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HLA-DQ Genotyping of Celiac Disease among Syrian patients

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ABSTRACT

Background
Celiac disease is an autoimmune enteropathic disease brought about by consuming gluten in people who are genetically predisposed by carrying HLA DQ2 or/and HLA DQ8 genotypes.
Objectives
To evaluate the distribution of human leukocyte antigen (HLA)-DQ2 and -DQ8 and their relative riskwith celiac disease (CeD) in Syrian patients comparedto healthy Syrian controls.
Subjects &Methods
Genotyping for unrelated CeD patients (n = 100) and unrelated matched healthy controls (n = 100) was performed to identify DQ2 and DQ8 alleles by SSP-PCR
Results:
In this case control study 82% of CeD cases and 24.5 % of controls were carriers of HLA DQ2 whereas 18 % and 8.5 % ofCeD patients and controls respectively carried HLA DQ8. The highest risk for the development of CD relating to HLA-DQB was found in patients carrying DQ2.5/DQ8 genotype and DQ2.5/DQ2.5 (1/10) and the lowest risk was found in patients with DQ2.2/DQ8 genotype.
Conclusion:
In Syrian Patients, celiac disease is strongly related to HLA DQ 2 and DQ 8 genotypes and thus these possess a high positive predictive value for the diagnosis of celiac disease. Additionally, our study highlighted the integral part of these genotypes in the pathogenesis of celiac disease.

Keywords: celiac, Gluten, genotyping, Allele, Marsh, Syrian

INTRODUCTION

Genetically susceptible persons with HLA-DQ2 or DQ8 heterodimer are subject to develop celiac disease (CeD) (1-3).Globally, the presence of celiac disease (CeD) has risen lately. In the western population,Ced prevalence is believed to be between 1% to 3% (4–8).

The widespread use of gluten-free diet (GFD) among the inhabitants has restricted the serologic test usefulness in terms of the negative effect of gluten removal on the precision of CeD diagnostic test by reducing the sensibility of serology (5).

Considering that CeD mostly affects gluten consuming individuals and persons who have the HLADQ2 or DQ8 heterodimer,when diagnosis is unreliable, HLA genotyping might be necessary for CeD distinguishing in patients on GFD (9).The peptide-binding notch in antigen-presenting cells is formed from major histocompatibility complex class II molecules comprised of 1 α-chain and 1 β-chain and is encoded by HLA-DQ genes(10).

The two common molecularly distinguished heterodimer HLA-DQ2.5 and HLA-DQ2.2 are related to CeD and form the HLA-DQ2 according to serotype

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classification. HLA-DQA1*05 XX and HLA-DQB1*02;01 haplotype form the HLA-DQ2.5 which is produced mainly by either a cis haplotype (HLA-DQA1*05:01/HLA-DQB1*02:01) or a trans haplotype configuration (HLA-DQA1*05:XX/HLA-DQB1*03:01 and HLADQA1* 02:01/HLA-DQB1*02:02).The HLA-DQA1*02:01 and HLA-DQB1*02:02 haplotypes express the HLA-DQ2.2 heterodimers, and the HLA-DQA1*03:01 and HLA-DQB1*03:02 haplotype express the HLA-DQ8 heterodimer (1,11).

HLA test is considered an accurate tool in the diagnostic algorithm of CD, because the HLA genes are lifelong steady markers and have been used especiallyto recognize genetically susceptible andnon-susceptible persons for CD pre the occurrence of any clinical or serological signs (12).

This studywas carried out in Syria. 100 CeD patients were recruited for this study. Studies about celiac disease are limited in Syria but there was one study that discussed itsprevalence in Syria and which found that 1/62 persons have the disease, as well as another study that aimed to genotype the HLA Dq2 and Dq8 amongchildren. Thisstudy aimed to evaluate the diagnosis of pediatric and adult Ced patients with the use of DQ2 and DQ8 Haplotypes.

SUBJECTS & METHODS

Study design and population

The Ethics Committee of Damascus University in Syria had approved the protocol of this study and all participants submitted written informed consents prior to the commencement of this study. In addition to that, parental approval was obtained for minor participants.

The design of this case-control study was used to test the research hypothesis. The research sample consisted of 100 unrelated CeD patients (both pediatric and adult patients included), with a similar ratio of males to females (51, 49 respectively). The average age was 19.12 ± 13.71. Patients were recruited from gastroenterological centers at Damascus university hospitals – Syria.

For the control group, 100 healthy subjects corresponding in age(22 ±14) and gender (51 Males and 49 females) to the case group, with no previous systematic diseases and with the absence of previously diagnosed celiac disease, cancer, gastrointestinal complains or autoimmune disorders. All examined individuals yielded negative serological tests resultsfor CD. The subjects of this group were selected from private clinics inSyria. All those patients were tested positive for tTGA and/or EMA antibodies and histology based on Rostami Marsh classification.

Study process:

This study was carriedout at the biotechnology center at Al-Baath University in Syria. 5ml of venous EDTA-anti-coagulated blood had been drawn from each participant and put at 2-8 c till genomic DNA was extracted.

DNA extraction was done within 3 days at most, out of 200 µl whole blood specimen through the Wizard Genomic DNA Purification kit (GF1, Malaysia)

The concentrations of the DNA specimen had been determined spectrophotometrically by using BioSpec-nano Life Science Spectrophotometer .

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HLA DQ2 & DQ8 genotyping:

Using the polymerase chain reaction (PCR) stored and newly extracted DNA was amplified by the allele particular primer sets displayed in table 1 (13-15).

The amplified alleles were included DQA1*0201, DQA1*0301, DQA1*0501, DQA1*0502, DQB1*02, and DQB1*0302. For each reaction, Human growth hormone (HGH) primer sets had been used as an internalcontrol. In addition to the HGH endogenous monitor, amplification reactions had been setup for HLA-DQA1 and HLA-DQB1 individually and together. The data are demonstrated in table 1.

Thermal cycler (Techne,UK) was used for the Amplification reactions. Table2 represents the touchdown thermal profile. PCR test was used to generate the results, and it was confirmed by loading of the PCR yield onto 2% agarose gel, spotted with ethidium bromide and displayed using a UV- gel documentation system in order to exclude undefined amplification.

Table 1. Nucleotide sequence of the PCR primers and their characteristics.

Primer	Primers Sequence (5' to 3')	Amplicon Size (bp)	Reference
DQB1*02 Forward	GTGCGTCTTGTGAGCAGAAG	198	Bunce et al.,1995
DQB1*02 Reverse	CGTGCGGAGCTCCAACTG		
DQB1*0302 Forward	GTGCGTCTTGTGACCAGATA	119	
DQB1*0302 Reverse	CTGTTCCAGTACTCGGCGG		
DQA1*0201 Forward	ACGGTCCCTCTGGCCAGTT	120	Scola et al.,2008
DQA1*0201 Reverse	GCGGGTCAAATCTAAGTCTGT		
DQA1*0301 Forward	CCCTCGCCCTGACCACCG	195	
DQA1*0301 Reverse	TGCGGAACAGAGGCAACT		

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DQA1*0501 Forward	CTCAGACAATTTAGATTTGACCC	92	
DQA1*0501 Reverse	GAGTTGGAGCGTTTAATCAGAC		
DQA1*0502 Forward	CTCAGACAATTTAGATTTGACCG	92	
DQA1*0502 Reverse	GAGTTGGAGCGTTTAATCAGAC		
HGH Forward	GCC TTC CCA ACC ATT CCC TTA	439	(Profaizer et al., 2011)
HGH Reverse	TCA CGG ATT TCT GTT GTG TTT		

Table 2: Temperature profile for amplification of DQA and DQB Alleles

Amplification program	**DQB Allels**			**DQA Allels**		
	Cycles	**Temp**	**Time**	**Cycles**	**Temp**	**Time**
	1	96 C°	1 min	1	96 C°	5 min
	5	96 C°	25 sec	30	96 C°	30 sec
		70	45 sec		61 C°	30 sec
		72	45 sec		72 C°	45 sec
	31	96	25 sec	1	72 C°	2 min
		65	50 sec			
		72	45 sec			
	4	96	25 sec			
		55	60 sec			
		72	120 sec			

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Holding Stage	4 C̊	Holding Stage	4 C̊

Data analysis:

SPSS V.22 software(SPSS Inc., Chicago, Illinois, USA) was used to analyze the data. Confidence level was set at 95% (P value ≤0.05), calculation for the relative risk of developing the disease for specific genotypes was made as following ((the percentage of allele in patients / the percentage of allele in controls) x probability of being affected in the general population)(16).

RESULTS

For the purpose of this study, participants were split up into two groups. The case group was compromised of one hundred Syrian patients diagnosedwith CD(51 males and 49 females) and the control group contained a100 healthy Syrian individuals. The participants ages ranged from 4 years to 65 years. Everyonewithin both groupswere genotyped for HLA-DQ2 and DQ8 genes.

The allele and genotype frequencies of DQ2 and DQ8 genes in individuals affected by CeD were contrasted with the controls. The frequency of the DQB1*02:01 allele was significantly higher in the patients group juxtaposed with the controls' (82 % vs. 24.5 %; p value: <0.001). The frequency of the DQB1*03:02 allele was also higherin CDpatients, but uncorrected P value significance wasborderline (18 % vs. 8.5 %; p value: 0.005)as shown in table 3:

Table 3: HLA-DQ2 (DQB1*0201) and HLA-DQ8 (DQB1*0302) alleles frequency in patients and control

Allele	Patient (n= 100) (200 alleles)		Control (n= 100) (200 alleles)		P-value
	N	Fr (%)	N	Fr (%)	
DQB1*0201	164	82	49	24.5	< 0.001
DQB1*0302	36	18	17	8.5	0.005
DQX	0	0	134	67	< 0.001

Genotype analysis of DQ2 and DQ8 genes revealed that t 53 outof 100 patients (53%) carry DQ2.5 in homozygous genotype which can be considered a high ratio. On the other hand, this genotype was found only in 11 of the controls (11 %). The frequency of the DQ2.5/DQ2.2 or DQ2.5/DQ8 and DQ 2.2/Dq 2.2 was increased in patients compared to controls (11 % vs. 5 %), (17 % vs. 2 %), (8 % vs.2 %) respectively. More genotype analysis denoted that 11 % of patients carried another DQ genotypes, while 80 % of controls had these genotypes (Table 4).

The highest HLA-DQB relative risk for CD development was found in patients carrying DQ2.5/DQ8& DQ 2.5/DQ 2.5 genotype (1/10), while, the patients

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carryingDQ2.2/DQ2.2 or DQ2.5/DQ2.2 genotype had a relative risk about 1/ 12.5 and 1/25 respectively (Table 4). Based on the Marsh Score, 4 Patients were categorized as marsh 1 , 14 patients as Marsh II and others as Marsh III.

Table 4: Distribution of HLA-DQ genotypes in patients with celiac disease and control

HLA Genotype	Patient (n= 100)		Control (n= 100)		Odds ratio	95% CL	Relative risk	**	P-value
	N	Fr (%)	N	Fr (%)					
DQ2.5/DQ8	17	17	2	2	10.0361	2.2527 to 44.7132	8.5000	1/10	**0.0025**
DQ2.5/DQ2.5	53	53	11	11	9.1238	4.3560 to 19.1102	4.8182	1/10	**0.0001**
DQ2.5/DQ2.2	11	11	5	5	2.3483	0.7848 to 7.0264	2.2000	1/25	**0.1268**
DQ8/DQX	8	8	5	5	1.6522	0.5213 to 5.2365	1.6000	1/40	**0.3936**
DQ2.2/DQ8	3	3	5	5	0.5876	0.1366 to 2.5280	0.6000	1/100	**0.4751**
DQ2.2/DQ2.2	8	8	2	2	4.2609	0.8817 to 20.5919	4.0000	1/12.5	**0.0713**
DQ2.x	0	0	3	3	0.1386	0.0071 to 2.7185	0.1429	0	**0.1931**
DQX/DQX	0	0	67	67	0.0025	0.0001 to 0.0410	0.0074	0	< **0.0001**

**Relative risk of developing disease for specific genotypes were calculated as (% of allele in patients / % of allele in controls) x probability of being affected in general population

The frequency of patients who have mild form of CD (Marsh I) carrying DQ2 allele was 3 %, whereas, the frequency was 11 % for patients having DQ2 with Marsh II classification. 58 patients were classified into Marsh III.

The Frequency of patients who carry DQ2/DQ8 were 0%,1%,19% , whereas patients who carry DQ8 Allele were 1%,2%,5% for Marsh I, MarshII,Marsh III respectively.

These results suggest therole of DQ2 allele in the intensity ofmucosal lesions (Table 5).

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Table 5: Distribution of HLA-DQ2 and DQ8 alleles in relation with to severity of mucosal damage

Marsh classification	Patient (n= 100)						P-value
	DQ2		DQ8		DQ2 / DQ8		
	N	Fr (%)	N	Fr (%)	N	Fr (%)	
Marsh I	3	3	1	1	0	0	< 0.001
Marsh II	11	11	2	2	1	1	< 0.001
Marsh III	58	58	5	5	19	19	< 0.001

DISCUSSION

It was believed that celiac disease occurs rarely in childhood, but now the possibility of incurring the diseaseis recognized inany age. The range of clinical manifestations of this illness is wide; almost only half of celiac disease patients present with typical symptoms like chronic diarrhea and failure to thrive, while the remaining patients exhibit extra - gastrointestinal manifestations such as an isolated short stature, therapy – reluctant anemia, osteopenia, dental enamel disorders, late puberty and ataxia. (17,18) The lack or absence of gastrointestinal symptoms and signs with distinctive villous atrophy is defined as an irregular form of the disease(19) and is usually seen in older children and adult.

In Syria, the diagnostic method for CeD depends primarily on serological tests or small bowel biopsy. This method does not correspond to the published CeD diagnosis standards of the different global guidelines such as NASPGHAN, ESPGHAN and BSG (19,20,21).

One helpful method to exclude CeD or to confirm the diagnosis, especially in cases that are negative for both markers, is genotyping for HLA-DQ2. Thus, detecting the expression of (DQA1*05, DQB1*02) HLA DQ2 and (DQB1*03:02, HLA-DQA1*0301) provides a great tool to exclude the presence of CeD in dubious individuals.

This study reveals that 82% of Syrian CD patients carries the DQB1*0201 allele and 18 % carries the DQB1*0302 allele. It was found that all studied patients who carried two DQ2 or DQ8 alleles had onesevere symptom (Chronic diarrhea, weight loss, stunted growth, iron deficiency anemia or chronic abdominal ache), along a high titer of anti-transglutaminase antibody. Many of these patients had been classified depending on March classification with various degrees of severity regardingvillous atrophy.

An extremely important correlation was found in this study among DQB1*02:01 allele and CeD occurrence, whereas the bond with DQB1*03:02 allele was marginal. Those outcomes showed that there was a major statistically important correlation among these two specific alleles and the Syrians CeDpatients. These results corresponded with data fromseveralother studies for CeD diagnosed patients from many Arabic countries regarding HLA-DQ2 variants; in Syria (76%), Palestine

(84.6%); Jordan (100%) and Morocco (45.2%) (22-25). Moreover, these HLA-DQ2 variants have a large frequency in France (87%), Italy (84%), and UK (88%) CD patients (26).

The relative risk for different HLA genotype has been appraised. The HLA-DQ genotype with the most relative risk for CeD was DQ2.5/DQ8 or DQ2.5/DQ2.5 genotype (1/10). In addition, DQ2.2/DQ2.2 genotype carriers have been classified in the group with high relative risk (1/12.5). Also, a new available research proved that DQ2.5/DQ2.5genotype exhibited the highest risk for CD (27). Whereas, in the case group, the patients who carried DQ2.5/DQ2.2 or DQ8/DQX genotypes held intermediate risks for CD (1/25 and 1/40) respectively. Patients with DQ2.2/DQ8 genotype had the lowest risk.

Therefore, a higher risk was noticed for CeD occurrence among patients who have a single or doubled titers of HLA-DQ2.5. The prior published data discussing the effective dose of DQB1*02 allele necessary for CeD development were similar to the results of this study (28).

This research results revealed that the prevalence of CD patients with DQ2 allele and Marsh I is (3%),(11%) for Marsh II, and 3% for CD patients who had DQ8. Marsh III score was found to have an increasemore than eleven-fold inprevalence in CD patients who carry DQ2 (58 %) compared with CeD patients who carry DQ8 (5%). Patients who carried both DQ2 and DQ8 had the highest percentage (19%). These results suggest an important positive correlation of DQ2 allele with Marsh III in CeD individuals.Many previous studies displayed the part of the DQ2 allele in mucosal injury intensity, while Zamani et al., found a very important correlation between 02:01 allele and Marsh IIIc patients indicating the participation of this allele in the intensity of damage to the mucosa(29).

CONCLUSION

with HLA - DQ genotyping, celiac disease in Syrian patients was proved to be mainly associated with HLA DQ 2 and/or DQ8 genotype. Depending on the previousinformation, we recommendcarrying out a prospective research in the future to study the HLA genotype role when joined with serology in symptomatic subjects for positive predictive value.

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REFERENCES

[1] Murray J, Moore S, van Dyke C, Lahr B, Dierkhising R, Zinsmeister A, Melton L, Kroning C, El–Yousseff M, Czaja A: HLA DQ Gene Dosage and Risk and Severity of Celiac Disease. *Clinical Gastroenterology and Hepatology* 2007, 5:1406-1412.

[2] Pietzak M, Schofield T, McGinniss M, Nakamura R: Stratifying Risk for Celiac Disease in a Large At-Risk United States Population by Using HLA Alleles. *Clinical Gastroenterology and Hepatology* 2009, 7:966-971.

[3] Congia M, Cucca F, Frau F, Lampis R, Melis L, Clemente M, Cao A, De Virgiliis S: A gene dosage effect of the DQA1∗0501/DQB1∗0201 allelic combination influences the clinical heterogeneity of celiac disease. *Human Immunology* 1994, 40:138-142.

[4] Choung R, Ditah I, Nadeau A, Rubio-Tapia A, Marietta E, Brantner T, Camilleri M, Rajkumar V, Landgren O, Everhart J, Murray J: Trends and Racial/Ethnic Disparities in Gluten-Sensitive Problems in the United States: Findings from the National Health and Nutrition Examination Surveys From 1988 to 2012. *American Journal of Gastroenterology* 2015, 110:455-461.

[5] Choung R, Unalp-Arida A, Ruhl C, Brantner T, Everhart J, Murray J: Less Hidden Celiac Disease But Increased Gluten Avoidance Without a Diagnosis in the United States. *Mayo Clinic Proceedings* 2017, 92:30-38.

[6] Liu E, Dong F, Barón A, Taki I, Norris J, Frohnert B, Hoffenberg E, Rewers M: High Incidence of Celiac Disease in a Long-term Study of Adolescents With Susceptibility Genotypes. *Gastroenterology* 2017, 152:1329-1336.e1.

[7] Virta L, Saarinen M, Kolho K: Declining trend in the incidence of biopsy-verified coeliac disease in the adult population of Finland, 2005-2014. *Alimentary Pharmacology & Therapeutics* 2017, 46:1085-1093.

[8] West J, Fleming K, Tata L, Card T, Crooks C: Incidence and Prevalence of Celiac Disease and Dermatitis Herpetiformis in the UK Over Two Decades: Population-Based Study. *American Journal of Gastroenterology* 2014, 109:757-768.

[9] Kaukinen K, Partanen J, Maki M, Collin P: HLA-DQ typing in the diagnosis of celiac disease. *The American Journal of Gastroenterology* 2002, 97:695-699.

[10] Fallang L, Bergseng E, Hotta K, Berg-Larsen A, Kim C, Sollid L: Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nature Immunology* 2009, 10:1096-1101.

[11] Karinen H, Kärkkäinen P, Pihlajamäki J, Janatuinen E, Heikkinen M, Julkunen R, Kosma V, Naukkarinen A, Laakso M: Gene dose effect of theDQB1*0201allele contributes to severity of coeliac disease. *Scandinavian Journal of Gastroenterology* 2006, 41:191-199.

[12] Megiorni F, Pizzuti A: HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. *Journal of Biomedical Science* 2012, 19:88.

[13] Bunce M, O'Neill C, Barnardo M, Krausa P, Browning M, Morris P, Welsh K: Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 1995, 46:355-367.

[14] Scola L, Lio D, Candore G, Forte G, Crivello A, Colonna-Romano G, Pes M, Carru C, Ferrucci L, Deiana L, Baggio G, Franceschi C, Caruso C: Analysis of HLA-DRB1,DQA1,DQB1 haplotypes in Sardinian centenarians. Experimental Gerontology 2008, 43:114-118.

[15] Profaizer T, Eckels D, Delgado J: Celiac disease and HLA typing using real-time PCR with melting curve analysis. *Tissue Antigens* 2011, 78:31-37.

[16] Megiorni F, Mora B, Bonamico M, Barbato M, Nenna R, Maiella G, Lulli P, Mazzilli M: HLA-DQ and risk gradient for celiac disease. *Human Immunology* 2009, 70:55-59.

[17] Makharia GK, Baba CS, Khadgawat R et al. Celiac disease:variations of presentations in adults. *Indian J Gastroenterol*.2007;26:162–6.

[18] Cronin C, Shanahan F: Exploring the Iceberg-the Spectrum of Celiac Disease. The American Journal of Gastroenterology 2003, 98:518-520.

[19] Ludvigsson J, Leffler D, Bai J, Biagi F, Fasano A, Green P, Hadjivassiliou M, Kaukinen K, Kelly C, Leonard J, Lundin K, Murray J, Sanders D, Walker M, Zingone F, Ciacci C: The Oslo definitions for coeliac disease and related terms. *Gut* 2012, 62:43-52.

[20] Husby S, Koletzko S, Korponay-Szabó I, Mearin M, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer K: European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. Journal of Pediatric Gastroenterology and Nutrition 2012, 54:136-160.

[21] Hill I, Dirks M, Liptak G, Colletti R, Fasano A, Guandalini S, Hoffenberg E, Horvath K, Murray J, Pivor M, Seidman E: Guideline for the Diagnosis and Treatment of Celiac Disease in Children: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Journal of Pediatric Gastroenterology and Nutrition 2005, 40:1-19.

[22] Mohammed M, Omar N, Shebl A, Mansour A, Elmasry E, Othman G: Celiac Disease Prevalence and its HLA-genotypic Profile in Egyptian Patients with Type 1 Diabetes Mellitus. Trends in Medical Research 2014, 9:81-97.

[23] Ayesh B, Zaqout E, Yassin M: HLA-DQ2 and -DQ8 haplotypes frequency and diagnostic utility in celiac disease patients of Gaza strip, Palestine. Autoimmunity Highlights 2017, 8.

[24] El-Akawi Z, Al-Hattab D, Migdady M: Frequency of HLA-DQA1*0501 and DQB1*0201 alleles in patients with coeliac disease, their first-degree relatives and controls in Jordan. Annals of Tropical Paediatrics 2010, 30:305-309.

[25] Piancatelli D, Ben El Barhdadi I, Oumhani K, Sebastiani P, Colanardi A, Essaid A: HLA Typing and Celiac Disease in Moroccans. Medical Sciences 2017, 5:2.

[26] Margaritte-Jeannin P, Babron M, Bourgey M, Louka A, Clot F, Percopo S, Coto I, Hugot J, Ascher H, Sollid L, Greco L, Clerget-Darpoux F: HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease. *Tissue Antigens* 2004, 63:562-567.

[27] Liu E, Lee H, Aronsson C, Hagopian W, Koletzko S, Rewers M, Eisenbarth G, Bingley P, Bonifacio E, Simell V, Agardh D: Risk of Pediatric Celiac Disease According to HLA Haplotype and Country. New England Journal of Medicine 2014, 371:42-49.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4926 - 4936

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[28] Stanković B, Radlović N, Leković Z, Ristić D, Radlović V, Nikčević G, Kotur N, Vučićević K, Kostić T, Pavlović S, Zukic B: HLA genotyping in pediatric celiac disease patients. *Bosnian Journal of Basic Medical Sciences* 2014, 14:171.

[29] Zamani M, Modares-Sadegi M, Shirvani F, Zamani H, Emami MH. The involvement of the HLA-DQB1 alleles in the risk and the severity of Iranian coeliac disease patients. Int J Immunogenet. 2014;41:312–7.