Chapter

Aldosterone Synthase Gene (*CYP11B2*) Polymorphisms and Enhanced Cardiovascular Risk

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Abstract

Aldosterone, the principal human mineralocorticoid, acts mainly for sodium reabsorption with potassium and hydrogen excretion. The adrenal cortex is the main site of aldosterone synthesis; however, extra-adrenal tissues such as the nervous, the cardiovascular, and the adipose tissues may be involved. Therefore, its action is mediated via endocrine as well as paracrine or autocrine mode. Aldosterone receptors are distributed extensively in the renal distal nephron and other sites, such as the heart, brain, vessels, and liver. The aldosterone synthase catalyzes the conversion of deoxycorticosterone finally to aldosterone. *CYP11B2* gene occupies human chromosome 8q21-22 with nine exons and eight introns. Alteration of aldosterone synthase gene that is attributable to genetic polymorphisms can affect its transcription leading to several cardiovascular disorders such as essential hypertension, myocardial infarction, cardiomyopathies, and atrial fibrillations. Accordingly, it is important to illustrate these polymorphisms and the mechanisms by which they alter the aldosterone synthase gene and produce cardiovascular dysfunctions.

Keywords: aldosterone, *CYP11B2*, polymorphisms, risk, transcription

1. Introduction

CYP11B2 enzyme is one of the enzymes in the pathway of steroidogenesis and responsible for the catalysis of the last three steps in the aldosterone biosynthetic cascade. It is encoded by *CYP11B2* gene located on human chromosome 8q21-22. The genetic element of cardiovascular disorders has been emerged as a risk factor for the progression of these disorders. Among these genetic elements, *CYP11B2* genetic variants and haplotypes play a pivotal role in the susceptibility, progression, survival, and therapeutic response of many cardiovascular disorders such as hypertension, coronary heart disease (CAD), atrial fibrillation (AF), cardiomyopathy, heart failure (HF), and other disorders. It was suggested to influence the cardiovascular system via alteration of aldosterone production, which acts either directly on the heart or systemically via stimulating sodium and water reabsorption and increasing the blood pressure.

2. Aldosterone

2.1 Aldosterone biosynthesis

Aldosterone is the main human mineralocorticoid. The main site of aldosterone synthesis is the zona glomerulosa (ZG) in the adrenal cortex. However, it can be produced by extra-adrenal tissues such as the central nervous system, the cardiovascular system, and the adipose tissue with a non-detectable physiological relevance and a small contribution to circulating aldosterone levels [1].

It is synthesized from cholesterol by a group of enzymatic cascade (**Figure 1**). First, cholesterol is translocated into the mitochondria across its wall mediated by steroidogenic acute regulatory protein (StAR). Cholesterol is then converted to pregnenolone, through three reactions, a 20α-hydroxylation, a 22-hydroxylation, and cleavage of the bond between C-20 and C-22 catalyzed by the *CYP11A1* cleavage enzyme, encoded by the *CYP11A1* gene on human chromosome 15 [2].

The produced pregnenolone is then released into the cytoplasm where it undergoes dehydrogenation of the 3β-hydroxyl group and isomerization of the double bond at C-5 to Δ4 by 3β-hydroxysteroid dehydrogenase (3β-HSD) and converted to progesterone [3]. Furthermore, progesterone undergoes 21-hydroxylation by the *CYP21A* enzyme encoded by *CYP21A* mapped to human chromosome 6p21·3 and located on the cytoplasmic surface of the smooth endoplasmic reticulum, generating 11-deoxycorticosterone (DOC). Then, DOC passes through three consecutive reactions catalyzed by aldosterone synthase enzyme, located on the inner mitochondrial membrane and encoded by the *CYP11B2* gene, 11β-hydroxylation to corticosterone, 18-hydroxylation to 18-hydroxycorticosterone, and finally 18-methyloxidation to aldosterone [4].

2.2 Mechanism of action of aldosterone

The action of aldosterone is mediated though mineralocorticoid receptor (MR), a specific nuclear receptor that comprises N-terminal domain, DNA-binding domain, and C-terminal ligand-binding domain. It is present as a hetero-oligomeric

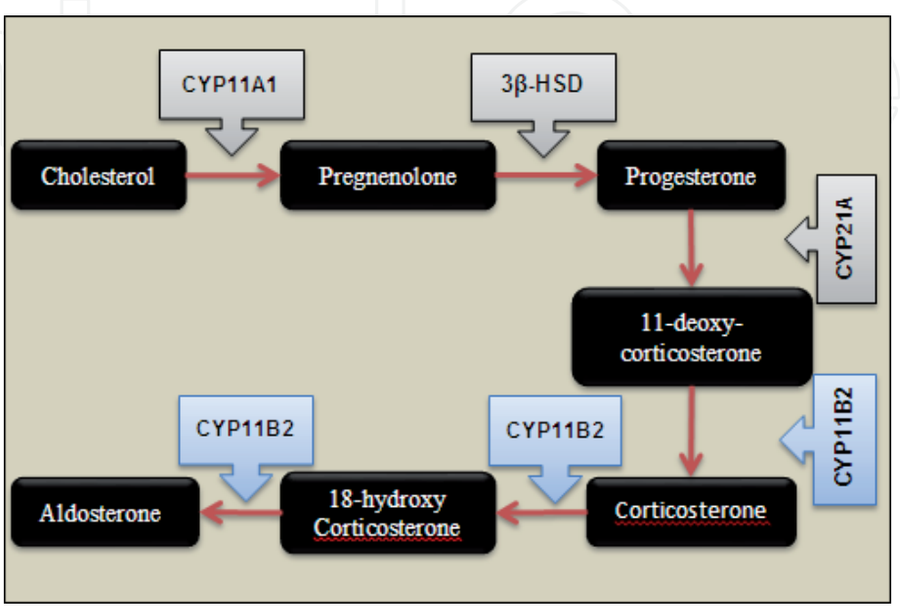

Figure 1.
Enzymatic cascade of the aldosterone biosynthesis.

complex with heat-shock proteins in the cell. Once aldosterone binds to its receptor, it undergoes a conformational change resulting in dissociation of the associated proteins, dimerization, and nuclear translocation [5]. This hormone receptor complex combines with the steroid responsive elements in the 5′-UTR of aldosterone-responsive genes with the release of aldosterone-induced proteins (AIP) that enhances or suppresses gene transcription [4].

The serine threonine glucocorticoid regulated kinase 1 (sgk1), which is one of the AIP proteins, has been phosphorylated and activated by the aldosterone, which in turn phosphorylates epithelial sodium channel (ENaC) regulatory protein, known as Nedd4–2, reducing its binding to ENaC [6] with subsequent increase in ENaC density and stability at the apical membrane resulting in increased ENaC-dependent Na^+ reabsorption [7]. The glucocorticoid-induced leucine zipper (GILZ) and the corticosteroid hormone-induced factor (CHIF) are also AIP proteins. GILZ interacts with aldosterone inhibiting the ERK signaling pathway, thus liberating ENaC from Nedd4 proteins; accordingly, its action in blocking sodium reabsorption is inhibited [8]. CHIF may affect the baso-lateral Na/K-pump, resulting in increased sodium reabsorption with potassium or hydrogen ion excretion (**Figure 2**) [9]. Aldosterone also exerts a genomic action via modulating the gene expression and subsequent protein production that result in a lag time of 1–2 h before a noticeable change in target cell activity occur [10].

2.3 Physiological action of aldosterone

As the kidneys are the main site of action for aldosterone, MRs are confined mainly in high concentration to the renal distal convoluted tubules and collecting duct controlling the apically located epithelial sodium channels at their luminal cells. Mineralocorticoid receptors also exist in other epithelial sites, such as the colon, sweat gland ducts, salivary glands [11], and non-epithelial sites including

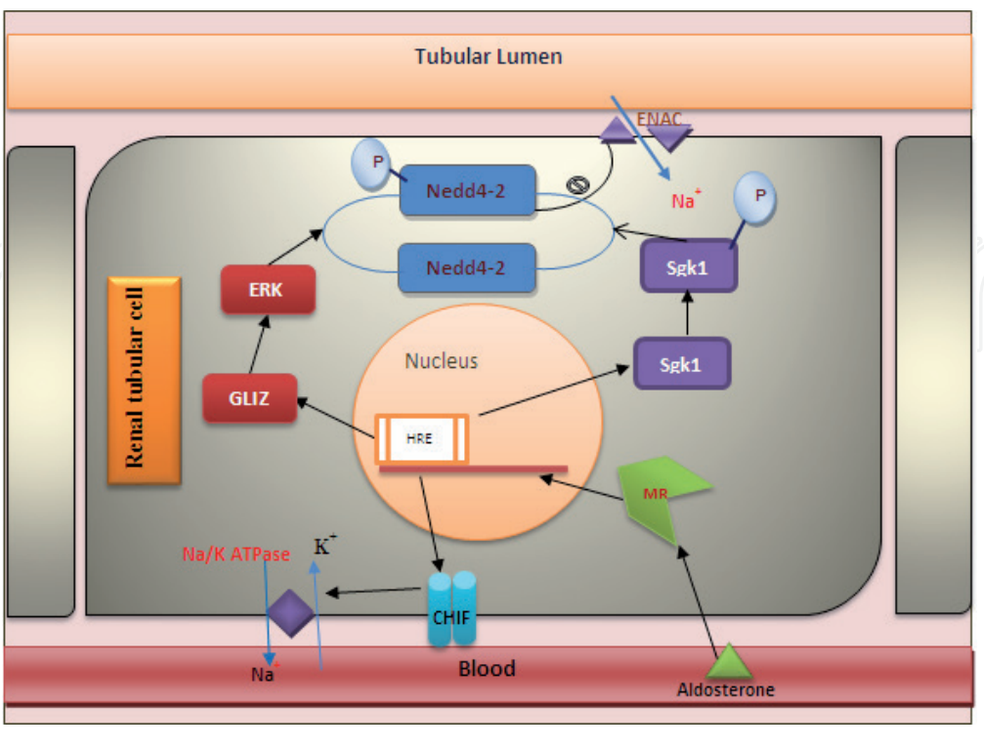

Figure 2.
Mechanism of action of aldosterone in epithelial cells. Abbreviations: ENaC, epithelial sodium channel; HRE, hormone response element; GILZ, glucocorticoid-induced leucine zipper protein; Nedd4-2, neuronal precursor cell-expressed, developmentally down-regulated protein; Sgk1, serine threonine glucocorticoid regulated kinase 1; CHIF, channel-inducing factor; MR, mineralocorticoid receptor.

myocytes, endocardium of the heart, brain, vascular smooth muscle, liver, and leukocytes [12]. The main action of aldosterone is stimulation of sodium reabsorption in the kidney and at other secretory epithelial sites with excretion of potassium and hydrogen ions [13] possibly mediated via increasing the opening periods of the existing ion channels or increasing their number [14].

In cardiovascular system, aldosterone promotes myocardial hypertrophy and fibrosis via increasing collagen I synthesis in cardiac fibroblasts and also elevating endothelin receptor numbers that further increases collagen synthesis [15]. So, increased expression of mineralocorticoid receptors in the heart may result in left-ventricular hypertrophy in normotensive subjects [16]. Aldosterone also stimulates vascular constriction via enhancing the pressor response to catecholamines and impairing the vasodilatory response to acetylcholine or by upregulation of angiotensin II receptors [4]. Also, aldosterone excess can trigger collagen deposition in blood vessels, enhancing vascular remodeling and reducing compliance [17]. In the CNS, it appears to regulate blood pressure, salt appetite, and sympathetic tone [4].

Under normal circumstances, it is likely that cardiac MRs are occupied by glucocorticoid due to its higher circulating concentration exerting antagonistic effect attenuating the rise in blood pressure and the cardiac fibrosis caused by aldosterone alone [18].

3. Cytochrome P450 11B2 (*CYP11B2*)

3.1 Reaction catalyzed

CYP11B2 enzyme is located in the inner mitochondrial membrane. Aldosterone synthesis from 11-deoxycorticosterone is catalyzed by *CYP11B2* enzyme, more commonly referred to as aldosterone synthase, which catalyzes three sequential reactions, each utilizing one molecule of NADPH, one molecule of oxygen, and the mitochondrial electron transfer system. The three sequential reactions are as follows: the 11β-hydroxylation of 11 deoxycorticosterone, the hydroxylation of carbon 18, followed by oxidation of the carbon 18 hydroxyl group to yield the carbon 18 aldehyde group resulting in the formation of aldosterone (**Figure 3**) [19].

3.2 Molecular structure

CYP11B2 gene is located on human chromosome 8q21-22. It consists of nine exons, eight introns, and comprises about 7 kb (**Figure 4**). Its coding region is 95% identical, and its intronic region is 90% identical with *CYP11B1*. However, its 5′ untranslated region is different from that of *CYP11B1* gene, thus accounting for the differences in regulation and expression pattern for each gene [20].

3.3 Protein structure

The human *CYP11B2* proteins are located in the inner mitochondrial membrane and consist of 503 amino acids, including a 24-residue N-terminal mitochondrial targeting sequence. At the protein level, the enzyme is 93% homologous with *CYP11B1* reflecting its shared 11β-hydroxylase and 18-hydroxylation activity. Only 37 amino acids differ between them which accounts for their different functions. Although *CYP11B1* and *B2* consist of the same number of amino acid, the apparent molecular mass of the human enzymes was reported as 51 and 49 kDa, respectively [20].

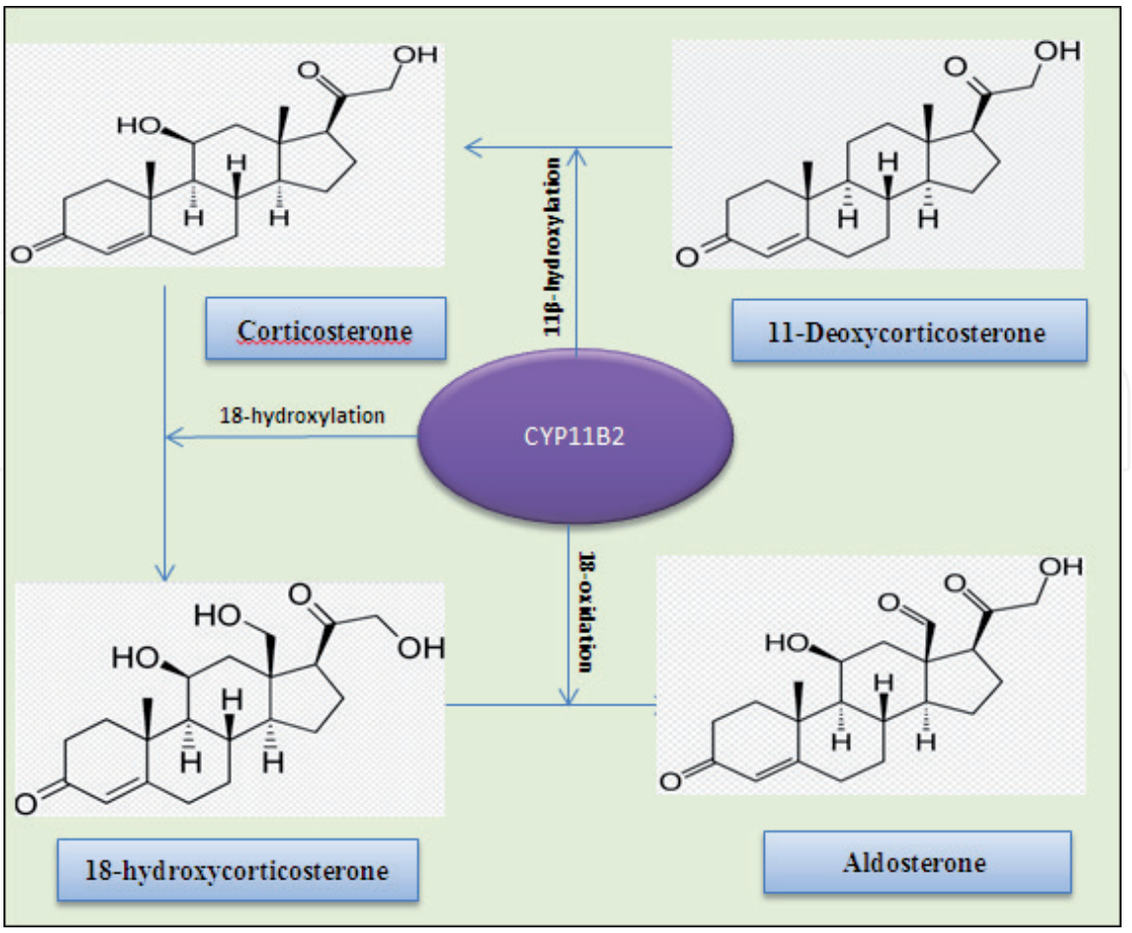

Figure 3.
Enzymatic reactions catalyzed by CYP11B2 enzyme.

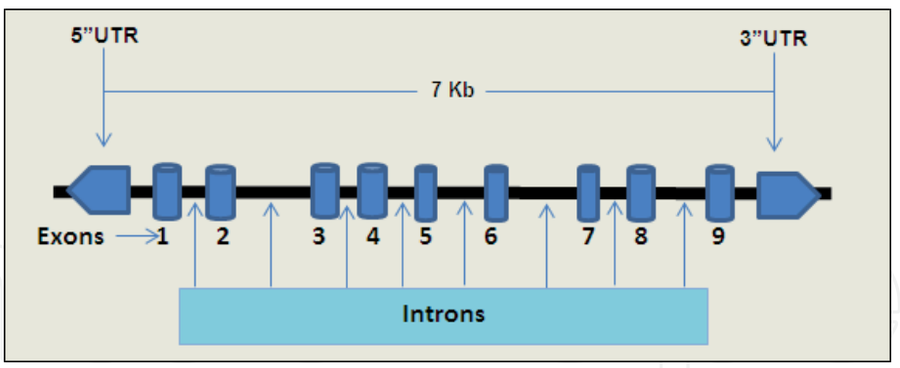

Figure 4.
Schematic diagram of the exonic-intronic arrangement of the CYP11B2 genes.

3.4 Tissue-specific expression

Expression of *CYP11B2* is limited to the adrenal cortex and appears to be exclusively in adrenal zona glomerulosa cells [21].

3.5 Regulation of *CYP11B2* expression

Adrenal steroidogenesis is under both acute and chronic regulation by tropic hormones. The acute response occurs within minutes and involves the mobilization of cholesterol from intracellular stores to the mitochondrial membrane in response to ACTH, angiotensin II, K^+, and their respective intracellular messenger pathways.

The chronic response takes several hours and involves the transcription of the genes encoding the steroidogenic enzymes [22].

3.5.1 Signaling pathways that regulate aldosterone production

3.5.1.1 ACTH

Adrenocorticotrophin (ACTH) is a 39 amino acid peptide released from the anterior pituitary in pulsatile and diurnal rhythm with the highest levels in the morning and the lowest at night [23]. ACTH exerts its effects by binding to its receptor (ACTH-R), a G-protein-coupled receptor. ACTH acutely stimulates aldosterone secretion but in the long term has an inhibitory effect on *CYP11B2* gene expression and aldosterone levels [24].

Acute stimulation of aldosterone production has suggested to be mediated via activation of StAR protein production. Also, ACTH after binding to its receptor can activate adenylate cyclase, resulting in an increased intracellular cAMP concentration, activation of protein kinase A (PKA), and calcium influx via calcium channels (**Figure 5**) [25].

On the other hand, chronic ACTH stimulation may depress serum aldosterone level as cyclic AMP, the second messenger for ACTH, desensitizes adrenocortical cells to angiotensin II by causing a reduction in the expression of angiotensin II receptors. ACTH may also decrease aldosterone production by stimulating the expression of *CYP11B1* and *CYP17*, thereby resulting in a removal of precursors from the aldosterone pathway and using them to

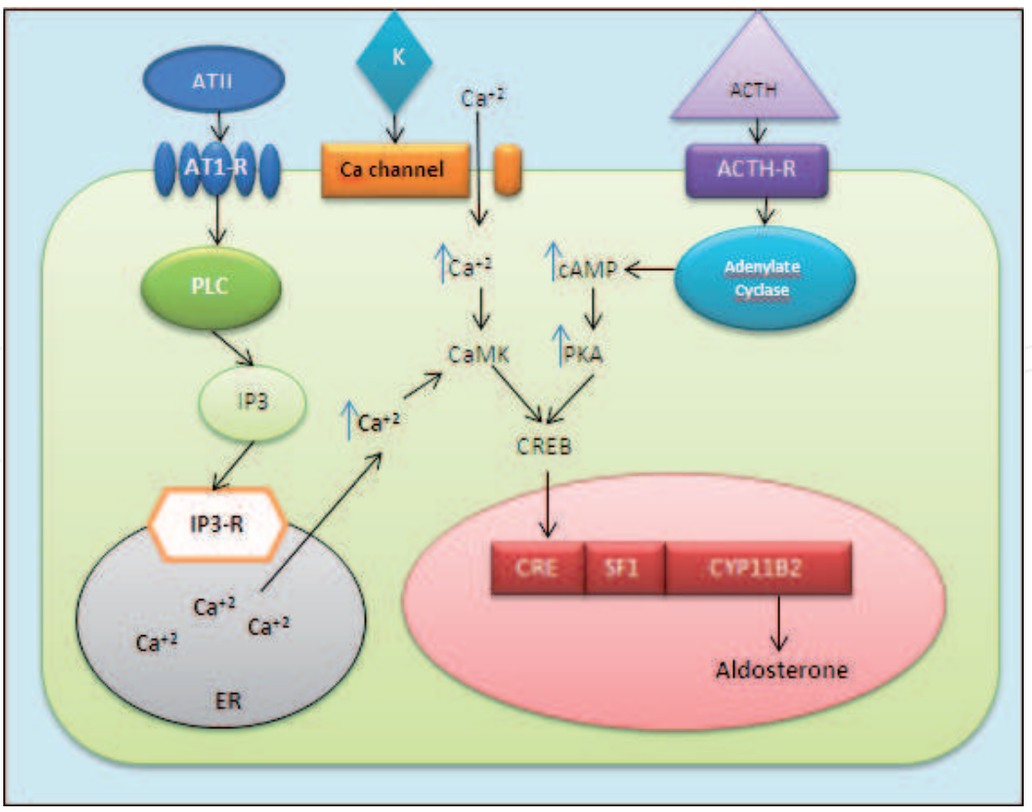

Figure 5.
Intracellular mechanisms of angiotensin II, K^+ and ACTH influencing gene expression. Abbreviations: ER, endoplasmic reticulum; AT1-R, angiotensin 1 receptor; PLC, phospholipase C; PKA, protein kinase A; IP3R, inositol triphosphate receptor; IP3, inositol triphosphate; CaMK, calmodulin kinase; SF-1, steroidogenic factor 1; CREB, cAMP regulatory element response element; ACTH, adrenocorticotrophic hormone.

synthesize cortisol. ACTH appears to specifically induce the proliferation of zona fasciculata cells while recruiting and transforming glomerulosa cells into fasciculata-like cells [26].

Under normal circulating ACTH levels, the glomerulosa maintains *CYP11B2* expression by at least two mechanisms. First, angiotensin II inhibits ACTH-stimulated cAMP production in glomerulosa but not fasciculata cells. Second, the glomerulosa expresses a type of adenyl cyclase that is inhibited by increasing intracellular Ca^{2+}, the second messenger for both angiotensin II and K^+ [27].

3.5.1.2 Angiotensin II

Angiotensin II is thought to stimulate aldosterone synthesis as result of sodium depletion and extracellular fluid volume reduction through various intracellular signaling pathways. However, the best characterized pathway is the activation of phospholipase C (**Figure 5**). It is mediated by acting on angiotensin 1 (AT1) receptor, a specific G-protein-coupled receptor that activates phospholipase C. Once activated, phospholipase C hydrolyses phosphatidyl inositol 4,5-biphosphate (PIP2) to 1,4,5 inositol triphosphate (IP3) and 1,2-diacylglycerol (DAG) resulting in release of Ca^{2+} from intracellular stores and activation of protein kinase C (PKC), respectively. The increased intracellular Ca^{2+} concentration activates calmodulin and Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases) [28, 29] to phosphorylate and activate transcription factors as activating transcription factor 1 (ATF-1), cAMP-response-element binding protein (CREB), nerve growth factor IB (NGFIB), and nuclear receptor related 1 protein (*NURR1*) that combined to specific cis-acting series in the 5′ region of *CYP11B2* [30]. The acute stimulation mediated by angiotensin II can stimulate rapid aldosterone synthesis either de novo or from intermediate compounds in the steroidogenic pathway. However, chronic stimulation may lead to ZG hypertrophy and hyperplasia with increased *CYP11B2* expression and subsequent aldosterone secretion [31].

3.5.1.3 Potassium

The level of potassium affects renin secretion as well as having a direct effect on the adrenal cortex to increase aldosterone secretion. Increased extracellular K^+ (like angiotensin II) stimulates aldosterone secretion through an increase in intracellular Ca^{2+} and activation of calmodulin kinases with consequent phosphorylation of transcription factors leading to stimulation of *CYP11B2* gene transcription. Potassium signaling is mediated through membrane depolarization, leading to an influx of calcium through T and L-type channels (**Figure 5**) [32].

3.5.2 Transcriptional regulation of CYP11B2 gene

As mentioned previously, chronic regulation of steroidogenesis involves transcription of the genes encoding the necessary steroidogenic enzymes. This is mediated by alteration of trans-acting factors that bind to the cis-regulatory elements within the 5′ regulatory regions of the target genes. Investigation of the 5′ regulatory regions of the *CYP11B* gene revealed six cis-acting elements (Ad1–6) (**Figure 6**). The most important cis-elements in the *hCYP11B2* promoter are CRE at −71/−64 (Ad1), Ad5 at −129/−114, Ad4 at −344/−336, and a cis-element termed NGFIB response element 1 (NBRE-1) −766/−759 (**Figure 7**) [33].

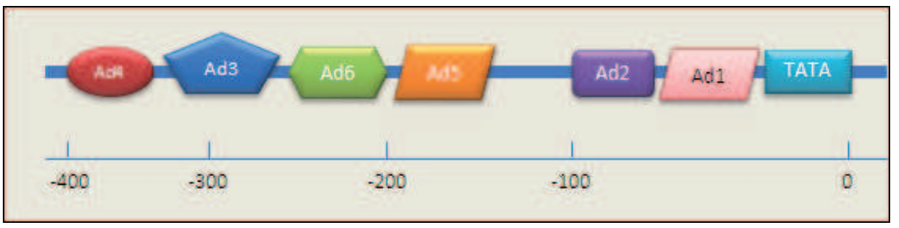

Figure 6.
Schematic diagram of the CYP11B2 promoter with the cis-elements (Ad1–Ad6).

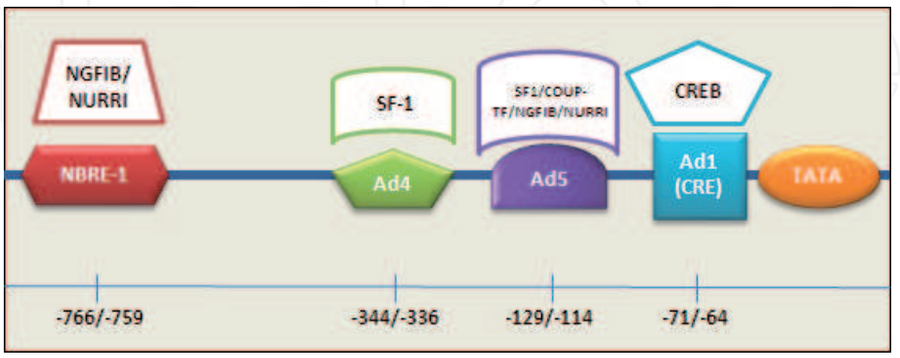

Figure 7.
Schematic diagram of the most important transcription factor binding sites in the hCYP11B2 5'UTR. Abbreviations: CREB, cAMP regulatory element response element; SF-1, steroidogenic factor 1; COUP-TF, chicken ovalbumin upstream promoter transcription factor; NGFIB, nerve growth factor IB; NURRI, nuclear receptor-related 1 protein; NBRE-1, NGFI-B response element.

3.5.2.1 Ad1 (CRE)

The Ad1 element closely resembles a consensus cAMP regulatory element (CRE) site. CREs plays an essential role in cAMP-dependent gene expression of a wide variety of genes. Proteins, such as the CRE-binding protein (CREB) and the highly related activating transcription factors (ATF), bind to CRE sites to initiate transcription. CREB binds to DNA as a dimer and has a conserved region of leucine residues (leucine zipper) at its C terminus that enables dimerization and sequence specific DNA binding [34].

CREB functions not only as a component of a variety of signaling pathways, particularly PKA, but also mitogen-activated protein kinases (MAPKs) and CaMKs. All these pathways mediate CREB-induced transcription by phosphorylating CREB at residue serine 133 [35]. The phosphorylated serine 133 binds another protein referred to as the CREB-binding protein (CBP). CBP is a 265-kDa nuclear protein, which binds to phosphorylated CREB and allows recruitment and stabilization of the RNA polymerase II transcription complex on the promoter of CREB target genes [36].

Using electrophoretic mobility shift assay, the *CYP11B2* CRE has been shown to bind members of the activating transcription factor (ATF-1 and ATF-2) and CRE-binding protein (CREB) families. The ability of these transcription factors, particularly ATF-1 and CREB, to enhance transcription is partially regulated by their state of phosphorylation. Thus, one possibility is that activated CaMK I or CaMK IV phosphorylates CREB or ATF-1 leading to increased *CYP11B2* transcription [28].

3.5.2.2 AD4 (SF-1)

The Ad4 site (CCAAGGTC) is also found to be important in the regulation of the *CYP11B* gene. This Ad4 site or homologous sequences have been identified in the regulatory regions of all other steroid P450 genes (*CYP11A1*, *CYP21*, *CYPI7*, *CYP11B1*,

CYP11B2, and *CYPI9*), suggesting an important functional role in steroidogenesis. An Ad4-binding protein (Ad4BP) has been identified and cloned from bovine adrenal cortex nuclear extract. Ad4BP is a homolog of the steroidogenic factor 1 (SF-1) identified in the human [37].

SF-1 is an orphan member of the nuclear hormone receptor superfamily, with potential phosphorylation sites for cAMP-dependent kinases, CaMK or PKC, suggesting a role for SF-1 in cAMP-dependent transcription. SF-1 is a 53 kDa protein consisting of a zinc finger domain and ligand binding/dimerization domain. The hydroxyl cholesterols enhance SF-1-dependent transcriptional activity in vitro, suggesting that SF-1 is a ligand-activated receptor. However, this is a controversial finding and needs further clarification [38].

SF-1 is expressed exclusively in steroidogenic tissues and plays an essential role in the development and function of the primary steroidogenic tissues [39]. Within the adrenal, SF-1 has been found to play a key role in the transcriptional regulation of most of the steroid hydroxylase genes (*CYP11A1*, *CYP21*, *CYP11B1*, *CYP17*, and *CYP 19*) as well as three *β HSD* and steroidogenic *acute regulatory protein* (StAR). SF-1 regulation of transcription is mediated by interaction with various co-activator proteins, including steroid receptor coactivator 1 (SRC1), glucocorticoid receptor interacting protein (GRIP), and also through repressors such as dosage-sensitive adrenal hypoplasia congenita of the X chromosome 1 (DAX1) that inhibits SF-1-mediated steroidogenesis [40].

The Ad4 or SF-1 site has been identified in all steroid P450s based on sequence alignments, including at position −351/−343 (AGGTCC) of *CYP11B2.* Although this site binds strongly to SF-1, deletion studies suggest that it is not essential for basal or stimulus-induced expression of *CYP11B2*. In fact, co-expression of *CYP11B2* reporter gene constructs with SF-1 has a negative effect on gene transcription, making the regulation of *CYP11B2* different to the other steroidogenic genes, *StAR, CYP11A*, *CYP11B1*, and *CYP 17* that are all induced by SF-1. The effects of both NGFIB and NURRl on *CYP11B2* expression can be inhibited by SF-1 supporting a negative effect of SF-1 [33]. Thus, *hCYP11B2* expression is regulated quite differently from genes for other proteins involved in steroid biosynthesis including *StAR*, *CYP11A*, *CYP11B1*, and *CYP17*, which are all positively regulated by SF-1 [41].

3.5.2.3 AD5

Electrophoretic mobility shift assay (EMSA) analysis of the −129/−114 (Ad5) element (CTCCAGCCTTGACCTT) has shown that it binds several nuclear proteins, including SF-1 and another orphan nuclear receptor, chicken ovalbumin upstream promoter transcription factor (COUP-TF). On the bovine *CYP 17* and the mouse *CYP21* gene, COUP-TF and SF-1 bind competitively to a common site. Deletion of this site decreases basal activity by approximately 80% and also reduces the maximal response to Ca^{2+} and cAMP stimulation [42].

3.5.2.4 NBRE

The transcription factors nerve growth factor IB (NGFIB) and nuclear receptor-related 1 protein (NURRl) are members of the NGFIB family of orphan nuclear receptors that bind to a consensus sequence NGFI-B response element (NBRE) (AAAGGTCA). These factors can also bind to the Ad5 element as well as a novel NBRE-1 site at −766/−759. Both these transcription factors increase *CYP11B2* expression by angiotensin II or K^+ stimulation. Mutation of the NBRE-1 site decreases basal expression as well as the response to angiotensin II and K^+.

Therefore, the CRE, Ad5, and NBRE-1 sites interact to regulate basal transcription as well as the response to each signaling pathway (cAMP or Ca^{2+}) [33].

4. Genotypic variants and haplotypes in *CYP11B2* gene

Several polymorphisms have been identified in *CYP11B2* and are associated with enhanced *CYP11B2* transcription, increased aldosterone production, and the progression of many cardiovascular disorders (**Figure 8**). About 227 single-nucleotide polymorphisms (SNPs) have been detected in different population worldwide according to National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) [43]. However, only few SNPs are evaluated in different studies. The first and most extensively studied polymorphism is −344 C/T which exists in the 5′ promoter region of *CYP11B2* gene. It occupies the putative binding site for the transcription factor SF-1 which is responsible for the expression of several enzymes involved in steroid biosynthesis framework in the adrenal cortex. The two alternative bases at this position are thymine (T) and cytosine (C) [44]. *CYP11B2* −344C/T polymorphism is associated with increased aldosterone synthesis and secretion in serum [45] or urine [46] and increased aldosterone renin ratio [47, 48] with either C allele or T allele were suggested to be involved among different studies. The mechanisms that stand behind this association are conflicting. Functional studies showed that T allele binds to SF-1 with four folds lower affinity than the C allele. This results in stimulation of the expression of enzymes responsible for steroidogenesis leading to increased aldosterone synthase activity in C allele carriers [41]. However, in T allele carrier, the increased aldosterone production was explained by lower affinity for SF1 binding leading to the increased availability of this transcription factor in other parts of the gene with subsequent activation of the steroidogenic acute regulatory gene [45]. On the other hand, others suggest that this polymorphism exert its effect via a strong linkage disequilibrium with functional polymorphisms in *CYP11B1*, a substitution (T to C) in codon 75 and (G to A) in intron 6 that result in 11 β hydroxylase deficiency [49]. This results in sustained elevation of ACTH [50] with subsequent increases in the expression of a number of genes required for aldosterone synthesis including StAR, *CYP11A*, and *CYP21* leading to enhanced aldosterone synthesis [31, 50].

The second polymorphism is a gene intronic conversion (IC) whereby a fragment of the "wild-type (Wt)" intron 2 of *CYP11B2* is replaced by the corresponding intronic fragment of *CYP11B1* conversion (Conv). Due to the nature of this variation and difficulty in genotyping it, its frequency has not been reported in

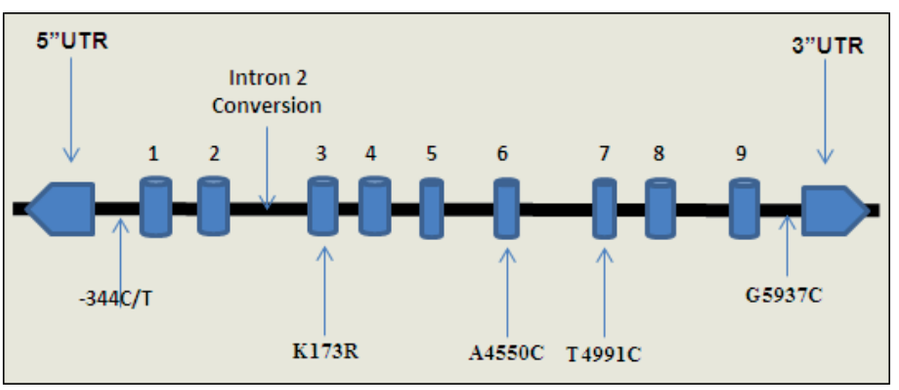

Figure 8.
Common CYP11B2 polymorphisms associated with increased aldosterone production.

public databases. The −344 T/C and intron conversion polymorphisms are in tight linkage disequilibrium and three common haplotypes have been reported: T/Conv (38%), C/Wt (45%), and T/Wt (13%), where the first allele corresponds to the −344 polymorphism (T or C) and the second to the intron 2 conversion (Wt or Conv) [31].

Beside the previously described polymorphisms of *CYP11B2*, other interesting polymorphisms have been reported and associated with increased aldosterone production as missense mutation in exon 3, where a lysine is replaced by an arginine (K173R) and *CYP11B2* (T4991C) SNP in exon 7 [51]. However, the haplotype including −344T and K173 was associated with higher *CYP11B2* expression in adrenal tissue than the −344C and R173 haplotype [52]. Moreover, other polymorphisms have been studied as G5937C and A4550C and were associated with heart size [53].

4.1 *CYP11B2* genotypic variants and hypertension

Hypertension is a complex progressive cardiovascular disorder, not just a scale of threshold blood pressure values, with many causes that result in both functional and structural cardiac and vascular system abnormalities that damage the heart, kidneys, brain, vasculature, and other organs, and lead to premature morbidity and death [54]. Essential hypertension is a multifactorial disorder, predisposed by genetic and environmental factors [55]. Growing evidences suggest the potential role of genetic alteration affecting genes encoding the aldosterone synthesis pathway enzymes with associated enhanced aldosterone production resulting in progression to essential hypertension [56].

Several polymorphic variants of *CYP11B2* have been identified as potential genetic contributor in patients with essential hypertension with −344C/T was extensively studied among different ethnic groups, with conflicting results. Public databases reported that the T allele is more frequent than the C allele in highlanders compared with lowlanders Indian [57], African Americans and Japanese subjects [48], Caucasian [58], Tamil population [59], Chinese Han population [38], Taiwanese females [60], and recently in the Egyptian population [61]. C allele has been suggested to be associated with hypertension by Tsukada et al. [62], Kumar et al. [63], Ji et al. [64], Li et al. [65], and in the meta-analysis by Cheng et al. [66]. No association was detected in the Japanese population [67] and in the meta-analysis by Chen et al. [68]. These discrepancies reflect the influence of different genetic and environmental factors as well as the age and gender in geographically separated populations [61].

The *CYP11B2* gene intronic conversion (IC) was also described in a small study of essential hypertensive subjects; the conversion allele frequency was much lower in black subjects compared with white [69]. However, several studies proved the more frequent association of *CYB11B2* −344T/IC haplotype with hypertensive subjects than normotensives, especially those with a high aldosterone renin ratio (ARR) and increased aldosterone levels in plasma [45, 47], although this finding is not supported in all studies [67]. IC has also associated with a better therapeutic response to anti-hypertensive treatment [70].

On the other hand, *CYP11B2* (K173R) and *CYP11B2* (T4991C) polymorphisms were found to be associated with hypertension [51, 52]. A strong synergistic effect has existed among different genotypes of *CYP11B2* C−344T, IC, and K173R polymorphisms with the haplotype (−344T-Conv-K173) associated with a higher risk for essential hypertension progression [43]. Despite the use of several polymorphisms in *CYP11B2,* their causal relationship with hypertension and inappropriate aldosterone production remains unclear.

4.2 *CYP11B2* genotypic variants and atrial fibrillation

Atrial fibrillation (AF) is the most clinically prevalent type of cardiac arrhythmia which may be precipitated due to the presence of underlying heart disease such as valvular dysfunction, ventricular dysfunction, and hypertension. However, AF does not exist in some patients with one or more of these risk factors and presents in others without any risk factors. Accordingly, the genetic role had been emerged in the predisposition for AF. A positive family history of AF in at least one parent was suggested by a recent Framingham Heart study on 2243 participants to be associated with an 85% increased relative risk for AF [71].

Taking together this genetic role, two types of AF have been identified regarding the heredity characteristics as familial and non-familial AF. Recent studies have detected several candidate genes which were suggested to be associated with the familial AF type such as genes encoding for subunits of potassium or sodium channels, sarcolipin, connexin 40, endothelial nitric oxide synthase, interleukin 10, and RAAS [72].

Association of genetic variants of renin angiotensin aldosterone system (RAAS) system with non-familial AF was suggested by Tsai et al. [73] using a risk-factor matched design. *CYP11B2* −344C/T polymorphism was reported to be associated with the susceptibility of AF by Amir et al. [74] in a cohort of 196 patients with symptomatic systolic heart failure. They found that the −344 CC genotype to be a strong independent predictor for AF (adjusted OR 2.35, 95% CI 1.57–3.51, $P = 0.03$). Therefore, *CYP11B2* −344C/T polymorphism may predispose to AF in patients with HF. Another study by Lu et al. [75] on 359 of Han and Kazak population with non-valvular AF and 527 non-AF patients as a control reported that −344C/T polymorphism of *CYP11B2* was associated with AF risk as the frequencies of TT genotype, and co-dominant model (CC + TT genotype) in Han population and of TT genotype, and dominant model (CT + TT genotype) in Kasak population were significantly higher in AF group than in the control group. Furthermore, a meta-analysis by Li et al. [76] involving 2758 subjects from six distinct studies reported that *CYP11B2* T−344C gene polymorphism was significantly associated with AF in all genetic models; allelic (OR: 1.26, $P = 0.0002$), recessive (OR: 1.99, $P = 0.003$), dominant (OR: 0.903, $P = 0.036$), and homozygous (OR: 1.356, 95% CI: 1.130–1.628, $P = 0.001$), and additive (OR: 1.153, $P = 1.0 \times 10^{-10}$). On the other hand, no significant association was detected by Zhang et al. [77] between different genotype and alleles of −344 T/C polymorphism and lone AF patients.

The possible mechanisms for the association of *CYP11B2* polymorphism and AF are mainly related to increased aldosterone production. Aldosterone exerts its effect via direct and indirect ways. Indirect effect of aldosterone on the heart arise from its role on increasing blood volume, blood pressure, left ventricular pressure, left ventricular hypertrophy, left atrial pressure, and left atrial volume. However, aldosterone can directly act on the heart inducing cardiac hypertrophy and fibrosis [78, 79] via the proliferation and differentiation of myocardial cells, vascular smooth muscle cells, and fibroblasts, leading to a significant increase in collagen production. These effects lead to cardiac fibrosis and structural remodeling leading to heart rhythm disorders. Specifically, in the atrium, aldosterone may directly or indirectly cause atrial enlargement and fibrosis, leading to structural and electrical atrial remodeling resulting in atrial fibrillation. Based on these findings, aldosterone antagonists as angiotensin II receptor antagonists may be used clinically in the patients with AF to control and minimize the incidence and persistence of AF [80].

4.3 *CYP11B2* genotypic variants and coronary artery disease

Coronary artery disease (CAD) is a complex disorder comprised two major subsequent events: coronary atherosclerosis and myocardial infarction (MI). Despite the major progress in diagnosis of CAD, the pathogenesis and possible risk factors need further evaluation. The classical risk factors including positive family history, smoking, high body mass index, and disorders as hyperlipidemia, hypertension, and diabetes mellitus have been reported to be responsible for no more than 50% of total risk factors for CAD. Accordingly, genetic background seems also to participate in the predisposition for CAD [81].

Among the different polymorphisms described for *CYP11B2* gene, several studies have shown that the *CYP11B2* gene -344T>C polymorphism is associated with CAD in different ethnic groups with controversial results. Previous study on 201 CAD patients and 201 controls form Italian population have detected that *CYP11B2* polymorphism and CC genotype were associated with CAD risk in crude analysis with borderline significance which is lost by stratification to the confounding factors as smoking and family history [82]. A meta-analysis suggested that the −344T>C polymorphism in the *CYP11B2* gene might be associated with susceptibility to CAD in Caucasians and Asians [83]. A study on 609 Taiwanese male and female subjects who were unrelated and received coronary catheterization found that the C/C allele occurred more frequently in females who had CAD, and that it was associated with higher left ventricular mass (LVM) and left ventricular end diastolic diameter (LVEDD) [84]. Sharma et al. [85] also reported the association of the *CYP11B2* −344C>T polymorphism with the size of atherosclerotic plaque in the carotid artery. Neal et al. [86] suggested that −344C/T polymorphism is a cardiovascular risk factor due to its association with LV hypertrophy and decreased baroreflex sensitivity which predict the morbidity and mortality rates of MI. Others failed to find any significant association of *CYP11B2* with CAD in different populations as in an Indian population and other populations [87–89].

The underlying mechanism by which the *CYP11B2* -344T>C polymorphism can increase the risk for CAD remains unclear. However, it is related to its effect on increasing expression of *CYP11B2*, thereby increasing aldosterone secretion [83]. Also, the influence of gene-environment interaction has been involved as an etiological factor for CAD risk. Growing evidence has suggested the interaction between -344T>C polymorphism and positive smoking for enhanced CAD risk [90]. Also, Hautanen et al. [91] detected that smoking and dyslipidemia are associated risk factors for non-fatal MI in males who were carriers for *CYP11B2* −344C/T allele.

4.4 *CYP11B2* genotypic variants and hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a clinical cardiac dysfunction characterized mainly by hypertrophy of left ventricle. HCM is an autosomal dominant disorder and its diagnosis needs to exclude other cardiac or systemic causes of increased ventricular wall thickening. HCM comprises different histological features as cardiomyocyte hypertrophy, myofibrillar disarray, and fibrosis. Several factors are involved in the pathogenesis of HCM, with the genetic element has an upper hand. Mutations and polymorphisms in genes encoding the sarcomere proteins and renin-angiotensin-aldosterone system (RAAS) seem to be related to the predisposition for left ventricular hypertrophy (LVH) and HCM [92].

Evidences suggested that aldosterone seems to play a major role in the progression of LVH and HCM as it is produced locally in the heart and *CYP11B2*

mRNA levels show sevenfold increase in the cardiac tissue of HCM patients when compared normal cardiac tissue [93]. It exerts its action via stimulation of mineralocorticoid receptor resulting in enhancement of myocardial cell hypertrophy, progressive myocardial fibrosis leading to ventricular and septal remodeling, and hypertrophy, resulting in elevated ventricular mass observed in HCM patients [94, 95]. Therefore, genetic variations involving *CYP11B2* gene might influence the structure and function of the left ventricle via increasing aldosterone secretion [93].

Several polymorphisms in the *CYP11B2* had been described in the HCM with −344C>T and intron 2 conversion have significant associations with left ventricular size, mass, and function in different population studies. However, the −344C>T promoter polymorphism is a much better predictor of left ventricular size than is the intron 2 gene conversion [96]. The −344C>T polymorphism have proved to be associated with the risk for HCM progression in many previous studies [93, 94, 97–99], whereas no association has suggested by other studies [100, 101].This controversy extents to the risk allele, which may be the −344C in some studies [96, 98–100, 102, 103] or −344T in another study [97]. Moreover, the risk allele for increased ventricular size and thickness differs regarding gender as with a study by Chai et al., [97] in which the T allele carrier is associated with a significantly higher intra-ventricular septum thickness in males, whereas in females, CC genotype carriers had a higher risk for increased intra-ventricular mass thickness, left ventricular mass index, and Wigle score.

4.5 *CYP11B2* genotypic variants and heart failure

Heart failure (HF) is a cardiac disorder characterized by cardiac remodeling with subsequent cardiac dysfunction [104]. It is a multifactorial disease, which is precipitated due to underlying cardiac disorders as hypertension, CAD, valvular dysfunction, arrhythmia, and cardiomyopathy. Integrated neuronal and hormonal elements are involved in the pathogenesis of HF including activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system, and other mediators as endothelin, vascular endothelial growth factor, and inflammatory cytokines. Based on the previously described neurohormonal factors, therapeutic approaches for HF have developed to block these factors including angiotensin-converting enzyme inhibitors (ACEI), and aldosterone antagonists [105]. Several risk and prognostic factors have influenced the progression of chronic congestive heart failure (CHF) including age, New York Heart Association (NYHA) class, renal function, and comorbidities such as atrial fibrillation, diabetes mellitus, and ischemic heart disease [106]. However, genetic elements seem to affect the risk, severity, and therapeutic response of HF [107]. *CYP11B2* polymorphisms specifically −344C/T is among these genetic factors and has been associated with the susceptibility, therapeutic response, or prognosis of HF.

A study by McNamara et al. [108] was performed on a total of 354 subjects from A-HeFT (African American Heart Failure Trial). They detected a higher frequency of −344 TT genotypes carriers (61%) than CC genotypes carriers (6.2%) in African Americans. Also, the −344 C allele was associated with significantly poorer HF hospitalization-free survival and a higher rate of death. Moreover, the therapeutic influence of nitric oxide donor (isosorbide dinitrate and hydralazine) was augmented in the presence of the TT genotype.

Another study by Feola et al. [109] on 175 patients from the European continental ancestry congestive heart failure (CHF) population and revealed that no significant differences between −344 CC and −344 TT genotypes carriers regarding cardiac output, end-systolic or diastolic left ventricle diameter, left ventricular ejection fraction (LVEF), and pro-natriuretic peptide (BNP). Also, −344 C allele

carriers have a higher degree of disability (Barthel Index), NYHA class, and a lower cardiac index. Moreover, the two groups showed a similar clinical outcomes either death or re-hospitalization after 4 years follow up period.

In 107 Black South African patients with dilated cardiomyopathy in functional class II–III, Tiago et al. [110] detected that the −344C allele was associated with improvement of LVEF after traditional therapy despite it is not related to the baseline LVEF as well as left ventricular dimension. Also, the allele distribution has proven to be different among races as the C allele was significantly more frequent in non-African-American HF patients if compared with African-American patients and associated only in the African-American with a lower end-systolic left ventricular diameter at 1 year follow-up [111]. Moreover, the association of −344C allele was confirmed as a risk factor for the progression of AF in a cohort of 194 African-American HF patients [112].

5. Conclusions

CYP11B2 gene polymorphism is associated with increased cardiovascular risk via enhancing aldosterone synthesis and production which acts either systemically or locally on the heart. There is a large number of polymorphisms has been identified for *CYP11B2*. Among them, −344C/T polymorphism was extensively studied in different ethnic groups and has proved to be associated either solely or synergistic with other *CYP11B2* polymorphisms in the risk for progression and susceptibility of several cardiovascular disorders as hypertension, AF, CAD, cardiomyopathy, and HF. Also, *CYP11B2* polymorphisms were confirmed as a predictor for survival and therapeutic response in some cardiovascular disorders as hypertension and HF. Moreover, gene-environment interactions of *CYP11B2* were suggested in several studies but still need to be confirmed.

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