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ORIGINAL ARTICLE

Association of *IL-10* and *TGF-beta* cytokine gene polymorphisms with autoimmune hepatitis

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Summary
Background and aims: Autoimmune hepatitis is a chronic immune-mediated liver injury caused by dysregulated immune response to liver antigens. Genetic susceptibility is affected by multiple single nucleotide polymorphisms in immune-related genes. There are few reports on the association of *TGF-β* and *IL-10* genetic variants with autoimmune hepatitis.
Methods: Allele frequency and genotype status of *IL-10* −1082, −819, −592 and *TGF-β* +869 and +915 polymorphisms were investigated in 57 unrelated patients with autoimmune hepatitis and 140 healthy controls by polymerase chain reaction with sequence-specific primers.

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Results: *IL-10* −592 and −819 allele frequencies and genotypes were not associated with autoimmune hepatitis in our population, while *IL-10* −1082 genotypes were. *IL-10* −1082/−819/−592 ''high-producing'' haplotype GCC was significantly less frequent in patients. *TGF-β* +869 ''high-producing'' allele C and genotype CC were significantly more in autoimmune hepatitis, compared to controls; whereas, *TGF-β* +915 ''low-producing'' allele C and genotype CC were significantly more in autoimmune hepatitis compared to control. *TGF-β* +869/+915 haplotype TG was significantly less frequent in patients while CC haplotype was significantly more frequently observed in patients.
Conclusion: We identified a significant association between *IL-10* −1082/−819 and *TGF-β* +869/+915 genotypes and haplotypes with autoimmune hepatitis in Iranians.

Introduction

Autoimmune hepatitis (AIH) is a chronic immune-mediated liver injury caused by dysregulated immune response to liver antigens. AIH is clinically manifested by elevated serum transaminases, hypergammaglobulinaemia, and circulating autoantibodies. It is histologically characterised by interface hepatitis. From the immunologic perspective, AIH develops as a result of a disruption in immune self-tolerance; which results in aberrant immune response to hepatocyte antigens. T cells, specifically Th17 and Treg are implicated in the pathogenesis of the disease [1]. Although, the underlying mechanisms of the self-intolerance are not fully understood, molecular mimicry is speculated to be partly responsible [2,3]. In addition, it is suggested that hepatotropic viral infections can facilitate the breach of the self-tolerance [4].

Little is known about the Immunogenetics of AIH. Single nucleotide polymorphisms (SNPs) in human leukocyte antigens (HLAs) have been previously studied which confer susceptibility to AIH albeit, with different results in different populations [5—10]. One genome-wide association study has been performed on AIH in Europeans. The most significant associations were detected in chromosomal regions 6p21.32, 12q24.12, and 22q13.1; which contain reported genes of *HLA-DQA1*, *SH2B3*, and *CARD10*, respectively [11].

SNPs in specific cytokines have been shown to be associated with immune-mediated diseases like autoimmune hepatitis. *Transforming growth factorβ* (*TGF-β*) and*Interleukin 10 (IL-10*) polymorphisms seem to be of utmost importance due to the studies regarding their association with many immune-mediated diseases including autoimmune diseases [12—15], inflammatory diseases [16—19] and immunodeficiencies [20].

TGF-β is a major cytokine, which is implicated in a wide range of cellular processes and disease pathogenesis. TGF-β1-deficient mice spontaneously develop extensive hepatitis; which is significantly dependent on the mice genetic background [21]. In accordance, overexpression of inhibitory TGF-β receptor type II (TGF-βRII) results in increased susceptibility to experimental AIH induced by intraperitoneal injection of syngeneic liver lysate [22]. The serum level of TGF-β1 is increased in AIH compared to healthy control and in active disease compared to remission. Peripheral blood mononuclear cells (PBMC) expression of TGF-βRII is also decreased in AIH compared to control. Hepatocytes and infiltrating cells in the liver are also a source of TGF-β in AIH [23,24]. SNPs in *TGF-β1* have been comprehensively catalogued [25]. *TGF-β* +869 T/C (rs1982073) and +915 G/C (rs1800471) are coding SNPs, which result in amino acid substitution at codons 10 (Leucine to Proline) and 25 (Arginine to Proline), respectively. *TGF-β* +869 T allele and +915 G allele results in higher TGF-β1 protein expression. There is one report on the association of *TGF-β1* +869 and +915 SNPs with pediatric and adult AIH in Argentina [26]. However, there is currently no report on the association of *TGF-β1* SNPs with AIH in Iranians.

IL-10 is one of the major cytokines, which plays a crucial role in immune-mediated diseases including AIH. IL-10 is significantly more produced by cultured bone marrow cells and peripheral lymphocytes of AIH patients compared to healthy controls [28,29]. *IL-10* −592 (rs1800872), −819 (rs1800871), −1082 (rs1800896) are promoter SNPs in IL-10 upstream sequence. *IL-10* −1082 A allele results in higher IL-10 expression. Along with similar cytokines [27], *IL-10* promoter SNPs have been investigated in adult AIH1 [30—32] and AIH [26,33,34] in Argentinians, Chinese and Europeans; and pediatric AIH in Argentina [26]. However, the association of*IL-10* promoter polymorphism with AIH has not been studied in Iranians. Therefore, the main objective of this study was to investigate *IL-10* -1082 G/A, -819 C/T, and -592 C/A; and *TGF-β* +869 T/C and +915 G/C SNPs in Iranian patients with AIH.

Patients and methods

Patients

Fifty-seven unrelated patients with AIH were recruited from Gastroenterology Department, Children's medical center, Tehran University of Medical Sciences. Diagnosis of AIH was made based on the International Autoimmune Hepatitis Group criteria [35]. The patients were Iranians with the mean age of 6.2 years old and female to male ratio of 2.5:1. Six patients were from consanguineous parents. Among the 25 patient whom we have had access to their

medical records, 9 patients had AIH type 1 (positive for ANA or ASMA), 2 patients had AIH type 2 (positive for anti-LKM), 5 patients had autoantibody negative AIH and the remainders were diagnosed based on other criteria. Patients were negative for HBsAg, HBsAb, HBcAb, and HCVAb. The control group was comprised of 140 unrelated, relative age- and sex-matched, healthy blood donors with no history of AIH or systemic diseases as previously described [36]. The study was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tehran University of Medical Sciences. Written informed consent was obtained from patients' parents at enrolment.

Genotyping

Five mL of the peripheral venous blood was collected in EDTA anti-coagulated tubes and stored at −20 °C until further processing. Genomic DNA was extracted by salting out method and stored at −20 °C. Genotyping was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP assay kit; Heidelberg University, Heidelberg, Germany) as previously described [36]. PCR was performed in primer pair-coated 96-well plates with a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). Amplification condition was as follows: initial denaturation 94 °C, 2 min; denaturation 94 °C, 10 sec; annealing + extension 65 °C, 1 min (10 cycles); denaturation 94 °C, 10 sec; annealing 61 °C, 50 sec; extension 72 °C, 30 sec (20 cycles). Each well also contained primer pairs for housekeeping genes β-globin or C-reactive protein. PCR products were visualised on 2% agarose gel with an ultraviolet transilluminator.

Statistical analysis

Allele, genotype and haplotype frequencies were compared by χ^2 test. Statistical analyses were performed with SPSS (version 25; SPSS, Chicago, IL, USA). *P*-values of less than 0.05 were considered statistically significant.

Results

Overall 57 AIH and 140 healthy controls were investigated. Among these 198 children, the following SNPs were investigated in *IL-10*: −1082 G/A, −819 C/T, and −592 C/A. Allele frequencies of each *IL-10* SNP was not associated with AIH. However, *IL-10* −819 TT genotype ($P = 0.021$; OR = 2.86 [1.17—6.97]) and −1082 AA ($P = 0.004$; OR = 3.28 [1.47—7.31]), GA ($P = <0.001$; OR = 0.12 [0.04—0.36]), GG ($P = 0.043$; OR = 2.87 [1.03—8.00]) genotype were significantly associated, where −592 C/A was not significantly associated with AIH in our population. All *IL-10*haplotypes were observed in patients with AIH. However, only GCC, ACC, and ATA were detected in the controls. While *IL-10* −1082/−819/−592 haplotypes GCC ($P = 0.004$; OR = 0.27 [0.11—0.67]) was significantly less frequent in patients; ACA, GCA, ATC, GTC, and GTA were exclusively detected in patients with statistical significance. We observed a strong association of the investigated SNPs in *TGFβ* with AIH. *TGF-β* +869 allele C ($P < 0.001$; OR = 4.02 [2.3—7.04]) and *TGF-β* +915 allele C ($P < 0.001$; OR = 5.56 [2.94—10.51]) were significantly more in AIH compared to control. *TGF-β* +869 CC genotype ($P < 0.001$; OR = 14.52 [6.52—32.32]) was significantly more in AIH while CT genotype was significantly underrepresented in AIH, ($P < 0.001$; OR = 0.1 [0.04—0.24]). *TGF-β* +915 CC genotype ($P < 0.001$; OR = 20.61 [4.31—98.56]) was significantly more frequent among patients while GG genotype ($P < 0.001$; OR = 0.24 [0.11—0.53]) was significantly less frequent among the patient group. *TGF-β* +869/+915 haplotype TG was significantly less frequent in patients ($P < 0.001$; OR = 0.078 [0.03—0.20]) while CC haplotype was significantly more frequently observed in patients ($P < 0.001$; OR = 7.99 [4.08—15.66]). The results are summarized in Table 1 and Table 2.

Discussion

Cytokines play a major role in the progression and clinical manifestation of AIH. However, little is known about the genetic susceptibility to AIH in general, and the association of *IL-10* and *TGF-β* SNPs to AIH predisposition in particular. We did not detect a strong significant difference in *IL-10* −592 and −819 SNPs genotypes in cases vs. controls. This is in accordance with previous reports in AIH1 [30—32] and AIH [26,33,34]. However, according to the significant association found among *IL-10* −1082 genotypes and AIH, we suggest GA genotype is higher in AIH patients whereas AIH patients harbour AA genotype less than healthy individuals. Among *IL-10*haplotypes only GCC, ACC, and ATA were detected in the controls. While *IL-10* −1082/−819/−592 haplotypes GCC, ACC, and ATA were significantly less frequent in patients; ACA, GCA, ATC, GTC, and GTA were exclusively detected in patients with statistical significance. However, no significant association of *IL-10* −1082/−819/−592 haplotype with AIH has been previously reported [26,30,31]. *IL-10* −1082/−819/−592 haplotype is known to alter the level of *IL-10* gene expression. ACC and ATA are associated with low IL-10 production while GCC is associated with high production [37—39]. In this regard, we suggest that the ''high-producing'' haplotype is associated with AIH, while ''low-producing'' haplotypes are not associated with disease status. There is no report on the effect of other *IL-10* haplotypes on IL-10 production. However, since the allele A at position −1082 is associated with low production and allele G at position −1082 is associated with high production; it is speculated that ACA and ATC are also low-producing while GCA, GTC, and GTA are high-producing. We have detected ATC haplotype in AIH patients with a significant association compared to healthy controls. The overall frequency of high-producing haplotype vs. low-producing haplotype is 15.2% and 84.8% in AIH and 35.4% and 64.6% in controls.

A wide variety of cell types can secrete TGF-β and this pleiotropic cytokine controls proliferation, differentiation and other cell functions in many cell types. Abrogation of TGF-β1 results in extensive hepatitis in mice [22]; which suggests that TGF-β plays a protective role against AIH. Previous reports suggest that TGF-β level is altered by the coding SNPs at codon 10 (+896 C/T). Individuals with CC genotype had higher levels of TGF-β in the serum [40,41]. We observed

Table 1 Frequency of *IL-10* and *TGF-β* single nucleotide polymorphisms alleles and genotypes in patients with AIH compared to the controls.

Gene	Allele/genotype	AIH*n*, (%)	Control*n*, (%)	*P*-value	OR, (95% CI)
TGF-β +869	C	69 (78.4)	131 (47.5)	**<0.001**	4.02 (2.3–7.04)
	T	19 (21.6)	145 (52.5)	**<0.001**	0.25 (0.14–0.44)
	CC	31 (70.5)	20 (14.5)	**<0.001**	14.52 (6.52–32.32)
	CT	7 (15.9)	91 (65.9)	**<0.001**	0.1 (0.04–0.24)
	TT	6 (13.6)	27 (19.6)	0.377	0.65 (0.25–1.70)
TGF-β +915	C	27 (31.4)	21 (7.6)	**<0.001**	5.56 (2.94–10.51)
	G	59 (68.6)	255 (92.4)	**<0.001**	0.18 (0.09–0.34)
	CC	10 (23.3)	2 (1.5)	**<0.001**	20.61 (4.31–98.56)
	CG	7 (16.3)	17 (12.3)	0.505	1.38 (0.53–3.60)
	GG	26 (60.5)	119 (86.2)	**<0.001**	0.24 (0.11–0.53)
IL-10 −592	A	25 (27.2)	81 (28.9)	0.746	0.92 (0.54–1.55)
	C	67 (72.8)	199 (71.1)	0.746	1.09 (0.64–1.85)
	AA	4 (8.7)	12 (8.6)	0.979	1.02 (0.31–3.32)
	CA	17 (37)	57 (40.7)	0.652	0.85 (0.43–1.70)
	CC	25 (54.3)	71 (50.7)	0.669	1.16 (0.59–2.26)
IL-10 −819	C	68 (65.4)	199 (71.1)	0.283	0.77 (0.48–1.24)
	T	36 (34.6)	81 (28.9)	0.283	1.30 (0.80–2.10)
	CC	27 (51.9)	71 (50.7)	0.882	1.05 (0.55–1.98)
	CT	14 (26.9)	57 (40.7)	0.081	0.54 (0.27–1.08)
	TT	11 (21.1)	12 (8.6)	**0.021**	2.86 (1.17–6.97)
IL-10 −1082	A	48 (72.7)	181 (64.6)	0.213	1.46 (0.80–2.64)
	G	18 (27.3)	99 (35.4)	0.213	0.69 (0.38–1.24)
	AA	22 (66.7)	53 (37.8)	**0.004**	3.28 (1.47–7.31)
	GA	4 (12.1)	75 (53.6)	**<0.001**	0.12 (0.04–0.36)
	GG	7 (21.2)	12 (8.6)	**0.043**	2.87 (1.03–8.00)

AIH: autoimmune hepatitis; OR: odds ratio; 95% CI: Wald's 95% confidence interval. The values in bold (*P*-value < 0.05) indicate a significant association.

Table 2 Frequency of *IL-10* and *TGF-β* haplotypes in patients with AIH compared to the controls.

Gene	Haplotype	AIH*n*, (%)	Control*n*, (%)	*P*-value	OR, (95% CI)
TGF-β +869/+915	CG	33 (52.4)	110 (39.9)	0.071	1.66 (0.96–2.88)
	TG	5 (7.9)	145 (52.5)	**<0.001**	0.078 (0.03–0.20)
	CC	25 (39.7)	21 (7.6)	**<0.001**	7.99 (4.08–15.66)
	TC	0	0 (0)	**<0.001**	–
IL-10 −1082/−819/−592	GCC	6 (13)	99 (35.4)	**0.004**	0.27 (0.11–0.67)
	ACC	16 (34.8)	100 (35.7)	0.903	0.96 (0.5–1.85)
	ATA	14 (30)	81 (28.9)	0.835	1.075 (0.54–2.12)
	ACA	1 (2.2)	0 (0)	**<0.001**	–
	GCA	0 (0)	0 (0)	**<0.001**	–
	ATC	8 (17.4)	0 (0)	**<0.001**	–
	GTC	1 (2.2)	0 (0)	**<0.001**	–
	GTA	0 (0)	0 (0)	**<0.001**	–

AIH: autoimmune hepatitis; OR: odds ratio; 95% CI: Wald's 95% confidence interval. The values in bold (*P*-value < 0.05) indicate a significant association.

a significant increase in the high-producing genotype of *TGF-β* +896 in AIH compared to control. This is in accordance with the previous report in adult AIH in Argentina [26]. *TGF-β1* +915 G allele and GG genotype are associated with higher production of TGF-β [42]. We observed a significant reduction in the high-producing GG genotype in AIH. In the previous report, the GG genotype was decreased in adult AIH compared to control. However, it did not reach statistical significance. In contrast, the low-producing CC genotype was significantly increased in adult AIH compared to control. Interestingly, paediatric AIH demonstrated distinct genetic pattern. While *TGF-β1* +869 allele frequency and genotype did not differ between paediatric AIH and healthy control, the high-producing G allele and GG genotype were significantly increased in paediatric AIH compared to adult AIH and healthy control [26]. In our study, *TGF-β* +869/+915

high-producing haplotype CG was increased in AIH but did not reach statistical significance. *TGF-β* +869/+915 TG was significantly less frequent in patients while CC haplotype was significantly more frequently observed in patients.

Our study is limited by not classifying the patients according to their profile of autoantibodies. This might result in masking small but significant results in one subtype and not the other. Nevertheless, we detected significant association between*IL-10*haplotypes and *TGF-β* genotype with AIH.

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Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Oo YH, Hubscher SG, Adams DH. Autoimmune hepatitis: new paradigms in the pathogenesis, diagnosis, and management. Hepatol Int 2010;4(2):475—93.
[2] Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. Clin Rev Allergy Immunol 2012;42(1):102—11.
[3] Fujinami RS, et al. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev 2006;19(1):80—94.
[4] Holdener M, et al. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. J Exp Med 2008;205(6):1409—22.
[5] Djilali-Saiah I, et al. Linkage disequilibrium between HLA class II region and autoimmune hepatitis in pediatric patients. J Hepatol 2004;40(6):904—9.
[6] Ngu JH, et al. Association of the HLA locus and TNF with type I autoimmune hepatitis susceptibility in New Zealand Caucasians. Springerplus 2013;2:355.
[7] Tanwandee T, et al. Association between HLA class II alleles and autoimmune hepatitis type 1 in Thai patients. J Med Assoc Thai 2006;89(5):S73—8.
[8] Ferucci ED, et al. Autoimmune hepatitis in the Alaska Native population: autoantibody profile and HLA associations. Liver Int 2014;34(8):1241—9.
[9] Hassan N, et al. Clinical profile and HLA typing of autoimmune Hepatitis from Pakistan. Hepat Mon 2013;13(12):e13598.
[10] Suzuki Y, et al. Association of HLA-DR14 with the treatment response in Japanese patients with autoimmune hepatitis. Dig Dis Sci 2010;55(7):2070—6.
[11] de Boer YS, et al. Genome-wide association study identifies variants associated with autoimmune hepatitis type 1. Gastroenterology 2014;147(2):443e5—52e5.
[12] Jung JH, et al. Association of Interleukin 10 Gene Polymorphisms with autoimmune thyroid disease: meta-analysis. Scand J Immunol 2016;84(5):272—7.
[13] Gerenova J, Stanilova S. IL-12B and IL-10 gene polymorphisms in the development of Hashimoto's thyroiditis. Int J Immunogenet 2016;43(6):397—403.
[14] Xavier JM, et al. Association study of IL10 and IL23R-IL12RB2 in Iranian patients with Behcet's disease. Arthritis Rheum 2012;64(8):2761—72.
[15] Shahram F, et al. Association of interleukin-2, interleukin-4 and transforming growth factor-beta gene polymorphisms with Behcet's disease. Clin Exp Rheumatol 2011;29:S28—31 [4 Suppl 67].
[16] Movahedi M, et al. IL-10, TGF-beta, IL-2, IL-12, and IFN-gamma cytokine gene polymorphisms in asthma. J Asthma 2008;45(9):790—4.
[17] Tavakol M, et al. Association of interleukin 10 and transforming growth factor beta gene polymorphisms with chronic idiopathic urticaria. Acta Dermatovenerol Croat 2014;22(4):239—45.
[18] Nasiri R, et al. Gene polymorphisms of interleukin-10 and transforming growth factor beta in allergic rhinitis. Allergol Immunopathol (Madr) 2016;44(2):125—30.
[19] Harsini S, et al. Interleukin 10 and transforming growth factor beta 1 gene polymorphisms in juvenile idiopathic arthritis. Bratisl Lek Listy 2016;117(5):258—62.
[20] Rezaei N, et al. Cytokine gene polymorphisms in common variable immunodeficiency. Int Arch Allergy Immunol 2009;150(1):1—7.
[21] Gorham JD. Transforming growth factor-beta1, Th1 responses, and autoimmune liver disease. Transfusion 2005;45(2): 51S—9S.
[22] Schramm C, et al. Impairment of TGF-beta signaling in T cells increases susceptibility to experimental autoimmune hepatitis in mice. Am J Physiol Gastrointest Liver Physiol 2003;284(3):G525—35.
[23] Sakaguchi K, et al. Serum level of transforming growth factor-beta1 (TGF-beta1) and the expression of TGF-beta receptor type II in peripheral blood mononuclear cells in patients with autoimmune hepatitis. Hepatogastroenterology 2004;51(60):1780—3.
[24] Bayer EM, et al. Transforming growth factor-beta1 in autoimmune hepatitis: correlation of liver tissue expression and serum levels with disease activity. J Hepatol 1998;28(5):803—11.
[25] Watanabe Y, et al. A catalog of 106 single-nucleotide polymorphisms (SNPs) and 11 other types of variations in genes for transforming growth factor-beta1 (TGF-beta1) and its signaling pathway. J Hum Genet 2002;47(9):478—83.
[26] Paladino N, et al. The most severe forms of type I autoimmune hepatitis are associated with genetically determined levels of TGF-beta1. Clin Immunol 2010;134(3):305—12.
[27] Yousefi A, et al. IL4 gene polymorphisms in Iranian patients with autoimmune hepatitis. Expert Rev Gastroenterol Hepatol 2016;10(5):659—63.
[28] Zachou K, et al. Autoimmune hepatitis type 1 and primary biliary cirrhosis have distinct bone marrow cytokine production. J Autoimmun 2005;25(4):283—8.
[29] Zgair AK. Involvement of (IgG and IgM)-secreting B lymphocytes in severity of autoimmune hepatitis type 1. Med Microbiol Immunol 2013;202(3):229—37.
[30] Cookson S, et al. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. Hepatology 1999;30(4):851—6.
[31] Czaja AJ, et al. Cytokine polymorphisms associated with clinical features and treatment outcome in type 1 autoimmune hepatitis. Gastroenterology 1999;117(3):645—52.
[32] Ma X, Qiu DK. Cytokine gene polymorphisms in Chinese patients with type I autoimmune hepatitis. Zhonghua Gan Zang Bing Za Zhi 2004;12(5):296—8.
[33] Chen QB, et al. A study on the relationship between interleukin-10 promoter polymorphism and autoimmune liver disease. Zhonghua Gan Zang Bing Za Zhi 2004;12(6):356—8.
[34] Fan LY, et al. Genetic association of cytokines polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. World J Gastroenterol 2005;11(18):2768—72.
[35] Alvarez F, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31(5):929—38.

[36] Amirzargar AA, et al. Cytokine single nucleotide polymorphisms in Iranian populations. Eur Cytokine Netw 2008;19(2): 104—12.
[37] Sharma S, et al. Haplotype-dependent differential activation of the human IL-10 gene promoter in macrophages and trophoblasts: implications for placental IL-10 deficiency and pregnancy complications. Am J Reprod Immunol 2010;64(3):179—87.
[38] Temple SE, et al. Alleles carried at positions —819 and —592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with Streptococcus pneumoniae. Immunogenetics 2003;55(9):629—32.
[39] Koss K, et al. Cytokine (TNF alpha LT alpha and IL-10) polymorphisms in inflammatory bowel diseases and normal controls: differential effects on production and allele frequencies. Genes Immun 2000;1(3):185—90.
[40] Yamada Y, et al. Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res 1998;13(10):1569—76.
[41] Yamada Y, et al. Association of transforming growth factor beta1 genotype with spinal osteophytosis in Japanese women. Arthritis Rheum 2000;43(2):452—60.
[42] Awad MR, et al. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 1998;66(8):1014—20.