Computational Identification of the Paralogs and Orthologs of Human Cytochrome P450 Superfamily and the Implication in Drug Discovery

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Abstract: The human cytochrome P450 (CYP) superfamily consisting of 57 functional genes is the most important group of Phase I drug metabolizing enzymes that oxidize a large number of xenobiotics and endogenous compounds, including therapeutic drugs and environmental toxicants. The CYP superfamily has been shown to expand itself through gene duplication, and some of them become pseudogenes due to gene mutations. Orthologs and paralogs are homologous genes resulting from speciation or duplication, respectively. To explore the evolutionary and functional relationships of human CYPs, we conducted this bioinformatic study to identify their corresponding paralogs, homologs, and orthologs. The functional implications and implications in drug discovery and evolutionary biology were then discussed. GeneCards and Ensembl were used to identify the paralogs of human CYPs. We have used a panel of online databases to identify the orthologs of human CYP genes: NCBI, Ensembl Compara, GeneCards, OMA (“Orthologous MAtrix”) Browser, PATHER, TreeFam, EggNOG, and Roundup. The results show that each human CYP has various numbers of paralogs and orthologs using GeneCards and Ensembl. For example, the paralogs of CYP2A6 include CYP2A7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2F1, 2J2, 2R1, 2S1, 2U1, and 2W1; CYP11A1 has 6 paralogs including CYP11B1, 11B2, 24A1, 27A1, 27B1, and 27C1; CYP51A1 has only three paralogs: CYP26A1, 26B1, and 26C1; while CYP20A1 has no paralog. The majority of human CYPs are well conserved from plants, amphibians, fishes, or mammals to humans due to their important functions in physiology and xenobiotic disposition. The data from different approaches are also cross-validated and validated when experimental data are available. These findings facilitate our understanding of the evolutionary relationships and functional implications of the human CYP superfamily in drug discovery.
Keywords: human CYP; drug metabolism; paralog; homolog; ortholog; comparative genomics; bioinformatics

1. Introduction

The cytochrome P450s (CYPs) are a large heme-containing enzyme superfamily with a large number of members. They are found across all organisms from animals, plants, fungi, protists, bacteria, and Archaea to even viruses [1–3]. The CYP enzymes were first reported in 1958 since they displayed a Soret peak at 450 nm (therefore known as “P450” or “Pigment at 450 nm”). This featured peak is generated via a thiolate anion which serves as the fifth binding ligand to the heme moiety, and this unique peak is only found in P450s, chloroperoxidases, nitric oxide synthases, and protein H450 (all belong to the hemoprotein superfamily) [4–6]. CYPs are responsible for metabolizing numerous exogenous and endogenous compounds, including steroids, fatty acids, retinoids, clinical drugs, vitamins, procarcinogens/promutagens, and environmental compounds [7–9]. To date, at least 39,417 CYPs from 236 species have been reported, with 22,675 CYPs from 129 species of fungi (57.53%) and 16,742 CYPs from non-fungal species (42.47%). The CYP superfamily comprises a family, subfamilies, and individual members. As hemoproteins, CYPs often catalyze various modes of oxidative reactions such as hydroxylation, sulfoxidation, demethylation and dealkylation, deamination, dehalogenation, epoxidation, and peroxidation of their substrates [10]. In addition to these classical oxidative reactions, CYPs also catalyze some uncommon oxidative or reductive reactions of certain substrates such as oxidative cleavage of carboxylic acid esters, desaturation, 1- and 2-electron reductions, 1-electron oxidation, rearrangements of oxidized eicosanoids, deformylation of aldehydes, ring formation and aldoxime dehydration, etc.

The majority of CYPs are mixed function oxidases or monooxygenases, and electrons needed for reduction of the heme, and subsequently the oxygen substrates are provided by special partners [11,12]. For example, all microsomal CYPs are able to transfer electrons from nicotinamide adenine dinucleotide phosphate (NADPH) as the donor via cytochrome P450 reductase while cytochrome b₅ is a ubiquitous electron carrier which is reduced by cytochrome b₅ reductase (also called methemoglobin reductase) [11–15]. On the other hand, mitochondrial CYPs employ adrenodoxin reductase and adrenodoxin to transfer electrons from NADPH to the enzyme [16]. However, CYP5A1 (called thromboxane X₂ synthase, TBXAS1), CYP8A1 (called prostacyclin H₂ synthase, PTGIS), and CYP74A (called allene oxide synthase) do not require a protein partner for their enzymatic catalytic reactions [17]. The catalytic cycle by CYPs is similar and complicated, with the heme prosthetic group acting as the catalytic center of the enzyme. Upon initiation of the CYP-mediated catalytic reaction, a hypervalent oxo-iron protoporphyrin IX radical cation is produced, facilitating subsequent insertion of the iron-bound oxygen into a substrate bond. When the thiolate side chain of a conserved Cys attaches to the iron molecule opposite to the bound oxygen, the substrate molecule moves in and binds with multiple residues in a cavity (active site) above the heme moiety near the reactive radical cation. Although CYPs share a catalytic mechanism, they show remarkable differences in substrate specificity, regio-, and stereo-selectivity of metabolic reaction, and inhibition by molecules [18].

The gain and loss events are not evenly distributed among the CYP genes and CYP genes and pseudogenes are often present as clusters in the genome of organisms. Continuing and consecutive tandem gene duplication including unequal crossover, transposable element-induced non-allelic recombination, chromosome duplication, and retroposition may result in relatively large CYP clusters on some chromosomes, which often represent remarkable landmarks of the CYPomes in organisms. For instance, the mouse cluster of Cyp2 genes on chromosome 7 carries 12 functional genes and 10 pseudogenes [19]. The largest ancestral CYP6A cluster in fruit flies contains 8 CYP6A genes and CYP317A1 at 51D region of the right arm of chromosome 2 [20]. Among these genes, CYP6A17-21 is coordinately regulated by the circadian rhythm [21]. In the malaria vector Anopheles gambiae,
the largest CYP gene cluster contains 14 CYP6 genes and another cluster carries 12 CYP325 genes and 2 pseudogenes [22]. In mosquito genomes, there are 16 CYP gene clusters consisting of ≥4 genes while 22 CYP genes are present as singletons [22]. Rats contain 14 CYP2 genes and 4 pseudogenes and humans carry 6 CYP2 genes and 7 pseudogenes in the same cluster. The human CYP2C cluster located on chromosome 10 consists of only 4 functional genes, but it has expanded to 15 functional genes on chromosome 9 (Cyp2c29, 2c37-2c40, 2c44, 2c50, 2c54, 2c55, and 2c65-2c70) in mice. In the rat genome, the Cyp2c cluster on chromosome 1q contains 13 functional genes, including Cyp2c6/2c37, 2c7/2c39, 2c11, 2c12/2c40, 2c13/2c38, 2c22-2c24, 2c26, 2c29, 2c66, 2c79/2c65, and 2c80/2c55.

Human CYP members play an important role in oxidizing a number of endogenous and exogenous compounds, including >80% of clinically used drugs and physiologically important substrates such as retinoids and fatty acids [8,23]. According to the current human genome GRCh38.p6 assembly (Genome Reference Consortium Human Build 38 patch release 6) released on 23 December 2015, there are 57 functional CYP genes and 58 pseudogenes distributed into at least 18 families and 43 subfamilies. CYP1, 2, 3, and 4 families play a major role in the oxidative metabolism of most drugs while other CYP families are responsible for the metabolism of several groups of important endogenous compounds including steroids, fatty acids, and retinoids [7,8,24–26]. The relative abundance of human hepatic CYPs is as: CYP1A1 (<1%), 1A2 (4.4%–16.3%), 2A6 (3.5%–14%), 2B6 (1.7%–5.3%), 2C8 (~7.5%), 2C9 (4.5%–29%), 2C19 (0.9%–3.8%), 2D6 (1.3%–4.3%), 2E1 (5.5%–16.5%), 2J2 (~1%), 3A4 (14.5%–37%), and 3A5 (~1%) [27]. A study has shown that 66% of the 110 drugs examined are metabolized by one or more CYPs, and 44% of these drugs are metabolized by CYP3A4, 41% by 2D6, 26% by 2C19, 9% by 1A2, and 4% by 2C9 [28]. As functionally important enzymes, CYPs have been well studied in humans, mice, and rats, but data are limited in other species. It is important to identify suitable animal models for CYP studies in drug discovery and toxicological studies since the data can be readily extrapolated to humans with reasonable accuracy, although there are marked differences in the anatomical, biochemical, and physiological characteristics between these animals and human beings.

A multigene family or superfamily may arise from gene duplication and amplification, expression of overlapping genes, exon shuffling, programmed frame shifting, alternative RNA splicing, and gene sharing (also called “protein moonlighting” where the same protein fulfills at least two strikingly different functions) [29–33]. Exon shuffling is a molecular process through which two or more exons from distinct genes are recombined ectopically, or the same exon can be duplicated to produce a novel intron-exon structure. This process may be transposon-mediated, or it can arise from crossover during meiosis and recombination between non-homologous or short homologous DNA sequences. Gene duplication/amplification results from ectopic homologous recombination, aneuaploidy, whole genome duplication, retrotransposition, and replication slippage (also called slipped-strand mispairing resulting in either a trinucleotide or dinucleotide expansion or contraction during DNA replication) [34,35]. Based on currently available molecular genetic evidence from comparative genomic and phylogenetic studies, it is speculated that modern CYPs stem from an ancestral gene that was present ~3.5 billion years ago before eukaryotes appeared and an oxygen-rich atmosphere existed [1,3,36,37]. The CYP superfamily may have undertaken repetitive rounds of expansion via gene duplication events [1,3,36–38]. The first gene expansion might occur ~1.5 billion years ago, resulting in several CYP families such as CYP4 and 11 that primarily metabolized endogenous cholesterol and fatty acids. Another CYP gene expansion might occur ~900 million years ago (Mya), giving rise to several CYP families such as CYP19, 21, and 27 that are responsible for steroid biosynthesis. Around 400 Mya, there was a striking expansion of CYP genes, resulting in several CYP families (e.g., CYP2, 3, 4, and 6) that participated in xenobiotic metabolism. These CYP genes are rapidly evolving since the cells need protection from the damage when exposed to increasing toxic xenobiotics [3,36,37]. A well-defined distinction between orthologs and paralogs is important for the robust evolutionary classification of CYP genes and it is also critical for making reliable functional annotation of newly sequenced genomes from various organisms. Orthologs and paralogs are homologous genes arising from speciation or duplication event, respectively. Orthologs are
generally considered to preserve equivalent or similar functions across different organisms [39,40].

A function-oriented group of orthologous genes comprises orthologs that retain the same functions in different species and recently evolved paralogous genes that have the same biological activities (also called “in-paralogs”) [41]. Construction of orthologous groups is essential when conducting phylogenetic and comparative genomic studies and transferring verified annotations to newly sequenced genomes in other species [42]. In addition, identification of orthologous genes across different species can facilitate delineation of the gene genealogy to probe the driving forces and mechanisms through which to generate orthologous genes. On the other hand, pseudogenes are produced during evolution of genomes, which are characterized by a combination of homology to a known functional gene and lack of functionality (inability to code a protein) due to premature stop codons and frameshifts [43–45]. In human genome GRCh38.p5 assembly, there are 14,453 pseudogenes including 58 CYP pseudogenes. Vertebrate genomes typically contain 57–120 CYP genes. For example, Rhesus monkeys have 114 CYP genes, pigs contain 58 CYP genes, dogs embrace 48 CYP genes, mice carry 102 Cyp genes and 88 pseudogenes, rats retain 89 Cyp genes and 79 pseudogenes, and zebrafish carries 81 Cyp genes. Caenorhabditis elegans contains 83 Cyp genes and fruit flies retain 84 CYP genes and 6 pseudogenes. Oryza sativa (rice) carries 457 Cyp genes and Arabidopsis thaliana contains 272 Cyp genes. To further explore the evolutionary and functional relationships of human CYPs, we conducted this study to identify their corresponding paralogs, homologs, and orthologs. The data from different approaches were cross-validated and validated when experimental data are available. Finally, the implications in drug discovery and toxicological studies were then briefly discussed.

2. Results

2.1. Alignment of 57 Human CYPs

The alignment of the 57 human CYP proteins has identified four conserved amino acids, namely Glu242, Arg245, Phe310, and Cys316 (Figure 1A). Phe310 and Cys316 are located near the heme-binding region and so play an important role in the catalysis. Cys316 is near the iron ion in the heme-binding region, acting as a critical thiolate ligand in the active site of CYPs. Glu242 and Arg245 located about 80 amino acids upstream from the proximal Cys316 may also play a role in enzymatic catalysis.

![Figure 1. Cont.](image-url)
Figure 1. (A) Alignment of 57 human CYP proteins which are retrieved from Swiss-Prot. Multiple sequence alignment of human CYPs is carried out using Clustal W v2.0; (B) The phylogenetic tree of human CYPs which can infer the evolutionary relationships among human CYPs; (C) MEME (Multiple EM for Motif Elicitation) version 4.10.1 is employed to identify important conserved motifs present in human CYP proteins.
Next, we constructed the phylogenetic tree of human CYP genes (Figure 1B), in which each monophyletic group was reinforced by a relatively high bootstrap value. In this phylogenetic tree, an ancestral gene of CYP17A1 and 21A1 seemed to be duplicated, resulting in the ancestor of the CYP1 and 2 families. CYP3 and 5 families appeared to have a common ancestor, while CYP3 family appeared to be the ancestors of CYP4 family. The ancestors of the CYP1, 2, 3, 4, 11, and 26 families were generated by gene duplication events of CYP11A1 and 11B1. Furthermore, CYP4V2 was formed from the duplication of the ancestors of CYP46A and 22A, which was then duplicated to generate the whole CYP4 family.

Finally, we searched the conserved motifs in the human CYP proteins by sequence alignment (Figure 1C). We have identified at least 3 well conserved motifs in human CYPs: “FXXGXRXCXG” located in the heme-binding domain, “AGXDTT”, and “EXXR” located in helix K in C-terminal. The terminal Thr residue in “AGXDTT” is involved in the formation of the enzyme’s critical oxygen-binding pocket, and “EXXR” can interact with the loop of ~14 amino acid C-terminal. These motifs are functionally essential for the enzymatic activity.

2.2. The Paralogs, Homologs and Orthologs of CYP1A1, 1B1, 1A2, 17A1, and 21A2

The human genome contains three functional CYP1 genes including CYP1A1, 1A2, and 1B1 and one pseudogene CYP1D1P/1A8P. CYP1D1 became a pseudogene in human and cattle due to five nonsense mutations in the putative coding region; however, several other mammals including chimpanzee, Rhesus monkey, and cynomolgus monkey possess a functional CYP1D1. CYP1D1 is also conserved in the zebrafish, frog, Magnaporthe oryzae (M. oryzae), and rice. In cynomolgus monkey, CYP1D1 is 95% identical to human CYP1D1 sequence and is mainly expressed in the liver, kidney, and jejunum. Cynomolgus monkey CYP1D1 heterologously expressed in E. coli catalyzes ethoxyresorufin O-deethylation and caffeine 8-hydroxylation, which human CYP1A1/1A2 also catalyze. Based on both GeneCards 4.1.1 and Ensembl 84, CYP17A1 and 21A2 are the paralogs of CYP1A1, 1A2 and 1B1 (Figure 2 and Table 1).

CYP1A1 is primarily distributed in extrahepatic tissues, but it is involved in the metabolism of a number of clinical drugs (e.g., acetaminophen, caffeine, propranolol, and theophylline), certain procarcinogens (e.g., polycyclic aromatic hydrocarbons and aristolochic acid), toxicants and environmental compounds, and some endogenous substrates such as arachidonic acid (AA) and 17β-estradiol. The gene is highly inducible and mainly expressed in fetal liver. It has a low level in adult liver, lung, skin, intestine, skin, and gallbladder. In NCBI HomoloGene 68, CYP1A1 has 14 homologenes in 11 species, including chimpanzee, Rhesus monkey, dog, mouse, rat, Xenopus, zebrafish, etc. Based on NCBI Annotation Pipeline, 98 organisms have orthologs with CYP1A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP1A1 has 71 orthologs from 63 species of chordates including 11 species of non-human primates, 7 species of rodents, 12 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 2 and Table S3). In GeneCards version 4.1.1, CYP1A1 has orthologs in 15 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4). Similar results with remarkable differences are observed with OMA, PANTHER, and EggNOG (Tables S5–S7).
Figure 2. Cont.
Figure 2. Gene tree for human CYP1A1, 1A2, 1B1, 17A1, and 21A2 built using Ensembl 84. These five genes are paralogs to each other derived from the same ancestral gene via duplication events. The gene tree includes a total of 537 genes from various species. The total number of speciation nodes is 370, and the number of duplication is 143. The number of ambiguous nodes is 21, and the number of gene split events is 2.

Table 1. A list of 57 human functional CYP genes and their corresponding paralogs based on Ensembl 84 and GeneCards 4.1.1.

| Gene | Chromosomal Location | Substrates/Function | Number of Amino Acids | Paralogs by Ensembl 84 | Paralogs by GeneCards 4.1.1 |
|------|----------------------|---------------------|-----------------------|------------------------|-----------------------------|
| CYP1A1 | 15q24.1 | Drugs, procarcinogens, steroids, and fatty acids | 512 | CYP1A/18, 17A1 and 21A1 |
| CYP1A2 | 15q24.1 | Drugs, fatty acids, and steroids | 516 |
| CYP1B1 | 2p22.2 | Drugs, procarcinogens, steroids, and fatty acids | 543 |
| CYP2A6 | 19q13.2 | Drugs and steroids | 494 |
| CYP2A7 | 19q13.2 | Unknown (orphan) | 494 |
| CYP2A13 | 19q13.2 | Drugs and other xenobiotics | 494 |
| CYP2B6 | 19q13.2 | Drugs, steroids and fatty acids | 491 |
| CYP2C8 | 10q23.33 | Drugs, steroids and fatty acids | 490 |
| CYP2C9 | 10q24 | Drugs, steroids and fatty acids | 490 |
| CYP2C18 | 10q24 | Drugs, steroids and fatty acids | 490 |
| CYP2C19 | 10q24.1-q24.3 | Drugs | 490 |
| CYP2D6 | 22q13.1 | Drugs | 497 |
| CYP2E1 | 10q26.3 | Drugs, ethanol, and procarcinogens | 493 |
| CYP2F1 | 19q13.2 | Drugs and coumarins | 491 |
| CYP2J2 | 1p31.3-p31.2 | Fatty acid (e.g., AA) | 502 |
| CYP2R1 | 11p15.2 | Vitamin D | 501 |
| CYP2S1 | 19q13.1 | Xenobiotics | 504 |
| CYP2U1 | 4q25 | AA, DHEA, and long chain fatty acids | 544 |
| CYP2W1 | 7p22.3 | Unknown | 490 |
| CYP3A4 | 7q21.1 | Drugs, steroids and fatty acids | 503 |
| CYP3A5 | 7q21.1 | Drugs, steroids and fatty acids | 503 |
| CYP3A7 | 7q21-q22.1 | Drugs, steroids and fatty acids | 503 |
| CYP3A43 | 7q21.1 | Low level of testosterone 6β-hydroxylase activity | 503 |
| CYP4A11 | 1p33 | Medium-chain fatty acids such as laureate and myristate | 519 |
| CYP4A22 | 1p33 | Unknown (orphan) | 519 |
| CYP4B1 | 1p33 | Xenobiotics, steroids and fatty acids | 511 |
| CYP4F2 | p13.12 | Eicosanoids | 520 |
| CYP4F3 | 19p13.2 | Eicosanoids (e.g., LTB4) | 520 |
| CYP4F8 | 19p13.1 | Eicosanoids | 520 |
| CYP4F22 | 19p13.1 | Unknown (orphan) | 524 |
| CYP4F22 | 19p13.12 | Unknown (orphan) | 531 |

CYP2 members excluding CYP2D7

Gene tree for human CYP1A1, 1A2, 1B1, 17A1, and 21A2 built using Ensembl 84. These five genes are paralogs to each other derived from the same ancestral gene via duplication events. The gene tree includes a total of 537 genes from various species. The total number of speciation nodes is 370, and the number of duplication is 143. The number of ambiguous nodes is 21, and the number of gene split events is 2.

Table 1. A list of 57 human functional CYP genes and their corresponding paralogs based on Ensembl 84 and GeneCards 4.1.1.

| Gene | Chromosomal Location | Substrates/Function | Number of Amino Acids | Paralogs by Ensembl 84 | Paralogs by GeneCards 4.1.1 |
|------|----------------------|---------------------|-----------------------|------------------------|-----------------------------|
| CYP1A1 | 15q24.1 | Drugs, procarcinogens, steroids, and fatty acids | 512 | CYP1A/18, 17A1 and 21A1 |
| CYP1A2 | 15q24.1 | Drugs, fatty acids, and steroids | 516 |
| CYP1B1 | 2p22.2 | Drugs, procarcinogens, steroids, and fatty acids | 543 |
| CYP2A6 | 19q13.2 | Drugs and steroids | 494 |
| CYP2A7 | 19q13.2 | Unknown (orphan) | 494 |
| CYP2A13 | 19q13.2 | Drugs and other xenobiotics | 494 |
| CYP2B6 | 19q13.2 | Drugs, steroids and fatty acids | 491 |
| CYP2C8 | 10q23.33 | Drugs, steroids and fatty acids | 490 |
| CYP2C9 | 10q24 | Drugs, steroids and fatty acids | 490 |
| CYP2C18 | 10q24 | Drugs, steroids and fatty acids | 490 |
| CYP2C19 | 10q24.1-q24.3 | Drugs | 490 |
| CYP2D6 | 22q13.1 | Drugs | 497 |
| CYP2E1 | 10q26.3 | Drugs, ethanol, and procarcinogens | 493 |
| CYP2F1 | 19q13.2 | Drugs and coumarins | 491 |
| CYP2J2 | 1p31.3-p31.2 | Fatty acid (e.g., AA) | 502 |
| CYP2R1 | 11p15.2 | Vitamin D | 501 |
| CYP2S1 | 19q13.1 | Xenobiotics | 504 |
| CYP2U1 | 4q25 | AA, DHEA, and long chain fatty acids | 544 |
| CYP2W1 | 7p22.3 | Unknown | 490 |
| CYP3A4 | 7q21.1 | Drugs, steroids and fatty acids | 503 |
| CYP3A5 | 7q21.1 | Drugs, steroids and fatty acids | 502 |
| CYP3A7 | 7q21-q22.1 | Drugs, steroids and fatty acids | 503 |
| CYP3A43 | 7q21.1 | Low level of testosterone 6β-hydroxylase activity | 503 |
| CYP4A11 | 1p33 | Medium-chain fatty acids such as laureate and myristate | 519 |
| CYP4A22 | 1p33 | Unknown (orphan) | 519 |
| CYP4B1 | 1p33 | Xenobiotics, steroids and fatty acids | 511 |
| CYP4F2 | p13.12 | Eicosanoids | 520 |
| CYP4F3 | 19p13.2 | Eicosanoids (e.g., LTB4) | 520 |
| CYP4F8 | 19p13.1 | Eicosanoids | 520 |
| CYP4F22 | 19p13.1 | Unknown (orphan) | 524 |
| CYP4F22 | 19p13.12 | Unknown (orphan) | 531 |

CYP3, 4, 5 and 46 members and CYP3A7, 3A51P

CYP3, 4, and 5 members
CYP1A2 is a hepatic enzyme that oxidizes drugs (e.g., caffeine, clozapine, tacrine, theophylline, propranolol, and acetaminophen), procarcinogens and environmental compounds (e.g., benzopyrene, aflatoxin B\textsubscript{1}, and nicotine), and several groups of endogenous substrates (e.g., steroids and AA). Hepatic CYP1A2 is highly inducible by proton pump inhibitors such as omeprazole, smoking, polyamine hydrocarbons from grilled meats, and dietary cruciferous vegetables. Furafylline is a selective and potent inhibitor for human CYP1A2, but it is a weak inhibitor for mouse, rat and dog CYP1A2/1a2 and no inhibitory effect on monkey CYP1A2. In NCBI HomoloGene 68, CYP1A2 has 7 homologenes in 7 species, including chimpanzee, dog, cattle, mouse, rat, etc. Based on NCBI Annotation Pipeline, 69 organisms have orthologs with CYP1A2 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc.
In Ensembl release 84, CYP1A2 has 69 orthologs from 60 species of chordates including 9 species of non-human primates, 8 species of rodents, 11 species of Laurasiatheria, 33 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 2 and Table S3). In GeneCards 4.1.1, CYP1A2 has orthologs in 15 species including chimpanzee, dog, mouse, rat, etc. (Table S4).

The CYP1B1 gene located on chromosome 2p22.2 contains 3 exons and 2 introns, encoding a 543-amino acid protein. CYP1B1 can metabolize some drugs and several endogenous substances, but its role points to the bioactivation of certain procarcinogens such as polycyclic aromatic hydrocarbons. CYP1B1 is highly expressed in the nasal epithelium and lung, with a low expression in the liver, intestine, and kidney. In NCBI HomoloGene 68, CYP1B1 has 11 homologenes in 11 species, including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, Xenopus, zebrafish, etc. Based on NCBI Annotation Pipeline, 156 organisms have orthologs with CYP1B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP1B1 has 64 orthologs from 61 species of chordates including 11 species of non-human primates, 8 species of rodents, 11 species of Laurasiatheria, 33 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 2 and Table S3). In GeneCards 4.1.1, CYP1B1 has orthologs in 15 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP17A1 converts pregnenolone and progesterone to their corresponding 17α-hydroxylated metabolites and then to dehydroepiandrosterone (DHEA) and androstenedione. CYP17A1 maps to chromosome 10q24.3. CYP17A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, Arabidopsis thaliana and rice. In NCBI HomoloGene 68, CYP17A1 has 15 homologs in 8 species including chimpanzee, Rhesus monkey, dog, chicken, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 155 organisms have orthologs with CYP17A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP17A1 has 129 orthologs from 62 species including 10 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 36 species of placental mammals, 6 species of Sauropsida, and 11 species of fishes (Figure 2 and Table S3). In GeneCards 4.1.1, CYP17A1 has orthologs in 15 species including chimpanzee, cattle, dog, mouse, rat, opossum, etc. (Table S4).

CYP21A2 catalyzes steroid 21-hydroxylation, which is needed for the synthesis of mineralocorticoids and glucocorticoids in adrenal gland. CYP21A2 contains 10 exons and 9 introns and displays relatively low sequence identity compared to other CYP members. CYP21A2 resides a multiallelic, complex and tandem copy number variation of the major histocompatibility complex region on chromosome 6p21.3. CYP21A2 is expressed primarily in the adrenal cortex but has a low level in brain and lymphocytes. Mutations in CYP21A2 cause congenital adrenal hyperplasia. CYP21A1P is pseudogene also located on 6p21.3. CYP21A2 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog (Figure 2). In NCBI HomoloGene 68, CYP21A2 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, mouse, rat, etc. Based on NCBI Annotation Pipeline, 106 organisms have orthologs with CYP21A2 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP21A2 has 120 orthologs from 53 species including 10 species of non-human primates, 7 species of rodents, 8 species of Laurasiatheria, 29 species of placental mammals, 6 species of Sauropsida, and 10 species of fish (Figure 2 and Table S3). In GeneCards 4.1.1, CYP21A2 has orthologs in 11 species including chimpanzee, cattle, dog, mouse, rat, opossum, chicken, lizard, tropical clawed frog, zebrafish, and fruitfly (Table S4).

2.3. The Paralogs, Homologs and Orthologs of CYP2 Family

The CYP2ABFGST cluster located on chromosome 19q13.2 includes CYP2A, 2B, 2F, 2G, 2S, and 2T subfamilies. It contains six functional genes including CYP2A6, 2A7, 2A13, 2B6, 2F1, and 2S1 and seven pseudogenes including CYP2A7P1, 2B7P, 2F2P, 2G1P, 2G2P, 2T2P, and 2T3P. CYP2G1P carries a single nucleotide deletion in exon 2 and a 2.4-kb deletion between exons 3 and 7. CYP2G2P harbors two nonsense mutations in exons 1 and 3. The CYP2ABFGST cluster diverged through duplication events and inversions in the 80 Mya since the human and rodent lineages separated, resulting in 14 genes and 4 pseudogenes in rats, 12 active genes and 10 pseudogenes in mice, and 6 genes and 7 pseudogenes in humans. All the CYP2 members are paralogs of each other (Figure 3 and Table 1).
Figure 3. Cont.
CYP2A13 is a coumarin 7-hydroxylase that hydroxylates many drugs (e.g., tegafur, efavirenz, pilocarpine, and cyclophosphamide) and environmental and toxic compounds such as coumarin, nicotine, and nitrosamines. CYP2A6 maps to chromosome 19q13.2 and consists of 9 exons. This gene is located within a 350-kb gene cluster on chromosome 19q13 together with CYP2A7 and 2A13, two CYP2A7P pseudogenes, and CYP2B and CYP2F subfamilies. CYP2A6 is mainly expressed in the liver. In NCBI HomoloGene 68, CYP2A6 has 14 homologenes in 6 species including human CYP2A7 and 2A13, Rhesus monkey CYP2A24, mouse Cyp2a4 and 2a5, rat Cyp2a3, Xenopus cyp2/jf and 2a6, etc. Based on NCBI Annotation Pipeline, 3 species have orthologs with CYP2A6 (Table S2). These include crab-eating macaque, Rhesus monkey, and western gorilla. In Ensembl 84, CYP2A6 has 71 orthologs from 50 species of chordates including 7 species of non-human primates, 7 species of rodents, 12 species of Laurasiatheria, 29 species of placental mammals, 3 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2A6 has orthologs in 9 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP2A7 is a hepatic enzyme, but its substrate specificity is unclear. The gene is located about 25 kb upstream of CYP2A6. It maps to chromosome 19q13.2 consisting of 9 exons. In NCBI HomoloGene 68, CYP2A7 has 14 homologs in 6 species including human CYP2A6 and 2A13, Rhesus monkey CYP2A24, mouse Cyp2a4 and 2a5, rat Cyp2a3, etc. Based on NCBI Annotation Pipeline, 2 organisms have orthologs with CYP2A7, namely pig-tailed macaque and pygmy chimpanzee. In Ensembl 84, CYP2A7 has 71 orthologs from 50 species of chordates including 7 species of non-human primates, 7 species of rodents, 12 species of Laurasiatheria, 29 species of placental mammals, 3 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2A7 has orthologs in 9 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP2A13 is responsible for metabolic activation of hexamethylphosphoramide, N,N-dimethylaniline, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane, a tobacco-specific procarcinogen in the liver and other tissues. CYP2A13 located in the CYP2 cluster maps to chromosome 19q13.2. The nucleotide and protein sequences of CYP2A13 are highly similar to CYP2A6 with 93.5%–95.3% identity. CYP2A13 is mainly expressed in the liver and also in nasal mucosa, lung, brain, and trachea. In NCBI HomoloGene 68, CYP2A13 has 14 homologs in 6 species, including human CYP2A6 and 2A7, Rhesus monkey CYP2A24, mouse Cyp2a4 and 2a5, rat Cyp2a3, Xenopus cyp2/jf and 2a6, etc. Based on NCBI Annotation Pipeline, 3 species have orthologs with CYP2A13, namely Weddell seal, pygmy chimpanzee, and western gorilla (Table S2). In Ensembl 84, CYP2A13 has 75 orthologs from 53 species of chordates including 10 species of non-human primates, 7 species of rodents, 12 species of Laurasiatheria, 32 species of placental mammals, 3 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2A13 has orthologs in 12 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP2B6 metabolizes many drugs such as efavirenz, nevirapine, bupropion, and cyclophosphamide and activate certain procarcinogens and environmental compounds such as benzo(a)pyrene and some herbicides and pesticides. The gene maps to chromosome 19q13.2 together with CYP2B7P. It contains 9 exons encoding a 491-amino acid protein. CYP2B6 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. In NCBI HomoloGene 68, CYP2B6 has 7 homologs in 7 species, including chimpanzee, Rhesus monkey, dog, cattle, mouse, and rat.
on NCBI Annotation Pipeline, 25 organisms have orthologs with CYP2B6 (Table S2). These include the CYP2B6-like or CYP2B4 gene in non-human primates, pika, hedgehog, Arabian camel, degu, etc. In Ensembl 84, CYP2B6 has 79 orthologs from 48 species of chordates including 8 species of non-human primates, 6 species of rodents, 11 species of Laurasiatheria, 29 species of placental mammals, 1 species of Sauropsida (Chinese softshell turtle), and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2B6 has orthologs in 9 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP2F1 catalyzes the dehydrogenation of 3-methylindole, an endogenous toxin derived from the fermentation of tryptophan and bioactivates lung toxicants 4-ipomeanol, naphthalene, and styrene. This gene is mainly expressed in the lung, with very low or no expression in the liver, kidney, and intestine. CYP2F1 was present in the common ancestor of chordates and is conserved in chimpanzee, Rhesus monkey, dog, mouse, and rat. In NCBI HomoloGene release 68, CYP2F1 has 5 homologs in 5 species, including chimpanzee, Rhesus monkey, dog, mouse, and rat. Based on NCBI Annotation Pipeline, 46 organisms have orthologs with CYP2F1 (Table S2). These include non-human primates, rodents, other mammals, fishes, other vertebrates, etc. In Ensembl 84, CYP2F1 has 45 orthologs from 36 species of chordates including 3 species of non-human primates, 5 species of rodents, 9 species of Laurasiatheria, 17 species of placental mammals, 1 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2F1 has orthologs in 9 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

Human CYP2S1 oxidizes a number of carcinogens via the peroxide shunt. CYP2S1 also metabolizes PGG2, PGH2, hydroperoxyicosatetraenoic acids, and 13S-hydroperoxyoctadecadienoic acid, resulting in thromboxane A2 and other epoxyalcohols. The gene is mainly expressed in nasal epithelium, trachea, lung, stomach, small intestine, and spleen. CYP2S1 is conserved in chimpanzee, dog, cow, mouse, and rat. In NCBI HomoloGene release 68, CYP2S1 has 5 homologs in 5 species, including chimpanzee, dog, cattle, mouse, and rat. Based on NCBI Annotation Pipeline, 83 organisms have orthologs with CYP2S1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2S1 has 56 orthologs from 47 species of chordates including 8 species of non-human primates, 13 species of rodents, 4 species of Laurasiatheria, 28 species of placental mammals, 1 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2S1 has orthologs in 10 species including chimpanzee, cattle, dog, mouse, rat, opossum, etc. (Table S4).

The human CYP2C genes located on chromosome 10q24 align with an order of Centriole–2C18–2C19–2C9–2C8–Telomere. The human CYP2C genes have a significant potential to recombine, since they contain many L1 LINE repetitive DNA sequences located primarily in intron 5. Both CYP2C9 and 2C19 contain L1PA7, L1M4, L1MB5 and L1PA16 repeats in this intron. CYP2C18 and 2C19 share L1PA5 repeats. Both CYP2C8 and 2C19 carry an L1P repeat, but the two genes are on opposite strands. In mice, 14 of the 15 Cyp2c genes are located within a single cluster except for Cyp2c44 which is 3.8 Mb away from the locus and has unique catalytic property, expression profile and regulation. In the current rat genome assembly Rnor_6.0, there are 13 Cyp2c genes including 2c6/2c37, 2c7/2c39, 2c11, 2c12/2c40, 2c13/2c38, 2c22-2c24, 2c26, 2c29, 2c66, 2c79/2c65, and 2c80/2c55, which are located in a single cluster on rat chromosome 1q.

CYP2C8 metabolizes many drugs such as paclitaxel, amodiaquine, and methadone and several endogenous compounds such as AA and retinoid acid (RA). CYP2C8 is conserved in chimpanzee, Rhesus monkey, mouse, and rat. In NCBI HomoloGene release 68, CYP2C8 has 6 homologs in 4 species, including chimpanzee, Rhesus monkey, mouse, and rat. Based on NCBI Annotation Pipeline, 10 organisms have orthologs with CYP2C8 (Table S2). These include chimpanzee, pygmy chimpanzee, Bolivian squirrel monkey, horse, etc. In Ensembl 84, CYP2C8 has 163 orthologs from 59 species of chordates including 10 species of non-human primates, 7 species of rodents, 13 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2C8 has orthologs in 10 species including chimpanzee, cattle, dog, mouse, rat, opossum, etc. (Table S4).
CYP2C9 is abundant in the liver and metabolizes 15% of drugs that are metabolized by CYPs such as S-flurbiprofen, S-warfarin, tolbutamide, phenytoin, and diclofenac. CYP2C9 is conserved in chimpanzee, Rhesus monkey, cow, mouse, and rat. In NCBI HomoloGene 68, CYP2C9 has 8 homologs in 5 species, including chimpanzee, Rhesus monkey, cattle, mouse, rat, etc. Based on NCBI Annotation Pipeline, 7 organisms have orthologs with CYP2C9. These include chimpanzee, pygmy chimpanzee, Rhesus monkey, macaque, etc. In Ensembl 84, CYP2C9 has 160 orthologs from 58 species of chordates including 8 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 34 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2C9 has orthologs in 7 species including chimpanzee, cattle, mouse, rat, etc. (Table S4).

CYP2C18 can metabolize several drugs including tolbutamide, phenytoin, and verapamil. The gene is mainly expressed in the liver, esophagus, stomach, and small intestine. The CYP2C18 gene is conserved in Rhesus monkey, cow, mouse, rat, chicken, and mosquito. In NCBI HomoloGene 68, CYP2C18 has 7 homologs in 6 species, including Rhesus monkey, cattle, mouse, rat, etc. Based on NCBI Annotation Pipeline, 27 organisms have orthologs with CYP2C18 (Table S2). These include non-human primates, even-toed ungulates and whales, other mammals, etc. In Ensembl 84, CYP2C18 has 123 orthologs from 55 species of chordates including 8 species of non-human primates, 5 species of rodents, 11 species of Laurasiatheria, 29 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2C18 has orthologs in 10 species including chimpanzee, dog, mouse, rat, etc. (Table S4).

CYP2C19 metabolizes about 10% of drugs that are metabolized by CYPs, such as phenytoin, omeprazole, and voriconazole. The gene is mainly expressed in the liver, small intestine, and gallbladder. CYP2C19 is conserved in chimpanzee, Rhesus monkey, dog, cow, and rat. In NCBI HomoloGene 68, CYP2C19 has 7 homologs in 6 species. These include chimpanzee, Rhesus monkey, dog, cow, rat, etc. Based on NCBI Annotation Pipeline, 7 organisms have orthologs with CYP2C19 (Table S2). These include gray short-tailed opossum, pig, goat, etc. In Ensembl 84, CYP2C19 has 157 orthologs from 55 species of chordates including 5 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 31 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2C19 has orthologs in 8 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).

CYP2D6 belongs to a gene cluster consisting of highly homologous 2 functional genes and 1 pseudogene on chromosome 22q13. CYP2D8P encompasses multiple deletions and insertions in its exons. In Ensembl 84, this pseudogene produces one single transcript only that does not encode any functional proteins. The evolution of the human CYP2D locus results in inactivation of CYP2D7 and 2D8P and partial inactivation of CYP2D6 (in ~10% Caucasian). Based on the identification and characterization of a non-functional CYP2D7 gene and a 2D8P pseudogene, gene duplication events may give rise to CYP2D6 and 2D7, and that gene conversion events occur later to generate CYP2D8P.

CYP2D6 accounts for only a small percentage of total hepatic CYPs (1.3%–4.3%), however, it metabolizes ~25% of the drugs that are metabolized by CYPs such as tamoxifen, imipramine, codeine, and dextromethorphan. CYP2D6 is conserved in chimpanzee, Rhesus monkey, rat, chicken, and frog. In NCBI HomoloGene 68, CYP2D6 has 8 homologs in 5 species. These include chimpanzee, Rhesus monkey, rat, chicken, and frog. Based on NCBI Annotation Pipeline, 77 organisms have orthologs with CYP2D6 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2D6 has 97 orthologs from 49 species of chordates including 11 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 0 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2D6 has orthologs in 12 species including chimpanzee, mouse, rat, etc. (Table S4).

CYP2D7 is primarily expressed in brain cortex. In NCBI HomoloGene 68 and Orthologs from Annotation Pipeline, CYP2D7 has no homolog and ortholog. In Ensembl 84, CYP2D7 has 97 orthologs
from 49 species of chordates including 11 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 0 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2D7 has only one ortholog in mouse (Cyp2d37-ps).

The origin of the CYP2D subfamily could be traced back to before the divergence between amniotes and amphibia about 312 Mya. CYP2D7 was derived from CYP2D6 duplication in a stem lineage of humans and great apes. In fact, the origin of CYP2D6 and 2D8P in humans can be tracked back to a stem lineage of the New World monkeys and Catarrhini at the latest. Two functional CYP2Ds have been found in marmosets and macaques.

CYP2E1 metabolizes many low molecular weight xenobiotics such as acetaminophen, chlorzoxazone, halothane, and benzene. CYP2E1 is located on chromosome 10q26.3 with 9 exons. CYP2E1 is mainly expressed in the liver. CYP2E1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. In NCBI HomoloGene release 68, CYP2E1 has 6 homologs in 6 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, and rat. Based on NCBI Annotation Pipeline, 71 organisms have orthologs with CYP2E1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2E1 has 84 orthologs from 55 species of chordates including 9 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 32 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2E1 has orthologs in 6 species including chimpanzee, cattle, dog, mouse, rat, and opossum (Table S4).

CYP2J2 metabolizes AA, linoleic acid, and various drugs including certain antihistamine drugs (e.g., ebastine and terfenadine), mesoridazine, danazol, certain tyrosine kinase inhibitors (e.g., imatinib and sunitinitib), fenbendazole, etc. In the human genome and GRCh38.p2, there is only one single CYP2J2 gene, which maps to chromosome 1p31.3-p31.2. CYP2J2 is highly expressed in heart, present at a lower level in the liver, intestine, brain, bladder, pancreas, placenta, and kidney. CYP2J2 is conserved in chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, chicken, zebrafish, frog, fruitfly, mosquito, and C. elegans. In NCBI HomoloGene 68, CYP2J2 has as many as 51 homologs in 12 species, including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, etc. Based on NCBI Annotation Pipeline, 81 organisms have orthologs with CYP2J2 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2J2 has 188 orthologs from 60 species of chordates including 11 species of non-human primates, 7 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2J2 has orthologs in 6 species including chimpanzee, cattle, dog, mouse, rat, and opossum (Table S4).

CYP2R1 is also known as vitamin D3 25-hydroxylase that converts vitamin D into 25-hydroxyvitamin D3 (calcidiol). Calcidiol is subsequently converted by CYP27B1 (i.e., 25-hydroxyvitamin D3 1-α-hydroxylase) to calcitriol, the active form of vitamin D3 that binds to vitamin D3 receptor which mediates most of the physiological actions of vitamin D3. CYP2R1 maps to chromosome 11p15.2. CYP2R1 is mainly expressed in the liver and pancreas with the highest expression in the testes. CYP2R1 is conserved from zebrafish and frog to human. In NCBI HomoloGene 68, CYP2R1 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 161 organisms have orthologs with CYP2R1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2R1 has 66 orthologs from 62 species of chordates including 10 species of non-human primates, 7 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2J2 has orthologs in 11 species including chimpanzee, cattle, dog, mouse, etc. (Table S4).
CYP2U1 metabolizes AA, docosahexaenoic acid, other long-chain fatty acids, and endogenous N-arachidonoylserotonin. CYP2U1 maps to chromosome 4q25. There is a high mRNA expression of CYP2U1 in human thymus, with lesser expression in the heart, brain (mainly amygdala and prefrontal cortex), and platelets. The CYP2U1 gene is conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, frog, fruit fly, and A. thaliana. In NCBI HomoloGene 68, CYP2U1 has 11 homologs in 10 species including chimpanzee, dog, cattle, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 160 organisms have orthologs with CYP2U1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2U1 has 66 orthologs from 62 species of chordates including 11 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2U1 has orthologs in 13 species including chimpanzee, dog, mouse, rat, etc. (Table S4).

CYP2W1 catalyzes the oxidation of indole and certain lipids including lysolecithin and their stereoisomers and shows monooxygenase activity towards 3-methylindole and chlorzoxazone, but not AA. CYP2W1 maps to chromosome 7p22.3. The gene contains 10 exons and encode a 490-amino acid protein. The 5-prime flanking region, first exon, and first intron of CYP2W1 carry abundant CpG dinucleotides including 2 CpG islands. CYP2W1 is mainly expressed in colorectal, hepatic and adrenal gland tumors, but it is rarely detected in normal tissues. CYP2W1 is an ancient member of the CYP superfamily and it is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, and chicken. In NCBI HomoloGene 68, CYP2W1 has 7 homologs in 7 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, and chicken. Based on NCBI Annotation Pipeline, 108 organisms have orthologs with CYP2W1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP2W1 has 93 orthologs from 52 species of chordates including 10 species of non-human primates, 7 species of rodents, 9 species of Laurasiatheria, 28 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 3 and Table S3). In GeneCards 4.1.1, CYP2W1 has orthologs in 10 species including chimpanzee, cattle, dog, mouse, etc. (Table S4).

2.4. The Paralogs, Homologs and Orthologs of CYP3, 4, 5, and 46 Families

The CYP3A gene cluster is located on chromosome 7q21.1 (Ensembl cytogenetic band: 7q22.1) and spans ~231 kb, containing 4 CYP3A genes: CYP3A4, 3A5, 3A7 and 3A43, as well as 2 pseudogenes including (CYP3A51P/3A5P1 and 3A52P/3A5P2). CYP3A54P and 3A137P are two additional pseudogenes in CYP3 family, which map to chromosome 7q22.1. The human CYP3A subfamily is involved in the oxidative metabolism of a wide range of substrates, including more than 50% of all currently marketed drugs, endogenous steroids and xenobiotics. CYP3A4 and 3A5 are mainly expressed in the liver and intestine, while CYP3A5 appears to be primarily expressed in extrahepatic tissues. CYP3A4 is most abundantly expressed in the liver while CYP3A5 expression at the protein level is only about 10.6% of that of CYP3A4. Both CYP3A4 and 3A5 share substrate specificity and so it is often difficult to identify their relative contribution to the overall metabolism of a substrate. CYP3A4, 3A5, 3A7, and 3A43 share paralogs from human CYP superfamily and orthologs from various species with slight differences only. CYP3A7 is a fetal-specific CYP. CYP3A43 has very low expression in the liver. In GeneCards 4.1.1, CYP3, 4, and 5 members are paralogs to each other (Table 1). Ensembl 84 also includes CYP46A1 as the paralog of CYP3, 4, and 5 families (Figure 4 and Table 1).

CYP3A4 is conserved in chimpanzee, Rhesus monkey, dog, cow, rat, chicken, frog, fruitfly, mosquito, C. elegans, and M. oryzae. In NCBI HomoloGene 68, CYP3A4 has 27 homologs in 11 species, including chimpanzee, Rhesus monkey, dog, cattle, rat, etc. Based on NCBI Annotation Pipeline, 6 organisms have orthologs with CYP3A4 (Table S2). These include chimpanzee, white-tufted-ear marmoset, drill, Bolivian squirrel monkey, etc. In Ensembl 84, CYP3A4 has 148 orthologs from 60 species of chordates including 8 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP3A4 has orthologs in 16 species including chimpanzee, cattle, dog, mouse, rat, etc. (Table S4).
rodents, 14 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP3A43 has orthologs in 12 species including chimpanzee, dog, mouse, zebrafish, fruitfly, C. elegans, etc. (Table S4).

Figure 4. Cont.
Figure 4. Gene tree for human CYP3A4, 3A5, 3A7, 3A43, 4A11, 4A22, 4B1, 4F2, 4F3, 4F8, 4F11, 4F12, 4F22, 4V2, 4X1, 4Z1, 5A1/TBX5A1, and 46A1 built using Ensembl 84. These CYP3, 4, 5 and 46 family genes are paralogs to each other derived from the same ancestral gene via duplication events. The gene tree includes a total of 1008 genes from various species. The total number of speciation nodes is 558, and the number of duplication is 384. The number of ambiguous nodes is 31, and there are 4 gene split events.

CYP3A5 is conserved in chimpanzee, Rhesus monkey, mouse, rat, and fruitfly. In NCBI HomoloGene 68, CYP3A5 has 13 homologs in 5 species including chimpanzee, Rhesus monkey, mouse, rat, etc. Based on NCBI Annotation Pipeline, 10 organisms have orthologs with CYP3A5 (Table S2). These include chimpanzee, Rhesus monkey, etc. In Ensembl 84, CYP3A5 has 153 orthologs from 62 species of chordates including 10 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP3A5 has orthologs in 13 species including chimpanzee, cattle, dog, mouse, rat, opossum, etc. (Table S4).

CYP3A7 is conserved in chimpanzee, mouse, rat, chicken, zebrafish, frog, and rice. In NCBI HomoloGene 68, CYP3A7 has 9 homologs in 7 species including chimpanzee, mouse, rat, chicken, Xenopus, etc. Based on NCBI Annotation Pipeline, 7 organisms have orthologs with CYP3A7, including chimpanzee, Rhesus monkey, macaque, and gorilla, etc. In Ensembl 84, CYP3A7 has 150 orthologs from 61 species of chordates including 9 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 36 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP3A7 has orthologs in 14 species including chimpanzee, cattle, dog, mouse, rat, opossum, C. elegans, etc. (Table S4). CYP3A43 is conserved in primates only. In NCBI HomoloGene 68, CYP3A43 has only 2 homologs in two primates including chimpanzee and Rhesus monkey. Based on NCBI Annotation Pipeline, 13 organisms have orthologs with CYP3A43 (Table S2). These mainly include non-human primates and Ord’s kangaroo rat. In Ensembl 84, CYP3A43 has 148 orthologs from 60 species of chordates including 8 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP3A43 has orthologs in 12 species including chimpanzee, dog, mouse, zebrafish, fruitfly, C. elegans, etc. (Table S4).

There is a cluster of human CYP4 genes on chromosome 1p33, including CYP4A11, 4A22, 4B1, 4A26P, 4A27P, 4A43P, 4A44P, 4X1, 4Z1, and 4Z2P. The CYP4 proteins are the major fatty acid ω-hydroxylase which can hydroxylate the terminal ω-carbon and the ω-1 position of saturated and unsaturated fatty acids. They can also catalyze the ω-hydroxylation of various prostaglandins (PGs). CYP4A11 is conserved in chimpanzee, mouse, and rat. In NCBI HomoloGene 68, CYP4A11 has 5 homologs in 3 species including chimpanzee, mouse, and rat. Based on NCBI Annotation Pipeline, 26 organisms have orthologs with CYP4A11 (Table S2). These mainly include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4A11 has 134 orthologs from 57 species of chordates including 10 species of non-human primates, 8 species of rodents, 11 species of Laurasiatheria, 32 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4A11 has orthologs in 13 species including chimpanzee, dog, mouse, rat, fruitfly, C. elegans, etc. (Table S4).

CYP4A22 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, A. thaliana, and rice. In NCBI HomoloGene 68, CYP4A22 has 13 homologs in 9 species, including chimpanzee, Rhesus monkey, dog, cow, mouse, rat, etc. Based on NCBI Annotation Pipeline, 3 organisms have
orthologs with CYP4A22, including chimpanzee, pygmy chimpanzee, and Rhesus monkey (Table S2). In Ensembl 84, CYP4A22 has 136 orthologs from 58 species of chordates including 11 species of non-human primates, 8 species of rodents, 11 species of Laurasiatheria, 33 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GenCards 4.1.1, CYP4A22 has orthologs in 11 species including chimpanzee, cattle, dog, mouse, rat, *A. thaliana*, etc. (Table S4).

CYP4B1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP4B1 has 12 homologs in 9 species, including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, chicken, zebrafish, etc. Based on NCBI Annotation Pipeline, 79 organisms have orthologs with CYP4B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4B1 has 67 orthologs from 54 species of chordates including 10 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 35 species of placental mammals, 1 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GenCards 4.1.1, CYP4B1 has orthologs in 13 species including chimpanzee, cattle, dog, mouse, rat, zebrafish, *C. elegans*, etc. (Table S4).

CYP4X1 is a so-called “orphan” enzyme, but it converts the natural endocannabinoid anandamide to a single monoxygenated product and also metabolizes AA. CYP4X1 is mainly detected in the trachea, aorta, heart, liver, breast, brain, and prostate. CYP4X1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, *A. thaliana*, and rice. In NCBI HomoloGene 68, CYP4X1 has 13 homologs in 8 species, including chimpanzee, dog, mouse, rat, etc. Based on NCBI Annotation Pipeline, 53 organisms have orthologs with CYP4X1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4X1 has 86 orthologs from 62 species of chordates including 10 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GenCards 4.1.1, CYP4X1 has orthologs in 10 species including chimpanzee, cattle, dog, mouse, rat, *A. thaliana*, etc. (Table S4).

CYP4Z1 is responsible for the in-chain hydroxylation of myristic acid and lauric acid. CYP4Z1 is primarily expressed in mammary tissue. CYP4Z1 is conserved in fish, mammal, and primate. In NCBI HomoloGene 68, CYP4Z1 has only one homolog—chimpanzee CYP4Z1. Based on NCBI Annotation Pipeline, 14 organisms have orthologs with CYP4Z1 (Table S2). These mainly include non-human primates, bottlenosed dolphin, etc. In Ensembl 84, CYP4Z1 has 54 orthologs from 38 species of chordates including 5 species of non-human primates, 0 species of rodents, 0 species of Laurasiatheria, 5 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 4 and Table S3). In GenCards 4.1.1, CYP4Z1 has orthologs in 5 species including chimpanzee, mouse, opossum, platypus, and lizard (Table S4).

The human genome has 6 functional CYP4F genes including CYP4F2, 4F3, 4F8, 4F11, 4F12, and 4F22 and 5 pseudogenes including 4F9P, 4F10P, 4F23P, 4F24P, and 4F36P, which are located at chromosome 19p13.12. The Entrez Gene cytogenetic band is chromosome 19p13.1. The CYP4F subfamily is able to metabolize several important endogenous eicosanoids such as AA, PGs, and leukotriene B₄ (LTB₄). These compounds can regulate many physiological functions such as inflammation and vasoconstriction. Both CYP4F3A and 4F3B catalyze LTB₄ and AA ω-hydroxylation. CYP4Fs can convert AA to 20-HETE that regulates renal tubular and vascular functions. The ω-hydroxylated LTB₄ is further metabolized to form 20-carboxy-LTB₄, which can undergo B-oxidation from its ω-side and along with traditional β-oxidation from the C1 carbon, thus inactivating this pro-inflammatory agent. CYP4Fs appear to have a minor role in the biotransformation of therapeutic drugs. CYP4F2 can ω-hydroxylate vitamin E and vitamin K1 phytyl side chains, suggesting that this enzyme may play a role in the regulation of vitamin E status and synthesis of vitamin K-dependent clotting factors. CYP4F11 metabolizes erythromycin and ethylmorphine and CYP4F12 metabolizes ebastine with contribution from CYP2J2.
CYP4F2 is a major hepatic ω-hydroxylase that hydroxylates LTβ₄ and AA, inactivating LTβ₄ and forming the potent vasconstrictor 20-hydroxyecosatetraenoic acid (HETE), respectively. CYP4F2 is part of a cluster of the CYP4F genes located on the band of chromosome 19p13.12. CYP4F2 is conserved in chimpanzee, Rhesus monkey, cow, mouse, rat, and zebrafish. In NCBI HomoloGene 68, CYP4F2 has 8 homologs, including chimpanzee, Rhesus monkey, cattle, mouse, rat, zebrafish, etc. Based on NCBI Annotation Pipeline, 5 organisms have orthologs with CYP4F2 (Table S2). These include Rhesus monkey, yak, western gorilla, pygmy chimpanzee, and horse. In Ensembl 84, CYP4F2 has 56 orthologs from 27 species of chordates including 6 species of non-human primates, 4 species of rodents, 8 species of Laurasiatheria, 18 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F2 has orthologs in 12 species including chimpanzee, cattle, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP4F3 catalyzes the ω-hydroxylation of LTβ₄ and AA. The gene produces two tissue-specific splice variants, CYP4F3A (myeloid form) and CYP4F3B (liver form), but both transcripts are present in other tissues. CYP4F3 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. In NCBI HomoloGene 68, CYP4F3 has 6 homologs in 6 species including chimpanzee, Rhesus monkey, cow, dog, mouse, and rat. Based on NCBI Annotation Pipeline, 15 organisms have orthologs with CYP4F3 (Table S2). These mainly include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4F3 has 30 orthologs from 19 species of chordates including 2 species of non-human primates, 4 species of rodents, 3 species of Laurasiatheria, 10 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F3 has orthologs in 12 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP4F8 catalyzes the ω-2 hydroxylation of AA and three stable PGH₂ analogs but not PGD₂, E₂, F₂α, and LTβ₄. CYP4F8 is mainly expressed in epidermis, hair follicles, sweat glands, corneal epithelium, proximal renal tubules, and epithelial lining of the gut and urinary tract. CYP4F8 is conserved in chimpanzee, Rhesus monkey, mouse, rat, and A. thaliana. In NCBI HomoloGene 68, CYP4F8 has 12 homologs in 5 species including chimpanzee, Rhesus monkey, mouse, rat, etc. Based on NCBI Annotation Pipeline, 7 organisms have orthologs with CYP4F8 (Table S2). These include Rhesus monkey, chimpanzee, green monkey, etc. In Ensembl 84, CYP4F8 has 56 orthologs from 28 species of chordates including 7 species of non-human primates, 4 species of rodents, 8 species of Laurasiatheria, 19 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F8 has orthologs in 6 species including chimpanzee, mouse, rat, fruitfly, C. elegans, and A. thaliana (Table S4).

CYP4F11 metabolizes LTβ₄ and AA but at a much lower activity than CYP4F2 and 4F3A/B. The CYP4F11 gene is conserved in chimpanzee, mouse, rat, and chicken. In NCBI HomoloGene 68, CYP4F11 has 4 homologs in 4 species including chimpanzee CYP4F11, mouse and rat Cyp4f40, and chicken CYP4F22. Based on NCBI Annotation Pipeline, 12 organisms have orthologs with CYP4F11 (Table S2). These include chimpanzee CYP4F11, Sumatran orangutan, sooty mangabey, pygmy chimpanzee, etc. In Ensembl 84, CYP4F11 has 58 orthologs from 28 species of chordates including 7 species of non-human primates, 4 species of rodents, 8 species of Laurasiatheria, 19 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F11 has orthologs in 11 species including chimpanzee, cattle, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP4F12 catalyzes the hydroxylation of AA at C18 and C20, ω1 through ω3-hydroxylations of 9,11-diazo-PGH₂ and 9,11-methanoepoxy-PGH₂, and ω-oxidation of LTβ₄. In addition, CYP4F12 contributes to astemizole O-demethylation and hydroxylation of ebastine and terfenadine, with contributions from CYP3A4 and 2J2. CYP4F12 is mainly expressed in the liver, kidney, colon, small intestine, urogenital tract, and heart. CYP4F12 is conserved in chimpanzee, Rhesus monkey, mouse, rat, A. thaliana, and rice. In NCBI HomoloGene 68, CYP4F12 has 7 homologs in 6 species including chimpanzee, Rhesus monkey, mouse, rat, etc. Based on NCBI Annotation Pipeline, 7 organisms...
have orthologs with CYP4F12 (Table S2). These include chimpanzee, golden snub-nosed monkey, western gorilla, olive baboon, pygmy chimpanzee, etc. In Ensembl 84, CYP4F12 has 58 orthologs from 28 species of chordates including 7 species of non-human primates, 4 species of rodents, 8 species of Laurasiatheria, 19 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F12 has orthologs in 14 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP4F22 catalyzes the ω-3 hydroxylation of 20:4n-6 and synthesizes the ω-hydroxy fatty acids in the ceramide. CYP4F22 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, frog, A. thaliana, and rice. In NCBI HomoloGene 68, CYP4F22 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, cow, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 92 organisms have orthologs with CYP4F22 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4F22 has 44 orthologs from 43 species of chordates including 9 species of non-human primates, 6 species of rodents, 13 species of Laurasiatheria, 33 species of placental mammals, 6 species of Sauropsida, and 0 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4F22 has orthologs in 15 species including chimpanzee, dog, mouse, rat, zebrafish, A. thaliana, etc. (Table S4).

CYP4V2 is a selective ω-hydroxylase of saturated, medium-chain fatty acids with relatively high catalytic efficiency toward myristic acid and also hydroxylates the ω-3 polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid. CYP4V2 is an unusual CYP4 member in that it resides on chromosome 4q35.2, separate from the CYP4ABXZ and CYP4F clusters on chromosomes 1 and 19. The mRNA of CYP4V2 is found in the heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, retina, retinal pigment epithelium, and lymphocytes. The protein has very low sequence identity (31%–37%) to other CYP4 members. CYP4V2 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, fruitfly, mosquito, frog, and C. elegans. In NCBI HomoloGene 68, CYP4V2 has 12 homologs in 11 species including chimpanzee, Rhesus monkey, dog, cow, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 153 organisms have orthologs with CYP4V2 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP4V2 has 76 orthologs from 61 species of chordates including 10 non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 34 species of placental mammals, 7 species of Sauropsida, and 10 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, CYP4V2 has orthologs in 16 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

TBXAS1 catalyzes the conversion of PGH₂ to thromboxane, which causes vasoconstriction and platelet aggregation. TBXAS1 maps to chromosome 7q34-q35. TBXAS1 is expressed in platelets, erythroleukemia cells, human monocytes, leukocytes, and kidney interstitial dendritic reticulum cells, with a low expression in the liver and lung. TBXAS1 is conserved in dog, cow, mouse, rat, chicken, zebrafish, fruitfly, A. thaliana, rice, and frog. In NCBI HomoloGene 68, TBXAS1 has 18 homologs in 10 species including dog, cattle, mouse, rat, chicken, Xenopus, zebrafish etc. Based on NCBI Annotation Pipeline, 154 organisms have orthologs with TBXAS1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, TBXAS1 has 106 orthologous genes from 61 species of chordates including 11 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 10 species of fishes (Figure 4 and Table S3). In GeneCards 4.1.1, TBXAS1 has orthologs in 16 species including chimpanzee, cattle, dog, mouse, zebrafish, fruitfly, C. elegans, etc. (Table S4).

2.5. The Paralogs, Homologs and Orthologs of CYP7, 8, and 39 Families

CYP7B1 shares 40% amino acid sequence identity with CYP7A1. Human CYP7A1 and 7B1 share identical paralogs and orthologs. CYP7A1 (called cholesterol 7α-hydroxylase) catalyzes the first and major rate-limiting step in the classical, neutral pathway for bile acid biosynthesis. The CYP7A1 gene
maps to chromosome 8q11-q12 and its promoter region contains recognition sequences for a number of liver-specific transcription factors. The gene spans 10,059 bases with 7 exons, encoding a 504-amino acid enzyme. In both GeneCards 4.1.1 and Ensembl 84, CYP7A1 has 4 paralogs: CYP7B1, PTGIS, 8B1, and 39A1 (Figure 5 and Table 1).

CYP7A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP7A1 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, Xenopus, and zebrafish. Based on NCBI Annotation Pipeline, 163 organisms have orthologs with CYP7A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP7A1 has 68 orthologs from 64 species including 11 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 38 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 5 and Table S3). In GeneCards 4.1.1, CYP7A1 has orthologs in 12 species including chimpanzee, cattle, dog, mouse, rat, zebrafish, etc. (Table S4).

CYP7B1 is involved in metabolism of oxysterols, sex hormones and neurosteroids. The gene spans 211,029 bases with 6 exons, encoding a 506-amino acid enzyme. CYP7B1 shares 40% amino acid sequence identity with CYP7A1. CYP7B1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP7B1 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, mouse, rat, etc. Based on NCBI Annotation Pipeline, 150 organisms have orthologs with CYP7B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP7A1 has 70 orthologs from 65 species including 11 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 38 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 5 and Table S3). In GeneCards 4.1.1, CYP7B1 has orthologs in 12 species including chimpanzee, cattle, dog, mouse, rat, zebrafish, etc. (Table S4).

CYP8B1 is involved in bile acid synthesis and is responsible for the conversion of 7α-hydroxy-4-cholesten-3-one into 7α,12α-dihydroxy-4-cholesten-3-one. CYP8B1 maps to chromosome 3p22.1 and it contains only one single exon and lacks introns. CYP8B1 is conserved in chimpanzee, Rhesus monkey, dog, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP8B1 has 11 homologs in 8 species including chimpanzee, Rhesus monkey, dog, mouse, rat, etc. Based on NCBI Annotation Pipeline, 120 organisms have orthologs with CYP8B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, PTGIS has 70 orthologs from 56 species including 9 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 35 species of placental mammals, 2 species of Sauropsida (Anole lizard and Chinese softshell turtle), and 11 species of fishes (Figure 5 and Table S3). In GeneCards 4.1.1, human PTGIS has orthologs in 11 species including chimpanzee, cattle, dog, mouse, rat, tropical clawed frog, zebrafish, etc. (Table S4).

CYP8B1 is involved in bile acid synthesis and is responsible for the conversion of 7α-hydroxy-4-cholesten-3-one into 7α,12α-dihydroxy-4-cholesten-3-one. CYP8B1 maps to chromosome 3p22.1 and it contains only one single exon and lacks introns. CYP8B1 is conserved in chimpanzee, Rhesus monkey, dog, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP8B1 has 11 homologs in 8 species including chimpanzee, Rhesus monkey, dog, mouse, rat, etc. Based on NCBI Annotation Pipeline, 120 organisms have orthologs with CYP8B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP8B1 has 71 orthologs from 50 species including 8 species of non-human primates, 8 species of rodents, 8 species of Laurasiatheria, 27 species of placental mammals, 6 species of Sauropsida, 10 species of fishes (Figure 5 and Table S3). In GeneCards 4.1.1, CYP8B1 has orthologs in 13 species including chimpanzee, cattle, dog, mouse, rat, opossum, zebrafish, etc. (Table S4).
has orthologs in 11 species including chimpanzee, cattle, dog, mouse, rat, tropical clawed frog, zebrafish, etc. (Table S4).
CYP39A1 catalyzes 7α-hydroxylation of oxysterols including 25-hydroxycholesterol, 27-hydroxycholesterol and 24-hydroxycholesterol, while CYP7B1 is called oxysterol 7α-hydroxylase 1. CYP39A1 maps to chromosome 6p12.3, spans 103,079 bases, and contains 14 exons. CYP39A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, *M. oryzae*, and frog. In NCBI HomoloGene 68, CYP39A1 has 10 homologs in 10 species including chimpanzee, Rhesus monkey, dog, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 145 organisms have orthologs with CYP39A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP39A1 has 58 orthologs from 55 species including 10 species of non-human primates, 7 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida (birds and reptiles), and 3 species of fishes (Figure 5 and Table S3). In GeneCards 4.1.1, CYP39A1 has orthologs in 13 species including chimpanzee, dog, mouse, rat, zebrafish, etc. (Table S4).

2.6. The Paralogs, Homologs and Orthologs of CYP11, 19, 24, 27, and 46 Families

The CYP11 members are important enzymes that participate in steroid biosynthesis and metabolism. The production of glucocorticoids and mineralocorticoids occurs in the adrenal gland and the final steps are catalyzed by three mitochondrial CYPs, namely CYP11A1, 11B1, and 11B2. CYP11B1 shows close homology to the CYP11B2 gene, which encodes aldosterone synthase and is normally expressed only in the zona glomerulosa. Both CYP11B genes map to chromosome 8q21, while CYP11A1 is located on chromosome 15q24.1. All CYP11 members are mitochondrial enzymes. CYP19A1, 24A1, CYP27 family, and 46A1 are the paralogs of CYP11 family members.

CYP11A1 catalyzes the conversion of cholesterol to pregnenolone, which is the first and rate-limiting step in the synthesis of the steroid hormones. CYP11A1 maps to chromosome 15q23-q24. CYP11A1 is conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP11A1 has 9 homologs in 8 species including chimpanzee, dog, cow, mouse, rat, etc. Based on NCBI Annotation Pipeline, 148 organisms have orthologs with CYP11A1. These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP11A1 has 61 orthologs from 58 species including 8 species of non-human primates, 7 species of rodents, 12 species of Laurasiatheria, 31 species of placental mammals, 6 species of Sauropsida, 11 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP11A1 has orthologs in 14 species including chimpanzee, dog, mouse, rat, zebrafish, *C. elegans*, etc. (Table S4).

CYP11B1 catalyzes the 11β-hydroxylation of deoxycortisol to form cortisol and also catalyzes 18 or 19-hydroxylation of steroids and the aromatization of androstenedione to estrone. CYP11B1 maps to chromosome 8q21. The gene spans 7,491 bases with 11 exons and 10 introns. CYP11B1 is conserved in chimpanzee, Rhesus monkey, mouse, rat, zebrafish, and frog. In NCBI HomoloGene release 68, CYP11B1 has 6 homologs: chimpanzee, Rhesus monkey, mouse, rat, frog, and zebrafish. Based on NCBI Annotation Pipeline, 23 organisms have orthologs with CYP11B1. These include chimpanzee, macaque, Rhesus monkey, green monkey, etc. In Ensembl 84, CYP11B1 has 64 orthologs from 52 species including 11 species of non-human primates, 8 species of rodents, 9 species of Laurasiatheria, 32 species...
of placental mammals, 1 species of Sauropsida (Anole lizard), and 10 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP11B1 has orthologs in 13 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).
CYP11B2 catalyzes the conversion of 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxy corticosterone. The gene spans 7285 bases with 10 exons and 9 introns, encoding a 503-amino acid enzyme. CYP11B2 is expressed in the adrenal cortex. CYP11B2 is conserved in Rhesus monkey, dog, cow, mouse, and rat. In NCBI HomoloGene 68, CYP11B2 has 6 homologs in 5 species including Rhesus monkey, dog, cattle, mouse, and rat. Based on NCBI Annotation Pipeline, 13 organisms have orthologs with CYP11B2 (Table S2). These include Rhesus monkey, crab-eating macaque, pygmy chimpanzee, domestic cat, cattle, etc. In Ensembl 84, CYP11B2 has 62 orthologs from 50 species including 9 species of non-human primates, 8 species of rodents, 9 species of Laurasiatheria, 30 species of placental mammals, 1 species of Sauropsida (Anole lizard), and 10 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP11B2 has orthologs in 12 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP19A1 catalyzes the formation of aromatic C18 estrogens from C19 androgens. CYP19A1 maps to chromosome 15q21.1 and contains 13 exons. CYP19A1 is expressed in the ovaries, testes, placenta, fetal liver, adipose tissue, bone, vasculature smooth muscle, and brain. CYP19A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP19A1 has 10 homologs in 9 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, etc. Based on NCBI Annotation Pipeline, 155 organisms have orthologs with CYP19A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP19A1 has 75 orthologs from 62 species including 11 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 38 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Table S3). In GeneCards 4.1.1, CYP19A1 has orthologs in 14 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP24A1 is called vitamin D₃ 24-hydroxylase that catalyzes the 24-hydroxylation of calcidiol (25-hydroxyvitamin D₃) and calcitriol (1α,25-dihydroxyvitamin D₃). This gene maps to chromosome 20q13.2 and contains 12 exons. The enzyme is present in both proximal and distal kidney tubules. CYP24A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, frog, fruitfly, mosquito, and C. elegans. In NCBI HomoloGene 68, CYP24A1 has 15 homologs in 12 species, including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 163 organisms have orthologs with CYP24A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP24A1 has 68 orthologs from 64 species including 11 species of non-human primates, 8 species of rodents, 12 species of Laurasiatheria, 36 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP24A1 has orthologs in 14 species including chimpanzee, cattle, dog, mouse, rat, fruitfly, C. elegans, etc. (Table S4).

The human CYP27 family contains three functional members: CYP27A1, 27B1, and 27C1. Both sterol 27-hydroxylase and 25-hydroxy-D₃ 1α-hydroxylase are assigned to the CYP27 family since they share >40% sequence identity, while sterol 27-hydroxylase is assigned to the A subfamily and 25-hydroxy-D₃ 1α-hydroxylase to the B subfamily of CYP27 since their protein sequences are <55%
identical. The paralogs of CYP27 family include CYP11 family, 19A1, 24A1, and 46A1 (Figure 6). CYP27A1 is called vitamin D$_3$ 25-hydroxylase and sterol 27-hydroxylase catalyzing the first step in the oxidation of the side chain of sterol intermediates. CYP27A1 maps to chromosome 2q35 and contains 9 exons. Mutations in CYP27A1 cause cerebrotendinous xanthomatosis, a rare autosomal recessive lipid storage disease. CYP27A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, frog, fruit fly, and mosquito. In NCBI HomoloGene 68, CYP27A1 has 16 homologs in 11 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, zebrafish etc. Based on NCBI Annotation Pipeline, 139 organisms have orthologs with CYP27A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP27A1 has 78 orthologs from 65 species including 11 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP27A1 has orthologs in 14 species including chimpanzee, cattle, dog, mouse, rat, zebrafish, fruitfly, etc. (Table S4).

CYP27B1 is called 25-hydroxyvitamin D$_3$ 1α-hydroxylase that hydroxylates 25-hydroxyvitamin D$_3$ at the 1α position, resulting in 1α,25-dihydroxyvitamin D$_3$, the active form of vitamin D$_3$. CYP27B1 maps to chromosome 12q12-q14 and has 9 exons. Mutations in this gene causes vitamin D-dependent rickets, type I and hypocalcemic vitamin D-dependent rickets. CYP27B1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, zebrafish, and frog. In NCBI HomoloGene 68, CYP27B1 has 9 homologs in 9 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, frog, etc. Based on NCBI Annotation Pipeline, 116 organisms have orthologs with CYP27B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP27B1 has 61 orthologs from 58 species including 10 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 36 species of placental mammals, 2 species of Sauropsida, and 11 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP27B1 has orthologs in 12 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP27C1 maps to chromosome 2q14.3 and contains 14 exons. The gene spans 36,243 bases and encodes a 372-amino acid protein. CYP27C1 is conserved in chimpanzee, Rhesus monkey, dog, cow, chicken, zebrafish, frog, and M. oryzae. In NCBI HomoloGene 68, CYP27C1 has 11 homologs in 10 species including chimpanzee, Rhesus monkey, dog, mouse, rat, zebrafish, etc. Based on NCBI Annotation Pipeline, 132 organisms have orthologs with CYP27C1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP27C1 has 53 orthologs from 51 species including 9 species of non-human primates, 4 species of rodents, 11 species of Laurasiatheria, 25 species of placental mammals, 7 species of Sauropsida, and 10 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP27C1 has orthologs in 11 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, C. elegans, etc. (Table S4).

CYP46A1 (called cholesterol 24-hydroxylase) converts cholesterol to 24S-hydroxycholesterol in the brain. Cholesterol cannot pass the blood-brain barrier, but 24S-hydroxycholesterol can be secreted in the brain into the circulation to be returned to the liver for catabolism. CYP46A1 maps to chromosome 14q32.1. CYP46A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, frog, M. oryzae, A. thaliana, and rice. In NCBI HomoloGene 68, CYP46A1 has 15 homologs in 12 species including chimpanzee, Rhesus monkey, dog, mouse, rat, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 148 organisms have orthologs with CYP46A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP46A1 has 53 orthologs from 51 species including 9 species of non-human primates, 4 species of rodents, 11 species of Laurasiatheria, 25 species of placental mammals, 7 species of Sauropsida, and 10 species of fishes (Figure 6 and Table S3). In GeneCards 4.1.1, CYP46A1 has orthologs in 11 species including chimpanzee, cattle, dog, zebrafish, fruitfly, etc. (Table S4).
2.7. The Paralogs, Homologs and Orthologs of CYP26 and 51 Families

In the human genome, there are three members of in the CYP26 family: 26A1, 26B1, and 26C1. These three members are all RA hydroxylases with similar substrate specificity but different tissue-specific expression patterns. CYP26A1 is called retinoic acid 4-hydroxylase with both 4-hydroxylation and 18-hydroxylation activities, acting on all-trans-RA and its stereoisomer 9-cis-RA. CYP26A1 maps to chromosome 10q23-q24 and has 8 exons. In both GeneCards 4.1.1 and Ensembl 84, CYP26 members are the paralogs of CYP51A1 (Figure 7 and Table 1).

CYP26A1 has been detected in different cell lines with different tissue origins including kidney, liver, breast, intestine, and lung. Mutations in CYP26A1 causes keratomalacia and caudal regression syndrome. CYP26A1 is conserved in the chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, frog, A. thaliana, and rice. In NCBI HomoloGene 68, CYP26A1 has 19 homologs in 11 species including chimpanzee, Rhesus monkey, dog, mouse, rat, frog, zebrafish etc. Based on NCBI Annotation Pipeline, 156 organisms have orthologs with CYP26A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP26A1 has 62 orthologs from 61 species including 9 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 35 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 7 and Table S3). In GeneCards 4.1.1, CYP26A1 has orthologs in 16 species including chimpanzee, dog, mouse, rat, African clawed frog, zebrafish, fruitfly, baker’s yeast, A. thaliana, etc. (Table S4).

CYP26B1 is a critical regulator of all-trans-RA levels by the specific inactivation of all-trans-RA to hydroxylated forms. This gene maps to chromosome 2p13.2 and contains 8 exons. The gene spans 18,801 bases with 6 exons and encodes a 512-amino acid protein. Mutations in this gene are associated with radiohumeral fusions and other skeletal and craniofacial anomalies and lethal occipital encephalocele-skeletal dysplasia syndrome. CYP26B1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, frog, zebrafish, and A. thaliana. In NCBI HomoloGene 68, CYP26B1 has 19 homologs in 10 species including chimpanzee, Rhesus monkey, dog, mouse, rat, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 153 organisms have orthologs with CYP26B1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP26B1 has 64 orthologs from 62 species including 11 species of non-human primates, 8 species of rodents, 13 species of Laurasiatheria, 36 species of placental mammals, 6 species of Sauropsida, and 11 species of fishes (Figure 7 and Table S3). In GeneCards 4.1.1, CYP26B1 has orthologs in 13 species including chimpanzee, cattle, dog, mouse, rat, tropical clawed frog, zebrafish, fruitfly, baker’s yeast, A. thaliana, etc. (Table S4).

CYP26C1 is involved in the catabolism of all-trans- and 9-cis-RA, and thus contributes to the regulation of RA levels in cells and tissues. Like CYP26A1, this gene maps to chromosome 10q23.33. The gene spans 7,434 bases with 6 exons and encodes a 522-amino acid protein. Mutations of CYP26C1 causes focal facial dermal dysplasia 4 and focal facial dermal dysplasia. CYP26C1 is the conserved in chimpanzee, cow, mouse, rat, chicken, zebrafish, frog, and A. thaliana. In NCBI HomoloGene 68, CYP26C1 has 8 homologs in 8 species including chimpanzee, cattle, mouse, rat, chicken, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 139 organisms have orthologs with CYP26C1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP26C1 has 63 orthologs from 60 species including 10 species of non-human primates, 8 species of rodents, 11 species of Laurasiatheria, 34 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 7 and Table S3). In GeneCards 4.1.1, CYP26C1 has orthologs in 14 species including chimpanzee, dog, mouse, rat, zebrafish, fruitfly, A. thaliana, etc. (Table S4).
Figure 7. Cont.
CYP20A1 is called lanosterol 14α-demethylase/sterol 14α-demethylase which are found in yeast, plants, fungi, animals and even prokaryotes, suggesting this is among the oldest of the CYP genes. CYP51A1 is a common target of antifungal drugs (e.g., miconazole and ketoconazole), which inhibit CYP51A1 activity and formation of ergosterol. This gene has 11 exons and maps to chromosome 7q21.2. CYP51A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, frog, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Eremothecium gossypii*, *Schizosaccharomyces pombe*, *M. oryzae*, *A. thaliana*, and rice. In NCBI HomoloGene 68, CYP51A1 has 16 homologs in 16 species including chimpanzee, Rhesus monkey, dog, cattle, mouse, rat, frog, zebrafish, etc. Based on NCBI Annotation Pipeline, 162 organisms have orthologs with CYP51A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP51A1 has 64 orthologs from 63 species including 10 species of non-human primates, 8 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 11 species of fishes (Figure 7 and Table S3). In GeneCards 4.1.1, CYP51A1 has orthologs in 19 species including chimpanzee, cattle, dog, mouse, rat, zebrafish, baker’s yeast, etc. (Table S4).

### 2.8. The Paralogs and Homologs of CYP20A1

CYP20A1 maps to chromosome 2q33.2 and contains 14 exons. The gene spans 67,400 bases, encoding a 462-amino acid protein. This protein lacks one amino acid of the conserved heme binding site and also lacks the conserved I-helix motif AGX(D,E)T, suggesting that its substrate may carry its own oxygen. As an “orphan” CYP, the substrate specificity, structure, function and regulation of CYP20A1 are still unknown. In both GeneCards 4.1.1 and Ensembl 84, there is no paralog for CYP20A1. CYP20A1 is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. In NCBI HomoloGene 68, CYP20A1 has 9 homologs in 9 species, including chimpanzee, Rhesus monkey, dog, mouse, rat, frog, and zebrafish. Based on NCBI Annotation Pipeline, 160 organisms have orthologs with CYP20A1 (Table S2). These include non-human primates, rodents, even-toed ungulates and whales, other mammals, birds, fishes, other vertebrates, etc. In Ensembl 84, CYP20A1 has 66 orthologs from 62 species including 11 species of non-human primates, 7 species of rodents, 14 species of Laurasiatheria, 37 species of placental mammals, 7 species of Sauropsida, and 10 species of fishes (Table S3). In GeneCards 4.1.1, CYP20A1 has orthologs in 14 species including chimpanzee, dog, mouse, rat, zebrafish, etc. (Table S4).

### 3. Discussion

After 3.5 billion years of evolution, the number of species on the earth has expanded considerably. Each genome consists of a unique gene inventory, which determines the specific phenotype and interactions with the environment. Genotypic and phenotypic diversity have been observed in all species at the protein, DNA, and organismal levels, and this diversity is correlated with environmental variation and stress. The time frame for the evolution of the genus Homo out of the chimpanzee–human last common ancestor is roughly 10 to 2 Mya, that of *Homo sapiens* out of *Homo erectus* roughly 1.8 to 0.2 Mya. According to genetic and fossil evidence, archaic *Homo sapiens* evolved to anatomically...
modern humans solely in Africa, between 200,000 and 100,000 years ago, with members of one branch leaving Africa by 60,000 years ago and over time replacing earlier human populations such as Neanderthals and *Homo erectus*. Humans, and presumably most vertebrates, have genes not found in invertebrate animals like Drosophila and *C. elegans*. These include genes encoding antibodies and T cell receptors for antigen, the transplantation antigens of the major histocompatibility complex, cell-signaling molecules including the many types of cytokines, the molecules that participate in blood clotting, and mediators of apoptosis.

The human genome includes 57 protein-coding CYP genes, which play a key role in the biotransformation of a large number of xenobiotics such as drugs and environmental compounds and physiologically important endogenous compounds. Most human CYP genes are scattered widely across their genomes, but there are some exceptions. Human CYP genes often occur in clusters, with several related genes, pseudogenes and detritus exons aligned in tandem [10]. Mouse and human each have 30 CYP genes that lie outside the seven gene clusters. These CYP genes are distributed on all chromosomes except chromosomes 5, 16, and 17. Five clusters of closely related genes are located on chromosomes 1, 7, and 10 (one cluster each) and chromosome 19 (two clusters). Clusters of human CYP genes are found at different chromosome regions including 1p31 (CYP2J), 1p33 (CYP4ABXZ cluster), 7q22 (CYP3A cluster), 10q24 (CYP2C cluster), 19q13 (CYP2ABFGST cluster), and 22q13 (CYP2D cluster). In each of these clusters, the CYP genes are adjacent, with no other confirmed genes interspersed among them. Within each cluster, all the genes encode closely related enzymes with many cases of apparent gene duplication since the split from the rodent lineage [46,47]. Almost all xenobiotic-metabolizing CYP enzymes belonging to CYP1-4 families are located in these gene clusters. Each of the human CYP clusters had a syntenic counterpart cluster in mice. Very few of the genes in these clusters could be assigned as one-to-one orthologs due to continuing gene duplication and deletion events on both lineages. These syntenic gene clusters must have originated from a shared ancestral gene or genes, with gene duplications and losses resulting in lineage-specific groups of related genes. Genomic clustering of structurally and functionally related genes such as CYPs are also found in other species.

It is postulated that approximately one and a half billion years ago, the first of the gene expansions gave rise to the families of CYPs that are primarily involved in the metabolism of endogenous fatty acids and cholesterol (e.g., CYP4 and 11 families). Around 900 Mya, another expansion of the gene family is speculated to have resulted in several of the endogenous steroid-synthesizing CYP families (e.g., CYP19, 21 and 27 families). A dramatic expansion of several CYP families, including those known or suspected of being involved in xenobiotic metabolism (e.g., CYP2, 3, 4 and 6), commenced about 400 Mya. Phylogenetic analyses of CYPs suggest that they are also among the most rapidly evolving of genes which is a characteristic that is needed to protect the cells from the injuries when exposed to increasing toxic xenobiotic compounds [3,36,37].

It is generally assumed that orthologs have the same biological functions in different species, and duplication events produce paralogs that evolve new functions [39,40]. Clear delineation of orthologous relationships between CYP genes is obviously indispensable for the reconstruction of the evolution of species and their genomes in the post-genomic era. To achieve this objective, we have systematically studied the relationships of 57 human CYPs with those from other species. A sequence alignment and phylogenetic study have clearly shown the evolution of human CYPs from one ancestral gene and the key features as a functional group of heme-containing oxidative enzymes. The structural motifs identified include “AGXDTT”, “EXXR”, and “CXG”. Several residues including Glu242, Arg245, Phe310, and Cys316 are found to be well conserved in all human CYPs. In particular, Cys316 plays a central role in heme-binding where iron acts as a source/sink of electrons for reduction/oxidation reactions.

We have applied two approaches to identify the paralogs of human CYPs: GeneCards and Ensembl. Both methods produce similar results with slight differences (Table 2). Both GeneCards and Ensembl have identified CYP3, 4, and 5 members are paralogs to each other, but Ensembl predicts that
**CYP46A1** shares the ancestor with these genes. GeneCards predicts that CYP46A1 shares the ancestor with CYP11, 24, and 27 members. Both GeneCards and Ensembl have found that CYP7 and 8 members are the paralogs of CYP39A1. GeneCards predicts that CYP11, 24, 27 and 46 members are the paralogs of CYP19A1, but Ensembl has told us that CYP19A1 has no paralogs at all. Both GeneCards and Ensembl predict that CYP26 members are the paralogs of CYP51A1. The differences in the predicted paralogs of human CYPs may reflect the differences in the algorithms and cutoff values for inclusion and exclusion used by the two approaches.

Many important genes are conserved across species despite billions of years of intervening evolution and exposure to dramatically changed environment. The wide application of comparative genomics is essential in order to map knowledge across different species. Sequences of genes that share a common ancestry are typically refined into orthologs, which are pairs of genes that started diverging via speciation event, and paralogues, which are pairs of genes that started diverging via gene duplication [39,40]. Many approaches and databases have been developed to identify orthologs. We have adopted six approaches/databases to predict the orthologs of human CYPs, including NCBI, Ensembl Compara, GeneCards, OMA, PANTHER, and TreeFam. Since the species sets used by these six databases are different, it is not surprising to see a very different number of species and orthologs of a human CYP gene. However, the predicted orthologs of human CYPs based on these approaches are comparable. For example, they all predict that CYP46A1 and 51A1 have orthologs in non-human primate, rodent, placental mammal, fish, frog, rice, rice blast fungus, and *A. thaliana*, suggesting these two enzymes play an essential role in maintaining the cellular functions and biotransformation of key endogenous compounds across animal, yeast, and plant. CYP46A1 is a cholesterol 24-hydroxylase while CYP51A1 is a lanosterol 14α-demethylase [48]. Other CYPs that are significantly involved in the metabolism of important endogenous compounds are also well conserved across species based on our data. CYP4 members are major fatty acid ω-hydroxylases [49]. These enzymes remove excess free fatty acids to prevent lipotoxicity, catabolize leukotrienes and prostanoids including prostaglandins, thromboxanes and prostacyclins, and result in bioactive metabolites from arachidonic acid ω-hydroxylation. CYP7A1 and 7B1 are 7α-hydroxylases of steroids. CYP11A1, 11B1, and 11B2 are involved in steroid biosynthesis. CYP17A1 is present in adrenal cortex and has steroid 17α-hydroxylase and 17,20-lyase activities for steroids. CYP19A1 is an aromatase present in gonads, brain, and adipose tissue that catalyzes aromatization of androgens to estrogens. CYP21A2 is detected in adrenal cortex and has 21-hydroxylase activity toward steroids [50,51]. CYP26A1, 26B1, and 26C1 are retinoid acid hydroxylases. Moreover, CYP39A1 catalyzes 7α-hydroxylation of 24-hydroxycholesterol. All these enzymes are conserved in various species and indicate their key role in the survival of these species. CYP51 is involved in cholesterol biosynthesis, whereas CYP 7A1, 27A1, 46A1, 7B1, 39A1, and 8B1 are the key enzymes in cholesterol catabolism to bile acids, the major route of cholesterol elimination [52]. Conversion of cholesterol to steroids is initiated by CYP11A1, and CYP3A4 contributes to bile acid biosynthesis as well [52]. Six CYPs including CYP11 family and three type II CYPs including CYP17A1, 19A1 and 21A2 play indispensable roles in the biosynthesis of steroids. The key CYP enzymes in the bile acid biosynthetic pathways are CYP7A1, 8B1, 27A1 and 7B1. Biosynthesis and metabolism of cholesterol, bile acids and oxysterols involve CYP3A4, 7A1, 7B1, 8B1, 27A1, 39A1, 46A1, and 51A1. CYPs have many physiologically relevant functions including regulation of vascular tone in the cardiovascular system, ion transport in the kidney, inflammation and immune system, the secretion of pancreatic peptide hormones, cell proliferation and programmed cell death [8,26,53–55]. CYPs participate in cellular functions such as the metabolism of eicosanoids, the biosynthesis of cholesterol and bile acids, synthesis and metabolism of steroids and vitamin D₃, synthesis and degradation of biogenic amines, and the hydroxylation of RA and presumably other morphogens [49,56,57]. The metabolites of these endogenous compounds often have important physiological activities that regulate cellular metabolism, death and survival.

Clusters of CYP genes are often present in the genomes of various species. The processes of sequential tandem gene duplication events can lead to large clusters of CYP genes on chromosomes,
and these are often striking landmarks of the CYPomes in various species. Clusters of related CYP families are called “clans”. There are 10 CYP clans in humans: clans 2, 3, 4, 7, 19, 20, 26, 46, and 51, and the mitochondrial clan [47]. CYP families within a single clan have likely been diverged from a common ancestor gene. Clusters of human CYP genes are found at different chromosome regions including 1p31 (CYP2J), 1p33 (CYP4ABXZ cluster), 7q22 (CYP3A cluster), 10q24 (CYP2C cluster), 19p13 (CYP4F cluster), 19q13 (CYP2ABFGST cluster), and 22q13 (CYP2D cluster). In each of these clusters, the CYP genes are adjacent, with no other confirmed genes interspersed among them. Within each cluster, all the genes encode closely related enzymes with many cases of apparent gene duplication since the split from the rodent lineage [47]. Almost all xenobiotic-metabolizing CYP enzymes belonging to CYP1-4 families are located in these gene clusters. Each of the human CYP clusters had a syntenic counterpart cluster in mice. Very few of the genes in these clusters could be assigned as one-to-one orthologs due to continuing gene duplication and deletion events on both lineages. These syntenic gene clusters must have originated from a shared ancestral gene or genes, with gene duplications and losses resulting in lineage-specific groups of related genes. Genomic clustering of structurally and functionally related genes such as CYPs are also found in the other species.

The CYP1A2 gene may arise via duplication of CYP1A1 about 350 Mya during the evolution of mammals and birds based on phylogenetic analysis of CYP1A genes. The human CYP2 family has 4 clusters: CYP2ABFGST, CYP2C, CYP2D, and CYP2J. The CYP2ABFGST cluster diverged through duplication events and inversions in the 80 Mya since the human and rodent lineages separated, resulting in 14 genes and 4 pseudogenes in rats, 12 active genes and 10 pseudogenes in mice, and 6 genes and 7 pseudogenes in humans. Both CYP1 and CYP2 families belong to the CYP2 clan [58]. The CYP1 family is considered to diverge from the CYP2 family more than 420 Mya. The CYP1 family has four subfamilies (1A, 1B, 1C, and 1D), and these subfamilies diverge in the ancestor of vertebrates. Fish and amphibians express all these 4 subfamily members. The CYP1A1 and 1B subfamilies are conserved from fish to humans, whereas primates lack CYP1D, and mammals lack CYP1C [33]. Birds have two CYP1A genes, CYP1A4 and 1A5, which are orthologous to mammalian CYP1A1 and 1A2 [59]. CYP1C members are found in several bird genomes, but not in quail [60]. The CYP1C genomic region is highly conserved among vertebrates. CYP1B and 1C genes derive from duplication of a common ancestor gene. Tissue distribution of CYP1B and 1C transcripts in birds resembles that found in zebrafish, suggesting that these genes have similar functions in diverse vertebrates [60]. The CYP1A and 1B subfamilies are conserved from the fish to human, whereas primates lack CYP1D, and mammals lack CYP1C [61,62].

The CYP2 family, comprising at least 42 subfamilies (2A–2H, 2J–2N, 2P–2Z, 2AA–2AH, 2AJ–2AK, 2AM, 2AN, and 2AP–2AU), is the most dominant in Clan 2. The CYP2 family is considered to arise from a single ancestral vertebrate CYP2 gene. CYP2B, 2E and 2S subfamilies are specific to mammals, while the CYP2A/G and 2F subfamilies are present only in mammals and reptiles [63]. About half of the CYP2 subfamilies are non-mammalian: 2H derives from chicken; 2K, 2M, 2N and 2P are from fish; 2L is from lobster; 2Q, 2AC, 2AM, 2AN, 2AP, 2AQ, 2AR, 2AS, and 2AT from Xenopus; 2AA and 2AD from fish; 2AG, 2AH, 2AJ, and 2AK from green anole lizard; and 2AU from oyster. The first five (2A–2E) are present in mammalian liver with differing levels, while CYP2F members are selectively expressed in lung tissues, and have been implicated as important catalysts in the formation of reactive intermediates from several pneumotoxic chemicals. Avian CYP2Hs are orthologous to human CYP2C62P, rat Cyp2c23, and mouse Cyp2c44 [64]. CYP2R and 2U are present in all vertebrates. CYP2B, 2E and 2S are specific to mammals, while the 2A, 2G and 2F subfamilies are present only in mammals and reptiles. These five subfamilies (except the CYP2E subfamily) diverged successively to result in the CYP2 cluster in an ancestor of mammals.

Rat Cyp2a family includes Cyp2a1, 2a2 and 2a3. Rat Cyp2a1 (female dominant) and Cyp2a2 (male dominant) are expressed in the liver (2%). In contrast, CYP2A3 is not expressed in the rat liver and is constitutively expressed in the esophagus, lung and nasal epithelium, but not in the liver, intestine, and kidney. The rat cyp2a1/2 show about 60% homology in amino acid sequence to human
CYP2A6. In contrast to human, several endogenous steroids are good substrates for rat Cyp2a1/2. Rat Cyp2a1 catalyzes 7α-hydroxylation of testosterone and Cyp2a2 is involved in testosterone 15α- and 7α-hydroxylation. Mouse Cyp2a family includes Cyp2a4, 2a5, 2a12, and 2a22. Mouse Cyp2a5 resembles the human orthologue in catalyzing 7-hydroxylation of coumarin. Dogs have CYP2A13 and 2A25, rabbits express CYP2A10 and 2A11, and monkeys contain CYP2A23 and 2A24. These CYP2A/2a members from various species demonstrate different substrate specificity, tissue expression, and inhibition profiles.

The mammalian CYP3 and 5 families belong to clan 3 as insect CYP6 and 9 families, mollusk CYP30 family, and C. elegans CYP13 and 25 families. The CYP3 family contains 6 subfamilies, CYP3A, 3B, 3C, 3D, 3E, and 3F [65]. CYP3B, 3C, 3D and 3F are fish-specific. CYP3A exists in all classes of vertebrates, comprising amphibian-, bird-, and mammal-specific clades. Members of the CYP3A subfamily appear to have been duplicated independently. The CYP3 clan contains vertebrate CYP3 and CYP5 families, insect CYP6 and 9 families, the clam CYP30 family and C. elegans CYP25 and 13 families, as well as other named or unnamed families from various species. The common ancestor of the CYP3 clan was likely to occur 800–1100 Mya. The ancestral vertebrates had a single CYP3A gene that underwent independent diversification in bony fishes, reptiles and mammals [65]. The ancestral amniota genome had two CYP3A genes, one of which was lost at the origin of eutherian mammals, and the other underwent gene translocation. Most CYP3A genes in mammals resulted from recent gene duplication events. For example, there were two Cyp3a gene duplication events in rodents, while rapid evolutionary changes occurred in primates and the expansion of the CYP3A subfamily significantly differed among species.

The CYP4 family includes at least 127 subfamilies (4A–4H, 4J–4N, 4P–4Z, 4AA–4AF, 4AH, 4AJ–4AN, 4AP–4AZ, 4BA–4BH, 4BJ–4BN, 4BP–4BZ, 4CA–4CN, 4CD–4CZ, 4DA–4DH, 4D–4DN, 4D–4DZ, and 4FA). CYP4A and 4X/Z are specific to mammals, whereas 4B, 4F, and 4V are common to all vertebrates. The members of the 4F subfamily have formed several species-specific clusters, except CYP4F22. CYP4AA, 4BA, 4CA, 4FA, etc., are all found in insects only. The human CYP4 family is large with 6 subfamilies, consisting of 12 protein-coding genes and 26 pseudogenes. The 12 functional genes include CYP4A11, 4A22, 4B1, 4F2, 4F3, 4F8, 4F11, 4F12, 4F22, 4V2, 4X1, and 4Z1. The 26 pseudogenes include CYP4A26P, 4A27P, 4A43P, 4A44P, 4F9P, 4F10P, 4F23P–4F27P, 4F29P–4F36P, 4F44P, 4F45P, 4F59P–4F62P, and 4Z2P. The 21 CYP4F pseudogenes are dispersed in chromosomes 1, 2, 8, 9, 13, 18, 19, and 21.

The CYP5 family has only a single member in each species, namely CYP5A1, which is also known as thromboxane A synthase 1 (TBXAS). The TBXAS1 gene is conserved in the dog, cow, mouse, rat, chicken, zebrafish, fruit fly, A. thaliana, rice, and frog; it has an orthologous gene in 151 organisms. The CYP5 family is large and present in insects. CYP6 family contains at least 162 subfamilies. These include CYP6A–6H, 6J–6N, 6P–6Z, 6AA–6AH, 6AJ–6AZ, 6BA–6BH, 6BJ–6BN, 6BP–6BZ, 6CA–6CH, 6CD–6CN, 6CP–6CZ, 6DA–6DH, 6DJ–6DN, 6DP–6DZ, 6EA–6EH, 6EJ–6EN, 6EP–6EZ, 6FA–6FH, 6FJ–6FN, and 6FP–6FU.

Both CYP7 and 19 (aromatase) families are chordate-specific, but they are extremely sequence divergent from other CYP clans, suggesting that they are either rapidly evolving or that they may be much older than the chordate line [10]. CYP7 may have diverged from a CYP39 precursor. CYP7 family contain 7A, 7B, 7C, and 7D subfamilies. CYP7A and 7B are present in animals, while CYP7C and 7D are fish-specific. CYP7A1, a cholesterol 7α-hydroxylase, is conserved in the human, chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog; 157 organisms have orthologs with human CYP7A1. CYP7B1 is a 25-hydroxycholesterol 7α-hydroxylase which is expressed from frog to human.

The CYP8 family contains only CYP8A and 8B subfamilies. Both CYP8A1 and 8B1 are conserved from frog and fish to human. CYP8B2–8B4 are present in the fish only. CYP9 family is present in insects only. This family contains at least 48 subfamilies, including CYP9A–H, 9J–9N, 9P–9Z, 9AB–9AH,
CYP10 family present in insects only contains CYP10A, 10B and 10C subfamilies. CYP11 family has CYP11A, 11B and 11C subfamilies. CYP11A and 11B are conserved from the frog to human, while CYP11C is fish-specific. CYP12 family is present in insects only, consisting of 13 subfamilies including CYP12A–12H and 12J–12N. CYP13 family has only one subfamily CYP13B present in *C. elegans*. CYP14 family is present in *C. elegans*, with only one CYP14A subfamily.

CYP20A1 does not show any catalytic activity toward a number of potential steroids and biogenic amines. CYP21A2 is required for the synthesis of steroid hormones including cortisol and aldosterone. CYP21A2 is an important enzyme that is required for the glucocorticoids and mineralocorticoids synthesis. CYP24A1 involves in deactivation of the active form of vitamin D$_3$ through the C24 oxidation pathway. 24-Hydroxylcholesterol is a better substrate for CYP46A1 than cholesterol. Mutations of CYP46A1 may be associated with Alzheimer’s disease. CYP51A1 catalyzes a complex 14α-demethylation reaction with the aid of cytochrome P450 reductase. CYP51A1 in mammals is also responsible for production of the follicular fluid meiosis-activating sterol. Mutations of CYP51A1 are associated with pregnancy pathologies.

Identification of the paralogs and orthologs of human CYPs has important implications in drug discovery and toxicological studies. A panel of species including mouse, rat, rabbit, dog, etc. are commonly used in these fields. However, there are remarkable species-specific differences in CYP ortholog expression and tissue distribution patterns, substrate specificity and activities, and inhibitor profiles. This may make the extrapolation form animal models to humans difficult or inaccurate. For example, rodents are not proper models for human CYP2A6 studies due to species-specific CYP2A6 ortholog expression patterns and substrate specificity and activities. Rats have little or no coumarin 7-hydroxylation activity and it is the ortholog Cyp2a1 catalyzes 3,4-epoxidation of coumarin [66]. Human CYP2A6 converts nicotine to cotinine, but rat Cyp2b1, not Cyp2a1, catalyzes this reaction. Mice have 4 orthologs of human CYP2A6: Cyp2a4, 2a5, 2a12, and 2a22. The choice of proper animal models for human CYP2D6 studies is also difficult because there are significant differences between rodents and humans in the structure and number of active CYP2D genes in the CYP2D/Cyp2d locus. The mouse has nine different active Cyp2d genes [47] and the rat harbors six functional Cyp2D genes, whereas the human carries only one (CYP2D6), which indeed is absent from 7% of the Caucasian population. Mice contain Cyp2d9–2d13, 2d22, 2d26, 2d34, and 2d40 and seven pseudogenes (2d32p, 2d33p, 2d35p–2d39p and 2d41p) [47,67–70]. All mouse Cyp2ds have high amino acid sequence identity (65%–75%) compared with human CYP2D6. Cyp2d22 has been suggested to be the functional ortholog of human CYP2D6. Five Cyp2d genes, namely Cyp2d1–2d5, have been identified in rats by genomic analysis [47,70]. Rat Cyp2d5 has >95% similarities in amino acid sequence to Cyp2d1 and Cyp2d4. Rat Cyp2d3, but not Cyp2d1, 2d2 or 2d4, is the homolog of human, chimpanzee, Rhesus monkey and chicken CYP2D6 and frog Cyp2d6 and 2d20. In NCBI Gene database and the assembly Cavpor3.0, the guinea pig genome contains 4 active Cyp2d member including Cyp2d6, 2d16, 2d17 and 2d27 and 1 pseudogene (Cyp2d3p). The rabbit genome Cyp2d locus contain 4 Cyp2d members, Cyp2d24–2d17–2d23–2d4–ps. Except Cyp2d4–ps, other three genes are functionally active. Thus, caution should be taken when extrapolating the results involving CYP2D studies from animals to humans.

In summary, the identification of orthologs is a central problem in the field of comparative genomics and phylogenetic analysis and accurate prediction of the orthologs and paralogs of human CYP genes is fundamental to understand the evolutionary relationships and functional implications of this superfamily of important enzymes that are involved in the biotransformation of a large number of therapeutic drugs, environmental compounds and endogenous substances. The delineation of the human CYP orthologs in other species also has important implications in drug discovery and biomedical research when animal models are widely used. On the other hand, phylogeny-based orthologous relationships may not be enough to describe the evolutionary and functional relationships of human CYPs, other factors such as the protein 3D structures and protein interaction networks should be taken into account.
4. Methods

4.1. Human Cytochrome P450s (CYPs) in Current Human Assembly GRCh38.p6 and Sequence Alignment of Human CYPs

The current human genome assembly is GRCh38.p6 (GenBank assembly accession: GCA_000001405.21) which was released on 21 December 2015 by the Genome Reference Consortium. GRCh38.p2 was released in December 2014, which has offered a data set based on the Homo sapiens high-coverage assembly GRCh38 released by the Genome Reference Consortium in December 2013. In this assembly, there are 20,300 coding genes with 198,457 transcripts, 25,159 non-coding genes, and 14,424 pseudogenes. Identification of CYPs in any organisms is critical based on featured motifs in their protein sequences. For example, almost all CYPs carry two CYP signature motifs: one is “FXXGXRXCXG” (also known as “CXG”) located in the heme-binding domain and another one is the “EXXR” motif located in helix K. The genome of human beings carries 57 functional CYP genes and 58 pseudogenes as well. Previously, we thought CYP2D7 as a pseudogene, but now it has been considered as a functional gene in humans by HGNC (ID: 2624), UniProtKB (A0A087X1C5), Ensembl 84 (ENSG00000205702), and GeneCards 4.1.1 (GCID: GC22M042140). However, NCBI GenBank (ID: 1564) still lists CYP2D7 as a pseudogene.

The primary protein sequences of 57 functional CYPs present in humans were retrieved from the UniProtKB/Swiss-Prot database (http://www.uniprot.org/). Multiple sequence alignment of the human CYPs was carried out using Clustal W v2.0 (http://www.clustal.org) with all parameters set as default. The phylogenic tree of human CYPs was also built up in order to deduce the evolutionary relationships among these human CYP sequences. In addition, the MEME program version 4.10.1 (http://meme-suite.org) was employed to identify characteristic motifs present in human CYPs.

4.2. Computational Identification of the Paralogs, Homologs, and Orthologs of Human CYPs

Paralogs are defined as homologous genes in one species which arise from a gene duplication event in the genome [71–73]. Different from orthologous genes, a paralog is a novel gene with new function, although the new function is always associated with the biological role of the ancestral gene. If the mutations produce stop codons or frameshift, paralogs may eventually become pseudogenes. In GeneCards 4.1.1 (Table 2), GeneDecks is used to predict functional paralogs based on combinatorial similarity of attributes [74]. Paralogs in GeneCards are from HomoloGene, Ensembl, and SIMAP (http://liferay.csb.univie.ac.at), and pseudogenes from Pseudogene.org. In Ensembl release 84, paralogs are identified by a multi-step approach where the maximum likelihood phylogenetic gene trees are built [75]. In Ensembl, paralogous genes are defined as those for which the most common ancestor node is a duplication event (see below).

A homolog is a gene similar in structure and evolutionary origin to a gene in another species [73,76,77]. The term “homolog” may apply to the evolutionary relationship between genes split by speciation event (i.e., ortholog), or to the one between genes arising from a duplication event (i.e., paralog). As such, orthologous genes are defined as homologous genes separated by a speciation event in the genome during evolution, and these genes largely retain a similar function to that of the ancestral gene [41,78–81]. Speciation, an evolutionary process, gives rise to new species that can live in a new way from the parent species. There are four geographic types of speciation in nature, based on the extent to which speciating populations are separated from one another, namely allopatric, peripatric, parapatric, and sympatric. Speciation has obtained some barriers to genetic exchange with the parent species. Orthologous genes generally show $\geq 70\%$ of DNA or protein sequence identity. Homologous genes often maintain the function of their ancestral gene through a speciation event, although genetic variations may arise after the new species arises. Therefore, functions may be lost or gained when comparing a pair of orthologs. There are difficulties in confirming the exact ancestry of homologous genes in various organisms due to the frequent occurrence of gene duplication and
genome rearrangement. Phylogenetic analysis of the gene lineage always provides evidence whether two similar genes from distinct organisms are orthologous.

In NCBI, HomoloGene 68 released in April 2014 was used as an automated system for building up putative homologous groups based on the complete genomes of 21 eukaryotic species (Table 2 and Table S1). The protein sequences are compared to one another using the blastp (protein-protein BLAST) program and then are matched up to give rise to groups, using a tree developed from sequence similarity to guide the constructing process. During the process, closely related organisms are matched up first, and then more organisms are added as the tree is traversed toward the root. Thereafter, the protein sequence alignments are mapped back to their corresponding DNA sequences by which distance metrics such as molecular distance and the nonsynonymous (Ka) to synonymous (Ks) ratio (Ka/Ks) can be determined. Sequences are aligned using synteny when appropriate. In a bipartite matching, residual sequences from other organisms are aligned using an algorithm that will force to maximize the global score. Cutoff values on bits per position and Ks values are predetermined to avoid incorrect grouping of “unlikely” orthologs. The cutoff values are determined using the score distribution for a given group of organisms. In addition, paralogous genes are also found via matching sequences.

We further employed 8 online databases to identify the orthologs of human CYP genes: NCBI, Ensembl Compara, GeneCards, OMA (“Orthologous MAtrix”) Browser, PATHER, TreeFam, EggNOG, and Roundup (Table 2). In NCBI, the Annotation Pipeline method is used to identify the orthologous genes in selected vertebrae genomes [80]. In this approach developed by NCBI, a process flow has been created using the vertebrate RefSeq sequences to investigate the genes, protein sequence conservation, and annotation consistency. Briefly, the NCBI approach discovers sets of comparable proteins present in various vertebrates, including orthologs and similar proteins among alternatively splicing products. This protocol combines sequence, protein-coding regions, and functional annotation via identification of featured conserved domains to discover conservation from multiple levels [80]. Protein sequence alignments are efficiently conducted using BLAST. As such, mRNA transcript sequences and annotated protein-coding regions of the genes can be mapped onto their respective protein sequence alignments to identify splice conservation across different orthologous genes. The RefSeq database can identify sets of orthologs via best hits to corresponding Swiss-Prot proteins as sets of potential homologous genes and the orthology is finally confirmed through local synteny. In NCBI, the genomes of 187 vertebrate species including 22 primates, 16 rodents, 17 even-toed ungulates and whales (Cetartiodactyla), 36 other mammals, 58 birds, 27 fishes, and 11 other vertebrates, 50 insects, 15 other invertebrates, and 39 plants have been annotated completely (Table S1). These species have been included to identify orthologs of human CYP genes. Presently, all the model species and organisms from the Homologene database are included for ortholog identification in GeneCards version 4.1.1 released in March 2016 (Table S1).

In Ensembl 84, released in March 2016, orthologous and paralogous genes are predicted using the TreeBeST protocol that will finally build up maximum likelihood phylogenetic trees of 68 chordates (Table S1) [75,81–83]. The resultant phylogenetic trees merged with their species tree carry internal nodes that have been annotated to discern duplication or speciation events. To begin the prediction process, the TreeBeST protocol will load a representative translated protein of each gene from species used in Ensembl. From each gene tree, gene pairwise relations of orthologs and paralogs are inferred, and orthologs are finally verified using the model species and organisms of chordates in Ensembl (Table S1). GeneCards 4.1.1 contains orthologs from several databases including HomoloGene, Ensembl Pan Taxonomic Compara, SGD, MGI Flybase, WormBase (through Ensembl), and euGenes. The species from Ensembl Pan Taxonomic Compara are selected to create a diverse panel of taxa including model organisms and species of interest. In addition, all available species from Homologene are incorporated in GeneCards 4.1.1.
Table 2. Databases and programs used to predict the orthologs of human cytochrome P450 (CYP) genes.

| Database         | URL                                | Current Release          | Primary Application                                                                 | Number of Organisms                | Taxonomic Range | Update Frequency |
|------------------|------------------------------------|--------------------------|-------------------------------------------------------------------------------------|------------------------------------|------------------|------------------|
| NCBI             | http://www.ncbi.nlm.nih.gov/       | RefSeq release 75 (14 Mar 2016) | NCBI’s genome annotation pipeline                                                   | 862 mammalian vertebrates and 3145 other vertebrates (RefSeq covers 58,776 organisms) | Vertebrates      | Daily            |
| Ensembl Compara  | http://useast.ensembl.org/index.html | Release 84 (14 Mar 2016)  | To build phylogenetic trees across the whole set of protein-coding genes with one pipeline | 68 chordates plus 240 others       | All domains of life | Quarterly        |
| GeneCards        | http://www.genecards.org/          | Version 4.1.1 (13 Mar 2016) | To provide comprehensive, user-friendly information on all annotated and predicted human genes (39,629 HGNC approved, 21,976 protein-coding genes, 104,578 RNA genes, and 16,329 pseudogenes) | 58                                 | All domains of life | Yearly           |
| OMA              | http://omabrowser.org/oma/home/    | Release 17 (13 Sep 2014)  | To identify orthologous genes via massive cross-comparison of complete genomes     | 1706 (226 Eukaryota, 1353 Bacteria, and 127 Archaea)                             | All domains of life | Twice per year   |
| PANTHER          | http://pantherdb.org/              | Version 10.0 (25 Apr 2015) | Inference of gene function using GO terms and evolutionary relationships of genes among organisms (11,926 protein families, divided into 83,190 functionally distinct protein subfamilies) | 104                                 | All domains of life | Yearly           |
| TreeFam          | http://www.treefam.org             | Release 9 (3 May 2013)    | Phylogenetic tree construction and providing orthology/paralogy predictions as well the evolutionary history of genes | 109 (vs. 79 in TreeFam 8)          | Metazoans + model eukaryotes | Once every 2 years |
| EggNOG           | http://eggnogdb.embl.de/#/app/home | 4.1 (5 May 2015)          | A database of orthologous groups and functional annotation                          | 2031                               | All domains of life | Once every 3–4 years |
| RoundUp          | http://roundup.hms.harvard.edu/    | 2.0 (January 2012)        | An online database of gene orthologs for over 1800 genomes                         | 1807                               | All domains of life | 2–4 times per year |
OMA Browser is a large database that can be used to infer orthologous genes amongst species with known complete genomes and translated proteomes [84] (http://omabrowser.org). To calculate homologous sequences, all-against-all Smith-Waterman alignments are performed and significant matches are kept. The orthologous genes are discovered based on evolutionary distances, in view of distance inference uncertainty and possible differential gene losses [85]. In OMA, homologous genes are defined as pairs of homologous genes that have commenced diverging through speciation events between the progenitor genomes and then merged back into the same genome by hybridization. Thus, homologs can be considered as “orthologs between subgenomes”. OMA Browser covers all domains of life including 226 species of Eukaryota, 1353 species of Bacteria, and 127 species of Archaea. In the current release, (release 17), the database includes a total of 883,176 OMA groups and 8,798,758 proteins. Notably, OMA now has made 442,376,477 function annotations for a total of 7,947,728 proteins.

PANTHER is also used to predict the orthologs of human CYP genes via analyses of evolutionary relationships among 104 model organisms and inference of gene function using a total of 41,603 GO terms (Table S1) [86,87] (http://pantherdb.org/). In PANTHER, the phylogenetic trees are constructed to exhibit gene family evolution with incorporation of evolutionary events (e.g., speciation and duplication) [86]. The current version 10.0 released in May 2015 contains a total of 11,928 protein families which are further divided into 83,190 functionally distinct subfamilies.

The TreeFam database carries phylogenetic trees inferred from the genomes of animals and thus perform predictions of orthologous and paralogous genes (http://www.treefam.org/). The current release (release 9) contains 109 species (Table S1) and 15,736 gene families. In TreeFam, a gene family is defined as a group of genes arising from speciation of single-metazoan animals. TreeFam uses protein identifiers from Ensembl, Ensembl Genomes, Wormbase, and JGI (http://genome.jgi.doe.gov). In EggNOG (“evolutionary genealogy of genes: Non-supervised Orthologous Groups”) 4.1, orthologous genes are automatically inferred by splitting species space into “core” and “periphery” species [88] (http://eggno gdb.embl.de). The core species are critical for finding orthologous genes using the strict triangular criterion. Most of the phylogenetic trees in EggNOG are reconstructed using a strategy similar to the one described by Huerta-Cepas et al. [89], which uses a combination of multiple sequence aligners, alignment trimming techniques, model testing, and maximum likelihood inference. The model yielding the best maximum likelihood value is applied to infer a final tree with Phyml and full Maximum Likelihood optimization. The current 4.1 release contains 2031 organisms, 9.6 millions of proteins, and 190,000 orthologous groups. Finally, RoundUp 2.0 is a large-scale on-line database of orthologous genes (http://roundup.hms.harvard.edu). The orthologous genes across species are identified using the Reciprocal Smallest Distance (RSD) algorithm [90]. This algorithm used is able to discover more and more precise orthologous genes than reciprocal best blast hits and assigns each orthologous gene a score according to its maximum likelihood evolutionary distance. Roundup contains more than 1800 genomes that are from 226 eukaryota, 1447 bacteria, 113 archaea, and 21 viruses.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/17/7/1020/s1.

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