

MULTIPLEX REAL TIME PCR EVALUATION OF GENETIC CELIAC DISEASE PREDISPOSITION (HLA-DQ2 AND HLA-DQ8) IN 102 CASES AND RELATIVE CORRELATION WITH HLA-DRB1 HAPLOTYPE INVESTIGATED BY REVERSE DOT-BLOT HYBRIDIZATION

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INTRODUCTION

Celiac disease is a systemic immune disorder characterized by mild to severe gastrointestinal symptoms (such as diarrhea, weight loss, abdominal pain, abdominal distention, etc). This disease is a multifactorial disorder resulting from the interaction of specific pairs of allelic variants in two HLA genes, HLA-DQA1 and HLA-DQB1, associated with celiac disease susceptibility, and other environmental factors. Because of 30% of the general population has one of the celiac disease-associated HLA alleles and only 3% of individuals with one or both of these alleles develop celiac disease, the presence of celiac disease-associated HLA alleles is not diagnostic of celiac disease; however, their absence excludes a diagnosis of celiac disease. The loci within the DR-DQ region are especially valuable for their tight linkage and high linkage disequilibrium. The HLA-DRB1 gene is very important to identify the presence of homozygous or heterozygous alpha or beta chain in DQ-heterodimer and the susceptibility to celiac disease.

AIM

Evaluation of Clonit Multiplex Real Time PCR kit and relative correlation with HLA-DRB1 haplotype investigated by reverse dot-blot hybridization.

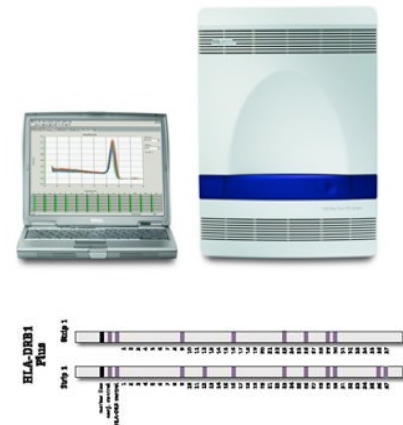
MATERIALS AND METHODS

The DNA of 102 random patients was extracted from 200µl of whole blood using QIAamp mini kit on QIAcube instruments.

The Real Time reaction was performed by multiplex TaqMan real-time PCR kit, supplied by Clonit srl, which allows the simultaneous amplification and characterization of the specific allelic variants of DQA1 (DQA1*02, DQA1*03 and DQA1*05) and DQB1 (DQB1*02 and DQB1*0302). The kit is composed by 2 ready-to-use amplification master mixes, one for each gene (DQA1 and DQB1), the controls for each allelic variant and the internal control (β-globin) for the evaluation of correct DNA extraction. The real time amplification was performed on AB 7000 series instrument.

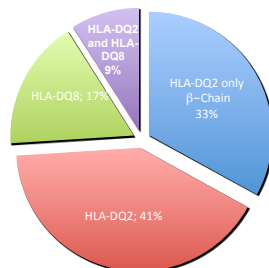
To evaluate the assay results, we correlated the real time experiments with DRB1 haplotype analysis. The DRB1 haplotype was investigated with Innolipa HLA-DRB1 Plus Kit (Innogenetics) by reverse dot-blot assay followed by automatic scanning of nitrocellulose strip and LiRAS software analysis to obtain the final results.

All the tests were performed at Service Lab Fleming Research.



RESULTS

The evaluation of 102 random samples shows that 40% of the tested patients (N=39) presents genetic predisposition to celiac disease. 33% (N=13) of these samples shows only the DQ2 β-chain, 41% (N=16) has got the allelic variants of HLA-DQ2, 17% (N=7) expresses the allelic variants of HLA-DQ8 and 9% (N=3) is characterized by the two HLA genes allelic variants of HLA-DQ2 and HLA-DQ8.



The table shows the correlation of our assay data between serologic variants associated at celiac disease predisposition (HLA-DQ2 and HLA-DQ8) and tight linkage HLA-DRB1 haplotype. The uncommon results were confirmed by automatic sequencing.

	DQ2-βchain	DQ2	DQ8	DQ2-bchain DQ8	DQ2 DQ8	Not Predisposed
DRB1*03	-	13 (6*)	-	-	3 (3)	-
DRB1*04	1	1 (*1)	7 (*1)	-	3 (3)	5 (*1)
DRB1*05	-	5 (*5)	1 (*1)	-	-	33 (*3)
DRB1*07	11	-	-	-	-	4 (*2)
DRB1*05 DRB1*07	-	3	-	-	-	-
Others	1	-	-	-	-	24

CONCLUSIONS

The final results obtained from both analysis (real time and reverse dot blot) performed simultaneously have demonstrated that the correlation between the HLA-DQ2/DQ8 expression and HLA-DRB1 haplotype is tight linked. A few uncommon cases reveal the necessity of a specific kit for discover specific allelic variants celiac disease associated of DQA1 and DQB1 genes.

The evaluation of multiplex real time PCR kit, supplied by Clonit, confirms the usefulness of this rapid and userfriendly test as first screening to exclude the celiac disease predisposition in a symptomatic patient.