

Expression of bovine ABC transporters: Potential role of ABCA1, ABCG5 and ABCG8 in cholesterol transport in the mammary gland

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the cell for transport to other organs, or secretion from the body. Whereas ABC-transporters play a considerable role in hereditary human diseases, only scarce information is available about their expression and function in farm animals. Thus, we analysed the mRNA expression of ABCA1, ABCG5 and ABCG8, candidate ABC transporters involved in lipid metabolism, in bovine tissues.

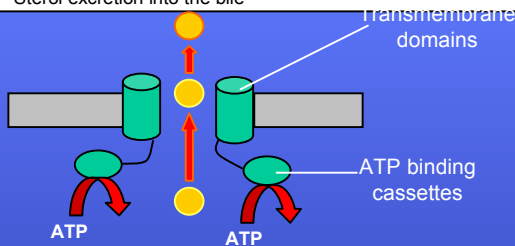
MATERIAL AND METHODS: Total RNA from 18 different bovine tissues was extracted using the RNeasy Midi Kit (Qiagen) and reversely transcribed into cDNA with SuperScript III (Invitrogen). Quantitative real-time PCR with gene specific primers was performed for the quantification of ABCA1, ABCG5, ABCG8 using LightCycler® technology. In addition, ubiquitin, GAPDH, 18S and β -actin were measured as housekeeping genes to normalize the data. Amplified products underwent melting curve analysis after the last cycle to specify the integrity of amplification. All runs included a negative control consisting of PCR-grade water; each sample was measured in duplicates. The relative quantification in the diverse tissues was calculated using bovine liver as reference. Data were analysed using the 2nd Derivate Maximum calculation described in the LightCycler® Relative Quantification Software.

RESULTS: We identified diverse bovine tissues with transcriptional activity for ABCA1, ABCG5 and ABCG8. These tissues are mainly involved in secretory function (mammary gland), metabolic function (liver), barrier function (lung, intestine), and trophic function (placenta, uterus). ABCA1 was amplified in all bovine tissues measured. High expression of ABCA1 was found in lung, esophagus, uterus, spleen and muscle. ABCG5 and ABCG8 expression was found in abomasus, jejunum and colon, in liver, in the lymph nodes of jejunum, in mammary gland, blood and placenta. Both genes showed very high expression in colon and liver.

CONCLUSIONS: In concordance with other mammalian species, ubiquitous expression of ABCA1 and high expression of ABCG5 and ABCG8 in samples from liver and digestive tract was found. However important data arises from their expression in the mammary gland, opening new avenues for elucidating a potential role of these genes in cholesterol homeostasis and milk lipid secretion.

INTRODUCTION

- ATP binding cassette (ABC) transporter proteins pump substrates through biological membranes at the cost of ATP.
- Three members of the family are involved in lipid homeostasis
 - **ABCA1** (expressed ubiquitously):
 - Reverse cholesterol transport from peripheral tissues to the liver
 - **ABCG5 and ABCG8** (expressed specifically in liver and digestive tract):
 - Dietary sterol absorption
 - Sterol excretion into the bile



OBJECTIVES

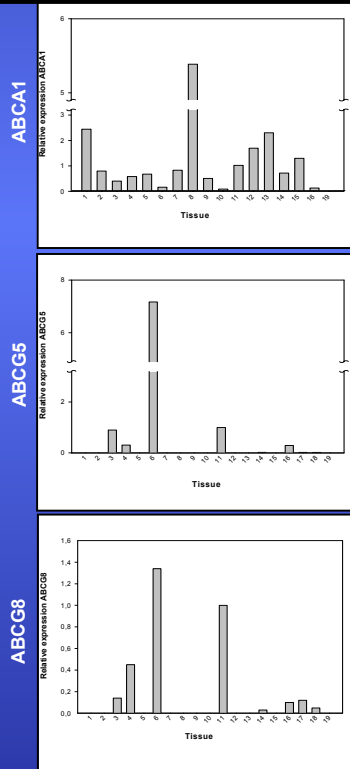
Identification and relative quantification of ABCA1, ABCG5 and ABCG8 in bovine tissues

MATERIAL AND METHODS

- RNA was extracted from 18 bovine tissues (RNeasy Midi Kit, Qiagen)
- cDNA was generated with SuperScript III (Invitrogen)
- ABCA1, ABCG5 and ABCG8 were measured using LightCycler® Technology.
 - Each sample was measured in duplicates
 - Negative control: PCR grade water
 - Relative quantification calculated using liver as reference
- Housekeeping genes: Ubiquitin, GAPDH, 18S and β -Actin

RESULTS

ABCA1, ABCG5 and ABCG8 were found and quantified in the bovine tissue bank



Bovine tissue bank	
1.	Esophagus
2.	Rumen
3.	Abomasum
4.	Jejunum
5.	Caecum
6.	Colon
7.	Tongue
8.	Lung
9.	Heart
10.	Kidney
11.	Liver
12.	Spleen
13.	Uterus
14.	Lymphatic nodes
15.	Muscle
16.	Mammary gland
17.	Blood
18.	Placenta
19.	Negative control (water)

ABCA1 expression was detected in all tissues

- Data consistent with previous literature in other mammalian species
- Potential implication in reverse cholesterol transport to the liver

ABCG5 and G8 expression was more specific

- High expression in liver and digestive tract (abomasum, jejunum and colon)
- Significant expression in mammary gland → **Potential role in milk lipid transport**

CONCLUSIONS

In concordance with other mammalian species, ubiquitous expression of ABCA1 and high expression of ABCG5 and ABCG8 in samples from liver and digestive tract was found. Important data arise from the expression of the three lipid transporters in the mammary gland, opening new avenues for elucidating a potential role of these genes in cholesterol homeostasis and milk lipid secretion.