Journal of Advances in Biology & Biotechnology
7(4): 1-7, 2016; Article no.JABB.26641
ISSN: 2394-1081

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In Silico Analysis and Comparison of Alpha Tubulin Gene with Other Tubulin Families

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/26641
Editor(s):
(1) Margaret Tzaphlidou, Department of Medical Physics, School of Health Sciences, Ioannina University, Greece.
(2) Ram Shanmugam, Professor, School of Health Administration, Texas State University, 601 University Drive, San Marcos, TX 78666, USA.
Reviewers:
(1) Joanna Zarzynska, Warsaw University of Life Sciences, Poland.
(2) Dipak Kumar Sahoo, Iowa State University, USA.
Complete Peer review History: http://sciencedomain.org/review-history/15357

Original Research Article

Received 26th April 2016
Accepted 26th June 2016
Published 11th July 2016

ABSTRACT

Microtubules are composed of a heterodimer of alpha and beta tubulins and performs many diverse functions. The genes encoding these microtubule constituents are members of the tubulin superfamily. Studies shown the existence of genes from the alpha, beta and gamma tubulin families in all eukaryotes.

The two main classes includes alpha and beta which mainly represents the tubulin family. There are multiple alpha and beta tubulin genes, which are highly conserved among species. The selected gene encodes alpha tubulin which belongs to its associated protein superfamily. All tubulin genes differ in configuration just because of few amino acid substitutions. The study was performed to analyze the alpha tubulin (TUBA8) gene and its associated protein by using various bioinformatics tools. The gene was matched in HGNC against its particular entry to obtain the HGNC gene ID. The variant analysis shown 3 coding exons, 276 genetic variants and few UTR regions at 3'end of gene. Protein domains were analyzed by protein InterPro domain analysis software.

Keywords: Microtubule; TUBA8; substitutions; HGNC; UTR; domain; InterPro.

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1. INTRODUCTION

Microtubules are structural filamentous intracellular elements that are involved in variety of movement processes. They are cylindrical tubes of 20-25 nm in diameter and play an important role in nuclear and cellular division processes. Their assembly is found to be complexed. Mainly, their role in intracellular material organization is ambiguous.

Studies shown their importance in the disruption of normal microtubule assembly can affects cell motility, division, secretion and mainly intracellular transport machinery [1]. The diversity of this type of microtubular structure and its function suggests that different microtubule subunits assemble into specialized microtubules [2]. They are composed of some proto filaments which are in turn composed of alpha- and beta-tubulin polymers. Heterodimers of alpha and beta tubulin proteins are the major components of microtubules. According to some studies, each microtubule is polarized showing at one end alpha-subunits are exposed as (-) and beta-subunits are exposed (+) at the other end. The basic subunit of all microtubules is a heterodimer of both alpha and Beta-tubulin polypeptides. The alpha beta subunit was found to co assemble with different species including some tissue specific microtubule associated proteins to build microtubules [3]. Various studies of tubulin (TUB) proteins and genes from number of different species indicate that although tubulin structure is highly conserved among species.

According to some previous studies, tubulin is known to be the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain. All the tubulin proteins are structural proteins with conserved amino acid sequence between species. The tubulin gene family consists of six but highly conserved and distinct subfamilies that possess alpha, beta, gamma, delta, epsilon, and zeta-tubulins, each with specific conserved sequences and are widely distributed among eukaryotes [3,4].

1.1 TUBA8 Gene

The TUBA8 (Tubulin, Alpha 8) gene belongs to the alpha tubulin protein family. Alpha tubulins are one of two core protein families namely, alpha and beta tubulins that heterodimerize and assemble to form microtubules. Several mutations in this gene are found to be associated with polymicrogyria and optic nerve hypoplasia. Various studies suggested that TUBA8 is a Protein Coding gene. Sequence analysis of human, mouse, and rat α-tubulin genes has enabled to get the new details related to tubulin protein families. According to recent studies, α-tubulin mutations have been associated with some impaired neuronal migration [5,6]. Moreover, the γ-tubulin is essential for nucleation of the microtubule assembly. Similarly, details about other families are not known yet [7,8]. Multiple copies of the α-tubulin genes are found in the human genome. Tissue specific alpha tubulins are also found which gives different expression patterns in mouse and humans [9,10].

The present study was performed to analyze the tubulin gene with its associated protein. For, this the alpha tubulin gene was selected. The analysis was done by using various bioinformatics tools. The genetic variants were analyzed. The protein domains shown many conserved regions with coding leading towards formation of functional protein molecule. Results obtained are discussed below.

2. MATERIALS AND METHODS

2.1 Sequence Retrieval of TUBA8 Gene

Sequence of TUBA8 gene was retrieved from NCBI-Genbank data repository.

2.2 Chromosomal Map Analysis

Chromosomal Map of the TUBA8 gene was analyzed by using Ensemble software.

2.3 Reported Homolog

Homology searching shows the predicted homologs of TUBA8 gene.

2.4 Transcript Analysis

Transcript maps were obtained through Ensemble software.

2.5 Protein Domain Analysis through InterPro Tool

InterPro was used to study various protein matches and signatures. The putative conserved domain of TUBA8 gene was found by using InterPro data repository.

3. RESULTS

Gene analysis of TUBA8 shows multiple genetic variants with coding regions. The homology search shown the predicted gene homologs found in mouse and rats. Moreover, the study of protein signatures in conserved regions have given number of putative domains. Results obtained are given in Fig. 1.

3.1 Sequence Retrieval

The alpha tubulin gene 8 sequence was retrieved from NCBI - Genbank with accession Id >gi|7594609|.

3.2 Chromosome Map

Chromosome Map was obtained by using ensemble software. Map shows the tubulin gene is located at chromosome number 22, q arm and position 18,110,331-18,146,554. The map is shown in Fig. 2.

3.3 Homologs

The homology searches was performed to find the similar and paralogous genes of tubulin alpha 8 gene. Results shown 2 reported homologs of tubulin 8 gene in rats and mouse.

3.4 Transcript Analysis

Transcript analysis shown the highlighted regions with different colors. All the highlighted regions shows the frameshift, missense mutations, the coding regions and 3' UTR. The ensemble Id for selected tubulin alpha gene was ENSE00003704038. The sequence of 328bp from upstream region of gene was analyzed to identify these mutations. The results are shown in the Fig. 3.

>gi|7594609|emb|AJ245922.1| Homo sapiens mRNA for alpha-tubulin 8 (TUBA8gene)

AGGCGGCTGTATCTGGAGCAGTCGGGGCGGGCAGGCCCAGCTGAGAGGTGCGCGGGCGAGGACAGCGGCAGCGATGCGGAGCATATCAGTCCACGTGGGCCAAGCGGGAGTTCAGATTGGCAATGCCTGCTGGGAGCTCTTCTGCCTGGAACACGGCATCCCAGACG
GCACTTTTGATGCTCAAGCTAGCAAGATCAACGATGATGACTCCTTCACCACCTTTTTCAGCGAGACTGGCAATGGAGCATGTGCCCCGGGCCGTCATGATAGATCTGGAGCCTACTGTAGTGGATGAGGTTCGGGCAGGAACCTACCGCCAGCTCTTCCATCCAGA
GCAGCTGATCACAGGAAAGGAGGATGCAGCCAACAACTATGCCCGGGGCCACTACACGGTGGGCAAGGAGAGCATTGACCTGGTGCTGGACCGCATACGGAAGCTGACAGATGCTTGCTCTGGCCTGCAGGGCTTCCTGATTTTCCACAGTTTTGGTGGGGGCACTG
GCTCCGGCTTCACTTCTCTGCTGATGGAACGCCTCTCCCTGGATTATGGCAAGAAATCCAAGCTGGAGTTTGCCATCTACCCAGCCCCCCAGGTCTCTACTGCAGTGGTGGAGCCCTACAACTCCATCCTGACCACCCACACCACACTGGAAATTCAGATTGTGCTT
TCATGGTGGACAACGAAGCCATCTATGACATCTGCCGCAGGAACCTTGACATTGAGCGCCCTACCTATACCAACCTCAACCGCCTCATCAGTCAGATTGTGTCCTCAATCACTGCTTCTCTCCGCTTTGACGGGGCCCTCAATGTGGACCTCACTGAGTTCCAGACC
AACCTGGTGCCCTACCCCCGCATCCACTTCCCGCTGGTCACCTACGCGCCCATCATCTCTGCCGAGAAAGCCTATCACGAACAGCTCTCTGTGGCCGAGATAACCAGCTCCTGCTTTGAGCCCAACAGCCAGATGGTGAAGTGCGACCCGAGACATGGCAAGTACAT
GGCCTGCTGCATGCTCTACCGGGGCGACGTGGTGCCCAAGGATGTGAATGTCGCTATTGCTGCCATCAAGACCAAGAGGACCATCCAGTTTGTAGACTGGTGTCCCACAGGCTTCAAGGTGGGCATCAACTACCAGCCCCCGACCGTGGTCCCCGGGGGAGACCTGG
CCAAGGTGCAGCGGGCCGTCTGCATGCTCAGCAACACCACGGCCATTGCGGAGGCCTGGGCCCGCCTCGACCACACAAGTTCGACCTCATGTACGCCAAGCGGGCCTTTGTGCATTGGTATGTGGGAGAGGGGATGGAAGAAGGAGAATTTTCTGAGGCCAGGGAAGAC
TTAGCTGCCCTGGAGAAGGATTATGAAGAAGTGGGGACTGATTCGTTTGAAGAAGAAAATGAAGGGGAGGAATTTTAAATATATACCTTCCCCTTGGCTGTGTCTCTTTATTTATGCTGTGCCATTCAAAGCACATGTTCAAGAGAACAGAACACTCTCCCCGCCCC
AGCCTGATTCCTGCCTTACCCAGGAGGAGGGTGCCTGGCCCCAGTACCCAGGGTGGCACGACTGGGCTAAGTGGACACTGAGCTTCATCAGGCCCTCCCTGGGTAGGAGCAGCTTTGTGTCACTAAAGAAAGTGAGGGCCACTGTCTCCGGCGGGTGAGGCTGCAGC
CCAGTTTCACATGCGAGGAGGCCTAATCAGGAGTTTCAATTCCAGATCGGGCTGGGTCCAGCCCCAAAACATGGCCTGCTGGCTGGGGAGTGGGAACACTCAGAGAAAGGGGAGATCTGGGCCTGGGAAGATTCCGGGGCAGGGGTGAGCGGACGTTCACATGAGTG
GGTCTATAGCCCGCTCCGGGCATCATCTAGACTTAGCATGCATTCACTCCCCCATCACATATTCCAATACACACCCTGCCCTAGGCACTGAGAGCTGGAGAGTTGGTGATAAGCGAGACGAACACATTTCCTGCCCTCATACACACATAAATAAAGTGAATGAGATC
ATGTG

Fig. 1. Shows the nucleotide sequence of TUBA 8 gene obtained from NCBI – Genbank

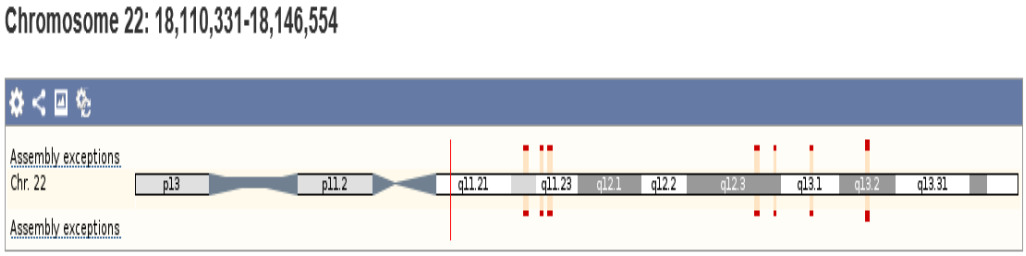
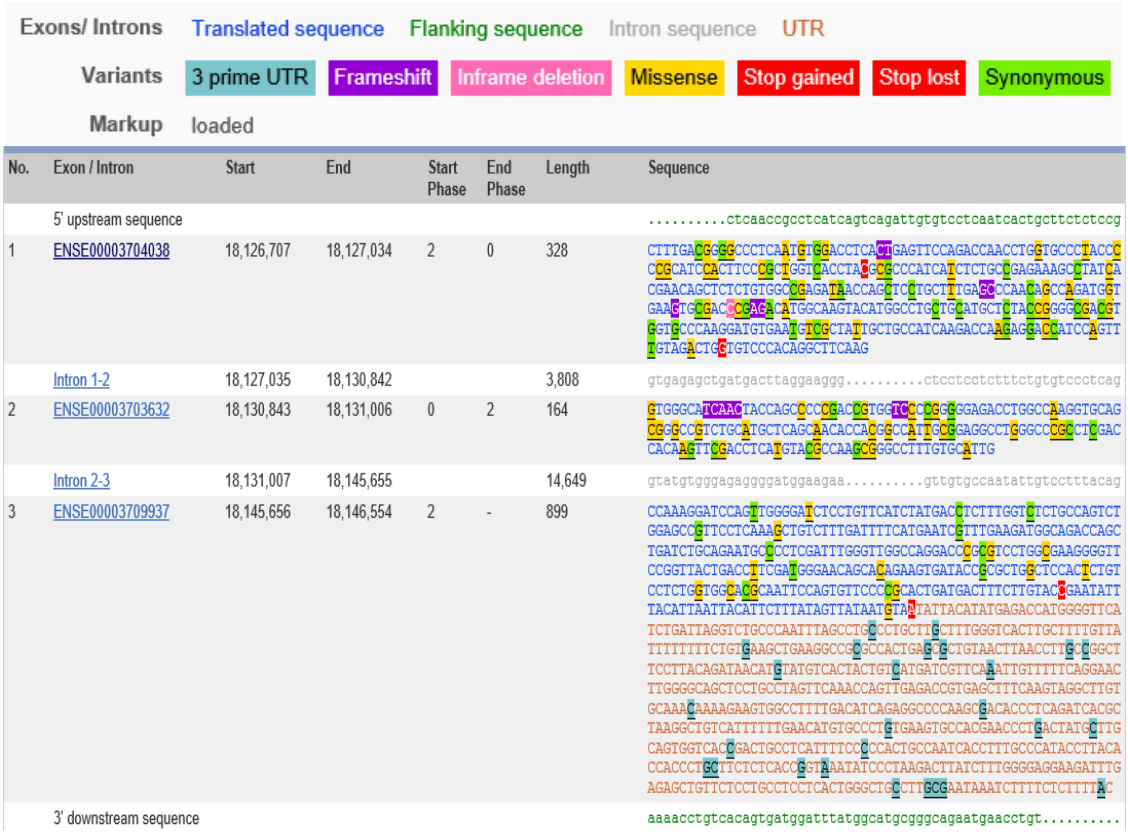


Fig. 2. Shows the chromosome map of TUBA8 gene

Table 1. Shows the homologs of TUBA8 gene

Name	Symbols	Database entry
Rattas norvegicus	TUBA8	MGI : 1858225
Mus musculus	TUBA8	RGD: 1566041



No.	Exon / Intron	Start	End	Start Phase	End Phase	Length	Sequence
	5' upstream sequence						.....ctcaaccgcctcatcagtcagattgtgtcctcaatcactgcttctctccg
1	ENSE00003704038	18,126,707	18,127,034	2	0	328	CTTTGACGGGGCCCTCAATGTGGACCTCACTGAGTTCCAGACCAACCTGGTGCCCTACCC CCGCATCCACTTCCCGCTGGTCACCTAGGCGCCCATCATCTCTGCCGAGAAAGCCTATCA CGAACAGCTCTCTGTGGCCGAGATAACCAGCTCCTGCTTTGAGGCCAACAGCCAGATGGT GAAGTGCGACCGAGACATGGCAAGTACATGGCCTGCTGCATGCTCTACCGGGGCGACGT GGTGCCCAAGGATGTGAATGTCGCTATTGCTGCCATCAAGACCAAGAGGACCATCCAGTT TGTAGACTGCTGTCCCACAGGCTTCAAG
	Intron 1-2	18,127,035	18,130,842			3,808	gtgagagctgatgacttaggaaggg.....ctcctcctctttctgtgtccctcag
2	ENSE00003703632	18,130,843	18,131,006	0	2	164	GTGGGCATCAACTACCAGCCCCGACCGTGGTGCCCGGGGAGACCTGGCCAAGGTGCAG CGGGCCGTCTGCATGCTCAGCAACACCACGGCCATTGCGAGGCCTGGGCCCGCCTCGAC CACAAGTTCGACCTCATGTACGCCAAGCGGGCCTTTGTGCATTG
	Intron 2-3	18,131,007	18,145,655			14,649	gtatgtgggagaggggatggaagaa.....gttgtgccaatattgtcctttacag
3	ENSE00003709937	18,145,656	18,146,554	2	-	899	CCAAAGGATCCAGTTGGGGATCTCCTGTTCATCTATGACCTCTTTGGTCTCTGCCAGTCT GGAGCCGTTCCTCAAAGCTGTCTTTGATTTTCATGAATCGTTTGAAGATGGCAGACCAGC TGATCTGCAGAATGCCCCTCGATTTGGGTTGGCCAGGACCCGCGTCCTGGCGAAGGGGTT CCGGTTACTGACCTTCGATGGGAACAGCACAGAAGTGATACCGCGCTGGCTCCACTCTGT CCTCTGGTGGCACGCAATTCCAGTGTTCCCCGCACTGATGACTTTCTTGTACCGAATATT TACATTAATTACATTCTTTATAGTTATAATGTAATATTACATATGAGACCATGGGGTTCA TCTGATTAGGTCTGCCCAATTTAGCCTGCCCTGCTTGCTTTGGGTCACTTGCTTTTGTTA TTTTTTTTCTGTGAAGCTGAAGGCCGCGCCACTGAGCGCTGTAACTTAACCTTGCCGGCT TCCTTACAGATAACATGTATGTCACTACTGTCATGATCGTTCAAATTGTTTTTCAGGAAC TTGGGGCAGCTCCTGCCTAGTTCAAACCAGTTGAGACCGTGAGCTTTCAAGTAGGCTTGT GCAAACAAAAGAAGTGGCCTTTTGACATCAGAGGCCCCAAGCGACACCCTCAGATCACGC TAAGGCTGTCATTTTTTGAACATGTGCCCTGTGAAGTGCCACGAACCCTGACTATGCTTG CAGTGGTCACCGACTGCCTCATTTTCCCCCACTGCCAATCACCTTTGCCCATACCTTACA CCACCCTGCTTCTCTCACCGGTAAATATCCCTAAGACTTATCTTTGGGGAGGAAGATTTG AGAGCTGTTCTCCTGCCTCCTCACTGGGCTGCCTTGCGAATAAATCTTTTCTCTTTTAC
	3' downstream sequence						aaaacctgtcacagtgatggatttatggcatgcgggcagaatgaacctgt.....

Fig. 3. Shows the complete transcript with exons, UTR and frameshift mutations

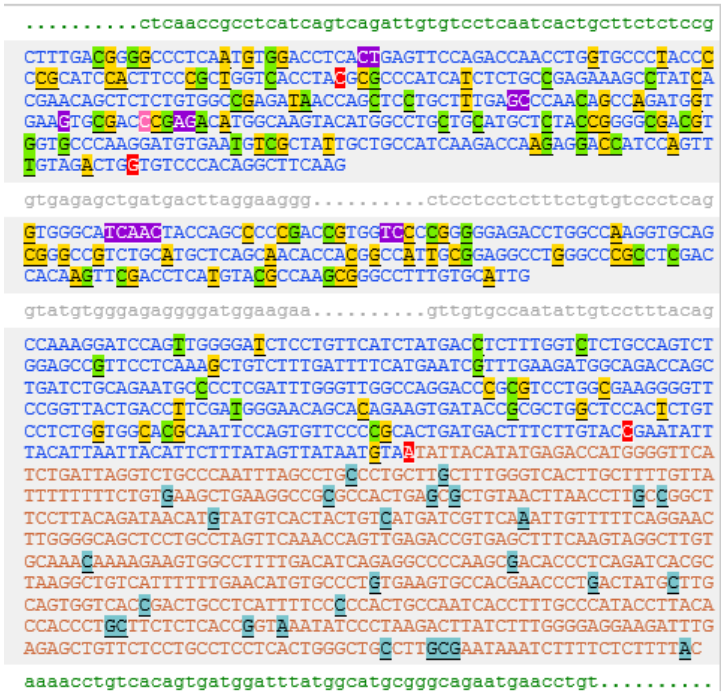


Fig. 4. Shows the clear view of coding regions with 3'UTR

3.5 Gene Variants

Variant analysis shown 276 reported variants for TUBA8 gene. Out of which few are given below. The results show all codons covering and their potential residues. This will help to study their evolutionary relationships. All variants have different variant Id's indicating different codons which have been changed due to missense or nonsense mutations.

3.6 Protein Domains

All proteins are comprised of some functional conserved parts or regions which can fold independently. Domain analysis shown the detailed signatures of alpha tubulin protein family. Results are shown in Fig. 6.

Studies shown different expression levels of tubulin alpha 8 gene in human tissues. Different expression levels can be checked by techniques like microarray, RNA sequencing and SAGE. The results obtained from gene cards human gene database are shown in Fig. 7.

4. DISCUSSION

Tubulin belongs to a family of globular gene and member of protein superfamily. Microtubule structure of these genes help to control the cellular movement. They are categorized into multiple classes. But the two most common are alpha and beta tubulins. α- and β-tubulins polymerize into microtubules which are major components of the eukaryotic cytoskeleton. They are also involved in division processes. According to previous studies, six alpha tubulin (TUA) genes from *Arabidopsis* and five *TUA* genes from cotton were reported with somewhat similar function. The functional analysis of these

Residue	Variation ID	Type	Evidence	Alleles	Ambig. code	Residues	Codons	SIFT	PolyPhen
3	rs193124477	Synonymous variant		C/T	Y	D	GAC, GAT	-	-
4	rs185024084	Missense variant		G/A	R	G, R	GGG, AGG	0.01	0.985
4	rs766440703	Synonymous variant		G/A	R	G	GGG, GGA	-	-
5	rs754011193	Missense variant		G/T	K	A, S	GCC, TCC	0.06	0.407
7	rs755134046	Missense variant		A/C	M	N, T	AAT, ACT	0	0.759
8	rs145621219	Missense variant		G/A	R	V, M	GTG, ATG	0	0.651
8	rs751418447	Synonymous variant		G/A	R	V	GTG, GTA	-	-
9	rs757012050	Missense variant		G/A	R	D, N	GAC, AAC	0.01	0.845
11	rs765400078	Frameshift variant		CT/-	-	T, X	ACT, A	-	-
18	rs781037898	Missense variant		G/C/T	B	V, L	GTG, CTG	0	0.145
18	COSM4624570	Coding sequence variant		COSMIC_M...	-	-	-	-	-
20	rs769194966	Missense variant		T/G	K	Y, D	TAC, GAC	0	0.848
20	COSM4765069	Coding sequence variant		COSMIC_M...	-	-	-	-	-
21	rs779563209	Missense variant		C/T	Y	P, L	CCC, CTC	0	0.883
22	rs748797090	Missense variant		C/G/T	B	R, G	CGC, GGC	0	0.663

Fig. 5. Shows the genetic variants of TUBA8 gene

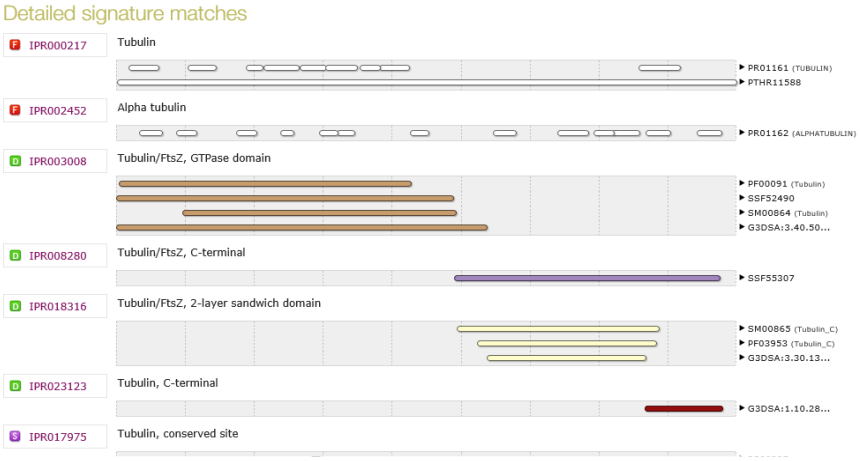


Fig. 6. Shows the protein domains and signature obtained through InterPro analysis software

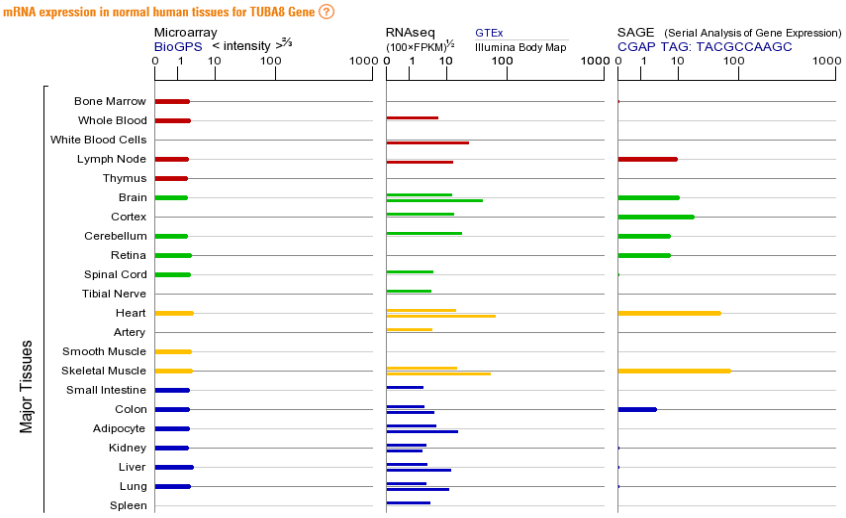


Fig. 7. Shows the different expression levels of TUBA8 gene obtained from Gene cards

genes deduced amino acid sequence, with some gene duplication events, translocations and chromosomal rearrangements. Some of the TUA genes are found to be rich with similar characteristics performing various similar functions. Analysis of these genes through bioinformatics software's shown results with similar gene structure and high level of sequence identity [11]. Similarly, in case of plants, it was difficult to balance these TUA genes with other TUB genes within the cytoplasm to tolerate large imbalances in the ratio of TUA to TUB within the cytoplasm. Also, in maize, the regeneration event in plants stops if a single tubulin gene was transformed and overexpressed [12,13].

In cotton, α-tubulin transcripts in fibers were found in much higher levels in other tissues reflecting the rapid cell elongation occurring in developing fibers. The higher levels are known to be consistent with abundance of tubulin proteins [14]. Studies suggested the characterization of fiber α-tubulin promoters may be a useful to study the tubulin expression levels. Many gene specific differences in transcript accumulation in developing fibers was observed. Transcripts of several genes have been shown to accumulate at high levels. Studies proposed that transcripts of another α-tubulin gene shown rapid expansion and declined during the onset of secondary wall synthesis [15].

Studies shown multiple copies of the α-tubulin genes in the human and rodent genomes exits. Alpha tubulin genes are commonly involved in formation of microtubules with beta tubulin and functions in processes like cellular movement and cell division. Studies about copy number variation of alpha tubulin in other organisms revealed many contrasting patterns which highlighted this gene to be studied in bdelloids [16]. Similarly, in many animals, it was found in low copy number and highly conserved. With this function, it can be used as a marker for phylogenetic relationships [17]. However, the different roles of microtubules have are important to study the functional divergence in many cases. Four distinct copies are found in *Drosophila melanogaster* and expressed in different tissues [18,19]. The selected gene was matched in HUGO nomenclature which assigns a unique number to each human gene. However, previously it was not known about the alpha tubulin gene in its nomenclature. The transcript comparison of TUBA8 gene, when viewed I n Ensemble Gene View, identifies that there were 3 exons, 14 conserved domain and 169 genetic variants present in this gene. The first exon of the *TUBA8* genes contains only untranslated region (UTR) sequences. Similarly, various protein signature were found to be associated with this alpha tubulin gene. The results were obtained from InterPro domain analysis software.

5. CONCLUSION

Transcripts of the α-tubulin gene mostly appear to accumulate in rapidly elongating tissues. The analysis of tubulin protein will help to identify the conserved domains and also to predict the 3D structure of protein. Sequence analysis depicts various sequence variations in different tubulin protein families. Also, this will lead to inner insights of particular functions in cellular movements and interactions. Moreover, the intracellular reactions, cellular junctions, gene

expression studies and genetic variations can be well studied to overcome multiple disease related problems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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