**SUMMARY**

An important aim in animal breeding is the improvement of growth and meat quality traits. Previous studies have demonstrated that genetic variants in the *fat mass and obesity associated* (*FTO*) gene have a relatively large effect on human obesity as well as on body composition in rodents and, more recently, in livestock. Here, we examined the effects of the *FTO* gene variants on growth and carcass traits in the Slovenian population of Simmental (SS) and Brown (SB) cattle. To validate and identify new polymorphisms, we used sequencing, PCR-RFLP analysis and TaqMan assays in the SS breed and *FTO* gene variants data from the Illumina BovineSNP50 v1 array for the SB breed. Sequencing of the eight samples of progeny-tested SS sires detected 108 single nucleotide polymorphisms (SNPs) in the bovine *FTO* gene. Statistical analyses between growth and carcass traits and 34 *FTO* polymorphisms revealed significant association of *FTO* variants with lean meat percentage in both breeds. Additionally, *FTO* SNPs analyzed in SS cattle were associated with fat percentage, bone weight and live weight at slaughter. The *FTO* gene can thus be regarded as a candidate gene for the marker-assisted selection programs in our and possibly other populations of cattle. Future studies in cattle might reveal novel roles for the *FTO* gene in shaping carcass traits in livestock species as well as body composition control in other mammals.

**Keywords** *fat mass and obesity associated*, haplotype, QTL, single nucleotide polymorphism, single nucleotide polymorphism array

Many economically important traits in livestock, such as growth and carcass traits, are under the control of multiple genes. Due to growing consumer demand for products with lower fat content, animals with higher growth rate and better carcass composition are of great significance. In addition, excessive fat deposition has an impact on animal productivity and, not least, on consumers’ health. Therefore, molecular markers associated with these traits would be very useful. For biomarker development, web servers are of great assistance. For example, the current version of the obesity gene atlas includes 1736 fat deposition associated loci, including the *FTO* gene (Kunej et al. 2012). The *FTO* gene has been studied extensively since 2007, when it was described as a gene affecting body mass index (BMI) in humans in three independent studies (Dina et al. 2007; Frayling et al. 2007; Loos & Bouchard 2008). These association studies confirmed that genetic variants in the *FTO* gene have a relatively large effect on some growth and carcass traits in chickens, pigs, rabbits, sheep and cattle (Table S1). The link between the *FTO* gene and body composition traits appears to be one of the strongest genotype–phenotype associations detected by genome-wide screening techniques (Barabási 2007). The objective of this study was to experimentally validate the already-published *FTO* polymorphisms, identify new SNPs and test their associations with growth and carcass traits in the Slovenian population of Simmental (SS) and Brown (SB) cattle (Table S2).

To evaluate polymorphisms residing within the *FTO* gene in the two studied populations, two different approaches were used: (i) sequencing, PCR-RFLP analysis and TaqMan
assays (Applied Biosystems) in the SS breed and (ii) data extraction from the BovineSNP50 v1 array (Illumina) in the SB breed (Fig. S1). The DNA sequence of the bovine FTO gene was downloaded from the Ensembl database e82 (September 2015). Primers (Table S3) and restriction enzymes for the PCR-RFLP assays were designed using the PRIMER3 (http://frodo.wi.mit.edu/primer3) and WEBCUTTER v2.0 programs (http://bio.lundberg.gu.se/cutter2/). TaqMan probes were designed in unique target sequences (Table S4) using FILEBUILDER (http://tools.lifetechnologies.com/content/sfs/manuals/cms_042457.pdf). To determine which SNPs from the Illumina BovineSNP50 v1 array data map to the FTO gene, the SNPchiMp v.3 database was used (Nicolazzi et al. 2014). The DNA samples were extracted from frozen semen of 31 SS sires and muscle tissue samples of 160 progeny-tested half-sibs using the DNeasy Blood & Tissue Kit (Qiagen). Conditions for the PCR were as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 59–63 °C for 30 s and 72 °C for 1 min; followed by a further 7 min extension at 72 °C. Three SNPs were genotyped using TaqMan in accordance with the manufacturer’s protocol (Applied Biosystems).

The genotype–phenotype association analysis for the SS breed was performed on 31 sires and their half-sibs (mean = 148, min = 134, max = 160). The following linear model was used to estimate the association between the SNP (one at a time) and measured traits in SS cattle:

\[ y_{ijkl} = \mu + S_i + G_j + b(x_{ijkl} - \bar{x}) + s_k + e_{ijkl} \]  

where \( y_{ijkl} \) is the phenotype observation for the analyzed trait, \( \mu \) represents trait average, \( S_i \) is the fixed effect of slaughter season (\( i = 1–7 \)), \( G_j \) is the fixed effect of genotype (\( j = 1–3 \)), \( b \) is the regression coefficient for age, \( x_{ijkl} \) is the fixed effect of age, \( s_k \) is the random effect for sire (\( k = 1–31 \)), and \( e_{ijkl} \) is the random error. In the SB breed, the dependent variable was the estimated breeding value for 469 sires from

Figure 1 Genomic organization of the bovine FTO gene, linkage disequilibrium (\( r^2 \)) plot for the 34 polymorphisms. Framed SNPs: SNPs associated with growth and carcass traits in population of SS or SB breed (see also Table 1). Light blue: nine SNPs present on the SNP array. Green star: SNPs from other studies associated with back fat thickness and longissimus muscle area (Wei et al. 2011). Blue stars: SNPs associated with final body weight, average daily gain and hot carcass weight (Rempel et al. 2012). The rectangle at the bottom left displays the three haplotype blocks with frequencies shown next to each haplotype.
the national genetic evaluation, and thus model (2) included only the effect of the SNP genotype: heterogeneity of residuals was accounted for through weighted analysis, with weights being proportional to the accuracy of estimated breeding values.

The Ensembl database e82 reports 11,491 polymorphisms mapping to the bovine FTO locus located on BTA18: 22,118,201–22,541,532 (GenBank Accession no. AC_000175.1). However, it was expected that a large portion would not be polymorphic in our studied populations or that some reported SNPs may be the result of sequencing errors. We therefore sequenced 106 regions encompassing 70,540 bp of the FTO gene and validated 108 SNPs in eight samples of SS cattle breed sires (Fig. 1, Table S3). A total of 627 SB sires from the national genetic evaluation were genotyped using the BovineSNP50 v1 array (Illumina), whereby nine of 54,001 SNPs mapped to the FTO gene region: g.22124137G>T (rs41636327), g.22201521T>C (rs41636322), g.22256619T>C (rs109987221), g.22300133T>C (rs109068316), g.22339904C>T (rs14579976), g.22379853G>A (rs1110638993), g.22437134T>G (rs109039421), g.22489013A>C (rs109068316) and g.22510315T>G (rs41636306).

The HAPLOVIEW 4.2 program (Barrett et al. 2005) was used to determine the linkage disequilibrium of 34 SNP markers located on the bovine FTO gene, based on r2 measurements (Fig. 1). A total of three haplotype blocks among 16 SNPs were identified in the SS breed population, with the largest one (block 2), spanning approximately 11 kb. The haplotypes are displayed in the left bottom corner of Fig. 1, which shows the population frequency of each haplotype in a block and the connections from one block to the next. In the crossing areas, multiallelic D’ values are displayed between blocks 1 and 2 (0.31) and between blocks 2 and 3 (0.51), indicating a moderate recombination level between the blocks. The most frequent haplotypes within the blocks comprised linkage of nucleotides TCT (47.5 %) for block 1, ACAT (43.2 %) for block 2 and AACCACACC (67.1 %) for block 3.

Table 1
Result of the association analysis between the FTO SNPs and growth and carcass-related traits with significant effect in the population of Slovenian Simmental and Brown breed.

<table>
<thead>
<tr>
<th>SNP ID and allele substitution</th>
<th>Trait</th>
<th>Genotype</th>
<th>BB</th>
<th>Bb</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simmental breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22277498G&gt;C (Bb)</td>
<td>Lean meat percentage1 (%)</td>
<td></td>
<td>68.7</td>
<td>69.9</td>
<td>0.038</td>
</tr>
<tr>
<td>g.22321335C&gt;T (Bb)</td>
<td>Fat percentage1 (%)</td>
<td></td>
<td>13.0</td>
<td>11.9</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Bone weight (kg)</td>
<td></td>
<td>27.3</td>
<td>28.3</td>
<td>0.016</td>
</tr>
<tr>
<td>g.22342594A&gt;G (Bb)</td>
<td>Lean meat percentage1 (%)</td>
<td></td>
<td>70.1</td>
<td>69.4</td>
<td>0.043</td>
</tr>
<tr>
<td>g.22347936G&gt;A (Bb)</td>
<td>Live weight at slaughter (kg)</td>
<td></td>
<td>655.3</td>
<td>645.8</td>
<td>0.045</td>
</tr>
<tr>
<td>g.22348014T&gt;C (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348032T&gt;C (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348033G&gt;A (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348151C&gt;T (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348158G&gt;A (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348243C&gt;T (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g.22348499C&gt;T (Bb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown breed</td>
<td>Estimated breeding value for lean meat percentage (%)</td>
<td>98.3 ± 1.0</td>
<td>105.0 ± 3.2</td>
<td>n.p.</td>
<td>0.044</td>
</tr>
</tbody>
</table>

B, first allele [e.g. Guanine (G) for SNP g.22277498G>C]; b, second allele [e.g. Cytosine (C) for SNP g.22277498G>C]; n.p., not present.

1Right side of carcass.

© 2015 Stichting International Foundation for Animal Genetics, 47, 219–222
detected significant SNPs are causal for the observed phenotypic effects. Namely, two recent reports indicate that obesity-associated intronic FTO SNPs appear functionally connected with two neighboring genes, IRX3 and RPGRIP1L (Smemo et al. 2014; Stratigopoulos et al. 2014), but the FTO gene itself plays an important role in determining growth and body composition in mammals. In future studies in our and other livestock populations, it would therefore be useful to explore a wider region around FTO to identify a composite haplotype at the IRX3–FTO–RPGRIP1L genomic segment as a potential marker for leaner and more efficient growth. Nevertheless, our study revealed that FTO is significantly associated with growth and carcass-related traits, suggesting that identified SNPs can serve as candidate genetic variants for the marker-assisted selection programs in our and possibly other populations of cattle.

Acknowledgements
This work was supported by the Slovenian Research Agency projects J4-2528, P4-0220, J4-3006, V4-1084, and Common Basic Breeding Program. We thank Assistant Professor Gregor Gorjanc, PhD for help and consultations on the data analyses of the SB breed population.

The authors declare that there are no conflicts of interest.

References

Supporting information
Additional supporting information may be found in the online version of this article.

Figure S1 Workflow of the study and the results.
Table S1 Review of published data on FTO-associated traits in livestock animals.
Table S2 Descriptive statistics and data structure.
Table S3 Primers used for identification of FTO gene polymorphisms.
Table S4 Real-time TaqMan primer and probe sequences.
Table S5 Summary statistics of 34 FTO SNPs calculated for Simmental and Brown cattle breed in Slovenia.

© 2015 Stichting International Foundation for Animal Genetics, 47, 219–222