Journal of Animal and Veterinary Advances 9 (12): 1745-1752, 2010
ISSN: 1680-5593

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/Ca2+dependent protein Kinase II (CaMKII) is an autonomous activity protein. Activated CaMKII protein can stimulate many pathways by phsophorylation 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-teriminal 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 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).

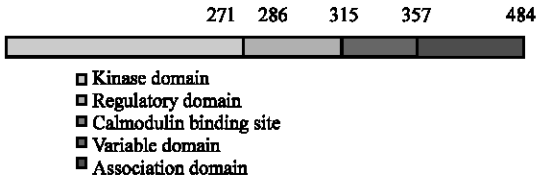

Fig. 1: The diagram of CaMKII functional domain

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

[a]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                MATTVTCTRFTDEYQLYEDIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_hsa_ENSP00000258682    MATTVTCTRFTDEYQLYEDIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_mmu_ENSMUSP00000019133 MATTVTCTRFTDEYQLYEDIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_gga_46048967           MATTVPCTRFTDEYQLYEEIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2B_xtr_118404282          --MAVTCTRFTDEYQLYEEIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 48
CaMK2B_dre_189518995          -MATTTCTRFTDEYQLYEELGKGAFSVVRRCVKLSTGQEYAAKIINTKKL 49
CaMKII_cel_K11E8.1c.2         MMN--ASTKFSDNYDVKEELGKGAFSVVRRCVHKTTGLEFAAKIINTKKL 48
CaMKII_dme_FBpp0099496        MAAPAACTRFSDNYDIKEELGKGAFSIVKRCVQKSTGFEFAAKIINTKKL 50
                              ..*:*:*:*:: *:.*******:*:***:  ** *:**********

CaMK2B_ssc_new                SARDHQKLEREARICRLLKHSNIGERPSEGGGGGGPAFLYSRQRDPKGVP 100
CaMK2B_hsa_ENSP00000258682    SARDHQKLEREARICRLLKHSNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMK2B_mmu_ENSMUSP00000019133 SARDHQKLEREARICRLLKHSNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMK2B_gga_46048967           SARDHQKLEREARICRLLKHSNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMK2B_xtr_118404282          SARDHQKLEREARICRLLKHPNIVRLHDSISEEGFHYLIFDLVTGGELFE 98
CaMK2B_dre_189518995          SARDHQKLEREARICRLLKHPNIVRLHDSISEEGFHYLLFDLVTGGELFE 99
CaMKII_cel_K11E8.1c.2         SARDFQKLEREARICRKLQHPNIVRLHDSIQEESFHYLVFDLVTGGELFE 98
CaMKII_dme_FBpp0099496        TARDFQKLEREARICRKLHHPNIVRLHDSIQEENYHYLVFDLVTGGELFE 100
                              :***.********** *:*.** . ..   .  :::.  . : .

CaMK2B_ssc_new                -KVSLSFPTEGSYPQGLCLLLPAEALGQPPGAGDQGWAPPGPVPAHRCVA 149
CaMK2B_hsa_ENSP00000258682    DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKG 150
CaMK2B_mmu_ENSMUSP00000019133 DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKG 150
CaMK2B_gga_46048967           DLVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKG 150
CaMK2B_xtr_118404282          DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKG 148
CaMK2B_dre_189518995          DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKN 149
CaMKII_cel_K11E8.1c.2         DIVAREFYSEADASHCIQQILESIAYCHSNGIVHRDLKPENLLLASKAKG 148
CaMKII_dme_FBpp0099496        DIVAREFYSEADASHCIQQILESVNHCHQNGVVHRDLKPENLLLASKAKG 150
                              *:.: :*.. .: : :* :   : * .:.  * . : * :.

CaMK2B_ssc_new                LSPDSAEDTQGSHVLWLQKSHRELP--SWHRAPELLSGAGISCPLGAHGA 197
CaMK2B_hsa_ENSP00000258682    AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 200
CaMK2B_mmu_ENSMUSP00000019133 AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 200
CaMK2B_gga_46048967           AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 200
CaMK2B_xtr_118404282          AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 198
CaMK2B_dre_189518995          AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 199
CaMKII_cel_K11E8.1c.2         AAVKLADFGLAIEVN-DSEAWHGFAGTPGYLSPEVLKKDPYSKPVDIWAC 197
CaMKII_dme_FBpp0099496        AAVKLADFGLAIEVQGDHQAWFGFAGTPGYLSPEVLKKEPYGKSVDIWAC 200
                              :. *:  . .*   ::  :. . : :**:*    . .:.  ..

CaMK2B_ssc_new                PGTRVLVALPASSSLLLKPQAKSLWATQRRSCWD-PRPRKLCRCPGASQP 246
CaMK2B_hsa_ENSP00000258682    G--VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKNL 248
CaMK2B_mmu_ENSMUSP00000019133 G--VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKNL 248
CaMK2B_gga_46048967           G--VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPDWDTVTPEAKNL 248
CaMK2B_xtr_118404282          G--VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKNL 246
CaMK2B_dre_189518995          G--VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKNL 247
CaMKII_cel_K11E8.1c.2         G--VILYILLVGYPPFWDEDQHRLYAQIKAGAYDYPSPEWDTVTPEAKSL 245
CaMKII_dme_FBpp0099496        G--VILYILLVGYPPFWDEDQHRLYSQIKAGAYDYPSPEWDTVTPEAKNL 248
                              :*  * .. : . : : :*:  : ..:* * *     * *..

CaMK2B_ssc_new                PPVPLSVRPLSPLYSCRSFSTSLPFGDFRDPDLAHVWKPPGGTRDSGQLR 296
CaMK2B_hsa_ENSP00000258682    INQMLTINPAKRITAHEALKHPWVCQRSTVASMMHRQETVECLKKFNARR 298
CaMK2B_mmu_ENSMUSP00000019133 INQMLTINPAKRITAHEALKHPWVCQRSTVASMMHRQETVECLKKFNARR 298
CaMK2B_gga_46048967           INQMLTINPAKRITAHEALKHPWVCQRSTVASMMHRQETVECLKKFNARR 298
CaMK2B_xtr_118404282          INQMLTINPAKRITAHEALKHPWVCQRSTVASMMHRQETVECLKKFNARR 296
CaMK2B_dre_189518995          INQMLTINPAKRITAQEALKHPWVCQRSTVASMMHRQETVECLKKFNARR 297
CaMKII_cel_K11E8.1c.2         IDSMLTVNPKKRITADQALKVPWICNRERVASAIHRQDTVDCLKKFNARR 295
CaMKII_dme_FBpp0099496        INQMLTVNPNKRITAAEALKHPWICQRERVASVVHRQETVDCLKKFNARR 298
                              *::.* . : : .::. .       ..  * ..   :. .  *

CaMK2B_ssc_new                SLSGWIPGPLLPTFGFSLGRQTTAPATMSTAASGT-TMGLVEQAKSLLNK 345
CaMK2B_hsa_ENSP00000258682    KLKGAILTTMLATRNFSVGRQTTAPATMSTAASGT-TMGLVEQAKSLLNK 347
CaMK2B_mmu_ENSMUSP00000019133 KLKGAILTTMLATRNFSVGRQTTAPATMSTAASGT-TMGLVEQAKSLLNK 347
CaMK2B_gga_46048967           KLKGAILTTMLATRNFSVGRQTTAPPSMSAAAA---PLGLVEQAKSLLNK 345
CaMK2B_xtr_118404282          KLKGAILTTMLATRNFSVGRQTTAPATITTAATST-ALGLVEQAKSLLNK 345
CaMK2B_dre_189518995          KLKGAILTTMLVSRNFSA----------AKTLLNK 322
CaMKII_cel_K11E8.1c.2         KLKAAISAVKMVTRMSGVLRTSDSTGSVASNGSTTHDASQVAGTSSQPTS 345
CaMKII_dme_FBpp0099496        KLKGAILTTMLATRN---------- 313
                              .*.. *    : :
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Fig. 2: Continue

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CaMK2B_ssc_new                KADGVKPQT---NSTKNSAAATSPKGTLP---------- 371
CaMK2B_hsa_ENSP00000258682    KADGVKPQT---NSTKNSAAATSPKGTLP---------- 373
CaMK2B_mmu_ENSMUSP00000019133 KADGVKPQT---NSTKNSSAITSPKGSLP---------- 373
CaMK2B_gga_46048967           KADGVKPQT---NSTKGSAGVTSPKGTLP---------- 371
CaMK2B_xtr_118404282          KTDVGKPQT---NSTKNSAGVTSPKGPIP---------- 371
CaMK2B_dre_189518995          KAD-VKPQT---NSTKNS-IVTSPKGNLP---------- 346
CaMKII_cel_K11E8.1c.2         PAAEVYPNVLLFNPQKFPRNCVHPFTTHPYYSPKESSKKKLFFTLLFEVC 395
CaMKII_dme_FBpp0099496        ---------------------------------------------


CaMK2B_ssc_new                ------PAALEPQTTVIHNPVDGIKE---------SSDSTHTTIEDEDT 405
CaMK2B_hsa_ENSP00000258682    ------PAALEPQTTVIHNPVDGIKE---------SSDSANTTIEDEDA 407
CaMK2B_mmu_ENSMUSP00000019133 ------PAALEPQTTVIHNPVDGIKE---------SSDSTNTTIEDEDA 407
CaMK2B_gga_46048967           ------PAALEPQTTVIHNPADGIKE---------SSDSTNTTIEDEDT 405
CaMK2B_xtr_118404282          ------PAALETQTTVIHNLVDGIKE---------SSDSTHTNPEDEEM 405
CaMK2B_dre_189518995          ------SPALEAQTTVIHNAVDGIKE---------SSDSSNATVEDEEM 380
CaMKII_cel_K11E8.1c.2         PHTSRSHILLRDNTKNIYHPYHCFTNKMSNYERAAPSSHGSSTTKKIANA 445
CaMKII_dme_FBpp0099496        ----------------------FS---------------------- 315
                                :.


CaMK2B_ssc_new                KAP-RVPDILSSVRRGSG-------------------------TPDVEG 428
CaMK2B_hsa_ENSP00000258682    KAP-RVPDILSSVRRGSG-------------------------APEAEG 430
CaMK2B_mmu_ENSMUSP00000019133 KAP-RVPDVLSLVRRASG-------------------------APEAEG 430
CaMK2B_gga_46048967           KA---------------------------------------------- 407
CaMK2B_xtr_118404282          KA---------------------------------------------- 407
CaMK2B_dre_189518995          KAATKFTDLLGVVRRGSVP-------------------------TSDAEG 405
CaMKII_cel_K11E8.1c.2         IADLVIRRSSPSIRRKTEADVHNSNRNRKVSAPANLQHALVPVIDVVVAT 495
CaMKII_dme_FBpp0099496        ---------------------------------------------


CaMK2B_ssc_new                PPPCLSPAPISPLPTPSPRISDLLSSVRRGSGTPEAEG----------- 466
CaMK2B_hsa_ENSP00000258682    PLPCPSPAPFSPLPAPSPRISDILNSVRRGSGTPEAEGPLSAGPPPCLSP 480
CaMK2B_mmu_ENSMUSP00000019133 PLSCQSPVPISPLPTPSPRISDILNSVRRGCGTPEAEGPLSVGPPPCLSP 480
CaMK2B_gga_46048967           ---------------------------------------------
CaMK2B_xtr_118404282          ---------------------------------------------
CaMK2B_dre_189518995          GTTTTPAVVAAPSTPQTPSIPTQMSRLTDLVSSVRR------------- 441
CaMKII_cel_K11E8.1c.2         GALASSSVDNLSASTSSDLGRNLLNKKEQGPPSTIKES----------- 533
CaMKII_dme_FBpp0099496        ----------------SRSMITKKGEG--SQVKES----------- 332


CaMK2B_ssc_new                ----PLPTPSPRISNILNTVRRGSGTPEAQGP----------PPCPSPA 501
CaMK2B_hsa_ENSP00000258682    ALLGPLSSPSPRISDILNSVRRGSGTPEAEGPSP------VGPPPCPSPT 524
CaMK2B_mmu_ENSMUSP00000019133 GLLGPLPTPSPRISDILNSVRRGSGTPEAEGLPP------VGPPPCPSPT 524
CaMK2B_gga_46048967           ---------------------------------------------
CaMK2B_xtr_118404282          ---------------------------------------------
CaMK2B_dre_189518995          -------PTVPQTDSEPSAASRALSPPVSVPS----------HPSPSPA 473
CaMKII_cel_K11E8.1c.2         ------SESS-QTIDDNDS-EKGGGQLKHENTVVRADG-ATGIVSSSNSS 574
CaMKII_dme_FBpp0099496        ------TDSSSTTLEDDDIKEDKKGTVDRSTTVVSKEPEDIRILCPAKTY 376


CaMK2B_ssc_new                LPGPPPTP---TRKQEIIKITEQLIEAVNNGDFEAYAKICDPGLTSFEPE 548
CaMK2B_hsa_ENSP00000258682    IPGPLPTP---SRKQEIIKTTEQLIEAVNNGDFEAYAKICDPGLTSFEPE 571
CaMK2B_mmu_ENSMUSP00000019133 LPGPLPTP---SRKQEIIKTTEQLIEAVNNGDFEAYAKICDPGLTSFEPE 571
CaMK2B_gga_46048967           -----------RKQEIIKITEQLIEAVNNGDFEAYAKICDPGLTSFEPE 445
CaMK2B_xtr_118404282          -----------RKQEIIKITEQLIEAVNNGDFEAYAKICDPGLTTFEPE 445
CaMK2B_dre_189518995          QVSSSPLPSAHSRKQEIIKITEQLIEAINNGDFEAYAKICDPGLTCFEPE 523
CaMKII_cel_K11E8.1c.2         TASKSSSTNLSAQKQDIVRVTQTLLDAISCKDFETYTRLCDTSMTCFEPE 624
CaMKII_dme_FBpp0099496          QQNIGNSQCSSARRQEIIKITEQLIEAINSGDFDGYTKICDPHLTAFEPE 426
                              ::*:*:: *: *::*:.  **: *:::** .:* ****


CaMK2B_ssc_new                ALGNLVEGMDFHRFYFENLLAKNSKPIHTTILNPHVHVIGEDAACIAYIR 598
CaMK2B_hsa_ENSP00000258682    ALGNLVEGMDFHRFYFENLLAKNSKPIHTTILNPHVHVIGEDAACIAYIR 621
CaMK2B_mmu_ENSMUSP00000019133 ALGNLVEGMDFHRFYFENLLAKNSKPIHTTILNPHVHVIGEDAACIAYIR 621
CaMK2B_gga_46048967           ALGNLVEGMDFHRFYFENLLSKNNKPIHTTILNPHVHVIGEDAACIAYIR 495
CaMK2B_xtr_118404282          ALGNLVEGIDFHRFYFENLLSKNNKPIHTTILNPHVHVVGEDAACIAYIR 495
CaMK2B_dre_189518995          ALGNLVEGMDFHRFYFENLLSKNSKPIHTTILNPHVHLIGEEAACIAYIR 573
CaMKII_cel_K11E8.1c.2         ALGNLIEGIEFHRFYFD---GNRKNQVHTTMLNPNVHIIGEDAACVAYVK 671
CaMKII_dme_FBpp0099496        ALGNLVEGIDFHKFYFENVLGKNCKAINTTILNPHVHLLGEEAACIAYVR 476
                      *****:**::**:***:  .:. : ::**:***:**::**:***:**::


CaMK2B_ssc_new                LTQYIDGQGRPRTSQSKETRVWHRRDGKWQNVHFHCSGAPVAPLQ----- 643
```

Fig. 2: Continue

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CaMK2B_hsa_ENSP00000258682       LTQYIDGQGRPRTSQSEETRVWHRRDGKWQNVHFHCSGAPVAPLQ----- 666
CaMK2B_mmu_ENSMUSP00000019133    LTQYIDGQGRPRTSQSEETRVWHRRDGKWQNVHFHCSGAPVAPLQ----- 666
CaMK2B_gga_46048967              LTQYLDAQGRPRTSQSEETRVWHRRDGKWQNVHFHGSGAPVAPLQ----- 540
CaMK2B_xtr_118404282             LTQYIDTQGRPRTSQSEETRVWHRRDGKWQNVHFHCSGAPVAPLQ----- 540
CaMK2B_dre_189518995             LTQYVDGQGRPRSSQSEETRVWHRRDSKWQNVHFHCSGAPAAPLQ----- 618
CaMKII_cel_K11E8.1c.2            LTQFLDRNGEAHTRQSQESRVWSKKQGRWVCVHVHRSTQPSTN-TTVSEF 720
CaMKII_dme_FBpp0099496           LTQYIDKQGHAHTHQSEETRVWHKRDNKWQNVHFHRSASAKISGATTFDF 526
                                 ***:.* :*.:: **:*:*** :::.:*  **.* * .

CaMK2B_ssc_new                   ----
CaMK2B_hsa_ENSP00000258682       ----
CaMK2B_mmu_ENSMUSP00000019133    ----
CaMK2B_gga_46048967              ----
CaMK2B_xtr_118404282             ----
CaMK2B_dre_189518995             ----
CaMKII_cel_K11E8.1c.2            ----
CaMKII_dme_FBpp0099496           IPQK 530
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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
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Fig. 3: Homology analysis of CaM/Cz2+binding domain sequence

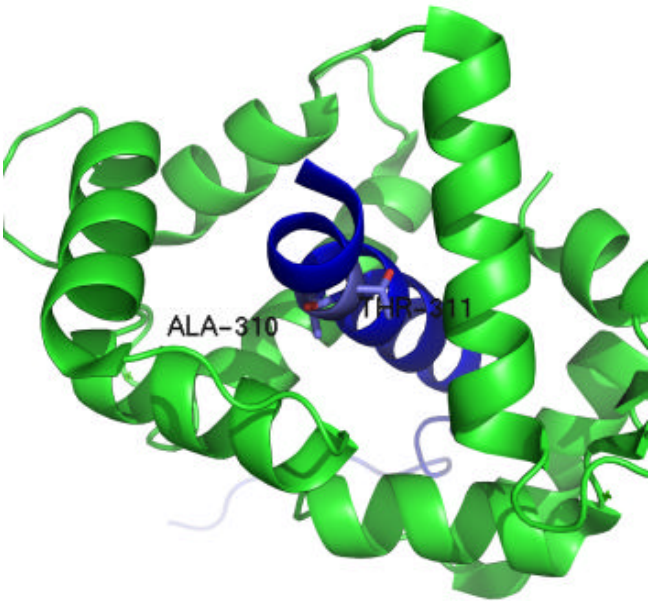

Fig. 4: 3D structure of CaMK2D binding with calmodulin/Ca2+

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. elegant*) and dme

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ATP binding site
CaMK2A_hsa_ENSP00000381412      -MATITCTRFTEEYQLFEELGKGAFSVVRRCVKVLAGQEYAAKIINTKKL 49
CaMK2B_hsa_ENSP00000258682      MATTVTCTRFTDEYQLYEDIGKGAFSVVRRCVKLCTGHEYAAKIINTKKL 50
CaMK2G_hsa_ENSP00000410298      MATTATCTRFTDDYQLFEELGKGAFSVVRRCVKKTSTQEYAAKIINTKKL 50
CaMK2D_hsa_ENSP00000378030      MASTTTCTRFTDEYQLFEELGKGAFSVVRRCMKIPTGQEYAAKIINTKKL 50
CaMKII_dme_FBpp0099496          MAAPAACTRFSDNYDIKEELGKGAFSIVKRCVQKSTGFEFAAKIINTKKL 50
CaMKII_cel_K11E8.1c.2           --MMNASTKFSDNYDVKEELGKGAFSVVRRCVHKTTGLEFAAKIINTKKL 48
                                     :.*:*:::*:: *::******:*:**::  :  *:**********

CaMK2A_hsa_ENSP00000381412      SARDHQKLEREARICRLLKHPNIVRLHDSISEEGHHYLIFDLVTGGELFE 99
CaMK2B_hsa_ENSP00000258682      SARDHQKLEREARICRLLKHSNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMK2G_hsa_ENSP00000410298      SARDHQKLEREARICRLLKHPNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMK2D_hsa_ENSP00000378030      SARDHQKLEREARICRLLKHPNIVRLHDSISEEGFHYLVFDLVTGGELFE 100
CaMKII_dme_FBpp0099496          TARDFQKLEREARICRKLHHPNIVRLHDSIQEENYHYLVFDLVTGGELFE 100
CaMKII_cel_K11E8.1c.2           SARDFQKLEREARICRKLQHPNIVRLHDSIQEESFHYLVFDLVTGGELFE 98
                                :***.********** *:*.*********.**..***:**********
                          Serine/Threonine protein kinases active-site
CaMK2A_hsa_ENSP00000381412      DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKLKG 149
CaMK2G_hsa_ENSP00000410298      DIVAREYYSEADASHCIHQILESVNHIHQHDIVHRDLKPENLLLASKCKG 150
CaMK2B_hsa_ENSP00000258682      DIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKG 150
CaMK2D_hsa_ENSP00000378030      DIVAREYYSEADASHCIQQILESVNHCHLNGIVHRDLKPENLLLASKSKG 150
CaMKII_dme_FBpp0099496          DIVAREFYSEADASHCIQQILESVNHCHQNGVVHRDLKPENLLLASKAKG 150
CaMKII_cel_K11E8.1c.2           DIVAREFYSEADASHCIQQILESIAYCHSNGIVHRDLKPENLLLASKAKG 148
                                ******:**********:****:: : *  .:*************** **
                                Activation loop
CaMK2A_hsa_ENSP00000381412      AAVKLADFGLAIEVEGEQQAWFGFAGTPGYLSPEVLRKDPYGKPVDLWAC 199
CaMK2B_hsa_ENSP00000258682      AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWAC 200
CaMK2G_hsa_ENSP00000410298      AAVKLADFGLAIEVQGEQQAWFGFAGTPGYLSPEVLRKDPYGKPVDIWAC 200
CaMK2D_hsa_ENSP00000378030      AAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKDPYGKPVDMWAC 200
CaMKII_dme_FBpp0099496          AAVKLADFGLAIEVQGDHQAWFGFAGTPGYLSPEVLKKEPYGKSVDIWAC 200
CaMKII_cel_K11E8.1c.2           AAVKLADFGLAIEVN-DSEAWHGFAGTPGYLSPEVLKKDPYSKPVDIWAC 197
                                **************: : :**.***************:*:.*.*.**:***
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Fig. 5: Homology analysis of kinase domain of CaMKII four gene production

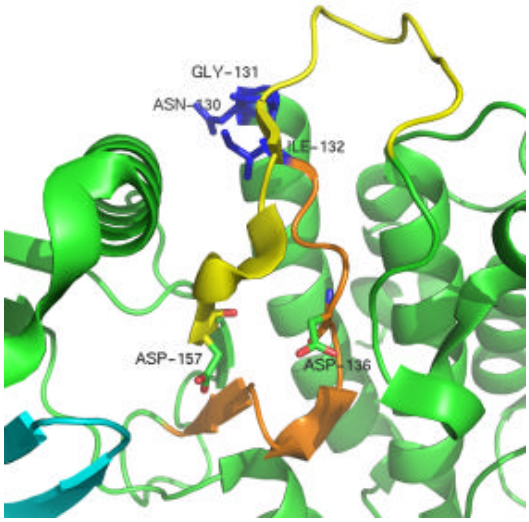

Fig. 6: The structure of peptide NGI and active center of CaMK2D

(*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 predicate protein sequence

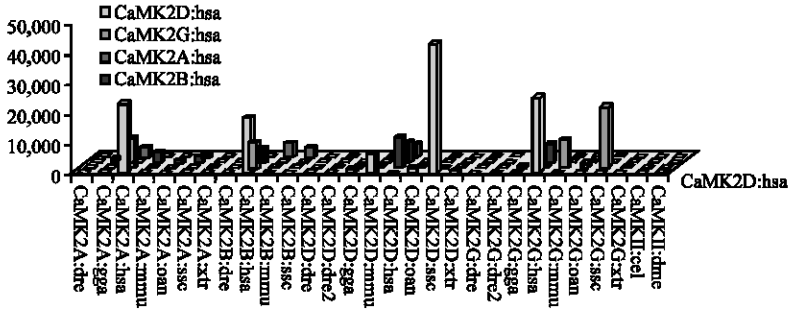

Fig. 7: Pairwise alignment of human four CaMKII gene and nine species

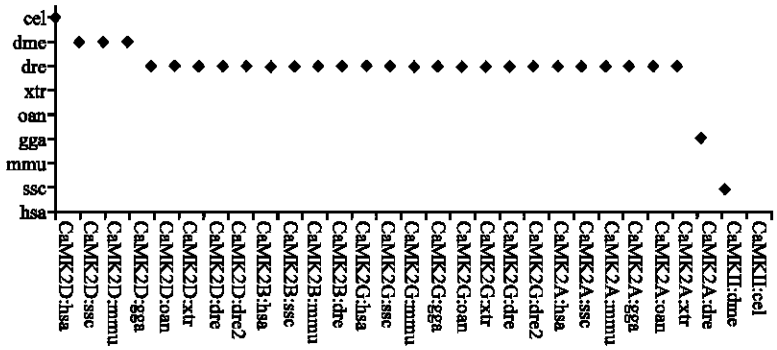

Fig. 8: Homology analysis of CaMKII in nine species

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 has the maximum phosphrylation 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 Ca2+/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

This research was financially supported by the National High Science and Technology Foundation of China 863 (2006AA10Z195 and 2008AA101008), the NSFC-Guangdong Key Project (U0631005), the Key Project of National Basic Research and Developmental Plan (2006CB102105) of China, the creative team project of education ministry.

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