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ORIGINAL ARTICLE

Non-coding Single Nucleotide Variants of Renin and the (Pro)renin Receptor are Associated with Polygenic Diseases in a Bangladeshi Population

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Abstract

Non-coding variants or single-nucleotide polymorphisms (SNPs) play pivotal roles in orchestrating pathogeneses of polygenic diseases, including hypertension (HTN) and diabetes. Renin–angiotensin system (RAS) components—renin and (pro)renin receptor [(P)RR]—maintain homeostasis of body fluids. Genetic variants of RAS components are associated with risk of HTN and type 2 diabetes (T2D) in different ethnic groups. We identified associations of SNPs within the renin and (P)RR genes with HTN, T2D, and T2D-associated hypertension in 911 unrelated Bangladeshi individuals. Five non-coding SNPs were involved in modulating regulatory elements in diverse cell types when tagged with other SNPs. rs61827960 was not associated with any disease; rs3730102 was associated with increased risk of HTN and T2D while under dominant model, it showed protective role against T2D-associated HTN. SNP rs11571079 was associated with increased risk of HTN and T2D-associated HTN and decreased risk of T2D, exerting a protective effect. Renin haplotypes GCA and GTG were related to increased risk of T2D and T2D-associated HTN, respectively. Heterogeneous linkage of genotypic and allelic frequencies of rs2968915 and rs3112298 of (P)RR was observed. The (P)RR haplotype GA was associated with increased risk of HTN and significantly decreased risk of T2D. These findings highlight important roles of non-coding variants of renin and (P)RR genes in the etiology of several polygenic diseases.

Keywords Renin and (Pro)renin receptor · Type 2 diabetes · Hypertension (HTN) · Diabetes-associated hypertension · Bangladeshi population

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Introduction

The multifaceted renin–angiotensin system (RAS) controls homeostasis of body fluids and ultimately regulates blood pressure, and renin is its key enzyme. The (pro)renin receptor [(P)RR], ubiquitously expressed in different tissues, has also been recognized to be a key member of the RAS family. Both renin and (P)RR take part in tissue-specific RAS in the heart, blood vessels, kidney, adrenal gland, pancreas, central nervous system, reproductive system, and lymphatic and adipose tissue (Lavoie and Sigmund 2003; Spät and Hunyady 2004). Abnormality in RAS causes high blood pressure that significantly contributes to cardiovascular disease, stroke, kidney failure, disability, and premature death (Alwan et al. 2010; Kuhlemeier 1994). Researchers have suggested the use of circulating renin as a diagnostic and prognostic biomarker for cardiovascular disease (Volpe et al. 2012). By contrast, plasma levels of prorenin, the pre-active form of renin, are considered to be a powerful early marker of diabetic nephropathy and retinopathy (Stankovic et al. 2006), whereas altered synthesis of renin was reported in insulin-dependent diabetes (Amemiya et al. 1990).

Diabetes, specially T2D, HTN, and T2D-associated HTN are global health burden. The global frequency of HTN has been predicted to increase by 60% during the first 25 years of the twenty-first century (Kearney et al. 2005). According to the World Health Organization, the prevalence of diabetes increased from 4.7% in 1980 to 8.5% in 2014, with 1.6 million deaths being directly caused by diabetes worldwide in 2015 (American Diabetes Association 2016). Bangladesh is no exception. Studies have claimed that approximately 20% of adults and 40–60% of elderly people in Bangladesh suffer from HTN (Monwarul Islam and Majumder 2012; Chowdhury et al. 2016), while Bangladesh has the third largest diabetic population (8.4 million, or 10% of the population) in the world, with the prevalence of these non-communicable diseases (NCDs) increasing (Akter et al. 2014; Khalequzzaman et al. 2017).

Single-nucleotide polymorphisms (SNPs) play pivotal roles in pathogenesis of genetic disease (Chaudhary et al. 2015). With the advent of genomics research, SNPs residing within exonic sequences received top priority for the identification of the effect on a gene function. Genome-wide association studies demonstrated relation of many risk SNPs to T2D and HTN (Xue et al. 2018; Wang and Wang 2018). However, intronic sequences also harbour functional polymorphisms that may influence the expression of the respective gene (Cooper 2010). Genome-wide association studies (GWAS) revealed that most disease-associated SNPs are present within the non-coding sequences or outside the protein-coding sequences (McCarthy and Hirschhorn 2008). Evidence suggested that these variants may modify functions of enhancer elements, promoter regions, DNase hypersensitivity regions, and chromatin marks (McCarthy and Hirschhorn 2008). Fewer than 10% of the GWAS SNPs affect coding sequences, with most non-coding variants being concentrated in DNA stretches marked by deoxyribonuclease I (DNase I) hypersensitive regions where they seem to disturb transcription factor-binding sites that may affect the expression level of the gene (Hrdlickova et al. 2014).

Generally, both environmental and genetic factors contribute to the development of HTN and diabetes. Both diseases are polygenic. Also, there is a substantial overlap between diabetes and HTN in terms of etiology and disease mechanisms (Cheung and Li 2012). Studies have reported associations of high blood pressure or HTN with diabetes in South Korean, Japanese, and European populations, including Germans (Cho et al. 2015; Hayashi et al. 1999; Meisinger et al. 2008; Ellencweig and Grafstein 1989; Cheung 2010). HTN occurs in approximately 30% of patients with type 1 diabetes and in 50% to 80% of patients with type 2 diabetes in the US population (Tsimihodimos et al. 2018).

It has been demonstrated that 30–60% of HTN cases are linked to genetic causes (Franceschini and Le 2014; Hirose et al. 2011; Ott et al. 2011; Satofuka et al. 2009), whereas genetic factors are also closely associated with the development of diabetes (Ghafar et al. 2020; Montesanto et al. 2018; Saha et al. 2019; Huda et al. 2018). Evidence revealed associations of gene polymorphisms of the renin–angiotensin aldosterone system with diabetes and diabetes-associated complications, such as HTN (Ghafar 2018), retinopathy (Luo et al. 2016), nephropathy (Wang et al. 2012), and cardiovascular complications (Ghafar 2020a, b). Animal model studies have clearly demonstrated that the development of HTN is associated with variants of the renin gene (Dene et al. 1989). Statistically significant associations between the incidence of allelic variants of renin and essential HTN have been reported in populations in the United Arab Emirates and among US whites (Frossard et al. 1999). In addition, Deinum et al. reported a relationship between diabetes caused nephropathy and a *Bgl*I RFLP in the first intron of the renin gene (Deinum et al. 1999). A significant association between the presence of the non-coding IVS5 + 169C>T variant of the (P)RR gene with increased ambulatory blood pressure was reported in Japanese men but not in women (Hirose et al. 2011). The significantly higher ambulatory blood pressure of T allele carriers was also confirmed in Caucasian men for the systolic but not the diastolic blood pressure (Ott et al. 2011). Satofuka et al. provided evidence that (pro)renin receptor-mediated signal transduction and tissue RAS contributed to diabetes-induced retinal inflammation (Satofuka et al. 2009).

The incidences of diabetes and HTN as well as associated diseases are increasing in Bangladesh. Thus, it is important to understand the genetics of these diseases in this population. Though studies have reported an association between gene polymorphisms in the RAS components and HTN in different populations with varying results (Dene et al. 1989; Frossard et al. 1999; Deinum et al. 1999), but association studies of renin and (P)RR gene polymorphisms with HTN, type 2 diabetes (T2D), and diabetes-associated HTN among Bangladeshi populations are rare. Thus, the present study was undertaken to investigate the association of the renin genotypes with regard to SNPs rs61827960, rs11571079, and rs3730102 and (P)RR genotypes with regard to SNPs rs2968915 and rs3112298 and their respective haplotypes with the incidence of the polygenic diseases T2D, HTN, and T2D-associated HTN to shed light on the genetics of these diseases with specific reference to a Bangladeshi population.

Methods

Subject Selection and Sample Collection

A total of 911 unrelated Bangladeshi individuals were enrolled in the present study. Among them, 299 individuals were healthy controls, 169 had HTN, 288 had T2D, and 155 suffered from both T2D and HTN. The study was conducted according to the guidelines approved by the ethical review committees of the Department of Biochemistry and Molecular Biology; the Faculty of Biological Sciences, University of Dhaka, Bangladesh (Ref. No. 16/Biol. Sci/2015) as well as Bangladesh University of Health Sciences (Memo No. BUHS/BIO/EA/18/148). Ethical approvals were taken for collecting blood samples to extract DNA samples for performing genetic association studies using polymorphism from candidate genes identifying risk SNPs for T2D, HTN, and T2D-associated HTN. All experiments were performed in accordance with relevant guidelines and regulations. Each participant was explained about the nature of the study and requested to donate blood for the sake of this research work. After obtaining their consent, approximately 5 mL of blood was collected from each subject. Blood samples were used to determine the concentration of glycated haemoglobin (HbA1c) using a standard method. T2D patients and HTN patients were confirmed using the concentrations of fasting plasma glucose (measured by standard glucose oxidase, phenol and aminophenazone or GOD-PAP colorimetric method) and HbA1c (by high-performance liquid chromatography (HPLC)-based ion exchange chromatography in Bio-Rad 10 system from Bio-Rad Laboratories, USA) and SBP/DBP, respectively, according to the criteria set by the World Health Organization (plasma glucose level (>7.0 mmol/L), HbA1c level of $>6.5\%$, SBP >140 mm Hg, and DBP >90 mm Hg) (World Health Organization 1999, pp. 539–553; World Heart Federation). The healthy individuals were people such as students, university employees, and hospital personnel who did not show clinical features of diabetes or HTN and did not exhibit other complications such as acute or chronic infection, kidney, liver, and heart diseases. Pregnant women were also excluded from the present study. Anthropometric and demographic data such as age, gender, height, weight, SBP, and DBP were collected and recorded using a well-defined questionnaire.

The durations of T2D and HTN among the randomly selected patients were 0.5–10 years and 1–8 years, respectively. In the case of T2D-associated HTN, almost all the patients were first diagnosed with T2D, with HTN being diagnosed between 4 and 8 years later. To control blood sugar level, patients were under treatment by drugs such as metformin and sulfonylureas, whereas a few of the patients diagnosed with very high levels of blood sugar were under insulin therapy to control excess glucose level. For controlling high blood pressure, patients were advised to take anti-hypertensive drugs such as angiotensin-converting enzyme (ACE) inhibitors (e.g. captopril and ramipril), or angiotensin receptor blockers (e.g. valsartan and losartan).

Extraction of Genomic DNA and Genotyping

White blood cells from the collected blood were used to extract genomic DNA, with the quantity and quality of the extracted DNA being verified according to our previous method (Saha et al. 2019; Huda et al. 2018; Afruza et al. 2014). Briefly, DNA was extracted using organic method employing EDTA (0.5 M, pH 8.0), Tris-HC1 (1 M, pH 7.6), red blood cell lysis buffer (1 M Tris, sucrose and $MgC1_2$, pH 8.0), Triton X-100, and SDS (Afruza et al. 2014).

To perform genotyping analyses, Applied Biosystems™ TaqMan® SNP Genotyping Assay Mixes (Applied Biosystems) were used with the following TaqMan IDs: rs61827960 (C__88357500_10), rs11571079 (C__31567084_10), rs3730102 (C__27472649_10) for the renin gene, and rs2968915 (C__15881558_20) and rs3112298 (C__31014389_10) for the (pro)renin receptor gene. Genotyping analyses were performed on an ABI 7500 Real-Time PCR system (Applied Biosystems) according to the manufacturer's guidelines. To control the quality of each experiment, negative controls were included for each plate used for the qPCR reaction. The overall genotyping call rate was 98%.

Analysis of Linkage Disequilibrium

Non-random association of SNPs at multiple loci, i.e. linkage disequilibrium (LD), was analysed using the LDlink web-based tool available at https://ldlink.nci.nih.gov/?tab=home. The values of D' indicate coinheritance of alleles. By contrast, values of the correlation coefficient (R^2) take account of allele frequency. As the 'R' measure is more commonly used to determine how well an SNP can act as a replacement for another SNP, and is sensitive to allele frequencies, this measure was accorded greater emphasis in determining the LD.

Determination of Sample Size

To determine the number of samples required to perform the study or for power calculations, a standard formula, $n = Z^2 \times p\ (1 - p)/d^2$, was used where n = required number of samples, 'Z' = 1.96, 'p' denotes expected minor allele frequencies of each SNP selected (obtained from 1000 genome project), and 'd' stands for the minimum degree of risk association. Being the common diseases worldwide, the heritability of T2D, HTN, or T2D-associated HTN varied from population to population with up to 80% in case of monozygotic twin-based studies. Recent genome-wide association studies demonstrated relation of many risk SNPs to T2D and HTN (Xue et al. 2018; Wang and Wang 2018) even using stringent threshold p values (5×10^{-9}), and degree of risk association varied at the level of 5% to 7%. In the present case–control study, we considered that association of the SNPs of interest will be at the average level of 6% for calculating the number of samples or for power calculations. Thus, the value of 'd' was 0.06. Considering these facts and above formula, the minimum

number of samples required for each SNPs varied from 20 to 177 while 80% power was considered at 5% confidence interval. Here, we analysed a total of 911 samples that include 299 healthy controls as well as 288 T2D, 169 HTN, and 155 T2D-associated HTN patients.

Statistical Analyses

Demographic data obtained from the structured questionnaire and clinical parameters were analysed using SPSS v21.0 (IBM) in which the results were expressed as mean ± SD for continuous variables and % for categorical variables. To compare the differences between different variables from the control and patients, Student's independent *t* test was performed.

For association studies and haplotype frequency determination, a web-based tool SNPStats (https://www.snpstats.net/start.htm) was used based on R packages (Solé et al. 2006) and haplo.stats (Schaid et al. 2005). Logistic regression analyses were performed to determine the association of each SNP with HTN, T2D, and T2D-associated HTN under following genetic models: codominant, dominant, recessive, over-dominant, and log-additive models. The data have been summarized as genotype and allele frequencies, proportions, and OR with a 95% confidence interval. Selection of the best models for interpreting association studies was carried out according to the values of the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) that scrutinize the quality of each model used. The models with the lowest AIC and BIC values were preferred. Haplotype frequency and its association with diseases were analysed using logistic regression analyses, shown as OR at 95% confidence interval. All the association analyses presented in this study were performed after adjusting the important covariates in a multivariate model: age, BMI, SBP, and DBP. A p value ≤ 0.05 was considered to be statistically significant.

Results

Demographic, Anthropometric, and Clinical Data of the Study Participants

The demographic, anthropometric, and clinical data of the study participants are summarized in Table 1. Out of 299 healthy individuals, 150 (50.17%) were male, and 149 (49.83%) were female. Of 169 hypertensive subjects, 118 (69.8%) were male, and 51 (30.2%) were female. Among the 288 T2D subjects, 143 (49.65%) were male, and 145 (50.35%) were female.

Results of statistical analyses and the levels of significance between the different parameters of the study subjects and the healthy individuals are also presented in Table 1. Statistical analyses revealed that all the demographic, anthropometric, and clinical parameters varied significantly between healthy controls and patients with type 2 diabetes, hypertension, T2D-associated HTN. However, the levels of fasting

Table 1 Demographic and biochemical characteristics of the study subjects

Parameters	CN (299)	*p* values of gender difference	HTN (169)	*p* values of gender difference	*p* values (CN vs HTN)	T2D (288)	*p* values of gender difference	*p* values (CN vs T2D)	T2D + HTN (155)	*p* values of gender difference	*p* values (CN vs T2D + HTN)
Demographic parameters											
Sex (male or female)	*M* = 150 *F* = 149		*M* = 118 *F* = 51			*M* = 143 *F* = 145			*M* = 129 *F* = 26		
Age (year)	50.06 ± 10.36	0.695	56.34 ± 11.24	0.863	< 0.001	50.72 ± 11.16	0.103	> 0.05	57.21 ± 9.83	0.539	< 0.001
Height (m)	1.65 ± 0.10	0.013	1.61 ± 0.08	0.349	< 0.001	1.54 ± 0.09	0.766	< 0.001	1.53 ± 0.09	0.001	< 0.001
Body weight (kg)	62.69 ± 10.60	0.107	66.65 ± 7.04	0.505	< 0.001	58.73 ± 4.76	0.788	< 0.001	55.28 ± 9.10	0.339	< 0.001
BMI (kg/m^2)	23.03 ± 4.20	0.705	25.60 ± 2.97	0.781	< 0.001	24.82 ± 3.43	0.687	< 0.001	23.77 ± 4.02	0.001	= 0.063
Systolic BP (mm Hg)	108.23 ± 11.39	0.070	133.43 ± 21.12	0.080	< 0.001	123.26 ± 8.12	0.089	< 0.001	141.32 ± 22.48	0.056	< 0.001
Diastolic BP (mm Hg)	71.69 ± 7.77	0.330	84.35 ± 11.58	0.226	< 0.001	82.07 ± 7.22	0.288	< 0.001	90.74 ± 14.06	0.001	< 0.001
Biochemical parameters											
FBG (mmol/L)	5.51 ± 0.69	0.248	5.66 ± 0.78	0.032	= 0.052	10.82 ± 4.10	0.038		11.45 ± 1.74	0.178	< 0.001
HbA1c (%)	5.39 ± 0.35	0.239	5.98 ± 0.23	0.366	= 0.053	8.74 ± 1.69	0.288		11.31 ± 4.33	0.262	< 0.001

Values are mean ± SD

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control individuals, *FPG* fasting plasma glucose, *HbA1C* glycated haemoglobin, *p* probability

plasma glucose and HbA1c between healthy controls and patients with HTN as well as the values of BMI between healthy controls and patients with T2D-associated HTN did not vary significantly (Table 1).

Frequency Distribution and Disease Risk Assessment of Renin rs61827960 Genotypes and Alleles

It was noticed that the frequency of genotypes containing homozygous for the mutant alleles (TT) was more than fivefold higher in the patients with HTN than in both the populations with T2D and T2D-associated HTN. Also, frequency of TT genotype was more than fourfold higher in HTN population compared to healthy individuals. Data are presented in Table 2.

Association analyses under any of the models of inheritance revealed that neither of the genotypes was associated with increased risk of any disease (Table 2). However, mutant allele T was found to be associated with increased risk of HTN. Additionally, no association was observed with an increased risk of any disease under the log-additive model.

Frequency Distribution and Disease Risk Assessment of Renin rs11571079 Genotypes and Alleles

A genotypic frequency analysis revealed that the heterozygotes (CT) were the least frequent genotype in the all three study groups, except in diabetic subjects. The frequency of the mutant allele containing genotype was almost threefold higher in the HTN subpopulation than in their healthy counterparts, and we did not find individuals of the TT genotype in the study participants with T2D (Table 3).

Statistical analyses revealed that the mutant T allele was associated with an increased risk of HTN and T2D-associated HTN and found to play a protective role in subpopulations with T2D (Table 3). Under the codominant model, the TT genotype in rs11571079 was significantly associated with an increased risk of HTN and T2D-associated HTN. Notably, under this model, the CT genotype exhibited a protective role against T2D. Under the recessive model, this variant showed an association with increased risk of both HTN and T2D-associated HTN. In the case of the dominant model, rs11571079 demonstrated an association with the risk of developing T2D-associated HTN. Under the log-additive model, rs11571079 showed an association of increased risk of all the diseases considered in the present study (Table 3).

Frequency Distribution and Disease Risk Assessment of (P)RR rs3730102 Genotypes and Alleles

Distribution patterns of genotypic and allelic frequencies with respect to rs3730102 are presented in Table 4.

With respect to the renin SNP rs3730102, genotypic frequencies under each of the genetic models were associated with an increased risk of T2D. Furthermore,

Table 2 Genotypic frequencies with respect to the genetic models of SNP rs61827960 of the human renin gene in subpopulations with hypertension, type 2 diabetes (T2D) and T2D-associated hypertension

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Codominant	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
GG	115 (65)	214 (73.8)	OR: 0.89 (0.47–1.69) $p=0.22$	197 (70.4)	214 (73.8)	OR: 1.03 (0.62–1.71) $p=0.86$	110 (71)	214(73.8)	OR: 1.10 (0.52–2.32) $p=0.7$
vs	vs			vs			vs		
GT	37 (21.9)	69 (23.8)		78 (27.9)	69 (23.8)		42 (27.1)	69 (23.8)	
GG	115 (65)	214 (73.8)	OR: 2.82 (0.81–9.78) $p=0.22$	197 (70.4)	214 (73.8)	OR: 0.68 (0.16–2.83) $p>0.05$	110 (71)	214 (73.8)	OR: 0.43 (0.05–3.64) $p>0.05$
vs	vs			vs			vs		
TT	17 (10.1)	7 (2.4)		5 (1.8)	7 (2.4)		3 (1.9)	7(2.4)	
Dominant									
GG	115 (65)	214 (73.8)	OR: 1.09 (0.60–1.97) $p=0.78$	197 (70.4)	214 (73.8)	OR: 0.99 (0.60–1.62) $p=0.97$	110 (71)	214 (73.8)	OR: 1.02 (0.49–2.09) $p=0.97$
vs	vs			vs			vs		
TT + GT	54 (31.9)	76 (26.2)		83 (29.6)	76 (26.2)		45 (29)	76 (26.2)	
Recessive									
GG + GT	152 (89.9)	283 (97.6)	OR: 2.90 (0.84–9.97) $p=0.08$	275 (98.2)	283 (97.6)	OR: 0.67 (0.16–2.79) $p=0.59$	152 (98.1)	283(97.6)	OR: 0.42 (0.05–3.51) $p=0.42$
vs	vs			vs			vs		
TT	17 (10.1)	7 (2.4)		5 (1.8)	7 (2.4)		3 (1.9)	7 (2.4)	
Over-dominant									
GG + TT	132 (78.1)	221 (76.2)	OR: 0.83 (0.44–1.57) $p=0.56$	202 (72.1)	221 (76.2)	OR: 1.04 (0.63–1.73) $p=0.87$	113 (72.9)	221 (76.2)	OR: 1.13 (0.54–3.51) $p=0.74$
vs	vs			vs			vs		
GT	37 (21.9)	69 (23.8)		78 (27.9)	69 (23.8)		42 (27.1)	69 (23.8)	
Log-wadditive	1.23 (0.77–1.97) $p=0.38$			0.96 (0.62–1.48) $p=0.84$			0.93 (0.49–1.77) $p=0.83$		

Table 2 (continued)

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Alleles	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
G	267	498	OR: 1.61 (1.14–2.29) $p=0.00$*	474	498	OR: 1.10 (0.79–1.53) $p=0.56$	262	498	OR: 1.11 (0.76–1.64) $p=0.59$
vs	vs			vs			vs		
T	71	82		86	82		48	82	

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control subpopulation

Numbers within the parentheses denote percent distribution of each genotype. '*n*' indicates the number of individuals in each genotype; *indicates statistically significant relation

Table 3 Genotypic frequencies with respect to SNP rs11571079 of the human renin gene in populations with hypertension, type 2 diabetes (T2D) and T2D-associated hypertension

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Codominant	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
CC	152 (89.9)	271 (93.5)	–	276 (98.6)	271 (93.5)	OR: 0.53 (0.09–3.17) $p=0.039$*	136 (87.7)	271 (93.5)	OR:2.12 (0.33–13.53) $p=0.078$
vs	vs			vs			vs		
CT	0	9 (3.1)		4 (1.4)	9 (3.1)		6 (3.9)	9 (3.1)	
CC	152 (89.9)	271 (93.5)	OR: 4.67 (1.44–15.16) $p=0.0085$*	276 (98.6)	271 (93.5)	–	136 (87.7)	271 (93.5)	OR: 5.27 (1.16–23.97) $p<0.01$*
vs	vs			vs			vs		
TT	17 (10.1)	10 (3.5)		0	10 (3.5)		13 (8.4)	10 (3.5)	
Dominant									
CC	152 (89.9)	271 (93.5)	OR: 2.44 (0.89–6.69) $p=0.085$	276 (98.6)	271 (93.5)	OR: 0.25 (0.05–1.11) $p=0.054$	136 (87.7)	271 (93.5)	OR: 3.67 (1.10–12.19) $p=0.034$*
vs	vs			vs			vs		
TT + CT	17 (10.1)	19 (6.5)		4 (1.4)	19 (6.5)		19 (12.3)	19 (6.5)	
Recessive									
CC + CT	152 (89.9)	280 (96.5)	OR: 4.80 (1.47–15.62) $p=0.0084$*	280 (100)	280 (96.5)	–	142 (91.6)	80 (96.5)	OR: 5.11 (1.12–23.23) $p=0.035$*
vs	vs			vs			vs		
TT	17 (10.1)	10 (3.5)		0	10 (3.5)		13 (8.4)	10 (3.5)	
Over-dominant									
CC + TT	169 (100)	281 (96.9)	–	276 (98.6)	281 (96.9)	OR: 0.54 (0.09–3.26) $p=0.19$	149 (96.1)	281 (96.9)	OR: 1.19 (0.30–12.05) $p=0.67$
vs	vs			vs			vs		
CT	0	9 (3.1)		4 (1.4)	9 (3.1)		6 (3.9)	9 (3.1)	
Log-additive	1.90 (1.083.36) $p=0.026$*			0.28 (0.08–1.03) $p=0.022$*			2.27 (1.11–4.64) $p=0.024$*		

Table 3 (continued)

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Alleles	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
C	304	551	OR: 2.13 (1.27–3.56) $p = 0.003$*	556	551	OR: 0.14 (0.05–0.39) $p < 0.01$*	278	551	OR: 2.19 (1.29–3.69) $p = 0.002$*
vs	vs			vs			vs		
T	34	29		4	29		32	29	

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control subpopulation

Numbers within the parentheses denote percent distribution of each genotype. '*n*' indicates the number of individuals in each genotype; *indicates statistically significant relation

Table 4 Genotypic frequencies with respect to the SNP rs3730102 of the human renin gene in subpopulations with hypertension, type 2 diabetes (T2D) and T2D-associated hypertension

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Codominant	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
GG	156 (92.3)	252 (86.9)	–	101 (36.1)	252 (86.9)	OR: 13.12 (7.10–24.25) $p < 0.0001$*	151 (97.4)	252 (86.9)	–
vs	vs			vs			vs		
GA	0	36 (12.4)		160 (57.1)	36 (12.4)		0	36 (12.4)	
GG	156 (92.3)	252 (86.9)	OR: 26.41 (4.13–168.74) $p < 0.0001$*	101 (36.1)	252 (86.9)	OR: 18.31 (3.60–93.24) $p < 0.0001$*	151 (97.4)	252 (86.9)	OR: 3.34 (0.60–18.44) $p = 0.17$
vs	vs			vs			vs		
AA	13 (7.7)	2 (0.7)		19 (6.8)	2 (0.7)		4 (2.6)	2 (0.7)	
Dominant									
GG	156 (92.3)	252 (86.9)	OR: 0.84 (0.36–1.95) $p = 0.69$	101 (36.1)	252 (86.9)	OR: 13.57 (7.49–24.59) $p < 0.0001$*	151 (97.4)	252 (86.9)	OR: 0.17 (0.06–0.50) $p = 0.001$*
vs	vs			vs			vs		
AA + GA	13 (7.7)	38 (13.1)		179 (63.9)	38 (13.1)		4 (2.6)	38 (13.1)	
Recessive									
GG + GA	156 (92.3)	288 (99.3)	OR: 32.45 (4.99–210.86) $p < 0.0001$*	261 (93.2)	288 (99.3)	OR: 7.75 (1.57–38.31) $p = 0.0026$*	151 (97.5)	288 (99.3)	OR: 16.26 (0.74–359.39) $p = 0.058$
vs	vs			vs			vs		
AA	13 (7.7)	2 (0.7)		19 (6.8)	2 (0.7)		4 (2.6)	2 (0.7)	
Over-dominant									
GG + AA	169 (100)	254 (87.6)	–	120 (42.9)	54 (87.6)	OR:10.92 (5.99–19.92) $p < 0.0001$*	155 (100)	254 (87.6)	–
vs	vs			vs			vs		
GA	0	36 (12.4)		160 (57.1)	36 (12.4)		0	36 (12.4)	
Log-additive	1.56 (0.88–2.79) $p = 0.13$			10.27 (5.86–18.01) $p < 0.0001$			0.68 (0.27–1.67) $p = 0.39$		

Table 4 (continued)

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Alleles	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
G	312	540	OR: 1.12 (0.67–1.88) $p = 0.65$	362	540	OR: 7.38 (5.13–10.64) $p \leq 0.001$*	302	540	OR: 0.18 (0.06–0.50) $p \leq 0.001$*
vs	vs			vs			vs		
A	26	40		198	40		4	40	

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control subpopulation

Numbers within the parentheses denote percent distribution of each genotype. '*n*' indicates the number of individuals in each genotype; *indicates statistically significant relation

under the codominant model, the AA genotype showed an association with an increased risk of HTN while under dominant model, rs3730102 showed protective role against T2D-associated HTN. Under the recessive model, rs3730102 showed a strong association with an increased risk of HTN. Further analyses revealed that the A allele was associated with an increased risk of T2D while it was associated with a protective role against T2D-associated HTN. In the log-additive model, rs3730102 appeared to be associated with an increased risk of only T2D but not with HTN or T2D-associated HTN (Table 4).

Frequency Distribution and Disease Risk Assessment of (P)RR rs2968915 Genotypes and Alleles

A genotypic frequency analysis revealed absence of heterozygous genotype from the subpopulation with T2D-associated HTN (Table 5). Statistical analyses revealed that no genotype or allele of rs2968915 within the non-coding region of the human (pro) renin receptor gene was associated with an increased risk of T2D. However, under the codominant, dominant and over-dominant genetic models, the heterozygous GA genotype showed protection against HTN and T2D-associated HTN (Table 5). The minor allele A also displayed a protective role against HTN and T2D-associated HTN. By contrast, under the log-additive model, this SNP was found to be not associated with an increased risk of any of the diseases studied.

Frequency Distribution and Disease Risk Assessment of (P)RR rs3112298 Genotypes and Alleles

The subpopulation with HTN had more than threefold and the subpopulation with T2D-associated HTN had almost threefold higher frequencies of the GA genotype than that of the healthy subpopulation (Table 6). T2D individuals had 2.5-fold lower frequency of GA genotype compared to their healthy counterparts.

Statistical analyses demonstrated a heterogeneous association of rs3112298 with the different diseases considered in the present study (Table 6). This non-coding variant revealed protective characteristics against T2D when the mutant allele frequency and any of the four models of inheritance (except recessive model) were considered. Under the codominant model, the GA genotype showed a significant association with an increased risk of both HTN and T2D-associated HTN, whereas AA genotype showed protective role against HTN and T2D under the same model. With the dominant and over-dominant models, the mutant A allele showed an association with an increased risk of HTN, but under the recessive model, this SNP showed a protective role against HTN. In the log-additive model, this SNP showed a protective role against T2D, and no association was observed with the other two diseases considered in the present study.

Table 5 Genotypic frequencies with respect to the SNP rs2968915 of the human (P)RR gene in populations with hypertension, type 2 diabetes (T2D) and T2D-associated hypertension

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Codominant	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
GG	159 (95.8)	266 (89.6)	OR: 0.11 (0.02–0.77) $p=0.03$*	258 (90.5)	266 (89.6)	OR: 0.62 (0.24–1.61) $p=0.62$	152 (98.1)	266 (89.6)	–
vs	vs			vs			vs		
GA	2 (1.2)	18 (6.1)		16 (5.6)	18 (6.1)		0	18 (6.1)	
GG	159 (95.8)	266 (89.6)	OR: 0.56 (0.13–2.42) $p=0.03$*	258 (90.5)	266 (89.6)	OR: 0.99 (0.32–3.05) $p=0.62$	152 (98.1)	266 (89.6)	OR: 0.82 (0.14–4.71) $p=0.016$*
vs	vs			vs			vs		
AA	5 (3.0)	13 (4.4)		11 (3.9)	13 (4.4)		3 (1.9)	13 (4.4)	
Dominant									
GG	159 (95.8)	266 (89.6)	OR: 0.28 (0.09–0.90) $p=0.022$*	258 (90.5)	266 (89.6)	OR: 0.75 (0.36–1.59) $p=0.45$	152 (98.1)	266 (89.6)	OR: 0.26 (0.06–1.12) $p=0.043$*
vs	vs			vs			vs		
AA + GA	7 (4.2)	31 (10.4)		27 (9.5)	31 (10.4)		3 (1.9)	31 (1.9)	
Recessive									
GG + GA	161 (97.07)	284 (95.6)	OR: 0.59 (0.14–2.57) $p=0.48$	274 (96.1)	284 (95.6)	OR: 1.01 (0.33–3.12) $p=0.98$	152 (98.1)	284 (95.6)	OR: 0.89 (0.15–5.13) $p=0.89$
vs	vs			vs			vs		
AA	5 (3.0)	13 (4.4)		11 (3.9)	13 (4.4)		3 (1.9)	13 (4.4)	
Over-dominant									
GG + AA	164 (98.9)	279 (93.9)	OR: 0.11 (0.02–0.79) $p=0.011$*	269 (94.4)	279 (93.9)	OR: 0.62 (0.24–1.61) $p=0.33$	155 (100)	279 (93.9)	–
vs	vs			vs			vs		
GA	2 (1.2)	18 (6.1)		16 (5.6)	18 (6.1)		0	18 (6.1)	
Log-additive	0.54 (0.26–1.11) $p=0.075$			0.88 (0.54–1.45) $p=0.63$			0.56 (0.23–1.33) $p=0.16$		

Table 5 (continued)

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Alleles	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
G	320	552	OR: 0.47 (0.24–0.90) $p = 0.02$*	532	552	OR: 0.89 (0.57–1.41) $p = 0.83$	304	552	OR: 0.25 (0.10–0.59) $p < 0.001$*
vs	vs			vs			vs		
A	12	44		38	44		6	44	

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control subpopulation

Numbers within the parentheses denote percent distribution of each genotype. '*n*' indicates the number of individuals in each genotype; *indicates statistically significant relation. The models of inheritances with regard to heterozygosity presented here were analysed using only female individuals

Table 6 Genotypic frequencies with respect to the SNP rs3112298 of the human (P)RR gene in populations with hypertension, type 2 diabetes and type 2 diabetes-associated hypertension

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Codominant	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
GG	120 (71.0)	254 (84.9)	OR: 5.04 (2.46–10.32) $p < 0.0001$*	269 (93.4)	254 (84.9)	OR: 0.21 (0.08–0.58) $p = 0.0015$*	122 (78.7)	254 (84.9)	OR: 1.65 (0.67–4.09) $p = 0.0054$*
vs	vs			vs			vs		
GA	47 (27.8)	26 (8.7)		10 (3.5)	26 (8.7)		33 (21.3)	26 (8.7)	
GG	120 (71.0)	254 (84.9)	OR: 0.26 (0.05–1.48) $p = 0.03$*	269 (93.4)	254 (84.9)	OR: 0.31 (0.10–0.97) $p = 0.0015$*	122 (78.7)	254 (84.9)	–
vs	vs			vs			vs		
AA	2 (1.2)	19 (6.3)		9 (3.1)	19 (6.3)		0	19 (6.3)	
Dominant									
GG	120 (71)	254 (84.9)	OR: 3.01 (1.59–5.70) $p < 0.0001$*	269 (93.4)	254 (84.9)	OR: 0.25 (0.11–0.97) $p < 0.0001$*	122 (78.7)	254 (84.9)	OR: 0.99 (0.43–2.29) $p = 0.51$
vs	vs			vs			vs		
AA + GA	49 (29.0)	4 (15.1)		19 (6.6)	45 (15.1)		33 (21.3)	45 (15.1)	
Recessive									
GG + GA	167(98.8)	280 (93.7)	OR: 0.18 (0.03–1.04) $p = 0.032$*	279 (96.9)	280 (93.7)	OR: 0.35 (0.11–1.08) $p = 0.062$	155 (100)	280 (93.7)	–
vs	vs			vs			vs		
AA	2 (1.2)	19 (6.3)		9 (3.1)	19 (6.3)		0	19 (6.3)	
Over-dominant									
GG + AA	122 (72.2)	273 (91.3)	OR: 5.35 (2.62–10.92) $p < 0.0001$*	278 (96.5)	273 (91.3)	OR: 0.23 (0.08–0.62) $p = 0.0031$*	122 (78.7)	273 (91.3)	OR: 2.83 (1.62–4.92) $p = 0.053$
vs	vs			vs			vs		
GA	47 (27.8)	26 (8.7)		10 (3.5)	26 (8.7)		33 (21.3) 33 (21.3)	26 (8.7)	
w	1.61 (0.99–2.62) $p = 0.059$			0.44 (0.26–0.74) $p = 0.0014$*			0.70 (0.35–1.40) $p = 0.74$		

Table 6 (continued)

Genotype models	HTN vs CN			T2D vs CN			HTN + T2D vs CN		
Alleles	Cases vs controls, *n*			Cases vs controls, *n*			Cases vs controls, *n*		
G	287	536	OR: 1.49 (1.00–2.21) $p < 0.001$	548	536	OR: 0.43 (0.27–0.68) $p < 0.001$*	279	536	OR: 1.83 (0.75–4.49) $p = 0.31$
vs	vs			vs			vs		
A	51	64		28	64		33	64	

HTN subpopulation with hypertension, *T2D* subpopulation with type 2 diabetes, *HTN + T2D* subpopulation with hypertension and type 2 diabetes, *CN* healthy control subpopulation

Numbers within the parentheses denote percent distribution of each genotype. '*n*' indicates the number of individuals in each genotype; *indicates statistically significant relation. The models of inheritances with regard to heterozygosity presented here were analysed using only female individuals

Table 7 Common haplotype frequencies and association analyses for rs61827960, rs11571079, rs3730102, rs2968915 and rs3112298 SNP variants of the human renin and (pro)renin receptor genes, respectively

Haplotypes	Haplotypes frequencies				HTN vs CN		T2D vs CN		HTN + T2D vs CN	
	HTN	T2D	T2D + HTN	CN	OR 95% CI	*p*	OR 95% CI	*p*	OR 95%CI	*p*
GCG	0.669	0.565	0.748	0.756	1.00	–	1.00	–	1.00	–
TCG	0.172	0.078	0.136	0.128	1.14 (0.67–1.96)	0.63	0.84 (0.46–1.53)	0.58	0.97 (0.48–1.98)	0.94
GCA	0.044	0.275	0.013	0.060	1.16 (0.56–2.43)	0.69	10.45(5.40–20.23)	< 0.0001	0.46 (0.15–1.45)	0.18
GTG	0.065	0	0.072	0.038	1.84 (0.85–4.01)	0.12	0.31 (0.03–3.23)	0.33	2.51 (1.04–6.04)	0.041
TTG	0.018	0.006	0.019	0.008	1.37 (0.35–5.34)	0.65	0.41 (0.03–5.39)	0.5	0.94 (0.10–8.78)	0.96
TCA	0.015	0.076	NA	0.005	2.86 (0.51–16.18)	0.23	12.21 (2.66–56.08)	0.0014	–	–
Global haplotype association *p*-values					0.19		< 0.0001*		0.19	
GG	0.83	0.912	0.874	0.842	1.00	–	1.00	–	1.00	–
GA	0.134	0.021	0.107	0.084	1.90 (1.11–3.27)	0.02	0.25 (0.12–0.53)	0.0003	1.23 (0.62–2.42)	0.55
AG	0.019	0.039	0.019	0.051	0.51 (0.19–1.38)	0.18	0.89 (0.47–1.68)	0.71	0.83 (0.33–2.11)	0.70
AA	0.017	0.028	NA	0.023	0.71 (0.24–2.11)	0.54	0.81 (0.36–1.82)	0.62	0.00 (− Inf–Inf)	1
Global haplotype association *p*-values					0.033*		0.0019*		0.0.046*	

HTN hypertension, *T2D* type 2 diabetes, *HTN + T2D* type 2 diabetes-associated hypertension, *CN* control, *OR* odds ratio, *CI* confidence interval

*indicates statistically significant relation

Linkage Disequilibrium and Association of Haplotypes with Diseases

Non-random association of SNPs at multiple loci, i.e. linkage disequilibrium (LD) revealed that the three SNPs within the renin gene (rs61827960, rs11571079, and rs3730102) were coinherited, but, due to the lower frequency of such rare alleles, R values were very low (Supplementary Fig. 1). Of all the haplotypes identified for rs61827960, rs11571079, and rs3730102 (Table 7), GCG was found to be the most common haplotype in all groups of study participants and considered to be the reference haplotype. Other than GCG, the frequencies of haplotypes GCA and TCA were higher in subpopulations with T2D than those in the other two subpopulations, exhibiting either HTN or T2D-associated HTN. Statistical analyses revealed that both the GCA and TCA haplotypes were associated with an increased risk of T2D and the GTG haplotype was found to be associated with an increased risk of T2D-associated HTN. However, statistical analyses could not be performed with the TCA haplotype data with respect to T2D-associated HTN as this haplotype was absent in this group of participants (Table 7).

Considering the rs2968915 and rs3112298 SNP variants within the (P)RR gene, four haplotypes were recognized, of which GG was the most common with the highest frequencies of all the genotypes in all the subpopulations. Haplotype GA was found to be associated with an increased risk of HTN and this haplotype was also found to play a protective role against development of T2D (Table 7).

Discussion

In the present study, not only associations with increased risk but also protective roles of SNPs were identified with respect to the diseases in question, after adjusting the data on the basis of the age, BMI, gender, SBP and DBP of the study participants. To perform the study, five SNPs [three from renin gene and two from (P)RR gene] were taken into consideration. For selecting these SNPs, the 1000-Genome browser was used (1000 Genomes Project Consortium et al. 2015). Polymorphisms with a minor allele frequency (≤ 0.10) were identified as having a minimum r^2 of 0.8, using the 'tagger' program in 'Haploview'. SNPs identified from the renin and (P)RR genes were identified and tagged, 'before being evaluated using HaploReg' (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) and 3DSNP (http://cbportal.org/3dsnp/) databases. Initially, 89 SNPs from the renin gene were identified; after tagging, the number was reduced to 65. Among the 65, three SNPs, rs61827960, rs11571079 and rs3730102, which were within the non-coding region of the human renin gene. In the case of the (P)RR gene, 48 SNPs were identified, and the number was reduced to 15 after tagging. Finally, two SNPs (rs3112298 and rs2968915) were selected within human (P)RR gene.

For analyzing frequency and association of genotypes in the study subjects, five SNPs were chosen. The variants rs61827960, rs11571079 and rs3730102 within the human renin gene were mapped to chromosome 1 at positions 204,163,894, 204,163,662 and 204,157,250, respectively. SNP rs61827960 was found to be linked to 29 SNPs of which five were intronic variants and the rest were several

kilobases upstream of the renin gene (Supplementary Table 1). This SNP was found to be linked to eight genes via chromatin loops, and it was located in the promoter sequence, where it alters sequence motifs. This SNP itself may modulate the following four histone marks: Histone 3 Lysine 4 monomethylation enhancer (H3K4me1_Enh), Histone 3 Lysine 27 acetylation enhancer (H3K27ac_Enh), Histone 3 Lysine 4 trimethylation promoter (H3K4me3_Pro), and Histone 3 Lysine 9 acetylation promoter (H3K9ac_Pro). Tagging with other SNPs, it also modulates regulatory elements such as enhancer histone marks and DNAse hypersensitive sites in several tissue types. The SNP rs11571079 was tagged with 10 SNPs of which two are intronic variants, two are several kilobases downstream of the 3′-end of K1SS1 and the rest are several kilobases upstream from the 5′-end. Functional annotation of this SNP predicted its association with chromatin states (Core 15-state model) and modulatory effects on H3K4me1_Enh, H3K4me3_Pro, H3K27ac_Enh, and H3K9ac_Pro. This SNP is also linked to eight genes by chromatin loops and alters sequence motifs and modulates enhancer histone marks, and DNAse hypersensitivity in several tissue types (Supplementary Table 2) via tagging with other SNPs. SNP rs3730102 was also found to be associated with seven intronic variants within the human renin gene that are linked to chromatin loops, which alter sequence motifs and modulate enhancer histone marks (Supplementary Table 3). In addition, functional annotation revealed its association with chromatin states histone marks. One of the linked SNPs was also found to cause synonymous mutations within the renin gene.

The intronic variants rs3112298 and rs2968915 within the human (pro) renin receptor gene were located on X chromosome at positions 40,580,182 and 40,594,652, respectively. Both variants have been identified to be linked with a total of 36 and 53 single-nucleotide variants located within the intronic region, respectively of (P)RR gene. Both rs2968915 and rs3112298 predicted to be linked to two histone marks (H3K4me1_Enh and H3K9ac_Pro, respectively) as well as four histone marks (H3K4me1_Enh, H3K4me3_Pro, H3K27ac_Enh, and H3K9ac_Pro) along with chromatin states (Core 15-state model and 25-state model using 12 imputed marks). Tagging with different SNPs, these two variants are also linked to several genes by chromatin loops, alter sequence motifs and modulate histone marks in different tissue types (Supplementary Table 4 and 5, respectively).

We report here that replacement of G by A (rs3730102) in the non-coding region of the renin gene is associated with increased risk of T2D, whereas replacement of A by G (rs3112298) in the non-coding region of the (pro)renin receptor gene is associated with an increased risk of HTN in the Bangladeshi population under investigation. In addition, SNPs rs2968915 and rs3112298 in the (P)RR gene were found to play protective roles against the development of HTN and T2D, respectively. Associations between the risk of HTN, T2D and related complications and both genotypic and allelic frequencies of major genes involved in regulating RAS have been reported in different populations, albeit with inconsistent findings (Franceschini and Le 2014; Hirose et al. 2011; Ott et al. 2011; Satofuka et al. 2009; Wang et al. 2012; Ghafar 2020a; Afruza et al. 2014; Chowdhury et al. 1998; Morshed et al. 2002). Notably, contrasting results with respect to the association of renin gene polymorphisms with HTN have been reported even within the same population, indicating low genetic diversity (Haga et al. 2002; Hammer et al. 2006; Kosachunhanun et al.

2003). Introns are widely considered to be a part of the gene sequence without function. However, most of the trait-associated single variants have been identified in the intronic rather than exonic regions through GWAS (Welter et al. 2013; Li et al. 2011). Investigation of the functional implication of intronic variants may resolve many of the biological mysteries related to diseases, in particular polygenic diseases.

Overactive or inappropriate effects of the RAS have been found to be linked with HTN and T2D (Andraws and Brown 2007; Velloso et al. 1996; Scheen 2004; Joyce-Tan et al. 2016). Genetic factors are known to play a significant role in the development of HTN (Franceschini and Le 2014; Hirose et al. 2011; Ott et al. 2011; Satofuka et al. 2009). Blood pressure, the cause of HTN, is also a complex genetic trait with heritability estimates of 30–50% (Franceschini and Le 2014; Yang et al. 2010). The SNPs rs6696954 and rs5705 within the human renin gene in Caucasians and the intronic *Bgl*I site of the renin gene in a United Arab Emirates population showed associations with HTN (Dene et al. 1989; Levy et al. 2000). An association between the renin gene *Mbo*I RFLP and essential or primary HTN in a Japanese population (Sun et al. 2011) was reported. Individuals with moderate hyperglycemia showed increased plasma renin activity, arterial pressure and renal vascular resistance with the activation of both local and circulating RAS (Miller et al. 1996; Miller 1999). Moreover, a direct correlation was established among the levels of glucose, p53 glycosylation and Ang-II production (Fiordaliso et al. 2001). Thus, relating SNPs in the renin gene to associated diseases is also of great potential interest.

In the case of rs61827960, the allelic frequencies demonstrated a 1.5-fold higher frequency of the T allele in the subpopulation with HTN compared to that in the healthy subpopulation, while frequencies of this allele were evenly distributed in T2D and T2D-associated hypertensive individuals and with healthy control subjects (Table 2). A significant association was found between the T allele and the risk of HTN, but not with other diseases (Table 2). When rs11571079 was considered, a twofold higher frequency of the mutant allele was observed in the subpopulations with either HTN or T2D-associated HTN than the healthy subpopulation, while a fivefold lower frequency of the mutant allele was found in the subpopulation with T2D than the healthy control subpopulation. Though the mutant allele of this SNP indicated a significant association with increased risks of HTN and T2D-associated HTN, it exhibited a protective role against the development of T2D (Table 3). The frequency of the heterozygous GA genotype of SNP rs3730102 was almost 58% of that of the total T2D individuals, resulting in the occurrence of a very high frequency of the mutant allele in this subpopulation, which was fivefold higher than that in the healthy subpopulation. However, in the subpopulation with HTN, the frequency of the mutant allele of this SNP was very similar to that in the healthy subpopulation, while the frequency in the T2D-associated HTN subpopulation was more than twofold lower than that in the healthy subpopulation. In the case of rs3730102, the frequency of the mutant allele in the African, admixed American, and European populations was around half the frequency in South Asian or East Asian populations. However, our study revealed that the frequency of the mutant allele of rs3730102 was closer to those of the American, African and European populations than to those reported for Asian populations (Supplementary Table 6). The frequency of the mutant allele of rs3730102 in the hypertensive and control

subpopulations matched the reported frequency from the 1000-Genome project (Supplementary Table 7), and this frequency was more than fourfold higher than that in the T2D Bangladeshi subpopulation. There was a strong association between this allele and the risk of T2D, but this allele also exhibited a protective role in T2D-associated HTN (Table 4).

Though our previous study (Afruza et al. 2014) did not find any relationship between high blood pressure in the Bangladeshi population and the SNPs present at intron 1, intron 9, and 4063 bp upstream of the promoter region of the renin gene, the present study demonstrated varying associations between the non-coding variants of the renin gene with the polygenic diseases under investigation under different models of inheritance. One of the plausible reasons for this discrepancy might be the different choices of statistical analyses used in the two studies. In the earlier study, we emphasized the patterns of allelic and genotypic frequencies and the Hardy–Weinberg Equilibrium equation, but logistic regression analyses were not considered to evaluate the association study (Afruza et al. 2014). An association of the genotypic and allelic frequencies of the RAS components with the risk of T2D has been studied in different ethnic groups. RAS components were reported to be clearly activated in diabetes (Deinum et al. 1999), with increased tissue angiotensin II (Franken et al. 1990) that leads to the development of diabetic nephropathy (DN). Meta analyses revealed ACE I/D polymorphism may contribute to the development of DN and diabetic retinopathy in Asian populations with T2D (Chowdhury et al. 1998; Morshed et al. 2002). DN was also found to be weakly associated with the bb genotype of a *Bgl*I RFLP in the first intron of renin (Afruza et al. 2014). Multi-locus association between the genes encoding ACE, ACE2, Mas, and angiotensinogen (AGT) with the increased risk of T2D was reported in a Chinese population (Germain et al. 1997), while AGT 235T/T and—6G/G genotypes and 235T and—6G alleles were shown to be associated with increased DN risk in a Tunisian population (Mtiraoui et al. 2011). Furthermore, Joyce-Tan et al. (2016) reported that two AGT variants, rs699 and rs4762, were associated with reduced risk of T2D and that rs5051 was associated with increased risk in Malay population (Joyce-Tan et al. 2016). In the present study, rs3730102 exhibited an association of increased risk with T2D (Table 4).

According to allelic frequency data from the 1000-Genome project phase 3, South Asian and American populations had the lowest frequency of the wild-type allele 'G' allele with respect to SNP rs2968915. Our data produced a similar result with 6% of the study participants carrying the ancestral 'G' allele (Supplementary Table 6). The frequency of the mutant allele of SNP rs2968915 in the T2D subpopulation was found to be similar to that in the healthy control subpopulation, but the frequency was twofold lower in the HTN subpopulation and threefold lower in the T2D-associated HTN subpopulation. With respect to frequencies reported in the 1000-Genome project (Phase 3), it was observed that the HTN subpopulation had a frequency of the mutant allele of SNP rs2968915 more than 1.5-fold higher, and the T2D-associated HTN subpopulation had a frequency twofold lower than that of the healthy subpopulation (Table 5). The T2D subpopulation had the lowest frequency of the mutant A allele of SNP rs3112298, and the HTN and T2D-associated HTN subpopulations had similar frequencies when compared with the healthy subpopulation (Table 6). Though the frequency of the

minor allele A of rs3112298 was reported to be only 2% in South Asian individuals, the present study found a frequency of this allele to be five times higher over the total study population. As (P)RR is present on X chromosome thus, before identifying genotypic frequencies with respect to SNPs in (P)RR gene, the participants were stratified between male and female subgroups. The models of inheritances with regard to heterozygosity presented in Tables 5 and 6 were analysed using only female individuals.

Several animal studies have shown that (P)RR contributes to blood pressure regulation (Hirose et al. 2011; Satofuka et al. 2009; Burcklé et al. 2006) or development of glomerulosclerosis (Kaneshiro et al. 2007), nephropathy (Ichihara et al. 2004), retinopathy (Luo et al. 2016; Hase et al. 2017). Burcklé et al. reported that elevated blood pressure and heart rate were observed in rats with overexpression of the human (P)RR gene (Burcklé et al. 2006), while the Ohasama study showed the potential importance of the (P)RR gene in blood pressure regulation in humans (Hirose et al. 2011). The study found that the IVS5+169C>T polymorphism of the (P)RR gene was associated with increased ambulatory blood pressure in Japanese men. This association suggests that (P)RR has a role in blood pressure regulation.

Haplotype-based analyses, which have been suggested as a more powerful strategy for analyzing associations of multiple alleles at different loci on the same chromosome, are important because of their ability to associate disease incidence with closely linked SNPs, due to their independent association. Single variants situated close together on the same chromosome exhibit high LD. In the present study, the rs61827960 locus is 232 bp downstream from that of rs11571079 and 6644 bp downstream from that of rs3730102, and locus rs11571079 is 6412 bp upstream from that of rs3730102 in the human renin gene on chromosome 1. Also, rs2968915 is 14470 bp upstream from that of rs40594652 of the human (P)RR gene on X chromosome. Data revealed that three SNPs within the renin gene were coinherited, but, due to the low frequencies of such rare alleles, R^2 values were estimated to be very low (Supplementary Figures. 1A and 1B), with the rare allele always being inherited with one particular allele of higher frequency. Thus, independent haplotypes themselves may be the causal variant for diseases, such as the situation in which the renin GCA and TCA haplotypes are associated with increased risk of T2D. Notably, the (P)RR haplotype GA exhibited a protective role against T2D in the study population.

A limitation of the present study is the inclusion of few SNPs from two genes of the RAS. Though multiple studies have shown associations of angiotensinogen, ACE gene, and angiotensin type 1 and type 2 receptor gene polymorphisms with HTN and T2D in different ethnic groups, inclusion of more variants and genes in the present study would have facilitated genetic underpinning of these diseases. Three SNPs (rs11571079, rs2968915 and rs3112298) tested in this study did not follow Hardy–Weinberg Equation (HWE) due to significant p values obtained by Chi-square test. However, if we consider the stringent p values (p-HWE $< 10^{-5}$), which is the statistical criteria to be departed from HWE tested by Chi-square test of goodness of fit between the observed and expected genotypes, the level of deviation could be circumvented in this association study. Also, allelic distribution pattern of each of the SNPs selected matched with the pattern of East Asian and South Asian population and even with the average frequency of all population reported by the

1000 genome project Phase 3 (data not shown). Further, while deviation from HWE may indicate false-positive outcome, reports also demonstrated that some true associations are expected to be out of HWE, and thus, SNPs severely out of HWE should not therefore be neglected for future analysis rather selected for additional investigation after completing association analyses (Turner et al. 2011).

In conclusion, the identification of specific genes associated with multi-factorial human diseases remain challenges to be answered. In the present study, we have identified non-coding variants within the renin and (pro)renin receptor genes that exhibited multiple patterns of association with polygenic diseases in a Bangladeshi population.

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Author contributions AHMNN conceived the idea. AHMNN and AE designed the experiments. JA conducted all the experiments in the laboratory of population genetics. MAR interviewed the patients and collected the samples. JA, TN, and SKS performed data analyses. MIH and AD performed the bioinformatics analyses and haplotype frequency analyses. JA, AHMNN, and FS wrote the manuscript. All authors approved submission of the final manuscript.

Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Compliance with ethical standards

Conflict of interest The author(s) declare that there no competing interests.

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