Immunological and Immunogenetic Changes in Children with Autistic Disorder in Republic of Macedonia

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Abstract

AIM: The aim of the study was to present our results about immunological and immunogenetic investigations in children with autistic disorder in Republic of Macedonia.

METHODS: Infantile autism was diagnosed by DSM-IV and ICD-10 criteria. Plasma samples were collected from 35 autistic subjects, and their 21 siblings (biological brothers and sisters) who served as healthy controls. Plasma samples were separated by centrifugation and stored at −20°C until the determination. Plasma immunoglobulin classes (IgM, IgA, IgG) and subclasses (IgG1, IgG2, IgG3, IgG4) were determined using a nephelometer Analyzer. Specific IgA and IgG antibodies against some food allergens, as well as total IgE have been determined with automated immunofluorescent device with solid phase - UniCAP 100 (AmershamBiosciences). HLA DNA typing of class I genes was performed using a Reverse Line Strip method (RLS), and the Sequencing Based Typing method (SBT) was used for typing of class II genes.

RESULTS: Children with autism had significantly higher plasma concentrations of IgG4 (p<0.001) compared to their siblings (healthy brothers or sisters). IgE specific antibodies, as well as plasma concentration of total IgE were statistically significant higher in plasma of participants with autism. Multiple comparisons for the IgA variable have shown statistically significant differences between children with autistic disorder from the fathers and mothers (p < 0.001), and healthy brothers and sisters from the fathers and mothers (p < 0.001). Our results showed significantly increased frequencies of HLA-C*03 (OR = 2.74*; χ^2= 4.68; p = 0.03), and HLA-DRB1*01 (OR = 3.10*; χ^2= 6.26; p = 0.012) alleles in autistic patients when compared to the controls.

CONCLUSION: Children with autism have increased plasma concentration of immunoglobulines. Our results demonstrate an association of HLA-C*03 and HLA-DRB1*01 alleles with Macedonian autistic patients. Comparison between healthy children and children with autistic disorder from the same family should be tested for immunoglobulin classes and subclasses in order to avoid differences between generations.

Introduction

Autism is a severe neurodevelopmental disorder characterized by a triad of impairments in reciprocal social interaction, verbal and nonverbal communication, and a pattern of repetitive stereotyped activities, behaviors, and interests [1].

A number of factors have been implicated in the pathogenesis of autism including genetic [2], environmental [3], and immunological factors [4]. There are strong lines of evidence to suggest that the immune system plays an important role in the pathogenesis of autism. These include changes in lymphocyte subsets [5], alteration in serum concentration of immunoglobulin classes and subclasses [6, 7] and cytokine production [8], presence of autoantibodies to neural antigens [9], increased frequency of the C4b null allele [10], and linkage to some immune response genes [11].

Abnormal immunoglobulins (low IgA, increased IgE), decreased natural killer cells and other T-cell abnormalities may reflect the "disregulation" of the immune system in persons with

autism [12]. The alteration in the immune system may also occur in parallel to changes in the developing central nervous system (CNS), and both may have the same aetiologies that underlie autism [9, 13-15]. Therefore the immune abnormalities would appear to be causative [16].

The aim of the study was to present our results about immunological and immunogenetic investigations in children with autistic disorder in Republic of Macedonia.

Material and Methods

Subjects

Infantile autistic disorder was diagnosed by DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1992) criteria. Thirty-five Macedonian patients (25 boys and 10 girls) with autistic disorder were studied, as well as 21 of their siblings (7 boys, and 14 girls). The mean age was 11 ± 5.90 years with minimum 4, and maximum 25 years old. The survey was realized on the territory of the Republic of Macedonia.

Immunoglobulin determination

Blood samples were separated by centrifugation, and stored at - 20°C until the determination. Serum immunoglobulin classes (IgA, IgG, and IgM) and subclasses (IgG1, IgG2, IgG3, and IgG4) were determined immunonephelometricallly by an automated Nephelometer Analyzer BN-100 (Dade-Behring, Vienna, Austria) as anonymous samples.

Determination of Specific Food Antibodies

Ten milliliters of venous blood was drawn from each donor by the standard venipuncture in a vacutainer with EDTA(K_3) after parental consent. At the time of blood drawing, none of autistic children were receiving any prescription medication or antipsychotic drug. Plasma samples were separated by centrifugation and stored at -20^0C till the determination. Specific antibodies, as well as total IgE have been determined with automated immunofluorescent device with solid phase - UniCAP 100 (AmershamBiosciences). The determinations were performed 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 United Kingdom National External Quality Assessment Service for Immunology and Immunochemistry (UKNEQAS).

Genotyping of HLA

Genomic DNA was extracted from whole blood using standard proteinase K digestion method followed by salting-out extraction and ethanol precipitation [17]. HLA DNA typing of class I genes was performed using a Reverse Line Strip method (RLS), and the Sequencing Based Typing method (SBT) was used for typing of class II genes [18-20]. Statistical analysis was performed with Arlequin v.2.000 software kindly provided by Excoffier and Slatkin [21, 22]. In summary, this program calculated HLA-A, -C, -B, and –DRB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between two alleles at two different loci. The number of autistic subjects and controls from Macedonian population positive for an allele or a haplotype were compared. The Odds Ratio (OR) expressed the strength of the statistical association between the syndrome and genetic markers. The statistical significance was examined by Chi-square test.

Statistics

The research data have been stored, classified and processed with standard statistical program Statgraphics Plus for Windows 2.1 version. Standardized skewness and stardized kurtosis were used to determine whether the samples come from normal distribution. Values of these statistics outside the range of -2 to +2 indicate significant departure from normality. Kolmogorov-Smirnov test was used to compare the distribution of the two samples. This test is performed by computing the maximum distance between the cumulative distributions of the two samples. P values of 0.05 or less were considered significant.

Results

Immunological findings in autistic disorder

Plasma concentration of IgM in autistic chlidren was significantly higher (p = 0.031) in comparison with their healthy brothers or sisters (1.36 $\pm$ 0.31 g/L and 1.20 $\pm$ 0.15 g/L, respectively). Plasma concentration of total IgG in autistic children was significantly higher (p = 0.023) in comparison with their siblings (13.14 $\pm$ 1.27 g/L and 12.39 $\pm$ 0.96 g/L, respectively). The mean value of plasma concentration for IgG4 in autistic group was 0.69 $\pm$ 0.14 g/L, and in control group 0.45 $\pm$ 0.14 g/L, and was significantly increased in autistic patients (p < 0.001). Plasma concentrations of immunoglobulin classes and subclasses were similar in autistic children and their healthy brothers or sisters for IgA, IgG1, IgG2, and IgG3 (Table 1).

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Table 1: Age and plasma concentration of immunoglobulin classes and subclasses in children with autism and their healthy broders or sisters in the Republic of Macedonia. Data are expressed as means ± SD [23].

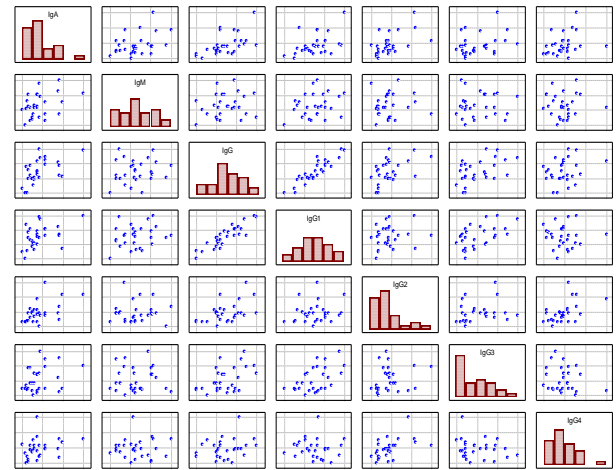| Parameters	Autistic children (n=35)	Healthy brothers or sisters (n=21)	p*
Age (year)	10.14±5.81	13.14±6.45	0.078
Immunoglobulins (g/L)			
IgA	1.63±0.33	1.52±0.30	0.217
IgM	1.36±0.31	1.20±0.15	0.031
IgG	13.14±1.27	12.39±0.96	0.023
IgG1	8.45±0.82	8.09±0.60	0.086
IgG2	2.44±0.38	2.34±0.52	0.441
IgG3	0.46±0.07	0.45±0.09	0.644
IgG4	0.69±0.14	0.45±0.14	<0.001

Student's t test.

Distribution of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) in probands, fathers and mothers are presented on the matrix plot, where the one-dimensional distribution is shown by diagonal and two-dimensional distribution by symmetrical matrix (Figure 1).

In the probands (n = 28) we can see that the individual classes and subclasses correlate between each other, there is very strong positive correlation between IgG and IgG1, and smaller correlation between the rest of variables. We can see one extreme value of IgA (4.133 g/L), and one of IgG4 (1.999 g/L), which are probably biological variations (Fig. 1.A). In the fathers (n = 24) we can see good correlation between the IgG, IgG1, IgG3, and IgG4, but also the other variables correlate to the some degree. We can see one extreme value of IgG, IgG1, IgG3, and IgG4 in the very same father, and one extreme value of IgM (Fig. 1B). In the mothers (n = 28) we can see good correlation between the IgG, IgG1, IgG2 and IgG3, but the rest of variables are also with good correlation. There are no extreme values of immunoglobulin classes and subclasses in the mothers (Fig. 1.C).
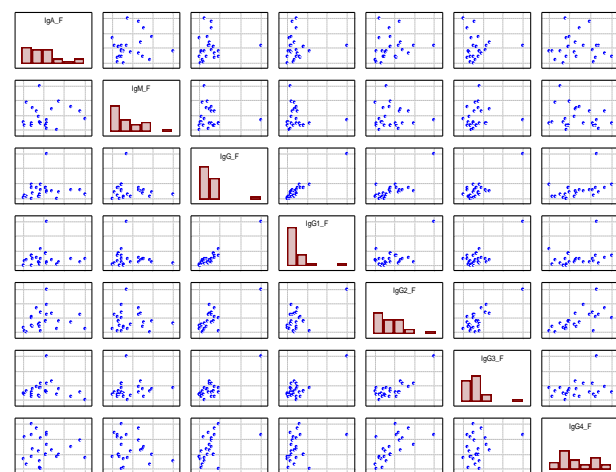
Combination of distribution of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) between probands and their parents (fathers and mothers) are presented on the matrix plot, where the distribution of probands is shown horizontally, and distribution of parent is shown vertically. We can see that the distribution of immunoglobulin classes and subclasses between probands and their fathers (n = 24), as well as with their mothers (n = 28) are dispersed, without any correlation (Figure 2).

Correlations of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) in all probands, fathers and mothers are presented on the matrix plot (Figure 3). We can see high positive correlation between IgG and IgG1, a little bit smaller positive correlation between IgG and IgG2, as well between IgG and IgG3. We can see that IgA and IgM do not correlate between themselves.
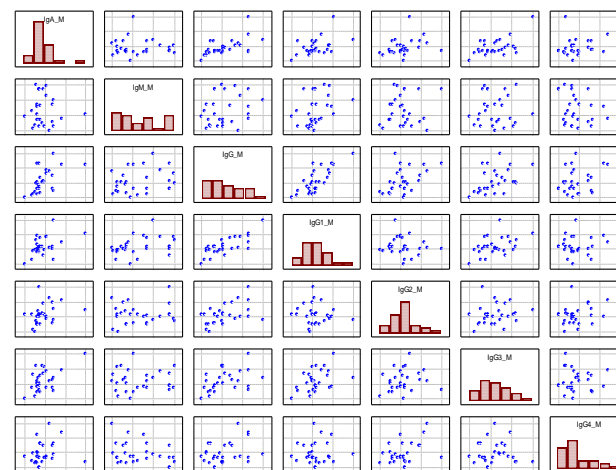

Figure 1: Distribution of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) in probands, fathers and mothers.
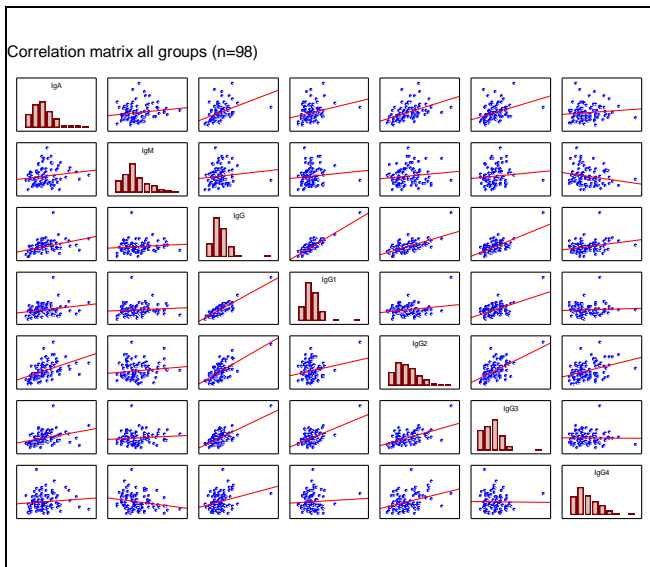
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Figure 2: Correlations of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) in all probands, fathers and mothers presented on the matrix plot.

Seven variables (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) entered into factorial multivariate analysis of variance (MANOVA) with two factors, sex (male/female), and three groups regarding autistic disorder (person with autistic disorder, father/mother of a person with autistic disorder, and brother/sister), giving altogether six groups. _Post hoc_ multiple comparisons with Tukey HSD test were made to reveal statistically significant pairs of groups for the IgA variable. We can see statistically significant differences between three pairs: male autistic from the fathers (p = 0.001), male autistic from the mothers (p = 0.008), as well as healthy sisters from the fathers (p = 0.011) (Table 2).

Table 2: Multivariate analysis of variance (MANOVA) for 6 variables, factorial model for sex (male/female), and group membership regarding autistic disorder (person with autistic disorder, father/mother of a person with autistic disorder, and brother/sister) [24].

Turkey HSD test, variable IgA for all autistic disorder (Probabilities for post hoc tests; error: between MS = 0.827, df = 92.000)

Sex*Family member	Male autistic 1.374	Father 2.531	Healthy brother 1.562	Female autistic 1.505	Mother 2.330	Healthy sister 1.472
Male autistic						
Father	0.001*					
Healthy brother	0.998	0.196				
Female autistic	0.999	0.076	0.999			
Mother	0.008*	0.971	0.427	0.224		
Healthy sister	0.999	0.011*	0.999	1.000	0.056	

*, Statistically significant.

Mean plasma concentration of total IgE in participants with autistic disorder was more than seven times higher (246.312 kU/L) than in their siblings (37.209 kU/L) with standard deviation twice bigger than average value, and standardized skewness and kurtosis far above normal distribution.
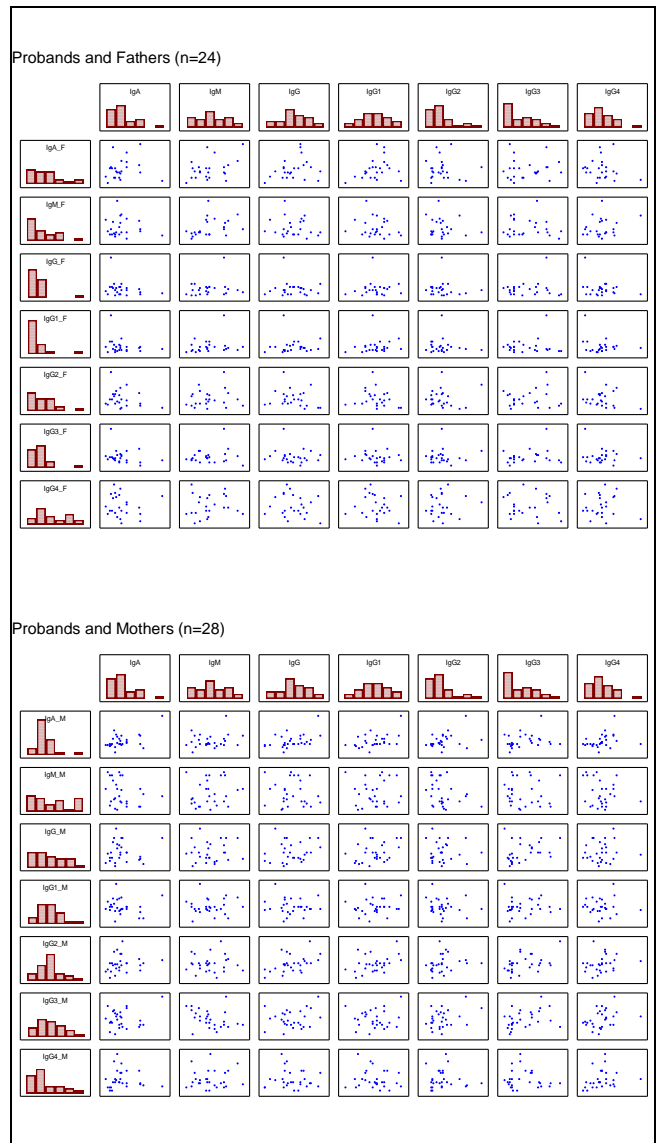

Figure 3: Combination of distribution of immunoglobulin classes and subclasses (IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4) between probands and their parents (fathers and mothers) presented on the matrix plot.

Statistical analysis with Kolmogorov-Smirnov test was significant (p = 0.044) (see Table 3).

Table 3: Plasma concentration of total IgE, and logarithmic transformation of total IgE (kU/L) in the participants with autistic disorder (n = 35) and their siblings (n = 21) in the Republic of Macedonia [25].

Allergen	Group	Average	Standard deviation	Standardized skewness	Standardized kurtosis	p
Total IgE	Autistic disorder	246.31	639.23	11.54&	30.33&	0.044
	Siblings	37.21	33.50	2.54&	1.20	
Log total IgE	Autistic disorder	1.79	0.67	1.50	0.45	0.044
	Siblings	1.38	0.46	-1.12	0.06	

&, Specific departure from normal distribution; p, statistically significant difference between the two distributions (participants with autistic disorder and siblings) tested by Kolmogorov-Smirnov test.

Immunogenetic findings in autistic disorder

Eleven different HLA-C alleles have been found in autistic group from which the most frequent were C*12 (20%), C*07 (17.1%) and C*03 (12.9%). Statistical analysis with Chi-square test have shown significantly higher frequency of C*03 allele, which can be found 2.5 times more in autistic patients than in healthy population (OR = 2.74; χ^2 = 4.68; p = 0.03;) (Table 4).

Table 4: HLA-C allele frequencies in Macedonian autistic patients and controls [26].

HLA-C allele	Allele frequency N (%) controls	Allele frequency N (%) autism	Odds ratio OR	χ^2	p-value
C*01	9 (4.6)	2 (2.9)	0.61	0.08	NS
C*02	19 (9.7)	6 (8.6)	0.87	0.08	NS
C*03	10 (5.1)	9 (12.9)	2.74*	4.68	0.03
C*04	27 (13.8)	6 (8.6)	0.59	1.29	NS
C*05	8 (4.1)	0	2.71	1.71	NS
C*06	8 (4.1)	6 (8.6)	2.20	2.09	NS
C*07	53 (27.0)	12 (17.1)	0.56	2.74	NS
C*08	1 (0.5)	3 (4.3)	8.73	2.74	NS
C*12	32 (16.3)	14 (20.0)	1.28	0.49	NS
C*14	8 (4.1)	5 (7.1)	1.81	1.04	NS
C*15	15 (7.6)	5 (7.1)	0.93	0.02	NS
C*16	3 (1.5)	2 (2.9)	1.89	0.04	NS
C*17	3 (1.5)	0	0	0.15	NS
Total	**196 (100.0)**	**70 (100.0)**			

Data from class II, specifically alleles from DRB1 locus are shown in Table 5. Ten different alleles have been found in autistic patients, with most frequency of DRB1*11 (21.4%), DRB1*01 (14.3%), DRB1*15 (12.9%) and DRB1*16 (12.9%). Significantly higher frequency of DRB1*01 allele compared to control subjects have been found (OR = 3.10*; χ^2 = 6.26; p = 0.012).

Table 5: HLA-DRB1 allele frequencies in Macedonian autistic patients and controls [26].

HLA-DRB1 allele	Allele frequency N (%) Controls	Allele frequency N (%) autism	Odds ratio OR	χ^2	p-value
DRB1*01	10 (5.1)	10 (14.3)	3.10*	6.26	0.012
DRB1*03	14 (7.1)	5 (7.1)	1.00	0.00	NS
DRB1*04	15 (7.6)	3 (4.3)	0.54	0.47	NS
DRB1*07	13 (6.6)	4 (5.7)	0.85	0.00	NS
DRB1*08	6 (3.1)	0	0	1.02	NS
DRB1*09	1 (0.5)	0	0	0.29	NS
DRB1*10	0	2 (2.9)	0	2.46	NS
DRB1*11	47 (24.0)	15 (21.4)	0.86	0.19	NS
DRB1*12	3 (1.5)	0	0	0.15	NS
DRB1*13	18 (9.2)	8 (11.4)	1.28	0.29	NS
DRB1*14	6 (3.1)	5 (7.1)	2.44	2.17	NS
DRB1*15	25 (12.7)	9 (12.9)	1.01	0.00	NS
DRB1*16	38 (19.4)	9 (12.9)	0.61	1.51	NS
Total	**196 (100.0)**	**70 (100.0)**			

Discussion

Our study has shown that children with autism had significantly increased values of IgG4 compared with their healthy siblings. This could be a consequence of enhanced autoimmunity and/or allergy in persons with autism. When autistic males were compared with their healthy brothers and autistic females with their healthy sisters, there was a statistical increase in plasma concentration of IgG1. In addition, autistic females had significantly higher plasma concentration of total IgG in comparison with their healthy sisters. These results differ from the cumulative data for all the children with autism.

Studies of immunoglobulins or antibodies-titers in autistic patients have yielded contradictory results so far. Some investigators found no abnormal increase in immunoglobulin levels in either serum or cerebrospinal fluid [27]. Ferrari et al [28] reported elevated IgG, IgM, and IgA antibody-titers in the serum of autistic patients, although significance was only reached for IgG titers. In contrast, in the study of Gupta et al [29], 20% of children with autism had a deficiency of IgA and 8% lacked it completely, and 20% had an IgG subclass deficiency. Serum levels of IgM and IgE were increased in 56% of patients, and high levels of IgG1 subclass were found in only 2 patients. Comparing our results with those of Gupta et al [29], we found significantly higher concentration of IgA, IgG1, IgG2, and IgG4. In 8 of 35 persons with autism (23%) we found lower levels of IgA compared to the normal values (data are not shown). Wareen et al [12] reported that 20% of individuals with autism had low serum IgA. Thus, IgA deficiency is more common in autism than in normal Caucasian population.

Low levels of IgG, IgA, and IgM and subfractions of IgG were found by Zimmerman et al [6]. They reported several autistic patients, positive for antinuclear antibodies, characteristic for autoimmune disorders like lupus or rheumatoid arthritis [6]. Croonenberghs et al [7], found increased serum concentrations of IgG, IgG2, and IgG4. They reported positive correlations between social problems and total serum proteins and serum gamma globulins, as well as between the withdrawal of the symptoms and total serum proteins and serum albumin and IgG.

Our study showed that statistically significant differences found with factorial multivariate analysis of variance with two factors, sex (male/female), and three groups regarding autistic disorder (person with autistic disorder, father/mother of a person with autistic disorder, and brother/sister) belongs to IgA, IgM, and IgG2 variables. _Post hoc_ multiple comparisons with Tukey HSD reveal statistically significant pairs of groups for the IgA variable. We found statistically significant differences between three pairs: male autistic from the fathers, female autistic from the mothers, as well as healthy sisters from the fathers. The results should be interpreted very carefully. We can interpret that IgA, IgM, and IgG2 are connected somehow with the autistic disorder in the male and female persons, but we cannot interpret connection of healthy sisters with the fathers. There is also possibility that found differences are rather between generations (older and younger generations), and/or because of the small number of the groups (there are only 8 female with autistic disorder, and only 7 healthy brothers) [23].

We found that mean plasma concentration of total IgE in participants with autistic disorder was more than seven times higher than in their siblings. We examined 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. We found statistically significant higher plasma concentration of IgA antibodies against alpha-lactalbumin, beta-lactoglobulin, casein, and gliadin 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 [25]. 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.

The strongest evidence for a link between autism and immune system comes from immunogenetic studies. HLA antigens are involved in the qualitative and quantitative aspects of immune system response. If autism is in some cases the result of immune system attack, then particular HLA antigens might be involved as a sufficient or predisposing factor [30]. The immunogenetic profile of the Macedonian population represented by HLA genes is published for HLA-A, -B, -DR, -DQ allele frequencies [31], for HLA-DRB1, DRB3/4/5 and DQB1 polymorphism [32], for HLA-A and HLA-B epidemiology [33], for high-resolution sequence-based method for direct HLA-DRB1 typing [34], for ambiguous allele combinations at the allele group level of HLA-A, -C and -B loci [35], and for HLA-DQB1 typing [36]. Our results showed significantly increased frequencies of HLA-C*03 and HLA-DRB1*01 alleles in autistic patients when compared to the controls.

Rogers et al. in 1999 have tested a hypothesis about a role for the HLA loci in the genetic susceptibility to autism. They did that by linkage analysis using genetic marker loci in the HLA region on chromosome 6p in multiplex families with autism. They have examined sharing of alleles identical by descent in 97 affected sib pairs from 90 families. Their results demonstrated no deviation from null expectation of 50% sharing of alleles in that region. Thus, it was unlikely that loci in that region contribute to the genetic etiology of autism to any significant extent in our families [37].

Our results for the connection between class I and II alleles in persons with autism in the Macedonian population are different from those published in the literature. While Warren R. et al. [38] found association with alleles DRB1*0401, DRB1*0101 and DRB1*0701, we found association with DRB1*01 alleles. His team found association with HLA-B* allele [10], which we couldn't register in the Macedonian sample of children with autism. We detected increased frequency of the HLA-C*03 allele and association with autism, a data we did not succeed t find in the literature. These differences could be due to a different genetic structure of the Macedonian population or to different association of the HLA alleles with the autism in the Macedonian population. Additional examinations of the HLA alleles in the families with autism are necessary to get more answers about the Macedonian population.

Several investigations of transmission disequilibrium (TDT) for HLA in families with autism were investigated. Significant transmission disequilibrium for HLA-DR4 was seen (odds ratio, 4.67; 95% confidence interval, 1.34-16.24; P = 0.008) for transmissions from maternal grandparents to mothers of probands, supporting a role for HLA-DR4 as an autism risk factor acting in mothers during pregnancy. Transmission disequilibrium was not seen for HLA-DR4 transmissions from parents to probands or from mothers to probands. The HLA-DR4 gene may act in mothers of children with autism during pregnancy to contribute to autism in their offspring [39]. The transmission disequilibrium test indicated that the ASD probands inherited the DR4 allele more frequently than expected (p = 0.026) from the fathers. The TDT also revealed that fewer DR13 alleles than expected were inherited from the mother by ASD probands (p = 0.006) [40]. The transmission disequilibrium test for the A2 allele revealed an increased frequency of inheritance for autistic children (p = 0.033). There were no significant associations of autism with HLA-B alleles; however, the A2-B44 and A2-B51 haplotypes were two times more frequent in autistic subjects [41].

Killer-cell immunoglobulin-like receptor (KIR) proteins are expressed on natural killer (NK) cells and appear important in innate and adaptive immunity. There are about 14 KIR genes on chromosome 19q13.4, composed of those that inhibit and those that activate NK cell killing. Haplotypes have different combinations of these genes meaning that not all genes are present in a subject. There are two main classes of cognate human leukocyte antigen (HLA) ligands (HLABw4 and HLA-C1/C2) that bind to the inhibitory/activating receptors. As a general rule, the inhibitory state is maintained except when virally infected or tumor cells are encountered; however, both increased activation and inhibition states have been associated with susceptibility and protection against numerous disease states including cancer, arthritis, and psoriasis. Utilizing DNA from 158 Caucasian subjects with autism and 176 KIR control subjects it was shown for the first time a highly significant increase in four activating KIR genes

(2DS5, 3DS1, 2DS1 and 2DS4) as measured by chi square values and odds ratios. In addition, published data suggests a highly significant increase in the activating KIR gene 2DS1 and its cognate HLA-C2 ligand (2DS1+C2; p=0.00003 [Odds Ratio=2.87]). This information ties together two major immune gene complexes, the Human Leukocyte Complex and the Leukocyte Receptor Complex, and may partially explain immune abnormalities observed in many subjects with autism [42]. Activating KIR/HLA complexes (aKIR/HLA) were recently suggested to prevail in children with autism spectrum disorders (ASD), a neurodevelopmental syndrome character-rized by brain and behavioral abnormalities and associated with a degree of inflammation. It was verified whether such findings could be confirmed by analyzing two sample cohorts of Sardinian and continental Italian ASD children and their mothers. Results showed that aKIR/HLA are increased whereas inhibitory KIR/HLA complexes are reduced in ASD children in two sample cohorts of Sardinian and continental Italian ASD children and their mothers. KIR and HLA molecules are expressed by placental cells and by the trophoblast and their interactions result in immune activation and influence fetal, as well as central nervous system development and plasticity. Published data suggest that in utero KIR/HLA immune interactions favor immune activation in ASD and this may play a role in the pathogenesis of the disease [43].

In conclusion, we can say that children with autism have increased plasma concentration of immunoglobulines. Multiple comparisons for the IgA variable have shown statistically significant differences between children with autistic disorder from the fathers and mothers (p < 0.001), and healthy brothers and sisters from the fathers and mothers (p < 0.001). Our results demonstrate an association of HLA-C*03 and HLA-DRB1*01 alleles with Macedonian autistic patients.

Acknowledgements

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