

## Characterization and Evolution Analysis of CaMKII Gene in Pig and Other Species

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**Abstract:** Using CaMK2B gene cds of human and mouse to blast *Sus scrofa* 9 genome, the porcine CaMK2B gene sequence was obtained. Homologous protein sequence between four isoforms in 9 species was analyzed. The results indicate that some key residues differences are responsible for different enzymatic properties of different isoforms.

**Key words:** CaMKII, swine, genewise, gene evolution, enzymatic properties, isoforms

### INTRODUCTION

CaM/Ca<sup>2+</sup>-dependent protein Kinase II (CaMKII) is an autonomous activity protein. Activated CaMKII protein can stimulate many pathways by phosphorylation substrate such as CREB. CaMKII has been considered to relate to neuron memory (Lisman *et al.*, 2002) immune memory (Bui *et al.*, 2000; Ishiguro *et al.*, 2006) and events of egg activation (Ducibella and Fissore, 2008). There are alpha, beta, gamma and delta isoforms of CaMKII in mammals. The protein structures of these isoforms are very similar: an N-terminal catalytic domain and C-terminal associated domain which used to form oligomeric complex are connected by regulatory domain and variable domain (Fig. 1).

The catalytic domain regulatory domain and associated domain is conserved in four isoforms. The experiment prove that CaMK2G is more sensitive and CaMK2A is less sensitive to changes in CaM2+ concentration than the other isoforms.

Gaertner *et al.* (2004)'s study also suggest that the rank order of rates of autophosphorylation is CaMK2D>CaMK2B>CaMK2A>CaMK2G. What reason should be responsible for these differences of enzymatic properties of CaMKII? In this study, the protein sequence for four isoforms was compared and find several residues differences could be responsible for these enzymatic properties difference. The gene homology and gene

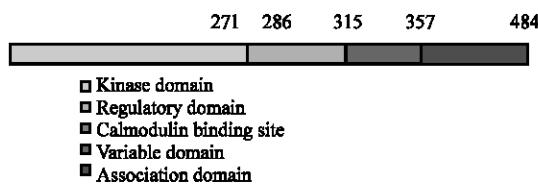


Fig. 1: The diagram of CaMKII functional domain

evolution of CaMKII was analyze. CaMK2D gene should be the earliest diverging vertebrate gene was found. This result is agreement with previous report (Tombes *et al.*, 2003).

### MATERIALS AND METHODS

CaMK2B cds of human was used and mouse to blast *Sus scrofa* 9 genome (Altschul *et al.*, 1990) then analysis blast result and construct possible gene fragment of *Sus scrofa*. The cds (protein coding sequence) and protein sequences of pig CaMK2B were predicted by Genewise (Birney *et al.*, 2004). All process is worked by Perl program (details Perl program available upon request to researchers). Four isoforms of CaMKII protein between nine species (Table 1) were analyzed using clustraw2 (Thompson *et al.*, 1994) to analyze homology to find the reason for enzymatic properties differences. In order to find the conservation properties of four isoforms of

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Table 1: CaMKII gene information

Gene name	Abbreviation	Species	Ensemble version	Chromosome	Strand	Start	End	Length
CaMK2A	hsa	<i>Homo sapiens</i>		5	+	149602254	149669192	66938
CaMK2B	hsa	<i>Homo sapiens</i>	GRCh37.56	7	+	44256749	44365230	108481
CaMK2D	hsa	<i>Homo sapiens</i>		4	+	114378344	114683083	304739
CaMK2G	hsa	<i>Homo sapiens</i>		10	+	75572259	75634343	62084
CaMK2A	ssc	<i>Sus scrofa</i>		2	+	136606174	136697322	91148
CaMK2B*	ssc	<i>Sus scrofa</i>	Sscrofa9.56	18	+	49413659*	49498413*	84754
CaMK2D	ssc	<i>Sus scrofa</i>		8	+	93598105	93903603	305498
CaMK2G	ssc	<i>Sus scrofa</i>		14	+	79839232	79894954	55722
CaMK2A	mmu	<i>Mus musculus</i>	NCBIM37.56	18	+	61085286	61146061	60775
CaMK2B	mmu	<i>Mus musculus</i>		11	+	5869675	5965751	96076
CaMK2D	mmu	<i>Mus musculus</i>		3	+	126299891	126547972	248081
CaMK2G	mmu	<i>Mus musculus</i>		14	+	21554105	21613252	59147
CaMK2A	gga	<i>Gallus gallus</i>	WASHUC2.56	13	+	13271379	13292367	20988
CaMK2D	gga	<i>Gallus gallus</i>		4	+	58100025	58259685	159660
CaMK2G	gga	<i>Gallus gallus</i>		6	+	16662302	16772557	110255
CaMK2A	oan	<i>Ornithorhynchus anatinus</i>	OANA5.56	X1	+	29054905	29112150	57245
CaMK2D	oan	<i>Ornithorhynchus anatinus</i>		Ultra445	-	3694254	3776888	82634
CaMK2G	oan	<i>Ornithorhynchus anatinus</i>		Contig3062	+	7868	43279	35411
CaMK2A	xtr	<i>Xenopus tropicalis</i>	JGI4.1.56	scaffold_559	+	562193	607649	45456
CaMK2D	xtr	<i>Xenopus tropicalis</i>		scaffold_89	+	1416999	1466884	49885
CaMK2G	xtr	<i>Xenopus tropicalis</i>		scaffold_168	+	1133889	1299037	165148
CaMK2A	dre	<i>Danio rerio</i>	Zv8.56	21	+	44429097	44467686	38589
CaMK2B	dre	<i>Danio rerio</i>		5	+	18876203	18969725	93522
CaMK2D	dre	<i>Danio rerio</i>		7	+	60248499	60402880	154381
CaMK2G	dre	<i>Danio rerio</i>		12	+	34604628	34758796	154168
CaMKII	dme	<i>Drosophila melanogaster</i>	BDGP5.13.56	4	+	1056643	1074329	17686
CaMKII	cel	<i>Caenorhabditis elegans</i>	WS200.56	IV	+	10324967	10348937	23970

\*which is predicted by blast and genewise software. \*which is the position of prediction start and end on genome

CaMKII, the genome sequences of four isoforms of CaMKII was compared between nine species by blast software. Then blast results were parsed (Parsed standard: alignment length >100 bp identity probability >80%). Sum the alignment length of pairwise homologous genes. Then the gene homology between species and assigned a conservation index to every gene was analyzed. For example CaMK2D: hsa gene is homologous to other eight species, so 9 was assigned to CaMK2D: hsa gene.

## RESULTS AND DISCUSSION

**CaMKII prediction:** CaMK2B gene of *Sus scrofa* was predicted by Genewise software. It locates on plus strand of chromosome 18 of *Sus scrofa* 9. The length of sus CaMK2B gene is around 84 kb (Table 1). Protein sequences of CaMK2B have been compared and shown in Fig. 2.

The predicted CaMK2B\_ssc\_new protein sequence is similar to other species except a fragment in kinase domain (red region in Fig. 2). There should have two possible reasons for this result, the predicted CaMK2B is correct, the porcine coding sequence is different with other species. The genome of *Sus scrofa* is not complete, so Genewise predicated a wrong protein sequence.

**CaMKII protein conservation:** The regulatory domain is conserved between CaMK2D: hsa, CaMK2A: hsa, CaMK2B: hsa and CaMKII: dme (Fig. 3). There are three residue differences (red mark in Fig. 3) for CaMK2G: hsa. VS residues located at the pocket of calmodulin complex (Fig. 4). The property difference between VS and AT residues must led calmodulin to has different binding affinity for regulatory domain. Thus, it can deduce that CaMK2G's special residues are responsible for its sensitivity to the calmodulin stimulation.

The protein sequence in the activation loop and Serine/Threonine protein kinases active-site (Fig. 5) was compared. Beside Serine/Threonine protein kinases active-site, there is a NGI peptide which interacts with activation loop (Fig. 5).

The NGI peptide of four isoforms was compared to cel and dme and then find that the rank order of similarity to CaMKII:cel is CaMK2D>CaMK2B> = CaMK2A >CaMK2G. This similarity order is the same as phosphorylation rate order which reported by Gaertner *et al.* (2004).

Activation loop control the phosphorylation rate of CaMKII (Fig. 6, Adams, 2003). So it can deduce these residue differences lead to that CaMK2D has a higher phosphorylation rate.

CaMK2B_ssc_new	MATTVTCTRFTDEYQLYEDIGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_hsa_ESNP00000258682	MATTVTCTRFTDEYQLYEDIGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_mmu_ESMUSP0000019133	MATTVTCTRFTDEYQLYEDIGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_gga_46048967	MATTVPCTRFTDEYQLYEEIGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_xtr_118404282	-MAVTCTRFTDEYQLYEEIGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 48
CaMK2B_dre_189518995	-MATTCTRFTDEYQLYEELGKGA FSVVRRCVKLSTGQEYAAKIINTKKL 49
CaMKII_cel_K11E8.1c.2	MNN-ASTKFSNDYDVK KEELGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 48
CaMKII_dme_FBpp0099496	MAAPAACTRFSNDYDI KEELGKGA FSVVRRCVKLCTGHEYAAKIINTKKL 50
.....***.....*****.....***.....*****.....	
CaMK2B_ssc_new	SARDHQKL EREARICRLLKHSNIGERPSEG GGGGGPAFLYSRQRDPKGVP 100
CaMK2B_hsa_ESNP00000258682	SARDHQKL EREARICRLLKHSNIVRLHDSI SEEGFH YLVFDLVTGGELFE 100
CaMK2B_mmu_ESMUSP0000019133	SARDHQKL EREARICRLLKHSNIVRLHDSI SEEGFH YLVFDLVTGGELFE 100
CaMK2B_gga_46048967	SARDHQKL EREARICRLLKHSNIVRLHDSI SEEGFH YLVFDLVTGGELFE 100
CaMK2B_xtr_118404282	SARDHQKL EREARICRLLKHPNIVRLHDSI SEEGFH YLFDLV TGELFE 98
CaMK2B_dre_189518995	SARDHQKL EREARICRLLKHPNIVRLHDSI SEEGFH YLFDLV TGELFE 99
CaMKII_cel_K11E8.1c.2	SARDHQKL EREARICRKLQHPNIVRLHDSI QEE SFHYL VFDL TGELFE 98
CaMKII_dme_FBpp0099496	TARDFQKL EREARICRKLH HPNIVRLHDSI QEEN YHYL VFDL TGELFE 100
.....*****.....***.....***.....	
CaMK2B_ssc_new	-KVSLSFPTEGSY PQGLCLLP AEALGQPPGA GDQGWAPP GPVPAHRCVA 149
CaMK2B_hsa_ESNP00000258682	DIVAREYY SEADASHC IQQILEA VLHCHQM GVVH RDLK PENLL ASKCKG 150
CaMK2B_mmu_ESMUSP0000019133	DIVAREYY SEADASHC IQQILEA VLHCHQM GVVH RDLK PENLL ASKCKG 150
CaMK2B_gga_46048967	DLVAREYY SEADASHC IQQILEA VLHCHQM GVVH RDLK PENLL ASKCKG 150
CaMK2B_xtr_118404282	DIVAREYY SEADASHC IQQILEA VLHCHQM GVVH RDLK PENLL ASKCKG 148
CaMK2B_dre_189518995	DIVAREYY SEADASHC IQQILEA VLHCHQM GVVH RDLK PENLL ASKCKG 149
CaMKII_cel_K11E8.1c.2	DIVAREFY SEADASHC IQQILESIA YCHSNG VH RDLK PENLL ASKAKG 148
CaMKII_dme_FBpp0099496	DIVAREFY SEADASHC IQQILESV NHCH HQNG VH RDLK PENLL ASKAKG 150
.....**.....**.....**.....**.....	
CaMK2B_ssc_new	LSPDSA EDTQGSHV WLQKSHRE LP--SWHRAPE LLSGAG ISCP LA GA HA 197
CaMK2B_hsa_ESNP00000258682	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LR KEA Y G K P V D I W A C 200
CaMK2B_mmu_ESMUSP0000019133	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LR KEA Y G K P V D I W A C 200
CaMK2B_gga_46048967	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LR KEA Y G K P V D I W A C 200
CaMK2B_xtr_118404282	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LR KEA Y G K P V D I W A C 198
CaMK2B_dre_189518995	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LR KEA Y G K P V D I W A C 199
CaMKII_cel_K11E8.1c.2	AAVKLADFG LAI EV VQGD QQAW WFG AG TPG YLS PEV LK KD PY SK P V D I W A C 197
CaMKII_dme_FBpp0099496	AAVKLADFG LAI EV VQGD HQAW WFG AG TPG YLS PEV LK KE Y G K S V D I W A C 200
.....**.....**.....**.....**.....	
CaMK2B_ssc_new	PGTRVL VALPASS LLL KPQAKSL WAT QRR CWD -PR PR KL CR CP GAS QP 246
CaMK2B_hsa_ESNP00000258682	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 248
CaMK2B_mmu_ESMUSP0000019133	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 248
CaMK2B_gga_46048967	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 248
CaMK2B_xtr_118404282	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 246
CaMK2B_dre_189518995	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 247
CaMKII_cel_K11E8.1c.2	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 245
CaMKII_dme_FBpp0099496	G-VILY ILL VGYP FW DED QH KLY QQ IK AG A Y DF PS PE W D T V T P E A K N L 248
.....**.....**.....**.....**.....	
CaMK2B_ssc_new	PPVPLSVRPLS PLY SCRS FS TSL P L FG DF R P D L A H V W K P P G G T R D S G Q L R 296
CaMK2B_hsa_ESNP00000258682	INQMLT INPA KRITA A HEAL K H P W V C Q R S T V A S M M H R Q E T V E C L K K F N A R 298
CaMK2B_mmu_ESMUSP0000019133	INQMLT INPA KRITA A HEAL K H P W V C Q R S T V A S M M H R Q E T V E C L K K F N A R 298
CaMK2B_gga_46048967	INQMLT INPA KRITA A HEAL K H P W V C Q R S T V A S M M H R Q E T V E C L K K F N A R 298
CaMK2B_xtr_118404282	INQMLT INPA KRITA A HEAL K H P W V C Q R S T V A S M M H R Q E T V E C L K K F N A R 296
CaMK2B_dre_189518995	INQMLT INPA KRITA A HEAL K H P W V C Q R S T V A S M M H R Q E T V E C L K K F N A R 297
CaMKII_cel_K11E8.1c.2	IDSMLT VNPKKR ITAD QALK WP C N R V A S A I R Q D T V D C L K F N A R 295
CaMKII_dme_FBpp0099496	INQMLT VNPKKR ITAA E AL K H P W C Q R E R V A S V V H R Q E T V D C L K F N A R 298
.....**.....**.....**.....**.....	
CaMK2B_ssc_new	SLSGWIP GPLL PTFG FS L GR QT TA P T M S A G T -TM GL V E Q A K S L N K 345
CaMK2B_hsa_ESNP00000258682	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T -TM GL V E Q A K S L N K 347
CaMK2B_mmu_ESMUSP0000019133	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T -TM GL V E Q A K S L N K 347
CaMK2B_gga_46048967	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T -PL GL V E Q A K S L N K 345
CaMK2B_xtr_118404282	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T -AL GL V E Q A K S L N K 345
CaMK2B_dre_189518995	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T -AK T L N K 322
CaMKII_cel_K11E8.1c.2	KLKAA I S A V K M V T R M S G V L R T S D G S V A S N G S T H D A S Q V A G T S S Q P T S 345
CaMKII_dme_FBpp0099496	KLKG A I L T T M L A T R N F S V G R Q T T A P T M S A G T - 313
.....**.....**.....**.....**.....	

Fig. 2: Continue

CaMK2B_ssc_new	KADGVKPQT--NSTKNSAAATSPKGTL-----	371
CaMK2B_hsa_ESNP00000258682	KADGVKPQT--NSTKNSAAATSPKGTL-----	373
CaMK2B_mmu_ENSMUSP0000019133	KADGVKPQT--NSTKNSAAITSPKGSLP-----	373
CaMK2B_gga_46048967	KADGVKPQT--NSTKGSAGVTSPKGTL-----	371
CaMK2B_xtr_118404282	KTDVGKPQT--NSTKNSAGVTSPKGPIP-----	371
CaMK2B_dre_189518995	KAD-VKPQT--NSTKNS-IVTSPKGNL-----	346
CaMKII_cel_K11E8.1c.2	PAAEVYPNVLLFNPQKFPRNCVHPFTTHPYYSPESSKKLFFTLLFEVC	395
CaMKII_dme_FBpp0099496	-----	
CaMK2B_ssc_new	-----PAALEPQTTVIHNPVDGIKE-----SSDSTHTTIEDEDT	405
CaMK2B_hsa_ESNP00000258682	-----PAALEPQTTVIHNPVDGIKE-----SSDSANTTIEDEDA	407
CaMK2B_mmu_ENSMUSP0000019133	-----PAALEPQTTVIHNPVDGIKE-----SSDSTNTTIEDEDA	407
CaMK2B_gga_46048967	-----PAALEPQTTVIHNPADGIKE-----SSDSTNTTIEDEDT	405
CaMK2B_xtr_118404282	-----PAALETQTTVIHLVDGIKE-----SSDSTHTNPEDEEM	405
CaMK2B_dre_189518995	-----SPALEAQTTVIHNAVDGIKE-----SSDSSNATVEDEEM	380
CaMKII_cel_K11E8.1c.2	PHTSRSHILLRDNTKNIYHPYHCFTNKMSNYERAAPSSHGSSTTKIANA	445
CaMKII_dme_FBpp0099496	-----FS-----	315
CaMK2B_ssc_new	KAP-RVPDILSSVRRGSG-----TPDVEG	428
CaMK2B_hsa_ESNP00000258682	KAP-RVPDILSSVRRGSG-----APEAEG	430
CaMK2B_mmu_ENSMUSP0000019133	KAP-RVPDVSLVRRASG-----APEAEG	430
CaMK2B_gga_46048967	KA-----	407
CaMK2B_xtr_118404282	KA-----	407
CaMK2B_dre_189518995	KAATKFTDLLGVVRRGSVP-----TSDAEG	405
CaMKII_cel_K11E8.1c.2	IADL VIRRSSPSIRRTEADVHSNRRKVSAPANLQHALVPVIDVVAT	495
CaMKII_dme_FBpp0099496	-----	
CaMK2B_ssc_new	PPPCLSPAPIPLPTPSPRISDLSSVRRGSGTPEAEG-----	466
CaMK2B_hsa_ESNP00000258682	PLPCPSPAPFSPLPAPSPRISDLNSVRRGSGTPEAEGPLSAGPPPCCLSP	480
CaMK2B_mmu_ENSMUSP0000019133	PLSCQSPVPISPLPTPSPRISDLNSVRRGCGTPEAEGPLSVGPPPCCLSP	480
CaMK2B_gga_46048967	-----	
CaMK2B_xtr_118404282	-----	
CaMK2B_dre_189518995	GTTTTPAVVAAPSTPQTPTSIPTQMSRLTDLVSSVRR-----	441
CaMKII_cel_K11E8.1c.2	GALASSSVNDLNLASTSSDLGRNLLNKKEQGPPIKES-----	533
CaMKII_dme_FBpp0099496	SRSMITKKGEG-SQVKES-----	332
CaMK2B_ssc_new	PLPTPSPRISNILNTVRRGSGTPEAQP-----PPCPSPA	501
CaMK2B_hsa_ESNP00000258682	ALLGPLSSPSPRISDLNSVRRGSGTPEAEGPSP-----VGPPPCPSPT	524
CaMK2B_mmu_ENSMUSP0000019133	GLLGPLPTPSPRISDLNSVRRGSGTPEAEGLPP-----VGPPPCPSPT	524
CaMK2B_gga_46048967	-----	
CaMK2B_xtr_118404282	-----	
CaMK2B_dre_189518995	PTVPTDSEPSAASRALSPPVSVPS-----HPSPSPA	473
CaMKII_cel_K11E8.1c.2	SESS-QTIDDNDS-EKGGGQLKHENTVVRADG-ATGIVSSSNSS	574
CaMKII_dme_FBpp0099496	TDSSTTLEDDDIKEDKKGTVDRSTTVSKEPEDIRLCPAKTY	376
CaMK2B_ssc_new	LPGPPPTP---TRKQEIIKITEQLIEAVNNGDFEA YAKICDPGLTSFEPE	548
CaMK2B_hsa_ESNP00000258682	IPGPLPTP---SRKQEIIKTTTEQLIEAVNNGDFEA YAKICDPGLTSFEPE	571
CaMK2B_mmu_ENSMUSP0000019133	LPGPLPTP---SRKQEIIKTTTEQLIEAVNNGDFEA YAKICDPGLTSFEPE	571
CaMK2B_gga_46048967	RKQEIIKITEQLIEAVNNGDFEA YAKICDPGLTSFEPE	445
CaMK2B_xtr_118404282	RKQEIIKITEQLIEAVNNGDFEA YAKICDPGLTTFEPE	445
CaMK2B_dre_189518995	QVSSSPLPSAHSRKQKEIIKITEQLIEAINNGDFEA YAKICDPGLTCFEPE	523
CaMKII_cel_K11E8.1c.2	TASKSSTTNLSAQKQDIFVRTQTLDAISCKDFETYTRLCDTSMTCFEPE	624
CaMKII_dme_FBpp0099496	QQNIGNSQCSSARRQEIIKITEQLIEAINS GDFDGYTKICDPHLTAFPE	426
CaMK2B_ssc_new	*****.*****.*****.*****.*****.*****.*****.	
CaMK2B_hsa_ESNP00000258682	ALGNLVEGMDFHRFYFENLLAKNSKPIHTTILNPVHVIGEDAACIA YIR	598
CaMK2B_mmu_ENSMUSP0000019133	ALGNLVEGMDFHRFYFENLLAKNSKPIHTTILNPVHVIGEDAACIA YIR	621
CaMK2B_gga_46048967	ALGNLVEGMDFHRFYFENLLSKNNKPIHTTILNPVHVIGEDAACIA YIR	495
CaMK2B_xtr_118404282	ALGNLVEGIDFHRFYFENLLSKNNKPIHTTILNPVHVIGEDAACIA YIR	495
CaMK2B_dre_189518995	ALGNLVEGMDFHRFYFENLLSKNSKPIHTTILNPVHVIGEDAACIA YIR	573
CaMKII_cel_K11E8.1c.2	ALGNLIEHEFHRFYFD---GNRKNQVHITMLNPVNVIIGEDAACVAYVK	671
CaMKII_dme_FBpp0099496	ALGNLVEGIDFHFKFYFENVLGKNCKAINTTILNPVHVILLGEEAACIAYVR	476
CaMK2B_ssc_new	*****.*****.*****.*****.*****.*****.*****.	
	LTQYIDGQGRRTSQSKETRVWHRRDGKWQNVHFHCSGAPVAPLQ-----	643

Fig. 2: Continue

CaMK2B_hsa_ENSP00000258682	LTQYIDGQGRPRTSQSEETRVWHRDGKWQNVHFHCSGAPVAPLQ----	666
CaMK2B_mmu_ENSMUSP0000019133	LTQYIDGQGRPRTSQSEETRVWHRDGKWQNVHFHCSGAPVAPLQ----	666
CaMK2B_gga_46048967	LTQYLDQAQGRPRTSQSEETRVWHRDGKWQNVHFHGSGAPVAPLQ----	540
CaMK2B_xtr_118404282	LTQYIDTQGRPRTSQSEETRVWHRDGKWQNVHFHCSGAPVAPLQ----	540
CaMK2B_dre_189518995	LTQYVDGQGRPRSSQSEETRVWHRDLSKWQNVHFHCSGAPAAPLQ----	618
CaMKII_cel_K11E8.1c.2	LTQFLDRNGEAHTRQSQESRVWSKKQGRWVCVHVRSTQPSTN-TTVSEF	720
CaMKII_dme_FBpp0099496	LTQYIDKQGHATHHQSEETRVWHRDNKWQNVHFHRSASAKISGATTDFD	526
	*****: * ..: ***:*** : ..: * ***: * .	
CaMK2B_ssc_new	---	
CaMK2B_hsa_ENSP00000258682	---	
CaMK2B_mmu_ENSMUSP0000019133	---	
CaMK2B_gga_46048967	---	
CaMK2B_xtr_118404282	---	
CaMK2B_dre_189518995	---	
CaMKII_cel_K11E8.1c.2	---	
CaMKII_dme_FBpp0099496	IPQK 530	

Fig. 2: CaMK2B homology analysis. CaMK2B\_ssc\_new is predicted by Genewise. Red region means different residues

CaMK2G_hsa_ENSP00000410298	RKFNARRKLKGAILTTMLVSRNFS 315
CaMK2B_hsa_ENSP00000258682	KKFNARRKLKGAILTTMLATRNFS 314
CaMK2D_hsa_ENSP00000378030	KKFNARRKLKGAILTTMLATRNFS 315
CaMK2A_hsa_ENSP00000381412	KKFNARRKLKGAILTTMLATRNFS 314
CaMKII_dme_FBpp0099496	KKFNARRKLKGAILTTMLATRNFS 315
CaMKII_cel_K11E8.1c.2	KKFNARRKLKAAISAVKMVTRMSG 312

Fig. 3: Homology analysis of CaM/Cz2+binding domain sequence

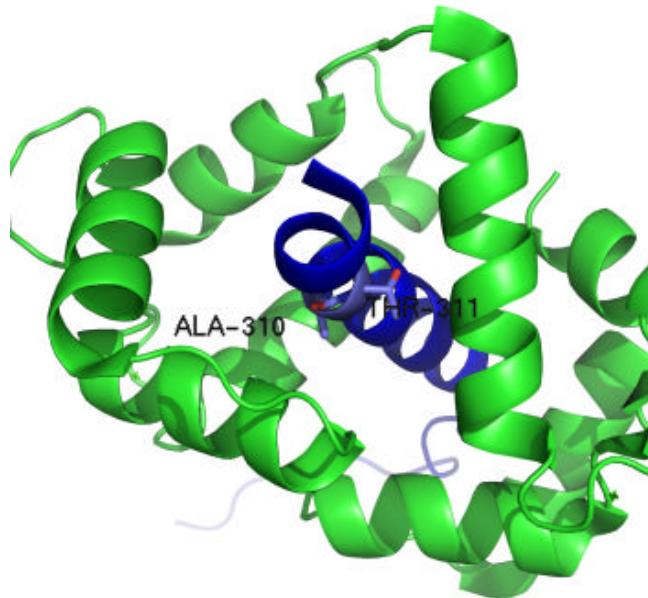


Fig. 4: 3D structure of CaMK2D binding with calmodulin/Ca2+

**CaMKII gene evolution:** Human CaMK2D:hsa has many homologous segment to other three genes (Fig. 7). Other species such as ssc (*Sus scrofa*), oan (*Ornithorhynchus anatinus*) and dre (*Danio rerio*) have similar results. The

CaMK2D gene has three to four times more intronic sequences than other three genes. So we can deduce that CaMK2D and other three genes have the same ancient gene origin. If CaMKII gene in cel (*C. elegans*) and dme

ATP binding site	
CaMK2A_hsa_ENSP00000381412	-MATITCTRFTEEYQLFEE <span style="color:red">LGKGAFSVRRCVKVLAGQEYA</span> <span style="color:blue">AKIINTK</span> KL 49
CaMK2B_hsa_ENSP00000258682	MATTVTCTRFTDEYQLYEDIGKGAFSVRRCVK <span style="color:red">LCTG</span> <span style="color:blue">H</span> EYAAKIINTKKL 50
CaMK2G_hsa_ENSP00000410298	MATTATCTRFTDDYQLFEELGKGAFSVRRCVK <span style="color:red">KTSTQ</span> <span style="color:blue">EY</span> AAKIINTKKL 50
CaMK2D_hsa_ENSP00000378030	MASITTCTRFTDEYQLFEELGKGAFSVRRC <span style="color:red">CMKIPTG</span> <span style="color:blue">QEY</span> AAKIINTKKL 50
CaMKII_dme_FBpp0099496	MAAPAACTRFSDNYDIKEELGKGAFSIV <span style="color:red">V</span> KRC <span style="color:red">VQK</span> <span style="color:red">S</span> TGF <span style="color:red">EF</span> FAAKIINTKKL 50
CaMKII_cel_K11E8.1c.2	--MMNASTKFSDNYDVKEELGKGAFSVRRC <span style="color:red">VHKTTG</span> <span style="color:red">LE</span> FAAKIINTKKL 48 :.*:***:***: *:*****:***: : *:*****
CaMK2A_hsa_ENSP00000381412	SARDHQKLEREARICRLLKH <span style="color:red">PN</span> I <span style="color:red">V</span> R <span style="color:red">L</span> HDSI <span style="color:red">SEE</span> G <span style="color:red">H</span> YL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 99
CaMK2B_hsa_ENSP00000258682	SARDHQKLEREARICRLLKH <span style="color:red">SN</span> I <span style="color:red">V</span> R <span style="color:red">L</span> HDSI <span style="color:red">SEE</span> G <span style="color:red">H</span> YL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 100
CaMK2G_hsa_ENSP00000410298	SARDHQKLEREARICRLLKH <span style="color:red">PN</span> I <span style="color:red">V</span> R <span style="color:red">L</span> HDSI <span style="color:red">SEE</span> G <span style="color:red">H</span> YL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 100
CaMK2D_hsa_ENSP00000378030	SARDHQKLEREARICRLLKH <span style="color:red">PN</span> I <span style="color:red">V</span> R <span style="color:red">L</span> HDSI <span style="color:red">SEE</span> G <span style="color:red">H</span> YL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 100
CaMKII_dme_FBpp0099496	TARD <span style="color:red">F</span> QKLEREARICR <span style="color:red">K</span> L <span style="color:red">H</span> <span style="color:red">P</span> N <span style="color:red">I</span> <span style="color:red">V</span> R <span style="color:red">L</span> HDS <span style="color:red">I</span> <span style="color:red">Q</span> <span style="color:red">E</span> EN <span style="color:red">Y</span> HYL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 100
CaMKII_cel_K11E8.1c.2	SARD <span style="color:red">F</span> QKLEREARICR <span style="color:red">K</span> L <span style="color:red">H</span> <span style="color:red">P</span> N <span style="color:red">I</span> <span style="color:red">V</span> R <span style="color:red">L</span> HDS <span style="color:red">I</span> <span style="color:red">Q</span> <span style="color:red">E</span> EN <span style="color:red">Y</span> HYL <span style="color:red">F</span> D <span style="color:red">L</span> V <span style="color:red">T</span> G <span style="color:red">G</span> E <span style="color:red">L</span> F <span style="color:red">E</span> 98 :****.*****:***: *:*****:***..***:*****
Serine/Threonine protein kinases active-site	
CaMK2A_hsa_ENSP00000381412	DIVAREYYSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">MGV</span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">L</span> K <span style="color:red">G</span> 149
CaMK2G_hsa_ENSP00000410298	DIVAREYYSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">MGV</span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">C</span> K <span style="color:red">G</span> 150
CaMK2B_hsa_ENSP00000258682	DIVAREYYSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">MGV</span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">C</span> K <span style="color:red">G</span> 150
CaMK2D_hsa_ENSP00000378030	DIVAREYYSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">MGV</span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">K</span> G 150
CaMKII_dme_FBpp0099496	DIVARE <span style="color:red">F</span> YSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">NGV</span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">A</span> K <span style="color:red">G</span> 150
CaMKII_cel_K11E8.1c.2	DIVARE <span style="color:red">F</span> YSEADASHCI <span style="color:red">QQ</span> ILEAVL <span style="color:red">L</span> H <span style="color:red">C</span> H <span style="color:red">Q</span> <span style="color:red">IAY</span> <span style="color:red">CHS<span style="color:red">NGI</span></span> <span style="color:red">V</span> <span style="color:red">H</span> R <span style="color:red">D</span> <span style="color:red">I</span> K <span style="color:red">P</span> E <span style="color:red">N</span> LLL <span style="color:red">A</span> SK <span style="color:red">A</span> K <span style="color:red">G</span> 148 *****:*****:*****: : * .:*****:*****: **
Activation loop	
CaMK2A_hsa_ENSP00000381412	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">EG</span> <span style="color:red">QQ</span> AWFG <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> D <span style="color:red">P</span> Y <span style="color:red">G</span> K <span style="color:red">P</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 199
CaMK2B_hsa_ENSP00000258682	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">QG</span> <span style="color:red">DQ</span> Q <span style="color:red">Q</span> AWFG <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> E <span style="color:red">A</span> Y <span style="color:red">G</span> K <span style="color:red">P</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 200
CaMK2G_hsa_ENSP00000410298	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">QG</span> <span style="color:red">E</span> <span style="color:red">QQ</span> AWFG <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> D <span style="color:red">P</span> Y <span style="color:red">G</span> K <span style="color:red">P</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 200
CaMK2D_hsa_ENSP00000378030	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">QG</span> <span style="color:red">DQ</span> Q <span style="color:red">Q</span> AWFG <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> D <span style="color:red">P</span> Y <span style="color:red">G</span> K <span style="color:red">P</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 200
CaMKII_dme_FBpp0099496	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">QG</span> <span style="color:red">DH</span> Q <span style="color:red">Q</span> AWFG <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> E <span style="color:red">P</span> Y <span style="color:red">G</span> K <span style="color:red">S</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 200
CaMKII_cel_K11E8.1c.2	AAVKLA <span style="color:red">D</span> FGLAIE <span style="color:red">EV</span> <span style="color:red">N</span> <span style="color:red">-D</span> <span style="color:red">S</span> B <span style="color:red">A</span> W <span style="color:red">H</span> G <span style="color:red">F</span> AGT <span style="color:red">P</span> GYLSPE <span style="color:red">V</span> LR <span style="color:red">K</span> D <span style="color:red">P</span> Y <span style="color:red">G</span> K <span style="color:red">S</span> V <span style="color:red">D</span> WL <span style="color:red">A</span> C 197 *****:*****: : : * .:*****:*****: *:..*.**:***

Fig. 5: Homology analysis of kinase domain of CaMKII four gene production

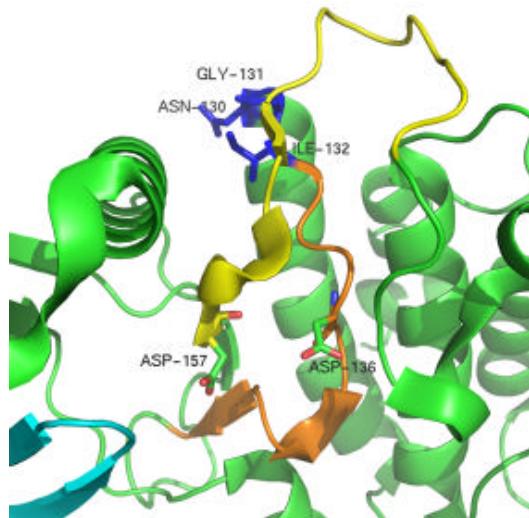


Fig. 6: The structure of peptide NGI and active center of CaMK2D

(*D. melanogaster*) could be considered as the earliest gene, the gene which is most similar to cel or dme CaMKII

gene could be considered as earliest gene. CaMK2D: hsa is similar to CaMKII: cel and CaMKII: dme. CaMK2D: mmu, CaMK2D: ssc and CaMK2D: gga is similar to CaMKII: dme. CaMK2D is the only gene which is similar to CaMKII: cel or CaMKII: dme (Fig. 8). CaMK2A, CaMK2B and CaMK2G gene can only find homologous genes in vertebrate species. CaMK2D has so many homology segment to CaMKII: cel and CaMKII: dme, this fact indicate that CaMK2D, CaMKII: cel and CaMKII: dme have the same ancient gene origin. CaMK2A, CaMK2B and CaMK2G gene emerge later than CaMK2D. This result is agreement with previous report (Tombes *et al.*, 2003).

CaMK2B: sus has been released in genebank (gi: 194042930) but homology analysis indicate the released version is wrong (details available upon request to authors), so we predicted it by blast human and mouse CaMK2B CDs to *Sus scrofa* 9 genome. It have successfully predicted the CaMK2A protein of *Sus scrofa*. So we think the predicted CaMK2B: sus is correct on chromosome 18 of *Sus scrofa* but due to uncompleted genome, Genewise could not predict protein sequence

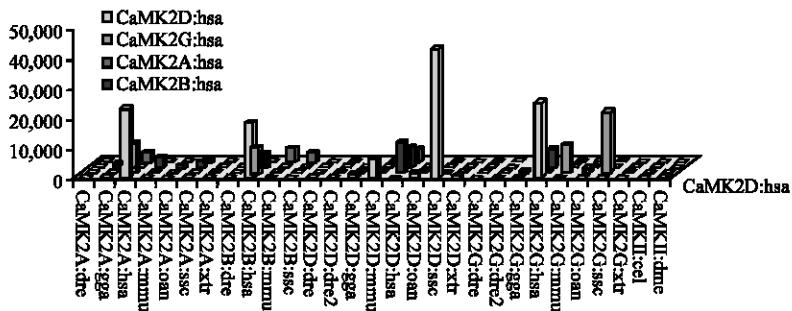


Fig. 7: Pairwise alignment of human four CaMKII gene and nine species

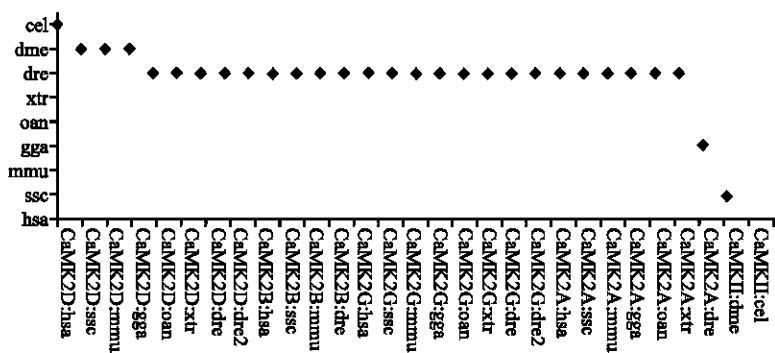


Fig. 8: Homology analysis of CaMKII in nine species

correctly (Fig. 2). Then we analyzed the relationship between protein sequence differences and enzymatic properties. It is known that activation loop of CaMKII control the phosphorylation rate (Adams, 2003). CaMK2D's activation loop and key residues which interact with activation loop is most similar to CaMKII: cel (Fig 5 and 6). *C. elegans* CaMKII has maximum phosphorylation rate (Griffith *et al.*, 2003; Chao *et al.*, 2010). So it can deduce that CaMK2D should have the maximum phosphorylation rate. CaMK2D have been reports to have maximum phosphorylation rate. The result is in agreement with previous reports (Gaertner *et al.*, 2004). KKFN residues in regulatory domain of CaMKII were found to be sufficient to maintain an inhibited state in a truncate form of the kinase (Cruzalegui *et al.*, 1992). CaMK2G mutant from KKFN-RKFN (Fig. 3), this mutation must influence the capability of KKFN segment to inhibit kinase activity. So RKFN lead CaMK2G was deduced to be more sensitive to changes of Ca<sup>2+</sup>/CaM concentration. This result is in agreement with previous reports (Gaertner *et al.*, 2004). The homology analyses of four isoforms indicate that CaMK2D is the earliest emergence gene (Fig. 8) and CaMK2D and other three genes have the same ancient gene origin (Fig. 7). This result is agreement with previous report (Tombes *et al.*, 2003).

## CONCLUSION

By homology analysis of nine species, it prove that CaMK2D is the earliest emergence gene than other three genes in the CaMKII family.

## ACKNOWLEDGEMENTS

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