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GENOME-WIDE ASSOCIATION STUDY FOR THE RELATIONSHIP BETWEEN TEMPERATURE AND FEED INTAKE IN BEEF CATTLE

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By

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Environmental conditions, such as changes in ambient temperature, can cause changes in animal behavior and performance. In general, it is believed that as ambient temperature increases, dry matter intake (DMI) of beef cattle decreases. However, our hypothesis was that the degree to which animals adjust their daily DMI due to changes in ambient temperature is partially controlled by genetic effects. Consequently, the objective of this study was to estimate the genetic component of the regression of DMI on ambient temperature using an admixed beef cattle population consisting of various crosses of Angus, Simmental, and Piedmontese (n = 239). Ambient temperatures were received from a local weather station and DMI was collected via Calen gates. The feeding period averaged 155 d with a range of 114 d to 189 d depending on the management group. Individual animal regressions of DMI on average daily ambient temperature were performed using either daily high or low temperatures over the entirety of the feeding period. Daily high temperatures (°C) averaged 15.07 with a range of -17.21 to 38.25. Daily low temperatures (°C) averaged 2.37 with a range of -28.33 to 15.26. The corresponding intercept and regression coefficient for each animal were used as phenotypes for a genome-wide association study (GWAS). Animals were genotyped with the BovineSNP50 Beadchip. Data were analyzed using a BayesC model with the GenSel software fitting contemporary group (n = 4) and initial body weight (IBW) as fixed effects. A MCMC chain of 100,000 iterations were used with the first

40,000 samples discarded as burn-in. The proportion of SNPs having null effect (π) was set to 0.995. Posterior mean heritability estimates (PSD) for the analysis when daily high temperature was considered in the regression were 0.68 (0.06) and 0.45 (0.08) for the intercept and slope, respectively. Similarly, posterior mean heritability estimates (PSD) for the intercept and slope when the daily low temperature was considered in the regression were 0.76 (0.05) and 0.48 (0.08), respectively. These results suggest that changes in DMI due to changes in ambient temperature are under genetic control. Admittedly the population under study is small and admixed, suggesting that the genomic heritability estimates contained herein are potentially biased upward. However, the concept of applying this same procedure in larger populations warrants further investigation as a means of identifying animals that are less sensitive to environmental extremes.

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Introduction

The environment in which farm animals are kept plays a significant role in their production performance. With the exception of some intensive production systems, beef production in the U.S. is often in extensive production systems whereby beef cattle are reared in complex environments in which they continuously face short and long term environmental change. As a result, beef cattle are susceptible to different environmental conditions and can experience both heat and cold stress. Environmental factors contributing to heat stress are daily high ambient temperature, high relative humidity, low air movement, solar radiation and heat wave. Moreover, due to their poor sweating mechanism, beef cattle also acquire heat through the process of fermentation during digestion. Even though animals are forced to adapt to certain environmental changes, these changes can be very detrimental to their performance and production ability. Reduction of feed intake and growth are among the common signs of beef cattle under heat stress. However, in extreme cases heat stress can also cause death contributing to a significant revenue loss to producers as well as animal welfare concerns.

Heat stress can be defined as the disturbance of a body system from its resting state due to the high level of an external force. Cattle maintain normal body temperature through balancing heat gain and loss, a process known as thermoregulation. Heat stress is a situation that occurs when animals are unable to dissipate their heat load efficiently. Based on the duration and severity of the stress, it can be described as acute or chronic. Acute heat stress is characterized by short and sudden periods of extremely high temperature; on the other hand, chronic heat stress is a condition when there is are long periods of elevated temperature. Beef cattle experience heat stress when an imbalance between the internal heat production and their ability to dissipate it efficiently exist. Decreased dry matter intake, reduced metabolic rate, increased respiratory rates

and sweating are some of the physiological signs that contribute negatively to the production ability of the animals. Generally, as the ambient temperature increases animals eat less, which negatively impacts the usage of energy for production. This negative correlation between dry matter intake and high ambient temperature is well-documented. However, differences in an animal's physiological response and production performance under extreme heat/cold stress is also partially controlled by their genetic makeup. Therefore, our hypothesis was that the degree to which animals adjust their daily DMI due to changes in ambient temperature is partially controlled by their genetic effects. Consequently, the objective of this study was to estimate the genetic component of the regression of DMI on ambient temperature via a genome-wide association study using an admixed beef cattle population consisting of various crosses of Angus, Simmental, and Piedmontese (n = 239).

Literature Review

Global projections of climate change have been one of the most critical issues facing the agricultural sector worldwide. Research studies show that there will still be an increase in temperature, precipitation and concentration of carbon dioxide globally (Hatfield et al., 2008, 2011). Similar projections for the United States over the next 30 years support this evidence that there will most likely be an increase of temperature of 1.5-2 ℃ (Tebaldi et al., 2006; Karl et al., 2009). This puts climate change as one of the top issues that challenges and threatens the future well-being of humans and animals. The forecast of climate change prompts us to consider the inevitable consequences of the climate change on agricultural production in particular.

Demographic changes of the world population is another serious challenge facing the agricultural sector. According to the United Nations Department of Economic and Social Affairs (UN DESA) 2015 report, the world population is expected to reach 8.5 billion by 2030, 9.7 billion by 2050 and 11.2 billion by 2100. Moreover, projection of demand for animal-source food as a result of diet change such as meat and milk are expected to grow by 73 and 58 %, respectively, by 2050. Both climate change and population growth combine to pose an unprecedented challenge that cannot be overlooked. Therefore, in order to feed more people by 2050, the scientific community must find a way to increase the level of current agricultural production given projected climate change.

Livestock production has been recognized as one of the main components of the agricultural sector given its key role in food security by providing protein. Protein is one of the three important nutrient requirements of humans. Despite its role in food security, livestock production is expected to change given the current forecast of climate change (Hatfield et al., 2008). Key et al. (2014) showed that the impact of environmental temperature on livestock is one

of the four major ways that livestock production could be impacted due to climate change by affecting animal health, reproduction and animal products (meat and milk). Furthermore, livestock production could be altered from an increase production costs and productivity losses incurred by climate change. For example, climate change further increase costs and availability of feed crops. In addition, pasture, rangeland places and yield could also be affected by climate change which in turn influences livestock production costs and profits. Climate change could also increase production costs incurred from the distribution of parasites and pathogens as a result of extreme temperature. The before mentioned examples are part of the consequences of climate change that could significantly affect livestock production. Climate change could contribute to increases in daily high ambient temperature and humidity, which together could result in heat stress for livestock. The stressor factors such as heat, gaseous contaminants, dust, mud, and/or crowding play an influential role on animals' performance whether in beef or milk production. Freeman (1987) pointed out that heat stress is one of the most critical stress factors, which most likely reduces the welfare and performance of animals particularly in the hot regions of the world. In light of such climate changes, animals' meat and milk production are deteriorating on a daily basis (Key et al., 2014).

Animals Response to Heat Stress

According to Yousef (1985), heat stress is defined as the disturbance of a body system from its resting state due to the high level of an external force. Therefore, heat stress is a situation that occurs when animals are unable to dissipate their heat load efficiently. Based on the duration and severity of the stress, it can be described as acute or chronic. Acute heat stress is characterized by short and sudden periods of extremely high temperature; on the other hand,

chronic heat stress is a condition when there are long periods of elevated temperature (Emery, 2004).

Environmental conditions where animals are producing at optimal level are known as the comfort or thermoneutral zone. The thermoneutral zone is a range of temperature where animals maintain their normal body temperature and are able to perform and produce without a need for a behavioral or physiological adjustment. This means that it is a range of temperature within which animal's production is optimum with minimum cost (Du Prezz et al., 1990). Depending on the species and breed, animal comfort zone varies. For example, cattle in general have a zone of comfort that ranges between 5℃ – 20℃, calves between 10℃ – 20℃, sheep between 21℃ – 31℃, goats 10℃ – 20℃, respectively (Kerr, 2015). However, environmental conditions that exceed either the upper or lower bound of the thermoneutral zone of an animal will induce stress which in turn alters the physiological and behavioral system of the animal. As mentioned above, heat stress is one of the main sources of stress caused by climatic conditions that has a direct physical impact on the performance of the animal. However, an animal can also experience stress in their life time due to other sources as well, such as infection, nutritional deficiency and metabolic disease. Depending on the genetic background of the animal and the environmental factors, the strength and duration of animal's response to stress varies (Freeman, 1987). Animals under heat stress exhibit various physiological and behavioral responses that include reduction in urinary water losses, reduction in feed intake and production, increased sweating, increased respiration, increased rectal temperature and heart rates. Animals exhibit these physiological and behavioral responses in order to maintain thermal equilibrium. Animals maintain their internal body temperature in a state of equilibrium by physical, physiological and biochemical responses a process called thermoregulation (Aggarwal and Upadhayay, 2012). Animals under heat stress

are unable to maintain a normal thermoregulation process. During such abnormal thermoregulatory process, animals cannot avoid heat from their body effectively. They will also experience poor sweating mechanisms. Often beef cattle are exposed to climatic conditions that occur naturally due to the extensive production system. Beef cattle that experience heat stress can be identified by using typical non-observable and observable signs. Some of the visible signs are listed as slobbering, panting, open mouth breathing, decreased activity, refusal to lie down, agitation and restlessness. On the other hand, lowered conception rate, appearance of stress hormones in the blood, lower fertility in bulls, increased peripheral blood flow, and a lowered ruminal pH value are typical invisible signs to recognize beef cattle that are suffering from heat stress.

Cattle can experience heat stress differently because of various contributing factors such as animals' characteristics and/or genetic factors. *Bos indicus* cattle are well known for their genetic adaptation to heat stress as compared to *Bos taurus* breeds. Moreover, cattle within the same breed can also experience higher heat stress if they absorb and produce more heat from the environment but dissipate less of their body heat. Animals with higher levels of performance can also experience more heat stress than animals with lower production performance. Because of their inherent higher level of productivity, such animals are able to produce more heat than those with lower productivity (Blackshaw and Blackshaw, 1994; West, 1994).

Similarly, there are well identified animal characteristics that impact animals experience with higher levels of heat stress. For example, a hide color is one of the significant characteristics that differentiate animals' ability to cope with heat stress. Animals with dark hair have lower reflectance ability and also absorb greater solar radiation than animals with white hair and as a result dark hair animals experience higher heat stress than others (Da Silva et al., 2003).

According to Brown-Brandle et al. (2006), the animals' level of fatness, history of respiratory pneumonia, and temperament are also other factors that distinguish animals in experiencing different levels of heat stress; for example, calm animals experience less heat stress than excitable animals. Feedlot cattle that are treated for pneumonia have better respiratory rate under heat stress than untreated animals. The age of animals also impacts their ability to cope with heat stress; very young and very old animals are more vulnerable to heat stress than others. Moreover, how cattle are kept in a feedlot and what they eat determines animals' ability to tolerate heat stress. Heavy feedlot cattle are at risk to experience heat stress. Similarly, animals that are being fed excessive protein levels are prone to experience heat stress, particularly in pastures and feedlots. The kind and quality of feed also creates differences among animals to experience heat stress. For example, feeds such as hay contribute to the occurrence of heat stress more than corn-based feeds. This is because corn-based feeds are known for their low heat production during fermentation or digestion. Generally, animals' responses to heat stress vary based on four important factors: differences in genetics, health, production status, and previous exposure to heat. The response of cattle to heat/wave and hot/dry conditions is a twofold process. The first process is directed towards the reduction of metabolic heat; whereas, the second process is directed towards the utilization of all mechanism to enhance the loss of heat from their body. During such conditions, animals do have lowered appetite and increased water consumption.

Measuring Heat Stress

Ambient temperature is not the only environmental factor that has an impact on the development of heat stress in cattle. Humidity and air movement also have a large impact on the occurrence of heat stress (Armstrong, 1994). For example, high humidity negatively influences the ability of animals to cool their body and in turn contributes to the occurrence of heat stress.

Under lower humidity conditions, animals can experience less heat stress; however, the risk for heat stress rises when the humidity increases, even though at lower ambient temperature. Animals will experience the most sever heat stress when both the ambient temperature and relative humidity are high. In addition to those day time conditions, night time conditions such as minimum wind speed, minimum solar radiation, and minimum Temperature Humidity Index (THI) also impact heat stress in cattle (Mader et al., 2006). The THI is an index that collectively measures ambient temperature, relative humidity, and evaporation rate (Dikmen and Hansen, 2009). "THI is an index for assessment of the potential of an environment to induce heat stress in humans and farm animals" (Aggarwal and Upadhayay, 2012). Dikmen and Hansen (2009) asserted that THI is a reliable indicator of heat stress in cattle. However, THI has some drawbacks; the ability of THI in predicting heat stress in extensive grazing systems may not be accurate, since THI does not account for accumulated heat load. The THI also cannot account for solar radiation and wind speed (Gaughan et al., 2008).

Economic Impact of Heat Stress

There is substantial evidence that shows significant economic losses incurred due to heat stress in livestock production. In general, economic damage of livestock production as a result of heat stress come from different sources of the production system such as reduction of feed intake and growth, decreased milk, meat and egg production, decreased fertility or reproduction efficiency, reduction in immunity and ultimately during an extreme stress event from mortality of the animal. Moreover, an additional source of economic loss can also come from an investment made to mitigate heat stress. Estimates of economic losses imposed by heat stress vary depending on several other factors such as type of production systems and its environmental location, tolerance and response of species and breeds to heat stress. Even though it is difficult to

have accurate estimates of costs caused by heat stress, St-Pierre et al. (2003) estimated the economic loss on the major livestock divisions of the U.S. The analysis was based on the assumption of animal performance, reproduction and mortality at an imaginary thermoneutral zone with a 2002 climate condition. The authors reported that the aggregate annual cost as a result of heat stress in the production of dairy, beef, swine and poultry ranged approximately from $1.7 to $2.4 billion (Table 1). Moreover, Rosenweig et al. (2007) conducted an extensive research review on the impact of climate change emphasizing the rise of temperature and its consequences. The authors reported an annual increase of 8% in economic losses due to natural disasters between the years of 1960 and 1990; the estimate of the aggregate increase was $584.4 billion.

Table 1. Total cost of heat stress in major U.S. livestock sectors

Livestock category	Total annual cost in millions
Beef	$370.1
Dairy	$896.7
Swine	$299.2
Poultry	$127.3
Aggregate cost of all livestock	$1,693.3

Mitigation Strategies of Heat Stress

Environmental Modification

Livestock producers and scientists use multidisciplinary management approaches to lessen the economic loss induced by heat stress (Collier et al., 2003). Environmental modification is the first and most common strategy implemented by livestock producers to help animals cope with heat stress. Hahn (1981) pointed out that the different alternative environmental modifications available for livestock producers to choose from are based on either shielding the animals from the variables contributing to heat stress or increasing the animal's ability of evaporative heat loss.

Often, farmers provide shade through buildings, trees and housing thereby protecting animals from the exposure of direct solar radiation (Blackshaw and Blackshaw, 1994; Buffington et al., 1983). Buffington et al. (1983) reported that tree shades are very effective in comforting animals that are reared outside. Insuring that there is enough shade available is also important as cattle tend to look for a cooler place during high temperatures and gather themselves to use shade provided by other animals. However, in some instances natural shades provided by trees may not be enough or appropriate. Therefore, livestock producers may use artificial shelters. In such cases, it is important to take into consideration not only the design but also the materials used, as both play a significant role in minimizing heat stress (Armstrong, 1994; Smith et al., 2002).

Usage of cooling equipment is another key method of environmental management intervention strategy to mitigate heat stress and help animals maintain their performance. Cooling devices such as sprinklers, ventilators or water nozzles are used separately or in combination during extreme high temperatures to cool down animals directly and/or the

environment. Applying sprinklers can help to reduce ground temperatures and raise evaporative cooling (Gaughan et al., 2008; Means et al., 1992; Morrison et al., 1973). Moreover, utilization of ventilators or fans can also help to increase the movement of air especially for cattle that are kept inside barns or pens. Opening of windows and sides of the barn are another option that can improve sufficient air movement through natural ventilation (Bryant et al., 2007).

Nutritional Modification

Combating heat stress through improved nutrition is another important mitigation strategy. During high temperatures livestock lose water through respiration and sweating and as a result consumption of water increases. Therefore, animals' access to adequate, available, cool and clean water is very critical. Increasing the number of water troughs and rate of refill during heat stress can keep cattle with sufficient quantities of water and help to avoid competition for access and crowding. Cattle drink more water during heat stress to regulate and maintain their body temperature. However, an increase in water consumption will also enhance urine production which results in the loss of minerals such as sodium, potassium and magnesium. As a result, additional supply of minerals during heat stress is necessary.

Understanding of nutrient requirements and timing of feeding can also be as critical as providing water in helping cattle cope with heat stress. In general, cattle react with lower dry matter intake during heat stress subsequently affecting animals' performance and productivity. Often, it is not advisable to make a sudden ration change during heat stress, but providing improved forage quality and palatable feeds can reduce the impact of heat stress (Beede and Shearer, 1996). It has been reported that cattle accumulate heat load from consuming diets that have high energy content or from feeds that contribute to the production of heat during fermentation. Feeds such as hay or straw are known for their low energy content but contribute

significantly to the production of heat during fermentation. Moreover, feeds such as corn and other concentrates are known for their higher energy content but with less heat production during digestion. However, feeding cattle with more concentrates can also lead to acidosis problems. Carstens et al. (1989) suggested that controlling high energy feeds can help lower metabolic heat production thereby reducing heat load that can be acquired by cattle. Therefore, it is very important to act with caution while adjusting feed rations during hot weather (NRC 2001). In addition, feeding patterns also contribute in determining whether cattle acquire high heat load or not during the day. Brosh et al. (1998) reported that feeding animals during the morning will lead to the peak of heat production from feed during which the environmental temperature is also high. The authors reported that in general heat production from feed intake reaches its peak after 4 to 6 hours of feeding. Therefore, it has been suggested that feeding cattle during the evening or night may reduce acquiring of heat load from metabolic heat (Reinhardt and Brandt 1994; Brosh et al., 1998).

Developing Genetically Improved Breeds

Heat stress mitigation strategies mentioned above often require financial investments. Moreover, it may exacerbate the situation sometimes if it has not been done properly instead of helping animals to cope with environmental stress. As a result, mitigation strategies may not provide a sustainable solution by themselves in the long run relative to the current projection of climate change and global warming. Therefore, utilizing genetic diversity to develop breeds that are genetically adaptive to harsh environments or improve current breeds should be an emphasis in order to address the situation in a sustainable manner.

It is a well-documented fact that certain breeds are better suited and perform better than others in a specific environment. Breeds can survive, be productive, and reproduce in a particular

environment because they have developed adaptation mechanisms pertinent to the environment that they live in that enables the population to continue for generations to come (Barker, 2009). For example, *Bos indicus* are a prominent sub-species of cattle in some regions of the world with the ability to perform as well as reproduce in tropical and arid areas. Moreover, they are also known for being a multipurpose breed; often farmers use them for ploughing and transportation. Body conformation, coat color, better sweating mechanism and sebaceous glands are some of the factors that contributed to *Bos indicus* heat tolerance capacity. More importantly it is believed that *Bos indicus* adaptability to heat stress and harsh environments is the result of their thermotolerant genes acquired from their exposure and interaction of the environment from where they descended (Bonsman, 1973; Hansen, 2004; Turner, 1980). In contrast to *Bos indicus* cattle, *Bos taurus* breeds are regarded as breeds of temperate environments because of their adaptability to cold environments. *Bos taurus* cattle are known for their high milk and meat production ability and are generally less tolerant to heat stress as well as harsh environments. However, there is still variation within *Bos taurus* cattle in response to heat stress. For example Jersey cattle have been identified as more heat tolerant than Holstein (Da Silva, 2006).

The existence of genetic variation for heat tolerance between and within breeds can give breeders the option to make genetic improvement of cattle thereby bringing a sustainable and long-lasting solution to ever evolving climate change. Crossbreeding and selection have been the two important breeding strategies that have been practiced by animal and plant breeders for decades to exploit genetic variation between and within breeds, respectively. Evolution has played a major role in distinguishing certain species and subspecies to be adapted to specific environments. As a result they have developed genes that help them thrive through harsh environments. Therefore, breeding tools can be used to make genetic improvement of cattle by

selecting animals within adapted breeds to improve economically important traits (i.e., growth and carcass merit, milk yield) or select within more productive breeds to make them more adapted. In addition, it is important to exploit the benefit from implementing crossbreeding by introgressing adapted genes from local breeds while avoiding the undesirable ones.

Crossbreeding

Crossbreeding is a breeding strategy used to exploit genetic variation that exists between breeds or lines. The two main advantages of crossbreeding are breed complementarity and heterosis (hybrid vigour). Often crossbred animals have improved performance as compared to the average performance of their parents as a result of heterosis. Because heritability estimates of adaptive traits such as heat/cold tolerance are often characterized as low to moderate, crossbreeding can be utilized for an improvement of such traits to attain and benefit from heterosis. As part of using a structured crossbreeding system to improve adaptation and capitalize on breed complementarity, improvement of other lowly heritable and economically important traits such as fertility (Stonaker, 1973; Venter et al., 1986) would be expected.

The Brahman breed was developed in the southern part of the U.S. from numerous humped cattle of *Bos indicus* origin from India (Philips, 1963; Yturria, 1973). The Brahman breed was developed due to the fact that the European breeds were not adaptive to the environment and production with the indigenous cattle was low. Koger (1963) and Randel (2005) have pointed out that Brahman animals have been used for crossbreeding in the beef industry for their adaptive traits. Turner (1980) also summarized that zebu cattle have been beneficial in the beef industry for their large heterosis effects when crossed with *Bos taurus* cattle for growth, adaptive traits, maternal effects and reproductive traits. Moreover, in a review paper, Turner (1980) presented different research studies performed on the contribution of zebu

cattle in the beef production system. For example, both Howes (1963) and Evans (1963) concluded that Brahman cattle have better adaptation to heat stress than Hereford due to their ability of maintaining lower respiration rates. However, Howes (1963) reported that Brahman cattle have lower ovulation rates than Hereford, which the author cited as part of the reason for better heat tolerance but lower reproductive efficiency than Hereford. Heat tolerance comparison between Brahman, Brahman x Hereford and Hereford by Cartwright (1955) identified the superiority of the former two over Hereford cattle. Research studies for tick resistance shows that crossbred of *Bos indicus x Bos taurus* are more resistant than *Bos taurus* cattle (Rick 1962; Strother et al., 1974). A study of energy comparison by Lofgreen et al. (1975) reported that Brahman x British crossbred steers utilized energy more efficiently than British steers. Brahman cattle was also found to be more energy efficient than Hereford cattle on low energy diets such as high roughage (Bonsma 1973; Moore et al., 1975). Crossbred cattle of British x Brahman were found to have increased carcass weight compared to straightbred Brahman cattle (Carpenter 1973).

Selection

In order to select animals to be parents of the next generation, one needs to estimate the breeding value of an animal for the desired economically important traits of interest, in this case heat tolerance (Dekkers, 2012). The breeding value of an animal can be defined as the sum of the average effect of all alleles (quantitative trait loci) that control the desired trait of interest (Falconer and Mackay, 1996). Unfortunately, most of quantitative trait loci that affect a trait of interest are unknown, and thus selection based on Estimated Breeding Values (EBV) using animal kinship and performance data is necessary. For traits that may be expressed late in life or

that are difficult or expensive to measure, using traditional pedigree-based EBV may result in slow rates of annual genetic gain.

However, with the current development of new technologies in molecular biology for the past couple of decades, the animal breeding and genetics industry has been revolutionized, subsequently increasing the need for incorporating molecular information into existing genetic selection tools. The advent of new technologies in the industry has opened the opportunity for new traits that have been once considered as difficult and complex to be incorporated into breeding goals. However, the challenge to identify mutations that are truly associated with heat stress and integrating it into breeding goals still remains.

It is a well-known fact that conventional selection tools (pedigree-based EBV) are effective at generating genetic change. Often, economically important traits are quantitative and breeding objectives are dictated by market needs (Hetzel et al., 1986). A breeding objective specifies the desired traits of interest to be improved in the population and shows the direction for genetic change (Kinghorn et al., 2015). However, conventional selection programs have been focused more on production traits, such as milk, meat and egg production. In addition, improving livestock production through environmental intervention was once seen as the simple way of countering the problem. As a result, adaptation traits have been ignored and their fundamental genetic mechanisms remain unclear. Several reasons could be available for why adaptation traits have received less attention, but foremost is a general lack of a clear phenotype that can be easily recorded. Therefore, integrating adaptation traits (i.e., heat/cold tolerance) into breeding objectives to select animals to be parents of the next generation will be necessary. To do so will require identifying a phenotype that can be relatively easily measured and quantifying the degree to which this phenotype is under genetic control.

Genetic parameter estimates of heat tolerance for beef cattle were published by Da Silva (1973), three decades after Rhoad (1940) first suggested selecting cattle for adaptation traits, especially for heat resistance. Da Silva (1973) reported heritability estimates of heat tolerance related traits in 192 Brazilian composite cattle from what is known as the Canchin breed. The author reported heritability estimates for initial rectal temperature and respiratory rate of 0.11 and 0.59, respectively. However, exposing them to direct sun light during the hottest time of the day, Da Silva (1973) reported a moderate heritability estimate (0.44) for rectal temperature and a very high negative genetic correlation (-0.895) with average daily gain. Based on these findings the author suggested that it should be possible for breeders to select cattle for heat tolerance and average daily gain simultaneously. The findings of Da Silva (1973) confirmed similar previous studies in dairy cows. Seath (1947) studied heat tolerance in 52 Jersey and 68 Holstein cows and reported heritability estimates of 0.15 to 0.31 and 0.77 to 0.84 for rectal temperature and respiratory rate, respectively. Legates (1953) also reported heritability estimates of 0.22 to 0.30 and 0.34 to 0.54 for rectal temperature and respiratory rate, respectively. Turner (1982) studied rectal temperature in relation to fertility in cows and reported a heritability estimate of rectal temperature of 0.25. Mackinnon et al. (1991) reported a similar heritability estimate of 0.19 for rectal temperature from a study of adaptation traits and growth in tropical cattle. Burrow (2001) performed a study between production, adaptation and temperament traits of tropical beef cattle and reported a low heritability estimate of 0.18 for repeated rectal temperature. Ravagnolo and Misztal (2000) reported a heritability estimate of 0.17 for heat tolerance using more than 15,000 Holsteins. Dikmen et al. (2012) also reported heritability estimate of rectal temperature of 0.17 in dairy cows which falls into the range of that reported by Seath (1947). Nguyen et al. (2016) performed a study of heat tolerance to phenotypic variation for milk, fat and protein yields on

366, 835 Holstein and 76, 852 Jersey cows in Australia. The authors defined heat tolerance as the rate of reduction of production during heat stress and reported a heritability estimate for heat tolerance of 0.19, 0.17, 0.17 for Holsteins and 0.24, 0.18 and 0.18 for Jerseys, respectively. Howard et al. (2014) reported a posterior heritability estimate of 0.68 and 0.21 for summer and winter measurements of hourly tympanic and vaginal body temperature of 239 crossbred beef cattle. All the aforementioned studies have confirmed the existence of genetic variation for adaptation traits, especially for heat tolerance. Variation exists both within and between breeds, which ultimately shows that there is a room for genetic improvement of livestock for adaptation using either structured crossbreeding or within-breed selection.

Genomic Selection

Quantitative genetics is known as the study of complex traits based on an infinitesimal model, which states that a trait is controlled by many genes with each one contributing a small amount, but also recognizing that environmental factors play a role (Bulmer, 1980; Dekkers et al., 2002). For more than four decades, domestic animals of agricultural importance have been artificially selected to be parents of the next generation based on their phenotype record and pedigree for the traits of economically important (Dekkers, 2012). Statistical methods of best linear unbiased prediction (BLUP) are used to estimate the breeding value of animals. Animals are ranked and selected according to their EBV (Dekkers, 2012; Dekkers et al., 2002). Enormous genetic improvement of livestock through conventional breeding schemes has been possible due to these methods, but genetic gain can be slow and time consuming. Moreover, conventional breeding schemes present limitations relative to desired economic traits that are difficult/expensive to measure, traits with low heritability and those that take a longer period of time to measure (Dekkers, 2012). However, with the recent advancement of molecular high-

throughput technology and low cost of genotyping, genomic selection came to play a significant role in overcoming the shortcomings of conventional breeding schemes.

Selecting animals based on their estimated genomic breeding values (GBV) is known as genomic selection. The name genomic selection was first presented by Haley and Visscher in 1998 (Meuwissen, 2007), but Meuwissen et al. (2001) introduced and showed the methodologies first. It is a form of marker-assisted selection, where breeding schemes are designed to develop prediction equations using phenotype and genotype information from a reference population which ultimately will be used to estimate genomic breeding values of livestock with limited phenotype and genotype records (Pryce and Daetwyler, 2012). The fundamental concept of genomic selection is based on selecting animals/plants using dense markers of single nucleotide polymorphisms (SNPs) that cover the whole genome and at least some of them are assumed to be in linkage disequilibrium (LD) with the quantitative trait locus (QTL). The main advantage of genomic selection over conventional selection is the ability to increase the accuracy of identifying genetically superior animals' earlier in life (Berry, 2014). The three major factors that determine the accuracy of the prediction of true genetic value of an animal/plant through genomic selection are the heritability of the trait, the number of animals in the reference population as well as the extent of linkage disequilibrium (LD) between markers and quantitative trait loci (QTL) (Daetwyler et al., 2008; Goddard, 2009; Meuwissen, 2009).

Since Meuwissen et al. (2001) demonstrated how to estimate the genetic value of an animal from genetic markers across the whole genome using simulation, genomic selection has become pervasive across many livestock and plant industries. Implementation of genomic selection required the redesign of existing breeding programs. The effect of genomic selection has varied across all major livestock sectors, but the dairy industry was the first livestock

industry to implement genomic selection on a wide-scale and is the most recognized for the progress made by incorporating genomic information in breeding value estimation. Being able to select animals at an early stage of life thereby shortening the generation interval is one of the benefits of genomic selection over conventional breeding strategies. Schaeffer (2006) reported based on a cost-benefit analysis that replacing traditional EBV-based selection by genomic selection in the dairy industry could result in a reduction of costs by 92% while attaining double the genetic improvement compared to selecting animals using progeny-testing methods. De Roos et al. (2011) studied the rate of genetic gain due to selecting animals earlier in life using genomic selection in comparison with selection of proven bulls in dairy cows and showed that rate of genetic gain could be doubled through implementation of the former method. Similar research was also performed by König et al. (2009). The authors compared progeny testing dairy breeding scheme with genomic selection breeding scheme approach and suggested that the ultimate economic benefit of dairy industry from implementing genomic breeding scheme came from the reduction of generation interval and costs associated with it**.** It is a well-known fact that dairy industry adopted genomic selection ahead of most of the other livestock sectors mainly due to its massive and well organized phenotypic and pedigree database as well as breeding structure, which subsequently led into a successful implementation and integration of national genetic evaluations of various countries since 2009 (Berry et al., 2016; Spelman et al., 2013).

Often, selection of animals in beef cattle breeding is performed based on market specific demand (Jonas and Koning, 2015). As a result, selection in beef cattle has led to increased rib eye area, marbling scores as well early growth (Garrick, 2011). However, for a trait complex that is much more difficult to measure and is lower in heritability, such as reproduction, there was no evidence to support any genetic change (Garrick, 2011). However, adoption and implementation

of genomic selection in the U.S. beef industry is not as broadly implemented as in dairy cattle breeding (Meuwissen et al., 2016), perhaps because of its unique breeding structure. It is common knowledge that selection candidates in beef cattle breeding have some of their individual phenotypes recorded before selection decisions are made (Boerner et al., 2014; Johnston et al., 2012). Moreover, beef cattle breeding often uses natural service bulls which in turn has restricted the impact of genomic selection in contrast to the dairy breeding structure, in which usage of artificial insemination is very common (Todd et al., 2011). Schaeffer (2006) and König et al. (2009) have shown that most of the economic benefit of genomic selection came from increasing the accuracy of selection thereby shortening the generation interval and costs associated with it, which is one of the main driving forces of genomic selection success along with other factors in dairy cattle breeding. Therefore, the beef cattle industry will likely benefit to a lesser extent as compared to dairy cattle breeding from the implementation of genomic selection relative to reducing the generation interval. However, beef cattle breeding will definitely benefit from other advantages that genomic selection has to offer. For example, it can benefit from incorporating genomic information into breeding goals thereby increasing selection accuracy for traits that are difficult/expensive to measure and/or require slaughtering the animal or for traits measured late in life and those that are lowly heritable. Traits such as feed efficiency, carcass quality and reproduction are some of the desired economic traits that beef cattle breeding can benefit from the implementation of genomic selection (Swan et al., 2012). One of the challenges of beef cattle breeding is that breeding values are still less accurate than dairy cattle (Johnston et al., 2012). Moreover, genetic markers in beef used to improve desired economic traits fail to show reliable result across populations (Allan and Smith, 2008). In addition, limited number of training populations and effective population size are part of the reasons for lagging

behind the implementation of genomic selection in beef cattle breeding (Johnston et al., 2012). Attaining higher prediction accuracy requires measurement of novel phenotypes from large populations (Pollak et al., 2012), otherwise significant SNP identified could be spurious. De Roos et al. (2009) pointed out that in order to increase the prediction accuracy, merging genomic data of different countries as well as breeds is necessary. Moreover, Pollak et al. (2012) also suggested that usage of high density markers could improve accuracy of prediction in populations that are distantly related. Therefore, beef cattle breeding perhaps can take the advantage of increasing the accuracy of estimated breeding values through genomic predictions of desired economic traits that are already in continuous genetic evaluations as well as traits that are expensive/difficult to measure, sex-limited and those measured late in life, or require the death of the animal (MacNeil, 2016).

Genome-wide association study (GWAS)

With the development of new technologies in molecular genetics as well as affordable genotyping costs associated with it, GWAS have been made feasible by the identification of thousands of SNPs across the whole genome of humans, livestock and plants species. Implementation of GWAS requires analyzing DNA sequence variants (mainly SNPs) across whole-genome of an organism along with its phenotype in order to identify genomic regions that are truly associated with the desired trait of interest. The fundamental concept of GWAS implementation is based on the assumption that a significant association can be detected between the genetic variants and the economic trait of interest because the SNPs are in LD with the QTL.

GWAS is a relatively new technique in agricultural livestock compared with other mapping techniques that have been used before. Linkage analysis and candidate gene techniques were used to decipher genes that affect complex economic traits of interest in domestic animals

before GWAS projects first started in humans. However, the availability and discovery of large numbers of genetic variants in different livestock species helped enormously the implementation of GWAS in the animal breeding and genetics field.

Genes involved in heat/cold stress response

As many other desired economic traits, heat tolerance seems to fall into category of complex traits which are influenced by many genes across the whole genome. Several research studies showed that response to environmental stress (heat/cold) are controlled by many genes in livestock species as well as humans (Dikmen et al., 2012, 2013; Hayes et al., 2009). Page et al. (2006) performed a genome-wide analysis in humans and found that heat shock factors (HSFs) have been involved as significant first responders to a rise in cell temperature and they are also associated to cellular adaptation and survival. The HSFs are transcription factors that control heat shock proteins (HSPs) expression through interaction with a specific DNA sequence in the promotor, which are known as heat shock element (HSE) (Akerfelt et al., 2010; Anckar and Sistonen, 2011; Morimoto, 1998). Heat shock proteins (HSPs) are recognized as proteins expressed during a significant heat shock (Lindquist 1986). Families of heat shock proteins that are associated to thermal regulation are HSP40, HSP60, HSP70 and HSP90. Moreover, HSPs found to be the main proteins synthesized by cells during both extreme temperature elevation and shortly after (Lindquist, 1986). Hansen (1999, 2015) described that the molecular basis of thermotolerance is not yet well-known, but there are suggestions for heat shock proteins involvement during heat stress which directly affects the function of the oocyte and embryo.

Olson et al. (2003) also discovered that a *slick hair gene* plays a role in producing short sleek hair coat in cattle. The *slick hair gene* is the only gene identified at the SLICK locus in Senepol and Criollo breeds and is inherited as a single dominant gene. Mariasegaram et al.

(2007) and Flori et al. (2012) were able to map the SLICK gene to bovine chromosome 20. Cattle with *slick gene* are recognized as thermotolerant. The following table adopted from the literature review by Rolf (2015), describes the different pathways and/or genes identified in genome-wide association studies.

Table 2. List of pathways and/or genes that have been identified in genomic studies as potential candidate genes for body temperature regulation, (Adapted from Rolf, 2015).

Pathway/Function	Gene (s)	Publication
Cellular response to stress	STAC, WRNIP1, MLH1, RIPK1, SMC6, GEM1	Howard et al., 2014
Response to heat	STAC	Howard et al., 2014
Gap junction	TUBB2A, TUBB2B	Howard et al., 2014
Cellular response to stress	CCNG, TNRC6A	Howard et al., 2014
Apoptosis	FGD3, G2E3, RASA1, CSTB, DAPK1, MLH1, RIPK1, SERPINB9, HMGB1	Howard et al., 2014
Ion transport	CACNG3, CLCN4, PRKCB, TRPC5, KCNS3, SLC22A23, TRPC4	Howard et al., 2014
Thyroid hormone regulation	DIO2	Howard et al., 2014
Body weight and feed intake	NBEA	Howard et al., 2014
Heat shock protein response	HSPH1, TRAP1	Howard et al., 2014
Respiration	ITGA9	Howard et al., 2014
Calcium ion and protein binding	NCAD	Dikmen et al., 2012
Protein ubiquitination	RFWD12, KBTBD2, CEP170, PLD5	Dikmen et al., 2012

Thyroid hormone regulation	SLCO1C1	Dikmen et al., 2012
Insulin signaling	PDE3A	Dikmen et al., 2012
RNA metabolism	LSM5, SNORD14, SNORA19, U1, SCARNA3	Dikmen et al., 2012
Transaminase activity	GOT1	Dikmen et al., 2012
Apoptosis, cell signaling	FGF4	Hayes et al., 2009
	XM_865508 (G3PD-like)	Hayes et al., 2009

Summary

Research evidence shows that heat/cold stress is becoming one of the main limitations on animal productivity, as well as a major contributor of production costs associated with it. As a result, it has drawn the attention of the scientific community to look for different ways of minimizing or if possible, avoiding the negative consequences of it through the implementation of different mitigation strategies as well as through the development of selection tools. Exploiting the current development of technology in molecular biology and incorporating genomic information into conventional breeding programs for the purpose of selecting parents of the next generation could possibly increase the accuracy of selection for novel traits such as tolerance to extreme climates. Several studies showed that the ability of animals to withstand heat/cold stress is low to moderately heritable which suggests that reconsideration of current breeding goals is necessary in order to incorporate this important trait complex. Without selection tools for adaptation, the genetic diversity of economically important agricultural species in the future may be restricted to those that are regionally adapted via natural selection and thus production potential could be limited.

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GENOME-WIDE ASSOCIATION STUDY FOR THE RELATIONSHIP BETWEEN TEMPERATURE AND FEED INTAKE IN BEEF CATTLE

Abstract

The interaction of livestock with the environment they live in is complex and plays a significant role in their production performance, which also depends on location and management practices. Climate change is projected to increase temperature globally. As a result, climate change will most likely aggravate the pressure from different sources of stressors on the current agricultural production system in general and in livestock specifically. Environmental conditions, such as changes in ambient temperature, can cause changes in animal behavior and performance. In general, it is believed that as ambient temperature increases, dry matter intake (DMI) of beef cattle decreases. However, our hypothesis was that the degree to which animals adjust their daily DMI due to changes in ambient temperature is partially controlled by genetic effects. Consequently, the objective of this study was to estimate the genetic component of the regression of DMI on ambient temperature using an admixed beef cattle population consisting of various crosses of Angus, Simmental, and Piedmontese (n = 239). Ambient temperatures were received from a local weather station and DMI was collected via Calen gates. The feeding period averaged 155 d with a range of 114 d to 189 d depending on the management group. Individual animal regressions of DMI on average daily ambient temperature were performed using either daily high or low temperatures over the entirety of the feeding period. Daily high temperatures (°C) averaged 15.07 with a range of -17.21 to 38.25. Daily low temperatures (°C) averaged 2.37 with a range of -28.33 to 15.26. The corresponding intercept and regression coefficient for each animal were used as phenotypes for a genome-wide association study (GWAS). Animals were genotyped with the BovineSNP50 Beadchip. Data were analyzed using GenSel software and a

BayesC model fitting contemporary group (n = 4) and initial body weight (IBW) as fixed effects. A MCMC chain of 100,000 iterations were used with the first 40,000 samples discarded as burn-in. The proportion of SNPs having null effect (π) was set to 0.995. Posterior mean heritability estimates (PSD) for the analysis when daily high temperature was considered in the regression and myostatin genotype (MG) was included as fixed effect in the model (model-1) were 0.27 (0.07) and 0.25 (0.08) for the intercept and slope, respectively Posterior mean heritability estimates (PSD) for the analysis when daily high temperature was considered in the regression and MG was not included as fixed effect in the model (model-2) were 0.68 (0.06) and 0.45 (0.08) for the intercept and slope, respectively. Similarly, posterior mean heritability estimates (PSD) for the analysis when daily low temperature was considered in the regression for model-1 were 0.29 (0.09) and 0.27 (0.08) for the intercept and slope, respectively. Posterior mean heritability estimates (PSD) for the intercept and slope when the daily low temperature was considered in the regression were 0.76 (0.05) and 0.48 (0.08), respectively. These results suggest that changes in DMI due to changes in ambient temperature are under genetic control. Admittedly the population under study is small and admixed, suggesting that the genomic heritability estimates contained herein are potentially biased upward. However, the concept of applying this same procedure in larger populations warrants further investigation as a means of identifying animals that are less sensitive to environmental extremes.

Key Words: beef cattle, dry matter intake, environmental stress, genome-wide association study

Introduction

The interaction of livestock with the environment they live in is complex and plays a significant role in their production performance, which also depends on location and management practices. Climate change is projected to increase temperature globally (Tebaldi et al., 2006; Walthall et al., 2013). As a result, climate change will most likely aggravate the pressure from different sources of stressors on the current agricultural production system in general (Hatfield et al., 2008, 2011). With the exception of some intensive production systems, beef production in the U.S. is often in extensive production systems whereby beef cattle are reared in complex environments in which they continuously face short and long term environmental changes such as ambient temperature. As a result, beef cattle are vulnerable to different environmental conditions and can experience both heat and cold stress.

Environmental factors contributing to heat stress are daily high ambient temperature, high relative humidity, low air movement, solar radiation and heat wave. Moreover, due to their poor sweating mechanism, beef cattle also acquire heat through the process of fermentation during digestion. Even though animals are forced to adapt to certain environmental changes, these changes can be very detrimental to their performance and production ability. Reduction of feed intake and growth are among the common signs of beef cattle under heat stress. However, in extreme cases, heat stress can also cause death contributing to a significant revenue loss to producers.

Cattle maintain normal body temperature through balancing heat gain and loss, a process known as thermoregulation. However, it is also a well-known fact that animals' capacity to

acclimatize its metabolic rate to cope with temperature extremes can cause not only production loss but also animal death (Walthall et al., 2013). Heat stress is a situation that occurs when animals are unable to dissipate their heat load efficiently. Based on the duration and severity of the stress, it can be described as acute or chronic. Beef cattle experience heat stress when an imbalance between the internal heat production and their ability to dissipate it efficiently exist. Decreased dry matter intake, reduced metabolic rate, increased respiratory rates and sweating are some of the physiological signs that contribute negatively to the production ability of the animals. Generally, as the ambient temperature increases animals eat less, which negatively impacts the usage of energy for production. This negative correlation between dry matter intake and high ambient temperature is well-documented. However, differences in an animal's physiological response and production performance under extreme heat stress is also partially controlled by their underlying genetic makeup. Therefore, our hypothesis was that the degree to which animals adjust their daily DMI due to changes in ambient temperature is partially controlled by underlying genetic effects. Consequently, the objective of this study was to estimate the genetic component of the regression of DMI on ambient temperature via a genome-wide association study using an admixed beef cattle population consisting of various crosses of Angus, Simmental, and Piedmontese.

Materials and Methods

Data

An admixed beef cattle population of steers and heifers (n = 239) of unknown pedigree consisting of various percentages of Angus, Simmental and Piedmontese with serial body weights and dry matter intake records were used in this study. The project was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

As described by Howard et al. (2013), animals were genotyped before arrival to confirm presence of the Piedmontese-derived myostatin mutation (C313Y). Animals with 0-copy (n= 84), 1-copy (n=96) and 2-copy (n=59) of the C313Y genotype (MG) were labelled as homozygous normal, heterozygous and homozygous for inactive MG, respectively. All animals were genotyped with the Illumina BovineSNP50 panel that included over 50,000 single nucleotide polymorphisms (SNP). Cattle were fed in four groups over a 2-year period and dry matter intake (DMI) were recorded via Calen a gate facility at the University of Nebraska Agricultural Research and Development Center (ARDC). Individual feed bunks were filled each day and refusals were calculated on average every 6 days with a range of 1 to 9 days. The time between 2 successive feed refusal collections were defined as a feeding period. Groups 1 (S1) and 2 (S2) contained calf-fed steers and S1 were on feed from December 16, 2009 to June 24, 2010 and S2 were on feed from December 23, 2010 to August 12, 2011. Groups 3 (H2) and 4 (H1) contained yearling heifers. H1 heifer group were on feed from July 28, 2010 to November 19, 2010 and H2 were on feed from July 20, 2011 to January 27, 2012. Each group was randomly assigned into 2 pens with approximately 30 cattle per pen. Cattle had access to water and were fed a diet that

satisfied NRC requirements. The finishing ration for group H1 and S1 contained wet distillers' grain and soluble, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 35, 52, 8 and 5 percent of the diet.

The finishing ration for H2 and S2 contained modified distillers grain with soluble sweet bran, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 20, 20, 48, 8, and 4 percent of the diet. Over the 2-year feeding period cattle were neither implanted with nor fed growth-promoting additives. The feeding period for groups S1 and S2 averaged 185.5 days (d) with a range of 182 to 189 d and for groups H1 and H2 averaged 124.5 d with a range of 114 to 135 d. Average body weight and visually appraised external fat were used to determine when groups of cattle were ready for harvest. Individual animal DMI was recorded periodically ranging between 3 to 9 d for steers and between 2 to 10 d for heifers.

Statistical Analysis

Daily high (DH) and low (DL) ambient temperatures were received from a local public weather station for the feeding periods of each group. Average daily high and low ambient temperatures were centered to improve the biological interpretability of regression coefficients (Schielzeth, 2010). Individual animal DMI were summed across four consecutive measurement events and this sum was considered as a DMI phenotype for an individual. Individual animal regressions of DMI on centered ambient temperature (either DH or DL) corresponding to the feed intake period for each DMI phenotype were fitted using DMI phenotypes as described above. These regressions were performed over the entirety of the feeding period. Model fit summaries by group can be found in tables 1-8. Three animals (two steers and one heifer) were removed from the analysis due to missing initial body weight (IBW) observation. Therefore, a

total number of 236 animals were used for the GWAS analysis. A summary of the phenotypes and ambient temperatures used for the analysis are reported in tables 9 - 14.

Genome-wide association studies to estimate the proportion of phenotypic variation due to additive genetic variation using a total of four traits were conducted: DH intercept and slope, and DL intercept and slope. Illumina data analysis software was used to assign quality scores (GenCall) for each genotype. If genotypes were missing or a GenCall score was below 0.20, they were replaced with the mean allele frequency across all animals (Illumina, Inc 2010; Edriss et al. 2012). Exclusion of markers based on minor allelic frequency and deviations from Hardy–Weinberg proportions were shown to have little impact on genetic prediction (Edriss et al., 2012). As a result, all single nucleotide polymorphisms (SNP) were utilized for analysis in this study and none were culled based on MAF. Data were analyzed and estimates of marker effects and variances were attained by fitting all markers simultaneously and contemporary group (n = 4) and initial body weight (IBW) as fixed effects using a BayesC model (Habier et al., 2011) via GenSel software (Version 0.9.2.045; Fernando and Garrick, 2008). Each trait was analyzed with (Model-1) and without (Model-2) MG in the GWAS model as a fixed effect. The proportion of SNP having null effect (π) was set to 0.995. A chain length of 100,000 iterations were run with the first 40,000 discarded as burn-in. The results reported herein are the averages of 60,000 samples from a Markov Chain Monte Carlo chain.

Convergence of each analysis was met by starting with high and low *a priori* heritability estimates until the posterior heritability estimates were trending down and up, respectively, and an average value between them was taken as the *a priori* heritability for the final analysis. SNPs were blocked into 1-Megabase (1-Mb) non-overlapping windows and the marker specific

posterior variance across SNP within a window was summed to give an estimate of the total genetic variance for each window (n = 2,681).

Gene Ontology

The top 0.5% of 1-Mb windows (n ~ 13) that accounted for the largest proportion of the additive genetic variance were extended by 0.5-Mb in both directions and a positional candidate gene approach was conducted using cow genes build UMD_3.1 assembly (Zimin et al., 2009). Human orthologues of beef cattle positional candidate genes were obtained using Ensembl Genes 90 database and the BioMart data mining tool (https://www.ensembl.org/biomart/martview/e5a2abeadd0cfb919f0e1f493f388748). Furthermore, functional annotation of human orthologues, identification of overrepresented gene ontology terms, and pathway analysis was performed using bioinformatics tool of the database for annotation, visualization and integrated discovery (DAVID v6.8) (https://david.ncifcrf.gov/).

Results and Discussion

Genetic Parameters

Mean posterior heritability estimates are presented in table 15. When MG was not fitted in the model, the posterior mean heritability (PSD) estimates for the intercept and slope when the daily high temperature was considered in the regression were 0.68 (0.06) and 0.45 (0.08), respectively. Similarly, posterior mean heritability (PSD) estimates for the intercept and slope when the daily low temperature was considered in the regression were 0.76 (0.05) and 0.48 (0.08), respectively. The mean posterior heritability estimates (PSD) when MG was fitted as a fixed effect for DH were 0.27 (0.07) and 0.25 (0.08) for the intercept and slope, respectively.

Likewise, the mean posterior heritability estimates (PSD) for DL when MG was fitted in the model were 0.29 (0.09) and 0.27 (0.08) for the intercept and slope, respectively. When MG was fitted in the model the posterior heritability estimates decreased substantially. This was not unexpected given previous studies using this population illustrated the impact of MG on individual animal body temperature regulation (Howard et al., 2013). The sizable reduction in the genomic heritability estimates are likely reflective of not only the impact of MG on this trait complex, but also potentially due to having corrected for Piedmontese background more generally when fitting MG as a fixed effect.

In general, the posterior mean heritability estimates of this study were within the range of heritability estimates of body temperature regulation and respiration rate reported from previous similar studies which ranged from 0.11 to 0.68 (Burrow, 2001; Da Silva, 1973; Dikmen et al., 2012; Howard et al., 2014; Mackinnon et al., 1991; Seath and Miller, 1947; Turner, 1984). Burrow (2001) used rectal temperatures as a measure of heat resistance in addition to other phenotype measures used to estimate variances and co-variances between productive and adaptive traits as well as temperament in a composite breed of tropical beef cattle. Burrow (2001) showed a low heritability estimate of rectal temperature 0.18. Da Silva (1973) used both respiration rate and rectal temperature record to estimate heritabilities and correlations of weight and heat tolerance traits for a group of 192 bullocks and heifers of tropical beef cattle. Da Silva (1973) found a heritability estimate for respiratory rate ranged from 0.44 to 0.59. Likewise, Da Silva (1973) showed estimates of heritability of rectal temperature ranging from 0.11 to 0.44. Dikmen et al. (2012) used a total of 1,695 Holstein cows record and estimated a moderate heritability of rectal temperature of 0.17. Mackinnon et al. (1991) also estimated the heritability of rectal temperature in zebu cross cattle to be moderate (0.19). Seath and Miller (1947) used

both body temperature and respiration rates as a measure of heat tolerance for Jersey and Holstein cows of data collected on the years of 1944 and 1945 and found a heritability estimate of body temperature of 0.15 and 0.31 for data collected in 1944 and 1945, respectively. Similarly, the authors found 0.76 and 0.84 heritability estimates of respiration rates for the years of 1944 and 1945, respectively. Turner (1984) used 200 heifers of *Bos indicus*, *Bos taurus* and crossbred lines to estimate a moderate heritability estimate of rectal temperature 0.33. Phenotypes of respiration rate and body temperature regulation are the two most common phenotypes used as an indicator of heat/cold stress (Rolf, 2015). However, decrease in production has also used as an alternative phenotype measurement for heat stress (Nguyen et al., 2016). As mentioned above, previous studies utilized respiration rate measured as breaths per minute (Da Silva, 1973; Seath and Miller, 1947) as well as one-time measurements of rectal temperature as phenotypes of heat stress or both (Da Silva, 1973; Seath and Miller, 1947). One of the reasons for the variation of the heritability estimates among different studies could be the choice of the phenotype for heat stress. Developing a standard phenotype measurement of heat stress is one of the challenges of the scientific community, previous studies have used body temperatures measured from either tympanic (in the ear), rectal or intravaginal and showed heritability estimates of their result. However, in this study we have developed and used regression coefficients of averaged daily ambient temperature on total DMI for the entire feeding period of each group. The other reason that could contribute to the different heritability estimates of different studies is that the use of different breeds reared in very different environments.

Results of posterior heritability estimates presented by Howard et al. (2014) are the closest to the current study, though the authors developed and used a different phenotype measurement for heat stress. Howard et al. (2014) found a posterior mean heritability estimate of

0.68 and 0.21 for summer and winter phenotypes developed using area under the curve of body temperature measured from tympanic and intravaginal tissue measurements. Admittedly, the population under the current study is relatively small and admixed, suggesting that the genomic heritability estimates contained herein could be potentially biased upward. However, the heritability estimates of this study indicate that feed intake changes in response to temperature related stress is partly controlled by the underlying genetic makeup of the animal.

Candidate genes

Genomic regions/gene names and function are detailed in tables 16 to 23. Functional annotation, enrichment and pathway analysis of the extended top 0.5% 1-Mb windows resulted in significant enrichments for multiple biological processes and pathways for both models and traits (DH and DL intercept and slope phenotypes) and unveiled genomic regions/genes with functions of heat shock protein binding (GBP1, LMAN2, DNAJC2, DNAJC9, HSPB1, DNAJB12, UNC45B, BAG6, STUB1, TELO2, STIP1), response to cold/heat and external temperature stimulus (VGF, CCL2, P2RX3, AMICA, MICB, HSPA1A, HSPA1B, PRKAA1, MLST8, HSPA1L, HSPB1, MROH2B, POLR2D, MSTN, ADORA1,PLAC8, REN, TRPM8, CPB2, PIRT), regulation of response to appetite (BBS4), response to feeding and eating behaviors (NAPEPLD, NPW, REN, DACH1), and temperature homeostasis (EDNRB, TNF). Expression of heat shock proteins (HSPs) is regulated by heat shock factors (HSF) known as a transcription factor family (Page et al., 2006). As described by Page et al. (2006) and Morimoto (1998) HSF regulate the expression of HSPs through the interaction with heat shock element (HSE). The HSE are a specific DNA sequence found in the promotor. Akerfelt et al. (2007) also illustrated that HSF organize the cellular response to heat/cold stress and control HSPs. Winter et al. (2004) mapped HSF1 to chromosome 14 in cattle and are known to take part in the acute

response to heat shock. Khazzaka et al. (2006) showed the relationship between HSP70 and Halothane genotypes response to heat stress in pigs. Sonna et al. (2002) have also identified approximately 50 genes that have not been previously considered as heat shock proteins that plays a role during response to heat stress. Charoensook et al. (2012) also identified polymorphism in the bovine HSP90AB1 gene that are associated with heat stress in an indigenous Thai cattle. The authors used respiration rate, rectal temperature, pack cell volume as well as individual heat tolerance coefficient as heat stress indicators (phenotypes). Liu et al. (2010, 2011) also identified a significant association between a polymorphism in the ATP1A1 gene and heat tolerance in 160 Chinese Holstein.

The largest effect 1-Mb chromosomal windows for model-1 (with MG fitted) were on chromosome 3 at 49-Mb for DH intercept and on chromosome 25 at 41-Mb for DL intercept and chromosome 25 at 1-Mb for both the DH and DL slope. The largest effect 1-Mb chromosomal windows for model-2 (without MG fitted) were on chromosome 2 at 8-Mb for DH and DL intercept and chromosome 25 at 1-Mb for DH and DL slope, respectively. The SNP name and location that explained the highest proportion of additive genetic variance within each of the top 0.5% 1-Mb windows for model-1 and model-2 of each DH and DL intercept and slope can be found in tables 24 and 25.

Implications

Application of regression coefficients of DMI on ambient temperature as phenotype for genome-wide association studies (GWAS) in the current study unveiled possible genomic regions and candidate genes that may have a significant association with both heat and cold stress. Furthermore, extending the genomic regions for functional annotation, enrichment and pathway analysis of the top 0.5% 1-Mb windows revealed significant enrichments for multiple biological processes and pathways that could potentially contribute to heat/cold tolerance. In addition, the genomic heritability estimates suggest that genomic information were able to explain a moderate to large proportion of the phenotypic variation for DH and DL intercept and slope. Moreover, estimates of posterior genomic heritability suggested that information of heat/cold stress tolerance of animals could be incorporated into breeding objectives to help selection decisions of current animals to be parents of the next generations. However, the current study also reveals that there is a need for additional investigations to develop a better and standardized measurement of heat/cold stress. Given the moderate to high heritability estimates reported herein and previous studies, this trait complex would respond favorably to selection and breeders could select for more robust individuals. However, the concept of applying this same procedure in larger populations warrants further investigation as a means of identifying animals that are less sensitive to environmental extremes.

Table 1. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for steer1 (S1) group of daily high ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First Order	AIC-Second Order	AIC-Third Order
Mean	0.245333	81.50909	78.09695	78.96899
Minimum	0.000124	69.50591	68.46136	63.31235
Maximum	0.837864	92.11029	86.40022	88.37352
SD	0.198399	4.700271	3.888438	4.362816

Table 2. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for heifer1 (H1) group of daily high ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First Order	AIC-Second Order	AIC-Third Order
Mean	0.488850715	81.37227544	82.83060207	83.96427198
Minimum	0.035164266	73.67868096	74.69280938	75.83834176
Maximum	0.783955982	88.34582399	87.10266737	89.09079118
SD	0.153103506	2.8827529	2.760734441	2.717777429

Table 3. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for group steer2 (S2) of daily high ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First Order	AIC-Second Order	AIC-Third Order
Mean	0.066086882	89.94814687	89.51098667	90.89819458
Minimum	0.00023907	78.7056886	79.69970268	78.06969669
Maximum	0.573615215	97.87825874	95.58704785	97.39146665
SD	0.121504175	3.713548607	3.131123685	3.491915785

Table 4. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for group heifer2 (H2) of daily high ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First-Order	AIC-Second	AIC-Third
Mean	0.533528415	59.861189	59.92463929	58.42559182
Minimum	0.059060676	51.37569144	48.08685882	32.48168491
Maximum	0.968539097	66.50988197	64.44284548	65.0249957
SD	0.23476167	2.936444244	2.93417505	5.745442924

Table 5. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for steer1 (S1) group of daily low ambient temperatures (DL) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First	AIC-Second	AIC-Third
Mean	0.221021526	81.78796238	77.25949103	77.90253294
Minimum	0.00013846	69.30531355	65.06357193	59.48729772
Maximum	0.763770168	92.4659131	85.94649748	87.50673534
SD	0.191188526	4.760319143	4.378242282	4.88303427

Table 6. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for heifer1 (H1) group of daily high ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First Order	AIC-Second Order	AIC-Third Order
Mean	0.518404803	80.93599917	82.50314079	83.67697333
Minimum	0.115883505	73.59341246	75.5453475	72.01297335
Maximum	0.810246854	85.96597672	86.95687809	88.75044468
SD	0.14666218	2.601986493	2.590254682	3.317428264

Table 7. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for steer2 (S2) group of daily low ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First	AIC-Second	AIC-Third
Mean	0.07747878	89.83133851	88.43792819	88.2774012
Minimum	9.49978E-05	78.60884614	80.07891189	77.19633363
Maximum	0.614493537	97.9336911	94.439036	95.46314202
SD	0.13953637	3.737603986	3.120187698	3.749574239

Table 8. Descriptive statistics of coefficient of determination and akaike information criterion (AIC) for heifer2 (H2) group of daily low ambient temperatures (DH) averaged over 4-week period on dry matter intake

Descriptive statistics	Coefficient of determination	AIC-First	AIC-Second	AIC-Third
Mean	0.689347307	56.9935386	54.84458836	54.8579066
Minimum	0.101614618	44.3710793	33.57139902	27.77909677
Maximum	0.99224899	64.9067043	63.31530074	64.22373171
SD	0.223698692	3.63072618	5.454864188	6.86851409

Table 9. Descriptive statistics of the intercept of the regression of centered daily high ambient temperatures averaged over 4-week period on dry matter intake

Group[1]				
Descriptive statistics of Intercept (kg)	S1	H1	S2	H2
Mean	208.70	141.25	204.48	232.78
Minimum	154.90	93.58	140.11	164.11
Maximum	261	168.60	274.83	300.78
Standard deviation	27.61	15.90	28.57	33.51

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 10. Descriptive statistics of the slope of the regression of centered daily high ambient temperatures averaged over 4-week period on dry matter intake

Group[1]				
Descriptive statistics of slope (kg/℃)	S1	H1	S2	H2
Mean	1.07	-4.56	-0.26	-4.49
Minimum	-0.605	-7.72	-5.27	-10.44
Maximum	4.03	-1.18	1.60	-0.87
Standard deviation	0.81	1.48	1.25	2.39

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 11. Descriptive statistics of the intercept of the regression of centered daily low ambient temperatures averaged over 4-week period on dry matter intake

Group[1]				
Descriptive statistics of intercept (kg)	S1	H1	S2	H2
Mean	208.70	177.67	204.48	232.78
Minimum	154.90	102.87	140.11	164.11
Maximum	261	232.42	274.83	300.78
Standard deviation	27.61	25.02	28.57	33.51

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 12. Descriptive statistics of the slope of the regression of centered daily low ambient temperatures averaged over 4-week period on dry matter intake

Group[1]				
Descriptive statistics of slope (kg/℃)	S1	H1	S2	H2
Mean	1.09	-3.76	-0.65	-4.64
Minimum	-0.52	-6.89	-5.63	-9.36
Maximum	4.22	-0.96	1.09	-1.19
Standard deviation	0.87	1.19	1.21	2.19

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 13. Descriptive statistics of initial body weight

Group[1]				
Descriptive statistics	S1	H1	S2	H2
Mean	276.15 kg	394.31 kg	259.14 kg	309.17 kg
Minimum	225.57 kg	342.60 kg	157.26 kg	254.01 kg
Maximum	319.33 kg	454.64 kg	339.60 kg	403.11 kg
Standard deviation	27.44 kg	27.20 kg	30.20 kg	37.79 kg

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 14. Descriptive statistics of dry matter intake over the entirety of the feeding period

Group[1]				
Descriptive statistics	S1	H1	S2	H2
Mean	1460.93 kg	988.67 kg	1413.27 kg	1163.93 kg
Minimum	1084.35 kg	655.16 kg	980.71 kg	820.55 kg
Maximum	1826.83 kg	1180.20 kg	1923.95 kg	1503.98 kg
Standard deviation	193.24 kg	111.31 kg	200 kg	167.55 kg
Number of animals per group	59	60	58	59
Feeding period	December 16, 2009 – June 24, 2010	July 28, 2010- November19, 2010	December 23, 2010 – August 12, 2011	July 20, 2011 – January 27, 2012
Total number of days	189	114	182	135

[1] Group = refers to a set of animals that were placed in the Calan gate feeding facility,

Where:

S1 = Steer1 group, H1 = Heifer1 group, S2 = Steer2 group, H2 = Heifer2 group

Table 15. Posterior heritability (Posterior Standard Deviation) estimates of average daily high and low regression coefficients

Trait	Heritability Estimates	
	High Temperature	Low Temperature
[1]Model-2 (Intercept)	0.68 (0.06)	0.76 (0.05)
[2]Model-1 (Intercept)	0.27 (0.07)	0.29 (0.09)
[1]Model-2 (Slope)	0.45 (0.08)	0.48 (0.08)
[2]Model-1 (Slope)	0.25 (0.08)	0.27 (0.08)

[1]Model-1 = refers to the exclusion of MG as fixed effect in the genome-wide association model

[2]Model-1 = refers to the inclusion of MG as a fixed effect in the genome-wide association model

Table 16. Functional annotation, enrichment and pathway analysis

Gene Name	Gene function and analysis type[1]
	Model- 1[2] (Intercept with MG included in the analysis)
guanylate binding protein 1(GBP1)	Heat shock protein binding, Hsp90 protein binding
lectin, mannose binding 2(LMAN2)	Heat shock protein binding, protein binding
VGF nerve growth factor inducible(VGF)	Response to cold, external stimulus, stress, temperature stimulus
C-C motif chemokine ligand 2(CCL2)	Response to temperature, heat, external stimulus, radiation.
DnaJ heat shock protein family (Hsp40) member C2(DNAJC2)	Response to stress, regulation of cellular response to heat, regulation of cellular response to stress, heat shock protein binding, Hsp70 protein binding.
DnaJ heat shock protein family (Hsp40) member C9(DNAJC9)	Positive regulation of ATPase activity, social behavior, positive regulation of catalytic activity, regulation of ATPase activity, positive regulation of molecular function, regulation of catalytic activity, regulation of hydrolase activity, positive regulation of hydrolase activity, intraspecies interaction between organisms, heat shock protein binding.
HEAT repeat containing 9(HEATR9)	hematopoietic progenitor cell differentiation, immune system process, immune system development, multicellular organism development, cellular process, hemopoiesis, cell differentiation, multicellular organismal process, developmental process, single-organism process, single-multicellular organism process, single-organism cellular process, single-organism developmental process, animal organ development, hematopoietic or lymphoid

	organ development, system development, anatomical structure development, cellular developmental process.
Heat shock protein family B (small) member 1(HSPB1)	Response to oxidative stress, regulation of cellular response to stress, regulation of primary metabolic process of response to stress, regulation of response to stress, protein binding.
Bardet-Biedl syndrome 4(BBS4)	Response to stimulus, regulation of response to food, negative regulation of response to food, regulation of appetite, regulation of response to external stimulus, negative regulation of response to external stimulus, negative regulation of appetite by leptin-mediated signaling pathway.
N-acyl phosphatidylethanolamine phospholipase D(NAPEPLD)	Response to stress, aging, behavior, feeding behavior, metabolic process, catabolic process, phospholipid catabolic process, developmental process, response to isolation stress, eating behavior, cellular metabolic process, primary metabolic process, negative regulation of biological process, negative regulation of behavior, regulation of biological process, regulation of behavior, response to stimulus, regulation of feeding behavior, biological regulation, organic substance metabolic process, organic substance catabolic process, regulation of eating behavior, negative regulation of eating behavior, negative regulation of feeding behavior.
DnaJ heat shock protein family (Hsp40) member B12(DNAJB12)	Membrane, integral component of membrane, intrinsic component of membrane, membrane part.
Unc-45 myosin chaperone B(UNC45B)	Binding, protein binding, heat shock protein binding, Hsp90 protein binding

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-1 = refers to the inclusion of myostatin genotype (MG) as a fixed effect during GWAS

Table 17. Functional annotation, enrichment and pathway analysis for model-1 analysis of DH slope

Gene Name	Gene function and analysis type[1]
	Model- 1[2] (DH slope phenotype with MG included in the analysis)
Neuropeptide W(NPW)	Feeding behavior, regulation of biological process, response to stimulus, cellular response to stimulus.
Endothelin receptor type B(EDNRB)	Temperature homeostasis.
Purinergic receptor P2X 3(P2RX3)	Response to stress, response to heat, temperature stimulus, cold, abiotic stimulus, external stimulus, and endogenous stimulus.
MHC class I polypeptide-related sequence A(MICA)	Response to stress, response to temperature stimulus, response to heat, external stimulus.
MHC class I polypeptide-related sequence B(MICB)	Response to temperature stimulus, external stimulus, heat, regulation of response to external stimulus.
VGF nerve growth factor inducible(VGF)	Response to temperature stimulus, cold, external stimulus, abiotic stimulus, endogenous stimulus.
Heat shock protein family A (Hsp70) member 1A(HSPA1A)	Response to temperature stimulus, heat, abiotic stimulus, endogenous stimulus, heat acclimation, cellular response to heat, stress.
Heat shock protein family A (Hsp70) member 1B(HSPA1B)	Response to heat, temperature stimulus, abiotic stimulus, heat acclimation, and regulation of cellular response to heat, heat shock protein binding.

Protein kinase AMP-activated catalytic subunit alpha 1(PRKAA1)	Response to stress, temperature stimulus, cellular response to starvation, endogenous stimulus, cold acclimation, response to radiation, response to cold, UV, light stimulus.
Tumor necrosis factor(TNF)	Temperature homeostasis, fever generation morphogenesis of a branching structure, regulation of response to external stimulus, regulation of fever generation, regulation of heat generation, positive regulation to external stimulus, positive regulation of heat generation.
MTOR associated protein, LST8 homolog(MLST8)	Cellular response to stress, response to stimulus, regulation of cellular response to heat, heat generation, regulation of heat generation, regulation of response to external stimulus.
Heat shock protein family A (Hsp70) member 1 like(HSPA1L)	Response to stress, regulation of response to stress, regulation of cellular response to stress, regulation of cellular response to heat.
Heat shock protein family B (small) member 1(HSPB1)	Regulation of response to external stimulus, cellular response to stress.
Maestro heat like repeat family member 2B(MROH2B)	Cellular response to stimulus, response to stimulus, biological regulation, regulation of biological process.
BCL2 associated athanogene 6(BAG6)	Binding, protein binding, Hsp70 protein binding, heat shock protein binding, protein complex binding.
STIP1 homology and U-box containing protein 1(STUB1)	Receptor binding, binding, protein binding, Hsp70 protein binding, Hsp90 protein binding.
Telomere maintenance 2(TELO2)	Protein binding, heat shock protein binding, protein complex binding, Hsp90 protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-1 = refers to the inclusion of MG as a fixed effect during GWAS

Table 18. Functional annotation, enrichment and pathway analysis for model-2 analysis of DH intercept

Gene Name	Gene function and analysis type[1]
	Model- 2[2] (Intercept with-out MG in the analysis)
RNA polymerase II subunit D(POLR2D)	Response to temperature stimulus, response to heat.
Myostatin (MSTN)	Response to temperature stimulus, response to heat.
Guanylate binding protein 1(GBP1)	Heat shock protein binding, Hsp90 protein binding.
Lectin, mannose binding 2(LMAN2)	Heat shock protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-2 = refers to the exclusion of MG as a fixed effect during GWAS

Table 19. Functional annotation, enrichment and pathway analysis for model-2 analysis of DH slope

Gene Name	Gene function and analysis type[1]
	Model- 2[2] (Slope with-out MG in the analysis)
Neuropeptide W(NPW)	Feeding behavior, regulation of biological process, response to stimulus, cellular response to stimulus.
Endothelin receptor type B(EDNRB)	Temperature homeostasis, heat generation, regulation of heat generation, regulation of response to external stimulus, regulation of fever generation.
Purinergic receptor P2X 3(P2RX3)	Response to stress, response to heat, temperature stimulus, cold, abiotic stimulus, external stimulus, and endogenous stimulus.
MTOR associated protein, LST8 homolog(MLST8)	Regulation of cellular response to heat, stress.
STIP1 homology and U-box containing protein 1(STUB1).	Heat shocking protein binding, Hsp70 protein binding, Hsp90 protein binding.
Telomere maintenance 2(TELO2)	Heat shock protein binding, protein complex binding, and Hsp90 protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-2 = refers to the exclusion of MG as a fixed effect during GWAS

Table 20. Functional annotation, enrichment and pathway analysis for model-1 analysis of DL intercept

Gene Name	Gene function and analysis type[1]
	Model- 1[2] (Intercept with MG included in the analysis)
Guanylate binding protein 1(GBP1)	Protein binding, Hsp90 protein binding, and heat shock protein binding.
Adenosine A1 receptor (ADORA1)	Response to temperature stimulus, detection of external stimulus, detection of abiotic stimulus, response to external stimulus, response to abiotic stimulus, negative regulation of metabolic process, positive regulation of metabolic process, detection of temperature stimulus involved in sensory perception of pain.
Placenta specific 8(PLAC8)	Response to stress, defense response, metabolic process, response to temperature stimulus, response to cold, external stimulus, abiotic stimulus, positive regulation of metabolic process.
Renin(REN)	Response to external stimulus, response to stress, feeding behavior.
Lectin, mannose binding 2(LMAN2)	Heat shock protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-1 = refers to the inclusion of MG as a fixed effect during GWAS

Table 21. Functional annotation, enrichment and pathway analysis for model-1 analysis of DL slope

Gene Name	Gene function and analysis type[1]
	Model- 1[2] (Slope with MG included in the analysis)
Endothelin receptor type B(EDNRB)	Temperature homeostasis, regulation of fever generation, heat generation, regulation of heat generation, regulation of response to external stimulus.
Purinergic receptor P2X 3(P2RX3)	Response to stress, temperature stimulus, heat, cold, external stimulus, endogenous stimulus.
Neuropeptide W(NPW)	Behavior, Feeding behavior, cellular process, regulation of cellular process, response to stimulus, cellular response to stimulus, biological regulation.
MTOR associated protein, LST8 homolog (MLST8).	Regulation of response to stress, regulation of cellular response to stress, cell-cell adhesion regulation of cellular response to heat, response to cold, detection of external stimulus, response to external stimulus, response to temperature stimulus, detection of temperature stimulus, sensory perception of temperature stimulus.
STIP1 homology and U-box containing protein 1(STUB1).	Protein binding, Hsp70 protein binding, heat shock protein binding, Hsp90 protein binding.
Telomere maintenance 2(TELO2)	Protein binding, heat shock protein binding, protein complex binding, Hsp90 protein binding.

Transient receptor potential cation channel subfamily M member 8(TRPM8)	Response to stress, temperature stimulus, cold, external stimulus, detection of external stimulus, abiotic stimulus.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-1 = refers to the inclusion of MG as a fixed effect during GWAS

Table 22. Functional annotation, enrichment and pathway analysis for model-2 analysis of DL intercept

Gene Name	Gene function and analysis type[1]
	Model- 2[2] (Intercept with-out MG in the analysis)
RNA polymerase II subunit D(POLR2D)	Response to temperature stimulus, heat, abiotic stimulus, endogenous stimulus, negative regulation of metabolic process, positive regulation of positive metabolic process, cellular response to heat, cellular response to stress.
Carboxypeptidase B2(CPB2)	Response to stress, temperature stimulus, heat, external stimulus, metabolic process, response to abiotic stimulus.
Myostatin (MSTN)	Response to temperature stimulus, heat, abiotic stimulus, endogenous stimulus, negative regulation of metabolic.
Guanylate binding protein 1(GBP1)	Heat shocking protein binding, Hsp90 protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-2 = refers to the exclusion of MG as a fixed effect during GWAS

Table 23. Functional annotation, enrichment and pathway analysis for model-2 analysis of DL slope

Gene Name	Gene function and analysis type[1]
	Model- 2[2] (Slope with-out MG in the analysis)
Endothelin receptor type B(EDNRB)	Temperature homeostasis, regulation of fever generation, heat generation, regulation of heat generation, regulation of response to external stimulus.
Phosphoinositide interacting regulator of transient receptor potential channels(PIRT)	Response to stress, behavior, response to temperature stimulus, heat, cellular process, abiotic stimulus.
Purinergic receptor P2X 3(P2RX3)	Response to stress, response to temperature stimulus, behavior, response to heat and cold, external stimulus, endogenous stimulus.
Dachshund family transcription factor 1(DACH1)	Behavior, feeding behavior, metabolic process, cellular aromatic compound metabolic process.
Neuropeptide W(NPW)	Behavior, feeding behavior, cellular process, regulation of biological process, regulation of cellular process, response to stimulus, cellular response to stimulus, biological regulation.
MTOR associated protein, LST8 homolog(MLST8)	Regulation of response to stress, regulation of cellular response to stress, cell-cell adhesion regulation of cellular response to heat.
STIP1 homology and U-box containing protein 1(STUB1)	Protein binding, Hsp70 protein binding, heat shock protein binding, Hsp90 protein binding.
Telomere maintenance 2(TELO2)	Protein binding, heat shock protein binding, protein complex binding, Hsp90 protein binding.

[1]Analysis type = refers to the model used during the GWAS analysis.

[2]Model-2 = refers to the exclusion of MG as a fixed effect during GWAS

Table 24. Largest SNP effect of daily high average temperature

Ilumina BovineSNP50 SNP ID	Trait	Analysis Model	Chr_Mb
ARS-BFGL-NGS-42373	DH Intercept	Model-1	3_49
Hapmap49413-BTA-102772	DH Intercept	Model-1	3_49
Hapmap48045-BTA-67779	DH Intercept	Model-1	3_48
Hapmap48939-BTA-90484	DH Intercept	Model-1	3_48
INRA-510	DH Intercept	Model-1	3_53
ARS-BFGL-NGS-112952	DH Intercept	Model-1	3_53
ARS-BFGL-NGS-18669	DH Intercept	Model-1	7_39
ARS-BFGL-NGS-116385	DH Intercept	Model-1	7_39
Hapmap53960-rs29016796	DH Intercept	Model-1	1_93
Hapmap42952-BTA-48143	DH Intercept	Model-1	1_93
ARS-BFGL-NGS-54279	DH Intercept	Model-1	25_35
ARS-BFGL-NGS-79606	DH Intercept	Model-1	25_35
ARS-BFGL-NGS-32007	DH Intercept	Model-1	13_34
ARS-BFGL-NGS-1661	DH Intercept	Model-2	2_8
BTB-00079285	DH Intercept	Model-2	2_8
ARS-BFGL-BAC-36882	DH Intercept	Model-2	1_94
BTB-01086791	DH Intercept	Model-2	1_94
BTA-97386-no-rs	DH Intercept	Model-2	2_5
BTA-47839-no-rs	DH Intercept	Model-2	2_5
Hapmap43083-BTA-86781	DH Intercept	Model-2	2_4

ARS-BFGL-NGS-6152	DH Intercept	Model-2	2_4
ARS-BFGL-NGS-100268	DH Intercept	Model-2	16_79
Hapmap25860-BTA-150580	DH Intercept	Model-2	16_79
ARS-BFGL-NGS-42373	DH Intercept	Model-2	3_49
Hapmap49413-BTA-102772	DH Intercept	Model-2	3_49
ARS-BFGL-NGS-81865	DH Intercept	Model-2	2_7
ARS-BFGL-NGS-110520	DH Slope	Model-1	25_1
Hapmap23849-BTC-016077	DH Slope	Model-1	25_1
ARS-BFGL-NGS-36563	DH Slope	Model-1	12_52
Hapmap30611-BTA-24813	DH Slope	Model-1	12_52
BTB-01885735	DH Slope	Model-1	10_44
Hapmap9514-BTA-67200	DH Slope	Model-1	10_44
ARS-BFGL-NGS-15341	DH Slope	Model-1	3_87
ARS-BFGL-NGS-40956	DH Slope	Model-1	3_87
ARS-BFGL-NGS-16109	DH Slope	Model-1	14_8
ARS-BFGL-NGS-84397	DH Slope	Model-1	14_8
BTA-122625-no-rs	DH Slope	Model-1	12_58
BTB-00266340	DH Slope	Model-1	12_58
ARS-BFGL-NGS-24349	DH Slope	Model-1	23_27
ARS-BFGL-NGS-110520	DH Slope	Model-2	25_1
Hapmap23849-BTC-016077	DH Slope	Model-2	25_1
ARS-BFGL-NGS-102158	DH Slope	Model-2	2_0
Hapmap55208-ss46526613	DH Slope	Model-2	2_0

ARS-BFGL-NGS-36563	DH Slope	Model-2	12_52
Hapmap30611-BTA-24813	DH Slope	Model-2	12_52
ARS-BFGL-NGS-117794	DH Slope	Model-2	2_1
ARS-BFGL-NGS-115117	DH Slope	Model-2	2_1
ARS-BFGL-NGS-29024	DH Slope	Model-2	15_82
BTB-01665549	DH Slope	Model-2	15_82
ARS-BFGL-NGS-15341	DH Slope	Model-2	3_87
ARS-BFGL-NGS-40956	DH Slope	Model-2	3_87
BTB-01885735	DH Slope	Model-2	10_44

Table 25. Largest SNP effect of daily low average temperature

Ilumina BovineSNP50 SNP ID	Trait	Analysis Model	Chr_Mb
Hapmap30960-BTC-030209	DL Intercept	Model-1	25_41
ARS-BFGL-NGS-43920	DL Intercept	Model-1	25_41
ARS-BFGL-NGS-42373	DL Intercept	Model-1	3_49
Hapmap49413-BTA-102772	DL Intercept	Model-1	3_49
INRA-510	DL Intercept	Model-1	3_53
ARS-BFGL-NGS-112952	DL Intercept	Model-1	3_53
Hapmap48045-BTA-67779	DL Intercept	Model-1	3_48
Hapmap48939-BTA-90484	DL Intercept	Model-1	3_48
Hapmap25446-BTC-054694	DL Intercept	Model-1	14_26
Hapmap25761-BTC-065280	DL Intercept	Model-1	14_26

Hapmap41308-BTA-60333	DL Intercept	Model-1	10_21
ARS-BFGL-NGS-64602	DL Intercept	Model-1	10_21
ARS-BFGL-NGS-110120	DL Intercept	Model-1	6_101
ARS-BFGL-NGS-1661	DL Intercept	Model-2	2_8
BTB-00079285	DL Intercept	Model-2	2_8
ARS-BFGL-BAC-36882	DL Intercept	Model-2	1_94
BTB-01086791	DL Intercept	Model-2	1_94
BTA-97386-no-rs	DL Intercept	Model-2	2_5
BTA-47839-no-rs	DL Intercept	Model-2	2_5
ARS-BFGL-NGS-100268	DL Intercept	Model-2	16_79
Hapmap25860-BTA-150580	DL Intercept	Model-2	16_79
Hapmap43083-BTA-86781	DL Intercept	Model-2	2_4
ARS-BFGL-NGS-6152	DL Intercept	Model-2	2_4
ARS-BFGL-NGS-42373	DL Intercept	Model-2	3_49
Hapmap49413-BTA-102772	DL Intercept	Model-2	3_49
BTB-00455305	DL Intercept	Model-2	11_8
ARS-BFGL-NGS-110520	DL Slope	Model-1	25_1
Hapmap23849-BTC-016077	DL Slope	Model-1	25_1
ARS-BFGL-NGS-29024	DL Slope	Model-1	15_82
BTB-01665549	DL Slope	Model-1	15_82
ARS-BFGL-NGS-36563	DL Slope	Model-1	12_52
Hapmap30611-BTA-24813	DL Slope	Model-1	12_52
ARS-BFGL-NGS-16109	DL Slope	Model-1	14_8

ARS-BFGL-NGS-84397	DL Slope	Model-1	14_8
BTA-56736-no-rs	DL Slope	Model-1	23_45
BTB-00867539	DL Slope	Model-1	23_45
ARS-BFGL-NGS-98604	DL Slope	Model-1	17_33
BTB-01786459	DL Slope	Model-1	17_33
ARS-BFGL-NGS-107859	DL Slope	Model-1	8_63
ARS-BFGL-NGS-110520	DL Slope	Model-2	25_1
Hapmap23849-BTC-016077	DL Slope	Model-2	25_1
ARS-BFGL-NGS-102158	DL Slope	Model-2	2_0
Hapmap55208-ss46526613	DL Slope	Model-2	2_0
ARS-BFGL-NGS-36563	DL Slope	Model-2	12_52
Hapmap30611-BTA-24813	DL Slope	Model-2	12_52
ARS-BFGL-NGS-102370	DL Slope	Model-2	28_27
ARS-BFGL-NGS-30132	DL Slope	Model-2	28_27
ARS-BFGL-NGS-117794	DL Slope	Model-2	2_1
ARS-BFGL-NGS-115117	DL Slope	Model-2	2_1
ARS-BFGL-NGS-29024	DL Slope	Model-2	15_82
BTB-01665549	DL Slope	Model-2	15_82
BTA-122625-no-rs	DL Slope	Model-2	12_58

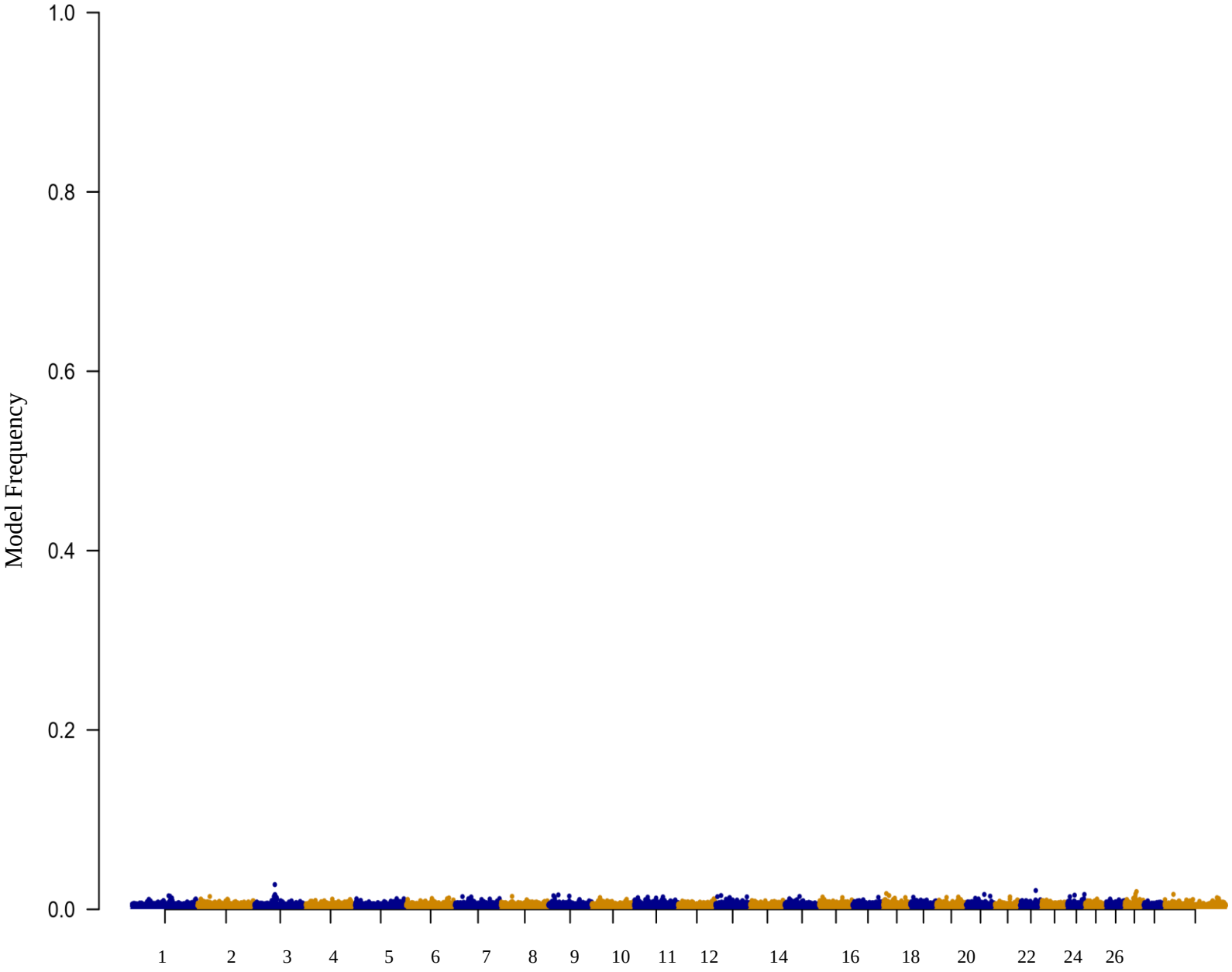

Figure 1. Genome-wide association analysis between SNP genotypes and daily high (DH) temperature intercept from model-1 analysis, when myostatin genotype (MG) was included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

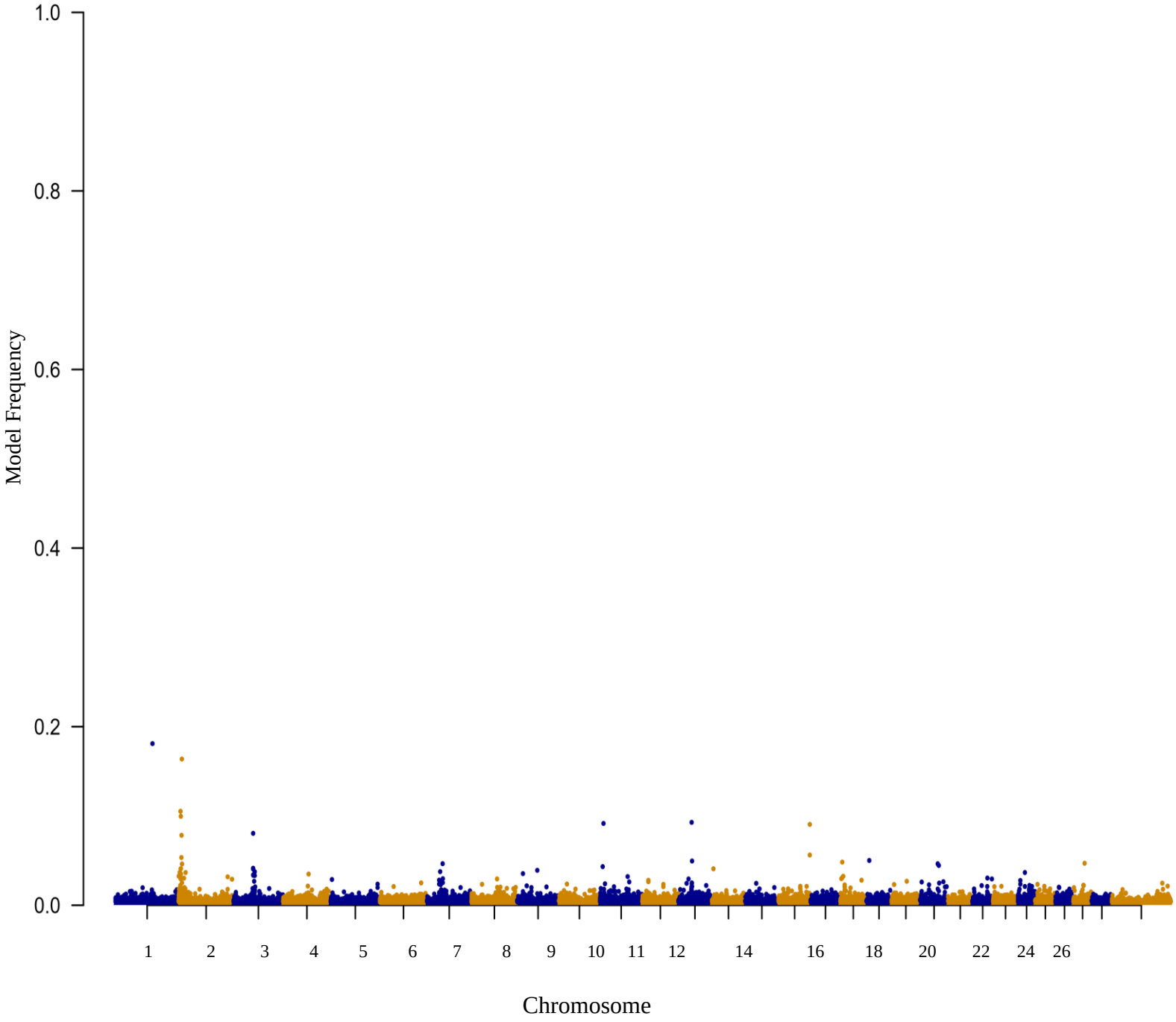

Figure 2. Genome-wide association analysis between SNP genotypes and daily high (DH) temperature intercept from model-2 analysis, when myostatin genotype (MG) was not included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

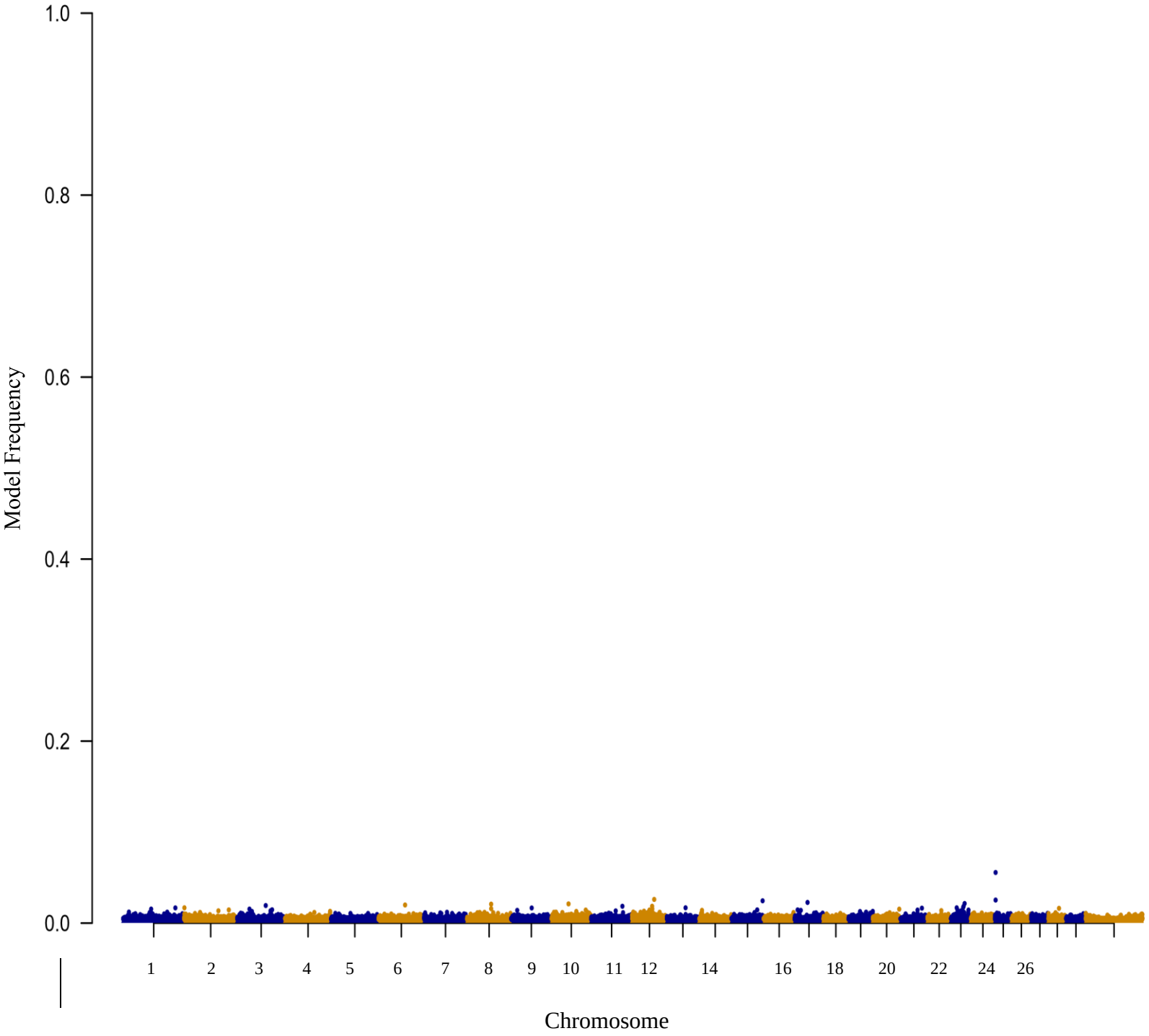

Figure 3. Genome-wide association analysis between SNP genotypes and daily high (DH) temperature slope from model-1 analysis, when myostatin genotype (MG) was included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

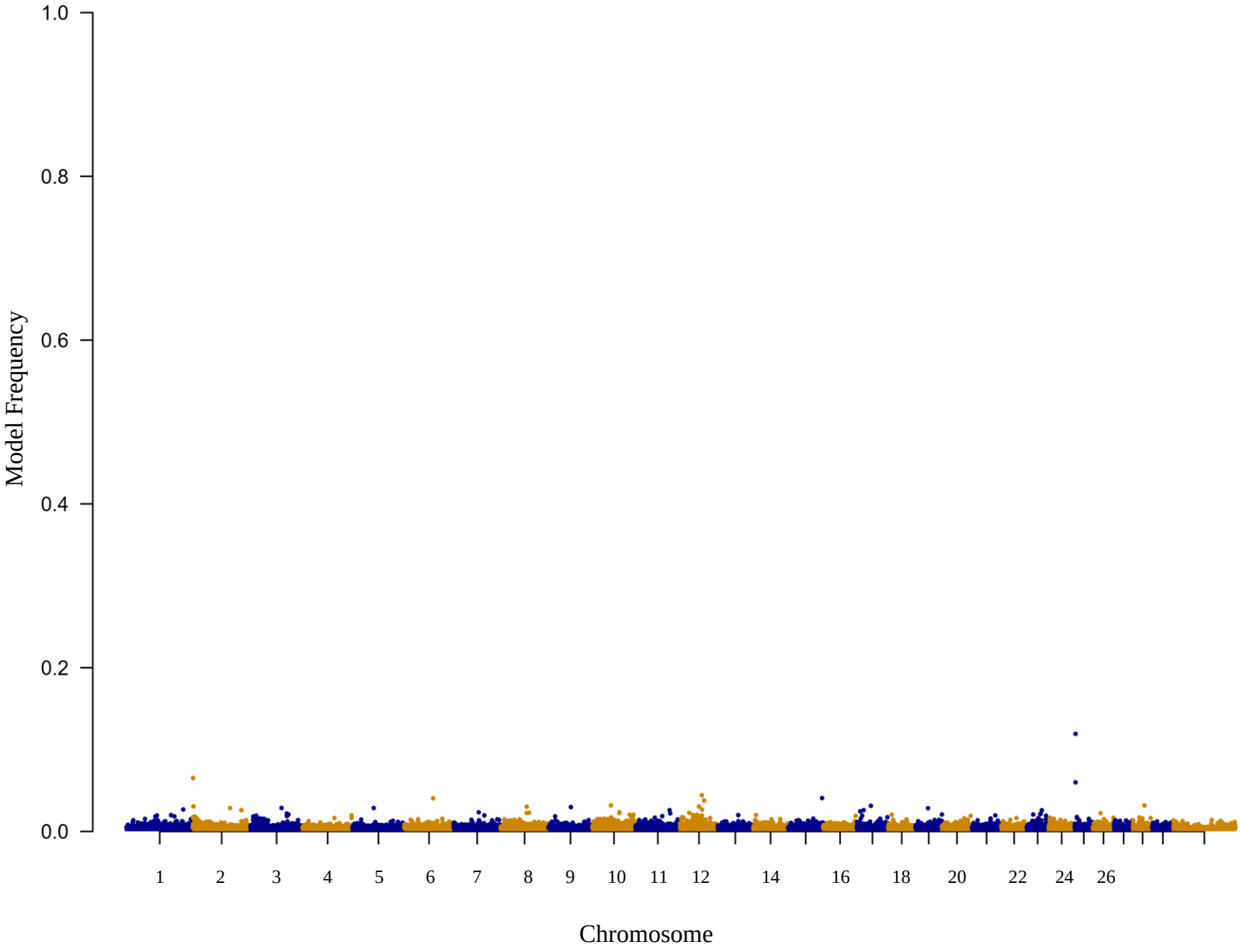

Figure 4. Genome-wide association analysis between SNP genotypes and daily high (DH) temperature slope from model-2 analysis, when myostatin genotype (MG) was not included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

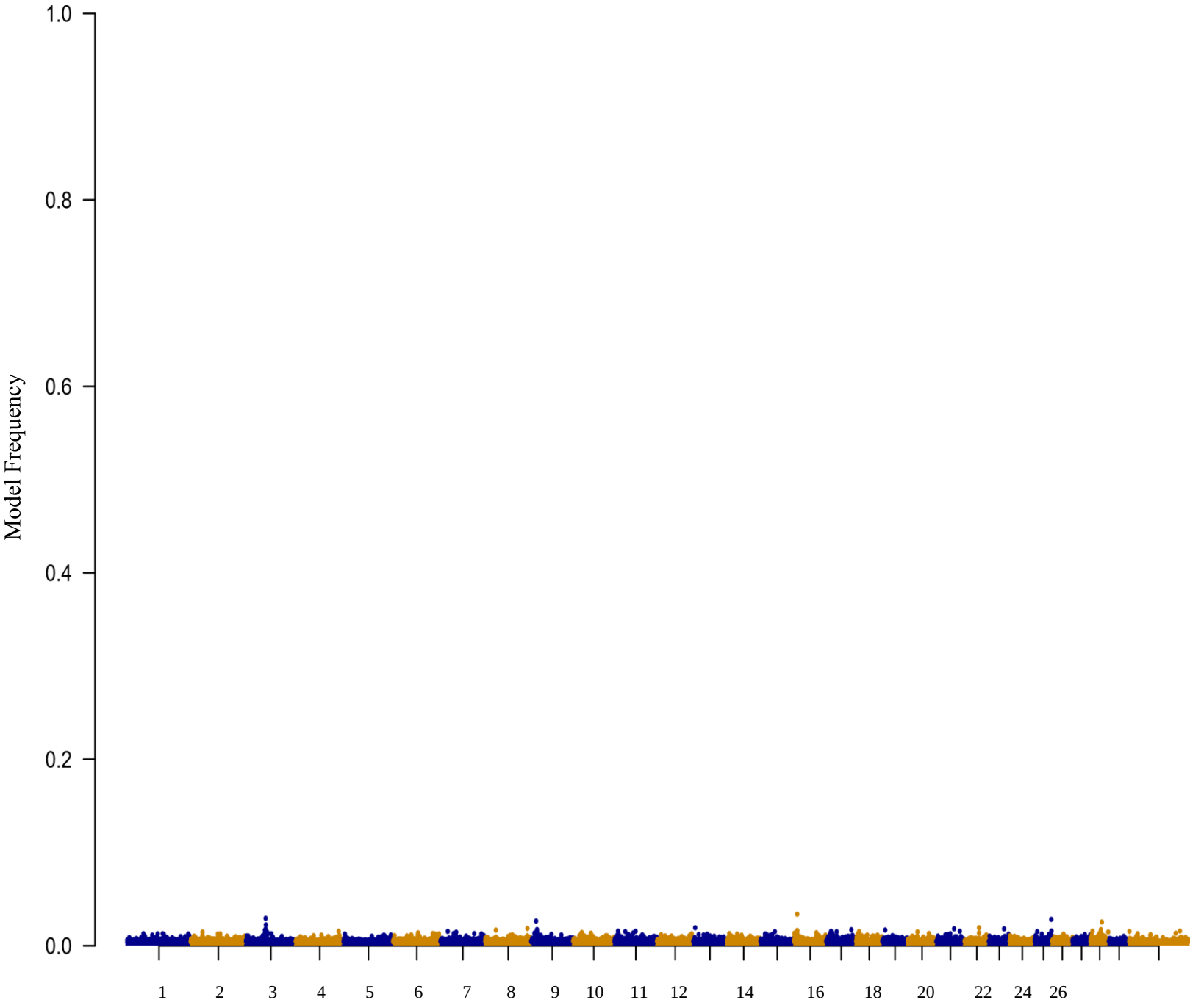

Figure 5. Genome-wide association analysis between SNP genotypes and daily low (DL) temperature intercept from model-1 analysis, when myostatin genotype (MG) was included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

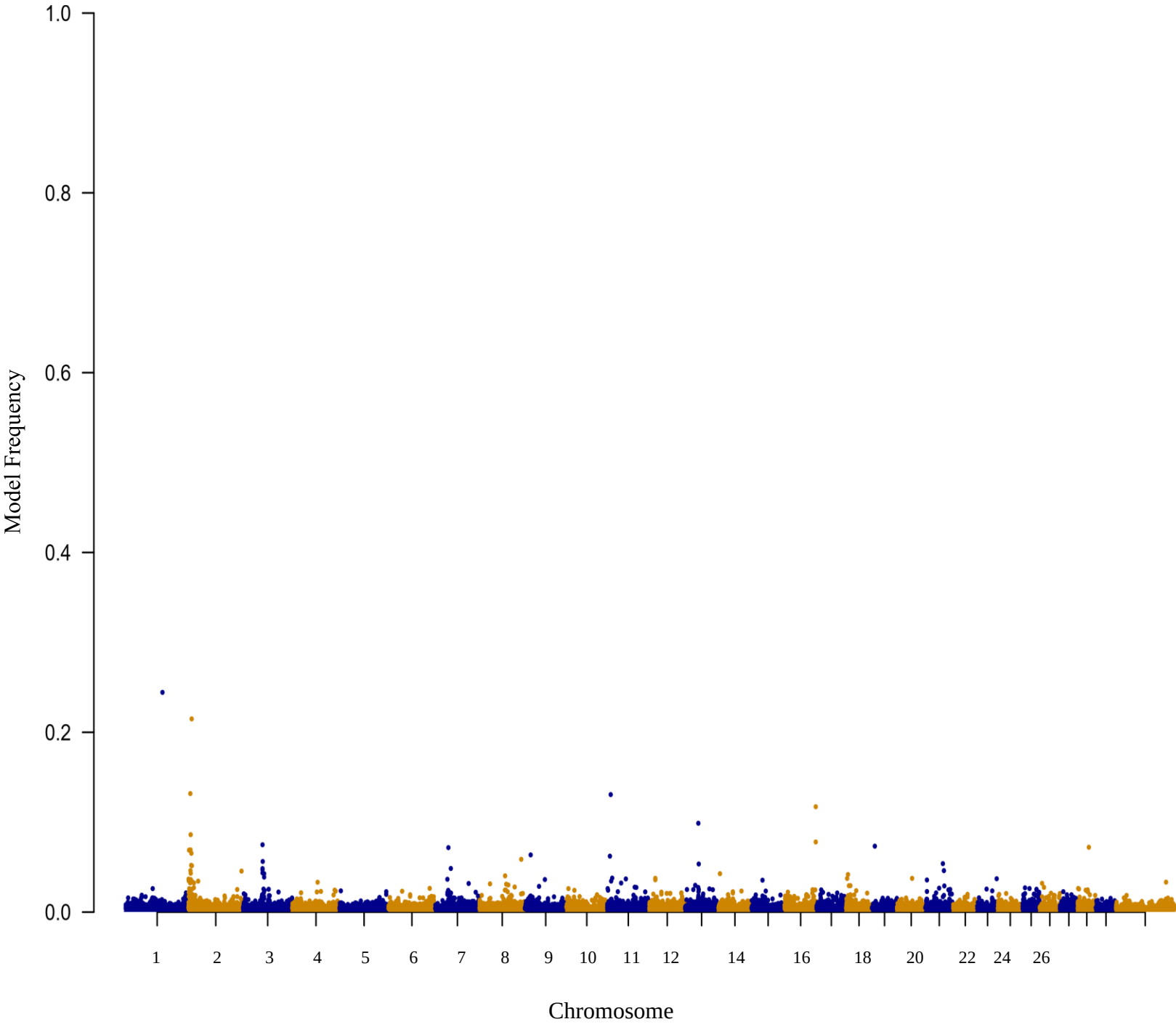

Figure 6. Genome-wide association analysis between SNP genotypes and daily low (DL) temperature intercept from model-2 analysis, when myostatin genotype (MG) was not included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

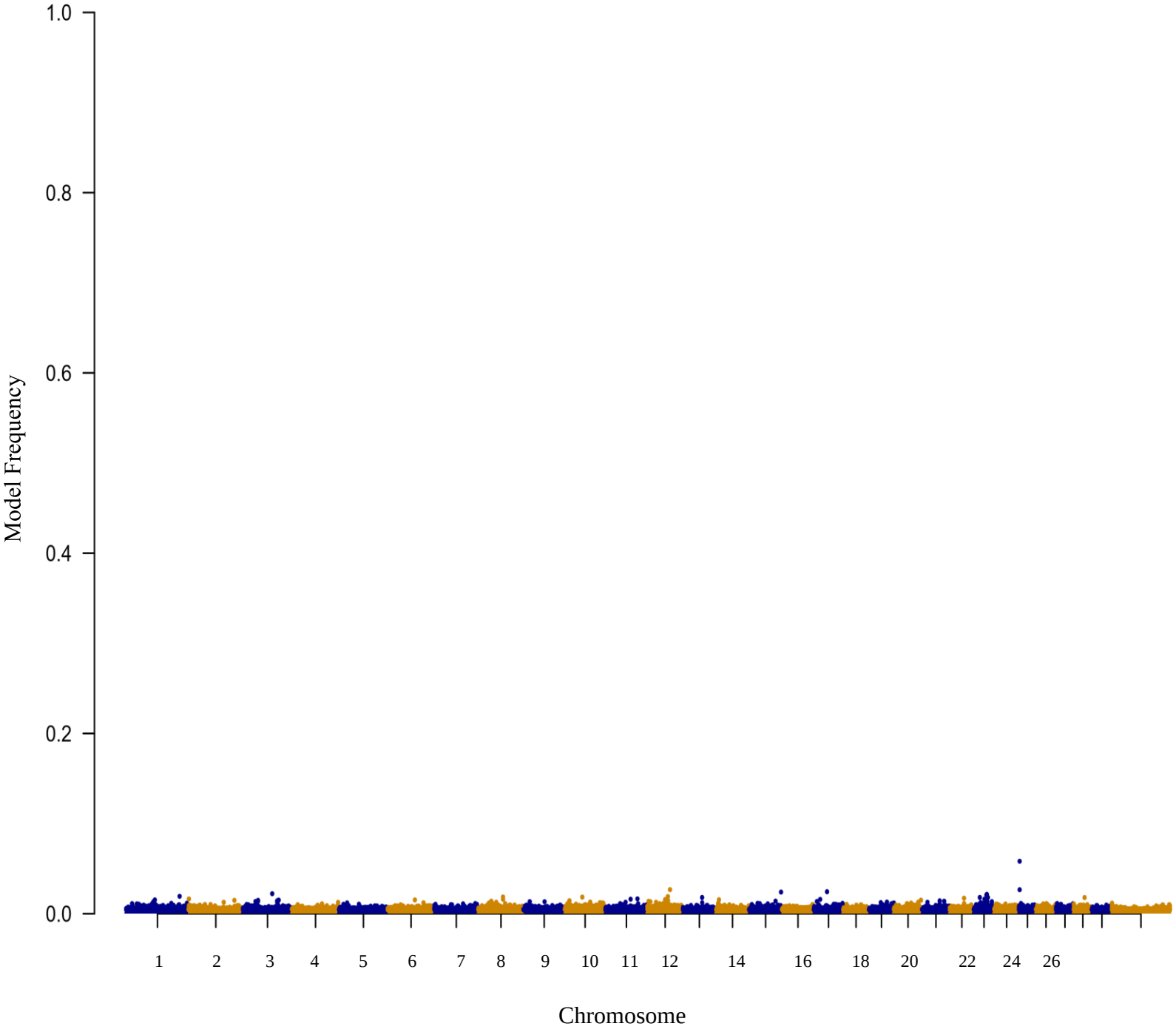

Figure 7. Genome-wide association analysis between SNP genotypes and daily low (DL) temperature slope from model-1 analysis, when myostatin genotype (MG) was included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

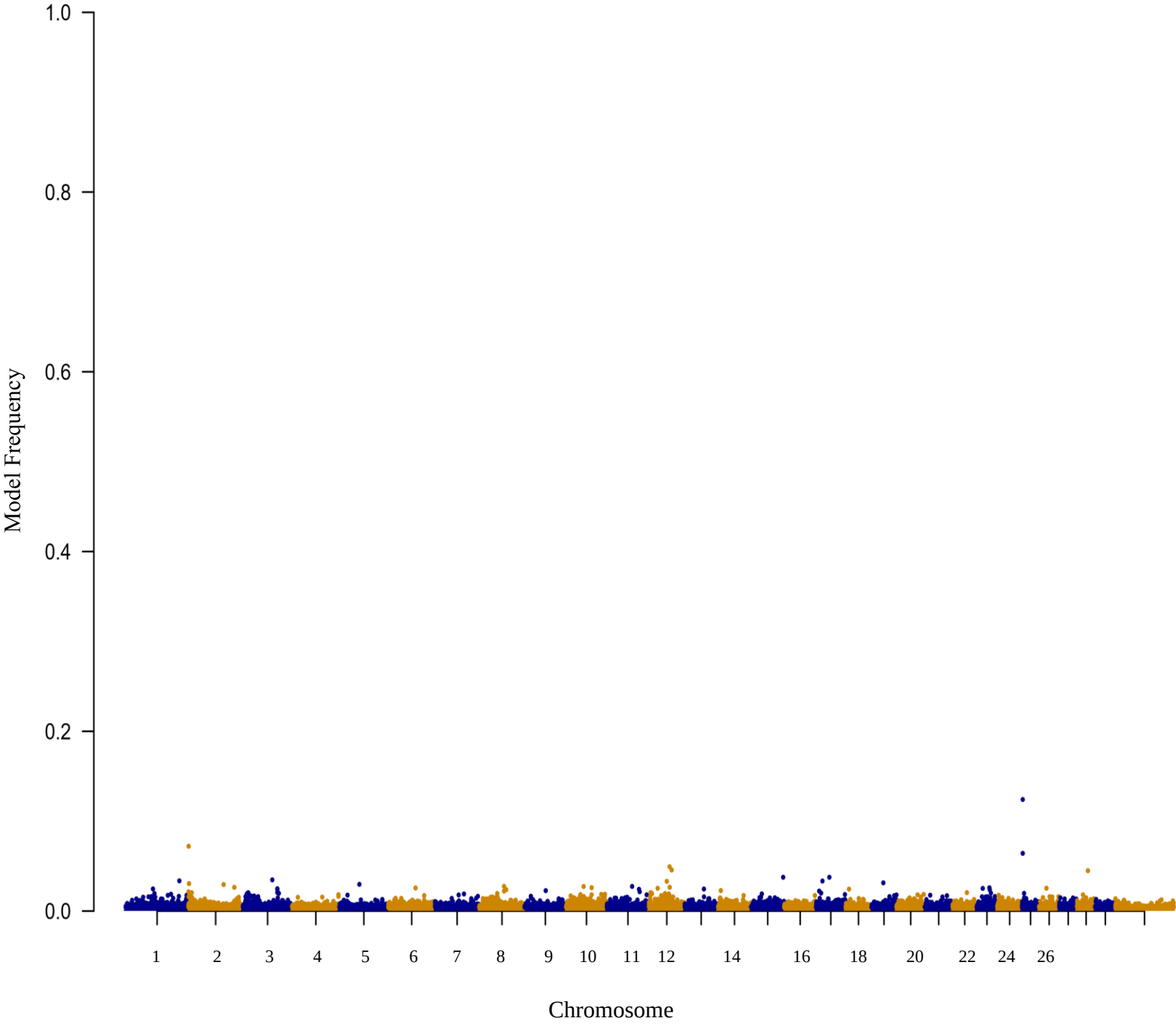

Figure 8. Genome-wide association analysis between SNP genotypes and daily low (DL) temperature slope from model-2 analysis, when myostatin genotype (MG) was not included as a fixed effect in the model. The Y-axis represents the model frequency of each marker. The X-axis represents number of chromosomes and each dot colors represent SNPs.

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