Int J Clin Exp Pathol 2015;8(7):8358-8366
www.ijcep.com /ISSN:1936-2625/IJCEP0003427

Original Article

Relationship between the single nucleotide polymorphisms of β_2-adrenergic receptor 5'-regulatory region and essential hypertension in Chinese Kazakh ethnic minority group

Weijuan Cai[1*], Liang Yin[2*], Jiang Cheng[3], Shaoliang Wang[1], Yu Wei[1], Wenjiang Cao[1], Jiang Cheng[1]

*Departments of [1]Clinical Laboratory, [2]Endocrinology and Metabolism, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi 832002, Xinjiang, P. R. China; [3]Department of Emergency, Burqin County People's Hospital, Burqin 836600, Xinjiang, P. R. China. *Equal contributors.*

Received October 27, 2014; Accepted June 29, 2015; Epub July 1, 2015; Published July 15, 2015

Abstract: Objective: To study the correlation of β_2-AR gene 5'-regulatory region SNPs and essential hypertension (EH) in Chinese Kazakh ethnic minority group. Methods: The Sequenom MassArray® SNP detection technology was used to detect β_2-AR gene 5'-regulatory region SNPs in 150 Xinjiang Kazakh EH patients and 150 controls. Biochemical analyzer was used to detect lipid and other related biochemical parameters. SHEsis and other software were used to analyze linkage disequilibrium and haplotype. Results: Six loci rs205304 (-1023G/A), rs17108803 (-893T/G), rs12654778 (-654G/A), rs11168070 (-468C/G), rs11959427 (-367C/T) and rs2895795 (-1429T/A) polymorphisms of β_2-AR gene 5'-regulatory region were found in the Xinjiang Kazakh populations. While, there was no significant difference between EH group and NH in genotypes and allele frequency of rs2053044, rs12654778, rs2895795, rs17108803 and rs11959427 ($P>0.05$). However; significant differences were detected of rs11168070 genotypes and allele frequency in two groups ($P<0.05$). Analysis of the linkage disequilibrium and haplotype in Kazakh population, there is a strong linkage disequilibrium of rs11168070, rs2053044, rs2895795 gene polymorphism in the EH group, and rs11168070, rs12654778, rs17108803 gene polymorphism in controls. Frequency of haplotype GTCCAT, GACTGT and ATGCGT in EH group was higher ($P<0.05$), while frequency of ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT and GTCTGT in the EH group was significantly lower than the control ($P<0.05$). Conclusions: β_2-AR gene 5'-regulatory region of rs11168070, rs2053044, rs17108803, rs12654778, rs11959427 and rs2895795 genetic polymorphism exists in Kazakh. Among them, rs11168070 locus genotype and allele frequency distribution in the two groups are significant differences. In six polymorphic loci, there is a strong linkage disequilibrium, which haplotypes GTCCAT, GACTGT, ATGCGT are risk factors of EH, and the ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT, GTCTGT are protective factors.

Keywords: β_2-adrenergic receptor, gene polymorphism, hypertension, Kazakh

Introduction

Essential hypertension (EH) is the most common risk factor for cardiovascular, accounting for over 90% of all cases of high blood pressure which is a common effect of genetic factors and environmental factors polygenic disease [1]. The potential mechanism underlying the origin and development of EH origin remains unclear. Data shows that the heritability of hypertension in about 30% to 50%, and has a significant familial aggregation and ethnic heterogeneity. So searching for EH genetic susceptibility genes has been a focus of the work of the human research.

Xinjiang is located in the hinterland of Eurasia, vast in territory, which is home to 47 ethnic groups, including 13 major nations which have a long history. The Kazakh is the main ethnic minority which dwells north of Xinjiang in northwest China and in which 99% are herdsman, is characterized by a higher prevalence of hypertension and higher BP levels compared to other ethnic populations residing in the same area [2]. Moreover, the hypertension in this population is salt-sensitive and BP decreases significantly after limiting salt intake [2]. Kazakh herdsmen living in remote areas, traffic inconvenience, communicate less, culture level is low, slow economic development, disease prevention awareness is poor, with a low awareness of the incidence of hypertension, low treatment rate and low control rate of hypertension, is a relatively ideal genetic segregation population. Moreover, the results are less affected by genetic heterogeneity, as its object of study, can obviously improve the correct rate of EH diagnosis, genetic analysis of effectiveness and reliability.

Sympathetic-catecholamine system to play in the regulation of cardiac and vascular activity in an important role, both involved in the pathogenesis of hypertension, but also plays an important role in maintaining blood pressure, but these effects are completed by adrenergic receptor (AR) on the heart and vascular. Beta-adrenergic receptor (β-AR) single nucleotide polymorphisms (SNPs) have been associated with hypertension and responses to antihypertensive medications [3, 4] as well as survival following acute coronary syndrome [5], and metabolic outcomes in cardiac patients [6]. Kazakh, Uygur correlation between β_2-AR gene polymorphism and EH are mostly located in the coding region of polymorphic loci studied [7, 8], but their relationship with the β_2-AR gene regulatory region SNPs polymorphic loci has not been explored.

Therefore, the aim of the present study is to evaluated β_2-AR gene 5'-regulatory region SNPs in Xinjiang Kazakh EH patients and controls by MALDI-TOF-MS (Matrix assisted laser desorption ionization time of flight mass spectrometry) technique.

Materials and methods

Epidemiological investigation

The experts in department of preventive medicine and department of epidemiology design the questionnaire. Written informed consent was obtained from all participating patients before enrollment in the study. This study was approved by the institutional ethics committee at the First Affiliated Hospital of Shihezi University School of Medicine and conducted in accordance with the ethical guidelines of the declaration of Helsinki.

Study subjects

EH group: Kazakh 150 patients of EH from Qingshui River township, West Gobi township and Boertonggu township of Xinjiang Shawan. (male 70, female 80), mean age 49.46±9.38 years old. Diagnostic use of "1999 WHO/ISH Hypertension treatment guidelines", not taking antihypertensive medication , sitting systolic blood pressure (SBP) ≥140 mm Hg (18.7 kPa) and/or diastolic blood pressure (DBP) ≥90 mm Hg (12.0 kPa); Exclusion of secondary hypertension, coronary heart disease, kidney disease, diabetes, thyroid disease and other diseases.

NH group: Kazakh normal blood pressure in 150 cases from the Xinjiang Shawan County, (male 64, female 86), mean age 47.96±10.29 years old. Inclusion criteria: systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg, without taking antihypertensive drugs, no family history of hypertension, excluding liver and kidney, thyroid, diabetes history and so on. All samples were from unrelated Xinjiang Kazakh population, and more than five years in a local residence. Before blood all epidemiological study to answer the questionnaire, with the consent of all subjects signed informed consent and informed consent.

General information and measurement of blood pressure

Physical measurements: including height (m), weight (kg), waist circumference (cm) and hip (cm). Blood pressure measurement: all of the subjects were measured blood pressure, using a mercury sphygmomanometer correction (scale in mm Hg), repeat for each measure blood pressure three times and each interval 2 min. Every time blood pressure measurement with the same blood pressure meter, measured by hand, taking the average of three measurements as systolic and diastolic blood pressure. Specimen Collection: Fasting 12~14 h elbow venous blood 4 ml. An effective EDTA anticoagulant, mix stored at -80°C for extraction of genomic DNA using.

DNA extraction

DNA was extracted using whole blood genomic DNA extraction kit (Beijing Tiangen biomass).

Table 1. Basic and clinic characteristics in Chinese Kazakh group w/o essential hypertension

Variable	Grouping	EH (%)	NH (%)	χ^2 value	*P* value
smoking	Yes	88 (64.0)	84 (56.0)	0.218	0.641
	No	62 (36.0)	66 (44.0)		
drinking	Yes	96 (64.0)	90 (60.0)	0.509	0.475
	No	54 (36.0)	60 (40.0)		
Gender	Male (%)	70 (47.0)	64 (41.0)	0.42	0.573
	Female (%)	80 (53.0)	86 (59.0)		
Intake of vegetables	Often (%)	130 (87.0)	138 (92.0)	4.98	0.039
	Occasionally and rarely (%)	20 (13.0)	12 (8.0)		

Note Often: including every day to eat, eat 4-6 days a week; Occasionally: at least 1-3 days a week to eat; Rarely eat: eat several times per month and rarely eat, do not eat.

Table 2. Comparison of clinically relevant indicators between EH and control groups ($\bar{x}$±S)

Index	EH (n=150)	Controls (n=150)	t	*P*
Age (years)	49.46±9.38	47.96±10.29	2.26	0.643
Systolic blood pressure (mm Hg)	158.64±22.45	117.24±10.20	31.39	0.000^{Δ}
Diastolic blood pressure (mm Hg)	98.41±13.19	73.51±7.69	28.99	0.000^{Δ}
Mean arterial pressure (mm Hg)	119.31±15.47	87.74±9.26	35.14	0.000^{Δ}
BMI	28.45±5.82	23.28±4.75	8.91	0.000^{Δ}
WHR	0.92±0.06	0.82±0.07	2.89	0.015^{Δ}
Fasting blood glucose (mmol/L)	5.60±1.10	5.26±0.90	3.99	0.018^{Δ}
Total cholesterol (mmol/L)	5.21±1.07	4.47±1.05	5.92	0.000^{Δ}
Triglycerides (mmol/L)	1.58±0.72	0.96±0.82	6.74	0.000^{Δ}
Low-density lipoprotein (mmol/L)	3.20±1.05	2.72±1.05	4.50	0.000^{Δ}
High-density lipoprotein (mmol/L)	1.36±0.43	1.47±0.43	-0.37	0.707

Note: "$^{\Delta}$" indicates statistical significance.

Table 3. EH-related risk factors for non-conditional Logistic regression analysis

Variable	B	SE	χ^2	*P*	OR	95% CI
Even to eat or not to eat fresh vegetables	-0.263	0.211	1.735	0.128	0.661	0.613-1.230
Overweight	0.989	0.176	21.468	0.000^{Δ}	2.782	1.575-3.684
Obesity	1.656	0.213	62.318	0.000^{Δ}	5.478	3.789-7.568
Abdominal obesity	0.756	0.165	30.582	0.000^{Δ}	2.785	1.485-3.478
Smoking	-0.085	0.201	0.235	0.690	0.889	0.601-1.402
Drinking	0.501	0.278	2.523	0.189	0.901	0.772-1.801
Total cholesterol	0.345	0.278	1.852	0.185	1.854	0.841-2.224
Triglycerides	0.874	0.340	6.701	0.012^{Δ}	2.402	1.285-4.698
Fasting plasma glucose	0.662	0.226	9.758	0.006^{Δ}	1.995	1.297-2.987
High-density lipoprotein	0.789	0.485	2.401	0.126	2.178	0.825-5.598
Low-density lipoprotein	0.289	0.228	1.187	0.279	1.262	0.878-1.957

Note: B, the correlation coefficient; SE, standard error; "$^{\Delta}$" indicates statistical significance.

PCR amplification

Reaction system 5 μl, including templates 10 ng/μl DNA 1 μl, the downstream primer (10 μmol) each 0.5 μl, PCR master mix 1.25 μl (including PCR buffer, $MgCl_2$, dNTP Mix and Taq enzyme) and dd H_2O 1.75 μl. PCR amplification conditions: 95°C denaturation for 3 min, 95°C denaturation 30 s, 56°C annealing 30 s, 72°C extension 60 s, 45 cycles. Cycle is completed,

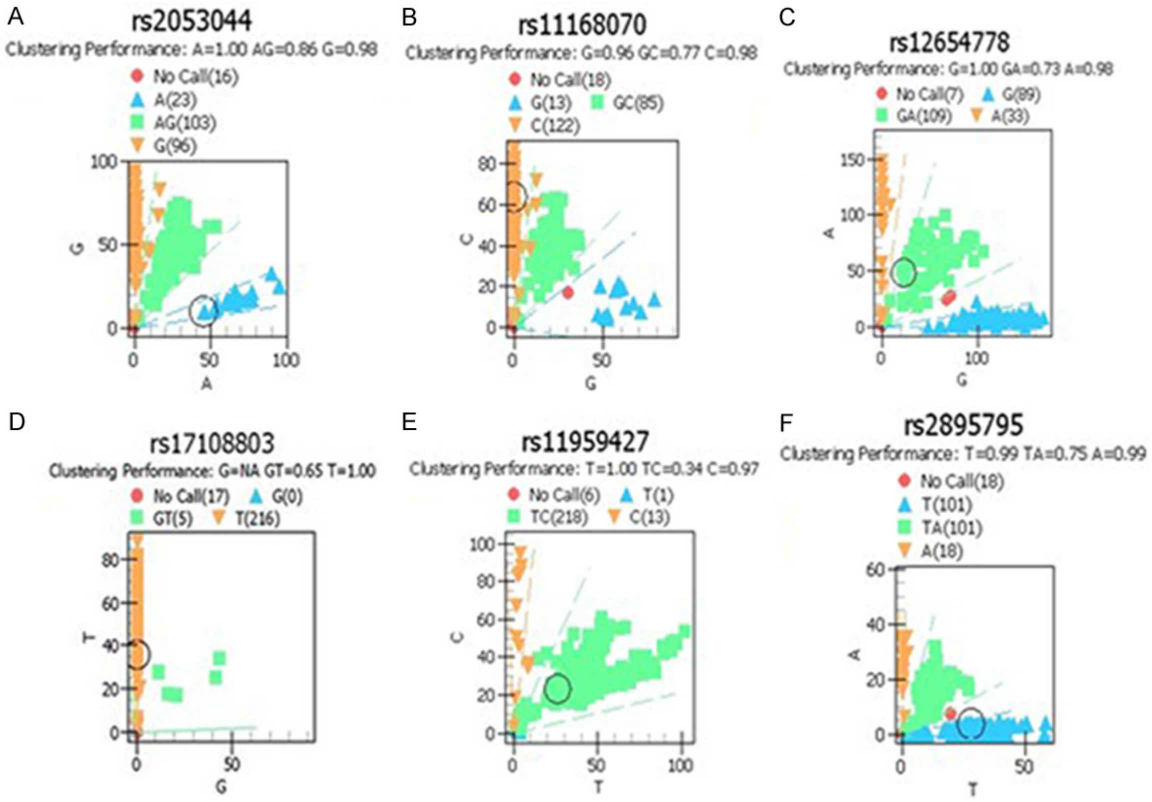

Figure 1. β_2-AR gene polymorphism in the 5'-regulatory region. A. AA, AG and GG of rs2053044, B. GG, GC, CC of rs11168070, C. AA, AG and GG of rs12654778, D. TT, GT of rs17108803, E. TT, TC and CC of rs11959427, F. AA, TA and TT of rs2895795.

72°C and then extended 5 min. Take 1 μl PCR products on 2% agarose gel electrophoresis to test results. Primers were designed by Sequenom Co. Inc, and synthezed by Dalian Treasure Biological Co.

The 384 well plate was removed from the PCR machine, and then 16 μl sterile double distilled water was added to each well. Take 6 mg ion adsorption resins (CLEAN Resin) flat on the 384 hole groove plate drying 10 min. Cover locking groove plate and the ion adsorption resin landing in the hole, reverse mixing 10-15 min. 384 well plate sealing membrane enclosed, centrifuge at 5000 rpm for 5 minutes, gently turn off the seal film ready to chip sample.

Information input

Input the relevant experimental information in the mass spectrometer computer experimental record database (such as: the primer sequence synthesis and sample number) according to the iPLEX Application Guide.

Chip sample

The specific operation in accordance with the MassARRAY Nanodispenser RS1000 User's Guide. After spotting complete, with a mass spectrometer to detect. The results analyzed according to Typer v4.0 User's Guide.

Statistical analysis

Unpaired t-test/chi-square test/Fisher's tests were used to compare the characteristics of two groups. To compare the aldosterone levels of two groups, Mann-Whitney U test and to compare aldosterone levels according to different genotypes, Kruskal-Wallis test were used since the distribution of the values deviated from the normal. Genotypes and alleles were compared using χ^2 test or Fisher's exact test as applicable. For the analysis of β_2-AR gene 5'-regulatory region polymorphisms and binary logistic regression analysis was carried out to identify the independent risk factors using SPSS ver. 19.0 (SPSS Inc, USA).

Table 4. The distribution of genotypes and alleles in Xinjiang Kazakh EH and control group

genotypes/alleles	EH n=150	NH n=150	χ^2	*P*	OR	95% CI
rs2053044 (n, %)						
AA	11 (7.0)	17 (11.0)	1.558	0.459	-	-
AG	70 (47.0)	70 (47.0)			0.647	0.283-1.480
GG	69 (46.0)	63 (42.0)			0.591	0.257-1.357
A	92 (31.0)	104 (35.0)	1.091	0.296	-	-
G	208 (69.0)	196 (65.0)			0.834	0.592-1.173
rs12654778 (n, %)						
GG	63 (42.0)	59 (39.0)	1.313	0.519	-	-
GA	63 (42.0)	72 (48.0)			1.220	0.747-1.993
AA	24 (16.0)	19 (13.0)			0.845	0.420-1.700
G	189 (63.0)	190 (63.3)	0.007	0.933	-	-
A	111 (37.0)	110 (36.7)			0.986	0.707-1.374
rs2895795 (n, %)						
TT	57 (38.0%)	69 (46.0%)	1.990	0.370	-	-
TA	75 (50.0%)	66 (44.0%)			0.727	0.449-1.177
AA	18 (12.0%)	15 (10.0%)			0.688	0.319-1.486
T	189 (63.0%)	204 (68.0%)	1.659	0.198	-	-
A	111 (37.0%)	96 (32.0%)			0.801	0.572-1.123
rs17108803 (n, %)						
TT	147 (98.0)	145 (97.0)	0.514	0.474	-	-
TG	3 (2.0)	5 (3.0)			1.690	0.396-7.200
GG	0 (0.0)	0 (0.0)			-	-
T	297 (99.0)	295 (98.0)	0.507	0.477	-	-
G	3 (1.0)	5 (2.0)			1.007	0.202-5.029
rs11959427 (n, %)						
CC	8 (5.3)	10 (6.7)	1.280	0.527	-	-
TC	141 (94.0)	137 (91.3)			0.777	0.298-2.028
TT	1 (0.7%)	3 (2.0%)			2.4	0.208-27.72
C	157 (98.49)	157 (98.77%)	0.000	1.000	-	-
T	143 (1.52%)	143 (1.23%)			1.000	0.726-1.378
rs11168070 (n, %)						
CC	106 (71.0%)	78 (52.0%)	11.021	0.004	-	-
GC	38 (25.0%)	62 (41.0%)			2.217	1.347-3.650
GG	6 (4.0%)	10 (7.0%)			2.265	0.790-6.495
C	248 (83.0%)	218 (73.0%)	8.648	0.003	-	-
G	52 (17.0%)	82 (27.0%)			1.794	1.212-2.655

Results

Comparison of clinical data

There is no significant difference in EH group for smoking and drinking rates when compared to healthy controls (shown in **Table 1**). Gender ratio is similar in two group, however, EH group has significant less vegetable intake than the control ($P<0.05$, **Table 1**). Laboratory test show that EH group has significant higher systolic blood pressure, diastolic blood pressure, mean arterial pressure, body mass index, waist-hip ratio, fasting glucose, total cholesterol, triglycerides, low-density lipoprotein average ($P<0.05$, **Table 2**). Other indicators such as age, high-density lipoprotein exhibited no difference between the two groups ($P>0.05$, **Table 2**). In addition, logistic regression analysis showed that the OR of overweight, obesity, abdominal obesity, triglycerides, blood glucose for increased the risk EH were 2.782 (95% CI

Table 5. EH group β_2-AR gene 5'-regulatory region polymorphism of linkage disequilibrium analysis

	rs2053044	rs2895795	rs11168070	rs11959427	rs12654778	rs17108803
rs2053044	0	0.7689	0.9122	0	0.5540	0.5540
rs2895795	0.1606	0	1	0	0.6471	0.6471
rs11168070	0.3900	0.1273	0	0	0.4161	0.4161
rs11959427	0	0	0	0	0	0
rs12654778	0.0803	0.1566	0.0212	0	0	0
rs17108803	-1	-1	-1	-1	-1	0

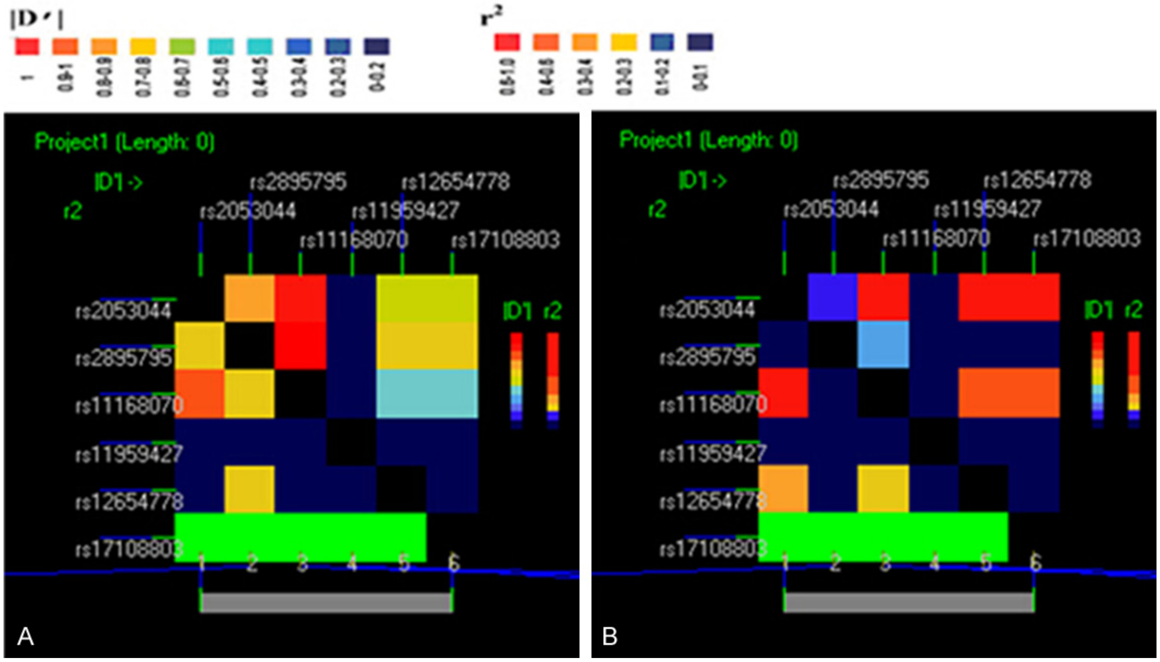

Figure 2. β_2-AR gene 5'-regulatory region polymorphisms in linkage disequilibrium (LD) analysis. A. EH group; B. NH group.

1.575-3.684), 5.478 (95% CI 3.789-7.568), 2.785 (95% CI 1.485-3.478), 2.402 (95% CI 1.285-4.698), 1.995 (95% CI 1.297-2.987) **Table 3**.

Determination of β_2-AR gene polymorphism in the 5'-regulatory region

Polymorphic loci rs2053044, rs17108803, rs12654778, rs11168070, rs11959427 and rs2895795, all the PCR products amplified fragment length were 200 bp. Single base extension and MALDI-TOF-MS analysis technology to detect the sample genotypes are shown in **Figure 1**. There was no correlation between rs2053044, rs17108803, rs12654778, rs11959427, rs2895795 and EH in Xinjiang. In the Kazakh EH and control group, the distribution of rs11168070 genotype CC, CG, GG and frequency of allele C and G have significant difference ($P<0.05$, **Table 4**). The G allele relative risk analysis showed: OR=1.794; 95% CI=1.212-2.655.

β_2-AR gene 5'-regulatory region polymorphisms in linkage disequilibrium (LD) analysis

There are some linkage disequilibrium between rs11168070, rs2053044, rs2895795 polymorphism in EH group (**Table 5**; **Figure 2A**), and rs11168070, rs12654778, rs17108803 polymorphism in NH group (**Table 6**; **Figure 2B**).

β_2-AR gene 5'-regulatory region polymorphisms in haplotype analysis

Using of SHEsis software for haploid analysis, six polymorphic loci were composed of twelve

Table 6. NH group β_2-AR gene 5'-regulatory region polymorphism of linkage disequilibrium analysis

	rs2053044	rs2895795	rs11168070	rs11959427	rs12654778	rs17108803
rs2053044	0	0.1084	0.9295	0	0.9022	0.9022
rs2895795	0.0028	0	0.3096	0	0.0775	0.0775
rs11168070	0.6232	0.0164	0	0	0.8616	0.8616
rs11959427	0	0	0	0	0	0
rs12654778	0.2490	0.0017	0.1638	0	0	0
rs17108803	-1	-1	-1	-1	-1	0

Table 7. Haplotype distribution in hypertension group and control group

Haplotype	EH	NH	χ^2	*P* value	OR (95% IC)
	N (%)	N (%)			
GTCCAT	96 (30.2)	56 (15.9)	19.592	0.000^{Δ}	2.293 (1.580-3.329)
GACTGT	84 (26.4)	8 (2.3)	82.46	0.000^{Δ}	15.481 (7.357-32.575)
ATCTGT	36 (11.3)	73 (20.7)	10.77	0.001^{Δ}	0.490 (0.318-0.754)
ATGCGT	30 (9.4)	7 (2)	17.83	0.000^{Δ}	5.149 (2.229-11.896)
GACCGT	27 (8.5)	44 (12.5)	2.792	0.095	0.652 (0.393-1.080)
ATGTGT	18 (5.7)	73 (20.7)	32.193	0.000^{Δ}	0.230 (0.134-0.395)
GTCCGT	6 (1.9)	19 (5.4)	5.699	0.017^{Δ}	0.338 (0.133-0.857)
GTCTAT	9 (2.8)	37 (10.5)	15.338	0.000^{Δ}	0.249 (0.118-0.524)
GACCAT	3 (0.9)	29 (8.2)	19.479	0.000^{Δ}	0.106 (0.032-0.353)
AACTAT	3 (0.9)	2 (0.6)	0.321	0.571	1.671 (0.277-10.068)
GACTAT	3 (0.9)	2 (0.6)	0.321	0.571	1.671 (0.277-10.068)
GTCTGT	3 (0.9)	31 (8.8)	21.368	0.000^{Δ}	0.099 (0.030-0.327)
Total	318	353			

Note: In the following order constituted haplotypes: rs2053044, rs2895795, rs11168070, rs11959427, rs12654778, rs1710880.

haplotypes, the highest frequency is GTCCAT (30.2%), GACTGT (26.4%). Frequencies of haplotype GTCCAT, GACTGT and ATGCGT in EH group were higher (*P*<0.05), and frequencies of ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT and GTCTGT in EH group were significantly lower than control group (*P*<0.05, shown in **Table 7**).

Discussion

β_2-adrenergic receptor is seven transmembrane receptor protein family which with G protein-coupled, the relative molecular mass is about 64 kDa. β_2-AR and its agonist binding activates adenylate cyclase (AC), so that an increase in cAMP generation, thus the activation of protein kinase A, which role in a variety of lipid metabolism related enzymes, ion channels, and transcription factors, and may play a physiological role in the heart, blood vessels, respiratory, metabolic, endocrine and central nervous system. Association between the expression and quantity of β_2-AR gene polymorphism and receptor, water salt metabolism and vascular reactivity change mechanisms that may be involved in hypertension.

Essential hypertension (EH) is regarded as a multifactorial and polygenic disease, and its underlying mechanism is very complex. In this study, the survey showed vegetable intake numbers are different, the proportion of occasionally and rarely eat vegetables in EH group is higher than the control, there was statistically significant difference (*P*<0.05). Moreover, systolic blood pressure, diastolic blood pressure, mean arterial pressure, body mass index, waist-hip ratio, fasting glucose, total cholesterol, triglycerides, low-density lipoprotein average, EH group were significantly higher (*P*<0.05). In addition, logistic regression analysis showed that overweight, obesity, abdominal obesity, triglycerides, blood glucose increased relative risk of suffering from EH were 2.782 (95% CI 1.575-3.684), 5.478 (95% CI 3.789-7.568), 2.785 (95% CI 1.485-3.478), 2.402 (95% CI 1.285-4.698), 1.995 (95% CI 1.297-2.987), which is risk factors and others are not associated with EH (P>0.05).

In recent years, many studies have confirmed the beta 2-AR gene polymorphism is related to different races of hypertension [9-14]. However,these studies are did not find an association with with the β_2-AR gene regulatory region SNPs polymorphic loci. In this study, analysis of β_2-AR gene 5'-regulatory region of

SNPs in the Kazakh EH and control group, the distribution of rs11168070 genotype CC, CG, GG and frequency of allele C and G have significant difference (P<0.05). The G allele relative risk analysis showed: OR=1.794; 95% CI=1.212-2.655. There is a certain correlation between β_2-AR gene polymorphism of rs11168070 and EH. It is also important to note, the C allele may be a susceptible gene genetic susceptibility to EH in Xinjiang Kazakh population.

The distribution of β_2-AR gene polymorphism and its relationship with EH in different races, different crowd there is a big difference, this suggests that the role of polymorphisms in the pathogenesis of EH in different races and groups have certain difference. Some scholars believe that another possible cause of inconsistent results is that some of the research has focused role of single or a few polymorphic loci, thereby underestimating multiple loci on expression of β_2-AR effect. Haplotype is locus gene consisting of genetic unit in the same chromosome, with the amount of genetic information more than SNP. So the subject of linkage disequilibrium and haplotype analysis of β_2-AR gene SNPs, LD results showed: there is a strong linkage disequilibrium in the Kazakh EH and control group. There are some linkage disequilibrium between rs11168070, rs2053044, rs2895795 polymorphism in EH group, and rs11168070, rs12654778, rs17108803 polymorphism in NH group. In addition, using of SHEsis software for haplotype analysis, six polymorphic loci were composed of twelve haplotypes, the highest frequency is GTCCAT and GACTGT. Frequencies of haplotype GTCCAT, GACTGT and ATGCGT in EH group were higher ($P<0.05$), and frequencies of ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT and GTCTGT in EH group were significantly lower than control group ($P<0.05$). Thus it can be seen haplotypes GTCCAT, GACTGT, ATGCGT, ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT and GTCTGT may be associated with EH, which GTCCAT, GACTGT and ATGCGT are risk factors, and ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT and GTCTGT is a protective factor.

In conclusion, these preliminary results show that there are gene polymorphisms of β_2-AR gene 5'-regulatory region of rs2053044, rs17108803, rs12654778, rs11959427, rs2895795 and rs11168070 in Xinjiang Kazakh population, and there were statistically significant difference in the EH and NH group of rs11168070 genotype and allele frequency distribution. There is a strong linkage disequilibrium in genetic polymorphism of six DNA loci in the EH group and controls. Our results indicate that haplotype GTCCAT, GACTGT, ATGCGT in the Kazakh population is a risk factor of EH, and ATCTGT, ATGTGT, GTCCGT, GTCTAT, GACCAT, GTCTGT is a protective factor. However, due to the single nucleotide polymorphism is affected by many factors, such as sample selection, sample size and statistical methods, in order to further understand the relationship between β_2-AR polymorphism and essential hypertension, and it needs to be multiregional, multiethnic diversity and the extensive research.

Acknowledgements

This study was supported by the National High Technology Research and Development Program (863 Program Grant No. 2011AA02A111).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jiang Cheng, Department of Clinical Laboratory, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi 832002, Xinjiang, P. R. China. E-mail: chengjiang931715@sina.com

References

[1] Hamet P, Pausova Z, Adarichev V, Adaricheva K, Tremblay J. Hypertension: genes and environment. J Hypertens 1998; 16: 397-418.

[2] Nan Fang L, Ling Z, Mei C, De Lian Z. Epidemiological Report of Hypertension in the Hebukesel Pastoral Area in Xinjiang. Modern Preventive Medicine 2003; 30: 141-143.

[3] Filigheddu F, Reid JE, Troffa C, PinnaParpaglia P, Argiolas G, Testa A, Skolnick M, Glorioso N. Genetic polymorphisms of the beta-adrenergic system: association with essential hypertension and response to beta-blockade. Pharmacogenomics J 2004; 4: 154-160.

[4] Pacanowski MA, Gong Y, Cooper-Dehoff RM, Schork NJ, Shriver MD, Langaee TY, Pepine CJ, Johnson JA. INVEST Investigators: beta-adrenergic receptor gene polymorphisms and beta-blocker treatment outcomes in hypertension. Clin Pharmacol Ther 2008; 84: 715-721.

[5] Lanfear DE, Jones PG, Marsh S, Cresci S, McLeod HL, Spertus JA. Beta2-adrenergic receptor genotype and survival among patients

receiving beta-blocker therapy after an acute coronary syndrome. JAMA 2005; 294: 1526-1533.

[6] Vardeny O, Detry MA, Moran JJ, Johnson MR, Sweitzer NK. The beta2 adrenergic receptor Gln27Glu polymorphism affects insulin resistance in patients with heart failure: possible modulation by choice of beta blocker. J Cardiovasc Pharmacol 2008; 52: 500-506.

[7] Hui L, Nan Fang L, Tao L, Ling Z, Qing Z. Association between Gln27Glu polymorphism in the bata2-adrenergic receptor gene and essential hypertension in the Kazakh Chinese Population. J Clin Cardiol 2005; 21: 125-129.

[8] Qing L, Nan-Fang L, Ling Z, Hong-Jian L. Association between +46 polymorphism in the bata2-adrenergic receptor gene and essential hypertension in the Kazakh Chinese Population. Science Technology and Engineering 2005; 9: 132-135.

[9] Ranade K, Shue WH, Hung YJ, Hsuing CA, Chiang FT, Pesich R, Hebert J, Olivier M, Chen YD, Pratt R, Olshen R, Curb D, Botstein D, Risch N, Cox DR. The glycine allele of a glycine/arginine Polymorphism in the bata2-adrenergic receptor gene is associated with essential hypertension in a population of Chinese origin. Am J Hypertens 2001; 14: 1196-1200.

[10] Kato N, sugiyama T, Morita H, Kurihara H, Sato T, Yamori Y, Yazaki Y. Association analysis of bata2-adrenergic receptor ploymorphisms with hypertension Japanese. Hypertension 2001; 37: 286-292.

[11] Xie HG, Stein CM, Kim RB, Gainer JV, Sofowora G, Dishy V, Brown NJ, Goree RE, Haines JL, Wood AJ. Human beta2-adrenergic receptor polymorphisms: no association with essential hypertension in black or white Americans. Clin Pharmaco Ther 2000; 67: 670-675.

[12] Jia H, Sharma P, Hopper R, Dickerson C, Lloyd DD, Brown MJ. Beta2-adrenoceptor gene polymorphisms and blood pressure variations in East Anglian Caucasians. J Hypertens 2000; 18: 687-693.

[13] Tomaszewski M, Brain NJ, Charchar FJ, Wang WY, Lacka B, Padmanabahn S, Clark JS, Anderson NH, Edwards HV, Zukowska-Szczechowska E, Grzeszczak W, Dominiczak AF. Essential hypertension and β2-adrenergic receptor gene: linkage and association analysis. Hypertension 2002; 40: 286-291.

[14] Herrmann SM, Nicaud V, Tiret L, Evans A, Kee F, Ruidavets JB, Arveiler D, Luc G, Morrison C, Hoehe MR, Paul M, Cambien F. Polymorphisms of the beta2-adreno receptor (ADRB2) gene and essential hypertension: the ECTIM and PEGASE Studies. J Hypertens 2002; 20: 229-235.