Full Length Article



A Genome-Wide Survey, Model Selection, Phylogenetic Analysis and Protein-Protein Interactional Network Profile of the Metazoan PRKAG Genes from 22 Vertebrate Genomes

Wuyi Liu^{1*}, Huifang Lv¹ and Alireza Seidavi^{2*}

¹Department of Biology Sciences, Biological and Food Engineering Faculty, Fuyang Normal University, China ²Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

*For correspondence: lwui@163.com; lwycau@163.com; alirezaseidavi@iaurasht.ac.ir

Abstract

AMP-activated protein kinase (AMPK) is a cellular energy sensor which regulates organizational energy and phosphorylates diverse enzymes, playing key roles in determining different metabolic phenotypes and the energy metabolism equilibrium. This genome-wide survey identified and analyzed 266 unique AMPK gamma subunit (PRKAG) gene sequences from the recently updated genomes of human and 21 animals. After the model selection of phylogenetic analysis parameters, all the 266 protein sequences of these identified human and animal PRKAG genes were used to compute ML (Maximum Likelihood) phylogenetic tree under the evolutionary WAG+G and the protein-protein interactional network analysis using the STRING database. Subsequently, the phylogenetic analysis verified and found all these PRKAG gene sub-families from the amphibian, fish, avian, and mammal genomes formed large monotonous phylogenetic clusters except for three frog PRKAG sequences. Particularly, all the primate PRKAG genes formed the super-class of their sole phylogenetic clusters. Furthermore, the mapped network profile analysis revealed that these PRKAG genes were functionally assembled and/or enriched in the energy metabolism, immune responsive and/or inflammatory and myosin or myosin heavy chain interacting proteins related signaling pathways. In addition, most of these proteins are interrelated and formed a tight protein interactional network of energy metabolism. In conclusion, the observed data and information of protein-protein interaction will contribute to understanding and analyzing the possible protein functions and interacted pathways of animal PRKAG genes and/or other AMPK subunit genes. The metazoan PRKAG gene sequences and identified dataset may be beneficial for related developmental and physiological research topics. These findings may also be potentially applied to explore and resolve the critical issues of disease-induced loss that many medical researchers are taking into research. © 2019 Friends Science Publishers

Keywords: Genome-Wide Survey; Phylogenetic Analysis; Evolutionary Algorithm; Protein-Protein Interactional network

Introduction

AMP-activated protein kinase (AMPK) is implicated as a cellular energy sensor which regulates organizational energy and phosphorylates diverse enzymes, including protein effectors and metabolic enzymes. In many studies and reports, AMPK plays key roles in determining different metabolic phenotypes and the energy metabolism equilibrium of biological organisms as well as other ATPbinding cassette proteins (Li et al., 2019) or ADG related uncoupling proteins (Jiang et al., 2019). Particularly, it appears as the primary energy inductor and regulator of cellular energy homeostasis acting in the long term and/or the short term in eukaryotes and to ameliorate the metabolic post-translational distress bv metabolic enzyme modifications (Hardie, 2007; Hardie et al., 2012; Ahmad et al., 2015; Garcia and Shaw, 2017; Neumann and Viollet, 2019; Rashtchizadeh et al., 2019). In fact, AMPK and its

subunits are also regarded the potential sensor molecules concerned in human and animal diseases and biomedicine targets for treating metabolic decline in aging and metabolism-dependent disorders (Burkewitz *et al.*, 2014; Ahmad *et al.*, 2015; Li *et al.*, 2015; Carling, 2017; Troncone *et al.*, 2017; Willows *et al.*, 2017; Neumann and Viollet, 2019; Rashtchizadeh *et al.*, 2019).

In structure, AMPK is made up of a catalytic alpha (α) subunit, a non-catalytic regulatory beta (β) subunit, and a connector gamma (γ) subunit. AMPK is actually a particularly economic three-dimensional complex with its effective α and γ core subunits and the additional β Cterminal domain, in which the β C-terminal domain is shuttled in the other two subunits. The α subunit has the main site threonine 172 (T172) of phospho-dephospho regulation, whereas the β subunit is associated with glycogen metabolism in a phosphorylation-dependent manner via its carbohydrate-binding module (Ahmad *et al.*, 2015;

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Garcia and Shaw, 2017; Neumann and Viollet, 2019; Rashtchizadeh *et al.*, 2019). On the other hand, there are the cystathionine- β -synthase (CBS) motifs and other internal domains indwelling in the AMPK γ subunit (PRKAG) to form those two binding sites required for the enzymatic activities of specific allosteric activators of AMP (Burkewitz *et al.*, 2014; Carling, 2017; Troncone *et al.*, 2017; Neumann and Viollet, 2019; Rashtchizadeh *et al.*, 2019).

At present, many studies in the human and animal genetics and development research suggested that these AMPK subunit genes were functionally important in the nutrient intake and physiological and pathological changes in the energy metabolism equilibrium of skeletal and cardiac muscle tissues of human and pig and chicken with both physiological and genetic evidences (Milan et al., 2000; Andersson, 2003; Zhao et al., 2006; Scheffler et al., 2011; Yin et al., 2012; Scheffler et al., 2016; Yang et al., 2016). Overall, PRKAG2 mutations lead to a decrease in the ability of AMP to bind AMPK, resulting in excessive storage of glycogen and causing disease (Gollob et al., 2001; Burwinkel et al., 2005; Carling, 2017; Rashtchizadeh et al., 2019). For example, in human genetics, previous studies have shown that the genetic mutated locus from Arg 302 to Gln 302 in the PRKAG2 gene usually rendered the human AMPK $\gamma 2$ subunit to be a structurally inactive or deficient kinase, resulting in the pre-excitation syndrome (Gollob et al., 2001), *i.e.*, Wolff-Parkinson-White syndrome. Furthermore, the fatal congenital glycogen hyperactivity was reported as mainly caused by the R531Q locus mutation of human PRKAG2 gene (Burwinkel et al., 2005). In the Hampshire pigs, Milan et al. (2000) found and reported a non-conservative single nucleotide polymorphism (i.e., a dominant RN-mutation) of R200Q locus of the swine PRKAG3 alleles (Milan et al., 2000; Andersson, 2003; Scheffler et al., 2011, 2016), which was identified as significantly associated with the excess glycogen content in pork. Later, many mutant substitutions like the V199I and AMPKv3R2000 loci of the swine PRKAG3 alleles and mutant substitutions of the bovine PRKAG1 alleles were also identified and evaluated in different pig populations in recent years (Scheffler et al., 2011, 2016). Since then, similar mutant allele cases of PRKAG genes reported in animal muscle tissues were also characterized in the allele loci of chicken PRKAG3 gene (Zhao et al., 2006; Yin et al., 2012; Yang et al., 2016) and dairy cows' PRKAG1 (Ahmad et al., 2015). Therefore, the genetic mutations of many PRKAG alleles have been the hotspot subject of domestic animal researchers in the genomics era.

Previously, an initial study has been conducted to mainly identify avian PRKAG genes using the genomewide BLAST searches (Liu, 2017). However, more and more sequences of PRKAG and related genes remain to be further explored and classified, whereas their functional interactional network and related signaling pathways remain to be characterized, especially these in domestic animal genomes. The present study conducted an updated genome-wide survey and the large-scale phylogenetic analysis to further analyze and characterize the human and animal PRKAG genes identified from 22 vertebrate genomes. Furthermore, the study also carried out the mapped interaction network profile analysis of PRKAG genes.

Materials and Methods

Data Retrieval and Sequence Alignment of Human and Animal PRKAG Genes from the Genomic Databases

The genome-wide survey was carried out using human PRKAG genes as input query sequence of BLAST searches. That is, with a few pair nucleotide and protein sequences of human PRKAG genes, we systematically performed previously used PSI-BLAST searches (Luo *et al.*, 2019) to retrieve all the putative PRKAG genes against the latest released genomes of 22 vertebrates in the NCBI genome resources (URL:

http://www.ncbi.nlm.nih.gov/genome/guide/build.shtml), i.e., Acinonyx jubatus, Bos taurus, B. mutus, Bubalus bubalis, Canis lupus, C. lupus familiaris, Capra hircus, Gorilla gorilla, Homo sapiens, Mus musculus, Ovis aries, Pongo abelii, Rattus norvegicus, Sus scrofa, Panthera tigris, Gallus gallus, Danio rerio, Meleagris gallopavo, Anas platyrhynchos, Taeniopygia guttata, Xenopus tropicalis, X. laevis. In total, we had retrieved more than 600 sequences of the nucleotide and protein sequences of the putative PRKAG genes (i.e., PRKAG1, PRKAG2, and PRKAG3) and their isoform proteins. All of the retrieved nucleotide and protein sequences of vertebrate PRKAG genes were subsequently used in the sequence alignments that were transformed into FASTA sequence files with the Windows software package of ClustalX version 2.0 (Larkin et al., 2007). Meanwhile, the incomplete sequences and highly divergent sequences were excluded from the further analysis, because the incomplete sequences and sequence indels and/or gaps those sequences of would result in uncertain alignments.

Model Selection of Phylogenetic Tree Parameters of Vertebrate PRKAG Genes

MEGA version 6.06 (Tamura *et al.*, 2013) was used to make model selection analysis of phylogenetic tree parameters for nucleotide and amino acid sequences. By default, MEGA presents for each model the estimated values of Bayesian information criterion (BIC), AICc (Akaike Information Criterion, corrected), Maximum Likelihood ratio (InL), the estimated values of shape parameter of the discrete Gamma distribution (+G) and other parameters (including invariant sites and tree branch lengths) as applicable (Nei and Kumar, 2000; Tamura *et al.*, 2013). Whenever applicable, the software package MEGA shows the estimates of gamma shape parameter and/or the estimated fraction of invariant sites (Nei and Kumar, 2000; Tamura *et al.*, 2013), whereas the non-uniformity of evolutionary rates among sites are computed under a discrete distribution of Gamma (+G) in the establishing of evolutionary phylogenetic trees (Tamura *et al.*, 2013). Depending on the model, the goodness-of-fit of each model is mainly measured by the Bayesian information criterion (BIC) and the corrected Akaike information criterion (AICc) in calculation (Tamura *et al.*, 2013). The simulated model parameters of phylogenetic analysis are briefly shown in Table 1 and the detailed phylogenetic model parameters provided in Supplementary Table 2.

Phylogenetic Analyses of Vertebrate PRKAG Genes

The evolutionary fit of major substitution models of phylogenetic tree parameters were compared and selected for nucleotide and amino acid sequences of vertebrate PRKAG genes (see the model selection part or simulated phylogenetic model parameters provided in Supplementary Table 2 for details). The finally identified putative protein sequences of vertebrate PRKAG genes were also used to compute the ML phylogenetic trees under the optimized evolutionary substitution model measured by the dual criterions of BIC and AICc (Nei and Kumar, 2000; Tamura *et al.*, 2013), respectively. Among the subsequent analyses, ML phylogenetic trees were computed and reconstructed with the subtree-pruningregrafting search heuristic method in all the established trees (Tamura *et al.*, 2013).

Protein Interactional Network Analysis using the STRING Database

The Search Tool for the Retrieval of Interacting Genes (STRING; URL: https://string-db.org/) is a pre-built database of abundant known experimental and predicted protein interactions (Szklarczyk et al., 2015, 2019). To investigate all the possible protein-protein interactions between the vertebrate PRKAG genes retrieved and identified from the updated genome-wide survey in 22 vertebrates, the STRING database was used for the creation of protein-protein interactional network (PIN) files as previously described (Szklarczyk et al., 2015, 2019). In practice, the STRING database version 11 online was used to evaluate and analyze the protein-protein interactions of vertebrate PRKAG1 and PRKAG2 and PRKAG3 protein sequences (Szklarczyk et al., 2019). The finally created and extracted PIN network files were explored and compared based on the PRKAG gene expression products (protein sequences) and their PIN network interactions and/or topological characteristics reported by more than 20 reports or studies of vertebrate PRKAG genes.

Results

Genome-wide Survey and Identification of Vertebrate PRKAG Genes

In total, more than 600 sequences were initially retrieved and screened from the NCBI genome resources (URL: http://www.ncbi.nlm.nih.gov/genome/guide/build.shtml). The nucleotide and protein sequences of vertebrate PRKAG genes were subsequently used and aligned by ClustalX 2.0 (Larkin et al., 2007). Those incomplete sequences and highly divergent sequences were excluded from the further analysis, since the incomplete sequences and sequence indels and/or gaps those sequences of would result in uncertain alignments. Furthermore, other removed records are predicted transcription factors or hypothetical proteins. After removing these incomplete sequences and uncertain and/or redundant sequence records, we then obtained 266 unique protein sequences and 266 unique nucleotide sequences of the putative PRKAG genes of human and animals for further analyses. It should be noted that all the 266 pairs of unique protein and nucleotide sequences retrieved from the genomes of 22 vertebrate species were carefully checked and identified and verified again. Finally, these 266 pairs of unique PRKAG protein and nucleotide sequences were further checked and examine by all the members of our group. These final used sequence dataset included a total of 266 unique protein sequences from the genomes of 22 different animal species (Supplementary Table 1), including 16 sequences from A. jubatus (Cheetah), 18 sequences from *B. taurus* (cattle, bovine), 5 sequences from B. mutus (wild yak), 18 sequences from B. bubalis (water buffalo), 15 sequences from C. lupus (gray wolf), 18 sequences from C. lupus familiaris (dog), 18 sequences from C. hircus (goat), 10 sequences from G. gorilla (western gorilla), 22 sequences from H. sapiens (human), 15 sequences from *M. musculus* (mouse), 13 sequences from O. aries (sheep), 8 sequences from P. abelii (Sumatran orangutan), 13 sequences from R. norvegicus (rat), 14 sequences from S. scrofa (pig), 4 sequences from P. tigris (tiger), 13 sequences from G. gallus (chicken), 13 sequences from D. rerio (zebrafish), 3 sequences from M. gallopavo (turkey), 11 sequences from A. platyrhynchos (duck), 3 sequences from T. guttata (zebra finch), 10 sequences from X. tropicalis (Western clawed frog) and 6 sequences from X. laevis (African clawed frog).

The corresponding information of these 266 pairs of unique protein and nucleotide sequences identified for further analyses of the putative PRKAG genes of human and animals are summarized in Supplementary Table 1.

Model Selection and Phylogenetic Analyses of Vertebrate PRKAG Genes

In the study, the analyzed ML phylogenetic trees were used to identify and elucidate the evolutionary and functional

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Table 1: Model selection of phy	vlogenetic analysis parameters o	f animal PPKAG protein sec	juences by the MEGA software	e package
			1	

WAGi Gi 180 988.83107 8414.4922 -4025.6445 nin 4.2327 WAGi Gi 179 986.64203 8450.2963 -4025.6445 0.0000 4.3237 WAGi Gi 180 987.63491 8452.2317 -4044.5637 0.0000 nin 4.2164 WAGi Gi 180 987.7250 844.6183 -4004.3892 0.0000 nin 3.9697 LGi-G 180 9881.7111 8457.9294 -4047.2447 nin 3.9697 LGi-G 180 981.2518 8473.2374 -4002.0166 0.0027 nin nin LG 177 991.12548 8487.2374 -4002.0166 0.0027 nin< nin LG 180 992.2346 898.4232 -4070.7055 0.0000 nin nin nin nin LGH 180 9942.5346 850.4232 -4077.656 nin	Model	Parameter	BIC	AICc	lnL	Invariant	Gamma
WAG-G-H 181 948.4397 8416.5290 -402.5445 0.000 4.3237 WAG 179 9866.4203 8440.9825 -400.3892 n/a 4.2164 WAG-I 180 9868.000 8443.9825 -400.3892 0.0000 n/a JTT-G-I 181 9877.3491 8446.0183 -400.3892 0.0000 3.0697 LG+G-I 181 981.711 847.9563 -4047.2447 0.0000 3.0697 JTT-I 179 9901.0844 844.9644 -4062.0166 0.0027 n/a LG 179 991.87117 8502.5287 -4007.0955 n/a 4.2164 LG+I 180 992.8407 8504.6222 -4007.0955 0.0000 n/a Dayhoff-G-I 181 992.8407 8504.333 -4088.871 n/a 3.8518 Dayhoff-G-I 180 992.8449 8503.5329 -4007.0950 0.0000 n/a RtEV-G-H 180 994.9559 850.0333 -4089.8348	WAG+G	180	9838.5107	8414.4932	-4025.6445	n/a	4.5237
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rtREV-IG 180 9922.5346 8498.5171 -4067.6565 n'a 4.2164 IGH 180 9923.6467 8504.622 -4070.7095 0.0000 4.2164 nREV-G-I 181 9932.4636 8500.5529 -4078.8948 0.0019 3.8518 Dayhoff-G-I 181 9954.9403 8520.9355 -4078.8948 0.0019 3.8907 rREV-I 179 9956.4594 8540.3357 -4089.5834 0.0000 n'a Dayhoff-GI 179 9961.5271 8564.031 -4102.1172 n'a n'a Dayhoff-H 180 9998.181 8565.1606 -4100.9782 0.0036 n'a Dayhoff-H 180 9990.8810 8563.6666 -4105.227 0.0000 n'a pREV-G-FF 199 10047.7906 8633.183 -4135.3071 0.0000 n'a pQREV-G-FF 199 10142.5227 8568.678 -4083.3250 0.0000 n'a LG-G-FF 199 10142.5227 8570.674	LG	179	9918.7117	8502.5877	-4070.7095	n/a	n/a
LG+1 180 9928.6407 8504.6222 44070.7095 0.0000 n'a Dayhoff-G 180 9944.9559 8500.5529 44078.8671 n'a 3.8518 Dayhoff-G-I 181 9954.9403 8523.0296 44078.8671 n'a 3.8907 MEV 179 9956.4594 8540.3253 44089.5334 n'a n'a mREV-I 180 9967.0431 8543.0257 40689.9107 0.0000 n'a ophoff-1 179 9981.781 8565.403 4100.9782 n'a 3.5901 ophoff-1 180 9999.1071 8567.1964 4100.9782 n'a n'a opREV-G 179 10047.7906 8631.6666 4135.2400 n'a n'a opREV-H 180 10057.8358 8633.8183 4103.270 0.0000 n'a 44785 WAG-G-F 199 10170.2020 8604.1368 4102.1291 n'a 4.39300 LF-G-F 199 10170.8223 8597.0274	rtREV+G	180	9922.5346	8498.5171	-4067.6565	n/a	4.2164
ntREV-G-I 181 9932.4636 8500.5529 4067.6565 0.0000 4.2164 Dayhoff-G-I 180 9949.4559 8520.9385 4078.8948 0.0019 3.8907 ntREV 179 9956.4594 8540.333 4089.5834 n/a n/a n/a Dayhoff-I 179 9956.4594 8540.333 4089.5834 n/a n/a n/a Dayhoff-I 179 9981.5271 8565.4031 4102.1172 n/a n/a S550.1000 n/a OREV-G-I 180 9980.1781 8565.1606 4100.9782 n/a 3.5501 Dayhoff-I 180 9990.810 8568.8636 4101.8297 0.0036 n/a OREV-G-I 181 9990.810 8568.8636 4101.8297 0.0036 n/a OREV-G-I 181 9990.810 8568.8636 4101.8297 0.0036 n/a OREV-G-I 180 100578.858 8633.8183 4135.2071 0.0000 n/a VAG-G-I-F 199 10142.5227 8568.5678 4083.3250 n/a 4/a85 VAG-G-I-F 199 10142.5227 8568.5678 4083.3250 n/a 4/a85 VAG-G-I-F 199 10142.5227 8570.6074 4068.3250 n/a 4/a85 VAG-G-I-F 199 10170.2020 8601.136 4102.1291 n/a n/a LG-G-F 199 10172.2870 8598.633.183 4135.3071 n/a 4/a785 VAG-G-I-F 199 10170.2202 8604.1368 4102.1291 n/a n/a A1743 TTF-G-FF 199 10172.2870 8598.6321 4098.3571 n/a 4.0503 VAG-G-I-F 199 10172.870 8598.6321 4098.3571 n/a 4.0503 VAG-G-I-F 199 10174.0040 8600.0491 4099.0656 n/a 4.0503 TTF-G-I-F 200 10180.313 8590.670 4097.5548 0.0000 A.0503 TREV-G-I-F 199 10174.0040 8600.191 4099.0657 0.0000 n/a CG-G-I-F 200 10180.913 8590.670 4097.5548 0.0000 A.0503 TREV-G-I-F 199 10173.0598.8621.4129.1718 0.0000 n/a CG-G-I-F 199 10120.0586 8603.4950 4099.7687 0.0000 A.0503 TREV-G-I-F 199 10220.5047 8656.498 4127.3159 n/a 3.8518 Dayhoff-I-F 199 10220.5047 8656.5498 4127.3159 n/a n/a CG-G-I-F 199 10220.5047 8656.5498 4127.3159 n/a n/a CG-G-I-F 199 10220.5048 8694.976 4149.7985 n/a n/a CG-G-I-F 199 10220.5047 8756.5418 4129.7182 0.0038 3.8907 TTF-G-I-F 199 10220.548 8794.756 4149.7986 n/a 3.5901 Dayhoff-I-F 199 10230.548 8704.4756 4149.7986 n/a 3.5901 Dayhoff-I+F 199 10234.5486 8704.4756 4149.7986 n/a 3.5901 Dayhoff-I+F 199 10234.5488 8704.4756 4149.7986 n/a 3.5901 Dayhoff-I+F 199 1034.5436 8704.587 4149.5786 n/a 3.5901 Dayhoff-I+F 199 1034.5436 8704.587 4149.5467 n/a 3.5901 Dayhoff-I+F 199 1034.5436 8704.587 4155.5444 n/a n/a N/a NREV24-G-I 181 10405.5487 8776.783 4198.41	LG+I	180	9928.6407	8504.6232	-4070.7095	0.0000	n/a
Dayboff-G 180 9944.9559 8520.326 4078.8671 na 3.8518 Dayhoff-G-H 181 9956.4594 8523.026 4078.8948 0.0019 3.8907 REV 179 9956.4594 8540.3253 4089.5834 n/a n'a nREV-1 180 9967.0431 8543.0227 4089.9107 0.0000 n'a ophoff-1 179 9981.5271 8565.4031 4110.2172 n'a 3.5901 ophoff-1 180 9998.1781 8565.4636 4101.8277 0.0036 n'a ophoff-1 180 9999.1071 8567.1964 4100.9782 0.0000 n'a opREV-1 179 10047.7906 8631.6666 4135.2490 n'a 4.4785 WAG-G-FF 199 10170.2220 8604.1368 4102.1291 n/a n'a LG-G-FF 199 10172.870 8596.631 -4099.5548 n/a 3.9300 TFT-G-FF 199 10174.0408 8600.0491 -4099.0656	rtREV+G+I	181	9932.4636	8500.5529	-4067.6565	0.0000	4.2164
Dayboff:-G-I. 181 9954.9403 852.0296 -4078.8948 0.0019 3.8907 rREV 179 9956.4594 8540.3353 -4089.9107 0.0000 n/a Dayhoff 179 9981.5271 8565.4031 -4102.1172 n/a n/a Dayhoff 179 9981.5271 8565.4031 -4102.1172 n/a n/a Dayhoff 179 9981.5271 8565.4066 -4100.9782 n/a 3.5901 Dayhoff-1 180 9990.810 8565.1666 -4100.9782 0.0000 n/a OREV-61 181 9990.1071 8567.1964 -4100.9782 0.0000 n/a OREV-1 179 10047.7906 863.16666 -4135.2490 n/a 4.4785 WAG+F 190 1012.24517 857.0674 -4083.3250 0.0000 -4.4785 WAG+F 199 10170.0202 8694.136 -4102.1291 n/a 4.1743 TPT-G+F 199 10174.0404 8600.491 -40	Dayhoff+G	180	9944.9559	8520.9385	-4078.8671	n/a	3.8518
nHE 179 9956.4594 840.3353 -4089.9167 0.0000 n'a nREV-I 180 9967.0431 8543.0257 -4089.9107 0.0000 n'a ophoff 179 9981.5271 8565.4051 -4102.1722 n'a 3.5901 ophoff-1 180 9998.1281 8565.6166 -4103.782 0.0036 n'a ophoff-1 180 9999.1071 8567.1964 -4100.9782 0.0000 3.5901 opREV-G-I 181 9999.1071 8567.1964 -4103.5240 n'a n'a WAG-G+F 199 10057.8358 863.8183 -4135.3071 0.0000 4.4785 WAG-G+F 199 10170.2020 8604.1368 -4102.1291 n'a 4.4785 WAG-G+F 199 10170.2020 8606.1761 -4102.1291 n/a 4.0503 WAG-G+I+F 199 1017.0404 8600.491 -4099.5548 0.0000 n'a IREV-G-I+F 199 10180.1310 8606.1761 <	Dayhoff+G+I	181	9954.9403	8523.0296	-4078.8948	0.0019	3.8907
nREV-1 180 9967.0431 8543.0257 -4089.0107 0.0000 n/a Dayhoff 179 9981.5271 8565.4031 -4102.1172 n/a n/a Dayhoff 180 9989.1781 8565.1606 -4100.9782 n/a 3.5901 Dayhoff-1 180 9990.8810 8565.863 -4101.8277 0.0036 n/a cpREV-41 181 9990.1071 8567.1964 -4100.9782 0.0000 n/a cpREV-41 180 10047.7906 8631.6666 -4135.2490 n/a 4.4785 WAG-67+F 199 10142.5227 8568.5678 -4083.3250 n/a 4.4785 WAG-67+F 199 10170.2020 8604.1368 -4102.1291 n/a 1/a ITF-67+F 199 10170.823 8597.0274 -4097.5548 n/a 4.0503 UAG-67+F 199 10170.9823 8600.0491 -4099.656 n/a 4.0503 UTF-67+F 199 10170.8235 8602.0870 <t< td=""><td>rtREV</td><td>179</td><td>9956.4594</td><td>8540.3353</td><td>-4089.5834</td><td>n/a</td><td>n/a</td></t<>	rtREV	179	9956.4594	8540.3353	-4089.5834	n/a	n/a
Dayhoff 179 9981.5271 8565.4031 -4102.1172 n/a	rtREV+I	180	9967.0431	8543.0257	-4089.9107	0.0000	n/a
cpREV+G 180 9989.1781 856.1606 -4100.9782 n/a 3.5901 Dayhoff+I 180 9990.8810 8566.8636 -4101.8297 0.0036 n/a opREV-1 181 9990.1071 8567.1964 -4100.9782 0.0000 n/a opREV-1 180 10057.8358 8633.813 -4135.33701 0.0000 n/a WAG-G+F 199 10142.5227 8568.5678 -4083.3250 n/a 4.4785 WAG+G-FF 198 10170.2020 8604.1368 -4102.1291 n/a n/a LG-G-FF 199 10172.5870 8598.6321 -4098.3571 n/a 4.0503 WAG+F 199 10174.0040 8600.0491 -4099.0565 n/a 4.0503 WAG+H+F 199 10180.9113 8599.0670 -4097.5548 0.0000 1.325 LG-G+F 199 10180.9135 8602.0892 -4099.0587 0.0000 4.132.5 LG+F 200 10183.9335 8602.0892	Davhoff	179	9981.5271	8565.4031	-4102.1172	n/a	n/a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	cpREV+G	180	9989.1781	8565.1606	-4100.9782	n/a	3.5901
cp, EV+G+I 181 9999.1071 8567.1964 -4100.9782 0.0000 3.5901 cp, REV 179 10047.7906 8631.6666 -4135.3071 0.0000 n'a n'a cp, REV+1 180 10057.8358 8633.8183 -4135.3071 0.0000 4.4785 WAG+G+F 199 10142.5227 8568.5678 -4003.3250 n/a 4.4785 WAG+F 198 10170.2020 8604.1368 -4102.1291 n'a n'a JTF-G+F 199 10170.2823 8597.0274 -4097.5548 n'a 3.9300 JTF-G+F 199 10170.2823 8597.0274 -4097.5548 n'a 4.0503 WAG+FF 199 10170.4040 8600.491 -4099.0556 n'a 4.0503 UF-G+F 199 10180.310 8606.1761 -4102.1291 0.0000 n'a LG-H+F 200 10183.9335 8602.0892 -4099.7687 0.0000 n'a Dayhoff-H+F 199 10220.1686 </td <td>Davhoff+I</td> <td>180</td> <td>9990.8810</td> <td>8566.8636</td> <td>-4101.8297</td> <td>0.0036</td> <td>n/a</td>	Davhoff+I	180	9990.8810	8566.8636	-4101.8297	0.0036	n/a
qPREV17910047.79068631.66664135.2490n/an/a q PREV+I18010057.83588633.81834135.30710.0000n/a q PREV+I18010057.83588633.81834135.30710.0000n/a q AG-FF1991012.52278568.56784083.3250n/a4.4785 w AG-FF19810170.0208604.13684102.1291n/an/a $LG+G+F$ 19910172.58708598.65214098.5571n/a4.0503 $TREV+G+F$ 19910172.58708598.65214098.3571n/a4.0503 $WAG-F+F$ 19910174.00408600.04914099.0656n/a4.0503 $REV+G+F$ 19910180.91138599.06704097.5480.00003.9300 $REV+G+F+$ 20010183.93358602.08924099.06580.00004.0503 $JTT+G+I+F$ 20010183.93358603.49504099.76870.00004.1325 $LG+F+$ 19910220.16868646.21364122.14790.0000n/a $LG+I+F$ 19910220.16868646.21364122.14790.0000n/a $Lg+I+F$ 19910220.54488704.47954149.7985n/a3.8907 $Dayhoff+G+F+19910240.03828658.19384127.11820.00383.8907Dayhoff+H+F19910240.03828658.19384127.11820.00383.8907Dayhoff+H+F19910240.03828758.19384149.7944<$	cpREV+G+I	181	9999.1071	8567.1964	-4100.9782	0.0000	3.5901
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	cpREV	179	10047.7906	8631.6666	-4135.2490	n/a	n/a
	cpREV+I	180	10057.8358	8633.8183	-4135.3071	0.0000	n/a
$\begin{array}{llllllllllllllllllllllllllllllllllll$	WAG+G+F	199	10142.5227	8568.5678	-4083.3250	n/a	4.4785
WAG+F 198 10170.2020 8604.1368 -4102.1291 n/a n/a LG-G+F 199 10170.9823 8597.0274 -4097.5548 n/a 3.9300 rtREV+G+F 199 10172.5870 8598.6321 -4098.3571 n/a 4.1743 rtREV+G+F 199 10174.0040 8600.0491 -4099.0656 n/a 4.0503 WAG+HF 199 10180.9113 8509.0670 -4097.5548 0.0000 n/a LG-G+HF 200 10183.3335 8602.0892 -4099.0658 0.0000 4.1325 LG+F 198 10209.4468 8643.3815 -4121.7515 n/a n/a LG+HF 199 10220.1686 8664.2136 -4122.1479 0.0000 n/a Dayhoff+G+F 199 10230.5047 8656.5498 -4127.3159 n/a n/a Dayhoff+G+F 198 10265.5408 8699.4756 -4149.7985 n/a n/a Dayhoff+HF 198 10270.0382 8658.1938	WAG+G+I+F	200	10152.4517	8570.6074	-4083.3250	0.0000	4.4785
	WAG+F	198	10170.2020	8604.1368	-4102,1291	n/a	n/a
JTT+G+F 199 10172.5870 8598.6321 -4098.3571 n/a 4.1743 rtREV+G-F 199 10174.0040 8600.0491 -4099.0556 n/a 4.0503 WAG+H+F 199 10180.9113 8599.0670 -4097.5548 0.0000 n/a LG+G+I+F 200 10183.9335 8602.0892 -4099.0588 0.0000 4.1325 LG+F 198 10209.4468 8643.3815 -4121.7515 n/a n/a LG+F+ 198 10209.4468 8643.3815 -4122.1479 0.0000 n/a Dayhoff+G+F 199 10220.1686 8646.2136 -4127.1182 0.0038 3.8518 Dayhoff+G+F 199 10240.0382 8658.1938 -4127.1182 0.0038 3.8907 Dayhoff+G+F 198 10265.5408 8699.4756 -4149.7085 n/a n/a Dayhoff+F 198 10270.5448 8704.4795 -4152.3005 n/a n/a Dayhoff+F 198 10274.4136 8700.	LG+G+F	199	10170.9823	8597.0274	-4097.5548	n/a	3.9300
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	JTT+G+F	199	10172.5870	8598.6321	-4098.3571	n/a	4.1743
$\begin{array}{llllllllllllllllllllllllllllllllllll$	rtREV+G+F	199	10174.0040	8600.0491	-4099.0656	n/a	4.0503
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	WAG+I+F	199	10180.1310	8606.1761	-4102.1291	0.0000	n/a
rtREV+G+I+F 200 10183.9335 8602.0892 -4099.0658 0.0000 4.0503 JTT+G+I+F 200 10185.3393 8603.4950 -4099.7687 0.0000 4.1325 LG+F 198 10209.4468 8643.3815 -4121.7515 n/a n/a Dayhoff+G+F 199 10230.5047 8656.5498 -4122.1479 0.0000 n/a Dayhoff+G+I+F 200 10240.0382 8658.1938 -4127.1182 0.0038 3.8907 Dayhoff+F 198 10265.5408 8699.4756 -4149.7985 n/a n/a Dayhoff+F 198 10270.5448 8704.4795 -4152.3005 n/a n/a Dayhoff+F 198 10281.9007 8715.8354 -4157.9784 n/a n/a CpREV+F 199 10284.2329 8710.2780 -4164.5436 0.0000 n/a cpREV+G+F 199 10304.9600 8731.0051 -4164.5436 0.0048 n/a cpREV+G+F+ 199 10304.9666 <t< td=""><td>LG+G+I+F</td><td>200</td><td>10180.9113</td><td>8599.0670</td><td>-4097.5548</td><td>0.0000</td><td>3.9300</td></t<>	LG+G+I+F	200	10180.9113	8599.0670	-4097.5548	0.0000	3.9300
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	rtREV+G+I+F	200	10183.9335	8602.0892	-4099.0658	0.0000	4.0503
LG+F 198 10209.4468 8643.3815 -4121.7515 n/a n/a LG+I+F 199 10220.1686 8646.2136 -4122.1479 0.0000 n/a Dayhoff+G+F 199 10230.5047 8656.5498 -4127.3159 n/a 3.8518 Dayhoff+G+I+F 200 10240.0382 8658.1938 -4127.1182 0.0038 3.8907 Dayhoff+F 198 10265.5408 8699.4756 -4149.7985 n/a n/a JTT+F 198 10270.5448 8704.4795 -4152.3005 n/a n/a Dayhoff+H+F 198 10281.9007 8715.8354 -4149.2704 0.0048 n/a rtREV+F 198 10284.2329 8710.2780 -4154.1800 0.0000 n/a cpREV+G+F 199 10344.9600 8731.0051 -4164.5436 0.0048 n/a rtREV-4+H+F 199 10335.5066 8761.5517 -4179.8169 n/a 3.5901 rtREV24+G+F 199 10335.5066 8761.	JTT+G+I+F	200	10185.3393	8603.4950	-4099.7687	0.0000	4.1325
LG+I+F19910220.16868646.2136 -4122.1479 0.0000n/aDayhoff+G+F19910230.50478656.5498 -4127.3159 n/a 3.8518 Dayhoff+G+I+F20010240.03828658.1938 -4127.3159 n/a n/a Dayhoff+F19810265.54088699.4756 -4149.7985 n/an/aJTT+F19810270.54488704.4795 -4152.3005 n/an/aDayhoff+I+F19910274.41368700.4587 -4149.2704 0.0048n/artREV+F19810281.90078715.8354 -4157.9784 n/an/artREV+FF19910284.23298710.2780 -4154.1800 0.0000n/acpREV+G+F19910304.96008731.0051 -4164.5436 0.0048n/acpREV+G+I+F2001036.74018724.8958 -4160.4692 0.0000 3.5901 mtREV24+G+I+F19910335.50668761.5517 -4179.8169 n/a 3.5901 mtREV24+G+I+F19910352.83368796.7883 -4198.4549 n/a n/a cpREV+F19810362.83368796.7883 -4160.4692 0.0000 3.5901 mtREV24+G18010395.61968971.6022 -4304.1990 n/a 3.5901 mtREV24+G18010395.61968971.6022 -4304.1990 n/a 3.5901 mtREV24+F19810362.8336880.0472 -4215.0844 n/an/amtREV24+F19910401.1	LG+F	198	10209.4468	8643.3815	-4121.7515	n/a	n/a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	LG+I+F	199	10220.1686	8646.2136	-4122,1479	0.0000	n/a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Davhoff+G+F	199	10230.5047	8656.5498	-4127.3159	n/a	3.8518
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Davhoff+G+I+F	200	10240.0382	8658.1938	-4127.1182	0.0038	3.8907
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Davhoff+F	198	10265,5408	8699.4756	-4149.7985	n/a	n/a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	JTŤ+F	198	10270.5448	8704.4795	-4152.3005	n/a	n/a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Dayhoff+I+F	199	10274.4136	8700.4587	-4149.2704	0.0048	n/a
rtREV+I+F19910284.23298710.2780-4154.18000.0000n/acpREV+G+F19910294.80428720.8492-4159.4657n/a3.5901JTT+I+F19910304.96008731.0051-4164.54360.0048n/acpREV+G+I+F20010306.74018724.8958-4160.46920.00003.5901mtREV24+G+F19910335.50668761.5517-4179.8169n/a3.5901mtREV24+G+I+F20010345.43568763.5912-4179.81690.00003.5901cpREV+F19810362.85368796.7883-4198.4549n/an/acpREV+F19810362.85368971.6022-4304.1990n/a3.5901mtREV24+G18010395.61968971.6022-4304.1990n/a3.5901mtREV24+F19810366.11258830.0472-4215.0844n/an/amtREV24+F19910401.19858827.2436-4212.66290.0000n/amtREV24+G+I18110405.54878973.6380-4304.19900.00003.5901mtREV24+G+I18010468.92699044.9094-4340.85260.0000n/amtREV24+I18010468.92699044.9094-4340.85260.0000n/amtREV24+I18010468.92699044.9094-4340.85260.0000n/amtREV24+I18010468.92699044.9094-4340.85260.0000n/amtREV24+I18010468.92699044.9094-	rtREV+F	198	10281,9007	8715.8354	-4157.9784	n/a	n/a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	rtREV+I+F	199	10284.2329	8710.2780	-4154.1800	0.0000	n/a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	cpREV+G+F	199	10294.8042	8720.8492	-4159.4657	n/a	3.5901
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	JTT+I+F	199	10304.9600	8731.0051	-4164.5436	0.0048	n/a
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	cpREV+G+I+F	200	10306.7401	8724.8958	-4160.4692	0.0000	3.5901
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	mtREV24+G+F	199	10335,5066	8761.5517	-4179.8169	n/a	3.5901
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	mtREV24+G+I+F	200	10345.4356	8763.5912	-4179.8169	0.0000	3.5901
cpREV+I+F 199 10376.2941 8802.3392 -4200.2106 0.0007 n/a mtREV24+G 180 10395.6196 8971.6022 -4304.1990 n/a 3.5901 mtREV24+F 198 10396.1125 8830.0472 -4215.0844 n/a n/a mtREV24+F 199 10401.1985 8827.2436 -4212.6629 0.0000 n/a mtREV24+G+I 181 10405.5487 8973.6380 -4304.1990 0.0000 3.5901 mtREV24+I 180 10468.9269 9044.9094 -4340.8526 0.0000 n/a mtREV24 179 10610.5703 9194.4462 -4416.6388 n/a n/a	cpREV+F	198	10362.8536	8796.7883	-4198.4549	n/a	n/a
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	cpREV+I+F	199	10376.2941	8802.3392	-4200.2106	0.0007	n/a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	mtREV24+G	180	10395.6196	8971.6022	-4304.1990	n/a	3.5901
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	mtREV24+F	198	10396.1125	8830.0472	-4215.0844	n/a	n/a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	mtREV24+I+F	199	10401.1985	8827.2436	-4212.6629	0.0000	n/a
mtREV24+I 180 10468.9269 9044.9094 -4340.8526 0.0000 n/a mtREV24 179 10610.5703 9194.4462 -4416.6388 n/a n/a	mtREV24+G+I	181	10405.5487	8973.6380	-4304.1990	0.0000	3.5901
mtREV24 179 10610 5703 9194 4462 -4416 6388 n/a n/a	mtREV24+I	180	10468.9269	9044.9094	-4340.8526	0.0000	n/a
	mtREV24	179	10610.5703	9194.4462	-4416.6388	n/a	n/a

Note: The model abbreviations or captions are interpreted as follows. GTR: General Time Reversible; JTT: Jones-Taylor-Thornton; rtREV: General Reverse Transcriptase; cpREV: General Reversible Chloroplast; mtREV24: General Reversible Mitochondrial; n/a: not available

divergence of these PRKAG genes in 22 different species. In the surveyed genomes of 22 vertebrate species, totally 266 pair unique putative PRKAG nucleotide and protein sequences were finally identified and used in the phylogenetic analysis (Supplementary Table 1). In the ML phylogenetic tree of the 266 unique putative protein sequences of vertebrate PRKAG genes, almost all the protein sequences were clustered into four large classes of amphibian, fish, avian, and mammal PRKAG genes accordingly (Fig. 1). However, a few exceptions do exist in the ML phylogenetic tree (Fig. 1). From all the three subfamilies of PRKAG sequences (*i.e.*, *PRKAG1*, *PRKAG2*, and *PRKAG3*), the PRKAG sequences identified from the amphibian, fish, avian, and mammal genomes formed large



Fig. 1: ML phylogenetic tree of the 266 protein sequences of vertebrate PRKAG genes

Note: It is the circular view of ML phylogenetic tree of human and animal PRKAG protein sequences. The phylogenetic tree was computed and reconstructed with these 266 putative protein sequences of PRKAG genes identified in the genomes of 22 vertebrate species

monotonous phylogenetic clusters except for three frog PRKAG sequences (NP_001096506.1, NP_001085968.1, and XP_012811772.1) that were close to those of fish. Furthermore, all the primate PRKAG sequences formed a sole super-class of their own phylogenetic clusters in blue circles (Fig. 1). In brief, the ML phylogenetic analysis of putative vertebrate PRKAG protein sequences revealed that these PRKAG gene subfamilies (*PRKAG1, PRKAG2* and *PRKAG3*) had their own independent common ancestor genes (Fig. 1).

Protein Interactional Network Analysis of Human and Animal PRKAG Protein Sequences

The protein-protein interaction of human and animal PRKAG protein sequences were analyzed and displayed using the STRING database (URL: http://string-db.org/) of experimental and predicted protein interactions. The STRING database is able to detect and find the molecular enrichment and functional correlation in a close protein interaction and experimentally validated contacted network among the genes implied the importance of any experimental subject pathways (Fig. 2). The picture was the evidence network view of key hub proteins (e.g., PRKAG1, PRKAG2 and PRKAG3) and their relevant partners (Figure 2). These interacted proteins mainly included GDE1 (Glycerophosphodiester phosphodiesterase 1), TCF3 (Transcription factor E2alpha, a basic helix-loop-helix transcription factor



Fig. 2: The protein–protein interactional network (PIN) of human and animal PRKAG protein sequences by the STRING database Note: It is the evidence view of protein–protein interactional network (PIN) built by the online bioinformatics tools of STRING database to probe the functional protein interactions and hub proteins of vertebrate PRKAG genes. Red line indicates the presence of fusion evidence, whereas green line represents neighborhood evidence. Blue line represents co-occurrence evidence, whereas purple line represents experimental evidence. Yellow line represents text mining evidence, whereas light blue line represents database evidence and a black line represents co-expression evidence. The average confidence scores for all of the protein– protein interactions is above 0.15 and those different line colors represent different types of protein functional associations (see the illustration shown the online website of the STRING database)

involved in the initiation of neuronal differentiation), TJP1 (Tight junction protein ZO-1 belonging to the MAGUK family whose N and C terminals may be involved in the cellular tight junction assembly), GYPA (Glycophorin-A, a receptor for influenza virus and a major intrinsic membrane protein critical for the function of SLC4A1), APRT (Adenine phosphoribosyltransferase catalyzing salvage reactions resulting in the formation of AMP), ATP8A2 (Phospholipid-transporting ATPase IB, a catalytic component of a P4-ATPase flippase complex), MLLT4 (Afadin, belonging to an adhesion system playing a role in the organization of homotypic, interneuronal and heterotypic cell-cell adherens junctions), ENSG00000160200 (Cystathionine-beta-synthase), MYH6 (Myosin-6, belonging to the Myosin family), MYH14 (Myosin-14, belonging to the Myosin family), ACACA (Acetyl-CoA carboxylase 1), PRKAA1 (AMP-activated protein kinase catalytic subunit alpha-1, a catalytic subunit of AMPK), PRKAA2 (AMP-activated protein kinase catalytic subunit alpha-2, a catalytic subunit of AMPK), PRKAG1 (AMP-activated protein kinase subunit gamma-1, the AMP/ATP-binding subunit of AMPK), PRKAG2 (AMP-activated protein kinase subunit gamma-2, the AMP/ATP-binding subunit of AMPK), and PRKAG3 (AMP-activated protein kinase subunit gamma-3, the AMP/ATP-binding subunit of AMPK). Besides GYPA (Glycophorin A), most of these protein partners (encoded by corresponding genes) are interrelated and formed a tight protein interactional network of energy metabolism (Fig. 2). In particular, the main hub proteins of the protein-protein interactional network are PRKAG1, PRKAG2, PRKAG3, PRKAA1, PRKAA2, APRT, ATP8A2, ENSG00000160200, MYH6, MYH14 and ACACA, interacting with no less than 3 protein partners or enzymes. Interestingly, they also linked to many immune responsive and/or inflammatory factors and myosin proteins (Fig. 2), such as MYH6 (myosin 6) and MYH14 (myosin 14).

Discussion

In this study, we identified and characterized 266 unique PRKAG genes from the updated genomes of human and 21 animals. In the present study, accurate ML phylogenetic analysis was used to identify putative homologous and/or orthologous relationships of these PRKAG genes in 22 different species with the presupposed corresponding model selection of phylogenetic trees. The phylogenetic clusters of PRKAG gene family analyzed here were in agreement much with the physiological and genetics classification of previous studies and reports with a more diversity observed among different vertebrate species. As mentioned in the corresponding part of materials and methods, these 266 finally identified putative protein sequences of human and animal PRKAG gene were also used to compute the ML phylogenetic trees under the optimized evolutionary WAG+G model measured by the dual criterions of BIC and AICc as shown in Table 1 and Supplementary Table 2 (Nei and Kumar, 2000; Tamura et al., 2013), respectively.

In practice, the protein–protein interaction of human and animal PRKAG protein sequences were analyzed using the STRING database of experimental and predicted protein interactions. The mapped integrative profile of STRING protein network was built to probe into the functional protein interactions of vertebrate PRKAG genes (*e.g.*, PRKAG1, PRKAG2 and PRKAG3) and their relevant partners. Interestingly, these interacted proteins were linked to many immune responsive and/or inflammatory factors and myosin proteins (Fig. 2), such as MYH6 (myosin 6) and MYH14 (myosin 14), whereas the previous reports indicated that PRKAG results in phosphorylation of the myosin heavy chains (Williams and Coluccio, 1995; Rosenberg and Ravid, 2006; Yan et al., 2018) and there was a cross talk between circadian rhythm and coronary heart disease identified by multiple correlation analysis (Yan et al., 2018). This phenomenon is in accord with previous studies that both the muscle and non-muscle myosin proteins are involved in the phosphorylation of ATP (Adenosine Triphosphate) and responses in energy metabolism and inflammatory (Williams and Coluccio, 1995; Musi et al., 2003; Rosenberg and Ravid, 2006; Yan et al. 2018; Vilchinskaya et al., 2018), which is evident to be associated with the function and activities of PRKAG genes. A recent review regarded the AMP-activated protein kinase genes (especially the PRKAG genes) as the key triggers for the disuse-induced skeletal muscle remodeling on some molecular targets (Vilchinskaya et al., 2018). In addition, there was a significant association of AMPK subunit gamma subunit (PRKAG) gene polymorphisms with growth, feed intake, and feed efficiency in meat-type chickens (Jin et al., 2016).

Meanwhile, the online resources of STRING database provide both these experimental data as well as the bioinformatics data and those predicted interaction information with confidence scores. The PIN profile of PRKAG kinases and their protein partners suggest that these proteins associated with short distances to each other in the functionally interacted network are more likely to share the common biological functions (Szklarczyk et al., 2015; Yan et al., 2018; Szklarczyk et al., 2019), whereas those interactive neighbors are more likely to have identical biological function than non-interactive ones (Szklarczyk et al., 2015; Yan et al., 2018; Szklarczyk et al., 2019). It is inferred that the query kinases and their interactive protein partners may form a complex to perform particular functions or involved in the same functional pathways. In brief, these STRING experimental and predicted protein interaction data and corresponding resulted protein interactional network will contribute to understanding and analyzing the possible protein functions and interacted pathways of the PRKAG genes and/or other AMPK subunit genes.

Conclusion

In the present study, phylogenetic analyses indicated that all the identified gene sequences belong to three sub-families of PRKAG genes forming four large super-classes of monotonous phylogenetic clusters (*i.e.*, the amphibian, fish, avian, and mammal monotonous clusters). In particular, those PRKAG gene sequences identified from the amphibian, fish, avian and mammal genomes formed large monotonous phylogenetic clusters except for three frog PRKAG sequences that were close to those of fish. Furthermore, the mapped network profile of PIN analysis revealed that the PRKAG genes were functionally assembled and/or enriched in energy metabolism, immune responsive and/or inflammatory, and myosin interacting proteins related signaling pathways. These observed data and information of protein-protein interaction will contribute to understanding and analyzing the possible protein functions and interacted pathways of vertebrate PRKAG genes and/or other AMPK subunit genes. The resulted findings will also be potentially applied to explore and resolve the critical issues of disease-induced loss that many medical researchers are taking into research.

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