Genetic architecture of methane emissions from dairy cows

M. Pszczola, T. Strabel, S. Mucha, E. Sell-Kubiak

Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Wolynska 33, Poznan, Poland
strabel@up.poznan.pl (Corresponding Author)

Summary

We performed genome-wide association study for daily methane emissions recorded on 281 Polish Holstein-Friesian cows. In total, 25,871 daily CH_4 emissions repeated records spanning across lactation were obtained using non-invasive Fourier Transform Infrared Spectroscopy breath analyzer during milking in automated milking system. Cows were genotyped using Illumina BovineSNP50 v2.0 BeadChip and the multi-SNP genome-wide association was used with application of Bayesian Variable Selection to identify SNP affecting methane emissions. Our analyses revealed 50 SNP associated with the daily methane emissions (Bayes Factor >10) of which three had "very strong" association (Bayes Factor >30). The identified SNP explained, however, only 0.154% of the total genetic variance. Therefore, no genomic regions with a major effect on the emissions level have been found. Nonetheless, several identified candidate genes were potentially related to methane emissions.

Keywords: methane emissions, gwas

Introduction

Methane (CH_4) emissions is an important topic in ruminants' breeding due to the environmental impact and possibilities for improvement of production profitability (Johnson, et al., 1993, Steinfeld, et al., 2006). Current development in dairy cattle enabled for estimating CH_4 emissions from cows using non-invasive breath analyzing techniques in a commercial environment (Chagunda, et al., 2009, Garnsworthy, et al., 2012, Negussie, et al., 2016). This created opportunity to obtain data sets suitable for genetic analyses and already several studies indicated the existence of the genetic component controlling CH_4 emissions in cattle (de Haas, et al., 2011, Lassen and Lovendahl, 2016, Pszczola, et al., 2017). However, literature investigating the genetic architecture of CH_4emissions is scarce (Manzanilla-Pech, et al., 2016, van Engelen, et al., 2014) and based on inderict measurements. The knowledge of the genetic background of CH_4 emissions would increase the current knowledge and possibly reveal relationships between CH_4 emissions and other traits.

In this study, we employed non-invasive Fourier Transform Infrared Spectroscopy breath analyzer to collect phenotypes on CH_4 emissions from dairy cows with the purpose of performing genome-wide association study to unravel the genomic regions controlling CH_4 emissions from dairy cattle.

Material and methods

A total of 281 Polish Holstein-Friesian cows kept on two commercial farms located in western Poland were used for this study. CH_4 emissions were measured using non-invasive Fourier Transform Infrared Spectroscopy breath analyzer (GASMET 4030; Gasmet

Technologies Oy, Helsinki, Finland) during milking in an automated milking system (Lely Astronaut A4). The CH_4 was expressed in grams per day following Madsen et al. (2010) and Pedersen et al. (2008). The total of 25,871 daily CH_4 emissions were collected with an average of 92 (S.D.=55) observations per cow spanning the whole lactation (DIM 5 to 305). The cows were in up to 4th lactation. The details on farms, measuring set-up and data processing can be found in Pszczola et al. (2017).

The animals were genotyped using Illumina BovineSNP50 v2.0 BeadChip (Illumina Inc., San Diego, CA). The genotyped SNPs were removed from further analysis if they were not in Hardy-Weinberg equilibrium, had minor allele frequency below 0.05, were monomorphic, had a call rate of below 0.95, or if the Illumina GenCall (GC score) was below 0.6. Five animals were removed as they had call rate below 0.9. After quality control 39,680 SNP remained for the genome-wide association analysis.

To identify SNP affecting CH_4 emissions a multi-SNP genome-wide association was used with application of Bayesian Variable Selection method and implemented in software Bayz (Heuven and Janss, 2010). The daily methane emissions model was defined as in Pszczola et al. (2017) and was the following:

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where CH_4 - daily CH_4 emission levels (g/d); $\boldsymbol{\mu}$ - mean; $\mathbf{X}$ - design matrix of fixed effects: year-week-farm and cow's lactation number (levels 1 or 2+) fitted within general lactation curve and modelled using 3rd order Legendre polynomials; e – vector of residuals ~; L_k - animal effect, which was modelled using 2nd order Legendre polynomials.

The mapping of marker effects is constructed as hierarchical model on animal effect which at the next level allows disentangling permanent environmental and genetic variances independently for each level of 2nd order Legendre polynomials, and where $\mathbf{Z_u}$ - matrix with dimensions n by p, with p SNPs coded as 0, 1, 2 copies of specific allele vector; β_{ijk} is a p-vector with the random effects of markers; and ε_{ijk} accounts for permanent environment effect assumed to be normally distributed . On the marker effect, the Bernoulli distribution was applied:

where in the first distribution it is assumed that the SNPs have a small effect ($\sigma^2_{g_0}$) and in the second distribution that the SNPs have a large effect ($\sigma^2_{g_1}$) on the analyzed trait. In this study, a prior of π_1=0.001 was selected, thus on average only 1 in 1,000 SNPs was in the second distribution in each cycle. The posterior means were calculated with 500k MCMC iterations with burn-in of 5k iterations to secure that all the SNPs were used.

The Bayes Factor (BF) was calculated for each SNP to determine the significant associations.

Between the SNPs detected on one Bos Taurus Autosome (BTA), the linkage disequilibrium (LD) was tested in Haploview (Barrett, et al., 2005). The candidate gene search was performed with software BIOMART available in Ensembl Bos Taurus UMD 3.1 by entering the position of a possible QTL region or one of the most significant SNPs with ±500 kbp. BIOMART was also used to study Gene Ontology Terms (GO Terms).

Results and discussion

The GWAS analyses indicated 50 SNPs with BF>10 associated with the daily CH_4 emissions in dairy cattle, which were located on 18 different BTA. From detected SNPs, three had "very strong" association (BF>30). On BTA 1, 4, 9, 13 and 25 the LD analysis also indicated six

possible candidate QTL regions. Those regions explained 0.032% of the total genetic variance, whereas remaining SNPs with 10<BF<30 explained 0.122% of this variance. Overall low level of variance explained by the identified SNP (0.154%) may indicate a highly polygenic character of daily CH_4emissions. It also could be explained by the small sample size and therefore the low power of the analyses.

For the most promising genomic regions on BTA 1, 4, 9, 13, and 25, a total of 130 candidate genes were selected. Based on the GO Terms analysis, 5 candidate genes were selected as the most promising. The indicated candidate genes are involved in metabolic processes of lipids, steroids, and nitrogen, the establishment of endothelial barrier, positive regulation of blood vessel endothelial, cell proliferation involved in sprouting angiogenesis, blood vessel development, and digestive tract development.

Conclusion

The GWAS analyses for daily CH_4 emissions did not reveal genomic regions with a major effect on the emissions level, and explained 0.154% of the total genetic variance (868 g/d^2). Nonetheless, several identified candidate genes were potentially related to CH_4 emissions. Further, analyses using larger sample size would be required for confirming the presented findings.

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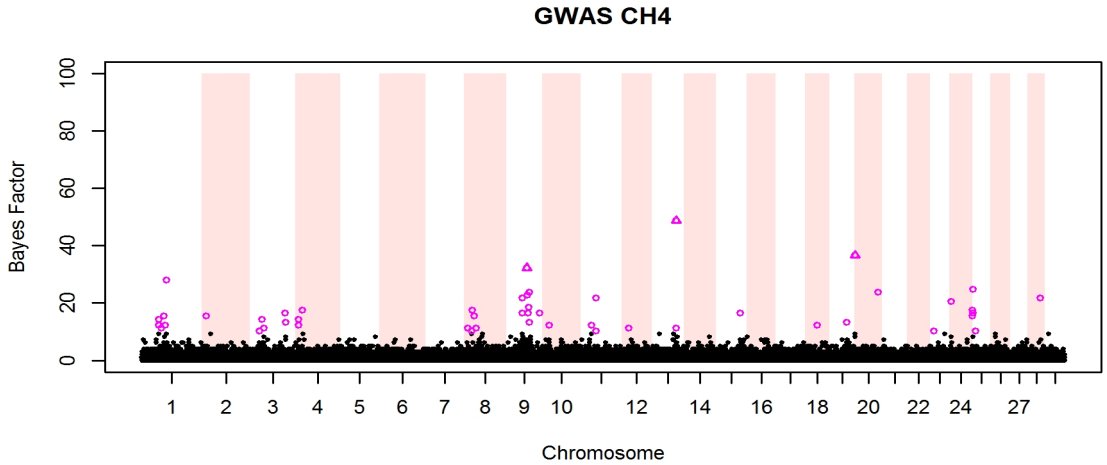

Figure 1. Results of genome-wide association study for raw phenotypic methane levels. Pink triangles indicate SNP with Bayesian Factor (BF) ≥30, pink circles SNPs with 10≤BF<30 and black dots non-significant SNP.

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