

ORIGINAL ARTICLE

HLA Class II Genotyping in Chronic HCV Patients

May Moheb Eldin Mohamed Raouf¹, Faika Mahmoud Ghoneim¹, Hala Abdel Salam Aly¹,
Dalal M. Nasr Eldin Alkafash², Nesrine Fathi Hanafi¹, Ahmed Elsayed Zid³

¹Medical Microbiology and Immunology; ²Clinical Pathology; ³Hepatology; Faculty of Medicine Alexandria University

ABSTRACT

Key words:
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***Corresponding Author:**
Nesrine Fathi Hanafi
Medical Microbiology and
Immunology
E-mail: drmesra1@hotmail.com
Tel.: 01223742472

Background: Response to therapy for HCV infection varies among individuals. Studies suggest that this variation may be linked to HLA profile. **Objectives:** We aimed to identifying the HLA class II DR and DQ genotypes in HCV patients with self limiting versus chronic infection undergoing treatment to reveal any association with HLA. **Methodology:** Three groups of Patients have been enrolled in the study: **Group A** 30 patients with self-limiting HCV infection. **Group B** 30 patients with Sustained virological response. **Group C** 30 chronic HCV patients non responders to therapy. Samples were subjected to HLA genotyping for 19 HLA-DRB (DRB1*01-16, DRB3-5) and 5 HLADQB (DQB1*02-06) alleles by PCR amplification. **Results** revealed a significantly higher detection of HLA-DRB1*11(60%, $p=0*002$) in subjects with self limiting infection in comparison to chronic cases, HLA DRB3 (93.3%, $p=0.01$) and HLA-DQB1*03 (66.7%, $p=0*001$). While, HLA-DRB4 detection was significantly higher in chronic HCV patients (80%, $p=0*0002$)and in patients that failed to respond to treatment (86.7%, $p=0.008$). Findings revealed that HLA-DRB1*11, DRB3 and DQB1*03 had significantly higher association with subjects with self limiting disease and sustained viral response to treatment. While the HLA- DRB4 showed higher association with non responders to therapy. **Conclusion** our results confirms that HLA class II type might affect outcome of HCV infection and response to treatment.

INTRODUCTION

Hepatitis C virus infection constitutes a universal problem with a global reported prevalence of 3 %¹. Egypt has been identified with the highest prevalence of around infection rate of 20% among the Egyptian population and a rate of chronicity of around 7.5%^{2,3}.

Researchers reported that 20–30% of HCV infected patients develop adequate immune response to clear the virus without antiviral therapy; while 70–80% will suffer from chronic HCV infection⁴.

The current guidelines for treatment of chronic HCV infection recommend a combination of pegylated interferon- α and the nucleoside analog, Ribavirin, as a regimen for 24 to 48 weeks, with a 30–80% effectiveness in viral clearance from patients sera⁵.

Still, successful viral clearance as response to therapy has been found highly variable between individuals. Studies have reported the effect of many factors on that response such as viral factors including viral genotype and viral load, and host factors including age, sex, environmental, associated medical conditions as well as genetic variations⁶.

Human Leukocyte Antigens (HLA) are under the control of a gene region on the short arm of chromosome 6, and are critical in the regulation and initiation of the cellular immune response. MHC class I and class II molecules present foreign antigens to CD8 and CD4 molecules on T-lymphocytes. Also, T-lymphocyte activation and expression of HLA molecules has been identified to be induced by immunomodulatory cytokine as Interferon- α ⁷. Many researchers have studied the HLA class II polymorphism in relation to diseases and to responses to therapy⁸⁻¹⁰.

Results revealed by studies investigating MHC polymorphisms and response to treatment by interferon combinations have been never been consistent. Differences in ethnicity, environmental and genotyping method could be a justification which raises the demand of more studies to resolve the dilemma^{11, 12}.

In this study we aim at identifying the HLA (Human Leukocytic Antigen) class II DR and DQ genotype in Hepatitis C virus (HCV) infection: in patients with self limiting infection and in chronic HCV patients undergoing treatment whether they were non responders or showed a sustained virologic response to therapy, to reveal any association between incidence of chronicity and response to treatment with HLA genotype.

METHODOLOGY

Patients selection

This study have been performed on patients attending gastroenterology unit, Internal Medicine Department, Faculty of Medicine, Alexandria University, during the period from January 2010 to August 2012 seeking for management of Hepatitis C virus infection. All patients subjected to antiviral therapy have been following the guidelines of the Egyptian national program for HCV patients' treatment. Patients included in the study have been categorized into three groups of patients matched for age and sex:

- **Group A** comprised 30 patient whose sera were HCV antibody positive and PCR negative for HCV-RNA, twice with 6 months interval. Patients enrolled in this group were considered to have self-limiting HCV infection.
- **Group B** comprised 30 that were HCV RT –PCR positive with baseline viral load $< 5 \times 10^6$ IU/ ml) and that showed undetectable serum HCV-RNA levels at 6 months after the end of treatment response (ETR). Those patients were recruited as subjects with Sustained virological response (SVR).
- **Group C** comprised 30 CHC patients that were recruited as non responders (NR) to therapy. Patients within this group had HCV baseline viral load $< 5 \times 10^6$ IU/ml) before start of therapy and had detectable viraemia at week 24 after conclusion of treatment.

All CHC patients had to undergo a liver biopsy to show degree of severity of the chronic hepatitis before starting PEG-IFN/ribavirin treatment. Liver histology was graded and staged according to the HAI scoring system.[14] Baseline haematological and liver function tests were done to all participants of the study. The study was approved by the Local Research Ethics Committee of Alexandria Main University Hospital and patients signed an informed consent.

Negative Serum HCV RNA twice with 6 months was used to recruit Self limiting infected seropositive subjects. Base line viral load $< 5 \times 10^6$ IU/ ml was used as a selection criterion to recruit CHC patients in this study as viral parameters including lower levels of viremia is reported to be associated with a better response to treatment.⁽⁵⁾

Treatment of CHC Patients

All CHC patients were treated with recombinant peginterferon alfa PEG-IFN α {PEG-IFN α -2a (PEGASYS[®], Hoffmann-La Roche) at a dose of 180 μ g/week or PEG-IFN α -2b (Peg-Intron, Schering Plough Corp.) at a dose of 1.5 μ g/kg/ week} subcutaneously and oral ribavirin daily in two divided doses for 48 weeks. The dose of ribavirin was based on body weight (1000 mg ribavirin for weight ≤ 75 kg and 1200 mg ribavirin for weight > 75 kg).[5] The presence of HCV RNA in the serum was assessed at 12 week, 24 weeks, 48 weeks of therapy (ETR) and 6 months after

ETR. Patients with undetectable serum HCV-RNA level at 6 months after ETR were grouped as sustained virological responders. Patients who still have detectable HCV RNA at week 24 of therapy were classified as non-responders.

Anti HCV antibody detection Anti-HCV was tested using third generation ELISA Abbott Imx; Abbott Diagnostics, Maidenhead, United Kingdom)[13] and HCV RNA viral load was estimated with real time PCR (COBAS Ampliprep[™]/COBAS TaqMan[™], “CAP/CTM” Roche Molecular Systems, Pleasanton, CA,USA).

HCV RNA RT PCR detection

Baseline Serum HCV RNA as well as at 12 week, 24 weeks, 48 weeks of therapy (ETR) and 6 months after ETR was determined by standardized automated qualitative real time PCR (COBAS Ampliprep[™]/COBAS TaqMan[™], “CAP/CTM” Roche Molecular Systems, Pleasanton, CA,USA; detection limit: 50 IU/ml).¹⁵

DNA extraction Genomic DNA was purified from whole blood by the QIAamp blood kit (Qiagen, Valencia, CA). The eluted DNA was measured for qualification and purity using NanoDrop[®] (ND-1000) spectrophotometer giving a ratio of (A260/A280) > 1.6 and DNA concentration of 75 ng (± 25 ng). The resulting DNA was preserved at a temperature of -20°C until performing PCR reactions.

HLA genotyping was performed for a total of 19 HLA-DRB(DRB1*01-16, DRB3-5) and 5 HLADQB(DQB1*02-06) alleles by PCR amplification with two digit intermediate/low resolution using the PCR–sequence-specific-primer method¹⁶. Primer pairs were designed for typing class II genes according to polymorphism in exon 2 including DQB (8 primer pairs), DRB1 (21 primer pairs), DRB3 (1 primer pair), BRB4 (1 primer pair), DRB5 (1 primer pair). Primers were presented in a pre-optimized dried 32 wells of a 96-well (designed for 3 cases/ tray) 0.2 ml thin-walled tube tray for PCR and are ready for the addition of 137 μ l of genomic DNA 50 μ g/reaction, 2.4 μ l deoxynucleoside triphosphate, Taq polymerase (5 U/ μ l), 148 μ l PCR mix and 182.6 μ l water DNase, RNase free. PCR amplifications were carried out in a QB-96 life science using quanto Biotech thermal cycle software.

Statistical analysis:

It was carried out by SPSS (statistical package of social science) version 16 (SPSS Inc., Chicago, IL, USA). The quantitative data were expressed using mean, median and SD while the qualitative data were expressed in the form of number and percentage. The quantitative data were examined by Kolmogorov Smirnov test for normality. The quantitative data were analysed using Anova test to test for significance then post hoc tukey test was used to compare between the groups. Chi square with Yates correction was used as a test of significance for qualitative data. Fisher exact test

was used when any cell value was less than 5. For all tests a P value <0.05 was considered to be significant, and significant P values were corrected (Bonferroni correction) for the number of alleles detected at each locus. Odds ratio and 95% confidence interval were calculated.¹⁷

RESULTS

In the present study sample, 10 alleles at the DRB1, 1 allele at the DRB3, 1 allele at the DRB4, 1 allele at the DRB5 and 5 alleles DQB1 within the MHC class II region were observed (**Table 1**).

Table 1: Distribution of MHC class II alleles among the three studied groups

<i>MHC class II</i>	<i>Group A (n=30)</i>	<i>Group B (n=30)</i>	<i>Group C (n=30)</i>	<i>P value</i>
DRB1*01	8 (26.7%)	12 (40%)	8 (26.7%)	P=0.882
DRB1*03	0 (0%)	2 (6.7%)	2 (6.7%)	P=0.936
DRB1*04	2 (6.7%)	2 (6.7%)	6 (20%)	P=0.772
DRB1*07	4 (13.3%)	4(13.3%)	4(13.3%)	P=1
DRB1*10	4 (13.3%)	4(13.3%)	0(0%)	P=0.73
DRB1*11	18 (60%)	8 (26.7%)	2(6.7%)	P=0.004
DRB1*13	8 (26.7%)	8 (26.7%)	12(40%)	P=0.882
DRB1*14	6 (20%)	6 (20%)	10 (33.3%)	P=0.867
DRB1*15	2 (6.7%)	6 (20%)	6 (20%)	P=0.822
DRB1*16	0 (0%)	0 (0%)	2 (6.7%)	P=0.879
DRB3	28 (93.3%)	22 (73.3%)	12 (40%)	P=0.002
DRB4	10 (33.3%)	22 (73.3%)	26 (86.7%)	P=0.005
DRB5	14 (46.7%)	8 (26.7%)	14 (46.7%)	P=0.653
DQB1*02	6 (20%)	8 (26.7%)	14 (46.7%)	P=0.452
DQB1*03	20 (66.7%)	6 (20%)	6 (20%)	P=0.005
DQB1*04	2 (6.7%)	10 (33.3%)	8 (26.7%)	P=0.383
DQB1*05	8 (26.7%)	12 (40%)	8 (26.7%)	P=0.882
DQB1*06	16 (53.3%)	16 (53.3%)	16 (53.3%)	P=1

Comparing detection of HLA-DRB1*11, it has been found to be significantly associated with group A 60% versus 26.7% and 6.7% in group B and C respectively, $p=0.004$ and HLA-DQB1*03 was positive in 66.7% of group A versus 20% and 20% in group B and C respectively, $p=0.005$. The statistical significance was preserved after correction of P value ($P_c < 0.05$).

Statistical difference was detected in HLA-DRB3 alleles among group A and B versus group C (93.3% and 73.3% versus 40% respectively, $p=0.002$). HLA-DRB4 showed higher frequency among the B and C groups versus group A (73.3% and 86.7% versus 33.3% respectively), statistical difference was detected ($p=0.005$). The statistical significance was preserved after correction of P value ($P_c < 0.05$).

Comparing detection of HLA class II alleles in Group A patients representing the self limiting infection versus group B+ C representing chronic hepatitis C (CHC), it has been revealed that: group A patients were significantly associated with detection of HLA-DRB1*11 (60%) versus 16.7% in group C+ B (CHC), $p=0.002$. Similarly, HLA DRB3 (93.3% versus 56.7%, $p=0.01$) and HLA-DQB1*03 (66.7% versus 20%, $p=0.001$) while HLA-DRB4 was significantly associated with CHC (80% versus 33.3%, $p=0.0002$). The statistical significance was preserved after correction of P value.

DRB3 was statistically significant more associated with group B patients group (73.3% versus 40% in

group C, $p=0.03$) but the statistical significance was lost after correction of P value ($P_c > 0.05$). It was observed HLA-DRB1*11 to be more frequent in group B while DRB4 to be more frequent in group C. Thus potential, though not statistically significant, differences in these MHC associations were observed.

HLA-DRB1*11 and DRB3 was found to be significantly associated with clearance of infection represented in patients group A+B (43.3%) versus 6.7% in group C, $p=0.009$ and 83.3% versus 40% in group C, $p=0.0007$ respectively). DRB4 was found to be significantly associated with group C (86.7% versus 53.3% in the cleared group, $p=0.008$, the statistical significance was preserved after correction of P value ($P_c < 0.05$).

linkage disequilibrium Coefficient of LD measure Δ_{ij} is 0.09, Standard errors SEs are 0.03765, 0.01528, 0.0148 magnitude of $\Delta_{ij} = 6.08$

Statistical significance of LD between DRB1*11 and DQB1*03 in the study population is admitted. Logistic regression analysis demonstrated that there is a strong protective association with the haplotype DRB1*11-DQB1*03 (OR = 0.22).

DISCUSSION

HCV is now recognized as one of the major causes of chronic liver diseases worldwide^{1,2}. Egypt

Demographic and Health Survey (EDHS) in 2008 reported that the incidence of HCV in Egypt is continuing at a rate of $\approx 6.9/1,000$ persons per year, indicative of possibly ongoing hyperepidemic transmission which motivated the government to start the national program for treatment of HCV patients.³

As more than 70 % of patients become chronically infected, Hepatitis C virus (HCV) RNA is becoming the most important parameter for diagnosis, management of antiviral therapy, and determination of virologic response to therapy in HCV infection. The performance evaluation of the fully automated COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] HCV Test presents an assay reliable and accurate with high sensitivity and specificity for identification and quantification of HCV RNA genotypes¹⁵.

Progression of the disease as well as response to treatment is a complex process in which immunological factors plays an important role. Effective presentation of viral antigens to CD4+ T cells and CD8+ T cells by HLA class II and class I molecules, respectively, is the key regulation of optimum immune response against viral infection^{7,8,10}. With the upregulated expression of immunogenetic molecules which enhances the immune response by IFN, the genetic variations at HLA loci with respect to antigen presentation might be a candidate related to response to IFN based therapy.^{6,9}

Several researchers studying the outcome of HCV infection compared individuals with chronic HCV infection to healthy controls. Still, The use of healthy individuals would affect the study design, as an estimated 80% of these controls, if exposed to the HCV, would develop chronic infections, while the others experience viral clearance. Ideally, individuals with documented self-limiting infections should be compared to individuals experiencing chronic infections (anti-HCV antibody and HCV-RNA positive). This study confirmed associations of self-limiting HCV infection with HLA DRB1*0301 and HLA-DQB1*1101. This observation was detected by Wang¹⁸, Harris¹⁹, Thio²⁰, Zavaglia²¹, Ali et al²², Cramp et al²³, and many other researchers. One of the strong features emphasizing the possible role of DQB1*0301 in self-limiting HCV infection is the fact that this allele, along with different extended haplotypes, has been correlated with self-limiting infection in several populations. A French study correlated the DRB1*1101- DQB1*0301 haplotype with HCV clearance. A British study found that the DRB1*0401-DQB1*0301 haplotype correlated with clearance. In Italy, two studies have shown an association for DRB1*11-DQB1*0301 with self-limiting HCV as well. The association of viral clearance with DRB1*01-DQB1*03 was recently reported in a Brazilian population.^{11,12,18} HLA DRB1*0301 and HLA-DQB1*1101 were observed to be in tight linkage disequilibrium in this study as well as in other studies.⁸

CD4 T-cell responses to the nonstructural proteins of HCV are raised in patients with self-limiting infection. This finding provides a potential mechanism underlying the consistent association of self limiting infection and MHC class II alleles whose products are pivotal in antigen presentation to CD4 T cells.²² Cramp et al found that CD4 Tcell responses to the non-structural protein 3 and core antigens of HCV were greater in patients with DQB1*0301²³. DRB1*0401, which was observed to be more associated with viral persistence in this study as well as in El-Chennawi 2008, two studies in Japan as well as two studies from United Kingdom¹⁸, was identified as additional allele associated with viral clearance in the Irish cohort.²⁴

It is difficult to determine whether HLA-DRB1*1101 or DQB1*0301 allele is the more relevant factor as HLADRB1* 11 is associated with DQB1*0301. Some studies found that DRB1*1101 rather than DQB1*0301 is closely associated with viral elimination. While some findings suggest that DQB1*0301 is dominant in determining the outcome of HCV infection. In a meta-analysis, the OR for the two alleles was virtually identical, but which one is responsible for the viral elimination is not clear.¹¹

While there are some consistent observations on DQB1*0301 and DRB1*1101 with self-limiting infection, many results are not uniform. These inconsistencies may be due to ethnic differences, patient selection, sample size, HCV-serotype, and HLA typing technique.

Many factors have been considered as predictors for the response to PEG-IFN/ribavirin therapy. This study on associations between alleles and response to combination treatment have been verified by adjusting and controlling the important virological and histologic criteria (pretreatment serum HCV RNA levels, Base line viral load $< 5 \times 10^6$ IU/ ml was used as a selection criterion, and mild to moderate liver fibrosis)

HLA DRB1*1101 allele and DQB1*0301 were also found with higher frequency in patients with a sustained response to interferon therapy than in those who did not respond. Although these associations were only just significant, the results are plausible, since interferon therapy in hepatitis C is thought to act by stimulation of the T-cell responses to the virus as well as inhibition of viral replication. Thus, the genetic profile of patients who respond to interferon therapy is probably similar to that of patients with self-limiting infection.²⁵ Heterozygous advantage was not shown for the MHC class II loci in our study or in many other studies.¹⁰⁻¹²

HCV infection outcomes is found to be determined by several genetic, environmental, and viral factors in a complex way. The type of MHC class II alleles is one determinant, but other immunogenetic factors should also be sought. Extension of these cellular immunology studies may help to elucidate the relative roles of

HLADR and HLA-DQ alleles in the elimination of HCV. More studies are needed to investigate the host genetic determinants for HCV infection. Finding an association between specific alleles and favorable clinical outcomes in HCV patients might open new avenues to explore and understand the pathogenesis of HCV and assists the hepatologists in their protocols in management of the disease. Study of MHC class II associations in HCV infection might assist in the clinical prediction of its course and would give evidence for the clinician to choose the proper antiviral agent especially with release of newer generation of anti HCV chemotherapy. Identification of conserved peptide epitopes bound and presented by these alleles might also provide vaccines candidates for boosting the T cell defence in patients who do not naturally mount a good response.

REFERENCES

- Centers for Disease Control and Prevention [online]. Chapter 5-Hepatitis C-2010 Yellow Book | CDC Travelers' Health. [cited March 19, 2012]. Available from URL: <http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-5/hepatitis-c.aspx>
- Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005;5: 558–67.
- El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008. Egyptian: Ministry of Health (El-Zanaty and Associates and Macro International, Cairo), 2009 pp 431.
- Bialek SR, Terrault NA. The changing epidemiology and natural history of hepatitis C virus infection. *Clin Liver Dis* 2006;10: 697–715.
- Ghany MG, Strader DB, Thomas DL, Seeff LB. AASLD PRACTICE GUIDELINES. Diagnosis, Management, and Treatment of Hepatitis C: An Update. *Hepatology* 2009;49(4):1335-74.
- Dai CY, Chuang WL, Hsieh MY, Huang JF, Lin YY, Chu PY, et al. Human leukocyte antigen alleles and the response to pegylated interferon/ribavirin therapy in chronic hepatitis C patients. *Antiviral Research* 2010;85(2): 396-402.
- Ishii S, Koziel MJ. Immune responses during acute and chronic infection with hepatitis C virus. *Clinical Immunology* 2008;128(2): 133-47.
- Bensch B, Thimme R, Blum HE. REVIEW Role of Host Genetic Factors in the Outcome of Hepatitis C Virus Infection. *Viruses* 2009;1: 104-25.
- Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol* 2007; 13(12): 1770-87.
- Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. *Lancet* 1999; 354: 2119–24.
- Hong X, Yu RB, Sun NX, Wang B, Xu YC, Wu GL. Human leukocyte antigen class II DQB1*0301, DRB1*1101 alleles and spontaneous clearance of hepatitis C virus infection: A metaanalysis. *World J Gastroenterol* 2005;11(46):7302-7.
- Yee LJ. REVIEW Host genetic determinants in hepatitis C virus infection. *Genes and Immunity* 2004;5: 237–45.
- Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J Viral Hepat* 2001;8:87-95.
- Ishak KG, Baptista A, Bianchi L. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; 22: 696–9.
- Sizmann D, Boeck C, Boelter J, Fischer D, Miethke M, Nicolaus S, et al. Fully automated quantification of hepatitis C virus (HCV) RNA in human plasma and human serum by the COBAS® AmpliPrep/COBAS® TaqMan® System. *J Clin Virol* 2007;38:326–33.
- Bunce M, O'Neill CM, Barnardo MC, et al. 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(5):355–67.
- Norussis Mx: Manual of statistical package for social sciences SPSS incorporation 2006.
- Wang LY, Lin HH, Lee TD, Wu YF, Hu CT, Cheng ML. Human leukocyte antigen phenotypes and hepatitis C viral load. *J Clin Virol* 2005 ; 32:144-50
- Harris R A , Sugimoto K, Kaplan D E, Ikeda F, Kamoun M, and Chang K M . Human Leukocyte Antigen Class II Associations with Hepatitis C Virus Clearance and Virus-Specific CD4 T Cell Response Among Caucasians and African Americans *HEPATOLOGY* 2008 :48 (1):70-9
- Thio CL, Thomas DL, Goedert JJ, Vlahov D, Nelson KE, Hilgartner MW, O'Brien SJ, Karacki P, Marti D, Astemborski J, Crrington M . Racial differences in HLA class II associations with Hepatitis C virus outcomes. *J Infect Dis* 2001 . 1 ;184(1):16-21.
- Zavaglia C , Martinetti M, Silini E et al. Association between HLA class II alleles and protection from or susceptibility to chronic hepatitis C . *J Hepatol*,1998;28: 1-7
- Ali L, Mansoor A, Ahmad N, Siddiqi S, Mazhar K, Muazzam AG et al. Patient HLA-DRB1* and -DQB1* allele and haplotype association with hepatitis C virus persistence and clearance. *Journal of General Virology* (2010), 91, 1931–8.

23. Cramp ME, Carucci P, Underhill J, Naoumov NV, Williams R, Donaldson PT. Association between HLA class II genotype and spontaneous clearance of hepatitis C viraemia. *J Hepatol* 1998; 29: 207-13.24-McKiernan SM, Hagan R, Curry M, McDonald GSA, Kelly A, Nolan N et al. Distinct MHC Class I and II Alleles Are Associated With Hepatitis C Viral Clearance, Originating From a Single Source. *Hepatology* 2004;40(1): 108-14.
24. Cramp ME, Rossol S, Chokshi S, Carucci P, Williams R, Naoumov NV et al. Hepatitis C virus-specific T-cell reactivity during interferon and ribavirin treatment in chronic hepatitis C. *Gastroenterology* 2000;118: 346–55.