In silico study of protein to protein interaction analysis of AMP-activated protein kinase and mitochondrial activity in three different farm animal species

S Prastowo and N Widyas
Animal Science Department, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

E-mail: prastowo@staff.uns.ac.id

Abstract. AMP-activated protein kinase (AMPK) is cellular energy censor which works based on ATP and AMP concentration. This protein interacts with mitochondria in determine its activity to generate energy for cell metabolism purposes. For that, this paper aims to compare the protein to protein interaction of AMPK and mitochondrial activity genes in the metabolism of known animal farm (domesticated) that are cattle (*Bos taurus*), pig (*Sus scrofa*) and chicken (*Gallus gallus*). In silico study was done using STRING V.10 as prominent protein interaction database, followed with biological function comparison in KEGG PATHWAY database. Set of genes (12 in total) were used as input analysis that are PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3, PPARGC1, ACC, CPT1B, NRF2 and SOD. The first 7 genes belong to gene in AMPK family, while the last 5 belong to mitochondrial activity genes. The protein interaction result shows 11, 8 and 5 metabolism pathways in *Bos taurus*, *Sus scrofa* and *Gallus gallus*, respectively. The top pathway in *Bos taurus* is AMPK signaling pathway (10 genes), *Sus scrofa* is Adipocytokine signaling pathway (8 genes) and *Gallus gallus* is FoxO signaling pathway (5 genes). Moreover, the common pathways found in those 3 species are Adipocytokine signaling pathway, Insulin signaling pathway and FoxO signaling pathway. Genes clustered in Adipocytokine and Insulin signaling pathway are PRKAA2, PPARGC1A, PRKAB1 and PRKAG2. While, in FoxO signaling pathway are PRKAA2, PRKAB1, PRKAG2. According to that, we found PRKAA2, PRKAB1 and PRKAG2 are the common genes. Based on the bioinformatics analysis, we can demonstrate that protein to protein interaction shows distinct different of metabolism in different species. However, further validation is needed to give a clear explanation.

1. Introduction

AMPK is a protein working as energy censor by sensing the balance of cellular ADP and AMP concentration [1,2]. It's known as protein kinase AMP-activated, abbreviated as PRAKA in the NCBI database as the official gene name, a heterotrimetric complex consist of α, β and γ subunit. According to the previous study, AMPK has a close relation with mitochondrial activity in serving cell with energy[3], any defect of its function resulted in metabolic disorder [4,5]. In addition, a wide range of AMPK expression have been found in many living organism such as cattle [6], pig [7], chicken [8] and mice [9].

As widely known, specific gene encode specific protein. In the multicellular organism activity of protein interact each other [10] to support its metabolic function, thus in specific organism/species

different protein express different metabolic function. A protein to protein interaction will show the direct link to gene expression and metabolism pattern [11]. Knowing its interaction could be a way to identify and understand the key player in the molecular level [12], such as identify gene or protein function which associated with specific defect of metabolism [13].

Considering the AMPK and mitochondria activity as cellular energy regulator and to know the specific function of interact proteins, therefore this study aim to reveal the AMPK protein to protein interaction using existing protein database in three different animal species. The result will give us a picture of different metabolism occurred in different species.

2. Methods

Three known farm animal species were selected that are cattle (*Bos taurus*), pig (*Sus scrofa*) and chicken (*Gallus gallus*). To compare metabolism in selected species, as the analysis input for this in silico study, set of genes related to AMPK and mitochondrial activity were taken from the previous studies[14,15]. Those genes are in AMPK family (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2 and PRKAG3) and mitochondrial activity (PPARGC1, ACC, CPT1B, NRF2 and SOD), genes abbreviation and accession number based on official name in NCBI database (www.ncbi.nlm.nih.gov). The protein to protein interaction was retrieved from functional protein association network using STRING V.10 (https://string-db.org/) according to the previous study [16], followed with protein biological function classification using KEEG PATHWAY database (http://www.kegg.jp/) to retrieve its metabolism pathway. Common genes in the same metabolism pathway were clustered with the help of Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/).

3. Result and Discussions

Retrieved metabolic pathway form protein to protein interaction in *Bos taurus*, *Sus scrofa* and *Gallus gallus* were listed in Table 1, 2 and 3 respectively. As listed, AMPK signaling pathway, Adipocytokine signaling pathway and Insulin signaling are in the top listed pathways in those three species. In each pathway there are different number of observed genes that match with clustered protein network. For example, there are 10 genes in protein network which belongs to AMPK signaling pathway in *Bos taurus*. This result shows that protein interact each other working together to support specific metabolism pathway [11].

Table 1. Observed metabolism pathways in *Bos taurus*

No	Pathway Description	Observed Gene Count	Matching proteins in your network
1	AMPK signaling pathway	10	ACACA,CPT1B,PPARGC1A,PRKAA1,PRKAA2,PRKAB1,PRKAB2, PRKAG1,PRKAG2,PRKAG3
2	Adipocytokine signaling pathway	9	CPT1B,PPARGC1A,PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1 ,PRKAG2,PRKAG3
3	Circadian rhythm	7	PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
4	Insulin signaling pathway	9	ACACA,PPARGC1A,PRKAA1,PRKAA2,PRKAB1,PRKAB2, PRKAG1,PRKAG2,PRKAG3
5	Hypertrophic cardiomyopathy (HCM)	7	PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2, PRKAG3
6	FoxO signaling pathway	7	PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
7	Oxytocin signaling pathway	7	PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
8	Non-alcoholic fatty liver disease (NAFLD)	7	PRKAA1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
9	Regulation of autophagy	2	PRKAA1,PRKAA2
10	Fatty acid metabolism	2	ACACA,CPT1B
11	mTOR signaling pathway	2	PRKAA1,PRKAA2

Table 2. Observed metabolism pathways in *Sus scrofa*

No	Pathway Description	Observed Gene Count	Matching proteins in your network
1	Adipocytokine signaling pathway	8	CPT1B,PPARGC1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
2	AMPK signaling pathway	8	CPT1B,PPARGC1,PRKAA2,PRKAB1,PRKAB2,PRKAG1, PRKAG2,PRKAG3
3	Circadian rhythm	6	PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
4	Insulin signaling pathway	7	PPARGC1,PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG,PRKAG3
5	Hypertrophic cardiomyopathy (HCM)	6	PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
6	FoxO signaling pathway	6	PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
7	Oxytocin signaling pathway	6	PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3
8	Non-alcoholic fatty liver disease (NAFLD)	6	PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3

Table 3. Observed metabolism pathways in *Gallus gallus*

No	Pathway Description	Observed Gene Count	Matching proteins in your network
1	Insulin signaling pathway	7	ENSGALG00000005439,PPARGC1A,PRKAA1,PRKAA2,PRKAB1,PRKAG2,TTLL4
2	Adipocytokine signaling pathway	6	PPARGC1A,PRKAA1,PRKAA2,PRKAB1,PRKAG2,TTLL4
3	FoxO signaling pathway	5	PRKAA1,PRKAA2,PRKAB1,PRKAG2,TTLL4
4	Regulation of autophagy	2	PRKAA1,PRKAA2
5	mTOR signaling pathway	2	PRKAA1,PRKAA2

According to the listed pathway in 3 species in this study, we clustered those pathways using Venn diagram and found 3 common pathways that are Adipocytokine signaling pathway, Insulin signaling pathway, FoxO signaling pathway (Table 4). In the classification of genes responsible in each of those 3 metabolism pathways, we found different set of distinct genes in specific species (Table 5). As we can see here, group of genes interact together in every specific pathway and species which support the previous report [11].

Table 4. Common pathways based on species group

Species	Observed Pathways	Pathway name
Bos taurus, *Sus scrofa* *Gallus gallus*	3	Adipocytokine signaling pathway, Insulin signaling pathway, FoxO signaling pathway
Bos taurus, *Sus scrofa*	5	Hypertrophic cardiomyopathy (HCM), Non-alcoholic fatty liver disease (NAFLD), AMPK signaling pathway, Oxytocin signaling pathway, Circadian rhythm
Bos taurus, *Gallus gallus*	2	mTOR signaling pathway, Regulation of autophagy
Bos Taurus	1	Fatty acid metabolism

Table 5. Distinct genes in the metabolism pathway in each species

Adipocytokine signaling pathway			Insulin signaling pathway			FoxO signaling pathway		
Bt	Ss	Gg	Bt	Ss	Gg	Bt	Ss	Gg
CPT1B	CPT1B	PPARGC1A	ACACA	PPARGC1A	ENSGALG00000005439	PRKAA1	PRKAA2	PRKAA1
PPARGC1A	PPARGC1A	PRKAA1	PPARGC1A	PRKAA2	PPARGC1A	PRKAA2	PRKAB1	PRKAA2
PRKAA1	PRKAA2	PRKAA2	PRKAA1	PRKAB1	PRKAA1	PRKAB1	PRKAB2	PRKAB1
PRKAA2	PRKAB1	PRKAB1	PRKAA2	PRKAB2	PRKAA2	PRKAB2	PRKAG1	PRKAG2
PRKAB1	PRKAB2	PRKAG2	PRKAB1	PRKAG1	PRKAB1	PRKAG1	PRKAG2	TTLL4
PRKAB2	PRKAG1	TTLL4	PRKAB2	PRKAG2	PRKAG2	PRKAG2	PRKAG3	
PRKAG1	PRKAG2		PRKAG1	PRKAG3	TTLL4	PRKAG3		
PRKAG2	PRKAG3		PRKAG2					
PRKAG3			PRKAG3					

Bt: *Bos taurus*, Ss: *Sus scrofa*, Gg: *Gallus gallus*

In the 3 species of this study, we found common genes that interact within Adipocytokine signaling pathway, Insulin signaling pathway and FoxO signaling pathway (Table 6), that are PRKAA2, PRKAB1 and PRKAG2. Whether these genes worked as the key player in the interact proteins was the question to be addressed and validated. As short explanation, those 3 pathways belong to cell signaling which determine intracellular signaling molecules for energy metabolism. Adipocytokine signaling is an important regulator of energy intake and metabolic rate through adipocyte cell and positively correlated with leptin production. This pathway related with glucose utilization and fatty acid oxidation via AMPK activation [17,18]. Insulin signaling pathway work in determine the insulin binding to its receptor results in the tyrosine phosphorylation of insulin receptor substrates by the insulin receptor tyrosine kinase for controlling glucose uptake into the cell [19], as we know glucose used as energy source. The last one is FoxO signaling pathway which regulates the expression of genes in cellular physiological events and act as the integrator of homeostasis maintenance signal [20].

Table 6. Common genes in the same pathway

Pathway	Total gene	Gene name
Adipocytokine signaling pathway	4	PRKAA2, PPARGC1A, PRKAB1, PRKAG2
Insulin signaling pathway	4	PRKAA2, PPARGC1A, PRKAB1, PRKAG2
FoxO signaling pathway	3	PRKAA2, PRKAB1, PRKAG2

The *in silico* work in this study shows the possibility of using common genes in the different species as metabolic marker, for example, according to the gene similarity name. However, the different in specific gene sequence in each species need to be considered. For that, carefully wet lab need to be employed to validate the results to give a distinct function in each species.

4. Conclusion

This study shows that AMPK plays in different metabolism pathway of *Bos taurus*, *Sus scrofa* and *Gallus gallus*. As the cellular metabolic regulator, AMPK may act in different pathway by expressing different protein to protein interaction in specific species, thus its expression need to be characterized individually.

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