HLA A*0201 ALLELE ACCOUNTS FOR DQ2/DQ8 NEGATIVE GENOME SCREENING IN CELIAC DISEASE PATIENTS.

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Background: HLA class II alleles DQA1*0501/DQB1*0201 are the antigen presenting cells surface receptors for deamidated toxic gliadin fragments. Despite that their presence is considered necessary for celiac disease (CD) pathogenesis, these two alleles account for only 90-95% of the genomic pattern of CD HLA class II DQ2/DQ8 haplotypes. It has been recently reported that HLA DQ2 haplotype can be coded by the previously undescribed A*0201 allele.

Aim: To verify whether patients classified as DQ2/DQ8 negative harbor the A*0201 allele both in the European and North American populations.

Methods: The HLA typing was performed in biopsy-proven CD patients using the Eu-DQ Kit (Eurospital, Trieste - Italy). This Kit contains multiplex PCR reactions for DQα 1*0501, DQα 1*0201, DQ β 1*0302, and DQ β 1*02 primers, with beta globin primer as internal control. The amplicons obtained were resolved on 2% agarose gel and stained using ethidium bromide. Results: The results are shown in the table. Both in the American and European population the A*0201 allele accounted for the previously-described DQ2/DQ8-negative CD patients.

Conclusion: Our data showed that A*0201 allele accounts for all DQ2/DQ8 negative, biopsy-proven CD patients. These results suggest that this allele should be included for the appropriate genomic screening of CD patients.

	U.S.A.				**Europe**			
	CD Patients		**Controls**		**CD Patients**		**Controls**	
Total (N)	**78**		**40**		**100**		**60**	
	N	%	N	%	N	%	N	%
DQ2 (A*0501/B*02)	45	57.69	9	22.50	58	58.00	14	23.33
DQ2 (A*0501-A*0201/B*02)	14	17.95	2	5.00	16	16.00	2	3.33
DQ2 (A*0201/B*02)	6	7.69	2	5.00	5	5.00	3	5.00
DQ2/DQ8 (A*0501/B*02 & B*0302)	6	7.69	4	10.00	8	8.00	3	5.00

DQ2/DQ8 (A*0201/B*02 & B*0302)	0	0.00	2	5.00	1	1.00	1	1.67
DQ8 (B*0302)	7	8.97	6	15.00	12	12.00	3	5.00
DQ2/DQ8 negative	0	0.00	15	37.50	0	0.00	34	56.67

Background I

Celiac Disease (CD) is an intestinal disorder with multifactorial etiology. This chronic inflammatory disease is triggered by the ingestion of wheat gluten or related proteins from rye and barely in genetically-predisposed individuals. The disease is associated with specific HLA alleles indicating that HLA genes contribute to CD genetic susceptibility. Given the indisputable role of gluten in causing inflammation and immune-mediated tissue damage, CD represents a unique model of autoimmunity in which, in contrast to the most other autoimmune diseases, a close association with HLA genes (DQ2 and/or DQ8), a highly specific humoral autoimmune response (autoantibodies to tissue transglutaminase), and, most importantly, the triggering environmental factor (gluten), are known. The disease shows a strong human HLA association predominantly to HLA-DQ2 (α1*0501, β1*0201) and/or DQ8 (β1*0302) heterodimer (Fig. 1).

Background II

The association between CD and HLA class II, is due to the fact that HLA DQ2 and, less efficiently, DQ8 are the antigen presenting cells surface receptors for deamidated toxic gliadin fragments (Fig 2). Despite that their presence is considered necessary for celiac disease (CD) pathogenesis, there is a low percentage of CD patients that are DQ2/DQ8 negative. However it has recently been reported that HLA DQ2 haplotype can be coded by the previously undescribed A*0201 allele.

Antigen Presenting Cell and its Role in CD

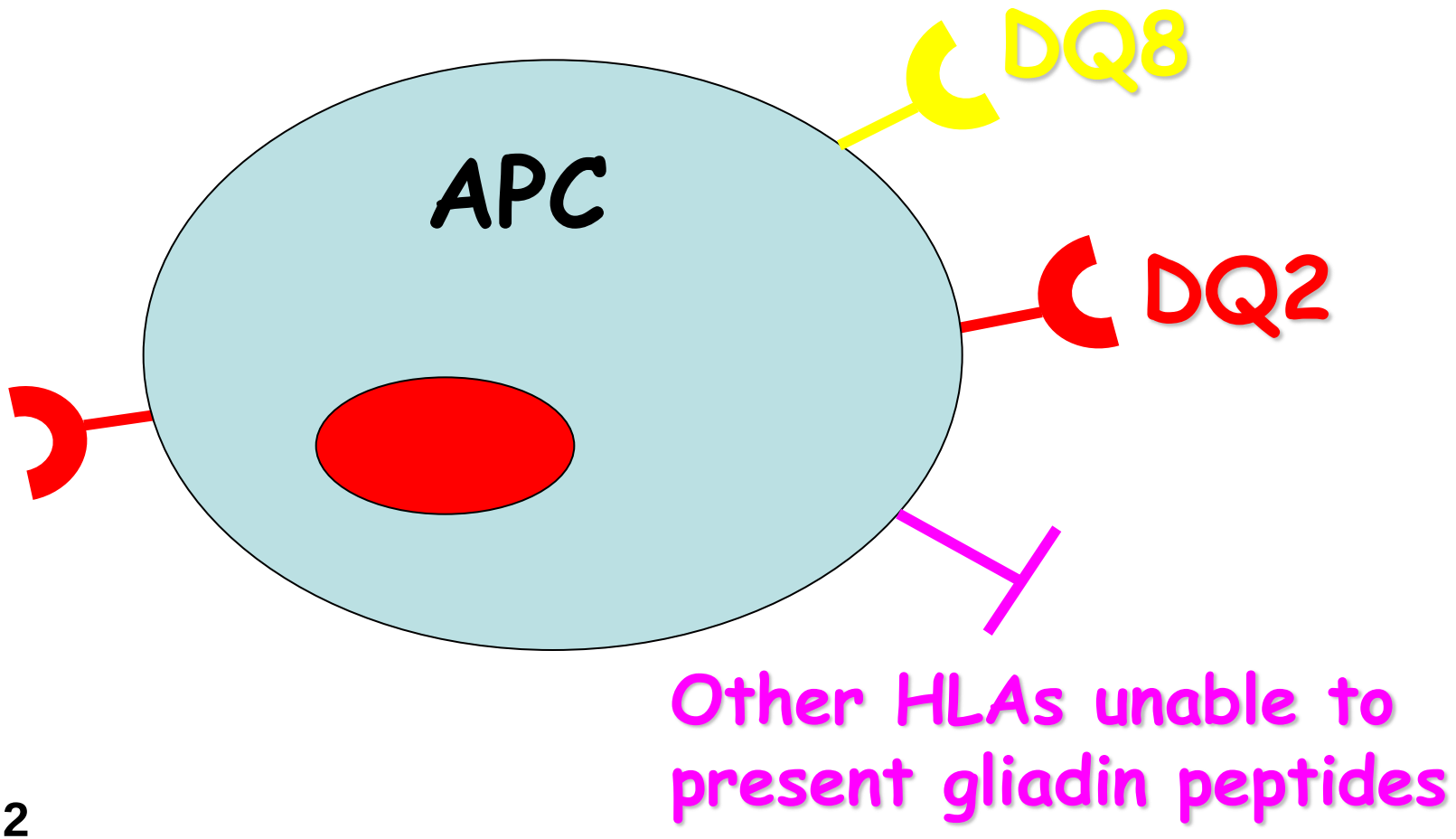

Figure 2

Aim

To verify whether patients classified as DQ2/DQ8 negative harbor the A*0201 allele both in European and North American populations

Methods

The HLA typing was performed in biopsy-proven CD patients.
Genomic DNA was extracted from whole blood samples; the DNA obtained was subjected to PCR using the Eu-DQ® Kit (Eurospital, Trieste - Italy). This Kit contains multiplex PCR reactions for DQα1*05, DQα1*0201, DQ β1*0302, and DQ β1*02 primers. Beta globin primers were used as internal control. The amplicons were resolved on 3% agarose gel and stained using ethidium bromide

Results I

	U.S.A.				Europe			
	CD Patients		Controls		CD Patients		Controls	
Total (N)	78		40		100		60	
	N	%	N	%	N	%	N	%
DQ2 (A*0501/B*02)	45	57.69	9	22.50	58	58.00	14	23.33
DQ2 (A*0501-A*0201/B*02)	14	17.95	2	5.00	16	16.00	2	3.33
DQ2 (A*0201/B*02)	6	7.69	2	5.00	5	5.00	3	5.00
DQ2/DQ8 (A*0501/B*02 & B*0302)	6	7.69	4	10.00	8	8.00	3	5.00
DQ2/DQ8 (A*0201/B*02 & B*0302)	0	0.00	2	5.00	1	1.00	1	1.67
DQ8 (B*0302)	7	8.97	6	15.00	12	12.00	3	5.00
DQ2/DQ8 negative	0	0.00	15	37.50	0	0.00	34	56.67

Table 1

Results II

As reported in literature 95% of European CD patients tested positive for DQ2 and/or DQ8 using conventionally genotyping. The remaining 5% harbored the A0201/B02 haplotype (see Table 1). Similarly in American CD patients 7.7% of DQ2/DQ8 negative subjects as established by the classical HLA genotyping also tested positive for A0201/B02 allele. (see Table 1).

Therefore the A0201 allele accounted for the previously described DQ2/DQ8 negative CD patients, irrespective of geographical differences.

Conclusions

Our data showed that A*0201 allele accounts for all DQ2/DQ8 negative, biopsy-proven CD patients.

These results suggest that this allele should be included for the appropriate genomic screening of CD patients

The presence of DQ2 and/or DQ8 is confirmed to be crucial for deamidated gliadin presentation by antigen presenting cells as a key step in the pathogenesis of the intestinal damage typical of celiac disease (figure 3).

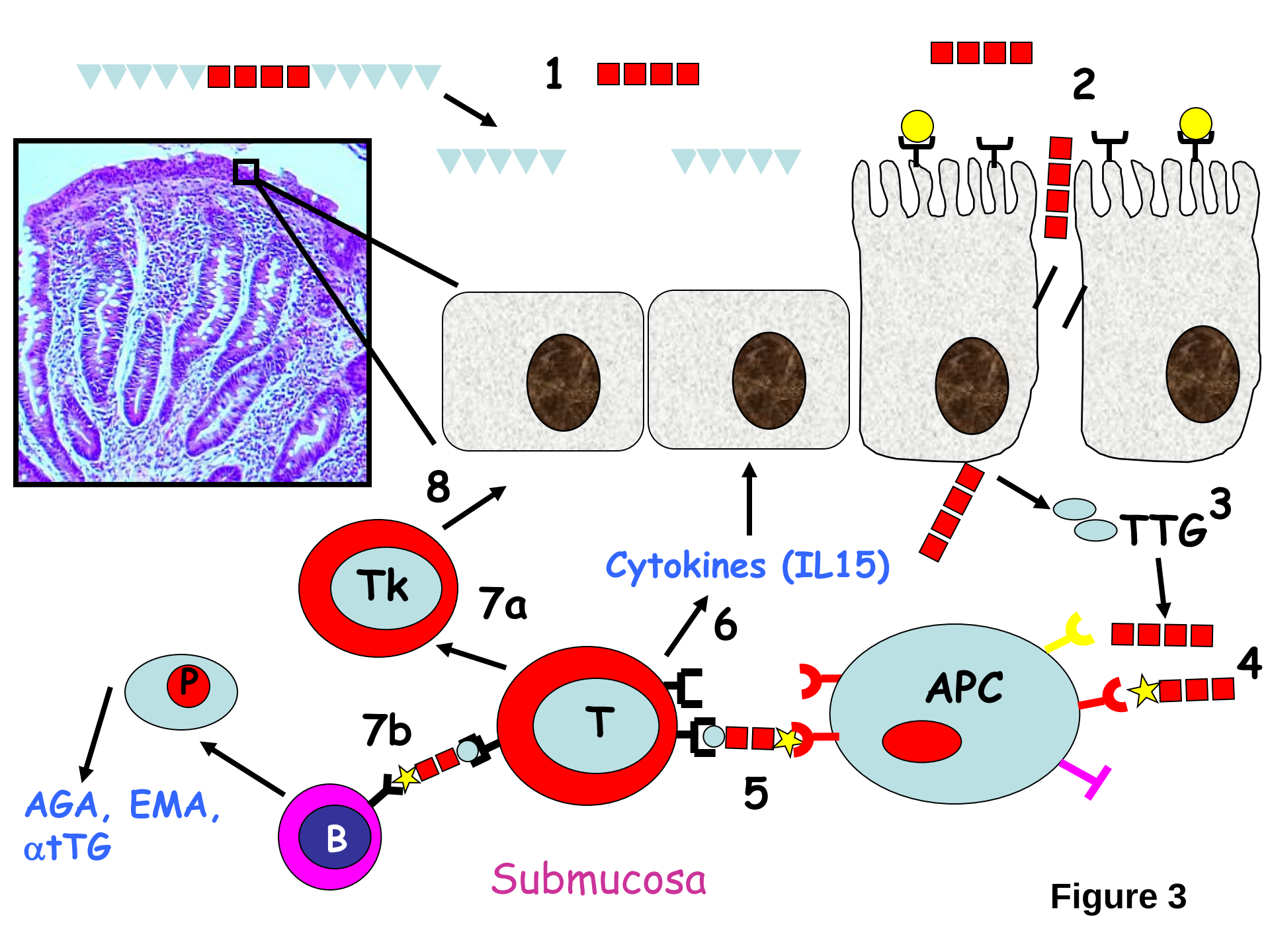
1
2
8
Tk
7a
Cytokines (IL15)
6
TTG
3
4
APC
P
7b
T
5
AGA, EMA,
αtTG
B
Submucosa

Figure 3