SUMMARY

Recent studies have highlighted the role of promoter methylation on expression of genes involved in hypertension. But as per our literature search negligible reports were found on human AT1R. However, study on functional activity of promoter CpG island of AT1R was altogether missing. Thus, main aim of present work was to study the effect of promoter DNA methylation of AT1R with respect to hypertension. CpG island within promoter of AT1R (CpG.P.AT1R) was identified using CpG Plot software. Functional role of CpG.P.AT1R was studied by cloning this region in promoter-less reporter vector (pGL4.10). Reporter assay results for this construct (pGL4.10-CpG.P.AT1R) showed promoter activity of CpG.P.AT1R region in comparison to pGL4.10 vector highlighting its functional significance. In addition to this, presence of methylation in the target region was also found to play significant role on functional activity of this region where reduction in expression of reporter gene (luciferase, luc2) was found by treatment with CpG methyltransferase enzyme (M.SssI) which methylates all cytosine residues (C$^{5}$) within double-stranded dinucleotide sequence (CG). Role of methylation on this region was also validated through sequencing and demethylation experiments. These in-vitro results were further studied through in-vivo experiment where methylation pattern of the targeted CpG island (CGI) was compared between hypertensive (HTN) and normotensive (NTN) individuals through bisulphite sequencing method. Peripheral blood samples were collected from volunteers falling in each individual group. Isolated DNA was bisulphite treated for PCR amplification and purified PCR products were sequenced to analyze the methylation pattern. No methylation difference was found at any CpG site within targeted region except for, one hemi-methylated CpG site in one of the hypertensive sample. This hemi-methylated site harboured binding site of transcription factor Sp1 (Specificity protein 1) which binds to GC rich motifs of promoters but reports suggest that CpG methylation does not affect its binding affinity. As per literature hypomethylation of promoter leads to hypertension through increased expression of AT1R but our study could not find hypomethylation of promoter among hypertensive (HTN) samples though our in-vitro results supported the literature findings.

AT1R gene was screened for novel as well as for functionally relevant SNPs. Two SNPs of AT1R namely, rs5186 and rs422858 along with ACE (I/D) polymorphism
were genotyped in the collected samples. This study is the first report on analysis of rs422858 polymorphism of \textit{AT1R} gene from Indian population. Frequency of allele A (rs422858, A-214C) in normotensive (NTN) individuals was found to be much higher (0.837) than that reported in Caucasians (0.74) indicative of population differences. Allele and genotype analysis between both groups did not yield much difference for rs5186 and rs422858. However, gender based segregation showed significant difference ($p=0.003$) between both alleles of \textit{ACE} (I/D) polymorphism in females where allele I was found to be more common in HTN and allele D among NTN. Lipid profile and lifestyle factors were also compared among HTN and NTN samples. Though different variables of lipid profile were almost comparable but statistically significant differences were found between Body mass index (BMI), Systolic and Diastolic blood pressure where higher values were found in HTN group as expected. In addition to this, mean onset age of hypertension showed significant difference between familial and sporadic group where onset of hypertension was much earlier in familial group.

Screening of exons of \textit{AT1R} was done to search for novel variations through sequencing of purified PCR products. No variation was found in exon 1 which forms part of CpG island present in promoter of \textit{AT1R}. Sequencing results of exon 2 depicted one already reported SNP in intronic region (A/G- rs2276736) whose function has been reported with respect to non-alcoholic fatty liver disease (NAFLD). However, in exon 3 neither novel nor reported SNP was found in the sequenced samples.

To conclude, present study highlights functional role of \textbf{CpG} island within \textbf{promoter} of \textit{AT1R (CpG.P.AT1R)} which is further affected by degree of DNA methylation. As tissue RAS also exists in addition to systemic RAS where components are synthesized locally, so this report highlighting role of DNA methylation on promoter activity of CpG.P.AT1R in systemic RAS suggests similar study targeting methylation pattern of studied region on isolated tissues as future aspect to cover its functional role in both systemic as well as tissue specific expression of \textit{AT1R}.