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 sensor 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, PPARC1, 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, PPARC1A, 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 sensor 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 heterotrimeric 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,PRKAG1,PRKAG2,PRKAG3 |
| 5  | Hypertrophic cardiomyopathy (HCM) | 6 | PRKAA2,PRKAB1,PRKAB2,PRKAG1,PRKAG2,PRKAG3 |
| 6  | FoxO signaling pathway | 6 | PRKAA2,PRKAB1,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,PRKAA2,PRKAB1,PRKAG1,PRKAG2,TTLL4 |
| 2  | Adipocytokine signaling pathway | 6 | PPARGC1A,PRKAA2,PRKAB1,PRKAG2,TTLL4 |
| 3  | FoxO signaling pathway | 5 | PRKAA1,PRKAA2,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*       | 3                 | Adipocytokine signaling pathway, Insulin signaling pathway, FoxO signaling pathway |
| *Gallus gallus*                | 3                 | Hypertrophic cardiomyopathy (HCM), Non-alcoholic fatty liver disease (NAFLD), AMPK signaling pathway, Oxytocin signaling pathway, Circadian rhythm |
| *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 |

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

| Adipocytokine signaling pathway | Insulin signaling pathway | FoxO signaling pathway |
|--------------------------------|----------------------------|-----------------------|
| Bt: *Bos taurus*               | Ss: *Sus scrofa*            | Gg: *Gallus gallus*   |

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].

| Pathway                  | Total gene | Gene name                  |
|--------------------------|------------|----------------------------|
| Adipocytokine signaling  | 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.

References
[1] Towler M C and Hardie D G 2007 AMP-activated protein kinase in metabolic control and insulin signaling Circ. Res. 100 328–41
[2] Hardie D G and Hawley S A 2001 AMP-activated protein kinase: the energy charge hypothesis revisited Bioessays 23 1112–9
[3] Dugan L L, You Y H, Ali S S, Diamond-Stanic M, Miyamoto S, DeCleves A E, Andreyev A, Quach T, Ly S, Shekhtman G, Nguyen W, Chepetan A, Le T P, Wang L, Xu M, Paik K P, Fogo A, Viollet B, Murphy A, Brosius F, Naviaux R K and Sharma K 2013 AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function J. Clin. Invest. 123 4888–99
[4] Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M and Andreelli F 2007 Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders Diabetes Metab. 33 395–402
[5] Viollet B, Ornman S, Leclerc J, Lantier L, Foretz M, Billaud M, Giri S and Andreelli F 2010 AMPK inhibition in health and disease Crit. Rev. Biochem. Mol. Biol. 45 276–95
[6] Tosca L, Chabrolle C, Uzbekova S and Dupont J 2007 Effects of metformin on bovine granulosa cells steroidogenesis: possible involvement of adenosine 5’ monophosphate-activated protein kinase (AMPK) Biol. Reprod. 76 368–78
[7] Park S K, Sheffler T L, Spurlock M E, Grant A L and Gerrard D E 2009 Chronic activation of
5′-AMP-activated protein kinase changes myosin heavy chain expression in growing pigs J. Anim. Sci. 87 3124–33

[8] Nguyen T M D, Alves S, Grasseau I, Métayer-Coustard S, Praud C, Froment P and Blesbois E 2014 Central role of 5′-AMP-activated protein kinase in chicken sperm functions1 Biol. Reprod. 91 121

[9] Chen J, Hudson E, Chi M M, Chang A S, Moley K H, Hardie D G and Downs S M 2006 AMPK regulation of mouse oocyte meiotic resumption in vitro Dev. Biol. 291 227–38

[10] Phizicky E M and Fields S 1995 Protein-protein interactions: methods for detection and analysis Microbiol. Rev. 59 94–123

[11] Van der Knaap J A and Verrijzer C P 2016 Undercover: gene control by metabolites and metabolic enzymes Genes Dev. 30 2345–69

[12] Xing S, Wallmeroth N, Berendzen K W and Grefen C 2016 Techniques for the analysis of protein-protein interactions in vivo Plant Physiol. 171 727–58

[13] Sevimoglu T and Arga K Y 2014 The role of protein interaction networks in systems biomedicine Comput. Struct. Biotechnol. J. 11 22–7

[14] Prastowo S, Amin A, Rings F, Held E, Wondim D S, Gad A, Neuhoff C, Tholen E, Looft C, Schellander K, Tesfaye D and Hoelker M 2017 Fateful triad of reactive oxygen species, mitochondrial dysfunction and lipid accumulation is associated with expression outline of the AMP-activated protein kinase pathway in bovine blastocysts Reprod. Fertil. Dev. 29 890–905

[15] Amin A, Gad A, Salilew-Wondim D, Prastowo S, Held E, Hoelker M, Rings F, Tholen E, Neuhoff C, Looft C, Schellander K and Tesfaye D 2014 Bovine embryo survival under oxidative-stress conditions is associated with activity of the NRF2-mediated oxidative-stress-response pathway Mol. Reprod. Dev. 81 497–513

[16] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou K P, Kuhn M, Bork P, Jensen L J and Von Mering C 2015 STRING v10: Protein-protein interaction networks, integrated over the tree of life Nucleic Acids Res. 43 D447–52

[17] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn B B and Kadowaki T 2002 Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase Nature Med. 8 1288–95

[18] Minokoshi Y, Kim Y-B, Peroni O D, Fryer L G D, Müller C, Carling D and Kahn B B 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase Nature 415 339–43

[19] Bevan P 2001 Insulin signalling J.Cell Sci. 114 1429–30

[20] Eijkelenboom A and Burgering B M T 2013 FOXOs: Signalling integrators for homeostasis maintenance Nature Rev. Mol. Cell Biol. 14 83–97