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Bioinformatics approach prediction of nonsynonymous and miRNA-binding site polymorphisms within human *SclA9* and *Scl22A12* genes

MOHAMMED A. ESHAGE
Faculty of Medical Laboratory Sciences
Sudan University of Science and Technology
Khartoum, Sudan
WASAL A. HAMID
Faculty of Science, Al-Neelain University
Khartoum, Sudan
ENTSSAR S. TAHA
Microbiology Department, Faculty of Science
Al-Neelain University, Khartoum, Sudan
REHAB A. ABDALLA
Faculty of Veterinary Medicine
University of Khartoum, Khartoum, Sudan
EMAN M. ABD ELHALIM
Faculty of Medical Laboratory Sciences
Sudan University of Science and technology
Khartoum, Sudan
MOHAMED M. HASSAN[1]
Faculty of Medical Laboratory Sciences
Al Zaiem Al Azhari University, Khartoum, Sudan
SOFIA B. MOHAMED
Tropical Medicine Research Institute
Khartoum, Sudan

Abstract:

Single nucleotide polymorphisms (SNPs) have been found in 3'UTRs that disrupt normal miRNA binding or introduce new binding sites and some of these have been associated with disease pathogenesis. This raises the importance of detecting miRNA targets and predicting the possible effects of SNPs on binding sites. Where non-synonymous

[1] Corresponding author: m_rbx@hotmail.com

single nucleotide polymorphisms (nsSNPs) in coding regions can lead to amino acid changes, may cause alteration protein function alteration, lead to variations in susceptibility to disease and altered drug response. Identification of deleterious nsSNPs is important for characterizing the genetic basis of human disease. Many insilico methods (SIFT, PolyPhen, I-mutant, CPH, Chimera) were used to identify functional from total pool SNPs, and that to explore the possibility relationships between genetic mutation and phenotypic variation. Through these methods, within exons regions, 14 damaging nsSNPs were found within Scl22A12 and SclA9 genes (seven SNPs in each gene). By the same token in 3-untranslated region (3'UTR), eight functional SNPs found within Scl22A12 and five in Slc2A9 genes. In conclude these previous functional identified SNPs, have a pathogenesis basis for -SclA9, Scl22A12- genes alteration, and could lead to gout disease. This prediction analysis of SNPs in human would be useful for further genotype-phenotype studies on individuals.

Key words: Nonsynonymous SNPs (nsSNPs), miRNA-binding site polymorphism (miSNPs), Slc2A9 and Slc22A12 genes, Bioinformatics methods, Gout disease.

Introduction:

The most common type of genetic variation in the human genome occurs as single nucleotide polymorphisms (SNPs) (Nadeau, 2002). Few SNPs have been found to be associated with some rare human diseases. However, not all the SNPs can cause amino acid changes and correlate with human diseases, nonsynonymous SNPs (nsSNPs) are SNPs that occur in exon regions and cause an amino acid change in the corresponding protein (Ramensky et al., 2002). According to the dataset of two online databases, online mendelian inheritance in man -OMIM- (http://www.ncbi.nlm.nih.gov/omim), and human gene mutation databases (http://www.hgmd.cf.ac.uk), nsSNP variants are the almost half of all the genetic changes related to

human diseases (Hamosh et al., 2005; Stenson et al., 2008). Hence, these nsSNPs are considered to be deleterious nsSNPs because they lead to dramatic phenotypic consequences. On the other hand, there are some nsSNPs that do not alter protein function even though the first-order structure, these called tolerant or benign nsSNPs. It is important to differentiate deleterious from tolerant nsSNPs to, characterize the genetic basis of human diseases, assess individual susceptibility to these diseases, understand the pathogenesis of diseases, identify molecular and potentially therapeutic targets, and predict clinical phenotypes (Wang et al., 2009). Over the past few years, many studies have attempted to predict the functional consequences of an nsSNPs whether it is disease-related or neutral, based on sequence information and structural attributes (Richard et al., 2006). Using computational algorithms such as SIFT and PolyPhen algorithms to screen for deleterious nsSNPs (Livingston et al., 2004). The structure of protein can change in various ways due to the biochemical differences of the amino acid variant (acidic, basic, or hydrophobic) and the location of variant in the protein sequence (affecting tertiary or quaternary structure or the active site where substrate binds), these could lead to deleterious effect on the structure and/or function of the proteins (Melissa et al., 2005). Therefore, determining the nsSNPs that affects the amino acid sequence of a gene product which alter protein function and contribute to disease will be a big challenge in the coming years (Karchin et al., 2005). That why, several studies tried to evaluate deleterious nsSNPs based on 3-dimensional (3D) structure information of proteins used insilico analysis (Sunyaev et al., 2001).

MicroRNAs (miRNAs) are short (19-23 nucleotides) non-coding RNAs that bind to target sites within messenger RNA (mRNA) sites in 3'UTR. Single nucleotide polymorphisms (SNPs) in 3'UTRs could disrupt or introduce new

miRNA/mRNA binding site, lead to trouble (decrease or increase) expression of target gene, and associated with disease. Those draw attention to the importance detect miRNA targets and predicts the possible effects of SNPs on these binding sites. In the last decade, many studies had been conducted to predict the location of miRNA binding sites used many logarithm methods. Moreover, the existing open source softwares have some shortcomings including the requirement for significant manual labor when working with huge lists of SNPs, and that algorithms work only for SNPs present in databases such as dbSNP. These limitations leading to large predicted number of novel variants in 3'UTR and other regions, results of used high resolution technology as next generation sequencing (NGS) (Mehmet et al., 2014). Recently, many databases are designed specifically to work in 3'UTR (miRNA) such as, microSNiPer, Patrocles, Mirsnpscore, miRd-SNP, MirSNP, PolymiRTS (Cheng et al., 2013).

SLC2A9 and *SLC22A12* genes are related to organic anion transporter family, located primarily in the proximal renal tubules, responsible for most of the renal handling of uric acid. Multiple renal tubular transporters have been identified, but the most important ones seem to be uric acid transporter (URAT 1) (Enomoto et al., 2002) and URAT1v (Anzai et al., 2008). Lack expression of these proteins leading to renal hypouricemia types 1 and 2, respectively. Polymorphisms encoding hyperactive variants of these renal tubular transporters have been associated with increased risk of hyperuricemia and gout (Kolz et al., 2009). Single nucleotide polymorphisms within URAT1 or *SLC22A12* gene(solute carrier family 22 member 12) are associated with altered (increased or decreased) reabsorption of uric acid by kidneys in different human populations. These altered of reabsorption could contribute to hyperuricemia or hypouricemia (Graessler et al., 2006). The Solute carrier family 2, facilitated glucose

transporter member 9 (Glut9) was also found to act as a voltage-driven urate transporter and is therefore also known as URATv1 (voltage-driven urate transporter 1) (Anzai et al., 2008). Glut9 is encoded by the *SCL2A9* gene, and recently found to be as uric acid transporter, by the same token genetic variants in this transporter get a linked to increase risk of developed, both hyperuricemia and gout (Vitart et al., 2008). The main goal of this work is to predict the functional non-synonymous SNPs (nsSNPs) and miSNP on 3UTR using computational methods.

Material and methods

Bioinformatics processing and data analysis

Total SNPs contain Homo sapiens SNPs of 3′UTR/5′UTR near to gene, noncoding 3′UTR/5′UTR, intron, coding synonymous and coding nonsynonymous (frame shift, missense, nonsense and stop gained) regions. For this study human nonsynonymous and 3′UTR SNPs had been selected for our investigation.

Datasets

SNPs on nucleotides sequences for *SLC2A9* and *SLC22A12* genes were obtained from the SNPs database (dbSNPs) (http://www.ncbi.nlm.nih.gov/SNP/), and UniProt database (http://www.uniprot.org) for SNPs related protein sequences.

Predicting damaging amino acid substitutions using SIFTv5.1 (Sorting Intolerant from Tolerant)

An online computational program used to detect a harmful nonsynonymous single-base nucleotide polymorphism (nsSNP), it performs an alignments between an order sequence and large number of homologous sequences to predict if an amino acid substitution affects protein function. The score result of each

residual ranges from zero to one, where the amino acid substitution is predicted damaging when the score is below or equal to 0.05, and tolerated if the score is greater than 0.05 (Ng and Henikoff, 2003). SIFT version 5.1 is available at: http://sift.bii.a-star.edu.sg/index.html.

Prediction of functional modification of coding nsSNPs using Polyphen (Polymorphism Phenotyping)

A tool used to predict possible impact of an amino acid substitution on the structure and function level of submitted proteins, by analysis multiple sequence alignment and protein 3D structure, in addition calculates and computes the position-specific independent count scores (PSIC) for each two variants. The higher a PSIC score difference, the higher functional impact on particular amino acid substitution is likely to have. Prediction outcome can be one of probably damaging, possibly damaging, or benign and also can be indicated by a vertical black marker inside a color gradient bar, where green is benign and red is damaging (Hassan et al., 2014). PolyPhen version 2.2.2r398 is available at: http://genetics.bwh.harvard.edu/pph2/index.shtml.

I-Mutant Suite (Predictor effects of single point protein mutation)

Protein stability change disturbs both protein structure and protein function (Daggett and Fersht, 2003). I-Mutant is a suite of support vector machine based predictors integrated in a unique web server, which used to predict the protein stability changes at single-site mutations starting from the protein structural or sequence information (Capriotti et al., 2005). I-mutant 3.0 is available at http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I Mutant3.0.cgi.

Protein 3D structural Modeling

Modeling SNPs on the 3D structure of proteins is a very helpful action in order to predict the effect that SNPs may cause on the structural level. Therefore we used CPHmodels 3.2 server to predict the 3D structure for those proteins with an unknown 3D structure model. It is in fact a protein homology modeling server, where the template recognition is based on profile-to-profile alignment, guided by secondary structure and exposure predictions (Nielsen et al., 2010). http://www.cbs.dtu.dk/services/CPHmodels/.

Explore amino acid substitution model

UCSF Chimera is a highly extensible program for interactive visualization, analysis of molecular structures and related data. Chimera (version 1.8) software was used to scan the 3D (three-dimensional) structure of specific protein, and hence modifies the original amino acid with the mutated one to see the impact that can be produced. The outcome is then a graphic model depicting the mutation (Hassan et al., 2014). Chimera (version 1.8) currently available within the Chimera package and available from the Chimera web site at: http://www.cgl.ucsf.edu/chimera/.

PolymiRTS database 3.0 to detect Polymorphism in microRNA Target Site

PolymiRTS database was designed specifically for 3'UTR analysis, identify single-nucleotide polymorphisms (SNPs) that affect miRNA targeting in human and mouse. In this study it used to determine SNPs that may alter miRNA target sites through 3′UTR SNPs located within *SCL2A9 and SCL22A12 genes* (Hassan et al., 2014).

Results

Table 1. Distribution of human total, 3′UTR and non-synonymous SNPs

SNPs	Slc22a12	Slc2a9
Total	856	13181
Coding-nonsynonymous	84 (~ 9.8% of total)	189 (~ 1.4% of total)
3′UTR	42 (~ 4.9% of total)	125 (~ 0.9% of total)

Table 2. Prediction results of SIFT and PolyPhen programs for deleterious nsSNPs

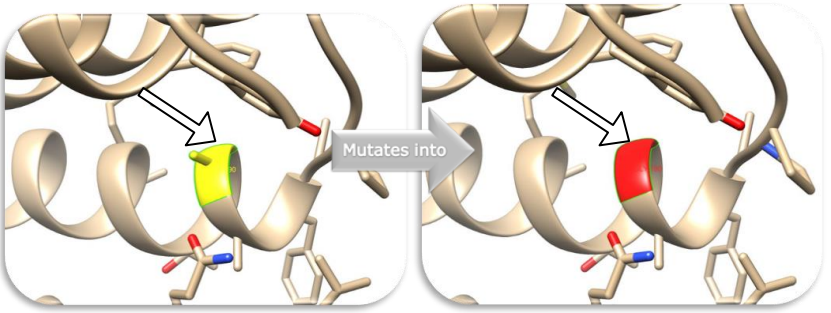
Gene name	SNP ID	Chromosome location	Nucleotide change	Amino Acid Change	PolyPhen-2 result	PSIC SD	SIFT result	Tolerance index
SCL22A12	rs121907893	11:64593548	C/T	T217M	Probably damaging	1	damaging	0.01
	rs121907894	11:64598579	A/G/T	E298d	Probably damaging	0.991	damaging	0.03
	rs121907895	11:64599858	G/T	L418R	Probably damaging	0.992	damaging	0
	rs121907896	11:64591825	A/G	R90H	Probably damaging	0.960	damaging	0.03
	rs12800450	11:64591749	G/T	G65W	Probably damaging	0.998	damaging	0
	rs61737613	11:64591861	A/G	N102S	possibly damaging	0.951	damaging	0.03
	rs80133078	11:64593467	C/G	A190G	Probably damaging	0.960	Damaging	0.01
SLC2A9	rs6282	4:9782292	G/A	L88R	Probably damaging	1	Damaging	0
	rs2227840	4:9782214	C/G	C62S	Possibly damaging	0.889	Damaging	0
	rs2227849	4:9782358	A/G	G110E	Possibly damaging	0.816	Damaging	0
	rs2227851	4:9782918	A/C	T297P	Probably damaging	1	Damaging	0
	rs73805846	4:9782833	C/G	H268Q	Probably damaging	0.998	Damaging	0.04
	rs112029473	4:9782993	C/T	C322R	Probably damaging	1	Damaging	0
	rs113223975	4:9782210	A/G	V61M	Probably damaging	0.970	Damaging	0

Table 3. Prediction result of I-Mutant software for deleterious nsSNPs.

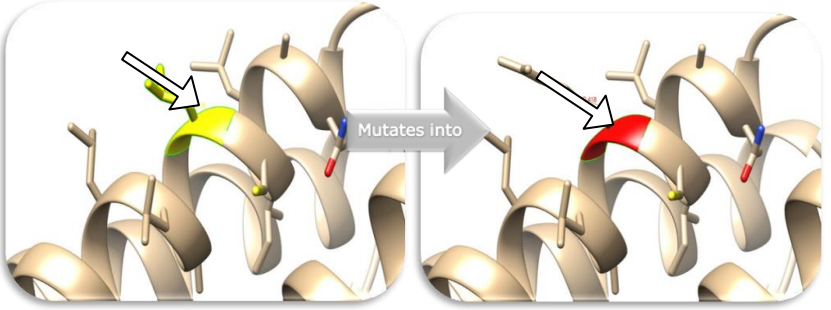
Gene name	SNP ID	Amino acid position	WT	MT	SVM2 Prediction Effect	DDG Value Prediction Kcal/mol	RI
Slc22A12	rs121907893	217	T	M	Decrease	-0.25	3
	rs121907894	298	E	D	Decrease	-0.34	5
	rs121907895	418	L	R	Decrease	-1.22	6
	rs121907896	90	R	H	Decrease	-0.65	6
	rs12800450	65	G	W	Decrease	-0.37	1
	rs61737613	102	N	S	Decrease	0.02	5
	rs80133078	190	A	G	Decrease	-1.19	8
SLC2A9	rs121907893	217	T	M	Decrease	-0.25	3
	rs121907894	298	E	D	Decrease	-0.34	5
	rs121907895	418	L	R	Decrease	-1.22	6
	rs121907896	90	R	H	Decrease	-0.65	6

	rs12800450	65	G	W	Decrease	-0.37	1
	rs61737613	102	N	S	Decrease	0.02	5
	rs80133078	190	A	G	Decrease	-1.19	8

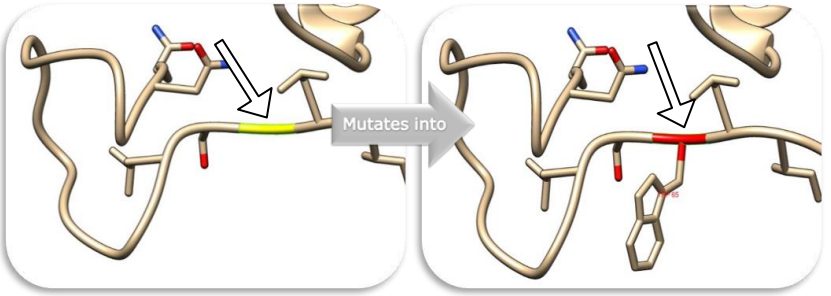
Where WT: Wild type amino acid, MT: Mutant type amino acid, DDG: DG (New Protein)-DG(Wild Type) in Kcal/mol (DDG<0: Decrease stability, DDG>0: Increase stability), RI: Reliability index



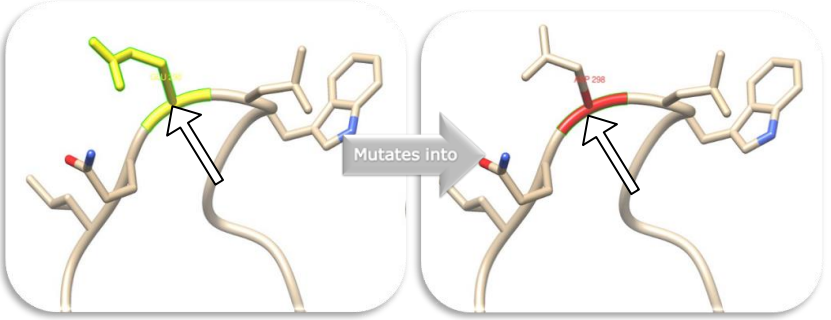
SNP ID: rs80133078. Protein position 190 changed from Alanine to Glycine



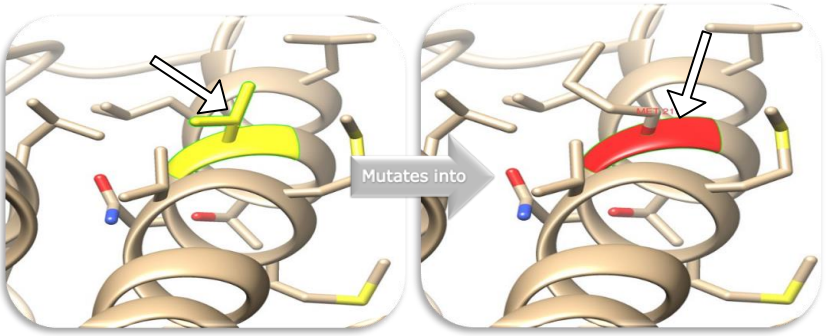
SNP ID: rs121907895. Protein position 418 changed from Leucine to Arginine



SNP ID: rs12800450. Protein position 65 changed from Glycine to Tryptophan

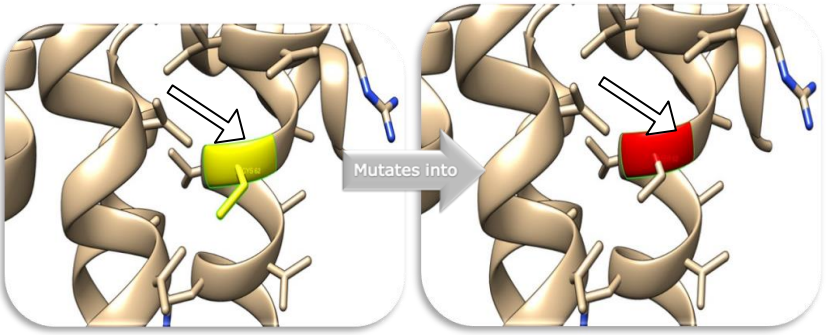


SNP ID: rs121907894 Protein position 293 changed from Glutamic acid to Aspartic acid

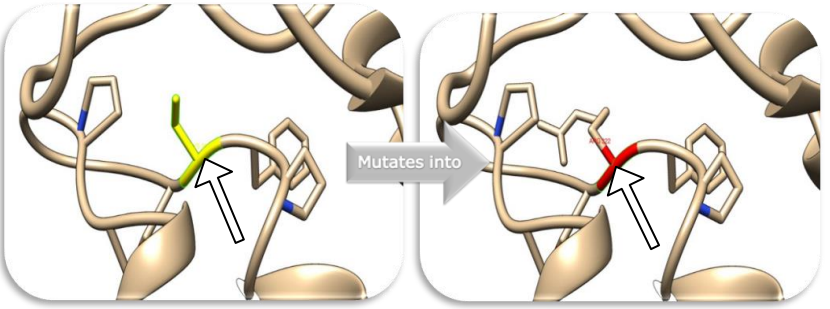


SNP ID: rs121907893. Protein position 217 changed from Threonine to Methionine

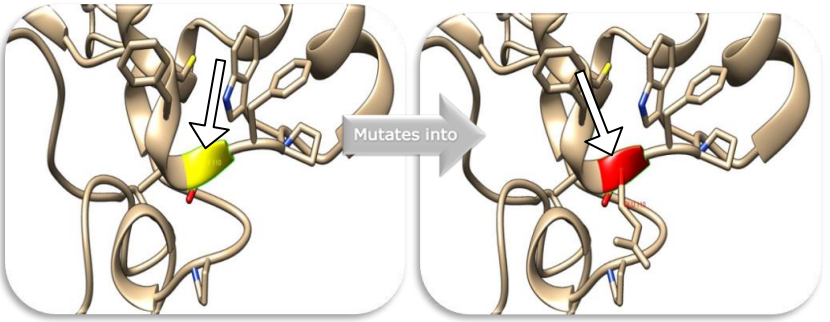
Fig. 1. Shows position of native (yellow) and new (red) amino acid residues used Chimera program v1.8 for (*Slc22a12* gene)



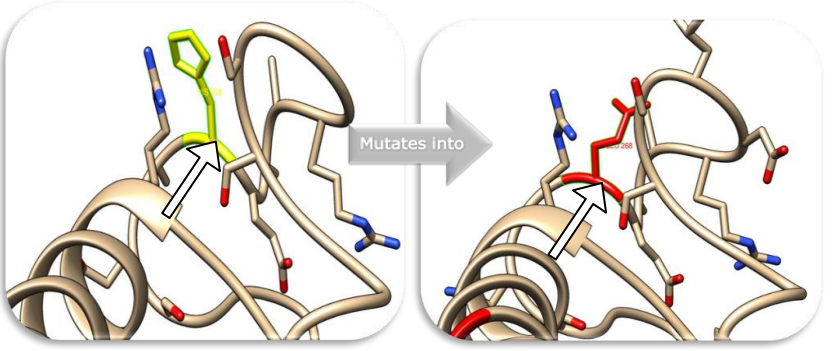
SNP ID: rs2227840 Protein position 62 changed from Cysteine to Serine



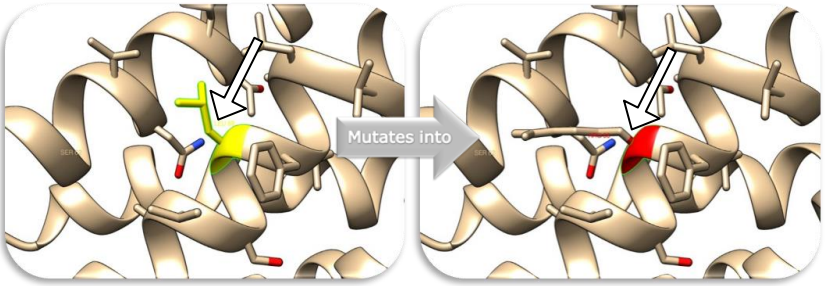
SNP ID: rs112029473 Protein position 322 changed from Cysteine to Arginine



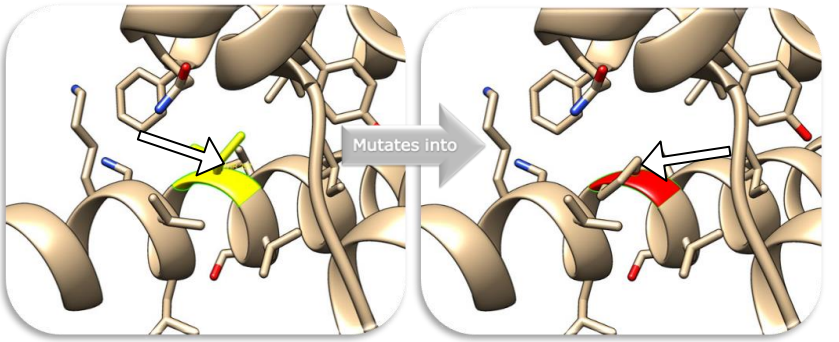
SNP ID: rs2227849 Protein position 110 changed from Glycine to Glutamic acid



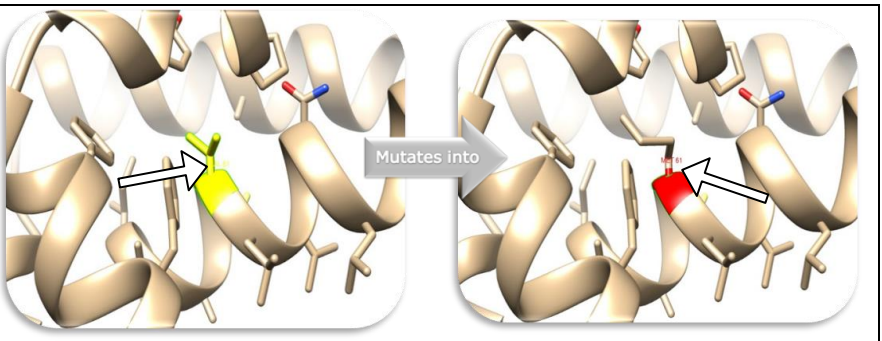
SNP ID: rs2227849 Protein position 110 changed from Glycine to Glutamic acid



SNP ID: rs6282 Protein position 88 changed from lysine to Arginine



SNP ID: rs2227851 Protein position 297 changed from Threonine to Proline



SNP ID: rs113223975 Protein position 61 changed from Valine to Methionine

Fig. 2. Shows position of native (yellow) and new (red) amino acid residues used Chimera program v1.8 for (*SLC2A9* gene)

Table 4. PolymiRTS prediction results of *Slc22a12* gene

dbSNP ID	Variant type	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	context+ score change
rs146221674	SNP	A	A	hsa-miR-5088-5p	3	caggtGAGCCCTg	D	-0.162
			G	hsa-miR-3918	3	caggtgGGCCCTG	C	-0.167
				hsa-miR-7160-3p	3	caggtGGGCCCTg	C	-0.197
rs148845071	SNP	G	G	hsa-miR-4671-5p	3	ggCTTCGGAgagc	D	-0.134
			A	hsa-miR-146a-3p	3	ggcTTCAGAGAgc	C	-0.086
				hsa-miR-301a-5p	3	ggcttCAGAGAgc	C	-0.023
				hsa-miR-3921	3	ggcttCAGAGAgc	C	-0.013
				hsa-miR-4653-5p	3	ggcttCAGAGAgc	C	-0.006
				hsa-miR-6817-3p	21	ggcttCAGAGAGc	C	-0.098
rs476037	SNP	G	G	hsa-miR-1908-5p	3	ctgCCCGCCACat	D	-0.193
				hsa-miR-663a	3	ctgCCCGCCACat	D	-0.193
				hsa-miR-6787-5p	3	ctgCCCGCCACat	D	-0.203
				hsa-miR-7112-5p	2	CTGCCCGccacat	D	-0.178
			A	hsa-miR-541-3p	3	ctGCCCACCACat	C	-0.383
				hsa-miR-654-5p	3	ctGCCCACCACat	C	-0.362
				hsa-miR-6769a-5p	3	ctgCCCACCACat	C	-0.133
				hsa-miR-6769b-5p	3	ctgCCCACCACat	C	-0.133
				hsa-miR-92a-2-5p	3	ctgCCCACCACat	C	-0.111

miR ID : Link to miRBase, Conservation : Occurrence of the miRNA site in other vertebrate genomes in addition to the query genome, miRSite : Sequence context of the miRNA site. Bases complementary to the seed region are in capital letters and SNPs are highlighted in red, FuncClass : D: The derived allele disrupts a conserved miRNA site (ancestral allele with support > 2), C: The derived allele creates a new miRNA site, Context score: increase negatively = increase SNP functionality.

Table 4. PolymiRTS prediction results of *SLC2A9* gene

dbSNP ID	Variant type	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	context+ score change
rs189072747	SNP	T	-					
			G	hsa-miR-155-3p	3	gTGTAGGAgctcg	C	-0.145
rs191141694	SNP	A	A	hsa-miR-3136-5p	4	aaacTCAGTCAAa	D	-0.326
				hsa-miR-4439	4	aaacTCAGTCAAa	D	-0.347
				hsa-miR-4513	4	aaacTCAGTCAaa	D	-0.151
				hsa-miR-4682	4	aAACTCAGtcaaa	D	-0.184
				hsa-miR-6855-3p	5	aaacTCAGTCAaa	D	-0.157

				hsa-miR-6857-3p	5	aaaCTCAGTCAaa	D	-0.389
				hsa-miR-943	5	aaactCAGTCAAa	D	-0.12
			G	hsa-miR-29c-5p	4	aaacTCGGTCAaa	C	-0.285
rs185764800	SNP	G	G	hsa-miR-634	3	tgTGCTGGTgggg	D	-0.145

miR ID : Link to miRBase, Conservation : Occurrence of the miRNA site in other vertebrate genomes in addition to the query genome, miRSite : Sequence context of the miRNA site. Bases complementary to the seed region are in capital letters and SNPs are highlighted in red, FuncClass : D: The derived allele disrupts a conserved miRNA site (ancestral allele with support > 2), C: The derived allele creates a new miRNA site, Context score: increase negatively = increase SNP functionality.

Discussion

Our current analysis focuses on SNPs in the coding regions, and study findings could explain a significant fraction of the Gout risk that has been detected. This approach might also be applied to a relationship between SNP conservation levels of diseases other than Gout. The use of these servers to select potentially polymorphic nsSNPs for studies can be an efficient way to explore the role of genetic variation. Polymorphisms in miRNA-binding sites not only offer a possibility of novel diagnostic in Gout disease, but can also help to understand complex regulatory networks of miRNAs in health and disease. With the development of more sophisticated bioinformatics algorithms and accumulation of data from genomes sequencing, identification of miRSNPs of clinical utility and functional relevance for human genetics disease should be facilitated.

A major interest in human genetics is to distinguish non function or neutral mutations from those that contribute to disease. Amino acid substitutions are responsible for approximately half of the known gene lesions lead to human inherited disease (Cooper et al., 1998). Identification of nsSNPs that affect protein functions and relate to disease becomes an importance task and big challenged (Yan et al., 2002). Currently, millions of human SNPs have been reported used high-throughput methods, Thus follows that a lot of work from

biologist and bioinformaticians to find the links between those SNPs and currently diseases (Doss and Sethumadhavan, 2009). Some limitation prediction found within CPHmodel server to get a homology modeling of two damaging SNPs (rs121907896, rs61737613) in *Slc22a12* gene, to show native and mutant residues.

Conclusion

Our successful Insilco prediction of identification of several pathogenic SNPs in anion transporter family genes suggests the application of bioinformatics analysis tools as well as publicly available databases such as NCBI, dbSNP and HapMap for efficiently selecting a functional SNP for the conduct of genetic association studies. Finally we hope that our comprehensive investigation provide an empirical guideline for researchers to prioritize the known nsSNPs on the basis of molecular analysis. It is obvious from the results that the deployment of Insilco tools for application in biomedical research is highly effective and has a great impact on the ability to uncover the cause of genetic variation in different genetic disease. Important for future studies is to confirm this result in wet lab in order to obtain more information regarding possible novel SNPs involved in gout disease.

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