Journal of Animal Research: v.11 n.6, p. 963-968. December 2021

DOI: 10.30954/2277-940X.06.2021.2

An Animal Model to Visualize Differential Expression of Genes Related to Metabolic Process and Immune Reaction between Large White Yorkshire (LWY) and Indigenous Pigs of Punjab

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Received: 26 Aug., 2021 **Revised:** 20 Oct., 2021 **Accepted:** 26 Oct., 2021

ABSTRACT

The pig rearing is renowned area of Indian livestock. It is mainly adopted by tribal folk and economically week classes of country. The pig meat known as pork is highly proteinaceous. The four genes (*ACAT2*, *ACAA2*, *APOA4* & *SOCS2*) are evaluated in the current study on the basis of their expression in indigenous pigs of Punjab in comparison to Large White Yorkshire (LWY) where the tissue used is liver. The quality and quantity of RNA transcript is assessed by gel-elctrophoresis and real-time quantitative PCR. All genes show significant difference ($P<0.05$) in both breeds except *ACAA2* ($P>0.05$). The expression of *ACAT2* is higher in indigenous breed whereas other three genes downregulates in indigenous breed of pigs. The mRNA expression of *APOA4* is negligible i.e. only 1% expression of LWY. On the behalf of this data, we can say that indigenous pigs have good cholesterol metabolism as *ACAT2* is higher in indigenous pigs but are more prone to cardiac diseases, gastric ulcers and other chronic diseases due to low expression of *APOA4* and *SOCS2*. This study can be helpful to find the genetic differences between LWY and indigenous pigs of Punjab.

HIGHLIGHTS

- We studied comparative expression profiling of genes in liver of indigenous and Large White Yorkshire breeds of pigs
- The present study will help in genetic profiling of genes responsible for regulating metabolism and immune response

Keywords: Large White Yorkshire (LWY), Indigenous, *ACAA2*, *ACAT2*, *APOA4*, *SOCS2*.

The Indian livestock distribution includes 1.69% of pigs with nine (9.06) million population. According to nineteenth livestock production statistics, India situated at rank five for pig farming in the world. The pig rearing is highly adopted by North-East India especially Assam (Singh, 2019). It is a better source of income and food for tribal people and other economically weaker communities (Haldar *et al*., 2017). The traits that attracts people towards it i.e., consuming low feed but gives output with early sexual maturity and short generation interval (Moanaro *et al*., 2011; Chauhan *et al*., 2016).

According to NBAGR, ten pig breeds are registered that belongs to different locations of country. The indigenous pigs provides highly proteinaceous meat (Pugliese *et al*., 2012). In India, Large White Yorkshire (LWY) as a exotic pig breed is characterized by better growth rate than local pigs providing tenderer and juicy meat (Sodhi *et al*., 2014). Therefore, cross-breeding has been adopted that produces pigs with good reproductive health, less fat that provides

How to cite this article: Kaur, A., Sodhi, S.S., Sethi, R.S. and Mukhopadhyay, C.S. (2021). An Animal Model to Visualize Differential Expression of Genes Related to Metabolic Process and Immune Reaction between Large White Yorkshire (LWY) and Indigenous Pigs of Punjab. *J. Anim. Res.,* **11**(06): 963-968.

Source of Support: None; **Conflict of Interest:** None

high grade meat (Čandek-Potokar *et al*., 2012; Zhang *et al*., 2019).

Liver performs various biological functions including metabolism. It has high content of lipid, protein and nutrients also (Srebernich *et al*., 2017). After transcription of genes, lipids regulates them and also play an important role in differentiation of tissues (Uddin *et al*., 2011).

The metabolism and immunity both plays vital role in cellular growth. The linked genes are Acetyl Coenzyme A acetyltransferase-2 (*ACAT2*), Acetyl Coenzyme A acyltransferase (*ACAA2*), Apolipoprotein A-4 *(APOA4)* and Suppressor of cytokine signaling (*SOCS*2). *ACAT2* has seven domains that acts as transferase and catalase enzyme (Sodhi *et al*., 2014). It is involved in cholesterol biosynthesis; induces absorption and transportation of lipids from liver to various tissues via chylomicrons (Cerqueira *et al*., 2016). *ACAA2* also linked with metabolic pathways; encodes thiolase enzyme (Kiema *et al*., 2014; Miltiadou *et al*., 2017). *APOA4* gene has dual functon, involved in metabolism and immunity also. It induces reverse cholesterol transport and acts as an antioxidant (Wang *et al*., 2015). Also, *SOCS2* prevents various tumor formations and liver diseases caused by fat buildup (Jian *et al*., 2021; Li *et al*., 2021).

There are a few reports available about transcriptomic analysis of genes in indigenous pigs of Punjab. The current study demonstrates the comparative expression of genes linked with metabolism and immunity in indigenous and LWY pigs.

MATERIALS AND METHODS

The study was conducted at (Department of animal biotechnology, College of aniaml biotechnology) Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab.

Tissue collection

The study committed twelve liver samples with six samples from each breed. The liver tissues samples were collected from adult pig slaughtered for meat purpose. The dry ice was used for the temperature maintenance during tissue collection and transportation. Then the samples were stored at -80°C. The Di-ethyl pyro-carbonate (DEPC) treated eppendorf and tips were used for RNA extraction.

The 120 mg of liver tissue was used for RNA extraction. One ml of Trizol reagent (Qiazol) was added with tissue in pestle and mortar and minsed to form paste. 0.2 ml of chloroform (Himedia) was used for phase separation. For the precipitation of RNA, 0.5 ml of isopropanol (Himedia) was added. The washing step includes the use of 1ml of 75% ethanol. The RNA was purified by RNase-free DNase set (QIAGEN, Hilden, Germany) and quantified with thermo scientific Nanodrop one, where the RNA integrity lied between 8.0 and 10.0 and purity ratio for 28S/18S was 1.8 to 2.0.

Qualitative analysis

The qualitative analysis involves use of agarose gel electrophoresis (Agilent Technologies Ireland, Dublin, Ireland). The normal PCR was run at specific conditions i.e., initial denaturation at 95ºC for 2 minute, denaturation at 95ºC for 30 seconds, annealing temperature as given in table 1 for 30 seconds, extension at 72ºC for 1 minute and final extension 72ºC for 5 minutes. Then PCR product was run in 1.5% agarose gel at 100 volt/cm. The UV- illumination (Bio-Rad, USA) and gel doc (G:Box Syngene) were used to visualize and capture gel image having bands.

Quantitative analysis

The primer used for quantitative real time-PCR were designed with Online Primer-3 (Table 1) (Rozen and Skaletsky, 2000). Real-time qRT-PCR (BIO RAD model CFX96^{TM} Optics Module real time PCR) was used for quantitative assessment of mRNA expression of *ACAT2, ACAA2, APOA4* and *SOCS2* genes; in indigenous and LWY pigs. The EvaGreen (Biotium, USA) dye was used to quantify the transcripts of target genes. The conditions given for real-time PCR were 94ºC for 5 min (initial denaturation), 94ºC for 30 sec (denaturation), 60ºC for 30 sec (annealing temperature), 72ºC for 1 minute (extension) and then melt curve was added. The samples were run in triplicates and the standard curve method was used to assess the real-time PCR efficiency. The amplified target genes were compared with endogenous control i.e. *β-actin* (Rebouças *et al*., 2013). The C_T method was used to quantify the transcript levels; selected gene's threshold cycle (C_T) were standardized with C_T value of *β-actin* (Erkens *et al*., 2006; Van Poucke *et al*., 2001).

Table 1: Primer sequences of *ACAA2, ACAT2, APOA4* and β- actin for quantitative and qualitative analysis

Gene	Primers	Size of Product	Annealing Temperature (Ta)	GenBank ID
ACAA2	F-TAGGCTCTGTGGCTCTGGTT	225 bp	61°C	NM_001167638
	R-GTAATTGCCATCGGGATTTG			
ACAT2	F-ATCACCAAGGAGCGAATCC	245 bp	58.5°C	NM_001243427
	R–CCTCTTCTGCTTGTCCCAAC			
APOA4	F-GATGAAGAAGCAGGCAGAGG	215 bp	61°C	NM_214388
	R-TGTTGAAGGTCTCCCCGTAG			
SOCS2	F-ATTGGTTTTGTGGCTGGAAG	188 bp	61°C	NC_010447
	R-AGCACAGAATCGGAACATCC			
β- actin	F-GACATCCGCAAGGACCTCTA	250 bp	60°C	XM_003124280
	R-ACACGGAGTACTTGCGCTCT			

Statistical analysis of the differential expression patterns of the genes

The genes were analyzed for their differential expression using ANOVA where the mean and standard error were given as mean ± SEM. The level of significance $P<0.05$ has been calculated by Tukey's b- test.

RESULTS AND DISCUSSION

The bands obtained from gel electrophoresis shows the significant expression of genes and actual product size as given in Table 1. with respect to DNA ladder (Fig. 1).

The mRNA transcript expression obtained from real-time quantitative PCR demonstrated that *ACAT2* has significantly ($p<0.05$) upregulated in indigenous pigs than in LWY (Fig. 2, Table 2). Whereas, the *ACAA2* gene shows no significant difference ($p>0.05$) having low expression in indigenous pigs (Fig 2, Table 3). The mRNA expression of *APOA4* and *SOCS2* had shown significantly ($p<0.05$) lower expression in indigenous pigs than LWY (Fig. 2, Table 4 & 5).

The normal expression of *ACAT2* regulates the metabolic pathways, lipid metabolism (Pedrelli *et al*., 2014) but the overexpression of gene results in various metabolic diseases i.e., atherosclerosis, hypercholesterolemia, IgA neuropathy and gallstone formation (Guo *et al*., 2018; Wu *et al*., 2020). The results for *ACAT2* gene are matching with previous study done by Sodhi *et al*. (2014). Similarly the upregulation of gene *ACAA2* results in glioma formation (Wu *et al*., 2020; Xia *et al*., 2016).

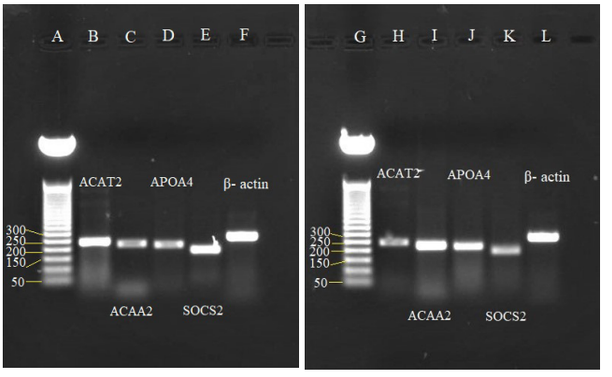

Fig. 1: The gel-doc image shows bands depicting PCR product with perfect primer ligation. The 50bp DNA ladder is loaded in "A" and "G" wells, ACAT2 gene in "B" and "H" wells, *ACAA2* gene in "C" and "I" wells, *APOA4* gene in "D" and "J" wells, *SOCS2* gene in "E" and "L" wells and Beta Actin gene in "F" and "L" wells respectively from indigenous and LWY pigs

In the current study *APOA4* expression is lower in indigenous pig which is in accordance with previous findings (Sodhi *et al*., 2014). The gene has specific role in immune system as an antioxidant, antiulcer and anti-inflammatory factor (Okumura *et al*., 1995; Natarajan *et al*., 2019). The *SOCS2* gene is also linked with immunity; reduces posibility of many disorders i.e. encephalomyelitis disseminata, lupus (SLE) and rheumatoid arthritis (Tsao

Table 2: Expression of *ACAT2* mRNA validated by using real time PCR (SYBR green)

Group	*ACAT2* (Average C_t)	*β-actin* (Average C_t)	ΔCT (C_t *ACAT2* - C_t *β-actin*)	ΔΔCT ΔCt of treated – ΔCt of untreated	Fold change
LWY	27.03 ± 0.17	23.80 ± 0.26	3.22 ± 0.26	0.00 ± 0.26	1.00
Indigenous	22.79 ± 0.13	21.47 ± 0.16	1.32 ± 0.18	-1.92 ± 0.36	3.78*

Calculations are Mean ± Standard Error. The significant difference ($p<0.05$) is shown by superscript *.

Table 3: Expression of *ACAA2* mRNA validated by using real time PCR (SYBR green)

Group	*ACAA2* (Average C_t)	*β-actin* (Average C_t)	ΔCT (C_t *ACAA2*- C_t *β-actin*)	ΔΔCT ΔCt of treated – ΔCt of untreated	Fold change
LWY	21.17 ± 0.19	19.43 ± 0.07	1.74 ± 0.17	0.00 ± 0.17	1.00
Indigenous	27.06 ± 0.16	25.05 ± 0.18	2.00 ± 0.22	0.26 ± 0.18	0.83

Calculations are Mean ± Standard Error.

Table 4: Expression of *APOA4* mRNA validated by using real time PCR (SYBR green)

Group	*APOA4* (Average C_t)	*β-actin* (Average C_t)	ΔCT (C_t *APOA4*- C_t *β-actin*)	ΔΔCT (ΔCt of treated – ΔCt of untreated)	Fold change
LWY	22.62 ± 0.21	23.80 ± 0.26	-1.18 ± 0.24	0.00 ± 0.24	1.00
Indigenous	25.79 ± 0.20	19.91 ± 0.15	5.88 ± 0.29	7.05 ± 0.36	0.01*

Calculations are Mean ± Standard Error. The significant difference ($p<0.05$) is shown by superscript *.

Table 5: Expression of *SOCS2* mRNA validated by using real time PCR (SYBR green)

Group	*SOCS2* (Average C_t)	*β-actin* (Average C_t)	ΔCT (C_t *SOCS2*- C_t *β-actin*)	ΔΔCT (ΔCt of treated – ΔCt of untreated)	Fold change
LWY	26.71 ± 0.21	24.68 ± 0.19	2.03 ± 0.29	0.00 ± 0.29	1.00
Indigenous	23.41 ± 0.23	20.87 ± 0.14	2.53 ± 0.22	0.51 ± 0.22	0.70*

Calculations are Mean ± Standard Error. The significant difference ($p<0.05$) is shown by superscript *.

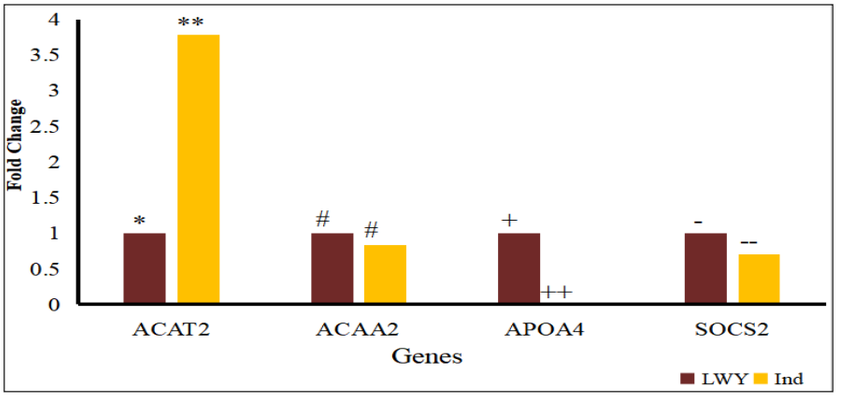

Fig. 2: The comparative mRNA expression of genes *ACAT2*, *ACAA2, APOA4* and *SOCS2* in Indigenous pigs of Punjab and Large White Yorkshire (LWY). The amplified target genes were normalized with *β-actin* relative to LWY. The different superscripts on bars demonstrates that transcript levels differs significantly ($p < 0.05$)

et al., 2008; Cramer *et al*., 2019), controls lipogenesis (Yang *et al*., 2013) and central nervous system (Esper *et al*., 2012; Brant *et al*., 2016). Immune response has a great impact on the growth of animal as it is linked with weight gain (Alder *et al*., 2013). Therefore, the animal having high immune response must be choose for better breeding programs (Mallard *et al*., 1998; Clapperton *et al*., 2006).

On the behalf of this data, we can say that indigenous pigs have good cholesterol metabolism as *ACAT2* is higher in indigenous pigs but are more prone to cardiac diseases, gastric ulcers and other chronic diseases due to low expression of *APOA4* and *SOCS2*.

CONCLUSION

The genes linked with metabolism and immune reaction were analyzed comparatively. This same analysis can be further used for assessment of genetic mechanisms that are responsible for genetic differences between exotic and indigenous breeds of pigs. The current study will become roots for the further investigation of other genes related with various metabolic pathways and immune system. The present findings can be accounted for the betterment of breeding programs.

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