Research Article

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

Wei-Jiang Hu,1 Sheng-Mei Zhou,2 Joshua SungWoo Yang,3, 4 and Fan-Guo Meng 1

1 Zhejiang Provincial Key Laboratory of Applied Enzymology, Yangtze Delta Region Institute of Tsinghua University, Jiaxing 314006, China
2 College of Biology and Chemical Engineering, Jiaxing University, Jiaxing 314001, China
3 Korean Bioinformation Center (KOBIC), Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon 305-806, Republic of Korea
4 Department of Bioinformatics, University of Sciences & Technology, Daejeon 205-305, Republic of Korea

Correspondence should be addressed to Fan-Guo Meng, mengfanguo@tsinghua.org.cn

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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 phospho-creatine 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
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.

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.

NFKB1 (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. NFKB1 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 NFKB1 requires binding of NF-kappaB essential modulator (NEMO) to ubiquitinated substrates [38]. With respect to an association with CK, it has been reported that NFKB1 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    | HMGBI1      | High-mobility group box 1 |
| 3301    | DNAJ1       | DnaJ (Hsp40) homolog, subfamily A, member 1 |
| 4221    | MEN1        | Multiple endocrine neoplasia 1 |
| 3312    | HSPA8       | Heat shock 70 kDa protein 8 |
| 3840    | KPN4        | 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    | HNRNPFI     | 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     | C1QBQP      | 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    | IKBKB       | 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 1 |
| 5966    | REL         | v-rel reticuloendotheliosis viral oncogene homolog (avian) |
| 1147    | CHUK        | Conserved helix-loop-helix ubiquitous kinase |
| 55922   | NKRFP       | NFKB repressing factor |
| 2113    | ETS1        | v-ets erythroleukemia virus E26 oncogene homolog 1 (avian) |
| 64332   | NFkBIZ      | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta |
| Gene ID | Gene symbol | Full name                                                                 |
|--------|-------------|---------------------------------------------------------------------------|
| 51773  | RSF1        | Remodeling and spacing factor 1                                           |
| 5971   | RELB        | v-rel reticulendotheliosis viral oncogene homolog B                        |
| 1832   | DSP         | Desmoplakin                                                               |
| 347733 | TUB2B       | 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                           |
| 9172   | MYOM2       | Myomesin (M-protein) 2, 165 kDa                                           |
| 10856  | RUVBL2      | RuB-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      | Dnal (Hsp40) homolog, subfamily A, member 3                               |
| 4628   | MYH10       | Myosin, heavy chain 10, non-muscle                                        |
| 4221   | MEN1        | Multiple endocrine neoplasia 1                                            |
| 6774   | STAT3       | Signal transducer and activator of trancription 3 (acute-phase response factor) |
| 3839   | KPN3        | 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 1                                            |
| 672    | BRCA1       | Breast cancer 1, early onset                                              |
| 4221   | MEN1        | Multiple endocrine neoplasia 1                                            |
| 3836   | KPN1A       | 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 reticulendotheliosis viral oncogene homolog A (avian)               |
| 9782   | MATR3       | Matrin 3                                                                  |
| 8600   | TNFSF11     | Tumor necrosis factor (ligand) superfamily, member 11                     |
| 8607   | RUVBL1      | RuB-like 1 (E. coli)                                                      |
| 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       | Coatamer protein complex, subunit beta 2 (beta prime) |
| 4221    | MEN1        | Multiple endocrine neoplasia 1 |
| 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    | HMGA2       | High-mobility group AT-hook 2 |
| 6202    | RPS8        | Ribosomal protein S8 |
| 1051    | CEBPB       | CCAAT/enhancer binding protein (C/EBP), beta |
| 222643  | UNCSCL      | 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 |
| 3520    | 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.
| 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    | C1QC        | Complement component 1, q subcomponent, B chain |
| 3688    | ITGB1       | Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) |
| 3688    | 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 |
| 7704    | ZBTB16      | Zinc finger and BTB domain containing 16 |
| 2200    | FB1         | Fibrillin 1 |
| 27332   | ZNF638      | Zinc finger protein 638 |
| 92086   | GGTLC1      | Gamma-glutamyltransferase light chain 1 |
| 713     | C1QC        | Complement component 1, q subcomponent, B chain |
| 3029    | HAGH        | Hydroxycyglutathione 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        | Hydroxycyglutathione 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 (S. cerevisiae) |
| 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 metallopeptidase 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   | CAP         | CAP, adenylate cyclase-associated protein 1 (yeast) |
| 7046    | TGFR1       | Transforming growth factor, beta receptor 1 (activin A receptor type II-like kinase, 53 kDa) |
| 3543    | IGL1        | Immunoglobulin lambda-like polypeptide 1 |
| 5313    | PKLR        | Pyruvate kinase, liver and RBC |
| 51455   | REV1        | REV1 homolog (S. cerevisiae) |
| 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
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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