PARMACOGENOMICS BASED DRUG DESIGN FOR ASTHMA

D. Rachel Evangelene Tulip[1], K. Krishnadevi[2], K.K. Surya[2], K.Sekar[3]

[1]Department of Civil Engineerimg, Bharath University.Chennai-73

[2]Anna University, Guindy, Chennai-25

[3]Indian Institute of Science, Bangalore.

Abstract

Each sub-population will respond to a particular drug in a different way based upon the genetic profile. Pharmacogenomics deals with the study of how individual's genetic make-up plays an important role in body's response to drugs by correlating gene expression with the drug efficacy or toxicity. Asthma is a very common chronic disease involving the respiratory system in which the airways constrict, become inflamed, and are lined with excessive amounts of mucus, often in response to one or more triggers. The β_2-adrenergic receptor is the most common adrenergic receptor in the lung, and associations between ADRB2 polymorphisms and intermediate phenotypes of asthma have been reported. The total number of SNPs in the ADRB2 gene was analyzed. According to the SNP profile, the ADRB2 protein was modeled with the help of Modeller 9V2 software. Docking studies were performed with the available candidate drugs with the help of Autodock. The application of pharmacogenomics approach to Asthma will be essential for understanding the preventive mechanisms and could lead to individualized drug therapies in future.

1. Introduction

Asthma is a very common chronic disease involving the respiratory system in which the airways constrict, become inflamed, and are lined with excessive amounts of mucus, often in response to one or more triggers. These episodes may be triggered by such things as exposure to an environmental stimulant such as an allergen, environmental tobacco smoke, cold or warm air, perfume, pet dander, moist air, exercise or exertion, or emotional stress. In children, the most common triggers are viral illnesses such as those that cause the common cold. This airway

narrowing causes symptoms such as wheezing, shortness of breath, chest tightness, and coughing. The airway constriction responds to bronchodilators. Between episodes, most patients feel well but can have mild symptoms and they may remain short of breath after exercise for longer periods of time than the unaffected individual. The symptoms of asthma, which can range from mild to life threatening, can usually, be controlled with a combination of drugs and environmental changes.

In some individuals asthma is characterized by chronic respiratory impairment. In others it is an intermittent illness marked by episodic symptoms that may result from a number of triggering events, including upper respiratory infection, stress, airborne allergens, air pollutants (such as smoke or traffic fumes), or exercise. Some or all of the following symptoms may be present in those with asthma: dyspnea, wheezing, stridor, coughing, tightness and itching of the chest or an inability for physical exertion. Some asthmatics who have severe shortness of breath and tightening of the lungs never wheeze or have stridor and their symptoms may be confused with a COPD-type disease.

An acute exacerbation of asthma is commonly referred to as an asthma attack. The clinical hallmarks of an attack are shortness of breath (dyspnea) and either wheezing or stridor. Although the former is "often regarded as the sine qua non of asthma", some patients present primarily with coughing, and in the late stages of an attack, air motion may be so impaired that no wheezing may be heard. When present the cough may sometimes produce clear sputum. The onset may be sudden, with a sense of constriction in the chest, breathing becomes difficult, and wheezing occurs (primarily upon expiration, but can be in both respiratory phases).

2. Materials and Methods

2.1 Tools

SNP analysis of the ADRB2 genes was analyzed using various tools such as GeneCards, Polyphen, SNPper, SIFT and even few databases were used such as dbSNP, MUTdb.

2.1.1 Polyphen

Polyphen was used to predict the possible impact of amino acid substitutions on the protein. The program is based on sequence comparison with homologous proteins: profile scores, position specific independent counts (PSIC) are generated for the allelic variants and

represent the logarithmic ratio of the likelihood of a given amino-acid occurring at any site (background frequency). PSIC score differences above 2 indicate a damaging effect: scores below 0.5 indicate that the variant is benign.

Polyphen (Polymorphism phenotyping), one of these algorithms, was used to study if the new variants might have a functional role. Polyphen is based on all the previous characteristics, but also values the location of the substitution within identified functional domains and known structural features available in the annotated database (SwissProt). Testing Polyphen using known variants confirmed its ability of discriminating between benign and deleterious variants and its high concordance with other algorithms.

2.1.2 Polydoms

Since the completion of the sequence for several genomes, there has been an increased focus on functional polymorphism. Databases containing huge numbers of SNPs are now available for the research community. Besides outlining genome architecture with gene location and description of polymorphisms, one of the major challenges is to infer the functional implications of these variations. It has been estimated that 20% of common human nsSNPs damage the protein. A large database for identification of human nsSNPs with potential impact on disease Polydoms.

2.1.3 SNPper

SNPper is a web-based application, developed in Common Lisp using the LispWeb development platform. It relies on a set of local databases containing information about genes and snps, and on remote access to the Draft Human Genome3 site to download up-to-date genomic sequences. The database currently contains information about 9,550 genes and over 1.2 million snps. Although the number of known snps is higher (currently close to 2 million) snpper only uses those whose exact position on a chromosome is known. snpper can be accessed at http://bio.chip.org/biotools/; its use is free for non-commercial research purposes.

2.1.4 GeneCards

GeneCards is a compendium of human genes and their encoded proteins, with major focus on functional genomics and medical aspects including involvement in diseases.

GeneCards offers concise information about the structure and function of human genes. It extracts and integrates a carefully selected subset of the gene information, obtained from major data sources, public and proprietary, successfully overcoming barriers of data format heterogeneity. GeneCards is unique in its combination of user-friendly interface, as wellas the organization and display of just the right mix of links and detailed information. Data are divided into "fields" such as "Aliases," "Location," "Ontologies," "Expression" and "SNPs."Some of GeneCards Specific advantages Identifies splice variants and SNPs for each gene.GeneCards' highly advanced search tool usually finds more genes associated with a specific disease and/or protein characteristic than other databases would.

2.1.5 MutDB

MutDB(http://mutdb.org/), to aid in determining which SNPs are likely to alter the function of their associated protein product. MutDB currently contains protein structure annotations and comparative genomic annotations for 8000 disease-associated mutations and SNPs found in the UC Santa Cruz (UCSC) Annotated Genome and the human RefSeq gene set. Normally multiple sequence alignments are used together in combination with protein structure to highlight functional mutations and functionally important protein regions.These data sets usually fall into two classes:Hand-curated, loci-specific databases that contain phenotypic annotations of rare variants and SNPs and Large unannotated data sets associated with many genes.

2.1.6 dbSNP

dbSNP Schema is very complex with well over 100 tables and many relationships among tables. One single ER (Entity Relationship) diagram with all dbSNP tables will be too huge to present useful information. Instead, separate tables can be made according to subject areas:Batch submission,Submitted SNP,population frequency and individual genotype. Frequency calculation by submitted SNP and population. SNP Mapping and Annotation.

2.1.7 Drugbank

The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with

comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains nearly 4800 drug entries including FDA-approved small molecule drugs, 123 FDA-approved biotech (protein/peptide) drugs, 71 nutraceuticals and >3,243 experimental drugs. Additionally, more than 2,500 non-redundant protein (i.e. drug target) sequences are linked to these FDA approved drug entries. Each DrugCard entry contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data.

2.1.8 PharmGKB

PharmGKB is a publicly available Internet research tool developed by Stanford University with funding from the National Institutes of Health (NIH) and is part of the NIH Pharmacogenetics Research Network (PGRN), a nationwide collaborative research consortium. Its aim is to aid researchers in understanding how genetic variation among individuals contributes to differences in reactions to drugs.The PharmGKB database is a central repository for genetic, genomic, molecular and cellular phenotype data and clinical information about people who have participated in pharmacogenomics research studies. The data includes, but is not limited to, clinical and basic pharmacokinetic and pharmacogenomic research in the cardiovascular, pulmonary, cancer, pathways, metabolic and transporter domains. The contributers tab contains the links to all of the projects submitting data to the PharmGKB.

2.1.9 MODELLER

MODELLER is used for homology or comparative modeling of protein three-dimensional structures. The user provides an alignment of a sequence to be modeled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints, and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. MODELLER is available for download for most Unix/Linux systems, Windows, and Mac.

2.1.10 Autodock

Autodock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure.Autodock actually consists of two main programs: Autodock performs the docking of the ligand to a set of grids describing the target protein; AutoGrid pre-calculates these grids.In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders.We have also developed a graphical user interface called AutoDockTools, or ADT for short, which amongst other things helps to set up which bonds will treated as rotatable in the ligand and to analyze dockings.

AutoDock has applications in:

- X-ray crystallography
- structure-based drug design
- lead optimization
- virtual screening
- combinatorial library design
- protein-protein docking
- Chemical mechanism studies

2.1.11 SIFT

SIFT is a sequence homology-based tool that Sorts Intolerant from Tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. SIFT is based on the premise that protein evolution is correlated with protein function. Positions important for function should be conserved in an alignment of the protein family, whereas unimportant positions should appear diverse in an alignment.

SIFT takes a query sequence and uses multiple alignment information to predict tolerated and deleterious substitutions for every position of the query sequence. SIFT is a multistep procedure that searches for similar sequences, chooses closely related sequences that may share similar function to the query sequence, obtains the alignment of these chosen sequences, and

calculates normalized probabilities for all possible substitutions from the alignment. Positions with normalized probabilities less than 0.05 are predicted to be deleterious; those greater than or equal to 0.05 are predicted to be tolerated.

2.1.12 Swiss-model

SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program DeepView (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modelling accessible to all biochemists and molecular biologists World Wide.

2.1.13 BLAST

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

2.1.14 Genbank

GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research, 2008 Jan;36(Database issue):D25-30). There are approximately 85,759,586,764 bases in 82,853,685 sequence records in the traditional GenBank divisions and 108,635,736,141 bases in 27,439,206 sequence records in the WGS division as of February 2008.

The complete release notes for the current version of GenBank are available on the NCBI ftp site. A new release is made every two months. GenBank is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI. These three organizations exchange data on a daily basis.

2.1.15 PDB (Protein Data Bank)

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the wwPDB, the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

3. Results and Discussions

3.1 SNP Tools Results

Beta 2 Adrenergic receptor has a pivotal role in the pathogenesis of Asthma. When the drug binds with this gene (ADRB2),it will reduce the bronchial inflammation, Asthma patients show good response to these drugs but the response is varying between patients due to the presence of SNPs. Now the ADRB2 gene is analyzed by various SNP tools to find the presence of SNPs in the gene. The result has been displayed.

3.1.1 SIFT output

Table 1: predicting the result of sift

Protein ID	Substitution	dbSNP ID	Prediction	Score	No. of Seqs. at Position
4501969	N15S	rs33973603	DAMAGING.	0.04	16
4501969	R16G	rs1042713	TOLERATED	1.00	16
4501969	Q27E	rs1042714	TOLERATED	1.00	16
4501969	A57P		DAMAGING	0.00	16
4501969	F104Y		DAMAGING	0.00	16
4501969	T164I	rs1800888	TOLERATED	0.08	16
4501969	S220C	rs3729943	DAMAGING	0.01	16
4501969	F240L	rs41320345	DAMAGING	0.00	16

4501969	Q247H	rs41358746	DAMAGING	0.00	16
4501969	G257R	rs56100672	DAMAGING	0.00	16

3.1.2 Polyphen

Table 2: predicting the result of polyphen

Swiss prot	Pos	Am1	Am2		
P07550	15	N	S	Benign	rs33973603
	16	G	R		rs1042713
	27	E	Q		rs1042714
	164	T	I	possibly damaging	rs1800888
	220	S	C	Benign	rs3729943
	240	F	L	Benign	rs41320345
	247	Q	H	Benign	rs41358746
	257	G	R	Benign	rs56100672

3.1.3 SNPer

Table 3: predicting the result of snper

SNP id	SNP position	Alleles	AA change	AA position	Validated
rs33973603	chr5:148186631	A/G	N/S	15	Y
rs1042713	chr5:148186633	A/G	G/R	16	Y

rs33957121	chr5:148186653	C/T	H/H	22	Y
rs35892629	chr5:148186665	A/G	Q/Q	26	N
rs1042714	chr5:148186666	C/G	E/Q	27	Y
rs1042717	chr5:148186839	A/G	L/L	84	Y
rs1800888	chr5:148187078	C/T	T/I	164	Y
rs1042718	chr5:148187110	A/C	R/R	175	Y
rs3729943	chr5:148187246	C/G	S/C	220	Y
rs35933628	chr5:148187427	C/T	G/G	280	N
rs1042719	chr5:148187640	C/G	G/G	351	Y
rs3729944	chr5:148187757	C/T	H/H	390	N
rs3730182	chr5:148187766	C/T	T/T	393	Y
rs1042720	chr5:148187826	A/G/T	L/?	413	Y

3.1.4 Polydom

Table 4: predicting the result of polydom

Query term	mRNA acc. no.	Prot. acc. no.	Polyphen prediction	SIFT prediction
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated

NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Tolerated
NP_000015	NM_000024	NP_000015	Possibly damaging	Tolerated
NP_000015	NM_000024	NP_000015	Possibly damaging	Tolerated
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Deleterious
NP_000015	NM_000024	NP_000015	Benign	Tolerated

3.1.5 Mutdb

Table 5: predicting the result of mutdb

Source ID	AA Position	WT->MT	Sequence	SIFT Score
DBSNP:rs1042713	15	G R	NCBI	Not scored
DBSNP:rs1042714	26	E Q	NCBI	Not scored
SWISS:VAR_003454	34	V M	Swiss-Prot	0.07
SWISS:VAR_009125	159	I F	Swiss-Prot	0.06
SWISS:VAR_009124	159	I L	Swiss-Prot	0.3
DBSNP:rs1800888	163	T I	NCBI	1.00
DBSNP:rs1042718	175	R G	NCBI	Not scored
DBSNP:rs3729943	219	S C	NCBI	0.24
SWISS:VAR_025101	220	S C	Swiss-Prot	0.16
SWISS:VAR_009394	375	K R	Swiss-Prot	0.25

3.1.6 Genecard

Table 6: predicting the result of genecard

SNP ID	Valid	Chr 10 pos	Sequence	AA Chg
rs1801704	A,C,F,H	148186568(+)	GCGCT**T**/**C**ACCTG	--
rs12654778	A,C,F,H	148185934(+)	AGTCT**G**/**A**AGCAT	--
rs2400707	A,C,F,H	148185245(+)	atggc**G**/**A**caacc	--
rs1042713	A,C,F,H	148186633(+)	CCAAT**G**/**A**GAAGC	R/G
rs1042714	A,C,F,H	148186666(+)	CGCAG**C**/**G**AAAGG	E/Q
rs2053044	A,C,F,H	148185565(+)	AAATC**G**/**A**GCAGC	--
rs1042711	A,C,F,H	148186541(+)	CCGCC**T**/**C**GCTGA	--
rs3729943	C,F,H	148187246(+)	CTACT**C**/**G**CAGGG	S/C
rs11959427	A,C,F	148186221(+)	CAGCC**T**/**C**CAGGA	--
rs11168070	C,F,H	148186120(+)	GTAAG**C**/**G**ACACC	--

3.1.7 dbSNP

Table 7: predicting the result of dbSNP

DbSNP cluster id	Function	DbSNP allele	Protein residue	Codon pos
	start codon			1

rs33973603	missense	G	Ser [S]	2
	contig reference	A	Asn [N]	2
rs1042713	missense	A	Arg [R]	1
	contig reference	G	Gly [G]	1
rs33957121	synonymous	T	His [H]	3
	contig reference	C	His [H]	3
rs35892629	synonymous	A	Gln [Q]	3
	contig reference	G	Gln [Q]	3
rs1042714	missense	C	Gln [Q]	1
	contig reference	G	Glu [E]	1
rs35336948	frame shift	C	Pro [P]	3
	contig reference	-		3
rs1042717	synonymous	A	Leu [L]	3
	contig reference	G	Leu [L]	3
rs35680672	frame shift	A	Tyr [Y]	2
	contig reference	-		2
rs1800888	missense	T	Ile [I]	2
	contig reference	C	Thr [T]	2
rs1042718	synonymous	A	Arg [R]	1
	contig reference	C	Arg [R]	1

rs3729943	missense	G	Cys [C]	2
	contig reference	C	Ser [S]	2
rs41320345	missense	C	Leu [L]	1
	contig reference	T	Phe [F]	1
rs41358746	missense	T	His [H]	3
	contig reference	G	Gln [Q]	3
rs56100672	missense	A	Arg [R]	1
	contig reference	G	Gly [G]	1
rs35933628	synonymous	T	Gly [G]	3
	contig reference	C	Gly [G]	3
rs1042719	synonymous	C	Gly [G]	3
	contig reference	G	Gly [G]	3
rs41354346	synonymous	C	Tyr [Y]	3
	contig reference	T	Tyr [Y]	3
rs3729944	synonymous	C	His [H]	3
	contig reference	T	His [H]	3
rs3730182	synonymous	C	Thr [T]	3
	contig reference	T	Thr [T]	3
rs1042720	synonymous	A	Leu [L]	3
	synonymous	T	Leu [L]	3

	contig reference	G	Leu [L]	3

The result obtained from the SNP tools, showed the presence of SNP in the gene ADRB2 at articular position also their prediction whether tolerated or deleterious .The information obtained from these tools helped to modeled the ADRB2 3D structure with SNP using modeller 9v6.

3.2 Normal Protein Sequence (ADRB2)

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

3.3 Altered Snp Sequences

Position: 15

Change: N/S

MGQPGNGSAFLLAP**S**RSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAIA
KFERLQTVTNY

FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK

YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV

PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEH
KALKTLGIIMG

TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY

GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 16

Change: R/G

MGQPGNGSAFLLAPN**G**SHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 27

Change: Q/E

MGQPGNGSAFLLAPNRSHAPDHDVTQ**E**RDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 57

Change: A/P

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVIT**P**I
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE

HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 104

Change: F/Y

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGN**Y**WCEFWTSIDVLCVTASIETLCVIA
VDRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 164

Change: T/I

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGL**I**SFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV

PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 220

Change: S/C

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVY**C**RVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 240

Change: F/L

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY

AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGR**L**HVQNLSQVEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 247

Change: Q/H

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK
YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLS**H**VEQDGRTGHGLRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

Position: 257

Change: G/R

MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAI
AKFERLQTVTNY

FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAV
DRYFAITSPFK

YQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAY
AIASSIVSFYV
PLVIMVFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGH**R**LRRSSKFCLKE
HKALKTLGIIMG
TFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCL
RRSSLKAY
GNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCST
NDSLL

3.4 Structures of Normal and SNP Changed Proteins

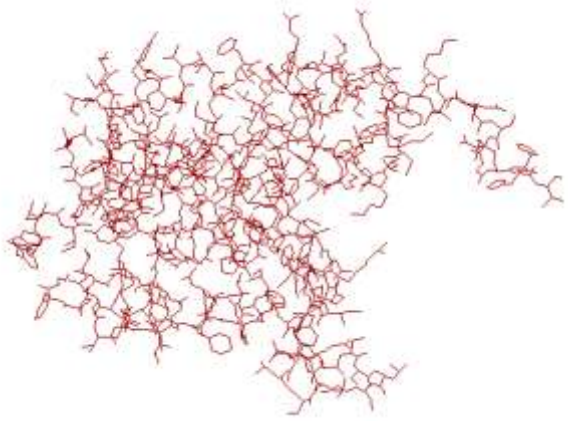


Figure 1: Structure of normal protein

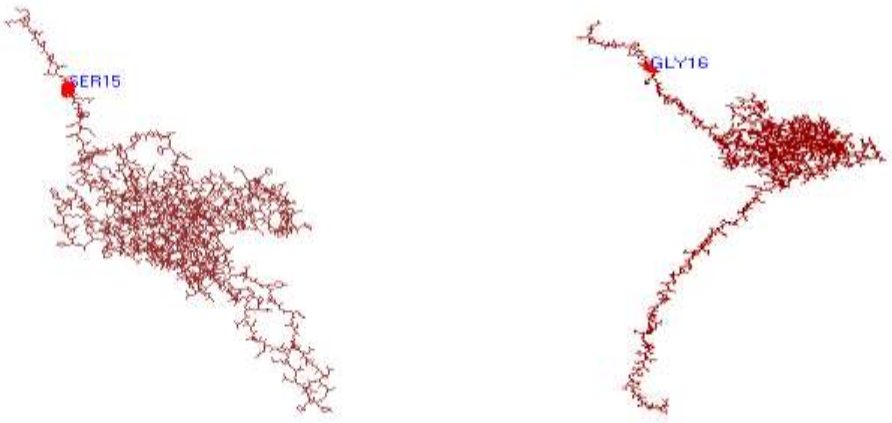
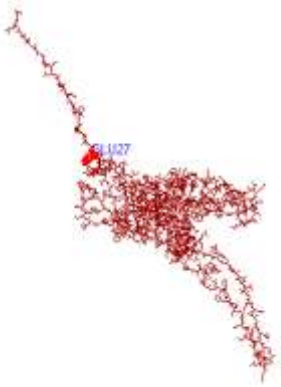


Figure 2: Snp in 15th position

Figure 3: Snp in 16th position



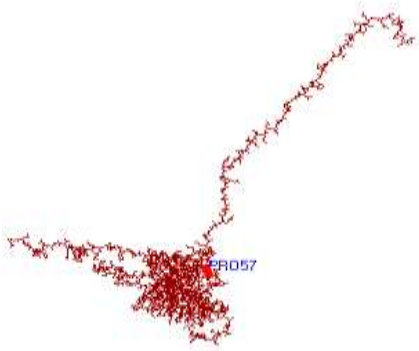
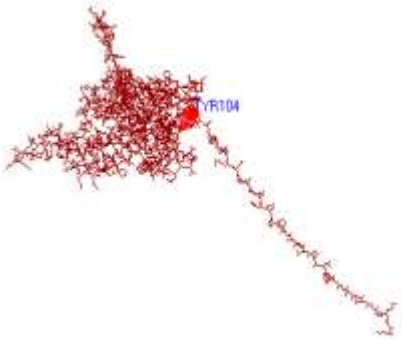


Figure 4: SNP in 27th position

Figure 5: SNP in 57th position



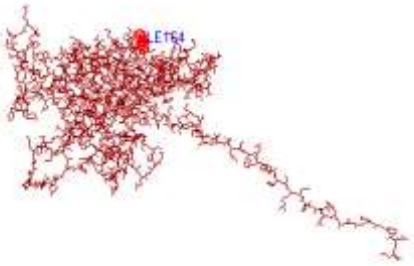


Figure 6: SNP in 104th position

Figure 7: SNP in 164th position

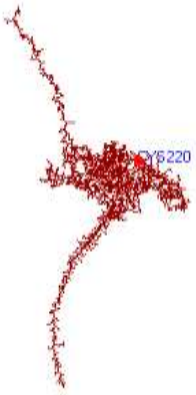


Figure 8: SNP in 220th position

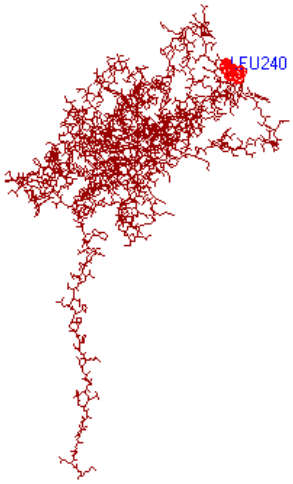


Figure 9:SNP in 240th position

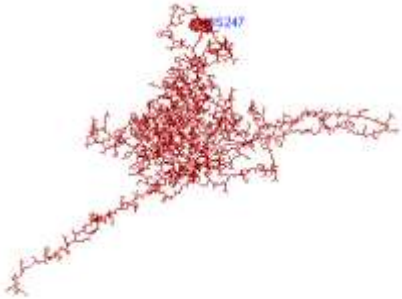


Figure 10: Snp in 247th position

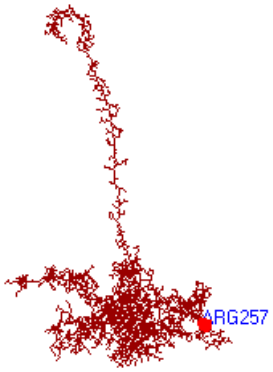


Figure 11: Snp in 257th position

3.5 Docking Results

Table 8: Estimated Free Energy of Binding for normal and SNP altered proteins

	Normal	15	16	27	57	104	164	220	240	247	257
Isoproterenol	-3.08 kcal/mol	-1.86 kcal/mol	-3.51 kcal/mol	-4.88 kcal/mol	-3.22 kcal/mol	-1.87 kcal/mol	-5.33 kcal/mol	-3.68 kcal/mol	-6.13 kcal/mol	-4.08 kcal/mol	-2.53 kcal/mol
Salbutemol	-2.94 kcal/mol	-2.26 kcal/mol	-2.86 kcal/mol	-2.85 kcal/mol	-3.18 kcal/mol	-1.76 kcal/mol	-5.06 kcal/mol	-3.97 kcal/mol	-5.86 kcal/mol	-4.75 kcal/mol	-3.16 kcal/mol

This table shows the comparative analysis of docking of two drugs with the normal protein and with the snp changed proteins.Here the normal represents the ADRB2 protein and the 15,16,27…….257 represents the different SNP changed proteins.The number represents the position where the SNP change has been made.

4.Conclusion

SNPs do not cause disease, but they help determine the likelihood that someone will develop a particular disease. All the SNPs in the ADRB2 gene were analyzed. The ADRB2 gene was modelled according to the SNP profile of ADRB2. Docking studies were performed for both normal ADRB2 and SNP-ADRB2 with the available candidate drugs using Autodock . Two drugs (Isoproterenol and Salbutemol) were taken and were docked with the normal protein and with the snp changed proteins. It has been found that

- Isoproterenol can be suggested to the patients having SNPs in 16, 27, 57, 164, 220, 240, 247 regions.

- Salbutemol can be suggested to patients having SNPs in 57,164,220,240,247,257 regions.

Hence, designing a drug based on the SNP profile will reduce the toxicity and improve the efficacy in the treatment. The application of pharmacogenomics approach to Asthma will be essential for understanding the preventive mechanisms and could lead to individualized drug therapies in future

5.References

1) Bach, P. B., Brown, C., Gelfand, S. E. and McCrory, D. C. (2001), "Management of acute exacerbations of chronic obstructive pulmonary disease: a summary andappraisal of published evidence". *Ann Intern Me.,* **134**, 600-620.

2) Ballester, E., Reyes, A., Roca, J., Guitart, R., Wagner, P, D. and Rodriguez-Roisin, R. (1989), "Ventilation-perfusion mismatching in acute severe asthma:effects of salbutamol and 100% oxygen", *Thorax.*, **44**, 258-267.

3) Barbera, J, A., Roca, J., Ferrer, A., Felez, M, A., Diaz, O, Roger, N. and Rodriguez-Roisin, R. (1997), "Mechanisms of worsening gas exchange during acute exacerbations of chronic obstructive pulmonary disease", *Eur Respir J.*, **10**, 1285-1291.

4) Bateman ED, Bousquet J, Braunstein GL. Is overall asthma control being achieved? A hypothesis-generating study. Eur Respir J 2001;17:589-595.

5) Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, Pedersen SE, and the GOAL Investigators Group. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med 2004;170:836-844.

6) Bernasconi, M., Brandolese, R., Poggi, R., Manzin, E. and Rossi, A.(1990), "Dose-response effects and time course of effects of inhaled fenoterol on respiratory mechanics and arterial oxygen tension in mechanically ventilated patients with chronic airflow obstruction", *Intensive Care Med.*, **16**, 108-114.

7) Cardus, J., Burgos, F., Diaz, O., Roca, J., Barbera, J, A., Marrades, RM., Rodriguez-Roisin, R. and Wagner PD. (1997), "Increase in pulmonary ventilation-perfusion inequality with age in healthy individuals", *Am J Respir Crit Care Me.,* **156**, 648-653.

8) Celli, B, R. and MacNee, W. (2004), "Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper", *Eur Respir J*, **23.**, 932-946.

9) Diaz, O., Barbera, J, A., Marrades, R., Chung, K, F., Roca, J. and Rodriguez-Roisin, R. (1997), "Inhibition of PAF-induced gas exchange defects by beta-adrenergic agonists in mild asthma is not due to bronchodilation", *Am J Respir Crit Care Me.,* **156**, 17-22.

10) Gabrijelcic, J., Casas, A., Rabinovich, R, A., Roca, J., Barbera, J,A., Chung, K, F. and Rodriguez-Roisin, R. (2004), "Formoterol protects against platelet-activating factor-induced effects in asthma", *Eur Respir J.,* **23**, 71-75.

11) Gale, G, E., Torre-Bueno, J, R., Moon, R, E., Saltzman, H, A. and Wagner, P, D. (1985), "Ventilation-perfusion inequality in normal humans during exercise at sea level and simulated altitude", *J Appl Physio.,* **58**, 978-988.

12) Karpel, J, P., Pesin, J., Greenberg, D. and Gentry, E. (1990), "A comparison of the effects of ipratropium bromide and metaproterenol sulfate in acute exacerbations of COPD", *Chest.*, **98**, 835-839.

13) Kessler, R., Faller, M., Fourgaut, G., Mennecier, B. and Weitzenblum, E. (1999), "Predictive factors of hospitalization for acute exacerbation in a series of 64 patients with chronic obstructive pulmonary disease", *Am J Respir Care Med*, **159**, 158-164.

14) McDonald, D, M. (1990), "The ultrastructure and permeability of tracheobronchial blood vessels in health and disease", *Eur Respir J Suppl.*,**12**, 572-585.

15) Mendes, E, S., Campos, M, A. and Wanner, A. (2006), "Airway blood flow reactivity in healthy smokers and in ex-smokers with or without COPD", *Chest.*, **129**, 893-898.

16) Parker, C, M., Voduc, N., Aaron, S, D., Webb, K, A. and O'Donnell, D, E. (2005), "Physiological changes during symptom recovery from moderate exacerbation of COPD", *Eur Respir J.*, **26**, 420-428.

17) Polverino, E., Gómez, F, P., Rodríguez, A, C., Peña, A., Valera, J, L., Soler, N., Barberà, J, A., Roca, J, R. and Rodríguez-Roisin, R. (2005), "Nebulized salbutamol transiently deteriorates pulmonary gas exchange in COPD exacerbations", *Proc Am Thorac Soc*, **2**, 406-408.

18) Polverino, E., Gómez, F, P., Valera, J, L., Soler, N., Roca, J., Barberà, J, A. and Rodriguez-Roisin, R. (2006), "Gas exchange response to nebulized salbutamol in exacerbations and stable COPD", *Proc Am Thorac So,*. **3**, 721-725.

19) Rabe, K, F., Hurd, S., Anzueto, A., Barnes, P, J., Buist, S, A., Calverley, P., Fukuchi, Y., Jenkins, C., Rodriguez-Roisin, R. and van Weel, C. (2006), "Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease", *Eur Respir J.*, **26**, 480-488.

20) Ringsted, C, V., Eliasen, K., Andersen, J, B., Heslet, L. and Qvist, J. (1989), "Ventilation-perfusion distributions and central hemodynamics in chronic obstructive pulmonary disease:effects of terbutaline administration", *Chest.*, **96**, 976-983.

21) Roca, J., Burgos, F., Barbera, JA., Sunyer, J., Rodriguez-Roisin, R., Castellsague, J., Sanchis, J., Anto, J, M., Casan, P. and Clausen, J, L. (1998), "Prediction equations for plethysmographic volumes", *Respir Me,* .**92**, 454-460.

22) Roca, J. and Wagner, P, D. (1994), "Contribution of multiple inert gas elimination technique to pulmonary medicine. 1: principles and information content of the multiple inert gas elimination technique", *Thora.*, **49**, 815-824.

23) Roca, J., Burgos, F., Sunyer, J., Saez, M., Chinn, S., Anto, J, M., Rodriguez-Roisin, R., Quanjer, P, H., Nowak, D. and Burney, P. (1998), "References values for forced spirometry: Group of the European Community Respiratory Health Survey", *Eur Respir.*, **11**, 1354-1362.

Rodriguez-Roisin, R. (2000), "Toward a consensus definition for COPD exacerbations", *Chest.*, **117**, 398S-401S.

Rodriguez-Roisin, R., Ballester, E., Roca, J., Torres, A. and Wagner, P, D. (1989), "Mechanisms of hypoxemia in patients with status asthmaticus requiring mechanical ventilation", *Am Rev Respir Di.,* **139**, 732-739.

Rodriguez-Roisin, R. (2006), "COPD exacerbations.5: management", *Thorax,* **61**, 535-544.

Stevenson, N, J., Walker, P, P., Costello, R, W. and Calverley, P, M. (2005), "Lung mechanics and dyspnea during exacerbations of chronic obstructive pulmonary disease", *Am J Respir Crit Care Med.,* **172**, 1510-1516.

Wagner, P, D. and Rodriguez-Roisin, R. (1991), "*Clinical advances in pulmonary gas exchange*", *Am Rev Respir Dis.,* **143**, 883-888.