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Hypothesis

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Genetic Variant of C-5312T Can Change Binding Pattern of Sp1 to Renin Enhancer that are Very Likely to Affect Renin Gene Expression

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Abstract:
Renin distal enhancer plays a pivotal role in renin gene expression, and the genetic variants C-5312T of renin enhancer can affect renin gene transcription level. However, the mechanism associated with the transcription level changes remains unknown. Therefore, it is of interest to investigate the possible role of distal enhancer in regulating the expression of renin gene. Single nucleotide polymorphism in renin distal enhancer was identified in 34 hypertensive patients by automatic sequencing. The data showed that the renin enhancer from the patients have genetic variants C-5312T or C-5312T SNP. Hence, the functionality of the renin enhancer and influence of the genetic variants C-5312T on binding to Sp1 is studied. These results from the binding study suggested that Sp1 binds to the DNA in GC rich region. Thus, the genetic variant C-5312T has changed the binding pattern of Sp1 to renin enhancer. This is likely to influence Sp1 activity to stimulate the expression of renin gene. The binding of Sp1 to the cis-element will enhance transcription of renin gene. Thus, polymorphism within C-5312T might contribute to the reduction of renin transcription.

Background:
Uncontrolled hypertension has become a major health problem worldwide. Previous study reveals that the prevalence of uncontrolled hypertension in both developed and developing countries remains high **[1].** Many studies suggest that uncontrolled hypertension leads to a half of coronary heart disease and cerebrovascular disease incidents **[2].** Moreover, it contributes to 7.5 million deaths per year (13% of all death causes) **[3].** Therefore, achieving blood pressure target is essential to reduce the mortality and morbidity of hypertension related diseases **[4, 5, 6].** Many factors may contribute to uncontrolled hypertension, such as antihypertensive drug combination, patients' compliance, and genetic variants in renin angiotensin aldosterone system.

Renin is an aspartyl protease produced and secreted by juxtaglomerular cell in kidney **[7].** This enzyme induces enzymatic cascade that generates angiotensin II peptide as the main effector in the system of renin angiotensin aldosterone as blood pressure regulator **[8].** Plasma renin level has been used for many years to determine the responses to antihypertensive therapy. The transcription level of renin is determined by many factors such as the presence of distal enhancer in renin gene and polymorphism in this area.

Studies on human chorionic cultured cell suggest that renin distal enhancer plays a pivotal role in renin gene expression. Deletion of this area resulted in 10 fold loss of enhancer activity **[9].** Fuchs, *et al.* suggest that SNP in C-5312T of renin enhancer

increases transcription level of renin gene by 45% in -5312T compared to -5312C9 **[10].** On the other hand, Pan L., *et al.* (2003) report that renin gene expression is regulated by Sp1/Sp3 proteins, mutations in these proteins decrease renin levels up to 40% in mice **[9].** This phenomenon suggests that Sp1 acts as a positive regulatory protein that binds renin enhancer. Thus, this study has been designed to investigate whether renin enhancer has a Sp1-binding site, and to examine the influence of genetic variant of C-5312T on Sp1 binding pattern to renin enhancer.

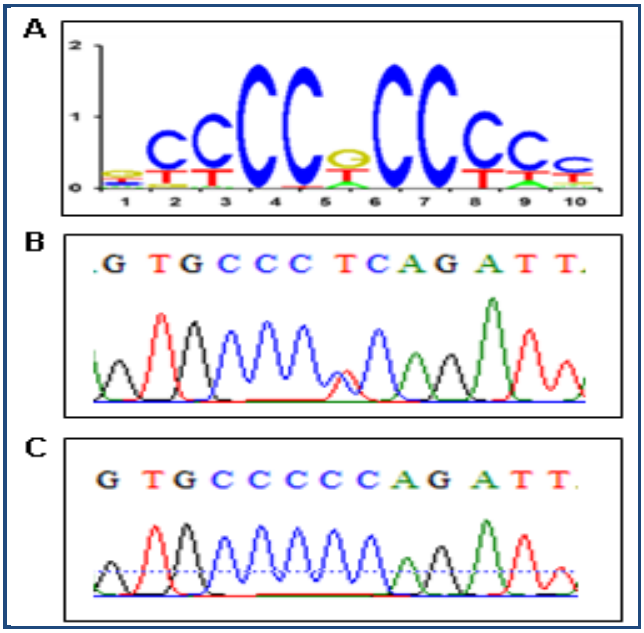

Figure 1: Genetic variation of Sp1-binding site at hypertensive patients. Consensus of Sp1-binding site was retrieved from Jaspar CORE database **(A),** Varian renin enhancer at -5312C **(B),** and variants of the renin enhancer at -5312T **(C).**

Methodology:

Detection of polymorphism

SNP within renin distal enhancer of human renin gene was identified by direct sequencing of genomic DNA obtained from 34 hypertensive patients. Each sample was amplified by these primers 5′-CGTAGTGCCATTTTTAGGAAC-3′ and 5′-AACACCA AAGCAGGCTTAA-3′. All samples were sequenced by automatic sequencing method (Macrogen) and the genetic variants were analyzed using Genescane software (Applied Biosystem). The existence of Sp1-binding site of these sequences was determined based on Sp1-binding site consensus from CORE Jaspar database (http://jaspar.genereg.net/cgi-bin/jaspar_db.pl).

DNA Modeling

We generated 3D structural models of DNA from sequences of renin enhancer (5′gtgcccccagattaagcctg3′) normal and (5′gtgccctcagattaagcctg3′) mutant using 3D-DART provided by haddock (http://haddock.science.uu.nl/services/3DDART/) **[11].**

Molecular Docking

Models of SP1 protein (1R8U) and ER-Estradiol (1A52) were retrieved from protein structure databases, PDB (http: //www.rcsb.org). Binding interaction among molecule complex of ER-Estradiol, Sp1, and DNA was done by using Escher NG in VEGAZZ. Further for detailed analysis, we used Hex Protein Docking to predict interactions between Zinc Finger Domain 2 (Zf-2) of SP1 and DNA of renin enhancer. Hex is FFT-based approach that was first used as a rapid way to calculate shape complementarity within a 3D Cartesian grid **[12].** Thereafter, the complex of docking result was analyzed using ligand Scout to know the amino acids and nucleotides responsible for the interaction **[13].**

Visualization

All the visualization of the structure files was done using PyMol (www.pymol.org) and YASARA molecular graphics system.

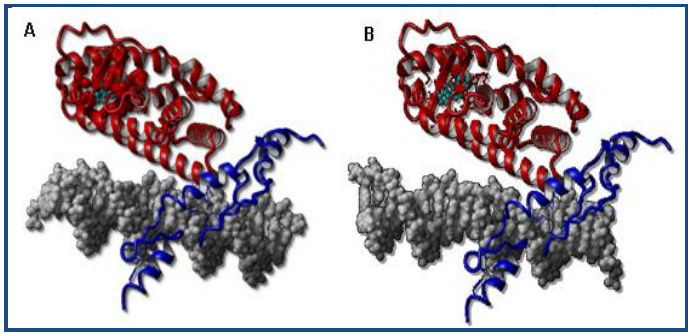

Figure 2: Complex molecules of 17β-Estradiol (cyan), ER (ed), Sp1 (blue) and the Renin Enhancer (gray) was resulted from docking analysis by using Escher NG in VEGAZZ. The presence of SNP in C-5312T has led to change the bonding pattern of Sp1 to renin enhancer. Sp1 bound to the major groove side of renin enhancer on-5312T **(A),** shifts to the minor grove on-5312C **(B).**

Results:

Proportions of genetic variant of the patients were CC (47.1%), CT (44.1%), and TT (8.8%). Sp1 protein is a trans-acting transcription factor-1, has 3 zinc finger domains which serves to bind DNA. Since the Sp1 bound to DNA in the GC rich motif, then we searched the motif in renin enhancer of 34 hypertensive patients that we analyzed. The data showed that renin enhancer of the hypertensive patients contained Sp1-DNA binding motif. Further, we found two genetic variations in Sp1-binding site from this study, SNPs in C-5312T **(Figure 1).**

Sp1 is activated by a complex of ER alpha receptor (ER) and 17β-Estradiol, and then binds DNA to stimulate transcription of the gene being regulated. Therefore, we used the ER-Estradiol-Sp1 complex to elucidate pattern of the regulator bind to renin enhancer by using docking methode. The docking results showed that Sp1 most preferably bind to DNA in GC rich sequence. Sp1 was bound in the center of the GC rich sequence and form bond with C base in -5312C variant. However, Sp1 was bound in downstream of the GC rich sequence in -5312T variant. Taken together the phenomenon, indicated that genetic variations C-5312T of renin enhancer causes differences of Sp1 binding to renin enhancer **(Figure 2).**

Furthermore, we analyzed more detail the differences of Sp1 binding pattern to SNPs in C-5312T of renin enhancer by docking Zinc Finger domain-2 of SP1 with 21 BP DNA containing the C-5312T SNP. The result indicated that change from T to C causing displacement of Sp1 binding site from

center to dowstream of GC rich sequence, and Sp1 loss of contact with bases -5312 **(Figure 3).** The shift in the Sp1 binding suggested that Sp1 loos contact with the center of rich GC sequence, which was very likely not be able to stimulate transcription of renin gene. This result explained why the transcription level of renin gene in -5312T was higher by 45% compared to -5312C.

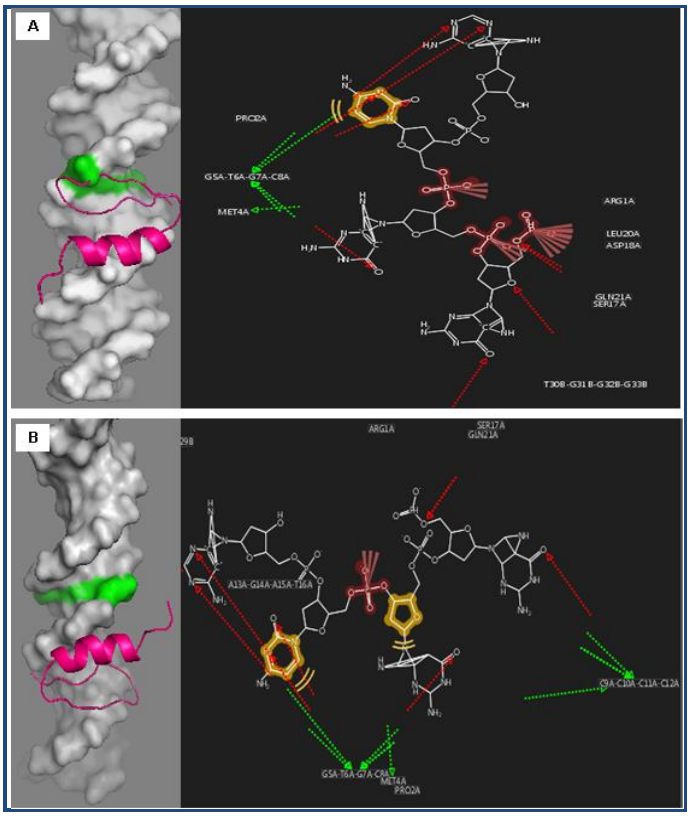

Figure 3: Binding pattern of Zinc Finger Domain of Sp1 (Pink) to renin enhancher (Grey) was identified by docking analysis using Hex 8, position of SNPs in C-5312T is shown in green (Left Panel). Interaction of Sp1 amino acids and DNA is indicated by arrows (Right Panel). Binding pattern of Zinc Finger Domain shifted from the middle of Sp1-binding site on -5312T **(A),** to downstream of Sp1-binding site on -5312C **(B).**

Discussion:

Renin angiotensin aldosterone system (RAAS) plays a pivotal role in blood pressure regulation **[8].** Renin, that catalyzes angiotensinogen to angiotensin I, plays an essential role on blood pressure regulation and electrolyte homeostasis **[14].** Plasma renin level has been used for many years to determine the responses to antihypertensive therapy. The transcription level of renin is determined by many factors such as the presence of distal enhancer in renin gene and polymorphism in this area. Several studies have been conducted to identify the single nucleotide polymorphism (SNP) in renin gene and its association with hypertension **[15, 14, 16].** These studies suggested that polymorphism in intron 1 of renin gene is strongly correlated with hypertension in Caucasian **[15].** Furthermore, SNP in renin distal enhancer at -5312 is associated with hypertension in whites **[14, 16].** This SNP also becomes a major determinant of patients' response to angiotensin-receptor blocker therapy in Ireland and Japan. This study shows two polymorphisms in renin distal enhancer, in -5190 site and -5312 site. Previous study has reported the functionality of polymorphism in -5312 in Japan and French **[10, 17].** Sp1 binds to GC rich promoter especially in CACCC box and -5312 area might be identified by Sp1. This transcription factor will be activated by ER alpha and 17β-Estradiol (E2) **[18].** Thus, polymorphism within C-5312T might contribute to the reduction of renin transcription. Fuchs, *et al.* stated that polymorphism in this site increases transcription level by 45% in -5312T compared to that of -5312C **[10].** However, these studies do not explain the mechanism of transcriptional level increase. Therefore, according to all of the results of the analyses, we concluded that Genetic variant of C-5312T has changed binding pattern of Sp1 to renin enhancer, which is very likely to influence Sp1 activity to stimulate expression of renin gene. Morever, this study helped to explain why SNP -5312C reduces expression levels of renin gene.

Conclusion:

This study shows that Sp1 most preferably binds to DNA at the GC rich region. The genetic variants C-5312T has changed binding pattern of Sp1 to renin enhancer. This is likely to influence Sp1 activity to stimulate the expression of renin gene.

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