

## Research Article

# *In Silico* Structural, Functional, and Phylogenetic Analysis of Cytochrome (CYPD) Protein Family

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Cytochrome (CYP) enzymes catalyze the metabolic reactions of endogenous and exogenous compounds. The superfamily of enzymes is found across many organisms, regardless of type, except for plants. Information was gathered about CYP2D enzymes through protein sequences of humans and other organisms. The secondary structure was predicted using the SOPMA. The structural and functional study of human CYP2D was conducted using ProtParam, SOPMA, Predotar 1.03, SignalP, TMHMM 2.0, and ExPASy. Most animals shared five central motifs according to motif analysis results. The tertiary structure of human CYP2D, as well as other animal species, was predicted by Phyre2. Human CYP2D proteins are heavily conserved across organisms, according to the findings. This indicates that they are descended from a single ancestor. They calculate the ratio of alpha-helices to extended strands to beta sheets to random coils. Most of the enzymes are alpha-helix, but small amounts of the random coil were also found. The data were obtained to provide us with a better understanding of mammalian proteins' functions and evolutionary relationships.

## 1. Introduction

Cytochrome P450 (CYP) is a unique heme-containing protein that has always been proven as a centrepiece of the organisms' defense system against toxicants [1, 2]. Various compounds, such as medications, steroid hormones, car-

cinogens, and environmental toxins, are metabolized by these enzymes. [3]. The CYPs are thought to be active in xenobiotic detoxification processes and the biosynthesis of a variety of endogenous compounds [4, 5]. CYPs are classified primarily into two distinct forms: membrane-bound forms found in eukaryotes and soluble forms found



FIGURE 1: *CYP2D* gene tree showing minimum evolution among organisms.

in prokaryotes [6, 7]. CYPs are most functional in the liver, where the levels of metabolites, systemic drug alteration, and metabolic rate are excessive [8]. However, it has been reported that the level of drugs in plasma never correlates with therapeutic effect, especially when centrally acting drugs are involved [9].

Among different P450 subfamilies in humans, one of them is the Cytochrome P450 2D (*CYP2D*) located on human chromosome 22 [10], comprised of one functional gene (*CYP2D6*) and two pseudogenes (*CYP2D7P* and *CYP2D8P*) [11, 12]. The *CYP2D* family of mammalian enzymes is involved in the synthesis of 20–25 percent of structurally distinct common medications such as dextromethorphan, bufuralol, and antiarrhythmic blockers and antidepressants [13] and accounts for about 2–4% of hepatic CYPs. Moreover, the *CYP2D6* proteins in humans have great affinity with alkaloids [14]. Both pseudogenes have similar nucleotide sequences with the *CYP2D6* gene. However, presence at the first exon *CYP2D7P* is due to a single mutational frameshift, responsible for an immature downstream of stop codons. From these pseudogenes, alternative mRNAs have been monitored in the breast, lungs, and brain of humans [15, 16]. Though a functional transcript of *CYP2D7P* has been investigated in few members of the Indian population, no evidence of mRNA from *CYP2D8P* was observed [17]. Despite this, the organization of the *CYP2D* subfamily genes has been discovered in nonhuman primates such as the rhesus monkey, chimp, white tufted ear marmoset, and Sumatran orangutan. They found that *CYP2D7* originated from *CYP2D6* in a stem lineage of great apes and humans [5]. As

a result, the *CYP2D6* and *CYP2D8P* genes in the human genome originated from the Catarrhine and New World monkeys' stem lineage. [12].

The relationship among different human *CYPs* is scarce and cannot be generalized easily. The human *CYP* subtype homology modeling based on the crystal structure can provide valuable information about their structural and functional association [18]. A high degree of protein sequence similarities is not enough to evaluate these enzymes' different indistinctive activities. According to their respective substrates and inhibitors, various studies regarding the classification of these enzymes based on structural and pharmacophoric characteristics have been done [19, 20]. Substrate recognition sites (SRSs) in the *CYP2* family are vital for the catalysis of target substrates; various functional sites can be monitored with a 3D structure modeling approach in humans and compared with the homology of bacterial *CYPs* [21–23].

Due to the clinical importance, human *CYP2D6* proteins have been studied in various disciplines like structure biology, medicine, and pharmaco-genetics. Therefore, the genetic variability of *CYP2D6* in human populations was investigated extensively [24, 25]. Hence, due to its complex structure and single-nucleotide polymorphism (SNP), *CYP2D6* structural, functional, and evolutionary analyses have yet to be explored more to enhance the clinical implications. To meet this requirement, this study was designed to explore more insights into *CYP2Ds* through various bioinformatics tools, which would help in a rational drug design in the future.

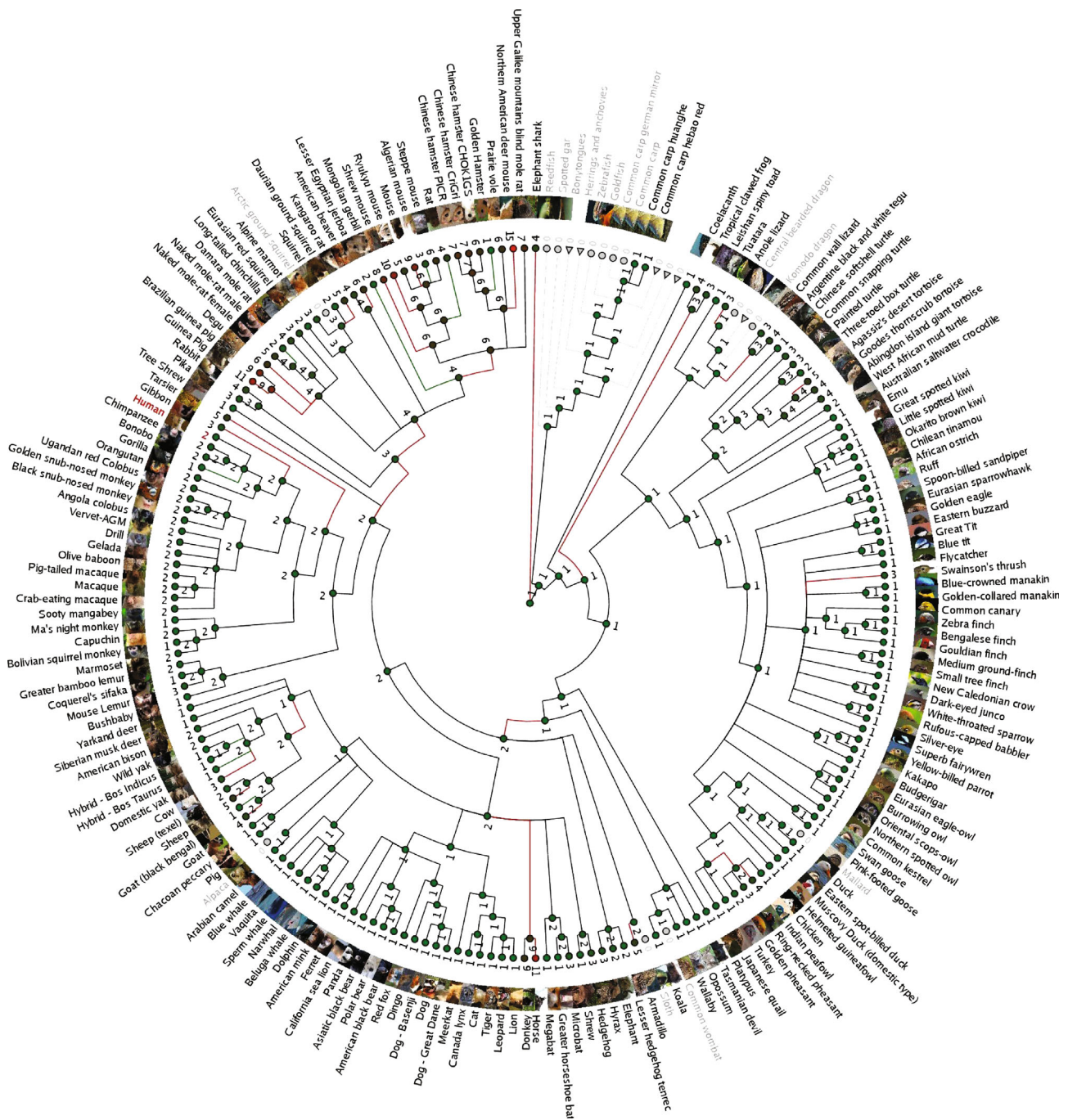


FIGURE 2: The Gene Gain/Loss Tree depicts the CYP2D gene family’s phylogenetic history by showing new gene additions and deletions.

## 2. Materials and Methods

**2.1. Exploration and Mining of Human and Mouse CYP2D Structure and Sequences.** The human CYP2D gene family’s amino acid sequence was searched from the Public Database of the National Center for Biotechnology Information (NCBI) <https://www.ncbi.nlm.nih.gov>, and the protein crystal structure was obtained from the Public Database of Protein Data Bank (PDB) <http://www.rcsb.org>. Further, we also searched the PubChem database for some structural proteins and ligands available at <https://pubchem.ncbi.nlm.nih.gov/search/search.cgi>.

**2.2. 3D Structure Prediction and Validation of Human CYP2D Genes.** The solved crystal structure of human and mouse protein CYP2D is not available in the PDB. Thus, the three-dimensional (3D) structures of CYP2D were predicted by homology modeling approaches. To prepare or predict precise 3D modeled structure of the target proteins, we employed several software programs, including Phyre2 [26], Swiss model [27], and iterative Threading assembly Refinement (I-TASSER) [28, 29], which works by multiple threading approaches through identifying the template from PDB. The 3D modeled target proteins were minimized by

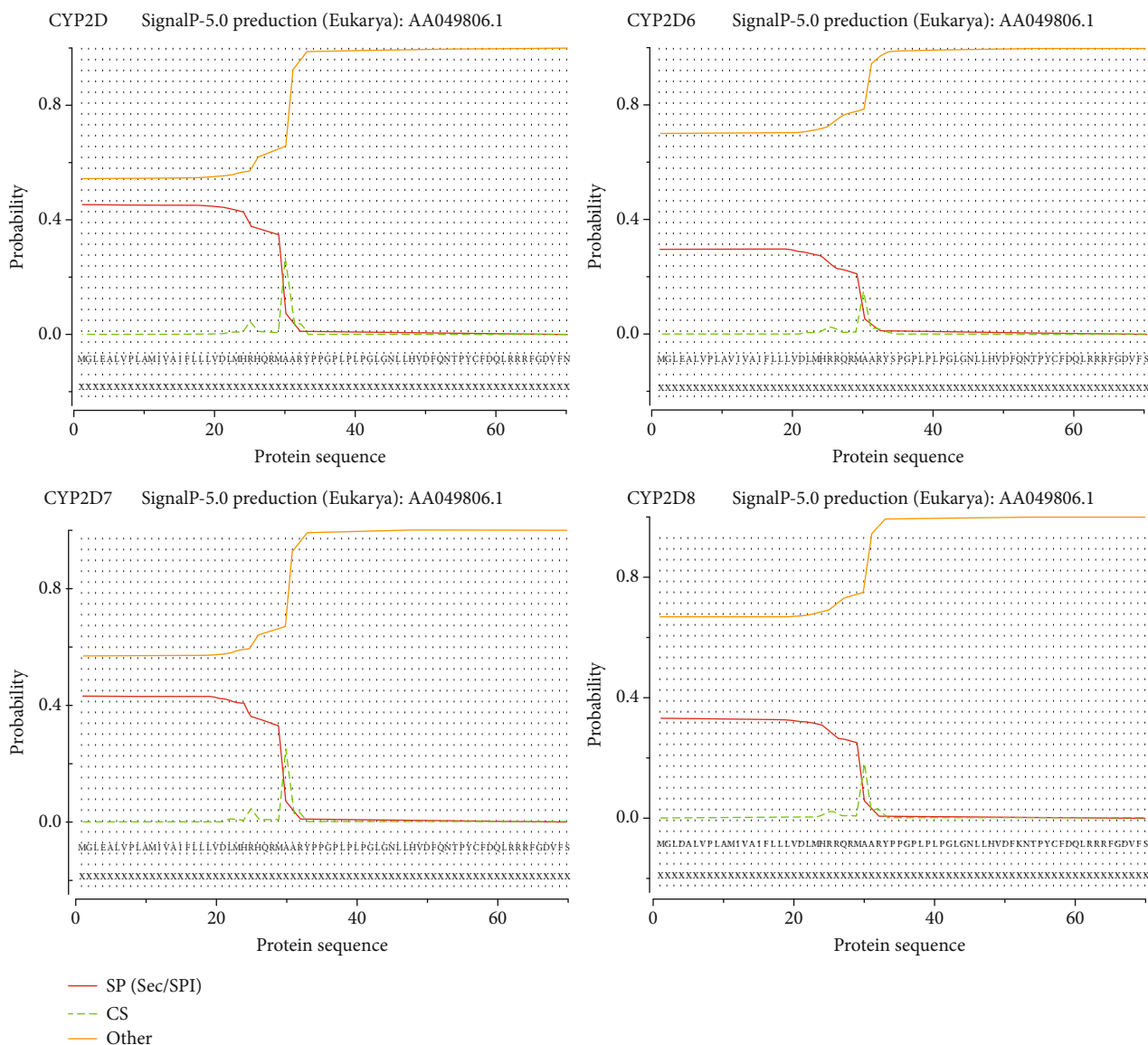


FIGURE 3: Signal peptide cleavage prediction by the SignalP 4.1 server. The signal peptide score and cleavage site score, cleavage site score, and cleavage site score are shown as combined scores (raw cleavage site score).

employing the conjugate gradient algorithm and the Amber force field in UCSF Chimera 1.10.1 [30]. Validation of the predicted protein structure remained bottlenecked problems. In our analysis, following 3D structure modeling, we used several protein structure validation tools, including protein structure analysis (ProSA), to identify errors in experimental and theoretical protein structure [31, 32]. The ProSA program language is intended to validate from the predicted atomic structure coordinates, and the results are evaluated by  $z$ -score value.

**2.3. Disordered Analysis of Human CYP2D Proteins.** Following the 3D homology modeling of the human and mouse CYP2D protein, we sought to identify the disordered protein segment or unstructured regions [33]. It is believed that these regions contribute to protein instability, thereby causing pathological conditions. To have precisely predicted unstruc-

tured protein segment of amino acid residues positions, we employed Cspritz version 1.2, <http://protein.bio.unipd.it/cspritz/> [34].

**2.4. Analysis of Human and Mouse CYP2D Protein Ligands and Domain.** In proteomics, understanding the structural property of the functional unit of protein is worthwhile. Thus, the protein-ligand, ligand-binding residue, ligand-binding regions, and domains of CYP2D were predicted by using several bioinformatics tools. Further, the protein ligands were clustered based on their functional similarity. RaportX predicted ligand-binding residues of the protein structures, a template-based robust 3D modeling web tool [35, 36] RaportX is known for its high-quality structural modeling output multiple targets by using one remote template. Other additional programs were used to crosscheck and validate the reliability and the accuracy of the predicted

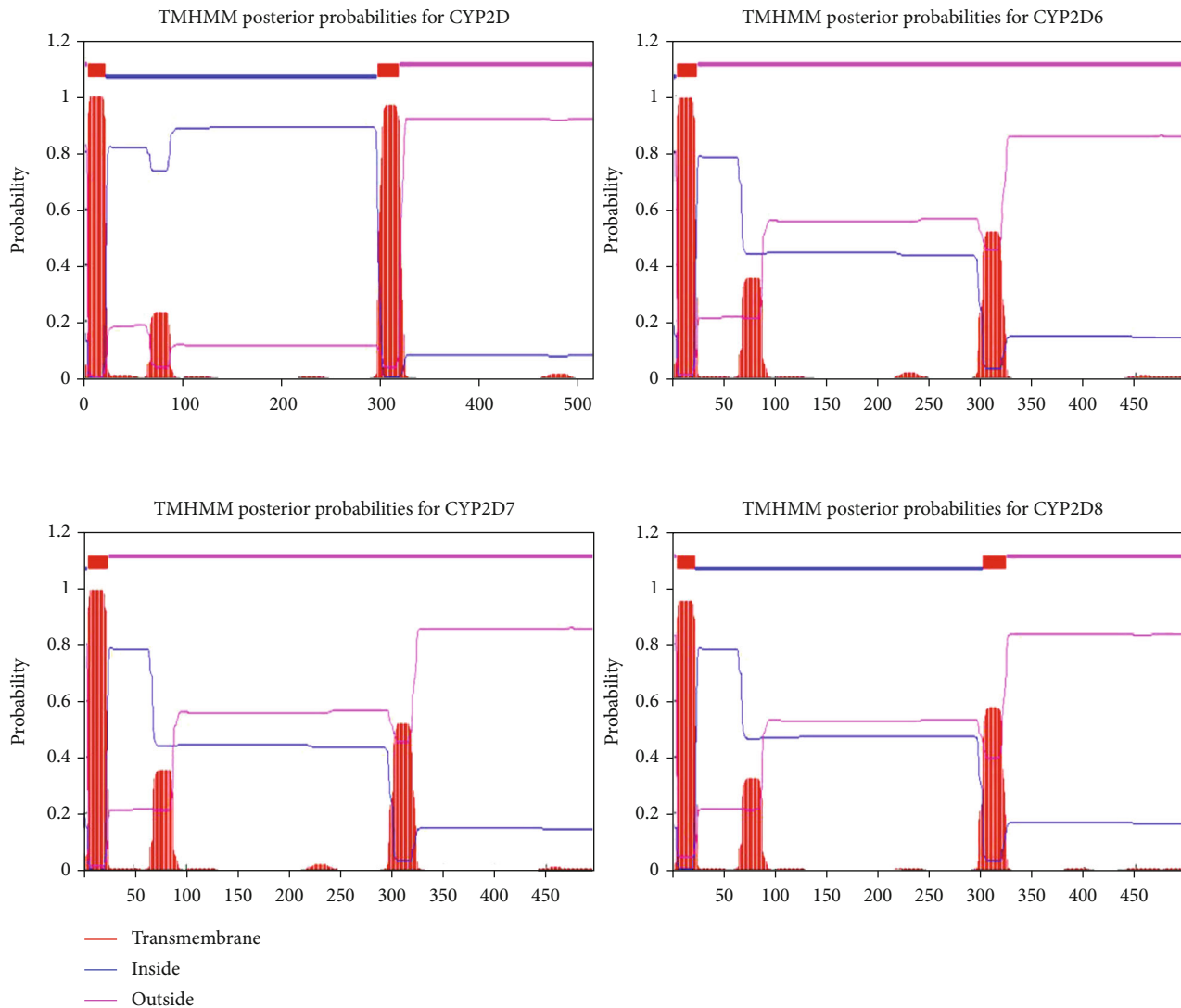


FIGURE 4: The prediction of transmembrane helices in *Phytophthora infestans* and other organisms by the TMHMM 2.0 server.

result, including COACH server [37], I-TASSER was used to determine the number of binding sites and their positions [28]. COACH is a metasearch approach for predicting ligand-binding targets through two comparative methods TM-SITE [28] and S-SITE. The BioLiP protein function database is used in these methods to recognize ligand-binding sites [38]. To understand the ligand-binding surface on nonbound of freely existing protein structure, we applied FTSite server ligand-binding prediction tools [39]. Its accuracy is reported to be comparable with experimental results [40]. The prediction principle of FTSite is template evolutionary-based; instead, it is based on evidence obtained from experiments [41]. Presumably, the prediction algorithm is designed for the mapping of protein regions [41]. To further understand the interaction between target protein's ligands and amino acids, ligand module clustering was done by LPIcom' web server [42] found at <http://crdd.osdd.net/raghava/lpicom>. Amino acid residues and ligand interactions were predicted using the LPIcom web server to classify residues' desired interaction and binding motif for a given ligand [43].

**2.5. Protein Interactions and Coexpression Analysis.** Protein-protein interaction analysis of human and mouse *CYP2D* was undertaken to assess the functional relationship and information flow networks among *CYP2D* and its neighbouring genes and other proteins. Protein-protein interactions are one of the physiological settings known to affect a multitude of biological functions. The interaction analysis was performed by STRING software [44] and visualized by commercial Cytoscape software [45]. The STRING software program considers both functional and physical interactions among proteins. The software algorithm was developed to capture the protein-protein interactions based on existing literature, experimental output, text mining, and cooccurrence [46].

**2.6. Predicting Consensus Sequence Alignment and Secondary Structure.** To understand the structural alignment of *CYP2D*, we employed ENDscript 2 open-access tool, which enables us to visualize multiple biochemical and structural alignments [47]. The web tool has been designed to recognize simple to

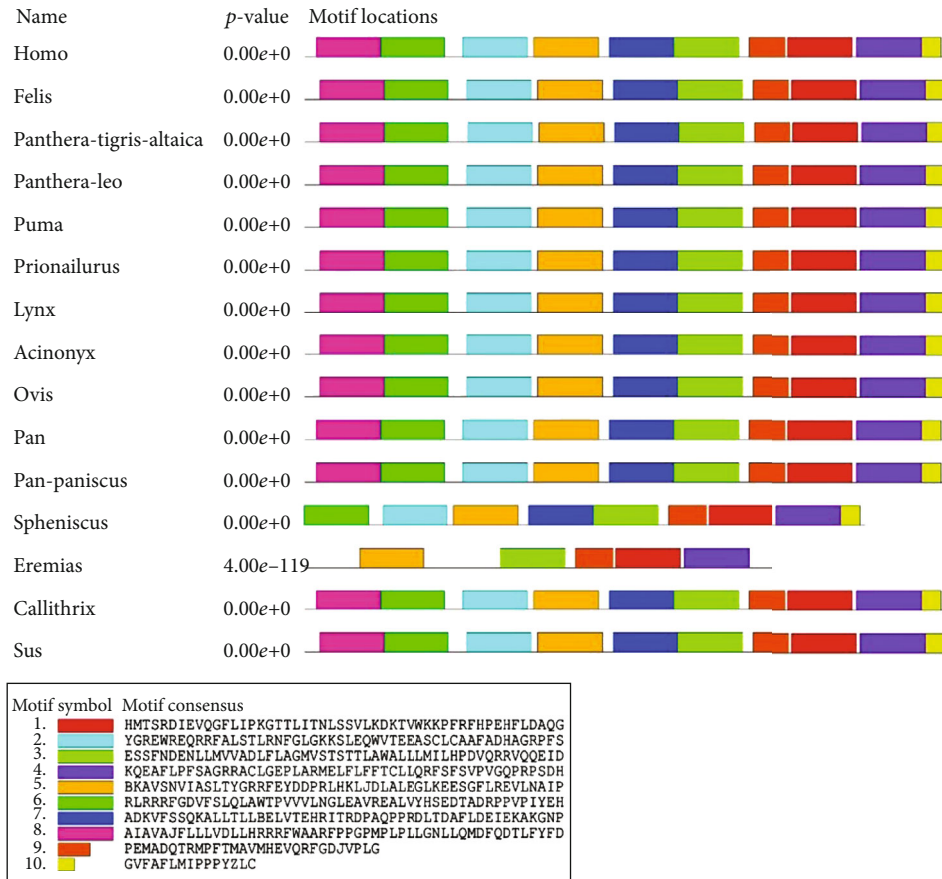


FIGURE 5: CYP2D motif distribution in the vertebrate species. Motifs of these genes from representatives of different species are predicted using the MEME suite based on amino acid sequences. Some conserved animal motifs have been found. The motifs are marked as grey boxes.

complex structures, i.e., primary to quaternary. Additionally, it utilizes the PDB input format and processes into several categories of outcomes that can be visualized in many structural interface tools. Further, the secondary structure of all target proteins was determined by online server PSIPRED version 3.3 [48], a web tool that combines protein sequences and structural analysis into one platform. Initially, the software utilizes protein sequence input data and performs PSI-BLAST [49].

### 3. Results

**3.1. Gene Cooccurrence and Evolutionary History.** Gene trees represent the evolutionary history of gene families that evolved from a common ancestor. Reconciliation of the gene tree with the species' tree enables us to ascertain speciation events by comparing their sequences' similarities and differences. In the case of unique orthologous genes, concordance between the best reciprocal best methods is quite evident. The Gene Tree pipeline can analyze the relationships between more complex gene trees that combine one-to-many and many-to-many. This greatly raises the number of Fly/Mammal and Worm/Mammal orthologues compared to the number of Mammal orthologues and has a much more drastic impact on the Fly/Mammal and Worm/Mammal orthologues. By determining the most recent ancestry of a

given internal tree node, we may also use this approach to "time" duplications of events (Figure 1). The tree merging algorithm is used to assemble a single consensus tree from the five trees. This enables TreeBeST to take advantage of the fact that DNA-based trees are often more accurate in determining relationships between distant parts of trees. Under some circumstances, a group of algorithms may outperform others.

The algorithm combines the output of the five models into a single model. The topology of consensus includes clades found in any input trees, minimizing the number of inferred losses and duplications and having the best bootstrap values. Because of the DNA alignment, branch lengths have been estimated according to the HKY model. The Gene Gain/Loss Tree depicts a gene family's phylogenetic history by showing new gene additions and deletions. A red branch on the tree represents a significant expansion of a gene's history, a green branch denotes a contraction, and a grey branch denotes no change (Figure 2). The numbers above each ancestral species refer to the number of distinct genes it once had. The color of each node reflects the gene's size, based on the number of people who have the gene. The species names are colored red, black, and grey to show the current genes of interest and the genes present in Ensembl (species with no current genes in this tree). Only a selected tree of humans, chimps, marmosets, mice, zebrafish, and zebra finches is

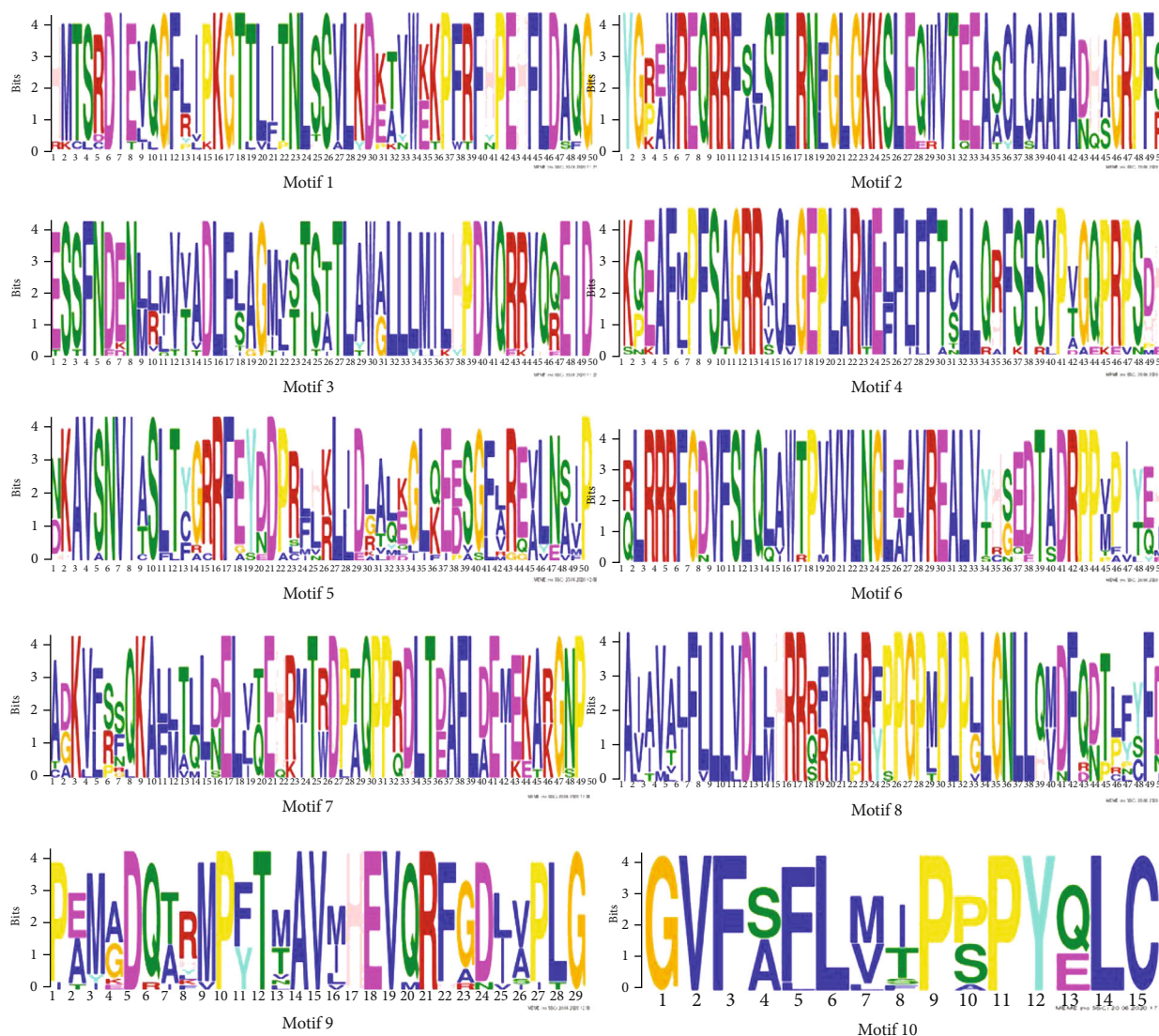


FIGURE 6: Sequence-specific MEME conserved motifs for *CYP2D* proteins.

displayed on this site. Be warned that the species of interest in these trees may not be included (Figure 2).

The SignalP 4.1 program was used to analyze the protein dataset, which revealed that the protein sequences are enzymatic proteins (Figure 3). Analysis of the cytochrome protein suggests that the amino acids from 1 to 26 have a *D*-score of 0.735 and an upper limit of 0.642 at the position. It is essential to note the *D*-score used in this study as it is used to measure the dissimilarity between signal and nonsignal peptides. In principle, N-terminal or amino-terminal signal peptides are known as short sequences that target and guide secretory proteins to the translocator and the ER. We used protein sequence representations according to the groups of amino acids and amino acids' properties with the TMHMM algorithm. We utilized the publicly available genome annotation parameters of TMHMM and compared them to our selected protein sequences. The possible observations in each state are collapsed into three possible states for polarity (nonpolar, polar, and neutral), three for the charge, three for aromaticity, three for size, and five for electronic properties. Once

the body absorbs unwanted proteins, they could be cleaved off from their original passenger protein sequence. Many proteins lacking the N-terminal signal peptide exist outside of cells. It can be exported from the cell without the use of a signal sequence. However, the other proteins analyzed were not found to have N-terminal signal peptides, which continued to pose a mystery on the matter (Figure 4).

**3.2. Motif Analysis.** The MEME (Multiple Em for Motif Elicitation) algorithms have been used to perform DNA and protein motif analysis. The sequences were compared, and similar sequence motifs were calculated (Figure 5). MEME tool identified the distribution of the motif in protein sequences. This combined high conservation patterns with motifs from one to five (Figure 6). All patterns in protein sequences of animal species have been observed. The homology of motifs in different species means that the structural gene characteristics differ concerning exon-intron relationships. These analyses showed that the variances in the sharing of motifs in these animal species'

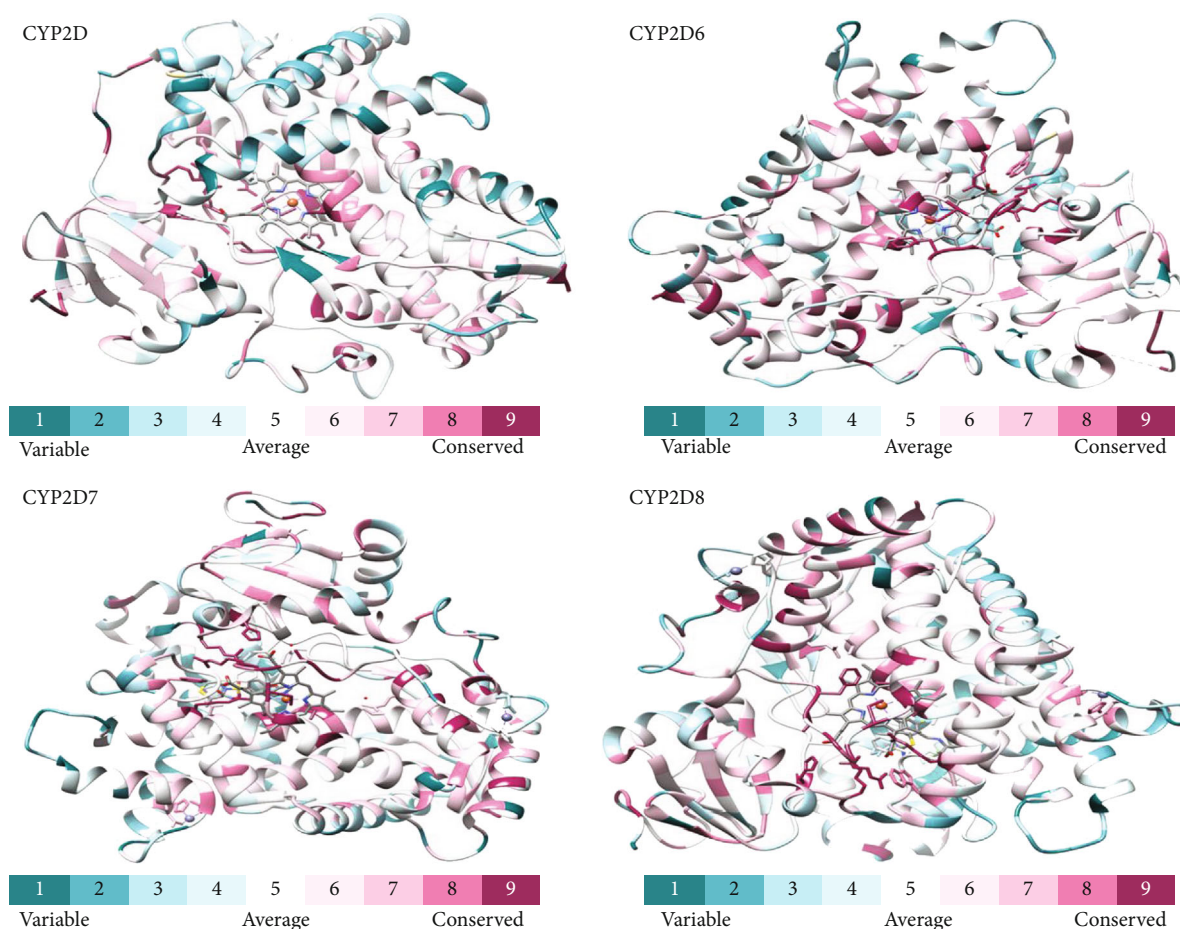


FIGURE 7: The crystal structure was determined using human protein as a reference species. The amino acids were predicted based on the value of conservation ranging from 1 to 9, with a value between 1 and 4 considered variable, a value of 5 and 6 average conservation, and a value between 7 and 9 highly conserved.

proteins might have deviated from those genes' functions during adaptive evolution.

The crystal protein structure was predicted by powerful online webserver I-TASSER (<https://zhanglab.ccmb.med.umich.edu/I-TASSER>), which usually utilizes the PDB template. The 3D protein structure of human cytochromes was predicted. After 3D modeling, UCSF Chimera was used in protein-energy by applying the Amber force field principle's conjugate gradient algorithm. We used the ConSurf server to predict nucleic acids' position and the level of evolutionary conservation of amino acids in these proteins to determine the degree of evolutionary conservation between bacterial strains in these two genes (Figure 7). The vast majority of sites positively selected throughout evolution have been maintained across the mammalian branches of evolution. Many amino acids were found to have residues visible or hidden in these proteins regarding the NNA (neural network algorithm).

Protein-protein interactions are a central part of the cellular network and are known to have various impacts. The information flow networks between all targeted proteins were analyzed to determine how much information flows between the cytochrome proteins and other proteins. A molecular-genetic interaction network was created using

online STRING software and visualized using Cytoscape software. Nodes, lines, and colors justify the interactive network. (Figure 8). Proteins whose genes are observed to be correlated in expression across a large number of experiments. The STRING performed a coexpression analysis database that showed coexpression scores based on RNA expression patterns and protein coregulation provided by ProteomeHD. Analysis of proteins signaling intensities was analyzed for cytochrome proteins. The result showed that more protein residues are involved in signal receiving as compared to signal communicating residues. The results are described by colors displayed on the predicted structures (Figure 9).

#### 4. Discussion

Bioinformatics can be instrumental in analyzing and interpreting the data of proteomics and genomics. It uses mathematical, statistical, computational, biological, and medical methods and technologies. It is a vital sophisticated tool for interpreting the protein functions from its amino acids sequence and has revolutionized organisms' metabolism. The P450 forms of humans and rats have always been considerable information for the best comparison of its structures





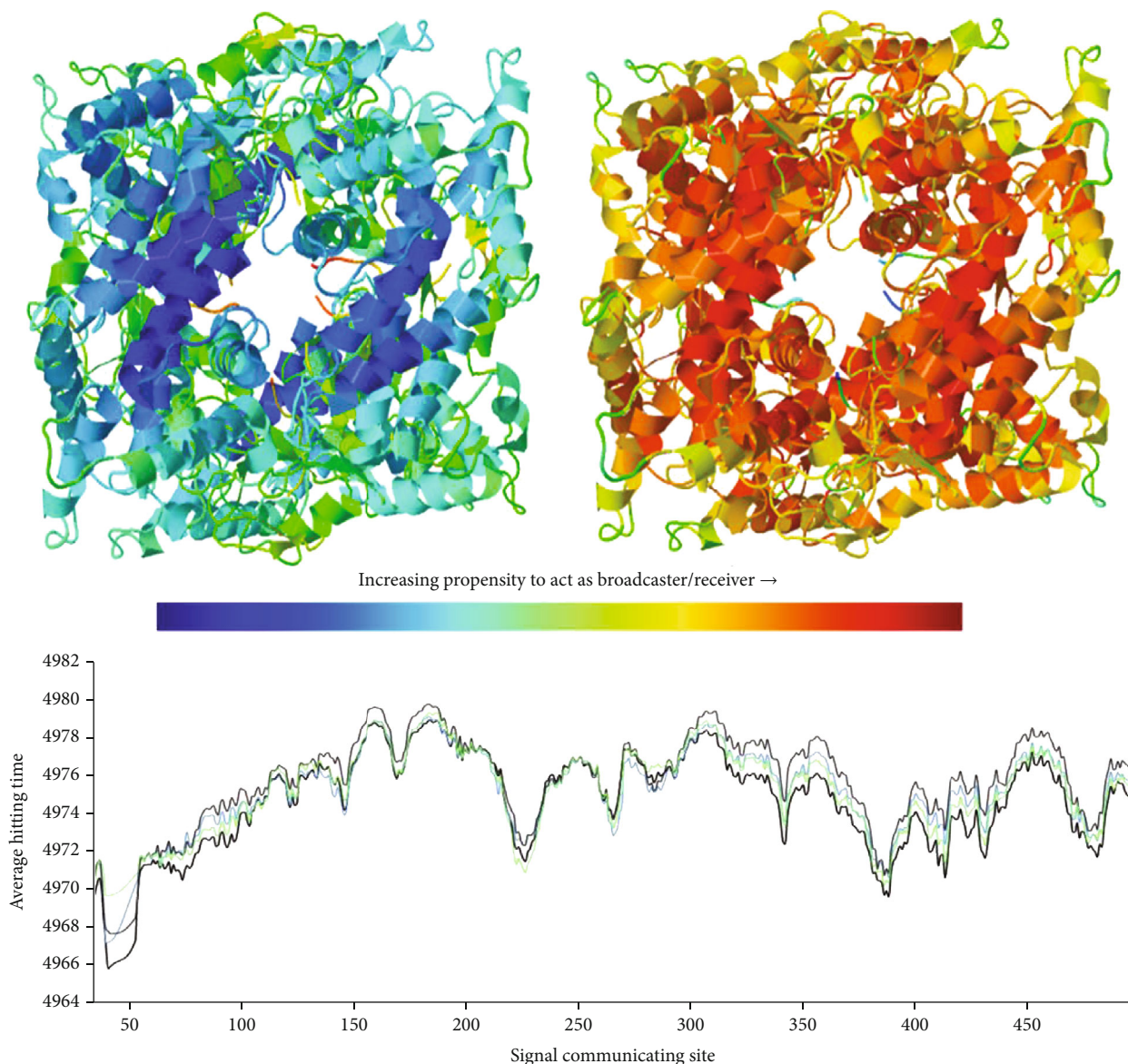


FIGURE 9: Predicted signal communication of *CYP2D* proteins. Signal communicating residue structure justified by blue color having less residue. The signal receiving residue structure is represented by the orange color with more residue. Signal communicating residue structure is explained by the blue color having less residue and signal receiving residue structure represented by the orange color with more residue.

(Figures 2 and 3). Most parts of the *CYPs* are alpha-helix and random coil, according to the results. Phyre2 server has predicted the tertiary structures of human *CYP2D* as a sample of other organisms. These findings have been very similar and supportive when *CYPs* were analyzed in different species like seed plants and other animal's protein sequences reported previously [50].

Substrate recognition sites (SRSs) in the *CYP2* family are necessary for the catalysis of substrates targeted. Various functional sites can be monitored with a 3D structure modeling approach in humans and compared with the homology of bacterial *CYPs* [1, 22]. Spectroscopic experiments showed that P450 active sites are variable in their flexibility and stability. According to one review report [3, 51, 52], all *CYPs* were different with different activities,

and their functional properties were substrate-dependent. The user can swap the active sites to get the desired function from *CYPs* [53]. Rowland et al. [54] reported the crystal structure of *CYP2D6* at the resolution of 3Å and noted that the primary structure of the *CYP2D6* is resampled with other *CYPs*.

Similarly, according to the previous study [22], they reported the reaction site centre for *CYP2D6* substrates based on the ligand-bound *CYP2C5* model. Besides, it was reported that the substrates of *CYP2D6* presented the essential nitrogen at a distance of about 5 to 7Å from the oxidation site and were also found interacting with Glu-216 Asp-301 [55]. The Rabbit *CYP2C5* crystal structure was used in *CYP2D6* homology modelling, which was present as Arg440 near the protein surface (Figure 4). Docking the FMN structure of

P450 reductase to the *CYP2D6* model, it was evident that Arg440 is a central cluster of amino acids basic residues [56].

According to one study, the ligand-binding characteristics of *CYP2D* isoforms *CYP2D1*–4 in rats and *CYP2D6* in humans were investigated. They measured IC<sub>50</sub> values of already known 11 *CYP2D6* ligands by using 7-methoxy-4-(aminomethyl) coumarin (MAMC) as substrate [57]. The crystallized rabbit *CYP2C5* based homology model was generated, and its capacity to reproduce binding sequences related to the substrates' metabolic orientation was maintained during direct molecular dynamics simulations [58]. The electrostatic potential of *CYP2D* and *CYP2D1*–4 active binding sites showed affinities towards ligands. Whereas the active sites residues and their binding pattern differences could help renationalize the IC<sub>50</sub> values of every ligand [59]. They concluded that *CYP2D2* was the most critical rat isoform (resembled with the human) in binding through IC<sub>50</sub> values of ligand [18]. According to Hasemann et al. [60], three enzymes, like the hemoprotein domain of P450<sub>BM-3</sub> and crystal structures of P450<sub>terp</sub> and P450<sub>cam</sub>, were compared. They reported that all enzymes' topology was quite similar; however, the substrate-binding region was highly variable. Similarly, compared to local differences in I helices, the heme-binding core structure was conserved well (Figure 7).

In this present study, five motifs were found in most mammalian species according to the conserved motifs obtained by MEME and MAST tools (Figures 5 and 6). Similar findings were reported previously [61]; they made a comparison among six major P450s in humans (*CYP3A4*, *CYP2D6*, *CYP2C9*, *CYP2C19*, *CYP1A2*, and *CYP2E1*) to monitor its structural, functional, and evolutionary relationships [50]. According to motif analysis, they suggested eight motifs across these six *CYPs* identified by MEME and interpreted by MAST tools. Among the six *CYPs*, the cytochromes 2C9, 2C19, and 2E1 possessed all eight motifs. However, *CYP2D6* was found lacking with the 8<sup>th</sup> number motif. Similarly, *CYP1A2* and *CYP3A4* were found lacking with 3<sup>rd</sup>, 6<sup>th</sup>, and 8<sup>th</sup> motifs (Figure 5). They concluded that *CYPs* (2C19, 2C9, 2E1, and 2D6) were similar and different from *CYP3A4* and *CYP1A2*. In this connection, Darabi et al. [50] analyzed *CYP* proteins to find conserved motif patterns using MEME and MAST tools. In a total of 39 different species, ten motifs were found highly conserved. However, motifs (1, 3, 4, and 7) were reported common in 35 species, and no common conserved motif was present in all, according to their results.

## 5. Conclusion

This study shows the mechanistic insights obtained from a comprehensive computational framework focused on evolutionary details and structure prediction. Bioinformatics methods used across fields such as computer science, mathematics, statistics, genetics, physics, and medicine can forecast and analyze genomic and proteomic results. It could be a novel way of interpreting protein structure, and it could revolutionize knowledge about an organism's metabolism. In humans and other mammals, these bioinformatics experiments have shown the structural-functional and evolutionary

understanding of *CYP2D*. Our findings suggest that the animal species' cytochrome protein family is very diverse, encompassing all species studied. Differences in cytochrome subunits are expected to be critical in deciding the unique substrates that allow this functional diversity.

## Data Availability

The data of this study will be available openly to readers, and data the data supporting the conclusions of the study can be accessible.

## Conflicts of Interest

There is no conflict of interest in the conduction of this study.

## Authors' Contributions

HIA, GA, and KM are responsible for the conceptualization; HIA and GA for the data curation; HIA and GA for the formal analysis; HIA, IA, SA, AA, and JH for the methodology; HIA, GA, SA, and KM for the software; MAK and ZK for the supervision; AJ, SK, AJ, and SK for the validation; HIA, GA, AA, and JH for the writing and original draft; and MAK, ZK, IA, SA, and SK for the writing, review, and editing.

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