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Genetic and genomic analysis of hyperthelia in Brown Swiss cattle

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ABSTRACT

Supernumerary teats (SNT) are any abnormal teats found on a calf in addition to the usual and functional 4 teats. The presence of SNT has also been termed hyperthelia since the end of the 19th century. Supernumerary teats can act as an incubator for bacteria, infecting the whole udder, and can interfere with the positioning of the milking machine, and consequently, have economic relevance. Different types of SNT are observed at different positions on the udder. Caudal teats are in the rear, ramal teats are attached to another teat, and intercalary teats are found between 2 regular teats. Not all teats are equally developed; some are completely functional but most are rudimentary and not attached to any mammary gland tissue. Recently, different studies showed the poly/oligogenic character of these malformations in cattle as well as in other mammalian species. The objective of this study was to analyze the genetic architecture and incidence of hyperthelia in Swiss Brown Swiss cattle using both traditional genetic evaluation as well as imputed whole genome sequence variant information. First, phenotypes collected over the last 20 yr were used together with pedigree information for estimation of genetic variance. Second, breeding values of Brown Swiss bulls were estimated applying the BLUP algorithm. The BLUP-EBV were deregressed and used as phenotypes in genome-wide association studies. The gene *LGR5* on chromosome 5 was identified as a candidate for the presence of SNT. Using alternative trait coding, genomic regions on chromosome 17 and 20 were also identified as being involved in the development of SNT with their own supernumerary mammary gland tissue. Implementing knowledge gained in this study as a routine application allows a more accurate evaluation of the trait and reduction of SNT prevalence in the Swiss Brown Swiss cattle population.

Key words: dairy cattle, hyperthelia, supernumerary teats, genome-wide association

INTRODUCTION

Supernumerary teats (**SNT**) are any abnormal teats found on a calf in addition to the usual and functional 4 teats. The presence of SNT has also been termed hyperthelia since the end of the 19th century (Schmidt, 1896). Studies on different populations report a prevalence of SNT of 40 to 44% in German Fleckvieh, 15% in Swiss Holstein, and 31% in German Brown Swiss (**BS**; Brka et al., 2002; Pausch et al., 2012; Joerg et al., 2014). Only 20% of the Swiss BS population is affected by SNT, the result of drastic selection for clear udders in the years before recording this trait (Lucas Casanova, Braunvieh Schweiz, Zug, Switzerland, personal communication). Prevalence differences between breeds for SNT indicate genetic variation within this trait and must be analyzed for each population individually. Supernumerary teats have been described at different locations: on the rear of the udder (caudal), between the front and rear teats (intercalary), or appended to a regular teat (ramal; Skjervold, 2010). The developmental stage of the SNT can also vary. In some cases SNT had their own supernumerary mammary gland tissue (live SNT), but in most cases no separate mammary gland tissue existed for the SNT (blind SNT; Pausch et al., 2012; Joerg et al., 2014). Brka et al. (2002) showed that cows whose mother was lactating during their embryonic development had SNT prevalence 5% higher than animals whose mother was a heifer during pregnancy. Development of mammary gland tissue has predominantly been studied in mice. Results of such studies indicate a 2-step development process. In a first phase, epithelial cells superimpose and placodes appear along the milk lines and develop into mammary buds. Later in pregnancy, the buds branch to form a rudimen-

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tary ductal tree, which grows further at puberty (Hens and Wysolmerski, 2005). Different signaling pathways may influence both phases; therefore, multiple genomic regions could be involved in the presence of SNT.

In the dairy industry, SNT are considered problematic for various reasons. First, they present a risk factor for mastitis. Bacteria present in the unused teat(s) can migrate to an active teat and induce an infection (Steiger and Grünenfelder, 1988). Second, SNT lower milking ability. This is not only true for the ramal SNT, which interfere with the correct placement of the conventional milking machine (Wiener, 1962), but for all types of SNT that may not be recognized as such by automatic milking systems (**AMS**). Swiss AI centers reacted to the second problem by creating a label for bulls whose daughters are well adapted to AMS (Bigler, 2015). In this context, better understanding of the genetic background of SNT becomes particularly relevant. Third, animals affected by SNT have fewer chances to perform well at exhibitions and have a lower price on the market (Pausch et al., 2012; Joerg et al., 2014). Finally, consumers have identified surgical elimination of SNT as a major risk for poor calf welfare (Vasseur et al., 2010). As consciousness for animal welfare rises, the surgical removal of SNT may be banned. Therefore, dairy cattle breeders should aim to quickly decrease the SNT prevalence in their population using highly accurate breeding values.

In this study, we performed a traditional genetic evaluation of SNT applying BLUP. Sire genotypes and deregressed EBV were used to discover QTL affecting the presence of blind or live SNT. Use of this information in routine genomic prediction will improve the accuracy of selection against SNT, help to decline their prevalence, and thus provide an alternative to surgical removal.

MATERIALS AND METHODS

Animals and Phenotypes

During linear scoring of BS cattle, SNT have been routinely recorded together with conformation traits for more than 20 yr in Switzerland. A genetic analysis has not been performed on these records before. The SNT are evaluated on a numerical scale from 1 to 9, where 9 indicates animals without SNT. Codes from 1 to 8 are applied depending on the form and severity of the SNT. However, the scale is not linearly proportional. The SNT with supernumerary mammary gland tissue have code 3. The SNT without supernumerary mammary gland tissue have code 4. Removed SNT (scars) have code 5 or 6 whether the presence of a supernumerary gland is detected or not. Differences in code for present or removed SNT were not taken into account as codes 3 and 5 but also 4 and 6 were always considered similarly. Code 7 describes a rudimentary SNT without supernumerary mammary gland tissue. Codes 4 and 7 are only differentiated by their length, as SNT shorter than 0.5 cm are considered rudimentary. Codes 1, 2, and 8 are given for ramal SNT, a second orifice on a main teat, or a hairless spot on the udder, respectively. These codes do not represent an independent SNT and therefore were not considered for this analysis.

Udder clearance (**UCT**) compared clear animals (code 9) to affected animals (codes 3 to 7). A second trait, the presence of supernumerary mammary gland tissue with an SNT (**PMG**) emphasized the live SNT by contrasting animals with a clear udder and animals with blind SNT (codes 4, 6, 7, and 9) to animals with live SNT (codes 3 and 5). Figure 1 shows the code repartition of both binary traits. Since the beginning of linear description in Switzerland, 863,997 records have been routinely registered. Only records of first lactating cows were kept. A year was defined from August to July to adhere to the seasonal recording rhythm. Groups were created for expert-by-year and farm-by-year. To ensure balanced groups, years with less than 30,000 records, expert groups with less than 200 entries, and farm groups with less than 6 animals were excluded. These cut-offs were empirically chosen on the basis of graphical observation. After editing, phenotypic data contained 383,873 SNT scores. Table 1 presents the proportions of raw SNT scores before and after filtering.

Genetic Parameter and Breeding Value Estimation

Genetic parameters were estimated with VCE version 6 (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010). Reducing the number of farm-by-year classes, we created 4 random samples of approximately 150,000 records. We fitted the model $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z_a a} + \mathbf{Z_p p} + \mathbf{e}$, where $\mathbf{y}$ is a vector of binary phenotypes of UCT or PMG, $\boldsymbol{\beta}$ is the vector of the fixed effect of the animal dam's life stage—heifer or cow, $\mathbf{X}$ is a design matrix that associates $\boldsymbol{\beta}$ with $\mathbf{y}$, $\mathbf{a}$ is the vector of direct genetic effects, $\mathbf{Z_a}$ is the design matrix that attributes $\mathbf{a}$ with $\mathbf{y}$, $\mathbf{p}$ is the vector of the random effects expert-by-year and farm-by-year, $\mathbf{Z_p}$ is the matrix that associates $\mathbf{p}$ with $\mathbf{y}$, and $\mathbf{e}$ represents the residuals effects not explained by the model. The fixed effect of the animal's dam's life stage had 2 classes. Records of animals whose dam was a heifer during pregnancy were divergent to records of animals whose dam was lactating during their embryological development. Variance component estimates presented are averages of the results of 4 runs per trait definition.