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Vladimir Trajkovski
Aleksandar Petlichkovski
Olivija Efinska-Mladenovska
Dejan Trajkov
Todor Arsov
Ana Strezova
Ljubomir Ajdinski
Mirko Spiroski
Institute of Immunobiology and Human Genetics, Faculty of Medicine, University Ss. Kiril and Metodij, Skopje, Republic of Macedonia

Specific IgA, IgG, and IgE antibodies to food antigens in 35 participants with autistic disorder and 21 of their siblings in the Republic of Macedonia were examined. Statistically significant higher plasma concentration of IgA antibodies against alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin were found in the children with autistic disorder. Plasma concentrations of IgG antibodies against alpha-lactalbumin, beta-lactoglobulin, and casein in participants with autistic disorder were significantly higher. IgE-specific antibodies (alpha-lactalbumin, beta-lactoglobulin, casein, and gluten), as well as plasma concentration of total IgE, also were statistically significantly higher in the participants with autistic disorder. Gender differences were found for select IgA, IgG, and IgE (but not for total IgE) food-specific antibodies (kU/L) in the participants with autistic disorder and their siblings.

Keywords: *autistic disorder; diet; family*

An antibody or immunoglobulin is a large *Y*-shaped glycoprotein belonging to the immunoglobulin super family that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The production of antibodies is the main function of the humoral immune system. In mammals, there are five antibody isotypes or classes, each named with an "Ig" prefix, which stands for immunoglobulin, and known as IgA, IgM, IgG, IgD, and IgE. These immunoglobulin classes differ in their biological properties, functional locations, and their ability to deal with different antigens (Woof & Burton, 2004). Some of the immunoglobulin classes and subclasses are different in children with autistic disorder (Ashwood, Wills, & Van De Water, 2006; Trajkovski, Ajdinski, & Spiroski, 2004).

Many people with autistic disorder are reported to be sensitive to certain food products. Food products most often suspected of inducing food allergy are cereals (wheat, oat, rye, barley) and milk products (milk, cheese, and yogurt). Gluten, a protein from wheat and other cereals, and casein, milk protein, are the main causes for allergic manifestations. Other food products consumed in spring and summer, such as strawberries and tropical fruits, can also cause an allergic reaction. Food allergy results in many somatic and behavioral problems, such

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as headache, abdominal pain, nausea, insomnia, hyperactivity, aggression, noise sensitivity, depression, fatigue, digestion problems (diarrhea, constipation), muscle pain, ear infections, and so forth (Egger, Stolla, & McEwen, 1992; Murch, 2005; Tryphonas & Trites, 1979).

Some have hypothesized that autistic disorder is caused by disturbances in the digestive system. Mucosal changes have been found especially in late-onset autistic disorder with regression and increased intestinal permeability (Horvath, Papadimitriou, Rbasztyn, Drachenberg, & Tildon, 1999; Horvath & Perman, 2002; Jyonouchi, Sun, & Itokazu, 2002; Knivsberg, Reichelt, Hoien, & Nodland, 2002; Lucarelli et al., 1995; Martin, Uhlmann, Killalea, Sheils, & O'Leary, 2002; Torrente et al., 2002; Uhlmann et al., 2002; Wakefield et al., 2000). It is proposed that persons with autistic disorder have metabolic disorders because some components of the food are not digested completely. It is well known that some of these peptides have biological activity, and some of the products of casein digestion are beta caseomorphins, which have an opioid activity (morphine-like), and are found with different chain lengths from caseomorphin 1-2 to 1-10. Similarly, grain-derived opioids are divided into gliadinomorphins or gluteomorphins. If their concentrations are very high in the intestines or intestinal wall, these peptides pass into the blood and reach the central nervous system (CNS). The exorphins pass the blood–brain barrier (Ermisch, Ruhle, Neubert, Hartrodt, & Landgraf, 1983), where they can interfere with brain functioning and may result in autistic symptoms (Nyberg et al., 1989). The exorphins have been found in the urine of patients with schizophrenia (Hole et al., 1979).

Gluten and casein are considered the most frequent causative proteins for differences in behavior. A gluten- and casein-free diet was accompanied by improvement in 81% of children with autism within 3 months in most of the behavior categories (Reichelt & Knivsberg, 2003). The data provide support for the proposal that many patients with autistic disorder or schizophrenia suffer because of absorption of exorphins formed in the intestine as a result of incomplete digestion of gluten and casein (Ermisch et al., 1983).

Strong connection between different types of food and CNS disturbances related to allergenic peptides present in the food has been found (Lucarelli et al., 1995). Whey protein is the name for a collection of globular proteins that can be isolated from whey, a by-product of cheese manufactured from cow's milk. Whey is typically a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), which are soluble in their native forms, independent of pH. Peptides in cow's milk and cereals could be allergens, which can cause noxious effects on the CNS interacting with neurotransmitters. Lucarelli et al. (1995) found that 36% of their participants with autism showed positive results on allergy skin tests and had significantly higher levels of IgA specific antibodies against casein, lactalbumin, and beta-lactoglobulin and IgG and IgM antibodies against casein, compared to the control group of 20 typically developing children. These results lead to a hypothetical relationship between food allergy and autistic disorder (Uhlmann et al., 2002), reflecting an increase in the normal protein uptake (Reichelt & Knivsberg, 2003).

Efficacy of diet without cow's milk was verified in 36 patients with autism (Lucarelli et al., 1995). Their results of immunological studies showed a moderate immediate immuno-allergic response in most of their patients with autism. Although only 36% of their patients had positive skin tests for food antigens and an even smaller percentage (33% and 15%, respectively) had high total IgE and specific IgE positivity, a large percentage of patients had higher levels of antigen-specific antibodies belonging to the IgA, IgG, and IgM classes for casein. The levels of these antibodies were significantly higher than in the control group. Lucarelli et al. (1995) found high levels of antigen-specific antibodies belonging to the IgA class against lactalbumin, beta-lactoglobulin, and ovalbumin and high IgM antibodies to lactalbumin. These findings allowed them to hypothesize that food allergies can have a certain role in the pathogenesis of autistic disorder and other diseases of the CNS (Lucarelli et al., 1995).

Immune aberrations consistent with a dysregulated immune response, which so far have been reported in children with autism (Plioplys, Greaves, Kazemi, & Silverman, 1994; Stubbs & Crawford, 1977; Warren, Margaretten, Pace, & Foster, 1986; Yonk et al., 1990), include abnormal or skewed T helper cell type 1 (TH1)/TH2 cytokine profiles (Gupta, Aggarwal, Rashanravan, & Lee, 1998; Singh, 1996), decreased lymphocyte numbers (Warren et al., 1990), decreased T cell mitogen response (Engstrom et al., 2003), and the imbalance of serum immunoglobulin levels (Croonenberghs et al., 2002; Trajkovski et al., 2004). In addition, autistic disorder has been linked with autoimmunity (Connolly et al., 1999; Silva et al., 2004) and an association with immune-based genes, including human leukocyte antigen-DRB1 and complement C4 alleles (Odell et al., 2005; Torres, Maciulis, & Odell, 2001). There is potential that such aberrant immune activity during vulnerable and critical periods of neurodevelopment could participate in the generation of neurological dysfunction characteristic of autistic disorder (Ashwood et al., 2006).

Autoimmune disorders, such as inflammatory bowel disease, rheumatoid arthritis, and lupus, are much more prevalent in families of children with autism (Sweeten, Bowyer, Posey, Halberstadt, & McDougle, 2003). A majority of the children with autism who have been tested have antibodies directed against the myelin sheath in their own brain cells (Singh, Warren, Averett, & Ghaziuddin, 1997; Singh, Warren, Odell, Warren, & Cole, 1993; Todd, Hickok, Anderson, & Cohen, 1988). The myelin sheath acts as an insulator on the neuron that increases the processing time of the signals along the brain cell. If this sheath is destroyed, brain processing time is slowed (Vojdani et al., 2002; Vojdani, O'Bryan, et al., 2004; Vojdani, Pangborn, Vojdani, & Cooper, 2003). This happens commonly in multiple sclerosis, another autoimmune disorder, but affects more of the peripheral nerves resulting in muscle weakness.

Analysis of previous studies reveals a high prevalence of excessive production of proinflammatory cytokines (most notably, tumor necrosis factor-alpha [TNF-alpha]) with stimulation of endotoxin (lipopolysaccharide [LPS]) in a substantial number of children with autistic disorder (Jyonouchi, Sun, & Le, 2001). Cytokines are a group of proteins and peptides that are used in organisms as signaling compounds. These chemical signals are similar to hormones and neurotransmitters and are used to allow one cell to communicate with another (Correa et al., 2007). Endotoxin is one of the major microbial products produced in the gastrointestinal mucosa and provokes potent innate immune responses through TLR4 and other pattern-recognition receptors (Pasare & Medzhitov, 2003). Children with autistic disorder with excessive TNF-alpha production with LPS often reveal cellular immune reactivity to common dietary proteins as observed in children with nonallergic food hypersensitivity (Jyonouchi et al., 2002). These findings suggest a role of innate immune abnormalities in the development of a cell-mediated immune reaction to common dietary proteins in children with autistic disorder (Jyonouchi, Geng, Ruby, & Zimmerman-Bier, 2005).

Several hypotheses are described that have been advanced to account for the intestinal pathophysiology observed in autistic disorder (Black, Kaye, & Jick, 2002; Kidd, 2002; Knivsberg, Reichelt, & Nodland, 2001; Wakefield, 1999; White, 2003). Research in gastrointestinal pathology must be extended to detail the alterations in morphology, metabolism, and transepithelial transport capacity at the cellular level and to evaluate the reputed pathology as a tool in the clinical diagnosis of autistic disorder. The causative agent(s), whether exogenous or endogenous, must be identified. It is important to extensively evaluate the efficacy of gluten- and casein-free diets in ameliorating autistic symptoms and whether such diets ameliorate the gut pathology similar to the manner in which a gluten-free diet reverses the extensive mucosal pathology in celiac disease.

The aim of this study was to examine plasma concentration of specific IgA, IgG, and IgE antibodies against food in the participants with autistic disorder and their siblings in the Republic of Macedonia to test the connection with intestinal disturbances.

Method

Participants

The investigation was performed retrospectively in children with autism registered in the social institutions of the Republic of Macedonia. Autistic disorder was diagnosed by *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; American Psychiatric Association, 1994) and International Classification of Diseases–10 (World Health Organization, 1992) criteria. The diagnosis of children with autistic disorder and their siblings was reached in all cases by a specialist assessment with a senior pediatrician and with contributions from a specialist in mental health care as appropriate.

Thirty-five Macedonian children (25 boys and 10 girls) with autistic disorder were studied as well as 21 of their siblings (7 boys and 14 girls). The families of four children with autistic disorder declined to participate in the investigation. None of the children was on special diets that included avoidance of casein or gluten. The mean age for children with autistic disorder was 10.14 ± 5.81 years and 13.14 ± 6.45 years for their siblings.

Procedures

Ten mL of venous blood was drawn from each donor by standard venipuncture in a vacutainer with EDTA(K_3) after parental consent. At the time of blood drawing, none of the children with autism was receiving any prescription medications or antipsychotic drugs. Plasma samples were separated by centrifugation and stored at –20°C until the determination. Specific IgA and IgG antibodies against some food allergens (alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin), specific IgE antibodies (alpha-lactalbumin, beta-lactoglobulin, casein, and gluten), and total IgE were determined with an automated immunofluorescent device with solid phase (UniCAP 100; AmershamBiosciences). The determinations were performed on deidentified samples at the Institute of Immunobiology and Human Genetics at the Faculty of Medicine in Skopje. External quality control of total serum IgE and allergen-specific IgE was performed by

Table 1
Plasma Concentration of IgA Food-Specific Antibodies (kU/L) in Participants With Autistic Disorder (n = 35) and Their Siblings (n = 21) in the Republic of Macedonia

Allergen	Group	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	p
α-lactalbumin (IgAf76)	Autistic disorder	1.19	0.38	8.22[a]	16.45[a]	< .001
	Siblings	1.10	0.20	3.28[a]	1.56	
β-lactoglobulin (IgAf77)	Autistic disorder	1.26	0.45	5.53[a]	7.43[a]	< .001
	Siblings	1.10	0.22	4.81[a]	6.34[a]	
Casein (IgAf78)	Autistic disorder	2.10	1.94	6.99[a]	11.45[a]	< .001
	Siblings	1.44	1.02	5.84[a]	9.88[a]	
Gliadin (IgAf98)	Autistic disorder	1.55	0.81	4.65[a]	4.53[a]	.005
	Siblings	1.37	0.80	6.97[a]	14.26[a]	

a. Specific departure from normal distribution.

Table 2
Plasma Concentration of IgG Food-Specific Antibodies (kU/L) in Participants With Autistic Disorder (n = 35) and Their Siblings (n = 21) in the Republic of Macedonia

Allergen	Group	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	p
α-lactalbumin (IgGf76)	Autistic disorder	10.43	13.24	4.82[a]	5.86[a]	.001
	Siblings	9.27	20.98	5.45[a]	7.79[a]	
β-lactoglobulin (IgGf77)	Autistic disorder	9.44	10.35	5.24[a]	8.19[a]	.001
	Siblings	5.44	8.84	3.63[a]	3.05[a]	
Casein (IgGf78)	Autistic disorder	22.84	26.79	8.13[a]	18.48[a]	.036
	Siblings	12.98	19.97	3.57[a]	2.48[a]	
Gliadin (IgGf98)	Autistic disorder	12.83	21.06	10.38[a]	25.75[a]	.310
	Siblings	7.70	8.40	2.85[a]	1.13	

a. Specific departure from normal distribution.

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Data Analysis

The research data were stored, classified, and processed with a standard statistical program (Statgraphics Plus for Windows Version 2.1). Standardized skewness and standardized kurtosis were used to determine whether the samples came from a normal distribution. Values of these statistics outside the range of –2 to +2 would indicate a significant departure from normality. A Kolmogorov-Smirnov test was used to compare the distribution of the two samples. This test was performed by computing the maximum distance between the cumulative distributions of the two samples. As IgE was significantly nonnormal (Kolmogorov-Smirnov test, $p < .044$), parallel statistical analyses were carried out using log10 IgE values (Pereira Vega et al., 1997; Sapigni et al., 1998). p values of .05 or less were considered significant.

Results

Specific IgA Antibodies Against Food

Plasma concentrations of specific IgA antibodies against four allergens in participants with autistic disorder and their siblings are presented in Table 1. Standardized skewness and standardized kurtosis for IgA antibodies to specific food allergens were statistically different between the participants with autistic disorder and their siblings, except for the alpha-lactalbumin in siblings. Kolmogorov-Smirnov results were significant for all IgA-specific food antibodies.

Specific IgG Antibodies Against Food

Plasma concentrations of IgG food-specific antibodies against four allergens in participants with autistic disorder and their siblings are presented in Table 2. Concentrations in participants with autistic disorder were higher than in siblings, but standard deviations were higher than the

Table 3
Plasma Concentration of IgE Food-Specific Antibodies (kU/L) in Participants With Autistic Disorder ($n = 35$) and Their Siblings ($n = 21$) in the Republic of Macedonia

Allergen	Group	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	*p*
α-lactalbumin (IgEf76)	Autistic disorder	0.45	0.43	12.15[a]	32.83[a]	.005
	Siblings	0.39	0.18	8.57[a]	19.64[a]	
β-lactoglobulin (IgEf77)	Autistic disorder	0.43	0.26	8.89[a]	16.67[a]	ND
	Siblings	0.35	0	0	0	
Casein (IgEf78)	Autistic disorder	0.45	0.35	9.34[a]	17.30[a]	.005
	Siblings	0.35	0.01	8.57[a]	19.64[a]	
Gluten (IgEf79)	Autistic disorder	0.52	0.74	12.23[a]	32.34[a]	ND
	Siblings	0.35	0	0	0	

Note: ND = not done because of the 0 standard deviation.
a. Specific departure from normal distribution.

Table 4
Plasma Concentration of Total IgE and Logarithmic Transformation of Total IgE (kU/L) in Participants With Autistic Disorder ($n = 35$) and Their Siblings ($n = 21$) in the Republic of Macedonia

Allergen	Group	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	*p*
Total IgE	Autistic disorder	246.31	639.23	11.54[a]	30.33[a]	.044
	Siblings	37.21	33.50	2.54[a]	1.20	
Log total IgE	Autistic disorder	1.79	0.67	1.50	0.45	.044
	Siblings	1.38	0.46	–1.12	0.06	

a. Specific departure from normal distribution.

average values. Standardized skewness and kurtosis were higher than 2.0, except for gliadin in siblings. Differences between participants with autistic disorder and their siblings were statistically significant for alpha-lactalbumin ($p = .001$), beta-lactoglobulin ($p = .001$), and casein ($p = .036$) but not for gliadin.

Specific IgE Antibodies Against Food

Plasma concentrations of IgE food-specific antibodies against four allergens in participants with autistic disorder and their siblings are presented in Table 3. Standardized skewness and kurtosis were above normal distribution, with statistically significant differences between the two distributions, tested by Kolmogorov-Smirnov test, for alpha-lactalbumin ($p = .005$) and casein ($p = .005$). Plasma concentration of IgE-specific antibodies against beta-lactoglobulin and gluten were on the bottom level for all of the siblings, which precluded performance of the Kolmogorov-Smirnov analysis.

Total IgE

Mean plasma concentration of total IgE in participants with autistic disorder was more than 7 times higher (246.32 ± 639.23 kU/L) than in their siblings (37.21 ± 33.50 kU/L), with standard deviation twice the size of the average value. Standardized skewness and kurtosis were far above normal distribution. The differences were significant according to Kolmogorov-Smirnov analysis (see Table 4).

Analysis by Gender

As shown in Table 5, comparison of the distributions of the male and female samples for IgA food-specific antibodies (kU/L) in the participants with autistic disorder (male, $n = 25$; female, $n = 10$) and their siblings (male, $n = 7$; female, $n = 14$) showed statistically significant differences between the two distributions at the 95.0% confidence level for alpha-lactalbumin and beta-lactoglobulin. However, only the siblings had significant differences by gender for casein and gliadin.

Statistically significant differences in plasma concentration of IgG food-specific antibodies (kU/L) between the two distributions of the male and female samples at the 95.0% confidence level were found only for beta-lactoglobulin ($p = .042$) in the siblings (see Table 6). None of the other parameters (alpha-lactalbumin, beta-lactoglobulin, casein, or gliadin) in the children with autistic

Table 5
Plasma Concentration of IgA Food-Specific Antibodies (kU/L) in Participants With Autistic Disorder (male, $n = 25$; female, $n = 10$) and Their Siblings (male, $n = 7$; female, $n = 14$), According to Gender, in the Republic of Macedonia

Allergen	Group	Gender	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	p
α-lactalbumin (IgAf76)	Autistic disorder	Male	1.19	0.43	6.84[a]	12.54[a]	.012
		Female	1.19	0.23	2.07[a]	2.03[a]	
	Siblings	Male	1.15	0.21	1.07	–0.46	.002
		Female	1.08	0.20	3.72[a]	3.75[a]	
β-lactoglobulin (IgAf77)	Autistic disorder	Male	1.25	0.48	5.05[a]	6.97[a]	.006
		Female	1.26	0.36	1.59	0.34	
	Siblings	Male	1.11	0.19	1.45	–0.08	.006
		Female	1.09	0.24	4.62[a]	7.12[a]	
Casein (IgAf78)	Autistic disorder	Male	2.34	2.25	4.85[a]	6.22[a]	.203
		Female	1.51	0.43	–0.04	–1.21	
	Siblings	Male	1.96	1.62	2.02[a]	1.65	.006
		Female	1.66	0.65	0.58	–1.11	
Gliadin (IgAf98)	Autistic disorder	Male	1.51	0.87	4.58[a]	4.86[a]	.203
		Female	1.66	0.65	0.58	–1.11	
	Siblings	Male	1.13	0.24	1.96	1.45	.017
		Female	1.50	0.95	4.80[a]	8.90[a]	

a. Specific departure from normal distribution.

disorder or their siblings revealed significant differences between the two distributions of male and female samples.

Distributions of the male and female samples for IgE food-specific antibodies (kU/L) in the participants with autistic disorder showed statistically significant differences between the two distributions for alpha-lactalbumin ($p \leq .001$), beta-lactoglobulin ($p \leq .001$), casein ($p \leq .001$), and gliadin ($p \leq .001$). All of the siblings, regardless of gender, had IgE-specific antibodies below the basal (detection) level for alpha-lactalbumin, so statistical analyses could not be conducted. Finally, statistical analysis of the distributions of the male and female samples for total IgE and logarithmic transformation of total IgE (kU/L) in the participants with autistic disorder and their siblings did not show any statistically significant differences between the two distributions at the 95.0% confidence level.

Discussion

In this study, the concentration of specific IgA, IgG, and IgE antibodies toward certain components of the food, such as alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin, was examined in participants with autistic disorder and their siblings (brothers and sisters without autism). The concentration of total IgE antibodies in the plasma of these participants was also examined.

Analysis of the results reveals statistically significant higher plasma concentrations of IgA antibodies against alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin in children with autism compared to their siblings who did not have autism. These findings are similar to the previously published results (Lucarelli et al., 1995) with higher values for IgA to casein, lactalbumin, beta-lactoglobulin, and ovalbumin.

The significantly higher plasma concentration of IgG antibodies against alpha-lactalbumin, beta-lactoglobulin, and casein in participants with autism in this study is in accordance with the high percentage of patients with specific IgG antibodies to casein (Lucarelli et al., 1995) and significantly higher levels of both gluten and cerebellar peptide antibodies in more than 80% of the cases with autistic disorder (Vojdani, O'Bryan, et al., 2004). Plasma concentration of IgG antibodies against gliadin in this study was not significantly different between the participants with autistic disorder and their siblings.

For the first time, it was found that IgE-specific antibodies (alpha-lactalbumin, beta-lactoglobulin, and casein) were significantly higher in plasma of participants with autistic disorder in comparison to their siblings.

Gender differences between the distributions of the male and female participants for alpha-lactalbumin and beta-lactoglobulin were found for IgA food-specific antibodies (kU/L) in the participants with autistic disorder and their siblings as well as for casein and gliadin in

Table 6
Plasma Concentration of IgG Food-Specific Antibodies (kU/L) in Participants With Autistic Disorder (male, $n = 25$; female, $n = 10$) and Their Siblings (male, $n = 7$; female, $n = 14$), According to Gender, in the Republic of Macedonia

Allergen	Group	Gender	Average	Standard Deviation	Standardized Skewness	Standardized Kurtosis	*p*
α-lactalbumin (IgGf76)	Autistic disorder	Male	9.73	13.98	4.93[a]	6.80[a]	.630
		Female	12.19	11.68	0.69	–0.82	
	Siblings	Male	17.22	30.11	2.46[a]	2.92[a]	.095
		Female	5.29	14.41	5.51[a]	10.11[a]	
β-lactoglobulin (IgGf77)	Autistic disorder	Male	9.67	11.39	4.66[a]	6.80[a]	.937
		Female	8.86	7.60	0.46	–0.81	
	Siblings	Male	9.28	8.98	0.50	–0.83	.042
		Female	3.51	8.43	5.10[a]	8.96[a]	
Casein (IgGf78)	Autistic disorder	Male	22.59	29.87	7.22[a]	15.31[a]	.559
		Female	23.46	18.24	0.11	–1.24	
	Siblings	Male	21.78	22.96	0.94	–0.34	.095
		Female	8.58	17.54	4.96[a]	8.46[a]	
Gliadin (IgGf98)	Autistic disorder	Male	13.93	24.06	8.14[a]	18.03[a]	.559
		Female	10.09	10.97	3.29[a]	4.34[a]	
	Siblings	Male	6.40	6.38	2.19[a]	2.27[a]	.983
		Female	8.36	9.41	2.11[a]	0.49	

a. Specific departure from normal distribution.

siblings. IgG food-specific antibodies against alpha-lactalbumin, casein, and gliadin were not statistically different between male and female participants (except for beta-lactoglobulin in siblings). IgE food-specific antibodies against alpha-lactalbumin, beta-lactoglobulin, casein, and gluten were statistically different between male and female participants with autistic disorder, but analyses could not be conducted for their siblings. Total IgE was not statistically different for males and females participants with autistic disorder or their siblings.

The results of gender differences should be analyzed with caution, because the subdivision of the participants with autistic disorder and their siblings according to gender produced smaller, unbalanced groups (25 males and 10 females with autistic disorder, 7 male and 14 female siblings). Meta-analysis of the published results from food allergens in participants with autistic disorder and their siblings is required for more precise analysis and conclusions.

Normal values of the total IgE in the serum of humans are highly variable and have asymmetrical (nonnormal) distribution. Because of that, logarithmic transformations of the data were used (Peova, 1993; Pereira Vega et al., 1997; Sapigni et al., 1998). Because the serum level of the total IgE in the normal population acts as a criterion in the evaluation of atopic (type of immediate [Type I] hypersensitivity to common environmental allergens in humans mediated by humoral antibodies of the IgE class) and many other diseases, it is necessary to define the normative values for every region. In Macedonia, there have been very few data published on the serum values of IgE. The results of the control group of children without autism show higher values than those referred for the same age groups (Peova, 1993). IgE levels of nonatopic immigrants from Asia, Africa, the Middle East, and South America were significantly higher than the IgE levels of nonatopic Swedes. In general, IgE levels of immigrants showed a decline with time and reached approximately the same levels as for the Swedish patients in 10.5 years (Kalyoncu & Stalenheim, 1992). Results of plasma total IgE in siblings in the current study are very similar to those published for the Macedonian population (Peova, 1993). About 33% of individuals with autism have been found to have high total values for IgE (Lucarelli et al., 1995). In the current study, 37% of patients with autistic disorder had higher IgE values from mean value.

Comparison of food allergens between the participants with autism and their siblings should be carefully interpreted and could not be directly compared with the general population, because genetic and other common factors connected with food allergens are possible in the families. Multifactorial analysis of the simplex families with autistic disorder (fathers, mothers, children with autistic disorder, and their siblings) could be a more precise model for investigating familial interactions of food allergens.

These results support the idea that in individuals with predisposing HLA molecules, dietary peptides bind to aminopeptidases and possibly other enzymes and induce antibodies to dietary peptides and tissue antigens. This autoantibody production and dysfunctional membrane peptidases may result in neuroimmune dysregulation and autoimmunity (Vojdani, Bazargan, et al., 2004; Vojdani et al., 2002; Vojdani et al., 2003).

In conclusion, the current results suggest that the increased plasma concentrations of IgA- and IgG-specific antibodies to alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin; IgE-specific antibodies to alpha-lactalbumin, beta-lactoglobulin, casein, and gluten; and total IgE in the participants with autism compared with their siblings in the Republic of Macedonia indicate intestinal immunological disturbances and the need for further studies in this area.

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Vladimir Trajkovski, MD, PhD, is a professor of genetics and physiology at the Institute of Special Education and Rehabilitation, Faculty of Philosophy, at the University Ss. Kiril and Metodij in Skopje, Republic of Macedonia. His current interests are several fields of autism spectrum disorders.

Aleksandar Petlichkovski, MD, MSc, is an assistant of immunology and human genetics at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia. His current interests are polymorphisms and mutations in immunology.

Olivija Efinska-Mladenovska, Biotechnolog, is working at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia, in the field of genetics.

Dejan Trajkov, MD, MSc, is an assistant of immunology and human genetics at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia. His current interests are humoral and cellular mechanisms in immunology.

Todor Arsov, MD, PhD, is an assistant of immunology and human genetics at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia. His current interests are mutations in some genes connected with obesity.

Ana Strezova, MD, is an assistant of immunology and human genetics at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia. Her current interests are HLA genotypings and transplantation immunology.

Ljubomir Ajdinski, PhD, is an professor emeritus of defectology at the Institute of Special Education and Rehabilitation, Faculty of Philosophy, at the University Ss. Kiril and Metodij in Skopje, Republic of Macedonia. His current interests are social problems of disabled persons.

Mirko Spiroski, MD, PhD, is a full professor of immunology and human genetics at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Republic of Macedonia. He is founder and director of the institute. His interests are immunology, molecular biology, and human genetics. Please address correspondence to Mirko Spiroski, 50 Divizija No 6, PO Box 60, Skopje 1109, Macedonia, The Former Yugoslav Republic of; e-mail: mspiroski@yahoo.com