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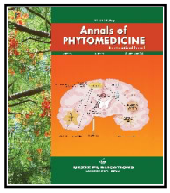

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Bioinformatics approach for the AHSG gene polymorphisms : An *in silico* analysis

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Abstract

Alpha-2 Heremans Schmid Glycoprotein (AHSG) is a glycoprotein with a known renoprotective role. The pathophysiology of kidney stones may be influenced by the gene's polymorphism. The purpose of the study is to investigate the potential role of AHSG gene mutations in the aetiology of kidney stones by applying bioinformatics methods to analyse the AHSG gene's silica analysis. Using its protein accession, IDs and their FASTA amino acid sequences, which were collected from NCBI, *in silico* analysis of SNPs of the AHSG gene was performed. The research was conducted using the bioinformatics programmes SIFT, PolyPhen-2, and I mutant 3.0.

SIFT analysis of AHSG gene SNPs showed that 39% of mutations were tolerable and 61% were harmful. A PolyPhen-2 analysis revealed 21% benign and 79% probably damaging. On I mutant analysis, 25% of the SNPs led to enhanced stability whereas 75% of them led to decreased thermodynamic stability. The data strongly suggests that the pathobiology of kidney stones may be influenced by the harmful effects of mutations on AHSG as well as their decreased stability as anticipated by the bioinformatics techniques. The examination of the AHSG gene by wet study in individuals with kidney stone disease may validate this.

1. Introduction

Kidney disease has increased exponentially over the last two decades. Kidney stones are one of the most common urinary tract problems (Gudulkar *et al*., 2020).

Alpha-2-Heremans Schmid Glycoprotein (human AHSG), a serum-based inhibitor of vascular calcification that is generated by the liver and secreted in high amounts (0.5-1.0 g/l), is responsible for about 50% of the inhibition of calcium and phosphorous precipitation (Jahnen-Dechent *et al*., 1997; Schinke *et al*., 1996). Patients with end-stage renal disease had decreased AHSG serum concentrations, and lower serum concentrations have been independently linked to an increased risk of cardiovascular and all-cause death in this population (Ketteler *et al*., 2003).

A significant inhibitor of extraosseous calcification is the serum glycoprotein known as AHSG. It has been shown that AHSG is significant because it causes extraosseous calcification in all parts of the body, including the kidney. It is still unclear exactly how AHSG protects the kidneys from nephrocalcinosis. Nephrocalcinosis in the proximal tubules can be prevented in part by the intratubular AHSG.

Single nucleotide polymorphisms (SNPs) are about 90% genetic variations in 3-billion base long human genome that result in alterations to the DNA sequence (A, T, C, or G). (Lee *et al*., 2005). Both coding and non-coding regions of the genome are affected by SNPs. SNPs can have consequences ranging from having no effect on cell function, to causing disease, to altering the way drugs interact with the body. They are of particular concern given that non-synonymous SNPs (nsSNPs) that cause amino acid substitutions in protein products are responsible for approximately half of all genetic variations associated with inherited diseases in humans (Krawczak *et al*., 2000).

Nevertheless, coding synonymous SNPs (sSNPs) and non-coding SNPs (sSNPs) can influence transcription factor binding, splicing and gene expression (Prokunina *et al*., 2004; Stenson *et al*., 2009).

SNPs detection essential due to their cause on particular traits.This is a challenging undertaking because it calls for the assessment of tens of thousands of SNPs in potential genes (Ramensky *et al*., 2002). Choosing SNPs to examine the significance of an SNP in disease is challenging descision. In such circumstances, separating functional from neutral SNPs may be possible using bioinformatics prediction algorithms. They might also reveal the structural basis of the mutations. Simply put, these bioinformatics algorithms rank SNPs according to their functional significance (Emahazion *et al*., 2001; Schork *et al*., 2000).

The use of bioinformatic techniques for *in silico* genetic analysis eliminates the need to screen large numbers of people to identify genetic disease associations with sufficient statistical significance. In other words, these techniques support SNP preselection (Ramensky *et al*., 2002).

It would be very helpful, if disease-associated SNPs could be separated from neutral SNPs before using wet lab-based approaches. *In silico* analysis is useful, if future independent studies fail to establish disease associations (Emahazion *et al*., 2001). As a result,

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additional resources can be deployed to distinguish between true positives and false positives using independent evidence of SNP function discovered through the application of predictive algorithms. *In silico* analysis of AHSG gene may be useful in establishing a cause and effect relationship of AHSG with kidney stone disease.The proposed work aimed to study all the single nucleotide polymorphisms (SNPs) of AHSG gene that resulted due to missense mutations and association with the renal stone disease. Analysis also reveals the structural basis of mutations. These bioinformatics tools are nothing but tools to prioritize SNPs according to their functional significance .

By using bioinformatic methods for *in silico* genetic analysis, it is possible to demonstrate associations between genes and diseases at statistically significant levels without having to screen significant numbers of individuals.These technologies aid in the pre-selection of SNPs, in other words.The purpose of the project is to analyse the AHSG gene *in silico* using bioinformatics tools including sorting the intolerant from tolerant (SIFT), PolyPhen-2, and I-mutant in order to investigate the potential role of these genes' mutations in the aetiology of kidney stone disease.

2. Materials and Methods

2.1 Methodology

2.1.1 Evaluation of the functional impact of coding nsSNPs using a sorting intolerant from tolerant (SIFT) sequence homology tool

SIFT can be accessed at sift.jcvi.org (Ng and Henikaff, 2003). Query sequences are analyzed, and various alignment information are used to predict legal and harmful substitutions at each point of the query sequence. This involves, for a given protein sequence, first searching for related sequences, then selecting closely related sequences that may have similar functions, and then multiplexing these selected sequences. It is a multi-step process of obtaining alignments and finally computing the normalized probabilities of all potential permutations, each position from the orientation. If the normalized probability is less than 0.05, intolerance or harmful substitution is predicted to occur. Acceptable substitutions are predicted, if the normalized probability is greater than 0.05 (and Henikaff, 2006).

The study was performed by allowing the algorithm to search for homologous sequences using default settings (UniProt-TrEMBL 39.6 database, average sequence conservation of 3.00, removing sequences with >90% match to query sequence function is identical). The SIFT approach determines whether amino acid changes affect protein function. It works by exploiting the physicochemical properties of amino acid residues and the sequence homology between related genes and domains. A web program was used to sort intolerance from tolerance and record the total number of non-intronic missense mutations, RS numbers, and positions of SNPs on the chromosome for AHSG in a format suitable for analysis (SIFT).

As the query sequence, the filtered nsSNPs from the dbSNP database were examined. The query sequence was the FASTA amino acid sequence of the NCBI protein accession id for AHSG.

2.1.2 Using PolyPhen-2, evaluate the functional impact of coding nsSNPs

PolyPhen-2 (Polymorphism Phenotyping v2) is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. The PolyPhen-2 score represents the probability that a substitution is damaging, so values nearer one are more confidently predicted to be deleterious. SIFT and PolyPhen-2, which rely on database selection because they use sequence comparisons from BLAST searches, work similarly to PROVEAN (Choi *et al*., 2012; Kono *et al*., 2018). PolyPhen-2, like SIFT, collects groups of highly similar sequences from the NCBI database for non-redundant protein sequences (nr).

Using the given query sequence, a BLAST (Altschu *et al*., 1990; Camacho *et al*., 2009). Search is conducted as the initial phase of PolyPhen-2. This software has acquired two points, "probably damaging," and " benign," respectively, based on scores ranging from "0" to "1". 0-0.5 indicates benign, 0.6 to 1 indicates probably damaging.

This typically yields thousands of matches for different taxa. These sequences are grouped into clusters based on a 75% sequence similarity threshold to prevent duplicates. Support sequence sets can be saved and analyzed individually. The computer reports the predicted functional category (harmful or potentially harmful) based on PolyPhen-2 scores and sensitivity and specificity.

There is no category for advantageous impacts, even though it is feasible for a mutant protein to have a higher mean alignment score than the wild type. When determining the functional impact of common versus disease-causing human protein variations, this threshold was set to maximise sensitivity (detection) and specificity (accuracy) (Choi and Chan, 2015).

2.1.3 Analyzing the functional effects of nsSNP coding I mutant 3.0

Mutant 3.0 is a tool that uses support vector machines (SVMs) to automatically predict the impact of single point mutations on protein stability. Both the protein structure and, more importantly, the protein sequence are used as starting points for the I-mutant 3.0 prediction. For all three tools, SIFT, PolyPhen-2 and I Mutant, amino acid sequences are obtained from protein accession IDs in in silico analysis of single nucleotide polymorphisms in the AHSG gene.

3. Results

In NCBI reference protein isoform 2 sequence: NP_001613.2.Out of the 274 number coding variants (100%) identified by SIFT analysis of the AHSG gene, 268 coding variations are anticipated (97%), 165 were tolerated (61%), 103 were harmful (39%), 268 were non-synonymous (97%), 6 were synonymous (3%), and 256 were novel (97%).

The SIFT scale runs from 0 to 1. SNPs with a SIFT score of less than or equal to 0.05 are deemed harmful, whereas those with a value higher than that are deemed tolerable. The optimal range for the median information is between 2.75 and 3.5. This is used to gauge the diversity of the prediction sequences. An indicator indicating the prediction was based on closely similar sequences is a number larger than 3.25. The number of sequences at a certain place is

known as the number of sequences at prediction. SIFT makes automatic sequence selections. If the substitution is located at the beginning or end of the protein, there may be only few sequences represented at that position and this column indicates this fact.

Proof ratings for the chosen SNPs that were less negative than -2.5 indicated neutral mutations. 50% of the mutations were neutral, and 50% were harmful. The number of SNPs discovered by PolyPhen-2 analysis to be harmful is higher than that discovered by SIFT analysis. This might be because the PolyPhen-2 tool can also evaluate amino acid changes and deletions in addition to insertions and deletions.

DDG values of binary classification of SNPs of genes with values 0 implied a lower stability in I mutant suite 3.0 study. Only 15% of alleles exhibited enhanced stability after mutation, while 85% of SNPs showed a decrease in stability. According to this investigation, the majority of mutations, whether harmful or neutral, led to a reduction in protein stability.

Table1 : Showing SIFT,PolyPhen-2 and I mutant 3.0 results of AHSG gene

SNPs	Coordinates	Amino acid Substitution	Prediction	SIFT Score	SVM2 Prediction Effect:	DDG Vale Predic-tion	Poly Phen-2 score	Sensitivity & Specificity
rs746165143	3,186333488,1,G/C	E76D	damaging	0.03	Decrease	-0.29	**0.61**	(sensitivity: **0.87**; specificity: **0.91**)
rs1018718165	3,186333532,1,T/G	V91G	damaging	0.01	Decrease	-2.4	**0.644**	(sensitivity: **0.87**; specificity: **0.91**)
rs775522111	3,186331139,1,C/G	P70R	damaging	0.02	Decrease	-0.61	**0.737**	(sensitivity: **0.85**; specificity: **0.92**)
rs756960189	3,186333483,1,G/A	G75R	damaging	0	Decrease	-0.29	**0.797**	(sensitivity: **0.84**; specificity: **0.93**)
rs766181399	3,186333505,1,T/C	I82T	damaging	0.01	Decrease	-1.9	**0.82**	(sensitivity: **0.84**; specificity: **0.93**)
rs754467302	3,186331106,1,C/A	T59N	damaging	0.03	Decrease	-1.08	**0.835**	(sensitivity: **0.84**; specificity: **0.93**)
rs551743520	3,186335109,1,G/C	Q181H	damaging	0.04	Decrease	-1.54	**0.865**	(sensitivity: **0.83**; specificity: **0.93**)
rs1282991554	3,186337718,1,T/C	F250L	damaging	0.04	Decrease	-0.56	**0.899**	(sensitivity: **0.82**; specificity: **0.94**)
rs754601222	3,186336346,1,G/C	V199L	damaging	0	Decrease	-0.4	**0.94**	(sensitivity: **0.80**; specificity: **0.94**)
rs140827890	3,186335056,1,G/A	A164T	damaging	0.04	Decrease	-0.71	**0.951**	(sensitivity: **0.79**; specificity: **0.95**)
rs1233480935	3,186335089,1,A/G	N175D	damaging	0.02	Decrease	-0.26	**0.963**	(sensitivity: **0.78**; specificity: **0.95**)
rs573820635	3,186336338,1,C/T	S196F	damaging	0.01	Decrease	-0.84	**0.972**	(sensitivity: **0.77**; specificity: **0.96**)
rs747550722	3,186338624,1,C/T	R337W	damaging	0.04	Decrease	-0.79	**0.978**	(sensitivity: **0.76**; specificity: **0.96**)
rs570498140	3,186338633,1,C/T	R340C	damaging	0.01	Decrease	-0.71	**0.978**	(sensitivity: **0.76**; specificity: **0.96**)
rs1031255291	3,186333565,1,T/C	V102A	damaging	0	Decrease	-1.36	**0.98**	(sensitivity: **0.75**; specificity: **0.96**)
rs960090053	3,186331137,1,G/T	W69C	damaging	0.02	Decrease	-1.46	**0.983**	(sensitivity: **0.74**; specificity: **0.96**)
rs781729678	3,186335116,1,G/A	E184K	damaging	0	Decrease	-0.93	**0.987**	(sensitivity: **0.73**; specificity: **0.96**)
rs775843967	3,186334235,1,G/A	V110I	damaging	0	Decrease	-0.3	**0.99**	(sensitivity: **0.72**; specificity: **0.97**)
rs1253935270	3,186331073,1,T/C	I48T	damaging	0	Decrease	-2.23	**0.992**	(sensitivity: **0.70**; specificity: **0.97**)

rs780389938	3,186334991,1,T/G	V142G	damaging	0	Decrease	-2.06	**0.997**	(sensitivity: **0.41**; specificity: **0.98**)
rs745960136	3,186338639,1,G/A	V342M	damaging	0.05	Decrease	-0.25	**0.997**	(sensitivity: **0.41**; specificity: **0.98**)
rs1420998178	3,186335033,1,A/C	N156T	damaging	0	Decrease	0.03	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs868152128	3,186338552,1,C/A	H313N	damaging	0.03	Decrease	-0.48	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs761898439	3,186338582,1,G/A	V323M	damaging	0.05	Decrease	-0.36	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs755179125	3,186335020,1,C/G	L152V	damaging	0	Decrease	-1.77	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs750831608	3,186331097,1,A/G	Y56C	damaging	0	Decrease	-1.02	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs750328046	3,186331061,1,C/A	A44D	damaging	0	Decrease	-0.96	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs1267794029	3,186333517,1,A/G	E86G	damaging	0	Decrease	-0.93	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs1255502310	3,186335082,1,C/G	N172K	damaging	0.02	Decrease	-0.13	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs1204732359	3,186335003,1,G/A	C146Y	damaging	0	Decrease	-0.28	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs1158090968	3,186338598,1,C/T	S328L	damaging	0	Decrease	-0.12	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs1003867227	3,186334985,1,A/G	E140G	damaging	0	Decrease	-1.27	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs771078303	3,186333529,1,A/G	H90R	damaging	0.03	Decrease	-0.03	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs770636541	3,186335044,1,G/A	V160M	damaging	0	Decrease	-0.8	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs762195916	3,186331076,1,A/G	N49S	damaging	0	Decrease	-0.24	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs760680324	3,186336406,1,T/G	C219G	damaging	0.02	Decrease	-0.54	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs754707768	3,186333568,1,G/A	R103K	damaging	0	Decrease	-0.74	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs752410579	3,186333558,1,T/C	C100R	damaging	0	Decrease	-0.31	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs752157937	3,186338691,1,C/T	P359L	damaging	0	Decrease	-0.07	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs374708959	3,186333516,1,G/A	E86K	damaging	0	Decrease	-0.29	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs35457250	3,186338564,1,C/T	R317C	damaging	0	Decrease	-2.33	**1**	(sensitivity: **0.00**; specificity: **1.00**)
rs1490650775	3,186335052,1,C/A	H162Q	damaging	0.05	Decrease	-0.13	**0.257**	(sensitivity: **0.91**; specificity: **0.88**)
rs763435308	3,186334977,1,C/G	D137E	damaging	0	Decrease	-0.47	**0.897**	(sensitivity: **0.82**; specificity: **0.94**)
rs568116304	3,186331094,1,G/A	G55E	damaging	0	Decrease	-0.85	**0.977**	(sensitivity: **0.76**; specificity: **0.96**)

rs758967731	3,186333555,1,A/G	R99G	damaging	0.04	Decrease	-1.38	**0.000**	(sensitivity: **1.00**; specificity: **0.00**)
rs1252870356	3,186338589,1,C/T	S325L	damaging	0	Decrease	-0.17	**0.018**	(sensitivity: **0.95**; specificity: **0.80**)
rs777587342	3,186338601,1,C/T	P329L	damaging	0.05	Decrease	-0.65	**0.037**	(sensitivity: **0.94**; specificity: **0.82**)
rs1370174701	3,186331083,1,C/A	N51K	damaging	0.02	Decrease	-0.21	**0.177**	(sensitivity: **0.92**; specificity: **0.87**)
rs769790254	3,186335091,1,C/G	N175K	damaging	0.01	Decrease	-0.24	**0.343**	(sensitivity: **0.90**; specificity: **0.89**)
rs141102051	3,186331129,1,A/G	K67E	damaging	0.05	Decrease	-0.13	**0.444**	(sensitivity: **0.89**; specificity: **0.90**)
rs992583883	3,186331127,1,T/C	V66A	damaging	0	Decrease	-1.01	**0.583**	(sensitivity: **0.88**; specificity: **0.91**)
rs772079865	3,186331033,1,C/A	P35T	damaging	0.01	Decrease	-1.16	**0.848**	(sensitivity: **0.83**; specificity: **0.93**)
rs965006114	3,186333583,1,A/G	H108R	damaging	0.01	Decrease	-0.05	**0.879**	(sensitivity: **0.82**; specificity: **0.94**)
rs1288177478	3,186335023,1,G/T	A153S	damaging	0.03	Decrease	-0.66	**0.913**	(sensitivity: **0.81**; specificity: **0.94**)
rs745891423	3,186331037,1,A/C	E36A	damaging	0.01	Decrease	-0.43	**0.945**	(sensitivity: **0.80**; specificity: **0.95**)
rs370989005	3,186333484,1,G/C	G75A	damaging	0.01	Decrease	-0.53	**0.945**	(sensitivity: **0.80**; specificity: **0.95**)
rs1163123976	3,186335039,1,C/T	T158I	damaging	0	Decrease	-0.31	**0.958**	(sensitivity: **0.78**; specificity: **0.95**)
rs371802506	3,186336335,1,C/A	P195H	damaging	0.02	Decrease	-1.79	**0.971**	(sensitivity: **0.77**; specificity: **0.96**)
rs375304468	3,186334238,1,G/A	E111K	damaging	0	Decrease	-0.87	**0.991**	(sensitivity: **0.71**; specificity: **0.97**)
rs1455432970	3,186331120,1,G/C	D64H	damaging	0	Decrease	-0.28	**0.994**	(sensitivity: **0.69**; specificity: **0.97**)
rs374704176	3,186331132,1,G/A	V68M	damaging	0	Decrease	-0.73	**0.996**	(sensitivity: **0.55**; specificity: **0.98**)
rs1221690609	3,186335069,1,T/A	L168Q	damaging	0	Decrease	-1.33	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs372639228	3,186335034,1,C/A	N156K	damaging	0	Decrease	-2.11	**0.999**	(sensitivity: **0.14**; specificity: **0.99**)
rs1192798742	3,186335080,1,A/G	N172D	damaging	0.03	Decrease	-0.14	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs776029982	3,186334286,1,G/A	V127M	damaging	0	Decrease	-1.18	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs772980949	3,186334302,1,G/A	C132Y	damaging	0	Decrease	-0.47	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs755530712	3,186338688,1,G/A	C358Y	damaging	0	Decrease	-0.83	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs753599978	3,186337658,1,T/C	C230R	damaging	0	Decrease	-0.99	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs749520427	3,186333564,1,G/A	V102M	damaging	0	Decrease	-0.91	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)
rs142793377	3,186338703,1,G/C	R363T	damaging	0.03	Decrease	-1.75	**1.000**	(sensitivity: **0.00**; specificity: **1.00**)

4. Disscussion

Protein structure, stability, and subsequent function are affected by mutations. Mutations are part of the "raw materials" of evolution. Negative purifying selection, on the other hand, eliminates most, if not all, protein mutations, reducing the potential for future adaptation. Only a small part of is fixed to take on new functionality. Due to randomness or "neutral drift", neutral mutations can be stochastically modified in small populations. The effects of mutations on fitness at the organismal level are complex and rarely correlate with single gene or protein traits. Redundancy, backups and resilience at multiple levels mask the impact of large numbers of changes (Wagner *et al*., 2013). Indeed, understanding and predicting the effects of mutations at the organismal level is a major challenge in evolutionary biology (Pál *et al*., 2006; Camps *et al*., 2007).

The amount of functional protein present affects protein stability. Studies of pathogenic mutations have found that stability and folding effects account for 80% of the deleterious consequences of pathogenic mutations (Yue *et al*., 2015). Mutations that destabilize beyond a certain threshold (or DDG value) by reducing levels of soluble functional protein are responsible for protein dysfunction (Yue *et al*., 2015). Experimental data show that the risk of deleterious mutations ranges from 33-40% for many proteins (average 36%). Therefore, protein fitness decreases dramatically as mutations increase. After the protein has gone through 5 mutations, its fitness is only 20%. Although, a protein's initial stability can buffer some of the destabilising effects of mutations (Pál *et al*., 2006), stability appears to be the primary (though obviously not the only) factor that affects how quickly proteins evolve (Bloom *et al*., 2005) , and perhaps how quickly entire organisms evolve (Zeldovich *et al*., 2007), especially but not exclusively in relation to the acquisition of new functions.

Experimental datasets are often provided for a small subset of proteins and are usually associated with changes in thermodynamic stability (DDG value) of mutations. Recent computational advances have made it possible to predict DDG values for various protein mutations. Some prediction methods use array structure and conformation (Huang *et al*., 2007; Parthiban *et al*., 2006).

The predictions exclude effects on folding intermediates and focus primarily on how mutations affect the native state. Although, likely to overlap with *in vivo* thermodynamic stability effects, predictions regarding dynamic stability effects are very useful. Overall, work is underway to provide more accurate and realistic estimates of how mutations affect protein levels *in vivo* (Vendruscolo and Tartaglia, 2008).

A small loss of stability in kcal/mol depletes a fraction of partially folded and/or misfolded species in sufficient quantities to cause irreversible aggregation or irreversible degradation. Production appears to lead to a significant reduction in protein levels.

Additional mutations reduce stability beyond acceptable limits and fitness is lost with changes in DG. The destabilizing effects of mutations prevent the emergence of new protein functions. On the other hand, we find that neutral or non-adaptive mutational drift is less destructive and tends to occur at buried residues (Tokuriki *et al*., 2008).

Regardless of whether SIFT and PolyPhen-2 analyses of AHSG gene, SNPs indicate that they are harmful or tolerable, I mutant analysis reveals that the proteins' thermodynamic stability has deteriorated. This might be a factor in the AHSG protein's altered functionality.This finding supports carrying out a wet lab based study on AHSG gene polymorphisms in kidney stone disease.

One SNP in particular, RS 4917 has been shown that T allele is the risk allele of calcification of heart valves and coronary arteries (Mohammadi-Noori *et al*., 2020). This SNP results in a single amino acid substitution (glutamic acid to lysine) in the protein sequence of fetuin A, which may affect its function by reducing a protein expression and leading to kidney stone (Mehrsai *et al*., 2017), potentially affecting its interaction with other molecules or cellular processes.

Although, there are already a number of studies demonstrating the relationship between SNPs in various genes and various disorders (Honnalli *et al*., 2022; Jovièiæ-Pavloviæ *et al*., 2022; Dai *et al*., 2023) A computational study of the functional effects of SNPs in this gene has not yet been performed. SIFT technology uses sequence homology and physicochemical properties of amino acid residues between related genes and domains across evolution to predict whether amino acid changes affect protein function. The "false negative" and "false positive" error rates of SIFT are estimated to be 31% and 20%, respectively. SIFT has shown approximately 80% success in benchmark studies using amino acid substitutions believed to have a significant negative impact on the residual activity of the mutant protein as a test set.

However, SIFT and PolyPhen-2 can be very helpful in predicting how a mutation will affect how a protein functions as well as the necessity of evaluating gene polymorphisms using wet lab techniques. I mutant evaluated the stability of the mutant proteins because the majority of disease mutations have an impact on protein stability.

5. Conclusion

The current silica analysis is intended to investigate how the expression of AHSG gene polymorphisms affects the pathobiology of renal stone disease. It makes a compelling case that the pathobiology of renal stone disease may be affected by the harmful effects of AHSG gene mutations as well as their decreased protein stability, as anticipated by bioinformatics techniques. Understanding the pathophysiology of kidney stone disease may benefit from wet research on this gene.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W and Lipman, D. J. (1990). Basic local alignment search tool. J. Mol. Biol., **215**(3):403-410.

Bloom, J. D.; Silberg, J. J.; Wilke, C. O.; Drummond, D. A.; Adami, C and Arnold, F. H. (2005). Thermodynamic prediction of protein neutrality.Proc. Natl. Acad. Sci. U. S. A., **102**(3):606-611.

Choi, Y.; Sims, G. E.; Murphy, S.; Miller, J. R and Chan, A. P. (2012). Predicting the functional effect of amino acid substitutions and indels. Journals. plos.org. e46688.

Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Bealer, K. and Madden, T.L(2009). BLAST+: Architecture and applications. BMC Bioinform., **10**:1-9.

Choi, Y and Chan, A. P. (2015). PROVEAN web server: Atool to predict the functional effect of amino acid substitutions and indels. Bioinformatics., **31**(16):2745-2747.

Camps, M.; Herman, A.; Loh, E. R. N and Loeb, L. A. (2007). Genetic constraints on protein evolution. Crit. Rev. Biochem. Mol., **42**(5):313-326.

Dai, S.; Chen, Y.; Shang, D.; Ge, X.; Yan, H.; Hao, C. and Zhu, T. (2023). Alpha 2 Heremans Schmid glycoprotein gene polymorphism (rs4918) is associated with coronary artery calcification in incident peritoneal dialysis patients. Nephrology, **28**(1): 28-35.

Emahazion, T.; Feuk, L.; Jobs, M.; Sawyer, S. L.; Fredman, D.; St Clair, D.; Prince, J.A. and Brookes, A.J(2001). SNP association studies in Alzheimer's disease highlight problems for complex disease analysis. Trends Genet., **17**(7):407-413.

Gudulkar, S.; Rajbhar, K.; Dawda, H. and Mukundan, U. (2020). Screening of selected plants for their effectiveness in the treatment of kidney stone. Ann. Phytomed., **9**(1):213-217.

Huang, L. T.; Gromiha, M. M and Ho, S. Y. (2007). iPTREE-STAB: interpretable decision tree based method for predicting protein stability changes upon mutations. Bioinformatics., **23**(10):1292-1293.

Honnalli, N. M., Adiga, U. and Adiga, S. (2022). Comparison of Serum Fetuin A Levels and Biochemical Parameters In Patients With and Without Calcium Oxalate Stones: A Cross Sectional Study. J. Sch. Psychol., **6**(7):1029-1034.

Jahnen-Dechent, W.; Schinke, T.; Trindl, A.; Muller-Esterl, W.; Sablitzky, F.; Kaiser S. and Blessing, M. (1997). Cloning and targeted deletion of the mouse fetuin gene. J. Biol. Chem., **272**(50):31496-31503.

Jovièiæ-Pavloviæ, S.; Simiæ-Ogrizoviæ, S.; Bukumiriæ, Z.; Eriæ, M.; Pavloviæ, N.; Kotlica, B. and Novakoviæ, I. (2022). Impact of the fetuin gene polymorphisms in coronary artery calcification and mortality of patients with chronic kidney disease and renal transplant. Genetika, **54**(1):457-472.

Ketteler, M.; Bongartz, P.; Westenfeld, R.; Wildberger, J. E.; Mahnken, A. H.; Böhm, R.; Metzger, T.; Wanner, C.; Jahnen-Dechent, W. and Floege, J. (2003). Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: A cross-sectional study. Lancet., **361**(9360):827-833.

Kono, T. J.; Lei, L.; Shih, C. H.; Hoffman, P. J.; Morrell, P. L and Fay, J. C. (2018). Comparative genomics approaches accurately predict deleterious variants in plants. G3-Genes Genom Genet., **8**(10):3321-3329.

Krawczak, M.; Ball, E. V.; Fenton, I.; Stenson, P. D.; Abeysinghe, S.; Thomas, N and Cooper, D.N. (2000). Human gene mutation database: A biomedical information and research resource. Hum. Mutat., **15**(1):45-51.

Lee, J. E.; Choi, J. H.; Lee, J. H and Lee, M. G. (2005). Gene SNPs and mutations in clinical genetic testing: Haplotype-based testing and analysis.Mutat Res-Fund Mol M., **573**(1-2):195-204.

Mohammadi-Noori, E., Salehi, N., Mozafari, H., Elieh Ali Komi, D., Saidi, M., Bahrehmand, F., Vaisi-Raygani, A., Elahirad, S., Moini, A., and Kiani, A. (2020). Association of AHSG gene polymorphisms with serum Fetuin-A levels in individuals with cardiovascular calcification in west of Iran. Mol. Biol. Rep., **47**:1809-1820.

Mehrsai, A., Guitynavard, F., Nikoobakht, M. R., Gooran, S and Ahmadi, A. (2017). The relationship between serum and urinary Fetuin-A levels and kidney stone formation among kidney stone patients. Cent European J. Urol., **70**(4):394-399.

Ng, P. C. and Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res., **31**(13):3812-3814.

Ng, P. C. and Henikoff, S. (2006). Predicting the effects of amino acid substitutions on protein function. Annu. Rev. Genomics Hum. Genet., **7**:61-80.

Prokunina, L and Alarcón-Riquelme, M. E. (2004). Regulatory SNPs in complex diseases: Their identification and functional validation. Expert Rev. Mol. Med., **6**(10):1-15.

Pál, C.; Papp, B.; and Lercher, M. J. (2006). An integrated view of protein evolution. Nat. Rev. Genet.,**7**(5):337-348.

Parthiban, V.; Gromiha, M. M and Schomburg, D. (2006). CUPSAT: Prediction of protein stability upon point mutations. Nucleic Acids Res., **34**(2):W239-W242.

Ramensky, V.; Bork, P and Sunyaev, S. (2002). Human non synonymous SNPs: Server and survey. Nucleic Acids Res., **30**(17):3894-3900.

Schinke, T.; Amendt, C.; Trindl, A.; Pöschke, O.; Müller-Esterl, W. and Jahnen-Dechent, W. (1996). The serum protein á2-HS glycoprotein/fetuin inhibits apatite formation *in vitro* and in mineralizing calvaria cells: A possible role in mineralization and calcium homeostasis. J. Biol. Chem., **271**(34):20789-20796.

Stenson, P. D.; Mort, M.; Ball, E. V.; Howells, K.; Phillips, A. D.; Thomas, N. S and Cooper, D.N.(2009). The human gene mutation database: 2008 update. Genome Med., **1**(1):1-6.

Schork, N. J.; Fallin, D and Lanchbury, J. S. (2000). Single nucleotide polymorphisms and the future of genetic epidemiology.Clin. Genet., **58**(4):250-264.

Tokuriki, N.; Stricher, F.; Serrano, L and Tawfik, D. S. (2008). How protein stability and new functions trade off. PLoS Comput. Biol., **4**(2): e1000002.

Vendruscolo, M and Tartaglia, G. G. (2008). Towards quantitative predictions in cell biology using chemical properties of proteins. Mol. Biosyst., **4**(12):1170-1175.

Wagner, A. (2013). Robustness and evolvability in living systems. Princeton university press.(15)

Yue, P.; Li, Z and Moult, J. (2015). Loss of protein structure stability as a major causative factor in monogenic disease. J. Mol. Biol., **353**(2): 459-473.

Zeldovich, K. B.; Chen, P. and Shakhnovich, E. I. (2007). Protein stability imposes limits on organism complexity and speed of molecular evolution. Proc. Natl. Acad. Sci. U. S. A., **104**(41):16152-16157.

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