Correlation between TCRCα -560 C/T polymorphism and the clinical presentation of Uygur IgA nephropathy patients in XinJiang

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Summary

Objective: **To investigate the relationship between T cell receptor alpha chain constant gene (TCRCα) -560 C /T polymorphism and the clinical presentation of Uygur IgA Nephropathy patients in XinJiang.**

Methods: **TCRCα -560 C/T genotypes were determined by PCR-RFLP in 300 Chinese Uygur IgAN patients and 600 healthy Chinese Uygur control subjects. All subjects were classified, based on their genotype, into TT, CT and CC groups and their corresponding clinical presentation was analyzed.**

Results: **No significant difference was observed in the frequency of CC/CT/TT genotypes in patients and control subjects (χ^2 =0.904, *P* =0.636). However, the incidence of intermittent microscopic hematuria and proteinuria is significantly higher in patients with CT genotype than CC and TT genotypes (χ^2 =33.978, *P* <0.05).**

Conclusion: **TCRCα-560 C / T gene polymerphism may be associated with the occurrence of intermittent microscopic hematuria and proteinuria in Chinese Uygur IgAN patients.** ***(Asian Pac J Allergy Immunol 2011;29:236-9)***

Key words: *IgA nephropathy, Uygur nationality, TCRCα, gene polymorphism, clinical presentation*

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Introduction

Immunoglobulin A nephropathy (IgAN), the most common type of glomerulonephritis worldwide, is a chronic glomerular disease with markedly different disease prevalence across the globe and highly variable clinical presentation[1-2]. In China, IgAN accounts for 30-40% of primary glomerulitis in the Han people and 13.19% in the Uygur people[3-4].

The pathology of IgAN varies and the prognostic factors for this disease remain to be defined. Although it may first appear benign, IgAN can progress to the end stage renal disease (ESRD). Currently, the pathogenesis of this disease is still unknown but immunological and genetic factors seem to be largely involved[5]. It was previously reported that the polymorphism of the T-cell receptor alpha chain constant gene upstream regulatory region (TCRCα -560 C/T) correlates with the proteinuria symptom of Chinese Han IgAN patients[6].Given the epidemiological variation of IgAN, we explored the correlation between TCRCα-560 C/T polymorphism and the clinical presentation of Chinese Uygur IgAN patients.

Methods

Patient

A total of 300 Uygur patients (178 males accounts for 59.33% and 122 females accounts for 40.67%) ageed between 17 and68 years old were enrolled for the study between 2006 and 2010. All patients had renal biopsy confirmed IgAN with a disease history of 3 to 31 months. Patients who were diagnosed with acute post-streptococcal glomerulonephritis (*APSGN*), non-IgA mesangial proliferative glomerulonephritis, Schonlein-Henoch purpura nephritis, systemic lupus erythematosus, Hepatitis-B-associated glomerulonephritis (HBGN), thin basement membrane nephropathy and other diseases were excluded from the study. 600 healthy Uygur volunteers were enrolled as control subjects, of whom 324 were males (54%) and 276 were females (46%) with the age from 22-59 years old.

Table 1. Correlation between TCRCα -560 C/T Polymorphism and the Clinical Presentation of IgA Nephropathy patients (n, $\bar{x}\pm s$)

	TT（n=104）	CT (n=148）	CC (n=48）
Systolic pressure (mHg）	117±15.0	121±13.5	120±16.3
Diastolic pressure (mmHg）	75.6±11.3	73.2±10.5	73.4±9.6
Proteinuria (g/24h）	2.30±1.20	2.10±1.30	2.40±1.10
Serum creatinine (umol/L）	104.23±70.61	112±82.31	98.23±79.23
Paroxysmal gross hematuria (%）	61.54%(64/104)	59.46%(88/148)	66.67%(32/48)
Microscopic hematuria and Proteinuria (%）	69.23%(72/104)	94.59%(140/148)*	66.67%(32/48)

* : The difference between CT and the other two genotypes is statistical significant. χ^2=33.978, P <0.05.

None of the control subjects showed any symptoms of infection, fever, injury or other stress responses within four weeks of enrollment. This research was approved by IRB in People's Hospital in Xinjiang Uygur Autonomous Region and written informed consent was obtained from each participant before data collection. Clinical information from the patients, including age, gender, systolic/diastolic blood pressure, gross hematuria history, serum creatinine, estimated glomerular filtration rate (eGFR), urinary protein excretion and time-average proteinuria were collected.

Specimen Collection

Patients and control subjects were advised to avoid strenuous exercise three days before specimen collection. On the day of collection, 10ml venous blood was drawn from fasting patients and control subjects for genotyping analysis.

Genotyping

Patients and control subjects were genotyped by a polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) assay. Briefly, genomic DNA was isolated from whole blood using UNIQ-10 blood genomic Extraction Kit (Songon) according to the manufacturer's instructions. PCR was used to amplify the upstream regulatory region of the TCRCα gene which contains C/T single nucleotide polymorphism. Amplification primers include: forward primer 5′-AGGGGCTGATT TCTTTGGTT-3′ and reverse primer 5′-TCGT GATGGACTGGGACTCA-3'. The PCR products were digested with HaeII (New England Biolabs) at 37°C overnight. Fragments were separated by electrophoresis on agarose gels and stained with ethidium bromide.

Statistical Analysis

We adopted a direct count method to calculate the frequency of TCRCα-560 C /T genotype and allele. Tested by the Hardy Weinberg Equilibrium, all genotype frequencies has reached genetic equilibrium and wererepresentative of their groups. Variables were shown as mean±SD. The Chi-square test was used for group comparisons. Statistical analyses were performed by SPSS version 13.0 (SPSS, Chicago, IL) and two-sided p-values less than 0.05 were considered significant.

Results

Determination of TCRCα-560C/T genotype

The PCR product spanning the upstream regulatory region of the TCRCα gene and its translation initiation site was 1027bp in length. Due to the presence of SNP, it could generate different digestion patterns based on the genotype. The TT genotype yielded one fragment of 1027bp; CC gave rise to two fragments of 622 and 405bp, respectively, and CT produces three fragments of 1027, 622 and 405bp, respectively.

Genotype frequency comparison between IgAN patients and control subjects

TT, TC, CC genotype frequencies of the TCRCα-560 C/T locus in Uygur IgAN patients were 34.70% (104/300), 49.30% (148/300) and 16.0% (48/300), respectively, and the corresponding genotype frequencies in control subjects were 36.70% (220/600), 46.0% (276/600) and 17.3% (104/600). There was no statistical difference in genotype frequency between Uygur IgAN patients and control subjects（χ^2 =0.904, P =0.636).

Clinical classification of patients

Based on the clinical presentation, 300 Uygur IgAN patients were first divided into recurrent gross hematuria (the most common) and asymptomatic abnormal urine test groups. The asymptomatic abnormal urine test group was further divided into two types: type A included asymptomatic microscopic hematuria without proteinuria and type B included asymptomatic microscopic hematuria and proteinuria.

Table 2. Association Analysis on Gene Polymorphism of TCRCα-560 C /T and IgAN Clinical Data *(n ,%)*

Type	Recurrent gross hematuria	Asymptomatic abnormal urine test (Type A)	Asymptomatic abnormal urine test (Type B)	Proteinuria (Type A)	Proteinuria (Type B)	Hypert-ension	Accurate renal failure	Total
TT (n=104)	34(32.69%)	24(23.08%)	12(11.54%)	4(3.85%)	4(3.85%)	22(21.15%)	4(3.85%)	104
CT (n=148)	68(45.95%)	20(13.51%)	12(8.11%)	12(8.11%)	6(4.05%)	24(16.22%)	6(4.05%)	148
CC (n=48)	24(50.00%)	0	10(20.83%)	2(4.17%)	2(4.17%)	4(8.33%)	6(12.50%)	48
Total	126(42.00%)	44(14.67%)	34(11.33%)	18(6.00%)	12(4.00%)	50(16.67%)	16(5.33%)	300

Analysis of the correlation between TCRCα-560C/T gene polymorphism and clinical presentation of IgAN patients

There was no statistical difference (P >0.05) in the hematuria incidence rate, blood pressure level, proteinuria and serum creatinine between Uygur IgAN patients with the three different genotypes. However, patients with the CT genotype had a significantly higher (P <0.05) incidence rate of Microscopic hematuria and Proteinuria than CC and TT patients (Table 1.). Moreover, in Chinese Uygur IgA patients, the TT genotype correlated with recurrent gross hematuria and asymptomatic abnormal urine test (type A), the CT genotype correlated with continuous microscopic hematuria and asymptomatic abnormal urine test (type B) and the CC genotype correlated with asymptomatic abnormal urine test (type B), Table 2.

Discussion

Immunoglobulin A nephropathy is characterized by a highly variable clinical presentation and course with an unpredictable ultimate outcome. Despite the fact that the pathogenetic mechanisms underlying IgAN remain elusive, considerable clinical and experimental evidence has suggested that IgAN is an autoimmune disease partly caused by an abnormal O-glycosylation of the IgA1 molecule, genetic factors, environmental stimuli and various inflammatory mediators[7-9,12]. Genome-wide screening identified several candidate gene loci which showed linkage to familial IgAN, including 6q22-q23, 2q36, 4q26-q31 and 17q12-q22[13]. Gene polymorphism studies of the human leukocyte antigen (HLA), the renin-angiotensin-aldosterone system and selectin gene clusters, suggests a certain degree of genetic predisposition in IgAN patients.

In IgAN patients, there is increased IgA-specific T and B cell activity and decreased IgA-specific suppressor T cell activity[9]. The T cell receptor (TCR) is present on the surface of T cells and is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules and T cell activation. Together with HLA antigens, complement factors, immunoglobulins and cytokines, the TCR is considered as one of the candidate genes involved in immune regulation in IgAN patients[11]. The human TCR alpha chain locus is located on chromosome 14q11-14q12 and consists of three gene segments: variable (V), joining (J) and constant (C)[10]. Recently, polymorphisms of the TCR genes have been identified and the polymorphism in TCR constant beta chain and TCR constant alpha chain have been linked to the progression of IgA nephropathy in Japanese and Chinese Han people, respectively[14].

In this study, we investigated whether a C/T single nucleotide polymorphism 560bp upstream of the TSS of TCRα constant region was important for the clinical presentation of Chinese Uygur IgAN patients. Our results showed that there was no statistical difference in genotype distribution between Uygur IgAN patients and control subjects, indicating that TCRCα -560 C/T polymorphism was not related to susceptibility to IgAN. In addition, consistent with a previous report, in which the TCRCα -560 CT genotype was associated with high proteinuria incidence rate in Chinese Han IgAN patients, we found that the incidence rate of proteinuria varied significantly in Chinese Uygur IgA patients, with lowest rate in TT and highest in CT genotypes. Moreover, in Chinese Uygur IgA patients, the TT genotype correlated with recurrent gross hematuria and asymptomatic abnormal urine test (type A), the CT genotype correlated with intermittent microscopic hematuria and asymptomatic abnormal urine test (type B) and the CC genotype correlated with asymptomatic abnormal urine test (type B).

Currently, TCRCα -560 SNP has not been characterized at molecular or biochemical levels.

We speculate that since the SNP is located in close proximity to TSS, it may cause transcription alteration of the TCRα constant region, which may lead to variations in the structure or regulatory elements of the TCR and ultimately to IgAN. In addition to TCRCα -560 SNP, a few other SNPs in TCR have been identified. It is not clear whether TCRCα -560 SNP is ethnic group specific or universal and whether the presence of TCRCα -560 SNP coincides with other SNPs. The subjects in this study were Uygur IgAN patients; the correlation between TCRCα -560 genotype and clinical presentation had a few differences with results reported from Han IgAN patients. Fewer samples were collected in this study; thus further studies are necessary to explore these details and to validate the use of TCRCα -560 SNP and other SNPs as prognostic factors of IgAN.

In conclusion, TCRCα- 560 C/T polymorphism in Chinese Uygur patients in Xinjiang may correlate with recurrent gross hematuria, intermittent microscopic hematuria and proteinuria. However, due to the sporadic nature and epidemiological variation of IgAN, these findings need to be further confirmed in different patient populations.

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