Association between polymorphism in the FTO gene and growth and carcass traits in pig crosses

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Abstract

Background: Independent studies have shown that several single nucleotide polymorphisms (SNP) in the human FTO (fat mass and obesity associated) gene are associated with obesity. SNP have also been identified in the pig FTO gene, among which some are associated with selected fat-deposition traits in F2 crosses and commercial populations. In this study, using both commercial pig populations and an experimental Meishan × Pietrain F2 population, we have investigated the association between one FTO SNP and several growth and carcass traits.

Association analyses were performed with the FTO polymorphism either alone or in combination with polymorphisms in flanking loci.

Methods: SNP (FM244720:g.400C>G) in exon 3 of porcine FTO was genotyped by PCR-RFLP and tested for associations with some growth, carcass and fat-related traits. Proportions of genetic variance of four pig chromosome 6 genes (FTO, RYR1, LIPE and TGFB1) on selected traits were evaluated using single- and multi-locus models.

Results: Linkage analysis placed FTO on the p arm of pig chromosome 6, approximately 22 cM from RYR1. In the commercial populations, allele C of the FTO SNP was significantly associated with back fat depth and allele G with muscling traits. In the Meishan × Pietrain F2 pigs, heterozygotes with allele C from the Pietrain sows and allele G from the Meishan boar were more significantly associated with fat-related traits compared to homozygotes with allele G from the Pietrain and allele G from the Meishan breed. In single- and multi-locus models, genes RYR1, TGFB1 and FTO showed high associations. The contribution in genetic variance from the polymorphism in the FTO gene was highest for back fat depth, meat area on the musculus longissimus lumborum et thoracis tissues and metabolite glucose-6-phosphate dehydrogenase.

Conclusions: Our results show that in pig, FTO influences back fat depth in the commercial populations, while in the Meishan × Pietrain F2 pigs with a CG genotype, heterosis occurs for several fat-related traits.

Background

The fat mass and obesity associated (FTO) gene encodes a protein of unknown function in an unknown pathway. The abbreviation FTO originates from a study in mice with a fused toes (Ft) phenotype and other abnormalities resulting from a 1.6 Mb deleted region on mouse chromosome 8 that includes this gene. It has been suggested that mouse Fto could be involved in programmed cell death, limb development, craniofacial development and the control of left-right asymmetry [1-3]. Fto mRNA is expressed in all murine tissues examined, with the highest signal detected in the brain and more specifically in the hypothalamus, which plays a key role in the control of energy balance [4].

The human FTO gene is more than 400 kb long and is located on human chromosome 16q12.2 http://www.ensembl.org/Homo_sapiens. Sequence analysis has shown that the encoded protein, FTO, shares amino-acid motifs with the Fe(II)- and 2-oxoglutarate-dependent oxygenases, which are involved in various processes, including DNA repair, fatty acid metabolism and posttranslational modifications [4,5]. Wu et al. [6] have reported that FTO is a transcriptional co-activator, which facilitates transcription from unmethylated and methylation-inhibited gene promoters and enhances C/EBPs binding to DNA, and that it may play a role in the regulation of adiposity.

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Studies on obesity-associated genes in humans [7-9] have identified several single nucleotide polymorphisms (SNP) in FTO intron 1 that are associated with obesity. Additional studies have confirmed these results e.g. [10,11] but not for all ethnic groups (see reviews [12,13]).

The porcine FTO gene has been mapped to the p arm of chromosome 6 or SSC6 (SSC for Sus scrofa) by radiation hybrid mapping and linkage analysis [14-16]. Whilst the porcine FTO gene is annotated in the Sscrofa9 draft genome assembly (see: http://www.ensembl.org/Sus_scrofa/), unfortunately this sequence is missing from the assembly Sscrofa10.2 on which the pig genome sequence paper will be based ([17]; A. L. Archibald, personal communication; http://www.ncbi.nlm.nih.gov/gene/100127165). Several polymorphisms have been detected in porcine FTO [15,18,19] and used in association analyses with selected growth and fat-related traits. Fan et al. [18] have reported the existence of two SNP in this gene, one in intron 1 and one in exon 3 (a synonymous mutation) that are significantly associated (P < 0.01) with average daily gain on test and total lipid percentage in muscle, respectively, in a Berkshire × Yorkshire F2 population. Fontanesi et al. [15] have identified another polymorphism in intron 4 associated with intermuscular fat deposition in the Duroc breed and with feed conversion rate in Italian Large White pigs. These results have been confirmed in subsequent analyses on Italian Duroc (P < 0.01) and commercial pig populations (P < 0.05; [16]). Finally, a significant association (P < 0.05) has been found between an SNP located in the 5’ flanking region of FTO and intramuscular fat content in the Jinhua × Pietrain F2 reference population [19].

Our study was primarily aimed at investigating the association between an SNP in porcine FTO exon 3 and growth, carcass and fat-related traits in Meishan × Pietrain F2 and commercial pig populations. We also estimated the proportions of variance of four linked genes on SSC6 (FTO, RYR1, LIPE and TGFBI) on selected traits in the Meishan × Pietrain F2 population using single- and multi-locus models.

In pig, the RYR1 (ryanodine receptor 1) gene has a role in stress resistance and also affects carcass and meat quality traits. Homozygotes carrying the 1843 T allele (stress sensitive) have heavier, shorter and leaner carcasses than homozygotes carrying the 1843 C allele (stress resistant). Allele 1843 T has a near additive effect on lean content, killing-out percentage and carcass length [20]. The protein encoded by the LIPE (hormone-sensitive lipase) gene catalyzes the intracellular hydrolysis of triacylglycerols and cholesteryl esters, and is involved in regulating body fat, steroidogenesis and insulin secretion. Population and genetic studies in humans have suggested that genetic variability in LIPE could be involved in lipid metabolism and risk for obesity and type 2 diabetes [21]. The TGFBI gene encodes a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. Rosmond et al. [22] and Long et al. [23] have reported associations between an SNP in TGFBI and variation in obesity phenotype, suggesting a possible effect of the gene on obesity.

**Methods**

**Animals and performance traits for association analysis**

DNA sampled from unrelated animals of eight pig breeds (Czech Large White, 28; Czech Landrace, 38; Czech Meat Pig, 15; Pietrain, 23; Black Pied Prestice, 17; Hampshire, 9; Duroc, 30; Meishan, 22) and wild boar (10) was used to estimate the frequencies of FTO alleles.

Linkage mapping was performed on the Hohenheim Meishan × Pietrain three-generation pedigree [24] using the CRI-MAP software, version 2.4 [25]. An association analysis between FTO genotypes and production traits was performed on two populations: (i) F2 animals from the Hohenheim Meishan × Pietrain pedigree (described in detail by [24], and (ii) commercial pigs, mostly originating from crosses (Table 1).

Briefly, the Meishan × Pietrain pedigree was obtained from one Meishan boar and eight Pietrain sows as founder animals and consisted of 22 F1 animals (three boars and 19 sows) and 316 F2 animals raised in uniform environmental conditions at an experimental station at the University of Hohenheim (Germany). Pigs were slaughtered at 210 days of age (SD ± 7.3 days) and had an average live weight of 96.1 kg. One hundred and forty-eight traits were recorded including eight growth and fattening traits, 11 fat deposition traits, eight muscling traits, 14 meat quality and stress resistance traits, and many other specific traits (muscle fibres, metabolites and protein content of the muscle, enzyme activities, fat cell number and volume, etc.).

The 469 commercial pigs were maintained in air-conditioned stables of the Test Station in Ploskov of the Czech University of Life Sciences in Prague under standard conditions. At the start of the test, pigs had a live weight of 25-30 kg and were slaughtered at a target

| Group | Pig crosses | n |
|-------|-------------|---|
| 1     | PNx(CZLxCZLW) | 156 |
| 2     | CZLW         | 22  |
| 3     | CZLWxCZL    | 23  |
| 4     | (CZLWxPNx)(CZLxCZLW) | 36 |
| 5     | PICx(CZLxCZLW) | 36  |
| 6     | FHxPIC      | 60  |
| 7     | (DxCZLW)x(CZLxCZLW) | 72 |
| 8     | PNxCZLx(CZLxCZLW) | 64 |

PN Pietrain; CZL Czech Landrace; CZLW Czech Large White; PIC Pig Improvement Company; FH French hybrids; D Duroc
weight of 113 kg (SD ± 10.5). They were fed a commercial diet (wheat, barley, soybean meal and a premix of supplements of essential elements) *ad libitum* and dosed feed. The following performance traits were recorded: average back fat depth (calculated from back fat depth 1 i.e. above the first thoracic vertebra, back fat depth 2 i.e. above the last thoracic vertebra, back fat depth 3 i.e. above the first lumbar vertebra); intramuscular fat in *m. longissimus lumborum et thoracis* (m.LLt) and fat content of belly 2 (for information on belly 2, see [26]; the method used a gravimetric determination according to the Czech norm ISO 1443); fat and muscle depth and lean meat (measured with Fat-O-Meter); feed conversion. The average daily gain during the test was calculated as the ratio between live weight gained from the beginning to the end of the test and corresponding days.

Due to practical and organizational reasons, individual performances were not recorded for all the animals, which explain the difference in numbers of pigs analysed for the different traits.

All pigs were slaughtered according to protocols for certified European (Germany) or national (Czech Republic) slaughterhouses under the control of an independent veterinarian.

**PCR amplification and genotyping of FTO, RYR1, LIPE and TGFB1**

To identify polymorphisms in the porcine *FTO* gene, polymerase chain reaction (PCR) primers were designed based on the porcine cDNA *FTO* sequence corresponding to exon 3 of human *FTO* [Ensembl:ENSG00000140718]. The SNP reported in *FTO* intron 4 by [15] was also genotyped in commercial pig breeds and crosses. Using single- and multi-locus models, the proportions of variance of three linked genes (*RYRI*, *LIPE* and *TGFB1*) were estimated in the Meishan × Pietrain pedigree. Additional information on PCR-RFLP conditions for *FTO*, *RYRI*, *LIPE* and *TGFB1* [see Additional file 1]. FTO PCR products obtained from wild boar, Pietrain and Meishan DNA were sequenced (ABI PRISM 3130 Sequencer; Applied Biosystem, Foster City, CA, USA) and the sequences were deposited in the public database [EMBL: FM244719 - FM244721].

**Association analysis**

Associations between *FTO* SNP FM244720g.400C>G and quantitative traits were analysed with SAS® (SAS® Inst. Inc., Cary, NC) using SAS® 8.2 Version and the results of the Type III sum of squares calculation for the Meishan × Pietrain pedigree, and the GLM procedure (Type IV) of SAS 9.1 for the commercial populations.

(i) For the statistical evaluation of the Meishan × Pietrain F₂ family, the following model A was used:

\[
Y_{ijklm} = \mu + FTO + month + sex_i + litterno_j + b(s_age)_{ijklm} + S_{age}^{m} + e_{ijklm} \quad (A)
\]

where *Y*_{ijklm} = value of a trait for animal *n*; *μ* = estimated mean value of a trait; *FTO* = the effect of genotype class for *FTO* (CmCm, CmGm, CmGp, GmGp, GpGp) with CmCm being: homozygotes with both alleles *C* of grandmaternal origin; CmGm: heterozygotes with alleles *CG* both of grandmaternal origin; CmGp: heterozygotes with alleles *CG* inherited from both grandparents; GmGp: homozygotes with alleles *GG* inherited from both grandparents; GpGp: homozygotes with both alleles *G* of grandpaternal origin (the genotype classes for *FTO* were created using the procedure Chrompic from the CRI-MAP package [25]. The maternal and paternal phase information for all animals is marked with signs for grandmaternal or grandpaternal origin for all loci included in the map calculation); *month* = the effect of two-month class created from slaughter date (*j* = 1-6); *sex* = gender and type class for *FTO*; *litterno* = litter number (*l* = 1, 2); *b* = linear regression value; *s_age*_{ijklm} = age at slaughter of animal *m*; *S_age* = estimated average of the age at slaughter; *e*_{ijklm} = random residual.

Models including different genes (*FTO*, as well as *RYRI*, *LIPE* and *TGFB1* - for more information, see [27]) with their genotype classes in the initial model (starting model including all non genetic independent variables) were tested stepwise to find the highest proportion of variance reduction (VR). In cases for which inclusion of another locus in the model did not result in any increase of VR (%), only a single-locus model was retained (e.g. for meat area on *m.LLt*). Genes that showed no significant association remained in a combined model if their contribution to the total VR reached about 2%.

(ii) For analyses on commercial pigs, the model included the *FTO* genotype with/without the *RYRI* CT genotype, a crossbred combination, gender and type of diet as fixed factors, and average of age at slaughter as a regression coefficient. The statistical model B was:

\[
Y_{ijklmn} = \mu + FTO + RYRI + cross + sex_i + diet_m + b(s_age)_{ijklmn} + S_{age}^{m} + e_{ijklmn} \quad (B)
\]

where: *Y*_{ijklmn} = value of a trait for animal *n*; *μ* = estimated mean value of a trait; *FTO* = the effect of *FTO* genotype (*i* = 1, 2, 3); *RYRI* = the effect of *RYRI* genotype (*i* = 1, 2); *cross* = the effect of crossbred combination (*k* = 1, 2, 3, 4, 5, 6, 7, 8); *sex* = gender and type of diet as fixed factors, and average of age at slaughter as a regression coefficient. The statistical model B did not include the effect of gene *RYRI* and ** means that the separate association analysis of animals with genotype CC at gene *RYRI* did not include the effect of gene *RYRI* and ** means that the separate association analysis of crossbred combination PNx
and the probable order (Kosambi cM; sex averaged) was: 1.4 - EAH
- 3 . 4 - S0087 - 1 5 . 7 - RYR1 - 1 9 - LIPE - 0 . 9 - TGBF1 - 1 . 2 - A1BG - 1 4 - EAH - 3 . 4 - SKI - 1 5 . 8 - FABP3 - 3 . 6 - ID3 - 1 4 . 2 - S0146 - 9 . 8 - S0003 - 1 6 . 3 - SW824 - 5 0 . 1 - P3 - 2 0 . 0 - EAO.

This assignment agrees with earlier mapping results [14-16,19] and the FTO locus is most probably situated in the proximal part of the p arm since microsatellites SW1057 and S0087 have been mapped in this region [29,30]. In both the Sscr09a and Sscr010a draft assemblies of the pig genome, the order is FTO - RYR1 - TGBF1 - LIPE - A1BG, which is consistent with that of the human orthologs on chromosome 19. In contrast, the order of TGBF1 and LIPE is reversed in our linkage map. This inconsistency could be due to the short distance between the two genes (~50 kb) and the difficulty of discriminating between alternative orders by linkage analysis with limited numbers of informative meioses. However, the order of tightly linked loci has no influence on the results of genotype effects in single- and multi-locus analyses.

**Table 2 Allele frequencies of FTO SNP FM244720: g.400C>G in eight pig breeds and the wild boar**

| Breed                  | n  | C   | G   |
|------------------------|----|-----|-----|
| Czech Large White      | 28 | 0.45| 0.55|
| Czech Landrace         | 36 | 0.66| 0.34|
| Czech Meat Pig         | 15 | 0.80| 0.20|
| Pietrain               | 23 | 0.65| 0.35|
| Black Pied Prestice    | 17 | 0.47| 0.53|
| Hampshire              | 9  | 0.94| 0.06|
| Duoc                   | 30 | 0.43| 0.57|
| Meishan                | 22 | 0.02| 0.98|
| Wild boar              | 10 | 0.10| 0.90|

1 Included are pigs that were genotyped for both SNP FM244720g.400C>G and SNP AM931150g.276T>G [15]; both SNP were in complete linkage disequilibrium (allele g.400 C with g.276 T, and g.400 G with g.276 G), with the exception of one Meishan pig, which was heterozygous g.400 CG and homozygous g.276 GG (repeatedly tested).

**Results and discussion**

**Polymorphism, allele frequencies and linkage analysis of the FTO gene**

Sequence analyses identified two SNP, FM244721: g.307C>T and FM244720-g400C>G that were detected by PCR - restriction fragment length polymorphism (RFLP) using conditions presented in Additional file 1 [see Additional file 1]. Both SNP are synonymous mutations localized in exon 3. SNP g.400C>G is identical to SNP c.594C>G as reported by Fan et al. [18].

Pigs of different breeds were analysed for SNP FM244720:g.400C>G (as well as for intron 4 SNP AM931150:g.276T>G [15]; both SNP were in complete linkage disequilibrium (allele g.400 C with g.276 T, and g.400 G with g.276 G), with the exception of one Meishan pig, which was heterozygous g.276 GG and SNP AM931150:g.276T>G [15]).

Table 3 attention should be paid to the differences between genotype classes according to the genotype at RYR1 of the grandparents and parents of the F2 animals combined with the FTO flanking phase information made it possible to identify the grandparental origin of alleles in the F2 animals. The results of the association analysis for the genotype classes according to the grandparental alleles’ origin are presented in Table 3. The genotype at RYR1 was not included as a co-factor in these analyses. If it was included as another factor in the model, the differences between estimates in the one-locus and two-locus models remained (data not shown). We consider that the recombination frequency (0.17) observed between FTO and RYR1 in this population makes it possible to detect specific associations between FTO and the traits analysed, mostly or totally independent from RYR1.

In Table 3 attention should be paid to the FTO genotype classes CmGp and GmGp. In both cases, allele Gp is of grandpaternal origin, and the two classes differ by Cm and Gm alleles originating from granddams (Pietrain). Table 3 shows that many fat-related traits (abdominal fat weight, back fat depth on m.l.l.t. average back fat depth, shoulder fat depth, fat depth at 10th rib, loin fat depth and fat area on m.l.l.t.) as well as feed consumption in the two classes differ significantly, with higher values for genotype CmGp (i.e. with the C allele coming from Pietrain). The values for fat-related traits in this class are also significantly higher than those in classes CmCm and GmGm (i.e. with both alleles of grandmaternal origin).

The high positive effects in the CmGp class most probably represent a heterosis effect, often overdominant in comparison with other classes. However, it may not necessarily be the effect of the FTO genotype itself, since extensive linkage disequilibrium in this population means that in the F2 generation extensive haplotypes are inherited intact from the Pietrain and Meishan grandparents in the observed chromosome region, and thus other...
linked genes may be responsible for the heterosis effect on the fat traits. The heterosis effect was apparent when allele Cm (or an allele in linkage disequilibrium with this) was present. However, the chromosome segments from both breeds led to heterosis, but if both FTO alleles (i.e. chromosome segments) come from the Pietrain breed (genotype CmGm), no heterosis occurred. This effect may be the result of the genetic background of the founders and consequently F2 pigs, and may be different in other populations.

Proportions of trait variance associated with the FTO gene and three distally located genes (RYR1, LIPE and TGBF1), in single- and multi-locus models for several traits are summarised in Additional file 2 [see Additional file 2]. Three genes (RYR1, TGBF1 and FTO) show strong associations in single- and multi-locus models. The covariance components (interaction effects) of RYR1 and TGBF1 are often very low and indicate that combining both loci does not increase the declared variance proportion. These genes are also similarly associated with some traits, e.g. fat cuts, lean cuts and meat quality traits. The most significant variance proportion is observed for FTO in combination with RYR1, LIPE and TGFB1 in the multi-locus model for back fat depth, whereas for meat area on m.lLt, and the metabolic trait G6P in fat tissue, it is highest in the single- and multi-locus models, respectively. TGFB1 shows the most significant gene effects on weight of ham relative to half carcass weight in both model types.

Commercial populations

Commercial crossbred pigs were evaluated in two analyses: (i) with the crossbred combination PNxCZLxCZLW alone and (ii) with the combined populations (Table 1). All animals were genotyped for SNP g.400C>G and SNP g.276T>G (see Additional file 1). There was complete linkage disequilibrium between the two loci, giving two haplotypes: g.400 C-g.276 T and g.400 G-g.276 G. We used SNP g.400C>G for the analysis.

Crossbred combination PNxCZLxCZLW

All three FTO SNP g.400C>G genotypes were observed. Using model (B) with the genotype at RYR1 included, FTO SNP g.400C>G was significantly associated with average back fat depth (P < 0.0001), fat in the belly 2 (P = 0.002), muscle depth (P = 0.02) and average daily gain (P = 0.02). The highest values for fat traits were observed in animals with genotype CC and for muscle depth in those with genotype GG (Table 4). If only the genotype CC at RYR1 was used in the FTO association analysis, the results were very similar (data not shown). For intramuscular fat in ham, the number of pigs analysed was rather low (Table 4), so the results are inconclusive.

The joint population

All three FTO SNP g.400C>G genotypes were also observed in the combined commercial pig populations. Using model (B), significant associations were found between the FTO SNP and average daily gain (P = 0.02), average back fat depth (P = 0.02), muscle traits (lean cuts, P = 0.013; muscle depth, P = 0.007) and feed conversion (P = 0.003). In this case too, higher values for back fat depth and fat depth were observed in animals with genotype CC and CG. For the muscling traits, genotype GG was the most favourable (Table 4). Significant associations were also found for fat in the belly 2 and intramuscular fat

### Table 3 Association analysis between FTO FM244720:g.400C>G and selected traits in Meishan × Pietrain F2 pigs

| Trait                        | CmCm1 ± SE | CmGm ± SE | CmGp ± SE | GmGp ± SE | GpGp ± SE | P   |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----|
| Average daily gain          |           |           |           |           |           |     |
| (110-210 days of age; g/day) | 579 ± 24.75 | 605 ± 19.87 | 598 ± 12.98 | 583 ± 17.38 | 579 ± 15.77 | 0.31 |
| Abdominal fat weight (kg)   | 0.67 ± 0.07a | 0.72 ± 0.05c | 0.97 ± 0.04b | 0.85 ± 0.05cd | 0.86 ± 0.04d | <0.0001 |
| Back fat depth on m.lLt.     | 18.94 ± 1.09a | 19.0 ± 0.87b | 23.58 ± 0.57b | 21.17 ± 0.76bc | 22.74 ± 0.69bc | <0.0001 |
| at 13th-14th rib (mm)       |           |           |           |           |           |     |
| Average back fat depth (mm) | 25.69 ± 1.08c | 25.09 ± 0.87a | 29.91 ± 0.57b | 26.93 ± 0.76bc | 27.98 ± 0.69bc | <0.0001 |
| Shoulder fat depth (mm)     | 33.49 ± 1.29a | 34.27 ± 1.03b | 38.64 ± 0.68b | 35.99 ± 0.90bc | 37.40 ± 0.82bc | 0.0004 |
| at 10th rib (mm)            |           |           |           |           |           |     |
| Fat depth at 10th rib (mm)  | 22.27 ± 1.03bc | 20.67 ± 0.82a | 25.30 ± 0.54b | 22.23 ± 0.72bc | 23.37 ± 0.65bc | <0.0001 |
| Loin fat depth (mm)         | 21.32 ± 1.27ac | 20.34 ± 1.02a | 25.80 ± 1.06b | 22.57 ± 0.89bc | 23.17 ± 0.81bc | <0.0001 |
| Fat area on m.lLt. at 13th-14th rib (cm³) | 18.43 ± 0.98a | 19.28 ± 0.78bc | 22.30 ± 0.51b | 20.01 ± 0.69bc | 21.18 ± 0.62bc | 0.0004 |
| Lean cuts (%)               | 47.57 ± 0.67a | 48.10 ± 0.57a | 44.68 ± 0.35b | 45.35 ± 0.47b | 44.20 ± 0.43b | <0.0001 |
| Feed consumption (110-210 days of age; kg) | 209 ± 7.06a | 214 ± 5.67a | 230 ± 3.70b | 218 ± 4.96b | 223 ± 4.50ab | 0.006 |
| Feed conversion (kg/kg)     | 3.73 ± 0.16ab | 3.60 ± 0.13a | 3.94 ± 0.09b | 3.82 ± 0.11de | 4.05 ± 0.10b | 0.008 |

Model (A) was used; for each genotype the LS mean ± SE are given

1 m: allele of grandmaternal origin (Pietrain); p: allele of grandpaternal origin (Meishan)

a,b,c: values with different letters in the rows differ significantly (P < 0.05)

m.l Lt: *musculus longissimus lumbarorum et thorasis*

P: most significant probability of t test between two genotype classes
Table 4 Association analysis between FTO SNP FM244720g.400C>G and carcass traits in crossbred pigs

| Trait                  | PNx(CZLxCZLW)1 | Joint population1 |
|------------------------|-----------------|-------------------|
|                        | CC ± SE(n)      | CG ± SE(n)      |
|                        | GG ± SE(n)      | P                | CC ± SE(n)      | CG ± SE(n)      | GG ± SE(n)      | P                |
| Average daily gain (g/day) | 801 ± 16.53a     | 825 ± 13.59a     | 0.02             | 873 ± 12.73     | 869 ± 10.92a     | 895 ± 12.35a     | 0.02             |
|                        | (64)            | (71)             |                  | (148)           | (218)            | (99)             |                  |
| Fat in the belly (%)   | 39.07 ± 2.68a   | 30.73 ± 1.72a   | 0.002            | 33.25 ± 1.49a   | 30.81 ± 1.13b    | 33.46 ± 1.25a    | 0.03             |
|                        | (14)            | (25)             |                  | (50)            | (99)             | (46)             |                  |
| Intramuscular fat (%)  | 1.58 ± 0.16 (14) | 1.47 ± 0.11 (27) | 0.46             | 1.67 ± 0.13 (38) | 1.68 ± 0.107 (78) | 1.93 ± 0.12 (28) | 0.04             |
|                        | 2.18 ± 0.52a     | 3.52 ± 0.30a     | 0.02             | 3.01 ± 0.39 (24) | 3.42 ± 0.27 (62) | 3.42 ± 0.31 (30) | 0.29             |
| Average back fat depth (mm) | 27.96 ± 0.98a   | 27.01 ± 0.69a   | < 0.0001         | 26.06 ± 0.65a   | 25.94 ± 0.54a   | 24.68 ± 0.62a   | 0.02             |
|                        | (30)           | (45)             |                  | (115)           | (188)           | (93)             |                  |
| Lean cuts (%)         | 15.76 ± 0.59 (64) | 15.30 ± 0.48 (71) | 0.41             | 17.78 ± 0.59 (107) | 17.85 ± 0.50 (149) | 16.76 ± 0.59 (67) | 0.05             |
| Muscle depth (FOM; mm)| 56.80 ± 0.46 (64) | 57.32 ± 0.38 (71) | 0.11             | 54.52 ± 0.45 (106) | 54.72 ± 0.38 (147) | 55.72 ± 0.45 (67) | 0.013            |
| Feed conversion (kg/kg)| 64.44 ± 1.15a   | 66.92 ± 0.95b   | 0.02             | 58.67 ± 1.00 (107) | 59.80 ± 0.84 (149) | 61.51 ± 1.00 (67) | 0.007            |

Model (B) RYR1 included was used; for each genotype the LS mean ± SE are given;

1 see Table 1; a, b, A, B - values with different letters in the rows per animal group differ significantly (P < 0.05; P < 0.01; P < 0.001, respectively); m.l.l.t: m. longissimus lumborum et thoracis; P - most significant probability of t test between two genotype classes

in the m.l.l.t. Values for average daily gain and feed conversion were higher in pigs with genotype GG (Table 4). If only the CC genotype at RYR1 was used in the FTO association analysis, the results were similar (data not shown).

Clearly, the quantitative traits studied here are under polygenic control. Tests for associations with a single gene can reveal both the effects on genetic variance of the genotypes at the gene of interest and at the genes in linkage disequilibrium with the gene of interest. However, if the animals in the population analysed have similar genetic backgrounds (e.g. pure bred populations) and are subject to standardized environmental influences. These requirements are not always met, mainly for economic reasons. In our experiment, we used the commercial crossbred population PNx(CZLxCZLW) separately, in which the heterogeneity in genetic background is not so high as in the population composed of several crossbreds. However, in both this crossbred population and in the joint population composed of several crossbreds, the FTO SNP g.400C>G genotypes CC and CG were significantly associated with high values for average back fat depth. A similar situation was observed for associations between genotype GG and high values for muscle depth.

Effect of genetic background can be observed on F2 pigs from the Meishan × Pietrain family, in which the highest effects on fat-related traits, apparently overdominance, are detected in heterozygotes with allele C inherited from the Pietrain and allele G inherited from the Meishan breed.

Since extended Pietrain and Meishan haplotype blocks are inherited intact in the F2 pigs, it was important to include in the models the genotypes of linked genes, which are known to have effects on the traits of interest, especially RYR1. We examined the proportion of genetic variance explained by FTO and the linked loci. In this case too, highly significant associations between FTO polymorphisms and back fat depth, and also meat area m.l.l.t. and G6P were observed. It is hypothesized that other loci on different chromosomes may also be significantly involved in the determination of each trait, as additional proportions of genetic variance (examples are given in [27]).

Previous association analyses between pig FTO genotypes and fatness and growth rate traits have been performed with SNP from various regions of the gene [15,16,18,19]. Although different populations were used and different traits were recorded, in all cases, genotypes at some of the SNP were associated mostly with fatness traits. In addition, this study revealed a significant effect of SNP g.400C>G (which is the same as SNP c.594C>G of Fan et al. [18]) on average back fat depth, with genotypes CC and CG having higher effects.

Associations between the intrinsic SNP (g.276T>G) and back fat thickness have been reported for Italian Duroc and commercial pig populations [16] and agree with our results on commercial populations. Allele g.400 C (and g.276 T) is associated with higher values for fat deposition, while allele g.400 G (and g.276 G) with higher values for meat traits. However, Fan et al. [18] and Du et al. [31] did not find any association between polymorphisms in exon 3 and the 5′ untranslated region of porcine FTO, respectively, and average back fat thickness in the Berkshire × Yorkshire F2 population. Zhang et al. [19] have
reported an association between an SNP in the 5’ flanking region of FTO and intramuscular fat, but not with average back fat thickness, leaf fat and average daily gain in the Jinhua × Pietrain F2 population.

There is no evidence that any of the mutations identified in the pig FTO gene cause the observed variation in the traits of interest. However, some of these appear to be in linkage disequilibrium with loci influencing fatness traits. Additional research is required to identify causative mutations in the chromosomal region within and flanking the FTO gene.

In addition to association studies involving the FTO gene in pig, several genome scans have identified QTL on chromosome 6 in the region harbouring the RYR1 gene with effects on fatness traits (see http://www.animalgenome.org/QTLdb/pig.html). However, additional genes linked to RYR1 such as FTO may be involved in the phenotypic variation of these traits. For example, Mohrmann et al. [32], using F2 pigs from crosses between two commercial pig lines, found QTL located close to RYR1 for several fatness traits (side fat thickness at the 13/14th rib, external shoulder fat weight, belly weight and loin fat depth) even when adjusting for RYR1 genotypes. Paszek et al. [33] detected several fatness QTL (for leaf fat, fat thickness at the 10th rib and average fat thickness) on SSC6, in the interval between markers SW1841 - SW1067 in F2 pigs of Meishan × Yorkshire breeds, and Ovilo et al. [34] have reported a QTL for back fat thickness at the 1st rib in the interval between SW1057 and S0087 in an Iberian × Landrace intercross. In addition to the FTO gene, there are several other candidate genes of interest (LIPE, TGFBI, SKI, FABP3 and ID3) linked to the RYR1 locus [27,35], which may exert effects on the production traits, as shown in Additional file 2 [see Additional file 2].

Conclusions

SNP FM244720g.400C>G in pig FTO gene was analysed for associations with carcass and growth traits. FTO genotypes differ in their effects on several fat deposition traits and some carcass traits, both in Meishan × Pietrain F2 family and in commercial pig populations. In the commercial populations, genotypes CC and CG were associated with a higher average back fat depth, while genotype GG was associated with a higher value for some muscling traits. In F2 pigs of a Meishan × Pietrain family, heterozygotes with allele C from the Pietrain sows and allele G from the Meishan boar had significantly higher values for fat-related traits in comparison with homozygotes G (from Pietrain) and G (from Meishan). The significance of the FTO polymorphism for these traits was further supported by the single- and multi-locus models. Results from different association studies between FTO polymorphisms and production traits indicate that this gene (and its flanking regions) has a major role in the variation of fatness traits. Further research will be needed to identify candidate/causative mutations in this chromosomal region.

Additional material

Additional file 1: Information on PCR and RFLP conditions for SNP analysis in FTO and three additional genes (RYR1, LIPE and TGFBI).

The file shows PCR primers for FTO, RYR1, LIPE and TGFBI, amplicon lengths, MgCl2 concentrations, annealing temperatures, SNP types and restriction enzymes for RFLP analyses [15,36-39].

Additional file 2: Associations of the FTO polymorphism in combination with linked genes on SSC6 (RYR1, LIPE and TGFBI) with several traits analysed for the Meishan × Pietrain F2 pigs (different models used, including single- and multi-locus information).

Proportions of genetic variance of the four genes (FTO, RYR1, LIPE and TGFBI) on selected traits analysed by single- and multi-locus models are shown. (DOCX 28 kb)

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