Research Article

Computational Simulations to Predict Creatine Kinase-Associated Factors: Protein-Protein Interaction Studies of Brain and Muscle Types of Creatine Kinases

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Creatine kinase (CK; EC 2.7.3.2) is related to several skin diseases such as psoriasis and dermatomyositis. CK is important in skin energy homeostasis because it catalyzes the reversible transfer of a phosphoryl group from MgATP to creatine. In this study, we predicted CK binding proteins via the use of bioinformatic tools such as protein-protein interaction (PPI) mappings and suggest the putative hub proteins for CK interactions. We obtained 123 proteins for brain type CK and 85 proteins for muscle type CK in the interaction networks. Among them, several hub proteins such as NFKB1, FHL2, MYOC, and ASB9 were predicted. Determination of the binding factors of CK can further promote our understanding of the roles of CK in physiological conditions.

1. Introduction

Creatine kinase (CK) (ATP: creatine kinase N-phosphotransferase, EC 2.7.3.2) is thought to be crucial for intracellular transport and the storage of high energy phosphate because it catalyzes the reversible transfer of a phosphoryl group from MgATP to creatine, which leads to the creation of phosphocreatine and MgADP [1]. CK plays an important role in the cellular energy metabolism of vertebrates, and it is widely distributed in tissues that require a lot of energy [2]. Several types of CK are expressed in various tissues: the muscle and brain types of CK are the most common, and three different isoenzymes that include CK-MM (the muscle type homodimer), CK-BB (the brain type homodimer), and CK-MB (the muscle plus brain type heterodimer) originate from these two common types. CK is an important serum marker for myocardial infarction. Various types of CKs (the muscle, brain, and mitochondrial types) are thought to be important not only in the diagnosis of myocardial infarction, cardiac hypertrophy, and muscular dystrophy but also for studies of

some other serious diseases, including Alzheimer's disease, Parkinson's disease, and psoriasis [3–8].

CK-BB is associated with several pathologies, including neurodegenerative and age-related diseases. Recently, Chang et al. [9] reported an important role for CK-BB in osteoclast-mediated bone resorption, which was found using a proteomics approach. They found that CK-BB is greatly increased during osteoclastogenesis and suggested that it represents a potential target for antiresorptive drug development. CK-BB interacts with the potassium-chloride cotransporter 3, which is involved in the pathophysiology of hereditary motor and sensory neuropathy with agenesis of the corpus callosum [10]. Previous studies [11, 12] have reported that CK-BB is involved in Alzheimer's disease (AD) as an oxidatively modified protein. This suggests that oxidatively damaged CK-BB may be associated with aging and age-related neurodegenerative disorders such as AD.

CK-MM is a good model to use for studying folding pathways because of several characteristics: (i) it is a dimer

FIGURE 1: PPI map for CKB as a target hub protein with the 80% identity. Labels with red color indicate the hub protein of targeting. The image was made by the aiSee program (<http://www.aisee.com/>).

that consists of two identical subunits, each with an N-terminal domain with about 100 residues and a C-terminal domain with about 250 residues connected by a long linker [13]; (ii) extensively denatured CK can be renatured spontaneously with restoration of its enzymatic activity in the absence of any external assistance [14]; (iii) its folding pathway is complicated and involves several intermediates [15, 16]; (iv) conformational changes of the secondary and tertiary structures can be easily measured by monitoring activity changes [14, 15]; (v) protein-protein interactions, including molecular chaperones, are observed during refolding [17, 18].

In this study, we obtained computational predictions of the binding proteins by using two types of CK (CK-BB and CK-MM) as hub proteins in bioinformatic algorithms. As a result, we obtained 208 protein lists in the interaction networks via application of both muscle and brain types of CK. Determination of the binding factors and functions of CK can further promote our understanding of the physiological roles of CK.

2. Materials and Methods

2.1. PPI Mappings: PEIMAP and PSIMAP Algorithms. We present the functionally classified protein-protein interactions on the basis of the cell cycle, cell transport, oxidoreductase, and apoptosis. PPI resources were assembled from

a combination of several experimental protein interaction databases. The protein interaction resources included six databases: DIP [19], BIND [20], IntAct [21], MINT [22], HPRD [23], and BioGrid [24]. We performed a redundancy test to remove identical protein sequences from the interaction databases. The databases contain 116,773 proteins and 229,799 interactions.

PPI prediction uses most of the major types of PPI algorithms. They are (1) Protein Structural Interactome MAP (PSIMAP), a method that uses the structural domain of the SCOP (Structural Classification of Proteins) database [25] and (2) Protein Experimental Interactome MAP (PEIMAP), a common method that uses public resources of experimental protein interaction information such as HPRD, BIND, DIP, MINT, IntAct; and BioGrid. The basic procedure of PSIMAP is to infer interactions between proteins by using their homologs. Interactions among domains or proteins for known PDB (Protein Data Bank) structures are the basis for the prediction. If an unknown protein has a homolog to a domain, then PSIMAP assumes that the query has the probability to interact with its homolog's partners. This concept is called "homologous interaction." The original interaction between two proteins or domains is based on the Euclidean distance. Therefore, PSIMAP gives a structure-based interaction prediction [26]. PEIMAP was constructed by combining several experimental protein-protein interaction databases. We carried out a redundancy check to remove identical protein sequences from the source interaction

FIGURE 2: PPI map for CKB as a target hub protein with the 100% identity. The methodological conditions were the same as for Figure 1 except the identity.

FIGURE 3: PPI map for CKM as a target hub protein with the 80% identity. Labels with red color indicate the hub protein of targeting. The image was made by the aiSee program (<http://www.aisee.com/>).

FIGURE 4: PPI map for CKM as a target hub protein with the 100% identity. The methodological conditions were the same as for Figure 3 except the identity.

databases. The image was made by the Pajak2.00 program (<http://vlado.fmf.uni-lj.si/pub/networks/pajek/>).

3. Results and Discussion

We identified potential candidates through protein-protein interaction predictions made using various protein interaction resources. By analyzing the hub protein of the networks with metrics such as degree and centrality, we detected 123 potential candidates for CKB interacting (direct or indirect) factors and 85 candidates for CKM.

In Figure 1, interacting factors such as NFKB1 (NP_003989, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1), MYOC (NP_000252; myocilin, trabecular meshwork inducible glucocorticoid response), MYOM2 (NP_003961; myomesin (M-protein) 2, 165 kDa), FHL2 (NP_001034581, four-and-a-half LIM domains 2), HIF1AN (NP_060372, hypoxia-inducible factor 1, alpha subunit inhibitor), ASB9 (NP_076992, ankyrin repeat and SOCS box-containing 9), and CKM (NP_001815, creatine kinase, muscle) were elucidated. Interestingly, NFKB1 was detected as a hub protein interacting with CK-BB in our results. In Figure 2, we obtained results similar to those from Figure 1, where NFKB1, MYOC, MYOM2, FHL2, HIF1AN, ASB9, and CKM were detected as interacting factors that were directly or indirectly associated with CKB. NFKB1, CKM, and ASB9 interacted with CKB directly.

In the same way, we detected the CKM-associated proteins as shown in Figure 3 with 80% sequence identity. As a result, we found that CKB, FHL2, MYOC, ASB9, HIF1AN, NFKB1, TTN (NP_596870, titin), MYH9 (NP_002464, myosin, heavy chain 9, non-muscle), and ITGA7 (NP_002197, integrin, alpha 7) mainly interacted with CKM at 80% sequence identity. At the level of 100% identity, we found that MYOM2, CKB, FHL2, and MYOC directly interacted with CKM as shown in Figure 4. In addition to these factors, complete lists of factors that interacted with CKB and CKM in a direct or indirect manner are shown in Tables 1 and 2. After overlapping the results from Figures 1 to 4, we found that NFKB1, FHL2, and MYOC were still detected as hub proteins in Figure 5.

NF κ B1 (also known as p50 or NF-kappaB) is a well-known transcription regulator that is responsible for the expression and regulation of many genes for immune response, cell adhesion, differentiation, proliferation, angiogenesis, and apoptosis [27–31]. It translocates into the nucleus and stimulates the expression of many genes involved in various biological functions. NF κ B1 is also associated with a number of inflammatory diseases such as lymphoma [32], Alzheimer disease [33], psoriatic arthritis [34], breast cancer [35, 36], and rheumatoid arthritis [37]. Activation of NF κ B1 requires binding of NF-kappaB essential modulator (NEMO) to ubiquitinated substrates [38]. With respect to an association with CK, it has been reported that NF κ B1 is mostly associated with myocardial ischemia/reperfusion.

TABLE 1: Gene lists for the analyses of the PEIMAP and PSIMAP using CK-BB as a hub protein with 100% identity.

Gene ID	Gene symbol	Full name
6256	RXRA	Retinoid X receptor, alpha
3309	HSPA5	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)
3320	HSP90AA1	Heat shock protein 90 kDa alpha (cytosolic), class A member 1
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
6778	STAT6	Signal transducer and activator of transcription 6, interleukin-4 induced
3146	HMGB1	High-mobility group box 1
3301	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1
4221	MEN1	Multiple endocrine neoplasia I
3312	HSPA8	Heat shock 70 kDa protein 8
3840	KPNA4	Karyopherin alpha 4 (importin alpha 3)
2274	FHL2	Four-and-a-half LIM domains 2
4792	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
57805	KIAA1967	KIAA1967
3185	HNRNPF	Heterogeneous nuclear ribonucleoprotein F
203068	TUBB	Tubulin, beta
6774	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
4670	HNRNPM	Heterogeneous nuclear ribonucleoprotein M
1997	ELF1	E74-like factor 1 (ets domain transcription factor)
113457	TUBA3D	Tubulin, alpha 3D
1999	ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)
5591	PRKDC	Protein kinase, DNA-activated, catalytic polypeptide
708	C1QBP	Complement component 1, q subcomponent binding protein
2274	FHL2	Four-and-a-half LIM domains 2
3313	HSPA9	Heat shock 70 kDa protein 9 (mortalin)
8600	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11
3659	IRF1	Interferon regulatory factor 1
84617	TUBB6	Tubulin, beta 6
7280	TUBB2A	Tubulin, beta 2A
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
8517	IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
7295	TXN	Thioredoxin
10318	TNIP1	TNFAIP3 interacting protein 1
4793	NFKBIB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
3065	HDAC1	Histone deacetylase 1
3551	IKKBK	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
1152	CKB	Creatine kinase, brain
4069	LYZ	Lysozyme (renal amyloidosis)
140462	ASB9	Ankyrin repeat and SOCS box-containing 9
4653	MYOC	Myocilin, trabecular meshwork inducible glucocorticoid response
6774	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
3660	IRF2	Interferon regulatory factor 2
7278	TUBA3C	Tubulin, alpha 3c
4221	MEN1	Multiple endocrine neoplasia I
5966	REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)
1147	CHUK	Conserved helix-loop-helix ubiquitous kinase
55922	NKRF	NFKB repressing factor
2113	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
64332	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta

TABLE 1: Continued.

Gene ID	Gene symbol	Full name
51773	RSF1	Remodeling and spacing factor 1
5971	RELB	v-rel reticuloendotheliosis viral oncogene homolog B
1832	DSP	Desmoplakin
347733	TUBB2B	Tubulin, beta 2B
2353	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
9325	TRIP4	Thyroid hormone receptor interactor 4
4435	CITED1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1
22984	PDCD11	Programmed cell death 11
790	CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
1326	MAP3K8	Mitogen-activated protein kinase kinase kinase 8
1917	EEF1A2	Eukaryotic translation elongation factor 1 alpha 2
9172	MYOM2	Myomesin (M-protein) 2, 165 kDa
10856	RUVBL2	RuvB-like 2 (E. coli)
1158	CKM	Creatine kinase, muscle
808	CALM3	Calmodulin 3 (phosphorylase kinase, delta)
672	BRCA1	Breast cancer 1, early onset
801	CALM1	Calmodulin 1 (phosphorylase kinase, delta)
293	SLC25A6	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
3310	HSPA6	Heat shock 70 kDa protein 6 (HSP70B')
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
136319	MTPN	Myotrophin
2274	FHL2	Four-and-a-half LIM domains 2
9093	DNAJA3	DnaJ (Hsp40) homolog, subfamily A, member 3
4628	MYH10	Myosin, heavy chain 10, non-muscle
4221	MEN1	Multiple endocrine neoplasia I
6774	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
3839	KPNA3	Karyopherin alpha 3 (importin alpha 4)
57805	KIAA1967	KIAA1967
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
1869	E2F1	E2F transcription factor 1
55662	HIF1AN	Hypoxia-inducible factor 1, alpha subunit inhibitor
79155	TNIP2	TNFAIP3 interacting protein 2
9532	BAG2	BCL2-associated athanogene 2
6421	SFPQ	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
10627	MRCL3	Myosin regulatory light chain MRCL3
7431	VIM	Vimentin
672	BRCA1	Breast cancer 1, early onset
2274	FHL2	Four-and-a-half LIM domains 2
4221	MEN1	Multiple endocrine neoplasia I
672	BRCA1	Breast cancer 1, early onset
4221	MEN1	Multiple endocrine neoplasia I
3836	KPNA1	Karyopherin alpha 1 (importin alpha 5)
3093	UBE2K	Ubiquitin-conjugating enzyme E2K (UBC1 homolog, yeast)
805	CALM2	Calmodulin 2 (phosphorylase kinase, delta)
5970	RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
9782	MATR3	Matrin 3
8600	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11
8607	RUVBL1	RuvB-like 1 (E. coli)

TABLE 1: Continued.

Gene ID	Gene symbol	Full name
4627	MYH9	Myosin, heavy chain 9, nonmuscle
23421	ITGB3BP	Integrin beta 3 binding protein (beta3-endonexin)
140462	ASB9	Ankyrin repeat and SOCS box-containing 9
4841	NONO	Non-POU domain containing, octamer-binding
9276	COPB2	Coatomer protein complex, subunit beta 2 (beta prime)
4221	MEN1	Multiple endocrine neoplasia I
1213	CLTC	Clathrin, heavy chain (Hc)
292	SLC25A5	Solute carrier family 25 (Mitochondrial carrier; adenine nucleotide translocator), member 5
4066	LYL1	Lymphoblastic leukemia-derived sequence 1
64332	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
5531	PPP4C	Protein phosphatase 4 (formerly X), catalytic subunit
8091	HMG2A	High-mobility group AT-hook 2
6202	RPS8	Ribosomal protein S8
1051	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
222643	UNC5CL	Unc-5 homolog C (C. elegans)-like
4790	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
71	ACTG1	Actin, gamma 1
3312	HSPA8	Heat shock 70 kDa protein 8
9782	MATR3	Matrin 3
3320	HSP90AA1	Heat shock protein 90 kDa alpha (cytosolic), class A member 1
4637	MYL6	Myosin, light chain 6, alkali, smooth muscle and nonmuscle
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
4793	NFKBIB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
688	KLF5	Kruppel-like factor 5 (intestinal)
672	BRCA1	Breast cancer 1, early onset

During reperfusion, the absence of poly(ADP-ribose) polymerase-1 (PARP-1) leads to a reduction of myocardial apoptosis, which is associated with reduced NFKB1 activation [39, 40], and proteasome inhibition ablates activation of NFKB1 in myocardial reperfusion and reduces reperfusion injury [41]. Myocardial injury was assessed by measuring the serum levels of CK, and CK was reduced in serum along with reduction of NFKB1 activation.

FHL2 is a member of the human four-and-a-half-LIM-only protein family, which consists of the members FHL1, FHL2, FHL3, FHL4, and ACT. These proteins function in various cellular processes, including regulation of cell survival, transcription, and signal transduction [42]. FHL2 contains an LIM domain, one of the protein-protein interaction motifs, which allows specific proteins to combine with certain partners. The specificity of a protein-protein interaction can be obtained by an interaction code predicted by conserved amino acid sequences. The interaction of FHL2 with transcription factors and other proteins involved in cancer development was examined. Since transcription factors control all fundamental developmental and homeostatic processes, transcriptional cofactors such as FHL2 are likely to contribute to human carcinogenesis and are of clinical importance in various forms of cancer [43], including leukemia [44]. With respect to an association with CK, Chung et al. [45] reported that FHL2 (developmentally enhanced

phosphotransfer enzyme-anchoring protein) amalgamated the myofibrillar CK metabolic signaling circuit, providing an energetic continuum between mitochondria and the nascent contractile machinery in a murine embryonic stem cell cardiac differentiation model. They reported that CK-M clustered around developing myofibrils, sarcolemma, and the perinuclear compartment, whereas CK-B was tightly associated with myofibrillar alpha-actinin, forming wire-like structures extending from the nuclear compartment to the sarcolemma. FHL2 was also increased in myocardial ischemia-reperfusion injury, where IL-6 and IL-8 mRNA are upregulated in human cardiac myocytes [46].

Recently, ASB9 was found to interact with ubiquitous mitochondrial CK [47]. The ankyrin repeat domains of ASB9 can associate with the substrate binding site of CK in a SOCS box-independent manner. The overexpression of ASB9 induces ubiquitination of CK. ASB9 reduces CK activities and cell growth and negatively regulates cell growth. ASB9 is a member of the ankyrin repeat and is a suppressor of the cytokine signaling (SOCS) box protein family. It can interact with the SOCS box domain of the elongin B-C adapter complex and can further complex with the cullin and ring box proteins to form E3 ubiquitin ligase complexes [48]. These complexes may be involved in specific substrate-recognition for ubiquitination and degradation and mediate the substrate-recognition of the E3 ubiquitin ligases.

TABLE 2: Gene lists for the analyses of the PEIMAP and PSIMAP using CK-MM as a hub protein with 100% identity.

Gene ID	Gene symbol	Full name
1889	ECE1	Endothelin-converting enzyme 1
5981	RFC1	Replication factor C (activator 1) 1, 145 kDa
226	ALDOA	Aldolase A, fructose-bisphosphate
2335	FN1	Fibronectin 1
9372	ZFYVE9	Zinc finger, FYVE domain containing 9
60	ACTB	Actin, beta
3688	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
7273	TTN	Titin
2274	FHL2	Four-and-a-half LIM domains 2
4853	NOTCH2	Notch homolog 2 (Drosophila)
2512	FTL	Ferritin, light polypeptide
1192	CLIC1	Chloride intracellular channel 1
2274	FHL2	Four-and-a-half LIM domains 2
5313	PKLR	Pyruvate kinase, liver and RBC
302	ANXA2	Annexin A2
7704	ZBTB16	Zinc finger and BTB domain containing 16
2200	FBN1	Fibrillin 1
27332	ZNF638	Zinc finger protein 638
92086	GGTLC1	Gamma-glutamyltransferase light chain 1
713	C1QB	Complement component 1, q subcomponent, B chain
3029	HAGH	Hydroxyacylglutathione hydrolase
5664	PSEN2	Presenilin 2 (Alzheimer disease 4)
7086	TKT	Transketolase (Wernicke-Korsakoff syndrome)
4176	MCM7	Minichromosome maintenance complex component 7
1152	CKB	Creatine kinase, brain
1499	CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
140462	ASB9	Ankyrin repeat and SOCS box-containing 9
9457	FHL5	Four-and-a-half LIM domains 5
4653	MYOC	Myocilin, trabecular meshwork inducible glucocorticoid response
3029	HAGH	Hydroxyacylglutathione hydrolase
7704	ZBTB16	Zinc finger and BTB domain containing 16
3675	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
226	ALDOA	Aldolase A, fructose-bisphosphate
3689	ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
3688	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
302	ANXA2	Annexin A2
92086	GGTLC1	Gamma-glutamyltransferase light chain 1
3679	ITGA7	Integrin, alpha 7
2023	ENO1	Enolase 1, (alpha)
9172	MYOM2	Myomesin (M-protein) 2, 165 kDa
1158	CKM	Creatine kinase, muscle
4790	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
2	A2M	Alpha-2-macroglobulin
3688	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
56944	OLFML3	Olfactomedin-like 3
1281	COL3A1	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
2274	FHL2	Four-and-a-half LIM domains 2
3688	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)

TABLE 2: Continued.

Gene ID	Gene symbol	Full name
118427	OLFM3	Olfactomedin 3
22900	CARD8	Caspase recruitment domain family, member 8
3488	IGFBP5	Insulin-like growth factor binding protein 5
7132	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
226	ALDOA	Aldolase A, fructose-bisphosphate
3675	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
51455	REV1	REV1 homolog (<i>S. cerevisiae</i>)
6421	SFPQ	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)
302	ANXA2	Annexin A2
4633	MYL2	Myosin, light chain 2, regulatory, cardiac, slow
8880	FUBP1	Far upstream element (FUSE) binding protein 1
2274	FHL2	Four-and-a-half LIM domains 2
3688	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
27332	ZNF638	Zinc finger protein 638
3673	ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
203	AK1	Adenylate kinase 1
4627	MYH9	Myosin, heavy chain 9, non-muscle
5213	PFKM	Phosphofructokinase, muscle
140462	ASB9	Ankyrin repeat and SOCS box-containing 9
7076	TIMP1	TIMP metalloproteinase inhibitor 1
5176	SERPINF1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1
3694	ITGB6	Integrin, beta 6
59	ACTA2	Actin, alpha 2, smooth muscle, aorta
4176	MCM7	Minichromosome maintenance complex component 7
10487	CAP1	CAP, adenylate cyclase-associated protein 1 (yeast)
7046	TGFBR1	Transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53 kDa)
3543	IGLL1	Immunoglobulin lambda-like polypeptide 1
5313	PKLR	Pyruvate kinase, liver and RBC
51455	REV1	REV1 homolog (<i>S. cerevisiae</i>)
10296	MAEA	Macrophage erythroblast attacher
3911	LAMA5	Laminin, alpha 5
2597	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
975	CD81	CD81 molecule
92086	GGTLC1	Gamma-glutamyltransferase light chain 1
1915	EEF1A1	Eukaryotic translation elongation factor 1 alpha 1
5664	PSEN2	Presenilin 2 (Alzheimer disease 4)
1278	COL1A2	Collagen, type I, alpha 2

The interaction between CK and MYOC has not been elucidated. However, MYOC has a cytoskeletal function, and this implies that it may interact with CK somehow. MYOC is expressed in many ocular tissues including the trabecular meshwork [49], which is a specialized eye tissue that is essential in regulating intraocular pressure. MYOC mutations have been identified as the cause of hereditary juvenile-onset open-angle glaucoma [50].

Researchers could apply computational prediction by PPI mapping to help determine target proteins. Since the next step in the functional study of interesting proteins/genes is

a time- and cost-consuming process, the number of target proteins is limited; hence, for the right choice, computational prediction on the basis of database information could be critical at this step. Functional studies can be further conducted using a mouse model and a large number of clinical samples. Final confirmation and CK mechanisms could then be more clearly evaluated for developing drugs to effectively treat CK-related diseases.

The functions of most of the candidate proteins predicted in this study have not been well reported in skin diseases or in the pathogenesis of other diseases. We provide new

FIGURE 5: Overlapping map between CKB and CKM PPI maps. Data were input by using the results from Figures 1 to 4.

information regarding these candidate proteins' interaction with CK, as well as the involvement of several hub proteins such as NFKB1, FHL2, ASB9, and MYOC. Although we do not suggest a direct role of any candidate protein in skin diseases, we provide candidate proteins to be targeted in further studies of CK-associated diagnostic markers and/or treatment of corresponding skin conditions. Furthermore, we also provide some insights into understanding the responses of CK in skin.

Abbreviations

PPI: Protein-protein interaction
CK-MM: Muscle type homodimer
CK-BB: Brain type homodimer.

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