Structure-Based Analysis of Single Nucleotide Variants in the Renin-Angiotensinogen Complex

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ABSTRACT

The renin-angiotensin system (RAS) plays an important role in regulating blood pressure and controlling sodium levels in the blood. Hyperactivity of this system has been linked to numerous conditions including hypertension, kidney disease, and congestive heart failure. As such, various classes of drugs have been developed to inhibit this system. These drugs are aimed at preventing angiotensin II from performing its function by inhibiting angiotensin II receptors or inhibiting angiotensin-converting enzyme from converting angiotensin I to angiotensin II. The last class of inhibitors is aimed at preventing angiotensin I from being formed by preventing renin from interacting with a plasma protein called angiotensinogen. In this study, we provide a structure-based analysis of the effect of single nucleotide variants on the interaction between renin and angiotensinogen with the aim of revealing important residues and potentially damaging variants.

The renin-angiotensin system (RAS) is responsible for the regulation of blood pressure and sodium homeostasis [1]. This is achieved by producing angiotensin II, a potent, 8-residue vasoconstrictor, which causes the arterioles to constrict, resulting in increased blood pressure. Angiotensin II also stimulates the release of aldosterone, which increases the rate at which sodium ions are reabsorbed into the blood [2].

When RAS is activated, the juxtaglomerular cells in the kidneys secrete renin into the blood. Once in the blood, renin cleaves angiotensin I, a 10-residue peptide, from a plasma protein known as angiotensinogen, which originates in the liver. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme, which cleaves a further 2 residues from the former [2].

Hyperactivity of RAS has been linked to high blood pressure (hypertension), congestive heart failure, and kidney disease. As such, various classes of drugs have been developed to inhibit this system including angiotensin-converting enzymeinhibitors [3], angiotensin receptor blockers [4], and renin inhibitors [5].

In this study, we have used structural bioinformatics and network analysis techniques to investigate the effect of nonsynonymous single nucleotide variants (SNVs) on renin-angiotensinogen interaction to identify important residues and potentially damaging SNVs.

MATERIAL AND METHODS

Data retrieval

Sequences and suitable Protein Data Bank (PDB) [6] structures for renin and angiotensinogen were identified via a search of the Human Mutation Analysis (HUMA) [7] database. The amino acid sequences were then downloaded from Uniprot. Structures were evaluated for suitability based on their coverage of the target sequences and their PDB validation metrics, before being downloaded from the PDB. All available SNVs for each protein were then downloaded from the HUMA database.

Homology modeling: wild type

To account for missing residues in existing experimental structures, the renin-angiotensinogen complex was modeled using MODELLER [8]. The complex has been solved in 2X0B, which was used as the main template. Renin was covered by chain A of 2X0B. Three additional templates, 2WXY, 2WXW, and 2WXZ were used to cover gaps in chain B (i.e., angiotensinogen). Alignment of the templates to the target sequences was performed using PROMALS3D [9].

After aligning, the first 73 residues of the renin target sequence and the first 32 residues and last 3 residues of the angiotensinogen target sequence were not covered by the templates. These residues were trimmed from the alignment as a result. One hundred models were then generated using very slow refinement.

Model evaluation. One hundred models of the renin-angiotensinogen complex were generated. The top 3 models were then selected based on their DOPE z-score [10], and further evaluated using PROCHECK [11], VERIFY3D [12], and PROSA [13] to validate that they were indeed accurate models. The best model was then chosen based on the combination of these results.

SNV filtering. The SNV data set, obtained from the HUMA database, contains all SNVs from dbSNP [14] that could be mapped to the renin and angiotensinogen protein

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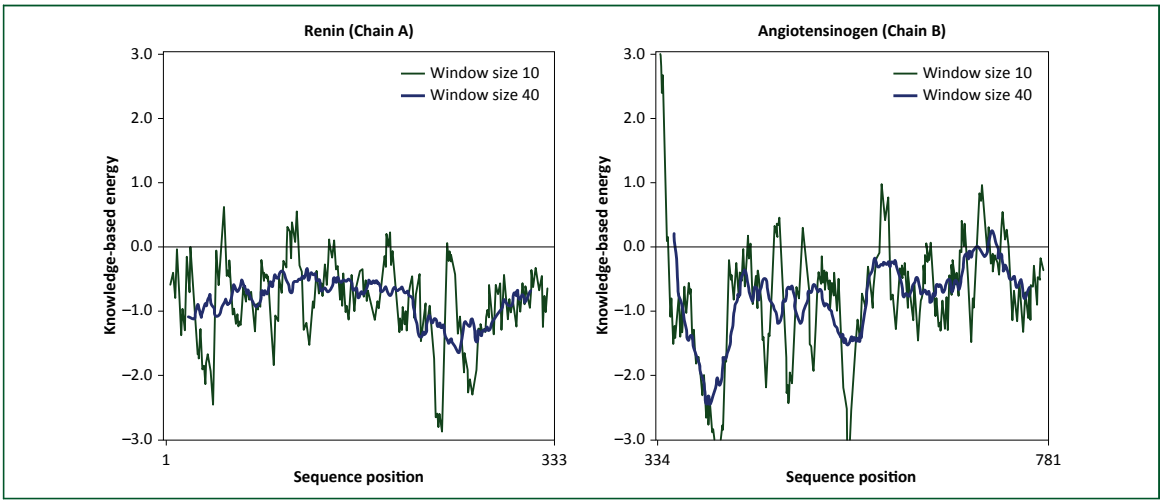

FIGURE 1. PROSA results for the renin-angiotensinogen complex.

sequences based on their chromosome coordinates. As such, the resulting data set contained synonymous and nonsense SNVs. Synonymous SNVs result in no change in the amino acid sequence. They are, therefore, unlikely to have any structural effects on the protein, and were removed from the data set. Nonsense SNVs, on the other hand, result in early stop codons. These SNVs are highly likely to be damaging as they usually result in nonfunctioning proteins. As such, there was little reason to further analyze these SNVs in this paper and they were also removed from the data set.

The Variant Analysis Portal (VAPOR) [15], a workflow that combines the results of PolyPhen-2 [16], Provean [17], PhD-SNP [18], PANTHER-PSEP [19], and FATHMM [20] to predict the functional effect of SNVs, was then used to predict which of the remaining SNVs were likely to cause conformational changes that would alter the functionality of the protein. If more than 1 program predicted that the SNV was neutral, we removed it from the data set. One exception to this is if there was a known disease-association in the HUMA database.

Lastly, we used the Protein Interactions Calculator [21] to determine which residues in the complex were interacting. The final data set contained all SNVs that were at the interface between the 2 proteins and were either predicted to be damaging or were interacting with a position at which a SNV occurred in the other protein.

Homology modeling: mutants

The SNVs identified in the previous step were introduced into the structures via homology modeling. Models were generated for each SNV individually. Additional models were then generated where SNVs occurred at the

TABLE 1. SNV data set for angiotensinogen

dbSNP ID	Residue Change	Location	Reason for Inclusion
rs539231427	H39R	Interface	• Highly damaging prediction by VAPOR • Interacts with position in renin where SNV occurs
rs746613821	P40L	Interface	• Highly damaging prediction by VAPOR • Interacts with position in renin where SNV occurs
rs41271499	L43F	Interface	• Highly damaging prediction by VAPOR • Interacts with position in renin where SNV occurs
rs760531325	E48K	Interface	• Interacts with position in renin where SNV occurs
rs751752211	S49G	Interface	• Interacts with position in renin where SNV occurs
rs377047370	S49N	Interface	• Highly damaging prediction by VAPOR • Interacts with position in renin where SNV occurs
rs201406560	A104T	Interface	• Interacts with position in renin where SNV occurs
rs767370325	M105V	Interface	• Interacts with position in renin where SNV occurs
rs756744141	D168Y	Interface	• Highly damaging prediction by VAPOR • Interacts with position in renin where SNV occurs

SNV, single nucleotide variation.