



PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS

Qingdao (China) - June 15-18, 2016

ISSN 2308-1910

Session Breeding and Genetics

Fu C.Y., Ning L.C., Liu L., Li F. C

IDENTIFICATION OF THE RABBIT GPR41 AND GPR43 GENES AND
THEIR EXPRESSION PATTERN IN DIFFERENT TISSUES
AND DEVELOPMENTAL STAGES

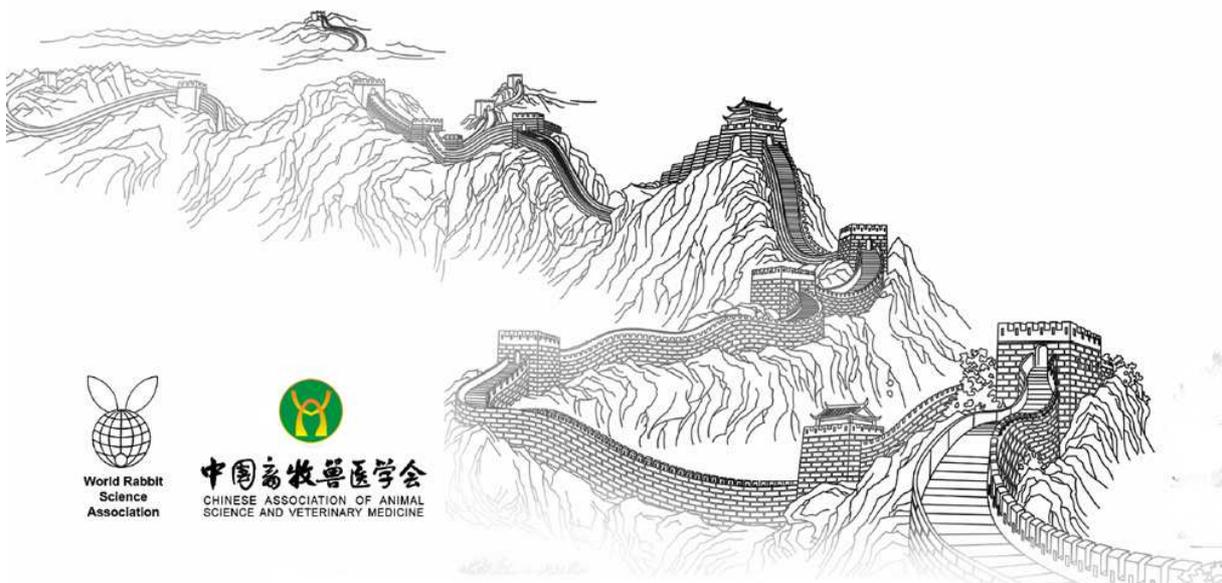
Full text of the communication

+

Poster

How to cite this paper :

Fu C.Y., Ning L.C., Liu L., Li F. C., 2016. Identification of the rabbit GPR41 and GPR43 genes and their expression pattern in different tissues and developmental stages Proceedings. Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 39-42 + Poster



IDENTIFICATION OF THE RABBIT GPR41 AND GPR43 GENES AND THEIR EXPRESSION PATTERN IN DIFFERENT TISSUES AND DEVELOPMENTAL STAGES

Fu C. Y., Ninh L.C., Liu L., Li F.*

College of Animal Science and Technology, Shandong Agricultural University, Tai'an 271018, China

*Corresponding author: Dr. F. C. Li, Professor, E-mail address: chlf@sdau.edu.cn .

ABSTRACT

Short-chain fatty acids (SCFAs) are not only an important energy source, but they also play a regulatory role in various physiological processes. Current studies, mostly in humans and rodents, have revealed that SCFAs acted as endogenous ligands for G protein-coupled receptor GPR41 and GPR43. Whether proteins similar to human GPR41 and GPR43 mediate the regulatory effects of SCFAs in rabbit remains unclear to date. The aims of this study were to determine whether GPR41 and GPR43 genes are expressed in rabbits; and whether the expression of GPR41 and GPR43 is tissue-specific and/or time-associated. The sequence similarity search showed that rabbit genome contained GPR41 and GPR43 genes. Reverse transcription polymerase chain reaction (RT-PCR) indicated that GPR41 and GPR43 were expressed in various tissues of rabbits. Real-time PCR results indicated that GPR41 and GPR43 were expressed throughout the developmental stages in tissue-specific and time-associated manner. GPR41 and GPR43 were most highly expressed in the pancreas ($p < 0.0001$). Meanwhile, we detected the expression of GPR41 and GPR43 mRNA in the subcutaneous adipose tissue ($p = 0.1296$). In conclusion, the rabbit genome encoded GPR41 and GPR43 genes, and these two genes were detected in a variety of rabbit tissues and expressed in tissue-specific and time-associated manner.

Keywords: SCFAs; Rabbit; GPR41; GPR43; RT-PCR

INTRODUCTION

Short-chain fatty acids (SCFAs), predominantly acetate, propionate, and butyrate, can supply energy for maintenance in rabbits. In addition to providing energy, SCFAs play a regulatory role in various physiological processes. The precise mechanisms underlying these regulatory effects of SCFAs are poorly understood. Several studies employing the “reverse pharmacology” approach have reported that SCFAs acted as endogenous ligands for orphan G protein-coupled receptor 41 and GPR43 (Brown et al., 2003; Nilsson et al., 2003). It has also been reported that GPR41 mediated the stimulatory effect of SCFAs on leptin production in adipocytes (Xiong et al., 2004) and the effect of gut microbiota on host adiposity and energy balance in mice (Samuel et al., 2008). GPR43 mediated the effect of SCFAs on the promotion of adipogenesis (Hong et al., 2005) and inhibition of lipolysis in vitro (Ge et al., 2008). Dewulf et al. (2011) demonstrated that inulin-type fructans (ITFs), which can be fermented by gut microbiota for SCFA production, counteracted GPR43 over-expression and PPAR γ activation induced by a high-fat diet in the adipose tissue of mice. Bjursell et al. (2011) found that GPR43-knockout protected mice from obesity and dyslipidemia induced by a high-fat diet. These rodents' original results indicated that GPR41 and GPR43 might be the underlying mechanism of SCFA-associated physiological processes.

To date, the majority of research studies on GPR41 and GPR43 have been on humans and rodents. The identification of rabbit GPR41 and GPR43 and their functions in physiological processes remains to be elucidated. In this study, we tried to determine whether and where GPR41 and GPR43 genes are expressed in rabbit, and the expression pattern of these two genes in different tissues and developmental stages.

MATERIALS AND METHODS

All surgical and animals care procedures in this study followed protocols approved by Experimental Animal Care and Use guidelines (Chinese Science and Technology Committee, 1988).

Tissue collection and RNA extraction

Various rabbit tissues (spleen, thymus, pancreas, lung, duodenum, jejunum, ileum, colon, cecum, adipose tissue) were collected from eight New Zealand rabbits slaughtered at 15 (newborn), 30 (weaning), 60 (growing), 90 (finishing) days, immediately frozen in liquid nitrogen and stored at -80°C until total RNA and membrane protein extraction. Total RNA was extracted with Trizol reagent (TransGen Biotech, Beijing), according to the manufacturers' instructions. The quality of the extracted RNA was determined by standard RNA gel electrophoresis. The concentration of RNA was quantified with a DeNovix DS-11 Spectrophotometer (DeNovix, Wilmington, Delaware, USA). The 260:280 ratio for all samples was greater than 1.85.

Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription (RT)-PCR was used to amplify the protein-coding regions of rabbit GPR41 and GPR43 mRNAs and to detect the expression of these mRNA in the rabbit tissues. One µg total RNA was reverse-transcribed to cDNA in a total volume of 20 µL using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's instructions.

The specific primers of GAPDH (housekeeping gene), GPR41, and GPR43 genes were designed using Premier Primer 5 based on the sequences of predicted rabbit GPR41 (Accession number: XM_002722237.2) and GPR43 (Accession number: XM_002722218.2), as well as glyceraldehydes-3-phosphate dehydrogenase (GAPDH, Accession number: NM_001082253.1). All primers used in this study were synthesized by Sangon (Shanghai, China) and presented in Table 1.

Table 1. Primers used in this study

Gene	Primers sequences(5'→3')	Product size(bp)
rGPR41	F:CCATCTATCTCACCTCCCTGTTC R:AACCAGCAGAGCCCACTGAC	130
rGPR43	F:CGTCCAACCTCCGCTGGTA R:CTTGTACTGCACGGGGTAGG	146
rGAPDH	F: TCACCATCTTCCAGGAGCGA R: CACAATGCCGAAGTGGTTCGT	293
rGPR41-1	F:TCGGGCTTCCTTCTTCTCAC R:CACCCCAAAGAGGACCACAG	315
rGPR41-2	F:TCCTCTTCTTACCACCATCTA R:ACCCCAAAGAGGACCACAG	307
rGPR43-1	F:GCCTACATCATCATCTTCTCACT R:CCTGCGTGAAGTTCTCGTAGC	475
rGPR43-2	F:GGCTTCTACAGCAGCATCTACT R:CCTGCGTGAAGTTCTCGTAG	250

To amplify the protein-coding regions of rabbit GPR41 and GPR43 mRNA, 2µL rabbit pancreas and adipose tissue cDNA was mixed with 12.5µL of PCR Master Mix (CW BIO, China) and 1mM of primers rGPR41-1-F/R and primers rGPR43-1-F/R respectively in a total volume of 20µL. The conditions of these PCR reactions were 94°C for 2min, 35 cycles of 94°C for 30s, 60°C for 30s and 72°C for 30s, followed by extension at 72°C for 2min. 2µL PCR products were amplified again after diluted 50 times with ddH₂O using 1mM of primers rGPR41-2-F/R and primers rGPR43-2-F/R respectively with the same mix and conditions. The quality of the second amplified PCR products were determined by 1% agarose gel electrophoresis. The amplified PCR products were sequenced by Sangon(Shanghai,China).

Real-time PCR

To determine the expression of GPR41 and GPR43 mRNA in rabbit tissues with respect to the different developmental stages (15d, 30d, 60d and 90d), the cDNA representing each tissue were determined with real-time fluorescent quantitative PCR method using FastStart Universal SYBR Green Master (ROX) (Roche, Germany) and amplified using primers rGPR41-F/R, or primers rGPR43-F/R respectively, using an ABI 7500 Sequence Detection System (Applied Biosystems, NY). In the PCR reactions, GAPDH cDNA was also amplified as housekeeping gene control, using primers rGAPDH-F/R. The conditions of these PCRs were 50°C for 2min, 95°C for 10min and 40 cycles of 95°C for 15s, 60°C for 60s. The cDNA from eight 90d rabbits were used to detect the expression of rabbit GPR41 and GPR43 in various tissues.

Statistical analysis

Luciferase data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). For mRNA quantification analysis, n=8. Multiple means were compared using Tukey's analysis. All the results are expressed as the mean \pm SEM (standard error of the mean). Differences were considered statistically significant with $p < 0.05$.

RESULTS

Rabbit genome contains GPR41 and GPR43 genes

A search of rabbit genome database in GenBank using the BLAST program revealed that rabbit genome contains GPR41 and GPR43 genes, which are highly similar to these genes in humans, bovines, rats, and mice. The similarities between rabbit GPR41 and human (NM_005304.3), bovine (NM_001145233.1), capra hircus (XM_005692314.1), porcine (XM_003127053.2), mouse (NM_001033316.2), and rat (NM_001108912.1) were 84%, 84%, 84%, 83%, 81%, and 80%, respectively. The similarities between rabbit GPR43 and human (NM_005306.2), porcine (NM_001278758.1), bovine (NM_001163784.1), rat (NM_001005877.1), and mouse (NM_146187.4), capra hircus (NM_001285655.1) were 80%, 82%, 79%, 84%, 84%, 80%, respectively.

Amplification and tissue expression of rabbit GPR41 and GPR43

Nucleotide sequences of 307 bp and 250 bp were obtained for GPR41 and GPR43 genes, respectively. The pooled RNA was used in this section. The rabbits sampled in this study were of the same breed, from different families, of identical age and comparable body weight, and were raised in the same house using the same feed to describe the distribution of GPR41 and GPR43 in various tissues would be acceptable. The RT-PCR analysis indicated that both GPR41 and GPR43 mRNA were expressed in the tested tissues, including spleen, thymus, pancreas, lung, duodenum, jejunum, ileum, colon, cecum, adipose tissue.

Expression level of rabbit GPR41 and GPR43 in different tissues and different developmental stages

GPR41 and GPR43 were expressed in a significant tissue-specific and time-associated manner. GPR41 was most adequately expressed in the pancreas, which had a significantly higher expression level than any other tested tissue ($p < 0.05$). Its expression level was higher in the lung than in the thymus ($p < 0.05$), and comparable in the other tissues ($p > 0.05$). The highest mRNA level of GPR43 was shown in the adipose tissue ($p < 0.05$) and its expression level was higher in the colon than in the thymus ($p < 0.05$). There were no differences in GPR43 expression level among the other tissues ($p > 0.05$).

Rabbit GPR41 and GPR43 were also differentially expressed in different developmental stages of several tissues. The highest expression levels of GPR41 in the duodenum, cecum ($p < 0.0001$) and pancreas ($p < 0.0001$) were at 15d, significantly higher than in the other developmental stages. After birth, the expression levels of GPR41 in the duodenum, cecum and pancreas were down-regulated. The expression levels of GPR41 in the jejunum, ileum, adipose tissue, spleen were up-regulated after birth, with peaks at 90d in the jejunum, ileum and adipose tissue and 60d in the spleen. However, rabbit GPR41 were not differentially expressed in different developmental stages of the colon, thymus

and lung. The expression pattern of GPR43 at different developmental stages was different from that of GPR41. GPR43 was highly expressed in the duodenum, jejunum, ileum, colon, cecum and lung at 15d, whereas expression levels in the pancreas and spleen increased after birth, with peaks both at 60d. But GPR43 expression in the thymus and adipose tissue was comparable at the different developmental stages.

DISCUSSION

As is known, SCFAs are generated by gut microbial fermentation of complex carbohydrates in the rabbit distal small intestine and large intestine. Acetate, propionate, butyrate are predominant SCFAs in the gut lumen of rabbit. In addition to acting as the substrate for energy generation, SCFAs act as signal molecules and play a regulatory role in a variety of physiological processes. Given their critical regulatory roles, SCFAs and their regulatory functions have drawn much attention. However, the underlying mechanism of SCFAs remains unclear. The identification of SCFAs receptors, GPR41 and GPR43, might clarify the mechanism of SCFAs in various physiological processes.

In summary, our study has shown that the rabbit genome encodes GPR41 and GPR43 genes, and these two genes are expressed in a variety of rabbit tissues, including spleen, thymus, pancreas, lung, duodenum, jejunum, ileum, colon, cecum and adipose tissue. The expression of GPR41 and GPR43 occurs in a significant tissue-specific and time-associated manner, suggesting that these two receptors may have different functions in different tissues and at different development stages. The SCFA-GPR41 and -GPR43 interactions might also represent a novel link between gut microbiota and physiological processes. However, further research is required to determine the precise mechanisms of action of GPR41 and GPR43 in various physiological pathways.

REFERENCES

- Bjursell M., Admyre T., Goransson M., Marley A.E., Smith D.M., Oscarsson J., Gohlöoly M., 2011. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *American J. Physiol. - Endocrinology and Metabolism*, 300: E211-E220.
- Brown A.J., Goldsworthy S.M., Barnes A.A., Eilert M.M., Tcheang L., Daniels D., Muir A., Wigglesworth M.J., Kinghorn I., Fraser N.J., Pike N.B., Strum J.C., Steplewski K.M., Murdock P.R., Holder J.C., Marshall F.H., Szekeres P.G., Wilson S., Ignar D.M., Foord S.M., Wise A., Dowell S.J., 2003. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278: 11312-11319.
- Dewulf E.M., Cani P.D., Neyrinck A.M., Possemiers S., Van Holle A., Mucciloi G.G., Deldicque L., Bindels L.B., Pachikian B.D., Sohet F.M., Mignolet E., Francaux M., Larondelle Y., Delzenne N.M., 2011. Inulin-type fructans with prebiotic properties counteract GPR43 overexpression and PPAR gamma-related adipogenesis in the white adipose tissue of high-fat diet-fed mice. *J. Nutr. Biochem.* 22: 712-722.
- Ge H, Li X, Weiszmann J, Wang P, Baribault H, Chen J.L., Tian H., Li Y., 2008. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology* 149: 4519-4526.
- Hong Y.H., Nishimura Y., Hishikawa D., Tsuzuki H., Miyahara H., Gotoh C., Choi K.C., Feng D.D., Chen C., Lee H.G., Roh S.G., Sasaki S., 2005. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* 146: 5092-5099.
- Le Poul E., Loison C., Struyf S., Springael J.Y., Lannoy V., Decobecq M.E., Brezillon S., Dupriez V., Vassart G., Van Damme J., Parmentier M., Dethoux M., 2003. Functional characterization of human receptors for fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* 278: 25481-25489.
- Nilsson N.E., Kotarsky K., Owman C., Olde B., 2003. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem. Biophys. Res. Commun.* 303: 1047-1052.
- Samuel B.S., Shaito A., Motoike T., Rey F.E., Backhed F., Manchester J.K., Hammer R.E., Willimas S.C., Crowley J., Yanagisawa M., Gordon J.I., 2008. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings Natl. Acad. Sci. USA*, 105: 16767-16772.
- Xiong Y, Miyamoto N, Shibata K, Valasek M.A., Motoike T., Kedziński R.M., Yanagisawa M., 2004. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings Natl Acad Sci USA*, 101: 1045-1050.



Identification of the rabbit GPR41 and GPR43 genes and their expression pattern in different tissues and developmental stages



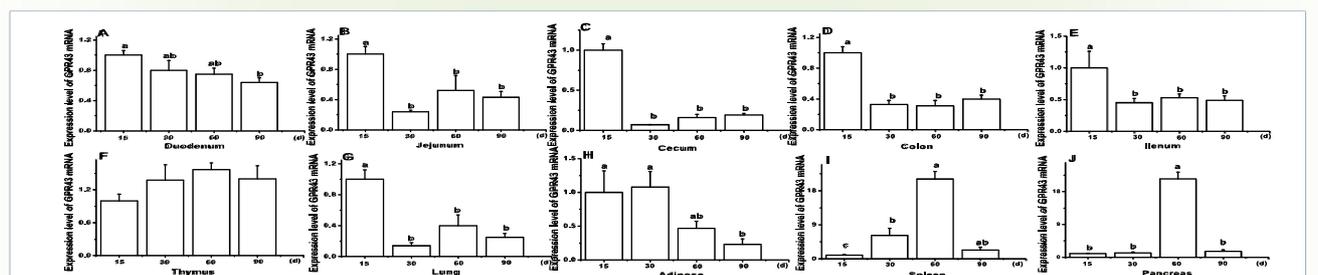
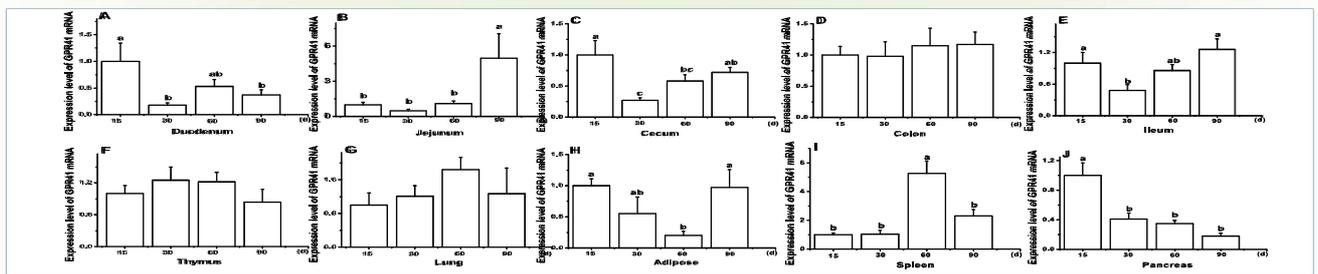
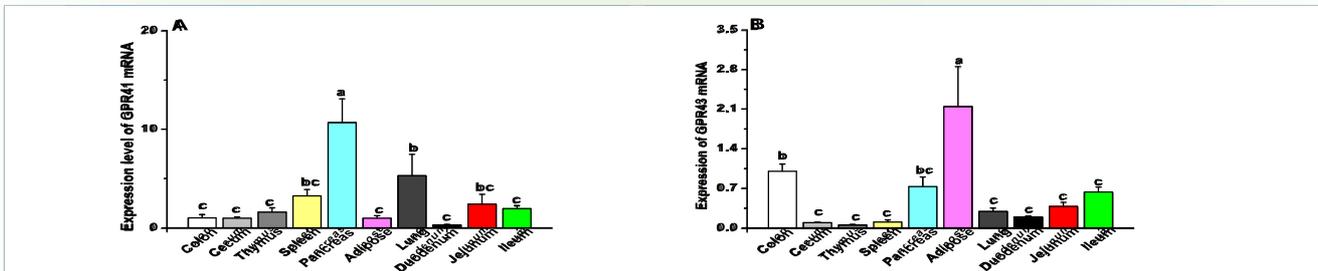
Fu C., Ning L., Liu L., Li F.*
College of Animal Science and Technology, Shandong Agricultural University,

Introduction

- Short-chain fatty acids (SCFAs), predominantly acetate, propionate, and butyrate, can supply energy for maintenance in rabbits. The precise mechanisms underlying these regulatory effects of SCFAs are poorly understood. Several studies have reported GPR41 and GPR43 might be the underlying mechanism of SCFA-associated physiological processes.
- To date, the majority of research studies on GPR41 and GPR43 have been on humans and rodents. The identification of rabbit GPR41 and GPR43 and their functions in physiological processes remains to be elucidated. In this study, we tried to determine whether and where GPR41 and GPR43 genes are expressed in rabbit, and the expression pattern of these two genes in different tissues and developmental stages.

Methods

- All surgical and animals care procedures in this study followed protocols approved by Experimental Animal Care and Use guidelines (Chinese Science and Technology Committee, 1988).
- Tissue collection and RNA extraction and RT-PCR



Results

- Figure 1. The mRNA expression levels of rabbit GPR41 (A) and GPR43 (B) in different tissues on 90d.
- Figure 2. The rabbit GPR41 mRNA levels in the duodenum (A), jejunum (B), cecum (C), colon (D), ileum (E), thymus (F), lung (G), adipose tissue (H), spleen (I), and pancreas (J) at different developmental stages (15, 30, 60, and 90 days).
- Figure 3. The rabbit GPR43 mRNA levels in the duodenum (A), jejunum (B), cecum (C), colon (D), ileum (E), thymus (F), lung (G), adipose tissue (H), spleen (I), and pancreas (J) at different developmental stages (15, 30, 60, and 90 days).

Conclusions

- In summary, our study has shown that the rabbit genome encodes GPR41 and GPR43 genes, and these two genes are expressed in a variety of rabbit tissues, including the spleen, thymus, pancreas, lung, duodenum, jejunum, ileum, colon, cecum, and adipose tissue.
- The expression of GPR41 and GPR43 occurs in a tissue-specific and time-associated manner, suggesting that these two receptors may have different functions in different tissues and at different developmental stages.
- The SCFA-GPR41 and -GPR43 interactions might also represent a novel link between gut microbiota and physiological processes in rabbit.