Hypertension, commonly known as high blood pressure is a condition in which blood vessels are under elevated pressure constantly. It is an indispensable risk factor for cardiovascular, cerebrovascular and renal disorders which cause vast extent of mortality in a cumulative manner worldwide. At present nearly 1 billion of total population is affected by hypertension (Kearney et al., 2005) and it has reached new heights in India too where present prevalence in urban population is 33% and in rural is 25% (Anchala et al., 2014). Blood pressure is measured in millimetres of mercury (mm Hg) and is recorded as systolic pressure (highest pressure in blood vessels at the time of contraction of heart) and diastolic pressure (lowest pressure when heart muscle relaxes) and normal pressure is defined as 120/80 mm Hg. As number of individuals affected with hypertension is increasing at an alarming rate so to generate awareness about this risk factor, May 17 every year is being observed as World Hypertension Day since 2006. Normal level of systolic and diastolic blood pressure is essential for efficient functioning of vital organs like heart, brain and kidney and perturbations from normal levels might lead to stroke, cardiovascular or end-stage renal disorders (Messerli et al., 2007). For this purpose Joint National Committee (JNC) for prevention, detection, evaluation and treatment of high blood pressure monitors blood pressure in the population and keeps updating its guidelines as per current scenario. Hypertension is usually a complex disorder but monogenic forms of hypertension also exists that include causes like gain of function mutation (eg. Liddle’s syndrome, Gordon’s syndrome), enzyme deficiency (eg. Apparent mineralocorticoid excess), excessive aldosterone synthesis (eg. Glucocorticoid remediable aldosteronism) (Simonetti et al., 2012). Such monogenic forms of hypertension are less prevalent at population level but the most common form usually develops as a result of complex interplay between various genetic and environmental factors. Hypertension is broadly classified as primary and secondary. Primary also known as essential hypertension is the more prevalent form which is without any known identifiable cause whereas secondary hypertension occurs due to any renal disorder or certain other primary ailments leading to high blood pressure.

Through genome wide association study (GWAS) various candidate genes have been discovered to play critical role in blood pressure regulation. These genes are essential components of various metabolic pathways, or are involved in sodium transportation, steroid or natriuretic peptide synthesis (Natekar et al., 2014). Foremost pathway to
regulate blood pressure is Renin angiotensin system (RAS) and any perturbation in the components of this pathway leads to alteration in blood pressure. Present medication for high blood pressure also targets key components of RAS i.e. Angiotensin converting enzyme (ACE) and Angiotensin II type 1 receptor (AT1R). Besides RAS, enzymes (Tyrosine hydroxylase, PNMT) of catecholamine synthesis pathway also play important part to generate critical target compounds involved in blood pressure regulation. Association studies of single nucleotide polymorphisms (SNPs) involving genes of RAS, catecholamine pathway and others have been targeted to understand the etiology of hypertension in different populations. Most of the time results from such SNP studies remain inconclusive which can be due to the differences in ethnicity, age or selection criteria among studied populations. Such results highlight important fact that no single gene individually plays critical role to develop hypertension rather it is an outcome of various genes working in a cumulative manner to cause diseased condition. It further highlights involvement of certain other additional factors. One such factor which needs further exploration is epigenetic mechanism that might be involved but not yet studied in depth with respect to hypertension. As epigenetic mechanisms like DNA methylation, histone modification (methylation, acetylation, phosphorylation) and non-coding RNAs are known to affect gene expression in cancer and other developmental genes, therefore such epigenetic mechanisms might be involved in targeting expression of blood pressure regulating genes in addition to SNPs. Involvement of such additional factor is further highlighted by the occurrence of a condition referred to as resistant hypertension in which blood pressure remains high even after having three antihypertensive drugs. Clinicians nowadays are also motivating people to change their lifestyle (daily routine and diet regime) to curb the effect of such unknown factors in order to prevent development of hypertension.

Studies involving role of epigenetic mechanisms in cancer causing genes were already present in literature but focus on eigenetic regulation of blood pressure controlling genes has recently gained interest. Pioneer studies in mice found role of diet and other factors on gene expression through altered methylation pattern culminating in hypertension. Low protein diet in pregnant rats lead to hypertension in their offsprings by upregulating AT1b expression through promoter hypomethylation (Bogdarina et al., 2007). Maternal low protein diet (MLPD) further caused altered expression of
RAS genes in fetal brain (Goyal et al., 2010) and development of hypertension in sex specific manner (Goyal and Longo, 2013). Along with MLPD, nicotine administration in pregnant rats enhanced AT1a expression in offsprings by promoter hypomethylation (Xiao et al., 2014). In addition to studies on mice, difference in global methylation pattern of 5-methyl cytosine (5mC) (Smolarek et al., 2010) and homocysteine levels have been studied in human samples (Kulkarni et al., 2011). Along with global methylation status, role of methylation to alter expression of individual genes in hypertensive patients have also been found which indicates significant role of DNA methylation in development of hypertension. Difference in methylation pattern at CpG sites in promoter of aldosterone synthase (Gu et al., 2016), glucokinase (Fan et al., 2015a), Inerleukin-6 (Mao et al., 2017) and some other genes was found in relation to essential hypertension. In recent studies, not just DNA methylation but histone modifications and non-coding RNAs have also caught interest of scientists working in this field of hypertension.

Taking a clue from innumerable SNP studies and recently realized involvement of DNA methylation in hypertension of animal models, we searched through published data and found almost no information on the epigenetic regulation mainly DNA methylation study on ATIR gene involving human samples. Keeping this in mind, the main aim of this study was to decipher the effect of promoter methylation of ATIR. To achieve this aim, following objectives were designed:

1) Explore presence of any CpG island in promoter of Angiotensin II type 1 receptor (AT1R) gene.

2) Study promoter activity of CpG island and effect of methylation on its functional activity.

3) Analysis of methylation pattern in targeted CpG island of AT1R promoter among human samples comprising of both hypertensive and normotensive individuals.

4) Determine the status of functionally relevant SNPs of AT1R in the sample set.