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Effects of candidate gene polymorphisms on the detailed fatty acids profile determined by gas chromatography in bovine milk

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ABSTRACT

Association analyses between candidate genes and bovine milk fatty acids can improve our understanding of genetic variation in milk fatty acid profiles and reveal potential opportunities to tailor milk fat composition through selection strategies. In this work, we investigated the association of 51 single nucleotide polymorphisms (SNP) selected from 37 candidate genes using a functional and positional approach, with 47 fatty acids, 9 fatty acid groups, and 5 Δ^9-desaturation indices in milk samples from Brown Swiss cows. Individual milk samples were collected from 1,158 Italian Brown Swiss cows, and gas chromatography was used to obtain detailed milk fatty acid compositions. A GoldenGate assay system (Illumina, San Diego, CA) was used to perform genotype 96 selected SNP located in 54 genes across 22 chromosomes. In total, 51 polymorphic SNP in 37 candidate genes were retained for the association analysis. A Bayesian linear animal model was used to estimate the contribution of each SNP. A total of 129 tests indicated relevant additive effects between a given SNP and a single fatty acid trait; 38 SNP belonging to 30 genes were relevant for a total of 57 fatty acid traits. Most of the studied fatty acid traits (~81%) were relevantly associated with multiple SNP. Relevantly associated SNP were mainly found in genes related to fat metabolism, linked to or contained in previously identified quantitative trait loci for fat yield or content, or associated with genes previously identified in association analyses with milk fatty acid profiles in other cow breeds. The most representative candidate genes were *LEP*, *PRL*, *STAT5A*, *CCL3*, *ACACA*, *GHR*, *ADRB2*, *LPIN1*, *STAT1*, *FABP4*, and *CSN2*. In particular, relevant associations with SNP located on bovine chromosome 19 (BTA19) were found. Two candidate genes on BTA19 (*CCL3* and *ACACA*) were relevantly associated with de novo short- and medium-chain fatty acids, likely explaining the high heritability values found for these fatty acids (with the exception of C6:0). Two additional genes on BTA19 (*CCL2* and *GH1*) showed associations with saturated and branched-chain fatty acids. Our findings provide basic information on genes and SNP affecting the milk fatty acid composition of dairy cows. These results may support the possibility of using genetic selection to modify milk fatty acid profiles to promote beneficial health-related effects.

Key words: association analyses, candidate genes, milk fatty acids, single nucleotide polymorphism

INTRODUCTION

Dairy products contain a wide range of saturated and unsaturated fatty acids with chain lengths ranging from 4 to >20 carbon atoms. Regulation of the fatty acid composition of milk has received attention because certain fatty acids are believed to have various beneficial or potentially harmful effects on human health. However, this issue is still very controversial, with recent studies seeming to contradict the traditional accepted role of specific fatty acids in health and disease. For instance, recent human meta-analyses found no association between SFA intake and the risk of cardiovascular disease or stroke (Mente et al., 2009; Siri-Tarino et al., 2010). Some epidemiological studies have suggested that coronary heart disease risk may be positively associated with industrial *trans* fatty acids but not those originating from biohydrogenation in ruminants, including vaccenic acid (**VA**) and the naturally occurring isomer of CLA or C18:2 *cis*-9,*trans*-11 (rumenic acid, **RA**; Gebauer et al., 2011). The biologically beneficial effects of CLA have been widely documented (Dilzer and Park, 2012), and both n-6 and n-3 PUFA are shown to reduce cardiovascular risk (Michas et al., 2014). However, the benefits of n-3 fatty acids [e.g., eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**)] seem to be not as pronounced as previously believed, warranting

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caution when recommending dietary supplementation of PUFA to the general population without considering the individual intake of total energy and fats.

Because of this increasing awareness of the relationships between diet and health, several feeding and genetic strategies have been evaluated for their ability to engineer milk fat toward a healthier composition (Mele, 2009; Shingfield et al., 2013). In a few European countries (e.g., France and the Netherlands), the dairy industry recently introduced supplementary premiums for farmers based on milk fatty acid compositions (e.g., SFA, n-3 fatty acids, and total C18:1 fatty acids), and contents of α-linolenic acid (C18:3 *cis*-9,*cis*-12,*cis*-15), oleic acid (C18:1 *cis*-9), and palmitic acid (C16:0; Borreani et al., 2013). Feeding trials have clearly demonstrated that altering the cow's nutrition is a very effective way to quickly change the milk fat composition; however, these effects are completely reversible when feeding conditions change and the necessary changes can significantly increase the feeding cost (Shingfield et al., 2013).

In contrast, genetic improvement can gradually induce smaller but permanent trait modifications. Genetic analyses have shown heritable variations in the fatty acid profile of bovine milk (e.g., Bastin et al., 2013; Krag et al., 2013; Pegolo et al., 2016). Moreover, candidate gene studies have shown that SNP in the genes encoding stearoyl-CoA desaturase (**SCD**), diacylglycerol o-acyltransferase 1 (**DGAT1**), and sterol regulatory element binding protein-1 (**SREBP1**) clearly influence the milk fat composition and explain part of the genetic variation of milk fat unsaturation indices seen in Holsteins (Schennink et al., 2007, 2008; Rincon et al., 2012) and Brown Swiss cows (Mele et al., 2007; Conte et al., 2010). Single nucleotide polymorphisms located in other genes involved in lipid biosynthesis have also been shown to affect the milk fatty acid compositions of different bovine breeds (Matsumoto et al., 2012; Marchitelli et al., 2013; Nafikov et al., 2014). Genome-wide studies in Holstein cows have allowed researchers to detect QTL harboring candidate polymorphic genes that are involved in fat synthesis or known to affect fat yield or content, and significantly associated with fatty acids (~20 fatty acid traits were considered) (e.g., Stoop et al., 2009; Bouwman et al., 2011). Recently, a selective DNA-pooling approach was used to identify QTL for milk content of RA, VA, and SCD (Strillacci et al., 2014).

These results collectively suggest that loci found to affect fatty acid profiles could be used in marker-assisted selection programs aimed at modifying the nutritional qualities of milk. However, relatively few fatty acids have been examined to date in this context, and several interesting fatty acids are not yet well known, especially those present at relatively low amounts in milk (and thus hard to detect) but that could have a role in human health.

This work is part of a broader project including the evaluation of the effects of polymorphisms in candidate genes on production traits, milk technological properties, and milk fatty acid profile in Brown Swiss cows. We previously analyzed 96 SNP in 54 candidate genes, and 51 polymorphic SNP in 37 candidate genes were retained for association analyses with productivity and technological traits (Cecchinato et al., 2014, 2015). Here, we extend our association analysis to a very detailed milk fatty acid profile, including 47 individual fatty acids, 9 fatty acid groups, and 5 Δ^9-desaturation indices, determined by GC in the same population. Our aim is to develop genetic markers that will allow breeders to select for animals that produce milk with a healthier fatty acid composition.

MATERIALS AND METHODS

Animals and Milk Sampling

The milk sampling procedure is detailed in Cipolat-Gotet et al. (2013). Briefly, milk samples were collected once per cow during the evening milking from 1,158 Brown Swiss cows from 85 herds (a maximum of 15 cows per herd) located in the Alpine province of Trento (Italy). Each farm was sampled once. The milk samples (without the addition of preservative) were immediately refrigerated at 4°C and transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Padova, Italy). Data regarding the cows and herds were provided by the Superbrown Consortium of Bolzano and Trento (Italy), and pedigree information was obtained from the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy). Each sampled cow had known ancestors for at least 4 generations and the pedigree file included 8,845 animals; there were 1,326 sires, 264 of which had progeny (between 2 and 80 daughters) with records in the data set.

GC Analysis

Fatty acid methyl esters were prepared by the direct extraction and alkali-catalyzed *trans*-methylation procedure previously described by Feng et al. (2004) and modified as in Pegolo et al. (2016). The fatty acid composition was determined using a ThermoQuest gas chromatograph (ThermoElectron Corp., Waltham, MA) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack