Revista ARACÊ
ISSN: 2358-2472

ASSOCIATION BETWEEN IL-1, IL-6, TGF-β AND IFN-γ SINGLE NUCLEOTIDE POLYMORPHISMS AND AUTISM SPECTRUM DISORDER: SYSTEMATIC REVIEW AND METANALYSIS

https://doi.org/10.56238/arev6n3-286

Date of submission: 20/10/2024 **Date of publication:** 20/11/2024

Maraiza Alves de Oliveira1, Renata Rocha da Silva2, Veruska Moreira de Queiroz3, Lucas Sousa Magalhães4, Débora dos Santos Tavares5, Camilla Natália Oliveira Santos6, Rosana de Souza Siqueira Barreto7 and Priscila Lima dos Santos8

ABSTRACT

Autism spectrum disorders (ASDs) constitute a group of neurodevelopmental disorders with increasing incidence. Cytokine changes may affect the integrity of the central nervous system, contributing to neuroinflammation and behavioral symptoms in patients with ASD. Single nucleotide polymorphisms (SNPs) can lead to immune disorders such as this. Therefore, the aim of the present study was to perform a systematic review and meta-analysis to determine whether cytokine SNPs are more common in individuals with ASD. A systematic search of seven databases was conducted on September 19, 2023. Odds ratios (ORs) were used in the meta-analysis to test the association between each SNP and ASD. Six studies were included. Four cytokines were investigated: interleukin-1 (IL-1), IL-6, transforming growth factor beta (TGF-β) and interferon-γ (IFN-γ). Although some studies alone have associated genotypic and allelic variants with ASD, the pooled results of the studies were not statistically significant in the meta-analysis. Our systematic review highlights important gaps and inspires new studies to investigate significant associations between genetic variations in different cytokines and the development of ASD.

Keywords: Autistic Disorder. Single Nucleotide Polymorphisms. Behavioral Symptoms. IL-1. IL-6. TGF. IFN-γ.

1 Master in Applied Health Sciences
Postgraduate Program in Health Sciences, Federal University of Sergipe.
2 Master in Health Sciences
Federal University of Sergipe.
3 Master in Nutrition Sciences
Federal University of Sergipe.
4 PhD in Health Sciences
Federal University of Alagoas
5 PhD in Materials Science-Biomaterials
Federal University of Sergipe.
6 PhD in Health Sciences
Federal University of Sergipe
7 PhD in Health Sciences
Federal University of Sergipe
8 PhD in Health Sciences
Postgraduate Program in Health Sciences, Federal University of Sergipe.

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impairments in social interaction, communication, and stereotypical patterns of behavior and interests[1]. The incidence has increased significantly in recent years[2]. At least one in every hundred children worldwide has autism[3].

It is a multifactorial disorder with an etiology associated with genetic, epigenetic, and environmental factors, such as parental conditions and intrauterine and perinatal factors. Hundreds of genes are involved in ASD, resulting in a spectrum with different phenotypes, including language and social deficits, along with several associated subphenotypes[4].

Immune disorders, especially neurological development deficits, seem to be prominent in the pathogenesis of ASD, especially in neurological development deficits[5]. Cytokines can alter the functional and structural integrity of the central nervous system (CNS), contributing to neuroinflammation and influencing behavior[6]. Altered levels of cytokines, such as interleukin-1 (IL-1), IL-2, IL-6, tumor necrosis factor-α (TNF-α), TNF-β and interferon-γ (IFN-γ), have been found in individuals with autism[7].

IL-1β, an important proinflammatory cytokine, is expressed during crucial early stages of development. However, increased IL-6 levels in autistic children are positively correlated with autism severity, behavioral abnormalities, and increased IL-6 production[8]. IL-6 and IFN-γ are related to neurodevelopment. Reduced levels contribute to normal physiological development, whereas elevated levels are associated with neuroinflammatory processes that can cause neural abnormalities[9]. Transforming growth factor beta (TGF-β) is considered a crucial regulator of CNS development. However, studies suggest that there is a decrease in TGF-β1 levels in the serum of patients with autism[10,11]. Lower levels are associated with worse behavioral symptoms[11].

Understanding the factors that increase susceptibility to ASD and its development can improve patients' quality of life and treatment[2]. Therefore, single nucleotide polymorphisms (SNPs) in cytokine genes can modify genetic transcription and cytokine secretion[12], thus influencing the behavioral symptoms of patients with ASD. Studies that evaluate genetic changes in patients with ASD analyze SNPs. However, the relationship between cytokine gene SNPs and autism is unclear[13]. Therefore, the objective of the present study was to carry out a systematic review and meta-analysis to determine whether cytokine SNPs are more common in individuals with ASD.

Revista ARACÊ
ISSN: 2358-2472

MATERIAL AND METHODS

The present study consists of a systematic review and meta-analysis performed and reported following the Meta-analysis Of Observational Studies in Epidemiology (MOOSE)[14] statement and the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines[15].

SURVEY QUESTION AND ELIGIBILITY CRITERIA

The guiding question of the research was “Are inflammatory cytokine polymorphisms more frequent in children with ASD?” To answer this question, case–control studies that evaluated the presence of polymorphisms in neurotypical individuals (control group) and in individuals with ASD (group) were selected. Family-based studies, editorials, comments and opinions, reflection articles, projects, technical reports, reviews, and articles that did not answer the question were excluded from this review.

DATA SEARCH STRATEGY

A systematic search of the Embase, PubMed, Web of Science, Scopus, Science Direct, and Google Scholar databases was carried out to identify all relevant publications without language restrictions. The search strategy was structured with the following terms: (Interleukin OR Chemokines) AND ("Autistic Disorder" OR Autism OR TEA) AND (“Single Nucleotide Polymorphism” OR Polymorphism OR variant OR gene).

STUDY SELECTION

Two reviewers (MAO and RRS) independently examined the search results and identified potentially relevant studies on the basis of the article titles and abstracts. The selected articles were read in full, and only the relevant ones, consistent with the eligibility criteria, were included in our study. Any disagreements were resolved after discussion with a third reviewer (PLS). Reference lists of the retrieved studies were consulted to identify additional relevant studies.

DATA EXTRACTION

Data extraction from the manuscripts was carried out in accordance with the eligibility criteria, and the extracted data were recorded in a spreadsheet. The following information was extracted from each article: name of the author(s), year of publication, total

Revista ARACÊ
ISSN: 2358-2472

number of subjects (cases/controls), country where the research was carried out, and cytokines and SNPs. Genotype and allele counts for cases/controls, along with p values, are listed.

QUALITY ASSESSMENT

The Newcastle–Ottawa Scale (NOS)[16] was used to evaluate the methodological quality of the selected studies. Two independent reviewers (MAO and the RSS) carried out the validity assessment. All discrepancies were resolved by discussion between the two reviewers. The NOS scale is based on three perspectives: selection of study groups (maximum of one star for each column), comparability of groups (maximum of two stars) and exposure (maximum of one star for each column). Studies that received a score equal to or greater than 6 were considered high quality and included in the review.

STATISTICAL ANALYSIS

Data were extracted from full-text articles into structured tables containing all the descriptive variables and relevant outcomes. Information such as author, country and year of study; number of cases, controls, and polymorphisms studied; and the OR and p value of each group was collected.

Odds ratios (ORs) were used in the meta-analysis to test the association between each SNP and autism spectrum disorder in case–control studies; 95% confidence intervals were included, and p values < 0.05 were considered statistically significant. Five genetic models for each SNP corresponding to dominant, recessive, homozygote, heterozygote, and allelic modes of inheritance were investigated. Heterogeneity was evaluated via the chi-square-based Cochran's Q statistic and the I2 index. When high heterogeneity ($p < 0.1$ for the Q test or $I2 > 50\%$) was detected, the random-effects model was employed for meta-analysis; otherwise, a fixed-effects model was applied.

Sensitivity analysis was performed by excluding individual studies from the pooled ORs and recalculating the statistical significance to assess the stability of findings and to ascertain whether final pooled effect sizes were affected by a single publication. Pearson's chi-square test was applied to examine Hardy–Weinberg equilibrium (HWE) in the healthy control group. HWE was reached if $p > 0.05$. For all the analyses, Review Manager Version 5.3 software (Cochrane Collaboration, Copenhagen) was used.

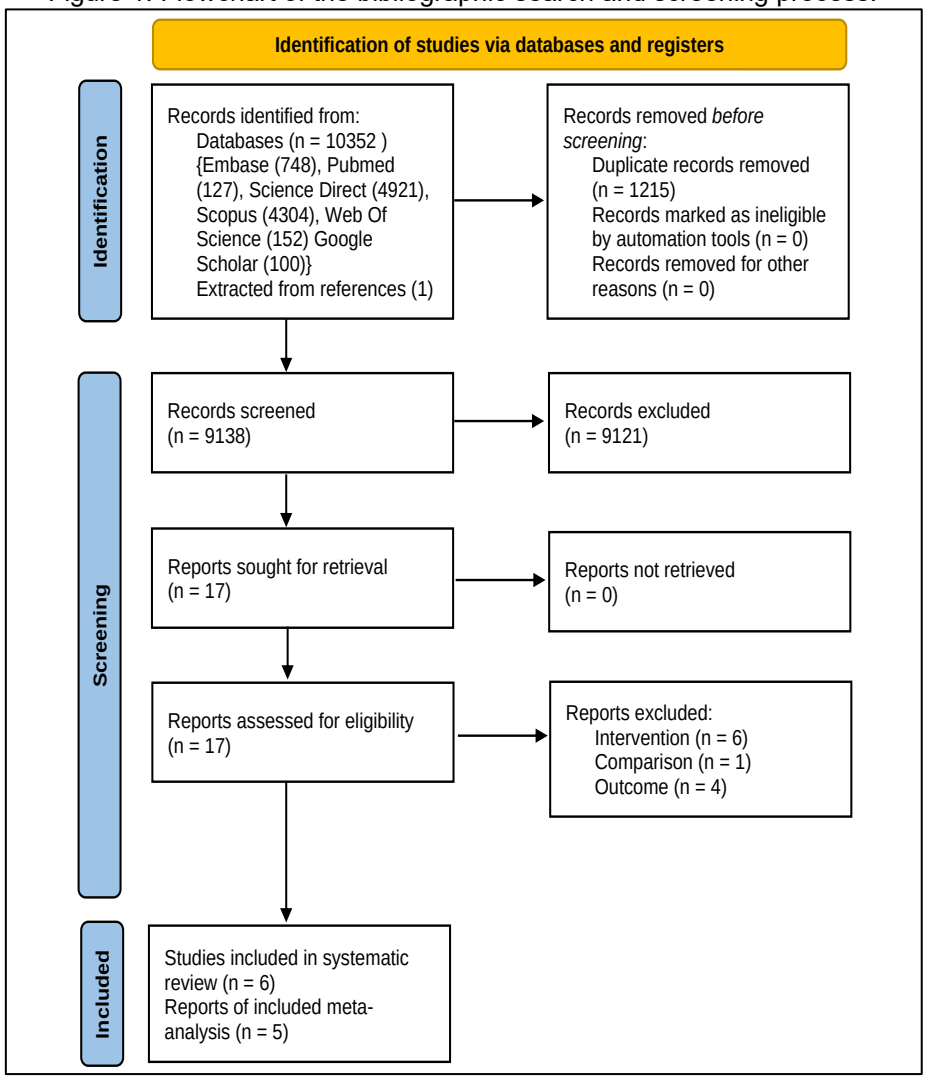
Revista ARACÊ
ISSN: 2358-2472

RESULTS

STUDY SELECTION

The search yielded 10,352 potentially relevant records. Of these, 748 were retrieved from Embase, 127 from PubMed, 4,921 from Science Direct, 4,304 from Scopus, 152 from Web of Science and the top 100 from Google Scholar. Additionally, one article was retrieved from a reference. After the titles and abstracts were analyzed, 17 full texts were assessed for eligibility, and 6 (six)[2,8,17–20] were included in the review. The flowchart of the study selection process and the specific reasons for exclusion are detailed in Figure 1.

Figure 1: Flowchart of the bibliographic search and screening process.



The selected studies were published between 2015 and 2022. Two studies were carried out in Iraq[18,20], one in China[2], Egypt[8], Iran[19] and one in Turkey[17]. The study populations ranged between 74[19] and 350[2] participants. Studies have evaluated

Revista ARACÊ
ISSN: 2358-2472

polymorphisms in IL-1β[2,8,17], IL-6[2,17], IFN-γ[20] and TGF-β1[18,19]. Table 1 summarizes the characteristics of the studies included in the systematic review. Table 2 shows their genotypic and allele frequencies.

Table 1. Characteristics of the included studies.

Study (Year)	Country	Cases	Control	Cytokine	Polymorphisms studied
Han et al (2021)	China	98	252	IL-1β	16944
Han et al (2021)	China	98	252	IL-6	1800796
Kaleel et al (2020)	Iraq	94	100	IFN-γ	2430561
Khakzad et al (2015)	Iran	39	35	TGF-β1	1982073
Khakzad et al (2015)	Iran	39	35	TGF-β1	1800471
Saad et al (2020)	Egypt	80	60	IL-1β	16944
Saad et al (2020)	Egypt	80	60	IL-1β	1143627
Smail et al (2020)	Iraq	40	40	TGF-β1	1982073
Smail et al (2020)	Iraq	40	40	TGF-β1	1800471
Uyanik et al (2022)	Turkey	65	62	IL-1β	1143634
Uyanik et al (2022)	Turkey	95	84	IL-6	1800796

Table 2. Genotypic and allelic frequencies present in the studies.

Study	Cases					Control					HWE p value
16944											
Han et al	AA	AG	GG	A	G	AA	AG	GG	A	G	
	28	51	19	107	89	67	140	45	274	230	0.0575
Saad et al	TT	TC	CC	T	C	TT	TC	CC	T	C	
	4	45	31	56	104	11	31	18	88	32	0.7124
1982073	TT	TC	CC	T	C	TT	TC	CC	T	C	
Khakzad et al	20	13	6	53	25	11	17	7	39	31	0.9259
Smail et al	11	25	4	47	33	13	21	6	47	33	0.5989
1800796											
Han et al	CC	CG	GG	C	G	CC	CG	GG	C	G	
	48	43	7	139	57	116	118	18	350	154	0.1008
	CC	CG	GG	C	G	CC	CG	GG	C	G	
Uyanik et al	56	36	3	148	42	61	21	2	143	25	0.941
1800471	GG	GC	CC	G	C	GG	GC	CC	G	C	
Khakzad et al	27	11	1	65	13	30	4	1	64	6	0.1091
Smail et al	32	6	2	70	10	16	18	6	50	30	0.8003
2430561											
Kaleel et al	TT	TA	AA	T	A	TT	TA	AA	T	A	
	19	3	72	22	75	15	52	33	67	85	0.4543
1143627	CC	CT	TT	C	T	CC	CT	TT	C	T	
Saad et al	20	35	25	84	76	14	27	19	66	54	0.4679
1143634	CC	CT	TT	C	T	CC	CT	TT	C	T	
Uyanik et al	30	34	1	94	36	37	18	7	92	32	0.0569

Revista ARACÊ
ISSN: 2358-2472

il-1β

Polymorphisms in IL-1β were investigated at the SNPs rs16944, rs1143627 and rs1143634. Saad et al. analyzed the rs16944 SNP for IL-1β-511 and detected significantly higher frequencies of homozygous (CC), heterozygous (TC) and allele (C) genotypic variants in the ASD group than in the control group (OR: 2.36, 95% CI: 1.06–5.18; OR: 2.04, 95% CI: 1.06–38; p = .039, respectively)[8]. In contrast, Han et al. reported no significant differences in the genotypic and allelic frequencies of polymorphisms between the ASD and control groups (p > 0.05)[2].

In the case of the rs1143634 polymorphism investigated by Uyanik et al., the TT variant genotype and the T allele were not associated with ASD (p > 0.05), but CT carriers were associated with an increased risk of developing ASD (OR: 2.330, 95% CI: 1.104–4.918; p = 0.02)[17]. On the other hand, when rs1143627 was analyzed for IL-1β-31C/T, Saad et al. reported no significant difference between the groups, neither for alleles nor for genotypes (p > 0.05)[8].

il-6

The IL-6 polymorphism was investigated at SNP rs1800796 in two studies[2,17]. The first study revealed no significant differences in the genotypic and allelic frequencies of the IL-6-572C/G polymorphism between the ASD and TD groups[2]. In contrast, another study associated GC carriers with an increased risk of developing ASD (OR: 1.867, 95% CI: 0.976–3.573; p = 0.05). However, the authors also reported no significant difference between the case and control groups for the alleles and other genotypes[17].

tgf-β

Two studies[18,19] investigated two TGF-β polymorphisms, the SNPs rs1982073 and rs1800471. Both studies did not detect a significant association between genotypes or alleles and ASD (p >0.05) when rs1982073 was analyzed. However, when rs1800471 was analyzed, the studies presented different results. While one[19] did not significantly differ, another[18] highlighted a significantly greater G allele frequency in the patient group than in the control group (OR: 4.200, 95% CI: 1.882–9.372; p = 0.0003) and a significant association in the recessive model (GG vs. GC/CC: OR: 6.00, 95% CI: 2.206–16.32; p = 0.0003).

Revista ARACÊ
ISSN: 2358-2472

ifn-ɣ

rs2430561 of IFN-ɣ +874A/T was analyzed in only one study[20]. The authors reported a greater number of T alleles (p = 0.00) in the control group and a greater number of AA genotypes in the ASD group. However, the work did not present statistical analyses of the genotype, making it difficult to infer significance.

QUALITY OF THE STUDIES

As depicted in Table 3, all studies included in this systematic review had good overall methodological quality, with NOS scores ranging from 6 to 7.

Table 3: Risk of bias of the included studies in the systematic review using the Newcastle–Ottawa (NOS) quality assessment scale for case–control studies.

Study	Selection				Comparability	Exposure		
	1	2	3	4	1	1	2	3
Han et al	☆	-	☆	☆	☆☆	☆	☆	
Kaleel et al	☆	-	☆	☆	☆☆	☆	-	-
Khakzad et al	☆	-	-	☆	☆☆	☆	☆	-
Saad et al	☆	☆	-	☆	☆☆	☆	☆	-
Smail et al	☆	-	-	☆	☆☆	☆	☆	
Uyanik et al	☆	☆	☆	☆	☆	☆	☆	-

Selection: 1. Adequate case definition; 2. Representativeness of the cases; 3. Selection of controls; 4. Definition of controls. Comparability: 1. Comparability of cases and controls on the basis of the design or analysis. Exposure: 1. Ascertainment of exposure; 2. The same method of ascertainment for cases and controls; 3; Nonresponse rate.

METANALYSIS

rs16944 polymorphism

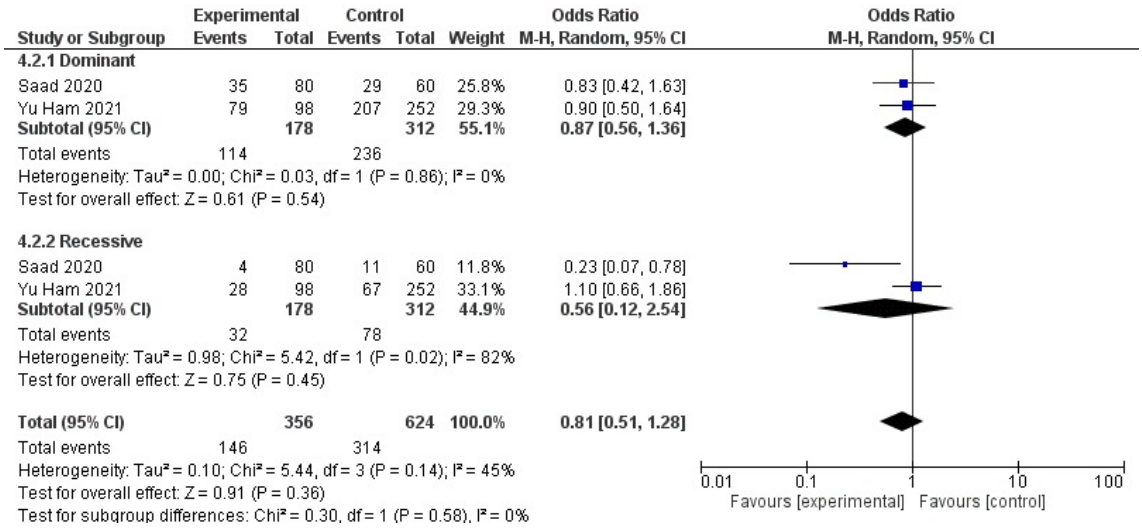
Overall, two studies[2,8] involving 178 cases and 312 healthy controls were found to be eligible for inclusion in the quantitative analysis of the association between the rs16944 SNP and autism spectrum disorder. The pooled OR revealed no significant associations between the rs16944 SNP and autism spectrum disorders across all the genotype models, including the dominant model (OR 0.87, 95% CI 0.56–1.36, P = 0.86), recessive model (OR 0.56, 95% CI 0.12–2.54, P = 0.02), allelic model (OR 0.45, 95% CI 0.09–2.25, P = <00001), homozygote model (OR 0.5, 95% CI 0.11–2.27, P = 0.04), and heterozygote model (OR 1.68, 95% CI 0.38–7.38, P = 0.03). The analyses of the recessive and heterozygous

Revista ARACÊ
ISSN: 2358-2472

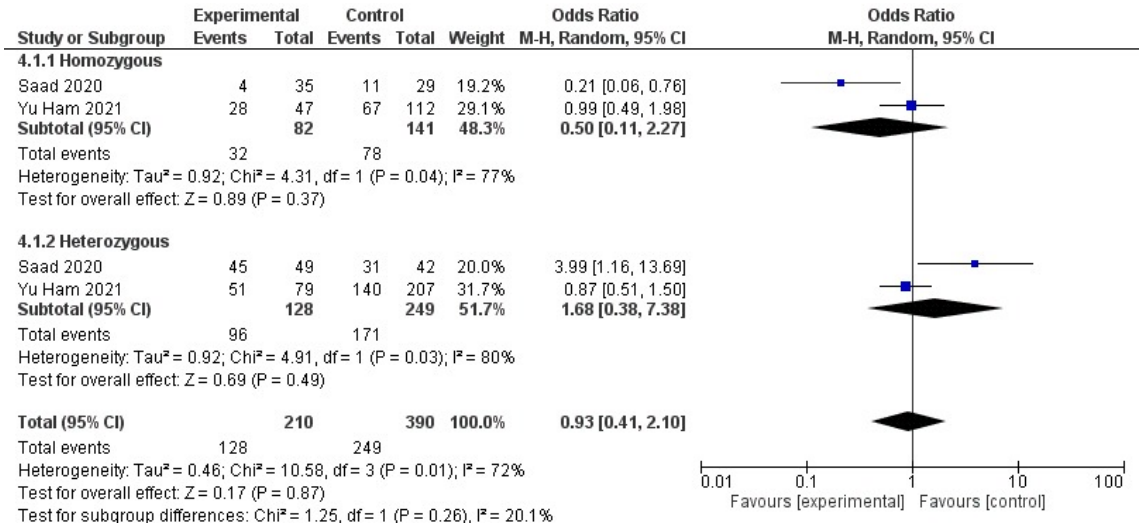
genotype groups revealed a moderate degree of heterogeneity (I^2 82%; I^2 80%, respectively) (Figure 2).

Figure 2: Pooled odds ratios and 95% confidence intervals for the association between the rs16944 and the development of ASD.

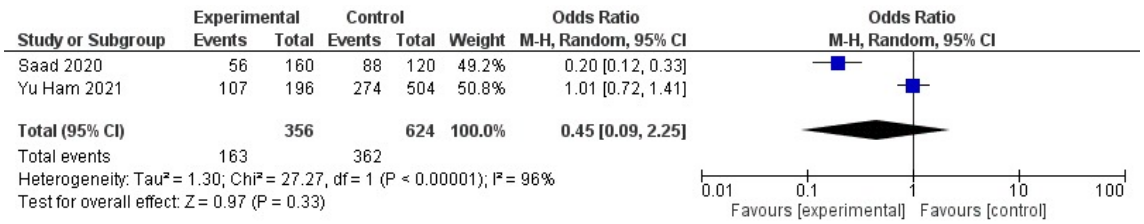
a)



b)



c)



(a) Dominant and recessive models. (b) Homozygous and heterozygous models. (c) Allelic model.

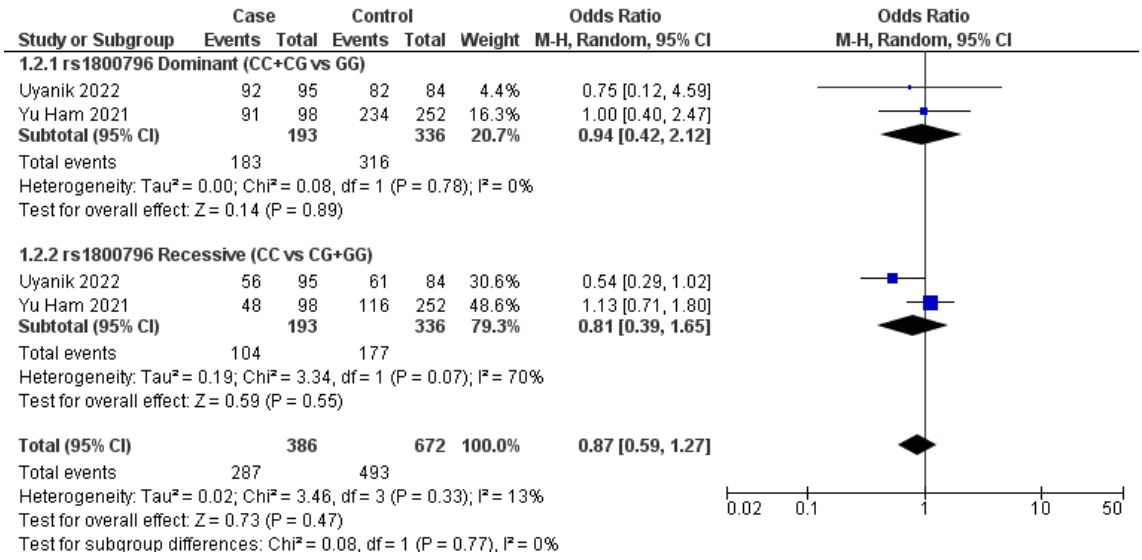
Revista ARACÊ
ISSN: 2358-2472

rs1800796 polymorphism

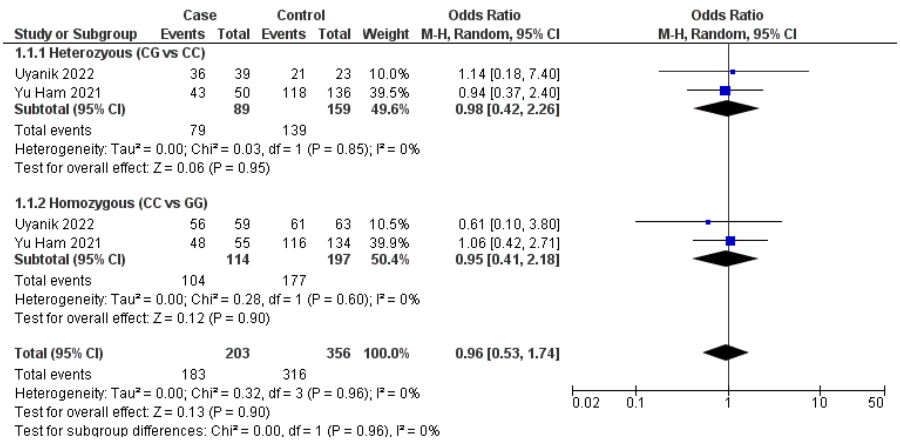
Two studies[2,17] involving 193 cases and 336 healthy controls were considered eligible and included for quantitative analysis of the association between the IL-6 SNP rs1800796 and ASD. The pooled OR revealed no significant association between the rs1800796 SNP and the risk of developing ASD in any of the genotype models, including the dominant model (OR 0.94, 95% CI 0.42–2.12; P = 0. 78), recessive (OR 0.39–1.65, P = 0.07), allelic (OR 0.85, 95% CI 0.49–1.45, P = 0.10), homozygous (OR 0.95, 95% CI 0.41–2.18, P = 0.60) and heterozygous (OR 0.98, 95% CI 0.42– 2.26, P = 0.85). The analyses of the recessive genotype and allele groups revealed a moderate degree of heterogeneity (I^2 70%; I^2 64%, respectively) (Figure 3).

Figure 3: Pooled odds ratios and 95% confidence intervals for the association between the rs1800796 and the development of ASD.

a)

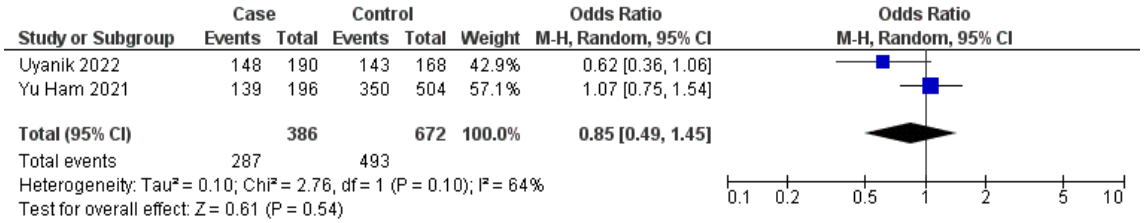


b)



Revista ARACÊ
ISSN: 2358-2472

c)



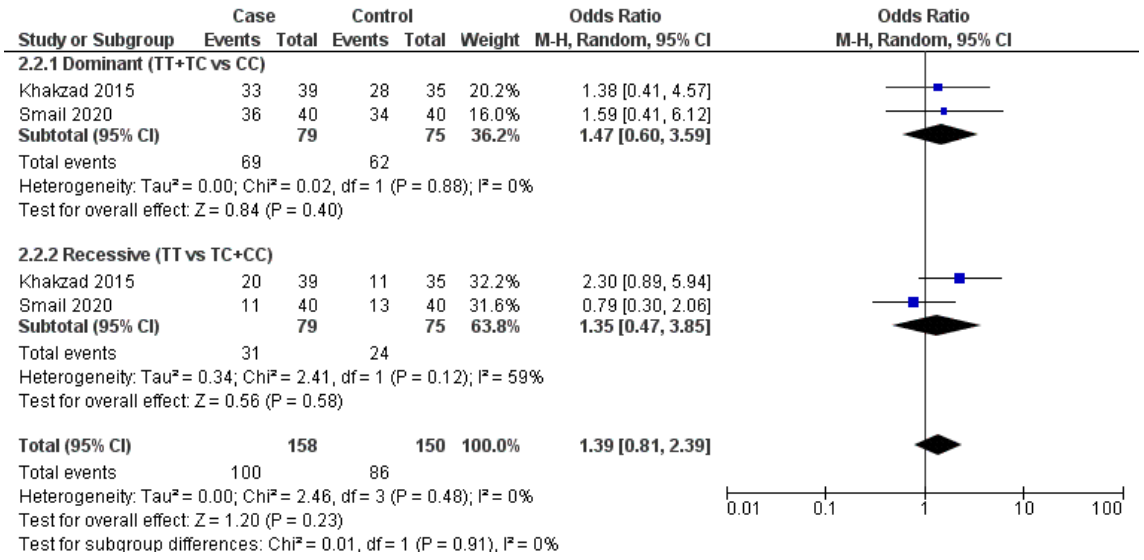
(a) Dominant and recessive models. (b) Homozygous and heterozygous models. (c) Allelic model.

rs1982073 and rs1800471 polymorphisms

Two studies[18,19] involving 79 cases and 75 healthy controls were considered eligible and included for quantitative analysis of the association between TGF-β1 polymorphisms and ASD. There was no significant association between the rs1982073 SNP and ASD patients in any of the analyzed models, including the dominant model (OR 1.47, 95% CI 0.60–3.59, P = 0.88), recessive model (OR 1.35, 95% CI 0.47–3.85, P = 0.12), allelic model (OR 1.55, 95% CI 0.97–2.50, P = 0.74), homozygous model (OR 1.70, 95% CI 0.63–4.56, P = 0.61) and heterozygous model (OR 0.78, 95% CI 0.24–2, 53, P = 0.10). The analyses of the recessive and heterozygous genotype groups revealed a moderate degree of heterogeneity (I^2 59%; I^2 64%, respectively) (Figure 4).

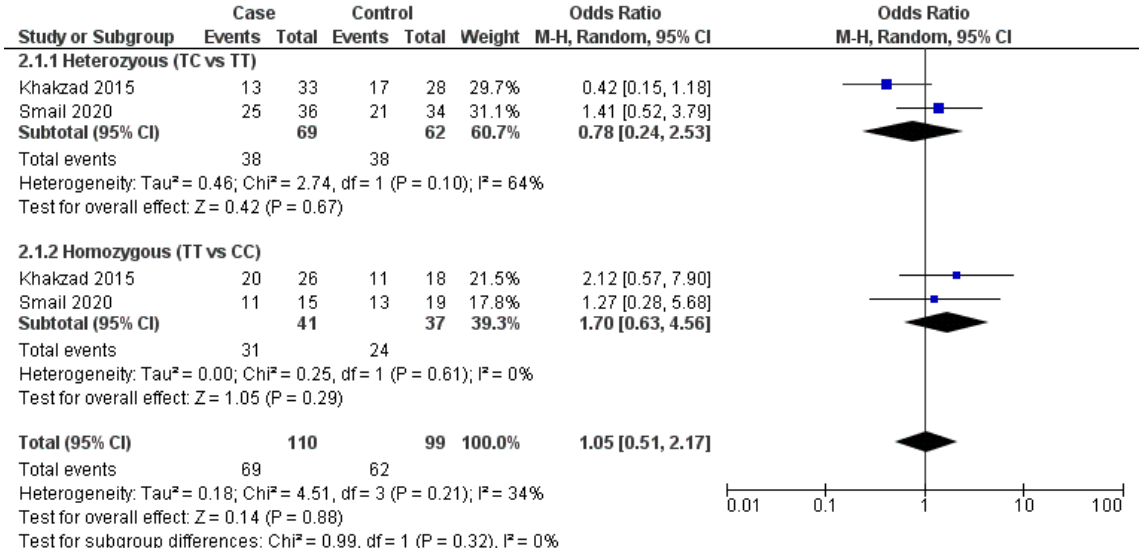
Figure 4: Pooled odds ratios and 95% confidence intervals for the association between the rs1982073 and the development of ASD.

a)

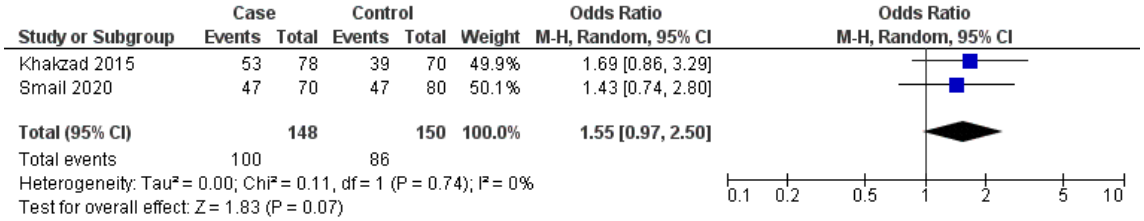


Revista ARACÊ
ISSN: 2358-2472

b)



c)



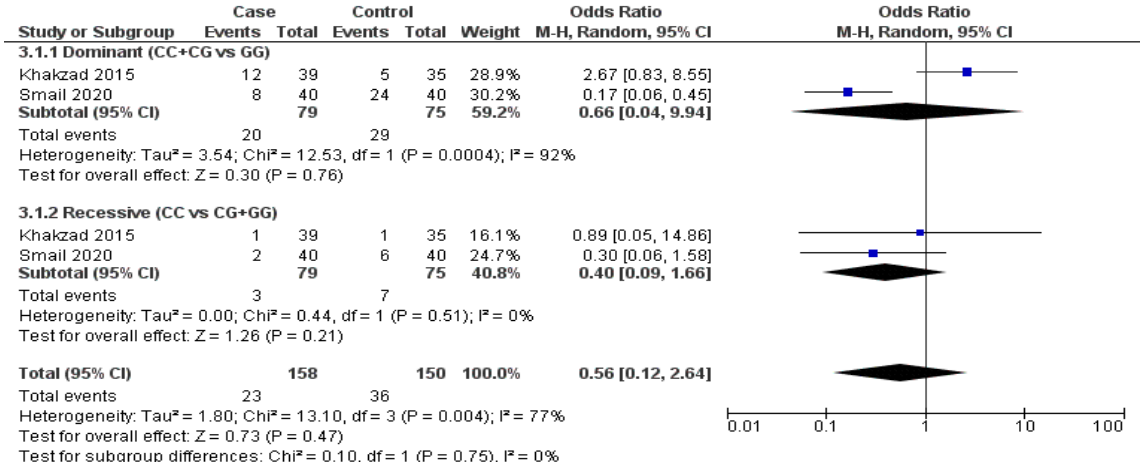
(a) Dominant and recessive models. (b) Homozygous and heterozygous models. (c) Allelic model.

Our results also revealed no significant associations between the rs1800471 SNP and ASD risk in any of the genotypic models, including the dominant model (OR 0.66, 95% CI 0.04–9.94, P = 0.0004), recessive model (OR 0.40, 95% CI 0.09–1.66, P = 0.51), allelic model (OR 0.70, 95% CI 0.08–5.96, P = 0.001), homozygous model (OR 0.30, 95% CI 0.05–1.72, P = 0.26) and heterozygous model (OR 1.32, 95% CI 0.27–6, 37, P = 0.57). The analyses of the dominant genotype and allele groups revealed a high degree of heterogeneity (I^2 92%; I^2 91%, respectively) (Figure 5).

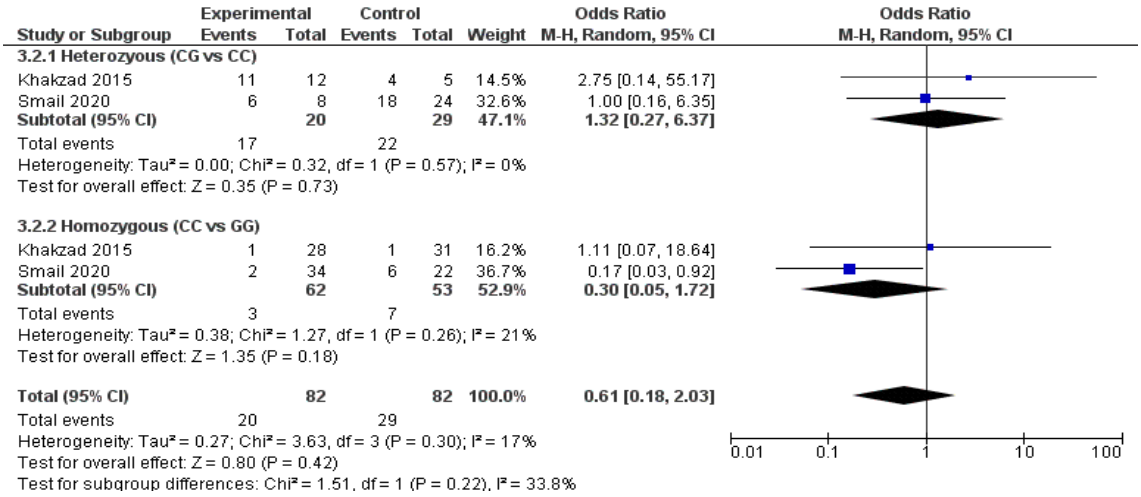
Revista ARACÊ
ISSN: 2358-2472

Figure 5: Pooled odds ratios and 95% confidence intervals for the association between the rs1800471 and the development of ASD.

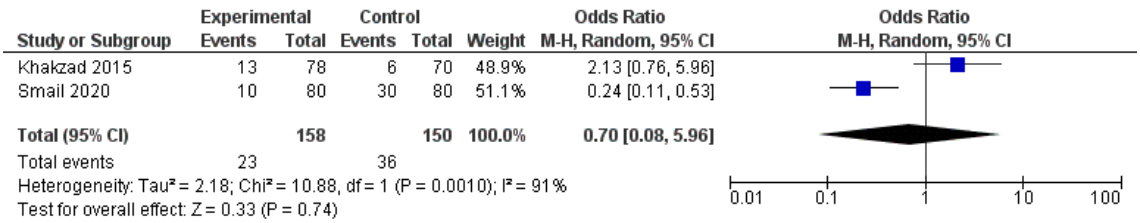
a)



b)



c)



(a) Dominant and recessive models. (b) Homozygous and heterozygous models. (c) Allelic model.

DISCUSSION

To our knowledge, this is the first systematic review and meta-analysis to examine whether inflammatory cytokine polymorphisms are more common in individuals with ASD.

Revista ARACÊ
ISSN: 2358-2472

Our results indicate that although some studies have associated genotypic and allelic variants with ASD in isolation, the pooled results of the studies were not statistically significant in the meta-analysis. Therefore, it is not possible to infer whether there is an association between the investigated SNPs and ASD. Studies investigating this issue are scarce, and the polymorphisms of many inflammatory cytokines have not yet been investigated. Consequently, this review highlights major gaps and important points that need to be explored in future research.

Cytokines regulate the type and magnitude of immune function, influencing neurodevelopment[21–23]. These molecules require adequate regulation to maintain a balance between pro- and anti-inflammatory responses. Recent systematic reviews involving 17[24] and 38[25] studies have shown that the levels of the proinflammatory cytokines IL-1β, IL-6, and IFN-γ are increased in individuals with ASD, providing evidence of innate activation and dysregulation. On the other hand, other studies have detected a decrease in the level of TGF-β1, which has anti-inflammatory activity, in patients with autism[10,11,26–28].

Increased levels of the cytokines IL-1β and IL-6 are associated with worse behavioral scores[29]. Similarly, lower levels of TGF-β1 have been associated with worse behavioral symptoms[11] and the severity of autism[10,27,28]. These findings suggest that there is a continuous proinflammatory state in patients with ASD, where disturbances in immune responses affect central behaviors. One of the factors that can influence these immunological disorders is the presence of polymorphisms[23,30].

SNPs are the most common form of genetic variation in the human genome and are becoming important markers in clinical diagnosis and genetic research[31,32]. Among the polymorphisms identified in this review, rs1800796 of IL-6 can alter the circulating levels of this cytokine through complex interactions according to the haplotype[33]. Conversely, the rs2430561 polymorphism of IFN-γ is associated with a decrease in the concentration of IFN-γ[20]. The IL-1β polymorphisms rs16944, rs1143634 and rs1143627 that were identified in the review are associated with increased expression of cytokines[34,35]. While rs16944 is related to increased transcriptional activity[36], rs1143627 is associated with reduced promoter activity, and the rs1143627 polymorphism increases transcription factor binding[37].

Thus, functional polymorphisms of cytokine genes are considered risk factors for Alzheimer's disease (IL-10 rs1800871)[38], Parkinson's disease (IL-6 rs1800795)[39], gastric cancer (TNF-α rs1799724, IL-8 rs2227532 and IL-10 rs1800872)[40] and bipolar disorder (IL-1 rs1143627, rs16944, rs1143623, and rs12621220)[41]. Despite their importance, SNPs

Revista ARACÊ
ISSN: 2358-2472

involve only a single type of genetic and environmental interaction, especially in complex disorders such as ASD. However, studies that explore this association with autism are still scarce, and new research could contribute to understanding the development of this disorder.

The present review revealed six studies that evaluated seven SNPs of four different cytokines. Although some of these studies revealed an association between genotypic variants and autism, this review is not able to prove such an association. The scarcity of studies and low number of participants contributed to a moderate to high degree of heterogeneity between studies, making it impossible to obtain conclusive results on this hypothesis. Therefore, considering that cytokines are important modulators of behavior and that polymorphisms can influence their expression, further studies investigating these associations must be carried out.

CONCLUSION

Although some studies have associated genotypic and allelic variants with ASD alone, the pooled results of these studies were not statistically significant in the meta-analysis. Furthermore, few studies have carried out these investigations; therefore, more studies are needed to validate the results presented herein. Our systematic review highlights important gaps and inspires new studies involving larger populations and different ethnic groups to investigate significant associations between genetic variations in different cytokines and the development of ASD.

CONFLICTS OF INTEREST

All the authors have read and approved the contents and manuscript.

FUNDING SOURCES

This work was supported by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Finance code 001.

Revista ARACÊ
ISSN: 2358-2472

REFERENCES

1. Belica, I., Janšáková, L., Celušáková, H., Kopčíková, M., Polónyiová, K., Rašková, B., ... & Polónyiová, K. (2023). Plasma cytokine concentrations of children with autism spectrum disorder and neurotypical siblings. *Cytokine, 170,* 156333. https://doi.org/10.1016/j.cyto.2023.156333

2. Han, Y., Xiong, W., Liu, J., Dai, W., Su, Y., Gao, L., ... & Gao, L. (2021). Associations of serum cytokine levels and interleukin-6-572C/G polymorphism with myelin damage in Chinese children with autism spectrum disorder. *Neuroscience, 465,* 95–10. https://doi.org/10.1016/j.neuroscience.2021.04.006

3. Zeidan, J., Fombonne, E., Scorah, J., Ibrahim, A., Durkin, M. S., Saxena, S., ... & Saxena, S. (2022). Global prevalence of autism: A systematic review update. *Autism Research, 15*(5), 778–790. https://doi.org/10.1002/aur.2696

4. Masini, E., Loi, E., Vega-Benedetti, A. F., Carta, M., Doneddu, G., Fadda, R., & Zavattari, P. (2020). An overview of the main genetic, epigenetic and environmental factors involved in autism spectrum disorder focusing on synaptic activity. *International Journal of Molecular Sciences, 21*(21), 8290. https://doi.org/10.3390/ijms21218290

5. Meltzer, A., & Van de Water, J. (2017). The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology, 42*(1), 284–298. https://doi.org/10.1038/npp.2016.158

6. Masi, A., Glozier, N., Dale, R., & Guastella, A. J. (2017). The immune system, cytokines, and biomarkers in autism spectrum disorder. *Neuroscience Bulletin, 33*(2), 194–204. https://doi.org/10.1007/s12264-017-0103-8

7. Xu, N., Li, X., & Zhong, Y. (2015). Inflammatory cytokines: Potential biomarkers of immunologic dysfunction in autism spectrum disorders. *Mediators of Inflammation, 2015,* 531518. https://doi.org/10.1155/2015/531518

8. Saad, K., Abdallah, A. M., Abdel-Rahman, A. A., Al-Atram, A. A., Abdel-Raheem, Y. F., Gad, E. F., ... & Gad, E. F. (2020). Polymorphism of interleukin-1β and interleukin-1 receptor antagonist genes in children with autism spectrum disorders. *Progress in Neuropsychopharmacology & Biological Psychiatry, 103,* 109999. https://doi.org/10.1016/j.pnpbp.2020.109999

9. Majerczyk, D., Ayad, E. G., Brewton, K. L., Saing, P., & Hart, P. C. (2022). Systemic maternal inflammation promotes ASD via IL-6 and IFN-γ. *Bioscience Reports, 42*(11), BSR20220713. https://doi.org/10.1042/BSR20220713

10. Yousefi, J., Khakzad, M. R., Hojati, M., Ebrahimi, S. A., Hosseinpour, M., & Akhondian, J. (2021). Is serum TGF-β1 and TGF-β2 levels correlated to children with autism intensity? *Iranian Journal of Child Neurology, 15*(2), 57–67. https://doi.org/10.22037/ijcn.v15i1.21826

Revista ARACÊ
ISSN: 2358-2472

11. Ashwood, P., Enstrom, A., Krakowiak, P., Hertz-Picciotto, I., Hansen, R. L., Croen, L. A., ... & Croen, L. A. (2008). Decreased transforming growth factor beta1 in autism: A potential link between immune dysregulation and impairment in clinical behavioral outcomes. *Journal of Neuroimmunology, 204*(1–2), 149–153. https://doi.org/10.1016/j.jneuroim.2008.07.006

12. Rao, M., Wong, C., Kanetsky, P., Girndt, M., Stenvinkel, P., Reilly, M., & Raj, D. S. (2007). Cytokine gene polymorphism and progression of renal and cardiovascular diseases. *Kidney International, 72*(5), 549–556. https://doi.org/10.1038/sj.ki.5002391

13. Jiao, Y., Chen, R., Ke, X., Cheng, L., Chu, K., Lu, Z., & Herskovits, E. H. (2012). Single nucleotide polymorphisms predict symptom severity of autism spectrum disorder. *Journal of Autism and Developmental Disorders, 42*(6), 971–983. https://doi.org/10.1007/s10803-011-1327-5

14. Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., ... & Rennie, D

. (2000). Meta-analysis of observational studies in epidemiology: A proposal for reporting. *JAMA, 283*(15), 2008–2012. https://doi.org/10.1001/jama.283.15.2008

15. Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., ... & Petticrew, M. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews, 4*(1), 1. https://doi.org/10.1186/2046-4053-4-1

16. Wells, G., Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., ... & Losos, M. (2014). The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Ottawa Hospital Research Institute. Retrieved from https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

17. Uyanik, K. C. P., Yigin, A. K., Dogangun, B., & Seven, M. (2022). Evaluation of IL1B rs1143634 and IL6 rs1800796 polymorphisms with autism spectrum disorder in Turkish children. *Immunological Investigations, 51*(4), 766–777. https://doi.org/10.1080/08820139.2020.187048

18. Smail, S. W., Qadir, M. K., Rajab, M. F., Ismail, I. I., Taha, O. S., Shekha, M. S., ... & Shekha, M. S. (2020). TGF-β1 polymorphism is an inflammatory disease specifier in autism spectrum disorders? *Gene Reports, 21,* 100843. https://doi.org/10.1016/j.genrep.2020.100843

19. Khakzad, M. R., Salari, F., Javanbakht, M., Hojati, M., Varasteh, A., Sankian, A., ... & Sankian, A. (2015). Transforming growth factor beta 1 869T/C and 915G/C polymorphisms and risk of autism spectrum disorders. *Reports of Biochemistry and Molecular Biology, 3*(2), 82–88.

20. Kaleel, K. A., Ibraheim, W. N., & Almaliki, Q. N. T. (2020). INF-γ plasma level and polymorphism significance in autistic spectrum disorder: A controlled study in Basrah.

Revista ARACÊ
ISSN: 2358-2472

Journal of Biotechnology and Bioinformatics Research, 2(3), 1–5. https://doi.org/10.47363/JBBR/2020

21. Ransohoff, R. M., Schafer, D., Vincent, A., Blachère, N. E., & Bar-Or, A. (2015). Neuroinflammation: Ways in which the immune system affects the brain. *Neurotherapeutics, 12*(4), 896–909. https://doi.org/10.1007/s13311-015-0385-3

22. Monet, M. C., & Quan, N. (2023). Complex neuroimmune involvement in neurodevelopment: A mini-review. *Journal of Inflammation Research, 16,* 2979–2991. https://doi.org/10.2147/JIR.S410562

23. Ross, O. A., Walton, R., Hinkle, K. M., Graff-Radford, N., & Rea, I. M. (2018). Cytokine polymorphisms, immunosenescence, and neurodegeneration. In *Handbook of Immunosenescence.* Springer, Cham. https://doi.org/10.1007/978-3-319-64597-1-33-1

24. Masi, A., Quintana, D. S., Glozier, N., Lloyd, A. R., Hickie, I. B., & Guastella, A. J. (2015). Cytokine aberrations in autism spectrum disorder: A systematic review and meta-analysis. *Molecular Psychiatry, 20*(4), 440–446. https://doi.org/10.1038/mp.2014.59

25. Saghazadeh, A., Ataeinia, B., Keynejad, K., Abdolalizadeh, A., Hirbod-Mobarakeh, A., & Rezaei, N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. *Journal of Psychiatric Research, 115,* 90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019

26. Okada, K., Hashimoto, K., Iwata, Y., Nakamura, K., Tsujii, M., Tsuchiya, K. J., ... & Tsuchiya, K. J. (2007). Decreased serum levels of transforming growth factor-beta1 in patients with autism. *Progress in Neuropsychopharmacology & Biological Psychiatry, 31*(1), 187–190. https://doi.org/10.1016/j.pnpbp.2006.08.020

27. El Gohary, T. M., Abd El Aziz, N., Darweesh, M., & Sadaa, E. S. (2015). Plasma level of transforming growth factor β 1 in children with autism spectrum disorder. *Egyptian Journal of Ear, Nose, Throat and Allied Sciences, 16*(1), 69–73. https://doi.org/10.1016/j.ejenta.2014.12.002

28. Hashim, H., Abdelrahman, H., Mohammed, D., & Karam, R. (2013). Association between plasma levels of transforming growth factor-β1, IL-23 and IL-17 and the severity of autism in Egyptian children. *Research in Autism Spectrum Disorders, 7*(1), 199–204. https://doi.org/10.1016/j.rasd.2012.08.007

29. Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., & Van de Water, J. (2011). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, Behavior, and Immunity, 25*(1), 40–45. https://doi.org/10.1016/j.bbi.2010.08.003

30. Dutra, W. O., Moreira, P. R., Souza, P. E. A., Gollob, K. J., & Gomez, R. S. (2009). Implications of cytokine gene polymorphisms on the orchestration of the immune response: Lessons learned from oral diseases. *Cytokine & Growth Factor Reviews, 20*(3), 223–232. https://doi.org/10.1016/j.cytogfr.2009.05.005

Revista ARACÊ
ISSN: 2358-2472

31. Manaz, M., Karasakal, Ö. F., Özkan Oktay, E., & Karahan, M. (2023). In silico analysis of missense SNPs in GABRA1, GABRB1, and GABRB3 genes associated with some diseases in neurodevelopmental disorders. *Egyptian Journal of Medical Human Genetics, 24,* 67. https://doi.org/10.1186/s43042-023-00446-6

32. Wu, K., Kong, F., Zhang, J., Tang, Y., Chen, Y., Chao, L., ... & Chao, L. (2023). Recent progress in single-nucleotide polymorphism biosensors. *Biosensors, 13*(9), 864. https://doi.org/10.3390/bios13090864

33. Rai, H., Colleran, R., Cassese, S., Joner, M., Kastrati, A., & Byrne, R. A. (2021). Association of interleukin 6 -174 G/C polymorphism with coronary artery disease and circulating IL-6 levels: A systematic review and meta-analysis. *Inflammation Research, 70,* 1075–1087. https://doi.org/10.1007/s00011-021-01505-7

34. Ma, X., Sun, L., Li, X., Xu, Y., & Zhang, Q. (2023). Polymorphism of IL-1B rs16944 (T/C) associated with serum levels of IL-1β affects seizure susceptibility in ischemic stroke patients. *Advances in Clinical and Experimental Medicine, 32*(1), 23–29. https://doi.org/10.17219/acem/152738

35. Fang, Y., Xie, H., & Lin, Z. (2018). Association between IL-1β+3954C/T polymorphism and myocardial infarction risk: A meta-analysis. *Medicine, 97*(30), e11645. https://doi.org/10.1097/MD.0000000000011645

36. Fu, L. Y., Qiu, X., Deng, Q. L., Huang, P., Pi, L., Xu, Y., ... & Xu, Y. (2019). The IL-1B gene polymorphisms rs16944 and rs1143627 contribute to an increased risk of coronary artery lesions in Southern Chinese children with Kawasaki disease. *Journal of Immunology Research, 2019,* 4730507. https://doi.org/10.1155/2019/4730507

37. Huang, H. Y., Yu, R. L., Tsai, W. F., Chuang, W. L., Huang, J. F., & Dai, C. Y. (2024). Impact of interleukin-1β single nucleotide polymorphisms and depressive symptoms in individuals with chronic viral hepatitis. *Kaohsiung Journal of Medical Sciences, 40*(1), 94–104. https://doi.org/10.1002/kjm2.12776

38. Magalhães, C. A., Carvalho, M. D. G., Sousa, L. P., Caramelli, P., & Gomes, K. B. (2017). Alzheimer's disease and cytokine IL-10 gene polymorphisms: Is there an association? *Arquivos de Neuro-Psiquiatria, 75*(9), 649–656. https://doi.org/10.1590/0004-282X20170110

39. Yi, M., Li, J., Jian, S., Li, B., Huang, Z., Shu, L., & Zhang, Y. (2023). Quantitative and causal analysis for inflammatory genes and the risk of Parkinson's disease. *Frontiers in Immunology, 14,* 1119315. https://doi.org/10.3389/fimmu.2023.1119315

40. De Oliveira, J. G., Rossi, A. F., Nizato, D. M., Cadamuro, A. C., Jorge, Y. C., Valsechi, M. C., ... & Valsechi, M. C. (2015). Influence of functional polymorphisms in TNF-α, IL-8, and IL-10 cytokine genes on mRNA expression levels and risk of gastric cancer. *Tumor Biology, 36*(12), 9159–9170. https://doi.org/10.1007/s13277-015-3593-x

Revista ARACÊ
ISSN: 2358-2472

41. Pu, X., Li, J., Ma, X., Yang, S., & Wang, L. (2021). The functional polymorphisms linked with interleukin-1β gene expression are associated with bipolar disorder. *Psychiatric Genetics, 31*(2), 72–78. https://doi.org/10.1097/YPG.0000000000000272