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Computational Analysis of Single nucleotide Polymorphism (SNP) in human MTNR1B gene

Insilico prediction of missense mutation in MTNR1B gene

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Abstract –

***Background**: The MTNR1B gene encodes melatonin receptor type 1B which belongs to the superfamily of G-protein coupled receptors. It is expressed in various peripheral tissues and maintains circadian rhythm. The MTNR1B gene mutations are seen in metabolic disorder such as diabetes.*

***Objective**: This study was undertaken to find the functional non-synonymous single nucleotide polymorphisms in MTNR1B.*

***Methods:** Combination of computational methods provides a way of sorting out the probably damaging non-synonymous single nucleotide polymorphisms and then analyzing the protein structure stability. We performed single nucleotide polymorphisms analysis for 77 missense non-synonymous single nucleotide polymorphisms by the SIFT, PolyPhen, SNPs3D, SNAP and PANTHER programs.*

***Results:** Among the predicted non-synonymous single nucleotide polymorphisms, rs377626851 and rs574453327 were identified as deleterious and damaging. Additionally, I-Mutant2.0 showed a decrease in stability for these non-synonymous single nucleotide polymorphisms upon mutation. Protein structural analysis with these amino acid variants was performed by using QMEAN6 score values and MUSTER (Multi-Sources Threader) was used for modelling mutant structures. The cis regulatory elements were also analysed.*

***Conclusion**: This study suggests that G109E and R222H variants of MTNR1B gene could directly or indirectly destabilize the amino acid interactions and hydrogen bond networks thus explaining the functional deviations of protein to some extent. So single nucleotide polymorphisms should be considered important candidates in causing diseases related to MTNR1B gene malfunction and disruption of circadian rhythm.*

Key Words: Melatonin receptor type 1B, Single nucleotide Polymorphism (SNP), mutant

INTRODUCTION

Single nucleotide substitutions in the DNA sequences at particular location are referred to as Single Nucleotide Polymorphisms, or SNPs. The DNA sequences are almost exactly the same, except at one nucleotide position resulting in biological variation between people. As a result, individuals differ in physical appearance, susceptibility to disease or environmental factors and response to medication. SNPs within the coding sequence are of two types, synonymous and non-synonymous. The non-synonymous substitutions changing the amino acid within a protein termed as missense mutation while a single base mutation that encodes a stop codon resulting in truncation of protein termed as nonsense mutation. The assembly of human SNPs is maintained and curated by the National Centre for Biotechnology Information (NCBI). DbSNP, the NCBI SNP database is linked to several Entrez resources .It contains information such as sequence location, function, cross-species homology, SNP validation status and degree of heterozygosity. It is a catalogue of genome variation to investigate a wide variety of genetically based natural phenomenon to promote biological researches(1).

MTNR1B gene encodes 362 amino acid protein, melatonin receptor type 1B. It is 60% identical to the MTNR1A amino acid sequence. MTNR1B gene has been mapped to human chromosome 11q21-22. The gene arrangement consist of 2 exons and one intron with a length of about 9.0 kb(2). Melatonin receptor belongs to the superfamily of G-protein coupled receptors. Melatonin is synthesized and secreted by the pineal gland in a circadian rhythm and a variety of its physiological actions are mediated via MT1 and MT2 receptors(formerly Mel1a and Mel1b)(3). Melatonin receptor type 1B are known to be expressed in the retina, brain, Superchiasmatic Nucleus(SCN), lung, cardiac, aortic and coronary tissue, myometrium and granulosa cells, immune cells, duodenum, adipocytes , pancreas and skin(2, 4-6). Melatonin activate or inhibit signal transduction pathways through melatonin receptors.

It has negative effect on adenylyl cyclase and guanylyl cyclase which decreases the formation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) respectively (2, 7). It activates phospholipase C/diacylglycerol signaling pathway via Gq protein (8). Melatonin type 2 receptors are widely distributed in the body. Table 1 summarize its expression and action in various peripheral tissues.

Over the past few years, considerable experiments have been performed to explore the relationships between the MTNR1B polymorphisms and metabolic disorders. Genome wide association studies (GWAS) and meta-analysis have revealed that a particular SNP (rs10830963) significantly increases the risk of type 2 diabetes in individuals of European descent. It is localised within the single 11.5kb intron of MTNR1B but does not interfere with consensus transcription factor binding or cryptic alternative splice sites (9). MTNR1B gene has a potential role in the pathophysiology of several disease. Due to the diversity of the melatonin response such as immunomodulation, regulation of endocrine functions, anti-cancer activity, circadian activity, cardiovascular activity, skin pigmentation, hair growth and aging via MT2 receptors, we aimed to examine SNPs within the coding sequence of the MTNR1B gene. We would analyze a wide array of missense variants that may affect protein structure and stability that possibly have an important role in disease susceptibility.

Table 1

Tissue distribution of MT2 receptor and its physiological effects.

Tissues	**Physiological Roles**
Superchiasmatic nucleus(SCN)	Participate in phase-shifting activities(10)
Retina	Enhance visual sensitivity(11)
Vasculature	Mediates vasodilation(12)
Immune System	Modulate leukocyte rolling(13)
	Proliferation of lymphocytes, erythrocytes, and leukocytes(14)
Pancreas	Improve insulin action and β-cell function(15)
Ovary	Regulate progesterone and GnRH (gonadotrophin releasing hormone)(16)
Skin	Hair cycle regulation(17)
Gastrointestinal tract	Stimulates pancreatic amylase secretion(18)
Kidney	Protection from inflammation that can cause renal damage(19)

METHODS

MTNR1B gene-specific information was retrieved through Entrez gene at the National Center for Biotechnology Information (NCBI) web site. The SNP data (reference identifier i.e. rs# and protein accession number) of the MTNR1B gene was retrived from the NCBI dbSNP(http://www.ncbi.nlm.nih.gov/snp).UniProtKB/Swiss-Prot provided multiple reports of Melatonin receptor type 1B accessed through ExPASy(http://expasy.org/).

1. Assessment of the functional effect of coding nsSNPs

a. SIFT

1) SIFT (Sorting Intolerant from Tolerant) uses sequence homology to predict whether an amino acid change will affect protein function. On providing a query sequence, it uses multiple alignment information to anticipate functionally tolerated and deleterious amino acid substitutions for each residue of the protein sequence (20).SIFT is available at http://sift.bii.a-star.edu.sg/.

2) The MTNR1B FASTA amino acid sequence of the NCBI Reference Sequence: NP_005950.1 was used as the query sequence. The analysis was performed by allowing the algorithm to search for homologous sequences using three parameters, (1) UniProt-TrEMBL 2009 March as database to search, (2) The value for the Median conservation of sequences was kept 3.0 as recommended, (3) Identity percentage was kept 90 as allowance to remove sequences more than 90% identical to query sequence.

b. PolyPhen-2

3) PolyPhen-2 (**Poly**morphism **Phen**otyping v**2**) is a tool for predicting damaging effects of missense mutations. It estimates possible impact of an amino acid substitution on the structure and function of a human protein using specific empirical rules(21). PolyPhen-2 web interface can be accessed at http://genetics.bwh.harvard.edu/pph2/ and the input is amino acid sequence of a protein or the protein or SNP identifier together with sequence position and two amino acid variants characterizing polymorphism(22). It calculates multiple alignment derived PSIC profile scores for two amino acid variants. Depending on the PSIC score difference the functional impact of a particular amino acid is predicted. The retrieved and calculated data characterize the substitutions as *probably damaging* (i.e., it is with high confidence supposed to affect protein function or structure), *possibly damaging* (i.e., it is supposed to affect protein function or structure), *benign*(most likely lacking any phenotypic effect) and *unknown* (when in some rare cases, the lack of data do not allow PolyPhen to make a prediction)(23).

c. SNPs3D

4) SNPs3D (http://www.snps3d.org/) is a website which assigns molecular functional effects of non-synonymous SNPs based on structure and sequence analysis. The SNP analysis module was implemented and it anticipates data on the relationship between non-synonymous SNPs and protein function(24). Providing the gene name and amino acid substitution it analyze the targeting SNP by using the profile model. A positive SVM score indicates a variant classified as non-deleterious, and a negative score indicates a deleterious case.

d. SNAP

5) SNAP (**s**creening for **n**on-acceptable **p**olymorphisms) (https://rostlab.org/services/snap/) is an insilico method that can classify nsSNPs of a protein into non-neutral (effect on function) and neutral (no effect). It is a neural network-derived tool and requires protein sequence as input.

e. PANTHER

6) The PANTHER (protein annotation through evolutionary relationship) classification system (http://www.pantherdb.org/) is a comprehensive system that combines gene function, ontology, pathways and statistical analysis tools that facilitate biologists to explore large-scale, genome-wide data from sequencing, proteomics or gene expression experiments(25). The cSNP scoring tool estimate the possibility of certain non-synonymous (amino-acid changing) coding SNP to cause a functional impact on the protein. The MTNR1B FASTA amino acid sequence of the NCBI Reference Sequence: NP_005950.1 was used as the query sequence and

then entered the substitutions relative to this input sequence in the standard amino acid substitution format. It calculates the subPSEC (substitution position-specific evolutionary conservation) score based on an alignment of evolutionarily related proteins. When subPSEC = 0, the substitution is interpreted as functionally neutral, whereas more negative values of subPSEC predict more deleterious substitutions (26).

2. Prediction of protein structure stability

7) I-Mutant

8) I-Mutant version 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html) is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single point mutations. It is trained and tested on a data set derived from ProTherm, which is most suited comprehensive available database of thermodynamic experimental data of free energy changes of protein stability upon mutation under different conditions.(27).It was operated by providing the MTNR1B protein sequence altogether with amino acid residue and its position in the peptide chain. The pH was kept 7 and temperature as 25°C as the experimental conditions. It act as a classifier, predicting sign of the free energy stability change upon mutation as well as regression estimator, predicting the value of free energy stability change ($\Delta\Delta G$) upon mutation(27).

3. Model Generation of protein

9) MUSTER

10) MUSTER stands for Multi-Source ThreadER is a template based server. It is an extension to the simple threading algorithm named, profile-profile alignments (PPA). The accuracy of PPA is further improved by incorporating (1) sequence-derived profiles; (2) secondary structures; (3) structured-derived profiles; (4) solvent accessibility; (5) torsion angles (psi and phi angles); (6) hydrophobic scoring matrix. It uses Needleman-Wunsch dynamic programming algorithm to identify the best match between the query and the template sequence. Subsequent to selection of suitable templates the models are built using the inbuilt MODELLER algorithm. A Z-score is defined for predicting the quality of threading alignments if it is greater than 7.5 it is considered to be good otherwise classified as bad(28).

4. Protein Model Quality Estimation

QMEAN

Model quality estimation is an essential component of protein structure prediction, since ultimately the accuracy of a model determines its usefulness for specific applications. The composite scoring function QMEAN, derives a quality estimate on the basis of the geometrical analysis of single models. The web server performs a ranking of the input models and highlights potentially problematic regions for each model(29). QMEAN stands for Qualitative Model Energy Analysis and the server is available at http://swissmodel.expasy.org/qmean.

5. Identification of cis regulatory elements

The FASTA sequence of Homo sapiens melatonin receptor 1B (MTNR1B) gene was used as a query sequence.

a. PROSCAN

PROSCAN Version 1.7(http://www-bimas.cit.nih.gov/molbio/proscan/) is an experimental tool to find putative eukaryotic Pol II promoter sequences in primary sequence data. It predicts regions of DNA that contain a significant number and type of transcriptional elements (TEs) that are usually associated with Pol II promoter sequences(30). It is developed by Dr. Dan Prestridge and maintained at the Advanced Biosciences Computing Center, University of Minnesota.

b. Promoter 2.0 Prediction Server

Promoter2.0 (http://www.cbs.dtu.dk/services/Promoter/) predicts eukaryotic Pol II promoters that provide start sites for the transcription of protein coding genes. It has been developed as an evolution of simulated transcription factors that interact with sequences in promoter regions. It combine methods similar to neural networks and genetic algorithms to recognize a discrete sub-patterns , with variable separation as one pattern i.e promoter sequence(31). It is developed by Steen Knudsen and maintained at The Center for Biological Sequence Analysis at the Technical University of Denmark.

c. TSSG

TSSG (www.softberry.com) is an online tool that recognizes human Pol II promoter region and transcription start sites. It is the most accurate promoter prediction program to identify statistically significant regulatory motifs in genomic sequences(32).

RESULTS

1. Assessment of the functional effect of coding nsSNPs

SNPs are expected to facilitate large-scale association genetics studies. We opted to avail the dbSNP because the allelic frequency of most of nsSNPs of MTNR1B has been recorded there and is most extensive current SNP database(33).We selected missense SNPs for our investigation.

Deleterious nsSNPs by SIFT program

Protein sequence with mutational position and amino acid residue variants associated to 77 missense nsSNPs were submitted as input to the SIFT server, and the results are shown in Table 2, along with the corresponding heterozygosity and validation status description for each SNP, when available from dbSNP.

Table 2 : List of nsSNPs that affect protein as analyzed by SIFT.

S.No.	SNP	Amino acid change	Score
1	rs8192552	G24E	0.02
2	rs369043907	R29W	0.02
3	rs532760946	S31C	0.04
4	rs541167036	V53L	0.04
5	rs148309052	V56M	0.02
6	rs61739452	V65A	0.00
7	rs144090735	A74T	0.03
8	rs377626851	G109E	0.04
9	rs190261791	S123R	0.00

10	rs145440211	V124I	0.00
11	rs370131617	G126S	0.01
12	rs367759317	A135T	0.00
13	rs188196440	N137T	0.00
14	rs192957400	R138L	0.00
15	rs201423081	I151T	0.01
16	rs536883305	V169M	0.03
17	rs555139096	V170M	0.03
18	rs372043726	E182D	0.02
19	rs150221232	Y188C	0.01
20	rs375405491	F192S	0.02
21	rs138675484	T201M	0.00
22	rs190464820	V206G	0.01
23	rs188825896	H208R	0.01
24	rs199694998	V215F	0.01
25	rs574453327	R222H	0.05
26	rs192325525	I223T	0.00
27	rs8192553	R231H	0.00
28	rs201744782	A234D	0.00
29	rs150751119	R248Q	0.01
30	rs200739086	A265V	0.04
31	rs147569774	V274M	0.02
32	rs61741965	N304S	0.00
33	rs199529640	R316L	0.00
34	rs555175869	I322T	0.00
35	rs532362784	L326R	0.00

36	rs145300624	R330Q	0.02
37	rs575839980	G339V	0.01

Protein sequence with mutational position and amino acid residue variants associated to nsSNPs were submitted as input to the SIFT server, and the results are shown in Table 2. According to the classification proposed by Ng and Henikoff (34) and the lower the tolerance index, the higher the functional impact a particular amino acid residue substitution is likely to have and vice versa. Among the total 77 missense nsSNPs analyzed, 37 nsSNPs were identified to be deleterious with a tolerance index score less than 0.05. The remaining nsSNPs were found to be tolerated with tolerance index scores greater than 0.05.

11) Damaged nsSNPs by PolyPhen server

All the 77 nsSNPs submitted to SIFT were also submitted to the PolyPhen server. A PSIC score difference of 1.5 and above is considered to be damaging. Eleven nsSNPs were considered to be possibly damaging and 15 nsSNPs were probably damaging.

Deleterious nsSNPs by SNPs3D, SNAP and PANTHER

Twenty amino acid variants were predicted deleterious on the basis of negative SVM score.

Forty two amino acids were predicted non-neutral by the SNAP.

Thirty two amino acid substitutions were predicted to cause a deleterious effect on protein function as estimated by $P_{deleterious.}$

On analyzing the coding region of gene we found two nonsynonymous SNPs rs377626851 (G109E) and rs574453327 (R222H) that were expected to affect protein. These were the two most common variations investigated by different SNP analyzing tools and supposed to alter protein structure and function.

2. Structural analysis of mutant structures

The two nonsynonymous SNPs rs377626851 (G109E) and rs574453327 (R222H) were mapped to the native structure by I mutant 2.0 server. Then, energy minimizations were performed by GROMACS for the native structure and the mutant modeled structures.

Table 3: Protein structural stability based on standard free energy change.

Mutation	Position	WT	New	pH	Temperature	Stability	DDG(Kcal/mol)
109: G-E	109	G	E	7	25°C	Decrease	-1.26
222: R-H	222	R	H	7	25°C	Decrease	-2.21

Where, WT: Amino acid in Wild-Type Protein
NEW: New Amino acid after Mutation
DDG: DG (New Protein)-DG (Wild Type) in Kcal/mol
DDG<0: Decrease Stability
DDG>0: Increase Stability
T: Temperature in Celsius degrees
pH:- log[H+]

I-Mutant is a neural network based routine tool used in the analysis of protein stability alterations by considering the single-site mutation. I-Mutant also provides the scores for free energy alterations, calculated with the FOLD-X energy based web server. By assimilating the FOLD-X estimations with those of I-Mutant, the 93% precision can achieved. The two mutations (109, G → E and 222, R → H) of MTNR1B gene have been selected on the basis of prediction scores of different SNP analyzers. These variants were given to I-Mutant web server to predict the DDG stability and reliability index (RI) upon mutation (Table 3).

3. Modelling of mutant structure

If Z-score is greater than 7.5, the corresponding template is considered good otherwise designated as bad. It was found that for all the alignments of MTNR1B Z score was > 7.5, which indicates that all the templates can be considered as good type(Table 4).

Table 4: Z score value of different templates analyzed by MUSTER.

Rank	Template	Align_length	Coverage	Zscore	Seq_id	Type
1	4n6hA2	296	0.817	19.500	0.223	Good
2	4iaqA1	278	0.767	18.275	0.237	Good
3	3d4sA1	270	0.745	17.195	0.204	Good
4	4mbsA1	284	0.784	17.190	0.201	Good
5	3rzeA1	264	0.729	17.153	0.265	Good
6	3sn6R2	281	0.776	17.110	0.196	Good
7	4grvA1	281	0.776	17.033	0.231	Good
8	3pblA1	267	0.737	17.027	0.266	Good
9	3uonA1	270	0.745	17.004	0.241	Good
10	2vt4B	270	0.745	16.945	0.256	Good

4. Model quality assessment of wild and mutant protein structures

QMEAN6 score was used to evaluate the generated models. It is a reliability score for the whole model which can be used in order to compare and rank alternative models of the same target. Information about mapping the deleterious nsSNPs into protein structure was obtained from dbSNP. Two nsSNPs were found to be the highest deleterious nature among all the nsSNPs. Hence we selected these nsSNPs for structural analysis. The mutational position and amino acid variant associated with this nsSNP is G → E at the residue position 109 and at the residue 222 R →H were estimated.

Table 5 : QMEAN6 Scores of wild and mutant protein.

Model	C_beta interaction energy	All-atom pairwise energy	Solvation energy	Torsion angle energy	Secondary structure agreement	Solvent accessibility agreement	QMEAN6 score
Wild type	179.68	3469.74	30.69	-12.23	87.8%	69.6%	0.525
Mutant G109E	175.41	3614.50	32.66	12.49	88.1%	68.5%	0.480
Mutant R222H	177.29	3677.97	37.94	0.45	88.4%	68.0%	0.493

Q-MEAN6 score is the composite score of all the terms in linear with estimated model reliability between 0-1. C_beta interaction and all-atom pairwise energy refer to two distance-dependent potentials. Solvation potential investigates the burial status of the residues. Torsion angle potential indicates over three consecutive amino acids in local geometry. Secondary structure and solvent accessibility agreement are percentage of agreement between predicted and measured features from sequence to model(35).

5. Analysis of cis regulatory elements

Proscan: Version 1.7

Promoter region predicted on reverse strand in 6749 to 6499 and 4654 to 4404.
Promoter Score: 63.32 and 54.72 (Promoter Cut off = 53.000000)
TATA found at 6515 and 4428

Softberry TSSG

3 promoters were predicted
Pos.: 14785 LDF- 14.78 TATA box predicted at 14758
Pos.: 4447 LDF- 4.34 TATA box predicted at 4420
Pos.: 5599 LDF- 4.18

Promoter 2.0 Prediction Server

Position	Score	Likelihood
800	0.567	Marginal prediction
2400	0.679	Marginal prediction
4900	0.754	Marginal prediction
6400	0.577	Marginal prediction
8400	0.658	Marginal prediction
9500	0.662	Marginal prediction
10900	0.647	Marginal prediction
12900	1.099	Highly likely prediction
14000	1.225	Highly likely prediction
15000	0.702	Marginal prediction
16600	1.054	Highly likely prediction
19200	0.675	Marginal prediction
19800	0.607	Marginal prediction

DISCUSSION

Melatonin is a neurohormone associated with the biological clock. It is mainly secreted by the pineal gland of mammals. It plays important role in modulation of circadian rhythms and of sleep wake cycle in humans(36).Other effects of melatonin include anti-inflammatory, vascular, antioxidant, pain modulatory, retinal, neuroprotective and stroke protective properties(37).The physiologic effects of melatonin result mainly from the activation of the high affinity G-protein-coupled receptors MT1 and MT2. MT2 receptors are expressed both centrally (suprachiasmatic nucleus, pars tuberalis, cortex, etc.) and peripherally (adipocytes, kidney, retina, blood vessels, etc.). MT2 receptors appear to play a major role in the resynchronizing activity of melatonin(38). It has been observed that risk allele for type 2 diabetes is located at rs10830963 in the unique MTNR1B inronic region(39). The meta-analysis for type 2 diabetes susceptibility related to the MTNR1B polymorphism provided evidence of rs10830963and rs1387153 locus increases the level of Fasting Plasma Glucose(40). It can be assumed that MTNR1B variants might impair melatonin receptor 1B function contribute to metabolic disorders implying that presence of DNA sequence variation causes the modification of protein. Thus, the insilico evaluation of newly identified rare but potentially damaging variants, can contribute to a more complete picture of the genetic architecture of various disease risk.

Computational approach provide an opportunity to understand association between genotype and phenotype based on the genomic sequence and proteome information. Therefore, an effort was made to identify SNPs that can modify the structure, function and expression of the MTNR1B gene. There were 77 missense nsSNPs submitted to the SIFT as well as to the PolyPhen server, out of these 77 SNPs, 37 nsSNPs were identified to be deleterious by SIFT and 11SNPs were possibly damaging as predicted by the PolyPhen. Apart from these 22 SNPs were predicted deleterious by SNPs3D; 42 predicted non-neutral by the SNAP and 32 were predicted deleterious by PANTHER. Associations between polymorphisms (rs10830963and rs1387153) and Fasting Plasma Glucose level leading to diabetic complications have been reported (40). The MTNR1B-rs4753426 SNP is associated with the pathogenesis of Gestational Diabetes Mellitus, and rs4753426 is the predisposing locus of Gestational Diabetes Mellitus(41). The two most common variants rs377626851 (G109E) and rs574453327 (R222H) have not been reported to be associated with any disease. Therefore the validation of these nsSNPs in any disease is required to complement this finding. The combination of the analysis of human genetic variations of the MTNR1B gene, together with the computational method to predict their possible functional impact, can facilitate the analysis of MTNR1B gene variant and their effects on protein functional characteristics. In conclusion, the presence of a non-complete concordance among clinical evidences, experimental investigations, and in silico analyses strongly suggested that improvements are necessary in all these fields, in order to enhance our understanding of MTNR1B nsSNPs and their role in the pathogenesis of the MTNR1B related disease.

The mutant structures were generated by the MUSTER. The structural analysis of mutant structures 109: G-E and 222: R-H by I mutant 2.0 server predicted decrease in protein stability. The QMEAN6 score of wild type protein was 0.525 while that of mutant G109E and mutant R222H was 0.480 and 0.493, respectively. This composite scoring function provide reference knowledge for the protein structure prediction. The mutated structures have less QMEAN6 score than native type. We compared QMEAN6 score for native structure and mutated modelled structure for MTNR1B gene variant. Two variants G109E and R222H were found to have decreased the stability of protein structure. This may be due to mutant residue which is bigger than the wild type and cannot fit within the available space. Due to carrying the less rigid behavior, the mutant residue can possibly disturb the original core structure of native protein. Two identified variants i.e.G109E and R222H could deregulate the adenylyl cyclase/cAMP pathway, the cGMP pathway or the phospholipase C/IP3 pathway, thus may participate in the pathogenesis of diabetes. Hence, G109E and R222H variants constitute a unique resource of genetic markers that may considerably increase the power of MTNR1B gene mutation-screening in disease epidemiological studies.

CONCLUSIONS

Functional and structural impact of SNPs in the MTNR1B gene was found out using computational prediction tools. The assessment of the functional effect of coding nsSNPS by different SNP analyzers i.e.SIFT,PolyPhen,SNPs3D,SNAP

and PANTHER, found two nonsynonymous SNPs rs377626851 (G109E) and rs574453327 (R222H) that altered protein structure and function. Structural analysis results showed that these amino acid residue substitutions had the greatest impact on the stability of the Melatonin type 2 receptor. Based on our results, we conclude that these SNPs should be considered important candidates in causing diseases related to MTNR1B gene malfunction and disruption of circadian rhythm.

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