Use of SNP Markers Within the *Fat Mass and Obesity-associated* (*FTO*) Gene to Verify Pedigrees and Determine Haplotypes in Paternal Half-sib Families of Slovenian Simmental Cattle

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Summary

The objective of this preliminary study was to identify SNP markers within the *FTO* gene for evaluation of pedigree data accuracy and determination of haplotypes in paternal half-sib families of Slovenian Simmental cattle. Out of 23 polymorphic SNPs identified ten most informative SNPs for genotyping 31 sires and 56 half-sib progeny were used. The ATLAS program was used for paternity testing. Haplotype analysis revealed three haplotype blocks. The effect of SNPs "ex2 T>C" and "int2 indel*>T" was significant on three correlated carcass traits: live weight at slaughter (P= 0.03), carcass weight (P= 0.038), and lean weight (P= 0.048). The *FTO* gene can thus be regarded as a candidate for the marker assisted selection programs in our and possibly other populations of cattle. Future studies in cattle might also reveal novel roles of the *FTO* gene in carcass traits on livestock species as well as fatness control in other mammals.

Key words

cattle, growth, *FTO*, SNP, haplotype

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Aim

For economic reasons and due to growing consumer demand for products with lower fat content, an important aim in animal breeding is to improve meat quality traits. Genetic variants in recently identified fat mass and obesity associated (*FTO*) gene have been shown to have a relatively large effect on human obesity and some carcass traits in model organisms, pigs and more recently also in cattle. The aim was to identify polymorphisms in various regions of the *FTO* gene to develop SNP markers for testing associations with growth and carcass traits in paternal half-sib families of the Slovenian Simmental cattle. Additionally, data were used to verify validity of pedigrees and to perform haplotype analysis.

Material and methods

Animals and traits. Animals were selected from the National progeny test for Slovenian Simmental cattle. The test was carried out on half-sibs in a central progeny testing stations in Rogoza and Lenart, Slovenia. Growth traits were evaluated under controlled environmental conditions for a period of approximately 15 months. At the end of test, bulls were slaughtered and one to six half-sib's carcasses per sire were transported into experimental slaughter facility, where carcasses were dissected into muscle, fat, bone, and tendon. The following carcass traits: carcass length, chest depth, dressing percentage, conformation index, forequarter weight, forequarter percentage, hindquarter weight, hindquarter percentage, lean weight, carcass fat weight, tendon weight, bone weight, meat percentage, carcass fat percentage, weight of more valuable cuts, percentage of more valuable cuts, live weight at slaughter, carcass weight, meat : bone ratio and subjective carcass fatness evaluation of 31 half-sib's carcasses were included in present study. For the genotype-phenotype association analysis presented here, 31 sires and 56 half-sibs were used.

SNP marker identification and genotyping. Sequencing of a DNA panel of sires was performed for various regions of the *FTO* gene (including already known SNP loci from NCBI or ENSEMBL databases) to identify polymorphisms in our population. Primers and probes for the TaqMan SNP assay (Applied Biosystems) were designed following BLAST and Repeat masker analysis. Primers and restriction enzymes for the PCR-RFLP assays were designed using Primer3 and Webcutter v2.0 programs (http://bio.lundberg.gu.se/cutter2/). DNA was extracted from frozen semen of sires or muscle tissues of progeny tested half – sibs using Qiagen DNA extraction kit.

Verifying data consistency. The ATLAS program was used for verifying data validity and paternity between 31 sires and 56 half-sibs (Pérez - Enciso et al., 2005).

Haplotype analysis. The Haploview 4.2 program was used to determine the linkage disequilibrium (LD) of 10 most informative SNP markers located on bovine *FTO* gene (Barrett et al., 2005). A haplotype analysis was performed in population of 31 sires and 56 half-sib progeny of Slovenian Simmental cattle.

Statistical analysis. Statistical package SAS/STAT (SAS User's Guide, 2002) was used for statistical analyses. The following linear model using single SNP analysis was used:

$$y_{ijk} = \mu + S_i + G_j + e_{ijk} \quad (1)$$

where y_{ijk} is the observation for the carcass traits, μ represents trait average, S season (i= 1-7), G genotype (for SNP j= 1-10) and e random error.

Results and discussion

A total of 23 polymorphic SNP markers were identified within the *FTO* gene in Slovenian Simmental study population. Eight of these have previously been reported in databases, whereas 15 were newly identified by sequencing a panel of DNAs from Slovenian Simmental sire-bulls. Ten of the most informative SNPs were selected for genotyping of 31 sires and their 56 half-sibs.

Genotype frequencies of the chosen *FTO* SNP polymorphisms in half-sibs are shown in Figure 1. The highest heterozy-

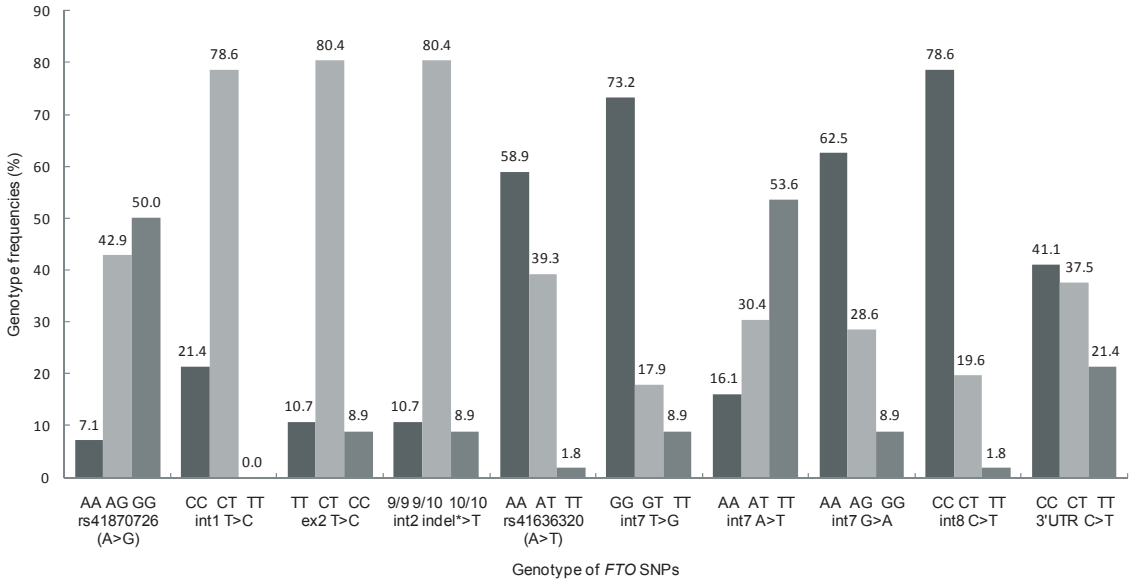

Figure 1. Genotype frequencies in half-sibs for 10 most informative *FTO* SNPs

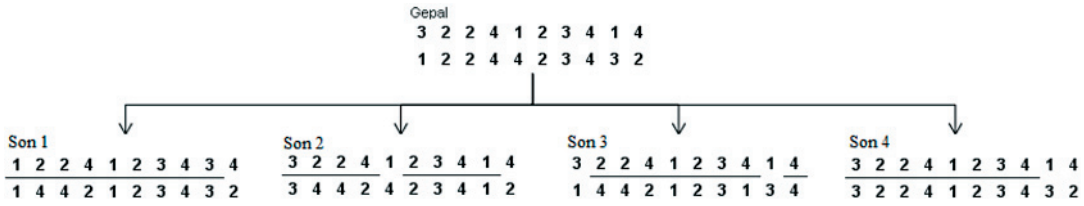

Figure 2. An example of paternity verification between a sire and his four half-sibs using ATLAS program (1: adenine (A), 2: cytosine (C), 3: guanine (G), 4: thymine (T)).

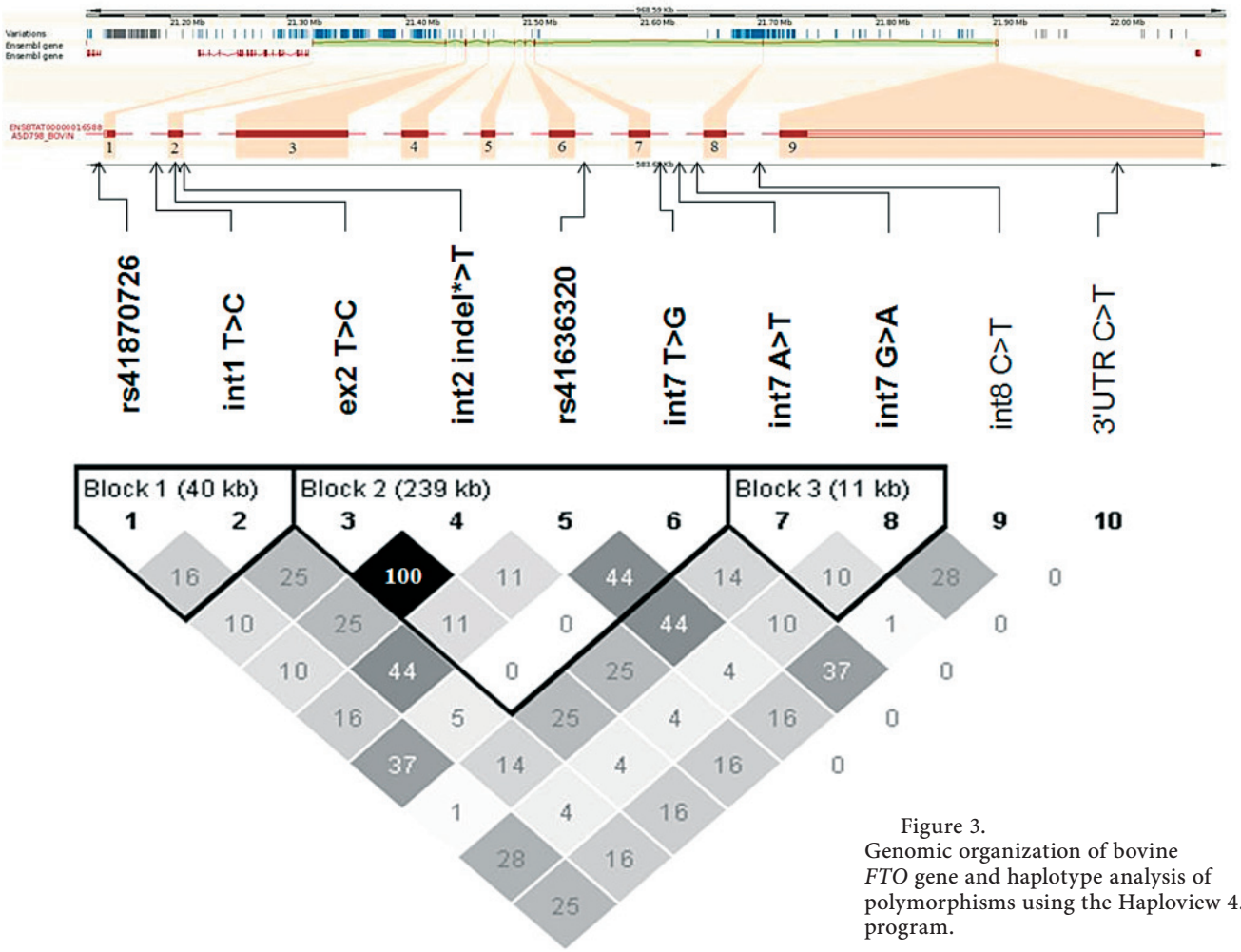

Figure 3.
Genomic organization of bovine *FTO* gene and haplotype analysis of polymorphisms using the Haploview 4.2 program.

gosity was observed for SNP "ex2 T>C" (exon 2) and SNP "int2 indel*>T" (intron 2). The lowest heterozygosity was observed for SNP "int7 T>G" (intron 7).

Parentage as recorded at testing stations was checked against genotypes using ATLAS program. The analysis was performed among half-sib groups and their sire. An example of matching in 10 markers between a sire and his four half-sibs is shown in Figure 2.

In the follow up analysis, only data from half-sib families with complete correspondence between the pedigree records from testing stations and genotype analysis were used.

Haploview uses a two marker expectation-maximization algorithm (ignoring missing data) to estimate the maximum-likelihood values of the four gamete frequencies, from which the D', LOD and r^2 calculations derive. Conformance with Hardy–Weinberg equilibrium is computed using an exact test (Barrett et al., 2005).

Genomic organization of the bovine *FTO* gene and pair wise linkage disequilibrium relationship for ten polymorphisms, based on r^2 measurements is shown in Figure 3. The bovine gene *FTO* contains nine exons and eight introns. A total of three haplotype blocks among ten SNPs were identified in the population

Table 1. Association analysis between the SNP genotypes "ex2 T>C" and carcass related traits with significant effect in the Slovenian Simmental cattle (standard deviation shown in parentheses).

Trait	Genotype			*P*-value
	CC	CT	TT	
Live weight at slaughter (kg)	601.20 (34.32)	651.38 (43.22)	658.00 (19.49)	0.030
Carcass weight (kg)	338.60 (24.62)	362.80 (27.23)	369.50 (10.89)	0.038
Lean weight (kg)	114.35 (8.69)	122.04 (9.73)	125.55 (6.18)	0.048

of 31 sires and 56 half-sibs of Slovenian Simmental cattle using the Haploview program. The SNPs "rs41870726" and "int1 T>C" were present in block 1. The SNPs "ex2 T>C", "int2 indel*>T", "rs41636320" and "int7 T>G" were in block 2 and the SNPs "int7 A>T" and "int7 G>A" were in block 3.

Statistical analyses were done for all carcass traits. Because SNPs "ex2 T>C" and "int2 indel*>T" have no historical recombination events in the population (r^2= 1), nine tagging SNPs, rs41870726, int1 T>C, ex2 T>C, rs41636320, int7 T>G, int7 A>T, int7 G>A, int8 C>T and 3'UTR C>T, were used in the association analysis. The SNP "ex2 T>C" showed a significant effect on live weight at slaughter (*P*= 0.03), carcass weight (*P*= 0.038), and lean weight (*P*= 0.048) (Table 1). As expected these traits are strongly correlated (r= 0.83 - 0.93). Further analyses are required to confirm the results in a larger sample size of these SNPs and newly identified closely linked SNPs.

Conclusions

Associations between the *FTO* gene polymorphisms and fatness have already been reported in human (Loos and Bouchard, 2008; Fischer et al., 2009), mouse (Peters et al., 2002), pigs (Fontanesi et al., 2009, 2010; Zhang et al., 2009) and cattle (Wei et al., 2011). No association was found in our experiment between *FTO* polymorphisms and fatness traits in Slovenian Simmental cattle; however, association between the *FTO* gene polymorphisms and growth/carcass traits was confirmed. Since the lack of association between *FTO* gene polymorphisms with the fatness traits could be attributed to the small sample size, further research should be done on a larger sample size using denser array of SNP markers and haplotype analysis instead of single marker analysis. However, since the main fatness trait parameter used here was "carcass fat percentage", which mainly includes subcutaneous fat, the statistically significant effect of *FTO* SNP with the lean and carcass weight (Table 1) could be due to *FTO* affecting other fat depots not measured in our study such as visceral fat (mesenterial, perirenal, retroperitoneal, gonadal depots) or intramuscular fat. The results of this study contribute to the development of molecular markers in cattle selection programs allowing more effective, marker assisted selection for carcass traits in livestock animals.

References

Barrett J.C., Fry B., Maller J., Daly M.J. (2005). Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263-265.

Fischer J., Koch L., Emmerling C., Vierkotten J., Peters T., Brüning J.C., Brüther U. (2009). Inactivation of the *FTO* gene protects from obesity. Nature, 458(7240): 894-898.

Fontanesi L., Scotti E., Buttazzoni L., Davoli R., Russo V. (2009). The porcine fat mass and obesity associated (*FTO*) gene is associated with fat deposition in Italian Duroc pigs. Animal Genetics. 40(1): 90-93.

Fontanesi L., Scotti E., Buttazzoni L., Dall'Olio S., Bagnato A., Fiego D.P.L., Davoli R., Russo V. (2010). Confirmed association between a single nucleotide polymorphism in the *FTO* gene and obesity-related traits in heavy pigs. Molecular Biology Reports. 37(1): 461-466.

Loos R.J.F. and Bouchard C. (2008). *FTO*: the first gene contributing to common forms of human obesity. Obesity Reviews, 9: 246–250.

Pérez - Enciso E., García-Bernal P.G., Pérez-Enciso M. (2005). Atlas: A Java-Based Tool for Managing Genotypes. Journal of Heredity, 96(5): 623-625.

Peters T., Ausmeier K., Dildrop R., Rüther U. (2002). The mouse Fused toes (Ft) mutation is the result of a 1.6-Mb deletion including the entire Iroquois β gene cluster. Mammalian Genome, 13(4): 186-188.

SAS Inst. Inc. (2002). The SAS System for Windows, Release 9.1. Cary, NC, SAS Institute.

Wei S., Zan L., Ujan J.A., Wang H., Yang Y., Adoligbe C. (2011). Novel polymorphism of the bovine fat mass and obesity-associated (*FTO*) gene are related to backfat thickness and longissimus muscle area in five Chinese native cattle breeds. African Journal of Biotechnology. 10(15): 2820-2824.

Zhang L.F., Miao X.T., Hua X.C., Jiang X.L., Lu Y.P., Xu N.Y. (2009). Polymorphism in 5' regulatory region of the porcine fat mass and obesity associated (FTO) gene is associated with intramuscular fat content in a Jinhua x Pietrain F2 reference population. Journal of Animal and Veterinary Advances 8(11): 2329–2334.

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