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Technical note: The role of circulating low-density lipoprotein levels as a phenotypic marker for Holstein cholesterol deficiency in dairy cattle

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ABSTRACT

With the recent discovery of a Holstein cholesterol deficiency (HCD) haplotype, the USDA has labeled many dairy animals as HCD carriers based on haplotype and pedigree analysis. We set out to investigate the effect of HCD status on various cholesterol transport molecules, namely low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, and triglycerides in both males and females. A genome-wide association study was also conducted to narrow down the genomic region correlated with varying LDL-C levels. In the study, 34 HCD carrier animals showed significantly lower cholesterol and LDL-C levels compared with their 34 closely related, non-HCD controls. The genome-wide association study based on 73 animals using 56,198 SNP markers revealed an association with chromosome 11 in the region of 66,218,925 to 66,946,746 bp. We also tested the effect of HCD status on sperm quality traits using fresh ejaculates and frozen-thawed semen samples, but did not find any discriminating effects. Our study has demonstrated the use of LDL-C as a key phenotypic marker for determining HCD status in dairy cattle and this is the first study that clearly shows a cause-effect relationship of the HCD haplotype on circulating LDL-C.
Key words: Holstein cholesterol deficiency, cholesterol, Holstein, low-density lipoprotein-cholesterol, sperm quality, dairy

Technical Note

In the genomics era, scientists and breeders are constantly searching for recessive alleles associated with phenotypes of interest. Identification of new phenotypes and their genetic components allow farmers to make informed decisions about their herds and raise healthy and productive animals. Many other recessive haplotypes have been discovered and a simple genomic test can reveal carriers versus noncarriers; for example, bovine leucocyte adhesion deficiency, JH1 haplotype in Jerseys (Sonstegard et al., 2013), and the JH2 haplotype (VanRaden et al., 2014). A recent report by Kipp et al. (2015) highlighted the presence of the Holstein cholesterol deficiency (**HCD**) haplotype, which affects 8.7% of the Holstein population in Germany alone. The key phenotypic traits associated with the HCD haplotype are nontreatable lethal diarrhea and insufficient development resulting in death within the first few months after birth (Kipp et al., 2015). Key findings also included decreasing levels of circulating cholesterol in heterozygous animals and low to no cholesterol in homozygous animals. Since their initial report, the USDA has officially assigned many animals with HCD carrier status based on haplotype tests and pedigree analysis (VanRaden and Null 2015). As a result, many animals at STGenetics (Navasota, TX) were also assigned the HCD carrier status. STGenetics is a US-based genetics company engaged in the development of dairy cattle genetics for sale of semen and embryos both domestically and internationally. The objective of the current study was to investigate if there was any association of the assigned HCD status of these animals to a phenotypic trait. Specifically, we set out to determine (1) if HCD status had an effect on circulating cholesterols, low-density lipoprotein cholesterol (**LDL-C**), high-density lipoprotein cholesterol (**HDL-C**), and triglycerides, (2) if a correlation existed between LDL-C levels independent of HCD status and a chromosomal location, and (3) if sperm quality features of fresh and frozen-thawed semen samples were affected by the HCD status of the male animals.

Primary analysis by Kipp et al. (2015) suggested lower levels of cholesterol in carriers for the HCD haplotype. Using haplotype and pedigree analysis, the USDA assigned several STGenetics animals in to different categories based on HCD status. A nominal assignment of the carrier status is HCD0 as a confirmed noncarrier, HCD1 as a confirmed carrier, HCD2 as a lethal homozygous, and HCD3 as an indeterminate carrier. We have used the information on HCD carrier status provided by the USDA to determine other phenotypic

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characteristics of the HCD carrier haplotype. To ascertain if animals of HCD1 status had specific phenotypic traits, males and females were tested for varying levels of cholesterol, and closely related HDL-C, LDL-C, and total triglycerides. Controls were selected as sibs of HCD1 animals with USDA HCD0 designation. Peripheral blood from a venipuncture was collected in serum separator tubes and centrifuged within an hour of collection. Cholesterol, HDL-C, LDL-C, and triglyceride levels were measured directly in serum samples at Marshfield Labs using commercial kits manufactured by Beckman Coulter Inc. (Brea, CA; personal communication with Frances Moore, Marshfield Labs, Veterinary Services, Marshfield, WI). Briefly, total cholesterol was measured through a series of enzymatic reactions. Cholesterol esters in the samples were hydrolyzed using a reagent containing cholesterol esterase to release free cholesterol, followed with oxidation by cholesterol oxidase to produce hydrogen peroxide, which further reacted with 4-aminoantipyrine and phenol, in the presence of peroxidase, to produce a red-colored complex. This complex was measured spectrophotometrically at 540/600 nm, with the increase in absorbance being directly proportional to the total cholesterol concentration in the sample. Similarly, triglycerides were assayed through a series of coupled enzymatic reactions. Triglycerides in the samples were hydrolyzed using lipases to produce glycerol. The glycerol was then phosphorylated to produce glycerol-3-phosphate, which was further oxidized by glycerol phosphate oxidase to produce hydrogen peroxide. The formed hydrogen peroxide reacted with 4-aminophenazone and *N*,*N*-bis(4-sulfobutyl)-3,5-dimethylaniline disodium salt, in the presence of peroxidase, to produce a blue-colored complex. This complex was measured spectrophotometrically at 660/800 nm, with the increase in absorbance being directly proportional to the triglyceride concentration in the sample. The LDL-C in the samples was separated from the other lipid fractions and selectively measured using a 2-step enzymatic process. In the first step, a unique detergent was used to solubilize cholesterol from non-LDL lipoprotein particles, and this cholesterol enzymatically consumed by cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrine to generate a colorless end product. In the second step, a different detergent was added to solubilize cholesterol specifically from the LDL, followed by an enzymatic reaction with cholesterol esterase, cholesterol oxidase, and a chromogen system to yield a blue-colored complex. This colored complex was measured bichromatically at 540/660 nm, with the increase in absorbance being directly proportional to the LDL-C concentration in the sample. A similar 2-step detergent solubilization and enzymatic reaction was used to measure HDL-C in our samples.

Table 1 describes the sample size, sex, and age of the selected animals; females used to test the metabolites were either virgin or pregnant heifers and were not lactating. Blood samples were collected from bulls in active semen production in August 2015 on a commercial dairy in Fond du Lac, Wisconsin, and from virgin and pregnant heifers at a commercial dairy farm in South Charleston, Ohio, in October 2015 (Table 1). To identify a causal mutation or genomic region associated with the low LDL-C levels, a genome-wide association study (**GWAS**) was performed on 73 animals (31 females and 42 males; Table 1). Animals were genotyped using a collection of the Illumina Bovine BeadChip (Illumina Inc., San Diego, CA) and GeneSeek Bovine BeadChip (GeneSeek, Lansing, MI) comprising 56,198 total SNP. The GWAS was conducted using a sliding window multiple regression of 15 markers at a time. Test statistics were computed based on likelihood ratio tests against the null model that only included an in-

Table 1. Sample size and age of the selected animals belonging to the different Holstein cholesterol deficiency (HCD) groups and to carry out the genome-wide association study (GWAS)

Item	HCD Status	Sample size	Average age (mo)	Median age (mo)
HCD groups				
Males	HCD 1	19	28.6	25.9
	HCD 3	3	19.7	19.1
	Control	22	28.4	23.5
Females	HCD 1	15	11	12.1
	HCD 3	16	15.2	17.2
	Control	12	14.6	17
GWAS				
Males	HCD 1	19	28.73684	26
	HCD 3	3	19.66667	19
	Control	20	28.84211	25
Females	HCD 1	14	13.78571	12
	HCD 3	13	15.92308	19
	Control	4	12.50000	11

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