Phylogenetic analysis of some mammals based on cytochrome P450 gene sequences

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ABSTRACT

A phylogenetic study was conducted to understand the evolutionary relationships among 14 species of mammals based on mitochondrial CYPS gene sequences. The alignment and analysis of the sequences showed variable numbers of substitutions in CYP gene and reconstructed tree was having satisfactory bootstrap support. The sequence polymorphisms resulted from the nucleotide sequence substitutions of the CYP gene indicates that all the mammals were evolved from a common ancestor. Some genotypic changes have taken place leading to the diversification of same orders into different clades. Canis familiaris has clustered with distantly related species of order Artiodactyla comprising of Sus scrofa, Bos taurus and Avis Ares which have resulted due to the sequence similarities among them. Overall result indicates that CYP gene provides well-defined relationships within and among the species; supporting its successful use in animal systematics.

Key words: Phylogenetic, CYPS, systematic, alignments, substitutions, bootstrap, mammals.

1. INTRODUCTION

Evolution is the change over time in one or more inherited traits found in populations of organisms (Futuyma and Douglas, 2005). All organisms share a common ancestry, but during evolution driven by inheritance, variation, mutation, recombination or selection, some phenotypic and genotypic changes have taken place which ultimately led to diversification of different families, genera and species. Thus, the major sources of such variations are mutation, genetic recombination and gene flow (Jain et al., 1999; Richardson et al., 2007; Margulis and Lynn, 1998; Sapp, 1994). The cytochrome P450 (YP) superfamily comprises over 2000 known genes and has bacterial, archaean and eukaryotic representatives. It has been suggested that all eukaryotic CYPS genes have evolved from a single ancestral gene (Nebert and Gonzalez, 1987). Within known CYP diversity, the CYP 3 gene family is believed to have diverged between 800 and 1100 million years ago (Nebert and Gonzalez, 1987; Gonzalez, 1990). Mammals are members of a class of air-breathing vertebrate animals characterized by the possession of hair, three middle ear bones, and mammary glands functional in mothers with young. Mammals have always been looked upon for the study of evolutionary patterns being a diverse group of chordates. In the present study, we made an attempt to analyze nucleotide data of CYPS gene of 14 species of mammals to resolve the diverse phylogenetic relationship within the species under study.

1.1. Mitochondrial DNA and CYPS Gene

Mitochondria are structures within eukaryotic cells that convert the chemical energy from food into a form that cells can use as adenosine triphosphate (ATP). Human mitochondrial DNA (mtDNA) has been completely sequenced and found to be circular double-stranded molecules containing 16,569 base pairs (Anderson et al., 1981).

CYPS gene is a part of mitochondrial genome which codes for a large and diverse group of enzymes. The cytochrome P450 (YP) gene superfamily is significant in a wide variety of disciplines, ranging from medical genetics to inorganic chemistry (Guengerich, 1991) due to CYPS roles in oxidative transformation of exogenous and endogenous organic compounds. CYPS are the major enzymes involved in drug metabolism and bioactivation, accounting for ~75% of the total number of different metabolic reactions (Guengerich, 2008).

2. MATERIALS AND METHODS

2.1. Data Collection

A total of 14 species of mammals have been taken under study and the nucleotide sequences of their CYPS gene were downloaded from NCBI-GenBank (table 1).

2.1.1. Sequence alignment and analysis

All the downloaded sequences were saved in fasta format and analysed using BioEdit software. The nucleotide compositions of CYPS gene of all the species were determined using this software. Sequences are further loaded in Clustal X2 for alignment which provides an indication of the quality of an alignment by plotting a ‘conservation score’ for each column of the alignment. The quality curve is drawn below the alignment. The alignment of the nucleotide sequences showed that there were variable
numbers of substitutions in CYP gene. The output of Clustal X2 (*.aln, *.msf and *.phy) become the input for MEGA, GenDoc and PHYLIP.

2.2. Phylogenetic tree construction

The phylogenetic tree was constructed to access the degree and pattern of intraspecific differences among the 14 species of class mammalia by analyzing their CYP gene sequences of mitochondrial genome. The tree construction was done by using neighbor-joining (NJ) method as implemented in MEGA 4.1 and analysis was conducted by using the "Kimura-2-parameter" model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (700 replicates) is shown next to the branches.

3. RESULTS AND DISCUSSION

The AT-GC % and the nucleotide sequence lengths of CYP genes from all the mammals were obtained by using BioEdit software (table 1). The lengths of most of the sequences were around 2000 base papers. The alignment of the nucleotide sequences showed that there were variable numbers of substitutions in nucleotide sequences of CYP gene of those mammals. The phylogenetic tree constructed based on CYP gene depicts the phylogenetic divergence of the species into two branches. Some factors like recombination, mutation etc. resulted in the diversification of different species from a common ancestor and their clustering into different clades. The species that are closer to each other are represented as a part of the same branch or as leaf of the same clad. The taxa clustered in the latter part of the NJ-tree were considered as the most ancient in respect to the CYP gene. Thus, it could be inferred that species on apex of the NJ tree are genetically distal from the other species and were assumed to be the most evolved taxa. Bootstrap support values for all of those families in phylogenetic tree were strong (most cases 70 - 100) indicating the resolution and reliability of the results. The optimal tree with the sum of branch length = 2.595 is shown.

In the present study, order primate including gibbon and chimpanzee form a clade on the bottom of the tree (fig.1) and considered as less evolved according to evolutionary time scale. The order Rodentia holding mouse, guneapig, golden hamster and rat have clustered together whereas rat and golden hamster formed a monophyletic group and having a bootstrapping value of 100 among themselves. Calitthrix, human and macaque that belong to the same order primate, have themselves clustered. Callithrix, human and macaque that belong to the same primate order, were present in a cluster along with rabit of order Lagomorpha. The species of lagomorph resemble rodents and are considered as distant relative but due to some change in genotypic sequence, lagomorphs have evolved as distinct order during the course of evolution at this point. Gibbon and chimpanzees also belong to the same primate order but they are in a clade in the base of tree instead of forming cluster with other mammals of same primate order (macaque, human and callithrix). Order Artiodactyla comprising pig, cow and sheep have come together in a cluster with the order carnivora (dog) boasting support of bootstrapping value of 95. Sheep has a close similarity with pig and cow showing high bootstrapping value of 99. Taken as a whole, the results summarized that pig is the most evolved species standing on the specific nucleotide sequence of CYP gene. It was pre-established that the species of carnivora and artiodactyla are geographically isolated but based on the NJ-tree reconstructed during the course of present study, it was observed that both the order have formed clade together and thus seems to be evolved from a single ancestor.

By analyzing the CYP gene of Sus scrofa and Canis familiaris, it has been observed that a total of 198 numbers of transitions and 169 numbers of transversions have taken place in the nucleotide sequence of their CYP gene (table 2) during
the course of evolution which might have resulted in the diversification of species into two different orders from a common ancestor. Andrew G. McArthur, et al., performed phylogenetic analyses of 45 vertebrate CYP3 amino acid sequences indicating that teleost, diapsid, and mammalian CYP3A genes have undergone independent diversification and that the ancestral vertebrate genome contained a single CYP3A gene. There is strong support for placement of the guinea pig CYP3A genes within the rodent CYP3A diversification. The rat, mouse, and hamster CYP3A genes are mixed among several rodent CYP3A subclades, indicative of a complex history involving speciation and gene duplication (Andrew G. McArthur, et al. 2003). Our study also shows a close genotypic relation among different mammals of same order rodentia. The present study was an attempt to find out the evolutionary link between different mammals focusing on the novel mitochondrial CYP gene sequences.

4. CONCLUSION

The study reveals that genotypic changes may have taken place among the species which gradually led to phenotypic changes as dog and pig having no geographical relation, branched off from a common ancestor. The order artiodactyla is considered as a distant relative of the order carnivora but the present study made to be known that they differ from carnivores CYP gene sequence which might be influenced by variable number of substitutions (transitions and transversions) during the track of evolution. The present work was an endeavor to resolve the phylogenetic relationship taking into consideration a diverse class of chordates and therefore, intense research in this area is highly appreciated so that the CYP gene may stimulate future evolutionary studies of animal species.

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