Fine mapping of quantitative trait loci for meat color on *Sus scrofa* chromosome 6: Analysis of the swine *NUDT7* gene

M. Taniguchi,* T. Hayashi,* M. Nii,† T. Yamaguchi,† N. Fujishima-Kanaya,‡ T. Awata,* and S. Mikawa*2

*Animal Genome Research Unit, National Institute of Agrobiological Sciences, 2 Ikenodai, Tsukuba, Ibaraki 305-0901, Japan; †Livestock Research Institute, Tokushima Agriculture, Forestry, and Fisheries Technology Support Center, Izumidani, Kamiita, Tokushima 771-1310, Japan; and ‡Second Research Division, Society for Techno-Innovation of Agriculture, Forestry and Fisheries (STAFF) Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan

ABSTRACT: In the livestock industry, meat color has become important because consumer acceptance is subject to the appearance of the product in the marketplace. Our previous analyses of a whole genome QTL scan for various meat qualities using 2 F2 families from Japanese wild boar (known as a red meat) × Large White and from Duroc × Chinese Jinhua suggested that a meat color (heme content) QTL is located on SSC6. The objective of this study was to fine-map this SSC6 meat color QTL and subsequently investigate positional candidate genes for polymorphisms that may cause changes in meat color. Therefore, we conducted interval mapping on SSC6 using an additional 9 gene markers through combined analyses of the 2 F2 families of Japanese wild boar × Large White (353 progeny) and Duroc × Chinese Jinhua (204 progeny). Comparative analysis with humans, mice, and cattle suggested that there were 10 functional genes in the region. Among these genes, we suggested that a novel pig gene encoding a nudix (nucleoside diphosphate linked moiety X)-type motif 7 (*NUDT7*, a member of the nudix hydrolases) is a strong candidate for the QTL because the mouse Nudt7 is reported to hydrolyze succinyl-CoA, a substrate of the reaction limiting the rate of heme biosynthesis. We therefore determined the pig *NUDT7* gene sequence including the 5′ promoter region and explored genetic polymorphisms between Japanese wild boar and Large White. We identified 116 polymorphisms within the *NUDT7* CDS or in the 5′ region. None of the AA substitutions were associated with the meat color QTL; however, 3 polymorphisms were found in putative transcription factor recognition sites. We then investigated the differential expression of *NUDT7* in Japanese wild boar and Large White by allele-specific quantitative real-time PCR. The expression level of the Large White type allele was greater than that of the Japanese wild-boar-type allele. Consequently, we speculated that the difference in meat color between Japanese wild boar and Large White is caused partly by differential expression of this candidate gene. Upregulation of *NUDT7* expression in muscle may reduce succinyl-CoA content and thus reduce the level of heme biosynthesis.

Key words: gene expression, meat color, meat quality, quantitative trait loci, swine

INTRODUCTION

To strengthen consumer acceptance, meat quality improvement has recently become a top priority of the pork industry worldwide. In particular, meat color determines initial acceptance or rejection in the marketplace. Moreover, pig meat color is correlated with quality traits: lighter color in pork is associated with more drip loss, poorer water-holding capacity, and decreased pH (Malek et al., 2001). In Japan, wild boar is prized for its favorable characteristics, including its dark red appearance (Nii et al., 2005). However, identification of the genes associated with meat quality QTL is considered more difficult than identification of those for growth and carcass composition traits because many different environmental and genetic factors can influence the quality of fresh pork products (Sellier and...
Monin, 1994). In fact, Suzuki et al. (2005) estimated the heritability of pork color standard to be 0.18.

Previously, our QTL study of a Japanese wild boar × Large White F\textsubscript{2} population revealed that a QTL for hematin content in LM was detected near microsatellite SW1353 on SSC6 with 1% genome-wise significance (Nii et al., 2005). To the hematin content (mg/100 g of LM), the Japanese wild boar allele had 0.37 of additive and −0.15 of dominance effect. The QTL explained 9% of the phenotypic variance. Another study of the Duroc × Chinese Jinhua F\textsubscript{2} family also found the same QTL of the phenotypic variance. Another study of the Duroc × Chinese Jinhua F\textsubscript{2} family also found the same QTL of the phenotypic variance.

In this study, we aimed to fine-map the QTL on SSC6 to identify a meat color candidate gene. After the fine-mapping, we found that the \textit{NUDT7} gene was close to the most likely position of the QTL. The \textit{NUDT7} gene is associated with suppression of heme biosynthesis, so we sought to determine its structure and investigate genetic polymorphisms in the whole gene sequence. We also analyzed the expression level of the candidate gene in the LM and compared expression of the 2 \textit{NUDT7} alleles in heterozygotes using allele-specific quantitative (q) PCR.

**MATERIALS AND METHODS**

The animals used in the present study received humane care as described in the Guidelines for the Care and Use of Experimental Animals (National Institute of Agrobiological Sciences Care Committee, Japan).

**Animal Samples**

We used the same F\textsubscript{2} families for fine-mapping as we used for the initial whole-genome scan: Japanese wild boar × Large White (n = 353 F\textsubscript{2} progeny) and Duroc × Chinese Jinhua (n = 204 F\textsubscript{2} progeny; Mikawa et al., 2005; Nii et al., 2005). Nucleotide polymorphisms of \textit{NUDT7} were detected by comparison of its sequence between 1 Japanese wild boar (sire) and 3 Large White sows at the parental generation. For gene expression analysis, a backcross family was constructed with the same Japanese wild boar as used in the development of the F\textsubscript{2} population. Pigs were confirmed to possess the Japanese wild boar allele and backcrossed to Large White (Figure 1). A total of 9 animals at the Tokushima Prefectural Agriculture, Forestry and Fisheries Technology Support Center were slaughtered at age 82 d at approximately 30 kg of BW.

**Fine-Mapping of QTL for Meat Color**

**Phenotype.** Hematin content in LM was measured as meat color. Hematin content in LM was determined by the acidified acetone extraction method developed by Hornsey (1956) as we previously measured (Nii et al., 2005).

**Marker Development for Linkage Map and Interval Mapping.** Comparative chromosomal maps of pigs and humans (Rettenberger et al., 1995; Frönicke et al., 1996; Goureau et al., 1996) were utilized to identify pig genes on the SSC6 p-arm, corresponding to the HSA16 q-arm. Sequence tagged sites (STS) were developed from the pig sequences homologous to the human gene sequences on HSA16q. Swine bacterial artificial chromosome (BAC) clones were screened using pig STS in accordance with a previously established method (Suzuki et al., 2000). In brief, 2-step PCR was performed on BAC library superpools to select BAC clones containing the targeted gene. The BAC end sequences were determined and the SNP in these sequences were explored using genomic DNA from the Japanese wild boar, Large White, Duroc, and Chinese Jinhua breeds. Detected SNP were genotyped using SNaPshot Ready Reaction Mix (Applied Biosystems, Foster City, CA). Details of pig STS and BAC end sequences are given in Supplemental Table 1 (http://jas.fass.org/content/vol88/issue1/). For fine-mapping of the QTL, a linkage map using SNP markers was constructed with CRI-Map software (Green, 1992), and interval mapping was performed according to the method of Haley et al. (1994). The statistical model was based on a linear regression of the phenotype as we previously conducted (Nii et al., 2005). As nongenetic fixed effects, general mean, sex, and parity were taken into account in the analysis. The least squares method was applied to detect the QTL. Genome-wise significant thresholds for the 2 F\textsubscript{2} families were independently obtained with 5,000 repetitions of the permutation test for the phenotype. Then, interval mapping was performed again with the combined population of the 2 F\textsubscript{2} families on SSC6 by using common markers consisting of 6 microsatellites and 9 genes.

**Comparative Map Analysis for the QTL.** To analyze the swine genome structure between SW2406 and GABARAPL2 [GABA (A) receptor-associated protein-like 2], genes possibly included in the region were explored in the relevant regions of the human, mouse, and cattle genomes for which gene information was available in the National Center for Biotechnology Information (NCBI) GenBank database. These genes were mapped by linkage analysis with SNP developed using pig expressed sequence tags and whole-genome shotgun sequences (Supplemental Table 2; http://jas.fass.org/content/vol88/issue1/). In addition, pig BAC fingerprint contigs and their sequence information, available at Pre Ensemble pig map view (updated in December 2008 at http://pre.ensembl.org/Sus_scrofa_map/mapview?chr=6), were used to confirm the chromosomal locations of these genes in pigs, together with those of the other mammalian species.
Identification of a Candidate Gene

Cloning the Candidate Gene. A BAC clone containing a pig gene encoding nudix (nucleoside diphosphate linked moiety X)-type motif 7 (NUDT7) was cloned from the pig genomic BAC library by the screening procedure (Suzuki et al., 2000). The gene structure was determined by sequence analysis of the BAC clone. For analysis of the homology of NUDT7 in the pig with that in other mammalian species, protein sequences and gene coding sequences (CDS) of humans, mice, cattle, and rats were used, namely human NUDT7 (NM_001105663, NP_001099133), mouse Nudt7α (NM_024437, NP_077757.2), mouse Nudt7β (NM_024446, NP_077766.3), cattle NUDT7 (BC153854, AA153855), and rat Nudt7 (NM_001108450, NP_001101920). Sequence identity of the CDS and protein sequences across these mammalian species was analyzed using GENETYX-WIN version 4.0.2 (GENETYX, Tokyo, Japan). Multiple sequences were aligned using CLUSTAL W (Thompson et al., 1994). Transcription factor recognition sites in the 5' region of the gene were detected by TFSEARCH version 1.3 (http://www.cbrc.jp/research/db/TFSEARCH.html), using the criteria of a vertebrate-specific matrix and a threshold identity score of 85.0% (Heinemeyer et al., 1998). Repeat sequences were identified by the software program CENSOR (Kohany et al., 2006). Primer sequences used for PCR amplification and sequence detection for polymorphism analysis of the pig NUDT7 gene are listed in Supplemental Table 3 (http://jas.fass.org/content/vol88/issue1/).

Quantitative Real-Time PCR and Data Analysis. Total RNA samples from LM tissues were prepared from individuals from matings between fourth-generation pigs (Figure 1). The LM was sampled between the sixth and seventh thoracic vertebrae of each animal and immediately immersed in RNAlater (Ambion, Austin, TX). Total RNA extraction was performed using TRIzol Reagent (Invitrogen, Carlsbad, CA) followed by reverse transcription to synthesize single-strand cDNA using ReverTra Ace (TOYOBO, Osaka, Japan) in accordance with the manufacturers’ instructions. To quantify the abundance of mRNA of NUDT7 in pig LM cells, TaqMan absolute gene expression assay was performed with the gene-specific TaqMan minor groove binder (MGB) probe and a primer pair designed with Primer Express software v3.0 (Applied Biosystems). A PCR fragment amplified by the primer pair was cloned into pCR2.1-TOPO plasmid vector and transformed into DH5α-T1R competent cells using a TOPO TA Cloning kit (Invitro-
Expression of the pig \textit{NUDT7} gene ranging from $1.00 \times 10^{-7}$ µg to $1.25 \times 10^{-11}$ µg, to detect absolute expression values using a method reported previously (Pfaffl et al., 2002). In brief, the gene copy number was calculated based on the standard of which DNA amount used for the quantification was known. The TaqMan system was used with the ABI PRISM 7500 Sequence detection system and SDS software v1.4 (Applied Biosystems). Number of replicates was at least 4 times for each sample. The relationship between genotype and mRNA abundance was examined by regression analysis.

**Allele-Specific Quantitative Real-Time PCR and Data Analysis.** To detect differential allele expression of the pig \textit{NUDT7} gene, the same gene-specific TaqMan MGB probe as mentioned above was applied, and PCR primer pairs were designed to have an allele-specific nucleotide at the 3′ end of the anti-sense primer so that each allele could be detected specifically (Supplemental Table 3; http://jas.fass.org/content/vol88/issue1/). Allele-specific primers were designed at the SNP on exon 2, which segregated between Japanese wild boar (T allele) and Large White (C allele). Plasmid clones derived from each allele type were produced by the same method as described above and used for standard curve generation with the same range of serial dilutions for each separate allele. In addition, a calibration curve for the quantification of gene expression was generated by a mixture of plasmid clones of both alleles; the ratios of the C allele (Large White-specific) to the T allele (Japanese wild boar-specific) were 8:1, 4:1, 2:1, 1:1, 1:2, 1:4, and 1:8. The calibration curve (linear regression line) was generated by the following formula:

$$y = a + bx,$$

where \(x\) is the logarithm of a given mixing ratio for the \(C\) allele and \(T\) allele; \(y\) is the logarithm of the ratio between quantities of \(C\) alleles and \(T\) alleles, as evaluated by TaqMan assay for a given mixture; \(a\) is the intercept; and \(b\) is the slope. Fluorescence intensity data from the TaqMan assays were transformed to quantities by application of the standard and calibration curves and exported from the SDS software. For each mixing ratio of a given gene, we calculated the logarithm of the ratio of \(C\) to \(T\) alleles between 1/8 and 8/1. Allele-specific mRNA abundance in the LM was measured by quantitative real-time PCR. The allele-specific expression ratio was estimated from the calibration curve described above, where the estimate of the logarithm of the true ratio (\(x\)) was obtained from the observed one (\(y\)). Statistical testing (\(t\)-test) to detect differences in expression between the \(C\) and \(T\) alleles was performed to confirm unequal expression of the 2 alleles in 5 female animals with the CT genotype (animal ID 6801, 6805, 302, 1701, and 11109 in Figure 1).

**RESULTS**

**Fine-Mapping of the Meat Color QTL**

Interval mapping with additional SNP markers on SSC6 revealed that Japanese wild boar × Large White and Duroc × Chinese Jinhua families both indicated the same QTL peak position at \textit{SW1353} (23.4-cM) with 1% (13.3 \(F\)-ratio) and 5% (8.9 \(F\)-ratio) genome-wise significance levels, respectively (Figure 2). According to the \(F\)-ratio of the map constructed with combined \(F_2\) populations, the meat color QTL peak was located near \textit{SW1353} (23.4-cM, 19.3 \(F\)-ratio) and the 1- and 1.5-logarithm of odds (LOD) support intervals were 7.8 and 9.8-cM between \textit{SW2406} and \textit{GABARAPL2}, respectively (Figure 2). Comparative mapping of humans, mice, and cattle revealed that the region between \textit{SW2406} and \textit{GABARAPL2} contained 10 functional genes, which were lined up in the same order within a 4-Mb range (Supplemental Figure 1; http://jas.fass.org/content/vol88/issue1/). In pigs, these 10 genes were mapped to the same region by linkage analysis with the SNP in the genes. Of these 10 genes, 8 and 5 genes were identified in the 1.5 and 1-LOD support intervals (Supplemental Figure 1; http://jas.fass.org/content/vol88/issue1/). In addition to the linkage map, the pig BAC fingerprint contig used by the International Swine Genome Sequencing Consortium (Schook et al., 2005) indicated that the 10 genes were physically lined up in the same order, although there were several BAC clones for which the sequences had not yet been finished.

**Cloning the Pig \textit{NUDT7} Gene**

From the 10 genes in the QTL region, we selected and cloned the pig \textit{NUDT7} gene to be investigated as the candidate gene. In mice, \textit{Nudt7} has been identified to specifically eliminate oxidized CoA and also to hydrolyze succinyl-CoA (Gasmi and McLennan, 2001). Because succinyl-CoA is one of the substrates for the reaction limiting the rate of biosynthesis of heme, which is the primary component of myoglobin, it is possible that \textit{NUDT7} activity in skeletal muscle is associated with degradation of heme content, leading to pale meat color. In pigs, \textit{NUDT7} was located in the 1-LOD interval of the QTL and was found in BAC clone 317J11 (available at GenBank since October 2008), which overlaps BAC clone 76H3, containing the QTL peak marker \textit{SW1353} (Supplemental Figure 1; http://jas.fass.org/content/vol88/issue1/).

We then isolated a BAC clone containing the pig \textit{NUDT7} gene and determined the structure of the gene, including the promoter region and CDS (GenBank accession No. AB473629). The CDS of the pig \textit{NUDT7} gene was 714 nucleotides long and translated into 237 AA. The identity of the CDS was from 73.1 to 81.3% with those of other species, and that of the AA sequence was from 63.2 to 79.2% (Table 1 and Supplemental Figure 2; http://jas.fass.org/content/vol88/issue1/).
Pig NUDT7 contained UPF0035 and nudix motifs that were highly conserved in the other species.

**Structure and Polymorphism Analysis of the Pig NUDT7 Gene**

Comparison of the overall sequence of the pig NUDT7 gene using genomic DNA of the Japanese wild boar and Large White enabled us to identify a total of 116 genetic polymorphisms, consisting of 113 SNP and 3 indels. Of the SNP, 7 were identified in the exons (Figure 3). Comparison of the pig NUDT7 CDS between the parental breed pigs of the F2 families revealed some non-synonymous substitutions, although none of them were associated with the QTL (Figure 3). In the 5’ promoter region, 3 SNP (indicated by gray triangles) were identified in 4 transcription factor recognition sites (Figure 3). Nucleotide substitutions from Japanese wild boar to Large White were identified within the recognition sites for c-Ets-1 (–7998T > C), CdxA (–4187G > A), MZF1 (–3064A > G), and Nkx-2 (–3064A > G) (Woods et al., 1992; Margalit et al., 1993; Morris et al., 1994; Chen and Schwartz, 1997). The sequences detected in Large White pigs were identical to the consensus sequences for transcription factor recognition, whereas the Japanese wild boar genotype was mismatched with them (Figure 3). Repetitive sequences such as short- and long-interspersed repetitive elements (SINE and...

**Table 1. Identities of nucleotide (bottom left) and AA (top right) sequences of nudix-type motif 7 (NUDT7) among mammalian species**

<table>
<thead>
<tr>
<th>Item, %</th>
<th>Pig</th>
<th>Human</th>
<th>Cattle</th>
<th>Mouse-α</th>
<th>Mouse-β</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 77.3</td>
<td>69.6</td>
<td>79.2</td>
<td>68.8</td>
<td>63.2</td>
<td>69.6</td>
<td></td>
</tr>
<tr>
<td>Human 81.3</td>
<td>76.3</td>
<td>68.5</td>
<td>67.4</td>
<td>61.2</td>
<td>64.9</td>
<td></td>
</tr>
<tr>
<td>Cattle 73.1</td>
<td>73.4</td>
<td>70.7</td>
<td>64.3</td>
<td>58.4</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
<td>Mouse-α 62.6</td>
<td>59.8</td>
<td>58.6</td>
<td>76.2</td>
<td>91.5</td>
<td>80.1</td>
<td></td>
</tr>
<tr>
<td>Mouse-β 73.5</td>
<td>72.6</td>
<td>70.2</td>
<td>86.8</td>
<td>65.7</td>
<td>72.9</td>
<td></td>
</tr>
</tbody>
</table>

1Mouse Nudt7 transcript variants were detected in the predominantly expressed form α and the shorter 60-nucleotide (21-AA) minor form β.

**Figure 2.** Interval mapping of meat color QTL on SSC6. Red, green, and blue lines represent the results of interval mapping for the meat color QTL on SSC6 in F2 families constructed with Japanese wild boar × Large White, Duroc × Chinese Jinhua, and in combination of the 2 families, respectively. The y-axis is the value of the F-ratio. The x-axis indicates the marker position on each linkage map. Blue horizontal lines indicate 1-logarithm of odds (LOD; 7.8-cM) and 1.5-LOD (9.8-cM) support intervals in the combined map. Red and green horizontal lines indicate genome-wise 1% (Japanese wild boar × Large White family) and 5% (Duroc × Chinese Jinhua family) significance levels.
LINE) were detected by CENSOR in the 5′-upstream region and in intron 1 in the pig NUDT7 gene (Table 2 and Figure 3).

Another sequence characteristic of the pig NUDT7 gene, an alternative splicing with a 21-nucleotide insertion, was found at the 5′ end of exon 3 (Figure 3), although the genotype frequency of the 21-nucleotide-inserted splicing variant was negligible in both alleles (data not shown). Therefore, the major 21-nucleotide shorter variant was selectively used for the following analyses.

**Gene Expression Analyses**

**Differential Expression of NUDT7 Genotypes.** Gene expression assays were performed with offspring from the backcrossings (Figure 1). One SNP (2633T > C) in exon 2 segregated between Japanese wild boar (T) and Large White (C), so it was used for the discrimination of allele type and genotype. Measurement of NUDT7 mRNA abundance by quantitative real-time PCR (qPCR) assay revealed that the number of Large White-specific alleles (C) was positively correlated with the expression level of the NUDT7 gene in pig LM (r = 0.899, P < 0.001). The C allele tended to show an additive effect on the increase in NUDT7 mRNA abundance in pig LM, although a statistically significant difference was not confirmed due to shortages of available pigs with CC and TT genotypes (Figure 4).

**Differential NUDT7 Gene Expression Between Alleles.** To investigate the genotype-dependent regulation of pig NUDT7 transcription, we aimed to detect allele-specific expression of the NUDT7 gene in the LM of heterozygote pigs. In so doing, we developed and applied the allele-specific-qPCR method with common forward primers and 2 types of allele-specific reverse primers. The ratios of C allele (Large White)-specific gene expression and T allele (Japanese wild boar)-specific gene expression were calculated using serial DNA dilutions for standard (8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8) and calibration curves prepared for the measurement. The calibration curve for the quantification of allele-specific gene expression was linear (y = 0.999x - 0.019, r = 0.999, P < 0.001) over the range for the ratio of C to T (Supplemental Figure 3; http://jas.fass.org/content/vol88/issue1/). The fact that the slope was almost 1 and the intercept almost 0 indicated that the allele-specific primers detected only their matched alleles, and that the reaction efficiency of allele-specific-qPCR was not affected by the allele type. Statistical testing (t-test) showed that expression of the C allele was significantly (P < 0.001) greater than that of the T allele in the 5 heterozygous female animals (denoting genotype CT in Figure 1); animal ID 6801, 6805, 302, 1701, and 11109 indicated the C/T ratio of 1.434 ± 0.023, 1.878

![Figure 3. Structure of pig nudix-type motif 7 (NUDT7) gene and polymorphisms. White boxes denote regions of repetitive nucleotide sequences detected with CENSOR (Kohany et al., 2006). Black boxes indicate exons. Triangles denote positions of genetic polymorphisms such as SNP and indels. Black triangle at exon 2 indicates the SNP that specifically identifies the breed type; gene expression analyses were performed by detecting this genotype. Gray-shaded boxes indicate transcription factor recognition sites, including Nkx-2 (–3064A > G), MZF1 (–3064A > G), CdxA (–4186G > A), and c-Ets-1 (–7996T > C); adenine at the initiation codon is +1. Consensus sequences for these transcription factors are matched with Large White genotypes. Triangle at exon 3 indicates an alternative splicing site at the 5′ end of the exon with a 21-nucleotide insertion. Actual length of intron 2 was 2,944 bp and of intron 3 was 5,445 bp.](http://jas.fass.org/content/vol88/issue1/)
Interval mapping analysis revealed that the meat color QTL was located between \textit{SW2406} and \textit{GABARAPL2} markers flanking a 9.8-cM region of 1.5-LOD support interval with 95\% confidence. Comparative map analysis of the QTL region showed that fortunately only 10 genes were included around the support interval. From among the 10 genes, we selected the \textit{NUDT7} gene as the positional and functional candidate because the map position of \textit{NUDT7} and the QTL peak marker \textit{SW1353} are physically close enough. From the point of view of function, a previous mouse study had revealed that \textit{NUDT7} specifically eliminates oxidized CoA in peroxisomes and hydrolyzes succinyl-CoA (Gasmi and McLennan, 2001). Because succinyl-CoA is a substrate of the reaction limiting the biosynthesis of heme, which is the primary component of myoglobin, we considered it possible that \textit{NUDT7} activity was associated with the degradation of heme content in pig LM cells, leading to paleness of meat. Furthermore, no other genes seemed to be related to the enrichment of redness in muscle. Hence, we analyzed genetic polymorphisms and gene expression of the pig \textit{NUDT7}.

Exploration of SNP in the \textit{NUDT7} gene sequence in Japanese wild boar and Large White breed pigs revealed that none of the SNP in the CDS caused AA substitutions associated with the QTL. However, because 3 SNP in the promoter region were detected at transcription factor recognition sites, gene expression might be affected by these SNP. We therefore investigated the difference in \textit{NUDT7} gene expression between alleles from the Japanese wild boar and those from the Large White. For the expression analysis, we used animals from the fourth generation of a backcrossed population in which the QTL region of a Japanese wild boar had been introduced into a Large White; the result suggested that the Large White-type allele of \textit{NUDT7} is more effectively transcribed than the Japanese wild boar-type allele in LD. In addition, characterization of

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
Name & Length, nucleotides & Class\(^1\) & Direction\(^2\) \\
\hline
L2A & 113 & Non-LTR/CR1 & COM \\
MER3 & 172 & DNA/hAT & COM \\
SINE1_SS & 119 & Non-LTR/SINE/SINE1 & COM \\
PRE1_SS & 238 & Non-LTR/SINE & COM \\
SINE1A_SS & 110 & Non-LTR/SINE/SINE1 & DIR \\
PRE1_SS & 226 & Non-LTR/SINE & COM \\
MIR3 & 99 & Non-LTR/SINE & DIR \\
SINE_SS & 124 & Non-LTR/SINE/SINE1 & COM \\
ORSL & 258 & DNA & COM \\
MER5A & 144 & DNA/hAT & COM \\
L1P_MA2 & 1,295 & Non-LTR/L1 (LINE) & DIR \\
MLT1C1 & 254 & ERV/ERV3 & COM \\
SINE1_SS & 49 & Non-LTR/SINE/SINE1 & COM \\
PRE1_SS & 136 & Non-LTR/SINE & COM \\
SINE1_SS & 124 & Non-LTR/SINE/SINE1 & COM \\
MLT1C1 & 244 & ERV/ERV3 & COM \\
MER5A & 71 & DNA/hAT & DIR \\
\hline
\end{tabular}
\caption{Repeat sequence analysis of the 5’ untranslated region of the pig nudix-type motif 7 (\textit{NUDT7}) gene}
\end{table}

\(^1\)Class/subclass of repeat, as specified in repeat annotation defined by CENSOR (Kohany et al., 2006). SINE and LINE = short- and long-interspersed repetitive elements, respectively.

\(^2\)Sequence orientation is indicated as DIR (direct) or COM (complementary).

\(^3\)The similarity value between 2 aligned fragments is calculated as \(Sim = \frac{\text{match count}}{\text{alignment length} - \text{query gap length} - \text{subject gap length} + \text{gap count}}\), where match count is the number of matching base positions in alignment; alignment length is the length of alignment, which is the number of matches + number of mismatches + length of gaps; query gap length is the total length of alignment gaps on submitted query sequence; subject gap length is the total length of alignment gaps on Repbase library sequence; and gap count is the number of uninterrupted alignment gaps of any length on the query or subject sequences.

\(^4\)Alignment score obtained from basic local alignment search tool (BLAST) search.
SNP on the transcription factor recognition sites indicated that the Large White-type allele matched more consistently with the consensus sequences than did the Japanese wild-type allele, which therefore might not be as well recognized as the Large White-type one. Given that one or more of the transcription factors work effectively for the expression of NUDT7 in pig muscle cells, the transcription efficiency of the Large White-type allele (C) may be upregulated relative to that of Japanese wild boar. However, no study investigated whether transcription factors regulate the efficiency of transcription of their downstream genes in muscle tissue.

From the QTL interval mapping to expression assays for the positional candidate gene, we used the identical sire of Japanese wild boar to establish the F2 and backcross families so that the Japanese wild boar alleles introduced into those populations should be identical. In addition, genotypes of 4 SNP in 5’ transcription sites and exon 2 segregated between Japanese wild boar and Large White in the current study, although an analysis of linkage disequilibrium might bring further insight into elucidation of breed differences of this QTL.

Recent studies suggest that repetitive sequence elements such as SINE and LINE affect transcriptional regulation and thus characterize regulation of species-specific or tissue-specific expression or both (Lunyak et al., 2007; Shephard et al., 2007). Repeat sequence analysis revealed the presence of PRE1 (swine genome-specific SINE), SINE, and LINE sequences in the pig NUDT7 gene sequence, although the Japanese wild boar and the Large White showed neither differences in composition of the repetitive elements nor polymorphisms effective for gene function. Moreover, we applied rapid amplification of the cDNA end PCR to detect the start site of transcription of the pig NUDT7 gene, but there was no difference in transcription initiation between the Japanese wild boar and Large White. These results suggested that the presence of repetitive sequence elements and the position of transcription initiation were not associated with differences in expression of the pig NUDT7 gene.

We successfully identified the meat color QTL on SSC6 and then cloned the candidate gene encoding NUDT7 by mapping analyses and investigation of the functions of available candidate genes in the NCBI Entrez Gene database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). However, there is still a slight possibility of the existence of unconfirmed causative gene(s) in unidentified BAC clones exclusively within the QTL region of the swine genome and not in those of humans, mice, or cattle; this will not be known until the first draft of the pig whole genome sequence becomes publicly available. Nevertheless, sequencing of the whole pig genome is likely to confirm that NUDT7 is, as expected, the most appropriate candidate for the meat color QTL. Allele-specific-qPCR identified differential allele expression of pig NUDT7, suggesting that the Large White genotype has a potentially greater transcription efficiency than the Japanese wild boar phenotype. Differential expression of the pig NUDT7 gene might be regulated through genotype-dependent transcription regulation. In a future study, we intend to perform a muscle cell model-based gene expression assay to validate this allele-specific difference in transcription efficiency and to confirm that expression of NUDT7 regulates heme content in cultured cells derived from muscle cells. Moreover, we intend to use the same approach as used here in the other pig population (Duroc × Chinese Jinhua) because it is important to investigate whether the pig NUDT7 gene is associated with meat color in different genetic backgrounds. The development of DNA marker(s) in the SNP found in the 5’ promoter region should enable us to use the favorable meat color traits on the Japanese wild boar allele in pig breeding programs to meet consumer demands.

LITERATURE CITED


