Unraveling the Structural Consequence of Pathogenic Single Nucleotide Polymorphisms in the Human Aldosterone Synthase: An *in silico* Approach

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Abstract

Aldosterone is an essential hormone secreted by adrenal glands playing a major role in the regulation of blood pressure. The secretion of aldosterone depends on the regulation of aldosterone synthase through the CYP11B2 (cytochrome P450 family 11 subfamily B member 2) gene in the zona glomerulosa layer of adrenal glands. The CYP11B2 gene plays a major role in the development of essential hypertension through the conversion of deoxycorticosterone to aldosterone. Recent studies have shown that polymorphism in CYP11B2 has a relationship with the development of hypertension. It also showed that the CYP11B2 gene single nucleotide polymorphism (SNP) may play a major role in the development of disorders like diabetes, hypertension, myocardial infarction etc.

The present study made an initial attempt to study the effect of selective SNP mutation in the CYP11B2 protein structure modifications and its impact on aldosterone synthase production. This present study uses a bioinformatics database (uniprot) for protein sequence retrieval of CYP11B2.

The retrieved protein sequence of CYP11B2 was used for the creation of mutant structures (pathogenic, benign and like benign) with the help of a molecular modeling approach (Schrödinger Molecular Modeling Suite). The effect of created single-point mutations on protein stability was studied with the help of a computational tool (Dynamut). The outcome of the present study showed that selective pathogenic mutations severely impact the function of CYP11B2 proteins compared to benign and likely begin structures. It also proved that pathogenic mutations may also lead to structural changes in the CYP11B2 protein. The outcome of the present study showed that SNP may have a varying impact on the CYP11B2 protein functions through structural differentiations.

Keywords: Aldosterone synthase, CYP11B2, Dynamut, Single nucleotide polymorphism, Pathogenic mutations.

Introduction

The mineralocorticoid pathway plays a major role in the regulation of blood pressure through the salt and water balance (SWB). This SWB plays a crucial responsibility in the elevation of blood pressure levels during regulated retention of sodium in the circulatory system[8,16]. Imbalances in this sodium retention may result in the development of a condition termed hypertension. In a host system, the formation of hypertension (high blood pressure) results in the elevated contraction and relaxation process of heart muscles. The elevation of contraction and relaxation process may result in the pumping of more amount of blood into the arteries and may cause hindrances in the flow of blood through the arterioles[8,11,16].

Based on its nature and cause, formed hypertension is divided into two different types i.e. primary and secondary hypertension. Recent studies also reported that causatives of hypertension determine the type of hypertension in its abundance as primary (95 %) and secondary hypertension (5 %). Compared to secondary hypertension, primary hypertension plays a major role in the development of high blood pressure through the increased release of aldosterone in the bloodstream[3,8,12].

In a normal condition, aldosterone is produced by a group of adrenal zona glomerulosa (ZG) cells located at the top of the adrenal cortex in the kidneys. The secretion of aldosterone depends on three sets of stimuli which include free-flowing adrenocorticotrophic hormone (FFAH), amount of serum potassium (ASP) and angiotensin II (A-II)[10,13,18]. This stated stimulus plays a major role in the synthesis and release of aldosterone in the circulatory system. Initially, aldosterone synthesis uses an acute phase protein steroidogenic acute regulatory protein (SARP) for the transport of cholesterol from blood to the ZG cells. The transported cholesterol level later on moves into the inner mitochondrial membrane of ZG cells[5,16,18]. Whenever ZG cells receive stimulus, exposure results in the increased expression of SARP through its active phosphorylation in a gradual manner.

The active phosphorylation of SARP may result in the production of aldosterone with the help of a rate-limiting enzyme aldosterone synthase (AS)[2,6]. The rate-limiting enzyme AS is produced by a cytochrome P450 family 11 subfamily B member 2 (CYP11B2) gene located on

chromosome 8 with 9 different exons. The CYP11B2 approximately has a length of 700 base pairs with 93 % homology to the CYP11B1 gene[19]. The CYP11B2 encodes for cytochrome P450 proteins and enzymes. Produced cytochrome P450 proteins and enzymes are involved in the synthesis and regulation of steroids, lipids and cholesterol.

However, mutations and single nucleotide polymorphism (SNP) in this CYP11B2 gene result in the development of cardiovascular disorders like coronary heart (CD) disease, hypertension, atrial fibrillation (AF), heart failure and cardiomyopathy[1,3,5]. Compared to mutations, CYP11B2 gene SNP plays an important role in the increased production of AS. Further, increased levels of AS may result in the development of hypertension through the renin-angiotensin-aldosterone system[2,4,6]. In the present study, we planned to study the effect of all CYP11B2 SNPs and their influential role in the development of hypertension through the visualization of intramolecular changes in the observed mutational changes with the help of three-dimensional structures.

Material and Methods

Collection of experimental data: Experimental data used in this present study was retrieved from the computational database UniProt. UniProt is a comprehensive resource for protein sequence and functional information retrieval which provide access to a vast collection of protein data from various organisms. It acts as a central hub for protein-related information, offering detailed annotations, functional classifications and cross-references to otherdatabases. We utilized cytochrome P450 protein variant viewer to retrieve the single nucleotide polymorphisms (SNPs) and extract relevant data from UniProt (https://www.uniprot.org/uniprotkb/P19099/entry). This allows us to comprehensively understand the impact of genetic variations on the structure and function of cytochrome P450 enzymes, leveraging the curated information available in UniProt for deeper insights into these variants. The amino acid sequence of *Homo sapiens* Cytochrome P450 11B2 (Accession Number: P19099) was retrieved from the UniProtKB database (http://www.uniprot.org/). The retrieved sequence of Cytochrome P450 11B2 (CYP11B2) consists of 503 amino acid residues.

Molecular Modeling: The retrieved amino acid sequence of *Homo sapiens* Cytochrome P450 11B2 (Accession Number: P19099) was used for the creation of mutant structures. The mutant structures like pathogenic mutant, benign mutant and likely benign mutant structures were modeled using Schrödinger Molecular Modeling Suite (Prime, version 3.5, 2014; Schrödinger Inc). The Schrödinger molecular modeling suite was most commonly used in molecular visualization, molecular dynamic simulations and structure-based drug design studies.

Stability analysis test: In the present study, DynaMut (http://biosig.unimelb.edu.au/dynamut) was used for studying the impact of base variants on the stability of a protein through a stability analysis test. Dynamut is a computational tool that predicts how single-point mutations affect protein stability using molecular dynamics simulations. Based on the input protein structure in PDB format, it allows the users to visualize the impact of the mutations. The selected pathogenic mutations are analyzed using DynaMut, which predicts the changes in structural integrity and stability caused by these mutations.

DynaMut evaluates alterations in intramolecular interactions like hydrogen bonds and hydrophobic interactions to determine the mutation's impact on overall protein stability. DynaMut compares themutated protein's dynamics to the wild-type structure, predicting increased, decreased, or unchanged stability. This tool offers valuable insights into mutation-induced structural changes, aiding insights into protein functions and potential therapeutic targets.

Results

Retrieval of CYP11B2 sequences and its single nucleotide polymorphism identification: At first, the UniProt protein variant viewer was used to retrieve the cytochrome P450 SNP along with relevant needed data. The obtained information was used to identify the impact of single nucleotide variations in the structure and function of cytochrome P450 proteins based on the available research reports (Fig. 1). Followed by information acquaintance, reported phenotypic variants associated with CYP11B2 proteins were identified as natural and mutagenic variants by comparing the retrieved sequences with the help of UniProtKB database (Fig. 2).

Identification of SNP phenotypic variants in CYP11B2 protein: The human CYP11B2 gene contains a total of 560 SNPs, of which 39 are likely beningn (LB) and benign (B), 28 are likely pathogenic (LP) and pathogenic (P) and 31 are variant of uncertain significance (VUS). The pathogenic SNPs contain 27 numbers of pathogenic SNPs with 5 clinical variants and 5 numbers of multiple replacements throughout the amino acid sequence of the CYP11B2 along with likely pathogenic SNPs (Table 1). Compared to the pathogenic SNPs, 39 benign SNPs include 28 numbers of LB, 18 numbers of B and 3 numbers of multiple replacements in the CYP11B2 amino acid sequences (Table 2). Compared to pathogenic and benign SNPs, 31 numbers of uncertain significance SNPs were observed along with the LBB and LPP. Observed SNPs may happen due to single base changes (SBC) in the CYP11B2 amino acid sequences (Table 3).

The resultant of this SBC may result in the formation of mutant structures like beningn and pathogenic SNPs in the CYP11B2. Following the identification of reported pathogenic, benign and VUS SNPs, SNPs were short-listed based on their clinical significance and resultant phenotypic changes in the function of the CYP11B2 protein. Short-listed selective SNPs are classified as pathogenic, benign and VUS based on the amino acid change in the specific position.

Selective SNPs include 1 LP, 4 LP and a repetitive P SNP (T>M) in the 318 position. Observed pathogenic SNP also contains a position change (T>R) in the 318 position compared to the repetitive LP. The SNP changes in the 318 position showed a moderate effect on the corticosterone 18-monooxygenase deficiency.

The observed SNPs may result in phenotypic changes in the CYP11B2 protein (Table 4) followed by the identification of pathogenic SNPs. Benign SNPs were identified as 38 in number with 25 LB and 13 B which comprise of 9 and 13 repetitions. The repetitive SNPs may act as a causative for glucocorticoid-remediable aldosteronism, corticosterone 18-monooxygenase and corticosterone methyloxidase type 2 deficiencies. However, it also proved that benign SNPs had a mild effect on corticosterone 18-monooxygenase deficiencies compared to pathogenic SNPs (Table 5). To prove the impact of VUS in the CYP11B2 phenotypic changes, a list of 14 VUS SNPs and 13 VUS SNPs was prepared with repetitions in the identified SNPs.

The identified SNPs were involved in the development of inborn genetic disorders like corticosterone methyl oxidase type II deficiency and the corticosterone 18-monooxygenase deficiency (Table 6). The overall outcome of the SNP identification results shows that pathogenic SNPs played a major role in the development of phenotypic changes of CYP11B2 with some structural changes in the CYP11B2 protein sequence.

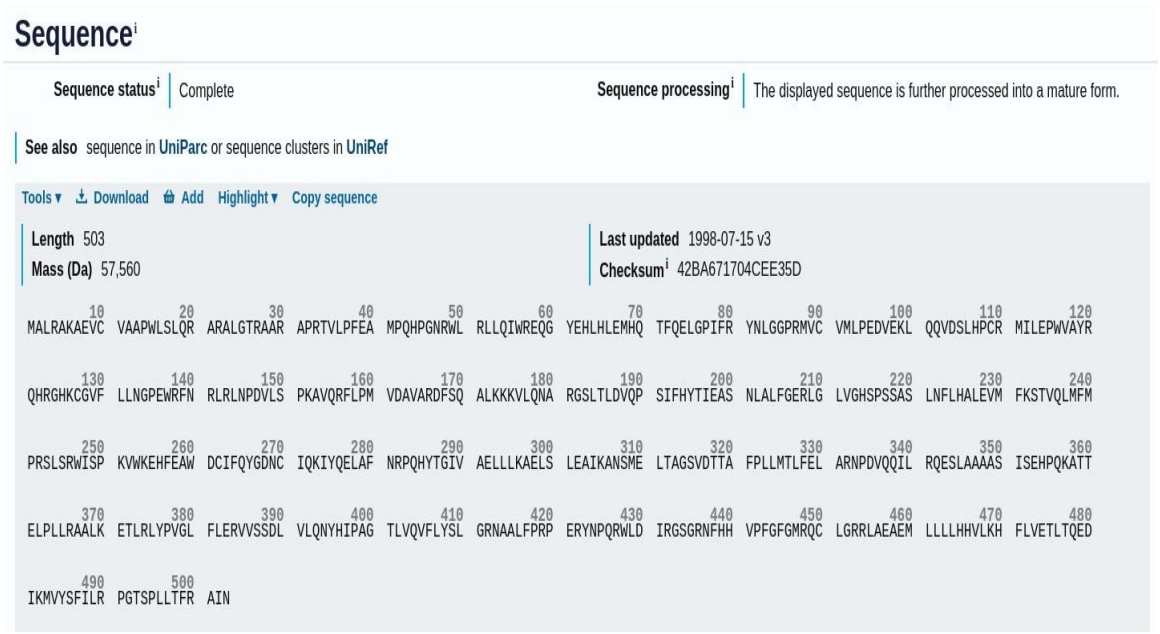

Figure 1: UniProtKB sequence retrieval showed the amino acid sequence of *Homo sapiens* Cytochrome P450 11B2 (Accession Number: P19099) with a length of 503 base pairs and molecular weight of 57.56 kilodaltons (kDa)

Figure 2: List of single nucleotide polymorphism (SNP) associated phenotypic variants reported in CYP11B2 protein

Table 1
List of SNP pathogenic variants reported in CYP11B2 protein sequence

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Likely pathogenic							
1.	rs1351295710	308	S>P				
Pathogenic							
1.	rs762727830	102	Q>*	10.	rs104894072	198	E>D
2.	rs765802331	141	R>*	11.	rs759384300	337	Q>*
3.	rs768685630	150	S>*	12.	rs752975169	384	R>*
4.	rs768685630	150	S>L	13.	rs61757294	386	V>A
5.	rs28931609	181	R>W	14.	rs121912977	255	E>*
6.	rs765921219	318	T>M	15.	rs121912979	272	Q>*
7.	rs1205192306	178	Q>*	16.	rs878852988	450	C>*
8.	rs771908700	183	S>*	17.	rs72554626	498	T>A
9.	rs121912978	185	T>I				
Pathogenic (ClinVar)				Pathogenic (Ensembl)			
1.	rs752745892	10	C>*	1.	rs752745892	10	C>W
2.	rs63748989	46-47	GN>*	2.	rs764756525	73	Q>*
3.	rs761855534	85	G>*	3.	rs765921219	318	T>R
4.	rs1817576999	339	I>*	4.	rs61757294	386	V>G
5.	rs1817574696	373	L>*	5.	rs759666789	254	K>*

Table 2
List of benign SNP variants reported in CYP11B2 protein sequence

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Likely Benign							
1.	rs750860338	21	A>T	15.	rs769090647	327	L>F
2.	rs200559721	22	R>Q	16.	rs149963901	332	R>W
3.	rs143027239	28	A>V	17.	rs562670189	341	R>C
4.	rs549023364	109	C>R	18.	rs562670189	341	R>S
5.	rs527956959	109	C>S	19.	rs377428697	225	H>Q
6.	rs527956959	109	C>Y	20.	rs144140791	225	H>R
7.	rs777780952	138	R>C	21.	rs149706111	235	V>I
8.	rs147109119	61	Y>F	22.	rs778544381	242	R>S
9.	rs760339298	143	R>W	23.	rs369953763	246	R>H
10.	rs151052374	147	D>E	24.	rs148659506	366	R>W
11.	rs563073392	159	P>L	25.	rs780392562	272	Q>H
12.	rs200283987	86	P>A	26.	rs763373087	281	N>K
13.	rs200283987	86	P>S	27.	rs759183694	422	R>W
14.	rs201830462	320	A>V	28.	rs544454389	447	M>I
Benign							
1.	rs6438	29	A>V	10.	rs140405063	339	I>V
2.	rs6441	30	R>P	11.	rs746708275	347	A>T
3.	rs6441	30	R>Q	12.	rs551933154	214	H>D
4.	rs372556807	118	A>T	13.	rs4547	248	I>T
5.	rs4539	173	K>R	14.	rs752962897	251	K>R
6.	rs373369254	336	V>L	15.	rs4537	281	N>S
7.	rs373369254	336	V>M	16.	rs774989997	282	R>C
8.	rs4544	339	I>N	17.	rs755139614	414	A>P
9.	rs4544	339	I>T	18.	rs4545	435	G>S
Benign (Ensembl)							
1.	rs4546	168	F>L	3.	rs61757295	382	L>V
2.	rs4538	374	R>W				

Table 3
List of VUS SNP variants reported in CYP11B2 protein sequence

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Variant of uncertain significance (VUS)							
1.	rs1463986346	20	R>K	16.	rs778346287	212	V>A
2.	rs753988592	34	T>M	17.	rs778346287	212	V>G
3.	rs529683430	165	A>S	18.	rs763034354	226	A>V
4.	rs755076671	13	A>V	19.	rs779486718	266	Y>D
5.	rs773477950	130	F>L	20.	rs1409744694	379	G>D
6.	rs778618899	87	R>H	21.	rs769533897	282	R>P
7.	rs886062744	197	I>M	22.	rs1817560561	406	F>V
8.	rs368296597	321	F>I	23.	rs150685467	418	P>L
9.	rs778618899	87	R>L	24.	rs776680043	422	R>L
10.	rs749800670	208	R>P	25.	rs769533897	283	R>H
11.	rs749800670	208	R>Q	26.	rs1817545728	481	I>L
12.	rs1374018568	94	P>S	27.	rs150685467	418	P>R
13.	rs775492721	197	I>T	28.	rs776680043	422	R>Q
14.	rs370011030	341	R>H	29.	rs1311444460	448	R>H
15.	rs770281644	344	S>G	30.	rs1817545422	485	Y>C
Variant of uncertain significance (ClinVar)							
1.	rs1409744694	379	G>V				

Table 4
List of selective pathogenic SNP mutations that influence the phenotypic changes in CYP11B2 protein function

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Likely Pathogenic							
1.	rs1351295710	308	S>P				
Pathogenic (Ensembl)							
1.	rs752745892	10	C>W	3.	rs765921219	318	T>R
2.	rs768685630	150	S>L	4.	rs61757294	386	V>G
Pathogenic(Ensembl, ClinVar)							
1.	rs765921219	318	T>M				

Impact of pathogenic SNPs in the CYP11B2 protein stability: Protein stability analysis was performed with the use of a Dynamut server to identify the balance between intramolecular interactions of functional groups and their interaction with the environment. This protein stability analysis was used in the study to identify the stabilized and destabilized state of CYP11B2 protein with ΔΔG values. The outcome result of protein stability analysis showed that certain amino acid replacements result in the formation of SNP in the CYP11B2 protein sequence. It also showed that stabilized and destabilized CYP11B2 protein result in the acquaintance of positive and negative ΔΔG values. In this protein stability analysis, a total of 65 amino acid replacements were categorized as stabilized and destabilized CYP11B2 protein structures in the numbers 33 and 32.

The stabilized CYP11B2 protein sequence has positive ΔΔG values between 0.009 – 1.158 kcal/mol. The resultant ΔΔG values of stabilized CYP11B2 protein showed lower value with the replacement of amino acid L with F and higher value observed at the replacement of A with T (Table 7). However, the destabilized CYP11B2 protein structure has a negative ΔΔG value compared to the stabilized structure. The ΔΔG values of destabilization studies showed negative values between - 0.191 – - 2.201 kcal/mol. The obtained results of the protein stability analysis showed that the stability of CYP11B2 protein plays a major role in the phenotypic determination of SNPs (Table 8).

Effect of intramolecular interaction in the identification of CYP11B2 pathogenic SNPs: Following protein stability analysis, the effects of intramolecular interactions were studied in the selective pathogenic SNPs by comparing wild-type and mutant structures. A list of five different molecular interactions like polar vanderwaal (PV), hydrophobic (H), carbon PI (C), methionine sulfur PI (M), donor PI (D) was employed in this study to identify the changes in the pathogenic SNPs of CYP11B2. The intermolecular interaction studies showed that amino acid change at 118 and 347 position results in the increased number of interactions in PV, H as a result of A replacement with T. The identified molecular interaction may result in the higher level of protein structural changes compared to the wild type. Compared to higher level protein structural changes, a moderate number of interactions were increased in the amino acid change at 143 positions as a result of W replacement instead of R. The moderate intermolecular interaction may result in the increased number of interactions in H, C, M and

D. Followed by moderate rise in intramolecular interaction, unchanged molecular interaction parameters were observed in the 485 position which has C instead of Y (Table 9). The observed intramolecular interaction may play a major role in the flexibility and rigidity of the CYP11B2 protein structure. To identify the impact of pathogenic SNPs on CYP11B2 flexibility and rigidity, the 3D structure of S103L, S308P, T318M, T318R and V386G was created using molecular modeling technique and showed the molecular interaction and rigidity of the representative pathogenic SNPs.

Discussion

In the present study, computational methods like Schrödinger suite and Dynamut were used to identify the SNP in the CYP11B2 protein sequences. In the initial phase of the study, the uniport database is used for the collection of cytochrome P45011B2 amino acid sequence (source – *Homo sapiens*, accession number – P19099). The collected CYP11B2 consisting of 530 amino acid residues was used for the identification of SNPs. Recent studies have shown that SNP in the aldosterone synthase-associated gene CYP11B2 results in the increased production of the aldosterone. The increased production of aldosterone results in the development of clinically visible phenotypes (CVP). The formed CVP showed the impairment in the development of hypertension which is associated with the cardiovascular system[7,14].

Recent studies have shown that non-synonymous single nucleotide polymorphism (nsSNPs) results in the amino acid sequence changes in the CYP11B2 protein. Observed amino acid changes in the CYP11B2 protein sequence may result in structural changes like intramolecular interaction (II) and stability changes (SC)[9,14,21]. Other than impairment in the II and SC, formed nsSNPs may result in the progression of disease through protein-protein interactions. So far, few studies opened up the role of SNPs in the promoter region of the CYP11B2 gene and reported protein structural changes (PSC)[1,9].

Observed PSC results in the deleterious effect of the CYP11B2 gene with the corticosterone methyl oxidase deficiency (CMO) associated with reduced aldosterone production. However, reported studies never tried to study the impact of whole SNP in the disease progression through the *in silico* studies. The *in silico* computational methods are flexible and reliable to predict the SNPs associated with the whole amino acid sequence of the CYP11B2 [6,15,17].

Table 5
List of selective benign SNP mutations that influence the phenotypic changes in CYP11B2 protein function

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Likely Benign							
1.	rs147109119	61	Y>F	9.	rs377428697	225	H>Q
2.	rs549023364	109	C>R	10.	rs149706111	235	V>I
3.	rs750860338	21	A>T	11.	rs778544381	242	R>S
4.	rs200559721	22	R>Q	12.	rs780392562	272	Q>H
5.	rs143027239	28	A>V	13.	rs763373087	281	N>K
6.	rs200283987	86	P>S	14.	rs149963901	332	R>W
7.	rs527956959	109	C>S	15.	rs148659506	366	R>W
8.	rs151052374	147	D>E	16.	rs759183694	422	R>W
Likely benign (NCI-TCGA)							
1.	rs777780952	138	R>C				
Likely benign(Ensembl,ClinVar,NCI-TCGA)							
1.	rs562670189	341	R>C				
Likely benign (Ensembl, ClinVar)							
1.	rs527956959	109	C>Y	5.	rs369953763	246	R>H
2.	rs760339298	143	R>W	6.	rs769090647	327	L>F
3.	rs563073392	159	P>L	7.	rs544454389	447	M>I
4.	rs144140791	225	H>R				
Benign							
1.	rs372556812	339	I>N	3.	rs372556814	347	A>T
2.	rs372556813	339	I>V				
Benign (Ensembl,ClinVar)							
1.	rs372556807	118	A>T	4.	RCV002058710	282	R>C
2.	rs372556808	214	H>D	5.	rs372556811	336	V>M
3.	rs372556809	251	K>R				
Benign (Ensembl)							
1.	rs372556815	30	R>P	4.	rs372556818	382	L>V
2.	rs372556816	168	F>L	5.	rs372556819	390	L>F
3.	rs372556817	336	V>L				

Table 6
List of selective VUS SNP mutations that influence the phenotypic changes in CYP11B2 protein function

S.N.	Variant	Position	Change	S.N.	Variant	Position	Change
Variant of uncertain Significance							
1.	rs755076671	13	A>V	8.	rs763034354	226	A>V
2.	rs1463986346	20	R>K	9.	rs779486718	266	Y>D
3.	rs753988592	34	T>M	10.	rs368296597	321	F>I
4.	rs778618899	87	R>H	11.	rs370011030	341	R>H
5.	rs529683430	160	A>S	12.	rs770281644	344	S>G
6.	rs775492721	197	I>T	13.	rs150685467	418	P>L
7.	rs778346287	212	V>A	14.	rs776680043	422	R>Q
Variant of uncertain Significance (Ensembl,ClinVar, NCI-TCGA)							
1.	rs1311444460	448	R>H				
Variant of uncertain Significance (Ensembl,ClinVar)							
1.	RCV001159610	94	P>S				
Variant of uncertain Significance (ClinVar)							
1.	rs1409744694	379	G>V	3.	rs1409744694	481	I>L
2.	rs1817560561	406	F>V	4.	rs1817545422	485	Y>C
Variant of uncertain Significance (Ensembl)							
1.	rs778618899	87	R>L	5.	rs769533897	282	R>P
2.	rs773477950	130	F>L	6.	rs1409744694	379	G>D
3.	rs749800670	208	R>P	7.	rs150685467	418	P>R
4.	rs778346287	212	V>G				

Table 7
The stability analysis of the selective pathogenic mutations in CYP11B2 shows positional change of amino acid and ΔΔG values

S.N.	Amino acid		Position	ΔΔG DynaMut	S.N.	Amino acid		Position	ΔΔG DynaMut
	From	To				From	To		
Stability analysis									
Stabilization									
1.	A	V	226	0.271 kcal/mol	18.	C	Y	109	0.958 kcal/mol
2.	N	K	281	0.339 kcal/mol	19.	V	I	235	0.402 kcal/mol
3.	Q	H	272	0.076 kcal/mol	20.	H	R	225	0.309 kcal/mol
4.	T	A	318	0.019 kcal/mol	21.	P	S	86	0.3 kcal/mol
5.	I	L	481	0.432 kcal/mol	22.	G	D	379	0.485 kcal/mol
6.	V	M	336	0.197 kcal/mol	23.	R	Q	422	0.341 kcal/mol
7.	G	V	379	0.36 kcal/mol	24.	V	L	336	0.252 kcal/mol
8.	P	R	418	0.487 kcal/mol	25.	I	N	339	0.178 kcal/mol
9.	R	W	143	0.468 kcal/mol	26.	P	L	418	0.497 kcal/mol
10.	R	P	208	0.266 kcal/mol	27.	L	F	390	0.793 kcal/mol
11.	T	R	318	1.055 kcal/mol	28.	K	R	251	0.034 kcal/mol
12.	A	T	118	0.705 kcal/mol	29.	T	M	318	1.229 kcal/mol
13.	M	I	447	0.805 kcal/mol	30.	C	R	109	0.978 kcal/mol
14.	H	Q	225	0.544 kcal/mol	31.	P	L	159	0.396 kcal/mol
15.	L	F	327	0.009 kcal/mol	32.	A	T	347	1.158 kcal/mol
16.	D	E	147	0.98 kcal/mol	33.	S	L	150	1.146 kcal/mol
17.	R	W	366	0.93 kcal/mol					

Initially, cytochrome P450 protein variant viewer was used to retrieve the SNP along with relevant data from UniProt database. Retrieved information was used as a blueprint to understand the impact of single nucleotide variations in the structure and function of cytochrome P450 enzymes. Followed by information acquaintance, CYP11B2 amino acid sequences were retrieved and used for the creation of mutagenic structures in the form of pathogenic, likely pathogenic, benign and likely benign structures with the use of the Schrödinger suite prime module. In observed mutant structures, pathogenic mutants may play a major role in the impaired CYP11B2 protein production through phenotypical changes. Followed by the phenotypical changes, stability analysis of selective pathogenic mutations

was studied with the help of dynamut server. The outcome of the stability analysis studies showed that most of the pathogenic mutations showed higher ΔG values with structural destabilization. Following stability analysis studies, the effect of SNPs was studied on the intramolecular interactions of CYP11B2 proteins. Observed results of the intramolecular interaction studies showed that pathogenic mutations played a major role in the development of structural changes in the CYP11B2 proteins which result in impaired protein functions. The overall outcome of the present study stated the SNP played a major role in the development of hypertension through the increased/decreased synthesis of aldosterone. The increased/decreased synthesis of aldosterone is regulated by the rate-limiting enzyme aldosterone synthase.

Table 8
The stability analysis of the selective pathogenic mutations in CYP11B2 shows positional change of amino acid and ΔΔG values during destabilization

S.N.	Amino acid		Position	ΔΔG DynaMut	S.N.	Amino acid		Position	ΔΔG DynaMut
	From	To				From	To		
Stability analysis									
Destabilization									
1.	R	H	87	-0.579 kcal/mol	17.	T	M	34	-0.812 kcal/mol
2.	R	L	87	-0.443 kcal/mol	18.	I	T	197	-2.911 kcal/mol
3.	V	G	386	-1.493 kcal/mol	19.	F	I	321	-0.812 kcal/mol
4.	Y	D	266	-0.195 kcal/mol	20.	I	V	339	-0.295 kcal/mol
5.	V	A	212	-2.207 kcal/mol	21.	Y	F	61	-0.692 kcal/mol
6.	R	P	282	-0.175 kcal/mol	22.	R	C	282	-0.401 kcal/mol
7.	F	V	406	-1.519 kcal/mol	23.	R	C	341	-1.543 kcal/mol
8.	P	S	94	-0.324 kcal/mol	24.	R	W	422	-0.014 kcal/mol
9.	H	D	214	-0.332 kcal/mol	25.	R	W	332	-0.228 kcal/mol
10.	V	G	212	-2.395 kcal/mol	26.	R	H	341	-1.424 kcal/mol
11.	Y	C	485	-0.54 kcal/mol	27.	R	Q	208	-0.196 kcal/mol
12.	F	L	130	-0.835 kcal/mol	28.	F	L	168	-0.845 kcal/mol
13.	R	S	242	-1.196 kcal/mol	29.	R	C	138	-0.659 kcal/mol
14.	R	H	448	-1.074 kcal/mol	30.	C	S	109	-0.144 kcal/mol
15.	S	G	344	-0.491 kcal/mol	31.	S	P	308	-0.289 kcal/mol
16.	L	V	382	-0.191 kcal/mol	32.	R	H	427	-1.531 kcal/mol

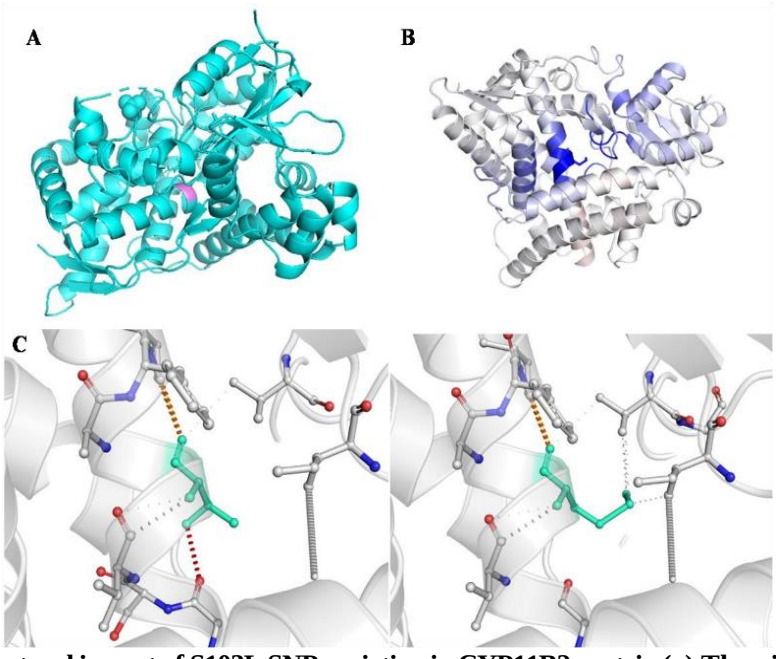

Figure 3: Influence and structural impact of S103L SNP variation in CYP11B2 protein (a) The pink color in the cyan color cartoon structure of CYP11B2 represents the SNP position (b) The regions influenced by SNP in the CYP11B2 structure, where the BLUE-colored areas represent the regions have become structurally RIGID due to this SNP (c) The comparison of intramolecular interactions observed in wild and SNP structures

Table 9
Changes in the intra-molecular interactions are influenced by selective pathogenic mutations, which lead to structural changes and thereby affect the function of the protein CYP11B2

	Wild Type					Mutant						Wild Type					Mutant				
Position	**PV**	**H**	**C**	**M**	**D**	**PV**	**H**	**C**	**M**	**D**	**Position**	**PV**	**H**	**C**	**M**	**D**	**PV**	**H**	**C**	**M**	**D**
A118T	3	4	-	3	4	6	3	-	3	3	R208P	3	-	-	-	-	-	1	-	1	-
A226V	2	3	-	3	-	2	2	2	4	-	R208Q	3	-	-	-	-	-	2	-	-	-
A347T	2	-	1	2	-	6	3	-	1	-	R242S	2	-	1	-	3	1	-	-	-	-
C109R	1	-	-	-	-	1	-	-	-	-	R282C	1	-	-	-	1	-	-	-	-	1
C109S	1	-	-	-	-	1	-	-	-	-	R282P	1	-	-	-	1	-	-	-	-	1
C109Y	1	-	-	-	-	1	4	11	1	-	R332W	3	-	-	2	1	2	-	1	1	2
D147E	-	1	1	-	-	2		1	1	-	R341C	3	-	4	1	1	4	-	1	-	1
F130L	2	-	5	-	2	2	-	-	1	3	R341H	3	-	4	1	1	2	-	5	-	1
F168L	2	-	4	-	-	2	1	4	-	-	R366W	1	1	1	1	1	2	-	2	-	1
F321I	1	1	3	1	-	-	1	4	-	-	R422Q	2	-	2	-	-	1	-	-	-	-
F406V	1	-	10	2	-	1	1	3	-	-	R422W	2	-	2	-	-	2	-	2	-	1
G379D	-	-	-	-	-	-	-	1	1	1	R427H	4	-	3	2	-	3	-	3	-	-
G379V	-	-	-	-	-	-	-	1	1	-	R448H	5	-	3	-	4	2	-	3	-	3
H214D		-	1	-	-	-	-	1	-	-	S150L	4	-	1	-	-	2	-	1	1	-
H225Q	3	-	1	1	-	3	-	-	-	-	S344G	2	-	1	-	-	2	-	-	-	-
H225R	3	-	1	-	-	2	-	1	2	-	T34M	1	-	-	-	-	1	-	-	-	-
I197T	1	-	1	1	2	4	-	1	-	2	T318A	1	-	1	-	-	-	-	1	-	-
I339N	1	-	-	-	-	1	-	-	-	-	T318M	1	-	1	-	-	-	-	1	-	-
I339V	4	-	-	1	-	2	-	1	-	-	T318R	1	-	1	-	-	-	-	2	1	-
I481L	-	1	2	3	-	-	1	2	3	-	V212A	-	-	2	-	-	-	-	1	-	-
K251R	-	-	1	1	-	1	-	1	-	-	V212G	-	-	2	-	-	-	-	1	-	-
L327F	1	-	1	1	-	1	4	3	-	-	V235I	1	-	4	2	2	1	3	5	2	2
L382V	1	-	3	1	-	1	2	3	-	-	V336L	-	-	1	1	-	-	-	1	1	-
L390F	2	-	1	-	1	2	3	1	-	1	V336M	-	-	1	1	-	-	-	1	-	-
M447I	-	-	1	-	-	-	2	2	-	-	V386G	2	-	-	-	1	2	-	-	-	-
N281K	1	-	1	-	1	1	-	1	1	-	Y61F	2	-	7	-	1	2	4	5	1	-
P86S	-	-	-	-	-	-	-	-	-	-	Y266D	2	1	2	1	-	-	-	2	-	-
P94S	1	-	2	-	1	-	-	2	-	1	Y485C	1	1	1	-	1	1	-	-	-	-
P159L	-	-	-	-	1	1	-	-	1	1	R87H	1	-	1	-	1	1	1	1	1	1
P418L	1	-	-	-	-	1	-	-	-	-	R87L	1	-	1	-	1	1	1	1	1	1
P418R	1	-	-	-	-	1	-	-	-	-	R138C	1	-	2	1	1	1	-	3	-	-
Q272H	2	-	-	1	-	2	-	-	-	-	R143W	2	-	1	-	3	-	3	3	3	2

PV - Polar Vander waal; H – Hydrophobic; C - Carbon PI; M - Metheonine Sulphur PI; D- Donor PI

Further, our studies also proved that the synthesis of aldosterone synthase may be affected by the established SNP in the CYP11B2 protein sequence. The 3D structure of CYP11B2 S103L SNP variation showed SNP position, structural rigid regions, compared to the intramolecular interactions observed in wild and SNP structures (Fig. 3). Like the 3D structure of CYP11B2 S103L SNP, SNP position, structural rigid regions and intramolecular interactions present among the wild and SNP structures were shown against the S308P, V386G and T318R SNP variations in this study.

Conclusion

The present study identified the presence of all SNPs in the entire sequence of CYP11B2 protein using computational methods. Initially, we have sorted out that pathogenic SNPs were reported to play an influential role in the functional defect of the CYP11B2 encoded protein aldosterone synthase. Further, we have modeled the three-dimensional structures for the identified 65 mutations. Based on the structural information, we have predicted the stabilization and destabilization parameters of the pathogenic mutations using delta G values.

We have compared the intramolecular changes that occurred in the protein sequence and associated intramolecular bonding differences that occurred between the wild type and mutant structures. Further, we evaluated the structural difference present between the wild type and mutant types for studying the effect of mutant types on the flexibility and rigidity of CYP11B2 protein structure. Thus, the outcome of the present study will help to design a common drug that can

work with wild and mutant types effectively. Our results also serve as a basis for structural pharmacogenomics studies and will pave the way for the development of personalized medicine.

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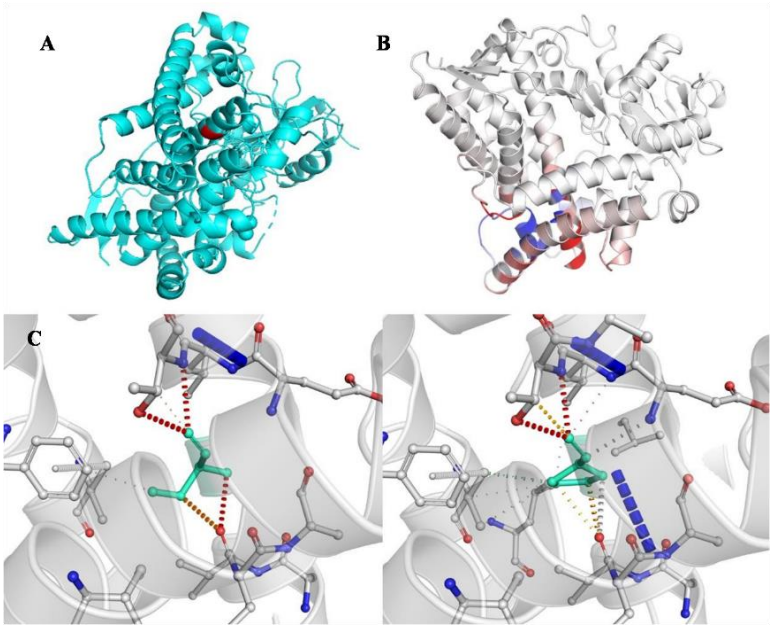

Figure 4: Influence and structural impact of S308P SNP variation in CYP11B2 protein (a) The pink color in the cyan color cartoon structure of CYP11B2 represents the SNP position (b) The regions influenced by SNP in the CYP11B2 structure, where the BLUE and RED-colored areas represent the regions have become structurally RIGID and FLEXIBLE due to change in amino acid (c) The comparison of intramolecular interactions observed in wild and SNP structures

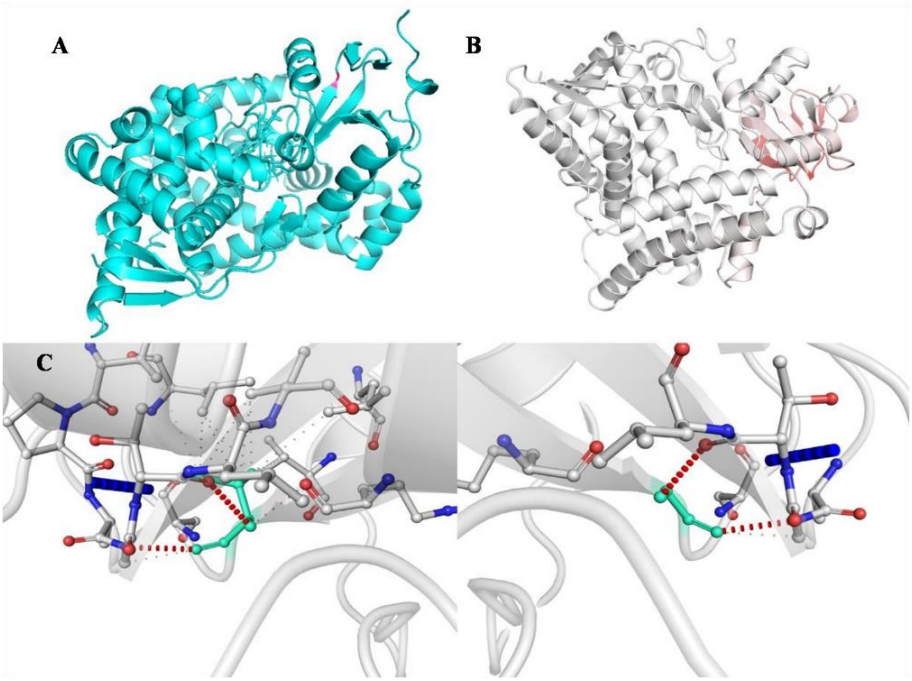

Figure 5: Influence and structural impact of V386G SNP variation in CYP11B2 protein (a) The pink color in the cyan color cartoon structure of CYP11B2 represents the SNP position (b) The regions influenced by SNP in the CYP11B2 structure, where the RED-colored areas represent the regions have become structurally FLEXIBLE due to this SNP (c) The comparison of intramolecular interactions observed in wild and SNP structures

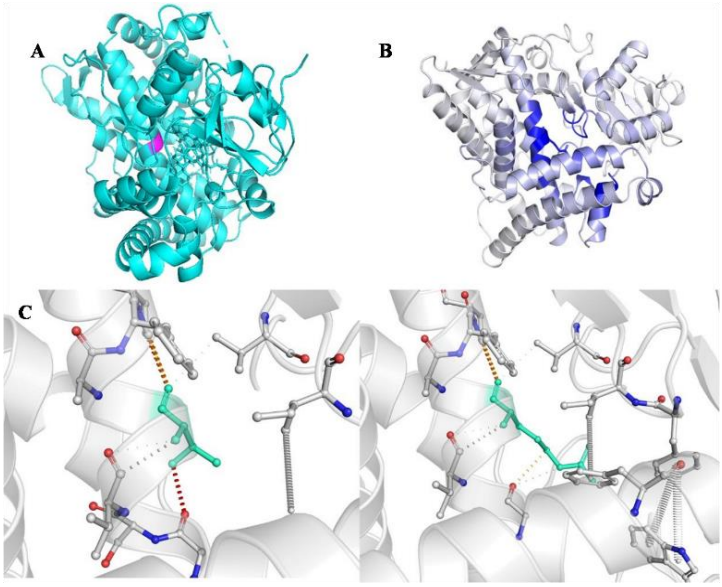

Figure 6: Influence and structural impact of T318R SNP variation in CYP11B2 protein (a) The pink color in the cyan color cartoon structure of CYP11B2 represents the SNP position (b) The regions influenced by SNP in the CYP11B2 structure, where the BLUE and RED-colored areas represent the regions have become structurally RIGID and FLEXIBLE due to change in amino acid (c) The comparison of intramolecular interactions observed in wild and SNP structures

References

1. Arooj A., Pervez M.T., Gillani Z., Chohan T.A., Chaudhry M.T., Babar M.E. and Shah A.T., *In-Silico* Analysis of nsSNPs Associated with CYP11B2 Gene, *BioRxiv*, doi: https:// doi.org/10.1101/602615 **(2019)**

2. Berber M., Leng S., Wengi A., Winter D.V., Odermatt A., Beuschlein F., Loffing J., Breault D.T. and Penton D., Calcineurin regulates aldosterone production via dephosphorylation of NFATC4, *JCI Insight*, **8(14)**, e157027 **(2023)**

3. Chow C.K. and Gupta R., Blood pressure control: a challenge to global health systems, *Lancet*, **394(10199)**, 613-615 **(2019)**

4. Corredor Z., Filho M.I.S., Rodríguez-Ribers L., Velázquez A., Hernández A., Catalano C., Hemminki K., Coll E., Silva I., Diaz J.M., Ballarin J., Prats M.V., Martínez J.C., Försti A., Marcos R. and Pastor S., Genetic Variants Associated with Chronic Kidney Disease in a Spanish Population, *Scientific Reports*, **10**, 144 **(2020)**

5. Crompton M., Skinner L.J., Satchell S.C. and Butler M.J., Aldosterone: Essential for Life but Damaging to the Vascular Endothelium, *Biomolecules*, **13(6)**, 1004 **(2023)**

6. Ghafar M.T.A., Aldosterone Synthase Gene (CYP11B2) Polymorphisms and Enhanced Cardiovascular Risk, The Recent Topics in Genetic Polymorphisms, Intech Open, DOI: 10.5772/intechopen.89133 **(2019)**

7. Ji X., Qi H., Li D., Liu R., Zheng Y., Chen H. and Guo J., Associations between human aldosterone synthase CYP11B2 (-344T/C) gene polymorphism and antihypertensive response to valsartan in Chinese patients with essential hypertension, *International Journal of Clinical and Experimental Medicine*, **8(1)**, 1173-1177 **(2015)**

8. Kim G., Primary Role of the Kidney in Pathogenesis of Hypertension, *Life*, **14(1)**, 119 **(2024)**

9. Lira S.S. and Ahammad I., A comprehensive in silico investigation into the nsSNPs of *Drd2* gene predicts significant functional consequences in dopamine signaling and pharmacotherapy, *Scientific Reports*, **11**, 23212 **(2021)**

10. Nanba K., Vaidya A. and Rainey W.E., Aging and Adrenal Aldosterone Production, *Hypertension*, **71(2)**, 218-223 **(2017)**

11. Oparil S., Acelajado M.C., Baris G.L., Berlowitz D.R., Cífková R., Dominiczak A.F., Grassi G., Jordan J., Poulter N.R., Rodgers A. and Whelton P.K., Hypertension, *Nature Reviews Disease Primers*, **4**, 18014 **(2018)**

12. Rossi G.P., Bisogni V., Rossitto G., Maiolino G., Cesari M., Zhu R. and Seccia T.M., Practice Recommendations for Diagnosis and Treatment of the Most Common Forms of Secondary Hypertension, *High Blood Pressure & Cardiovascular Prevention*, **27(6)**, 547-560 **(2020)**

13. Scott J.H., Menouar M.A. and Dunn R.J., Physiology, Aldosterone, Treasure Island (FL), StatPearls Publishing **(2024)**

14. Shah W.A., Jan A., Khan M.A., Saeed M., Rahman N., Zakiullah, Afridi M.S., Khuda F. and Akbar R., Association between Aldosterone Synthase (*CYP11B2*) Gene Polymorphism and Hypertension in Pashtun Ethnic Population of Khyber Pakhtunkwha, Pakistan, *Genes*, **1(6)**, 1184 **(2023)**

15. Shinwari K., Wu Y., Rehman H.M., Xiao N., Bolkov M., Tuzankina I. and Chereshnev V., *In-silico* assessment of high-risk non-synonymous SNPs in ADAMTS3 gene associated with Hennekam syndrome and their impact on protein stability and function, *BMC Bioinformatics*, **24**, 251 **(2023)**

16. Tsilosani A., Gao C. and Zhang W., Aldosterone-Regulated Sodium Transport and Blood Pressure, *Frontiers in Physiology*, **13**, 770375 **(2022)**

17. Turan H., Çakir A.D., Özer Y., Tarçin G., Özcabi B., Ceylaner S., Ercan O. and Evliyaoğlu S.O., Clinical and Genetic Characteristics of Patients with Corticosterone Methyloxidase Deficiency Type 2: Novel Mutations in *CYP11B2*, *Journal of Clinical Research in Pediatric Endocrinology*, **13(2)**, 232-238 **(2021)**

18. Xanthakis V. and Vasan R.S., Aldosterone and the Risk of Hypertension, *Current Hypertension Reports*, **15(2)**, 102-107 **(2013)**

19. Xie H., Yin H., Ye X., Liu Y., Liu N., Zhang Y., Chen X. and Chen X., Detection of Small CYP11B1 Deletions and One Founder Chimeric CYP11B2/CYP11B1 Gene in 11ß-Hydroxylase Deficiency, *Frontiers in Endocrinology*, **13**, 882863 **(2022)**

20. Zhang X., Wang Y., Zheng Y., Yuan J., Tong J., Xu J., Li Q., Li P., Jiang S., Wang Z., Chai F. and Li X., Effect of ACE, ACE 2 and CYP11B2 gene polymorphisms and noise on essential hypertension among steelworkers in china: a case-control study, *BMC Medical Genomics*, **15**, 22 **(2022)**

21. Żukowski Ł., Wawrusiewicz-Kurylonek N., Szumowski P., Mojsak M., Abdelrazek S. and Myśliwiec J., The Role of the CYP11B2 Promoter Polymorphism in the Diagnosis of Primary Aldosteronism, *Journal of Clinical Medicine*, **9(5)**, 1519 **(2020)**.

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