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## Clusters of CDK2, CCND1, and CMYC genes involved in cancers: Acute Lymphocytic Leukemia (ALL) as a model

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### Abstract

Cancer is not a single disease but it involves changes in multifunctional genes, the causes for these changes remain less understood. It is now becoming clear that multiple genes orchestrate to turn on the carcinogenesis process. These genes involve several signaling pathways which then characterize uncontrolled cell divisions. Our aim was to study cell cycle genes CDK2, CCND1, and c-MYC to determine their clustering in the evolutionary pathway and to understand their diversions leading to continued cell division processes. Since Acute Lymphoblastic Leukemia (ALL) is the most prevalent form of cancer in children we took this as a model for analyzing the role of these genes in the leukemia process. The prevalence/spread of these genes was found to be very limited in the animal kingdom; hence the question is whether this may be due to the fact that during evolution in time there could have been loss of some functions or mutations in these genes which relates to the switch function of these genes. Alternatively, have they evolved in a way which we are unable to trace due to limited methodology? Further, with the results analyzed so far we can imagine that these species in which we found the presence of these genes across the animal kingdom could have had cancer like diseases during their lifetime. We conclude that each of these genes formed several clusters which were typical of their role/functions in ALL.

Keywords: ALL; Cell cycle genes; Gene clusters; Phylogeny; CDK2; CCND1; c-Myc.

### Introduction

Acute Lymphoblastic Leukemia (ALL) is one of the most frequent types of cancer that afflicts children. It is characterized by accumulation of immature lymphocyte progenitor cells in the bone marrow. Although current long term survival rate in children is above 80%, this disease is not completely curable with the available treatment strategies (Crazzolara and Bendall 2009). A better understanding of the underlying mechanisms behind ALL requires information about the changes that occur during cell cycle and the genes that are involved in the process. Our earlier studies on leukemia in children determined the role of susceptibility biomarkers and risk factors (Reddy et al. 2006; Reddy and Jamil 2006; Jamil and Reddy 2007), further we also determined the SNP changes in drug metabolizing genes like GSTs and FLT3 which relate to drug-gene interactions (Reddy et al. 2006, Kumar et al. 2011), the signaling pathways and biomarkers of hematological malignancies were also determined (Mani et al. 2006, 2007). Cell division in organisms is regulated by a family of cyclin dependent kinases (CDKs), which consist of a subunit of CDK and an activating cyclin subunit. These

CDK complexes phosphorylate several substrates such as the Retinoblastoma family of proteins, which are negative regulators of cell cycle. The inactivation of these CDKs is also part of the typical cell cycle process. The inhibitors of CDK such as p16lnk4a, p15lnk4b, p27Kip1, and p21Cip1 negatively regulate CDK activities (D'Andrilli *et al.* 2004). The cell cycle process is regulated by the tumor suppressor gene, p53. Several other genes and proteins are also involved in the normal cell cycle process.

Several studies have been carried out to determine the changes in the cell cycle that lead to leukemogenesis. Homozygous inactivation of p16 INK4 gene has been reported in childhood ALL by several researchers (Okuda et al. 1995; Lemos et al. 2003). Aberrant p15 promoter methylation (Batova et al. 1997) and deletion of p15 (Okuda et al. 1995) have been reported in several cases of childhood ALL. A study based on a population of Chinese children has implicated polymorphism of cyclin D1 (CCND1) in relation to occurrence of ALL (Hou et al. 2005). Several studies have reported deletion of p27/Kip1 gene in childhood ALL (Markaki et al. 2006; Takeuchi et al. 2006). CDK2 catalytic activity was reported in a sample of childhood

ALL samples using in vitro kinase assays (Schmitz *et al.* 2005). Studies on Notch-1 regulatory mechanisms have suggested that c-Myc deregulation may be part of the early events in T-cell leukemogenesis (Palomero *et al.* 2006; Weng *et al.* 2006). Overexpression of MDM2 has been reported in 15–25% of ALL patients at the time of diagnosis (Hendy *et al.* 2009). Tumor suppressor gene, Tp53, mutations have been reported in some children with ALL, though it is more frequently associated with relapse patients (Kawamura *et al.* 1995).

In the present study, sequences of selected genes involved in the cell cycle pathway were selected to infer phylogeny as well as to determine their homology across various species in the animal kingdom to better understand how these genes contribute to leukemogenesis. Although the same cell cycle genes exist in various organisms across the animal kingdom, but they function differently in different organisms, while in humans when these genes develop mutations leukemogenesis occurs. A study of this nature might help in better understanding leukemogenic pathway in humans.

### Materials and Methods

### (a) Search for cell cycle genes in ALL

Literature databases were queried to devise a list of genes which are involved in cell cycle and have been reported in association with ALL (Table 1). Further information about each of the genes in the list was obtained by querying GeneCards database version 3 (Safran *et al.* 2010) (www.genecards.org).

# (b) DNA sequence data and sequence alignment

Three genes were selected for the study from the list of cell cycle genes. NCBI GenBank (Benson database et 2011) al. (at www.ncbi.nlm.nih.gov/) was gueried to retrieve all available nucleotide sequences, across various species, of the mRNA transcript of the genes. These sequences were saved as fasta file and were used for further analysis. The sequences were first imported into the alignment explorer of MEGA version 4 software (Tamura et al. 2007). An initial multiple sequence alignment

was carried out using the Clustal W (Thompson et al. 1994) algorithm. The aligned sequences were further manually edited and again aligned using Clustal W with default parameters for Gap Opening, Gap Extension Penalty and DNA weight matrix to obtain optimal global sequence alignment. This multiple sequence alignment file was then used to infer phylogeny.

### (c) Phylogenetic tree construction

Phylogeny was reconstructed using MEGA version 4.0. The distance based Neighbour-Joining (Saitou and Nei 1987) method was chosen for phylogeny reconstruction of the sequences. Kimura 2-parameter (Kimura 1980) distance model, which assumes uniform rate of substitution among sites, was selected as the nucleotide substitution model. To further increase the reliability of the phylogenetic tree obtained, 1000 Bootstrap replications were performed.

### (d) Functional divergence

Functional divergence is useful in identifying sites/residues that are subjected to functional constraints during evolution. In this study, functional divergence between the various species for each gene was calculated using Diverge 1.04 software (Gu and Velden 1999). Sequences of the corresponding proteins encoded by the genes were aligned using Clustal W in MEGA software using default parameters and this alignment was used as input for the software. Using this input, the software was used to build a Kimura 2 parameter tree to delineate clusters. These clusters were then used to estimate statistical parameters such as site specific profile, which is useful to predict the amino acid residues which are vital for functional divergence. Residues estimated to have a functional divergence value greater than 0.1 were highlighted in the sequence alignment.

### Results

### (i) Phylogenetic analysis

From the genes listed in Table 1 we selected three genes CDK2 (606 bp), CCND1 (526 bp) and c-MYC (509 bp) genes and determined their phylogeny after multiple sequence alignment.

S.No.	Gene Name	Function	Reference	GeneCards ID
1	p53	regulates target genes that induce cell cycle arrest	Wojcik <i>et al.</i> 2005	GC17M007565
2	p16INK4A (CDKN2A)	Capable of inducing cell cycle arrest in G1 and G2 phases.	Lemos <i>et al.</i> 2003	GC09M021957
3	p15 (CDKN2B)	Encodes a protein that functions as a cell growth regulator that controls cell cycle G1 progression	Iravani <i>et al</i> . 1997	GC09M021992
4	Cyclin D1 (CCND1)	Essential for the control of the cell cycle at the G1/S (start) transition	Hou <i>et al.</i> 2005; Aref <i>et al.</i> 2006	GC11P069455
5	c-MYB	play a critical role in regulating the G(1)/S cell cycle transition	Clappier <i>et al.</i> 2007	GC06P135544
6	CDK2	involved in the control of the cell cycle	Schmitz <i>et al.</i> 2005	GC12P056360
7	CDKN1B (p27, Kip1)	Important regulator of cell cycle progression	Markaki <i>et al.</i> 2006	GC12P012768
8	CDK6	Probably involved in the control of the cell cycle	Chilosi <i>et al.</i> 1998	GC07M092234
9	CDKN1A (p21, Cip1)	functions as a regulator of cell cycle progression at G1	Roman-Gomez et al. 2002	GC06P036645
10	CCND2	Essential for the control of the cell cycle at the G1/S (start) transition	Clappier et al. 2006	GC12P004382
11	ABL1	Regulates cytoskeleton remodeling during cell differentiation, cell division and cell adhesion.	Chiaretti <i>et al.</i> 2007	GC09P133589
12	CCND3	Essential for the control of the cell cycle at the G1/S (start) transition.	Sicinska <i>et al.</i> 2003	GC06M041949
13	CDKN1C (p57, Kip2)	Negative regulator of cell proliferation. May play a role in maintenance of the non-proliferative state throughout life	Gutiérrez <i>et al.</i> 2005	GC11M002861
14	c-MYC	plays a role in cell cycle progression, apoptosis and cellular transformation	Weng <i>et al.</i> 2006	GC08P128748
15	Rb1	key regulator of entry into cell division that acts as a tumor suppressor	Schmitz <i>et al.</i> 2005; Tsai <i>et al.</i> 1996	GC13P048877
16	MDM2	affects the cell cycle, apoptosis, and tumorigenesis through interactions with other proteins	Hendy <i>et al.</i> 2009; Zhou <i>et al.</i> 2003	GC12P069201
17	ATM	important cell cycle checkpoint kinase	Gumy et al. 2003	GC11P108127

### Table 1: Cell cycle genes associated with Acute Lymphoblastic Leukemia.

### CDK2

From Genbank database we obtained sequences of seventeen species, which were used in the construction of phylogenetic unrooted tree for CDK2 (Figure 1), which could be grouped in five clusters, one cluster with humans and other Mammals, an isolated cluster of Red Jungle Fowl, one cluster with different species of Fish, a cluster of Amphibians, a final cluster consisting of other organisms. Information about the gene was obtained from GeneCards database (GCid: GC12P056360).

<figure><figure></figure></figure>							
S.No.	Organism	Common Name	Accession Number	Accession			
			(Nucleotide)	Number (Protein)			
		Cluster 1	0//				
1 🔪	Homo sapiens	Human	X62071	CAA43985			
2	Bostaurus	Cattle	BT020790	AAX08807			
3	Cricetulusgriseus	Chinese Hamster	AJ223949	CAA11680			
4	Rattusnorvegicus	Norway Rat	NM_199501	NP_955795			
5	Musmusculus	House Mouse	NM_183417	NP_904326			
6	Ovisaries	Sheep	NM_001142509				
7	Mesocricetusauratus	Golden Hamster	D17350	NP_001135981			
8	Capra hircus	Cont	EE005044	NP_001135981 BAA04165			
	Capia militus	Guai	EF035041	NP_001135981 BAA04165 ABK34941			
9	Capra micus	Cluster 2	EF035041	NP_001135981 BAA04165 ABK34941			
	Gallus gallus	Cluster 2 Red Jungle Fowl	NM_001199857	NP_001135981 BAA04165 ABK34941 NP_001186786			
10	Gallus gallus	Cluster 2 Red Jungle Fowl Cluster 3	NM_001199857	NP_001135981 BAA04165 ABK34941 NP_001186786			
10	Gallus gallus Xenopuslaevis	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog	NM_001199857	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120			
11	Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog	NM_001199857 NM_001090651 NM_001008135	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136			
11	Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4	NM_001199857 NM_001090651 NM_001008135	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136			
11	Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish	NM_001199857 NM_001090651 NM_001008135 NM_213406	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571			
11 12 13	Gallus gallus Callus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio Salmosalar	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon	NM_001199857 NM_001090651 NM_001008135 NM_213406 NM_001141734	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571 NP_001135206			
11 12 13	Gallus gallus Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio Salmosalar	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon Cluster 5	NM_001199857     NM_001090651     NM_001008135     NM_213406     NM_001141734	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571 NP_001135206			
11 12 13 14	Gallus gallus Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio Salmosalar Sphaerechinusgranularis	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon Cluster 5 Purple Sea Urchin	NM_001199857     NM_001090651     NM_001008135     NM_213406     NM_001141734     AJ224917	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571 NP_001135206 CAA12223			
11 12 13 14 15	Gallus gallus Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio Salmosalar Sphaerechinusgranularis Patiriapectinifera	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon Cluster 5 Purple Sea Urchin Starfish	NM_001199857     NM_001090651     NM_001008135     NM_213406     NM_001141734     AJ224917     AB481376	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571 NP_001135206 CAA12223 BAH97197			
11 12 13 14 15 16	Gallus gallus Gallus gallus Xenopuslaevis Xenopus (Silurana) tropicalis Daniorerio Salmosalar Sphaerechinusgranularis Patiriapectinifera Nasoniavitripennis	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon Cluster 5 Purple Sea Urchin Starfish Jewel Wasp	NM_001199857     NM_001090651     NM_001008135     NM_001141734     AJ224917     AB481376     NM_001161462	NP_001135981 BAA04165 ABK34941 NP_001186786 NP_001084120 NP_001008136 NP_998571 NP_001135206 CAA12223 BAH97197 NP_001154934			
11 12 13 14 15 16 17	Gallus gallus   Xenopuslaevis   Xenopus (Silurana) tropicalis   Daniorerio   Salmosalar   Sphaerechinusgranularis   Patiriapectinifera   Nasoniavitripennis   Paramecium tetraurelia	Cluster 2 Red Jungle Fowl Cluster 3 African Clawed Frog Western Clawed Frog Cluster 4 Zebrafish Atlantic Salmon Cluster 5 Purple Sea Urchin Starfish Jewel Wasp Paramecium	NM_001199857     NM_001090651     NM_001008135     NM_001141734     AJ224917     AB481376     NM_001161462     AF126147	NP_001135981     BAA04165     ABK34941     NP_001186786     NP_001084120     NP_001008136     NP_001135206     CAA12223     BAH97197     NP_001154934     AAD34354			

### CCND1

Analyzing the phylogenetic tree constructed using sequences of cyclin D1 from thirteen species revealed four clusters - a cluster consisting of humans and few other Mammals,

an isolated cluster of Red Jungle Fowl, a cluster with two species of Amphibians and a cluster of Fish (Figure 2). Information about the gene was accessed from GeneCards database by querying with GCid: GC11P069455.



Figure 2: Phylogenetic tree of CCND1.

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Table 3: Sequence details of CCND1.									
S.No.	Organism	Common Name	Accession Number (Nucleotide)	Accession Number (Protein)					
	Cluster 1								
1	Homo sapiens	Human	NM_053056	NP_444284					
2	Musmusculus	House Mouse	S78355	AAB34495					
3	Rattusnorvegicus	Norway Rat	X75207	CAA53020					
4	Cricetulusgriseus	Chinese Hamster	EF524275	ABP73256					
5	Pongoabelii	Sumatran Orangutan	NM_001131301	NP_001124773					
6	Bostaurus	Cattle	BC112798	AAI12799					
7	Rattusrattus	Black Rat	D14014	BAA03115					
8	Canis lupus familiaris	Dog	NM_001005757	NP_001005757					
		Cluster 2							
9	Gallus gallus	Red Jungle Fowl	U40844	AAA83271					
		Cluster 3							
10	Xenopuslaevis	African Clawed Frog	X89475	CAA61664					
11	Xenopus (Silurana) tropicalis	Western Clawed Frog	NM_001005452	NP_001005452					
		Cluster 4							
12	Daniorerio	Zebrafish	AF365874	AAM00355					
13	Salmosalar	Atlantic Salmon	NM_001165391	NP_001158863					

### c-MYC

Sequences from seventeen species were used to infer phylogeny resulting in five clusters, a cluster of ten Mammalian species, a single isolated cluster of Red Jungle Fowl, a single cluster of Amphibians, a cluster consisting of Fish and a cluster with a species of hemichordate and Atlantic salmon (Figure 3). Information about the gene was accessed from GeneCards database (GCid: GC08P128748).





### (ii) Functional divergence

We used Diverge 1.04 to calculate the functional divergence of CDK2, CCND1 and c-MYC genes. In our analysis, CDK2 and CCND1 genes showed no significant functional divergence. This result could probably indicate that these two genes are highly conserved, especially in Mammalian species.For the functional divergence analysis, the c-MYC gene was designated into two clusters-the first cluster is composed of species other than Mammals and the second cluster contained all the Mammalian species. The coefficient of functional divergence between these two clusters was 0.41. We found 317 residues to have a posterior probability greater than 0.1. These residues showed a higher degree of variability in species belonging to cluster one than cluster two (Figure 4).

### Discussion

Loss of cell cycle regulation through changes in cell cycle gene in the bone marrow is a common cause for the progress of tumorigenesis process. ALL is a serious pediatric malignancy, exhibiting both normal and proliferative controls and blocking differentiation into functional cells. In ALL mostly cells reside in the G-1 phase, and only a few cells proceed to the next G0 phase. In normal cells during cell cycle progression early G1 cells respond to environmental stimuli inducing differentiation. However, in disease condition the cells do not respond or do not recognize the signals and no longer respond to differentiation process. It has been reported that cyclin dependent kinase CDK2 was active in ALL and contributed to the disease condition. The catalytic activity of CDK2 was reported to increase in childhood leukemia (Schmitz et al.

2005). Further it was suggested by these authors that CDK2 contributed to the functional inactivation of Retinoblastoma gene (Rb). In

view of the above findings it was important to determine the role of CDK2.

S.No.	Organism	Common Name	Accession Number(Nucleotide)	Accession Number (Protein)	
		Cluster 1			
1	Homo sapiens	Human	V00568	CAA23831	
2	Canis lupus familiaris	Dog	X95367	CAA64654	
3	Ovisaries	Sheep	Z68501	CAA92814	
4	Musmusculus	House Mouse	NM_010849	NP_034979	
5	Rattusnorvegicus	Norway Rat	NM_012603	NP_036735	
6	Feliscatus	Domestic Cat	NM_001173446	NP_001166917	
7	Susscrofa	Pig	FJ882404	ACQ76904	
8	Macacamulatta	Rhesus Monkey	NM_001142873	NP_001136345	
9	Bostaurus	Cattle	NM_001046074	NP_001039539	
10 🧹	Pan troglodytes	Chimpanzee	NM_001142794	NP_001136266	
		Cluster 2			
11	Gallus gallus	Red Jungle Fowl	NM_001030952	NP_001026123	
		Cluster 3			
12	Xenopuslaevis	African Clawed Frog	X14806	CAA32911	
		Cluster 4			
13	Takifugurubripes	Tiger Puffer	AB236413	BAE45315	
14	Oncorhynchusmykiss	Rainbow Trout	AJ627208	CAF25507	
15	Ictaluruspunctatus	Channel Catfish	AF283994	AF283994	
		Cluster 5			
16	Saccoglossuskowalevskii	Acorn Worm	NM_001164972	NP_001158444	
17	Salmosalar	Atlantic Salmon	NM_001173816	NP_001167287	

Table 4	Sequence	details	of	cMYC
1 abie 4.	Sequence	ucialis	UI.	CIVITO.

Cyclin D1 is an important cell cycle regulatory protein, which is involved in the transition of cell cycle from G1 phase to S phase during the process of cell division. Change in cell cycle kinetics and acceleration of G1 phase, which might lead to abnormal cell proliferation, has been associated with overexpression of this protein (Pabalan et al. 2008). During early G1 phase, Cyclin D1 binds to and activates CDK4 CDK6 kinases, which leads and to phosphorylation of Retinoblastoma protein, thus contributing to its inactivation. Studies have reported overexpression of cvclin D1 in patients with ALL and have suggested that cyclin D1 may play a role in mobilization of blast cell from the Bone Marrow to lymph nodes (Aref et al. 2006). These reports indicate that CCND1 could serve as a prognostic marker in the detection of ALL and hence needs to be investigated in more detail to elicit information regarding its role in The c-Myc proto-oncogene tumorigenesis. encodes a transcription factor that is essential for cell growth and proliferation. It has also been reported in the control of DNA replication. It dimerizes with a protein called Max, to bind Enhancer Box sequences (E-boxes) and recruits

histone acetyltransferases for regulation of gene expression. The c-Myc proto-oncogene is involved in transformation and cell proliferation partly through activation of cyclin D2 promoter and also induces programmed cell death which is mediated by nuclear respiratory factor 1 (NRF-1) and the Arf-p53 pathway (Luo et al.. 2005). In normal cells, c-MYC regulation is induced and regulated by mitogenic stimulation. In the absence of this induction, the cells revert back to the non-proliferative state. Studies suggest that in cancer cells, there is an absence of stringency in regulation attributed to mutations in the regulation of Myc genes and the persistent induction of Myc expression through oncogenic signals that lie upstream such as Wnt/β-catenin, Notch or RTK/Ras pathways (Sodir and Evan 2009). Translocations t(8;14), t(8;22), and t(2;8) involving MYC deregulation have been reported in 2%-5% of childhood ALL along with reports of aberrant c-Myc stability in cell lines and bone marrow samples in pediatric patients. Studies MYC is a have reported that direct transcriptional target of oncogenic Notch1, which is common in T-ALL. These studies indicate the need to delve further into the exact correlation

	111	1111112222	2222223333	3333444444	4455555566	6677777788	88899
	3456789012	3467890123	4567890145	6789012345	6723567847	8901234501	26801
Xenopus laevis	LNANFPSKNY	DYYDLOPCFF	FLEEENFY	HOOSRLOP	PAEWKFELLS	RRS	S
Homo sapiens	LNVSFTNRNY	DLYDSVOPYF	YCDEEEN-FY	OOOOOSELOP	PALWKFELLS	RRSGLCSPVT	PLGND
Canis lupus familiaris	LNVSFANRNY	DLYDSVOPYF	YCDEEEN-FY	OOOOOSELOP	PALWKFELLS	RESGLCSPVA	SPGDD
Ovis aries	LNVSFANRNY	DLYDSVOPYF	YCDEEEN-FY	HOOOOSELOP	PALWKFELLS	RRSGLCSPVA	SPGDD
Mus musculus	LNVNETNENY	DLYDSVOPYF	ICDEEEN-FY	HOOODSELOP	PALWKFELLS	RESGLCSEVA	TPEDD
Pattus porvegious	INVSFANDNY	DLYDSVORVE	ICDEEEN-EY	HOOOOSELOP	PATWKEELIS	PRCLCSDVA	TOFID
Falis Catus	LNVSFANDNY	DLYDSVOPYE	YCDEEEN EY	00000SELOP	PATWKERLIS	PRELCEPEA	SPGDD
Callue callue	LEAST DEKNY	DYVDSVORVE	VEFFFFFIIA	VOODCZETOD	DALMKEELIS	DOGGI AAA	51000
Takifugu rubrines	LUSSIASKNY	DYVDSLOPVE	VYDNEEED_E	XBOUTOB	DALWKEELIS	DDDGI	
Magaga mulatta	LINDEFENDING	DIVDEVODVE	YODEEEN EY	11 QQLQI	DALWKERLIC	DDCCLCCDV	DDCND
Sug agrafa	LINVEETNENT	DLIDSVQFIF	YCDEEEN FY	QQQQQSELQF	DALWKFELLS	DECL CEDVA	CDCDD
Bas tourne	LINVEENNINI	DLIDSVQFIF	ICDEEEN FI	VOODGELOP	PALWAFELLS	RESGLCSPVA	SPGDD
Ongerhunghus muking	LNVSPANKNI INCCIACINI	DUIDSVQFIF	YUDNEDED F	NHOODCOLOD	PALWAFELLS	RESGLOFVA	SFGDD
Dan tranladutas	LNSSLASANI	DIIDSIQFIF	IVDNEDED-F	INQQEGQLQE	PALWAFELLS	R-FSL	DICND
Pan_trogrodytes	EMEDBODIOG	TINDRYOPYE	ICDEEENFEI	QQQQQSELQP	PALWAFELLS	RESGLOSPVI	PLGND
Saccogiossus_kowalevskii	EMEPEQRIQS	LIDKIQPIF	LGHDENEL	FIGATHT	PSLWKFELKG	RPAPIP	
lctalurus_punctatus	MSSGLAWKNY	DYYDSVQPYF	YFDNDEED-F	XKÕTÕÕG	QALWRFELLS	R	
Salmo_salar	MLQSFSQ	SOWFYSEPLL	FDDEFCQS		LMDLQSQP	LKTGLN	
	11	11111111111	11111111111	11111111111	11111111111	11111111111	11111
	11	0000000111	11112222222	777777777777	7334444444	11555555555	56666
	2245679001	2456790124	E60001224E	6700010045	7000224567	44555555555	00122
V	2345 <u>678901</u>	2456/89124	3689012345	6/89012345	7890234567	8901234678	90123
Xenopus_laevis	QSSLEPSTAD	QEMVTERGGM	VNSFICEAD	DEALLKSIVI	DCMWGFSAAA	KLEKVVSKLA	SIQAS
Homo_sapiens	GGGGGFFSTAD	QEMVTELGGM	VNSTCDPD	DELFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Canis_lupus_familiaris	GGGGGFSTAD	QEMVTELGGM	VNSFICDPD	DELLIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Ovis_aries	GGGGGFSSAD	REMVTELGGM	VNSFICDPD	DETLIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Mus_musculus	GGGGNFSTAD	QEMMTELGGM	VNSFICDPD	DETFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Rattus_norvegicus	GGGGNFSTAD	QEMMTELGGM	VNSFICDPD-	DEFFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Felis_catus	GGGGGFSTAD	QEMVTELGGM	VNSFICDPD	DETFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Gallus_gallus	CFPSTAD	QEMVTELGGM	VNSFICDPD-	DESFVKSIII	DCMWGFSAAA	KLEKVVSKLA	TYQAS
Takifugu_rubripes	-SSLFPSVAD	QEMLTEFGDV	VNS-ICDADY	SQSFLKTIII	DCMWGFSAAA	KLEKVVSRLA	SLHAA
Macaca_mulatta	GGGGGFSTAD	QEMVTELGGM	VNSFICDPD-	DETFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Sus_scrofa	GGGGGFFSTAD	QEMVTELGGM	VNSFICDPD-	DETFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Bos taurus	GGGGSFSSAD	QEMVTELGGM	VNSFICDPD-	DETLIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Oncorhynchus_mykiss	-SSIFPSTAD	QEMVTEFGDV	VNSFICDADY	SQTFLKSIII	DCMWGFSATA	KLEKVVSRLA	SLQTA
Pan troglodytes	GGGGSFSTAD	QEMVTELGGM	VNSFICDPD-	DETFIKNIII	DCMWGFSAAA	KLVSKLA	SYQAA
Saccoglossus kowalevskii	LLSVVAE	AQQVSETDDN	HTTFVFPDL-	SNLKSNLI	DCMWGGNNGT	PEETTKGTTL	PAAIA
Ictalurus punctatus	RPSTAE	QEIVSEFGDA	VNSTICDSDS	SOTFLKSIII	DCMWGFSAVA	KLEKVVSSSR	RCTRR
Salmo_salar	AKPLSKED	OSYVSDILEO	DPLNWNCDFL	YDGSATETKD	OPDEDDCLWH	CLGDKSMKLS	SVLSS
	1111111111	1111111111	1111111222	2222222222	2222222222	2222222222	22222
	6666667778	8888888999	99999999000	0000000111	1111112222	2222223333	33333
	4567890120	2456789012	3456789012	3456789012	4567890123	4567890123	45678
Xenopus_laevis	RKESALSSSP	SLKSPSCHGS	LSLGGTHRSS	HGFLQDPSSD	VDPSVVFPYP	LNDSISNASS	PCQDL
Homo_sapiens	RKDSG	SPNPARG	HSVCSTSSLY	LODLSAAASE	IDPSVVFPYP	LNDSSSPKSC	ASQDS
Canis lupus familiaris	RKDSG	SPSPARG	PGGCSTSSLY	LQDLSAAASE	IDPSVVFPYP	LNDSSSPKPC	ASPDS
Ovis aries	RKDGG	SPSPARG	HGGCSTSSLY	LODLSAAASE	IDPSVVFPYP	LNDSSSPKPC	ASPDS
Mus musculus	RKDST	SLSPARG	HSVCSTSSLY	LODLTAAASE	IDPSVVFPYP	LNDSSSPKSC	TSSDS
Rattus norvegicus	RKDST	SLSPARG	HSVCSTSSLY	LODLTAAASE	IDPSVVFPYP	LNDSSSPKSC	TSSDS
Felis catus	RKDSG	SPSPARG	PGGCPTSSLY	LODLTAAASE	IDPSVVFPYP	LNDSSSPKPC	ASPDS
Gallus gallus	RREGGPAAAR	GPPSGPPPPP	AGPAASAGLY	LHDLGAAAAD	IDPSVVFPYP	LSERAP	
Takifugu rubripes	RKESAGG	DCTEPSG	AVSWKLNSSY	LODLNTSASE	IDPSVVFPYP	VALTPKHSAG	TVHSK
Macaca mulatta	RKDSG	SPNPARG	HSVCSTSSLY	LODLSAAASE	IDPSVVFPYP	LNDSSSPKSC	ASPDS
Sus scrofa	RKDSG	SPTPARG	HGGYSTSSLY	LODISAAASE	TDPSVVEPYP	LNDSSSPKPC	ASPDS
Bos taurus	RKDGG	SPSPARG	HGGCSTSSLY	LODLSAAASE	IDPSVVFPYP	LNDSSSPKPC	ASPDS
Oncorhynchus mykiss	RKDSAVG	DNAECP-	TRLNANY	LODPNTSASE	TDPSVVFPYP	TTTTPKPSK-	VAPPT
Pan troglodytes	RKDSG	SPNPAPC	HSVCSTSSLV	LODISAAASE	TDPSWVFPVP	LNDSSSPKSC	PSODS
Saccoglossus kowalevekii	ACLTP			-PPLDYAVAF	VDPTAVEPYP		
Tatalurus pupatatus	CV		DIAMODDECC	VVIUDICACE	TDDCMUEDVA	ITECDUCAUL	מגמגייי
Salmo salar	SULLS	AIFRL	DIDUGI	VILIIULSASE	HSTALACOAL	ENEDITIDEO	FOCSE
Saruo Sarar	21772		-1210121	TROPIDD	INTATACÁNT	Денциальны	569F
	2222222222	2222222222	2222222222	2222222222	2222222222	2222333333	33333
	3444444444	4555555555	5666666666	777777777777777777777777777777777777777	88888999999	9999000000	00001
	9012345678	9012345678	9012346789	0123456780	0123412345	6789012345	67893
Xenopus laevis	ILETPPISSN	SSSSESEEF-		PEDEDE	DCDEETVEKR	OSASKRVESS	SHS
Homo sapiens	SAFSPSSDSL	LSSTESSPOG	SPEPLVHEFT	PPTTSSDSFF	EOEDESVEKR	OAPGKRSESG	SPS
					-gv Little		

Canis_lupus_familiaris	AAFSPSSDSL	LSSAESSPRA	SPEPLAHEET	PPTTSSDSEE	EQEDESVEKR	QAPAKRSESG	SPS
Ovis_aries	TAFSPSSDSL	LSSAESSPRA	SPEPLAHEET	PPTISSDSEE	EQEDESVEKR	QPPAKRSESG	SPS
Mus_musculus	TAFSPSSDSL	LS-SESSPRA	SPEPLVHEET	PPTISSDSEE	EQEDESVEKR	QTPAKRSESG	SSP
Rattus_norvegicus	TAFSSSSDSL	LS-SESSPRA	TPEPLVHEET	PPTISSDSEE	EQDDESVEKR	QPPAKRSESG	SSP
Felis_catus	AAFSPSSDSL	LSSAESSPRA	SPEPLAHEET	PPTTSSDSEE	EQEEESVEKR	QPPAKRSESG	SPSAS
Gallus_gallus		RAAPPG	ANPAALGVDT	PPTTSSDSEE	EQEEDTLAEA	NESESSTESS	TEA
Takifugu_rubripes	DLG-LDTPPN	SGSSSSCSDS	DDGDDDDD	DDDDDDDDDQE	EEEEETVEKR	QAVKRCDPSP	SET
Macaca_mulatta	SAFSPSSDSL	LSSTESSPQA	SPEPLVHEET	PPTTSSDSEE	EQEEESVEKR	QAPGKRSESG	SPS
Sus_scrofa	TAFSPSSDSL	LSSAESSPRA	SPEPLAHEET	PPTTSSDSEE	EQEDESVEKR	QPPAKRSESG	SPS
Bos_taurus	TAFSPSSDSL	LSSAESSPRA	SPEPLAHEET	PPTTSSDSEE	EQEDESVEKR	QPPAKRSESG	SPS
Oncorhynchus_mykiss	DLA-LDTPPN	SGSSSSSGS-		-DSEDDDEEE	DDEDETVEKR	QAVKRCDPST	SET
Pan_troglodytes	SAFSPSSDSL	LSSTESSPQG	SPEPLVHEET	PPTTSSDSEE	EQEDESVEKR	QAPGKRSESG	SPS
Saccoglossus_kowalevskii				DPKDQLSGPS	QSDSETVAEK	LQQPPKRKVT	APATN
Ictalurus punctatus	HAPPVDTPPN	SGCSSDSDD-		EEEEDEED	EEDDETVEKR	QRRSEAEV	TES
Salmo_salar	STSDYGSAGG			EF <del>STYSSS</del>	ASDSETVKRT	TSPSSLSQSV	EES
	22222222222	22222222222	22222222222	22222222222	22222222222	22222222222	22222
	1111110000	000000000000000000000000000000000000000	2224444444	AEEEEEEEE	555555555555555555555555555555555555555	7000000000	00000
	1567900122	4560122456	7000122470	400000000000000000000000000000000000000	0012245679	0012245679	099999
Venerus lasuis	4307090123	4JUCHUDTHO	UNVAACDO	JUL234J078	3012343078	JULZ JAJOTON	DVCAD
Venopus_iaevis	QPSRENIS	PLVCHVPIHQ	INVAADDC	INVDIVSSAR	ARLESN	VENULATION	RICAP
Conic lunus familiania	AGGHSKEPHS	PLVCHVSINQ	INVAAPPS	IRADIPAARR	ADIDG	CONTROLONN	RACIP
Canis_iupus_iamiliaris	AGGHSKEPHS	PLVCHVSTHQ	HNYAAPPS	TRADIPAARR	ARLDS	GRVLKQISNN	RKCAP
Ovis_aries	AGSHSKEPHS	PLVCHVSTHQ	HNYAAPPS	TRKDIPAAKR	AKLDS	GRVLKQISNN	RKCAP
Mus_musculus	SRGHSKEPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPAAKR	AKLDS	GRVLKQISNN	RKCSP
Rattus_norvegicus	SRGHSKPPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPAAKR	AKLDS	GRVLKQISNN	RKCSP
Felis_catus	AGGHSKPPHS	PLVCHVPTHQ	HNYAAPPS	TRKDYPAAKR	AKLDS	GRVLKQISNN	RKCIP
Gallus_gallus	SEEHCKPHHS	PLVCHVNIHQ	HNYAAPPS	TKVEYPAAKR	LKLDS	GRVLKQISNN	RKCSP
Takifugu_rubripes	FLPS	PLVCHVSTHQ	HNYAAHPS	MRHEQPAVKR	LKLESGNGGH	SRVLKQISSN	RKCSP
Macaca_mulatta	AGGHSKPPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPAAKR	VKLDS	VRVLRQISNN	RKCTP
Sus_scrofa	AGGHSKPPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPSAKR	AKLDS	GRVLKQISNN	RKCAP
Bos_taurus	AGSHSKPPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPAAKR	AKLDS	GRVLKQISNN	RKCAP
Oncorhynchus_mykiss	FHHS	PLVCHVSTHQ	HNYAAHPS	TRHEQPAVKR	LRLENSS	SRVLKQISSN	RKCSP
Pan_troglodytes	AGGHSKPPHS	PLVCHVSTHQ	HNYAAPPS	TRKDYPAAKR	VKLDS	VRVLRQISNN	RKCTP
Saccoglossus_kowalevskii	TATTTTNTTS	SITVRPSHNH	HNHHSYSTKK	TKQELSMAEL	KALMQQSNGG	RRTPGNSRPG	SRSSR
Ictalurus_punctatus	FQPS	PLVCHVSTQQ	HNYAAQPS	TRHEHPVSKR	PRLETSSTG-	-HGTIRHSKP	RKCTP
Salmo_salar	RRR	ORAOHLEIOL	OHNYAAPCSP	LRSEPSSASY	HKRTRESDSH	SERHNLSSHO	HOSSR
	2222444444						A A A A A
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	5670122572	5022560124	5670014700	1224567000	1245670012	2456000224	56700
Venerus laovis	DECEMPTIVI	OFISENOVEV	JOIO 7 4 700	EVATELOEDE	DETERMENTY	DKEOKODOOL	DNEW
Nemo coniona	RESERVICE	QELSTAQVEV	FINERDARK	LIAISLQUDE	OKIGEEDIDK	REQUÓRÃÕT	DNCCD
Conia lunua familiaria	RSSIENVARL	QERSPAQIEL	ENNERPVRAI	ATTEVQALE	QRISEEDLRK	RREQUIREQU	RNSCA
Ovia arian	RSSIENLARL	QERSPAQIEL	ENNERPVARI	ATTEVQALE	QRESERVER	RREQUIREQU	RNSGA
OVIS_ALLES	RSSIENURRL	QERSFAQIEL	ENNERPVRKI	ATLSVQALE	QRISERDVRR	RREQUIREQI	RNSCA
Mus_musculus	RSSTENUKRL	QERSFAQIEL	ENNERPVKKT	AYILSIQADE	HKTSEKDLRK	RREQKHKEQL	RNSGA
Rattus_norvegicus	RSSTENLKRL	QERSFAQIEL	ENNERPVKKT	AYILSVQADE	HKISEKDLRK	RREQKHKEQL	RNSGA
Felis_catus	RSSTENDERL	QERSFAQIEL	ENNEKPVKKT	AYILSVQAGE	QKISEKDLRK	RREQKHKEQL	RNSCA
Gallus_gallus	RTSSENDKRL	QELSFAQIEV	ANNEKPVKKT	EYVLSIQSDE	HRIAEKEQRR	RREQKHKEQL	RNSRA
Takitugu_rubripes	RTSTDYDKRL	QELSFAEIEV	ANNEKAVKKT	ECIYSMQSDE	QRLLLKEQNR	KSELKQRAQL	QGSRV
Macaca_mulatta	RSSTENCKRL	QERSFAQIEL	ENNEKPVKKT	AYILSVQAEE	QKISEKDLRK	RREQKHKEQL	RNSCA
Sus_scrofa	RSSTENDERL	QERSFAQIEL	ENNEKPVKKT	AYILSVQAEE	QKVSEKDVRK	RREQKLKEQL	RNSCP
Bos_taurus	RSSTENDKRL	QERSFAQIEL	ENNEKPVKKT	AYILSVQAEQ	QKKSEIDVQK	RREQKLKEQI	RNSCA
Oncorhynchus_mykiss	RTSTDYDKRL	QELSFAEIDV	ANNEKAVKKT	ECIYSMQTDE	QRVNLKEQRR	KSEHKQKAQL	QNSCL
Pan_troglodytes	RSSTENEKRL	QERSFAQIEL	ENNEKPVKKT	AYILSVQAEE	QKISEEDLRK	RREQKHKEQL	RNSCA
Saccoglossus_kowalevskii	PSSSDNDKAL	KDTSLTNVEL	ENQERPVRKT	DHIQQITADE	LLVKDKEGKK	RNVILDKNRL	KNDLN
Ictalurus_punctatus	RTSSDNDKKL	QELSFAEIEV	ANNEKAMKKA	ECIHSMQADE	RRLSMKEQRR	KSELKHRQQL	RRSQL
Salmo_salar	<b>QSTVDEE</b> RHM	QENCLENVEL	SNNDKSVKRC	DSIRGLELAG	QRNVKRDKRE	RQEQKVKEQL	RRQRC

Figure 4: Significantly divergent residues highlighted in c-MYC sequence alignment.

between c-MYC and leukemogenesis (Delgado and León 2010).

Phylogenetic studies play an essential role in understanding evolutionary history of

genes and their impact on disease etiology. Several studies have calculated the functional divergence of genes based on phylogenetic reconstruction across various species and further implicate those sites which are subjected to functional constraints during evolution (Khan and Jamil 2008; Khan and Jamil 2010). Further, this information could be used to observe druggene interactions with the help of homology modeling and affinity modeling studies (Kotra *et al.* 2008). Our earlier studies on phylogeny of p53 and MDM2 revealed that these genes show a high degree of sequence similarity in Mammals, suggesting parallel carcinogenesis pathways involving these genes in the Mammalian species (Jayaraman *et al.* 2011).

Studies based on evidence from paleontology and genetics suggest that mechanisms of cancer are embedded deeply throughout evolution. Understanding the phylogenetic evolution of these genes could help in furthering our knowledge on the mechanisms involved in cancer (Davies and Lineweaver 2011).

In the present study, we applied bioinformatics approaches to mine databases to garner information regarding the CDK2, CCND1 and c-MYC cell cycle genes and their role in ALL. We inferred phylogeny of these genes across various species, for which sequence information is available in the databases. Analysis of the sequence alignments indicates that throughout the mammalian species, these genes are mostly similar/exhibit sequence homology and thus group under a single cluster. Though the avian species, the amphibians and the some species of fish tend to form three separate clusters due to the variation in their sequences, there appears to be a moderate degree of sequence similarity with those of mammalian species.

In our study of the phylogenetic analysis and tree constructed using sequences of these three genes, we observed that these gene sequences are more or less similar across these few taxa, this might indicate the presence of cancer like disease genes in the evolutionary history of these species.

In the future, when the sequence information for these genes across a wide range of taxa becomes available, a more intensive phylogenetic analysis would be possible which could help in delving further into the changes and the mechanisms of change through which these genes contribute to the evolution of leukemogenesis process and also assist in designing effective therapeutic measures.

Correlation between CDK2, CCND1, c-MYC

CDK2, cyclin D1 and c-MYC genes are important components in the cell cycle pathway. Alterations in these genes have been reported in association with malignant transformations. Studies have reported that c-MYC gene might be involved stimulating the activity of cyclin E/CDK2 complex. The phosphorylation of MYC by CDK2 is helpful in suppression of senescence (Hydbring and Larsson 2010). c-MYC gene has also been reported to regulate the expression of cyclin D1 at an early stage of the cell cycle process. Cyclin D1–CDK2 complex might indirectly promote cell proliferation by sequestering p21 and p27 genes this complex has been detected previously in breast cancer cell lines and was reported to exhibit several features of transformation (Chytil et al. 2004). These studies indicated that the three genes functionally interact with each other and play a role in direct/indirect regulation of the other genes. It is essential to better understand the association between these genes because their interaction might be a significant aspect in the tumorigenesis process.

### **Conflict of Interests**

Authors have no conflicting interests.

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