderate autism, whereas those with values between 37 and 60 had severe autism [26].

SRS, or the Social Responsiveness Scale

The SRS is a validated assessment of communication, interpersonal behavior, and stereotyped characteristics in autism. In order to separate clinically relevant ASD from other mental diseases with varied degrees of social impairment, it is employed as a diagnostic tool. There are five subscales that make up this scale: social awareness, social cognition, social communication, social motivation, and autistic mannerisms. Total SRS raw values vary from 0 to 195, with a score of 195 representing a profound social impairment as seen in ASD patients. A clinical diagnosis of autism is highly correlated with a score of 76 or above, which is considered severe. The mild-to-moderate range of social impairment is defined as a score between 60 and 75 [27].

Automated Battery for the Cambridge Neuropsychological Test (CANTAB)

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a touch-screen computer-based cognitive assessment system that includes a battery of neuropsychological tests that are given to individuals. Among the 25 tests in CANTAB, general memory and learning, working memory and executive function, visual memory, attention and reaction time (RT), semantic/verbal memory, decision-making, and response control are some of the cognitive functions that are tested.

Five subtest models—the Big/Little Circle (BLC), Intra/Extra Dimensional Set Shift (IED), Simple Reaction Time (SRT), Spatial Recognition Memory (SRM), and Motor Screening (MOT)—were used to evaluate the participants' performance on attention and memory tasks. Numerous investigations in the area of autism have employed CANTAB [28].

Collection of Blood Samples

After an overnight fast, blood was taken from 37 children with ASD and 37 similarly aged healthy children. For blood collection, blood was drawn into 3 mL EDTA-containing tubes. Following blood collection, the samples were centrifuged for 20 min. at 4 °C and 3000 g. The plasma was held at 80 °C prior to analysis. A commercial sandwich ELISA kit (Cusabio Biotech Co. Ltd., Wuhan, China) was used to assess the levels of AIRE-p in the plasma of autistic participants in accordance with the manufacturer's recommendations. The mean results of each biochemical analysis were reported after being carried out in triplicate. There was no discernible interference or cross-reactivity.

Analytical Statistics

Software from SPSS Inc., Chicago, Illinois, USA, called Statistical Package for Social Sciences, version 21, was used to analyze the data. Normality The Kolmogorov-Smirnov test was used to determine if the findings were normal. The Mann-Whitney test was employed to compare the non-parametric data. The median and interquartile range (IQR; 25th to 75th percentile) of the results were shown. The independent- samples t-test was used to the normally distributed data to compare the means between the control group and the autistic group. The result was described using descriptive statistics (mean and standard deviation). The Pearson correlation coefficient (r) was used to ascertain the relationship between the various variables (CARS, SRS, and CATAB). In order to explain any relationships between the biomarkers and clinical events by computing odds ratios (OR), multivariate logistic regression analysis of the biomarkers is utilized. A p-value of 0.05 or below was used to determine statistical significance for all tests.

Additionally, a Receiver Operating Characteristics (ROC) curve analysis for the assessment of biomarkers was performed. The area under the curve (AUC), cutoff values, and degree of specificity and sensitivity were all calculated. Predictiveness curves were also used to explain how the scores were distributed.

Results and Discussion

Table 1 summarizes the overall characteristics of the research subjects, plasma levels of AIRE-p in autistic (n=37) and normal children (n=37), as well as CARS and SARS scores. The results are shown as the median and IQR. AIRE-p levels were examined between autistic kids with various degrees of autism (mild, moderate, or severe), as well as controls. The recorded CARS scores of the ASD participants, which varied from 30 to 65, were used to categorize them. Children with autism's social traits were also noted by their SRS score.

The plasma level of AIRE-p in autistic children (n = 37) was considerably lower (p= 0.003) than that of normal children (1.144 (0.938) pg/ml).

Table 1. Plasma levels of AIRE-p protein in children with ASD and Controls with their relationship with Autism severity.

	Groups	N	AIR-p		P value
			Median	IQR	
All Patients	Control	37	1.144	0.938	0.003
	ASD	37	0.629	0.776	
CARS	Mild to Moderate	31	0.661	0.666	0.365
	Severe	6	0.365	1.114	

SRS	Mild to Moderate	10	0.585	0.995	0.639
	Severe	14	0.639	0.690	

AIRE-p and Childhood Autism Rating Scale (CARS)

The results of the childhood autism rating scale are shown in **Table 1**. Thirty-one individuals (83.80%) had mild to moderate autism according to the CARS scoring system, while six people (16.20%) had severe autism according to the mean (40.42 3.65) scores. According to the CARS score, there was no discernible difference between the plasma AIRE-p levels of severe autism (0.365 (1.114) pg/ml) and mild to moderate autism (0.661 (0.666) pg/ml).

The AIRE-p and the Social Responsiveness Scale (SRS)

Table 1 shows the findings of the social responsiveness scale. Using the SRS scoring system, it was determined that 10 individuals (41.70%) had mild to moderate impairment in social skills (mean: 71.30 2.11), and 14 subjects (58.30%) had severe impairment (mean: 78.50 3.11). Due to an age issue, 13 individuals with autism did not match the requirements for using the SRS. Based on the SRS score, there was no discernible difference in plasma AIRE p concentration between severe autism (0.639 (0.690) pg/ml) and mild to moderate autism (0.585 (0.995) pg/ml).

AIRE-p and the Automated Cambridge Neuropsychological Test Battery (CANTAB)

In this research, memory, attention, speed of reaction, and visual discrimination patterns were the five cognitive tasks examined. Median (IQR) values were used to display the results. AIRE-p levels and CANTAB cognitive tests did not significantly correlate in the ASD group compared to the control group. AIRE-p levels and The IED te 1 of CANTAB cognitive tests were found in the group of people with mild to moderate ASD, and they showed a significant positive association according to the CARS scoring system (CC=0.401, P = 0.028) **(Table 2)**.

Table 2. Plasma levels of AIRE-p in Autistic Children subgroups based on CARS scoring scale in Relation to CANTAB Cognitive Measures.

CARS	Parameters	R (Spearman Correlation Coefficient)	P value
Mild to Moderate	Age	-0.263	0.169
	BLC	0.057	0.763
	MOT	-0.022	0.907
	SRT (Mean)	-0.266	0.156
	SRT (Max)	-0.280	0.134
	SRT Correct Percent	-0.182	0.336
	SRT Correct Trials	-0.208	0.269
	IED te 1	0.401*	0.028
	IED te 2	-0.066	0.728
	SRM	0.078	0.684
Severe	Age	0.400	0.600
	BLC	0.000	1.000
	MOT	0.700	0.188
	SRT (Mean)	-0.410	0.493
	SRT (Max)	-0.410	0.493
	SRT Correct Percent	0.205	0.741
	SRT Correct Trials	-0.410	0.493
	IED te 1	-0.300	0.624
	IED te 2	0.700	0.188
	SRM	-0.667	0.219

"AIRE-p levels are not shown in the table but were taken into consideration.
* Correlation is significant at the 0.05 level.

Additionally, in the mild to moderate subgroup, a significant negative connection between AIRE-p levels and the SRT (mean) (CC=-0.730, P= 0.017) and the SRT (max) (CC=-0.644, P= 0.044) of CANTAB cognitive tests was discovered. This was by the ASD social character measured by the SRS social scale.

Additionally, a connection between the MOT test and ASD severe subgroups was positive (CC= 0.631, P= 0.015) **(Table 3)**.

Table 3. Plasma levels of AIRE-p in Autistic Children subgroups based on the SRS scoring scale in Relation to CANTAB Cognitive Measures.

SRS	Parameters	R (Spearman Correlation Coefficient)	P value
Mild to Moderate	Age	-0.314	0.377
	BLC	-0.626	0.053
	MOT	-0.018	0.960
	SRT (Mean)	-0.730*	0.017
	SRT (Max)	-0.644*	0.044
	SRT Correct Percent	-0.219	0.544
	SRT Correct Trials	-0.399	0.254
	IED te 1	0.437	0.207
	IED te 2	0.382	0.276
	SRM	0.165	0.648
Severe	Age	-0.047	0.879
	BLC	-0.009	0.976
	MOT	0.631*	0.015
	SRT (Mean)	-0.234	0.421
	SRT (Max)	-0.234	0.421
	SRT Correct Percent	-0.234	0.421
	SRT Correct Trials	-0.328	0.253
	IED te 1	-0.035	0.904
	IED te 2	0.031	0.916
	SRM	-0.293	0.310

"AIRE-p levels are not shown in the table but were taken into consideration.
*Correlation is significant at the 0.05 level.

The ROC curve applied for AIRE-p demonstrated 70.3 % Sensitivity and a Specificity of 67.6 %. The area under the curve was 0.700, which is considered a satisfactory value of accuracy with high specificity and sensitivity (P= 0.003) **(Table 4, Figure 1).** The predictive curve of AIRE-p is shown in **Figure 2.**

Table 4. ROC Curve for Autism group in AIRE-p

The area under the curve	Cutoff value	Sensitivity %	Specificity %	p-value
0.700	0.927	70.3 %	67.6 %	0.003

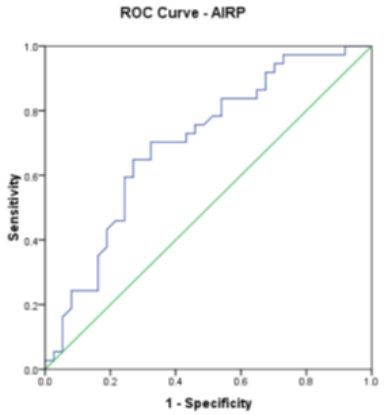

Figure 1. ROC Curve of AIRE-p for Autism group

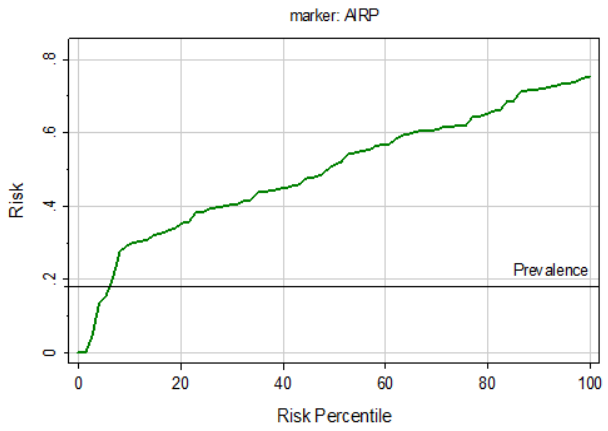

Figure 2. Predictiveness Curve of AIRE-p in ASD Group.

Research on novel biomarkers for ASD has been focused on peripheral tissues like blood. One of the most important analytical methods for identifying illnesses and categorizing people to benefit from certain pharmacotherapies is the use of blood biomarkers [29].

This research looked at AIRE-p as a biomarker for early autism diagnosis. AIRE-p has been associated to autoimmune disorders in people with type 1 diabetes and rheumatoid arthritis, according to prior studies [30, 31].

AIRE-p is a transcription factor that is involved in a number of biological processes, although it is unclear what pathophysiological function AIRE-p serves in ASD. The current study's findings suggest that the overexpression of AIRE-p in individuals with ASD may be related to autoimmune processes connected to microglial activation in ASD.

Numerous studies have shown evidence of inflammation in the brain and CNS, including notable microglia activation and elevated cytokine production in postmortem brain tissues from both young and elderly people with ASD. Additionally, large population population-based epidemiological studies [32, 33] have identified links between ASD and a family history of autoimmune diseases, Major Histocompatibility Complex (MHC) haplotypes association, and abnormal levels of various inflammatory cytokines and immunological markers in the blood.

AIRE-p is a vital protein in the maintenance of immunological tolerance because it plays a crucial role in T-cell negative selection [34]. The possible role of AIRE-p as an autoimmune inflammatory marker/protector biomarker for brain tissue was investigated and evaluated in the present research. Additionally, as a potential pathophysiological explanation of autism in Saudi children, the potential existence of a continuing immunological response was investigated.

We may learn more about the pathophysiology of ASDs by looking for different biomarkers to gauge the degree of social and cognitive deficits and other traits associated with autism [35]. The current research investigated the relationship between plasma AIRE-p levels and several social and behavioral measures (CARS SRS and CANTAB) in ASD-affected children. According to the findings of our investigation, the plasma level of AIRE-p in the ASD group was substantially lower than that in the control group (median 0.629, IQR 0.776 vs. 1.144, IQR 0.938; p = 0.003).

Although there was no significant correlation between plasma AIRE-p levels and CARS (r=0.024, p=0.88) or SRS (r=-0.003, p=0.94) scores among ASD patients, this suggests AIRE-p may not be connected to the severity or course of the condition. Additionally, ASD subgroups based on CARS and SRS scores showed a connection between AIRE-p levels and cognitive performance.

AIRE-p level or function variations have been linked to more prevalent autoimmune illness, according to a recent study [36]. Autoimmune disorders are more likely to strike individuals with a single gene mutation on only one AIRE allele at any point in their life [37]. Furthermore, several investigations have linked the AIRE gene mutation and altered protein function to a variety of autoimmune diseases, including rheumatoid arthritis and type 1 diabetes mellitus [31, 38].

According to the results of our investigation, it may be inferred that AIRE-p levels in autistic persons' plasma may be low due to thymic cell degeneration. This decline might result in the loss of both its significant function in T-cell negative selection and its function in the apoptotic process [11]. Additionally, the presence of self-reactive immunological T-cells in peripheral blood may raise the risk of the onset of an autoimmune response that might harm developing brain tissue [39]. As a result, it may result in changed social behavior, cognitive performance, and behavioral changes. Clarifying AIRE- p's role in peripheral organs might provide light on its participation in the onset or development of ASD since the precise function of AIRE- p in secondary lymphoid organs is still unclear. Therefore, further structural research is required to comprehend this recently suggested possible relationship.

AIRE-p's ROC curve showed 70.3% Sensitivity and 67.6% Specificity. Area under the curve: 0.700 (fair test), 0.003 (p-value). This curve and the related AUC demonstrated that AIRE-p may be used as a reliable predictive marker to distinguish the group of people with ASD from healthy individuals. Additionally, the predictiveness curve for AIRE-p shows sufficient predictive ability. This finding highlights the potential for employing AIRE-p plasma levels as a marker for early ASD case identification. Last but not least, the OR for AIRE-p is statistically significant (OR= 0.166, P= 0.002), indicating that this protein may operate as a protective factor

and that any decrease in its level may increase the risk of acquiring autistic characteristics as a result of autoimmune dysregulation.

As far as we are aware, AIRE-p's involvement in autism has never been researched. This is the first research to examine plasma AIRE-p levels in autistic children and their relationship to CARS, SRS, and CANTAB. To compare our findings with previous research, we were unable to find information in the literature on the function of the AIRE-p protein in a single group of autistic kids. Our results, however, were in line with other studies that found lower levels of AIRE- p in people with Down syndrome (DS) [23]. Although it is important to use care when interpreting our results, they do indicate that AIRE-p may have a role in ASD.

Given that AIRE-p is essential for autoimmunity, the current study's lower AIRE-p levels might have an adverse impact on ASD patients' anti-inflammatory activity in the brain since this activity relies on the availability of AIRE-p.

We might draw the conclusion that CNS inflammation in ASD patients may be caused by reduced plasma levels of AIRE-p. Potential Research is being done on the possible importance of AIRE-p in controlling inflammatory reactions. But it's probable that more processes than common pathogenic pathways are involved in the biology behind the AIRE-p relationship. This work offers proof that, in contrast to ASD sufferers, normal controls may likewise benefit from greater AIRE-p levels. Additionally, it supports the hypothesis that altered neurodevelopment may be related to reduced AIRE-p levels in ASD.

Since AIRE-p levels are modest, any changes in AIRE-p levels may indicate an abnormal immunological response given the protective immune role that these proteins provide while maintaining immune tolerance. It is probably going to result in the survival of auto-reactive T cells, which then starts auto-inflammation in ASD.

According to the first findings of this research, the preliminary findings are promising, and there appears to be suggestive evidence in favor of AIRE-p contributions to the pathophysiology of autism. It is necessary to do further research to build on these findings. Considering that AIRE-p plays a crucial part in autoimmunity in various disorders [31, 38]. our According to our research, reduced levels of AIRE-p may be a factor in the autoimmunity-induced autoinflammation response in ASD. To establish the AIRE-p's applicability and demonstrate its diagnostic efficacy in identifying ASD individuals at risk of rapid illness development, more research on a broader sample is necessary.

Limitations

The sample size and cross-sectional design of the current research are only two of its drawbacks. Thus, association rather than prediction or causality was discovered. This study's further flaw is that the control subjects' adaptive behavior was not evaluated. Therefore, it is unknown if AIRE-p level variations may be related to behavioral traits in healthy people. These results present preliminary direct evidence of altered AIRE-p protein in individuals with ASD, which may contribute to the disease's early development, provide useful biomarkers, and suggest potential therapy approaches.

Conclusion

In comparison to the control group, children with ASD were shown to have considerably decreased AIRE-p levels. According to our study, AIRE-p may contribute to the neuroinflammation brought on by autoimmunity in ASD. This protein may also be a potential for future studies on the molecular causes of ASD or the identification of ASD biomarkers. AIRE-p alterations in peripheral blood may also be useful for diagnostic and/or therapeutic purposes. To investigate AIRE-p's function in ASD, higher sample sizes are required for the studies. To ascertain whether the decline in AIRE-p levels is a direct result of autism or plays a pathogenic function in the condition, more research with a larger subject group is necessary.

ACKNOWLEDGMENTS: The text was revised by Dr. Nadra Elyass and Dr. Dost Muhammad Halepoto of the King Saud University's Autism Research and Treatment Center.
CONFLICT OF INTEREST: None
FINANCIAL SUPPORT: The National Plan for Science, Technology, and Innovation, King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award number: AI-35-355, provided the funding for this research.
ETHICS STATEMENT: The current research was authorized by the IBR Committee of the King Khalid Hospital at King Saud University in Riyadh, Saudi Arabia. All registered individuals had their parents or legal guardians sign informed written permission on their behalf. All procedures were carried out in compliance with the 1964 Helsinki Declaration and its subsequent revisions or equivalent ethical standards, as well as the ethical requirements of the institutional and/or national research committee.

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Arlington; 2013. 991 p. Available from: http://encore.llu.edu/iii/encore/record/C__Rb1280248__SDSM-V__P0,2__Orightresult__X3;jsessionid=ABB7428ECBC4BA66625EDD0E0C5AAFA5?lang=eng&suite=cobalt%5Cnhttp://books.google.com/books?id=EIbMlwEACAAJ&pgis=1
2. Thabtah F, Peebles D. Early Autism Screening: A Comprehensive Review. Int J Environ Res Public Health. 2019;16(18):3502. Available from: https://www.mdpi.com/1660-4601/16/18/3502
3. Pichitpunpong C, Thongkorn S, Kanlayaprasit S, Yuwattana W, Plaingam W, Sangsuthum S, et al. Phenotypic subgrouping and multi-omics analyses reveal reduced diazepam-binding inhibitor (DBI) protein levels in autism spectrum disorder with severe language impairment. Jacobs JM, editor. PLoS One. 2019;14(3):e0214198. doi:10.1371/journal.pone.0214198

4. Genovese A, Butler MG. Clinical Assessment, Genetics, and Treatment Approaches in Autism Spectrum Disorder (ASD). Int J Mol Sci. 2020;21(13):4726. Available from: https://www.mdpi.com/1422-0067/21/13/4726
5. Matta SM, Hill-Yardin EL, Crack PJ. The influence of neuroinflammation in Autism Spectrum Disorder. Brain Behav Immun. 2019;79:75-90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0889159118307025
6. Gray WA, Billock VA. Developmental neurotoxicity and autism: A potential link between indoor neuroactive pollutants and the curious birth order risk factor. Int J Dev Neurosci. 2017;62(1):32-6. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1016/j.ijdevneu.2017.07.004
7. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral D, Van De Water J. Autoantibodies in Autism Spectrum Disorders (ASD). Ann N Y Acad Sci. 2007;1107(1):79-91.
8. AlAyadhi LY, Hashmi JA, Iqbal M, Albalawi AM, Samman MI, Elamin NE, et al. High-resolution SNP genotyping platform identified recurrent and novel CNVs in autism multiplex families. Neuroscience. 2016;339:561-70. doi:10.1016/j.neuroscience.2016.10.030
9. El-Ansary A, Al-Ayadhi L. Lipid mediators in plasma of autism spectrum disorders. Lipids Health Dis. 2012;11(1):1.
10. Perniola R. Twenty years of AIRE. Front Immunol. 2018;9(FEB):98.
11. Conteduca G, Indiveri F, Filaci G, Negrini S. Beyond APECED: An update on the role of the autoimmune regulator gene (AIRE) in physiology and disease. Autoimmun Rev. 2018;17(4):325-30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568997218300296
12. Zhao B, Chang L, Fu H, Sun G, Yang W. The Role of Autoimmune Regulator (AIRE) in Peripheral Tolerance. J Immunol Res. 2018;2018.
13. Bruserud Ø, Oftedal BE, Wolff AB, Husebye ES. AIRE-mutations and autoimmune disease. Curr Opin Immunol. 2016;43:8-15.
14. Fierabracci A. Recent insights into the role and molecular mechanisms of the autoimmune regulator (AIRE) gene in autoimmunity. Autoimmun Rev. 2011;10(3):137-43. doi:10.1016/j.autrev.2010.08.019
15. Kont V, Laan M, Kisand K, Merits A, Scott HS, Peterson P. Modulation of Aire regulates the expression of tissue-restricted antigens. Mol Immunol. 2008;45(1):25-33.
16. Wang HX, Pan W, Zheng L, Zhong XP, Tan L, Liang Z, et al. Thymic Epithelial Cells Contribute to Thymopoiesis and T Cell Development. Front Immunol. 2020;10(January):1-10.
17. Abramson J, Giraud M, Benoist C, Mathis D. Aire's partners in the molecular control of immunological tolerance. Cell. 2010;140(1):123-35.
18. Abramson J, Husebye ES. Autoimmune regulator and self-tolerance - molecular and clinical aspects. Immunol Rev. 2016;271(1):127-40.
19. Macedo C, Evangelista AF, Magalhães DA, Fornari TA, Linhares LL, Junta CM, et al. Evidence for a network transcriptional control of promiscuous gene expression in medullary thymic epithelial cells. Mol Immunol. 2009;46(16):3240-4.
20. Meloni A, Fiorillo E, Corda D, Incani F, Serra ML, Contini A, et al. DAXX is a new AIRE-interacting protein. J Biol Chem. 2010;285(17):13012-21.
21. Zhu W, Hu Z, Liao X, Chen X, Huang W, Zhong Y, et al. A new mutation site in the AIRE gene causes autoimmune polyendocrine syndrome type 1. Immunogenetics. 2017;69(10):643-51.
22. Zhu ML, Bakhru P, Conley B, Nelson JS, Free M, Martin A, et al. Sex bias in CNS autoimmune disease mediated by androgen control of autoimmune regulator. Nat Commun. 2016;7(1):11350. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022202X20322764
23. Giménez-Barcons M, Casteràs A, Armengol M del P, Porta E, Correa PA, Marín A, et al. Autoimmune Predisposition in Down Syndrome May Result from a Partial Central Tolerance Failure due to Insufficient Intrathymic Expression of AIRE and Peripheral Antigens. J Immunol. 2014;193(8):3872-9.
24. American Psychiatric Association. Autism Spectrum Disorder. Am Psychiatr Assoc Washington. 2013;(October):2012-3.
25. Schopler E, Reichler R, Renner B. The Childhood Autism Rating Scale. Los Angeles, CA: Western Psychological Services; 1988.
26. Moon SJ, Hwang JS, Shin AL, Kim JY, Bae SM, Sheehy-Knight J, et al. Accuracy of the Childhood Autism Rating Scale: a systematic review and meta-analysis. Dev Med Child Neurol. 2019;61(9):1030–8.
27. Constantino JN, Gruber CP. Social Responsiveness Scale. Vol. 2. Los Angeles, CA: Western Psychological Services; 2012. 3-5 p. Available from: http://www.wpspublish.com/store/p/2994/social-responsiveness-scale-second-edition-srs-2
28. Cambridge Cognition. CANTAB The most sensitive and validated cognitive research software available. Cambridge Cognition. Cambridge Cognition. 2020. Available from: https://www.cambridgecognition.com/cantab.
29. Al-Ayadhi L, Halepoto DM. Role of proteomics in the discovery of autism biomarkers. J Coll Physicians Surg Pak. 2013;23(2):137-43.
30. Nermeen NH, Mansour MF, Omar HH, Fouad MM, Metwally L, El-Abaseri TB, et al. Association of autoimmune regulator gene polymorphism with susceptibility to rheumatoid arthritis in Egyptian population. Immunol Res. 2020;68(2):90-6.
31. Fornari TA, Donate PB, MacEdo C, Marques MMC, Magalhães DA, Passos GAS. Age-related deregulation of Aire and peripheral tissue antigen genes in the thymic stroma of non-obese diabetic (NOD) mice is associated with autoimmune type 1 diabetes mellitus (DM-1). Mol Cell Biochem. 2010;342(1-2):21-8.
32. Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Prochazka V, et al. Immunological and autoimmune considerations of Autism Spectrum Disorders. J Autoimmun. 2013;44:1-7.
33. Heidari A, Rostam–Abadi Y, Rezaei N. The immune system and autism spectrum disorder: association and therapeutic challenges. Acta Neurobiol Exp (Wars). 2021;81(3):249-63. Available from: https://www.exeley.com/acta_neurobiologiae_experimentalis/doi/10.21307/ane-2021-023
34. Marx A, Yamada Y, Simon-Keller K, Schalke B, Willcox N, Ströbel P, et al. Thymus and autoimmunity. Semin Immunopathol. 2021;43(1):45-64.
35. El-Ansary A, Hassan WM, Qasem H, Das UN. Identification of biomarkers of impaired sensory profiles among autistic patients. PLoS One. 2016;11(11):1-19.
36. Anderson MS, Su MA. AIRE expands: new roles in immune tolerance and beyond. Nat Rev Immunol. 2016;16(4):247-58. doi:10.1038/nri.2016.9
37. Oftedal BE, Hellesen A, Erichsen MM, Bratland E, Vardi A, Perheentupa J, et al. Dominant Mutations in the Autoimmune Regulator AIRE Are Associated with Common Organ-Specific Autoimmune Diseases. Immunity. 2015;42(6):1185-96.
38. Shao S, Li XR, Cen H, Yin ZS. Association of AIRE polymorphisms with genetic susceptibility to rheumatoid arthritis in a Chinese Population. Inflammation. 2014;37(2):495-9.
39. Khan U, Ghazanfar H. T Lymphocytes and Autoimmunity. Int Rev Cell Mol Biol. 2018;341:125-68.