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Original Article (Pages: 18586-18600)

Immunology and Genetics of Autism Spectrum Disorders and Retrospective Evaluation of Clinical Responses to Regular Immunoglobulin Replacement Therapy

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Abstract

Background: The results of the studies on the aetiology, pathogenesis, and treatment recommendations of Autism Spectrum Disorder (ASD) in different age groups are limited. We analysed ASD patients immunogenetically and analysed the effects of regular intravenous immunoglobulin (IVIG) treatment on their quality of life and clinical improvement.

Methods: Information about 96 patients was obtained from hospital system records and patient files.

Results: Seventy-seven patients were male (M/F-3.4/1; p=0.001). In lymphocyte subsets of the patients, mean gamma-delta (γδ) T cell percentages were high, whereas mean naive CD8 T cell percentages were low in all age groups. Parents of 51 patients completed the quality of life questionnaire. The mean score was 41 (±10.26) before IVIG and 72.67 (±9.79) after IVIG. They were significantly different (p=0.001), and the effect size was t = -23.001. Whole Exome Sequencing (WES) analysis was performed in 21 of 51 patients, and 27 mutations were associated with ASD.

Conclusions: Regular IVIG treatment significantly improved ASD symptoms and family quality of life.

Key Words: Autism spectrum disorder, Genetic, Immunology, Intravenous immunoglobulin, Quality of life in autism.

* Please cite this article as: Abdullayev E, Çepniler E, Yıldıran A. Immunology and Genetics of Autism Spectrum Disorders and Retrospective Evaluation of Clinical Responses to Regular Immunoglobulin Replacement Therapy. Int J Pediatr 2024; 12 (02):18586-18600. DOI: **10.22038/ijp.2024.78940.5439**

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Received date: Mar.26,2024; Accepted date: Feb.01,2024

1- INTRODUCTION

Studies have shown that approximately 1-2/100 children worldwide are diagnosed with Autism Spectrum Disorder (ASD) (1).

Genetic causes have been investigated in etiological studies (2). The most commonly observed duplication of chromosome 15q11-13, the second known microdeletion/micro-insertion on chromosome 16p11.2, has been observed in approximately 1% of sporadic ASD cases (3). Observations indicate that environmental factors contribute to ASD and support an epigenetic component in phenotypic variation. Examples of monogenic conditions are associated with ASD genes (syndromic), Tuberous Sclerosis (TSC1 and TSC2), Neurofibromatosis (NF1), Timothy Syndrome (CACNA1), and Cortical Dysplasia-Focal Epilepsy Syndrome (CNTNAP2) (4-6).

Some human and animal studies have shown that maternal immune activation during pregnancy increases the risk of ASD (7-10). In patients with central nervous system dysfunction, it is known to indicate the diagnosis of autoimmune encephalopathy (AIE) with or without autoantibodies directed against brain tissue (11). According to the case series, Intravenous immunoglobulin (IVIG) has been reported to help treat AIE. IVIG has been shown to heal ASD symptoms such as abnormal behaviour, speech, and social interaction (12).

The most important question is why we need to administer regular and long-term IVIG treatment for autistic people. First, we believe that chronic encephalitis could be the probable trigger. Second, some researchers tried and had some healing with IVIG. Third, we noticed that the mental faculties of patients we gave IVIG for other reasons improved.

In this study, patients with ASD were evaluated immunologically and genetically. We aimed to determine the efficacy of regular IVIG treatment as a promising and effective treatment to improve autism symptoms and the family's quality of life.

2- MATERIALS AND METHODS

In this retrospective study, 96 patients (aged 2-18 years) with a previous diagnosis of Autism Spectrum Disorder (according to DSM-5) were included in this study. They had been admitted to the Department of Pediatric Immunology and Allergy, Samsun Ondokuz Mayıs University Faculty of Medicine Hospital between January 1, 2014, and March 10, 2023. Information about the patients was obtained from hospital system records and patient files. All patients received IVIG therapy (500 mg/kg/month) for at least one year. Clinical response, improvement in behavioural disorders, and impact on family quality of life were retrospectively assessed in those who received regular IVIG treatment (51 of 96 patients). The primary endpoint for assessing life quality was one year after the beginning of the regular use.

Family history, consanguinity, history of deceased siblings, frequency of respiratory tract infections, height and weight percentiles, presence of tonsillar tissue, and BCG scar were evaluated, along with Lymphopenia, ANA (antinuclear antibody), serum immunoglobulin levels, CD4+, CD8+, CD19+, CD16/56, gamma-delta T cell, CD19+CD27+IgD-, CD8+CD45RA percentages, CD4+/CD8+ ratio. The normal ranges of plasma immunoglobulin levels and lymphocyte subgroups were classified and assessed based on the current references in our country (13, 14). In our country, WES (Whole Exome Sequencing) analysis is not covered by health insurance; the analyses were sent from patients who could afford the fee.

The patients' life quality was assessed after the regular IVIG replacement therapy. A questionnaire was administered to the parents using a 5-point Likert scale (each question ranging from one (not at all) to five (very much)). Part A of the questionnaire was the quality of life subscale with 28 items questioning the parent's quality of life in the last four weeks. Part B (Autism Questionnaire (QoL)) was the other subscale assessing the impact of ASD symptoms on parents with 20 items on how parents perceive autism-specific difficulties as problems. Higher scores indicated that the parents had fewer problems with their children's ASD-related behaviours (15). The Turkish validity and reliability study of the scale was conducted by Gürbüz et al., in 2016 (16).

Normally distributed data were presented as mean ± standard deviation (mean ± SD), while non-normal data were reported in medians (min-max). Fischer's Exact test and the Mann-Whitney U test were used for comparison. $p<0.05$ was considered statistically significant.

3- RESULTS

This study included 96 patients who were previously diagnosed with Autism Spectrum Disorder. Seventy-four (77.1%) of the patients were males, and 22 (22.9%) were females (M/F-3.4/1; $p<0.001$). At the first visit, the mean age was 77.6 months (±38.2). The anthropometric distribution is shown in **Tables 1** and **2**.

The parents of 15 (15.6%) patients were consanguine. A family history of the hereditary disease was present in 18 (18.8%) patients, frequent Respiratory Tract Infection (RTI) in 50 (52.1%) patients, and dysmorphic findings in 11 (11.5%) patients. On physical examination, tonsillar tissue (normotrophic) was seen in 83 (86.5%) patients, and BCG scar in 71 (74%) patients. When all the above mentioned conditions listed in **Table 3** were analysed categorically, no relationship was found between them ($p>0.05$). Serum immunoglobulin levels were in the normal reference range in all age groups at the first visit (**Table 4**). The percentage of gamma-delta T cells was high in all age groups, while the percentage of CD8+CD45RA+ (naive CD8 T cells) was low in all age groups. The age strata are grouped according to the data used as the baseline reference. The CD4+/CD8+ ratio was less than 1 in 18 (18.75%) patients (**Table 5**). When the above data from 96 patients with ASD were analysed for correlation, we found that with the increase in age and height, CD19+ cells decreased and memory B cells increased significantly; and there was a significant inverse correlation between IgA levels and CD4+, CD4+/CD8+, CD19+, CD19+CD27+IgD-.

Moreover, there was a significant negative correlation between CD8+ and CD19+. There was a significant negative correlation between gamma-delta T cells and CD8+CD45RA+. CD16/56 cells increased linearly along with the patient's growth, and there was a significant inverse correlation between CD4+ and CD19+ counts ($p<0.05$). The negative and positive significant correlations obtained were compared with the data from the studies on healthy children, used as reference, and the correlations were found to be similar (**Table 6**) (13, 14).

Fifty-one patients (51 of 96) who had used IVIG regularly (500 mg/kg/month) for at least one year were evaluated with a questionnaire (Q group). Eleven of the 51 patients (21.6%) were using other drugs (10 patients were using risperidone, and one patient aripiprazole) in addition to IVIG.

From among the patients who received IVIG for at least one year, 44 (86.3%) were males and 7 (13.7%) were females. In this group, seven (14%) patients had consanguinity in their parents.

Table-1: Anthropometric values of the children

Gender	M-77; F-22 *		M-44; F-7 *	
	Mean(±Std) N=96	Median (Min-max) N=96	Mean(±Std) N=51†	Median (Min-max) N=51 †
Age (month)	77,6 (±38,2)	71,5 (25 - 214)	78,86 (±35,7)	74 (25 - 180)
Weight (kg)	24,4 (±13,5)	20 (12 - 92)	25,6 (±14,8)	21 (12 - 92)
Height (cm)	115,5 (±18,1)	113 (87 - 168)	116,6 (±17,4)	118 (87 - 157)
BMI (kg/m^2)	17,17 (±3,59)	16,21 (12,02 – 37,32)	17,54 (±4,21) **	16,48 (13,29 – 37,32) **

† Anthropometric values of children on regular IVIG therapy for at least one year (51 of 96 patients, Group Q); * p<0.05; ** p>0.05

Table-2: Body weight percentile and height percentile distribution

Parameter	Body weight percentile		Height percentile		Body weight percentile†		Height percentile†		Body weight percentile‡		Height percentile‡	
	N(96)	%	N(96)	%	N(51)	%	N(51)	%	N(14)	%	N(14)	%
<3th	7	7,3	12	12,5	4	7,8	4	7,8	-	-	1	7,1
3-10th	9	9,4	7	7,3	7	13,7	7	13,7	1	7,1	-	-
10th	7	7,3	7	7,3	2	3,9	2	3,9	2	14,3	1	7,1
10-25th	4	4,2	7	7,3	1	2,0	1	2,0	-	-	2	14,3
25th	6	6,3	12	12,5	3	5,9	3	5,9	1	7,1	-	-
25-50th	10	10,4	7	7,3	4	7,8	4	7,8	-	-	1	7,1
50th	16	16,7	21	21,9	8	15,7	8	15,7	3	21,4	6	42,9
50-75th	10	10,4	6	6,3	5	9,8	5	9,8	2	14,3	-	-
75th	9	9,4	7	7,3	6	11,8	6	11,8	-	-	1	7,1
75-90th	5	5,2	4	4,2	2	3,9	2	3,9	2	14,3	-	-
90-97th	7	7,3	3	3,1	3	5,9	3	5,9	1	7,1	1	7,1
>97th	6	6,3	3	3,1	6	11,8	6	11,8	2	14,3	1	7,1

† Body weight percentile and height percentile distribution of children on regular IVIG therapy for at least one year (51 of 96 patients, GroupQ), ‡ Percentile distribution of 'syndromic' patients according to WES findings

Table-3: Medical history, family history, epilepsy, lymphopenia, ANA rates, and other factors

Parameter		N (96)	%	N (51) †	% †
Consanguinity	YES	15	15,6	9	17,6
	NO	81	84,4	42	82,4
Dead sibling	YES	3	3,1	1	2,0
	NO	93	96,9	50	98,0
Hereditary Disease	YES	18	18,8	7	13,7
	NO	78	81,3	44	86,3
Frequent RTI	YES	50	52,1	29	56,9
	NO	46	47,9	22	43,1
Tonsillar Tissue	YES	83	86,5	44	86,3
	NO	13	13,5	7	13,7

Parameter		N (96)	%	N (51) †	% †
BCG scar	YES	71	74	38	74,5
	NO	25	26	13	25,5
Anomaly/Dysmorphia	YES	11	11,5	6	11,8
	NO	85	88,5	45	88,2
Lymphopenia	YES	3	3,1	2	3,9
	NO	93	96,9	49	96,1
ANA	Neg.	66	68,8	34	66,7
	+1	19	19,8	11	21,6
	+2	8	8,3	4	7,8
	+3	2	2,1	2	3,9
	+4	0	0	0	0
Epilepsy	YES	-	-	7	13,7
	NO	-	-	44	86,3
Cigarette exposure	YES	-	-	28	54,9
	NO	-	-	23	45,1
Special Education	YES	-	-	38	74,5
	NO	-	-	13	25,5
Income and Expenditure	Income<Expenditure	-	-	30	58,8
	Income=Expenditure	-	-	15	29,4
	Income>Expenditure	-	-	6	11,8

† Children on regular IVIG therapy for at least one year (51 of 96 patients, Group Q)

Family history of hereditary disease was present in 7 (13.7%) patients, and respiratory tract infections were frequent in 29 (56.9%) patients. Epilepsy was present in 7 (13.7%) patients and dysmorphic findings in 6 (11.8%) patients.

The number of patients who attended special education (skills training programs) was 38 (74.5%). The income level of 30 (58.8%) families was low. Among the patients, 43 (84.3%) reported decreased respiratory tract infections. The remaining 8 (15.7%) patients stated no difference in the frequency of any condition. Blood immunoglobulin values of 51 patients were analysed and found normal. When the CD4+/CD8+ ratio was analysed, it was found to be below 1 in 13 (25.5%) patients, and the ratio was inverted. The median of this ratio was below 1.5 in all our patients (**Table 5**) (14).

Changes in the parent's quality of life in the Q group were evaluated. Forty-eight of the patients (94.1%) reported noticeable improvements shortly after the initiation of treatment. When the parents were asked for examples of progress, they stated that their children's 'fears decreased, perception increased, they more obeyed the given commands, vocabulary increased and speech improved, diarrhoea and constipation became regular, stereotypies decreased, self-expression skills increased, they took responsibility, toilet training accelerated, appetite returned to normal, and they learned to wear shoes.' The mean score of the QoL Parent Version Part B questionnaire was 41 (±10.26) before IVIG, and 72.67 (±9.79) after IVIG. Group averages were compared, the effect size t = -23.001 was found, and a significant difference was observed (p=0.001) between the groups (**Table 7**).

In the Q group, 21 patients had WES results. 27 mutations associated with ASD were found in 14 patients; they were searched in gene.sfari.org, genecards.org, and malacards.org databases.

Table-4: Plasma immunoglobulin levels according to age

Age (month)	N (96)	IgG±SD (mg/dL)	IgG (13) Reference (mean±SD)	IgA±Std (mg/dL)	IgA (13) Reference (mean±SD)	IgM±Std (mg/dL)	IgM (13) Reference (mean±SD)	N(51)†	IgG±SD† (mg/dL)	IgA±SD† (mg/dL)	IgM±SD† (mg/dL)
25-36	11	741±231	811±249	54,27±21	60±24	113±48,8	111±40	4	779±228	64,5±15,1	121,3±16,8
37-71	37	850±207	839±164	90±56,7	69±34	95,4±42,8	121±39	20	878±216	103±62,9	103±52,9
72-107	30	957±179	1014±255	131±66,8	106±49	95,3±41,6	114±41	17	957±182	115,4±50,4	80,8±40,5
108-143	12	1013±169	1055±322	133±49,7	115±47	102,1±33,3	113±43	8	1006±202	133±60,3	102±32,6
144-192	6	952±301	1142±203	175,3±116	120±48	160,7±84,3	125±39	2	1109±171	231±195,2	212,5±94

† Plasma immunoglobulin levels according to age of children on regular IVIG therapy for at least one year (51 of 96 patients, Group Q)

Table-5: Distribution of lymphocyte subgroups according to age

Age	2-5 (years)	2-5 (years)	5-10 (years)	5-10 (years)	10-16 (years)	10-16 (years)	2-5 (years)	5-10 (years)	10-16 (years)
	n=38 Med. (min-max)	Reference (14) Med. (min-max)	n=42 Med. (min-max)	Reference (14) Med. (min-max)	n=14 Med. (min-max)	Reference (14) Med. (min-max)	n=17 † Med. (min-max)	n=25 † Med. (min-max)	n=9 † Med. (min-max)
$CD3^+CD4^+$ %	38,5 (25-59)	38,3 (23,6-52,5)	37 (25-57)	35,8 (23,4-48,7)	39,5 (24-45)	36,8 (27,3-46,7)	38 (26-59)	36 (25-57)	39 (24-45)
$CD3^+CD8^+$ %	28 (17-44)	23,8 (12,1-35,7)	28,5 (15-41)	27,7 (16,8-46,5)	28,5 (23-34)	27,9 (16,5-39,4)	29 (18-44)	32 (15-41)	27 (23-34)
$CD4^+/CD8^+$	1,42 (0,63-3,28)	-	1,36 (0,66-2,71)	-	1,33 (0,8-1,87)	-	1,19 (0,72-3,28)	1,30 (0,66-2,71)	1,37 (0,8-1,87)
$CD19^+$ %	17 (7-34)	17,5 (8,4-28,5)	13 (7-23)	13 (6,5-20,3)	12 (6-16)	11 (5,1-21,9)	15 (9-25)	12 (7-23)	12 (6-16)
$CD3^-CD16/56$ %	6,5 (2-18)	7,3 (3,5-22,2)	10 (1-34)	9,8 (4-29)	14 (5-19)	13,6 (1,8-26,6)	11 (2-37)	14 (0-43)	22 (7-33)
$CD19^+CD27^+IgD^-$ %	14 (2-37)	9,6 (2,9-31,9)	18 (0-47)	14,4 (6,7-31,1)	20,5 (6-33)	15,5 (2,7-29)	7 (3-16)	9 (1-26)	15 (5-19)
Gamma-delta T cell %	22 (5-70)	9 (3,4-19,9)	22,5 (10-44)	11 (4,7-29,6)	15 (9-29)	11 (1,5-21)	22 (8-49)	21 (10-44)	15 (9-29)
$CD8^+CD45RA^+$ %	51 (10-84)	79,1 (34,3-90,3)	52 (21-78)	62,8 (36-87,8)	50 (36-64)	57,9 (28-86,2)	50 (27-53)	55 (29-76)	50 (36-64)

†Children on regular IVIG therapy for at least one year (51 of 96 patients, Group Q)

Table-6: Correlations

Variable		rho	p
Age	$CD19^+$	-0.40	<0.001
	$CD19^+CD27^+IgD^-$	0.36	0.002
	CD16/56	0,46	<0,001
Height	$CD19^+$	-0.36	<0.001
	$CD19^+CD27^+IgD^-$	0.21	0.04
	CD16/56	0,35	<0,001
Body weight	CD16/56	0,32	0,002
BW percentile	$CD19^+CD27^+IgD^-$	-0.23	0.03
Height percentile	IgE	-0.34	0.006
IgA	$CD4^+$	-0.23	0.023
	$CD4^+$/ $CD8^+$	-0.28	0.006
	$CD19^+$	-0.29	0.005
	CD16/56	0,30	0,003
	$CD19^+CD27^+IgD^-$	0.25	0.017
IgM	$CD4^+$	-0.21	0.045
$CD4^+$	$CD8^+$	-0.24	0.023
	CD16/56	-0,41	<0,001
	Gamma-delta T	-0.23	0.026
$CD8^+$	$CD19^+$	-0.40	<0.001
$CD19^+$	CD16/56	-0,35	<0,001
$CD4^+/CD8^+$	$CD19^+$	0.29	0.004
$CD8^+CD45RA^+$	IgG	-0.21	0.04
	Gamma-delta T	-0.30	0.003

rho: Spearman correlation coefficient (0-0.25 weak correlation; 0.25-0.50 moderate correlation; 0.50-0.75 strong correlation; 0.75-1.00 very strong correlation, negative value inverse correlation, positive value linear correlation), $p<0.05$ significant difference

Table-7: Quality of life questionnaire results (Group Q)

Variable	n	Mean Score (±Std)	t-test	SD	P
Before IVIG	51	41,00 (±10,26)	-23,001	50	<0,001
After IVIG	51	72,67 (±9,79)			

Dysmorphic findings were present in 6 (11.8%) patients at the first admission examination, and WES examination could be performed in 4 of 6 patients (P6, P10, P14, P15). There were additional genetic results from patients who could and could not undergo WES. Genes related to immunodeficiency and autoimmunity were also identified (**Table 8-9**).

Among the 21 patients who underwent WES, the number of "syndromic" patients according to the gene-phenotype relationship of WES findings was 14 (66.7%), and 12 (85.7%) of them were males. Consanguinity was present in 3 (21.4%) patients. Among the mutations found with the primary immunodeficiency panel performed in 11 of the 30 patients in whom WES was not performed, two were ASD-related, and 16 were immunodeficiency-related genes (**Table 10**).

Table-8: Outcomes of patients who underwent WES

Patient	Age (month)	WES results	Karyotype	Other mutations
P1	52	None	-	JAK3‡, TERT, TMC6, MTHFR, PAI1(hom)
P2	37	NF1†, FBXW11†	-	-
P3	131	TM4SF20†, KMT2C†, EDC3†	-	M694V, MTHFR
P4	141	NLGN1†, MAP2†	-	-
P5	130	None	-	MTHFR, PAI1
P6§	71	SHANK3†	-	TRMU, MTHFR (hom), ATM‡, NCF2‡, TTC7A‡, VPS13B†
P7	92	ERBB3, NFKBIA‡	46 XY	PIK3R1‡, NFKBIA‡
P8	81	FLG	-	PAI1 (hom), F13
P9	36	CHD1†	-	V626A, A1298C, F13, MAGT1‡, LİG4‡
P10§	74	SCN1A†	46 XY	A1298C (hom), PAI1, FXII, FV, M680I, SMARCAL1‡
P11	51	MVK	-	A1298C, PAI1
P12	117	KMT2E†, SCN2A†, KMT2A†, ZMIZ1†, QRICH1†, BAP1†	46 XY	A1298C, PAI1 (hom)
P13	84	NRXN1†	-	-
P14§	180	WARS1† (hom), TMEM237 (hom), MAT1A, FBLN5	46 XX	EZH2
P15§	79	GPC3† (Xl)	-	A1298C (hom), PAI1
P16	67	PRKAR1B†	-	M694V, LRBA†/‡, NHEJ1‡
P17	95	FDG1†	-	-
P18	41	DNMT3A†, TPM2, COL12A1	-	-
P19	102	None	-	AIRE‡, A1298C, FXIII
P20	88	None	-	R202Q
P21	72	CYP27A1†, EPB41L1†, NKX2-1, CHD1†	-	C677T (hom), PAI1, FXIII

† Mutation associated with ASD, ‡ Mutation associated with immunodeficiency, § Patient with dysmorphic findings Abbreviation: hom-homozygous

Table-9: Detailed information on mutations detected in WES

Mutation	Inheritance	Phenotype	Cytogenetic location	omim.org
DNMT3A	AD	Heyn-Sproul-Jackson Syndrome Tatton-Brown-Rahman Syndrome	2p23.3	602769
FBXW11	AD	Neurodevelopmental, jaw, eye, and digital syndrome	5q35.1	605651
TM4SF20	AD	Specific language impairment 5	2q36.3	615404
KMT2C	AD	Kleefstra syndrome 2	7q36.1	606833
EDC3	AR	Intellectual developmental disorder 50	15q24.1	609842
NLGN1	AD	Autism, susceptibility to, 20	3q26.31	600568
MAP2	del	Rett like Syndrome	2q34	157130
NF1	AD	Neurofibromatosis-Noonan syndrome Watson Syndrome Neurofibromatosis, type 1	17q11.2	613113
SHANK3 †	AD	Phelan-McDermid syndrome Schizophrenia 15	22q13.33	606230
NFKBIA	AD	Ectodermal dysplasia and immunodeficiency 2	14q13.2	164008
CHD1	AD	Pilarowski-Bjornsson syndrome	5q15-q21.1	602118
SCN1A†	AD	Developmental and epileptic encephalopathy 6B Dravet Syndrome	2q24.3	182389
KMT2E	AD	O'Donnell-Luria-Rodan Syndrome	7q22.3	608444
SCN2A	AD	Developmental and epileptic encephalopathy 11 Episodic ataxia, type 9 Seizures, benign familial infantile, 3	2q24.3	182390
KMT2A	AD	Wiedemann-Steiner Syndrome	11q23.3	159555
ZMIZ1	AD	Neurodevelopmental disorder with dysmorphic facies and distal skeletal anomalies	10q22.3	607159
QRICH1	AD	Ververi-Brady Syndrome	3p21.31	617387
BAP1	AD	Cury-İsidore Syndrome	3p21.1	603089
NRXN1	AD	Pitt-Hopkins-like syndrome 2 Schizophrenia, susceptibility to, 17	2p16.3	600565
WARS1†	AR AD	Neurodevelopmental disorder with microcephaly and speech delay, with or without brain abnormalities Neuronopathy, distal hereditary motor, type IX	14q32.2	191050
GPB3†	XR	Simpson-Golabi-Behmel Syndrome type 1	Xq26.2	300037
PRKAR1B	AD	Marbach-Schaaf neurodevelopmental syndrome	7p22.3	176911
FGD1	XR	Aarskog-Scott Syndrome Intellectual developmental disorder syndromic 16	Xp11.22	300546

Mutation	Inheritance	Phenotype	Cytogenetic location	omim.org
CYP27A1	AR	Cerebrotendinous xanthomatosis	2q35	606530
EPB41L1	AD	Intellectual developmental disorder 11	20q11.23	602879
ERBB3	AR AR AD	Lethal congenital contractural syndrome 2 Visceral neuropathy, familial, 1 Erythroleukemia, familial, susceptibility to	12q13.2	190151
TMP2	AD	Arthrogryposis, distal Congenital myopathy 23	9p13.3	190990
NKX2-1	AD	Chorea, hereditary benign Choreoathetosis, hypothyroidism, and neonatal respiratory distress Thyroid cancer, non medullary, 1	14q13.3	600635
COL12A1	OR AD	Ullrich congenital muscular dystrophy 2 Bethlem myopathy 2	6q13-q14.1	120320
MAT1A	AD/AR	Methionine adenosyltransferase deficiency	10q22.3	610550
FBLN5	AD	Cutis Laxa, 2 Charcot-Marie-Tooth disease, type 1H Neuropathy, age-related macular degeneration	14q32.12	604580
TMEM237	AR	Joubert Syndrome 14	2q33.1	614423
MVK	AR AR AD	Hiper-IgD Syndrome Mevalonic aciduria Porokeratosis 3, multiple types	12q24.11	251170
FLG	AD/AR	Ichthyosis vulgaris Dermatitis, atopic, susceptibility to, 2	1q21.3	135940

† Patient with dysmorphic findings. Abbreviation: AD- autosomal dominant, AR- autosomal recessive, XR- X-linked recessive.

Table-10: Genetic analysis results of patients who could not undergo WES

Patient	Age (month)	Karyotype	Other mutations
P22	123		CARD14, JAK3, PLCG2, TCF3
P23	47		PRF, SPINK5, TNFRSF6B, UNC13D
P24	78		MTHFR, PAI1, F13
P25	30		C677T, PAI1 (hom)
P26	71		E148Q
P27	69	46 XY	XIAP, TERT, C667T (hom), PAI1, F13, F2
P28	94		IL12B
P29	153	46 XY	V426A (hom), LYST, TERT
P30	60	46 XY	UNC119, ATM, CASP8, CSF2RA, IL7R, MSH5, NLRP12
P31	25		TCF3, FOXN1, NOD2, TNFSB13B, M680I
P32	38		AIRE, IL17RA, PRKDC, TMC6, A1298C

Abbreviation: hom-homozygous

4- DISCUSSION

In this study, the files of children admitted to the Pediatric Immunology Department with a diagnosis of ASD, receiving 500 mg/kg/month intravenous immunoglobulin replacement therapy, were evaluated retrospectively. IVIG was well tolerated; no subjects withdrew due to an adverse event.

ASD is a neurodevelopmental disorder in which many genetic, epigenetic, environmental, and perinatal factors play a role in its etiopathogenesis. The last report of the CDC (Centers for Disease Control), published in 2021, reported that the prevalence of autism is 1/44 (17). The increasing prevalence of ASD, the social, economic, and medical problems experienced by caregivers of ASD, and the education and rehabilitation process make it a candidate public health problem. Therefore, studies to clarify the etiopathogenesis of ASD are essential for the diagnosis, treatment, rehabilitation process, and prognosis of the disease.

One of the most consistent data on Autism Spectrum Disorder (ASD) is a higher prevalence in males than females like in our study (M/F-3.4/1; $p<0.001$) (17). Studies have shown anthropometric and body structure differences between children with ASD and typically developing children.

Immune system abnormalities have been widely reported to cause an increased risk of childhood infections among children with ASD. One study found that children with ASD were more likely to have neonatal and childhood infections than controls of the same age (18). In our research, 50 (52.1%) ASD patients had a history of frequent infections. The median age of these 50 patients was 68.5 months (25-175).

In a 2015 study by Mamidala et al., consanguinity was assessed as an independent risk factor for the development of ASD (19). Fifteen (15.6%) of our patients had consanguinity.

Autoimmunity against the central nervous system may play a role in the pathogenesis of Autism (20). In a study conducted by Mostafa et al., anti-dsDNA and ANA antibody positivity were found to be significantly higher in autistic children compared to healthy children ($p<0.001$) (21). In our study, ANA values were checked, and there were 66 (68.8%) patients with negative ANA, 19 (19.8%) with +1, 8 (8.3%) with +2, 2 (2.1%) with +3; in total, 29 (30.5%) patients were positive.

Some studies have looked at IgG concentrations in ASD. While some studies compared data obtained using the standard reference range, others compared data with a healthy child control group. In a survey by Grether et al., the serum IgG levels of 84 ASD patients were compared with the results of 159 typically developing and 49 developmentally retarded controls, and it was found that low IgG levels were associated with an increased risk of ASD (22). When Wasilewska et al., compared the IgG levels of 24 neurodevelopmentally delayed ASD patients with those of controls, no difference was found (23). Spiroksi et al., evaluated IgG and its subgroups. No difference was found between ASD and its relatives (24). Our study compared IgG, IgM, and IgA levels with reference values and found them normal in all age groups.

Several studies have shown the contribution of gamma-delta T cells to CNS inflammation, antitumor immunity, and CNS homeostasis. According to recent data, gamma-delta T cells in the meninges have been reported to have critical roles in brain function and homeostasis (25). Maternal autoinflammatory diseases, infection, or microbial dysbiosis have been shown to cause intrauterine immune activation, contributing to neurological disorders ranging from schizophrenia to

ASD in the offspring (26). In our study, gamma-delta T cells were elevated in all age groups.

Satterstrom et al., performed exome sequencing to detect underlying genetic mutations in ASDs. The study identified 102 risk genes. While 49 of these genes showed higher rates of destructive de novo variants in people with severe neurodevelopmental retardation, 53 of them were shown to be expressed at higher frequencies in ASDs. When ASD cases were compared with mutations in this group, phenotypic differences were found. Many suspected 'risk' genes expressed early in brain development were involved in regulating neuronal communication, and 13 of these were found to have copy number variants that repeatedly coincided with the same loci (27). 'Syndromic' refers to cases where additional phenotypes or dysmorphic findings accompany ASD. The term 'non-syndromic' typically refers to 'classic autism' as defined by Kanner, in which no other symptoms are present (28). Significant progress in the genetics of syndromic ASD began in the 1990s with identifying genes involved in the disease, such as FMR1, TSC1, and MECP2 (29). WES genetic analyses performed in our study revealed essential genes associated with ASD: NF1, FBXW11, TM4SF20, KMT2C, EDC3, NLGN1, MAP2, SHANK3, CHD1, SCN1A, KMT2E, SCN2A, KMT2A, ZMIZ1, QRICH1, BAP1, NRXN1, WARS1, GPC3, PRKAR1B, FDG1, DNMT3A, CYP27A1, EPB41L1, CHD1, VPS13B, LRBA, FOXN1, PRKDC. From among the 21 patients, the number of "syndromic" patients with gene-phenotype relationships, according to WES analysis, was 14 (66.7%), and 12 of them (85.7%) were males. Six patients had dysmorphic findings. WES could be requested from only four patients with dysmorphic findings, and the mutations detected were SHANK3, SCN1A, WARS1, and GPC3.

There are publications examining the therapeutic use of IVIG in patients with ASD. In a study by Niederhofer et al., 12 patients with ASD were treated with IVIG at 400 mg/kg, and improvement was seen in all subscales of the Autistic Behaviours Checklist (ABC) (30). Maltsev et al. treated 78 ASD children (2-10 years, 47 boys, 31 girls) with IVIG at 2g/kg/month for six months. The clinical characteristics of treated children were compared with those of a control group of 32 children with ASD without IVIG treatment. The authors reported that 77 (99%) patients improved with IVIG (31). In another prospective study, 14 ASD patients (mean age 7.6±3.0 years) with immune abnormalities were treated with 1g/kg IVIG, ten doses every 2 or 4 weeks. Basic behavioural measures were compared. Significant improvements were observed in total scores (32). Boris et al., treated 26 ASD patients (mean age 6.8 years (3-17)) with IVIG at 400mg/kg/month for six months. Only 6 (23%) treated patients had low immunoglobulin levels. The patients' general abnormal behaviour decreased dramatically shortly after receiving their first dose of IVIG, and significant improvements were seen at follow-up (33). In another study, 82 autoimmune encephalitis patients, 80 of whom had ASD, were evaluated.

The QoL Parent Version Part B questionnaire was completed in our Q group, and the results were compared. The group means were compared, the effect size was analysed, t = -23.001, and a significant difference was found. The improvement in the clinical status of the patients led to a considerable increase in the parent's quality of life (p=0.001).

One of the most common side effects of IVIG treatment is headache. Other side effects include chills, fever, flushing, flu-like muscle aches or joint pains, fatigue, nausea, vomiting, and rash. Three patients experienced headaches, two experienced

muscle aches, and three experienced nausea and vomiting. Other repeated doses did not cause the same symptoms. IVIG was well tolerated; no subjects withdrew due to an adverse event.

4-1. Limitations of the study

Our study had some analytical limitations. The first was its retrospective, and although treatment outcomes were compared over two different periods, the effect of change in the traditional treatment of ASD over time was not assessed. In addition, we could not establish a control group for the study, and there were no ASD patients who did not receive intravenous immunoglobulin and no healthy control group.

5- CONCLUSION

This study showed significant improvements in cognitive and behavioural disorders and the family's and patient's quality of life after immune-modulator (IVIG) treatment. These data and results support the strong link between children's immune conditions and neurodevelopmental outcomes.

6- ETHICAL CONSIDERATIONS

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ondokuz Mayis University (2023.03.08/ 2023000062). Written informed consent was obtained from the parents.

7- ACKNOWLEDGMENT

We are grateful to all patients and their families for their cooperation.

8- FUNDING

None.

9- CONFLICT OF INTEREST

None.

10- AUTHORSHIP CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by EA and AY. The first draft of the manuscript was written by EA, and all authors commented on previous versions. All authors read and approved the final manuscript.

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