Association of −394C>G and −420C>G polymorphisms in the RETN gene with T2DM and CHD and a new potential SNP might be exist in exon 3 of RETN gene in Chinese

Shuguang Chi · Cailian Lan · Sizhong Zhang · Hekun Liu · Xizhen Wang · Yuanzhong Chen · Xuexiang Chen · Suyun Chen · Wei Zhang

Abstract Genetic variations of the human RETN gene are associated with metabolic phenotypes, including obesity, insulin sensitivity, diabetes, and coronary heart disease (CHD). There are few studies of two gene variants, −394C>G and −420C>G, in Chinese population. This study investigated the distribution of RETN gene, single nucleotide polymorphisms (SNPs), in Chinese Han population and the association of the polymorphisms with type 2 diabetes mellitus (T2DM) and CHD. In a population-based, case–control genetic association study, a total of 961 subjects were recruited from the community, including 318 T2DM patients, 273 CHD patients, and 370 unrelated healthy control individuals. Serum lipid levels were detected. Two SNPs of RETN gene, −394C>G and −420C>G, were genotyped by PCR-RFLP. Unknown Polymorphisms were screened with the technique of denaturing high performance liquid chromatography (DHPLC). The frequencies of RETN −394G allele in T2DM group, CHD group, and control group were 0.3066, 0.3555, and 0.3481, respectively, which are met with the Hardy–Weinberg equilibrium. There is a significant difference of the comparison of sex in T2DM group of RETN gene SNP-394C>G ($P < 0.05$). Compared with controls, there was no significant difference in the distribution of genotypes and allele frequencies of −394C>G polymorphic site in T2DM patients and CHD patients, respectively. No direct association was found between the −394C>G polymorphism and T2DM or CHD. The frequencies of RETN −420G allele in T2DM group, CHD group, and control group were 0.4009, 0.3725, and 0.3859, respectively, which are met with the Hardy–Weinberg equilibrium. The frequencies of RETN −420G allele in T2DM groups and control groups of Chinese population are significantly different from those in European population (0.40 vs. 0.27, 0.39 vs. 0.26) ($P < 0.01$). Compared with controls, there was no significant difference in distribution of genotypes and allele frequencies of −420C>G polymorphic site in T2DM patients and CHD patients, respectively. No direct association was found between the −420C>G polymorphism and T2DM or CHD. In addition, we found new potential SNP +593G>C in exon 3 of RETN gene using DHPLC. The RETN gene exhibits sex and ethnic differences. +593G>C of RETN gene might be a new potential SNP in exon 3 of RETN gene. Association between SNP −394C>G and −420C>G of RETN gene with T2DM and CHD in Chinese needs more exploration.

S. Chi · C. Lan · H. Liu (✉) · X. Wang · X. Chen · S. Chen
Department of Cell Biology and Genetics, and Center of Neuroscience, Fujian Medical University, 88 Jiaotong Road, Fuzhou 350004, China
e-mail: fjlhk@163.com

S. Zhang · W. Zhang
Department of Medical Genetics, West China Hospital, Sichuan University, and Division of Human Morbid Genomics, State Key Laboratory of Biotherapy of Human Diseases, Chengdu, China

Y. Chen
Department of Hematology, Union Hospital, Fujian Medical University, Fuzhou, China

Introduction

In 2001, Steppan et al. [1] reported that they discovered a cystein-rich polypeptide hormone, termed as RETN, which was secreted by adipocytes in response to hypoglycemic drugs like thiazolidinediones (TZDs). The human resistin gene,

namely RETN gene, is located in 19p13.3 with a whole length of 1,369 bp, including four exons and three introns [2].

The RETN gene is a potential candidate for the etiology of insulin resistance and type 2 diabetes and has been implicated as the molecular link between type 2 diabetes and obesity [1]. Burnett et al. [3, 4] found that patients with premature coronary artery disease have higher plasma RETN levels compared to individuals with angiographically normal coronary arteries as well as prevalent CHD associated with higher resistin levels. There are several SNPs which are distinguished by different countries and populations located in the RETN gene. Meanwhile, the association of the two RETN genes SNPs, −394C>G and −420C>G, with T2DM and CHD in Chinese has not been known so far. We detected the distributions of the two RETN gene SNPs, −394C>G and −420C>G, in Chinese Han population and the relation of this polymorphism to T2DM and CHD by PCR-RFLP.

In addition, we took advantage of a highly sensitive and specific automated denaturing high performance liquid chromatography (DHPLC) technique to detect fragments that may harbor mutations prior to sequencing.

Subjects and methods

Subjects

Three hundred and seventy control subjects were unrelated age- and gender-matched individuals (Han people), selected via health screening at the Outpatient Department of West China Hospital of Sichuan University (224 males, 146 females, and age 58.8 ± 9.2 years old), and with exclusion of coronary vascular diseases and diabetes mellitus. None of the subjects were taking any medication. Three hundred and eighteen T2DM patients (179 males, 139 females, and age 58.2 ± 11.4 years old) diagnosed by the WHO criteria for diabetes mellitus from Department of Endocrinology of West China Hospital, Sichuan University were selected. 273 CHD patients (177 males, 96 females, age 60.4 ± 8.6 years old) with significant coronary stenosis by cardiovascular angiography (according to stenos ≥50%, at least one coronary artery) from the Department of Cardiology of West China Hospital, Sichuan University were selected. All the subjects mentioned above were unrelated Han people. The informed consent forms were obtained from all subjects studied. The study was approved by the Internal Ethical Review Board of West China Hospital.

Plasma lipid measurements

The blood sample after 12-h fasting was collected in a tube for measuring lipid. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) of all subjects were measured by Automatic Biochemistry Analyzer (Olympus 5400) and GmbH diagnostic kit (Roche).

PCR amplification

Genomic DNA was extracted from peripheral blood by salting out method, stored at 4°C. PCR primers were designed according to the reference [5]. For SNP −394C>G, a 561 bp sequence of the RETN gene was amplified by PCR in a DNA Thermal Cycler (PE 9600), using the upstream primer 5′-GCACCATGCCTAGCAAG AG-3′ and the downstream primer 5′-GCTGAAAG AGG GAACCAAGAG-3′. For SNP −420C>G, a 533 bp sequence of the RETN gene was amplified by PCR in a DNA Thermal Cycler (PE 9600), using the upstream primer 5′-TGT CATTCTCACCCAGAGACA-3′ and the downstream primer 5′-TGGGCTCAGCTAACCAAATC-3′. DNA templates were pre-denatured at 94°C for 5 min and then each PCR was subjected to 35 cycles with a temperature cycle consisting of 30 s of denaturation at 94°C, 30 s of annealing at 59°C, and 30 s of extension at 72°C, the last step was 10 min of extension at 72°C.

SNP genotyping

The PCR products (10 μl) of SNP −394C>G were digested with five units of Eam1104I at 37°C overnight, and the one of SNP −420C>G was digested with five units of BpiI at 37°C overnight. The fragments were separated by electrophoresis on 3% agarose gel. The DNA fragments were visualized by the gel analytic system (BIO-RAD Gel Doc 2000) and genotypes were determined. For SNP −394C>G, fragments of 561 bp for the CC wild homozygote (absence of Eam1104I cutting site) and of 150 and 411 bp for the GG mutant homozygote (presence of Eam1104I cutting site) were produced. The heterozygote CG includes three fragments of 150, 411, and 561 bp. For SNP −420C>G, fragments of 533 bp for the GG wild homozygote (absence of BpiI cutting site) and of 204 and 329 bp for the CC mutant homozygote (presence of BpiI cutting site) were produced. The heterozygote CG includes three fragments of 204, 329, and 533 bp.

Screening the new potential polymorphisms using DHPLC

Based on the sequence NT_011145, we designed seven pairs of primers of RETN gene by Primer 5.0. The amplified fragments include RETN gene's exons and introns. The PCR fragments were applied to a preheated reversed-phase column. An elution gradient was generated

by mixing buffer A (0.1 mmol/l EDTA) and buffer B (0.1 mol/l TEAA, 0.1 mmol/l EDTA, 25% acetonitrile). The predicted optimal column temperatures and elution gradient for the DHPLC analysis of each PCR amplicon were verified by confirming that the elution profile of each of the PCR amplicons generated from wild-type genomic DNA had a sharp and solitary peak. According to the different fragments' detecting temperature and lasting time, the automated program will control the gradient concentration of mixing buffers A and B in 1.5 ml/min velocity of flow to screen the PCR fragments sequence. Inspection of the DNA in a 260-nm wavelength will be transmitted into the DHPLC chromatogram.

Statistical analysis

Statistical analysis was performed with the SPSS 11.0 statistical software package (SPSS Inc., Chicago, IL). Observed frequencies in each of the three groups (T2DM, CHD, and control) were compared to the expected frequencies (Hardy–Weinberg equilibrium) by χ^2 test of HWE software. The distribution of genotypes and allele frequencies in T2DM, CHD, and control group was analyzed by χ^2 test. The difference of lipid levels in different genotypes of T2DM, CHD, and control group was evaluated by the one-way analysis of variance (ANOVA) test and LSD test. The association of RETN gene SNPs with T2DM and CHD was analyzed by binary logistic regression. The comparison of sex in T2DM was analyzed by χ^2 test.

Results

Genotypes and allele distributions of SNP −394C>G and −420C>G polymorphic sites in study population.

Table 1 shows the genotypes and allele distributions of SNP −394C>G and −420C>G polymorphic sites in the study population. The genotype distribution in each group was met with Hardy–Weinberg equilibrium. There was no significant difference of the genotypes and allele distributions in T2DM and CHD, compared with the control group, respectively ($P > 0.05$).

Comparison of sex in T2DM of RETN gene SNP −394C>G

Table 2 shows the genotypes distributions of SNP −394C>G polymorphic sites in T2DM group. It exhibits significant differences of the genotype distributions in female and male groups with T2DM ($P < 0.05$). There are obviously more women with the CG genotype than men in T2DM group.

Association of RETN gene SNP −394C>G and −420C>G with T2DM and CHD in study population

Table 3 shows the odds ratio (OR) and 95% confidence interval (CI) of RETN gene SNP −394C>G and −420C>G to T2DM and CHD. It shows that SNP −394C>G and −420C>G have no direct association with T2DM and CHD, respectively.

Comparison of plasma lipids levels in three genotypes in study population

Table 4 displays the comparison of lipid levels of three genotypes in three groups for RETN gene SNP −394C>G and −420C>G. No significant difference of lipid levels among three genotypes was found in both T2DM and CHD patients for two SNP sites ($P > 0.05$).

DHPLC chromatograms

Table 5 shows the PCR primers and the amplified fragments. Table 6 shows the DHPLC analysis conditions

Table 1 Genotype and allele frequencies in subjects with T2DM, CHD and controls

	Case (n)	Genotype frequencies			P-value[a]	Allele frequencies		P-value[a]
		CC (%)	GC (%)	GG (%)		C (%)	G (%)	
SNP-394								
T2DM	318	48.7	41.2	10.1	0.198	69	31	0.120
CHD	273	40.4	48.0	11.6	0.920	64	36	0.723
Controls	370	41.9	46.7	11.5	–	65	35	–
		GG (%)	GC (%)	CC (%)		G (%)	C (%)	
SNP-420								
T2DM	318	15.0	50.2	34.8	0.761	40	60	0.580
CHD	273	13.7	47.1	39.2	0.864	37	63	0.601
Controls	370	14.5	48.1	37.3	–	39	61	–

[a] Compared with controls

Table 2 The comparison of sex in T2DM of RETN gene SNP −394C>G

Genotype (n)	Case	Sex	
		Male (100%/n)	Female (100%/n)
CC	155	0.606(94)	0.394(61)
CG	131	0.481(63)	0.519(68)
GG	32	0.688(22)	0.312(10)
χ^2 value			
CC versus CG versus GG	6.793		
CC versus GG	0.740		
CC versus CG	4.519		
CG versus GG	4.398		
P-value			
CC versus CG versus GG	0.033*		
CC versus GG	0.390		
CC versus CG	0.034*		
CG versus GG	0.036*		

* $P < 0.05$

Table 3 Association of RETN gene SNP −394C>G with T2DM and CHD

	Genotype	χ^2	P	OR	95% CI
SNP −394C>G					
With T2DM	GG and CC	1.090	0.297	1.341	0.773–2.325
	GG and CC + GC	0.324	0.569	0.859	0.509–1.449
	GG and GC	0.001	0.980	1.007	0.580–1.748
With CHD	GG and CC	0.010	0.922	1.032	0.546–1.951
	GG and CC + GC	0.000	0.991	0.996	0.548–1.811
	GG and GC	0.004	0.948	0.979	0.523–1.833
SNP −420C>G					
With T2DM	GG and CC	0.262	0.609	0.863	0.490–1.518
	GG and CC + GC	0.019	0.890	1.037	0.622–1.729
	GG and GC	0.031	0.859	0.952	0.553–1.639
With CHD	GG and CC	0.132	0.716	0.889	0.472–1.675
	GG and CC + GC	0.019	0.889	0.960	0.543–1.698
	GG and GC	0.023	0.881	0.954	0.514–1.768

(temperature and concentration). Homozygote genomic DNA has a sharp and solitary peak contrast to heterozygote having double or anomalous peak (Figs. 1, 2).

PCR products sequence map

Based on the initial detection by DHPLC, five mutational samples were selected to be sequenced directly. And the results showed that 100% genotypes were consistent with their previous ones (Figs. 3, 4).

Discussion

This is, to our knowledge, the first study to analyze and identify an association in two loci, the −394C>G and SNP −420C>G polymorphisms, with T2DM, respectively, and find new potential SNP in exon 3 of RETN gene using DHPLC in West China. A search for genes that are downregulated by thiazolidinediones (TZDs) in mouse adipocytes led to the discovery of an adipose-specific secreted protein called resistin. Since Steppan et al. [1] reported that resistin gene, RETN, was discovered in 2001, it has attracted many scientists to research RETN gene. Resistin is a newly described circulating protein with no homology to any known hormone, cytokine, or other intercellular signaling molecule. It is secreted specifically by adipocytes and has actions that antagonize insulin action [6]. At the amino acid level, the human resistin proteins and that of the mouse exhibit 59% identity [2]. Reilly et al. [7] found that plasma resistin levels were associated with markers of inflammation, but not insulin resistance, both in SIRCA, a study of asymptomatic nondiabetic subjects, and in a type 2 diabetic sample. Furthermore, they found that resistin levels were significantly associated with coronary atherosclerosis in SIRCA even after control for multiple established risk factors and the presence of the metabolic syndrome.

The convergence of insulin resistance and inflammation in the pathogenesis of atherosclerotic CVD have been recognized over the last decade [8–11]. In Chinese Han population of Sichuan province, we analyzed the genotypes and allele frequencies of RETN gene SNP −394C>G in 318 T2DM patients, 273 CHD patients, and 370 unrelated healthy control individuals. There was no significant difference in the distribution of genotypes and allele frequencies of −394C>G polymorphic site in T2DM patients and CHD patients, respectively. In other studies, Wang et al. [5] reported in Caucasians population, the frequencies of minor allele G of RETN gene SNP −394C>G in T2DM group and control group were 0.36 and 0.29, respectively, in agreement with our findings (Table 1) in Chinese Han population. Besides, our result shows a significant difference in the comparison between male individuals and female individuals of T2DM group of RETN gene SNP −394C>G ($P < 0.05$). This findings suggest that the sex may play an important role in RETN gene SNP −394C>G in type 2 diabetes. As is known, T2DM is a complex metabolic disorder in which endogenous sex hormones may contribute to sex-dependent etiologies. We hypothesized that genetic variants related to T2DM might differ between men and women. We thus performed an association study to identify gene polymorphisms associated with T2DM in men and women separately. Our result revealed that the RETN gene −394

Table 4 The comparison of plasma lipids levels in genotypes of T2DM, CHD and the control group

Group and lipid (mmol/l)	Genotype			P-value		
	GG	GC	CC	GG versus GC	GG versus CC	GC versus CC
SNP −394C>G						
T2DM						
TG	1.72 ± 0.819	2.06 ± 1.847	2.08 ± 1.781	0.322	0.294	0.943
TC	4.42 ± 1.400	4.80 ± 1.607	4.68 ± 1.245	0.173	0.351	0.459
HDL-C	1.30 ± 0.357	1.37 ± 0.450	1.26 ± 0.400	0.391	0.663	0.333
LDL-C	2.74 ± 0.982	2.83 ± 1.022	2.85 ± 0.969	0.635	0.563	0.874
CHD						
TG	2.67 ± 1.320	2.81 ± 1.567	2.82 ± 1.283	0.694	0.683	0.972
TC	5.14 ± 1.024	5.18 ± 0.842	5.15 ± 0.891	0.841	0.971	0.800
HDL-C	1.10 ± 0.195	1.11 ± 0.234	1.12 ± 0.250	0.924	0.837	0.861
LDL-C	3.19 ± 0.998	3.28 ± 0.784	3.06 ± 1.366	0.679	0.556	0.119
Controls						
TG	1.97 ± 1.075	1.95 ± 1.139	1.83 ± 0.949	0.933	0.521	0.383
TC	3.45 ± 1.486	3.40 ± 1.389	3.21 ± 1.398	0.896	0.499	0.392
HDL-C	1.60 ± 0.808	1.47 ± 0.530	1.73 ± 1.120	0.465	0.446	0.221
LDL-C	2.18 ± 0.570	2.14 ± 0.658	2.13 ± 0.781	0.843	0.803	0.931
SNP −420C>G						
T2DM						
TG	1.75 ± 1.135	1.90 ± 1.626	1.68 ± 0.931	0.547	0.821	0.263
TC	4.46 ± 1.315	4.62 ± 1.259	4.57 ± 1.323	0.538	0.685	0.799
HDL-C	1.37 ± 0.390	1.32 ± 0.440	1.24 ± 0.402	0.479	0.124	0.226
LDL-C	2.74 ± 0.955	2.80 ± 1.020	2.87 ± 1.044	0.774	0.528	0.617
CHD						
TG	3.17 ± 1.435	2.80 ± 1.570	2.89 ± 1.328	0.312	0.451	0.732
TC	5.38 ± 0.926	5.15 ± 0.842	5.16 ± 0.888	0.174	0.212	0.924
HDL-C	1.09 ± 0.137	1.11 ± 0.330	1.12 ± 0.204	0.857	0.723	0.796
LDL-C	3.15 ± 0.953	3.19 ± 0.782	3.10 ± 1.481	0.863	0.835	0.595
Controls						
TG	1.95 ± 1.097	1.95 ± 1.097	1.76 ± 1.094	0.994	0.382	0.219
TC	3.11 ± 1.542	3.43 ± 1.588	3.30 ± 1.575	0.408	0.633	0.630
HDL-C	1.67 ± 0.942	1.59 ± 0.673	1.85 ± 1.258	0.679	0.348	0.058
LDL-C	2.20 ± 0.544	2.16 ± 0.654	2.15 ± 0.707	0.813	0.735	0.877

CG heterozygote was significantly associated with the prevalence of T2DM in women compared to the CC and GG homozygotes. That is to say, women with the CG genotype are more apt to develop T2DM. Further studies are needed to address the mechanism of RETN gene SNP −394C>G in humans.

We also analyzed the genotype and allele frequencies of RETN gene SNP −420C>G in 318 T2DM patients, 273 CHD patients and 370 unrelated healthy control individuals. Compared with controls, there was no significant difference in the distribution of genotype and allele frequencies of −420C>G polymorphic site in T2DM patients and CHD patients, respectively. However, our result is so close to the one of Cho [12] found in Korea. In addition,

the frequencies of G allele in T2DM groups and control groups of Chinese population are significantly different from those in European population [13] (0.40 vs. 0.27, 0.39 vs. 0.26) ($P < 0.01$). We can draw a conclusion that the distributions of −420C>G of RETN gene exhibit ethnic difference.
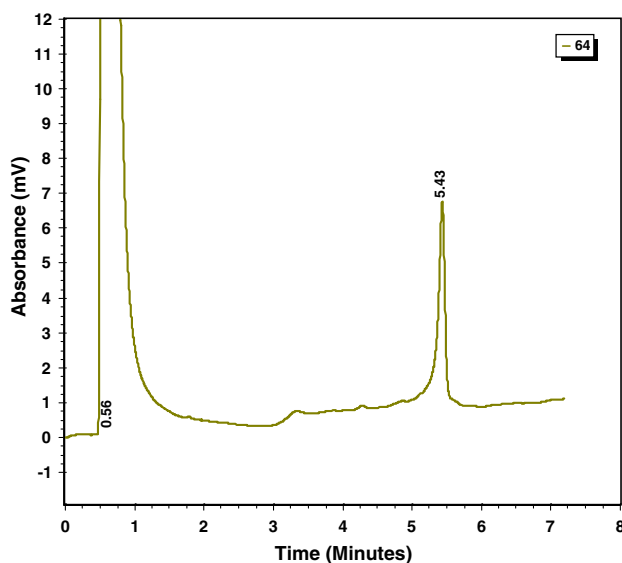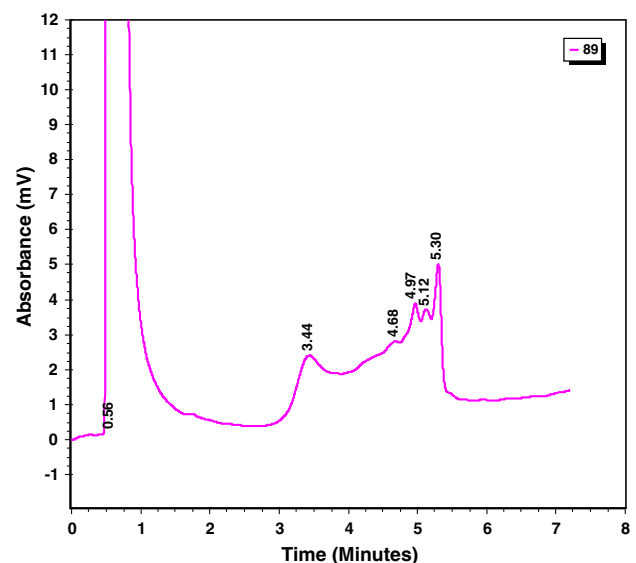
Resistin was initially suggested to be a link between obesity and insulin resistance in rodents [1, 6]. Evidence to confirm this in human has been raised by several study teams recently [1, 5, 12, 14–20]. Moreover, resistin expression was found to be abundant in monocytes/macrophages [19–21], which play an important role in atherosclerosis [22]. These cells infiltrate arteries and initiate or promote atherogenesis by secreting various pro-

Table 5 PCR Primer sequences and the lengths of the amplified fragments for RETN gene

Primer	Location	Primer sequence (5′→3′)	Length of fragments (bp)
1	Exon 1	F: CAGGGACTTATTAGCCAAGC	286
		R: AAGAGACCCACAGCTGGATC	
2	Exon 2	F: CAGATCCTACTCCCTCCATG	288
		R: TTGGAGTCAGGTCTGTGCCA	
3	Exon 3	F: GACCGTTTGGTCTCACAGCT	198
		R: AAGATCCTAGGGGAGTAGAGGCT	
4	Exon 4a	F: TCAGCCTCCCAGCTCAGAGT	261
		R: CTCCAGGTTTATTTCCAGCTC	
5	Exon 4b	F: GGAGGCGGCTCCAGGTCC	203
		R: GCAGTAGAAAGTCGCGGTGT	
6	−265 → −5	F: CTCTGCTTGTCTACCTGTTCC	260
		R: TCCTGCACCGCAGCTCTTTC	
7	−516 → −181	F: TGAATGTGGTATGTCATTCTCAC	335
		R: CTTCATGTCCAGAGACTGGTC	

Table 6 The DHPLC analysis conditions (temperature and concentration) for screening the new potential polymorphisms of RETN gene

Fragments	Temp (°C)	Concentration (%)	
		Buffer A	Buffer B
Exon 1	62.3	42.0	58.0
Exon 2	63.7	44.0	56.0
Exon 3	62.0	48.0	52.0
Exon 4a	61.3	43.0	57.0
Exon 4b	60.7	44.0	56.0
−265 → −5	64.2	43.0	57.0
−516 → −181	62.7	47.0	53.0

Fig. 2 The heterozygote genomic DNA of exon 3 of RETN gene shows the *double* and *anomalous peaks*

inflammatory cytokines [10]. Since resistin is expressed mainly in inflammatory cells [20, 21], regulated by and regulating inflammatory cytokines [23, 24], and increased in serum in subclinical inflammatory conditions [7, 25–30], it may be a link between inflammation and atherosclerosis. However, we cannot exclude the possibility that resistin might also be a contributory factor to insulin resistance or metabolic syndrome. The association between resistin and atherosclerosis remains controversial. Steppan et al. [18] also found resistin circulates in the mouse, with increased levels in obesity, and has effects on glucose homeostasis that oppose those of insulin. Resistin is a potential link among TZDs, obesity and insulin resistance in the mouse. Thus, resistin is a possible major risk factor for type 2

Fig. 1 The homozygote genomic DNA of exon 1 of RETN gene shows the *sharp* and *solitary peak*

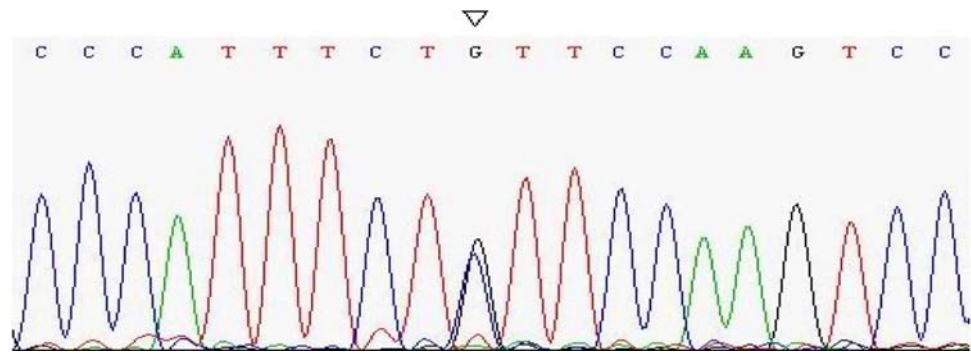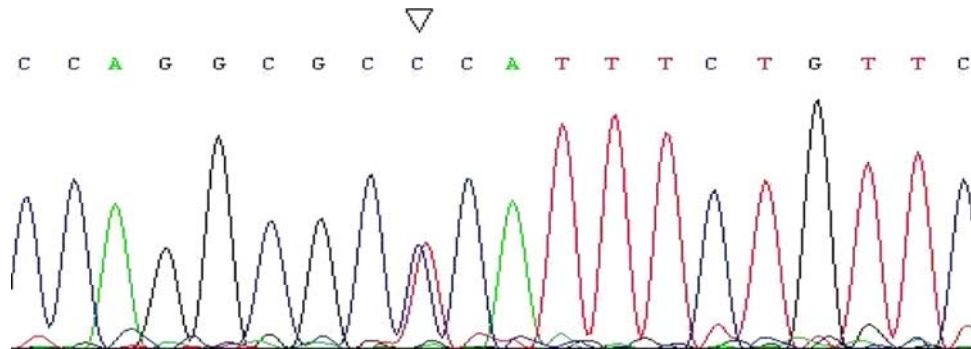**Fig. 3** The new potential SNP +593G>C in exon 3 of RETN gene

Fig. 4 The new potential SNP +585C>T in exon 3 of resistin gene. This SNP is also reported by Wang et al. [5] in Caucasians population

diabetes mellitus. Recently, high concentrations of resistin were shown to induce vascular endothelial dysfunction and vascular smooth muscle cell proliferation. Resistin secreted from macrophages may contribute to atherogenesis by virtue of its effects on vascular endothelial cells and smooth muscle cells in humans [31]. Patients diagnosed with premature coronary artery disease (PCAD) were found to have higher serum levels of resistin than normal controls. Resistin protein is present in both murine and human atherosclerotic lesions, and mRNA levels progressively increase in the aortas of mice developing atherosclerosis. Resistin induced increases in monocyte chemoattractant protein (MCP)-1 and soluble vascular cell adhesion molecule (sVCAM)-1 protein expression in murine vascular endothelial cells, suggesting a possible mechanism by which resistin might contribute to atherogenesis. PCAD patients exhibited increased serum levels of resistin when compared to controls [3]. Plasma resistin levels are correlated with markers of inflammation and are predictive of coronary atherosclerosis in humans, independent of plasma C-reactive protein (CRP). Resistin may represent a novel link among metabolic signals, inflammation, and atherosclerosis [7]. Satoh et al. [32] found that chronic hyper-resistinemia leads to whole-body insulin resistance involving impaired insulin signaling in skeletal muscle, liver, and adipose tissue, resulting in glucose intolerance, hyperinsulinemia, and hypertriglyceridemia.

Mutation analysis by DHPLC involves subjecting PCR products to ion-pair reversed-phase liquid chromatography in a column containing alkylated nonporous particles. At the proper temperature, the heteroduplexes formed in PCR samples with internal sequence variations have a lower column retention time relative to their homoduplex counterparts. For analysis of autosomal recessive and X-linked recessive disorders, genomic DNA from normal individuals is added to each well so that a heteroduplex is formed if the patients are homozygous or hemizygous for the mutant allele. The major advantages of this method include rapid analysis and automated instrumentation [33]. In our study, two mutant alleles, +593G>C and +585C>T in exon 3 of RETN gene were checked out using DHPLC and confirmed by direct sequencing. Since one of the mutation has been reported by Wang et al. [5] already, We found new potential SNP +593G>C in exon 3 of RETN gene. Further experiments and large population sample investigations are needed to fully confirm this result.

In summary, our data suggest that no direct association was found in the two loci, −394C>G and −420C>G, polymorphism with T2DM or CHD in Chinese Han population. However, there is a significant gender difference in the association of gene polymorphisms with T2DM. Ethic difference was also found in the RETN gene. In addition, new potential SNP +593G>C in exon 3 of RETN gene may exist according to our detection using DHPLC. Further functional studies are needed to identify the actual biological role of RETN gene polymorphisms.

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