PEDRO HENRIQUE FERREIRA FREITAS

GENOMIC ANALYSES FOR PREDICTED MILK FATTY ACID COMPOSITION IN DAIRY CATTLE: A LONGITUDINAL PERSPECTIVE

Dissertation presented to the Animal Science Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of Magister Scientiae.

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APPROVED: June 19th, 2019.

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(Adviser)

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Here I leave my thanks once again to all, and those who for my fault, I did not remember to quote.

BIOGRAPHY

Pedro Henrique Ferreira Freitas, son of Luis Claudio Cardoso de Freitas and Maria de Fátima Ferreira Freitas (in memoriam), was born on September 30, 1993, in Muriaé, Minas Gerais, Brazil. In March 2012, he started a Bachelor of Science program in Animal Science at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. During this time, he also participated in various research projects in the area of Statistics and Animal Breeding and extracurricular activities. For instance, from May 2014 to July 2015 he did an internship at the Goat Husbandry Research Unit at the Federal University of Vicosa, under the supervision of Prof. Dr. Fabyano Fonseca e Silva. From August 2015 to February 2017 he was granted a Scientific Initiation scholarship to develop two research projects entitled “Bayesian random regression models to describe milk, fat and protein yield in dairy goats” and “Methods of genetic evaluation for censured data applied to the age at first kidding in dairy goats”, under the mentorship of Prof. Dr. Fabyano Fonseca e Silva.

In March 2018 he started a Master of Science program in the area of Genetics and Animal Breeding in the Department of Animal Science at the Federal University of Vicosa, under the supervision of Prof. Fabyano Fonseca e Silva and Dr. Luiz F. Brito (Purdue University, United States). On June 19, 2019, he presented his thesis dissertation to the Examination Committee in order to obtain the title of Magister Scientiae in Animal Sciences.

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LIST OF ABBREVIATIONS

A – Pedigree-based Relationship Matrix

b_1 – Regression coefficient

BTA – Bovine chromosome

CDN – Canadian Dairy Network

DAVID – Database for Annotation, Visualization and Integrated Discovery

DHI – Dairy Herd Improvement

DIM – Days in milk

EBV – Estimated Breeding Values

FA – Fatty acid

GC – Gas chromatography

GEBV – Genomic estimated Breeding Values

GWAS – Genome-wide Association Studies

H – Hybrid matrix

h^2 – Heritability

HTD – Herd-Test-Day

LCFA – Long-chain fatty acid

MAF – Minor allele frequency

MCFA – Medium-chain fatty acid

MCMC – Markov Chain Monte Carlo

MIR – Mid-infrared spectroscopy

NCBI – National Center for Biotechnology Information database

PA – Parent Average

QTL – Quantitative trait loci

r^2 – Reliability

RRM – Random regression models

SCFA – Short-chain fatty acid

SFA – Saturated fatty acid

SNP – Single Nucleotide Polymorphism

ssGBLUP – Single-step Genomic Best Linear Unbiased Prediction

UFA – Unsaturated fatty acid

ABSTRACT

FREITAS, Pedro Henrique Ferreira, M.Sc., Universidade Federal de Viçosa, June, 2019. **Genomic analyses for predicted milk fatty acid composition in dairy cattle: a longitudinal perspective.** Adviser: Fabyano Fonseca e Silva.

Milk fat composition has important implications in the nutritional and processing properties of milk. In addition to nutritional and health aspects, milk fat composition can also be associated with cow physiological and health status. Milk fatty composition can be improved through various ways, including modification of the cow's diet and genetic selection. The main objectives of this study were: 1) to estimate genetic parameters for five milk fatty acid (FA) groups (i.e., short-chain, medium-chain, long-chain, saturated, and unsaturated) predicted based on milk mid-infrared spectroscopy, for Canadian Ayrshire, Holstein and Jersey breeds; 2) to perform genomic prediction of breeding values using a longitudinal single-step GBLUP approach for these five traits; 3) to conduct a single-step genome-wide association study aiming to identify genomic regions, candidate genes and metabolic pathways associated with milk FA, and consequently, to understand the underlying biology of these traits. We used 31,709 test-day records of 9,648 Ayrshire cows from 268 herds, 629,769 records of 201,465 Holstein cows from 6,105 herds, and 34,341 records of 11,479 Jersey cows from 883 herds. The genomic database contained a total of 2,330 Ayrshire, 8,865 Holstein, and 1,019 Jersey animals. The average daily h^2 ranged from 0.18 (long-chain FA) to 0.34 (medium-chain FA), from 0.24 (unsaturated FA) to 0.47 (medium-chain and saturated FAs) and from 0.25 (long-chain and unsaturated FAs) to 0.52 (medium-chain and saturated FAs) for Ayrshire, Holstein and Jersey, respectively. The reliability of the genomic prediction, when considering τ and ω equal to 1 (default), ranged from 0.540 (saturated) to 0.746 (unsaturated) in Ayrshire, and from 0.564 (long-chain) to 0.737 (medium-chain) in Holstein. When using the optimal τ and ω values, the Genomic Estimated Breeding Value' reliability ranged from 0.528 (saturated) to 0.786 (unsaturated) in Ayrshire, and from 0.583 (long-chain) to 0.732 (short-chain) in Holstein. Important genomic regions were identified in the chromosomes BTA3, BTA5, BTA12, BTA13, BTA14, BTA16, BTA18, BTA20, and BTA21. The proportion of the variance explained by 20 adjacent SNPs ranged from 0.70% (SFA) to 1.12% (SCFA) in Ayrshire, from 0.71% (SFA) to 15.12% (LCFA) in Holstein, and from 0.70% (UFA) to 3.23% (MCFA) in Jersey. Important candidate genes with respective pathways were also identified. Important candidate genes and pathways were also identified. The results of this study contribute to better understand the

genetic architecture of predicted milk FA in dairy cattle and will be of great value for the implementation of genomic selection for these traits.

RESUMO

FREITAS, Pedro Henrique Ferreira, M.Sc., Universidade Federal de Viçosa, junho de 2019. **Análises genômicas para composição predita de ácidos graxos do leite em bovinos leiteiros: uma perspectiva longitudinal.** Orientador: Fabyano Fonseca e Silva.

A composição da gordura do leite tem implicações importantes nas propriedades nutricionais e de processamento do leite. Além dos aspectos nutricionais e de saúde, a composição da gordura do leite também pode estar associada ao estado fisiológico e sanitário do animal. A composição de gordura do leite pode ser melhorada através de várias formas, incluindo modificação da dieta da vaca e seleção genética. Os principais objetivos deste estudo foram: 1) estimar parâmetros genéticos para cinco grupos de ácidos graxos do leite (AG) (isto é, cadeia curta, cadeia média, cadeia longa, saturados e insaturados) com base na espectroscopia do infravermelho médio do leite para as raças Ayrshire, Holandês e Jersey; 2) realizar predição genômica de valores genéticos usando uma abordagem longitudinal de um único passo GBLUP para estas cinco características; 3) conduzir um estudo de associação genômica ampla, com o objetivo de identificar regiões genômicas, genes candidatos e vias metabólicas associadas à AG do leite e, consequentemente, compreender os mecanismos biológicos de expressão dessas características. Utilizaram-se 31.709 registros de 9.488 registros de vacas Ayrshire de 268 rebanhos, 629.769 registros de 201.465 vacas Holandês de 6.105 rebanhos e 34.341 registros de 11.479 vacas Jersey de 883 rebanhos. Esses registros foram coletados (e usados para a previsão de AG de leite) entre janeiro de 2013 e julho de 2018. O banco de dados genômico continha um total de 2.330 animais Ayrshire, 8.865 Holandês e 1.019 Jersey. A h^2 média diária variou de 0,18 (AG de cadeia longa) a 0,34 (AG de cadeia média), de 0,24 (AG insaturado) a 0,47 (AGs de cadeia média e insaturado) e de 0,25 (AGs de cadeia longa e insaturado) a 0,52 (AGs de cadeia média e saturado) para Ayrshire, Holandês e Jersey, respectivamente. A confiabilidade da previsão genômica, considerando τ e ω igual a 1 (padrão), variou de 0,540 (AG saturado) a 0,746 (AG insaturado) em Ayrshire, e de 0,564 (AG de cadeia longa) a 0,737 (AG de cadeia média) em Holandês. Ao usar os valores τ e ω ótimos, a confiabilidade do valor genômico estimado variou de 0,528 (AG saturado) a 0,786 (AG insaturado) para Ayrshire, e de 0,583 (AG de cadeia longa) a 0,732 (AG de cadeia curta) para Holandês. Regiões genômicas importantes foram identificadas nos cromossomos BTA3, BTA5, BTA12, BTA13, BTA14, BTA16, BTA18, BTA20, e BTA21. A proporção da variação explicada para 20 SNPs adjacentes variou de 0,70% (AGS) a 1,12% (AGCC) para Ayrshire, de 0,71% (AGS) a 15,12% (AGCL) para Holandês, e de 0,70% (AGI) a 3.23% (AGCM) para Jersey. Importantes genes e vias metabólicas também

foram identificados. Os resultados deste estudo contribuem para um melhor entendimento da arquitetura genética de AG do leite em bovinos leiteiros e serão de grande valia para a implementação da seleção genômica para essas características.

INTRODUCTION

Cow milk has been part of the human diet since the cattle domestication, which occurred around 11,000 years ago (Helme et al., 2005). This is due to the high nutritional quality of milk and milk products (e.g. cheese, yogurt). The current world milk production (from dairy cattle) has been estimated to be 810,652 thousand tons (FAO, 2019). Cows' milk is constituted mainly by water (~ 87%), protein (~3.4%), fat (~ 4.2%), lactose (~ 4.6%), and vitamins and minerals (~ 0.8%) (Fox et al., 1998; Månsson, 2008). The fat proportion (i.e., lipids) has great economic and nutritional importance in the final milk composition, and it is directly related to flavor and chemical-physical characteristics of the milk and milk products, and cheese-making properties (Baer, 1991; Bergamaschi et al., 2016). In this regard, fatty acids (FA) are important components of milk fat, comprising 90% of its weight (Samková et al., 2012). Therefore, genetic selection for fat components is fundamental to improve milk fat composition.

The main sources of FA in milk are: 1) de novo synthesis within the mammary gland; 2) from the uptake of preformed plasma released from body fat stores, formed in the rumen from biohydrogenation or bacterial degradation; and 3) diet (Vlaeminck et al., 2006; Cozma et al., 2013; Fleming, 2016). The large majority of milk fat is triacylglycerols (i.e. 98% of total lipids) and the remaining milk lipids are: diacylglycerols, monoacylglycerols, phospholipids, cholesterol, and free FA (Jensen, 1995). The triacylglycerol FA vary in chain length (number of Carbon atoms), saturation, and arrangement. FA originated in the de novo synthesis occurring in the mammary gland are usually classified as short (from 4 to 8 or 10 Carbons) and medium (10 or 12 to 14 or 16 Carbons) chains, while FA from the blood stream and diet are usually classified as long chain (16 to 18 Carbons) (Cozma et al., 2013; Fleming et al., 2017). FA can also be classified as saturated (**SFA**), i.e., FA that have no double bonds between the individual Carbon atoms, and unsaturated (**UFA**), i.e., FA that have at least one double bond. In brief, SFA account for 70-75% of total fat in cows' milk and it is mainly composed by FA containing from 4 to 18 Carbons. The most common SFA are the palmitic (C16:0), stearic (C18:0) and myristic (C14:0) FA (Taylor, 2006). The UFA account for 25-30% of the total FA in milk and is characterized by the presence of cis-double bond between Carbons 9 and 10, in a Carbon chain length that ranges from 10 to 18 (Ntambi, 1995). The lower amount of UFA in milk compared to SFA is especially due to the biohydrogenation that occurs in the rumen (Sauer et al., 1998).

Nowadays consumers are more often choosing their food sources not only based on the nutritional aspects, but also based on products known to promote better health or prevent diseases. In this regards, the proportion of SFA in milk are a concern (Bilal et al., 2014), as SFA have been associated with increased risk of cardiovascular diseases, obesity and weight gain, as it can increase total and low-density lipoprotein cholesterol in the blood (Temme et al., 1996; Haug, 2007; Zong et al., 2016; Briggs et al., 2017). On the other side, some FA also have antiallergic, antimicrobial, anticarcinogenic and anti-inflammatory properties (Williams, 2000; Parodi, 2004; Haug 2007).

Milk fat composition can be altered through various ways, including dietary changes and genetic selection (Palmquist, 1993; Kęsek, 2014; Narayana et al., 2017). In this context, some countries (e.g. Canada and USA), have encouraged producers to select animals based on their genetic merit for fat content, i.e. fat content is included in the selection index and producers are remunerated based on that (CDC, 2017). In addition to nutritional and health aspects, milk fat composition can also be associated with cow physiological and health status (Overton et al., 2017).

The amount and proportion of different milk FA can be determined routinely in several laboratories around the world, in which two main technologies have been used: gas chromatography (**GC**) and mid-infrared spectroscopy (**MIR**) (Collomb and Buhler, 2000; Soyeurt et al., 2006). Although GC is adequate and reliable (gold-standard measurement technique), it requires skilled staff for the laboratorial analyses, it is time-consuming and more expensive (Soyeurt et al., 2006). On the other hand, the MIR spectroscopy technology allows multiple samples to be analyzed simultaneously, which decreases the analysis time and cost per sample. Due to all its advantages, the MIR technology has become the standard method to quantify milk fat and protein composition (Soyeurt et al., 2006; Narayana et al., 2017). MIR spectra data has also been used to phenotype various other traits, such as detailed milk composition, milk coagulation properties, cow energy status and efficiency, methane emissions and feed efficiency (Soyeurt et al., 2006; McParland et al., 2011, 2012, 2014; Marchi et al., 2014; McDermott et al., 2016; Kandel et al., 2017; Overton et al., 2017).

Fatty acids predicted based on MIR can be routinely used to evaluate animal performance, health status, and to calculate Estimated Breeding Values (**EBVs**) of the animals along the lactation curve using Random Regression Models (**RRM**) (e.g. Narayana et al., 2017). The calculation of EBVs for each lactation day enables modelling of the shape of the lactation curve (Schaeffer et al., 2000), in addition to more precisely identifying external factors that may

be affecting the animal production at different stages of the lactation (Jensen, 2001). With the advancement of genomics and the availability of high-density Single Nucleotide Polymorphism (**SNP**) panels, it became possible to predict more accurate genomic EBVs (**GEBVs**) for young animals compared to the traditional parent average (Meuwissen et al., 2001; Hayes et al., 2009). Therefore, especially for sex-limited (e.g. milk production related traits) and expensive-to-measure traits (e.g. direct individual FA), predicting more accurate GEBVs at a young age using phenotypes predicted based on MIR is a very promising approach.

Milk yield, and consequently FA production, are considered longitudinal traits, and therefore require a special statistical treatment, since the pattern of covariance between repeated measures is well structured (Oliveira et al., 2019). Thus, an alternative to work with such variables is through the application of RRM, proposed by Henderson Jr. (1982). The use of RRM has many advantages when compared to traditional models, including the fact that it does not require a minimum number of measurements per animal, it enables the estimation of (co)variance components between lactation time-points (e.g. test-days), and allows the trait heritability and the genetic value of each animal to be predicted at any point of the lactation curve (Oliveira et al., 2015).

Based on Henderson's traditional model, Legarra et al. (2009) proposed an approach in which the random effect (co)variance matrix simultaneously incorporate pedigree and marker-based SNPs information into one hybrid relationship matrix (**H**). This approach has been termed as single-step Genomic Best Linear Unbiased Prediction (**ssGBLUP**). ssGBLUP has been widely used for genomic predictions in various traits and species (Guarini et al., 2018; Piccoli et al., 2018). In general, it yields more accurate breeding values compared to traditional or two-step genomic predictions (Guarini et al., 2018; Piccoli et al., 2018). In addition to investigating the heritability of predicted FA through the lactation curve and predict GEBV, it is of utmost value to better understand the underlying genetic mechanisms associated with the phenotypic expression of milk FA. By performing Genome-wide Association Studies (**GWAS**), genomic regions and candidate genes associated with milk FA can be identified and used in selection schemes to alter milk FA composition (Palombo et al., 2018).

This study aimed to: 1) estimate genetic parameters for five milk FA groups (i.e., short-chain, medium-chain, long-chain, SFA, and UFA) predicted based on MIR spectroscopy information for the first parity of Ayrshire, Holstein and Jersey breeds; 2) perform genomic predictions over time for these five traits; 3) conduct single-step GWAS to identify genomic

regions and candidate genes associated with milk FA, and consequently, understand the underlying biology of these traits based on candidates genes and metabolic pathways.

MATERIALS AND METHODS

Data

The pedigree, phenotypic and genomic datasets were provided by the Canadian Dairy Network (**CDN**; Guelph, ON, Canada). The milk FA composition were predicted over time for the first lactation of Canadian Ayrshire, Holstein and Jersey breeds, according to the FA prediction equations developed by Fleming et al. (2017). No Animal Care Committee approval was necessary for the purposes of this study, as all information required was obtained from pre-existing databases.

Phenotypes

Milk FA composition used in this study was measured for specific days (i.e., test-days) using MIR spectra obtained from the routine milk recording systems, and predicted using the calibration equations developed by Fleming et al. (2017). In brief, Fleming et al. (2017) analyzed the FA profile of individual milk samples using gold standard GC methodologies and through Partial Least Squares Regression models, they developed equations to predict FA concentrations from the MIR spectra of milk samples. The FA were classified into five groups based on the length of the Carbon chain and degree of saturation: 1) short-chain (4 to 10 Carbons; **SCFA**); 2) medium-chain (12 to 16 Carbons; **MCFA**); 3) long-chain (**LCFA**, 17 to 22 Carbons); 4) saturated (**SFA**, no double bond); and 5) unsaturated (**UFA**, one or more double bonds).

The milk FA test-day records used in this study were for milk samples collected between January 2013 and July 2018. Only data from first-parity cows, recorded and/or predicted from 5 to 305 days in milk (**DIM**), were kept in the dataset for further analyses. Milk FA predictions for samples with MIR spectral data considered as outliers, and records dissimilar to those used to develop the calibrations were removed from the analyses. In addition, the test-day records with an observation removed for at least one FA group were removed, as it indicates that the FA groups for that specific sample could have been poorly predicted. Herd-Test-Day (**HTD**) with less than four cows, and records above or below three standard deviations from the mean,

within each HTD, were removed from the analysis. The final dataset contained 31,709 records of 9,648 Ayrshire cows from 268 herds, 629,769 records of 201,465 Holstein cows from 6,105 herds, and 34,341 records of 11,479 Jersey cows from 883 herds. The number of test-days per cow ranged from 2 to 10; 2 to 12; and 2 to 11 for Ayrshire, Holstein, and Jersey, respectively.

Pedigree and Genotypes

The pedigree files (for all available generations, i.e. no pedigree truncation) for the Ayrshire, Holstein, and Jersey breeds contained 687,070, 7,587,436, and 976,198 animals, respectively. From those, a total of 2,330 Ayrshire, 8,865 Holstein, and 1,019 Jersey animals were either genotyped with the Illumina BovineSNP50K BeadChip (Illumina, San Diego, CA, USA) or with a lower-density panel and accurately imputed to 50K using the FImpute software (Sargolzaei et al., 2014). Genotypic quality control was performed using the preGSf90 software (Aguilar et al., 2014; Misztal et al., 2014b), and SNPs with Mendelian conflicts, duplicated or unknown position, call rate less than 0.95, and minor allele frequency (**MAF**) less than 0.05 were removed. A total of 41,507, 44,368 and 38,248 SNPs remained for further analyses.

Variance Component Estimation

Variance components for all traits and breeds were estimated using a Bayesian approach and the pedigree-based relationship matrix (**A**). Analyses were performed using a single-trait RRM under a Markov chain Monte Carlo (**MCMC**) framework (using Gibbs sampler algorithm) implemented in the GIBBS2F90 software (Misztal, 2001). The general RRM used for each trait and breed can be described as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_h h} + \mathbf{Z_a a} + \mathbf{Z_p p} + \mathbf{e},$$

where: **y** is the vector of longitudinal observations [FA content (g/100 g of milk)]; **β** is the vector of systematic effects, which included HTD and the systematic regression coefficients for age-season of calving effect; **h** is the vector of random regression polynomial coefficients for herd-year of calving effect; **a** is the vector of random regression coefficients for animal additive genetic effect; **p** is the vector of regression coefficients for permanent environmental effect; and **e** is the residual vector. **X**, $\mathbf{Z_h}$, $\mathbf{Z_a}$, and $\mathbf{Z_p}$ are the corresponding incidence matrices for the mentioned effects. As suggested by Narayana et al. (2017), when analyzing genetic parameters for FA in the same Holstein cattle population (using a subset of the current dataset), both systematic and random effects were modeled based on third order Legendre orthogonal

polynomials (Kirkpatrick et al., 1990). The (co)variance structure for the normal assumed random effects was:

$$V\begin{bmatrix}\mathbf{h}\\ \mathbf{p}\\ \mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{D} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{P} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{T} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{E}\end{bmatrix},$$

where: $\mathbf{D} = \mathbf{I} \otimes \mathbf{D_0}$, $\mathbf{P} = \mathbf{I} \otimes \mathbf{P_0}$, and $\mathbf{T} = \mathbf{A} \otimes \mathbf{T_0}$; in which **I** is an identity matrix, **A** is the additive pedigree-based relationship matrix, $\otimes$ is the Kronecker product, and $\mathbf{D_0}$, $\mathbf{P_0}$, and $\mathbf{T_0}$ are (co)variance matrices for herd-year of calving, permanent environment, and additive genetic regression coefficients, respectively. Variances for different days were obtained by multiplying the matrix of Legendre orthogonal polynomials for all days (from 5 to 305 days) by the (co)variance matrices for regression coefficients, as described in Oliveira et al. (2019).

For all traits and breeds, the convergence of Gibbs sampling algorithm was verified through graphical analysis and Raftery and Lewis criterion (Raftery and Lewis, 1992), both available in the package Bayesian Output Analysis (Smith, 2007) of the R software (R Core Team, 2018). Therefore, a MCMC chain length of 700,000 cycles, considering a burn-in period of 250,000 cycles, and a sampling interval (thin) of 20 cycles were used in the Ayrshire's and Jersey's analyses. For the Holstein breed, a MCMC chain length of 270,000 cycles, considering a burn-in period of 30,000 cycles, and a sampling interval (thin) of 15 cycles were used, as it was enough to achieve the convergence criteria. Thereafter, daily heritability (h^2) were calculated for each lactation day (from 5 to 305 days) as follow:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_h^2 + \sigma_p^2 + \sigma_e^2},$$

where σ_g^2 is the additive genetic variance, σ_h^2 is the herd-year variance, σ_p^2 is the permanent environmental variance, and σ_e^2 is the residual variance. The general h^2 for each trait was obtained as the average daily h^2 estimates.

Genomic Predictions

Single-step Genomic Best Linear Unbiased Prediction approach (ssGBLUP)

In the ssGBLUP method, the **H** matrix is used to simultaneously combine the pedigree (i.e., the **A** matrix) and genomic (based on SNPs markers, i.e., on the **G** matrix) information. Thus, the same RRMs used for the estimation of variance components were used in the

ssGBLUP method, with the exception that **A** was replaced by the **H** matrix. As the direct estimation of **H** is computationally demanding, the $\mathbf{H^{-1}}$ was calculated as (Aguilar et al., 2010):

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{pmatrix} 0 & 0 \\ 0 & \tau(0.95\mathbf{G} + 0.05\mathbf{A_{22}})^{-1} - \omega\mathbf{A_{22}^{-1}} \end{pmatrix}$$

where: $\mathbf{A^{-1}}$ is the inverse of **A**, $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix (calculated using the first method presented in VanRaden, 2008), and $\mathbf{A_{22}^{-1}}$ is the inverse of the portion of **A** related to genotyped animals. As usually $\mathbf{G^{-1}}$ and $\mathbf{A_{22}^{-1}}$ are not in the same scale, scaling factors (e.g., τ and ω) are used to make both matrices more compatible. The values reported by Oliveira et al. (2019) for milk fat yield, in the same populations, were used in this study and considered for purposes of differentiation as the optimal value (i.e., $\tau = 2$ and $\omega = 0.6$ for the Ayrshire breed and $\tau = 1.5$ and $\omega = 0.6$ for Holstein).

The ssGBLUP analyses were performed for the Ayrshire and Holstein breeds using the BLUPF90 software (Misztal et al., 2014). The variance components previously estimated were assumed as the true variance components for the analyzed populations. Due to the small number of genotyped animals that had phenotypes (or daughters with phenotypes), no genomic prediction was performed for the Jersey breed.

Training and Validation Populations

Animals born between 1961 and 2011, and between 1957 and 2009, were defined as training populations for the Ayrshire and Holstein breeds, respectively. The number of genotyped animals in the training population was 1,816 and 8,489 for the Ayrshire and Holstein breeds, respectively. Genotyped animals born between 2012 and 2014, and between 2010 and 2012 were included in the validation population. The total number of validation animals for all traits was 514 and 376, for Ayrshire and Holstein, respectively. The phenotypes from all Ayrshire animals born on or after 2012 and Holstein animals born on or after 2010 were excluded from the analyses, in order to create a reduced dataset to be used to predict the GEBVs. The GEBVs of the validation animals were used to evaluate the reliability ($\mathbf{r^2}$) and bias of genomic predictions for each trait.

Reliability and Bias of Genomic Predictions

The reliability of genomic predictions was estimated as the squared Pearson correlation coefficient between daily GEBVs, predicted using the reduced dataset, with daily EBVs

predicted using the complete dataset (i.e., without excluding phenotypes for the validation animals or their descendants). The bias of the genomic predictions was calculated by obtaining the regression coefficient (**b_1**) estimated using a linear regression of EBVs (from the complete datasets) on GEBVs (from the reduced datasets), i.e.: EBV = b_0 + b_1xGEBV. Only animals from the validation population were used to calculate r^2 and b_1.

In order to analyze the advantages of including genomic information in the genetic evaluation of milk FA, the parent average (**PA**) was predicted for the validation animals. PA was used to calculate r^2 and b_1 using daily PAs (predicted using the reduced datasets) and daily EBVs (predicted using the complete datasets) for the animals in the validation population.

Genome-wide Association Studies and Functional Analyses

Genome-wide association analyses were performed using the postGSf90 software (Aguilar et al., 2014), considering the variance explained by 20 adjacent SNPs windows. The postGSf90 software back-solves the additive genomic random regression coefficients (i.e., the GEBVs for the additive random regression coefficients) to SNP effects and it can be described as follow (Wang et al., 2012):

$$\hat{\mathbf{u}}_c = \mathbf{M}'[\mathbf{M}\mathbf{M}']^{-1}\mathbf{G}\hat{\mathbf{E}}\mathbf{BV}_c,$$

where $\hat{\mathbf{u}}_c$ is the vector of estimated SNP solutions for the c^{th} random regression coefficient; **M** is the matrix that contains the centered genotypes (i.e., -1, 0 and 1 representing AA, Aa and aa, respectively), and $\mathbf{G\hat{E}BV}_c$ is the vector of GEBV for the c^{th} random regression coefficient, estimated in the ssGBLUP analyses, which contains the c^{th} random regression coefficients for all the genotyped animals.

Further, to calculate the SNP effects along the lactation, the SNP solutions for all random regression coefficients (c = 1, 2, 3, 4) of a same SNP k were combined into a vector ($\hat{\mathbf{u}}_k = [\hat{\mathbf{u}}_{k1}\ \hat{\mathbf{u}}_{k2}\ \hat{\mathbf{u}}_{k3}\ \hat{\mathbf{u}}_{k4}]$), and used to estimate the SNP effects for all DIM (from 5 to 305 days) as:

$$\mathbf{S\hat{N}P_k} = \mathbf{T}\hat{\mathbf{u}}_k,$$

where: $\mathbf{S\hat{N}P_k}$ is the vector that contains the SNP effects estimated for every DIM of the k^{th} SNP, **T** is a matrix of covariates for each DIM, associated with the third-order Legendre orthogonal

polynomials, and $\hat{\mathbf{u}}_k$ is the vector of SNP solutions for all random regression coefficients related to the k^{th} SNP.

The threshold of 0.70% of the total genetic variance explained by each genomic window was used to define the important genomic regions associated with the traits included in this study. This threshold was defined as the highest peak for the trait/breed with the lowest variance explained by each genomic window. Positional candidate genes were mapped using the Biomart tool (Kinsella et al., 2011) embedded in the Ensembl Genes database version 96 (http://useast.ensembl.org/index.html). Based on the start and end chromosomal positions, important genomic regions were further investigated to understand the biological processes related to the studied traits and to define the most likely functional candidate genes. Complete gene functions were obtained from the National Center for Biotechnology Information database (**NCBI**, www.ncbi.nlm.nih.gov/gene/) based on Bos taurus ARS-UCD1.2. The biological functions and KEGG pathways (Kenehisa et al., 2000; Kenehisa et al., 2016; Kenehisa et al., 2018) in which these genes are involved were assessed using the Database for Annotation, Visualization and Integrated Discovery (**DAVID**) v6.8. (Huang et al., 2009a, 2009b).

RESULTS

Descriptive Statistics

Table 1 shows the descriptive statistics for the different groups of milk FA in the three breeds. On average, the proportion of LCFA was 35.7%, 33.85% and 35.2% for Ayrshire, Holstein and Jersey, respectively. The proportion of MCFA was 53.15%, 55.5% and 53.2%, while for SCFA, the proportion was 11.15%, 10.60% and 11.61%, for Ayrshire, Holstein and Jersey, respectively. The proportion of SFA was 73.4%, 73.2% and 75.2%, while for UFA was 26.6%, 26.8% and 24,8% for Ayrshire, Holstein and Jersey, respectively. The highest average FA content was observed for the Jersey breed; however, the proportion of FA groups was similar among all breeds. In general, there were higher proportions of MCFA, followed by LCFA and SCFA (lowest).

Table 1. Descriptive statistics for the different groups of milk fatty acids in the Ayrshire, Holstein and Jersey breeds.

Breed	Fatty acid group (g/100 g of milk)	Mean	SD	Minimum	Maximum
Ayrshire	**LCFA**	1.492	0.38	0.363	4.266
	MCFA	2.220	0.51	0.233	4.972
	SCFA	0.466	0.07	0.117	0.914
	SFA	3.067	0.57	0.604	6.393
	UFA	1.114	0.26	0.332	3.494
Holstein	**LCFA**	1.331	0.39	0.143	4.48
	MCFA	2.183	0.44	0.513	4.497
	SCFA	0.417	0.09	0.063	0.848
	SFA	2.896	0.53	0.702	5.689
	UFA	1.065	0.26	0.171	3.596
Jersey	**LCFA**	1.759	0.41	0.238	4.656
	MCFA	2.656	0.59	0.436	5.401
	SCFA	0.578	0.10	0.121	1.052
	SFA	3.769	0.67	0.612	7.088
	UFA	1.242	0.27	0.259	3.731

SD: standard deviation. Fatty acids groups were defined as: long-chain fatty acids (LCFA; 17 to 22 Carbons), medium-chain fatty acids (MCFA; 12 to 16 Carbons), short-chain fatty acids (SCFA; 4 to 10 Carbons), saturated fatty acids (SFA; no double bond), and unsaturated fatty acids (UFA; one or more double bonds).

Genetic Parameters

The average daily h^2 estimates for each FA are shown in Table 2 and the complete pattern of daily h^2 for Ayrshire, Holstein and Jersey breeds are shown in Figure 1. The average daily h^2 ranged from 0.18 (LCFA) to 0.34 (MCFA), from 0.24 (UFA) to 0.47 (SFA and MCFA) and from 0.25 (LCFA and UFA) to 0.52 (MCFA and SFA) for Ayrshire, Holstein and Jersey, respectively. In general, h^2 observed at the beginning of the lactation was similar for all three breeds (~ 0.20). Especially for LCFA, a slight increase in the h^2 estimates was observed for Holstein and Jersey around the first third of the lactation and remained stable until the end of the lactation. In contrast, for the Ayrshire breed, h^2 remained almost constant until the end of

the second lactation stage, where it had a slight increase followed by a decrease in the estimates at the end of the lactation curve. For MCFA in Ayrshire, the h^2 daily values were similar across lactation, while for Holstein and Jersey there was an increase from the middle of the lactation to the end. The h^2 pattern for SCFA was similar to MCFA for the Holstein and Jersey breeds, but for Ayrshire there was a decrease in daily h^2 at the end of the lactation curve. For SFA the h^2 curve followed the same pattern for Ayrshire and Holstein, but with higher values for Holstein. Especially for UFA, the daily h^2 estimated for the three breeds followed a similar pattern over time.

Table 2. Average daily heritability (h^2) and standard deviation (SD) for five groups of milk fatty acids in the Ayrshire, Holstein and Jersey breeds.

	Ayrshire		**Holstein**		**Jersey**	
FA group	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
LCFA	0.18	0.04	0.25	0.07	0.25	0.08
MCFA	0.34	0.06	0.47	0.10	0.52	0.13
SCFA	0.33	0.07	0.31	0.09	0.51	0.11
SFA	0.31	0.06	0.47	0.09	0.52	0.13
UFA	0.20	0.05	0.24	0.07	0.25	0.09

SD: standard deviation along the lactation curve. Fatty acids groups were defined as: long-chain fatty acids (LCFA; 17 to 22 Carbons), medium-chain fatty acids (MCFA; 12 to 16 Carbons), short-chain fatty acids (SCFA; 4 to 10 Carbons), saturated fatty acids (SFA; no double bond), and unsaturated fatty acids (UFA; one or more double bonds).

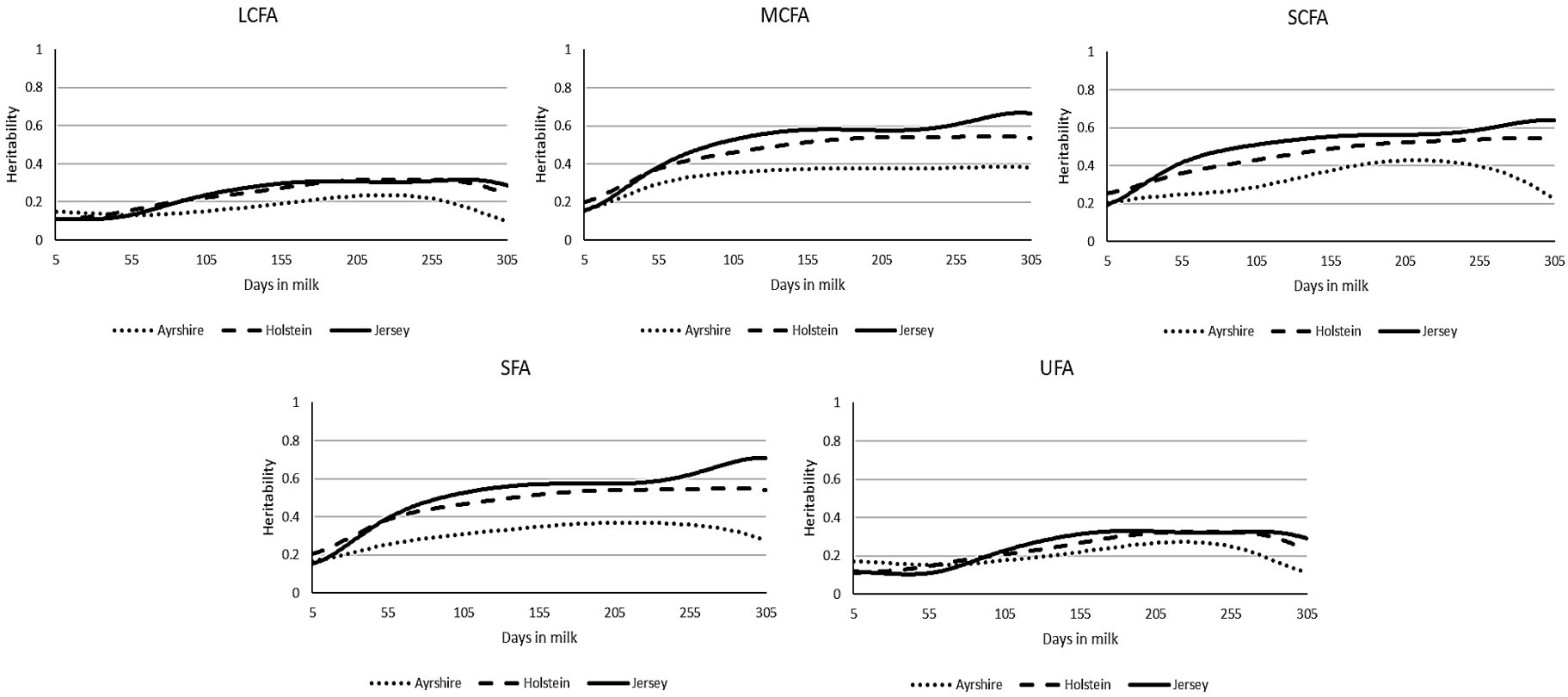

Figure 1. Daily heritability estimates of long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) milk fatty acid groups for the Ayrshire, Holsteins and Jersey breeds.

Reliability and Bias of Genomic Predictions

Average validation r^2 and their standard deviation for the Ayrshire and Holstein breeds, considering both analyzes, with and without (i.e., considering the default values) optimal τ and ω, are shown in Table 3. When considering τ and ω equal to 1 (default), the average r^2 in Ayrshire ranged from 0.540 (SFA) to 0.746 (UFA), and from 0.564 (LCFA) to 0.737 (SCFA) in Holstein. When using the optimal τ and ω values, the GEBV r^2 ranged from 0.528 (SFA) to 0.786 (UFA) in Ayrshire, and from 0.583 (LCFA) to 0.732 (SCFA) in Holstein. When compared to the Ayrshire breed, the GEBV r^2 estimated for Holstein were higher for all traits, except for UFA and LCFA. For both breeds, the inclusion of optimal values of τ and ω slightly changed (in both directions) the average r^2. An increase in r^2 was observed for LCFA and UFA when including the optimal values for τ and ω. However, a slight decrease in r^2 was observed for the other three traits. For all traits, the inclusion of genomic information increased the average r^2 for the Holstein breed, in both scenarios (with and without the optimal values for τ and ω). For the Ayrshire breed, the average GEBV r^2 were not higher than PA r^2. In addition, the PA r^2 were higher for the Ayrshire than for the Holstein breed.

The pattern of GEBV r^2 curves over time without and with the optimal values for τ and ω are shown in Figures 2 and 3, respectively. For Holstein, the GEBV r^2 were approximately constant over DIM, while they changed substantially across lactation for the Ayrshire breed. In summary, higher GEBV r^2 were estimated for SFA, SCFA and MCFA in the Holstein breed compared to the Ayrshire breed. On the other hand, higher GEBV r^2 were estimated for LCFA and UFA in the Ayrshire than for Holstein breed, in most lactation stages. Slight differences in the pattern of the GEBV r^2 curves were observed when including or not τ and ω in the analyses.

The average b_1 (indicator of bias), and its respective standard deviations, are shown in Table 4. The average b_1 for PA ranged from 0.949 (LCFA) to 1.032 (SFA) for the Ayrshire, and from 0.690 (UFA) to 1.062 (SCFA) for the Holstein breed. When no optimal values of τ and ω were used (i.e., the default values were applied), the b_1 coefficients ranged from 0.750 (SFA) to 0.862 (UFA) for the Ayrshire; and from 0.756 (LCFA) to 0.905 (SCFA), for the Holstein breed. When the optimal values of τ and ω were used, b_1 ranged from 1.091 (SFA) to 1.282 (UFA), and from 1.075 (LCFA) to 1.172 (SCFA), in Ayrshire and Holstein, respectively. In summary, an improvement in the bias (i.e., b_1 closer to 1) was observed for the majority of traits in Ayrshire (LCFA, MCFA, SCFA and SFA) when optimal τ and ω were used, and for all traits in the Holstein breed.

Table 3. Average validation reliability (and standard deviation) estimated for parent average (r^2_{PA}) and genomic breeding values predicted using ($r^2_{GEBV\tau\omega}$) or not (r^2_{GEBV}) the optimal values for τ and ω, for five groups of milk fatty acids in the Ayrshire and Holstein breeds.

Trait	Ayrshire			Holstein		
	r^2_{PA}	r^2_{GEBV}	$r^2_{GEBV\tau\omega}$	r^2_{PA}	r^2_{GEBV}	$r^2_{GEBV\tau\omega}$
SCFA	0.717(0.028)	0.604(0.026)	0.582(0.038)	0.587(0.019)	0.737(0.022)	0.732(0.012)
SFA	0.766(0.009)	0.540(0.039)	0.528(0.026)	0.551(0.018)	0.701(0.020)	0.680(0.008)
MCFA	0.782(0.016)	0.548(0.048)	0.535(0.027)	0.548(0.017)	0.698(0.016)	0.678(0.010)
UFA	0.696(0.023)	0.746(0.051)	0.786(0.056)	0.437(0.046)	0.587(0.043)	0.619(0.031)
LCFA	0.776 (0.080)	0.645(0.074)	0.665(0.090)	0.414(0.032)	0.564(0.032)	0.583(0.025)

The optimal values assumed for Ayrshire and Holstein were τ = 2.0 and ω = 0.6; and τ = 1.5 and ω = 0.6, respectively. LCFA: long-chain fatty acids; MCFA: medium-chain fatty acids; SCFA: short-chain fatty acids; SFA: saturated fatty acids; and UFA: unsaturated fatty acids.

The pattern of bias over time without and with the optimal values for τ and ω are shown in Figures 4 and 5, respectively. Similar pattern of bias over time were found when using or not the optimal values of τ and ω. In general, GEBVs predicted over time without the optimal values were deflated, and GEBVs predicted over time using the optimal values were inflated.

Table 4. Regression coefficients (and standard deviations) estimated for parent average (b_{PA}), and Genomic Estimated Breeding Values predicted using ($b_{GEBV\tau\omega}$) or not (b_{GEBV}) the optimal values for τ and ω, for five groups of milk fatty acids in the Ayrshire and Holstein breeds.

Trait	Ayrshire			Holstein		
	b_{PA}	b_{GEBV}	$b_{GEBV\tau\omega}$	b_{PA}	b_{GEBV}	$b_{GEBV\tau\omega}$
SCFA	1.020(0.018)	0.789(0.014)	1.133(0.028)	1.062(0.025)	0.905(0.046)	1.172(0.028)
SFA	1.032(0.025)	0.750(0.030)	1.091(0.036)	1.030(0.018)	0.883(0.035)	1.140(0.021)
MCFA	1.038(0.023)	0.754(0.034)	1.099(0.043)	1.054(0.018)	0.759(0.032)	1.164(0.020)
UFA	0.957(0.096)	0.862(0.118)	1.282(0.155)	0.690(0.052)	0.800(0.052)	1.120(0.033)
LCFA	0.949(0.125)	0.789(0.120)	1.190(0.169)	0.965(0.021)	0.756(0.045)	1.075(0.022)

The optimal values assumed for Ayrshire and Holstein were τ = 2.0 and ω = 0.6; and τ = 1.5 and ω = 0.6, respectively. LCFA: long-chain fatty acids; MCFA: medium-chain fatty acids; SCFA: short-chain fatty acids; SFA: saturated fatty acids; and UFA: unsaturated fatty acids.

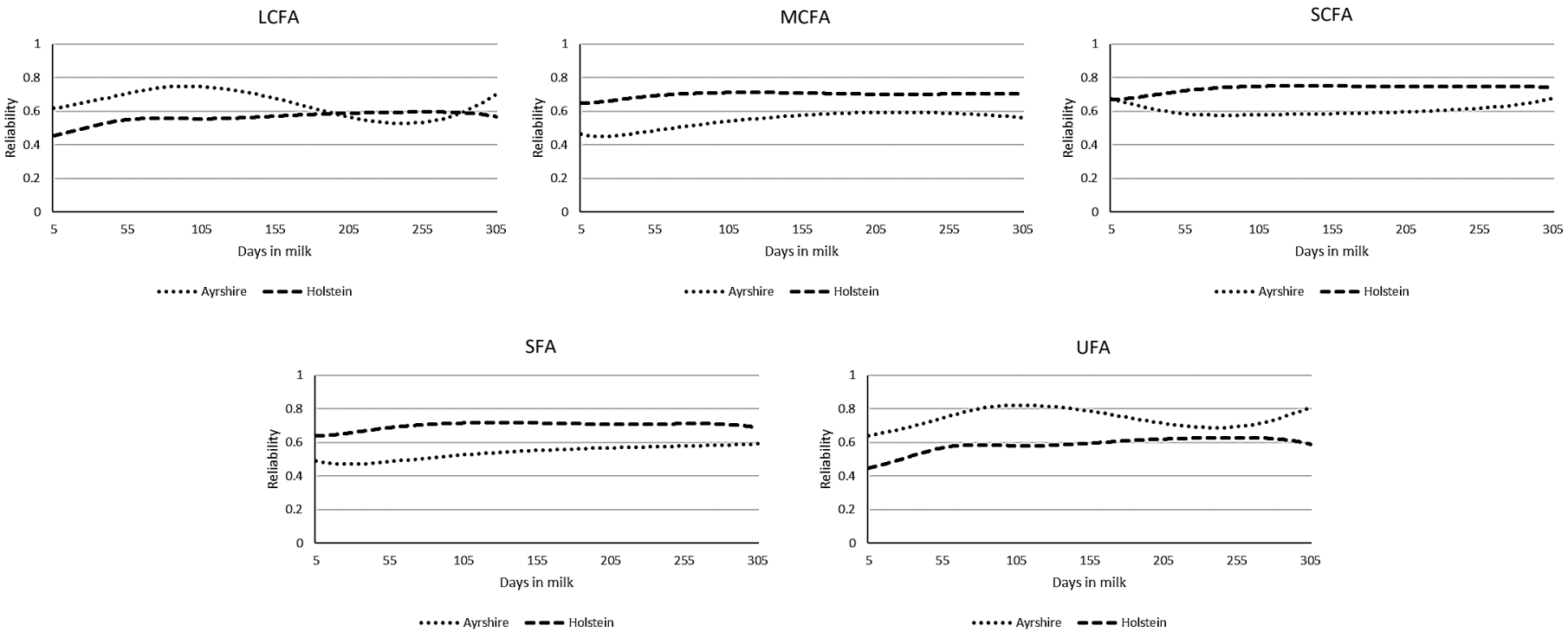

Figure 2. Daily reliabilities using τ and ω equal to 1 (default) for long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) milk fatty acid groups for the Ayrshire and Holstein breeds.

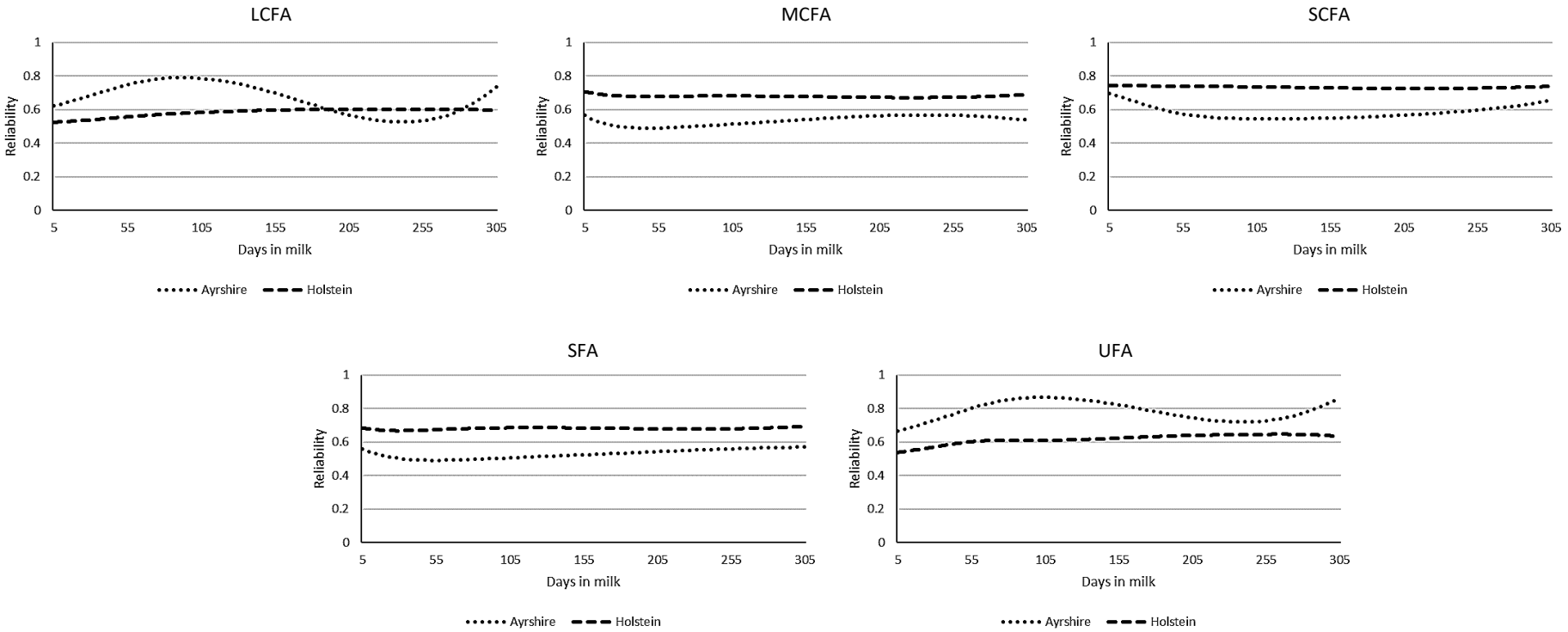

Figure 3. Daily reliabilities using optimal τ and ω for long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) milk fatty acid groups for the Ayrshire and Holstein breeds.

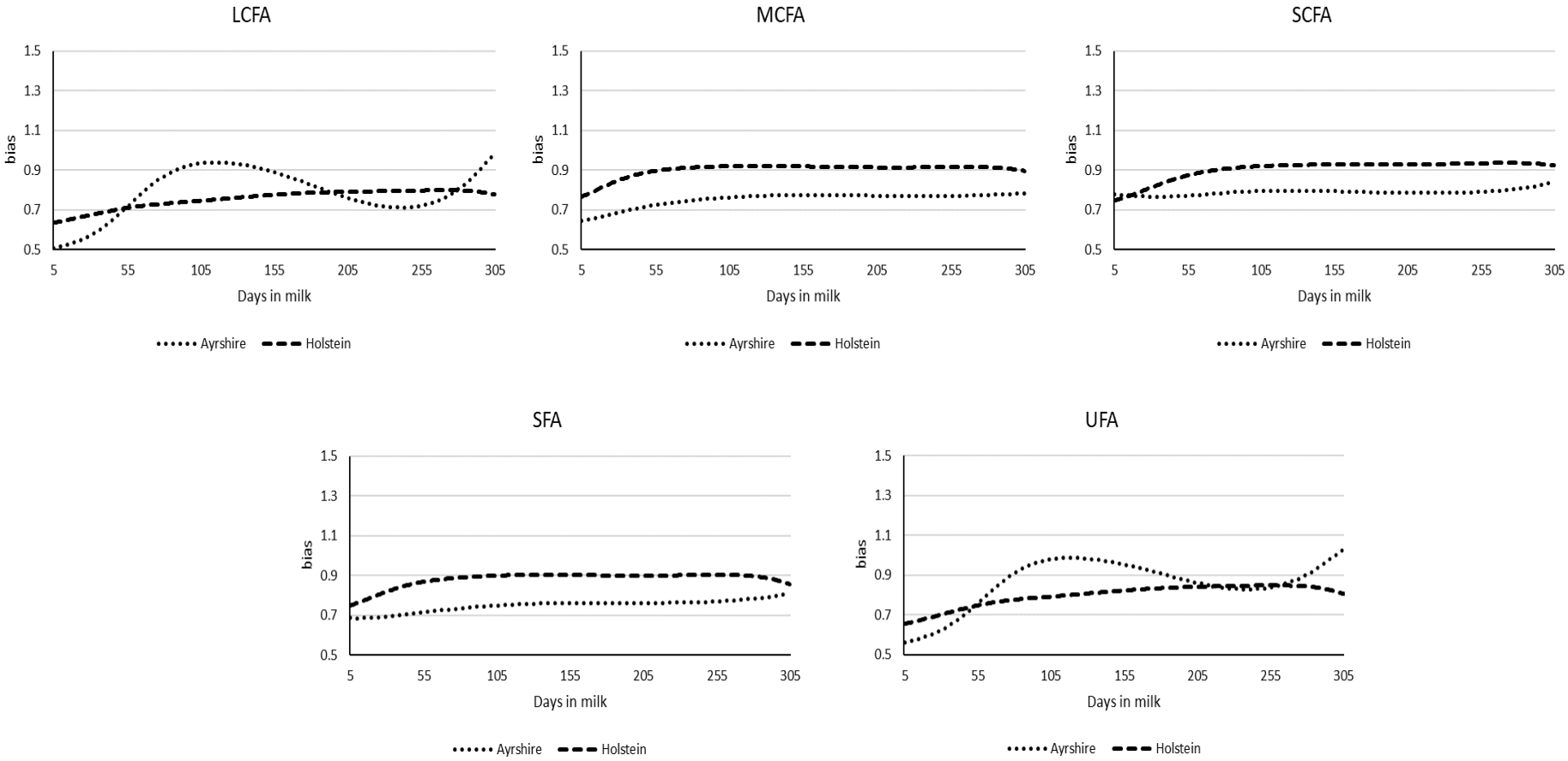

Figure 4. Daily bias (b_1) using τ and ω equal to 1 (default) for long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) fatty acid groups for the Ayrshire and Holstein breeds.

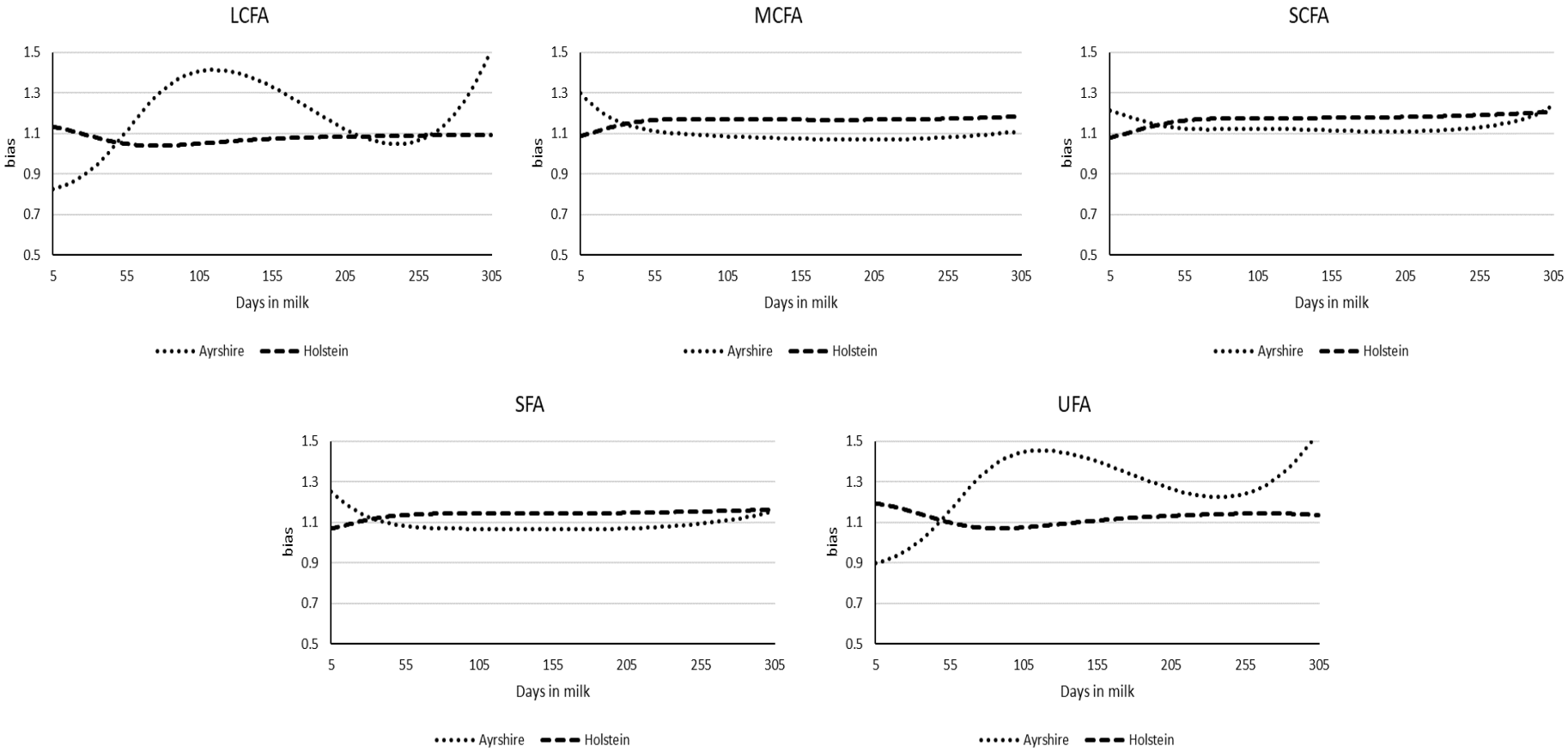

Figure 5. Daily bias (b_1) using optimal τ and ω for long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) milk fatty acid groups for the Ayrshire and Holstein breeds.

Genome-wide Association Studies and Functional Analyses

A total of 15, 39 and 16 genomic windows were associated with the five FA groups in Ayrshire, Holstein and Jersey, respectively. These genomic regions located in the chromosomes BTA3, BTA5, BTA12, BTA13, BTA18, BTA20 in Ayrshire, BTA5, BTA13, BTA14 in Holstein and BTA3, BTA14, BTA16, BTA20, BTA21 in Jersey. The maximum proportion of the additive genetic variance 1.12% (SCFA), 15.12% (LCFA) and 3.23% (MCFA) for Ayrshire, Holstein and Jersey, respectively. The Manhattan plots of the proportion of additive genetic variance explained for each third-order Legendre coefficients for Ayrshire, Holstein and Jersey are shown in Supplementary Material Figures S1 to S10, S11 to S20 and S21 to S30, respectively. The Manhattan plots show that the peaks of the markers were different when scaling factors with optimal values (tau and omega) or equal to 1 (default). With the use of the optimal values, some regions that had been identified as significant with the use of the default value, became less important (i.e. BTA5 on LCFA for Ayrshire).

A total of 415, 444 and 382 SNPs were selected as the most relevant (top 1%) for each trait of the Ayrshire, Holstein and Jersey breeds, respectively. When considering τ and ω equal to 1 (default), the proportion of variance explained by the 20-SNP windows ranged from 0.70% (SFA) to 1.12% (SCFA) in Ayrshire, from 0.72% (UFA) to 15.12% (LCFA) in Holstein, and from 0.71% (SFA) to 3.23% (MCFA) in Jersey. When using the optimal τ and ω values, the proportion of variance explained ranged from 0.71% (SFA) to 1.11% (LCFA) in Ayrshire, from 0.71% (SFA) to 15.10% (LCFA) in Holstein, and from 0.70% (UFA) to 3.09% (MCFA) in Jersey. In relation to SNPs effects over DIM, considering default values for τ and ω, it ranged (in module) from 0.016 (LCFA) to 0.902 (SFA) in Ayrshire, from 0.575 (SCFA) to 14.968 (SFA) in Holstein, and from 0.051 (LCFA) to 4.231 (SFA) in Jersey breeds. When using the optimal τ and ω values the SNP effect over DIM ranged 0.018 (LCFA) to 0.965 (SFA) in Ayrshire, from 0.596 (SCFA) to 14.974 (SFA) in Holstein, and from 0.058 (LCFA) to 4.240 (SFA) in Jersey breeds. For all traits, the largest SNP effects and genomic windows explaining the highest proportion of the additive genetic variance were found for the Holstein breed compared to Ayrshire and Jersey.

The trajectory of SNP effects over DIM, estimated for the top 10 SNPs of each trait considering optimal τ and ω are shown in Figures 9 to 11, for Ayrshire, Holstein and Jersey breeds, respectively. The figures for the SNP effects when considering the use of τ and ω equal to 1 are presented in the Supplementary Material Figures S31 to S33. The use default value of τ and ω or its optimal value did not alter the pattern of the effect curves of the SNPs over DIM.

The pattern of SNPs effects over lactation for LCFA, SCFA and UFA tended to remain constant, with little changes in their magnitude. Higher deviations in the pattern of SNP effects were observed for MCFA and SFA, where for the three breeds the effects of SNPs increased as lactation progressed. Moreover, higher values of SNP effect by lactation are observed for the Holstein breed, followed by Jersey and Ayrshire breeds.

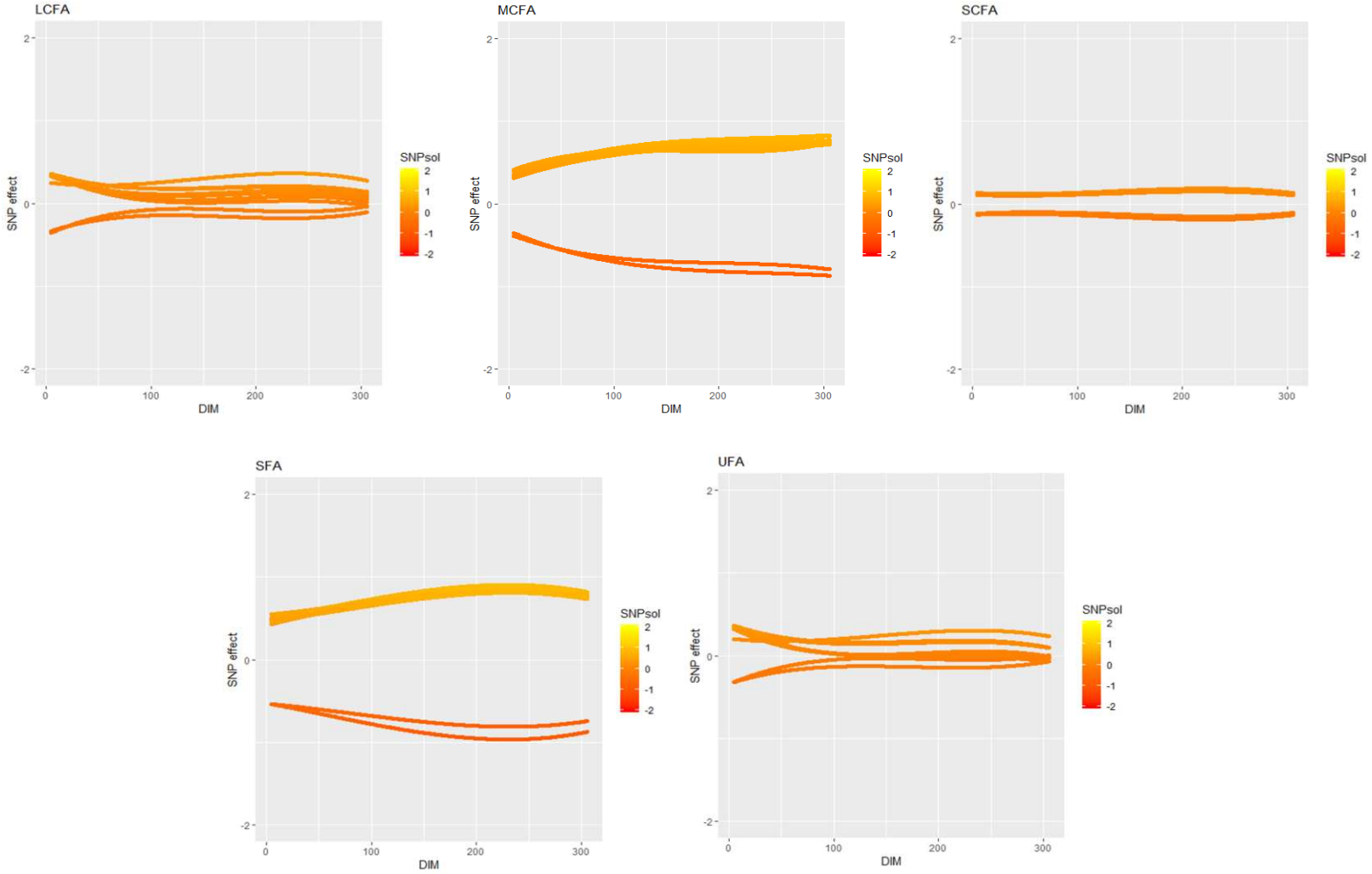

Figure 6. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated milk fatty acids using optimal τ and ω, in the Ayrshire breed.

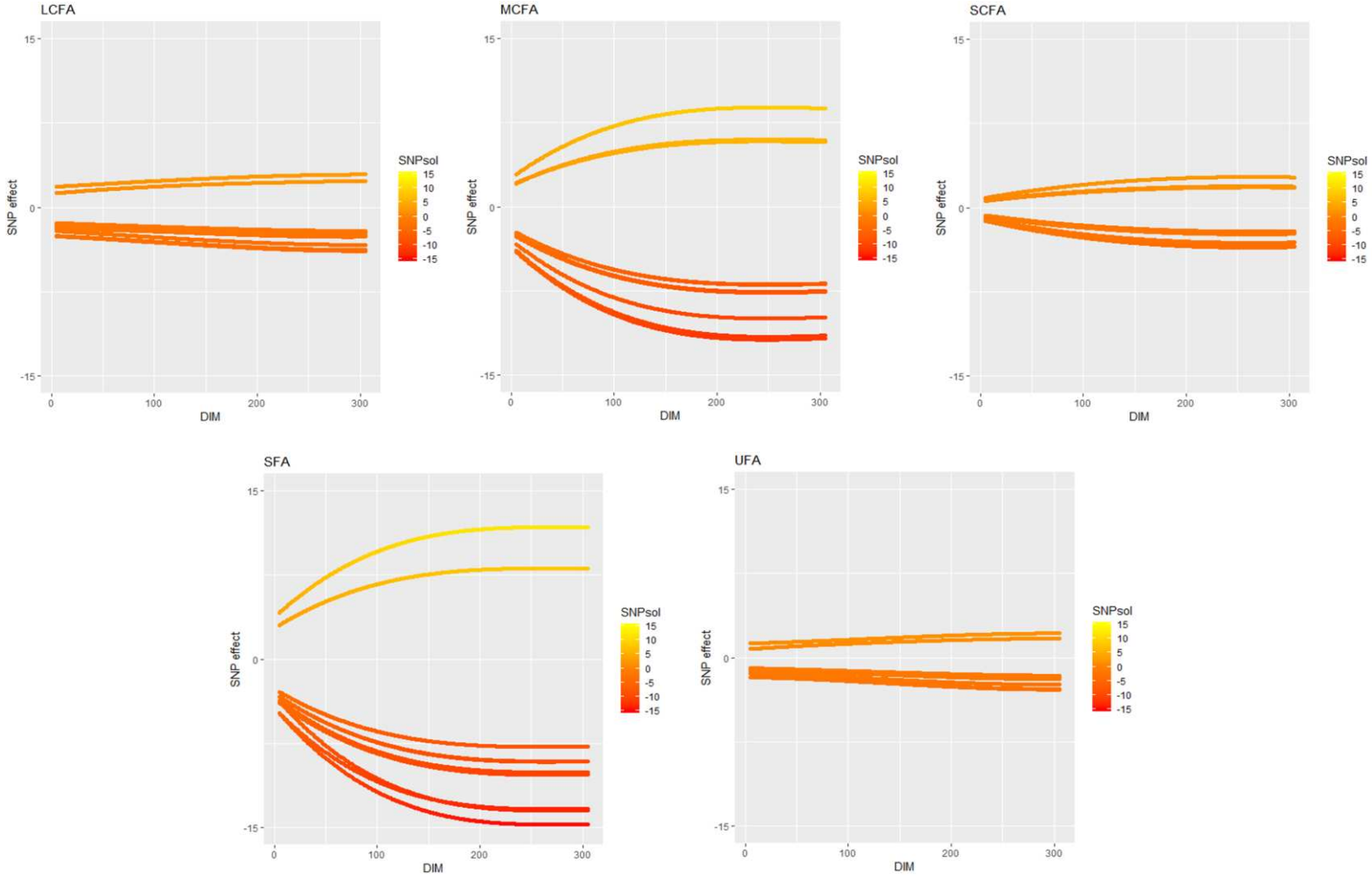

Figure 7. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated milk fatty acids using optimal τ and ω, in the Holstein breed.

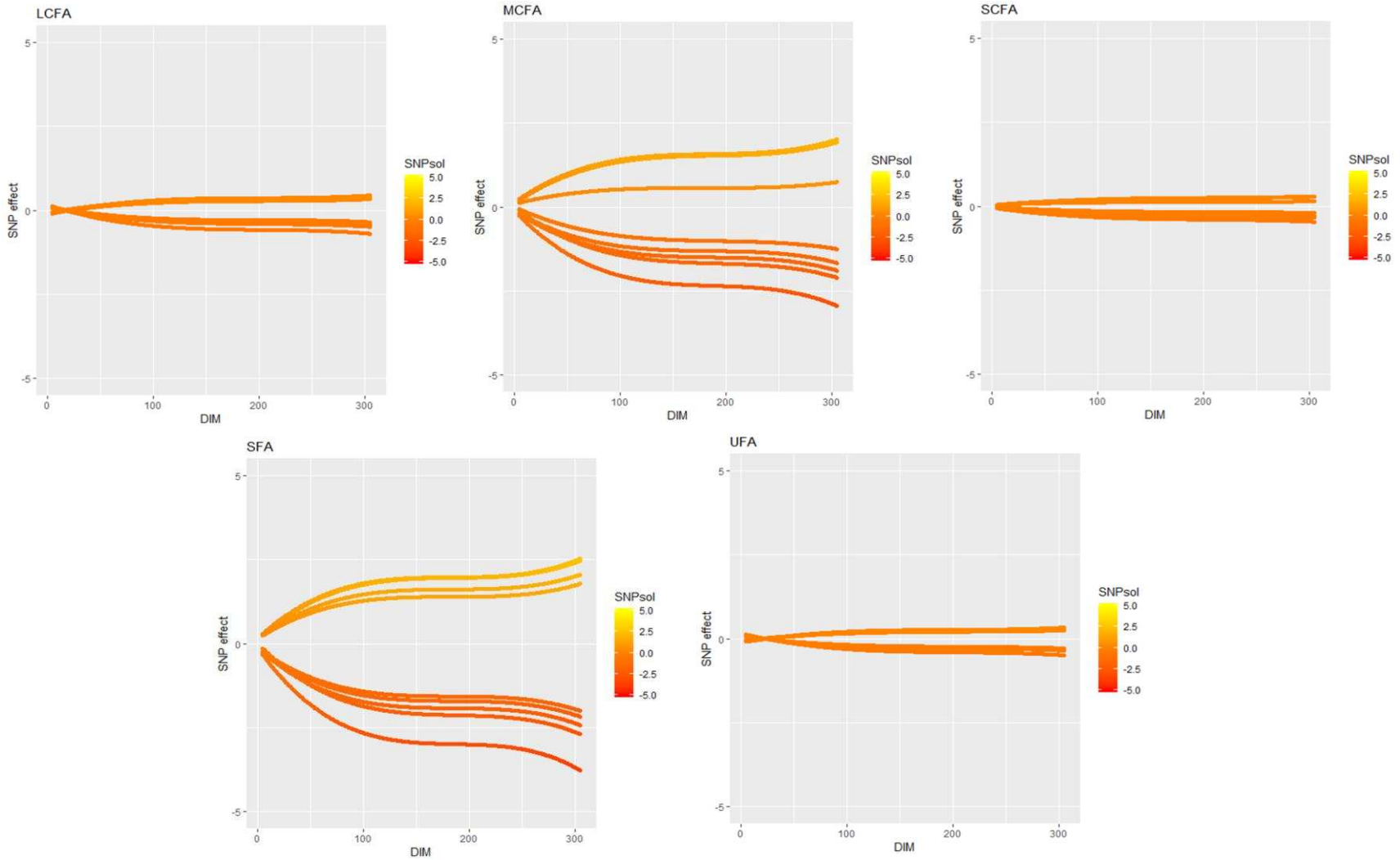

Figure 8. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated fatty acids using optimal τ and ω, of the Jersey breed.

For the Ayrshire breed, most of the important SNPs were located on the bovine chromosomes (**BTA**) BTA3, BTA5, BTA13, and BTA20. In the Holstein breed, important SNPs were located on BTA5 and BTA14. In Jersey, the important SNPs were located on BTA14, BTA16, BTA18, BTA20 and BTA21. The proportion of the variance explained by genomic windows for each trait was higher in the Holstein breed, followed by Jersey and Ayrshire breeds. In addition, the largest variances explained were found in the BTA14, indicating that this chromosome contain important genes associated with predicted milk FA groups, especially for the Holstein breed. A total of 2, 2, 4, 2 and 1 genomic region were in common between at least two breeds for SCFA, MCFA, LCFA, SFA and UFA, respectively.

All candidate genes mapped through Ensembl considering 20-SNP windows for the SNPs selected in each trait, for Ayrshire, Holsten and Jersey breed, using default and optimal τ and ω values, are shown in Table 5. The number of candidate genes identified to be associated with the five milk FA groups was, in general, higher for the Holstein breed, followed by the Jersey and Ayrshire breeds, respectively. The genes founded in this study are involved in many biological pathways, which are shown in Table 6.

Table 5. Chromosome information (BTA, inside brackets) and candidate gene symbols mapped through Ensembl for single nucleotide polymorphisms considering the 20-SNP windows selected in each trait of Ayrshire, Holstein and Jersey breeds*.

		τ and ω = 1	**Optimal τ and ω**
Ayrshire	**LCFA**	(BTA3) **LRP8**, MAGOH, CZIB, SLC1A7, POMGNT1, P3R3URF, IPP, GPBP1L1, CCDC17, PRDX1, TESK2; (BTA5) KIF21A, PTPRR, CPNE8; (BTA18) KIAA0513, GSE1, COX4I1, IRF8	(BTA3) TTLL7, **LRP8**, MAGOH, CZIB, SLC1A7, POMGNT1, P3R3URF, IPP, GPBP1L1, CCDC17, PRDX1, TESK2
	MCFA	(BTA3) **LRP8**, MAGOH, CZIB, SLC1A7; (BTA5) **ACO2** PTPRR; (BTA13) ZNF341, CHMP4B, RALY, ASIP, ITCH, MAP1LC3A, DYNLRB1; (BTA20) CCL28, PAIP1, C20H5orf34, TMEM267, HMGCS1	(BTA3) **LRP8**, MAGOH, CZIB, SLC1A7; (BTA5) **ACO2**, PTPRR; (BTA13) ZNF341, CHMP4B, RALY, ASIP, ITCH, MAP1LC3A, DYNLRB1; (BTA20) PAIP1, C20H5orf34, TMEM267 CCL28, HMGCS1, SELENOP
	SCFA	(BTA12) PTPRR; (BTA13) ZNF341, CHMP4B, RALY, ASIP, ITCH, MAP1LC3A; (BTA20) FGF10, PAIP1, C20H5orf34, TMEM267, HMGCS1	(BTA12) PTPRR; (BTA13) ZNF341, CHMP4B, RALY, ASIP, ITCH, MAP1LC3A; (BTA20) FGF10, PAIP1, C20H5orf34, TMEM267, HMGCS1
	SFA	(BTA3) **LRP8**, MAGOH, CZIB, SLC1A7; (BTA5) **ACO2**, KIF21A, PTPRR; (BTA13) CHMP4B, RALY, ASIP, ITCH	(BTA3) **LRP8**, MAGOH, CZIB, SLC1A7, LRRC41; (BTA5) **ACO2**, KIF21A, PTPRR; (BTA13) CHMP4B, RALY, ASIP, ITCH, MAP1LC3A
	UFA	(BTA3) PRDX1; (BTA18) KIAA0513, GSE1, COX4I1, IRF8	(BTA18) KIAA0513, GSE1, COX4I1, IRF8
Holstein	**LCFA**	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, ARC, JRK, **TRAPPC9**, **FAM135B**	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, PTK2, **AGO2**;

	MCFA	(BTA5) RERGL, EPS8, RERG, ARHGDIB, ERP27, **ACO2**, ART4, SMCO3, PLBD1; (BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, **TRAPPC9**, **FAM135B**	(BTA5) EPS8, RERG, **ACO2**, GUCY2C, PLBD1; (BTA14) LYNX1, SLURP1, ARC, JRK, TSNARE1, GPR20, **PTK2**, **AGO2**;
	SCFA	(BTA5) RERGL, EPS8, RERG, ARHGDIB, ERP27, ART4, PLBD1; (BTA13) DIP2C, PRNP, PRND; (BTA14) LY6D, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, ARC, JRK, **TRAPPC9**, **FAM135B**	(BTA5) ERP27, ART4, WBP11, GUCY2C, PLBD1; (BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, ARC, JRK, SLC45A4, **PTK2**, **AGO2**, **TRAPPC9**, **KCNK9**, **FAM135B**
	SFA	(BTA5) RERGL, EPS8, RERG, RERGL, EP27, ART4, SMCO3, PLBD1, ARHGDIB, GUCY2C; (BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, ARC, JRK, SLC45A4, **PTK2**, **AGO2**, **TRAPPC9**, **KCNK9**	(BTA5) RERGL, EPS8, RERG, ARHGDIB, GUCY2C; (BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, ARC, JRK, SLC45A4, **PTK2**, **AGO2**, **TRAPPC9**, **KCNK9**, ST3GAL1, NDRG1, SLA
	UFA	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, ARC, JRK, **TRAPPC9**, **KCNK9**, **FAM135B**	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, ARC, JRK, **TRAPPC9**, **KCNK9**
Jersey	**LCFA**	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, JRK, ARC; (BTA16) METTL11B, GORAB, PRRX1; (BTA20) PAIP1, C5orf34, TMEM267, CCL28, OXCT1; (BTA21) BTBD1, MORF4L1, ANKRD34C, TMED3, MINAR1	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1; (BTA16) METTL11B, GORAB, PRRX1
	MCFA	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, JRK, ARC, SLC45A4, **PTK2**, **AGO2,** ARC, JRK, **TRAPPC9, KCNK9**; (BTA18) IRF8, FOXF1, FOXC2, FOXL1; (BTA20) PAIP1, C5orf34, TMEM267, CCL28, OXCT1, PLCXD3	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1; (BTA20) PAIP1, C5orf34, TMEM267, CCL28, OXCT1

SCFA	(BTA14) LY6D, LYNX1, LYPD2, SLURP1, TSNARE1, JRK, ARC; (BTA16) METTL11B, GORAB, PRRX1; (BTA20) CCL28, OXCT1, C6, MROH2B, RPL37, PRKAA1, TTC33, LIFR, GDNF	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, SLC45A4, **PTK2**, **AGO2**, ARC, JRK; (BTA16) METTL11B, GORAB, PRRX1; (BTA20) PAIP1, C5orf34, TMEM267, CCL28, OXCT1, C6, MROH2B, RPL37, PRKAA1, TTC33
SFA	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, ARC, JRK; (BTA16) METTL11B, GORAB, PRRX1; (BTA20) PAIP1, C5orf34, TMEM267, SELENOP, OXCT1	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1, ARC, JRK; (BTA16) METTL11B, GORAB, PRRX1; (BTA20) PAIP1, C5orf34, TMEM267, SELENOP, OXCT1
UFA	(BTA3) DPYD; (BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1; (BTA20) PAIP1, C5orf34, TMEM267, CCL28, OXCT1; (BTA21) ANKRD34C, TMED3	(BTA14) **LY6D**, LYNX1, LYPD2, SLURP1, TSNARE1

*The DGAT1 gene was not identified because there were no genomic markers in the genomic datasets used that were close to the new position of the DGAT1 gene in the new reference genome. However, the high peaks identified in the beginning of BTA14 is likely due to linkage disequilibrium association with DGAT1 markers. LCFA: long-chain fatty acids; MCFA: medium-chain fatty acids; SCFA: short-chain fatty acids; SFA: saturated fatty acids; and UFA: unsaturated fatty acids. Some genes previously reported to be related to milk fat traits are highlighted in bold.

Table 6. Biological functions and pathways (KEGG database) of selected genes from long-chain (LCFA), medium-chain (MCFA), short-chain (SCFA), saturated (SFA) and unsaturated (UFA) milk fatty acids in the Ayrshire, Holstein and Jersey breeds.

Gene symbol	Gene name	Biological Pathway
ACO2	Aconitase 2	Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, **Carbon metabolism**, 2-Oxocarboxylic acid metabolism, biosynthesis of amino acids
ARC	Activity Regulated Cytoskeleton Associated Protein	Cytoskeleton organization, anterior/posterior pattern specification, cell migration, regulation of cell morphogenesis
ARHGDIB	Rho GDP Dissociation Inhibitor Beta	Neurotrophin signaling pathway, Vasopressin-regulated water reabsorption
ASIP	Agouti Signaling Protein	Generation of precursor metabolites and energy, hormone-mediated signaling pathway, melanosome transport, melanosome organization, regulation of molecular function, melanin biosynthetic process, positive regulation of melanin biosynthetic process
C6	Complement C6	In utero embryonic development, positive regulation of activation of membrane attack complex, **immune response**, complement activation, classical pathway, cytolysis, innate immune response, positive regulation of angiogenesis
CHMP4B	Charged Multivesicular Body Protein 4B	Endocytosis
COX4I1	Cytochrome C Oxidase Subunit 4I1	Oxidative phosphorylation, cardiac muscle contraction, non-alcoholic fatty liver disease (NAFLD)
DIP2C	Disco Interacting Protein 2 Homolog C	**Lipid metabolism**, secondary metabolites biosynthesis, transport, and catabolism
DPYD	Dihydropyrimidine Dehydrogenase	Pyrimidine metabolism, beta-Alanine metabolism, Pantothenate and CoA biosynthesis, metabolic pathways
FAM135B	Family With Sequence Similarity 135 Member B	**Cellular lipid metabolic process**
FGF10	Fibroblast Growth Factor 10	MAPK signaling pathway, Ras signaling pathway, Rap1 signaling pathway, PI3K-Akt signaling pathway, regulation of actin cytoskeleton, fibroblast growth factor receptor signaling pathway

HMGCS1	3-Hydroxy-3-Methylglutaryl-CoA Synthase 1	**Synthesis and degradation of ketone bodies**, Valine, leucine and isoleucine degradation, butanoate metabolism, **cholesterol biosynthesis, cholesterol metabolism**, **lipid biosynthesis**, l**ipid metabolism**, steroid biosynthesis, steroid metabolism
IRF8	Interferon Regulatory factor 8	Pertussis
ITCH	Itchy E3 Ubiquitin Protein Ligase	Ubiquitin mediated proteolysis, endocytosis, TNF signaling pathway, non-alcoholic fatty liver disease (NAFLD),
KCNK9	Potassium Two Pore Domain Channel Subfamily K Member 9	Aldosterone synthesis and secretion
LRP8	LDL Receptor Related Protein 8	**Very-low-density lipoprotein particle receptor activity**, **Low-density lipoprotein (LDL) receptor class A**, integral component of membrane, receptor complex
MAGOH	Mago Homolog, Exon Junction Complex Subunit	RNA transport, mRNA surveillance pathway, spliceosome
OXCT1	3-oxoacid CoA-transferase 1	**Synthesis and degradation of ketone bodies**, valine, leucine and isoleucine degradation, butanoate metabolism, **lipid metabolism**
PAIP1	Poly(A) Binding Protein Interacting Protein 1	RNA transport
PLBD1	Phospholipase B Domain Containing 1	**Lipid catabolic process**, Phospholipase B-like
PRDX1	Peroxiredoxin 1	Peroxisome
PRNP	Prion Protein	Prion diseases
PTK2	Protein Tyrosine Kinase 2	ErbB signaling pathway, Chemokine signaling pathway, PI3K-Akt signaling pathway, Axon guidance, VEGF signaling pathway, Focal adhesion, Leukocyte transendothelial migration, Regulation of actin cytoskeleton, Bacterial invasion of epithelial cells
PTPRR	Protein Tyrosine Phosphatase Receptor Type R	MAPK signaling pathway
RPL37	Ribosomal Protein L37	Ribosome
SLC1A7	Solute Carrier Family 1 Member 7	Glutamatergic synapse

DISCUSSION

Descriptive Statistics and Genetic Parameters

Current recommendations are for the public to reduce saturated fat intake and consume more unsaturated FA (FAO, 2010). A reduction in the proportion of saturated FA, together with an increase in the proportion of FA that promote good health, in the bovine milk can therefore be of great interest (Fleming, 2016). Previous authors have indicated that higher accuracies were obtained when defining the FA measurement unit on a per milk basis versus per fat basis (Soyeurt et al., 2006; Rutten et al., 2009; Fleming et al., 2017). Therefore, the unit used in this study was g/100g of milk. This might be due to the fact that milk samples contain different amounts of total fat and samples with the same relative concentrations of FA can contain very different total quantities of the FA (Fleming, 2016). Fleming et al. (2017) reported mean values of FA based on GC (gold standard method) of 2.81, 1.10, 0.461, 2.09 and 1.40 g/dL of milk for SFA, UFA, SCFA, MCFA and LCFA, respectively. These estimates are in a similar range with the averages (based on MIR predictions) observed in this study for the three breeds.

As observed in our study, the milk FA composition differs across breeds (Arnould and Soyeurt, 2009). For instance, DePeters et al. (1995) reported that Jersey has higher proportions of certain FA groups compared to Holstein, which was confirmed in this study. The heritability estimates obtained for all the five FA groups indicate that they are under moderate genetic control and therefore, can be improved through genetic selection. The higher h^2 estimates observed for de novo synthetized SCFA compared to dietary and adipose derived LCFA was expected. However, Fleming et al. (2018) using data from the same population had not clearly observed this difference. For the Holstein and Jersey breeds, our results were mostly in agreement with the literature (Bastin et al, 2011; Narayana et al. 2017; Hein et al., 2018). However, to our best knowledge, our study reports for the first time estimates of genetic parameters for FA groups in the Ayrshire breed. SCFA and MCFA have been shown to be more heritable than LCFA (Bobe et al., 2008; Stoop et al., 2008; Bastin et al., 2011; Narayana et al., 2017), which is in agreement with our results. Regarding to the h^2 values for Jersey, Hein et al. (2018), working with Danish Jersey, reported smaller values compared to the present study [LCFA: 0.09 (0.01); MCFA: 0.12 (0.01); SCFA: 0.16 (0.01); SFA: 0.10 (0.01)]. The differences in heritability estimates across studies are attributable to genetic differences among populations, sample size of the studies, methodology employed to measure (GC or MIR spectroscopy) or

predict FA, measurement unit (per gram of fat, per volume of milk, percentage values) and differences in the statistical models and methods to estimate variance components.

The h^2 estimates for the Holstein breed were similar to those found by Narayana et al. (2017), using data from the same population (data subset) and FA prediction equations, for LCFA and UFA (0.23 and 0.21, respectively), but were slightly higher for MCFA (0.32), SCFA (0.24) and SFA (0.33). In contrast, the values for these FA groups were similar to those reported by Bastin et al. (2011), in Holstein cattle from the Walloon Region of Belgium, that found 0.44 and 0.43, for MCFA and SFA, respectively. These differences may be due to the fact that the present study has more observations than the aforementioned and the distinct populations used in each study. In addition, the low to moderate accuracy of the calibration equations can also impact the results, as reported by Fleming et al. (2017). The R^2 (coefficient of determination of cross validation) of the prediction equations used to generate the phenotypes for this study were 0.94, 0.84, 0.72, 0.90, and 0.83 for SFA, UFA, SCFA, MCFA and LCFA, respectively (Fleming et al., 2017). In the current dataset, there were a limited number of test-days available for some animals because MIR spectra was recorded for only a proportion of all milk samples during Dairy Herd Improvement (**DHI**) milk recording and further laboratorial analyses. Consequently, there were fewer records per cow and the traits may not have been as well modeled across the lactation.

The h^2 peak found for Jersey may be due to a lower number of observations for this lactation range, which caused an overestimation of h^2. The h^2 values found for SFA were higher compared to UFA. This might be because the FAs that make up the SFA are mainly from de novo synthesis, which have enzymes that are likely genetically controlled (Karijord et al., 1982; Bastin et al. al., 2011). Strong genetic correlations between the FA groups and fat percentage were observed by Fleming et al. (2018). Out of the five FA groups, SFA and MCFA had the strongest genetic correlation with fat percentage at 0.973 and 0.964, respectively. In their study, the genetic correlation between UFA and fat percentage was lower at 0.814. This is in line with Penasa et al. (2016) whom also found that SFA were more strongly correlated with fat percentage than UFA (0.991 vs. 0.838). Negative genetic correlation between FA groups and milk yield, result of the effect of dilution, were observed by Bastin et al., (2011) and Fleming et al., (2018), ranging from -0.380 to -0.420 and -0.585 to -0.615, respectively.

Reliability and Bias of Genomic Predictions

The τ and ω parameters, are used to make $\mathbf{G^{-1}}$ compatible with $\mathbf{A_{22}^{-1}}$ and also $\mathbf{A^{-1}}$ (Misztal, 2017; Oliveira et al., 2018). In this context, Misztal (2017) has showed that τ is used to account for the reduced genetic variance, while ω is used to account for different depths of pedigree. For all traits, the GEBV r^2 calculated for the Holstein breed were higher than the GEBV r^2 calculated for PAs, even when no scaling factors were used. These results are in agreement with other studies, such as Oliveira et al. (2018) and Baba et al. (2016), which showed that the use of ssGBLUP increases the r^2 of breeding values. However, for the Ayrshire breed, the PA r^2 were usually higher than GEBV r^2. This fact may be related to the small size of the training population for the Ayrshire breed and the highly accurate EBVs (and PAs) of the animals included in the validation dataset. A larger and better structured training and validation populations will result in more accurate GEBVs. The fact that this study uses milk FA predicted using an equation developed mainly for Holstein and with reduced number of Ayrshire animals (Fleming et al., 2017) can also influence the reliability of GEBV. It is also worth pointing out that the animals used for these analyses have highly-accurate EBVs for these traits (and consequently highly-accurate PAs) and the average PA r^2 is considerably higher for Ayrshire in comparison to Holstein.

Regarding to b_1, the use of optimal τ and ω scaling factors yielded less biased GEBVs (i.e., b_1 closer to 1) when compared to the analyzes using the default values (1). In general, GEBVs predicted over time with and without the optimal values were inflated and deflated, respectively. However, GEBVs estimated for the majority of traits in this study would be accepted to participate in the international comparisons performed by Interbull (Interbull, 2016), if estimated using or not scaling factors. Compared to PA, GEBVs were more biased for the majority of traits, in both breeds. In this context, Lourenco et al. (2017) showed that the use of a genomic relationship matrix weighting markers according to the analyzed trait can generate more reliable and less biased estimates. The referred authors commented that using a weighted genomic relationship matrix can be beneficial especially in small-sized breeds, such as Ayrshire. Thus, posterior studies using the weighted ssGBLUP are needed, especially for small-sized populations.

Among the advantages of the method proposed in this study to evaluate FA is the fact that GEBVs and PAs are being predicted for all DIM. Thus, different selection criteria can be defined in the breeding schemes. In this regard, it becomes possible to change the pattern of the genetic (and phenotypic) FA lactation curves. Increase in GEBV r^2 can be achieve by increasing

the number of both genotyped and phenotyped animals and the use of more accurate prediction equations (developed using a larger number of animals).

Genome-wide Association Studies and Functional Analyses

Results found in this study support the polygenic nature of the studied traits, with many regions across the genome having small contributions to the total genetic variation, but with greater contributions of certain chromosomes (i.e. BTA5 and BTA14). The coefficients of the Legendre polynomial despite presenting differences in the patterns of markers identified have no biological explanation, since they are used for the modeling of the lactation curve. Thus, some coefficients allow the identification of different markers at different points in the curve. Important contributions were found on BTA14 for Holstein and Jersey breeds. This BTA is well known to have a substantial number of Quantitative Trait Loci (**QTL**s) for milk and fat production traits (Vitala et al., 2003; Wang et al., 2013), including DGAT1. DGAT1 is a gene involved in the last step of the synthesis of triacylglycerol and has a major effect on milk fat content (Grisart et al., 2002). Despite its importance, the DGAT1 gene was not found in this study due to the absence of markers located in the proximities of the DGAT1 and a change in its position in the new cattle reference genome (ARS-UCD1.2): BTA14:1,795,425-1,804,838 (Bos_taurus_UMD_3.1.1) to BTA14:603,981-614,153 (ARS-UCD1.2).

Various candidate genes were identified and shown to have important biological functions associated with the traits under study, including lipid metabolism and cholesterol synthesis. For instance, the LRP8 gene, identified to be associated with LCFA, MCFA and SFA in the Ayrshire breed, is involved with activation of cholesterol uptake (Argov et al., 2004). The PTK2, associated with all FA groups in Holstein breed and with MCFA in Jersey breed is related to several signal transduction pathways, such as cell motility and microtubule stability (Harte et al., 1996; Ezratty et al., 2005), and an important trait statistic related with fat percentage in Chinese Holstein cattle (Wang et al., 2013). Like the PTK2 gene, the TRAPPC9 gene is associated with all the FA traits for the Holstein breed and for MCFA in the Jersey breed. Wang et al. (2015) report that TRAPPC9 plays a role regulation of cell differentiation and was associated with mastitis susceptibility in Chinese Holstein cattle. The HMGCS1 and FGF10 genes are associated with reproductive traits. Bonsdorff et al. (2003) linked the gene HMGCS1 with hormone synthesis and angiogenesis and Castilho et al. (2014) associated

FGF10 with bovine ovarian development suggesting a regulating in early folliculogenesis and granulosa cell differentiation in preantral follicles.

CONCLUSIONS

Five groups of predicted milk fatty acids (SCFA, MCFA, LCFA, SFA, and UFA) are under moderate genetic control in Ayrshire, Holstein and Jersey, with heritability estimates ranging along the lactation. Genetic improvement can be made in all these traits through selection. Moderately to highly accurate genomic breeding values can be obtained for daily points throughout the lactation using the single-step GBLUP method. The use of scaling factors improved the accuracy and reduced bias for the majority of traits. Various genomic regions are associated with the traits, but many regions across the genome have small individual effects on the total genetic variation of each trait. Candidate genes and pathways were identified and helped to understand the genetic background of milk fatty acid production. These results contribute to better understand the genetic architecture of predicted milk FA in dairy cattle and will be of great value for the implementation of genomic selection for these traits.

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SUPPLEMENTARY MATERIAL

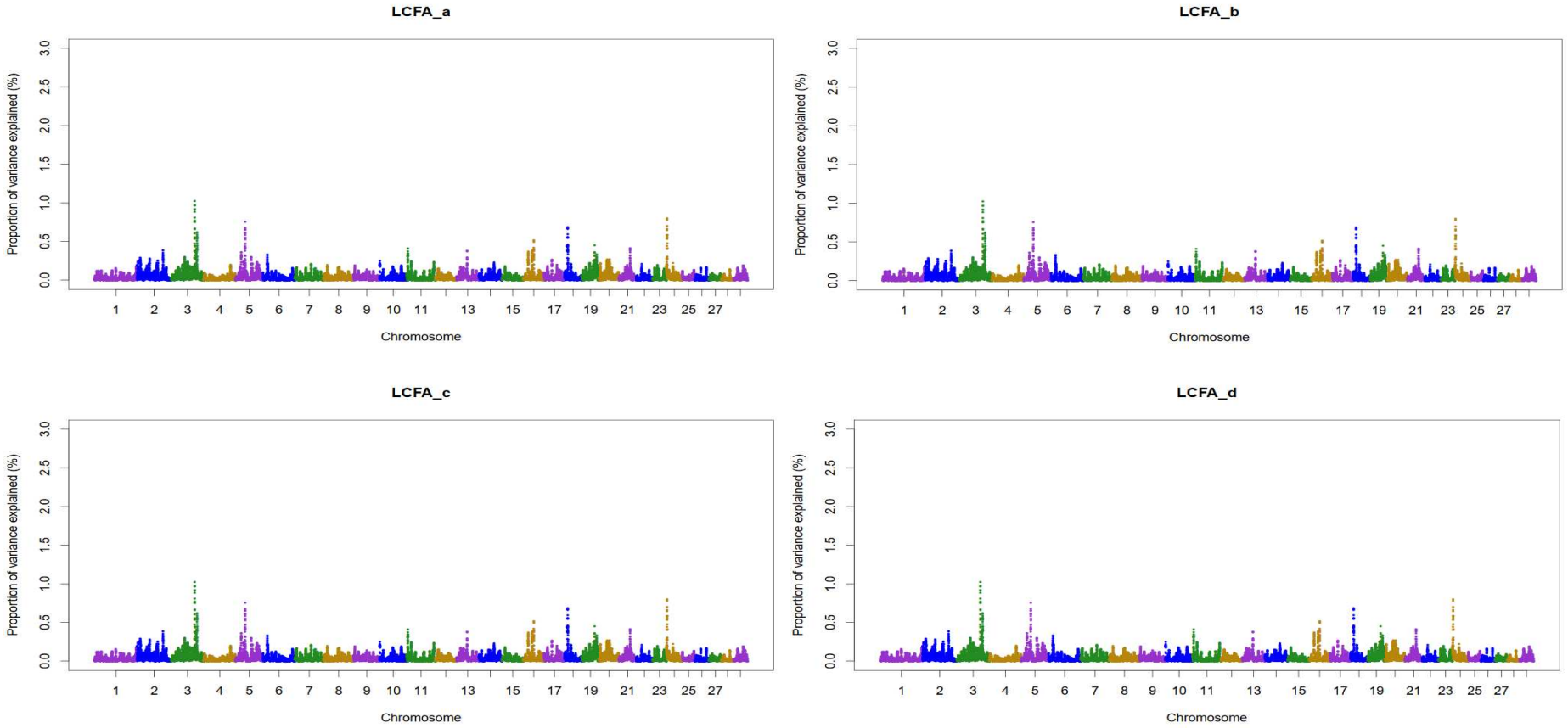

Figure S1. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Ayrshire using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

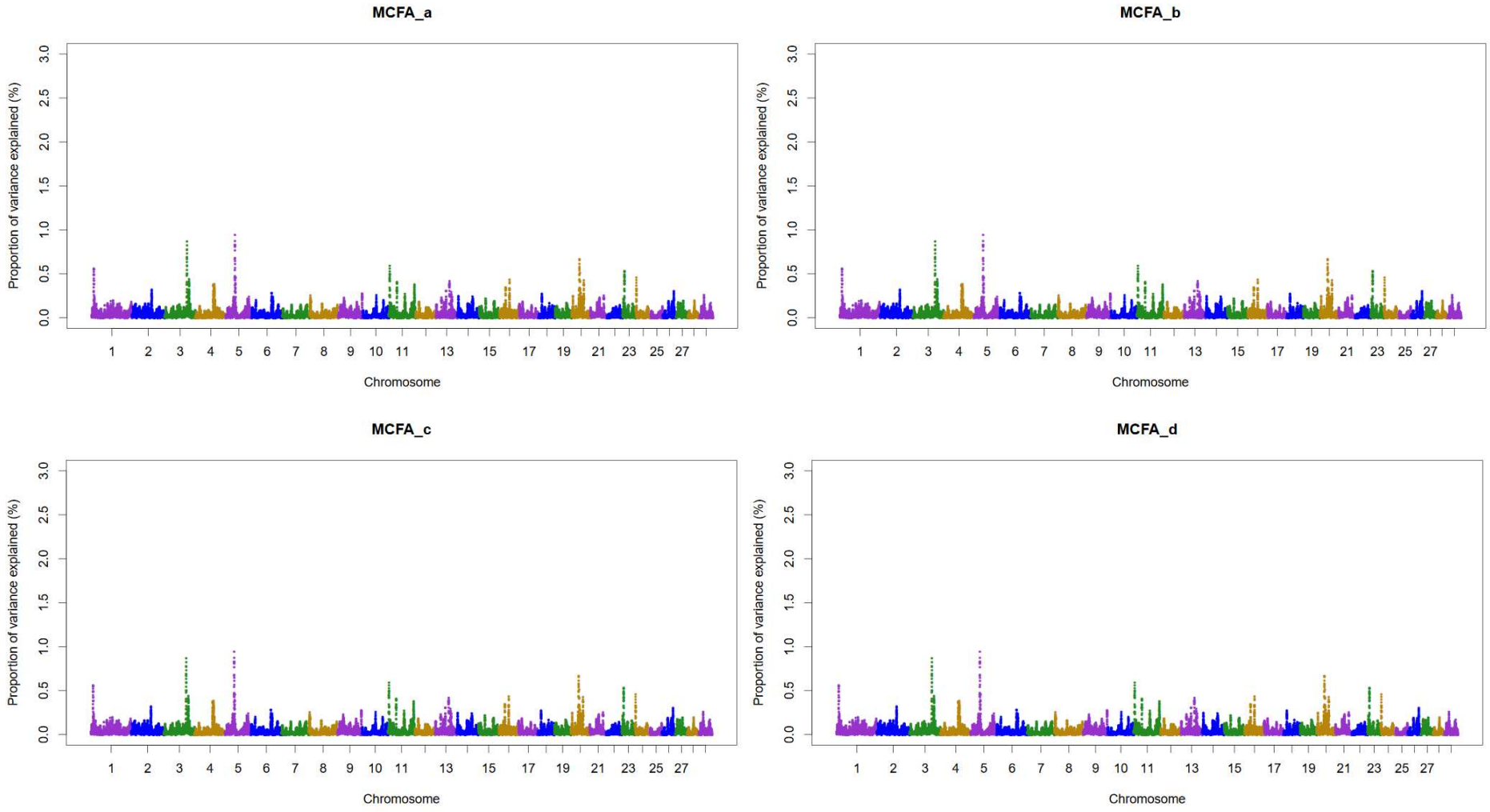

Figure S2. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Ayrshire using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

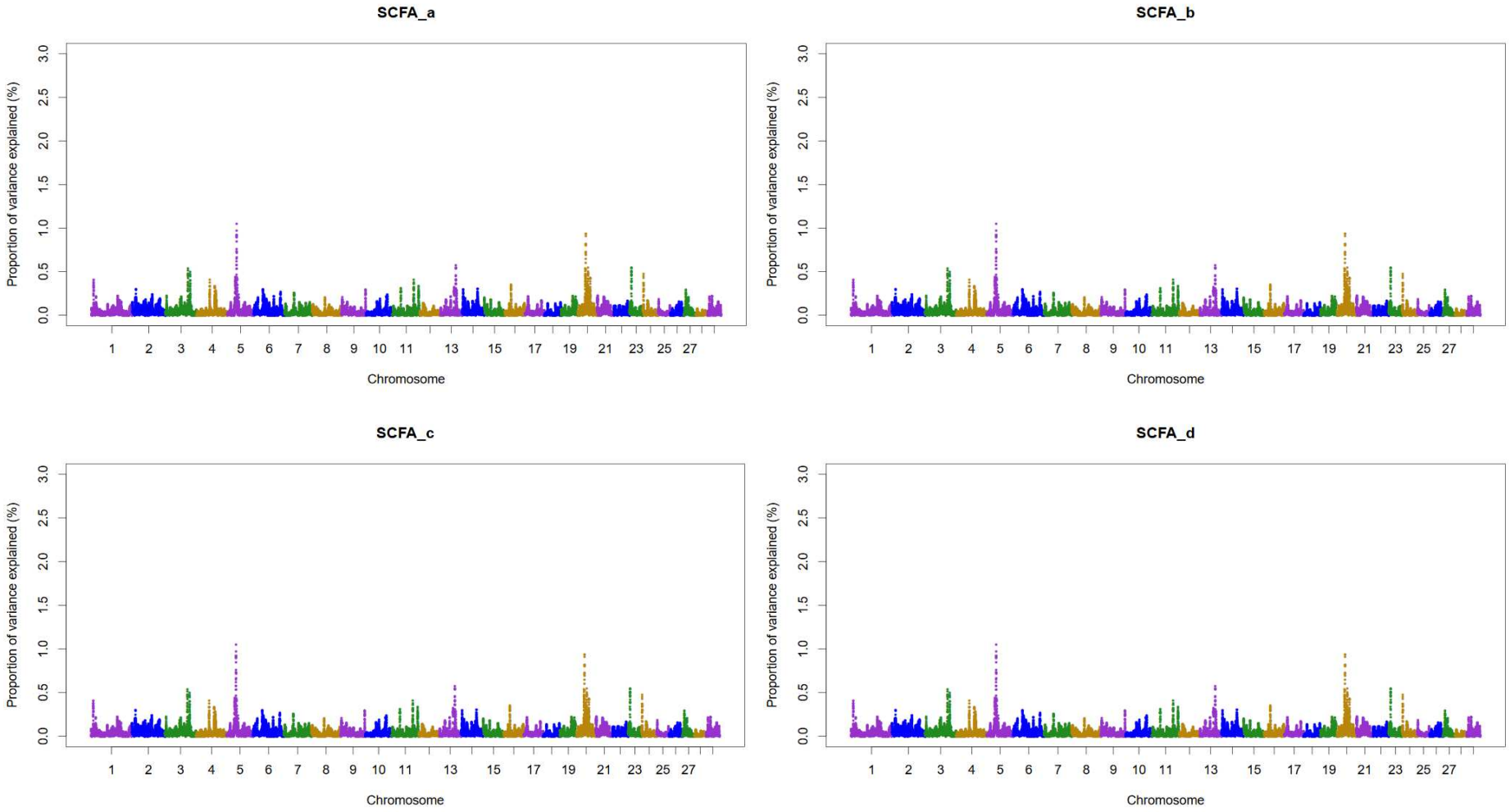

Figure S3. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Ayrshire using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

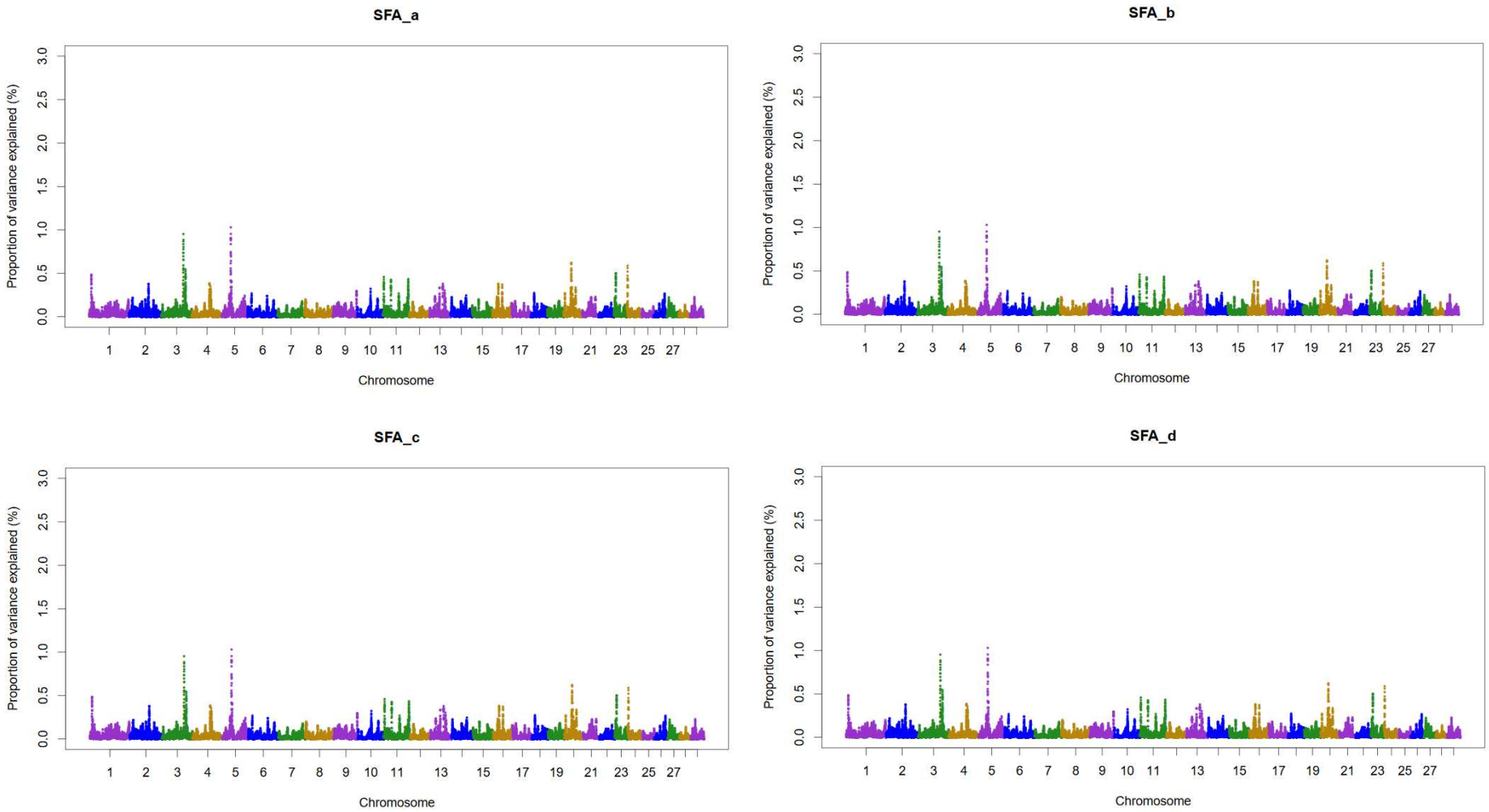

Figure S4. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Ayrshire using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

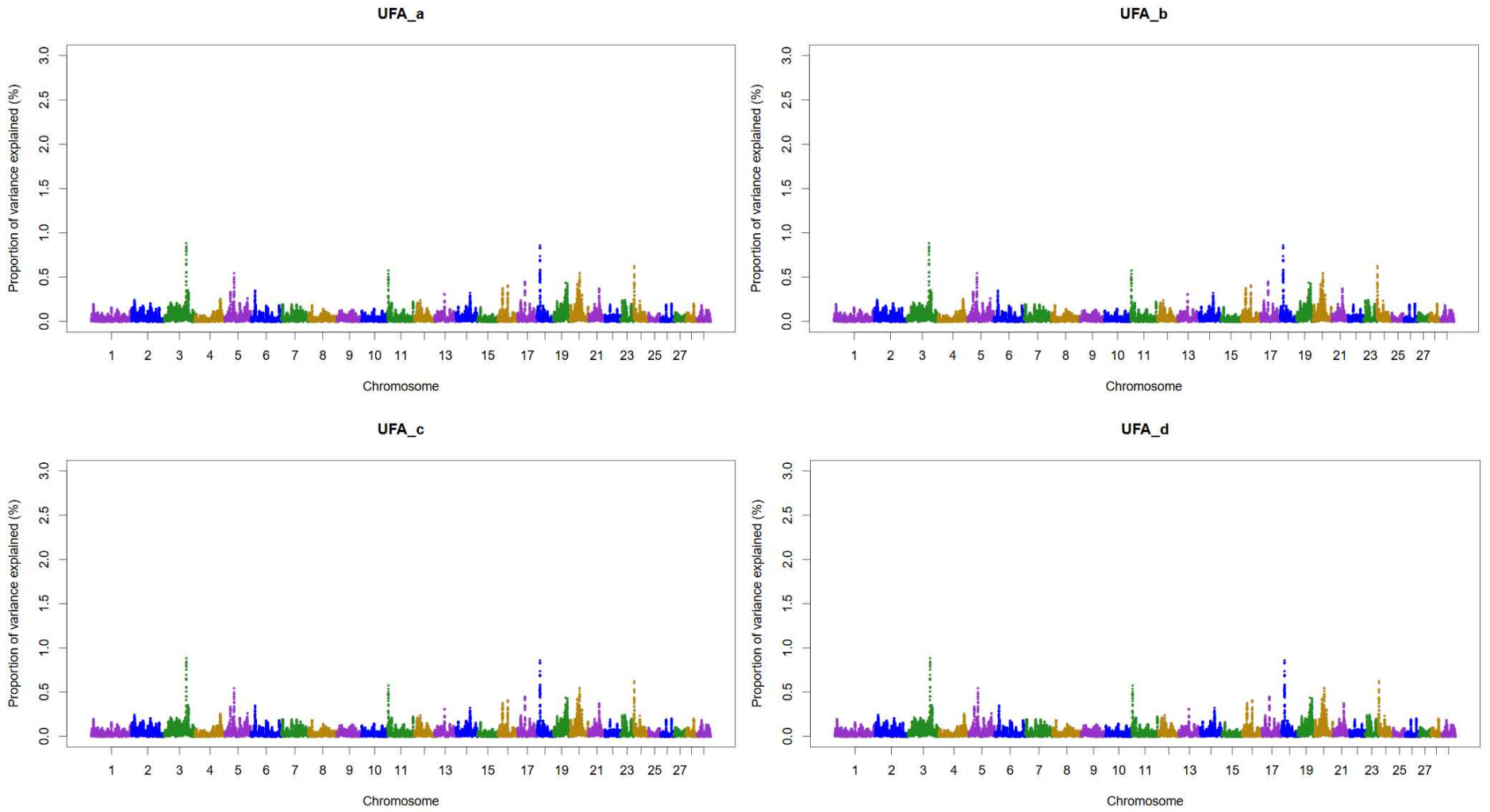

Figure S5. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Ayrshire using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

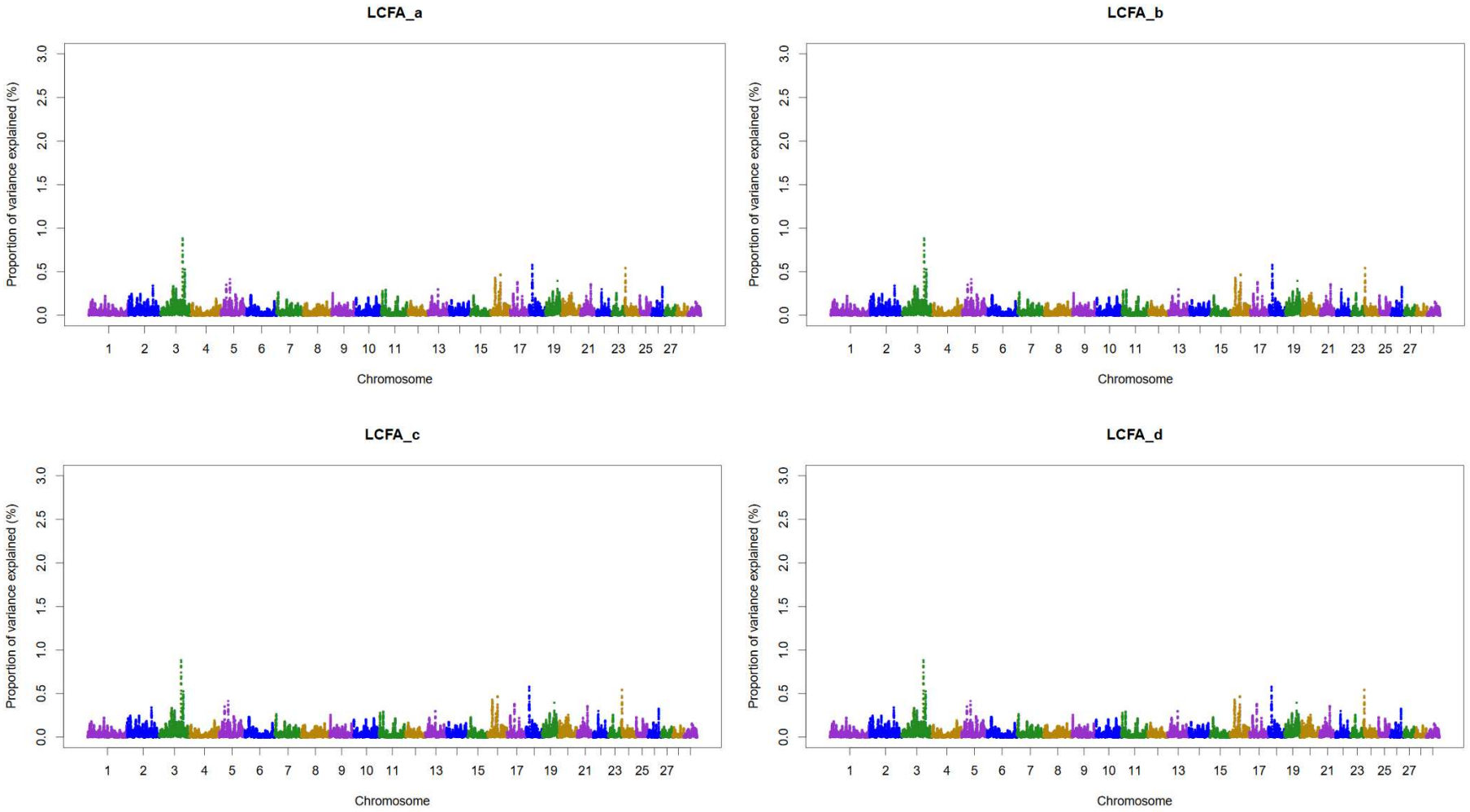

Figure S6. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Ayrshire using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

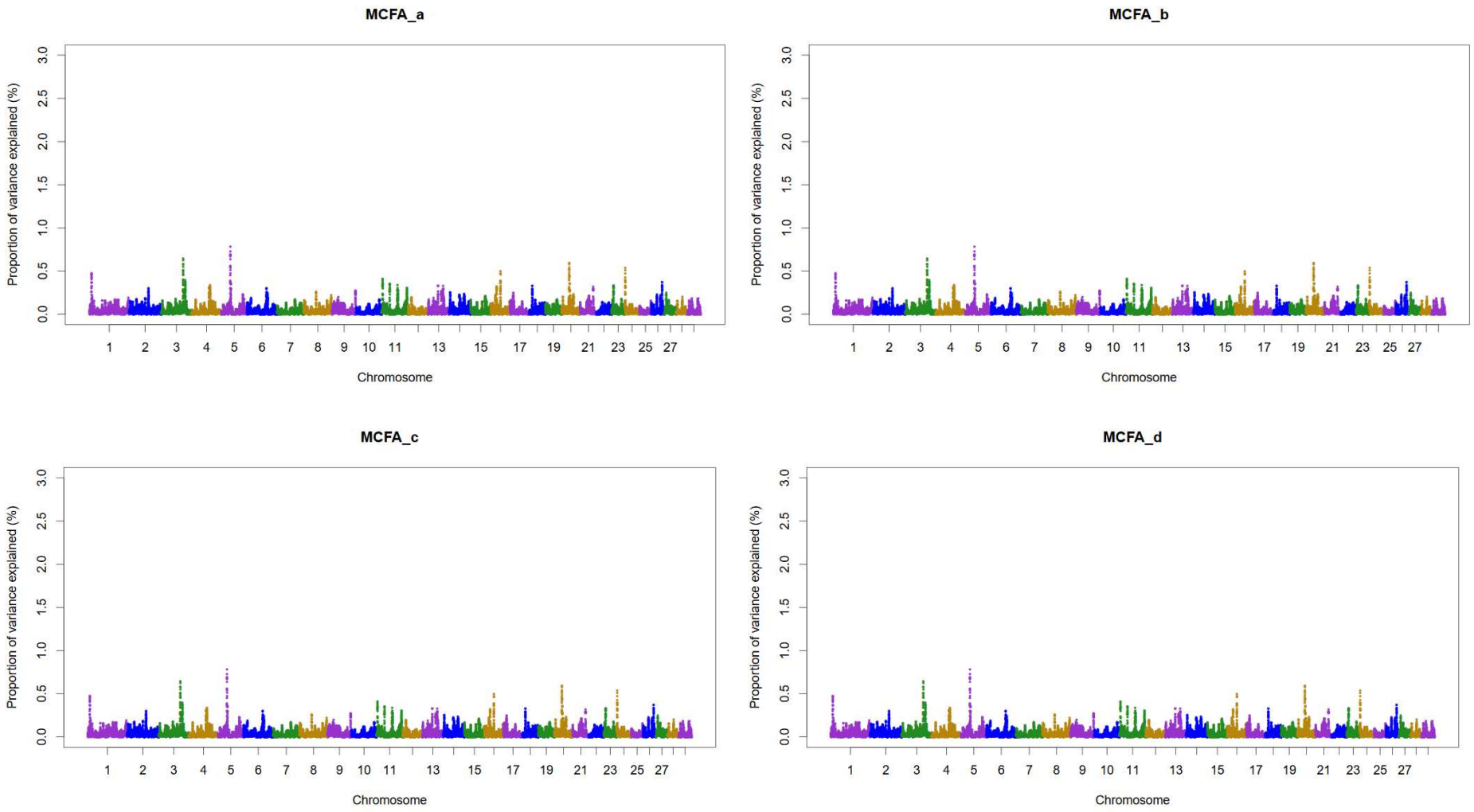

Figure S7. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Ayrshire using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

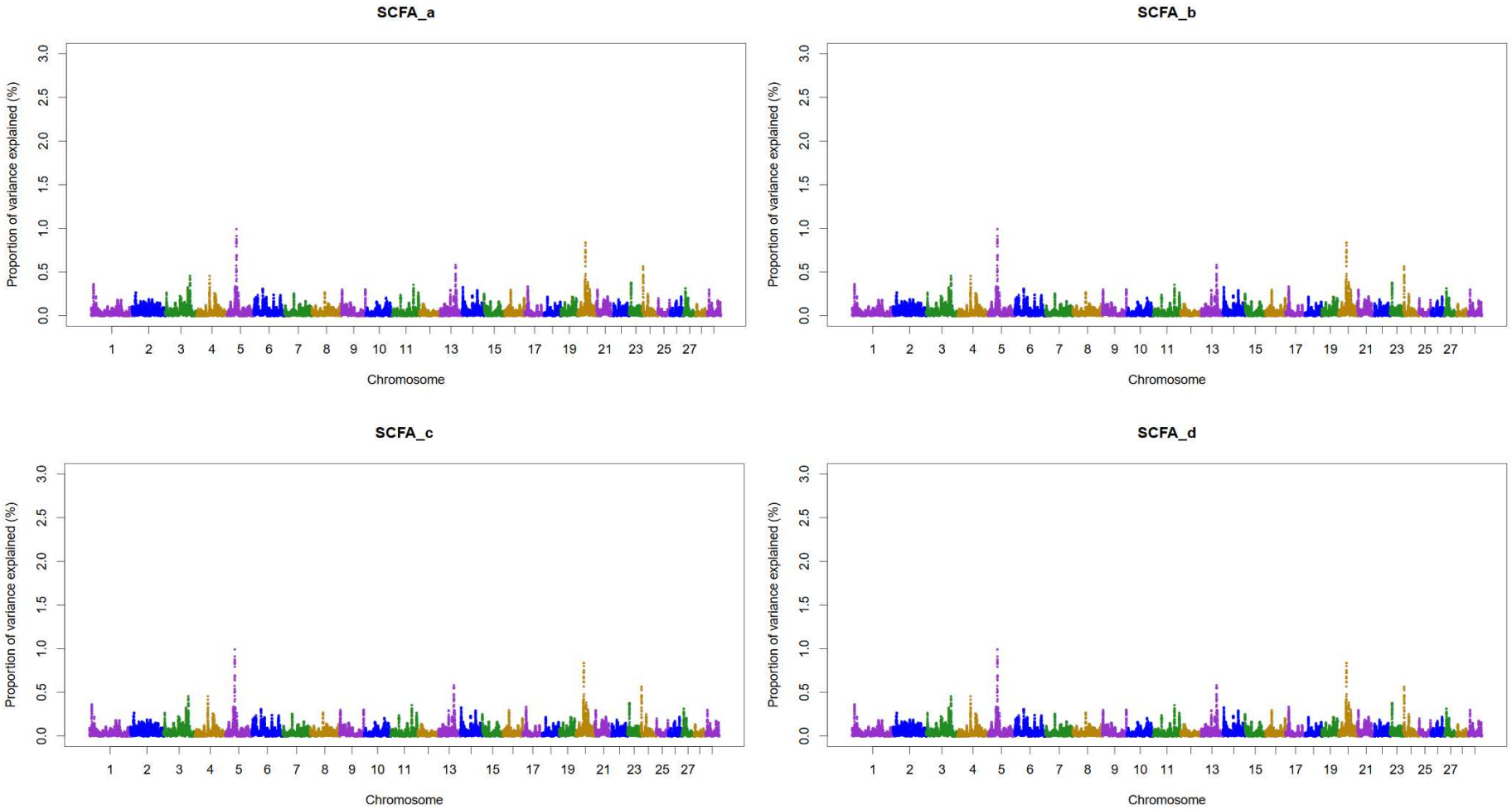

Figure S8. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Ayrshire using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

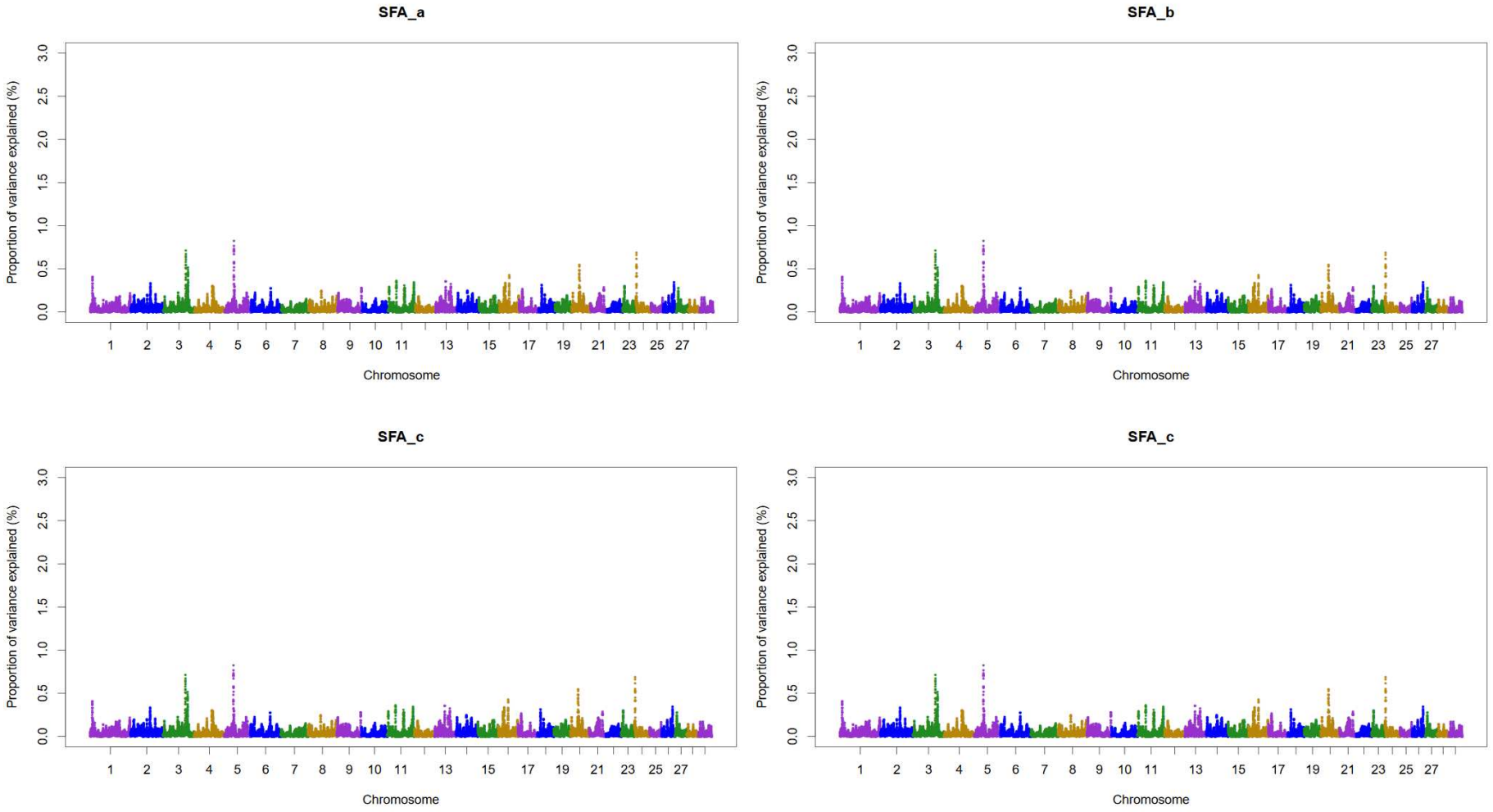

Figure S9. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Ayrshire using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

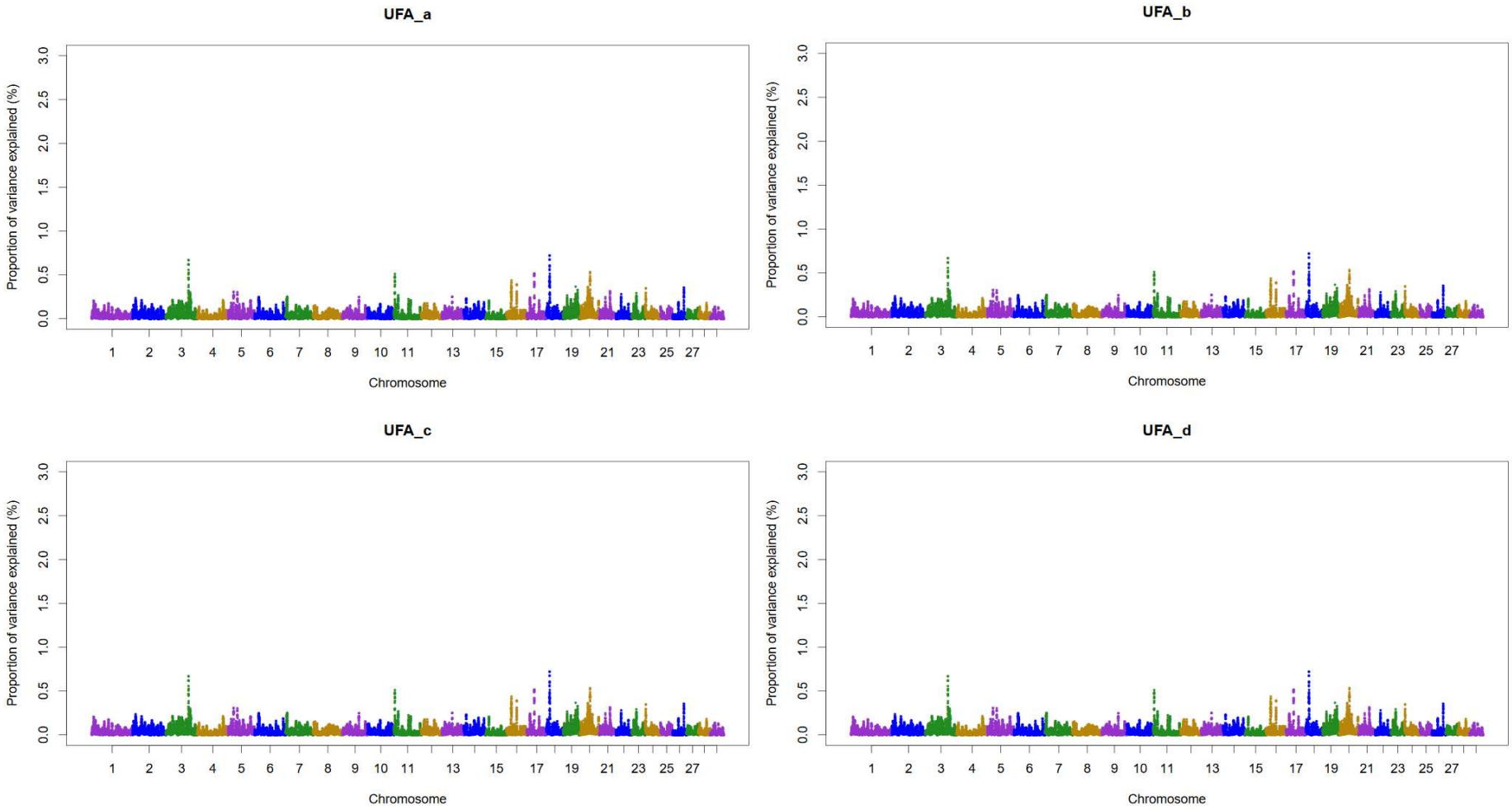

Figure S10. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Ayrshire using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

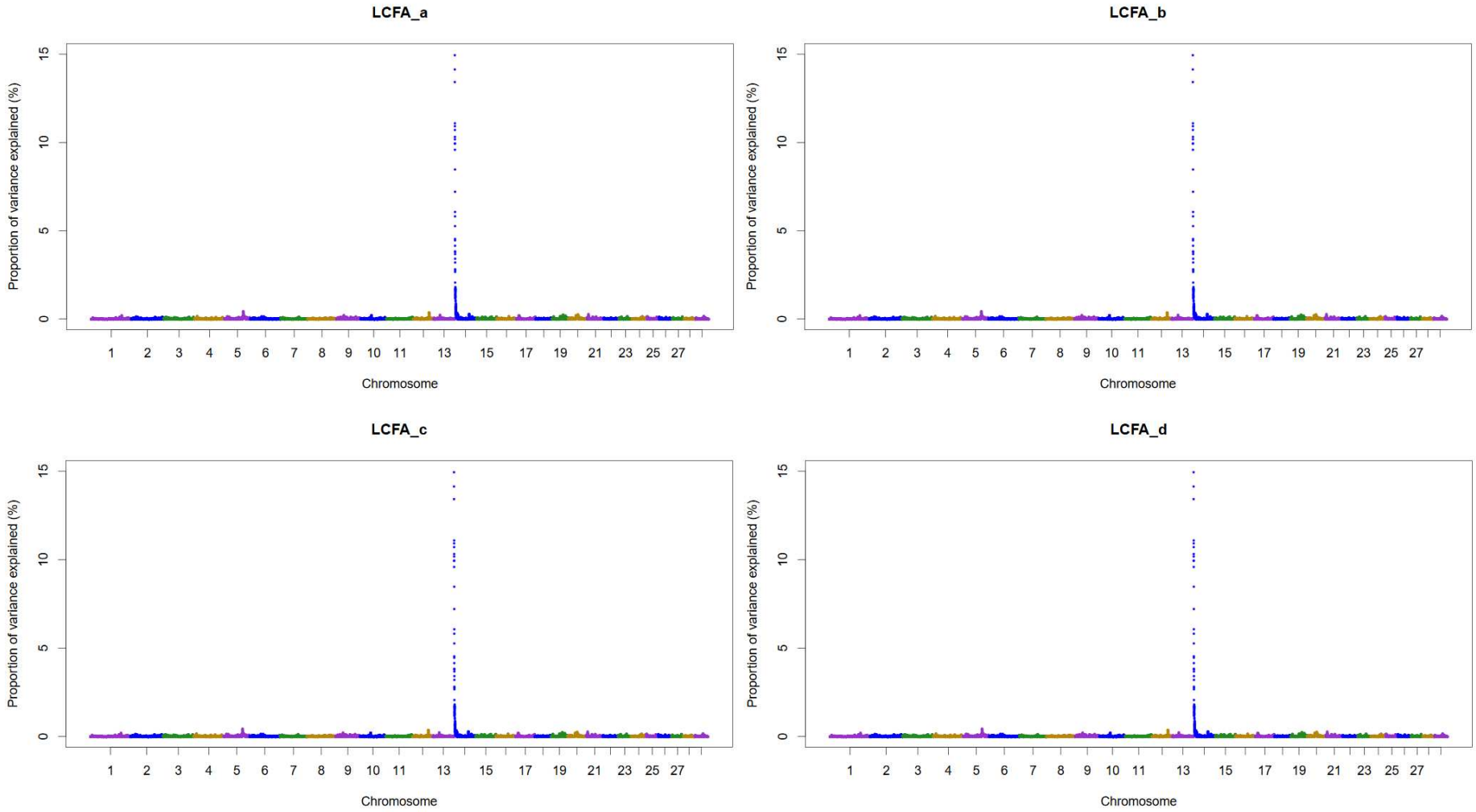

Figure S11. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Holstein using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

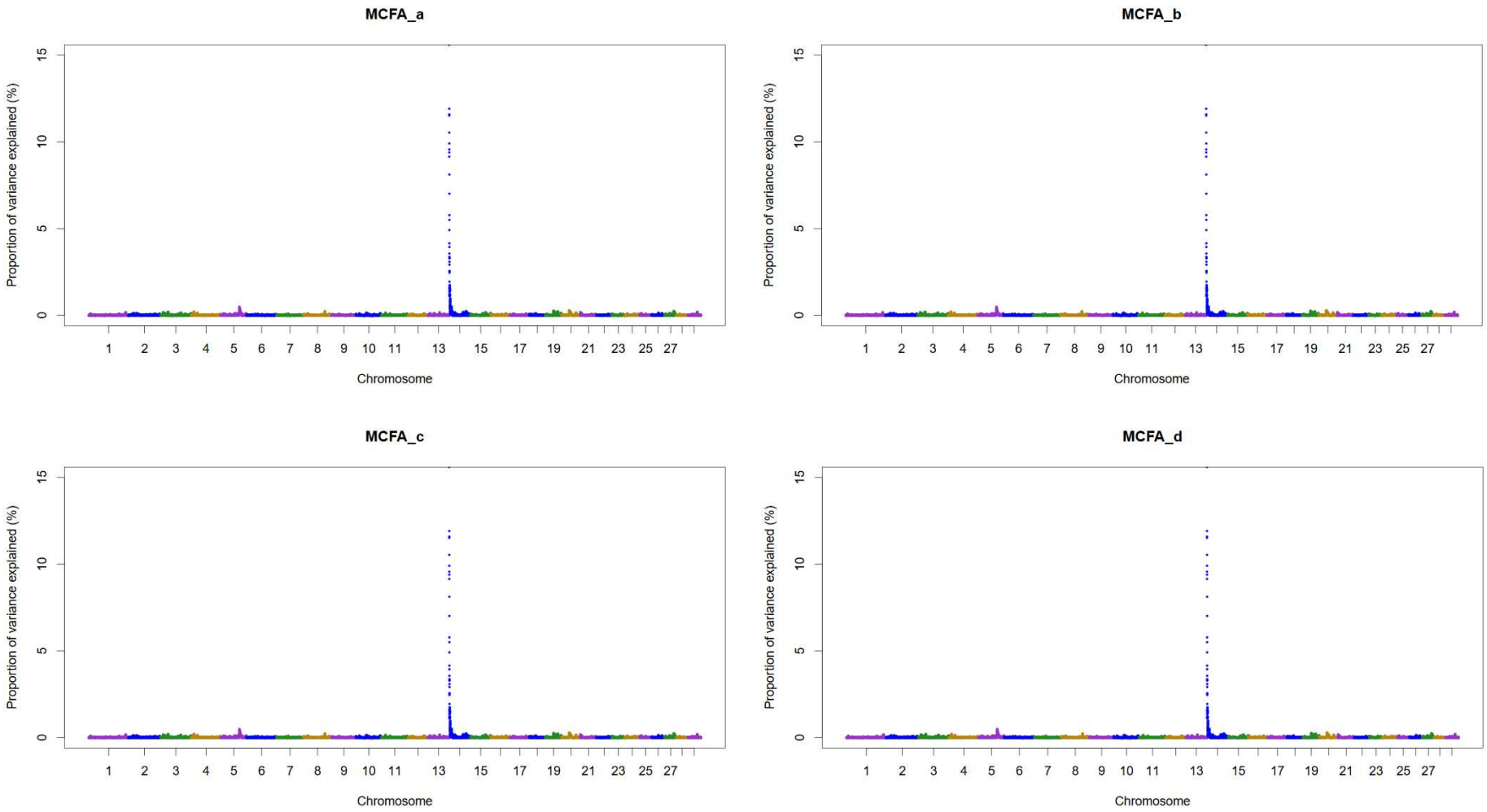

Figure S12. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Holstein using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

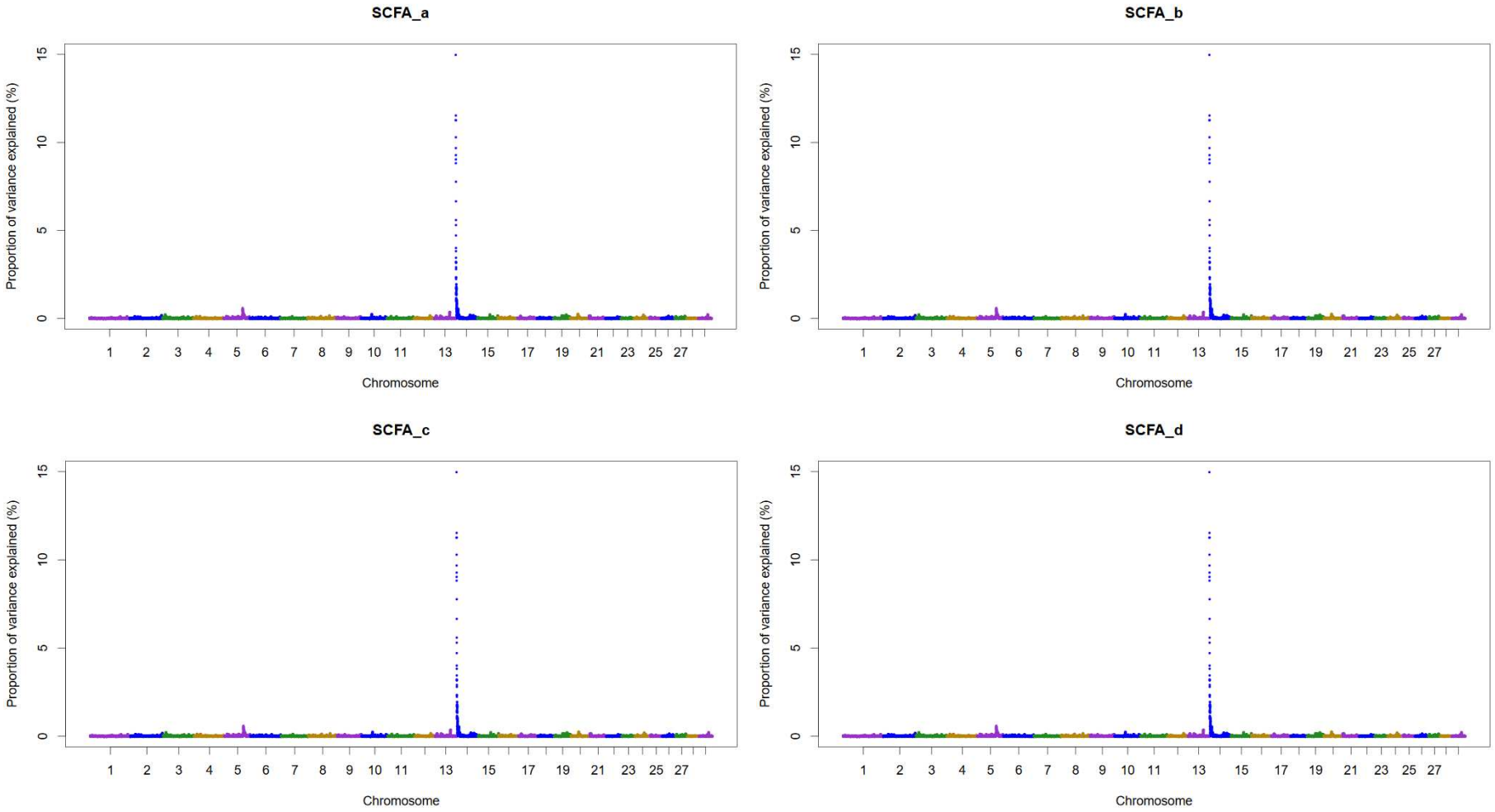

Figure S13. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Holstein using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

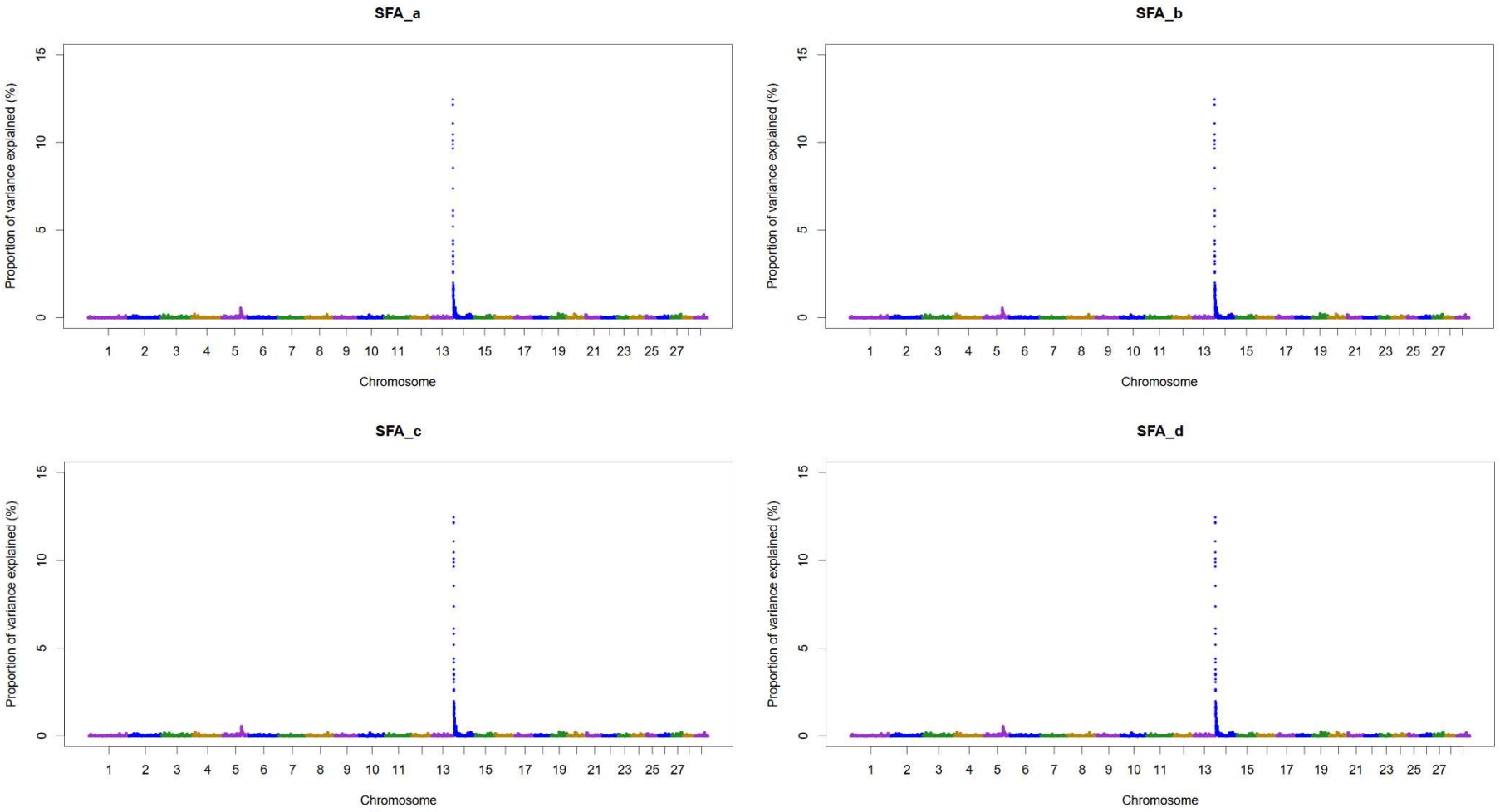

Figure S14. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Holstein using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

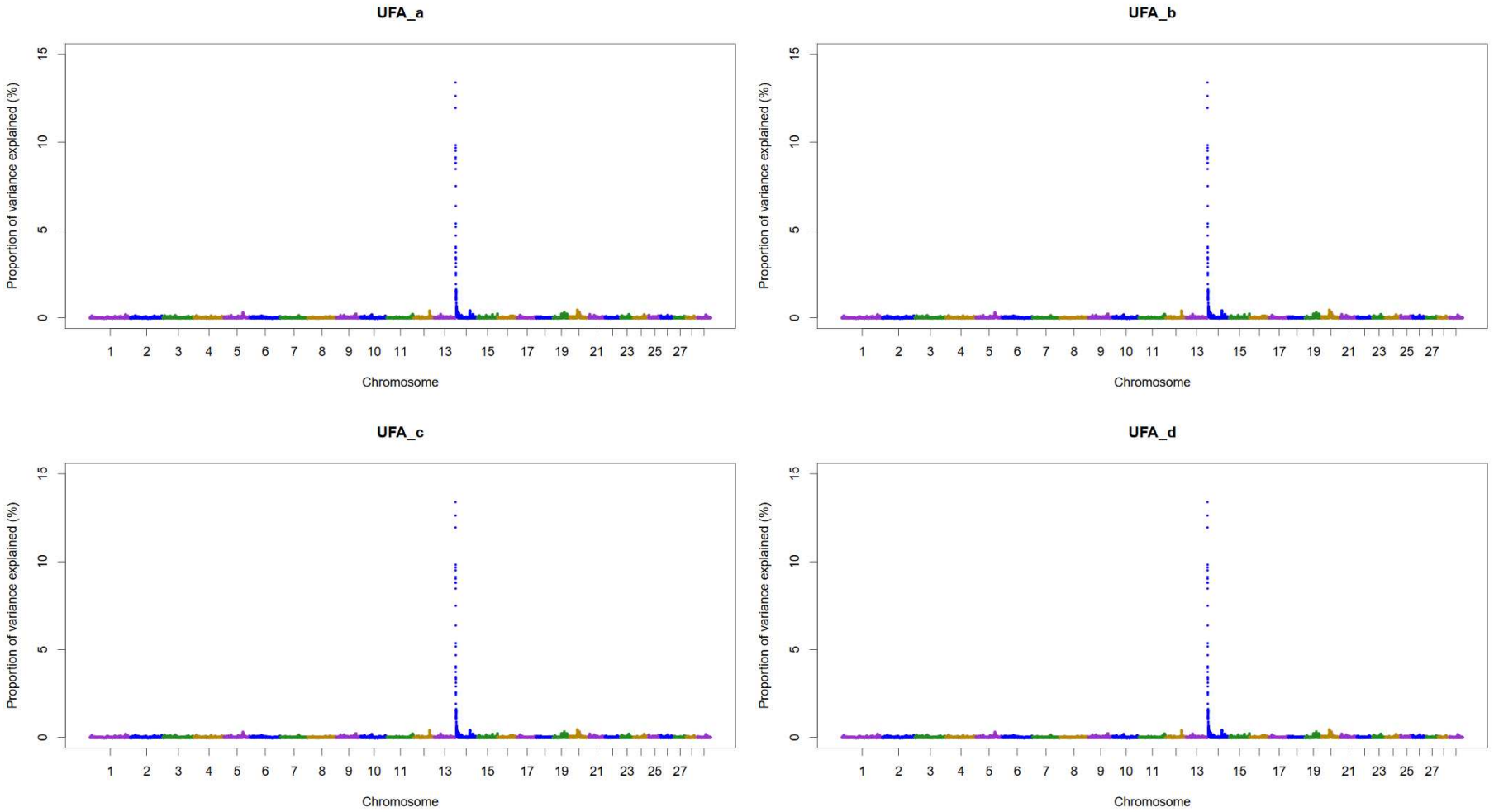

Figure S15. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Holstein using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

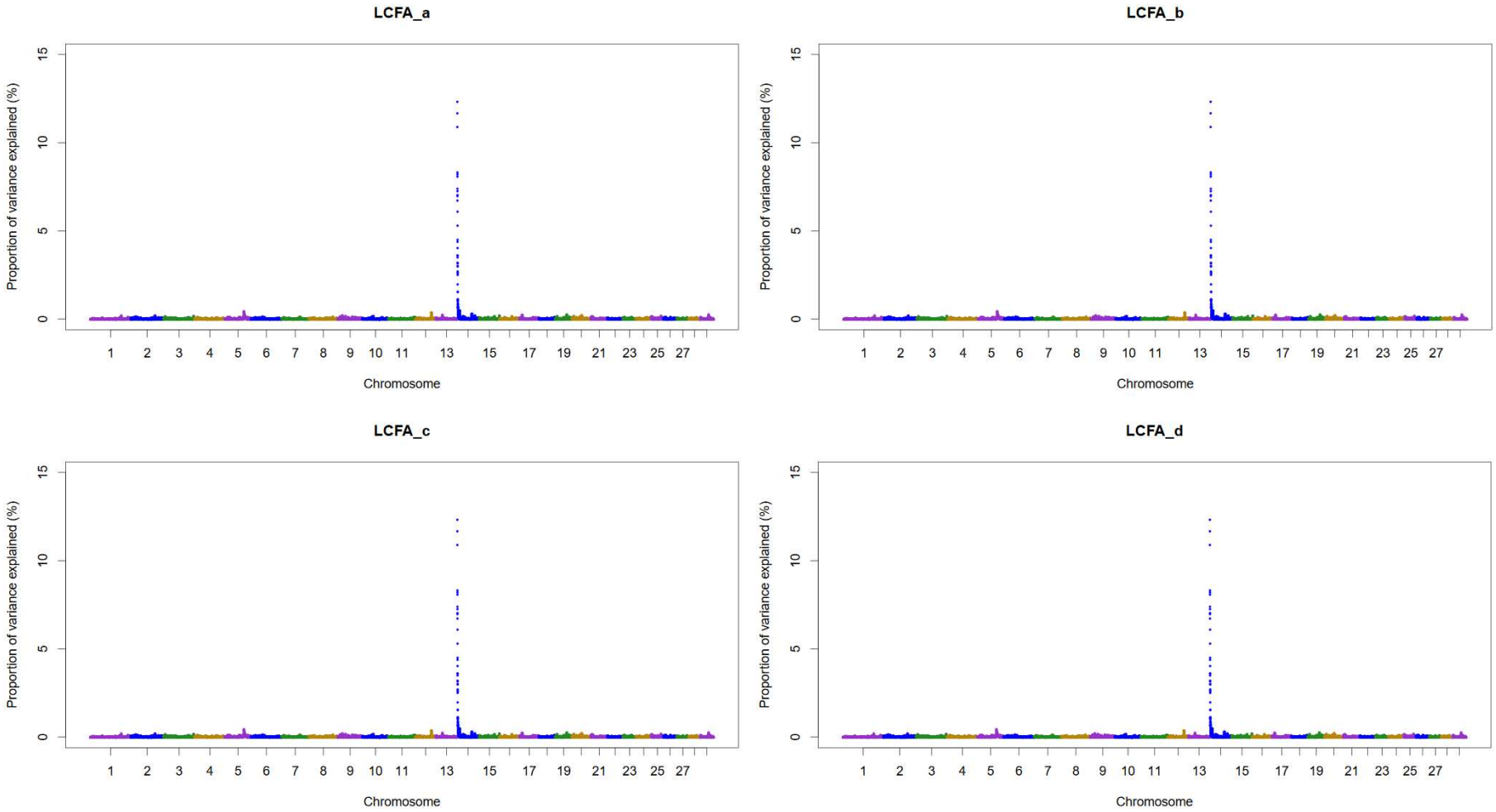

Figure S16. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Holstein using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

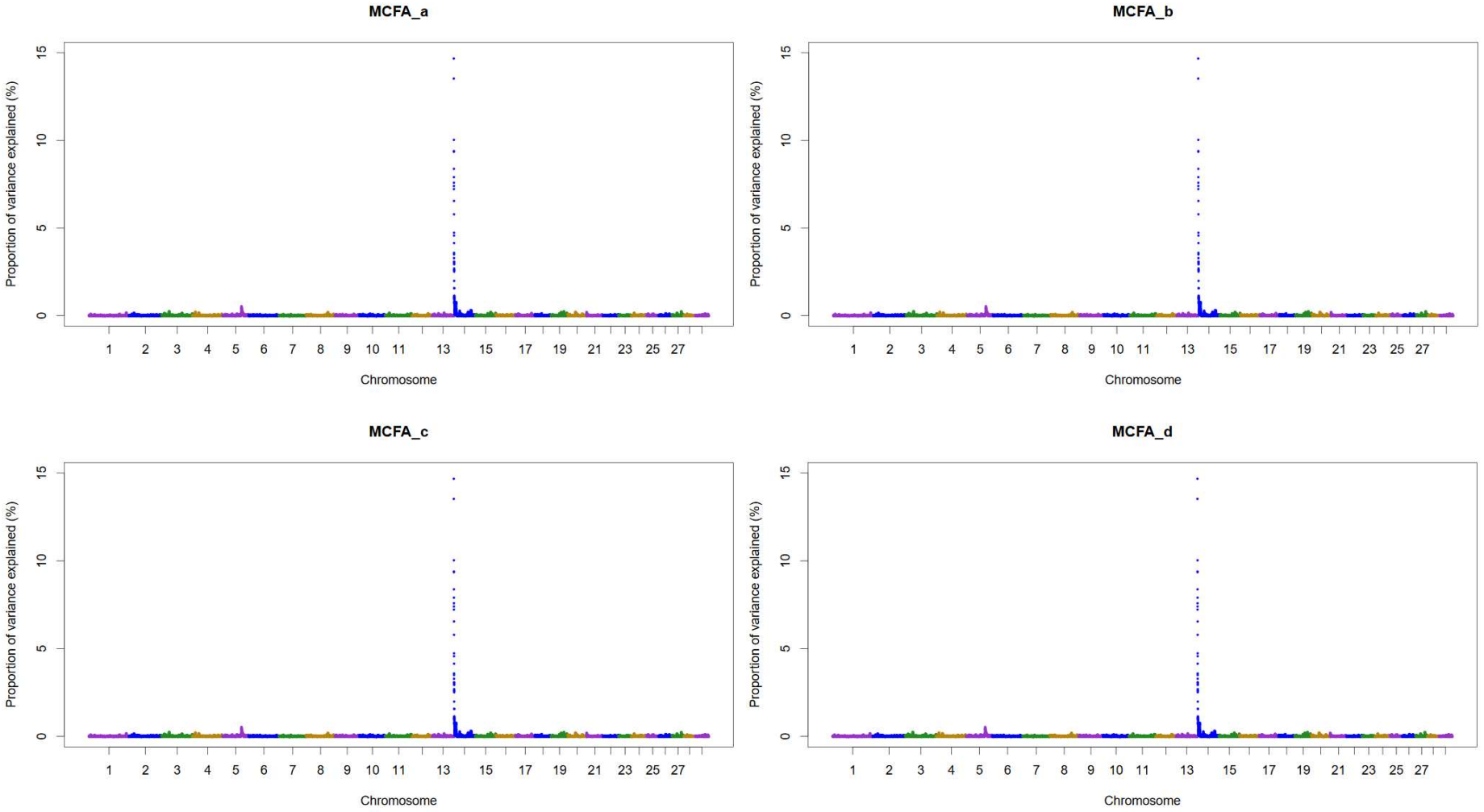

Figure S17. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Holstein using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

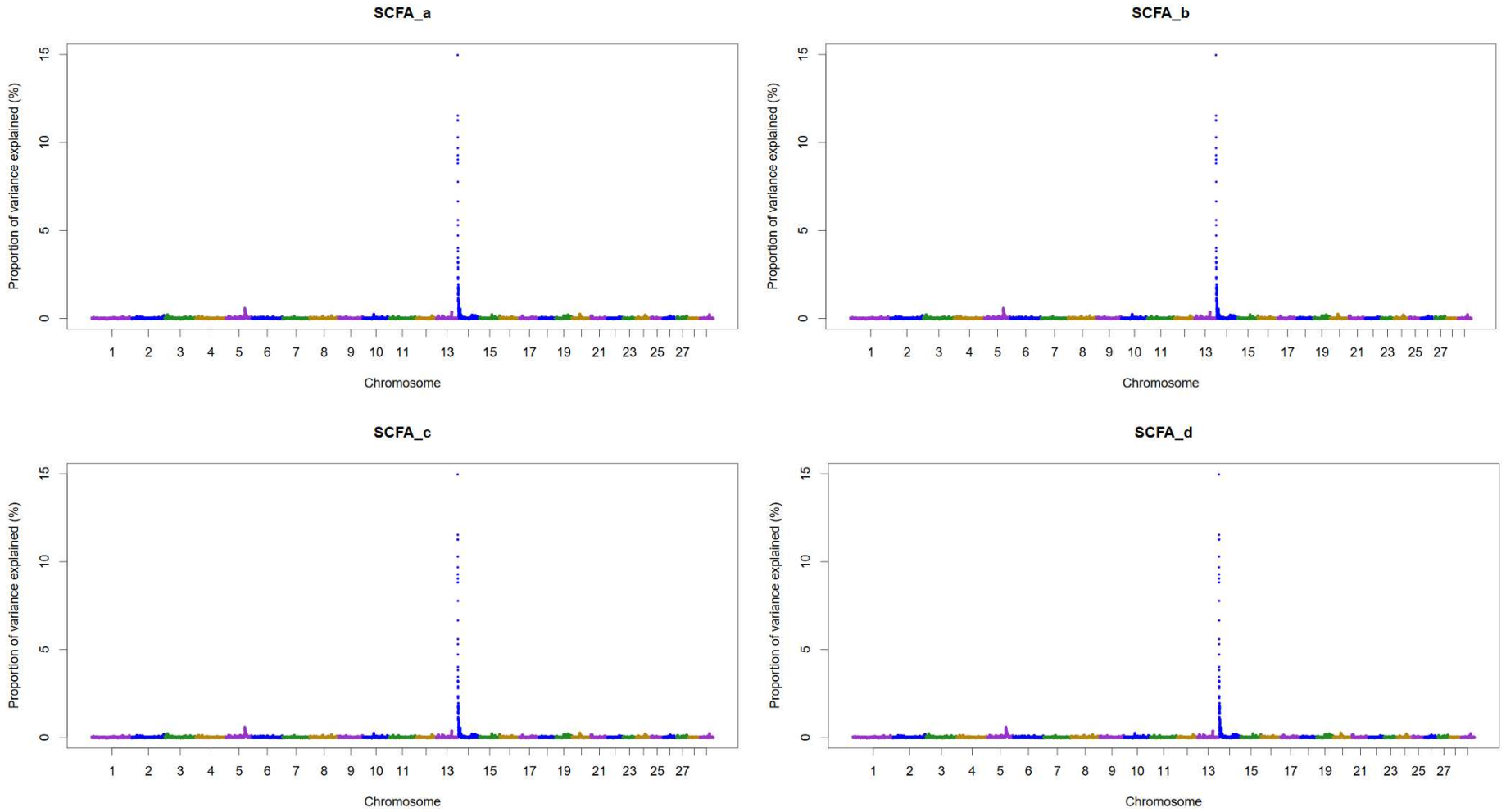

Figure S18. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Holstein using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

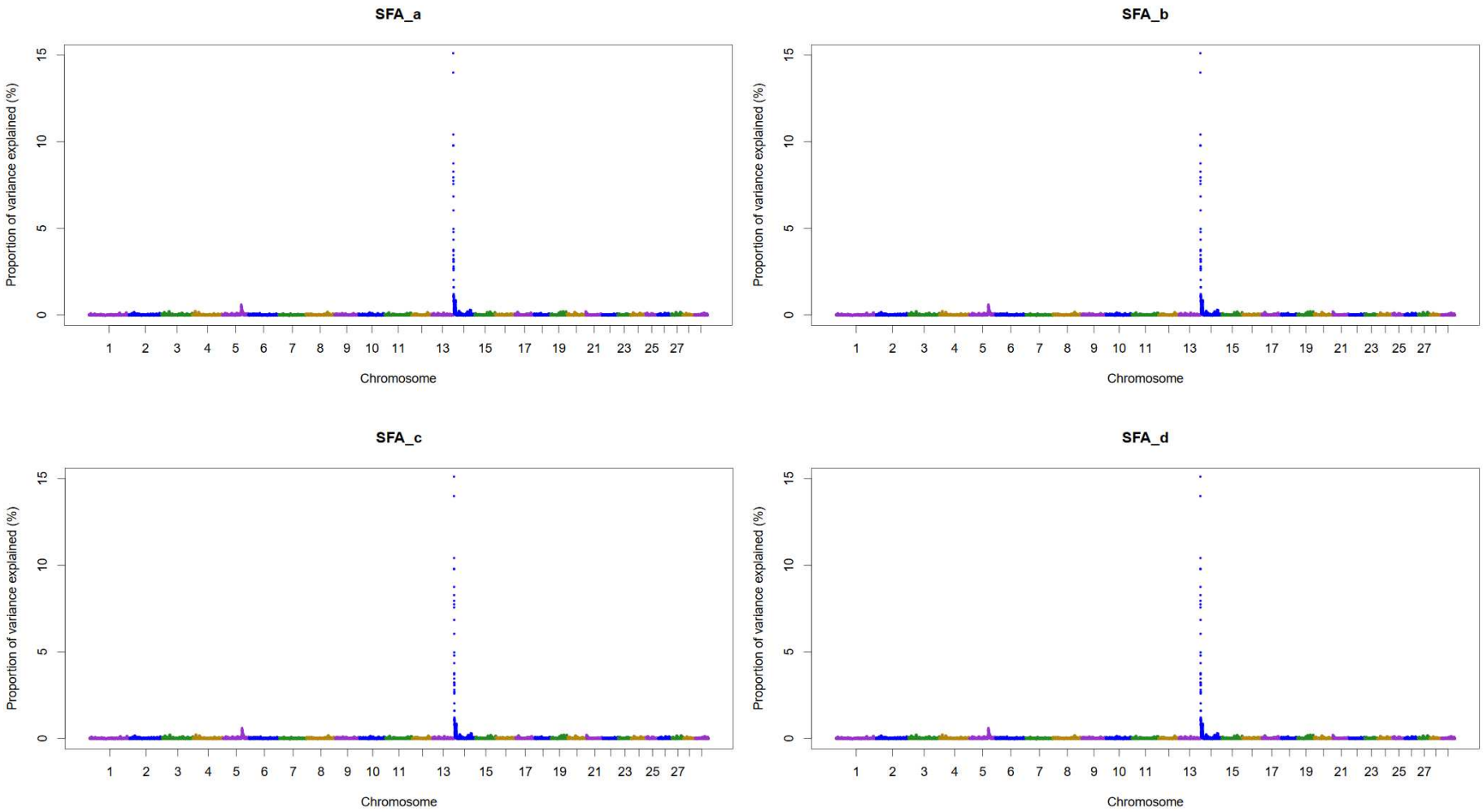

Figure S19. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Holstein using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

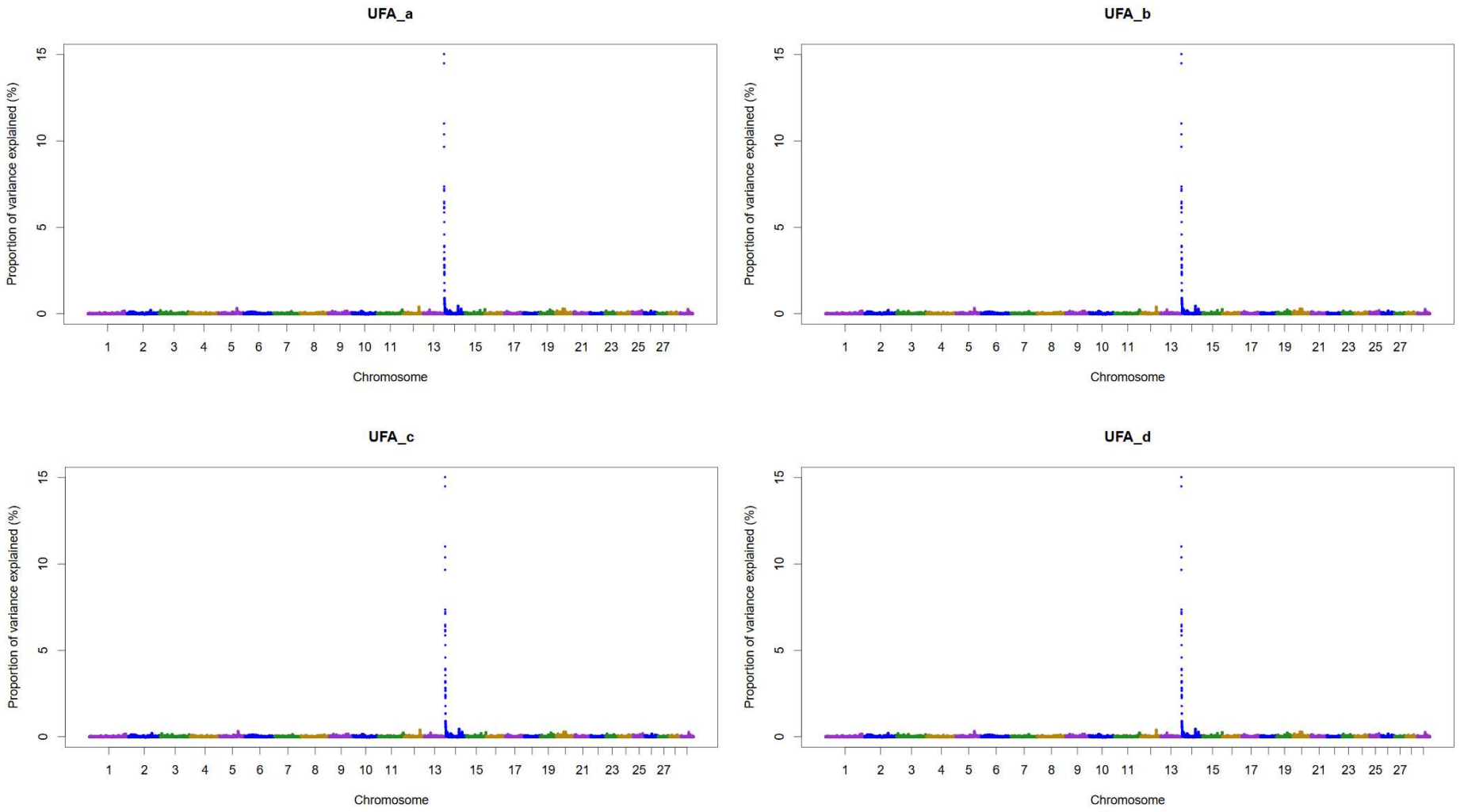

Figure S20. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Holstein using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

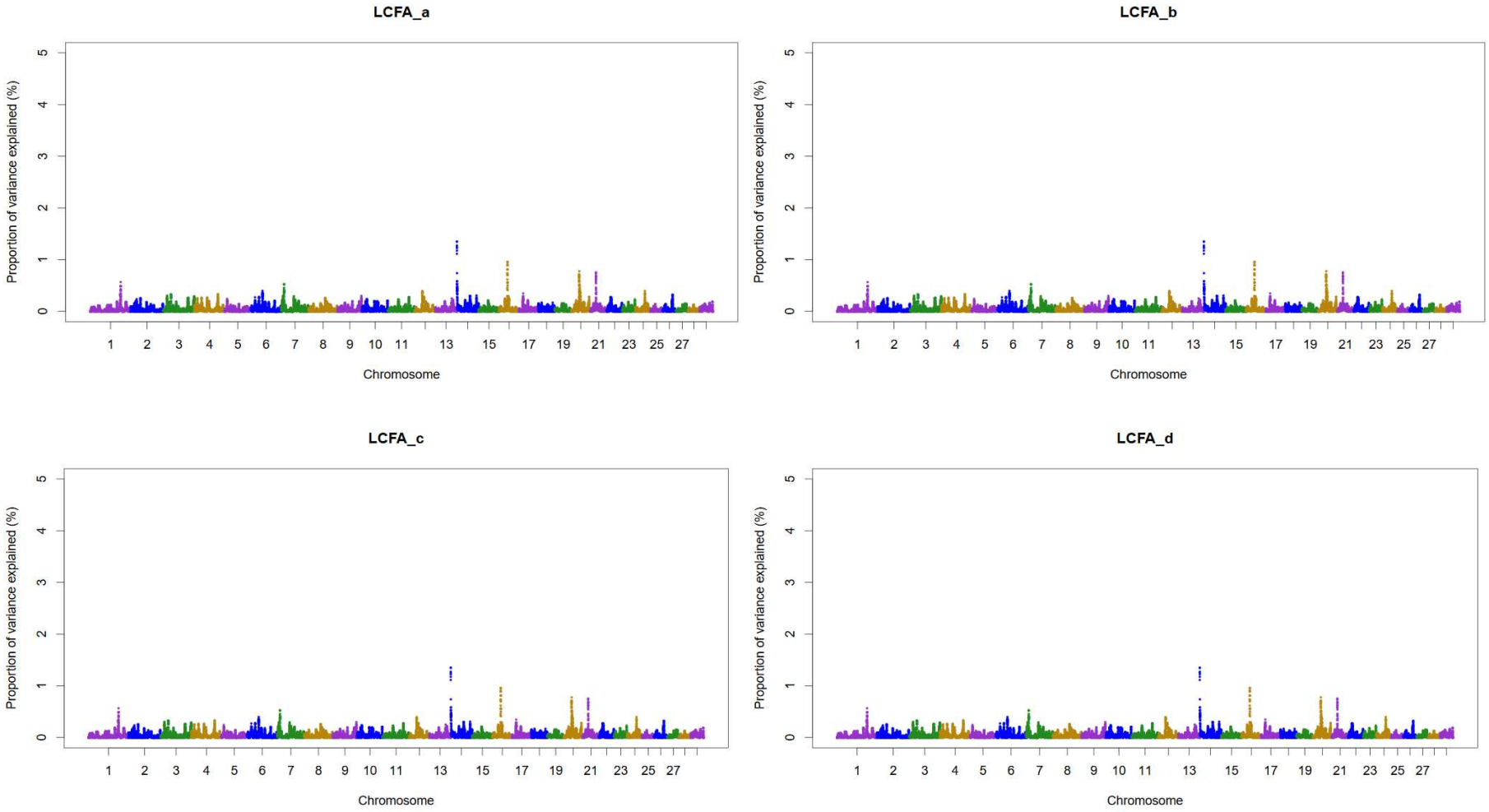

Figure S21. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Jersey using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

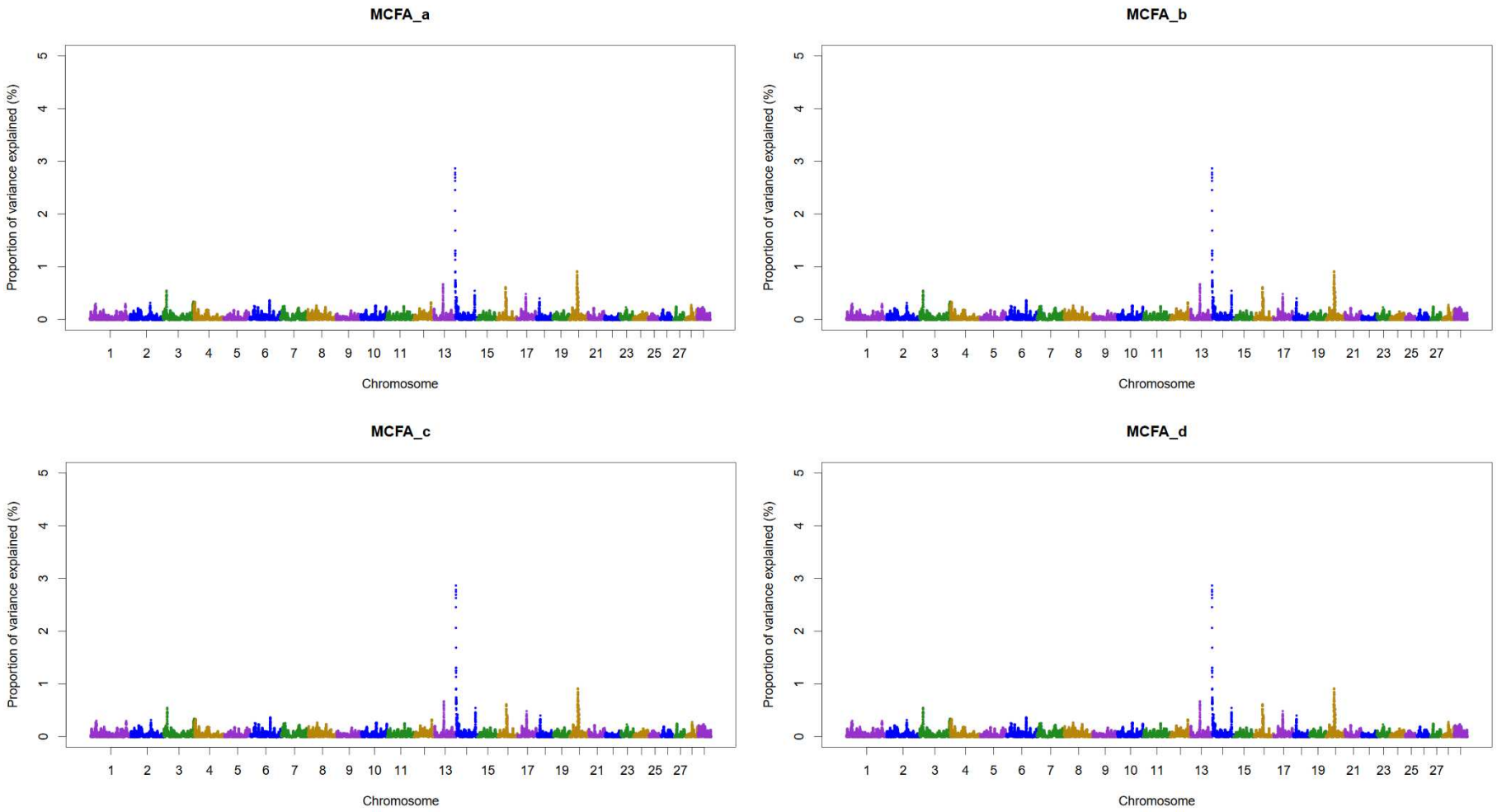

Figure S22. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Jersey using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

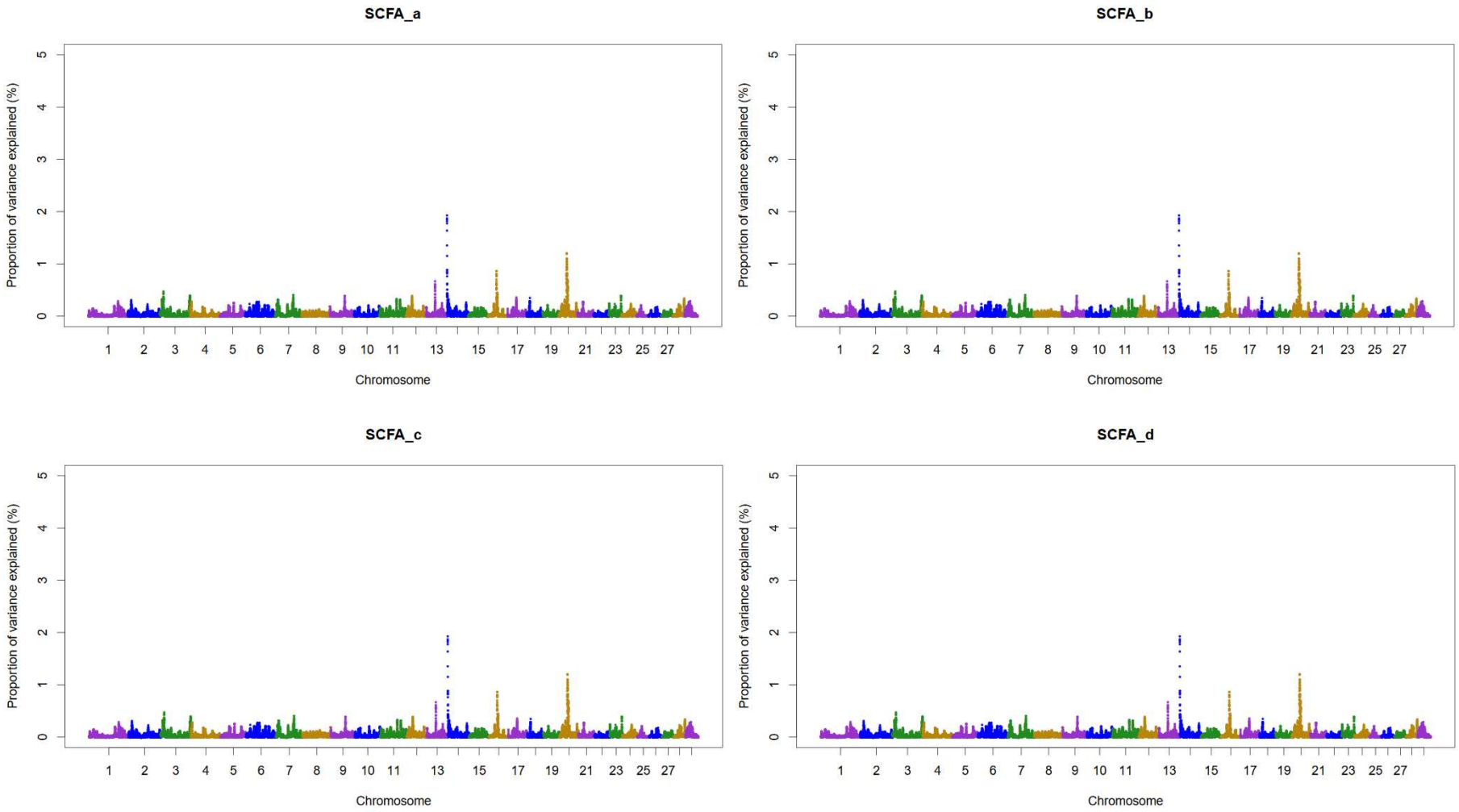

Figure S23. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Jersey using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

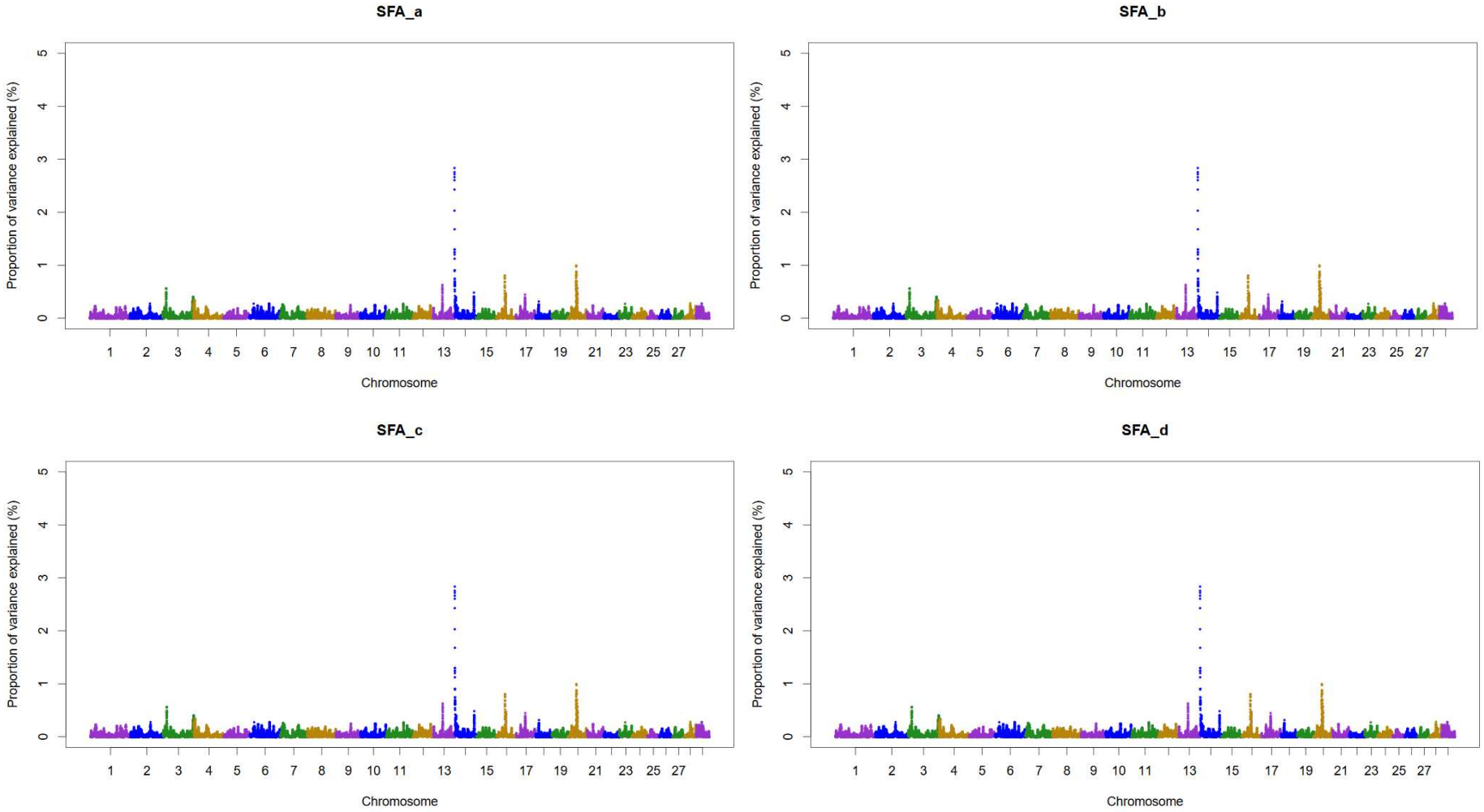

Figure S24. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Jersey using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

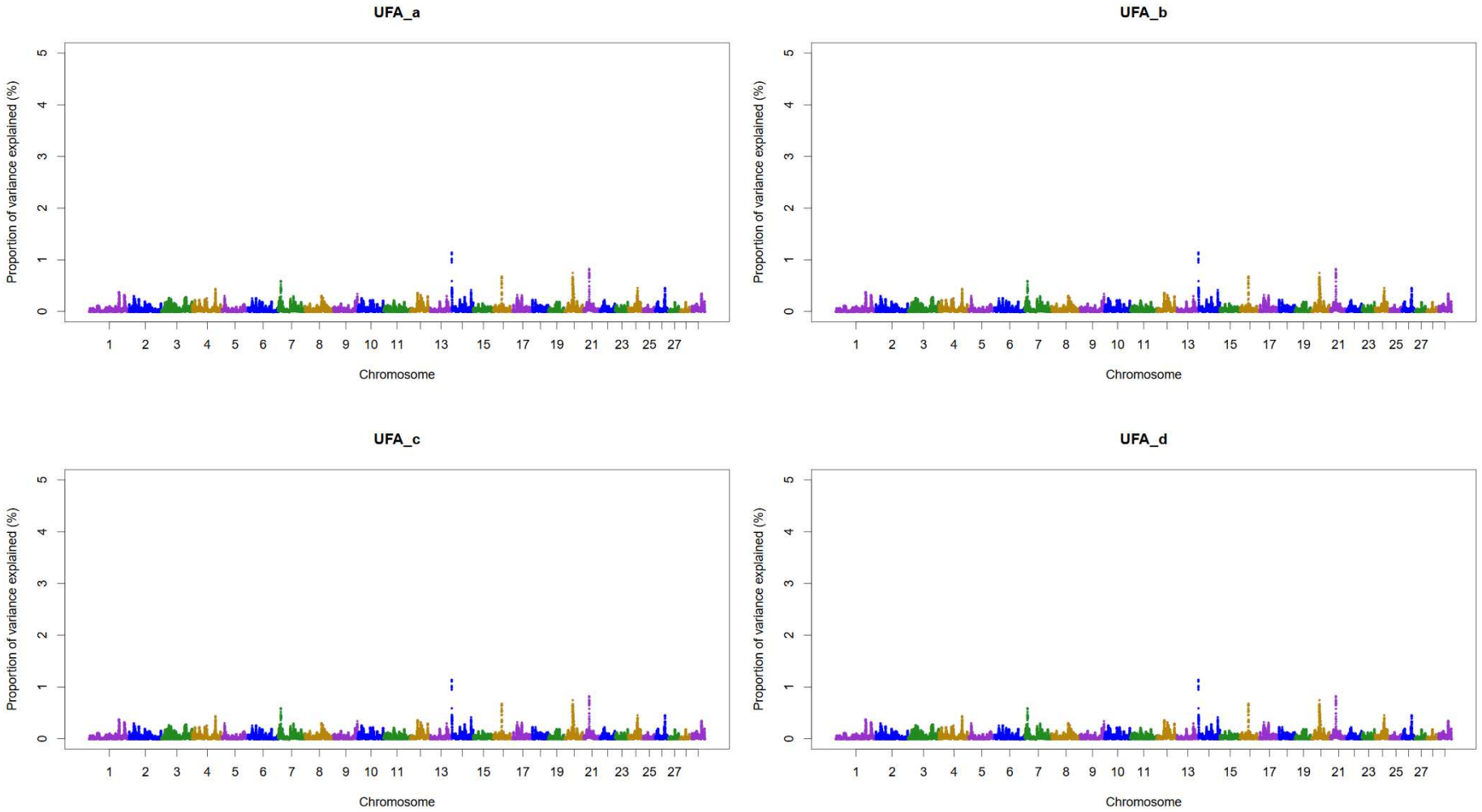

Figure S25. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Jersey using τ and ω equal to 1 (default), corresponding a, b, c and d to each third-order Legendre coefficients.

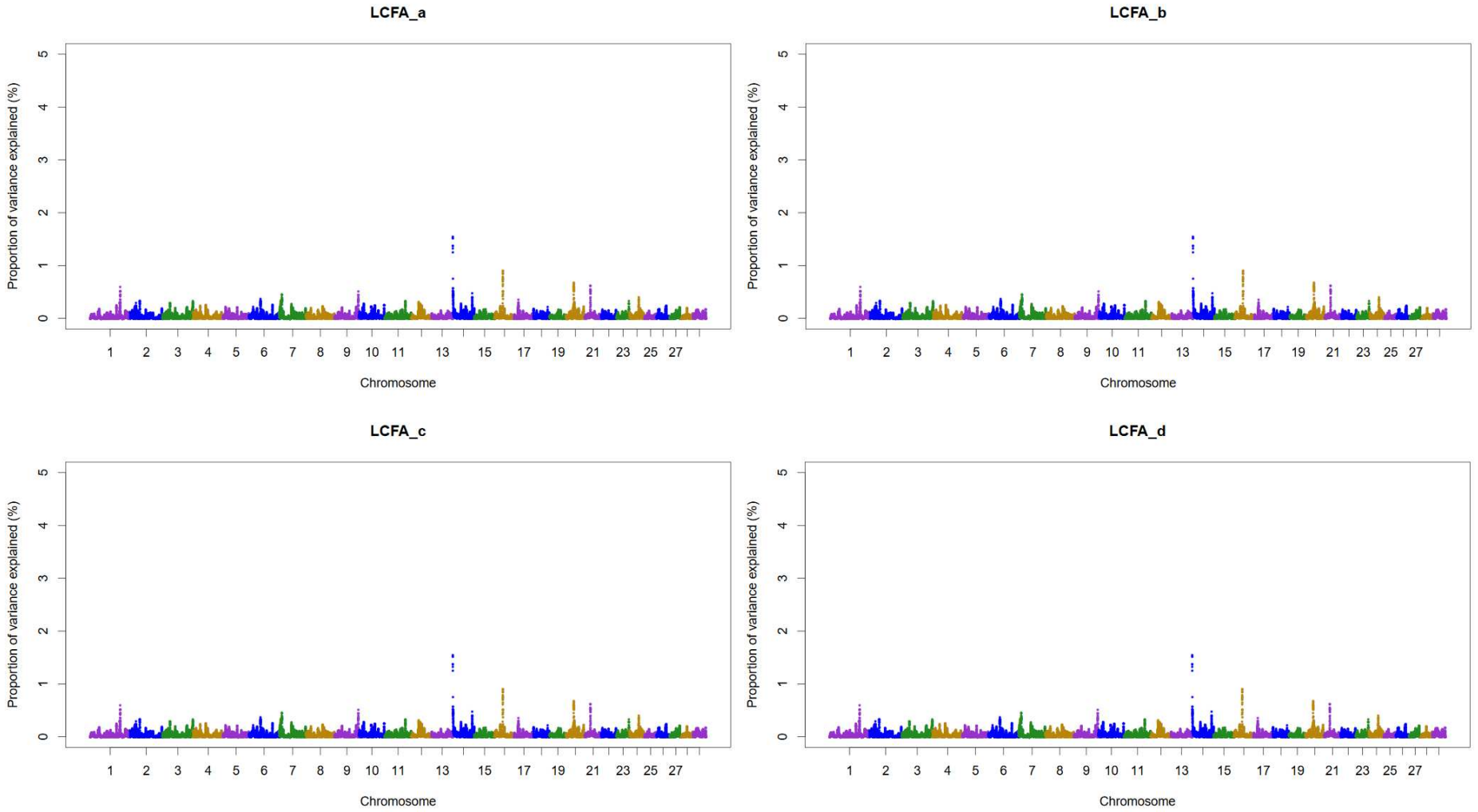

Figure S26. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for long-chain fatty acid for Jersey using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

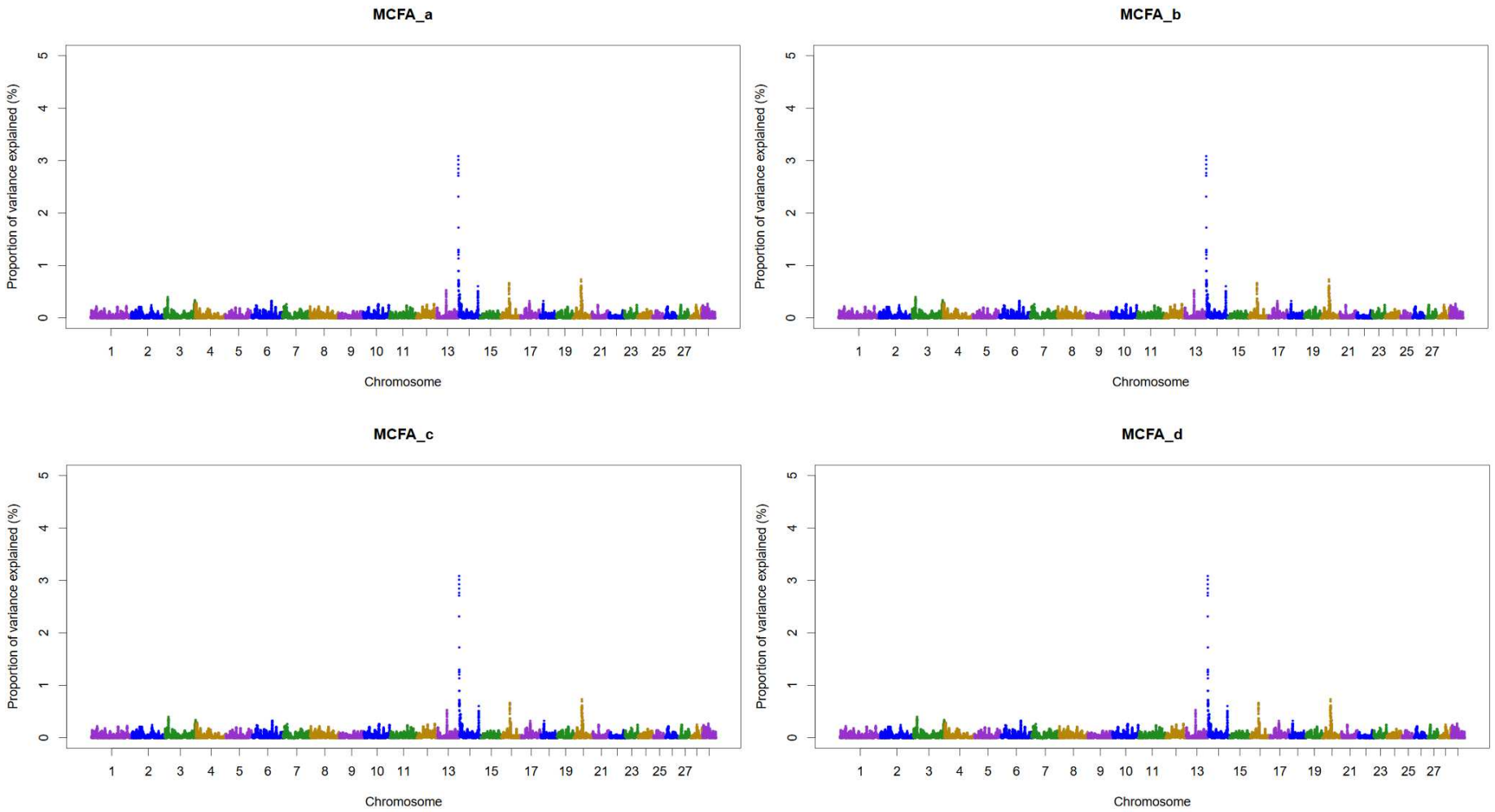

Figure S27. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for medium-chain fatty acid for Jersey using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

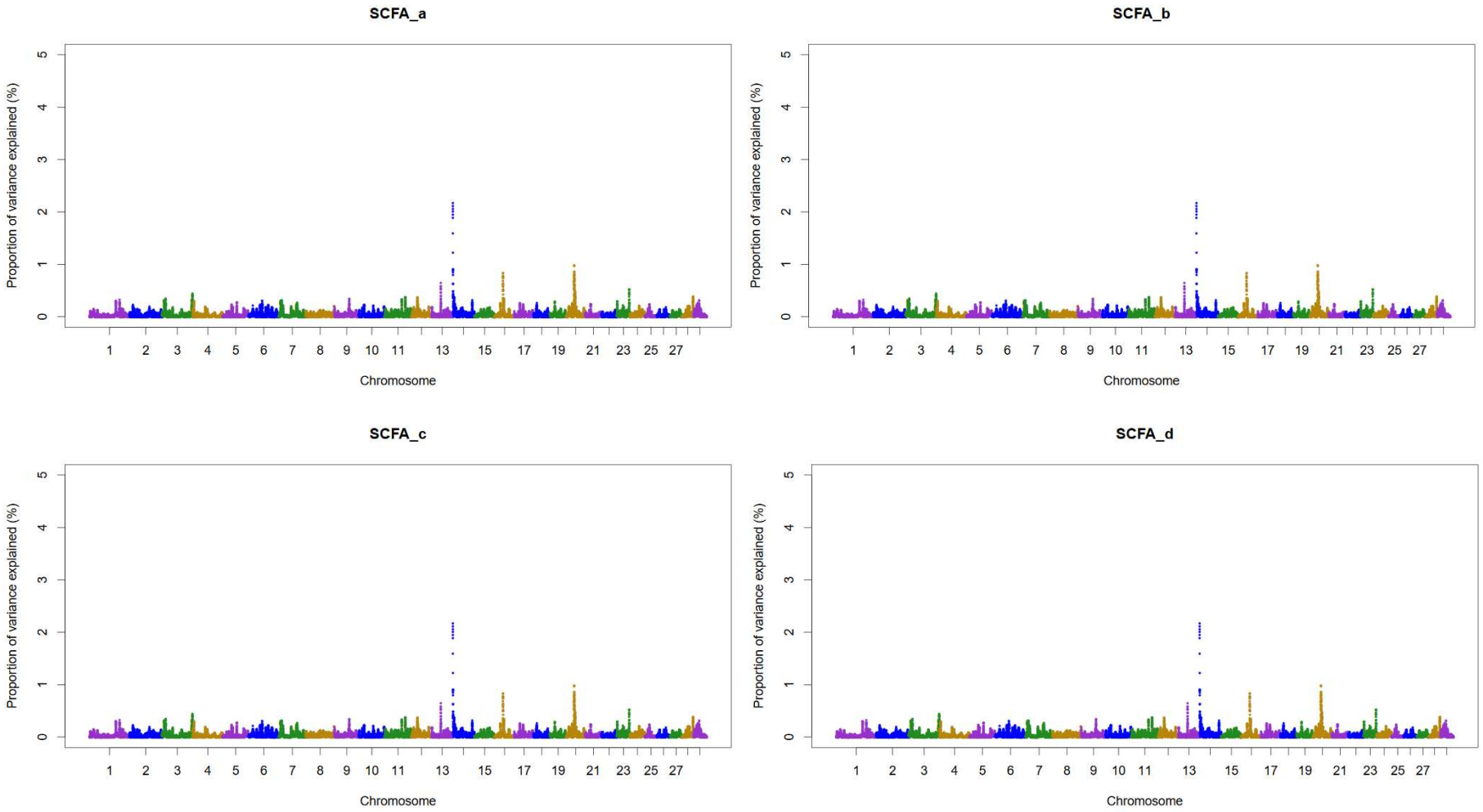

Figure S28. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for short-chain fatty acid for Jersey using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

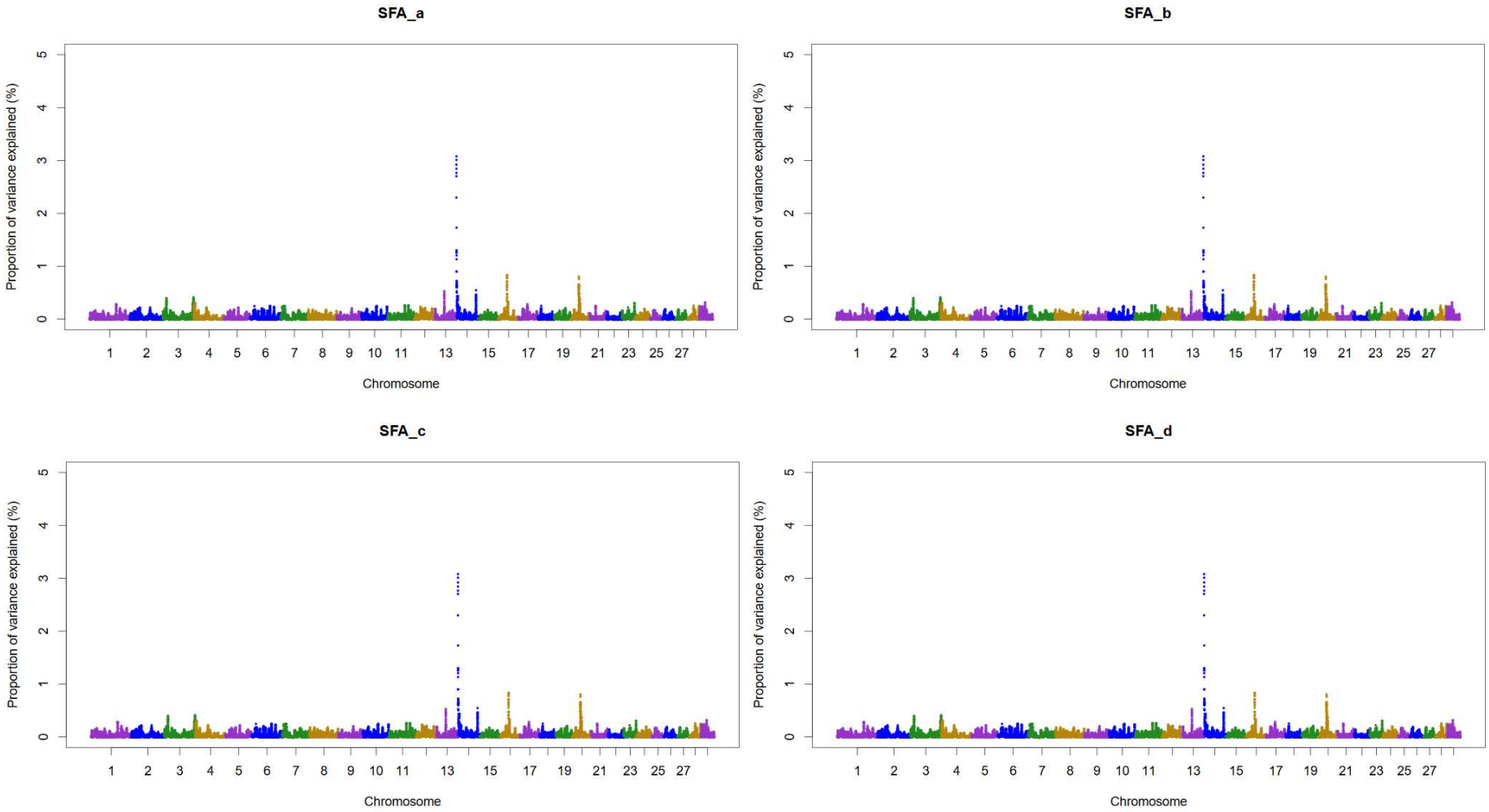

Figure S29. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for saturated fatty acid for Jersey using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

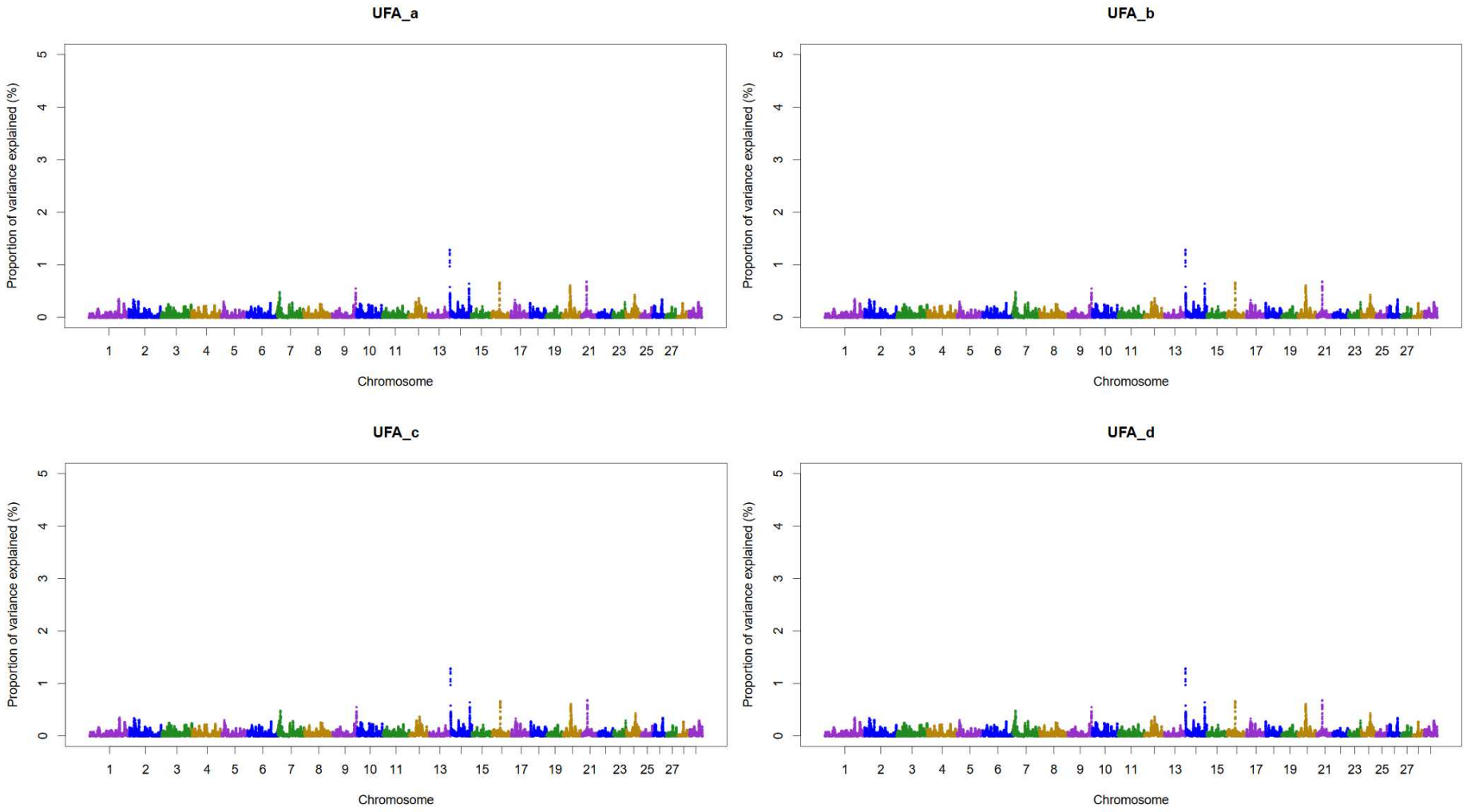

Figure S30. Manhattan plot for the proportion of genetic variance explained by the 20-SNP windows for unsaturated fatty acid for Jersey using optimal τ and ω, corresponding a, b, c and d to each third-order Legendre coefficients.

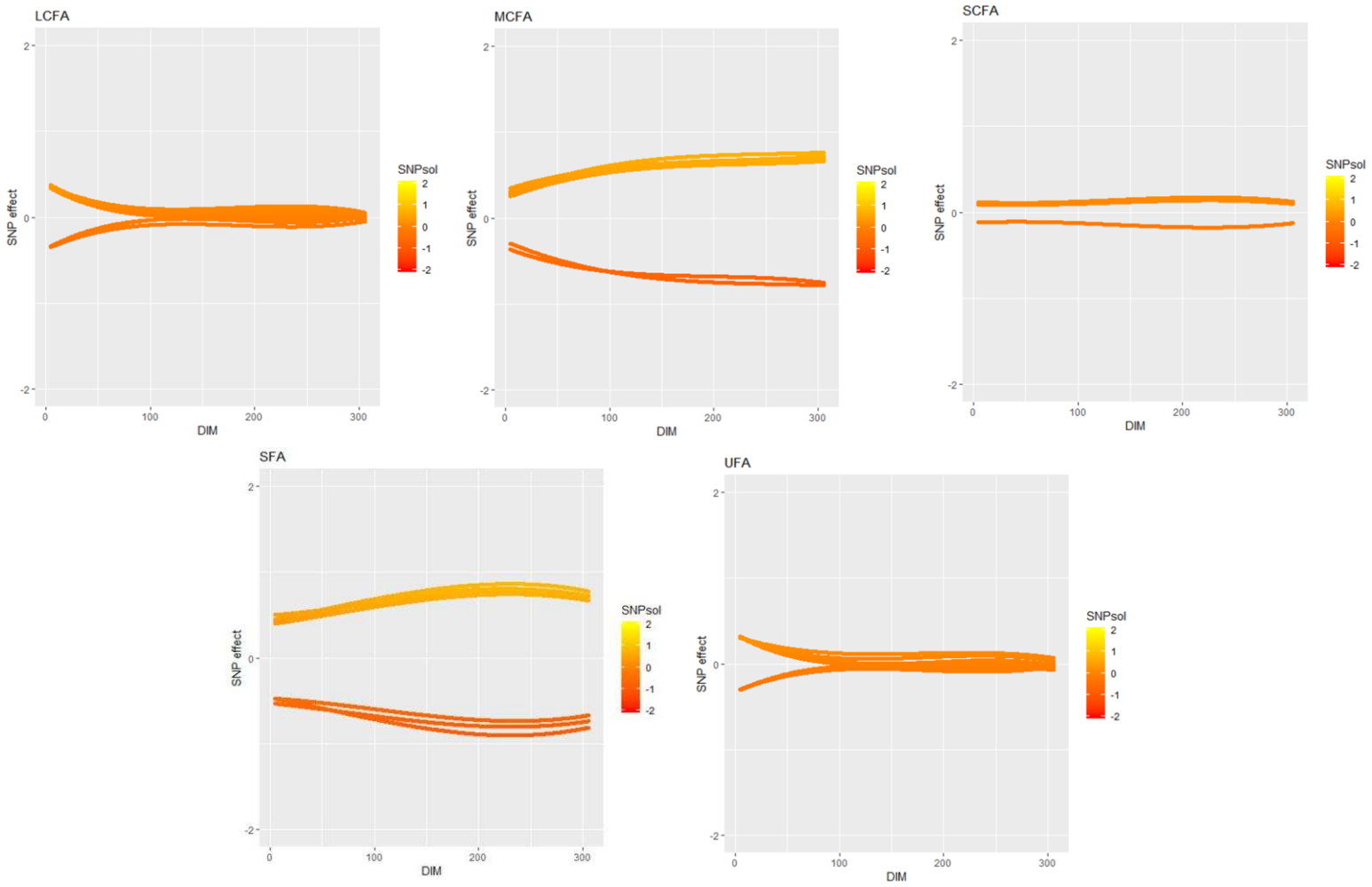

Figure 31. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated fatty acids using τ and ω equal 1 of the Ayrshire breed.

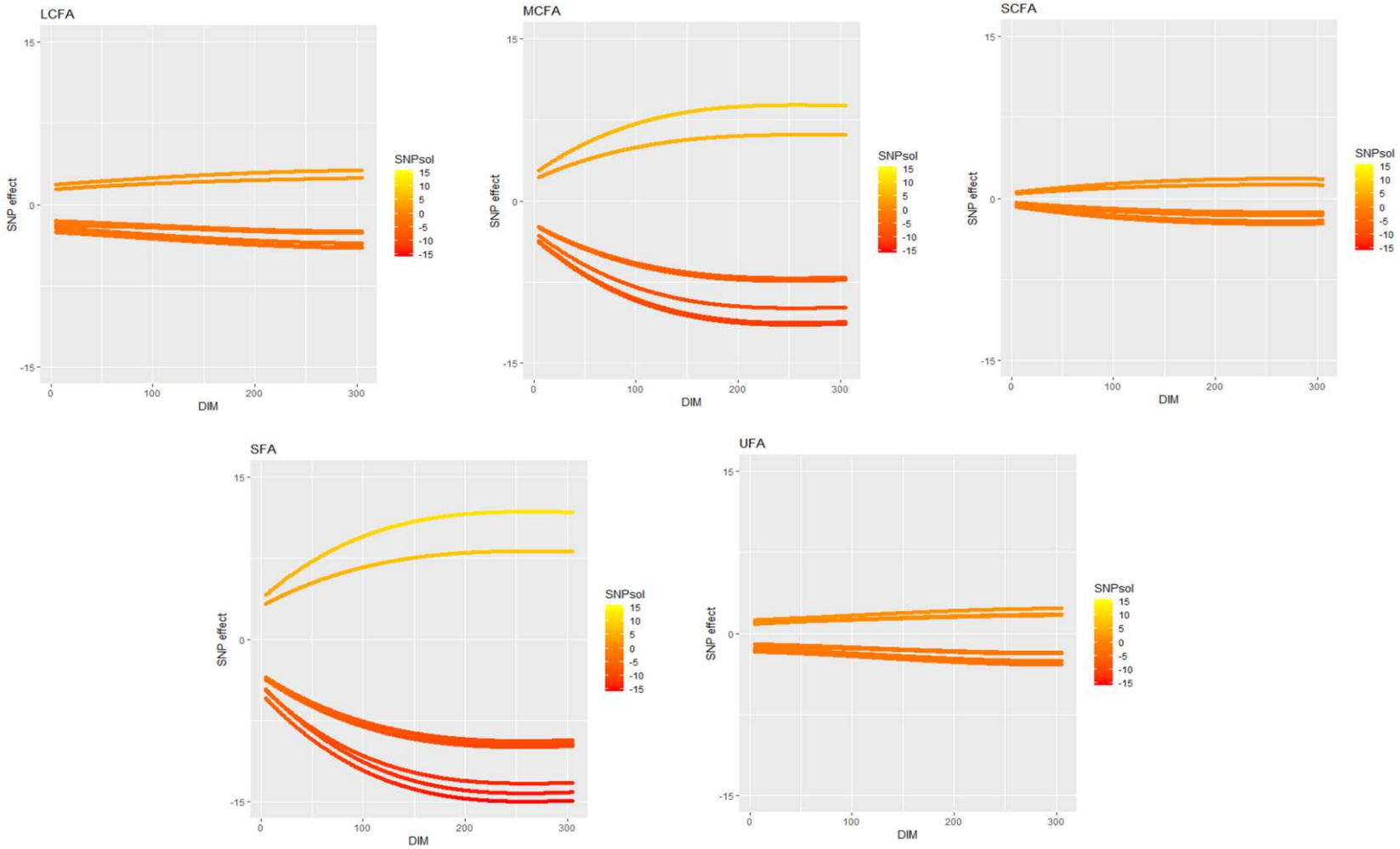

Figure 32. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated fatty acids using τ and ω equal 1 of the Holstein breed.

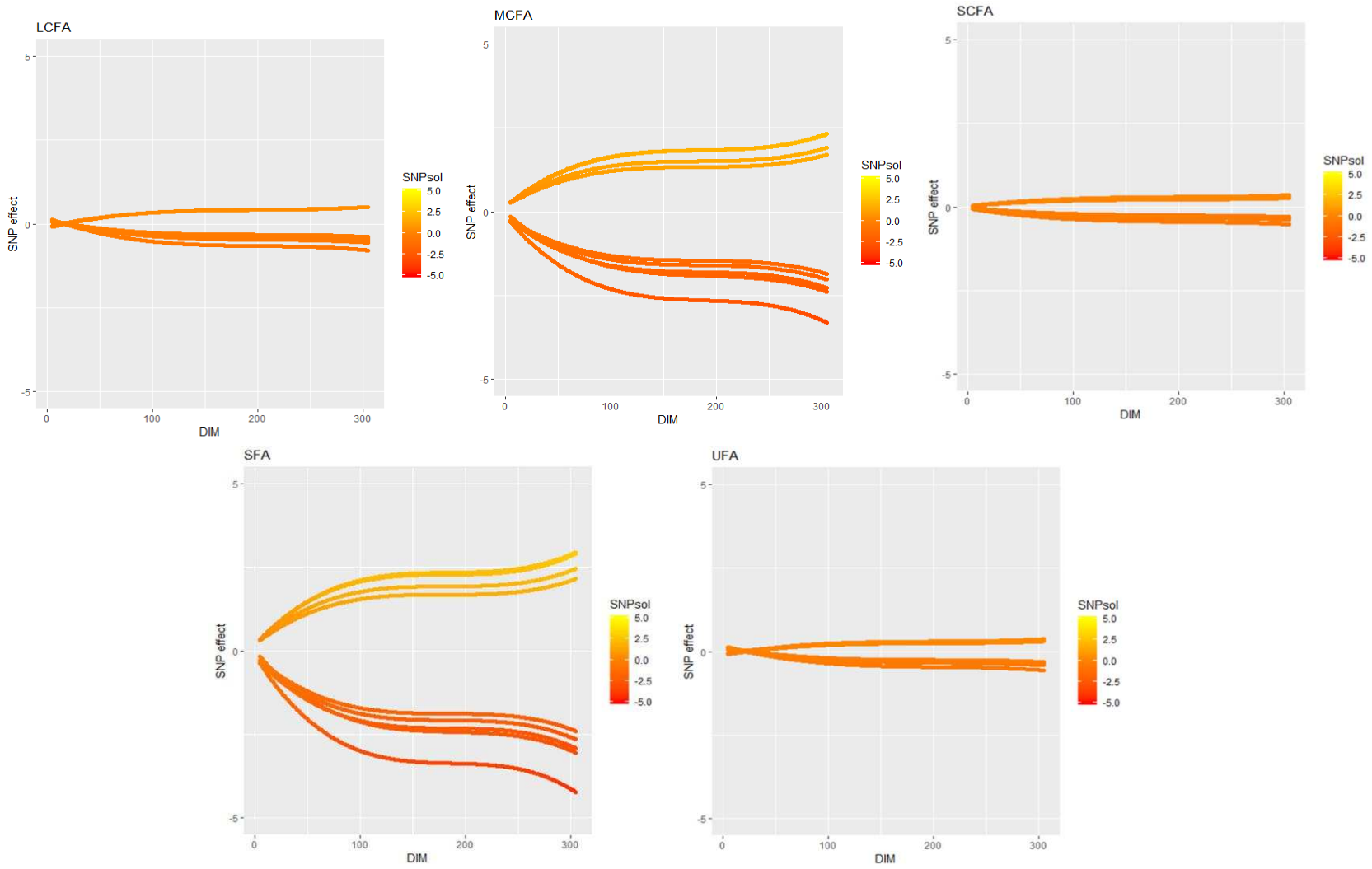

Figure 33. Single nucleotide polymorphism (SNP) effects pattern of the top 10 SNPs over days in milk for long-chain, medium-chain, short-chain saturated and unsaturated fatty acids using τ and ω equal 1 of the Jersey breed.