Research Article Molecular Evolution of the Vertebrate FK506 Binding Protein 25

Fei Liu,^{1,2} Xiao-Long Wei,³ Hao Li,^{1,2} Ji-Fu Wei,² Yong-Qing Wang,² and Xiao-Jian Gong¹

¹ Department of Pharmacology, China Pharmaceutical University, Nanjing 210009, China

² Research Division of Clinical Pharmacology, The First Affiliated Hospital, Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China

³ Department of Pathology, Cancer Hospital of Shantou University Medical College, Shantou, China

Correspondence should be addressed to Yong-Qing Wang; wyqjsh@hotmail.com and Xiao-Jian Gong; gongxj66@sina.com

Received 25 November 2013; Accepted 16 January 2014; Published 2 March 2014

Academic Editor: Huai-Rong Luo

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FK506 binding proteins (FKBPs) belong to immunophilins with peptidyl-prolyl isomerases (PPIases) activity. FKBP25 (also known as FKBP3) is one of the nuclear DNA-binding proteins in the FKBPs family, which plays an important role in regulating transcription and chromatin structure. The calculation of nonsynonymous and synonymous substitution rates suggested that FKBP25 undergoes purifying selection throughout the whole vertebrate evolution. Moreover, the result of site-specific tests showed that no sites were detected under positive selection. Only one PPIase domain was detected by searching FKBP25 sequences at Pfam and SMART domain databases. Mammalian FKBP25 possess exon-intron conservation, although conservation in the whole vertebrate lineage is incomplete. The result of this study suggests that the purifying selection triggers FKBP25 evolutionary history, which allows us to discover the complete role of the PPIase domain in the interaction between FKBP25 and nuclear proteins. Moreover, intron alterations during FKBP25 evolution that regulate gene splicing may be involved in the purifying selection.

1. Introduction

Immunophilins include three families with peptidyl-prolyl isomerases (PPIases) activity, FK506 binding proteins (FK-BPs), cyclophilins, and parvulins. FKBPs are named for binding to the immunosuppressive drug FK506, characterized by one or more PPIase domains. The 15 identified members of human FKBPs are divided into 4 groups: cytoplasmic, TPR domain, endoplasmic reticulum (ER), and nucleus. FKBP25 and FKBP133 locate in the nucleus, containing a single PPIase domain [1].

FKBP25 (also known as FKBP3) is the first mammalian FKBP with a calculated molecular mass of 25 kDa found in the nucleus, which plays a role in regulating transcription and chromatin structure. The FKBP25 comprises a conserved PPIase domain at its C-terminus with a 43% sequence identity to FKBP12 and a helix-loop-helix (HLH) motif at its unique hydrophilic N-terminal [2, 3]. This conserved PPIase domain functions in binding to the immunosuppressive agent FK506 or rapamycin. Unlike another FKBPs, FKBP25 shows a strong affinity for binding rapamycin (Ki = 0.9 nM) over

FK506 (Ki = 200 nM) [4]. The FKBP25 was reported to be associated with nuclear proteins including transcription factor Yin-Yang1 (YY1), mouse double minute 2 (MDM2), and histone deacetylases (HDACs) [5]. FKBP25 binds to YY1 at N-terminal and increases its DNA-binding activity without the involvement of the FK506/rapamycin binding domain [6]. In addition, the level and activity of the tumor suppressor protein p53 are negatively regulated by MDM2. The HLH motif of FKBP25 mediates protein-protein interaction to enhance ubiquitination and degradation of oncogene MDM2, increasing the expression of tumor suppressor p53 and its downstream effector p21 [7]. Moreover, the proteinprotein interaction contributes to form HDAC complexes, which is critical for the chromatin structure [2].

In 1992, Jin et al. reported the molecule cloning of human FKBP25 and performed a homology comparison between FKBP25 and FKBP12/FKBP13 [8]. Furthermore, Mas et al. showed the molecule cloning of mouse FKBP25 and expression pattern of FKBP25 gene during cerebral cortical neurogenesis [9]. However, the relationships between nuclear functions and evolution in FKBP25 are seldom reported.

Species	Models	Estimates of parameters	lnL	$2\Delta l$	Positively selected sites		
1	M7	$p = 0.91900 \ q = 8.19764$	-5463.938465		NA		
Vertebrate	M8	$p0 = 0.999999 \ p = 0.91899 \ q = 8.19758$ $(p1 = 0.00001) \ w = 1.86072$	-5463.940368	0.003806	None		
	M7	$p = 0.33823 \ q = 1.62046$	-2182.244789		NA		
Mammalian	M8	$p0 = 0.999999 \ p = 0.33824 \ q = 1.62055$ $(p1 = 0.00001) \ w = 1.00000$	-2182.244918	0.000258	None		
Primate	M7	$p = 4.13016 \ q = 99.00000$	-997.077389		NA		
	M8	$p0 = 0.99999 \ p = 4.12942 \ q = 99.00000$ $(p1 = 0.00001) \ w = 1.00000$	-997.077440	0.000102	None		
Mammalian excluding primate	M7	$p = 0.28229 \ q = 1.41420$	-2242.306222		NA		
	M8	$p0 = 0.999999 \ p = 0.28230 \ q = 1.41430$ $(p1 = 0.00001) \ w = 1.00000$	-2242.306302	0.000160	NS		
Rodent	M7	$p = 0.13287 \ q = 1.19752$	-1372.902164		NA		
	M8	$p0 = 0.999999 \ p = 0.13287 \ q = 1.19764$ $(p1 = 0.00001) \ w = 1.00000$	-1372.902193	0.000058	NS		
	M7	$p = 0.38691 \ q = 4.30540$	-2354.923181		NA		
Teleost	M8	$p0 = 0.999999 \ p = 0.38690 \ q = 4.30545$ $(p1 = 0.00001) \ w = 3.90806$	-2354.923385	0.000408	NS		

TABLE 1: Site-specific tests for positive selection of FKBP25.

In L: the log-likelihood difference between the two models; $2\Delta l$: twice the log-likelihood difference between the two models (In all the species, $2\Delta l < 9.21$, the *P*-value is more than the significance level 0.05, indicating that M8 model is not better than M7 model); NA: not allowed; NS: not shown (it means the sites under positive selection but not reaching the significance level of 0.9).

In this study, we exhibit an evolutional analysis not only on selective pressure but also on intron-exon conversion among vertebrate FKBP25 genes.

2. Materials and Methods

2.1. Sequence Data Collection. All the FKBP25 gene and amino acid sequences were obtained from the ENSEMBL (http://www.ensembl.org/index.html) [10], based on orthologous and paralogous relationships. The gained FKBP25 sequences were applied as queries to search known FKBP25 genes using BLAST at the National Center for Biotechnology Information (NCBI), in order to confirm whether their best hit was an FKBP25 gene [11].

Incomplete sequences of FKBP25 genes in four species (tree shrew, horse, platypus, and turkey) were retrieved from both ENSEMBL and NCBI. After eliminating these incomplete sequences, 28 sequences were applied for this study. The 28 sequences from 23 species comprised human (ENSG00000100442), chimpanzee (ENSPTRG000000063 05), gorilla (ENSGGOG00000013322), orangutan (ENSPP YG0000005778), macaque (ENSMMUG00000016512), marmoset (ENSCJAG00000015972), mouse (ENSMUSG00 000020949), rat (ENSRNOG0000004629), guinea pig (ENS CPOG0000001444), rabbit1 (ENSOCUG00000007535), rabbit2 (ENSOCUG0000026892), dog1 (ENSCAFG00000 014018), dog2 (ENSCAFG00000014093), dog3 (ENSCA FG00000024192), dog4 (ENSCAFG00000000 578), cow (ENSBTAG0000002610), elephant1 (ENSLAFG000000035 72), elephant2 (ENSLAFG00000027553), opossum (ENS MODG0000007352), chicken (ENSGALG00000012466),

zebra finch (ENSTGUG00000013231), *anole lizard* (ENS ACAG00000004080), *xenopus* (ENSXETG00000003052), *fugu* (ENSTRUG00000011887), *medaka* (ENSORLG00000 015070), *stickleback* (ENSGACG00000012834), *tetraodon* (ENSTNIG00000010980), and *zebrafish* (ENSDARG00000 079018).

2.2. Molecular Phylogenetic Analyses. The protein coding sequences of FKBP25 were aligned using CLUSTAL W program in MEGA 5.05. We constructed a maximum likelihood (ML) tree of FKBP25 amino acid sequences by MEGA 5.05 with the optimal model (Kimura 2-parameter model). The relative support of internal node was performed by bootstrap analyses with 1000 replications for ML reconstructions [12].

2.3. Selection Pressure Analyses. The numbers of nonsynonymous substitutions per nonsynonymous site (dN) and the numbers of synonymous substitutions per synonymous site (dS) were computed by MEGA 5.05 with the modified Nei-Gojobori method. The dN/dS <1, =1 and >1 demonstrate purifying selection, neutral selection, and positive selection, respectively [13]. The dN is the numbers of nonsynonymous substitutions per nonsynonymous site, and the dS is the numbers of synonymous substitutions per synonymous site. The transition/transversion ratio was 1.55 estimated using the ML method by MEGA 5.05 [14].

The FASTA format of FKBP25 sequences was converted to the PAML format using DAMBE software for subsequent site analyses [13]. The CODEML program implemented in the PAML 4.7 package was used to detect positive selection of individual sites. The site-specific model was exerted using

		Total exons	675	675	675	675	675	675	675	675	675	675	675	675	654	645	675	675	675	675	675	684	678	651	678	669	663	666	666	666			
		Exon8	1	Ι	I	Ι		I	Ι	Ι	Ι	Ι	Ι	Ι		Ι		I	Ι		Ι	Ι	55	Ι	Ι	Ι	Ι	Ι	I	11	Intron15	1784	
		Intron7	1	Ι	Ι	Ι	Ι	Ι		Ι	Ι	I			I		I	I	Ι	Ι	I		49	Ι	Ι	Ι	Ι	I	Ι	108	Exon15	76	
		Exon7	55	55	55	55	55	55	55	55	55	55		55	I		I	55	55	Ι	55	55	53	Ι	55	55	55	55	55	28	Intron14	26	
		Intron6	1775	1789	1778	1432	1818	2100	937	1118	1340	1266		1216			I	1309	1725	Ι	554	829	69	Ι	787	106	804	96	75	904	Exon14	15	
		Exon6	98	98	98	98	98	98	98	98	98	98		98		102	I	98	98	Ι	98	98	16	55	98	98	98	98	98	19	Intron13	107	
TABLE 2: Exon and intron lengths of FKBP25.		Intron5	2761	2725	2753	2457	2845	2537	1961	1667	1346	1826		1823		12		1706	1580	I	1261	1011	2054	610	186	68	70	81	70	359	Exon13	31	
	gth (bp)	Exon5	68	68	68	68	68	68	68	68	68	68	I	68		33	I	68	68	Ι	68	68	97	98	68	68	68	68	68	16	Intron12	98	
	Leng	Intron4	530	530	530	533	531	507	837	942	1416	1115	I	468		2	I	484	483	Ι	1051	1040	892	824	129	82	75	135	16	244	Exon12	10	
		Exon4	136	136	136	136	136	136	136	136	136	136		136		33	I	136	136	Ι	136	136	136	68	136	136	136	136	136	20	Intronll	66	
		Intron3	8173	8898	8214	8395	8273	5644	2224	2030	3600	4634		2088	I	2	I	2835	4756	Ι	2807	408	494	1078	418	65	738	93	75	1117	Exonll	14	
		Exon3	108	108	108	108	108	108	108	108	108	108		108	129	252	I	108	108	Ι	108	114	108	136	108	105	66	102	102	16	Intron10	708	
		Intron2	797	797	796	793	786	780	841	816	772	1082		1076	4	4		603	1089	Ι	1484	75	76	1333	403	78	71	76	80	447	Exon10	26	
		Exon2	102	102	102	102	102	102	102	102	102	102	55	102	229	195	248	102	102	Ι	102	102	102	108	102	102	102	102	102	102	Intron9	316	Exon17
		Intronl	3548	3524	3538	3498	3496	3592	3762	3528	3232	2189	40	2573	13	2	190	2332	3176	Ι	2560	76	112	1699	2319	375	109	294	305	2527	Exon9	24	Intron16
		Exonl	108	108	108	108	108	108	108	108	108	108	620	108	296	30	427	108	108	675	108	111	111	186	111	105	105	105	105	105	Intron8	1042	Exon16
		species	Нитап	Chimpanzee	Gorilla	Orangutan	Macaque	Marmoset	Mouse	Rat	Guinea pig	Rabbitl	Rabbit2	Dogl	Dog2	Dog3	Dog4	Сош	Elephant1	Elephant2	Opossum	Chicken	Zebra finch	Anole lizard	Xenopus	Fugu	Medaka	Stickleback	Tetraodon	Zebra fish			

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Human	NALEY DODANTUPOT DEPOT DEPOT VET OF HOSPICE AF HUT I ON TUNUARTEMP HT UTAVNHI FET P DEPOTEST	70
Chimpanzee	NAME AND ADDRESS OF A DEPARTMENT OF A DEPARTMENT AND A DEPARTMENTANT AND A DEPARTMENT AND A DEPARTMENT AND A	70
Gorilla		70
Orangutan	MAAAV. PÇRAWIVEÇIRSEÇIPREDITETI ÇENGSDIŞI ALEMELENI ENVARIANE DELVIA INFLIFETE, KERGILSI	78
Managutan	MAAAV. PÇRAWIVEÇIRSEÇIPERDIIKFIQEHGSDSFIAEHKLIGNIKNVARIANRDHIVIAINHLFEIK. KFRGIESI	78
Macaque	MAAAV.PÇRAWIVEQERSEQEPREDIIKFEQEHGSDSFEAEHKELGNIKNVARTANEDHEVIAYNHEFEIK.RFEGIESI	78
Marmoset	MAAAV.PCRAWIVEQLRSEQLPRRDIIKFLQDHGSDSFLAEHKLLGNIKNVAKTANRDHLVIAYNHLFEIK.RFKGTESI	78
Niouse	MAAAV. PQRAWIVEQLRSEQLPKKDIIKFLQDHGSDSFLAEHKLLGNIKNVAKTANKDHLVNAYNELFESK.RFKGTETI	78
Kat	MAAAV. PÇRAWIVEÇLRSEÇLPKKDIIKFLÇDHGSDSFLAEHKLLGNIKNVAKTANKDHLVTAYNELFESK.RFKGTETI	78
Guinea	MAAAV. PQRAWIVEQIRSEQIPKKDIIKFIQEHGSDSFIAEHKIIGNIKNVAKTANKDHIVIAYNHIFETK. RFKGIESV	78
Rabbit1	MAAAV.PQRAWIVEQIRSEQLPKKDIIKFLQDHGSDSFLAEHKLIGNIKNVAKTANKDHIVIAYNHIFETK.RFKGSENV	78
Rabbit2	MAAAV. PQRAWIVEQIRSEQIPKKDIIKFLQDHGSDSFLAEHKLIGNIKNVAKTANKDHIVTAYNHIFETK.HFKGSENV	78
Dog1	MAAAV.PQRAWIVEQIRSEQIPKKDIIKFIQDHGSDSFIAEHKLIGNIKNVAKTANKDHIVIAYNHIFESK.RFKGTESI	78
Dog2	NVAAV.FORAWIVEQIHSEQIPKKDIIEFIEDHGSDSFIVEHKLIGNIKNVAKTANHDHIVIAYNHIFEHK.RFKGTESI	78
Dog3	MAVAD. PCWAW.VECIPSECIPKKDIIRVLCDHGPDSFITEHKLLGNIKNVAKTANKDHIVTAHNHLFESK.RFKGTE.L	76
Dog4	MVVAV. PCCAWIMECTHSECIPKKDIIKFLCDHGSDLFLAECKLLGNIKNVAKTANEGHTVTAYNHLFESK.RFKGTESI	78
Cow	MAAAV. FCRAWIVECIRSECIPKKDIIKFLODHGSDSFIAEHKLIGNIKNVAKTANKDHIVTAYNHIFESK.RFKGTESI	78
Elephant1	MAANV. PCRAWIVECLRSECLPKKDIIKFLCSHGSDSFLAEHKLLGNIKNVAKTANKEHLVTAYNHLFETK.RFKGTESI	78
Elephant2	MAANV. PCRAWIVECLRSECLPKKDIIKFLCSHGSDSFLAEHKLLGNIENVAKTANKEHLVTAYNHLFETK. RFKDTESI	78
Opossum	MAAA.GPCRTWSAEOLRSEALPKKDIIKFLODNGSDSFLAEHKLLGNIKNVAKTANKDHLVTAYNHLFESK.RFKGTESV	78
Chicken	MAAATA FAOFWSAEELRSEALFKKDIIKFLOEHAAOAFLAEHRLLGOVKNVAKTANKEOLIAAVTOLFHTO. RFKGTDGA	79
Zebra	MAAAAGPAOPWSAEELRSEALAKKEIIKFLOEHAAOAFLAEHKLLGOVKNVAKTANKEOLIAAYTOLFHTÖ, BFKGTDGA	79
Anole	HTVL. GVUHSORPC. LRISFLV. SDEWFFFG FLARHKLIGGIKNVSKTASKOCI ITAVNCLERTK. SFKGSESP	70
Xenopus	TAS SEPAREWSNE OF HSEDI DEED I DEED I DEED OF MGSESELAEVELI ON VENU ANTAKEFOLATAVNELETE, DEEGSESU	70
Medaka	TO DEDMORNT PROTOS SUARINE THE OPATHTELSEVILLON THAN TO TANK OF UNA VNOLESSE DERGED	76
Fugu		70
Stickloback		70
Tatasadan	A DEPTRONDED FOR DEDEPTROTING IQUINANTS IN COLORIAN ANTAKE CLIAR INDEESS. RELEGIEPT	//
	HAAPIREWSDECKSDDLPRKDVINTIQURANSTISERKLEGNINNVALAKACCIIIAINQUESK.KEKGSEV	//
Zebransh	MAAAPERQWSDEQEKSEEVPRKELIKFIQDSAAHSFIAEHKLIGNIKMVSKIAKREQLIEAINQLEQSQ.RWK.PPLY	76
Consensus	m i fie iig nv kta k i a ir	
Human	skvseqvknvklnedkeketkseetldegpfkytksv ikkgdktn fpkkgevvhcwytgtlodgtvfetniqtsakkk	156
Chimpanzee	skvseqvknvklnedkpketkseetldegppkytksv ekkgdktn fpkkgevvhcwytgtlodgtvfetnigtsakkk	156
Gorilla	SKVSEQVKNVKLNEDKPKETKSEETLDEGPPKYTKSVLKKGDKTNFPKKGEVVHCWYTGTLQDGTVFDTNIQTSAKKK	156
Orangutan	SKVSEQVKNVKLNEDKPKETKSEETLDEGPPKYTKSVLKKGDKTNFPKKGDVVHCWYTGTLCDGTVFDTNIQTSAKKK	156
Macaque	SKVSECVKNVKLNEDKPKETKSEETLDEGPPKYTKSVLKKGDKTNFPKKGDVVHCWYTGTLCDGTVFDTNIOTSAKKK	156
Marmoset	SKVSECVKNVKLNEDKP., KETKSEETLDEGPPKYTKSVLKKGDKTNFPKKGDVHCWYTGTLODGTVFDTNIOTSAKKK	156
Mouse	SKUSFOUKNUKI SEDKE. KESKSFFTLDEGEPKYTKSTLKKGENTNEPKKGEUUHGWYTGTLPDGTUFETNIOTSSKKK	156
Rat	SUSPONENTED FOR A STATE OF A DESCRIPTION OF A DESCRIPTION OF A DESCRIPTION	156
Guinea		156
Rabbit1		156
Rabbit?		156
Dog1		150
Dog1	SKVSEQVRNVRLNEDRF., REIKCEELDEGPPATRSVRRGDRINFPRRGDVNRWIIGICOGIVFINIQISSRR	150
Dog2	CKV5EQVKNVKLNEDKPKEIKDEGPPKIIKPVLKRGLKINFPKKGLVHCWIGILQDGIVFEINIQISSKKK	151
Dog5	SKGSEQVKNVKLNEDKPKETKCEETPDEGPPKYTKPVLKKGLKINFPEKGEVVHCWHTGILCDGAVFEINIQISSKKK	154
Dog4	SKVSEQVKNVKLNEDKPRETKCEETLDEGPPKYTKSVLKKGLKINFPKKGDVHCWYTGTLQMGPVFDINIQTISKKK	156
Cow	SKVSEQVKNVKLNEDKPKETKSEETLDEGPPKYTKSVLKKGDKINFPKKGDVHCWYTGILQDGTVFDINIQTSSKKK	156
Elephanti	NKVSEQVKNVKVNDDKPKETKSEETLEEGPPKYTKSVLKKGDKTNFPKKGDVHCWYTGTLQDGTVFDTNIQTSSKKK	156
Elephant2	NNVLEQVKNVKVNDDKPREIKSEETLEEGPPKYIKSVLQKGDKINFPKKGLVVHCWYIGILQDGIVFEINIPISSKKK	156
Chieleen	AKVIEQVKSVKLDEEKSKEVKPEEILDEGPPKYIKSILKKGLKINFPKKGLVVHCWYIGILQDGIVFEINIQISAKKK	156
Zahara	ERAAEKARPGRAEGERERDRAARAEEPAELGPPRIIRSILERGERINFPRRGETVREWIIGELQDGIVFEINVQISSRR	159
Amala	EKAAEKAKPARALEAKGKAVKAELAVELGPPKIIKSILKKGLKINFPKKGLIVHCWIIGKLCDGIVFLINIQSSSKKK	157
Vananus	LELAEQVRSVRLDEGRAREAKQELAIDEGPPRIIKSVRRGLKVNPPRKGLVVNOWIGRLEDGIVPSNIQISSKRR	148
Medaka		157
Fugu		152
Stickleback	LEVIEVVAARIE.LEFEVII.EVVEEGEPIIASVANGULIIPPAGEIVSUUTSILEUGIVEINIPAARA	154
Tatraadan	EEVIE VRSVALE.ERFRDVFA.EVVDEGFFAIFSVIARGLAENFFARGENVSCWIIGSLEDGIVFUINFFIARA	153
Zahrafish	LEVIEQVRAARIE.EKPKEVKI.EAVDEGPPKIIKSVIRKGLKINFPKKGLIVSUWIGSLEUGIVFEINIPIAAKKK	153
Consensus	SPILQVLLIGIESAPIWIDISCVIEUPEWERILNINGELININGEKEMEN MOUCWLESARGESUULSAARE	153
Conscisus	p x i x x n gu v c g u xx	
		224
Chimmon		224
Chillipanzee		224
Gorilla	RNARPLSFRVGVGRVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGCPDAKIPPNARLIFEVELVDID	224
Orangutan	RNAKPLSFRVGVGRVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKLIFEVELVDID	224
Macaque	RNAKPLSEKVGVGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKIIFEVELVDID	224
Marmoset	KNAKPLSFKVGVGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGÇPDAKIPPNAKLIFEVELVDID	224
Mouse	KNAKPLSFKVGVGKVIRGWDEALLTMSKGEKARLEIEPEWAYGKKGQPDAKIPPNIKLIFEVELVDID	224
Rat	KNAKPLSFKVGVGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQPDAKIPPNIKLIFEVELVDID	224
Guinea	KNAKPLSFKVGVGKVIRGWDEALLTMSKGLKARLEIEPEWAYGKKGQPDAKIPPNAKLIFEVELVDID	224
RabbitI	RNAKPLSFRVGVGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKLIFEVELVDID	224
Rabbit2	KNAKPLSFKVGVGKVIRGWDEALLTMSKGEKALLEIEPEWAYGKKGQPDAKIPPNAKLIFEVELVDID	224
Dog1	KNAKPLSFRVGIGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKLIFEVELVDID	224
Dog2	.NAKPLSFKAGIDKVIRGWDEALLT.SKGEKARLEIEPEWAYGKKGQPDAKIPPNGKLIFEVELVDID	217
Dogs	RNARPRVGIGKVMRGW.KALLIMSKGEKIEPEWVYGKRGQPDAKIPPNAKIIFEVELVDID	214
Dog4	NNAKFLETRVEIGKVIRGWUEALLIMSKGEKARLEIEFEWAYGKKGQFUAKIPPNAKLIFEVELVDID	224
COW	KNAKFLSERVGIGKVIRGWDEALLIMSKGEKARLEIEFEWAYGKKGQFDAKIPFNAKLIFEVELVDID	224
Elephant1	KNAKFLSTRVGVGKVIRGWDEALLIMSKGEKARLEIEPEWAYGKKGQFDAKIPFNAKLIFEVELVDID	224
Elephant2	KNAKFLSERVEVGKVIRGWDEALLIMSKGEKAQLEIEPEWAYGKKGQFDAKIPPNATLIFEVELVDID	224
Opossum	KNAKFLSFRVGVGKVIRGWDEALLTMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKLNFEVELVDID	224
Chicken	KAAKPLSFRVGVGKVIRGWDEALLTMSKGEKAQLEIEPEWAYGKKGQPDAKIPPNAKLFFEVELVDIE	227
Zebra	KAAKFLSERVGVGKVIRGVSKVERALSATERSRHRAGGGWGAAGAALFREGIPPNAKLFFEVELVDIE	225
Anole	KIAKFLSPRVGVGKVIRGWDEALLIMSKGEKAHLEIEFEWAYGKKGQFDAKIPFNAKLFFEVELVDIE	216
Aenopus	KAAKFLSFRVGVGKVIRGWDEALLIMSKGEKAKLEIEPEWAYGRKGLPDAKIPPNAKLFFEVELVDID	225
медака	RQIKFLSFKVGLGRVIRGWDEALLIMSKGETARLEIEPEWAYGKKGLPDSKIPPNAKLIFEVELVSVD	220
rugu	KQAKFLSFKVGLGRVIRGWDEALLTMSKGETARLEIDPEWAYGRKGLPDSKIPPNAKIVFEVELVSVD	222
Stickleback	KQIKFLIFKVGLGRVIRGWDEGIMIMSKGETSRLEIEPEWAYGRKGLPDSKIPPNAKLIFEVELVAVD	221
Tetraodon	KÇAKFLSFKVGMGRVIRGWDEALLIMSKGEKAKLEIDPEWAYGKKGIPDSKIPPNAKLIFEVELVSVD	221
∠ebrafish	KÇSKELSEKVGMGKVIRGWDEGLLIMSKGETARLEIESEWAYGKKGIEDAKIPPNAKLIFEVELVAVD	221
Comercia		

FIGURE 1: Sequence and structural alignment of FKBP25.



FIGURE 2: Phylogenetic tree and motif distributions of FKBP25.



0.7 0.6 0.5 0.4 0.3 0.2 0.1 0 Bird and reptile and amphibian Mammalian Rodent Teleost Primate Vertebrate Nonprimate mammalian dN dS

FIGURE 3: Pairwise comparisons of *dN* and *dS* among 28 vertebrate FKBP25 sequences.

FIGURE 4: The average nonsynonymous (dN) and synonymous (dS) in FKBP25 from different vertebrate groups. The value of average dN was in blue, and the value of average dS was in red.

likelihood ratio tests (LRT) to compare M7 (null model) with M8 model. M7 is a null model that does not allow for any codons with $\omega > 1$, whereas M8 model allows for positively selective sites ($\omega > 1$). When the M8 model fitted the data significantly (*P*-value < 0.05) better than the null model (M7),

the presence of sites with $\omega > 1$ is suggested. On the contrary, the results of *P* value > 0.05 proved the absence of sites with $\omega > 1$. The twice log likelihood difference between the two compared models $(2\Delta l)$ is compared against χ^2 with



FIGURE 5: Sequence logos (MEME LOGOs) of conserved motifs identified in vertebrate FKBP25.

critical values 5.99 and 9.21 at 0.05 and 0.01 significance levels, respectively [15].

2.4. Protein Domain and Motif Analyses. Protein domain analyses of FKBP25 were shown at Pfam domains database (http://pfam.sanger.ac.uk) [16]. SMART (http://smart.emblheidelberg.de/) was used to make sure the presence of FKBP25 domains [17]. The motifs of FKBP25 were analyzed by the MEME software (http://meme.sdsc.edu/meme/website/intro.html) with a maximum of 10 motifs to find [18].

2.5. Exon-Intron Conservation Analyses. We collected elaborate information about FKBP25 exon and intron from ENSEMBL (http://www.ensembl.org/index.html) [19]. The number and length of FKBP25 exon and intron in 28 sequences were investigated for exon-intron conservation analyses.

3. Results

3.1. Phylogenetic Analyses of FKBP25. All the FKBP25 gene and protein sequences were collected from the ENSEMBL and checked by BLAST at NCBI. The sequence and structural alignment of FKBP25 was shown in Figure 1. The phylogenetic tree was constructed according to the protein coding sequences of FKBP25 using the maximum likelihood method (Figure 2, left panel). The FKBP25 genes from the primate lineage and teleost lineage form a species-specific cluster, respectively. Four FKBP25 isoforms of *dog* exhibited a close relationship and clustered together, according to the phylogenetic tree. There were similar phenomena in *rabbit* and *elephant*. 3.2. Selection Pressure Analyses. The nonsynonymous to synonymous rate ratio (dN/dS) may demonstrate the selective pressures of involved protein. We calculated the pairwise distance of FKBP25 sequences using MEGA 5.05. There was a significantly lower *dN* than *dS* in the pairwise comparisons of these sequences. Most values of dN/dS in these sequences were distributed blow the diagonal, showing that the presence of a purifying selection existed in the FKBP25 (Figure 3). The comparisons of average *dN* and *dS* in various vertebrate groups were shown in Figure 4, respectively. Furthermore, site-specific tests were performed for searching the positive selection sites in vertebrate, mammalian, primate, and mammalian excluding primate, rodent and teleost lineages. Although some positive selection sites were computed, each $2\Delta l$ of M7 and M8 <5.99 indicated that the M8 model was not significantly better than the M7 model to fit the data. Consequently, we concluded that the site-specific analyses also compute no positive selection sites acting on FKBP25 using PAML4.7 (Table 1).

3.3. Protein Domain and Motif Analyses. Early studies reported that mammalian FKBP25 have two portions: one is a putative helix-loop-helix motif within N-terminal unique sequence (Figure 5(a)) and the other is the PPIase domain at its C-terminus (Figure 5(b)) [20].

The domain distribution of FKBP25 was investigated using FKBP25 to search amino acid sequences at the Pfam database firstly. Only one domain (PPIase domain) was found in the Pfam database. The PPIase domain within FKBP25 sequences generally started at position 122 and ended at position 221. Similarly, we further make sure that the FKBP25 domain is at SMART, resulting in the single PPIase domain at position 119 to 221.



FIGURE 6: Exon-intron conservation among FKBP25 genes.

We then performed a detailed domain and motif analyses using the MEME software. Except two *dog* isoforms, *dog2* and *dog3*, the FKBP25 sequences used in this study contain a conversed PPIase domain within motif 1 (shown in Figure 2) at its C-terminus. In addition, the result implied that motif 2 located in the N-terminal contained an HLH motif [6], which was associated with DNA binding and dimerization [21]. However, HLH motif was not found in *dog3*, *anole lizard*, and *teleost* lineage, implying that these FKBP25 proteins may function on gene expression in another pathway.

3.4. Exon-Intron Conservation Analyses. The exon-intron information collected from the ENSEMBL database was shown in Table 2 and Figure 6. Most of the FKBP25 genes have 7 exons with similar length in different species (Table 2). Mammalian FKBP25 shows exon-intron conservation with 6 introns and similar sizes of each intron. Intron deletions existed in several isoforms of species. The *rabbit2* isoform had 2 exons, and *elephant2* isoform had only one exon. The exon numbers of *dog2*, *dog3*, and *dog4* isoforms were less

than seven. Except mammalian FKBP25 genes, *anole lizard* reduced one exon compared with mammalian and birds, but the *xenopus* and teleost maintained 7 exons. The intron deletions of FKBP25 genes may happen in the evolutionary process from amphibian to reptile. Then, a subsequent intron insertion occurred in the evolution from reptile to more advanced animals. The FKBP25 genes also had intron insertion in *zebra finch* and *zebra fish*.

4. Discussion

FKBP25 is a nuclear member of the FKBPs family that is associated with transcription and chromatin structure [2]. The interactions of FKBP25 with nuclear proteins are closely associated with HLH motif at the N-terminal of FKBP25. However, whether the PPIase domain at C-terminus is important for these interactions remains uncertain. The selection pressure analyses revealed that the purifying selection triggered a whole evolutionary history of FKBP25 in vertebrates, even in each lineage of vertebrates. Purifying selection is one of the natural selections that resist deleterious mutations with negative selective coefficients [22]. The mutations that disrupt the correct folding of the FKBP25 domain can weaken PPIase activity and may be the deleterious mutations [5]. It was hypothesized that the mutations of PPIase domain were one of explanations behind the purifying selection throughout FKBP25 evolution. Therefore, although the PPIase domain of FKBP25 was not found to be involved in the protein interactions previously, the PPIase domain might have some associations with the YY1 DNA-binding, MDM2 autoubiquitination and degradation, and HDACs complex formation. These inferences will become a potent direction for exploring the relationship between nuclear proteins and PPIase domain in the future.

The protein-coding sequence length of vertebrate FKBP25 is highly conversed that almost all the taxa are 224 bp; nevertheless the original gene length and exonintron status are tremendously various among vertebrate species. However, mammalian FKBP25 exhibit exon-intron conservation with 6 introns and similar sizes of each intron. Chicken FKBP25 maintains 6 introns, but zebra finch has one more intron that inserts in the gene. Similarly, a large variability of intron number and sizes among all the taxa shown in Figure 6 revealed that intron insertion and deletion events happened frequently during the FKBP25 evolutionary history from teleost to birds. In particular, zebrafish demonstrated the maximum number of introns in this study, and the size of exon is much smaller than other teleost species (Figure 6(g)). The intron loss of FKBP25 gene from species more advanced than zebrafish is likely to induce alterations of gene expression due to the absence of specific intron splicing. Under the purifying selection, the FKBP25 gene expression event continuously removes the pernicious mutations that may associate with intron splicing regulation [23]

FKBP25 gene knockdown declined the expression levels of p53 and p21, which emphasized the significance of FKBP25 in regulating p53 and subsequently p21 expression through controlling the ubiquitination of MDM2. Both the FKBP25 PPIase domain and its N-terminal portion were critical for the ubiquitination and degradation of MDM2 [2]. Moreover, Jin et al. reported that FKBP25 prefers to bind to rapamycin rather than FK506, implying that FKBP25 may be an important target molecule for immunosuppression by rapamycin [8]. All the evolution analyses indicated the conservation of FKBP25 gene in vertebrates. Therefore, FKBP25 possesses some basic functions in vertebrate species, like regulating p53 and p21 expression and binding to rapamycin for immunosuppression, reinforcing the suggestion that the purifying selection triggered the evolution of vertebrate FKBP25.

In conclusion, FKBP25 as a nuclear FKBP subjects to the purifying selection throughout the whole evolution, which implied the complete role of the PPIase domain involved in the interaction between FKBP25 and the nuclear proteins that are needed to be discovered continually. Additionally, incomplete exon-intron conservation of FKBP25 meets the vertebrate lineage. The intron gain or loss among the taxa is likely to be involved in the purifying selection.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Fei Liu and Xiao-Long Wei contributed to this paper equally.

Acknowledgments

This project was sponsored by the Grants from the National Natural Science Foundation of China (81273593, 81273274, and 81302331), the Priority Academic Program Development of Jiangsu Higher Education Institutions, National Major Scientific, Technological Special Project for "Significant New Drugs Development" (2011ZX09302-003-02), Jiangsu Province Major Scientific and Technological Special Project (BM2011017), Jiangsu Province's Key Provincial Talents Program (RC201170 and H201108), and the Foundation of the Nanjing Pharmaceutical Association, China (Nanjing, China) (Grant no. H2011YX001).

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