PO Box 2345, Beijing 100023, China
www.wjgnet.com
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RAPID COMMUNICATION

Cytotoxic T-lymphocyte antigen 4 gene polymorphisms and susceptibility to chronic hepatitis B

Amir Houshang Mohammad Alizadeh, Mehrdad Hajilooi, Mitra Ranjbar, Farahnaz Fallahian, Seyed Mohsen Mousavi

Amir Houshang Mohammad Alizadeh, Farahnaz Fallahian, Seyed Mohsen Mousavi, Research Center for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Mehrdad Hajilooi, Mithra Ranjbar, Hamedan University of Medical Sciences, Iran
Correspondence to: Dr. Amir Houshang Mohammad Alizadeh, Research Center for Gastroenterology and Liver disease, 7th Floor of Taleghani Hospital, Parvaneh Ave., Tabnak Str., Evin, Tehran 19857, Iran. ahmaliver@yahoo.com
Telephone: +98-21-2417283 Fax: +98-21-2412639
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Abstract

AIM: To assess the three polymorphism regions within cytotoxic T-lymphocyte antigen 4 (CTLA-4) gene, a C/T base exchange in the promoter region -318 (CTLA-4 -318C/T), an A/G substitution in the exon 1 position 49 (CTLA-4 49A/G), a T/C substitution in 1172 (CTLA-4 -1172T/C) in patients with chronic hepatitis B.

METHODS: Fifty-one patients with chronic hepatitis B virus infection and 150 healthy subjects were recruited sequentially as they presented to the hepatic clinic. Classification of chronic hepatitis B virus (HBV)-infected patients was as asymptomatic carrier state (26 patients) and chronic hepatitis B (25 patients). Genomic DNA was isolated from anti-coagulated peripheral blood Buffy coat using Miller's salting-out method. The presence of the CTLA-4 gene polymorphisms was determined using polymerase chain reaction amplification refractory mutation system (ARMS).

RESULTS: We observed a significant association between -318 genotypes frequency (T+C-, T+C+, T-C+) and susceptibility to chronic hepatitis B (P=0.012, OR = 0.49, 95%CI: 0.206-1.162). However, we did not observe a significant association for +49 genotype frequency (T+C+, T+C- T-C+) and -1172 genotype frequency (C+T+, T+C- C+T-) and state of disease.

CONCLUSION: Our results suggest that CTLA-4 gene polymorphisms may partially be involved in the susceptibility to chronic hepatitis B.

Key words: Cytotoxic T-lymphocyte antigen 4; Chronic hepatitis B; Gene polymorphism

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INTRODUCTION

The human cytotoxic T-lymphocyte antigen 4 (CTLA-4) gene was mapped to chromosome 2q33[1]. It consists of three exons; the first encodes a V-like domain of 116 amino acids. An A-to-G substitution at nucleotide 49 in exon 1 results in an amino acid substitution (Thr/Ala) in the leader peptide of the protein[2]. The Ala allele has been shown to predispose the individual carrying it to the development of various immune diseases, including Graves' disease[3], Hashimoto's thyroiditis[4], Addison's disease, rheumatoid arthritis[5,6], and celiac disease[7,8]. The existence of inactive hepatitis B carriers with normal liver histology and function suggests that the virus is not directly cytopathic. The fact that patients with defects in cellular immune competence are more likely to remain chronically infected rather than to clear the virus is cited to support the role of cellular immune responses in the pathogenesis of hepatitis B-related liver injury. Differences in the robustness of cytolytic T-cell responsiveness and in the elaboration of antiviral cytokines by T cells have been invoked to explain the differences in outcomes between those who recover after acute hepatitis or between those with mild and those with severe acute hepatitis B virus infection[9]. Chronic hepatitis is an important late complication of occult hepatitis B occurring in a small proportion of patients with chronic infection without having experienced an acute illness. Certain clinical and laboratory features suggest the progression of acute hepatitis to chronic hepatitis: (1) lack of complete resolution of clinical symptoms of anorexia, weight loss, and fatigue, and the persistence of hepatomegaly; (2) the presence of bridging or multilobular hepatic necrosis on liver biopsy during protracted, severe acute viral hepatitis; (3) failure of the serum aminotransferases, bilirubin and globulin levels to return to normal within 6-12 mo after the acute illness; and (4) the persistence of HBeAg beyond 3 mo after acute hepatitis. Under ordinary circumstances, none of the hepatitis viruses are known to be directly cytopathic to hepatocytes. Evidence suggests that the

clinical manifestations and outcomes after acute liver injury associated with viral hepatitis are determined by the immunologic responses of the host[9]. It is thought that the majority of autoimmune endocrinopathies, including Graves' disease, autoimmune hypothyroidism, type 1 diabetes mellitus and autoimmune Addison's disease (sporadic as well as autoimmune polyendocrinopathy syndrome type 2), are inherited as complex genetic traits. Multiple genetic and environmental factors interact with each other to confer susceptibility to these disorders. In recent years, there have been considerable efforts towards defining susceptibility genes for complex traits. These investigations have shown, with increasing evidence, that the CTLA-4 gene is an important susceptibility locus for autoimmune endocrinopathies and other autoimmune disorders[10]. Specific immunotherapy has moved into a new era with the introduction of soluble CTLA-4 protein into clinical trials. Treatment of bone marrow with CTLA-4 protein reduces rejection of the graft in HLA-mismatched bone marrow transplantation. In addition, promising results with soluble CTLA-4 have been reported in the down-regulation of autoimmune T-cell responses in the treatment of psoriasis[9]. Treatment with a blocking antibody against the CTLA-4 gene has been shown to enhance the effect of tumor rejection in mice vaccinated with irradiated tumor cells[11]. In this study, we have investigated whether genotypes of CTLA-4 –1172, -318, +49 polymorphisms as host factors predispose subjects to chronic hepatitis B.

MATERIALS AND METHODS

Subjects

Fifty-one patients with chronic hepatitis B virus infection (mean age, 36.23±12.65 years) and 150 healthy subjects (mean age, 32.64±7.12 years) were recruited sequentially as they presented to the hepatic clinic. Of chronic hepatitis B patients, 68.6% were males and 31.4% were females; and of healthy subjects (control group), 56% were males and 44% were females. Chronic HBV-infected patients were positive for HBV markers and if they had chronic hepatitis B, rising in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was seen for about 6 mo. Control groups were matched by age and sex; none of them were positive for hepatitis B serology. We did not regard any inflammatory conditions in the groups. All studies were carried out after the approval of Local Hospital Ethical Committees.

Hepatitis B was regarded if positive for HBsAg, anti-HBe, anti-HBc by ELISA HBsAg+, HBeAg (+, –), anti-HBe (+, –), HBV DNA (+, –), IgG anti-HBc+. Classification of chronic HBV-infected patients was carried out as follows: (A) Asymptomatic carrier state (26 patients), composed of chronically HBsAg-positive patients who had anti-HBc in serum, anti-HBs either undetected or detected at low titer against the opposite subtype specificity of the antigen regarded as inactive or asymptomatic carriers, HBV DNA load $<10^5$ copies/mL, HBeAg (+, –), liver transaminases of normal range; (B) chronic hepatitis B (25 patients) which comprised, based upon biochemical, virologic and histological activity, 17 patients who were still on interferon or lamivudine treatment, 5 non-responders to treatment (lack of virologic and or histologic response by first treatment course in whom sustained response was unlikely) and 3 chronic hepatitis B patients. Histologic classification was measured by modified histologic activity index (HAI) by Ishak score.

Treatment protocol

Management of chronic hepatitis B directed at suppressing the level of virus replication for patients who had HBV DNA detectable by hybridization assay and histological evidence of chronic hepatitis on liver biopsy. Interferon-α (IFN-α) or lamivudine was used for treatment. IFN-α registered as a 16-wk course was given subcutaneously at a daily dose of 5 million units or thrice a week at a dose of 10 million units. Lamivudine was administered orally at a daily dose of 100 mg for 12 mo. No treatment was recommended for inactive "non-replicative" hepatitis B carriers. Control subjects had no evidence of hepatitis B infection.

DNA isolation

Ten milliliters of venous blood was collected from each subject into tubes containing 50 mmol/L EDTA, and genomic DNA was isolated from anti-coagulated peripheral blood Buffy coat using Miller's salting-out method. All samples from cases and controls were handled in identical fashion as previously described[13].

Analysis of CTLA-4 gene polymorphisms

The presence of the CTLA-4 gene polymorphisms was determined by polymerase chain reaction (PCR) amplification refractory mutation system as previously described[6].

Statistical analysis

Allele and genotype frequencies among cases and controls were compared with values predicted by the Hardy-Weinberg equilibrium using the χ^2 test. All probability values were two-tailed. Distributions of alleles in patients and controls were compared using Fisher's exact test. The difference between susceptibility to hepatitis B was analyzed using Student's *t* test. A *P* value less than 0.05 was considered statistically significant. SPSS software version 11.05 was used for analysis.

RESULTS

Of chronic hepatitis B group, 26 cases (12.9%) were asymptomatic carriers, 3 cases (1.5%) were chronic hepatitis B, 17 cases (8.5%) were chronic hepatitis B on first course of treatment, and 5 cases (2.5%) were non-responders to first course of treatment. In addition, 86.3% of chronic hepatitis B patients were HBeAg-negative. No significant difference was observed between the mean age of HBeAg-positive patients (34.8 years) and HBeAg-negative patients (36.4 years). Of the 44 HBeAg-negative chronic hepatitis B patients, 25 cases were inactive carriers, 12 cases had received treatment, 4 cases were non-responders and 3 patients were still on treatment ($P<0.0001$).

Distribution of genotype frequency of CTLA-4 +49,

Table 1 Distribution of allele and genotype frequency of CTLA-4 +49, -318, -1172 polymorphisms in hepatitis B patients and controls

	Chronic hepatitis B *n* (%)	Controls *n* (%)
+49 genotype frequency ($P=0.211$)		
G+A–	9 (17.6)	41 (27.3)
G+A+	16 (31.4)	52 (34.7)
G–A+	26 (51)	57 (38)
+49 allele frequency		
G+	25 (49)	93 (62)
A+	42 (82.4)	109 (72.7)
–318 genotype frequency ($P=0.012$)		
T+C–	0 (0)	6 (4)
T+C+	10 (19.6)	10 (6.7)
T–C+	41 (80.4)	134 (89.3)
–318 allele frequency		
T+	10 (19.6)	16 (10.7)
C+	51 (100)	44 (96)
–1172 genotype frequency ($P=0.061$)		
T+C–	36 (70.6)	108 (72)
T+C+	13 (25.5)	42 (28)
T–C+	2 (3.9)	0 (0)
–1172 allele frequency		
C+	15 (29.4)	42 (28)
T+	49 (96.1)	150 (100)

Table 2 Relation between frequencies of genotypes of CTLA-4 +49, -318, -1172 CTLA-4 polymorphisms and mean AST and ALT levels in subjects with chronic hepatitis, chronic hepatitis on first course of treatment, inactive carrier, non-responders to first course of treatment

	TC 1172 genotype	Mean	SE
AST	1172 T+C–	35.1944	3.7008
	1172 T+C+	25.3077	2.3325
	1172 T–C+	35.0000	14.0000
	TC 318 genotype	**Mean**	**SE**
AST	318 T+C+	29.1000	5.8299
	318 T–C+	33.5366	3.1455
There are no valid cases for AST. Statistics cannot be computed.			
	AG 49 genotype	**Mean**	**SE**
AST	49 G+A–	32.6667	6.5362
	49 G+A+	32.3750	3.9811
	49 G–A+	32.8462	4.3960
	TC 1172 genotype	**Mean**	**SE**
ALT	1172 T+C–	40.9167	5.0528
	1172 T+C+	28.9231	4.3742
	1172 T–C+	50.0000	20.0000
	TC 318 genotype	**Mean**	**SE**
ALT	318 T+C+	33.5000	7.0683
	318 T–C+	39.3659	4.4751
There are no valid cases for ALT. Statistics cannot be computed.			
	AG 49 genotype	**Mean**	**SE**
ALT	49 G+A–	43.1111	9.3592
	49 G+A+	40.1875	5.7018
	49 G–A+	35.3077	5.9497

-318, -1172 polymorphisms of hepatitis B patients and controls is shown in Table 1. We observed a significant association between genotype frequency of -318T^+C^+ genotype and hepatitis B disease (19.6% *vs* 6.7%, $P=0.012$). On the contrary, no obvious difference was observed between HBeAg positive or negative hepatitis B disease and frequency of either studied genotypes. Moreover, we found a significant association between -318 genotypes frequency (T+C–, T+C+, T–C+) and susceptibility to chronic hepatitis B ($P=0.012$, OR = 0.490, 95%CI: 0.206-1.162).

There was no other association between genotype frequency of other genotypes frequencies and hepatitis B disease. Mean AST and ALT activities were 32.66 ± 19.71 and 38.21 ± 27.42 IU/L (normal range, 5-30 U/L), respectively. We observed significant differences in the mean AST levels among the inactive carriers (21.34 IU/L), chronic hepatitis B on treatment (37.76 IU/L), chronic hepatitis B who had received treatment (74.4 IU/L), and non-responders to treatment (31 IU/L) ($P<0.0001$). Moreover, significant differences were observed in the mean ALT levels among the inactive carriers (22.34 IU/L), chronic hepatitis B on treatment (45.7 IU/L), chronic hepatitis B who had received treatment (93 IU/L), and non-responders to treatment (42 IU/L) ($P<0.0001$). The relation between frequency of genotypes of CTLA-4 49, -318, -1172 CTLA-4 polymorphisms and mean AST and ALT levels in subjects with chronic hepatitis, chronic hepatitis on first course of treatment, inactive carrier, and non-responders to first course of treatment is shown in Table 2.

Percutaneous liver needle biopsy was performed in 24 cases. Mean pathologic grade and stage were 5.88 ± 1.84 and 2.11±1.13, respectively and mean score was 7.9 ± 2.48. Mean histology score for 1172 T+C– genotype was 7.68 (95%CI: 6.4-8.9), for 1172 T+C+ genotype was 8.75 (95%CI: 4.9-12.5), for –318 T+C+ genotype was 8.25 (95%CI: 6.7-9.7), for –318 T–C+ genotype was 8.75 (95%CI: 6.5-9.11), for 49 A+G+ genotype was 8 (95%CI: 5-10.9), and for 49 A+G+ genotype was 7.7 (95%CI: 5.2-10.3).

Association between CTLA-4 +49, –318, –1172 polymorphisms, and state of disease in chronic hepatitis B patients is shown in Table 3. We observed a significant association between +49 genotypes (G+A–, G+A+, G–A+), and state of disease in chronic hepatitis B patients ($P=0.043$). In contrast, no significant relation between -318 genotype frequency (T+C+, T+C–, T–C+), -1172 genotype frequency (C+T+, T+C–, C+T–) and state of disease was observed.

DISCUSSION

In a study[14] to test the hypothesis that single-nucleotide polymorphisms (SNPs) in the gene encoding CTLA-4 may affect the vigor of the T-cell response to hepatitis B virus (HBV) infection and thus influencing viral persistence, they studied genotyped six CTLA-4 SNPs, from which all frequent haplotypes can be determined, using a large, matched panel of subjects with known HBV outcomes. Haplotypes with these SNPs were constructed for each subject using PHASE software. The haplotype distribution

Table 3 Association between CTLA-4 +49 genotype polymorphism and state of disease in chronic hepatitis B patients

			State of disease				Total
			Healthy carrier	Under treatment	Refractory to treatment	Chronic hepatitis	
AG +49 genotype	+49 G+A–	Count	5	2	2	0	9
		% within state of disease	19.2%	11.8%	40.0%	.0%	17.6%
	+49 G+A+	Count	6	7	0	3	16
		% within state of disease	23.1%	41.2%	.0%	100.0%	31.4%
	+49 G–A+	Count	15	8	3	0	26
		% within state of disease	57.7%	47.1%	60.0%	.0%	51.0%
Total		Count	26	17	5	3	51
		% within state of disease	100.0%	100.0%	100.0%	100.0%	100.0%

[1]$P = 0.043$.

differed between those with viral persistence and those with clearance. Two haplotypes were associated with the clearance of HBV infection, which were most likely due to the associations with the SNPs -1722C (OR = 0.60, $P = 0.06$) and +49G (OR = 0.73, $P = 0.02$). The wild-type haplotype, which contains an SNP leading to a decreased T-cell response (+6230A), was associated with viral persistence (OR = 1.32, $P = 0.04$). These data suggest that CTLA-4 influences recovery from HBV infection, which is consistent with the emerging role of T regulatory cells in the pathogenesis of disease. The results of the present study showed a difference in CTLA-4 -318 genotype distribution between subjects and controls. In this study, we have demonstrated a significant association between -318 genotypes frequency (T+C–, T+C+, T–C+) and susceptibility to chronic hepatitis B ($P = 0.012$), whereas there was no significant relation between -318 genotype frequency (T+C+, T+C–, T–C+) and -1172 genotype frequency (C+T+, T+C–, C+T–) and state of disease.

Chronic hepatitis B is either HBeAg positive or negative. In the population studies, HBeAg-negative (anti-HBe-reactive) was found more common than HBeAg-reactive chronic hepatitis B [44 (86.3%) *vs* 7 (13.7%), $P = 0.082$]. In this study population, mean levels of both AST and ALT were significantly higher in HBeAg-positive patients as compared with HBeAg-negative patients. HBeAg-negative chronic hepatitis B patients tend to have progressive liver injury, complicated more frequently by cirrhosis and hepatocellular carcinoma, episodic reactivation of liver disease and more refractory to antiviral therapy. Most such cases represent precore or core-promoter mutations. Patients with HBeAg-negative phenotype or precore mutants are unable to secrete HBeAg and tend to have severe liver disease[9]. Precore genetic mutant of HBV is associated with the more severe outcome of HBV infection; therefore, relative pathogenicity is a property of the virus, and not the host[9]. In this study, we did not regard the influence of viral effects, such as hepatitis D virus, hepatitis B virus DNA load and YMDD variants, on the susceptibility and outcome of hepatitis B. However, as most of our patients were HBeAg-negative, this predominance was seen in all states of disease composed of asymptomatic carriers, subjects still on treatment, non-responders and subjects who finished treatment.

In other studies, especially those discussing about autoimmune diseases, a greater association between polymorphisms of this gene and disease has been demonstrated[15-18]. The strong genetic associations so far in PBC are with chromosomes 6p21.3 and 2q and include HLA DRBI*08 haplotypes, CTLA-4 G and IL1RN-IL1B haplotypes, CASP8, and nramp1. It is unlikely that only genes that influence disease susceptibility and progression in primary biliary cirrhosis (PBC) are immunoregulatory genes concerned with T-cell immunity. Recent studies have indicated a new era for immunogenetics, when genes encoding all immune active proteins may be considered as candidates. One should not concentrate solely on the immune response as recent investigations of mannose binding lectin and apolipoprotein-E have been testified. The authors stated the key issues for future investigators include defining the mechanisms whereby self tolerance is broken, defining the mechanisms that determine the rate of disease progression, and identifying genetic markers to predict progression and malignancy, and assessing the genetic basis of variability in disease progression. The significant variation in the rate of progression of PBC has led to the hypothesis that genes, in addition to contributing to disease susceptibility, may also determine the rate of disease progression. Several of the studies mentioned earlier have suggested associations between alleles at polymorphic loci and rate of progression. One problem inherent in such studies is that of definition of disease progression[15]. PBC is an autoimmune cholestatic liver disease thought to be developed through a complex interaction of genetic and environmental factors. It is characterized by T-cell-mediated non-suppurative destructive cholangitis. We have studied the polymorphic CTLA-4 gene, which encodes a molecule that is a vital negative regulator of T-cell activation, as a candidate susceptibility locus for PBC. This gene on chromosome 2q33 (designated IDDM12) is associated with susceptibility to both type 1 diabetes and autoimmune thyroid disease. The CTLA-4 exon 1 polymorphism (A/G encoding for threonine or alanine, respectively) was genotyped via PCR in 200 Caucasoid PBC patients and 200 non-related geographically matched Caucasoid controls. There was a significant over-representation of the G/A and G/G genotypes in PBC patients as compared with controls. Likewise, there was a significant difference in

allele frequencies. This association remained significant ($P = 0.00027$) when patients with autoimmune thyroid disease were excluded from the analysis. Thus, it was concluded that the CTLA-4 exon 1 polymorphism is the first non-major histocompatibility complex gene to be identified as a susceptibility locus for PBC. The data support the hypothesis that clinically distinct autoimmune disease may be controlled by a common set of susceptibility genes[16].

Latent autoimmune diabetes in adults (LADA) is identified by the presence of GAD65 autoantibodies in diabetic patients who do not require insulin treatment for at least 6 mo after the diagnosis. Previous studies have shown that the risk for LADA, similarly to type 1 diabetes mellitus (T1DM), is increased in subjects carrying the HLA-DRB1*03-DQA1*0501-DQB1*0201 and/or HLA-DRB1*04-DQA1*0301-DQB1*0302 haplotypes. Moreover, it has been investigated the association between LADA and the CTLA-4 A/G polymorphism, another gene polymorphism associated with T1DM and other autoimmune diseases. The heterozygous A/G genotype was significantly more frequent among 80 LADA (69%) than among 85 healthy subjects of similar age and geographical provenience (47%) (OR = 2.47, corrected $P = 0.023$). Conversely, the homozygous A/A genotype was significantly less frequent in LADA subjects compared to healthy controls (26% *vs* 47%, OR = 0.4, corrected $P = 0.028$). The results of our study showed that LADA was positively associated with the CTLA-4 A/G genotype, similarly to T1DM, thus providing further supporting evidence of the autoimmune origin of this form of diabetes mellitus in adults[17]. The CTLA-4 molecule plays an important role in immune regulation by down-regulating activation of T cells by antigen-presenting cells. Polymorphisms of the CTLA-4 gene have been shown to be associated with susceptibility to a number of autoimmune diseases. Some, but not all, studies suggest the association between the CTLA-4 gene and autoimmune hypothyroidism. The aim of this study was to determine whether allelic association was present between the A-G SNP at position 49 in exon 1 of the CTLA-4 gene and autoimmune hypothyroidism. The study was performed in 158 patients with autoimmune hypothyroidism and 384 control subjects. All subjects were white Caucasians from UK. There was a significant excess of the G allele in patients with autoimmune hypothyroidism as compared with controls. The GG and the AG genotypes were found to be more frequent in patients with autoimmune hypothyroidism than controls. These results suggested that the CTLA-4 gene region on chromosome 2q33 is a susceptibility locus for autoimmune hypothyroidism[18].

In conclusion, in the population that was studied, there is a significant difference in the frequencies of the -318 CTLA-4 gene polymorphisms ($T+C^-$, T^+C^+, T^-C^+) and susceptibility to chronic hepatitis B between controls and patients with hepatitis B. This association supports that this gene is partially the operating mechanism for genetic susceptibility to hepatitis B disease.

However, current knowledge of the genetic basis of susceptibility to hepatitis B is incomplete. Further investigations should be considered with caution until it is confirmed in independent large series. Elucidating predisposing genetic associations will markedly assist in understanding the susceptibility and pathophysiology of disease, the possibility of identifying the susceptibility of patients who are at elevated risk to hepatitis B infection or are in the early stages of disease or more rapid progression course of disease, would have obvious clinical benefit in terms of patient management and therapy.

REFERENCES

1 **Dariavach P,** Mattéi MG, Golstein P, Lefranc MP. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. *Eur J Immunol* 1988; **18**: 1901-1905

2 **Donner H,** Rau H, Walfish PG, Braun J, Siegmund T, Finke R, Herwig J, Usadel KH, Badenhoop K. CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *J Clin Endocrinol Metab* 1997; **82**: 143-146

3 **Yanagawa T,** Taniyama M, Enomoto S, Gomi K, Maruyama H, Ban Y, Saruta T. CTLA4 gene polymorphism confers susceptibility to Graves' disease in Japanese. *Thyroid* 1997; **7**: 843-846

4 **Donner H,** Braun J, Seidl C, Rau H, Finke R, Ventz M, Walfish PG, Usadel KH, Badenhoop K. Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease. *J Clin Endocrinol Metab* 1997; **82**: 4130-4132

5 **Seidl C,** Donner H, Fischer B, Usadel KH, Seifried E, Kaltwasser JP, Badenhoop K. CTLA4 codon 17 dimorphism in patients with rheumatoid arthritis. *Tissue Antigens* 1998; **51**: 62-66

6 **Gonzalez-Escribano MF,** Rodriguez R, Valenzuela A, Garcia A, Garcia-Lozano JR, Nuñez-Roldan A. CTLA4 polymorphisms in Spanish patients with rheumatoid arthritis. *Tissue Antigens* 1999; **53**: 296-300

7 **Djilali-Saiah I,** Schmitz J, Harfouch-Hammoud E, Mougenot JF, Bach JF, Caillat-Zucman S. CTLA-4 gene polymorphism is associated with predisposition to coeliac disease. *Gut* 1998; **43**: 187-189

8 **Naluai AT,** Nilsson S, Samuelsson L, Gudjónsdóttir AH, Ascher H, Ek J, Hallberg B, Kristiansson B, Martinsson T, Nerman O, Sollid LM, Wahlström J. The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders. *Tissue Antigens* 2000; **56**: 350-355

9 **Dienstag JL,** Isselbacher KJ. Acute viral hepatitis. In: Kasper LK, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL (eds). *Harrison's Principles of Internal Medicine*. 16th ed. New York: MacGraw-Hill; 2005: 1834-1930

10 **Vaidya B,** Pearce S. The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. *Eur J Endocrinol* 2004; **150**: 619-626

11 **Sutmuller RP,** van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, Toes RE, Offringa R, Melief CJ. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001; **194**: 823-832

12 **Bedossa P,** Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293

13 **Miller SA,** Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215

14 **Thio CL,** Mosbruger TL, Kaslow RA, Karp CL, Strathdee SA, Vlahov D, O'Brien SJ, Astemborski J, Thomas DL. Cytotoxic T-lymphocyte antigen 4 gene and recovery from hepatitis B virus infection. *J Virol* 2004; **78**: 11258-11262

15 **Jones DE,** Donaldson PT. Genetic factors in the pathogenesis of primary biliary cirrhosis. *Clin Liver Dis* 2003; **7**: 841-864
16 **Agarwal K,** Jones DE, Daly AK, James OF, Vaidya B, Pearce S, Bassendine MF. CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis. *J Hepatol* 2000; **32**: 538-541
17 **Cosentino A**, Gambelunghe G, Tortoioli C, Falorni A. CTLA-4 gene polymorphism contributes to the genetic risk for latent autoimmune diabetes in adults. *Ann N Y Acad Sci* 2002; **958**: 337-340
18 **Nithiyananthan R,** Heward JM, Allahabadia A, Franklyn JA, Gough SC. Polymorphism of the CTLA-4 gene is associated with autoimmune hypothyroidism in the United Kingdom. *Thyroid* 2002; **12**: 3-6

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