Analysis of Cytochrome P51A1 gene, its Sequence Variants and Protein Domains

Shahid Raza[*] **and Hira Mubeen**
Department of Biotechnology, University of South Asia, Lahore, Pakistan
dr.raza03@gmail.com

Abstract

The cytochrome gene CYP51 is a member of the cytochrome P450 (CYP) gene super family and is mainly involved in synthesis of sterolby chemical reactions in certain plants and animals. CYP51 is commonly known across eukaryotes. The study is focused on functional analysis of human CYP51 gene, which is 22 kb in size with coding region consisting of 10 exons and present on chromosome number7 long arm position 7q21.2–q21.3. However, the comparison of the human CYP51 gene with other CYP gene families from different species shows 64 different CYP51 orthologues which are located at unique chromosomal positions. Computational analysis of CYP51 gene was performed to analyze its various orthologues, genetic variants, protein domains and signatures.

Keywords: Exons, Biosynthetic Pathway, Genetic Variant.

Introduction

CYP51A1 is an important cytochrome P450 enzyme, also called as Lanosterol 14 α-demethylase. It converts lanosterol to 4,4-dimethylcholesta-8(9)[1]. Most of the cytochrome P450 associated enzymes belongs to some conserved group of proteins. Such proteins plays an important role in biosynthesis of some lipids and vitamins and also in metabolism of many organic substances[2,3].

Some of the previous studies shown the cytochrome P450 CYP from mammals is part of super family. It encodes several enzymes which are involved in the metabolism of certain foreign chemicals and various different pollutants[4]. Several mutations were observed in CYP gene. These mutations are responsible for multiple inborn errors of metabolism leading to multiple diseases. It was observed from previous studies, gene nomenclature was simpler before arrival of complete genome and gene names are not affected due to position of gene on the chromosome. Therefore, previously the names were assigned only on the basis of sequence similarity and evolutionary relationships[5,6].

According to some studies, genes occur in the form of clusters including many similar genes and some pseudogenes. Also, genes in subfamilies are found to be clustered with genes of other subfamilies[6]. Pseudogenes are found to be mainly involved in gene conversion and recombination events. Pseudogenes are found as not functional with no important activity reported[7,8].

Studies shown the CYP51 functions to catalyze the demethylation of lanosterolin fungi and alters the permeability by providing the rigidity to plasma membranes like animals[9,10].

CYP51A1 Gene Function: The cytochrome P450 proteins are associated with some specialized enzymes like monooxygenases. They play an important role to catalyze many reactions which are mainly involved in regulation of drug metabolism and cholesterol synthesis. The CYP protein is located in endoplasmic reticulum. Various homologous genes are found in fungi, plants, and animals. All of these homologs indicate the cytochrome P450 genes as oldest gene. Multiple diseases are associated with this gene. Mostly, related to some regulatory pathways.

In this study, we have selected the CYP51A1 gene from Homosapiens. The gene was compared with various orthologues. The sequences variants and structural variations related to CYP51A1 gene were studied. The gene tree was also obtained to study its evolutionary pathways. Various protein domain were analyzed by using various bioinformatics tools.

Materials and Methods

All cytochrome P450 genes are named assymbol CYP, and particularly belong to cytochrome P450 gene family. Each gene is given a specific number according to its group, gene family and sub family. Similarly, the cytochrome P450 gene is part of group 51, belongs to subfamily A and gene 1. Hence, it is written as CYP51A1. The selected gene CYP51A1 was analyzed by using various bioinformatics tools.

Identification of Orthologues: Orthologues for CYP51 gene was obtained from ensemble software.

Sequence Variations: Variant analysis shown number of different gene variants.

Gene hierarchy Map: Gene hierarchy map was obtained from HGNC.

Gene Tree: Gene tree was obtained from ensemble software.

Interacting Proteins Network for CYP51A1 Gene: Gene cluster analysis was performed to analyze the network of protein clusters.

Analysis of CYP51A1 protein domain: Protein domains were also analyzed using conserved domain identifier and CDD from NCBI and ensemble.

Results and Discussion

Identification of Orthologues: The analysis of CYP51 gene indicates 64 orthologues with different ensemble ID's. Out of which few are given below.

Table-1
Shows Human CYP51 Orthologues

Organism	Gene	Ensemble ID
Mouse (Mus Musculus)	Cyp51	ENSMUSG00000001467
Chicken (Gallus Gallus)	Cyp51A1	ENSGALG00000009365
Horse (*Equuscaballus*)	CYP51A1	ENSECAG00000006455
Gorilla (*Gorilla gorillagorilla*	CYP51A1	ENSGGOG00000011496
Cow (*Bostaurus*)	CYP51A1	ENSBTAG00000001992

Sequence Variations: Sequence analysis shown number of different variations within entire sequences near 3'end. The

reported variations are listed below in Table-2 with their chromosomal location.

Various numbers of structural variations were found. Few are listed below in Table-3 with their type and subtype.

Table-2
Shows the sequence variations with chromosomal locations

SNP ID	Sequence	Type	Chromosome Position
rs6[543]	AGAGA(C/T)GGGGT	intron-variant	92,117,817 (-)
rs11459[543]	TTTG(C/T)TTTGG	UTR-variant-3'	92,112,873 (-)
rs1135217[543]	GAGAC(A/G)GGGT	UTR-variant-3'	92,112,657 (-)
rs2106170[543]	CAAGG(C/T)GGGCA	intron-variant	92,121,060 (+)
rs2106171[543]	ATAC(A/G)AAAAT	intron-variant	92,121,136 (+)

Gene hierarchy Map: Gene hierarchy map was obtained by using HGNC database. Map is given in Figure-1.
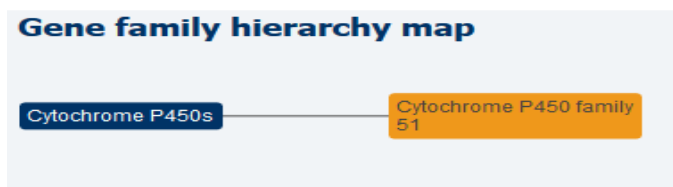

Figure-1
Shows the cytochrome P450 gene family map obtained from HGNC

Structure of Human CYP51 protein: The structure of CYP51 is found to be associated with various proteins which represent the complex with ketoconazole. The structure is shown in Figure-2.
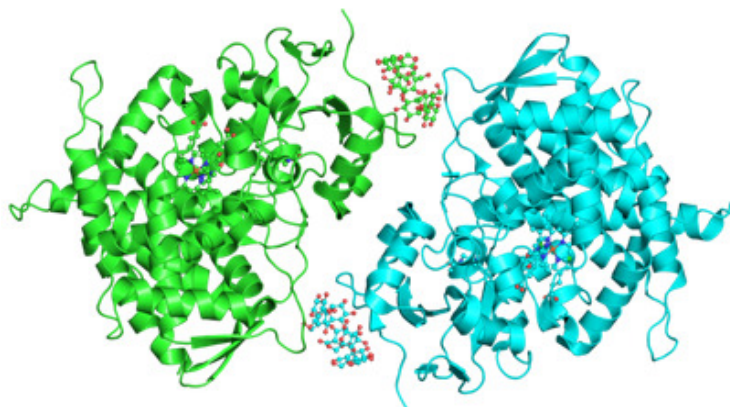

Figure-2
Shows the crystal representation of CYP51 protein obtained from InterPro

Gene Tree: The gene tree was obtained by using ensemble database. The human CYP51 gene was compared with its 64 reported orthologues. The ensemble gene ID assigned to CYP51 gene was ENSGT00800000124060.The tree shows 260 genes with 232 nodes of speciation with no split event. The results are shown in Figure-3.

Interacting Proteins Network for CYP51A1 Gene: The CYP51A1 gene was analyzed for studying protein network. Different string of clusters was found showing specific function with its associated protein in a network. The red cluster indicates the protein of humancytochrome P450 gene, which belongs to family 51, subfamily A, consisting of 509aa approximately. The results obtained from ProtoNet are shown in Figure-4.
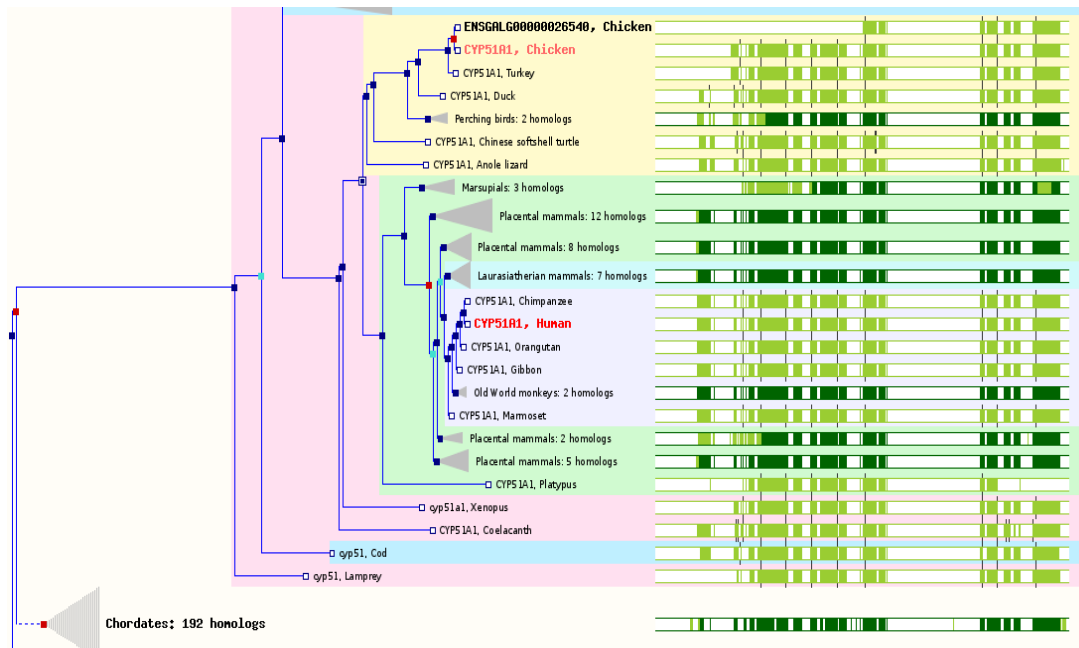

Figure-3
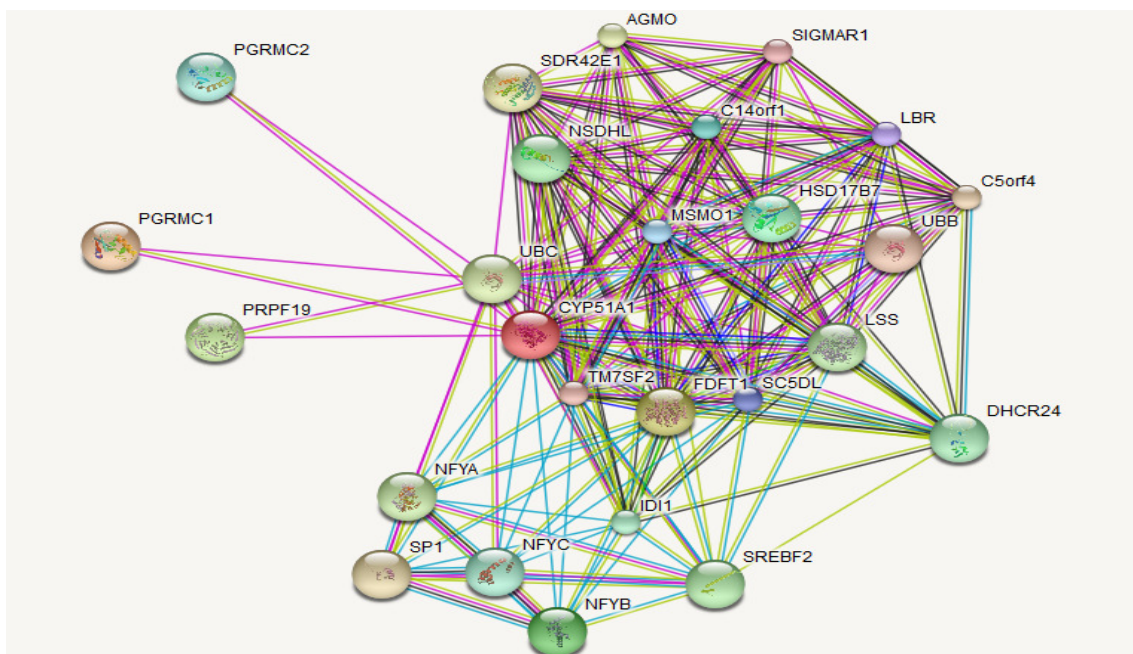**Shows the gene tree for CYP51 human gene and its orthologues obtained from ensemble software**

Figure-4
Shows the CYP51A1 gene cluster string in a protein network obtained from ProtoNet database

Figure-5
Shows the CYP51A1 protein domains with different families and patterns obtained from ensemble software

Analysis of CYP51A1 protein domain: Protein analysis shown number of putative domains with different protein signatures. The observed domains were taken from different databases like Pfam, Prints and PROSITE.

Discussion: The enzymes associated with Cytochrome P450 belongs to class of some liver cells and found commonly in some other bodycells. These enzymes play an important role in synthesis of some molecules and chemicals. Molecules like steroids are synthesized by these enzymes. Approximately, there are 60 CYP genes present in humans. Moreover, cytochrome P450 enzymes are mainly involved in synthesis and processing of protein and also in transport of various cells. Hence, genetic variations in cytochrome P450 genes can also affect the function of its associated enzymes. The effects of these genetic variations are seen frequently in the breakdown of various molecules. Therefore, drugs can be metabolized according to specific type of polymorphism.

Due to the importance of P450s in human physiology and drug metabolism, the genetic variations of these enzymes have been great importance. Many of these variations contribute to various differences in gene expression[11]. According to some studies, genetic polymorphisms including single nucleotide polymorphisms (SNPs) of CYP1, CYP2, and CYP3 family members, can be considered as an important candidate genes in pharmacogenetics. For example, sequence variations in CYP2D6 and CYP2C19 have been linked to the response and some adverse effects of various drugs used in the treatment of certain diseases[12-16]. According to previous studies, the regulatory mechanisms at transcriptional, translational, and post-translational levels related with genetic polymorphisms within P450 genes mainly contribute to the variability in P450 activities across various individuals[17,18].

Conclusion

This study provides broad insights into CYP51 gene regulation with sequence and structural variations. The gene analysis shown various orthologues among different species with specific functions. It was observed that all orthologues are located on different chromosomes. The gene clusters and putative protein domains were analyzed. It was found that the CYP cluster in humans is small, comprising of only four genes. Whereas in mouse it consists of 15 genes only. Studies shown the human CYP genes can recombine strongly due to many repetitive DNA sequences. Hence, computational analysis of CYP51 gene reveals many inner insights which will help to overcome gene silencing and also to avoid various diseases.

References

1. Wishart David S. (2007). HMDB: the Human Metabolome Database. Nucleic Acid Research, 35(Database issue), D521-D526.

2. Lepesheva Galina I., and Michael R. Waterman (2008). Sterol 14α-Demethylase Cytochrome P450 (CYP51), a P450 in All Biological Kingdoms. *BiochimBiophysActa.*, 1770(3), 467-77.

3. Daum G, Lees ND, Bard M and Dickson R. (1998). Biochemistry, Cell Biology and Molecular Biology of Lipids of Saccharomyces cervisiae. *Yeast.*, 14(16), 1471-1510.

4. Nebert DW and Russell DW (2002). Clinical importance of the cytochromes P450. *Lancet*, 360, 1155-1162.

5. Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ and Guengerich FP et al. (1987). The P450 gene superfamily. Recommended nomenclature. *DNA*, 6, 1-11.

6. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R and Waxman DJ et al. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, 6, 1-41.

7. Ranson R, Claudianos C, Ortelli F, Abgrall C, Hemingway J and Sharakhova MV et al. (2002). Evolution of supergene families associated with insecticide resistance. *Science*, 298, 179-181.

8. Hirotsune S, Yoshida N, Chen A, Garrett L, Sugiyama F, and Takahashi S et al. (2003). An expressed pseudogene regulates the messenger-RNA stability of its homologous coding gene. *Nature*, 423, 91-96.

9. Lepesheva GI and Waterman MR. (2004). CYP51 The Omnipotent P450. *Mol Cell Endocrinol.*, 215, 165-170.

10. Becher Rayko and Stefan G. R. Wirsel (2012). Fungal Cytochrome P450 Sterol 14α-demethylase (CY.P51) and Azole Resistance in Plant and Human Pathogens. *Applied Microbiology and Biotechnology.*, 95(4), 825-840.

11. Daly AK (2004). Pharmacogenetics of the cytochromes P450. *Curr Top Med Chem*, 4, 1733-1744.

12. Anderson JL, Carlquist JF, Horne BD and Muhlestein JB (2003). Cardiovascular pharmacogenomics: Current status, future prospects. *J CardiovascPharmacol*, 8, 71-83.

13. Van Schaik RH (2005). Cancer treatment and pharmacogenetics of cytochrome P450 enzymes. *Invest New Drugs*, 23, 513-522.

14. Vandel P, Talon JM, Haffen E and Sechter D (2007). Pharmacogenetics and drug therapy in psychiatry: The role of the CYP2D6 polymorphism. *Curr Pharm Des*, 13, 241-250.

15. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W and Braunwald E et al. (2009). Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*, 360, 354-362.

16. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N and Becquemont L (2009). Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*, 360, 363-375.

17. Aguiar M, Masse R and Gibbs BF (2005). Regulation of cytochrome P450 by posttranslational modification. *Drug Metab Rev*, 37, 379-404.

18. Lim YP and Huang JD (2008). Interplay of pregnane X receptor with other nuclear receptors on gene regulation. *Drug Metab Pharmacokinet*, 23, 14-21.