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Research Paper

Characterization of Olfactory Receptor Variants in Pakistani Population

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ABSTRACT

It has been reported that two olfactory receptor (OR) genes i.e. OR51B2 and OR51E2 are significantly enriched in variants in the Pakistani population. However, the inference of these variants on the OR-ligand interactions requires clarification. The current study was carried out to understand the olfactory receptor-ligands interactions in Pakistani individuals by application of bioinformatics tools and a 1000 genome database. The VCF files for these genes of Pakistani individuals were retrieved from the 1000 genome project. Homology models for human olfactory receptors were constructed by phyre2 program and by identifying the binding pockets for these receptors using the BIOVIA Discovery Studio program. Medium to long-range simulation studies of odor ligands in the receptor binding pocket using a Glaxy7TM were performed. Finally, non-covalent interactions of ligands with receptors were assessed by BIOVIA Discovery Studio. We retrieved Vcf data of two individual genes OR51E2 and OR51B2 for variant detections using BCF/VCFtools. BLAST was used to identify closely related structures of proteins encoded by OR51E2 and OR51B2 genes. The current study efficiently clarified changes in OR-ligand interactions due to OR gene variants in the Pakistani population.

KEYWORDS: BCF/VCF tools, BIOVIA Olfactory receptor.

INTRODUCTION

Among the five senses, the sense of smell in humans has been less studied and requires clarification on the molecular level [1]. The complex sensory processing system in the nose requires accurate detection of many structurally diverse odorants [2]. These Odorants include chemical assemblages such as alcohols, aldehydes, ketones, carboxylic acids, sulfur-containing compounds, and essential oils [3]. This diversity in their chemical structure leads to a unique odor response against each odorant.

In humans, chemosensation is one of the most critical functions of the nasal airway, which mediates safety, nutrition, the sensation of pleasure, and general well-being. Human olfaction-affecting factors include structural aspects of the nasal cavity that can modulate airflow. Therefore, odorant access to the olfactory cleft and inflammatory diseases affect both airflow and olfactory nerve function. After signals are generated, olfactory information is processed and coded in the olfactory bulb and disseminated to several areas in the brain. The precise mechanisms used by this system are still under great scrutiny due to the complexity of understanding how an enormous number of chemically diverse odorant molecules are coded into signals understood by the brain. [4].

Scientific literature typically claims that humans can discriminate 10,000 odors, but this number has never been empirically validated [5, 6, and 7].

Previous studies have also focused on receptor ligand-binding interactions, which are responsible for the sense of olfaction [8]. It demonstrates that the human olfactory system has a highly selective binding capacity, with its hundreds of different olfactory receptors, far outperforms the other senses in the number of physically different stimuli it can discriminate [9, 10,11].

An interaction of odorants with olfactory receptors is thought to be the initial step in odorant detection [12]. It is known that the process of olfaction is by recognizing

ligands, which potentially bind receptors and have robust effects leading to an example for olfactory receptor function.

Sensory receptors that detect and respond to light, taste, and smell primarily belong to the G-protein-coupled receptor (GPCR) superfamily [13]. A group of seven membrane-spanning hydrophobic domains, potential odorant-binding sites in the extracellular domain of the protein, and the ability to interact with G-proteins at the carboxyl-terminal region of their cytoplasmic domain. The amino acid sequences for these molecules also show substantial variability, particularly in regions that code for the membrane-spanning domains [14, 10, and 15]. Olfactory receptors (ORs) belong to the G protein-coupled receptor family includes β-adrenergic receptors and the photopigment rhodopsin and play a critical role in recognizing thousands of odorant molecules in the olfactory sensory system [16]. When an OR in an olfactory sensory neuron binds to its ligand, it activates an olfactory G-protein (Golf, similar to Gs), which subsequently activates adenylate cyclase 3 (AC3), leading to cAMP production. The resultant influx of cAMP activates cyclic nucleotide-gated channels, ultimately leading to a series of ion fluxes that produce an action potential [13]. Genome-wide searching for the OR family in various species has revealed the evolutional lineages that resulted in the dramatic changes in the numbers and sequences of the multigene family [17].

Functional characterization of ORs has progressed rapidly due to the development of various expression systems. The detailed pharmacology of ORs has revealed agonist and antagonist specificity and has led to the functional determination of the odorant-binding site. The three-dimensional configuration of the odorant-binding site, which is constructed by transmembrane helices, provides the molecular basis for odorant sensitivity and specificity. The one-neuron one-OR rule and OR-instructed axonal convergence, which have been explored by elegant genetic manipulation studies, produce neural circuits that ensure the precise transmission of the combinatorial receptor codes for odorants from the olfactory epithelium to the olfactory bulb. The function of ORs has turned out to be much more diverse, including participation in the guidance of olfactory neuron axons, chemoreception in tissues other than the olfactory epithelium, and sensing of pheromones that affects innate behavior or neuroendocrine systems [18, 19].

Studies on the sense of smell have advanced to the point of elucidating mechanisms underlying the integration of OR-mediated signals for odor perception related to survival and chemical communication [13]. It has been reported in previous studies that more than 1000 ORs in mice and about 350 ORs in humans are dedicated to smell, capable of detecting an enormous repertoire of chemical compounds by a combinatorial coding scheme. Typically, one OR recognizes multiple odorants and one odorant is recognized by multiple ORs, but different odorants are recognized by different combinations of ORs [20, 21].

Many studies also report that there are eight hundred genes encoding GPCRs in humans, out of which 396 genes represent functional olfactory receptor genes and are classified as the most diverse subclass [9]. There are 418 intact and potentially functional genes of olfactory receptors. This is the largest gene family in the human genome and can be divided into class-I plus class-II genes [22]. In humans, all class-I genes are bunched on chromosome 1, and class-II genes are expressed on all chromosomes except chromosomes 20 and Y [23]. Previous studies have reported that in the human

olfactory receptor set, 172 sub-sets are having 60% similarity in protein sequence, which can have a structural bias for ligand specificity according to the number of carbon atoms in the ligands [24, 25]. The diversity between the olfactory receptor genes at the odorant-binding site facilitates the recognition of different odorant ligands and disparity in these olfactory receptor gene sequences between individuals is excessive [8].

Sense of smell has a strong representation within the genome, allowing the existence of many types of receptors that allow us to capture multiple volatile odors-producing molecules, sending electrical signals to higher centers to report the outside world [26, 27].

In humans, the evolutionary source of genetic dissimilarity between the olfactory receptors is not completely known [8]. Humans use a family of more than 400 olfactory receptors (ORs) to detect odors, but each individual has a unique set of genetic variations that lead to variation in olfactory perception [28]. Genetic variation in human ORs is abundant and alters receptor function, allowing us to examine the relationship between receptor function and perception [29].

The olfactory receptor gene (OR) superfamily is the largest in the human genome presently, there are 390 putatively functional (protein-coding) OR genes that are intact in at least part of the population, but individuals have different repertoires of pseudogenes, copy number variations, and single nucleotide polymorphisms (SNPs) that can alter receptor responses [30].

The demand of current research demonstrates how natural variation can provide important clues to the normal translation of OR activation to odor information and places a constraint on the amount of redundancy in the olfactory code [25, 31, 29].

The effect of such variations on the phenotypic behavior of olfactory receptors can be assessed by utilizing computational methods. The combination of modeling and analysis pipelines can be used to understand the mechanism of interactions among olfactory receptors and ligands [9, 8, and 28].

Olfactory receptors are known to have a combinatorial response to odors and OR-ligand discrimination has been recorded in literature only for a few ORs through careful experiments [1]., for the advancement and to speed up research different bioinformatics methods like sequence alignment, homology modeling, MD simulations and a lot of biological databases containing information about olfactory receptors and their functional ligands, odorants were developed. These resources are the Olfactory Receptor Database (ORDB), the Human Olfactory Data Explorer (HORDE), and the ODOR actor. They are the repository of genomic and proteomic information related to ORs and other chemosensory receptors [1, 32, 33, 34, 35, and 36].

Understanding how the olfactory system detects odorants and translates its features into perceptual information is one of the fundamental questions in olfaction.

With the advent of next-generation genome sequencing to profile olfactory receptor (OR) genes and cell-based assays to identify ligands for ORs, however, receptor variation can now be matched to individuals and receptor responses can be directly observed [33].

It is reported in previous studies that two types of genomic variation leading to OR inactivation are relevant in this respect: segregating pseudogenes, owing to a specific type of single nucleotide polymorphism (SNP) that leads to gene inactivation and CNVs which involve deletion alleles [37].

Recently, several studies addressed the human olfactory subgenome based on available data from genome-wide surveys and databases. In the European population, the olfactory receptor OR6A2 detects aldehydes that may make cilantro smell soapy. Hence this receptor is a compelling candidate for the detection of the cilantro odors that give cilantro its divisive flavor [37, 36]. Variant analysis of Pakistani individuals indicated significant enrichment of variants in 2 Olfactory Receptor genes i.e. OR51B2 and OR51E2. However, the inference of this observation at the population level requires more genomic-level studies from these regions [38].

The current study has been designed to understand the structure-function relationship in olfactory receptors in the Pakistani population by applying available bioinformatics tools and databases.

MATERIALS AND METHODS

1000 Genomes Project is an international collaboration for whole genome sequence data analyses of more than 2,500 individuals from diverse worldwide populations. The purpose of this project was to discover genetic variants among the studied populations. For the retrieval of the 1000 genome vcf data, the online database of 1000 genome project was used (https://www.internationalgenome.org) [**39**]. In 1000 genome project phase3, genome of 2505 individuals belonging to different populations are present. Among these 2505 individuals, 159 belong to Pakistani population (code PJL). The genes of OR51B2, OR51B5 and OR51E2 olfactory receptors are present on chromosome 11. The VCF of chromosome 11 was be filtered using genomic coordinates of these genes by Vcf tools and data slicer [39, 40]. For the accurate retrieval of these VCF files, different filters and parameters available on the 1000 genome project website were applied. Different vcftools/Bcftool commands were used to perform operations on VCF files like filtering out specific variants, Comparing files, Summarizing variants, Converting to different file types, Validating and merge files and create intersections and subsets of variants. [40-42].

After literature review, odorants involved in the olfaction receptors of different spices and flavors were selected [43-45]. Phyre2 is an online tool that predicts and analyzes protein structure, function and mutations. It was used for understanding the structure of olfactory receptor [46].

For homology modeling of olfactory receptors OR51B2 and OR51E2, Phyre2 was utilized [**46**].

In the first step, BIOVIA Discovery Studio [**47]** was used to predict binding site of olfactory receptor by finding the amino acid residues involved in template structure making interactions with ligand(s). In the second step, EMBOSS Needle program was used; which reads two input sequences and provides their optimal global sequence alignment as output [48, 49]. In the third step, grid box residues were related with the global alignment sequence and selected only those residues present in conserved region.

DynaMut was used to analyze the changes occur in structure flexibility of olfactory receptors due to mutations by calculating the vibrational entropy change upon mutation and predicted the structure stabilization or destabilization based on the score [50].

Galaxy 7TM utilizes built-in Auto Dock and Rosetta MPrelax tools for refinement of structures followed by docking. This server perform well in both ligand docking and structure refinement than usually used programs Auto Dock Vina and Rosetta MPrelax, individually [51, 52]. BIOVIA Discovery Studio was used to analyze the noncovalent interactions between the ligand

and receptor binding pocket. There are different tools available in BIOVIA Discovery studio by which we can study non-covalent interactions (hydrogen bonds, ionic bonds, van der Waals interactions, and hydrophobic bonds) formed between ligand and receptors.

Effects of mutations on protein stability were predicted by bioinformatics tool namely DynaMut. Machine learning techniques was applied to validate effects of mutations on protein structure stability [53].

RESULTS

Genome VCF data of 1000 genome of Chromosome 11 was retrieved and 159 samples (both male/female) of Pakistan population (PJL) were filtered by using retrieved VCF tools. Furthermore, VCF data of OR51E2, OR51B2 genes were extracted for variant detection in using different BCF/VCF tools. The identified variants in the selected genes for Pakistani population are demonstrated in Table 1.

Table 1: variants identified in OR51E2, 0R51B2 genes in Pakistani populations.

Gene Name	Total variants	Non synonymous	synonymous
OR51E2	39	20	18
OR51B2	64	40	24

For understanding the secondary structure of olfactory receptor 51B2 Phyre2 was used. It predicts and analyze protein structure, function and mutations. It uses homology detection methods to build 3D models, predict ligand binding sites and analyze the effect of amino acid variants [1]. In the secondary structure and disorder prediction, the position in the sequence is indicated in the top line. The sequence is represented on the next line with residues colored according to a simple property-based scheme: (A,S,T,G,P: small/polar) are yellow, (M,I,L,V: hydrophobic) are green, (K,R,E,N,D,H,Q: charged) are red and (W,Y,F,C: aromatic + cysteine) are purple. The secondary structure prediction comprises three states: α-helix, β-strand or coil and in this condition TM helices. Green helices represent α-helices, blue arrows indicate β-strands, light brown helices indicate TM helices and faint lines indicate coils. The SS confidence line indicates the confidence in the prediction from PSIPRED, with red indicating high confidence and blue showing low confidence. Assess which regions are predicted with high and low confidence.The Disorder line contains the prediction of disordered regions in protein.The olfactory receptor 51B2 consists of 8% disordered regions, 76% alpha helices, 1% beta strand and 55% transmembrane helices.

Phyre2 program used 6 template structures to build the olfactory receptor 51B2 structure. The information regarding these templates is as follows:

c6kp6A: it is a membrane protein, residues position 23 -308 aligned with olfactory receptor 51B2 sequence with a coverage of 91%, percentage identity was 18% and confidence scores 100%.

c4zwjA: it is a signaling protein, residues position 2 -311 aligned with olfactory receptor 51B2 sequence with a coverage of 99%, percentage identity was 15% and a confidence score was 100%.

c4zwjC_: it is a signaling protein, residues position 2 -311 aligned with olfactory receptor 51B2 sequence with a coverage of 99%, percentage identity was 15% and a confidence score was 100%.

c5ndzA_: it is a membrane protein, residues position 23 -305 aligned with olfactory receptor 51B2 sequence with a coverage of 90%, percentage identity was 16% and a confidence score is 100%.

c3uonA_: it is a signaling protein/antagonist, residues position 23 -305 aligned with olfactory receptor 51B2 sequence with a coverage of 90%, percentage identity was 19% and a confidence score was 100%.
c6tpnA_: it is a membrane protein, residues position 19 -308 aligned with olfactory receptor 51B2 sequence with a coverage of 91%, percentage identity was 16% and a confidence score was 100%.

The tertiary structure of olfactory receptor 51B2 was built using sequence alignment of all the selected templates with 99% of residues modeled at >90% confidence. Figure 2 demonstrates the homology model of the Olfactory Receptor 51B2 gene.

BLAST was used to identify closely related sequences to Olfactory Receptor 51B2 gene. It was observed that Adenosine Receptor A2a [chain A] possessed highest similarity against OR51B2 gene sequence. Alignment showed that the modeled sequence has 32.4 and 33.3% percent identity with Adenosine Receptor A2a [chain A] PDB IDs 5G53_A and 2YDO_A respectively. Gene sequence of Olfactory Receptor 51E2 and Adenosine Receptor A2a [chain A] have 56 amino acids similar in their structures. Moreover, the e-value for alignment was 8x10^-7.

The structure of the adenosine A2A receptor bound to an engineered G protein, classified as signaling protein. It consists of two chains, A and B [2]. Amino acid sequence of chain A showed similarity with the sequence of Olfactory Receptor 51B2. The obtained structure comprised of eight alpha helices and two beta sheets which identified the binding pocket amino acids.

Non-Covalent interactions between ligand and Adenosine Receptor A2a was observed using BIOVIA Discovery studio Visualizer as demonstrated in figure 4. Five amino acids i.e. thr88, asn253, glu169, ser277, his278 were involved in the hydrogen bonding, shown in green color; his250 was bound with ligand with C-H bond. Pi-pi stacking (aromatic-aromatic) interaction and pi-sigma interaction of amino acid residues phe168 and leu249 was also observed. However, amino acid Ile274 was found to have pi-Alkyl interaction with ligand molecule.

There were 10 amino acids found in the binding site of the template structure. These amino acids were involved in non-covalent interactions .

We used the EMBOSS Needle program to perform the optimal global alignment between the sequence of OR51B2 and template Sequence adenosine receptor A2a [3]. It is found that nine amino acid residues namely (ile102, his103, ser106, phe258, ala188, val270, met274, gln180, and his179) used for the grid box of the binding site of the Olfactory Receptor 51B2. By using those amino acids docking was performed on, OR51B2 with acetic acid and it was seen that seven amino acids present.

Out of 40 non-synonymous mutations found in the OR51B2 gene in the PJL dataset of 1000 genome database, only one i.e. p.(His87Asp) was involved in receptor and ligand binding. Th mutations in Table 4 are then added to the Olfactory Receptor 51B2 structure was modeled with this mutation and eight odorant molecules were docked in the odorant binding site one by one.

A visual representation of the chain in which the mutation (H/D) occurs is shown in Figure 9. Amino acids are colored according to the vibrational entropy change upon mutation. BLUE represents a rigidification of the structure and RED represents a gain in flexibility. The vibrational Entropy difference ($\Delta\Delta S$) between wild-type and mutant structure is calculated as 0.096 kcal.mol-1.K-1 which shows an Increase of molecule flexibility.

To obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against the ACY structure. Figure 8-A provides an overview of the interaction between the predicted structure model and ACY. The affinity binding energy released during receptor-ligand binding was found to be -5.764.

It was observed that **a**mino acid residues his87 and ile90 were forming hydrogen bonds with ACY ligand molecule. Remaining amino acids on the pocked including, glu89, gly81, val85, arg88, cys95. Thr78 and ile82, developed the van der Waals interaction with ACY ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against acetic acid (ACY) structure. The affinity binding energy released during receptor-ligand binding was found to be -4.994 and as compared to the normal structure it decreased. After inserting the mutation It was observed in Figure, that number of an amino acids (thr13, leu12, leu11, and gly14)involved in forming a hydrogen bond with the ligand increased as compared to Figure 8-B , amino acid (thr13, leu12, leu11, and gly14) were forming a hydrogen bond with acetic acid (ACY). And the number of Amino acids (phe10 and phe15) involved in van der Waals interaction with acetic acid (ACY) decreased.

Due to the change of amino acid from HIS to ASP, which resulted that structure stability is not affected

It was observed that amino acid Glu89 were forming hydrogen bonds with BENZ ligand molecule. Amino acid Gly81 was forming amide-pi stacked with BENZ ligand molecule. Amino acid Gly81 was forming pi-sigma bond with BENZ ligand molecule. Remaining amino acids on the pocket site including, arg88, his87, val85, thr78 and gln98 developed the van der Waals interaction with Benzaldehyde ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against benzaldehyde (Benz) structure. The affinity binding energy released during receptor ligand binding was found to be -9.361 and as compared to the normal structure, it decreased.

After inserting the mutation It was observed that the number of amino acids (phe10, tyr260, asn265, leu11, and gly263) involved in van der Waals interaction with benzaldehyde (BENZ) decreased as compared to figure 12-B. And the number of Amino acids (cys187, pro9, ala186, val266, and lys264) involved in pi-alkyl interaction increases. Due to the change of amino acid from His to Asp, the destabilization of structure stability as shown in Figure 14a & Figure 14b. The presence of ionic interaction can be seen in figure 14b which was absent. To obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against Butanoic. The affinity binding energy released during receptor-ligand binding was found to be -7.837.

It was observed that amino acids Glu89 and Ile90 were forming hydrogen bonds with the BUA ligand molecule. Amino acid Trp2 was forming a pi-alkyl bond with the h BUA ligand molecule. The remaining amino acids on the pocket site including, arg88, his87, val85, thr78, cys96, ile82, and gly81 developed the van der Waals interaction with the Butanoic ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against the Butanoic acid (BUA) structure. The affinity binding energy released during receptor-ligand binding was found to be -6.825. It was

observed that amino acids cys187 and tyr260 were forming hydrogen bonds with the BUA ligand molecule. After inserting the mutation It was observed that number of amino acid (pro9, lys264, gly263, phe10 and met183) involved in van der Waals interaction with Butanoic acid (BUA) decreased. Due to the change of amino acid from His to Asp, the destabilization of structure stability. The absence ionic interactions in mutant structure as compared to wild type amino acid can be seen.In order to obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against Butyl acetate. Figure 18-A provides an overview of interaction between predicted structure model and Butyl acetate. The affinity binding energy released during receptor ligand binding was found to be -9.470.

It was observed that **a**mino acid Glu89 was forming hydrogen bond with butyl acetate ligand molecule, **a**mino acid Trp2 and Ile 90 were forming pi-alkyl bond with butyl acetate ligand molecule. Remaining amino acids on the pocket site including, arg88, his87, val85, thr78, gln98, ile82 and gly81 developed the van der Waals interaction with Butyl acetate ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against Butyl acetate structure. The affinity binding energy released during receptor ligand binding was found to be -9.491 and as compared to the normal structure it increased. It was observed that **a**mino acid val269 was forming hydrogen bonds with Butyl acetate ligand molecule. After inserting the mutation It was observed that the number of the amino acids (pro16, pro267, glu268, val270, ley18 and thr6) involved in van der Waals interaction with butyl acetate, decreased and the number of Amino acids (phe10, ile5, leu12 and val266) which were involved in pi-alkyl interaction increased. Due to the change of amino acid from His to Asp, resulted that the structure stability is not affected.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against citric acid. The affinity binding energy released during receptor-ligand binding was found to be **-9.330.**

It was observed that **a**mino acid Gly81, glu89, ile90 and his87 were forming hydrogen bond with citric acid ligand molecule. Remaining amino acids on the pocket site including, arg88, trp84, val85, thr6, gln98, ile82 and trp2 developed the van der Waals interaction with citric acid ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against citric acid structure. The affinity binding energy released during receptor ligand binding was found to be **-10.096** and as compared to the normal structure it increased.

It was observed that **a**mino acid val269, glu268 and pro16 were forming hydrogen bonds with citric acid ligand molecule. Amino acid (pro267, val270, ile5, leu18) involved in van der Waals interaction with citric acid.

Due to the change of amino acid from His to Asp, which resulted that the structure stability is not affected . In order to obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against butyrolactone. The affinity binding energy released during receptor ligand binding was found to be **-11.085.**

It was observed that **a**mino acid his87, glu89, ile90, pro77 and thr78 were forming hydrogen bond with butyrolactone ligand molecule. Remaining amino acids on the

pocket site including, arg88, trp2, gly81, val79, gln98, ile82 and cys95 developed the van der Waals interaction with butyrolactone ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against Butyrolactone structure. The affinity binding energy released during receptor ligand binding was found to be **-10.581** and as compared to the normal structure it decreased.

It was observed that **a**mino acid val269, val266, gly14 and ile5 were forming hydrogen bonds with butyrolactone ligand molecule. Amino acid (pro267, glu268, leu18, phe15, pro16, val270, thr6, leu18) involved in van der Waals interaction with butyrolactone.

Change of amino acid from HIS to ASP, absence of hydrogen bond could be seen in a mutant type, which affect the structure stability affect the structure stability. In order to obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against limonene. The affinity binding energy released during receptor ligand binding was found to be **-12.078.**

It was observed that **a**mino acid his87, glu89, gly81, thr6, cys95 and thr78 were forming van der Waals interaction with limonene ligand molecule. Remaining amino acids on the pocket site including, trp2, gly81, val85, ile90, ile82 and arg88 developed the pi-alkyl interaction with limonene ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against limonene structure. The affinity binding energy released during receptor ligand binding was found to be **-10.996** and as compared to the normal structure it decreased.

It was observed that **a**mino acid phe192, leu200 and ile199 were forming hydrogen bonds with limonene ligand molecule. Amino acid (pro197, asn193, leu185, arg194, val198, arg160, pro156 and val 157 were involved in van der Waals interaction with limonene. Amino acid glu181 involved in pi-anion bond while leu195 and pro198 forming covalent bond with limonene. Change of amino acid from HIS to ASP, the predicted outcome of free energy indicate the destabilization of structure stability.In order to obtain the ligand bindings and potentials of Olfactory Receptor 51B2 predicted structure, Olfactory Receptor 51B2 was docked against vanillin.The affinity binding energy released during receptor ligand binding was found to be -11.739.

It was observed that **a**mino acid his87 and glu89 were forming hydrogen bond with vanillin ligand molecule. Remaining amino acids on the pocket site including, trp2, gln98, val85, pro77, thr78 and arg88 were involved in van der Waals interaction with vanillin ligand molecule. Amino acid ile90 forming pi-sigma bond with vanillin molecule and gly81 is involved in pi-alkyl interaction.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51B2 predicted structure. It was docked against vanillin structure. The affinity binding energy released during receptor ligand binding was found to be -10.016 and as compared to the normal structure, it decreased.

It was observed that amino acid phe10 was forming hydrogen bonds with vanillin ligand molecule. Amino acid (leu11, leu12, gly263, argala186, lys264, were involved in van der Waals interaction with vanillin. Amino acid tyr260 involved in pi-sigma bond while cys187 and pro9 and val266 forming pi-alkyl bond with vanillin.

Due to the change of amino acid from His to Asp, the predicted outcome of free energy change indicate the destabilization of structure stability.

For understanding the secondary structure of olfactory receptor 51E2 Phyre2 was used.

Phyre2 program uses 6 templates to build the olfactory receptor 51E2 structure. The information regarding these template are as follows:

c6kp6A: it is a membrane protein, residues position 23 -308 aligned with olfactory receptor 51B2 sequence with the coverage of 88%, percentage identity was 18% and confidence score is 100%.

c4zwjA: it is a signaling protein, residues position 3 -314 aligned with olfactory receptor 51B2 sequence with the coverage of 97%, percentage identity was 15% and confidence score is 100%.

c4zwjC: it is a signaling protein, residues position 3 -314 aligned with olfactory receptor 51B2 sequence with the coverage of 97%, percentage identity was 15% and confidence score is 100%.

c5ndzA: it is a membrane protein, residues position 20 -306 aligned with olfactory receptor 51B2 sequence with the coverage of 89%, percentage identity was 16% and confidence score is 100%.

c3uonA: it is a signaling protein/antagonist, residues position 24 -306 aligned with olfactory receptor 51B2 sequence with the coverage of 88%, percentage identity was 16% and confidence score is 100%.

c6lflA: it is a membrane protein, residues position 23 -308 aligned with olfactory receptor 51B2 sequence with the coverage of 89%, percentage identity was 20% and confidence score is 100%.

The tertiary structure of olfactory receptor 51E2 was built using sequence alignment of all the selected template with 99% of residues modelled at >90% confidence.

BLAST was used to identify closely related gene sequence to Olfactory Receptor 51E2 gene. It was observed that Adenosine Receptor A2a [chain A] possessed highest similarity against OR51E2 gene sequence. Alignment result shows that the modeled sequence have 26 % percent identity with Adenosine Receptor A2a [chain A] PDBID 5G53_A respectively. Gene sequence of Olfactory Receptor 51E2 and Adenosine Receptor A2a [chain A] have 121 amino acids similar in their structures. Moreover, the e-value for alignment was 8x10^-5.

As it is shown that the template structure possessed highest similarity with both of the gene sequence (OR51B2 and OR51E2) so we studied both of gene structures and their sequence together to identify the similarity between them and characterized the binding pocket for olfactory receptor E12.

The similarity between these two genes is 63.2% and the percent identity is 40.5%. **The** nine amino acids namely (his23, val26, ser26, phe29 et80, trp84 and leu83) that used to provide grid box for pocket binding site of the Olfactory Receptor 51E2

Out of 20 non-synonymous mutations that are found in olfactory receptor 51E2 gene, only two of them were convoluted in contact between receptor and ligand binding.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against ACY structure. Figure 38-A provides an overview of interaction between predicted structure model and ACY. The affinity binding energy released during receptor ligand binding was found to be -5.078.

It was observed that amino acid Arg272 was forming hydrogen bond with ACY ligand

molecule. Remaining amino acids on the pocked including, phe6, ala9, met275, tyr279, asn5, pro253, leu257 and gly276 developed the van der Waals interaction with ACY ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2 predicted structure. It was docked against acetic acid (ACY) structure. The affinity binding energy released during receptor ligand binding was found to be -4.487 and as compared to the normal structure it decreased.

After inserting the mutation (276:G/D), It was observed that amino acid (val274) involved in forming hydrogen bond with the ACY as compared to figure 38-B in which amino acid (arg272) was involved in hydrogen bonding. And the remaining number of Amino acids involved in van der Waals interaction with acetic acid (ACY) increased

Due to the change of amino acid from GLY to ASP, the predicted outcome of free energy change indicate the destabilization of structure stability. The hydrophobic contacts, weak hydrogen bond and ionic interaction can be seen.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against benzaldehyde structure. The affinity binding energy released during receptor ligand binding was found to be -9.282.

It was observed that **a**mino acid val247 and val210 were forming pi-sigma bond with benzaldehyde ligand molecule, **a**mino acid leu114 was forming pi-alkyl bond with benzaldehyde ligand molecule. Remaining amino acids on the pocket site including, ile244, asp209, ile213, ser111, Met206, tyr251 and leu248 developed the van der Waals interaction with benzaldehyde ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2 predicted structure. It was docked against Benzaldehyde (Benz) structure. The affinity binding energy released during receptor ligand binding was found to be -9.208 and as compared to the normal structure it slightly decreased.

After inserting the mutation it was observed in Figure42-B, that amino acid leu280 was involved in pi-sigma bond, his 204 was involved in pi-pi interactions and amino acid ile103 and leu281was forming pi-alkyl interactions which were different in normal structure interaction

Due to the change of amino acid from MET to LEU, the predicted outcome of free energy change indicate the destabilization of structure stability. The presence of hydrophobic contacts and absence of ionic interaction.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against BUA structure. Figure 44-A provides an overview of interaction between predicted structure model and BUA. The affinity binding energy released during receptor ligand binding was found to be -**7**.785.

It was observed that amino acid phe6 was forming hydrogen bond with BUA ligand molecule, amino acid ala9, pro253 and tyr279 were forming pi-alkyl bond with BUA ligand molecule. Remaining amino acids on the pocket site including, asn5, leu257, thr10, arg272, gly276, met275 and ile14 developed the van der Waals interaction with BUA ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2

predicted structure. It was docked against Butanoic acid (BUA) structure. The affinity binding energy released during receptor ligand binding was found to be **-7.251** and as compared to the normal structure it slightly decreased.

After inserting the mutation it was observed that amino acid ser107 and glu110 were involved in hydrogen bonding, val201, val247, met206 and tyr251 were involved in pi-alkyl bond and amino acid leu114, ser111 and ile255 were forming van der waals interactions which were Due to the change of amino acid from Gly to Asp, the predicted outcome of free energy change indicate the destabilization of structure stability The presence of hydrophobic contacts, ionic interaction and weak hydrogen bonding can be seen in figure 46b which were absent.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against Butyl acetate structure. The affinity binding energy released during receptor ligand binding was found to be -9.459.

It was observed that **a**mino acid Met206, Tyr251 and Val247 were forming pi-alkyl bond with Butyl acetate ligand molecule. Remaining amino acids on the pocket site including, ser111, leu114, asp209, ile213, gly276, leu248 and ile244 developed the van der Waals interaction with Butyl acetate ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2 predicted structure. It was docked against butyl acetate structure. The affinity binding energy released during receptor ligand binding was found to be -9.459 and as compared to the normal structure it increased.

.After inserting the mutation it was observed that amino acid ile80, leu280 and ile103 were involved in pi-alkyl bond and remaining amino acids were forming van der Waals interactions which were different in normal structure interaction.

Due to the change of amino acid from MET to LEU, the predicted outcome of free energy change indicate the destabilization of structure stability. The presence of hydrophobic contacts in different

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against CIT structure. The affinity binding energy released during receptor ligand binding was found to be -11.533.

It was observed that amino acid val205 and ser111 were forming hydrogen bond with CIT ligand molecule, **a**mino acid met206 was forming weak hydrogen bond with CIT ligand molecule. Remaining amino acids on the pocket site including, val210, ile213, asp209, leu114, glu110, ser107, val247, ile244, leu248 and tyr251 developed the van der Waals interaction with CIT ligand molecule.

In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2 predicted structure. It was docked against citric acid (CIT) structure. The affinity binding energy released during receptor ligand binding was found to be -9.597 and as compared to the normal structure it decreased.

After inserting the mutation it was observed that amino acid thr98, lys79 and ala95 were involved in hydrogen bonding and remaining amino acids were forming van der Waals interactions which were different in normal structure interaction.

Due to the change of amino acid from Met to Leu, the predicted outcome of free energy change indicate the destabilization of structure stability. The presence of

hydrophobic contacts in different position as compared to wild type amino acid and absence of ionic interaction can be seen.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against Limonene structure. The affinity binding energy released during receptor-ligand binding was found to be -**12.517.**

It was observed that **a**mino acids val247, leu248, ile114, val210, met206 and ile244 were forming pi-alkyl bond with the Limonene ligand molecule. The remaining amino acids on the pocket site including, ser111, asp209, val205, arg272 and ile244 developed the van der Waals interaction with the Limonene ligand molecule. In order to obtain the impact of mutation and potentials of Olfactory Receptor 51E2 predicted structure. It was docked against acetic acid (ACY) structure. The affinity binding energy released during receptor-ligand binding was found to be -11.232 as compared to the normal structure it decreased.

After inserting the mutation it was observed in that amino acid ile103, leu280, pro284, tyr251, leu72, leu281 and val196 were involved in pi-alkyl bond, amino acid his104 were involved in pi-sigma bond and remaining amino acids were forming van der Waals interactions which were different in normal structure interaction .

Due to the change of amino acid from Met to Leu, the predicted outcome of free energy change indicate the destabilization of structure stability. The presence of hydrophobic contacts in different position as compared to wild type amino acid and absence of ionic interaction can be seen.

In order to obtain the ligand bindings and potentials of Olfactory Receptor 51E2 predicted structure, Olfactory Receptor 51E2 was docked against Vanillin structure. The affinity binding energy released during receptor ligand binding was found to be -11.204

It was observed that **a**mino acid tyr251was forming pi-pi bond, amino acid val210 was forming pi-alkyl bond with vanillin ligand molecule, **a**mino acid val247 was involved in 2 different non covalent interactions like hydrogen bonding and pi-sigma bond with the vanillin ligand molecule, amino acid met206 was involved in pi-sulfur bond. Remaining amino acids on the pocket site including, ile244, asp209, ile213, ser111 and leu115 developed the van der Waals interaction with vanillin ligand molecule.

DISCUSSION

This article gave an overview of approaches to analyzing protein Ligand interaction to discuss Human olfaction affecting factors included structural aspects of the nasal cavity and odorant access to the olfactory cleft where identified one of the most critical functions that is chemo sensation of the nasal airway, mediates safety, nutrition and sensation of pleasure. The olfactory receptor gene (OR) superfamily is the largest in the human genome, that are intact in at least part of the population, but individuals have different repertoires and single nucleotide polymorphisms (SNPs) can alter receptor responses.

An interaction of odorants with olfactory receptors is thought to be the initial step in odorant detection. However, ligands have been reported for only 6 out of 380 human olfactory receptors, with their structural determinants of odorant recognition just beginning to emerge[54]. There is tremendous functional variability in the human sense of smell, which may be attributable to population specific selective pressure to local environments or food. Significant population differentiation is a major indicator of local adaptation [55]. The

variation present in or genes alters perception of the odorant affect rendering those with mutations.

Current study analyzed the geographic distribution of genetic variants across OR51B2 and OR51E2 in Pakistani populations and identified possible evolutionary adaptive trends using bioinformatics approaches.

Four individual genes i.e., OR51B2, and OR51B5 was extracted for variant detection in selected genes where OR51B2 consists of 8% of disordered, 76% alpha helices, 1% beta strand and 55% transmembrane helices. BLAST was used to identify closely related gene sequence to Olfactory Receptor 51B2 gene and observed that Adenosine Receptor A2a [chain A] possessed highest similarity against OR51B2 gene sequence. Gene OR51E2 and A2a [chain A] have 56 amino acids similar in their structures.

Moreover, A2a [chain A] shows Non-Covalent interactions through five amino acids i.e. thr88, asn253, glu169, ser277, his278 with Ligand-receptors that are hydrogen bonding interaction, Pi-pi stacking (aromatic-aromatic) interaction, pi-sigma interaction and pi-Alkyl interaction. Furthermore, Global Alignment examine between OR51B2 and A2a with nine amino acids namely (ile102, his103, ser106, phe258, ala188, val270, met274, gln180 and his179), by using those amino acids docking was performed on, OR51B2 with acetic acid and it was seen that a list of seven amino acids (ile90, glu89, arg88, val85, his87, ile82 thr78) were involved in interaction with odorant molecule.

Out of 40 non-synonymous mutations that are found in OR51B2 gene, only one of them were convoluted in contact between receptor and ligand binding His87 with Asp where Entropy difference (ΔΔS is calculated as 0.096 kcal.mol-1.K-1 which shows Increase of molecule flexibility also observed that amino acid his87 and ile90 were forming hydrogen bonds and glu89, gly81, val85, arg88, cys95. Thr78 and ile82, developed the van der Waals interaction with acetic acid ACY ligand molecule. Changing effects of non-synonymous mutation (87: H/D studied with ligand binding, where affinity binding energy was found to be -4.994 that is decreased as compared to the normal structure where amino acid (thr13, leu12, leu11 and gly14) were forming hydrogen bond with acetic acid (ACY). (Phe10 and phe15) involved in van der Waals and no considerable Change in structure stability seen. The Entropy difference between wild-type and mutant structure shows increase of molecule flexibility.

Flexibility plays an important role in biological systems. Many molecular recognition processes depend on the participants' capacity to reorganize themselves to engender increased complementarity to their binding partner [56]. When wild type 51B2 was docked against ACY it was noticed that only two amino acids formed hydrogen bonds with it. While the mutated 51B2 gene showed higher number of amino acids involved in h-bonds and decrease in Van Der Waal's forces and affinity binding energy. This change leads to poor flexibility and rigid structure while it may cause some changes to binding pockets which lead to decrease in affinity binding[57]. Change of HIS to ASP lead to decrease in h-bond which affects the thermal stability of the protein [58].

Besides, OR51B2 gene binding with BENZ ligand studied, where affinity binding was found to be -9.490 where Gly81 was forming pi-sigma bond and the pocket site including, arg88, his87, val85, thr78, and gln98 developed the van der Waals interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -9.361 affinity

binding energy released during receptor ligand binding where amino acid (phe10, tyr260, asn265, leu11 and gly263) involved in van der Waals interaction and (cys187, pro9, ala186, val266 and lys264) were involved in pi-alkyl interaction. Change of amino acid from HIS to ASP shows effect the structure and ionic interaction was built and it was observed that affinity binding energy and Van Der Waal interactions decreased while pi-alkyl forces increased. Pi-alkyl bond helps to improve the hydrophobic interaction of the ligand in the binding pocket of the receptor [59].

In adding, OR51B2 gene binding with Butanoic acid (BUA) ligand studied, where affinity binding was found to be - 7.837 where Glu89 and Ile90 were forming hydrogen bonds and the pocket site including, arg88, his87, val85, thr78, cys96, ile82 and gly81 developed the van der Waals interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -6.825 affinity binding energy released during receptor ligand binding where amino acid cys187 and tyr260 were forming hydrogen bonds, ala186 and val266 were involved in pi-alkyl interaction and pro9, lys264, gly263, phe10 and met183 involved in van der Waals interaction. Change of amino acid from HIS to ASP shows effect the structure and absence of ionic interaction in mutant structure clearly observed and it was found that affinity binding energy and Van der Waal forces decreased while pi-alkyl interactions increased. As discussed in previous studies this change shows that the protein structure is rigid and the binding improved [59].

Moreover, OR51B2 gene binding with Butyl acetate ligand was studied, where affinity binding was found to be -9.470 where Glu89 was forming hydrogen bond, Trp2 and Ile 90 were forming pi-alkyl bond and the pocket site amino acid arg88, his87, val85, thr78, gln98, ile82 and gly81 developed the van der Waals interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -9.491 affinity binding energy released during receptor ligand binding where amino acid val269 were forming hydrogen bonds, and amino acid phe10, ile5, leu12 and val266 were involved in pi-alkyl interaction and pro16, pro267, glu268, val270, ley18 and thr6 involved in van der Waals interaction. Change in structure stability seen HIS to ASP in this non-synonymous mutant, it was found that affinity binding energy and pi-alkyl interactions increased while Van der Waal forces decreased. Several studies shows that mutations increase the binding affinity and they may have been selected to enhance cell survival and growth [60].

Additionally, OR51B2 gene binding with Citric acid (CIT) ligand studied, where affinity binding was found to be -9.330 where Gly81, glu89, ile90 and his87 were forming hydrogen bond and the pocket site including, arg88, trp84, val85, thr6, gln98, ile82 and trp2 developed the van der Waals interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -10.096 affinity binding energy released during receptor ligand binding where amino acid val269, glu268 and pro16 were forming hydrogen bonds and (pro267, val270, ile5, leu18) involved in van der Waals interaction. There is no considerable Change in structure stability seen when Amino acid HIS mutated to ASP in non-synonymous mutant.

Furthermore, OR51B2 gene binding with Butyrolactone ligand studied, where affinity binding was found to be -11.085 where his87, glu89, ile90, pro77 and thr78 were forming hydrogen bond and the pocket site including, arg88, trp2, gly81, val79, gln98, ile82 and cys95 developed the van der Waals interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -

10.581 affinity binding energy released during receptor ligand binding where amino acid val269, val266, gly14 and ile5 were forming hydrogen bonds and (pro267, glu268, leu18, phe15, pro16, val270, thr6, leu18) involved in van der Waals interaction. Change of amino acid from HIS to ASP shows effect the structure and absence of hydrogen bond could see in a mutant type.

Still two more ligands Limonene and Vanillin remained to study, OR51B2 gene with Limonene ligand studied, where affinity binding was found to be -12.078 where his87, glu89, gly81, thr6, cys95 and thr78 were forming van der Waals interaction and the pocket site including, trp2, gly81, val85, ile90, ile82 and arg88 developed the pi-alkyl interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -10.996 affinity binding energy released during receptor ligand binding where amino acid phe192, leu200 and ile199 were forming hydrogen bonds and pro197, asn193, leu185, arg194, val198, arg160, pro156 and val157 were involved in van der Waals interaction, whereas glu181 involved in pi-anion bond while leu195 and pro198 forming covalent bond. Change of amino acid from HIS to ASP shows effect the structure and absence of hydrogen bond can be seen in a mutant type but some amino acid formed pi-anion bonds with limonene.

OR51B2 gene with Vanillin ligand shows affinity binding around -11.739 his87 and glu89 were forming hydrogen bond and the pocket site including, trp2, gln98, val85, pro77, thr78 and arg88 were involved in van der Waals interaction, and ile90 forming pi-sigma bond and gly81 involved in pi-alkyl interaction. Whereas, non-synonymous mutation (87: H/D) in OR51B2 shows -10.016 affinity binding energy released during receptor ligand binding where amino acid leu11, leu12, gly263, argala186, lys264, were involved in van der Waals interaction and tyr260 involved in pi-sigma bond while cys187 and pro9 and val266 forming pi-alkyl bond. Change of amino acid from HIS to ASP shows effect the structure and absence of hydrogen bond can be seen in a mutant type.

Overall, OR51B5 gene binding with Ligands BENZ, BUA, Butyrolactone, Limonene and Vanillin effects the structure while ACY, Butyl acetate, CIT, do not shows any considerable effects in the structure.

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