ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 3, 2018

Online - 2455-3891
Print - 0974-2441

SHORT COMMUNICATION

RENIN SINGLE-NUCLEOTIDE POLYMORPHISM (rs5705) IN HEALTHY INDIAN POPULATION

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Received: 25 October 2017, Revised and Accepted: 30 November 2017

ABSTRACT

Objective: Renin gene (REN) polymorphisms are studied to understand its association with different diseases such as hypertension. The genetic variants have different frequencies in various populations. Therefore, we have established normal frequencies of rs5705 variants in healthy Indian population.

Methods: The genotype and allele frequencies of rs5705 were determined in 324 healthy Indian population using real-time polymerase chain reaction.

Results: Our study showed 91.7% T allele or TT genotype and 8.3% G allele or TG genotype. It is interesting to note that the minor allele (G) is found only in the form of heterozygous TG condition and the homozygous form GG was absent. These frequencies were compared with different populations.

Conclusions: The significant variations found between our population and others confirm interethnic variations. Future studies may use our established genotype frequencies of rs5705 to understand the risk of diseases such as hypertension.

Keywords: Renin, rs5705, Single-nucleotide polymorphisms, India.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i3.23250

SHORT COMMUNICATION

Renin (REN), an aspartyl protease, catalyzes the rate-limiting step of the REN–angiotensin system. It converts angiotensinogen to Angiotensin I, a decapeptide. It is then converted to octapeptide Angiotensin II by the angiotensin-converting enzyme. Angiotensin II is the main effector molecule resulting in physiological changes such as blood pressure regulation (a potent vasoconstrictor), electrolyte homeostasis, and extracellular volume balance [1]. REN is, therefore, associated with hypertension, myocardial infarction, and coronary artery disease. Moreover, the drugs used for the improvement of these conditions are on the rise [2–4].

REN gene is placed on chromosome 1q32.1 with 10 exons. Innumerable polymorphisms have been identified so far. However, very few are well studied. The REN single-nucleotide polymorphisms (SNPs) are found to be associated with risk of having the aforementioned diseases [5,6]. Some of these are studied in different populations such as Korea, Mexico, USA, and Europe [7,8]. rs5705 is an exonic synonymous variant whose frequency distribution is unavailable in Indian population. Therefore, we have conducted the study to evaluate the allelic frequency of rs5705 in healthy Indian population.

The study comprised a total 324 individuals, who were unrelated individuals of Indian origin. The individuals with chronic diseases of the kidney or liver or heart, diabetes mellitus, and hypertension were excluded from the study. We initiated the study after obtaining ethical approval by the Ethics Committee at National Institute of Nutrition and the study center, Gandhi Hospital. Written consent was obtained from subjects after orientation for the study. The demographic profile of the study population was recorded in a case report form. It includes information on medical history, smoking habits, alcohol consumption, and the use of medications. Anthropometric measurements were taken to calculate (body mass index, calculated as kg/m^2). After an overnight fast, blood was collected. Fasting glucose was immediately analyzed. Other clinical parameters were assessed in serum using the Roche Cobas c311 system. DNA was isolated from whole blood using the MasterPure™ DNA Purification Kit for Blood Version II kit (epicentre). The SNP belonging to REN, i.e., rs5705 was evaluated by allelic discrimination real-time polymerase chain reaction using predesigned TaqMan probes (Applied Biosystems, Foster City, CA).

Data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS version 16.0; SPSS, Chicago, IL). Clinical variables were assessed by t-test. Chi-square test was used for categorical variables. All p values were two-tailed, and significance was defined as $p \leq 0.05$.

The clinical characteristics of the study population are mentioned in Table 1. The polymorphism rs5705 was found to be in Hardy-Weinberg equilibrium (p=0.491). The frequencies of the REN rs5705 in healthy Indian population were evaluated (Table 2). It was compared with other populations of 1000 genome project [9]. In line with the data obtained, we found that the homozygous GG variant was not found in our population (0%). The same was observed in Gujarati Indian in Houston and Punjabi in Lahore, Pakistan. However, these two sub-populations were less in sample size. Moreover, the South Asian population which comprises Gujarati Indian in Houston and Punjabi in Lahore, Pakistan, along with Bengali in Bangladesh, Indian Telugu in the UK, and Sri Lankan Tamil in the UK, did show a very scanty GG frequency (0.006). In compliance with our study, even the women from Norway showed no GG genotype [10]. The African Americans and non-Hispanic white hypertensives were found to have 63% TT, 30.6% TG, and 6.3% GG variants [11]. Our study showed 91.7% T allele or TT genotype and 8.3% G allele or TG genotype. In another study, where 97 hypertensives were recruited for the "Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol" trial, 88% had T allele, and 12% G allele [12]. In the Chi-square analysis performed, African, American, and European were significantly different in carrying a varied percentage of alleles of rs5705 (Table 2). As expected, the South Asian population which our population also belongs to had shown no significant difference (p=0.55), whereas the sub-populations, Punjabi in Lahore, Pakistan, and Sri Lankan Tamil in the UK were different in the allele distribution ($p<0.05$). Sun *et al.* conducted a study between

Table 1: Characteristics of the study population

Variable	Male (n=158)	Female (n=166)	Total (n=324)	p value
Age (years)	44.58±13.39	41.75±11.66	43.1±12.58	NS
SBP, mmHg	122.17±14.53	118.2±15.68	120.1±15.22	NS
DBP, mmHg	81.97±9.44	77.93±9.97	79.9±9.9	0.009
BMI, Kg/m^2	25.48±2.99	26.74±4.13	26.1±3.67	0.028
FBS, mg/dL	100.25±12.63	101.27±16.63	100.8±14.79	NS
Total Cholesterol, mg/dL	188.84±41.58	182.36±38.29	185.5±39.93	NS
Triglycerides, mg/dL	177.14±133.61	123.51±72.41	149.6±109.74	0.002
HDL-C, mg/dL	37.29±9.59	40.07±10.53	38.7±10.15	NS
LDL-C, mg/dL	121.45±34.23	118.32.73	120.2±33.39	NS
Exercise	72 (45.6)	28 (16.9)	100 (30.9)	0.0001
Smoking	16 (10.1)	0 (0)	16 (4.9)	0.003
Alcohol intake	58 (36.7)	8 (4.8)	66 (20.4)	0.0001

Values are expressed as mean±SD, otherwise number of subjects (%). SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; FBS: Fasting blood sugar; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; NS, Not significant

Table 2: Comparison of frequencies of the REN rs5705 in healthy Indian population and other populations of 1000 genome project.

Population	N	Genotype Frequency; n (%)			Allele Frequency; n (%)		p
		TT	TG	GG	T	G	
Indian Population (Current study)	324	0.917 (270)	0.083 (54)	0 (0)	0.917 (594)	0.083 (54)	Reference
1000 genome data							
African	661	0.454 (300)	0.457 (302)	0.089 (59)	0.682 (902)	0.318 (420)	0.00
American	347	0.683 (237)	0.274 (95)	0.043 (15)	0.820 (569)	0.180 (125)	0.00
East Asian	504	0.849 (428)	0.145 (73)	0.006 (3)	0.922 (929)	0.078 (79)	0.72
European	503	0.746 (375)	0.235 (118)	0.020 (10)	0.863 (868)	0.137 (138)	0.00
South Asian	489	0.822 (402)	0.172 (84)	0.006 (3)	0.908 (888)	0.092 (90)	0.55
Bengali in Bangladesh	86	0.802 (69)	0.186 (16)	0.012 (1)	0.895 (154)	0.105 (18)	0.38
Gujarati Indian in Houston	103	0.874 (90)	0.126 (13)	0 (0)	0.937 (193)	0.063 (13)	0.07
Indian Telugu in the UK	102	0.873 (89)	0.118 (12)	0.010 (1)	0.931 (190)	0.069 (14)	0.50
Punjabi in Lahore, Pakistan	96	0.854 (82)	0.146 (14)	0 (0)	0.927 (178)	0.073 (14)	0.01
Sri Lankan Tamil in the UK	102	0.706 (72)	0.284 (29)	0.010 (1)	0.848 (173)	0.152 (31)	0.00

Values are expressed as number of subjects (%). Data are analyzed using Chi-square test. p value<0.05 is considered significant. TT: Wild Homozygous, TG: Heterozygous, GG: Recessive Homozygous

healthy and hypertensive individuals in the Hypertension Pathotype cohort; the minor allele frequencies were 0.10 and 0.16, respectively. The minor allele frequencies were found to be very less in our study (0.083), East Asian (0.078), Gujarati Indian in Houston (0.063), Indian Telugu in the UK (0.069), and Punjabi in Lahore, Pakistan (0.073). Although rs5705 occurs in the exonic region, it results in no amino acid change, i.e., synonymous variant. The A allele had shown an odds ratio of 2.04 in the risk of hypertension. Furthermore, the haplotype of rs5705, rs10900555, rs6693954, rs6676670, and rs11571078 had shown an overall risk toward of hypertension [13].

CONCLUSION

The allele frequencies established in our study will give a future input for the evaluation of the genetic association studies. The role of REN polymorphisms cannot be ignored as it plays a crucial role in the regulation of blood pressure and electrolyte homeostasis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge all the individuals who participated in this study. We are grateful to the Department of Science and Technology for according Inspire fellowship (Registration no.: IF110472) and thankful to NIN (Indian Council of Medical Research) for the facility to work (Project no.: 12FD-10). We also acknowledge the support of Gandhi Medical College, Hyderabad, India. We appreciate Mr. Nishanth Kumar for his contribution in sample collection and Ms. Kiranmayee Ale in sample processing.

AUTHORS CONTRIBUTIONS

The corresponding author (Dr. Dinesh Kumar Bharatraj) is the principal author who guided the project and reviewed the manuscript. The first author (Varsha Varakantham) carried out the complete experimental work, statistical analysis, and manuscript writing. Dr. Manjula Venkata helped in the identification, selection, and recruitment of appropriate patients.

CONFLICT OF INTERESTS

The author(s) declared no potential conflicts of interest.

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