行政院國家科學委員會專題研究計畫　成果報告

慢性 C 型肝炎病毒感染相關類風濕病變/自體免疫與第二型人類白血球抗原基因變異性之分析

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中文摘要

關鍵詞： C 型肝炎、肝臟外類風濕疾病、自體免疫抗體、第二型人類白血球抗原基因 DRB1

C 型肝炎病毒係引起慢性肝炎之重要病原。感染 C 型肝炎病毒者僅 10-15%可恢復，患者生成之特異性抗體無法清除體內病毒。85-90%感染者發展成為慢性肝炎，經二、三十載常有轉化為肝硬化情形，增高了轉變為肝細胞腫瘤之危險性。慢性 C 型肝炎患者亦常見類風濕免疫性病癥、血液中發現自體免疫抗體情形。為何部分慢性 C 型肝炎病患有自體免疫特徵、其他則無，原因尚不明瞭。最近的研究顯示自體免疫現象發生與其感染的 C 型肝炎病毒血清型之差異、臨床變化、及對治療的反應不同等因子並無明顯關聯。為探討與 C 型肝炎病毒感染相關的免疫病癥及自體抗體產生與個案本身第二型人類白血球抗原基因變異性是否有關聯；我們觀察 217 名曾遭 HCV 感染之個案，檢測其血清中是否有自體免疫抗體（抗細胞核抗體 ANA、類風濕因子 RF、抗甲狀腺抗體 ATG 與 AMC、抗嗜中性白血球抗體 PR3-ANCA 與 MPO-ANCA）、統計病例報告上有關類風濕症狀的描述，並做第二型人類白血球抗原基因 HLA-DRB1 之多型性分析交叉比對。結果發現 217 名 HCV 感染個案中 127 人（48.5%）血清中可測得至少一種自體免疫抗體。其中以抗細胞核抗體 ANA(27.4%)最常見，類風濕因子 RF(17.8%)次之，抗甲狀腺抗體(ATG: 4.4%, AMC: 7.6%)、抗嗜中性白血球抗體(PR3-ANCA:3.8%, MPO-ANCA: 2.7%)較少。個案臨床上表現類風濕症狀與其血清中自體免疫抗體出現與否沒有清楚關聯。HCV 感染者與健康未感染組相比結果，HLA-DRB1 基因之對偶基因型分佈並無顯著差異。比較 HCV 感染個案中，血清出現可測得自體免疫抗體 ANA, RF, ATG 者與無此類抗體個案群，可見 HLA-DR11 對偶基因型與自體免疫抗體發生呈負相關：血清中測得 ANA, RF, ATG 個案群中 HLA-DRB1 基因率顯著低於在無自體免疫抗體個案群中之基因率；血清中有任一（前述六種）自體免疫抗體者 HLA-DR11 對偶基因發生率亦明顯低於血清中皆無上述自體免疫抗體者。HLA-DR2 對偶基因型在無自體免疫抗體 RF, PR3-ANCA 的 HCV 感染個案群中也較在有自體免疫抗體個案群中為高。簡言之，HLA-DR11 及 HLA-DR2 基因型可能保護 HCV 感染個案，使較少發生自體免疫抗體。

Abstract
Keywords: hepatitis C, extrahepatic rheumatoid manifestations, autoantibody, HLA-DRB1

Hepatitis C virus (HCV) is recognized as a major cause of chronic hepatitis worldwide. Only 10-15% of the cases of acute hepatitis C resolved. Development of anti-HCV did not clear the virus. Chronic hepatitis develops in 85-90% of the HCV-infected individuals. Twenty to thirty percent of chronic hepatitis C progress to cirrhosis generally occurs over 2 to 3 decades, which will increase the risk for hepatocellular carcinoma (HCC). Patients with chronic viral hepatitis C commonly have immunologic manifestations, including circulating immune complex, autoantibodies, and concurrent extrahepatic autoimmune disorders. It is unclear why some patients with chronic hepatitis C virus infection develope autoantibodies/mixed cryoglobulinemia while the others do not. Both viral and host factors may contribute to development of autoantibodies and rheumatoid manifestations. Current studies indicated that autoantibodies and concurrent immune disorders were not associated with a particular HCV genotype, clinical profile, or treatment outcome. To investigate the rheumatic manifestations/autoimmune markers associated with chronic hepatitis C and find the possible link of the polymorphic HLA class II genotype(s) with factors determining the rheumatic manifestations/ autoimmunity in Taiwan, we studied 217 HCV-infected subjects for their serum autoantibodies (ANA, RF, anti-thyroid antibodies, anti-neutrophil antibodies), extrahepatic autoimmune manifestations and genetic polymorphism of HLA class II gene DRB1 locus. Of the 217 subjects, 127 (48.5%) had at least one of the autoantibodies investigated in this project. ANA was the most prevalent (27.4%) autoantibody found among the subjects. Rheumatoid factor was detectable in 52 (17.8%) of the subjects. The anti-thyroid (ATG: 4.4%, AMC: 7.6%) and anti-neutrophil (PR3-ANCA: 3.8%, MPO-ANCA: 2.7%) antibodies were less common. Presence of autoimmune manifestations correlated poorly with the presence of autoantibodies. There was no significant difference comparing the allelic distribution of DRB1 gene of the HCV-infected subjects with that of a control group of 98 healthy adults. HLA-DRB1*11 alleles were negatively associated with autoantibody production among HCV-infected individuals. Allelic frequency of HLA-DRB1*1101 was significantly lower in the HCV-infected subjects with ANA (3.1% of ANA (+) vs. 8.5% of ANA (-), OR: 0.38, $p \leq 0.0161$), RF (2.9% of RF (+) vs. 8% of RF (-), OR: 0.33, $p \leq 0.0439$), ATG (0% of ATG (+) vs. 7.4% of ATG (-), OR: 0.23, $p \leq 0.0486$) or among subjects with at least one autoantibody than those who without (3.9% of autoantibody (+) vs. 9.2% of autoantibody (-), OR: 0.42, $p \leq 0.0124$). HLA-DR2 was also noted being more prevalent in HCV-infected subjects without

autoantibodies RF (9.6% of RF (+) vs. 19.9% of RF (-), OR: 0.45, $p \leq 0.0088$), PR3-ANCA (9.1% of PR3-ANCA (+) vs. 19.1% of PR3-ANCA (-), OR: 0.54, $p \leq 0.0227$). In summary, we revealed possible protective effect conferred by HLA-DRB1*11 and –DR2 against autoantibody-production in chronic hepatitis C.

報告內容

Background introduction:

Hepatitis C virus (HCV) is recognized as a major cause of chronic hepatitis worldwide. HCV accounts for 20% of acute hepatitis in United States [1]. The estimated incubation period is 7 weeks, varies from 2 to 30 weeks. Children or Adults acquire HCV infection are usually asymptomatic or have non-specific clinical illness. Approximately 30% of HCV-infected adults present jaundice. Serum ALT (alanine amino-transferase) may be increased, with HCV RNA detectable by polymerase chain reaction (PCR) within days of infection. Anti-HCV antibodies may not be detected after development of symptom and occasionally is detectable only several weeks after acquiring acute HCV infection. Only 10-15% of the cases of acute hepatitis C resolved. Development of anti-HCV did not clear the virus. Chronic hepatitis develops in 85-90% of the HCV-infected individuals. Twenty to thirty percent of chronic hepatitis C progress to cirrhosis generally occurs over 2 to 3 decades, which will increase the risk for hepatocellular carcinoma (HCC). HCV is transmitted primarily through direct percutaneous exposure to blood. Groups with repeated exposure to blood products, such as intravenous drug abuser and haemophiliac patients have highest prevalence of HCV infection.

In Taiwan, prevalence of anti-HCV antibodies of voluntary blood donors was ranged from 0.8% [2] to 1.6% [3]. Before the overall anti-HCV screening of donated blood, about 7-12% of the blood transfusion recipients developed viral hepatitis irrespective of the fact that all the donors had been carefully screening for HBV and serum ALT level [3]. Although persistent Hepatitis B virus (HBV) infection plays the major role in chronic liver diseases (CLD) and HCC in Taiwan, in the HBsAg-negative CLD patients, the anti-HCV prevalence was high in each category of CLDs: 65% in chronic persistent hepatitis, 65% in chronic active hepatitis, 43% in cirrhosis, 63% in HCC. This indicates that HCV plays a key role in HBsAg-negative chronic liver diseases and HCC in Taiwan [4]. The anti-HCV prevalence was found to be highest in patients with non-A, non-B hepatitis (59% in sporadic, 73% in transfusion associated NANB hepatitis) [5].

Extrahepatic immunologic abnormalities have been shown to occur frequently in patients with chronic HCV infection. The extrahepatic manifestations can affect several different organ systems. Renal (membrane proliferative glomerulonephritis), pulmonary (idiopathic pulmonary fibrosis), dermatological (porphyria cutanea tarda, Lichen planus), haematological (aplastic anemia, lymphoproliferative disorders of B cell origin, non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukemia), immunologic (autoimmune thyroditis, Sjogren syndrome, idiopathic thrombocytopenic pupura), neurological, musculoskeletal manifestations have been noted to be associated with HCV infection. Although the evidence is incomplete, the extrahepatic syndromes seen with chronic hepatitis C appeared to be mediated by immune mechanisms [6]. Patients with chronic viral hepatitis C commonly have immunologic manifestations, including circulating immune complex, autoantibodies, and concurrent autoimmune disorders. The most documented extra-hepatic manifestation in HCV infection is mixed cryoglobulinemia (MC). Temperature-sensitive immune-complex of type II (monoclonal IgM rheumatoid factor against polyclonal IgGs) or of type III (polyclonal immunoglobins against polyclonal IgGs) were found in HCV-infected individuals as cryoglobulin. There found a very high prevalence of HCV markers (50-90%) in patients of essential mixed cryoglobulinemia [7-9]. Mixed cryoglobulinemia was commonly noted in chronic hepatitis C [10;11]. Anti-HCV antibodies were found in these cryoprecipitates [12]. Interestingly, anti-HCV antibodies could be found in the cryoprecipitates of chronic hepatitis C patients with MC. Mixed cryoglobulinemia development depends on length of infection [6]. However, individual inherited or acquired factors predisposing to lymphoproliferative disorders may accelerate the occurrence of cryoglobulinemia and enhance the corresponding clinical manifestations. Most of the studies agreed that no HCV genotype is specifically related to MC [13;14]. It was also found that high prevalence of autoantibodies in the chronic hepatitis C patients. Rheumatoid factor (RF), anti-smooth muscle antigen (ASMA), anti-nuclear antibodies (ANA) were detected in 76%, 66%, and 14% of the HCV-infected patient respectively [13]. Several documents also revealed a high prevalence of low titer autoantibodies ANA, ASMA, RF in chronic hepatitis C [10;13;15-17].

Recent interest was expressed in rheumatic manifestations (MC, vasculitis, sicca

syndrome, myalgia, arthritis and fibromyalgia etc.) in HCV infected populations [18;19]. Rheumatic, musculoskeletal manifestations were found in 31% of Israel patients with hepatitis C [10], and 69% of the patients had at least one of the autoantibodies RF, ANA, anti-cardiolipin 1 (ac1). The rheumatic complications were heterogeneous and were not associated with liver disease severity. The prevalence of autoantibodies was not associated with liver disease severity or with the presence of rheumatic disorders. In 49 Korean patients with HCV infection [11], 59% had cryoglobulinemia, 14% were RF(+), 3.4% were ANA (+). A high prevalence of rheumatological symptoms was documented: arthragia/arthritis (35%), cutaneous manifestations (37%), paresthesis (44%), dry eyes (22%), dry mouth (10%). A quarter of female HCV-infected patients were seropositive for thyroid autoantibody and had thyroid diseases [20]. It is unclear why some patients with chronic hepatitis C virus infection develop autoantibodies/MC while the others do not. Both viral and host factors may contribute to development of autoantibodies and autoimmune, rheumatoid manifestations. Current studies indicated that autoantibodies and concurrent immune disorders were not associated with a particular HCV genotype, clinical profile, or treatment outcome [13;21].

Several autoimmune diseases, such as SLE, rheumatoid arthritis [22;23], IDDM [24] are known to be closely associated with the highly polymorphic human leukocyte antigen (HLA) system. There was scarce information concerning the autoimmunity markers/ rheumatoid manifestations in chronic HCV infected patients in Taiwan. We are experienced in the PCR/SSOPH genetic typing system for HLA typing [24-26]. In this project, we intend to investigate the rheumatic manifestations/ autoimmune markers associated with chronic hepatitis C and find the possible link of the polymorphic HLA class II genotype(s) with factors determining the rheumatic manifestations/ autoimmunity in Taiwan.

Specific aims:

1. To investigate the prevalence of autoantibodies in chronic hepatitis C virus-infected subjects in Taiwan.
2. To investigate the prevalence of extrahepatic rheumatic manifestations in chronic hepatitis C virus-infected subjects in Taiwan.
3. To correlate the presence of autoantibodies and the rheumatic manifestations in chronic hepatitis infected subjects in Taiwan.
4. To explore the variation in genetic factor, the HLA class II polymorphism, in chronic hepatitis C-infected subjects and correlate it to the presence of autoantibodies as well as the extrahepatic manifestations.

Material and methods:

A. Study subjects:

Three hundred and seventeen patients who visited the Gastro-Intestinal clinics or hospitalised due to HCV-associated hepatic diseases in NTUH were observed in this project. All the patients were anti-HCV (+), confirmed by the third generation anti-HCV ELISA. Presence of extrahepatic autoimmune manifestations such as arthritis, glomerulonephritis, and vasculitis were referred to the records in the charts. Blood samples from 279 patients were obtained and analysed.

B. Detection of autoantibodies:

Rheumatoid factor (RF), anti-thyroglobulin (ATG) and anti-thyroid microsomal antigen (AMC) were detected by particle agglutination kits SERODIA-RF, SERODIA-ATG, SERODIA-AMC (SERODIA, FUJIREBIO, Taiwan) respectively. Samples gave clear agglutination in dilution of 1:40 in RF; 1:100 in ATG and AMC were regarded as positive. Anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antigens PR3-ANCA and MPO-ANCA were tested by ELISA kits VARELISA-ANA 8 screen, PR3-ANCA and MPO-ANCA (Pharmacia Deutschland GmbH, Diagnostic division, Freiburg, Germany).

C. Genetic typing of HLA class II DRB1 by polymerase chain reaction/sequence-specific oligonucleotide probe hybridisation (PCR/SSOPH)

The genetic typing by PCR/SSOPH was performed by methods previously described [25]. Briefly, the second exons of HLA-DRB genes encoding the most polymorphic sequences were amplified by specific primer sets. The PCR products were denatured and immobilized onto nylon membranes. Panels of 5'-biotinylated SSO probes were used to detect the hyper-variable regions within the polymorphic exon. The DRB1 genotype of each sample was

constructed.

D. Statistics:

The subjects were grouped according to presence of autoantibodies or extrahepatic autoimmune manifestations. The gene frequencies of each HLA-DRB1 allele of the individuals with or without autoantibodies or extrahepatic autoimmune manifestations were compared by log likelihood ratio Chi-square test using *JMP ver 5.0.1a (SAS Institute Inc.)*. The Odds ratio (OR) of each DRB allele predisposing autoantibody production or autoimmune manifestations in the HCV-infected subjects was calculated accordingly by Wolf's or Haldane's formula. A *p* value less than 0.05 was considered to be statistically significant.

Results

1. Autoantibodies were prevalent among HCV-infected subjects

A total of 271 HCV-infected subjects (140 males and 131 females, 12-89 years-old) were analyzed in this project. The serological profile of hepatitis viral infection was not complete in all subjects except for anti-HCV antibody. Autoantibodies ANA, RF, anti-thyroid (ATG, AMC) and anti-neutrophil cytoplasmic antigens (PR3-ANCA, MPO-ANCA) were determined in all subjects (Table 1). Of the 217 subjects, 127 (48.5%) had at least one of the autoantibodies investigated in this project. ANA was the most prevalent (27.4%) autoantibody found among the subjects. Rheumatoid factor was detectable in 52 (17.8%) of the subjects. The anti-thyroid (ATG: 4.4%, AMC: 7.6%) and anti-neutrophil (PR3-ANCA: 3.8%, MPO-ANCA: 2.7%) antibodies were less common. ATG and AMC were more prevalent among female (7.6%) than among male (4.4%) subjects ($p \leq 0.01$), while PR3-ANCA and MPO-ANCA showed male preference (PR3-ANCA: 1.5% of female vs. 5.7% of male, $P \leq 0.05$, MPO-ANCA: 0.8% of female vs. 4.3% of male, p: non-significant). Prevalence rate of ANA and RF did not show gender differences. After inspecting the medical charts, only a minor part of subjects had documented extra-hepatic autoimmune manifestations. Presence of autoimmune manifestations correlated poorly with the presence of autoantibodies (data not shown).

2. HLA-DRB1 genetic polymorphisms among the HCV-infected subjects

DNA samples from all the 271 patients were subjected to HLA-DRB1 genetic typing using PCR and sequence-specific oligonucleotide probe hybridization (PCR/SSOPH) technique. The primer UG138/UG139 amplify all the DRB genes, SSO probes used in this panel could distinguish most of the major DRB1 alleles corresponding to serologically-defined HLA-DR specificities except for all the DRB1*03 and some of the DRB1*13 and DRB1*14 alleles. The allelic distribution of DRB1 gene of the HCV-infected subjects was firstly compared with that of a control group of 98 healthy unrelated medical students. There was no significant difference noted (Table 2).

The HCV-infected subjects were further grouped according to whether or not presence of autoantibodies or extra-hepatic autoimmune manifestations. HLA-DRB1*11 alleles were negatively associated with autoantibody production among HCV-infected individuals (Table 3.1, 3.2). Allelic frequency of HLA-DRB1*1101 was significantly lower in the HCV-infected subjects with ANA (3.1% of ANA (+) vs. 8.5% of ANA (-), OR: 0.38, $p \leq 0.0161$), RF (2.9% of RF (+) vs. 8% of RF (-), OR: 0.33, $p \leq 0.0439$), ATG (0% of ATG (+) vs. 7.4% of ATG (-), OR: 0.23, $p \leq 0.0486$). DRB1*1101 allelic frequency was also significant lower among subjects with at least one autoantibody than those who didn't (3.9% of autoantibody (+) vs. 9.2% of autoantibody (-), OR: 0.42, $p \leq 0.0124$). HLA-DR2 was also noted being more prevalent in HCV-infected subjects without auto-antibodies RF (9.6% of RF (+) vs. 19.9% of RF (-), OR: 0.45, $p \leq 0.0088$), ATG (7.7% of ATG (+) vs. 18.7% of ATG (-), OR: 0.44, p: non-significant), AMC (9.1% of AMC (+) vs. 18.4% of AMC (-), OR: 0.447, p: non-significant), PR3-ANCA (9.1% of PR3-ANCA (+) vs. 19.1% of PR3-ANCA (-), OR: 0.54, $p \leq 0.0227$), and MPO-ANCA (6.3% of MPO-ANCA (+) vs. 18.8% of MPO-ANCA (-), OR: 0.42, p: non-significant). HLA-DRB1*1001 was correlated to the presence of thyroid antibodies ATG (7.7% of ATG (+) vs. 1.6% of ATG (-), OR: 5.9, $p \leq 0.0875$, non-significant) and AMC (4.5% of AMC (+) vs. 1.7% of AMC (-), OR: 3.25, *p*: non-significant) although the differences were statistically non-significant. Besides, DR4 was negatively associated with the presence of PR3-ANCA (9.1% of PR3-ANCA (+) vs. 15% of PR3-ANCA (-), OR: 0.69, $p \leq 0.0418$).

Discussion

Hepatitis C infection has broad range of outcomes. A minor part of patients are able to

clear the infection spontaneously by effective cell-mediated immunity [1]. Recently, genetic studies have indicated that HLA class II genotypes strongly influenced the outcome of HCV infection. It was interesting to observe a significant increase of HLA-DR2 (DRB1*1601/DQB1*0502) subtype in a group of 30 Sardinian thalassemic patients who despite 10.3±2.2 years of regular blood transfusion program did not show any evidence of HCV infection [27]. The frequency of HLA DRB1*0301/DQB1*0201 was significantly higher in patients with persistent HCV infection than in the transient-infected group in Thailand. HLA-DRB1*0701/DQB1*0201 were significantly decreased in all the HCV-infected patients compared to the non-infected normal control subjects [28]. In studying Turkish chronic hepatitis C and healthy blood donors, it was found that DRB*11 not only had a reduced frequency in patients with chronic hepatitis C compared to control, but also had a reduced frequency in all anti-HCV antibody (+) subjects compared to the control group [29]. HLA-DRB1*1101 /DQB1*0301 haplotype showed strong association with maintenance of multi-specific CD4+ T-helper cell response [30]. There were racial differences in HLA class II association with HCV infection. In an ethnically varied cohort of 200 HCV clearance and 374 matched HCV persistent infected subjects, viral clearance was associated with HLA DRB1*1101/DQB1*0301 haplotype, whereas viral persistence associated with DRB1*0301/DQB1*0201 haplotype [31]. It was noted that HLA class II alleles might contribute to the severity of HCV-related liver disease [32]. In HCV RNA (+) patients with chronic hepatitis, cirrhosis patients had higher frequency of DRB1*03/DQB1*0201. Mean index of fibrosis was higher in HLA-DR3 (+) than in HLA-DR11 (+) patients. HLA class II haplotype HLA-DRB1*11/DQB1*03 was noted to be associated with a reduced risk for the development of HCV-induced end stage liver diseases in German patients [33]. In a Japanese study Harune et. al. declared that HLA DRB1*1302 protected patients with chronic HCV infection from bile duct damage and portal lymphocyte infiltration [34].

HCV infection has been frequently detected in patients with immune complex-mediated diseases, such as mixed cryoglobulinemia, autoimmune hepatitis, Sjogren's syndrome, glomerulonephritis, polyarteritis nodosa, and immunologic abnormalities inducing autoantibodies. These immunologic abnormalities are more frequently found in patients with chronic HCV infection than in normal subjects, while the HCV-associated immune disorders seemed to be independent on HCV serotypes [13;21], relationship between HCV and host factors that involved in the immune responses were intensively investigated recently. It was reported that HLA-DR11 was significantly more frequent in patients with HCV-associated type II cryoglobulinemia (MC) than in those who without. HLA-DR7 was less frequent in HCV-infected patients with MC than those without [35]. Huang et al. reported that cryoglobulinemia and serum autoantibodies were frequently found in chronic hepatitis C. HLA-DR3 was positively associated with cryoglobulinemia and serum autoantibody production, and HLA-DR4 was more frequent among patients with serum autoantibodies [36].

In this project, we did not investigate HCV RNA in patients' serum samples. Whether the HCV was cleared or the infection persisted was not clear. However, as most (about 85%) of the HCV-infected subjects developed chronic hepatitis and the subjects in this study were recruited from the GI special clinics or the patients hospitalized for liver diseases in NTUH, it was quite reasonable to regard that most of the subjects we studied had chronic hepatitis rather than a well-being, viral clearance state. In our study, we did not find significant difference in DRB1 allelic distribution between the HCV-infected subjects and the non-infected control group. Presence of autoantibodies was commonly found in the HCV-infected subjects. Forty-five percent of the subjects had at least one detectable autoantibody in their sera. Anti-nuclear antibody (ANA, 27.4%) and rheumatoid factor (RF, 17.8%) were frequently found. Anti-thyroid antibodies and anti-neutrophil cytoplasmic antibodies were less frequent. Wu et. al. reported prevalence rates of 15.8%, 56%, 55.6%, 4.8% for ANA, RF, PR3-ANCA and MPO-ANCA respectively among 216 Taiwanese chronic hepatitis C patients[2], which showed an obvious difference in RF and PR3-ANCA prevalence from we observed. Differences in methodologies applied and patient composition might be attributed, which await further verification. Huang et. al. disclosed that HLA-DR4 being positively associated with autoantibody production (45.8% of autoantibody (+) vs. 19% of autoantibody (-)), whereas HLA-DR3 predisposed subjects to cryoglobulinemia [36]. Study in France revealed that HLA-DR11 was significantly associated with the increased risk for the development of type II mixed cryoglobulinemia in patients with chronic HCV infection. In contrast, HLA-DR7 appeared to confer protection against type II MC [35]. We found that HLA-DR2 seemed to have protective effect against production of

autoantibodies except for ANA, to a less extent. Previously, HLA-DR2 was reported to be associated with autoantibody production and clinical outcomes among HCV-infected subjects, HLA-DR2 subtype (DRB1*1601/DQB1*0502) seemed to protect subjects from transfusion associated HCV infection [27] and HLA-DRB1*1502 being more frequent in patients with than those without piecemeal hepatic necrosis and portal lymphocyte infiltration [34]. HLA-DR2 specificity can subdivide into DRB1*15 and DRB1*16, which differ in their DR/DQ haplotypes. It is suggested that DR2 subtype should be confirmed for the subjects we studies to see their potential relationship with autoantibody production.

It was impressing to see that HLA-DRB1*11 allele correlated to viral clearance, better outcome of liver disease and less necrosis, lymphocyte infiltration. In this report we revealed a protective effect conferred by HLA-DRB1*11 against autoantibody production in chronic hepatitis C. Correlation of HLA-DR11 with autoantibody production awaits further confirmation in other ethnic groups in the world. On the other way, all the previous studies encouraged us by hinting an important role HLA-DR11 may play in participating generation of effective and protective immunity against HCV infection. May it be the case the major T epitopes on HCV antigens and related issues would be valuable in developing preventive and protective strategies against chronic hepatitis C virus infection.

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Table 1 Prevalence of autoantibodies among the HCV-infected subjects.

	autoAb		ANA		RF	
Ab	n	%	n	%	n	%
positive	127	43.5	80	27.4	52	17.8
suspected	12	4.1	11	3.8	1	0.3
negative	153	52.4	201	68.8	239	81.8

	ATG		AMC		PR3-ANCA		MPO-ANCA	
Ab	n	%	n	%	n	%	n	%
positive	13	4.4	22	7.6	11	3.8	8	2.7
suspected	1	0.3	2	0.7	7	2.4	5	1.8
negative	278	95.2	267	91.7	274	93.8	279	95.5

Table 2 DRB1 allelic distribution of HCV-infected individual compared to 98 healthy control subjects

DRB1	Anti-HCV(+) (N=584)		Anti-HCV (-) (N=196)	
allele	n	%	n	%
**	104	17.8	10	5.1
0101	1	0.2	1	0.5
04	85	14.6	23	11.7
0701	19	3.3	9	4.6
0803	31	5.3	22	11.2
0901	83	14.2	34	17.3
1001	11	1.9	2	1
11	41	7.0	16	8.1
1201	22	3.8	9	4.6
1202	54	9.2	18	9.2
1302	11	1.9	6	3.1
1304	1	0.2	0	0
1312	1	0.2	0	0
14	14	2.4	9	4.6
DR2	106	18.2	30	15.3

OR: Odd's ratio
95% C.I.: 95% confidence interval of OR
** contain DRB1*03 and part of *13, *14, *08, *12 alleles, which await further characterization.

Table 3.1 Allelic distribution of HLA-DRB1 gene among HCV-infected subjects with and without autoantibodies.

	ANA				**RF**				**ATG**				**AMC**			
	(+)		(-)		(+)		(-)		(+)		(-)		(+)		(-)	
	N=160		N=402		N=104		N=478		N=26		N=556		N=44		N=534	
DRB1*	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
**	34	21.3	67	16.7	19	18.3	84	17.6	6	23.1	97	17.5	7	15.9	96	18.0
0101	0	0.0	1	0.3	0	0.0	1	0.2	0	0.0	1	0.2	0	0.0	1	0.9
04	20	12.5	60	14.9	11	10.6	74	15.5	7	26.9	78	14.0	8	18.2	76	14.2
0701	4	2.5	14	3.5	4	3.9	15	3.1	0	0.0	19	3.4	1	2.3	18	3.4
0803	8	5.0	21	5.2	7	6.7	24	5.0	2	7.7	28	5.0	3	6.8	27	5.1
0901	26	16.3	56	13.9	20	19.2	63	13.2	5	19.2	78	14.0	8	18.2	74	13.9
1001	4	2.5	7	1.7	2	1.9	9	1.9	2[5]	7.7	9	1.6	2[9]	4.6	9	1.7
1101	**5[1]**	**3.1**	**34**	**8.5**	**3[3]**	**2.9**	**38**	**8.0**	**0[6]**	**0.0**	**41**	**7.4**	3	6.8	38	7.1
1201	2[2]	1.3	18	4.5	4	3.9	18	3.8	0	0.0	22	4.0	2	4.6	20	3.2
1202	15	9.4	39	9.7	14	13.5	40	8.4	1[7]	3.9	53	9.5	5	11.4	48	9.0
1302	2	1.3	8	2.0	2	1.9	9	1.9	1	3.9	10	1.8	1	2.3	10	1.9
1304	1	0.6	0	0.0	1	1.0	0	0.0	0	0.0	1	0.2	0	0.0	1	0.2
1312	0	0.0	1	0.3	1	1.0	0	0.0	0	0.0	1	0.2	0	0.0	1	0.2
14	6	3.8	7	1.7	6	5.8	8	1.7	0	0.0	14	2.5	0	0.0	13	2.4
DR2	33	20.6	69	17.2	**10[4]**	**9.6**	**95**	**19.9**	2[8]	7.7	104	18.7	4[10]	9.1	102	19.1

OR	95% C.I.	G	p<	OR	95% C.I.	G	p<	OR	95% C.I.	G	p<
				[5]5.88	1.38-25.09	2.92	0.0875	[9]3.25	0.78-13.57	1.33	0.2488
[1]0.38	**0.15-0.95**	**5.79**	**0.0161**	**[6]0.23**	**0.01-3.29**	**3.89**	**0.0486**				
[2]0.33	0.09-1.24	4.21	0.0403	[7]0.55	0.1-2.94	1.189	0.2758				
[3]0.39	**0.13-1.2**	**4.062**	**0.0439**								
[4]0.45	**0.23-0.88**	**6.871**	**0.0088**	[8]0.44	0.12-1.65	2.451	0.1175	[10]0.47	0.17-1.27	3.174	0.0748

OR: Odd's ratio

95% C.I.: 95% confidence interval of OR

** contains DRB1*03 and part of *13, *14, *08, *12 alleles which awaits further characterization

Table 3.2 Allelic distribution of HLA-DRB1 gene among HCV-infected subjects with and without autoantibodies.

	Any auto Ab				**PR3-ANCA**				**MPO-ANCA**			
	(+)		(-)		(+)		(-)		(+)		(-)	
	N=254		N=306		N=22		N=548		N=16		N=558	
DRB1*	N	%	N	%	N	%	N	%	N	%	N	%
*	49	19.3	52	17.0	7	31.8	95	17.3	5	31.3	96	17.2
0101	0	0.0	1	0.3	0	0.0	1	0.2	0	0.0	1	0.2
04	32	12.6	47	15.4	**2**[2]	**9.1**	**82**	**15.0**	2	12.5	82	14.7
0701	7	2.8	11	3.6	1	4.6	17	3.1	1	6.3	18	3.2
0803	14	5.5	15	4.9	1	4.6	29	5.3	1	6.3	30	5.4
0901	40	15.8	41	13.4	3	13.6	77	14.1	3	18.8	78	14.0
1001	6	2.4	5	1.6	0	0.0	11	2.0	0	0.0	11	2.0
1101	**10**[1]	**3.9**	**28**	**9.2**	2	9.1	38	6.9	1	6.3	39	7.0
1201	8	3.2	12	3.9	0	0.0	21	3.8	0	0.0	21	3.8
1202	29	11.4	25	8.2	2	9.1	52	9.5	2	12.5	52	9.3
1302	5	2.0	5	1.6	0	0.0	10	1.8	0	0.0	11	2.0
1304	1	0.4	0	0.0	0	0.0	1	0.2	0	0.0	1	0.2
1312	1	0.4	0	0.0	1	4.6	0	0.0	0	0.0	0	0.0
14	9	3.5	5	1.6	1	4.6	13	2.4	0	0.0	13	2.3
DR2	43	16.9	59	19.3	**2**[3]	**9.1**	**101**	**18.4**	**1**[4]	**6.3**	**105**	**18.8**

RR	95% C.I.	G	p<	RR	95% C.I.	G	p<	RR	95% C.I.	G	p<
				[2]0.69	0.18-2.62	0.655	0.04184				
[1]0.42	0.2-0.87	6.258	0.0124	[3]0.54	0.14-2.03	1.46	0.0227	[4]0.42	0.08-2.25	2.067	0.1505

RR: relative risk

95% C.I.: 95% confidence interval of RR

* contains DRB1*03 and part of *13, *14, *08, *12 alleles which awaits further characterization

成果自評

1. 內容與計劃符合程度及達成目標狀況：
   本計劃執行情形與計劃內容相符，所收集個案數有達成預期目標，autoantibodies 之偵測大多已完成，限於免疫螢光測試法耗時、耗力甚鉅，不適用於大量檢體操作，故未再增加其他 auto-antibody 項目：如 anti-smooth muscle, anti-mitochondria 等；但僅計劃執行中所測 6 種自體免疫抗體即可看出有近一半慢性 C 型肝炎病患中具有至少一種 autoantibody。
   臨床病狀之統計方面，係透過免疫風濕專科醫師協助審視所有個案病例報告中有關免疫風濕相關症狀之紀錄。然或因此類症狀不明顯，或在腸胃專科醫療照護中較不注重免疫風濕症狀，大部份病人之病例報告中未紀錄免疫風濕相關癥候，資料嚴重不齊，以致此部份統計工作難以進行。
   HLA DRB1 基因分型工作尚有小部份未完成，DR3 及部份 DR13、 DR14 分型尚未解出，DR2 亞型 DR15、DR16 鑑定有待繼續完成之。
   由於本計畫中並未施行 HCV RNA 檢測，且由病歷記載中不易掌握 HCV 感染時間等因素，所選個案是否已清除 HCV 感染抑或為慢性肝炎狀態較難確定，若能進一步取得腸胃專科醫師之協助，瞭解病人 HCV 感染狀態對本計畫中各項統計結果更有幫助。
2. 搜尋文獻結果，目前 HCV 感染相關之自體免疫與個體本身 HLA 變異性間的相關性論文很少。此刻論文撰寫已同時進行中，待前述部分數據完成補強後應可做成相搭有價值論文發表於 SCI 雜誌。

總評

本計畫執行至今已達成絕大部分預期完成之目標，所得數據與去年度發表之中台灣地區相關報告有很大的差距。我們發現較有趣的部分是 HLA-DR11, DR2 對在慢性 C 型肝炎病患中發生自體免疫抗體似乎有保護作用。這與歐美各國去年度陸續發表報告，指出 HLA-DR11 與保護個體不受 HCV 感染、受 HCV 感染後能較有效清除病毒、及在慢性 C 型肝炎較佳癒後；較少發生肝硬化等正向因子相關有相當有趣及巧合的關聯。多瞭解 HCV 感染過程中宿主本身免疫反應的差異與其對於病毒感染能否產生有效的保護作用關係，將有助於發展預防感染及有效治療的方法，以避免慢性肝炎及後續肝臟疾病與腫瘤之發生，對於國人健康課題有重大的意義。