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Full Length Research Paper

Phylogenetic analysis of human Tp53 gene using computational approach

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The TP53 gene encoding p53 protein is involved in regulating a series of pathways. New discoveries about the function and control of p53 are still in progress and it is hoped to develop better therapeutics and diagnostics by exploiting this system. Evolutionary studies are of prime importance in the field of biological research since very long as provide the basis for comparative genomics. The sequence of Homo sapiens human TP53, transcript variant-1 mRNA sequence was retrieved from the NCBI in FASTA format and was studied for its relationships and percent similarity within human and others species. Genetic variation among TP53 found in human beings and other organisms were studied in detail. Multiple sequence alignment and phylogenetic analysis of the human TP53, transcript variant-1 mRNA sequence through UPGMA was performed which showed its relationship and pattern of variations among different organisms. This study will help in modern research strategies through the manipulation of p53 as its pathways are emerging rapidly and one can predict its extensive clinical use in the near future for the human benefit worldwide.

Key words: P53, tumour, cancer, phylogeny, sequence alignment.

INTRODUCTION

The *TP53* gene encoding p53 protein is involved in regulating a series of pathways including apoptosis, DNA repair, transcription, cell cycle control and genomic stability (Hussain and Harris, 2006). It has also been shown to act directly upon mitochondria through a transcription-independent pathway (Mihara et al., 2003; Park et al., 2005). The qualitative and quantitative activity of it depends on its integrity, amount and posttranslational modifications induced by different stress induced signaling pathways (Lacroix et al., 2009). Cancer research has reached an exciting phase of its evolution and investigation of the p53 tumour suppressor pathway, in particular, has become a key focus of current cancer research (Woods and Lane, 2003). New discoveries about the function and control of p53 are still in progress and it is hoped to develop better therapeutics and diagnostics by exploiting this system (Hupp et al., 2000; Lane and Hupp, 2003).

Evolutionary studies are of prime importance in the field of biological research since very long (Pavlopoulos et al., 2010). It has become an essential element combining diverse range of research fields investigating the patterns and mechanism of evolution (Brooks et al., 2007). Phylogeny reconstruction is not only important for the organisms that house genes but is also to the evolutionary history of the genes themselves (Soltis and Soltis, 2003). Phylogenetics also provides the basis for comparative genomics (Pryer et al., 2002; Doyle and Luckow, 2003) and thus more and more molecular biologists are using phylogenetic trees to guide their sampling of taxa for comparative research (Soltis and Soltis, 2003).

Evolution of transcription network is an important genetic component in diversification between species (Shapiro et al., 2004). The evolution pattern of the p53 and related family members was studied previously which provided insights into the phylogeny of these genes and their relations (Saccone et al., 2002; Waddell et al., 2001). Modern research strategies through manipulating the p53 pathway are emerging rapidly and one can predict its extensive clinical use in the near future for the human benefit worldwide. This study was focused to explore the distribution pattern of genetic variation in p53

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found in different organisms including man.

MATERIALS AND METHODS

Sequence retrieval

The sequence of *Homo sapiens* tumor protein p53 (TP53), transcript variant 1 mRNA was retrieved from the NCBI (http://www.ncbi.nlm.nih.gov) in FASTA format.

Local sequence alignment

BLAST (Altschul et al., 1990) was performed for the human TP53, transcript variant-1 mRNA sequence retrieved from NCBI to identify its relatives in different organisms including man using the online Geneious 4.8.3 software (http://www.geneious.com). This software takes the data in FASTA format and produces the BLAST table.

Phylogenetic analysis

Phylogenetic analysis of *H. sapiens* tumor protein p53 (TP53), transcript variant-1 mRNA sequence through UPGMA was carried out using Geneious software. Phylogenetic tree was constructed by the software showing the ancestral relationship among the sequences. The tree gives different clusters showing their relationship with each other. The sequences which lie in the same cluster are closely related.

RESULTS AND DISCUSSION

Sequence retrieval

H. sapiens tumor protein p53 (TP53), transcript variant-1 mRNA was retrieved from the NCBI in FASTA format. The sequence of the transcript variant-1 mRNA (NM_000546.4) is as the following:

>gi|187830767|ref|NM_000546.4| *Homo sapiens* tumor protein p53 (TP53), transcript variant 1, mRNA

GATTGGGGTTTTCCCCTCCCATGTGCTCAAGACTGG
CGCTAAAAGTTTTGAGCTTCTCAAAAGTCTAGAGCCA
CCGTCCAGGGAGCAGGTAGCTGCTGGGCTCCGGGG
ACACTTTGCGTTCGGGCTGGGAGCGTGCTTTCCACG
ACGGTGACACGCTTCCCTGGATTGGCAGCCAGACTG
CCTTCCGGGTCACTGCCATGGAGGAGCCGCAGTCA
GATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACA
TTTTCAGACCTATGGAAACTACTTCCTGAAAACAACG
TTCTGTCCCCCTTGCCGTCCCAAGCAATGGATGATTT
GATGCTGTCCCCGGACGATATTGAACAATGGTTCAC
TGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCC
AGAGGCTGCTCCCCCCGTGGCCCCTGCACCAGCAG
CTCCTACACCGGCGGCCCCTGCACCAGCCCCCTCCT
GGCCCCTGTCATCTTCTGTCCCTTCCCAGAAAACCTA
CCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCA
TTCTGGGACAGCCAAGTCTGTGACTTGCACGTACTC
CCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAA
GACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACC
CCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCT
ACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGC
GCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATG
GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAG
GAAATTTGCGTGTGGAGTATTTGGATGACAGAAACAC
TTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCC
TGAGGTTGGCTCTGACTGTACCACCATCCACTACAA
CTACATGTGTAACAGTTCCTGCATGGGCGGCATGAA
CCGGAGGCCCATCCTCACCATCATCACACTGGAAGA
CTCCAGTGGTAATCTACTGGGACGGAACAGCTTTGA
GGTGCGTGTTTGTGCCTGTCCTGGGAGAGACCGGC
GCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAG
CCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCG
AGCACTGCCCAACAACACCAGCTCCTCTCCCCAGCC
AAAGAAGAAACCACTGGATGGAGAATATTTCACCCTT
CAGATCCGTGGGCGTGAGCGCTTCGAGATGTTCCGA
GAGCTGAATGAGGCCTTGGAACTCAAGGATGCCCAG
GCTGGGAAGGAGCCAGGGGGGAGCAGGGCTCACTC
CAGCCACCTGAAGTCCAAAAAGGGTCAGTCTACCTC
CCGCCATAAAAAACTCATGTTCAAGACAGAAGGGCC
TGACTCAGACTGACATTCTCCACTTCTTGTTCCCCAC
TGACAGCCTCCCACCCCCATCTCTCCCTCCCCTGCC
ATTTTGGGTTTTGGGTCTTTGAACCCTTGCTTGCAAT
AGGTGTGCGTCAGAAGCACCCAGGACTTCCATTTGC
TTTGTCCCGGGGCTCCACTGAACAAGTTGGCCTGCA
CTGGTGTTTTGTTGTGGGGAGGAGGATGGGGAGTAG
GACATACCAGCTTAGATTTTAAGGTTTTTACTGTGAG
GGATGTTTGGGAGATGTAAGAAATGTTCTTGCAGTTA
AGGGTTAGTTTACAATCAGCCACATTCTAGGTAGGG
GCCCACTTCACCGTACTAACCAGGGAAGCTGTCCCT
CACTGTTGAATTTTCTCTAACTTCAAGGCCCATATCT
GTGAAATGCTGGCATTTGCACCTACCTCACAGAGTG
CATTGTGAGGGTTAATGAAATAATGTACATCTGGCCT
TGAAACCACCTTTTATTACATGGGGTCTAGAACTTGA
CCCCCTTGAGGGTGCTTGTTCCCTCTCCCTGTTGGT
CGGTGGGTTGGTAGTTTCTACAGTTGGGCAGCTGGT
TAGGTAGAGGGAGTTGTCAAGTCTCTGCTGGCCCAG
CCAAACCCTGTCTGACAACCTCTTGGTGAACCTTAGT
ACCTAAAAGGAAATCTCACCCCATCCCACACCCTGG
AGGATTTCATCTCTTGTATATGATGATCTGGATCCAC
CAAGACTTGTTTTATGCTCAGGGTCAATTTCTTTTTTC
TTTTTTTTTTTTTTTTTTCTTTTTCTTTGAGACTGGGTC
TCGCTTTGTTGCCCAGGCTGGAGTGGAGTGGCGTGA
TCTTGGCTTACTGCAGCCTTTGCCTCCCCGGCTCGA
GCAGTCCTGCCTCAGCCTCCGGAGTAGCTGGGACC
ACAGGTTCATGCCACCATGGCCAGCCAACTTTTGCA
TGTTTTGTAGAGATGGGGTCTCACAGTGTTGCCCAG
GCTGGTCTCAAACTCCTGGGCTCAGGCGATCCACCT
GTCTCAGCCTCCCAGAGTGCTGGGATTACAATTGTG
AGCCACCACGTCCAGCTGGAAGGGTCAACATCTTTT
ACATTCTGCAAGCACATCTGCATTTTCACCCCACCCT
TCCCCTCCTTCTCCCTTTTTATATCCCATTTTTATATC
GATCTCTTATTTTACAATAAAACTTTGCTGCCACCTGT
GTGTCTGAGGGGTG

Local sequence alignment

H. sapiens tumor protein p53 (TP53), transcript variant-1

Table 1. BLAST table of Human p53.

S/N	AN	E-Value	Organism	Description	Sequence Length	% Pairwise Identity
1	NM_000546	0	Homo sapiens	*Homo sapiens* tumor protein p53 (TP53), transcript variant 1, mRNA	2586	100.00
2	DQ191317	0	Homo sapiens	*Homo sapiens* p53 protein (TP53) mRNA, complete cds, alternatively spliced	2589	99.10
3	DQ286964	0	Homo sapiens	*Homo sapiens* p53 protein (TP53) mRNA, complete cds, alternatively spliced	2534	99.10
4	FJ207420	0	Homo sapiens	*Homo sapiens* mutant p53 mRNA, complete cds	2517	99.10
5	AB082923	0	Homo sapiens	*Homo sapiens* mRNA for P53, complete cds	2436	99.70
6	X01405	0	Human mRNA	Human mRNA fragment for phosphoprotein p53	2073	99.40
7	NM_001126115	0	Homo sapiens	*Homo sapiens* tumor protein p53 (TP53), transcript variant 5, mRNA	2015	100.00
8	XM_001172061	0	PREDICTED:Pan troglodytes	PREDICTED: Pan troglodytes tumor protein p53, transcript variant 2 (TP53), mRNA	2016	99.40
9	XM_001172050	0	PREDICTED:Pan troglodytes	PREDICTED: Pan troglodytes tumor protein p53, transcript variant 1 (TP53), mRNA	1997	99.30
10	K03199	0	Human p53	Human p53 cellular tumor antigen mRNA, complete cds	1684	99.90
11	AK223026	0	Homo sapiens	*Homo sapiens* mRNA for tumor protein p53 variant, clone: JTH07296	1665	99.90
12	AK225838	0	Homo sapiens	*Homo sapiens* mRNA for Cellular tumor antigen p53 variant, clone: FCC127A11	1618	99.90
13	NM_001126113	0	Homo sapiens	*Homo sapiens* tumor protein p53 (TP53), transcript variant 4, mRNA	1396	100.00
14	AK297462	0	Homo sapiens	*Homo sapiens* cDNA FLJ54297 complete cds, highly similar to Cellular tumor antigen p53	1362	99.80
15	DQ186649	0	Homo sapiens	*Homo sapiens* p53 gamma isoform (TP53) mRNA, complete cds	1406	98.60
16	DQ186648	0	Homo sapiens	*Homo sapiens* p53 beta isoform (TP53) mRNA, complete cds	1406	98.60
17	DQ186652	0	Homo sapiens	*Homo sapiens* del133 p53 gamma isoform (TP53) mRNA, complete cds	1408	98.50
18	DQ186651	0	Homo sapiens	*Homo sapiens* del133 p53 beta isoform (TP53) mRNA, complete cds	1408	98.50
19	M14694	0	Human p53	Human p53 cellular tumor antigen mRNA, complete cds	1308	99.80
20	M14695	0	Human p53	Human p53 cellular tumor antigen mRNA, complete cds	1305	99.80
21	AC007421	0	Homo sapiens	*Homo sapiens* chromosome 17, clone RP5-1030O14, complete sequence	1299	99.80
22	AY838896	0	Homo sapiens	*Homo sapiens* tumor protein p53 (Li-Fraumeni syndrome) (TP53) gene, complete cds	1299	99.80
23	BT019622	0	Homo sapiens	*Homo sapiens* tumor protein p53 (Li-Fraumeni syndrome) mRNA, complete cds	1180	99.80
24	AY888314	0	Synthetic construct	Synthetic construct *Homo sapiens* clone FLH008485.01X tumor protein p53 (TP53) mRNA, complete cds	1180	99.80
25	DQ895704	0	Synthetic construct	Synthetic construct *Homo sapiens* clone IMAGE:100010164; FLH186805.01L; RZPDo839A0962D tumor protein p53 (Li-Fraumeni syndrome) (TP53) gene, encodes complete protein	1182	99.70
26	X60011	0	Human mRNA	Human mRNA for mutated p53 transformation suppressor gene	1179	99.80
27	X60020	0	Human mRNA	Human mRNA for mutated p53 transformation suppressor gene	1180	99.70
28	AY429684	0	Homo sapiens	*Homo sapiens* tumor suppressor p53 (TP53) mRNA, partial cds	1137	99.60
29	AF456343	0	Macaca fascicularis	Macaca fascicularis p53 mRNA, complete cds	1182	96.40

Table 1. Contd.

30	U48956	0	Macaca mulatta	Macaca mulatta p53 gene, complete cds	1182	96.40
31	U48957	0	Macaca fascicularis	Macaca fascicularis p53 gene, complete cds	1182	96.10
32	EF101868	0	Homo sapiens	*Homo sapiens* cell-line L1236 nonfunctional tumor suppressor p53 (TP53) mRNA, complete cds	994	99.90
33	AF175893	0	Tupaia belangeri	Tupaia belangeri chinensis p53 tumor suppressor protein (p53) mRNA, complete cds	1206	91.10
34	EF101867	0	Homo sapiens	*Homo sapiens* cell-line L428 nonfunctional tumor suppressor p53 (TP53) mRNA, complete cds	818	100.00
35	AM076971	0	Homo sapiens	*Homo sapiens* partial mRNA for tumor protein p53 mutant form (TP53 gene), classical Hodgkin Lymphoma cell line HDLM-2	782	100.00
36	X60010	0	Human mRNA	Human mRNA for mutated p53 transformation suppressor gene	736	99.70
37	NM_001082404	0	Oryctolagus cuniculus	Oryctolagus cuniculus tumor protein p53 (TP53), mRNA >gi|1532043|emb|X90592.1| O.cuniculus mRNA for p53 protein	1464	83.50
38	AF475081	0	Delphinapterus leucas	Delphinapterus leucas P53 (p53) mRNA, complete cds	1198	86.60
39	NM_213824	0	Sus scrofa	Sus scrofa p53 protein (LOC396767), mRNA >gi|115550411|dbj|AK238676.1| Sus scrofa mRNA, clone:THY010016F09, expressed in thymus	1344	83.60
40	NM_214145	0	Sus scrofa	Sus scrofa tumor suppressor p53 (P53), mRNA >gi|6165622|gb|AF098067.1|AF098067 Sus scrofa tumor suppressor p53 (p53) mRNA, complete cds	1204	85.00
41	NM_001009294	0	Felis catus	Felis catus tumor protein p53 (TP53), mRNA >gi|538224|dbj|D26608.1|CATP53 Felis catus mRNA for p53, complete cds	1321	83.10
42	NM_174201	0	Bos taurus	*Bos taurus* tumor protein p53 (TP53), mRNA >gi|73587286|gb|BC102440.1| *Bos taurus* tumor protein p53, mRNA (cDNA clone MGC:127557 IMAGE:7953339), complete cds	1346	82.50
43	AJ009673	0	Cavia porcellus	Cavia porcellus mRNA for p53 protein	1721	79.30
44	FJ855223	0	Ovis aries	Ovis aries p53 mRNA, complete cds	946	87.60
45	AF124298	0	Sus scrofa	Sus scrofa p53 protein mRNA, complete cds	941	87.60
46	D49825	0	Bos primigenius	Bos primigenius p53 mRNA, partial cds	974	86.90
47	X81704	0	B.taurus p53	B.taurus p53 mRNA	972	86.70
48	DQ656490	0	Bos taurus	*Bos taurus* cell-line TaA288 p53 mRNA, complete cds	973	86.60
49	U07182	0	Mesocricetus auratus	Mesocricetus auratus tumor supressor p53 (p53) mRNA, complete cds	1428	80.70
50	DQ656491	0	Bos taurus	*Bos taurus* cell-line TpM803 p53 mRNA, complete cds	1173	83.60

AN = Accession number; EV = E-value; SL = Sequence length; PPI = Percent pair wise Identity.

mRNA sequence was studied for its similarity patterns and BLAST was therefore performed by feeding the FASTA format of sequence into the online Geneious 4.8.3 software. After performing BLAST, the software produced BLAST table showing the accession numbers, percent similarity, e-value etc (Table 1). The sequences having lowest e-value are more closely related while the difference in e-value shows the dissimilarity among

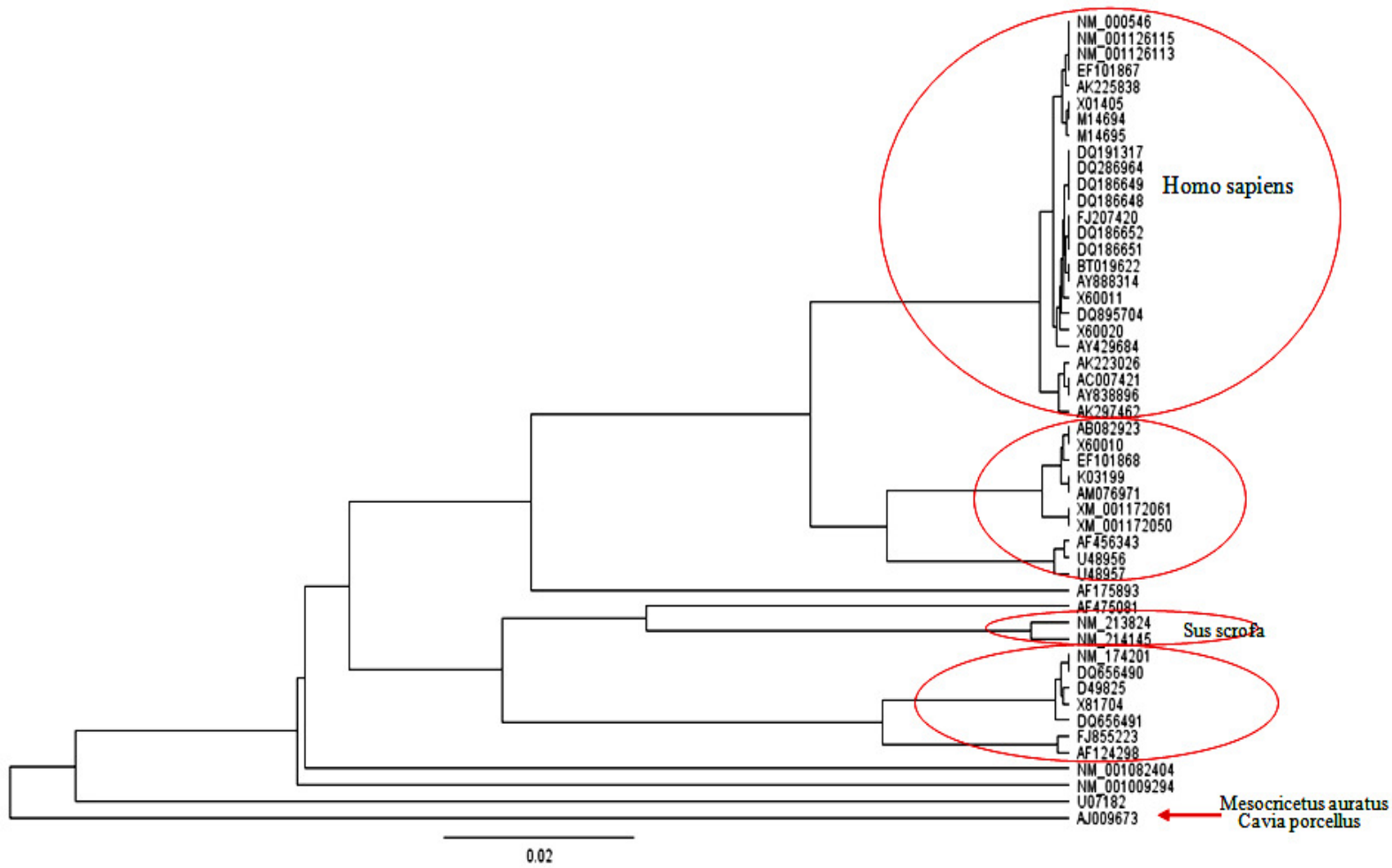

Figure 1. Phylogenetic analysis of human p53.

them.

It is clear from the results that *H. sapiens* tumor protein p53 (TP53), transcript variant 4 mRNA (NM_001126113), Expression vector Ad5CMV-p53 tumor suppressor protein p53 (p53) expression cassette, complete sequence (AF192534), *H. sapiens* cell-line L428 nonfunctional tumor suppressor p53 (TP53) mRNA, complete cds (EF101867), *H. sapiens* partial mRNA for tumor protein p53 mutant form (TP53 gene), classical Hodgkin Lymphoma cell line HDLM-2 (AM076971) and full-length cDNA clone CS0DC002YP20 of Neuroblastoma Cot 25-normalized of *H. sapiens* (CR608294) are 100% identical with *H. sapiens* tumor protein p53 (TP53), transcript variant 1(NM_000546.4), while *Bos taurus* tumor protein p53 (TP53), mRNA (cDNA clone MGC:127557 IMAGE:7953339), and complete cds (NM_174201)

are the most dissimilar sequences with 82% identity (Table) (Pintusa et al., 2006; Mills, 2005; Osnat et al., 2004; Kathryn et al., 2002; Shengkan et al., 2000).

Phylogenetic analysis

Phylogenetic analysis (Koref et al., 2003) often includes the search for evidence of directional selection in molecular evolution (Hsu et al., 2005; Hofmann et al., 2003; Yang and Bielawski, 2000). Evolution of the TP53 was studied in different organisms and adaptive changes were in the sequences. Phylogenetic analysis of *H. sapiens* tumor protein p53 (TP53), transcript variant-1 mRNA sequence was performed through the Geneious software using Tamura-Nei Algorithm (Tamura and Nei, 1993). The UPGMA rooted tree diagram of *H. sapiens* tumor protein p53 (TP53), transcript variant-1 mRNA sequence showed different clusters formation. Organism that originated from same ancestors having same e-value and 100% pair wise identity, are placed in same clusters whereas those which are distant from each other are placed in separate clusters.

Majority of human p53 sequences are lying in the same clusters. The genes lie in four distinct clusters while *Cavia porcellus* mRNA for p53 protein (AJ009673) is the most distinct one with 79.5% pair wise identity with the *H. sapiens* tumor protein p53. The phylogeny of the TP53 gene was also studied previously by different workers (Pintusa et al, 2006; Waddell et al, 2001; Ford, 2000). It can be concluded from the results that Human p53 may be evolved from the *C. porcellus* or both the species have evolved it from the same ancestor (Table). These results are confirmatory with the results of Villiard et al., 2007. Phylogenetic analysis was performed for the Pheromone-binding proteins (PBPs) in the genus Choristoneura (Lepidoptera: Tortricidae) to determine either selection has acted on these genes or not (Willett, 2000). Five fish TPI sequences obtained from GenBank were used for phylogenetic analyses to describe the TPI evolution in gnathostome vertebrates (Merritt and Quattro, 2001). (Fig. 1)

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