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THE BIOMOLECULAR CHARACTERISTICS OF ANGIOTENSIN II TYPE 1 RECEPTORS AS PARAMETERS IN KIDNEY FIBROSIS: A BIOINFORMATICS ANALYSIS

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ABSTRACT

AGTR1 is a modulator of angiotensin II-induced signal transduction. This article aims to examine the biomolecular characteristics of AGTR1 and the role of AGTR1 in the renal fibrosis pathway in Rattus norvegicus rats. The gene ID NC 051352.1 and protein ID NP 112271.2 for AGTR1 were retrieved from the National Center for Biotechnology Information's website. The 'NCBI' website has details on AGTR1's structure, location, and expression. Protease prediction using 'PEPTIDE CUTTER'; glycosylation index prediction using NETNGLYC; and target protein location evaluated on the 'TARGETP' homepage. AGTR1 in Rattus norvegicus is located on chromosome 17p12, this protein has a stability score of 34.48. The aliphatic index was 114.62. AGTR1 is found inside, outside, and on the transmembrane, as determined by the THMM. Only 21 enzymes were predicted to be capable of cleaving the AGTR1 protein, out of a total of 37 protease. AGTR1 protein has four glycosylated amino acid sequences. AGTR1 protein is primarily at other location 0.850, secretory pathway (0.590), and a little amount in mitochondria (0.018AGTR1/AT1R is a marker for renal fibrosis in the renin angiotensin system, which can lead in vasoconstriction, inflammation, fibrosis, sodium retention, and water retention. The AGTR1 protein's molecular feature is a determinant in the progression of renal fibrosis, revealed to a bioinformatics study

Keywords: AGTR1, AT1R, Bioinformatic, Biomolecular

1. INTRODUCTION

Renal fibrosis is a frequent complication of acute and chronic kidney injury, cytokine release, inflammatory cell infiltration, and epithelial-mesenchymal transition (EMT). Renal fibrosis is associated with glomerulosclerosis and interstitial fibrosis of the kidney. It is characterized by tubular atrophy, tubular dilatation, enhanced fibrogenesis, and extracellular matrix deposition (ECM). Numerous chemicals and proteins are implicated in the advancement of renal fibrosis, including angiotensin, particularly angiotensin II. [1,2]

Angiotensin is a hormone released by the endocrine system that plays a critical role in the renin system. Angiotensin aldosterone is an endocrine system that is critical for blood volume regulation and blood pressure control. Angiotensin is classified into four types: angiotensin I, angiotensin II, angiotensin III, and angiotensin IV. Angiotensin is obtained from angiotensinogen (formed in the liver and circulates in the plasma) and converted to Angiotensin I by renin. Angiotensin I is not biologically active but contributes as a precursor for Angiotensin II. [3,4]

The angiotensin converting enzyme (ACE) catalyzes the conversion of

angiotensin I to angiotensin II. ACE is mostly prevalent in the lungs and kidneys' vascular endothelium. Numerous effects occur when angiotensin I is converted to angiotensin II, including those on the kidneys, adrenal cortex, arterioles, and brain. Angiotensin II is a hormone that activates two distinct types of receptors: angiotensin II receptor type I (AGTR1) and angiotensin II receptor type II (AGTR2/AT2R). Angiotensin II has a half-life of 1-2 minutes in plasma before being degraded by peptidase to angiotensin III and angiotensin IV [4,5]

AGTR1 is closely attributed to renal fibrosis. This is because AGTR1 acts as a physiological and pathophysiological intermediate for its endogenous ligand, resulting in hypertension, kidney failure, and blood vascular remodeling when it is overexpressed.[6,7] While numerous studies on renal fibrosis are underway, the disease's etiology and treatment remain poorly understood. Thus, it is required to initiate research, commencing with examining the biomolecular characteristics of angiotensin II receptor type 1 as a parameter in renal fibrosis using bioinformatics analysis. Experimental animals are utilized in bioinformatics tracking reports because they have the same anatomical, hemodynamic, and fibrotic characteristics as humans. Therefore, this article aims to examine the biomolecular characteristics of AGTR1 and the role of AGTR1 in the renal fibrosis pathway in Rattus norvegicus rats.

2. METHOD

The genetic features of AGTR1 were obtained from the National Center for Biotechnology Information's website www.ncbi.org with the gene ID NC_051352.1 and the protein ID NP_112271.2. Bioinformatics media are used to explore AGTR1 and its role in the renal fibrosis pathway. The 'NCBI' website contains information about the structure, location, and expression of AGTR1.

'PROTPARAM' is performed in the chemical-physical analysis; on the 'PROTSCALE' website, hydrophobicity is highlighted; the 'TMHMM' website has details about transmembrane protein topology; protease prediction employing 'PEPTIDE CUTTER'; glycosylation index prediction utilizing NETNGLYC, and target protein location can be examined on the 'TARGETP' homepage.

3. RESULT

Location and structure of AGTR1

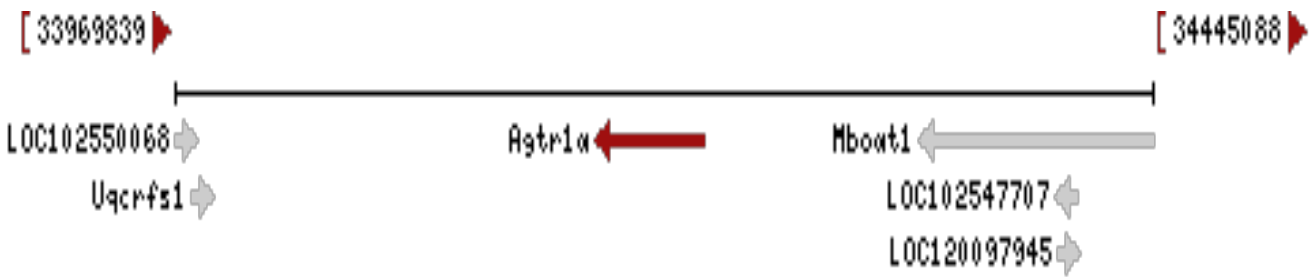

Figure 1. Location of AGTR1

Angiotensin II type 1a receptor is the official name obtained from the NCBI website

with code NC_051352.1. Also known as AT1; AT1A; AT1R; AGTR1. The location of AGTR1 in Rattus norvegicus is located on chromosome 17p12, which means AGTR1 is located on chromosome no. 17 with long arm (q), 1st arm band 2 and has 4 exons (Figure1).[8]

Expression of AGTR1

The transcriptomic RNA-Seq AGTR1 in Rattus norvegicus was prepared with RNA-Seq on 320 RNA samples isolated from 11 organs with 4 developmental stages from both sexes of juvenile and adult Fischer 344 (Figure 2). These organs are the adrenal glands, brain, heart, kidneys, liver, aorta, lungs, muscles, spleen, thymus, and testes or uterus. The four stages of development are (2-, 6-, 21-, and 104 weeks). RNA-Seq AGTR1 expression was high when >1000 TPM, moderate between 11 to 1000 TPM, low between 0.5 to 10 TPM, and lower limit <0.5 TPM. This expression shows a moderate limit in the renal system, namely 35 TPM.[8–10]

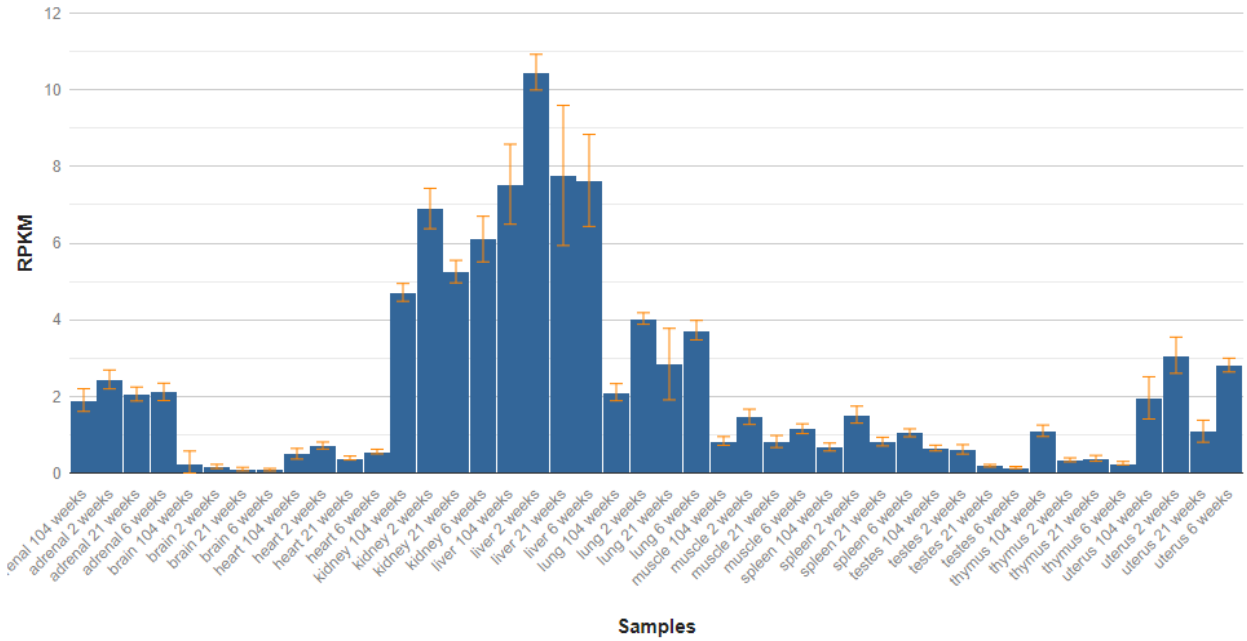

Figure 2. RPKM (Reads per kilobase of transcript per Milliom mapped reads) of ATGR1

AGTR1 Protein

The AGTR1 protein (NP_112271.2) has an amino acid (Figure 3) that readily pairs with a heterotrimeric guanine nucleotide-binding protein (Protein G). In various cell expressions, AGTR1 expression is endogenous or known as ectopic. Angiotensin II stimulation causes activation of phospholipase C-β, hydrolysis of membrane phospholipids and release of diacylglycerol. [10–12]

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  1 mtlnsstedg ikriqddcpk agrhnyifvm iptlysiifv vgifgnslvv iviyfymklk
 61 tvasvfllnl aladlcfllt lplwavytam eyrwpfgnhl ckiasasvsf nlyasvfllt
121 clsidrylai vhpmksrlrr tmlvakvtci iiwlmaglas lpaviyrnvy fientnitvc
181 afhyesqnst lpiglgltkn ilgfvfpfli iltsytliwk alkkaykiqk ntprnddifr
241 iimaivlfff fswvphqift fldvliqlgi irdceiadiv dtampitici ayfnnclnpl
301 fygflgkkfk kyflqllkyi pptakshagl stkmstlsyr psdnmsssak ksasffeve
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Figure 3. Sequence of AGTR1 (NP_112271.2)

Table 1. Physical-Chemical Characteristics of AGTR1

Characteristics	Protein AGTR1 isoform (**NC_051352.1)**
Number of amino acids	359
Molecular weight	40911.77
Theoretical pI	9.37
Amino acid composition	Ala (A) 25 7.0% Arg (R) 12 3.3% Asn (N) 17 4.7% Asp (D) 12 3.3% Cys (C) 9 2.5% Gln (Q) 6 1.7% Glu (E) 7 1.9% Gly (G) 13 3.6% His (H) 6 1.7% Ile (I) 38 10.6% Leu (L) 44 12.3% Lys (K) 22 6.1% Met (M) 11 3.1% Phe (F) 28 7.8% Pro (P) 15 4.2% Ser (S) 26 7.2% Thr (T) 22 6.1% Trp (W) 5 1.4% Tyr (Y) 18 5.0% Val (V) 23 6.4% Pyl (O) 0 0.0% Sec (U) 0 0.0%
Total number of negatively charged residues (Asp + Glu)	19
Total number of positively charged residues (Arg + Lys)	34
Atomic composition	Carbon C 1921 Hydrogen H 2981 Nitrogen N 457 Oxygen O 487 Sulfur S 20
Formula	$C_{1921}H_{2981}N_{457}O_{487}S_{20}$
Total number of atoms	5866
Instability index	34.48
Aliphatic index	114.62
Grand average of hydropathicity (GRAVY)	0.564

Physical-Chemical Characteristics of AGTR1 (PROTPARAM)

The amino acid composition below contains the atomic composition construction of AGTR1 ($C_{1921}H_{2981}N_{457}O_{487}S_{20}$) with a total atomic number of 5866. This atomic number is cut from the N-terminal region of AGTR1 in Methionine (Met) assuming all Cys residue pairs form cystine. The result of AGTR1 cleavage consists of a total of negatively charged residues, namely (Asp + Glu) 19 and positively charged residues (Arg + Lys) 34. This cleavage effect has the potential to alter the receptor's functional and pharmacological capabilities for ligand binding and signaling. This engineered AGTR1 exhibited macro-crystal M^{-1} cm^{-1} in water at 280 nm. The stability index for this protein was calculated to be 34.48, indicating that it is a stable protein (a stability index of 40 implies that the protein is stable). The aliphatic index of 114.62 indicates that the protein remains stable as the temperature rises; the greater the aliphatic index, the more resistant the protein is to rising temperatures, implying that the transportation temperature will not require cold chain transportation. [13,14]

Hydrophobicity (PROTSCALE)

Hydrophobicity study adopting the Hphob.APP (PROTSCALE) method and converted to scatter indicated that the amino acid ATRG1 protein had a hydrophobicity range of 10–359. On the Parker et al scale, the hydrophobicity of alanine was 1.8 while glycine was -0.4 (Figure 5).[15]

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Ala:  1.800  Arg: -4.500  Asn: -3.500  Asp: -3.500  Cys:  2.500  Gln: -3.500
Glu: -3.500  Gly: -0.400  His: -3.200  Ile:  4.500  Leu:  3.800  Lys: -3.900
Met:  1.900  Phe:  2.800  Pro: -1.600  Ser: -0.800  Thr: -0.700  Trp: -0.900
Tyr: -1.300  Val:  4.200  : -3.500  : -3.500  : -0.490
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Figure 4. Values of amino acid using the scale Hphob./Kyte&Doolttle

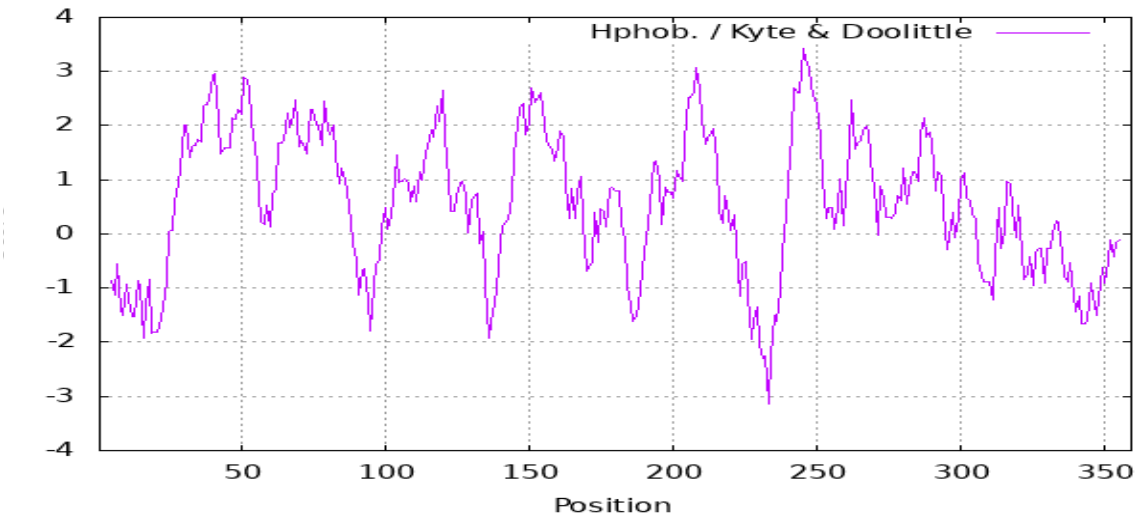

Figure 5. Proscale output of Hydrophobicity

Transmembrane helices in proteins (TMHMM)

The ATGR1 protein isoform is located outside the cell, inside the cell and transmembrane. Positions outside the cell are at amino acid positions 166 to 282, amino acids inside the cell at positions 54 to 359, transmembrane amino acids at positions 31 to 305 (Figure 6.)[16]

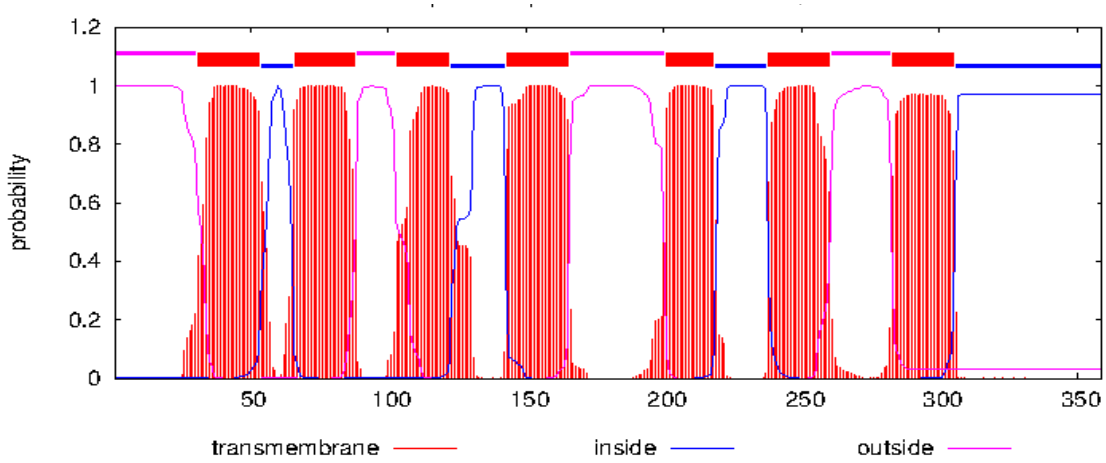

Figure 6. TMHMM protein ATGR1

AGTR1 as a transmembrane protein localized in intracellular inflammatory vesicles and plasma membranes that functions as a modulator of angiotensin II-induced signal transduction. Endogenous AGTR1 showed a particulate distribution. Electron microscopy revealed the presence of AGTR1 in prominent perinuclear vesicular membranes and colocalization analysis by immunofluorescence showed that AGTR1 colocalized in intracellular vesicular compartments corresponding to endoplasmic reticulum, Golgi, and endocytic vesicles. AGTR1 exhibits a constitutive translocation to the plasma membrane characterized by an epitope at the amino or carboxyl end of the molecule. The orientation of the amino ends as being outside the cell. [16,17]

Cutting Prediction by Protease (PEPTIDECUTTER)

Only 21 enzymes were predicted to be capable of cleaving the AGTR1 protein, out of a total of 37 protease enzymes listed on the website www.ezpasy.org/tools/#proteome. The enzymes Arg-C proteinase, Asp-N endopeptidase, Clostripain, and Formic acid can cleave the 12 amino acid site. AGTR1 can be cleaved at position 19 utilizing the enzyme Asp-N endopeptidase in conjunction with N-terminal Glu. BNPS-Skatole and Lodosobenzoic acid enzymes cleave site 5. CNBr cleaves the 11 amino acid site. The 105 site is cleaved by chymotrypsin-specific enzymes.[18]

Glutamyl endopeptidase enzyme can cleave AGTR1 at site 7. Site 22 is cleaved by enzymes LysC and LysN. The NTCB enzyme can cleave site number 9. Although both pepsin is the same, but pepsin pH 1.3 cleaves at site 106 and pepsin pH >2 cleaves site 131. Proline endopeptdase enzymes cleave at site number 2. Sites number 210, 7, 154, and 33 successively cut by the enzymes Proteinase K, Staphylococcal peptidase I, Thermolysn and Trypsin.[18]

The 16 enzymes that cannot cleave AGTR1 are: Caspase1, Caspase10, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7, Caspase8, Caspase9, Enterokinase, Factor Xa, GranzymeB, Hydroxylamine, Thrombin, Tobacco etch virus proteases.

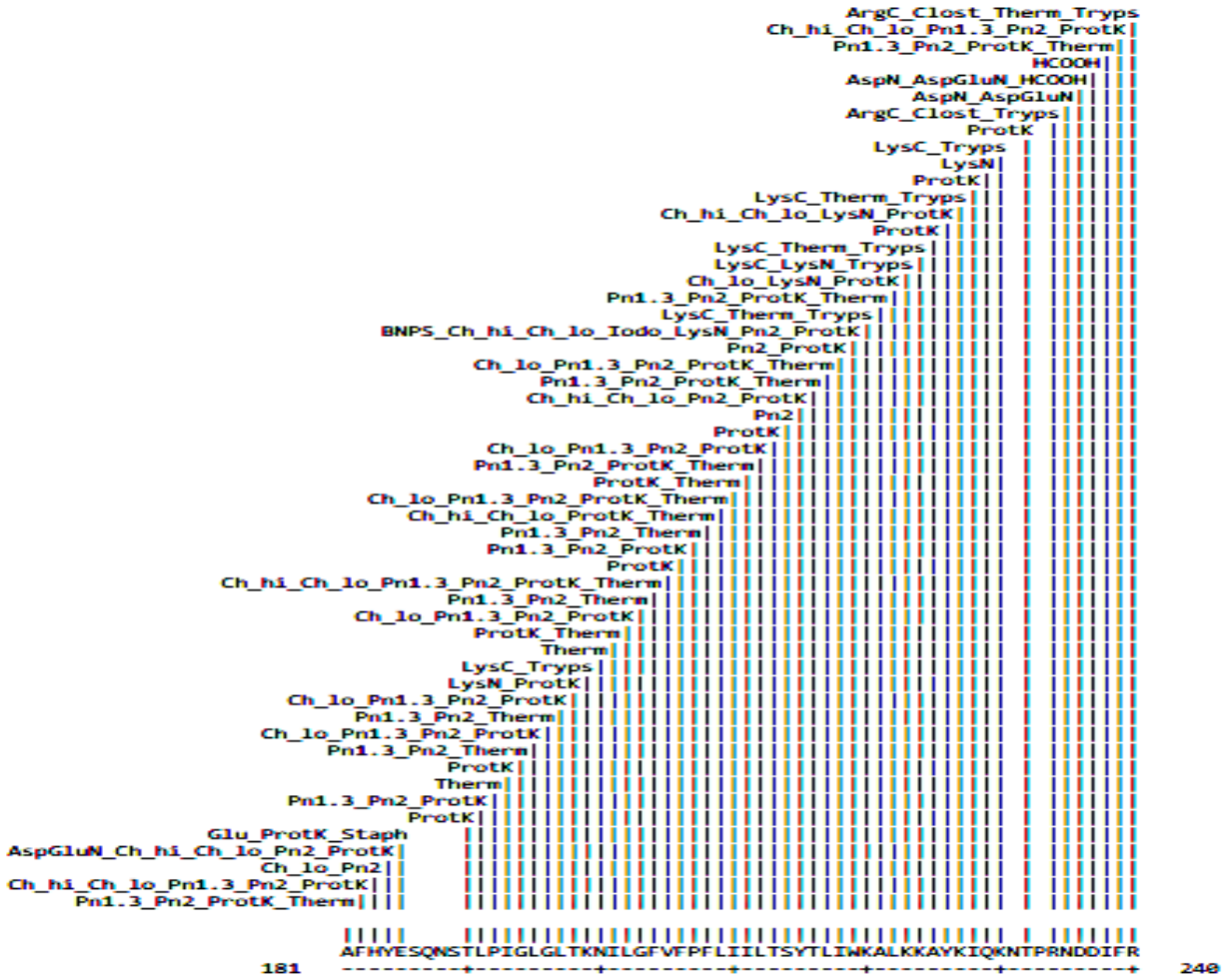

Figure 7. Cleavage sites of the chosen enzymes

Predict the Potential of Glycosylation Sites (NETNGLYC)

N-glycosylation is known to occur on Asparagines which occur in the Asn-Xaa-Ser/Thr stretch (where Xaa is any amino acid except Proline). While this consensus tripeptide (also called the N-glycosylation sequon in many texts) may be a requirement, it is not always sufficient for the Asparagine to be glycosylated. Furthermore, there are a few known instances of N-glycosylation occuring within Asn-Xaa-Cys (a Cysteine opposed to a Serine/Threonine at the N+2 position) e.g. plasma protein C (PRTC_HUMAN), von Willebrand factor (VWF_HUMAN).

NetNGlyc attempts to distinguish glycosylated sequons from non-glycosylated ones. By default, predictions are only shown on Asn-Xaa-Ser/Thr sequons. In the sequence output above, Asn-Xaa-Ser/Thr sequons are highlighted in blue, and N-glycosylated Asparagines are red. With the scores for each position, Asn-Xaa-Ser/Thr sequons can be identified (in case prediction is made on all Asparagines) by a 'SEQUON' note in the right margin.[19]

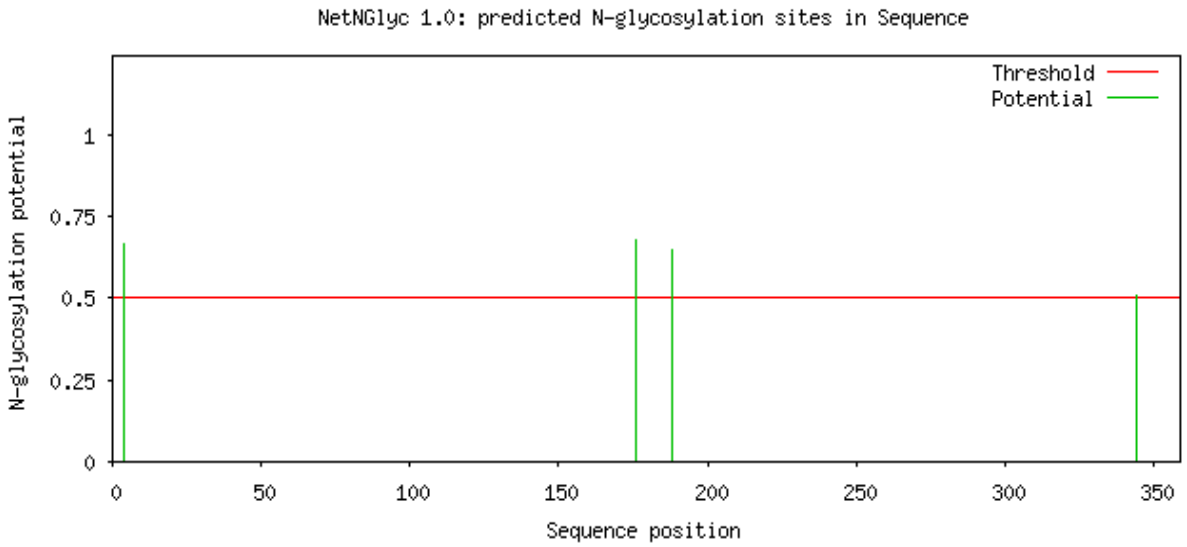

Figure 8. Predicted N-glycosylation sites og AGTR1

The graph illustrates predicted N-glyc sites across the protein chain (x-axis represents protein length from N- to C-terminal). A position with a potential (vertical lines) crossing the threshold (horizontal line at 0.5) is predicted glycosylated. The AGTR1 protein has 4 amino acid glycosylated sites, 4 (potential glycosylated 0.6), 176 (potential glycosylated 0.67), 188 (potential glycosylated 0.64) and 344 (potential glycosylated 0.50) (Figure 8)[19]

Prediction of Protein Location (TARGETP)

TargetP 1.1 predicts the subcellular location of eukaryotic proteins. The location assignment is based on the predicted presence of any of the N-terminal presequences: chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP) or secretory pathway signal peptide (SP).[20]

Name	Len	mTP	SP	other	Loc	RC
Sequence	359	0.018	0.590	0.850	_	4
cutoff		0.000	0.000	0.000		

Figure 9. Prediction of ATGR1 location

Predicted target location of AGTR1 protein, which is mostly at other location 0.850, secretory pathway (0.590) and a little in mitochondria (0.018). This is related to the function of the AGTR1 protein as a signal peptide receptor that must be on the cell surface, outside and inside the cell.[20]

Protein Structure

The structure of the AGTR1 amino acid sequence is encoded by the AGTR1 cDNA and the genomic DNA consists of 359 residues (Figure 13). Structural predictions suggested that the extracellular NH2 terminal is followed by seven transmembrane spanning domains helices linked by three extracellular and three intracellular loops linked to the carboxyl terminal. The three consensus sites for N-glycosylation can be found in Asn4 at the NH2 terminal and at Asn176 and Asn188 in the second extracellular loop. The carboxyl terminal region is rich in serine, threonine, and tyrosine residues, with three sites of protein kinase C (PKC) phosphorylation. [21,22]

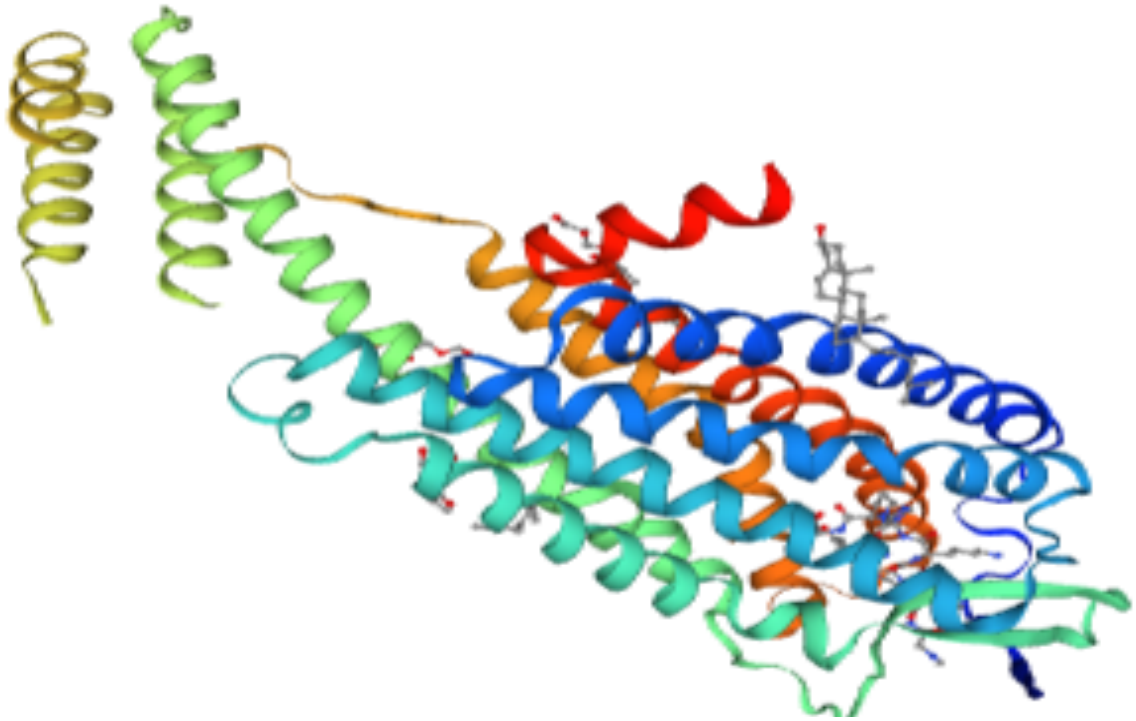

Figure 10. Protein AGTR1 structure

Hydropathic analysis of the amino acid sequence showed that the ATGR1 receptor has seven characteristic helical transmembrane domains of the paired G.-receptor super protein family, which is the largest member of the human genome based on the findings of the human genome project. Two pairs of disulfide bridges have been identified with four cysteine residues located in the extracellular domain. One of these bridges, which includes the second and third extracellular loops, is highly conserved in other G protein-coupled receptors. The second disulfide bridge connects the N-terminus and the last extracellular loop.[21,22]

The role of AGTR1 protein in metabolism

The renin-angiotensin system (RAS) is a peptidergic system with endocrine characteristics regarding to the regulation of the blood pressure and hydro-electrolytic balance. In the classical RAS, the enzyme renin cleaves its substrate angiotensinogen (Agt) forming the decapeptide angiotensin I that is in turn cleaved by angiotensin-converting enzyme (ACE) to produce the angiotensin II (Ang II), a key player of this system. Ang II activates its AT1 receptor (AT1R/ ATGR1), the principal receptor that mediates the majority of the known actions of Ang II in the kidney, including vasoconstriction, renal sodium (Na+)

reabsorption, and aldosterone secretion, increasing blood pressure and contributing to the development of hypertension. In addition to (ACE)/Ang II/AGTR1 and AT2R axis, other signaling pathways in the RAS, such as ACE2/angiotensin-(1-7)/Mas and Ang IV/IRAP, and other active peptide of the RAS, with physiological relevance as Ang III, Ang A and alamandine, are now widely recognized.[23]

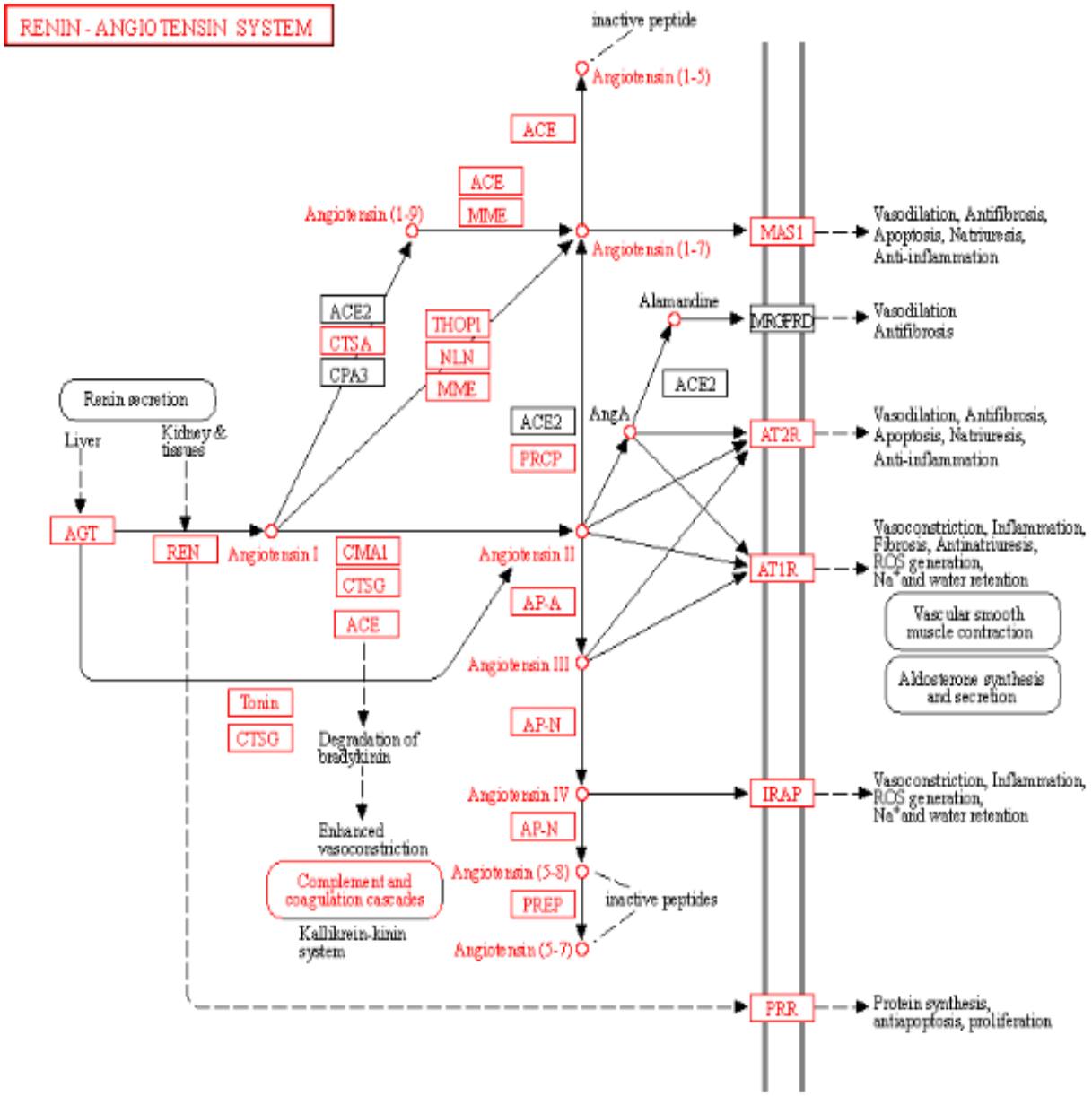

Figure 11. Renin angiotensin pathway[23]

4. DISCUSSION

AGTR1 in Rattus norvegicus is located on chromosome 17p12, which means AGTR1 is located on chromosome no. 17 with long arm (q), 1st arm band 2 and has 4 exons stated in NCBI website. AGTR1 was especially abundant in the rat liver and kidney, as stated in NCBI. This protein has a stability score of 34.48, indicating that it is a stable protein (a stability index of 40 implies that the protein is stable). According to the PROTPARAM website, the aliphatic index of 114.62 indicates that the protein retains its stability as the temperature rises; a higher aliphatic index indicates that the protein is more resistant to rising temperatures, implying that the transportation temperature will not require cold chain transportation.

The hydrophobicity of the amino acid ATRG1 protein was determined by using the Hphob.APP (PROTSCALE) approach. AGTR1 is found inside, outside, and on the transmembrane, as determined by the THMM. This is associated to the AGTR1 protein's function as a receptor that must be internal, external, and transmembrane.

Only 21 enzymes were predicted to be capable of cleaving the AGTR1 protein, out of a total of 37 protease enzymes listed on the website www.ezpasy.org/tools/#proteome. The enzymes are; Arg-C proteinase, Asp-N endopeptidase, Clostripain, Formic acid, Asp-N endopeptidase, N-terminal Glu, BNPS-Skatole, Lodosobenzoic acid, CNBr, Chymotrypsin-specific enzymes.

According to the NETNGLYC website, the AGTR1 protein has four glycosylated amino acid sequences: 4 (potentially glycosylated 0.6), 176 (potentially glycosylated 0.67), 188 (potentially glycosylated 0.64), and 344 (potentially glycosylated 0.64). (Potential glycosylated 0.50).

AGTR1 protein (TERGETP) predicted target location, which is primarily at other location 0.850, secretory pathway (0.590), and a little amount in mitochondria (0.018). This is relevant to the AGTR1 protein's function as a signal peptide receptor that must be present on the cell surface, both externally and internally.

AGTR1/AT1R is a marker for renal fibrosis in the renin-angiotensin system, which can result in vasoconstriction, inflammation, fibrosis, salt retention, and water retention, according to KEGG Pathway.

Angiotensin II has an important role in the pathology of renal fibrosis. Reinforcing physiological interactions are common, as occurs with fluid and salt retention, pressors, and the trophic action of Angiotensin II delivered via AGTR1 in vascular, cardiac, neuroendocrine, adrenocortical, central nervous, autonomic, and epithelial tissues. Receptor mapping also revealed high concentrations of AGTR1 where the action of Angiotensin II is less well understood, such as on renomedullary interstitial cells type 1 and various central nervous system sites including the nigrostriatal dopaminergic pathway and various sensory pathways.[24]

AGTR1 provides instructions for making a protein called the angiotensin II type 1 receptor. This protein is part of the renin-angiotensin system, which regulates blood pressure and fluid and salt balance in the body. Through a series of steps, the renin-angiotensin system produces a molecule called angiotensin II, which attaches (binds) to the AGTR1, stimulating a chemical signal. This signal causes blood vessels to constrict (narrow), which results in an increase in blood pressure.[25]

The binding of angiotensin II to the AGTR1 receptor also stimulates the production of the hormone aldosterone, which triggers the absorption of water and salt by the kidneys. An increase in the amount of fluid in the body also raises blood pressure. Proper blood pressure during fetal growth, which delivers oxygen to developing tissues, is necessary for the normal development of the kidneys, especially structures called the proximal

tubules, and other tissues. In addition, angiotensin II may play a more direct role in kidney development, possibly by influencing growth factors involved in the development of kidney structures.[5]

Overall receptor localization studies have served to confirm many suspected sites of action of Angiotensin II and to highlight some areas that may be useful in defining novel actions exerted via the AGTR1 receptor.

5. CONCLUSION

AGTR1 is a modulator of angiotensin II-induced signal transduction. On the basis of the bioinformatics study of the AGTR1 protein, it can determine that the molecular characteristic of AGTR1 is a factor in the progression of kidney fibrosis.

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