

Population genetic structure

UDC.2:575.174.015.3

doi: 10.15389/agrobiology.2017.6.1139rus

doi: 10.15389/agrobiology.2017.6.1139eng

ASSOCIATION OF POLYMORPHIC TYPES OF STEAROYL-CoA DESATURASE GENE (*SCD1*) WITH ECONOMICALLY VALUABLE TRAITS IN RUSSIAN POPULATION OF AYRSHIRE COWS

M.V. POZOVNIKOVA, G.N. SERDYUK, O.V. TULINOVA, V.P. TERLETSKIY,
N.V. DEMENTEVA, O.V. MITROFANOVA

All-Russian Research Institute for Farm Animal Genetics and Breeding — branch of L.K. Ernst Federal Science Center for Animal Husbandry, Federal Agency of Scientific Organizations, 55A, Moskovskoe sh., pos. Tyarlevo, St. Petersburg—Pushkin, 196625 Russia, e-mail labimngen@mail.ru, otulin@rambler.ru, valeriter@mail.ru, dementevan@mail.ru, mo1969@mail.ru, marina.qpr@gmail.com (corresponding author)

ORCID:

Pozovnikova M.V. orcid.org/0000-0002-8658-2026

Terletskiy V.P. orcid.org/0000-0003-4043-3823

Serdyuk G.N. orcid.org/0000-0002-4907-0742

Dementeva N.V. orcid.org/0000-0003-0210-9344

Tulinova O.V. orcid.org/0000-0002-7731-509X

Mitrofanova O.V. orcid.org/0000-0003-4702-2736

The authors declare no conflict of interests

Received March 6, 2017

Abstract

In modern animal breeding genes involved into biochemical and physiological pathways and having SNP polymorphisms in coding sequences (exon) leading to amino acid replacements in proteins, and in regulatory elements effecting transcription, are considered as promising marker genes. Significant attention is given to study of relations between polymorphisms in lipid metabolism genes such as leptin (*LEP*), thyroglobulin (*TG*), diacylglycerol O-acyltransferase 1 (*DGAT1*) with milk and beef productivity traits in cattle. Little is known about stearoyl-CoA desaturase gene polymorphism and its associations with cattle traits, particularly in Russian populations. Enzyme stearoyl-CoA desaturase plays an important role in fatty acids metabolism. For dairy cattle fat and protein content as well as live weight and rate of weight gain which make an impact on animal puberty age are among the most important traits. In our paper polymorphism of single nucleotide substitution (SNP) rs41255693 in *SCD1* gene has been revealed along with analysis of its link with economically important traits in first lactation cows ($n = 201$) of Ayrshire breed belonging to OAO PZ Novoladozhskii of Leningrad region. SNP in rs41255693 region is located at fifth exon of *SCD1* gene and characterized by amino acid Ala-Val substitution (allele $C > T$) and revealed by FauI restriction endonuclease. Allele *C* is characterized by the presence of restriction site. High frequency of allele *C* (0.858) and genotype *CC* (0.731) has been observed in the population under study. Allele *T* is rare with frequency of 0.142, and *TT* genotype has been detected in only 3 animals (frequency 0.015). In order to evaluate animal breeding value (BV) comparison with contemporaries has been employed showing deviation of each animal productive trait from that of contemporaries. Animal BV was calculated by the following traits: milk yield for 305 and 100 lactation days, percentage of fat and protein, yield of fat and protein, weight at birth, at 10, 12 and 18 month age, live weight at first service and at first lactation. Beyond, age of first service, first calving and days open were also taken into account. Association between genotypes of *SCD1* gene and such parameters as weight gain, animal development and reproductive traits (age at first service, age at first calving and days open) has not been established. Nevertheless, it has been demonstrated that data concerning *SCD1* gene polymorphism allows for QTL (Quantitative Trait Loci) high fidelity mapping for such traits as milk fat and protein content. Cows of the population under study having *CC* and *CT* genotypes exceeded with statistical significance the cows having *TT* genotype in BV milk yield for 305 days ($p < 0.01$), BV milk yield for 100 days ($p < 0.02$), yield of fat, kg ($p < 0.001$), and yield of protein, kg ($p < 0.001$).

Keywords: allele, *SCD1* genotype, Ayrshire breed, cattle, live weight, days open, calving, mutation, screening

Nowadays, genome-wide association study (GWAS) in various bovine breeds was unable to identify quantitative trait loci (QTL) universally associated

with desired economic useful traits. Usually, it is because of genotypic (bred-specific) influence, animal adaptation to environment, feeding and keeping conditions [1, 2]. SNPs (single nucleotide polymorphisms) related to genes, which are involved in control of productivity and quality of livestock products, could facilitate a prediction of cattle potential. Genes involved in lipid metabolism are of special interest because the fat content of milk has significantly higher heritability coefficient than the total amount of milk produced (0.60 vs. 0.18). Milk production is more often considered as combination of total milk yield, protein percentage and fat percentage. Systems governing mammal lactation are involved in body metabolism and immunity, which participate in animal adaptation to the environment [1]. Thus, one should consider association of a particular gene not only with milk production but also with all economically valuable traits.

Lipid metabolism of productive animals is of high importance. A total of 107 genes, 240 proteins, and 80 metabolites are involved in its regulation. Stearoyl-CoA-desaturase (SCD, EC 1.14.99.5) catalyzes desaturation of palmitic and stearic acids to monounsaturated fatty acids, the palmitoleic and oleinic acids, in mammal adipocytes [3]. SCD deficiency causes reduction of fat contents in tissues, an increased sensitivity to insulin and, consequently, accelerated metabolism. It was observed that gene *SCD1* knockout causes metabolic attenuation, atrophy of oil glands, intrusion of epidermal lipid barrier, disorder of thermal control and cold resistance [4, 5]. SCD ferment displays protective antiapoptotic effect by indirect regulation of triglyceride accumulation in cells [6].

Bovine *SCD1* gene is located on chromosome 26 and consists of 6 exons and 5 introns [7]. Information on associations of various SNPs, both intronic and exonic, of *SCD1* gene with economically valuable traits in various breeds is ambiguous. Thus, genome-wide association study of productive and reproductive traits of Holsteins [8] and northern red dairy cattle [9] did not find such association for *SCD1* gene. In other studies [10], with the use of two-stage association analysis of 50000 SNPs for identification of genome areas associated with certain fatty acids in cow milk, it was shown that only in two genes, *DGAT1* on chromosome BTA14 and *SCD1* on chromosome BTA26, such polymorphism strongly influences the unsaturated fatty acids ($C_{4:0}$ - $C_{18:0}$) and medium-chain fatty acids in bovine milk fat. Association of *SCD1* gene with milk level of unsaturated fatty acids had also been noticed [11]. In the search for associations of 51 SNPs in 37 candidate genes with 47 fatty acids in milk, positive correlation between *T* allele of *SCD1* gene (SNP rs41255693) and amount of $C_{20:0}$ and $C_{22:1}$ *cis*-9 in milk fat had been discovered [12]. It is fat composition, as well as proportion of saturated and unsaturated acids, which defines dietetic value of milk. Based on published data, it could be assumed that several SNPs in *SCD1* gene could be considered as potential markers upon forecasting the qualitative composition of milk. It should also be noted that a breed itself, due to inter-breed differences of genes, polymorphism of which is associated with milk production, is of particular importance. There are studies indicating on variation between breeds in milk composition, i.e. in percentage of saturated and unsaturated fatty acids. Herewith, negative genetic correlation between proportion of the unsaturated fatty acids and fat is seen [13].

Several breeds show high polymorphism of *SCD1*, which suggests location of this gene in the regions of imprints of natural selection. Japanese researchers had detected high polymorphism in exon 5 of the *SCD1* gene, with 8 SNPs revealed in this region in Japanese Black beef cattle [14]. As the same time, only 3 of these SNPs were found in Jersey and Holstein cattle [15] and amongst 12 Italian livestock breeds [16].

Various SNPs in *SCD1* gene are known to be associated with cow milk yield and composition. In Holstein cows of Chinese selection, evaluation of 5

SNPs both in intronic and exonic areas (g.6926A>G, intron 3; g.8646A>G, intron 4; g.10153A>G, g.10213T>C and g.10329C>T, exon 5) showed that the genotypes heterozygous for each of these SNPs had validly higher 305-day lactation yields and production of milk fat and protein [17].

This paper is the first to report the influence of polymorphism in exon 5 of the *SCD1* gene on economically valuable traits in a population of Ayrshire cows of Russian selection. These findings deepen the knowledge of biosynthesis of milk fat and could facilitate selection for improved dietetic milk quality in the studied population.

Our subjective was to examine *SCD1* gene polymorphism for SNP rs41255693C>T (NC 007327.6, position 2144708, https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=41255693) and its association with economically valuable traits of Ayrshires, the type Novoladozhskii selected in Russia.

Techniques. For DNA extraction, blood samples of first-calf Ayrshire cows ($n = 201$) (OAO PZ Novoladozhskii, Leningrad Province, 2015) was taken from tail vein in K3EDTA vials and stored at $-20\text{ }^{\circ}\text{C}$. DNA was extracted by phenol method [18].

PCR-RFLP (restriction fragment length polymorphism) was used for genotyping. Polymerase chain reaction (PCR) was conducted in $10\text{ }\mu\text{l}$ of reaction solution (67 mM Tris-HCl, pH 8.6; 2.5 mM MgCl₂, 16.6 mM NHOH, 0.125 mM of each dNTP, dATP, dGTP, dCTP, dTTP, 0.5 μM of each primer, 50-100 ng genomic DNA, and 2.5 IU Taq DNA-polymerase (SibEnzyme LTD, Russia) with the primer pair F — 5'-CCT-AAG-CAG-CAG-ACC-ACT-AG-3', R — 5'-TGG-GCT-CAA-CGT-CAC-CTG-3' (Eurogen CJSC, Russia) at an amplifier Thermal Cycler T1000 (Bio-Rad Laboratories, Inc., USA) according to the following protocol: initial denaturation for 1 min at $94\text{ }^{\circ}\text{C}$; 35 cycles: 30 s at $95\text{ }^{\circ}\text{C}$, 30 s at $56\text{ }^{\circ}\text{C}$, 30 s at $72\text{ }^{\circ}\text{C}$; final elongation for 10 min at $72\text{ }^{\circ}\text{C}$. For determination of animal genotypes, the obtained amplicates were treated by endonuclease *FauI* (SibEnzyme LTD, Russia). The reaction mixture was incubated at $55\text{ }^{\circ}\text{C}$ for 2 hours followed by electrophoretic separation of fragments in 3 % agarose gel containing 0.1 $\mu\text{g/ml}$ ethidium bromide. The signal was recorded using gel documentation system Gel Imager-2 (Helicon Company LLC, Russia). GeneRuler Ultra Low Range DNA Ladder (Fermentas, Lithuania) was used for sizing DNA restriction fragments.

Economic characters of dairy cows were taken from the electronic database IAS Selex of OAO PZ Novoladozhskii. Pedigree value (PV) of cows was estimated using SGS-VNIIGRG software [19]. The following parameters were taken into account: PV for 100- and 305-day lactation yield; percentage of milk fat and protein; milk fat and protein yield; PV for body weight at birth, at 10, 12 and 18 months of age; body weight at first insemination and after first calving; age at first insemination and first calving; service period.

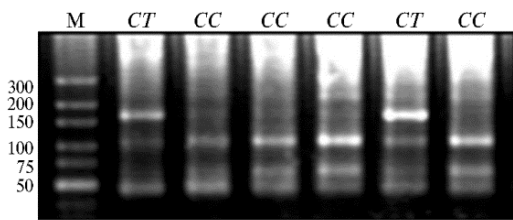
Statistical processing of data was performed using AtteStat software (http://www.studmed.ru/programma-attestat-1205_1778bebd8f9.html) and Microsoft Excel. Genotype and allele frequencies were quantitated. Deviations of these parameters from the Hardy-Weinberg equilibrium were estimated by χ^2 criterion. Means (M) and deviations from mean values ($\pm m$) were calculated. Statistical significance of the differences in mean values of economic characters between animal groups with different genotype was determined by Student's t -test [20].

Results. Nowadays, Ayrshire breed is widely spread in Russia, with 25 % of the total livestock concentrated in Leningrad region. Ayrshire cows are characterized by high milking, with high milk fat and protein content [21]. In our study, we selected a group of first-calf cows, type Novoladozhskii, owned by OAO PZ Novoladozhskii. For many years, in this farming unit, which remains

the leader by milk productivity in Ayrshire dairy cattle in Russia, the genetic potential of milk productivity in herd grows both from generation to generation and from year to year [22]. The herd is improved by engagement of pedigree resources from Scandinavian countries and nearly 80 % of the livestock has Finnish origin. Keeping type is free stall barns, animals have free access to silage and haylage all year round, concentrates are calculated individually depending on productivity of each cow.

So far as full realization of animal genetic potential for performance is ensured due to feeding and keeping conditions, gene alleles would most probably influence on productive traits. According to records at the beginning of 2016, milk productivity of first-calf cows for 305 days was 7619 kg with fat content of 4.25 % and protein content of 3.50 %; body weight was 563 kg. Milk of Ayrshire cows is highly appreciated for its technological properties, namely milk fat quality, and is recommended for production of cheese and butter [23].

Practically, dietetic milk properties and their improvement attract attention of stock-breeders due to healthy diet issues. A more detailed study of SNPs associated with genes of lipid metabolism in a population of Ayrshires which differ from other dairy breeds in high milk fat content allows for better understanding mechanisms of milk fat biosynthesis and regulation. Results of such studies allow identifying animals with the most valuable genotypes, which, in turn, allows breeders to improve effectiveness of selection.



FauI restriction of PCR-amplified fragment of stearyl-CoA-desaturase gene *SCD1* (SNP rs41255693) in Ayrshire breed: M — molecule weight marker Gene-Ruler Ultra Low Range DNA Ladder (Fermentas, Lithuania; from 10 to 300 bp), genotype *CC* — fragment sizes of 105 and 58 bp, genotype *CT* — 163, 105 and 58 bp (OAO PZ Novoladozhskii, Leningrad Province, 2015).

The figure shows electrophoretic separation of fragments resulted from restriction of the obtained PCR products. After treatment of 163 bp amplicons with FauI endonuclease, the presence of restriction site defined *C* allele, whereas the absence indicated *T* allele. Fragments of 105 and 58 bp corresponded to *CC* genotype, 163, 105 and 58 bp corresponded to *CT* genotype, and 163 bp corresponded to *TT* genotype.

Comparison of the obtained electrophoregrams revealed prevalence of certain SNP rs41255693 alleles and genotypes for the gene *SCD1* across the studied population of Ayrshires. Homozygotes *CC* and *C* allele were identified most frequently (0.731 and 0.858, respectively). Only three animals (0.015) had genotype *TT*, and frequency of *T* allele was 0.142. H_0 for heterozygotes *CT* was 0.254 at H_e of 0.243 ($p = 0.05$). The sample had not displayed any significant deviation of the actually observed genotype frequencies from those theoretically expected according to Hardy-Weinberg law ($\chi^2 = 0.338$).

In the studied sample, *CC* and *CT* genotypes appeared to be the most numerous and took precedence over animals with *TT* genotype by a number of indicators: PV for 305-day lactation yield ($p \leq 0.01$), PV for 100-day lactation yield ($p \leq 0.02$), yield of milk protein, kg ($p \leq 0.001$), and yield of milk fat, kg ($p \leq 0.001$) (Table). *TT* genotype was rare, and animals of such genotype were inferior to other cows by milk productivity indicators, and had low growth rate and longer service period. It should be noted however that such data was obtained from small sample, i.e. further studies are required for making final conclusions.

Economic characters of Ayrshires depending on genotypes for stearoyl-CoA-desaturase *SCD1* gene ($M\pm m$, OAO PZ Novoladozhskii, Leningrad Province, 2015)

Parameter	Genotype		
	<i>CC</i> ($n = 147$)	<i>CT</i> ($n = 51$)	<i>TT</i> ($n = 3$)
PV for 305-day milking yield	-20.25±23.47 ^a	11.76±37.76 ^b	-289.66±92.31 ^c
PV for 100-day milking yield	-4.02±6.38 ^d	-0.11±10.87 ^e	-58.00±23.11 ^f
PV for milk fat content (%)	0.007±0.008	0.010±0.010	-0.060±0.10
PV for milk protein content	-0.006±0.005	0.004±0.008	-0.060±0.040
Fat yield, kg	315.89±4.17 ^g	325.22±6.77 ^h	260.35±13.02 ⁱ
Protein yield, kg	265.14±3.21 ^g	273.90±5.10 ^h	215.36±11.86 ⁱ
PV for BW at birth, kg	0.29±0.25	-0.10±0.38	0.43±2.64
PV for BW at 10 months of age, kg	0.50±1.80	5.47±2.81	-12.80±18.40
PV for BW at 12 months of age, kg	0.79±1.89	4.50±3.01	-8.43±11.30
PV for BW at 18 months of age, kg	-0.72±2.69	7.26±4.34	-10.76±14.27
PV for BW at the 1 st insemination, kg	-1.23±1.78	-0.34±2.58	1.10±4.48
PV for BW after first calving, kg	-1.56±1.13	0.43±1.54	-6.00±4.04
Age at first insemination, months	17.18±0.13	16.90±0.24	17.07±0.09
Age at first calving, months	26.37±0.14	26.35±0.31	25.82±0.72
First lactation, service period, months	106.78±5.26	111.35±8.30	156.75±36.72

Note. *C* allele corresponds to the presence of restriction site for endonuclease *FauI* in 163 bp amplicons, *T* allele corresponds to absence of such site. PV — pedigree value, BW — body weight.

a, c; b, c Differences are statistically significant at $p \leq 0.01$.

d, f; e, f Differences are statistically significant at $p \leq 0.02$.

g, i; h, i Differences are statistically significant at $p \leq 0.001$.

We had not found reliable association of SNP rs41255693 in the gene *SCD1* with such traits as growth and development, reproductive properties (age at first insemination, age at first calving, and service period).

Frequency of gene alleles is one of the main characteristic of a breed or population. Evaluation of SNP rs41255693 in the gene *SCD1* across bovine breeds showed that differences in alleles and genotypes depend on the goal of selection for primary use. Thus, frequency of *T* allele in Brown Swiss cows comprised 0.15 [24]. With increase of Swiss breed proportion in Kostroma cows, their frequency of *T* allele had decreased from 0.611 to 0.231 [25]. In Holstein breed, animals of *TT* genotype have not been found while frequency of heterozygous genotype comprised 0.618 [26]. The prevalence of *T* allele increases in cows of meat and meat-and-milk breeds (e.g. 0.217 for Kalmyk breed vs. 0.321 for Mongolian hogorogo breed) [26]. Low frequency of *T* allele is observed in populations of milk cattle with high milk productivity, which is in line with our findings.

A number of papers show positive association of SNP in exon 5 of the gene *SCD1* with fat yield and milk fat percentage in Holsteins and Jersey cows [27], as well as with total milk fat percentage in Brown Swiss breed [28]. Holstein-Friesian breed of Polish selection displays positive influence of SNP g.10329C>T (exon 5) on of milk protein content ($p \leq 0.05$) [24]. Polymorphism in exon 3 of gene *SCD1* of dairy breeds, the Black-and-White and Ayrshire, was characterized by relatively low frequency of allele variant *A*, upon a decreased fat percentage in milk of Ayrshire cows [29].

There are studies in which no associations between *SCD1* gene and body weight have been identified [14, 30, 31], that is also confirmed by our data.

There was a report on negative impact of SNP T878C in *SCD1* on reproductive traits of Holstein cows that declines profitability in milk herds [32]. We did not found association between *SCD1* gene and reproductive properties of the examined population of Ayrshire cows, which could be explained by both the breed specificities and the external factors, e.g. feeding and keeping conditions.

Totalizing all above, it should be noted that most studies had revealed association between the gene *SCD1* polymorphic variants and milk composition in terms of saturated and unsaturated fatty acid content and total fat yield. It therefore may be said that realization of genetic potential could ensure produc-

tion of high-quality milk. This could also be promoted by proper feeding and keeping as factors maximizing phenotypic manifestation of a genotype.

Thus, we found out SNP rs41255693 polymorphism in the gene *SCD1* of Ayrshires from a population of Russian selection. In the population, *CC* homozygotes had prevailed with the frequency of 0.731 regardless of significant number of *CT* heterozygotes (0.254). The frequency of *TT* genotype found only in three cows was 0.015. High *C* allele frequency (0.858) was seen, whereas *T* allele was rare (0.142). Animals carrying *TT* genotype are few and differ from other groups in low growth rate and longer service period. *TT* genotype is also validly inferior to *CC* and *CT* genotypes with regard to PV for 305-day milking yield ($p \leq 0.01$), PV for 3100-day milking yield ($p \leq 0.02$), and the yield of milk protein and milk fat ($p \leq 0.001$). No valid relationship had been established between SNP rs41255693 in the gene *SCD1* and body weight gain in cows from the birth until the age of first lactation. So *CC* and *CT* genotypes are desirable, of which *CT* heterozygotes have better milk productivity and good growth rate. Selection for desired gene *SCD1* genotypes could significantly improve the effectiveness of breeding in the studied population. In this, molecular methods will facilitate revealing necessary genotypes at earlier age and accelerate formation of a herd with higher productivity potential.

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