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In Silico Phylogenetic Study of the Human Tp53 Gene: Insights into Evolutionary Dynamics

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Abstract: The tumour suppressor protein p53, sometimes known as the "protector of the genome," is widely recognised for its crucial function in averting cancer through the regulation of DNA repair, disruption of the cell cycle, and programmed cell death known as apoptosis. Recent research has shown that p53 plays a more complex role in genomic equilibrium than its conventional roles. The Tp53 gene's evolutionary linkages are examined in this work while evolutionary studies are crucial to contemporary scientific research. Studies on evolution provide links that are useful for current study. The Homo sapiens tumour protein Tp53 transcript variant-1 mRNA FASTA sequence used in this work was obtained from the NCBI, after the Multiple Sequence Alignment using UPGMA and MEGA were used to conduct evolutionary experiments with other species. Consequently, this research. Thus, this work will aid current biological research in developing and comprehending the evolutionary dynamics and pathways of the Tp53 gene in Clinical Research.

Keywords: p53, tumour suppressor gene, phylogenetics, evolutionary analysis, sequence alignment, BLAST

Introduction: The p53 gene, also known as TP53 or tumour protein, encodes a protein that controls the cell cycle and thereby inhibits cancer formation. In species with many cell lines, the capacity of cells to suppress cancer is essential. P53's role in preserving rigidity by preventing genome changes has given it the nickname "the guardian of the genome" (Strachan and Read, 1999). It has an atomic mass of 53 kilo Dalton, which is typical for cell proteins. It is located on the final region of the human seventeenth chromosome. The structure of p53 includes the p53 protein is made up of phosphoproteins that include 393 amino acids. The tumour silencer p53 is well-known for its role in cell cycle capture, apoptosis, senescence, and Ferro ptosis. As of late, more evidence has emerged suggesting p53 is also effectively involved in the reconstruction of the cellular digestive system. The quantity of scientific evidence supporting p53's multiple activities in metabolism, normal tissue functioning, and tumour progression is expanding at an incredible rate. Owing to the significance of this gene, further research into the regulation and function of p53 is still required in order to provide more effective treatments and diagnostics. Understanding the trends and mechanisms of evolution is crucial for current scientific study, and this gene's phylogenetic analysis plays a key role in that regard. It has shown to be a crucial component in bringing together a wide variety of scientific disciplines to analyse and explore evolutionary patterns. The distribution pattern of p53 genetic variation, which is present in several creatures including humans, is also investigated in this work.

MATERIALS AND METHODS

Retrieval of nucleotide sequences of p53 gene

The nucleotide sequence of Homosapiens tumour protein p53 (Tp53) transcript variant-1 mRNA was retrieved from the NCBI website (https://www.ncbi.nlm.nih.gov) with the accession number of gene bank in FASTA format.

Determination of identity and similarity (%)
Local Sequence Alignment

The BLAST (Basic Local Alignment Search Tool) technique was utilized to evaluate the identification and similarity (%) of the chosen animal. Homo sapiens cancer protein Tp53 transcript variant-1 mRNA was obtained from the NCBI website in the FASTA format, and the BLAST N (Nucleotide Blast) was carried out from the NCBI BLAST site (https://blast.ncbi.nlm.nih.gov) website. BLAST (Altschul et al., 1990) was carried out for the Tp53 gene.

Phylogenetic Analysis

The CLUSTALW tool was used to align the Tp53 gene sequences multiple and pairwise. Using the web program genome.jp, a phylogenetic analysis of the H. sapiens tumour protein p53 (TP53) and transcript variant-1 mRNA sequence was performed using CLUSTAL W. The program created a phylogenetic tree that illustrated the ancestral connection between the sequences. The 1000 replications of the bootstrap method were used to recreate the evolutionary tree. Sequences that are located in the same cluster have a close relationship. The tree displays many groupings that indicate how they relate to one another.

Results and Discussions

Sequence retrieval

H. sapiens tumour 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:

>NC_000017.11:c7687490-7668421 Homo sapiens chromosome 17, GRCh38.p14 Primary Assembly

GATTGGGGTTTTCCCCTCCCATGTGCTCAAGA
CTGG
CGCTAAAAGTTTTGAGCTTCTCAAAAGTCTA
GAGCCA
CCGTCCAGGGAGCAGGTAGCTGCTGGGCTCC
GGGG
ACACTTTGCGTTCGGGCTGGGAGCGTGCTTTC
CACG
ACGGTGACACGCTTCCCTGGATTGGCAGCCA
GACTG
CCTTCCGGGTCACTGCCATGGAGGAGCCGCA
GTCA
GATCCTAGCGTCGAGCCCCCTCTGAGTCAGG
AAACA
TTTTCAGACCTATGGAAACTACTTCCTGAAA
ACAACG
TTCTGTCCCCCTTGCCGTCCCAAGCAATGGAT
GATTT
GATGCTGTCCCCGGACGATATTGAACAATGG
TTCAC
TGAAGACCCAGGTCCAGATGAAGCTCCCAGA
ATGCC
AGAGGCTGCTCCCCCCGTGGCCCCTGCACCA
GCAG
CTCCTACACCGGCGGCCCCTGCACCAGCCCC
CTCCT
GGCCCCTGTCATCTTCTGTCCCTTCCCAGAAA
ACCTA
CCAGGGCAGCTACGGTTTCCGTCTGGGCTTCT
TGCA
TTCTGGGACAGCCAAGTCTGTGACTTGCACG
TACTC

CCCTGCCCTCAACAAGATGTTTTGCCAACTG
GCCAA
GACCTGCCCTGTGCAGCTGTGGGTTGATTCC
ACACC
CCCGCCCGGCACCCGCGTCCGCGCCATGGCC
ATCT
ACAAGCAGTCACAGCACATGACGGAGGTTGT
GAGGC
GCTGCCCCCACCATGAGCGCTGCTCAGATAG
CGATG
GTCTGGCCCCTCCTCAGCATCTTATCCGAGTG
GAAG
GAAATTTGCGTGTGGAGTATTTGGATGACAG
AAACAC
TTTTCGACATAGTGTGGTGGTGCCCTATGAGC
CGCC
TGAGGTTGGCTCTGACTGTACCACCATCCACT
ACAA
CTACATGTGTAACAGTTCCTGCATGGGCGGC
ATGAA
CCGGAGGCCCATCCTCACCATCATCACACTG
GAAGA
CTCCAGTGGTAATCTACTGGGACGGAACAGC
TTTGA
GGTGCGTGTTTGTGCCTGTCCTGGGAGAGAC
CGGC
GCACAGAGGAAGAGAATCTCCGCAAGAAAG
GGGAG
CCTCACCACGAGCTGCCCCCAGGGAGCACTA
AGCG
AGCACTGCCCAACAACACCAGCTCCTCTCCC
CAGCC
AAAGAAGAAACCACTGGATGGAGAATATTTC
ACCCTT
CAGATCCGTGGGCGTGAGCGCTTCGAGATGT
TCCGA
GAGCTGAATGAGGCCTTGGAACTCAAGGATG
CCCAG
TAGGTAGAGGGAGTTGTCAAGTCTCTGCTGG
CCCAG
CCAAACCCTGTCTGACAACCTCTTGGTGAAC
CTTAGT
ACCTAAAAGGAAATCTCACCCCATCCCACAC
CCTGG
AGGATTTCATCTCTTGTATATGATGATCTGGA
TCCAC
CAAGACTTGTTTTATGCTCAGGGTCAATTTCT
TTTTTC
TTTTTTTTTTTTTTTTTCTTTTTCTTTGAGACT
GGGTC
TCGCTTTGTTGCCCAGGCTGGAGTGGAGTGG
CGTGA
TCTTGGCTTACTGCAGCCTTTGCCTCCCCGGC
TCGA
GCAGTCCTGCCTCAGCCTCCGGAGTAGCTGG
GACC
ACAGGTTCATGCCACCATGGCCAGCCAACTT
TTGCA
TGTTTTGTAGAGATGGGGTCTCACAGTGTTGC
CCAG
GCTGGTCTCAAACTCCTGGGCTCAGGCGATC
CACCT
GTCTCAGCCTCCCAGAGTGCTGGGATTACAA
TTGTG
AGCCACCACGTCCAGCTGGAAGGGTCAACAT
CTTTT
ACATTCTGCAAGCACATCTGCATTTTCACCCC
ACCCT
TCCCCTCCTTCTCCCTTTTTATATCCCATTTTT
ATATC
GATCTCTTATTTTACAATAAAACTTTGCTGCC
ACCTGT
ACATTCTGCAAGCACATCTGCATTTTCACCCC
ACCCT
TCCCCTCCTTCTCCCTTTTTATATCCCATTTTT
ATATC
GATCTCTTATTTTACAATAAAACTTTGCTGCC
ACCTGT GTGTCTGAGGGGT

CACCT
GTCTCAGCCTCCCAGAGTGCTGGGATTACAA
TTGTG
AGCCACCACGTCCAGCTGGAAGGGTCAACAT
CTTTT

Local sequence alignment

Homo sapiens cancer protein Tp53 transcript variant-1 mRNA was obtained from the NCBI website in the FASTA format, and the BLAST N (Nucleotide Blast) was carried out from the NCBI BLAST site (https://blast.ncbi.nlm.nih.gov) website. BLAST (Altschul et al., 1990) was carried out for the Tp53 gene and the results of the BLAST were formulated in a table (table.1) showcasing the E-value, Accession Number, Percent Identity and organism and Sequence details.

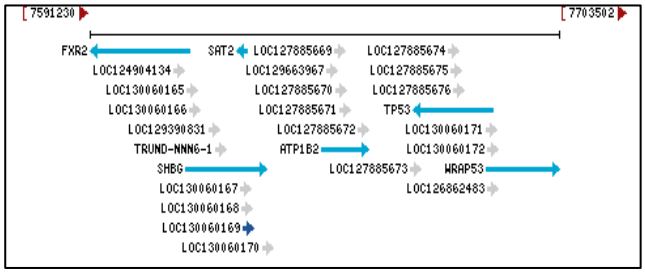


Fig.1 Genomic Context of Tp53 Gene

TAGGTAGAGGGAGTTGTCAAGTCTCTGCTGG
CCCAG
CCAAACCCTGTCTGACAACCTCTTGGTGAAC
CTTAGT
ACCTAAAAGGAAATCTCACCCCATCCCACAC
CCTGG
AGGATTTCATCTCTTGTATATGATGATCTGGA
TCCAC
CAAGACTTGTTTTATGCTCAGGGTCAATTTCT
TTTTTC
TTTTTTTTTTTTTTTTTTCTTTTTCTTTGAGACT
GGGTC
TCGCTTTGTTGCCCAGGCTGGAGTGGAGTGG
CGTGA
TCTTGGCTTACTGCAGCCTTTGCCTCCCCGGC
TCGA
GCAGTCCTGCCTCAGCCTCCGGAGTAGCTGG
GACC
ACAGGTTCATGCCACCATGGCCAGCCAACTT
TTGCA
TGTTTTGTAGAGATGGGGTCTCACAGTGTTGC
CCAG
GCTGGTCTCAAACTCCTGGGCTCAGGCGATC

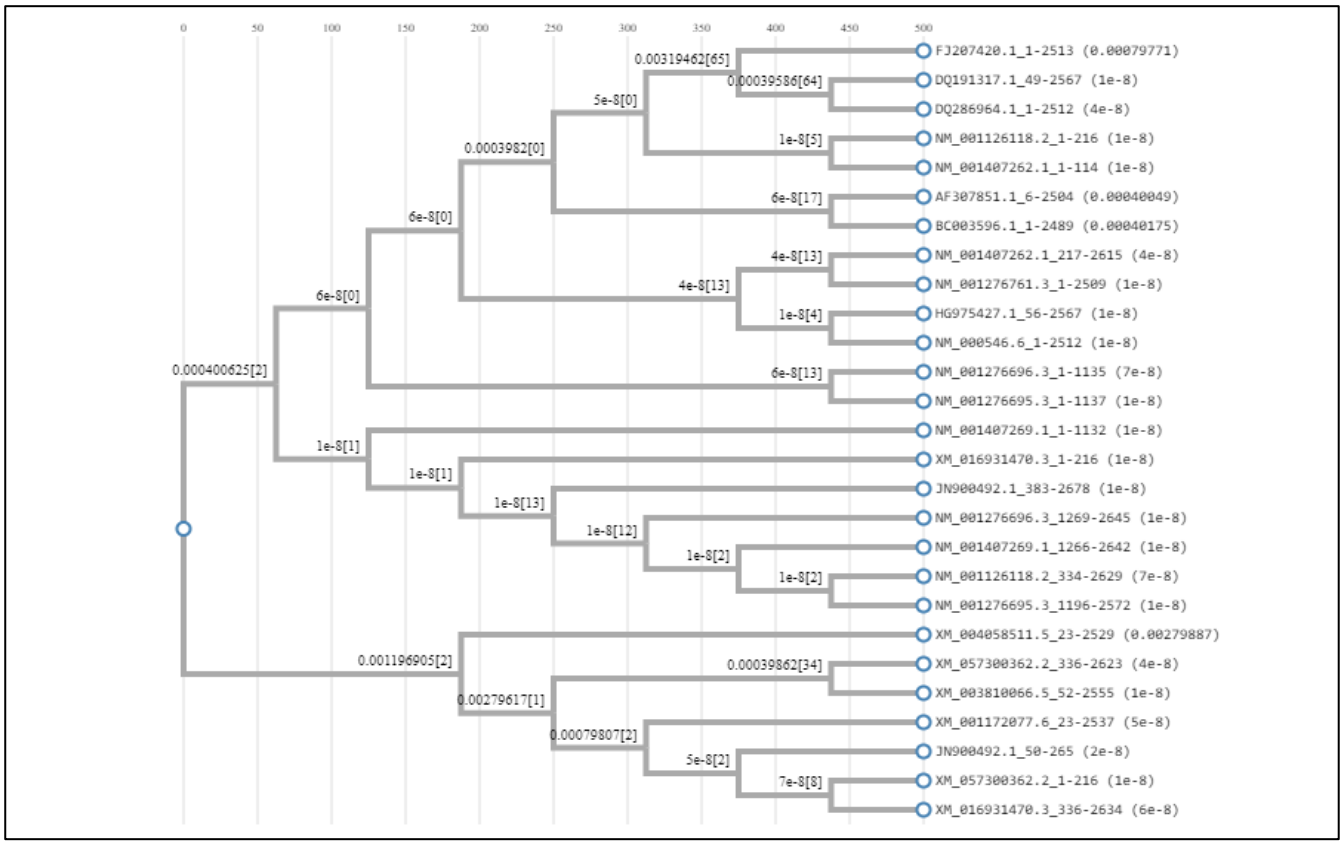
Table.1 BLAST Table of Homo sapiens cancer protein Tp53 transcript variant-1 mRNA Gene (Performed Analysis Using NCBI BLAST Program)

S.No	AC Number	E-Value	Organism	Description
1	NM_000546.6	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 1, mRNA
2	HG975427.1	0	Homo sapiens	TPA: Homo sapiens Processed transcript p53-mRNA (p53 gene)
3	NM_001276761.3	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 2, mRNA
4	AF307851.1	0	Homo sapiens	Homo sapiens p53 protein mRNA, complete cds
5	BC003596.1	0	Homo sapiens	Homo sapiens tumour protein p53, mRNA (cDNA clone MGC:646 IMAGE:3544714),
6	XM_001172077.6	0	Pan troglodytes	PREDICTED: Pan troglodytes tumour protein p53 (TP53), transcript variant X2, mRNA
7	XM_004058511.5	0	Gorilla gorilla gorilla	PREDICTED: Gorilla gorilla gorilla tumour protein p53 (TP53), transcript variant X2, mRNA
8	XM_003810066.5	0	Pan paniscus	PREDICTED: Pan paniscus tumour protein p53 (TP53), transcript variant X2, mRNA
9	DQ191317.1	0	Homo sapiens	Homo sapiens p53 protein (TP53) mRNA, complete cds, alternatively spliced
10	FJ207420.1	0	Homo sapiens	Homo sapiens mutant p53 mRNA, complete cds
11	DQ286964.1	0	Homo sapiens	Homo sapiens p53 protein (TP53) mRNA, complete cds, alternatively spliced
12	NM_001407262.1	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 9, mRNA
13	JN900492.1	0	Homo sapiens	Homo sapiens tumour suppressor TP53 (TP53) mRNA, complete cds, alternatively spliced
14	NM_001126118.2	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 8, mRNA
15	XM_016931470.3	0	Pan troglodytes	PREDICTED: Pan troglodytes tumour protein p53 (TP53), transcript variant X1, mRNA
16	XM_057300362.2	0	Pan paniscus	PREDICTED: Pan paniscus tumour protein p53 (TP53), transcript variant X1, mRNA
17	NM_001407269.1	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 12, mRNA
18	NM_001276696.3	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 3, mRNA
19	NM_001276695.3	0	Homo sapiens	Homo sapiens tumour protein p53 (TP53), transcript variant 4, mRNA

Phylogenetic Analysis

Finding evidence of directional selection in molecular evolution is a common step in phylogenetic research (Koref et al., 2003; Hsu et al., 2005; Hofmann et al., 2003; Yang and Bielawski, 2000). TP53 evolution was examined in a variety of taxa, and sequence modifications were shown to be adaptive. The UPGMA rooted tree diagram of H. sapiens tumour 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. Phylogenetic reconstructions and an alignment of the H. sapiens tumor protein p53 (TP53) transcript variant-1 mRNA sequence were carried out using the "build" function of ETE3 3.1.3 (Huerta-Cepas et al., 2016) as it was implemented on the GenomeNet (https://www.genome.jp/tools/ete/).The multiple sequence alignment was supplied by the user.
The PhyML v20160115 model and settings were used to infer the ML tree: -pinv e --alpha e --nclasses 4 -o tlr -f m --bootstrap 1000 (Guindon et al., 2010). Out of 1000, branch supports are calculated.

Fig.1 Phylogenetic Analysis of Human Tp53 Gene



Conclusion

We may infer that the p53 protein plays a significant role as a tumour suppressor in bovines as well as other species, including humans. Animals that have a high proportion of identity and resemblance have more conserved amino acid sequences. Therefore, this work can offer a platform for researchers to determine animal comparative genomics.

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