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Association of Angiotensin II Type I Receptor (AGTR1) Gene Polymorphism and Type 2 Diabetes & Nephropathy among the Eastern Indian Bengali Patients

Halder K[1] and Purkait P[2,3,4,5]*

[1]Department of Molecular Biology, Brahmananda Keshab Chandra College, India
[2]Origin LIFE Healthcare Solutions & Research Centre LLP, India
[3]Department of Anthropology (Molecular Anthropology Laboratory), Panjab University, India
[4]Department of Anthropology, Andhra University, India
[5]Anthropological Survey of India, India

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***Corresponding author:** Dr. Pulakes Purkait, Founder, Origin LIFE Healthcare Solutions & Research Centre LLP, SCO 181, Sector 38 C, Chandigarh, India, Tel: +91 9599877196; Email: pulakes.purkait28@gmail.com ; pp.diabetes@gmail.com

Abstract

Genetic polymorphisms of the angiotensin II type I receptor (AGTR1), has been reported to be the most probable candidate genes for hypertension, diabetes and complication of diabetes. Ethnic differences in the frequencies of these gene genotypes have also been reported. As there is no data available for AGTR1 polymorphism and T2DM in the Bengali population, it's our attempt to fill the scientific gap. To investigate whether the angiotensin II type I receptor gene A1166C (rs5186) polymorphism is associated with a risk of type 2 diabetes and nephropathy in Indian Bengali patients, in a case-control study, the AGTR1 gene (rs5186; A1166C) was examined in type 2 diabetic patients with and without nephropathy (T2DM: N=246; T2DNH: N= 168) and normal control (N=304) participants and genotyped using PCR-RFLP methods. Result of Fisher exact test for allelic association of SNP rs5186 exhibits significant difference in the allele frequencies between the control and T2DM groups (p= 0.0308). It is observed through the 4 genotypic model tests that additive model predicted significant association than basic genotype, dominant and recessive models and shows significant difference between CON vs. T2DM groups (p = 0.038). The present study reveals that the A1166C polymorphism (rs5186) of AGTR1 has a positive association with T2DM. This polymorphism with C allele may contribute to diabetic complication, nephropathy development particularly in T2DM patients.

Keywords: Angiotensin II type I receptor, Type 2 diabetes, Nephropathy, Eastern Indian, Bengali

Abbreviations: A1: Code for allele 1 (the more rare or 'minor' allele based on the entire sample frequencies); A2: Code for allele 2 (the more common or 'major' allele); CON: Control group; T2D: over all Type 2 diabetes with and without nephropathy group; T2DM: Type 2 Diabetes without nephropathy group; T2DNH: Type 2 diabetes with nephropathy who are on hemodialysis group; ObsHET: marker's observed heterozygosity; PredHET: marker's predicted heterozygosity (2*MAF*(1-MAF)) ; HWpval: Hardy-Weinberg equilibrium p value; MAF: Minor allele frequency; F_A: frequency of minor allele in affected individuals (case); F_U: frequency of minor allele in unaffected individuals (control); AFF: Affected group (Case); UNAFF: Unaffected group (Control); GENO: Genotype , basic genotypic test; TREND: Additive model ; DOM: Dominant model ; REC: Recessive model; CHISQ: Chi-squared value

for allelic association (with 1 df); p: the asymptotic p-value for chi-square test; OR: odds ratio; L95: lower bound of the 95% confidence;U95: upper bound of the 95% confidence; * :Significant] ACE: Angiotensin converting enzyme; ADD: Additive model; AGT: Angiotensinogen; Ang II: Angiotensin II; BMI: Body mass index; CI: Confidence interval; DBP: Diastolic blood pressure; HWE: Hardy-Weinberg equilibrium; NCBI: National Centre for Biotechnology Information; PCR: Polymerase chain reaction; RFLP : Restriction Fragment length polymorphism RAAS: Renin-angiotensin-aldosterone system; rs: Reference SNP ID number; SBP: Systolic blood pressure; SNP: Single nucleotide polymorphism; UTR: Un translated regions; ASN : East Asian ; CHB: Han Chinese in Bejing, China; JPT: Japanese in Tokyo, Japan; CHS: Southern Han Chinese ; EUR: European ; CEU :Utah Residents (CEPH); TSI: Toscani in Italia; FIN: Finnish in Finland; GBR: British in England and Scotland; IBS: Iberian population in Spain; AFR: African ; YRI: Yoruba in Ibadan, Nigera; ASW: Americans of African Ancestry in SW USA; AMR: Admixed American; MXL: Mexican Ancestry from Los Angeles USA; PUR: Puerto Ricans from Puerto Rico; CLM: Colombians from Medellin, Colombia.

Introduction

Type 2 Diabetes Mellitus (T2DM) constitutes the major mass of diabetes and India is the “Diabetes Capital of the World” with 69.2 million Indians having diabetes and with an estimated 72 million cases in 2017 [1-2] with every fifth diabetic in the world being an Indian. Nephropathy is a complication of diabetes and is related to the damage or disease of kidney. Diabetic nephropathy is impairment to the kidney caused by the hyperglycemia and characterized by consistent proteinuria (>300 mg/ 24 h) convoyed by increased arterial blood pressure and steady decline in renal function. In severe cases kidney can be failure. The kidneys filter waste from blood through its capillaries. Diabetes result of high blood sugar can destroy these tiny blood vessels. Renal failure or kidney disease in diabetes is intervened by various biochemical pathways such as renin-angiotensin-aldosterone system (RAAS) [3-4], aldose reductase-polyol [5], di-acyl glycerol-protein kinase C [6], hexosamine pathway [7] and advanced glycosylation end products (AGE) [8-9]. The renin-angiotensin-aldosterone system (RAAS) actually provides a signal transduction mechanism for regulating body’s blood pressure and water balance. When an individual confronted with low blood pressure or certain nerve impulse such as stressful situation, the kidneys release an enzyme known as renin. This triggers a signal transduction pathway: renin cleaves the circulating protein angiotensinogen to produce angiotensin I, which is later converted to angiotensin II by another enzyme angiotensin converting enzyme (ACE). Angiotensin II causes blood vessels to compress, which results in high blood pressure. It also stimulates the secretion of the water retaining hormone vasopressin in the pituitary gland as well as the release of adrenaline, noradrenaline and aldosterone in the adrenal gland [10-13].

The biological effects of angiotensin II are mediated via the angiotensin II receptor. By the help of ligand binding experiments two subtypes of cell surface receptors have been identified (AT1 & AT2) and are classified to the G-protein coupled receptor super family, which has seven transmembrane spanning domain[14]. In humans, the AT1 receptor is present predominantly in vascular smooth muscle cells, and the AT2 receptor is present in the uterus, brain and adrenal medulla [15-16]. Both subtypes are also expressed in the adrenal cortex and kidney [17]. But most of the physiologic and pathphysiologic signals of Ang II, such as vasoconstriction, aldosterone release, stimulation of tubular transport, renal sodium reabsorption, proinflmmatory effects and growth stimulatory actions are transduced by AGTR1 receptors [18]. The AGTR1 gene extents >55 kilo bases (kb) on chromosome 3q22 and comprises of five exons, four of which are untranslated and alternatively spliced [19]. It has been found that AGTR1 is highly polymorphic [20-23] but particularly rs5186 (A1166C) polymorphism of the AGTR1 gene, which was primarily identified by Bonnardeaux et al., a substitution of adenine (A) by cytosine (C) at position 1166, positioned at the 5’ end of the 3’ un-translated region of the gene, is the best evaluated [20] . Except this A1166C polymorphism, few other DNA polymorphisms have no significant impact on hypertension, diabetic nephropathy or myocardial infarction [20-22].

The renin-angiotensin-aldosterone system regulates not merely blood pressure but also the internal pressure of the glomerulus, and hypertension, which is an independent risk factor of diabetic nephropathy. The RAAS has been implicated in the pathogenesis of progressive renal disease [24-25], diabetic renal complications [26] and seem to be especially relevant both biologically and clinically to renal disease [27-28]. Therefore polymorphisms of RAAS candidate genes such as AGT, ACE, REN and AGTR1 are closely related to the progression of DN [13, 29-33]. Several studies suggest that the AGTR1 receptor might be involved in DN, type2 diabetes [21, 34-36], though many other investigator did not find any such relation between polymorphism of AGTR1 gene and diabetes as well as diabetic nephropathy [37-39]. Erstwhile we have identified that RAAS gene candidates ACE, AGT, CYP11B2, REN polymorphisms are significantly associated with type2 diabetes, hypertension, DN respectively, in eastern Indian Bengali population and western Indian Mewari population [13,33,40,41].

Due to controversial results about the role of AGTR1 gene locus in diabetes, DN and the lack of information in this

regard in Bengali population the aim of our present study was to scrutinize the role of AGTR1, in the development of DN in type 2 diabetes mellitus in Bengali population.

Materials and Methods

Study Patients

Recruitment of patients and study design describe elsewhere [13,33]. Patients were recruited from registered patients list of two participating medical institutions of Kolkata city, West Bengal. A total of 718 age and sex matched individuals were participated in the present study, out of which 55.40% (n=398) male and 44.60% (n= 320) female. The study included 304 healthy control (CON: Male = 180; Female = 124), 246 type 2 diabetes patients without nephropathy (T2DM: Male = 128; Female = 118)and 168 type 2 diabetic nephropathy patients on hemodialysis (T2DNH: Male = 90 ; Female = 78). The identification of Type 2 diabetic and nephropathy patients was based on physician's recommendation or registered patient for dialysis. The unrelated controls were randomly selected and recruited from local community centres. A standardized protocol was implemented to obtain detailed medical history and data from each of the study participants. Ethical committee clearance was obtained from the medical institutions prior to the recruitment of subjects in this study. An informed consent was obtained from all the participants prior to their recruitment for the study.

Genotyping

Genomic DNA was prepared from fresh whole blood by using the conventional phenol-chloroform extraction method followed by ethanol precipitation [42]. DNA working dilutions (100µl) were prepared at a concentration of 50ng/µl by dissolving required amount of stock DNA sample in TE buffer. After preparation of working dilutions the uniformity of the samples were checked by performing electrophoresis on a 1% agarose gel. In this study, previously published primers were used for the PCR based detections of SNPs [30,40]. PCR amplification was performed in a final volume of 10µL reaction mixture containing 50ng of genomic DNA, 20 pmol of each primer, 10X Taq PCR buffer, 25 mM MgCl2, 100 mM of each dNTPs and 0.5 U/uL of Red Taq polymerase. PCR amplification was performed in a DNA thermo cycler (Bio-Rad). The DNA was amplified for 35 cycles with denaturation at 94°C for 1 min, annealing at 61°C for 1:30 min and extension at 72°C for 1:30 min and final extension 72°C for 10 min. The PCR products were checked by 2% agarose gel electrophoresis with ethidium bromide staining and directly visualized in UV light. Only those PCR products that had a single amplification product with no evidence of non-specific amplification were used for PCR-RFLP, details about the PCR-RFLP of AGTR1 gene described in our previous article [43].

Statistical Analysis

Allele frequencies were calculated for all the SNPs and were tested for Hardy-Weinberg equilibrium (HWE) and allelic association with the disease (Chi-Square test / Fisher exact test). Allelic and genotype association with the phenotypes was tested under different genetic models for both quantitative and qualitative traits by regression analysis and Fisher Model Test. Allele frequencies were calculated for the SNPs and tested for Hardy-Weinberg equilibrium (HWE) and allelic association with disease (Fisher exact test, logistic regression and Fisher model tests) using PLINK software [44]. For comparing the allelic distributions between study groups, the odds ratio (OR) with 95% confidence interval (CI) were also calculated. Quantitative data were analyzed using SPSS Version 16.0 (SPSS Inc., Chicago,IL, USA), were expressed as Mean ± SD and ANOVA test were used to determine differences in means and significance levels. A level of p <0.05 was assumed statistically significant.

Results

Genotype and Allele Frequency

Distribution of study groups based on genotype of AGTR1 gene polymorphism presented in Table 1. CC genotype were found among only 10 patients, out of which 8 patients are belongs to T2DM group and only 2 patients in T2DNH group.

The SNP rs5186 is an established polymorphism of the AGTR1 gene on chromosome 3: 148459988. It occurred in three forms, wild type homozygous *AA*, heterozygous *AC* and mutant homozygous *CC* (Figure 1). Genotype distribution of this polymorphism is presented in Table 2 and statistical analyses are given in Table 3 to Table 6. . The results of Hardy-Weinberg Equilibrium (HWE) test for rs5186 (A>C) of AGTR1 gene among the present study groups are presented in Table 3. From the HWE test it is found that the SNP rs5186 is not in Hardy-Weinberg equilibrium among T2D and T2DM groups, whereas Control and T2DNH groups maintain the Hardy-Weinberg Equilibrium.

Result of Fisher exact test for allelic association of SNPrs5186 (A>C) of AGTR1 gene among the study groups is presented in Table 4. The results exhibit no significant difference in the allele frequencies of the SNP between the control and T2D groups. However, significant differences are observed between CON vs. T2DM ($\chi 2$= 4.664, OR= 1.593, p= 0.0308) and T2DM vs. T2DNH ($\chi 2$= 3.967, OR= 0.5928, p=0.04639) groups.

The associations were further verified through 4 genotypic model tests (Fisher model test) to confirm which of these models predict best associations between the study groups and the results are presented in table 5. It is observed through the analyses that additive model predicted significant association than basic genotype, dominant and recessive models and shows significant difference between CON vs. T2DM groups (TREND, $\chi 2 = 4.269$, $p = 0.03882$).

The mean values of quantitative variables, among study group based on AGTR1 gene genotype are presented in Table 6. The mean values of weight, BMI, triglyceride, total protein, globulin were significantly higher among the CC genotype group compared to AA and AC genotype groups of AGTR1 gene. The CC genotype individuals are relatively shorter while AA and AC genotype individuals are taller. Mean value of blood glucose comparatively higher among the CC genotype group, although not at significant level.

Comparison of Allele Frequency of AGTR1 Gene Variant Rs5186 with World Population

The allele frequency of the variant is calculated in the Indian Bengali population as well as in the World populations to check the utility of the SNP as a marker in our population as well as in the World populations (Table 7). The allele frequency of the rs5186 has been illustrated by the column graph in Figure 2. It is evident that the minor allele frequency is quite low in African population (YRI) where the 'C' allele shows a low frequency of 0.011, which is yielding a very low heterozygosity. The ancestral allele (A) frequency is quite high in all populations except in the European population and the lowest frequency among IBS population (0.679). Although European and American populations show quit higher frequency of minor allele, the two Mongoloid Asian populations CHB and JPT show similar profile, whereas the present study Indian Bengali population shows profile similar to the all Asian (ASN) and CHS populations.

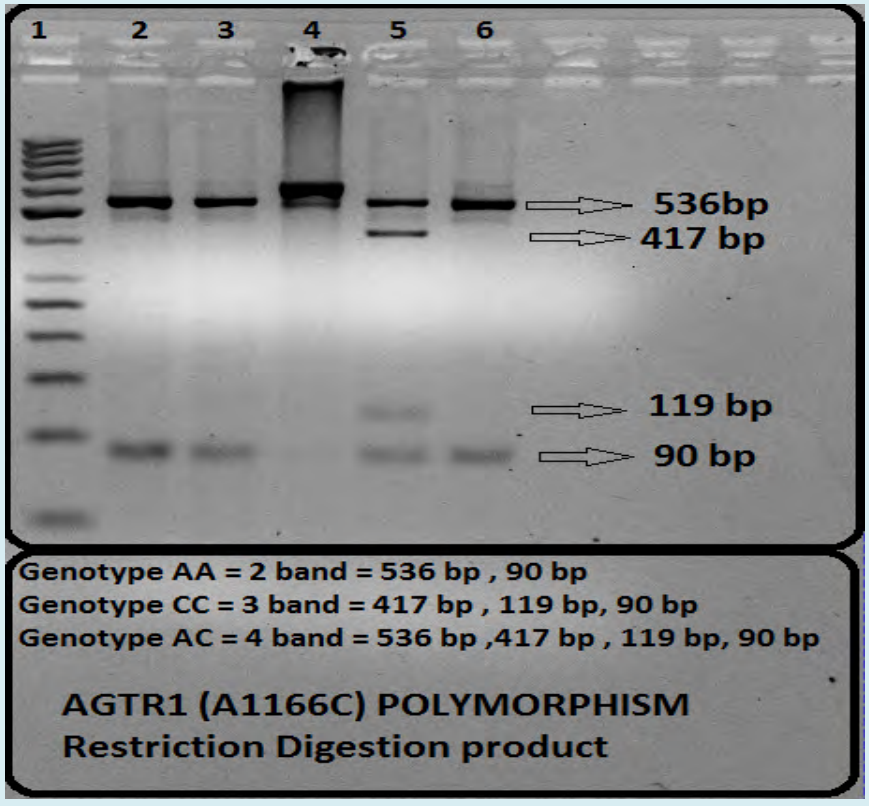

Figure 1: Ethedium bromide stained 2.5% agarose gel shows digested products for AGTR1 A1166C polymorphism(rs5186) by HpyF3I (DdeI) restriction enzyme .
Fragments for allele 'A' = 536 bp and 90 bp ; allele 'C '= 417 bp, 119 bp, and 90bp.
Lane 1: DNA marker.; **Lane 2,3,6 :** Genotype AA : 2 Fragments of 536 bp and 90 bp ; **Lane 4:** Undigested PCR product ; **Lane 5**: Genotype AC : 4 fragments of 536 bp, 417 bp , 119 bp and 90 bp.

AGTR1 Gene Polymorphismrs5186 (A1166C) Male Female				Sex		Total
A A	Study Group	Control	Count	154	108	262
			% within Study Group	58.8%	41.2%	100.0%
		T2DM	Count	108	94	202
			% within Study Group	53.5%	46.5%	100.0%
		T2DN	Count	76	72	148
			% within Study Group	51.4%	48.6%	100.0%
	Total % within Study Group		Count	338	274	612
			55.2%	44.8%	100.0%	
A C	Study Group	Control	Count	26	16	42
			% within Study Group	61.9%	38.1%	100.0%
		T2DM	Count	16	20	36
			% within Study Group	44.4%	55.6%	100.0%
		T2DN	Count	12	6	18
			% within Study Group	66.7%	33.3%	100.0%
	Total % within Study Group		Count	54	42	96
			56.2%	43.8%	100.0%	
C C	Study Group	T2DM	Count	4	4	8
			% within Study Group	50.0%	50.0%	100.0%
		T2DN	Count	2	0	2
			% within Study Group	100.0%	.0%	100.0%
	Total % within Study Group		Count	6	4	10
			60.0%	40.0%	100.0%	

Table 1: Sex wise distribution of study groups on the basis of AGTR1 Gene Polymorphism.

SNP	Genotype	Control		T2DM		T2DNH	
		N=304	%	N=246	%	N=168	%
rs5186	A A	262	86.18	202	82.11	148	88.10
	A C	42	13.82	36	14.63	18	10.71
	C C	0	0.00	8	3.25	2	1.19

Table 2: Genotype distribution of rs5186 (A1166C) polymorphism among the study groups.

SNP	Alleles	Study group	ObsHET	PredHET	HWpval	MAF
rs5186	A:C	CON	0.138	0.129	0.4369	0.069
		T2DM	0.146	0.189	**0.004***	0.106
		T2DNH	0.107	0.122	0.2876	0.065

***:**Significant

Table 3: Hardy-Weinberg Equilibrium (HWE) test for rs5186 (A>C) of AGTR1 gene among the study groups.

SNP	A1	A2	Between Study group	F_A	F_U	CHISQ	P	OR	L95	U95
rs5186	C	A	CON vs. T2DM	0.1057	0.0690	4.664	**0.0308***	1.593	1.041	2.437
			CON vs. T2DNH	0.0654	0.0690	0.0444	0.833	0.944	0.553	1.61
			T2DM vs. T2DNH	0.0654	0.1057	3.967	**0.0463***	0.592	0.352	0.996

***:**Significant

Table 4: Fisher exact test for allelic association of SNP rs5186 (A>C) of AGTR1 gene among the study groups.

SNP	A1	A2	Between Study group		GENO	TREND	DOM	REC
rs5186	C	A	CONVs.T2DM	AFF	8/36/202	52/440	44/202	8/238
				UNAFF	0/42/262	42/566	42/262	0/304
				CHISQ	NA	4.26	NA	NA
				p	NA	0.04*	NA	NA
			CONVs.T2DNH	AFF	2/18/148	22/314	20/148	2/166
				UNAFF	0/42/262	42/566	42/262	0/304
				CHISQ	NA	0.0447	NA	NA
				p	NA	0.8326	NA	NA
			T2DMVs.T2DNH	AFF	2/18/148	22/314	20/148	2/166
				UNAFF	8/36/202	52/440	44/202	8/238
				CHISQ	NA	3.31	NA	NA
				p	NA	0.06	NA	NA

*Significant

Table 5: Fisher model test for SNP rs5186 of AGTR1 gene among the study groups.

Quantitative variables	AGTR1 GENE POLYMORPHISM			ANOVA	
	AA (n=612)	**AC (n=96)**	**CC (n=10)**	**F**	**Sig.**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Age (Years)	54.91 ± 7.57	53.9 ± 7.099	55.8 ± 9.343	0.846	0.43
Height (cm)	160.47 ± 9.45	160.24 ± 10.29	157.72 ± 10.74	0.419	0.658
Weight (kg)	61.43 ± 12.31	61.18 ± 10.84	72.67 ± 15.75	4.245	0.015*
BMI (kg/m^2)	23.77 ± 3.83	23.85 ± 3.98	29.89 ± 8.910	11.787	0.000*
SBP(mmHg)	134.87 ± 24.80	134.94 ± 23.15	138 ± 25.29	0.08	0.923
DBP (mmHg)	84.33 ± 11.53	86.73 ± 10.94	84.00 ± 8.43	1.852	0.158
Glucose (mg/dl)	130.23 ± 53.986	126.58 ± 36.089	131.2 ± 38.142	0.209	0.811
Cholesterol (mg/dl)	176.10 ± 39.95	183.37 ± 33.14	164.27 ± 24.15	1.984	0.138
Triglyceride (mg/dl)	160.89 ± 76.39	180.83 ± 84.11	200.16 ± 64.21	3.857	0.022*
Creatinine (mg/dl)	2.33 ± 2.31	2.07 ± 2.00	2.11 ± 1.88	0.544	0.581
Total Protein (g/dl)	7.55 ± 0.96	7.67 ± 1.18	8.76 ± 0.86	7.747	0.000*
Albumin (g/dl)	4.21 ± 0.69	4.25 ± 0.69	4.62 ± 0.33	1.91	0.149
Globulin (g/dl)	3.34 ± 0.81	3.41 ± 0.81	4.14 ± 0.94	5.043	0.007*

*Significant

Table 6: Comparison of mean values of different quantitative variables among the study groups based on genotype of AGTR1 gene polymorphism.

POPULATION			Alleles A	Alleles C
PRESENT STUDY	INDIAN	BENGALEE	0.919	0.081
1000GENOMES	ASIAN	ASN	0.93	0.07
		CHB	0.948	0.052
		JPT	0.938	0.062
		CHS	0.905	0.095
	EUROPEAN	EUR	0.728	0.272
		CEU	0.694	0.306
		TSI	0.77	0.23
		FIN	0.769	0.231
		GBR	0.68	0.32
		IBS	0.679	0.321
	AFRICAN	AFR	0.974	0.026
		YRI	0.989	0.011
		ASW	0.91	0.09
	AMERICAN	AMR	0.765	0.235
		MXL	0.742	0.258
		PUR	0.791	0.209
		CLM	0.767	0.233

Table 7: Allele frequencies of AGTR1 gene variant rs5186 in present study groups and World population (1000 GENOME PROJECT) [45].

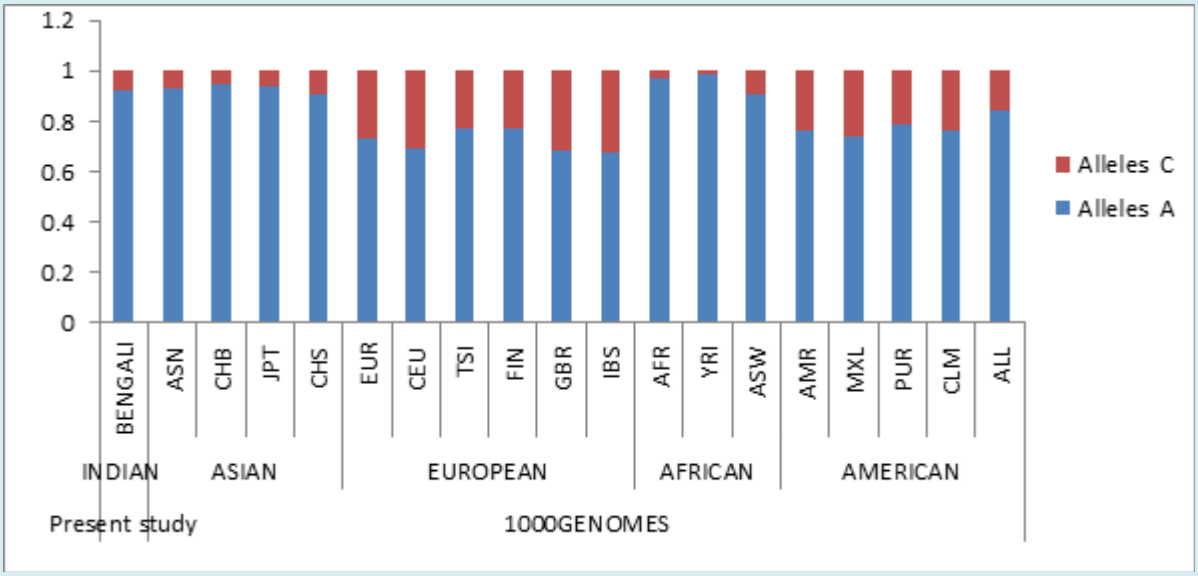

Figure 2: Allele frequencies of AGTR1 gene variant rs5186 among the study groups and World population (1000 GENOME PROJECT) [45].

Discussion

Insensitivity to insulin or type2 diabetes is diligently associated to the metabolic syndrome [45,46], and the RAAS have a pivotal role in the insulin sensitivity [47-49] as well as in the regulation of blood pressure, maintaining stable equilibrium of sodium ion and extracellular fluid volume [50-51].

In our preceding work we have found that RAAS polymorphisms are genetically susceptible for hypertension, and renal complication associated with diabetes [13,32,33,41]. Though various studies have found the association of RAAS variants with hypertension [52-56], atherosclerosis [57], progression of renal disease [35,53,58], diabetic nephropathy 29], the relevance of RAAS polymorphism regarding insulin resistance yet has not been fully illuminated. Polymorphisms of different candidate of the RAAS i.e. *REN*, *ACE*, *AGT*, *AGTR1* have been explained with controversial results [21,34-39,59,60] mainly elucidated by the diverse ethnic backgrounds of the study population.

Out of several single nucleotide polymorphisms within the *AGTR1* gene, the best evaluated polymorphism is rs5186 (A1166C) where nucleotide Adenine (A) is substituted by Cytosine (C). The A allele devoid of restriction enzyme site (DdeI) therefore produce larger fragment, whereas the C allele carries the enzyme-restriction site at nucleotide position 1166, therefore produce smaller fragment [54]. This rs5186 (A1166C) polymorphism of the AGTR1 gene was reported to be associated with a number of cardiovascular outcomes [61-64], essential hypertension [20,65-66], and has been proposed as a predictor of renal injury in T2DM [21]. The C allele (either in homozygous CC or heterozygous AC) carrying Type 2 diabetes patients showed rapid deterioration of renal function than those with the AA genotype and this was confirmed in a later study which showed that the A1166C polymorphism was associated with the development of renal disease and progression to end-stage renal failure [67-69]. A previous study reported that the A1166C polymorphism may contribute to nephropathy development, particularly in T2DM patients and the homozygote variant genotypes (CC) for the risk of DN [70]. Although a recent Japanese study reported that the impact of C allele on progression of DN in a small number of Japanese women T2DM patients [34]. However, a study from china reported that AGTR1 is not a contributing factor for DN in T2DM patient in Chinese population [71].

The result of our study have replicate findings of previous studies who have reported synergistic effect between the AGTR1 C1166 allele and poor glycemic control on risk of developing nephropathy in type 2 diabetic patients. The present study also reveals that the AGTR1 A1166C polymorphism has a positive association with T2DM (OR =1.593, $p < 0.05$). This polymorphism with C allele may contribute to diabetic nephropathy development particularly in T2DM patients and this result is in agreement with previous meta-analysis study [70].

Previously it was considered, the RAAS as an endocrine system resulting in the production of angiotensinogen

in the liver, which is split by renin released from the renal juxtaglomerular cells [72]. But over the past three decades several studies revealed that local RAAS independently operate from their systemic counterpart [73]. A local RAAS along with its all the members present in the proximal tubular cells of the kidney. Angiotensin II actively produced from the proximal tubular cell, which also secretes angiotensinogen into the urine [73]. Intraluminal angiotensinogen perhaps converted to Ang II in the distal tubules, which leads to the stimulation of sodium channels independent of aldosterone [74]. Poor glycemic control and abnormal quantities of protein in urine could stimulate the synthesis of local Ang II mainly by oxygen species as signal transducers [17]. AGTR1 expression is induced by various stimuli, but down regulated by high conc. of Ang II [17], a feedback mechanism of gene regulation. Now the polymorphic *AGTR1* may be insensitive to this down regulation by Ang II and therefore predisposed to renal dysfunction in hyperglycemic condition. It is beyond our scope to investigate this idea, but one can design an experimental set up to explore any such contribution of *AGTR1* gene polymorphism to the renal dysfunction in individual who is suffering from poor glycemic control.

Conclusion

To expose a particular disease and also to identify its tendency within a particular population in a particular ethnic background, the delineation of the responsible functional genes of the genome is of great importance which eventually be of support to the doctors for recommending personalized medicine.

The study reveals that the AGTR1 A1166C polymorphism has a positive association with T2DM and C allele may contribute to diabetic nephropathy development particularly in T2DM patients of Bengali population of Eastern India. The present study has to be taken under consideration within its limitations, that it was limited to a specific ethnic group (Eastern Indian Bengali population). The CC genotype individual are relatively shorter and shows higher mean values of weight, BMI, triglyceride, total protein, globulin compared to AA and AC genotype groups. A larger study from different ethnic groups will be needed to confirm for any contribution of AGTR1 gene polymorphism to T2DM complications for development of renal problem or nephropathy. It is to mention here that this research work only deals with the association study (irrespective of gender) between diabetic nephropathy and the genetic variation of AGTR1 gene within its 5' end of the 3'-untranslated region.

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Availability of Data Materials: Although the study is not a clinical trial, it is a genetic study. All data submitted to the Anthropological survey of India.

Conflicts of Interest

The authors declare that they have no competing interests.

Consent for Publication

Although the manuscript does not involve the use of live photographs of any of the participants, consent was obtained from them for the data to be published as at the time recruitment into the study.

Ethics Approval and Consent to Participate

Ethical committee clearance was obtained from the respective medical institutions and Ethical committee of the Anthropological Survey of India, Govt. of India.. Verbal and written well informed consent was obtained from all participants before they were eligible for recruitment into the study.

Authors' Contributions

PP was involved in the experiments, screening for gene mutations, performed the statistical analysis as well as participating in the write up of the manuscript; KH contributed to preparation of the manuscript. All authors read and approved the final manuscript.

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