Duplicated Spot 14 genes in the chicken: characterization and identification of polymorphisms associated with abdominal fat traits

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Abstract

In mammals, thyroid hormone responsive Spot 14 (THRSP) is a small acidic protein that is predominately expressed in lipogenic tissue (i.e., liver, abdominal fat and the mammary gland). This gene has been postulated to play a role in lipogenesis, since it responds to thyroid hormone stimulation, high glucose levels and it is localized to a chromosomal region implicated in obesity. In this paper, we report the identification and characterization of duplicated polymorphic paralogs of Spot 14 in the chicken, THRSP\textsubscript{a} and THRSP\textsubscript{b}. Despite low similarity in amino acid (aa) sequence between chickens and mammals, other properties of Spot 14 (i.e., pI, subcellular localization, transcriptional control and functional domains) appear to be highly conserved. Furthermore, a synteny group of THRSP and its flanking genes [NADH dehydrogenase (NDUFC2) and glucosyltransferase (ALG8)] appears to be conserved among chickens, humans, mice and rats.

Polymorphic alleles, involving a variable number of tandem repeats (VNTR), were discovered in the putative protein coding region of the duplicated chicken THRSP\textsubscript{a} (9 bp) and THRSP\textsubscript{b} (6 or 12 bp) genes. Our study shows that the THRSP\textsubscript{a} locus is associated with abdominal fat traits in a broiler \times Leghorn resource population.

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1. Introduction

The Spot 14 gene, also referred to as thyroid hormone responsive Spot 14 (THRSP), encodes a small acidic protein that was discovered in earlier studies of thyroid hormone action in hepatocytes (Seelig et al., 1981; Jump et al., 1984; Liaw and Towl, 1984). Although the exact molecular mechanism is not clear, THRSP is implicated as a transcription factor involved in control of lipogenic enzymes. For instance, THRSP is only expressed in lipogenic tissue such as liver, fat and the mammary gland (Liaw and Towl, 1984; Jump and Oppenheimer, 1985). THRSP mRNA levels are greatly increased by carbohydrate feeding or insulin-injection and decreased by high plasma glucagon levels or by feeding a diet rich in polyunsaturated fatty acids (Jump et al., 1993). Hepatocytes transfected with a THRSP antisense oligonucleotide express decreased mRNA levels in enzymes involved in the lipogenic pathway [i.e., ATP-citrate lyase (ACLY), fatty acid synthase (FAS) and malic enzyme (ME)] (Kinlaw et al., 1995; Brown et al., 1997). Although an
increase in lipogenesis was observed in the THRSP knockout mouse, this contradiction could be due to incomplete gene deletion or overcompensation by alternative pathways (Zhu et al., 2001). Homodimers of THRSP interact with and activate chicken ovalbumin upstream promoter-transcription factor 1 (COUP-TF1) in promoting transcription of L-type pyruvate kinase (L-PK) through its interaction with specificity protein 1 (Sp1) binding site (Compe et al., 2001). Furthermore, the THRSP promoter region contains three thyroid response elements (TREs) that work synergistically and interact with far upstream region (FUR) elements to maximize triiodothyronine (T₃) responses in hepatocytes (Liu and Towle, 1994). Apparently, the human THRSP promoter responds more robustly to T₃ than glucose, while the rat THRSP promoter region is more responsive to glucose than T₃ (Campbell et al., 2003).

Chicken Spot 14 (THRSP) was first identified by microarray analysis as a differentially regulated EST (pat. pk0032.c9.f) in livers of chickens divergently selected for fast or slow growth rate (Cogburn et al., 2000, 2003b). A related EST was revealed by differential mRNA display in liver of genetically fat and lean chickens and subsequently mapped to 1q41–44 (Carre et al., 2001). This chromosomal region in chickens also harbors quantitative trait loci (QTL) for skin fatness (Ikeobi et al., 2002) and abdominal fatness region in chickens also harbors quantitative trait loci (QTL) (Lagarrigue et al., 2003). Furthermore, expression of THRSP mRNA is regulated by thyroid hormone status in broiler chickens (Wang et al., 2002).

In this paper, we describe the identification, characterization and expression of duplicated, polymorphic Spot 14 (THRSPα and THRSPβ) genes in the chicken. We also report an association of the THRSPα locus with abdominal fat traits in a Leghorn x broiler resource population.

2. Materials and methods

2.1. Chicken EST assembly and DNA sequence analyses

The in silico cDNA sequence of THRSP was assembled from chicken EST sequences generated from two international chicken EST projects (Boardman et al., 2002; Cogburn et al., submitted for publication) and those found in public databases (GenBank). Contigs were assembled using CAP3 (Huang and Madan, 1999) with 40-bp overlap and 90% identity; the CAP3 assemblies and a chicken gene obtained from the Texas A&M University BAC Center and BAC DNA prepared using the Large Construct Kit (Qiagen). The primers for chicken NADH dehydrogenase (NDUFC2) and glucosyltransferase (ALG8) were designed from in silico cDNA sequences (UD CAP3 Contig_7797.2 and Contig_3078.1, respectively) which correspond to these chicken genes (see Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Quantitative RT-PCR (TaqMan) and PCR primers</th>
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<tbody>
<tr>
<td>Gene</td>
<td>Primer sequence</td>
</tr>
<tr>
<td>18S RNAa</td>
<td>Forward</td>
</tr>
<tr>
<td>THRSPα</td>
<td>GTGACATTATACAGC4AACACAA</td>
</tr>
<tr>
<td>Total</td>
<td>32F</td>
</tr>
<tr>
<td>THRSPβ</td>
<td>DeletionF</td>
</tr>
<tr>
<td>93R</td>
<td>AAGACCCCTCCGAGCAGG</td>
</tr>
<tr>
<td>THRSPβf</td>
<td>ParalogF</td>
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<td>127R</td>
<td>GCGTTCCTACGACGAGG</td>
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<td>NDUF2C2</td>
<td>465F</td>
</tr>
<tr>
<td>1053R</td>
<td>CAGCTGCGGCTTTTGCGCTGAT</td>
</tr>
<tr>
<td>ALG8</td>
<td>cTGGCTGTGTTGACG</td>
</tr>
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</table>

a These primer sets were used in TaqMan real-time qRT-PCR analysis.
b Determines both THRSPα and β. Generally, the PCR mixture contained 50 ng of template and 0.2 μM each primer (Table 1), 0.2 mM dNTP with 2 U Taq DNA polymerase and 1.5 mM MgCl₂ in 20 μl final volume. PCR was performed for 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 60 s at 72 °C after an initial denaturation at 95 °C for 2 min and a final extension for 7 min. Due to the high GC content, ThermalAce PCR kit (Invitrogen; Carlsbad, CA) was used to amplify fragments of THRSPβ. Thermal cycles are essentially the same for THRSPβ as those described above, except that the denaturation step was at 98 °C.

2.2. PCR conditions

2.3. Analysis of two chicken BAC clones

Two chicken BAC clones (65J23 and 94A1) which were positive for chicken THRSP (Carre et al., 2001) were obtained from the Texas A&M University BAC Center and BAC DNA prepared using the Large Construct Kit (Qiagen). The primers for chicken NADH dehydrogenase (NDUFC2) and glucosyltransferase (ALG8) were designed from in silico cDNA sequences (UD CAP3 Contig_7797.2 and Contig_3078.1, respectively) which correspond to these chicken genes (see Table 1).

2.4. RNA isolation and real-time quantitative RT-PCR

Tissues of interest were taken immediately after cervical dislocation, snap frozen in liquid nitrogen and stored at
− 80 °C until extraction of RNA. Total RNA was extracted using an RNeasy midi kit (Qiagen; Valencia, CA) and its concentration determined by reading the optical density at 260 nm. Samples were diluted in RNase-free water to a concentration of 20 ng/µl and stored in a 96-well plate at −80 °C. Real-time quantitative RT-PCR (qRT-PCR) was performed with a PRISM® 7900HT Sequence Detection System (TaqMan®) (ABI; Fullerton, CA). Primers were designed using Primer Express 2.0 software (ABI). The QuantiTech SYBR green RT-PCR kit (Qiagen) and gene-specific PCR primers (Table 1) were used in a 20-µl reaction following protocols recommended by the manufacturer. The concentration of total RNA in each sample was ensured by analyzing 18S RNA by qRT-PCR, which showed no significant difference among samples. A standard curve and conversion factor between primer sets 32F/93R (detects both THRSPα and β) and DeletionF/DeletionR (α specific) were generated using a plasmid from a THRSP EST clone (pg2ln.pk005.j11) as template, which was diluted to the optimal concentration range (4.26 × 10^4 to 1.75 × 10^6 copies per µl) in water containing 20 ng/µl yeast RNA. The template was then amplified following a standard TaqMan qRT-PCR protocol (ABI). The expression of THRSPβ in chicken tissue was calculated by taking the difference between total THRSP (32F/93R primers) and THRSPα-specific (DeletionF/DeletionR primers) measurements.

2.5. Genotyping and trait association analysis

The Iowa Growth and Composition Resources Population (IGCRP) was used to study the association of the THRSPα polymorphism with abdominal fat traits. This population was established by crossing a broiler sire (from a commercial broiler-breeder male line) with dams from two unrelated highly inbred lines (Leghorn G-B2 and Fayoumi M15.2). These two inbred lines are more than 99% inbred (Zhou and Lamont, 1999). Two F1 male offspring of the same sire, one from each genetic cross (F1 Leghorn and F1 Fayoumi) were randomly selected and each rooster mated with 20 half-sib F1 females, producing about 720 F2 offspring in three hatches. Abdominal fat weight (Fat) was measured and also expressed as a percentage of body weight (%Fat). For genotyping of THRSPα, genomic DNA samples were amplified by PCR using a fluorescence forward primer (6FAM-DeletionF) and reverse primer DeletionR. The PCR products were analyzed on an automated DNA sequencer with GeneScan program (ABI) used for calling alleles.

The JMP® program (SAS Institute; Cary, NC) (Sall and Lehman, 1996) was used to conduct the general linear model test for association between genotype and fat traits based on model for the whole F2 population: \( Y = \mu + G + Sex + Dam_{\text{random}}(\text{Cross}) + Hatch_{\text{random}} + e \). Where \( Y \) is the dependent variable, \( \mu \) is population mean, \( G \) is genotype, and \( e \) is the random error. For analysis of each genetic cross, the statistical model was the same except that \( Dam_{\text{random}} \) was substituted for \( Dam_{\text{random}}(\text{Cross}) \), because the crosses were analyzed separately.

3. Results

3.1. Identification of THRSPα and THRSPβ paralogs

An in silico cDNA sequence representing chicken THRSPα (Fig. 1A) was assembled from a total of 61 ESTs (UD CAP3 Contig_8452.1) found in the UD chicken EST (http://www.chicest.udel.edu/), the British Biotechnology and Biological Sciences Research Council (BBSRC) chick EST (http://www.chick.umist.ac.uk/) (Boardman et al., 2002) and GenBank databases. The THRSPα contig sequence is 874 bp and it includes two closely located poly(A) signals in the 3′-UTR. No additional sequence was found at the 5′-end of THRSPα by 5′-RACE analysis. Northern blot analysis shows that the THRSPα transcript is 1.1 kb (data not shown). The predicted size of the THRSPα peptide is either 129 or 132 amino acid (aa) (due to the 9-bp VNTR polymorphism in the coding region) with a molecular weight of either 14.5 or 14.2 kDa and a pI of either 4.61 or 4.53. As predicated by the PSORT II program (http://psort.ims.u-tokyo.ac.jp/), this peptide is localized in the nucleus and has a leucine zipper motif in the C terminus. The predicted chicken THRSPα peptide (Fig. 1A) has a low similarity (29% identities; 46% positives) to the human THRSP αa sequence (Grillasca et al., 1997) and to a gastrulation specific protein, G12 (33% identities; 45% positives) found in zebrafish (Conway, 1995). When a BlastX search of 1630 chicken protein sequences, derived from complete open reading frames in the UD CAP3 chicken EST assemblies, was made against the non-redundant human protein set in GenBank, the similarity of THRSPα was among the weakest 2%.

The chicken THRSPβ paralog (Fig. 1B) was identified from a tBlastX search of the chicken UD CAP3 contig database (Cogburn et al., submitted for publication) using THRSPα as an electronic probe. The in silico cDNA for THRSPβ (UD CAP3 Contig_8452.2) was assembled from eight ESTs found in the BBSRC collection. It is 670 bp long with a typical poly(A) signal sequence. The THRSPβ cDNA is almost identical to THRSPα isoform in the first 230 nt at the 5′-end, which encodes a nearly identical N-terminus. The overall similarity between THRSPα and THRSPβ paralogs is 70% identical and 79% positive (Fig. 1C). The THRSPβ cDNA is extremely GC-rich (74.7%), which makes it a difficult target for cloning and PCR amplification. Similar to the THRSPα isoform, the predicted THRSPβ protein is acidic (pI 5.1 or 4.96) with a molecular weight of 14.5 or 14.7 kDa and a leucine zipper motif in the C-terminus.

An additional chicken EST found in the BBSRC database (GenBank accession no. BU440998) has an exception-
**A. THRSPα**

```plaintext
32F
gagagagagccagaggagggccATGAGCATATCTTCTGGACACAGAAGAGATGAAGCAAGAGTGAATGCTGGCAGC
MEQYFSATQKMEEQEVMFPSL

93R
cGGTGGGCTCAGGAGGTCTTTCTCGAGCGGACGCGCGGACGCGCCCGGGGGACGCCGACACCTGCTACGGAGCTCCCACCGC
LLRGVFPOQDGAPADLYERYQL

DeletionF
CTCTAAACCCTACAAAGCCCGTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCG
LKAICKPVEVGLASVTDFQTPSNADAD

DeletionR
CTCTCAACCCTACAAAGCCCGTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCG
QVLTDLNTKNAARTLYRYSQILEEINLG
```

**B. THRSPβ**

```plaintext
32F
gccggagagccgcggggcggaATGAGCATATCTTCTGGACACAGAAGAGATGAAGCAAGAGTGAATGCTGGCAGC
MERYFSATQKMEEQEVMFPSL

93R
cGGTGGGCTCAGGAGGTCTTTCTCGAGGACGCGCGGACGCGCCCGGGGGACGCCGACACCTGCTACGGAGCTCCCACCGC
LLRGVFPOQDGAPADLYERYQL

ParalogF
CTCTCAACCCTACAAAGCCCGTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCG
LKAICKPVEVGLASFTERSAGHADA

ParalogR
CTCTCAACCCTACAAAGCCCGTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCGCTGGGACGCG
QVLTDLNTKNAARTLYRYSQILEEINLG
```

**C. Comparison of cTHRSPα and cTHRSPβ**

**Domain 1**

- **α**: MeqYFSAQKM-EQQVFMPSLSRGVFPOQDGAPADLYERYQLLKAICKPVEVGLASVTDFQTPSN 69
  
  **β**: MERYFSATQKM-EQQVFMPSLSRGVFPOQDGAPADLYERYQLLKAICKPVEVGLASFTERSAGH 69

**Domain 2**

- **α**: MEQYFSATQKM-EQQVFMPSLSRGVFPOQDGAPADLYERYQLLKAICKPVEVGLASVTDFQTPSN 69
  
  **β**: MERYFSATQKM-EQQVFMPSLSRGVFPOQDGAPADLYERYQLLKAICKPVEVGLASFTERSAGH 69

**α-VNTR**

- **α**: ADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADAD
  
  **β**: ADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADADAD

**β-VNTR**

- **α**: Leucine zipper motif
  
  **β**: Leucine zipper motif
al high homology (99% nucleotide sequence identity) to bovine THRSρ and probably represents a contaminating bovine cDNA sequence.

3.2. Sequence alignment and structural analysis of an extended Spot 14 protein family

A protein database search has revealed that the THRSρ family has three structurally related members in chickens and zebrafish, whereas mammals (i.e., human, mouse or rat) have only two members. A sequence comparison shows the structural similarity among Spot 14 (THRSP), the zebrafish gastrulation-specific protein (G12), and the hypothetical human protein (STRAIT11499) for chicken, human, mouse, rat and zebrafish (Fig. 2A). The Spot 14 protein family shares three conserved domains: a highly hydrophobic aa sequence (PSLLRDV) near N-terminus (Domain 1), a second hydrophobic region in the middle (Domain 2) and the distinct leucine zipper motif (Domain 3) in the carboxyl terminus (Fig. 1C).

A phylogenetic analysis shows that a common ancestor of birds, fishes and mammals could have expressed two genes that encode structurally related proteins (Fig. 2B). The THRSρ protein is found in chickens, humans, rodents and zebrafish (zTC192887). A second member of the THRSρ protein family found among these animals is represented by orthologs of a hypothetical human protein STRAIT11499, which includes two zebrafish orthologs (G12 and zTC194742). The THRSP gene is duplicated in chickens, whereas the gastrulation-specific G12 gene is duplicated in zebrafish.

3.3. Genomic organization

Genomic sequence in the vicinity of the THRSPα gene was carefully searched using the strategy described in Section 2.1. A single genomic DNA contig (14.4 kb) that includes the THRSPα gene and its flanking region was assembled by CAP3 from 185 chicken genomic shotgun sequences from the trace archive in GenBank (http://www.ncbi.nlm.nih.gov/Traces/traces.cgi). The alignment of the THRSPα cDNA and genomic sequences shows that this gene contains two exons and one intron. Similar to the human THRSPα gene, the first exon encodes the entire cTHRSP protein, while exon 2 represents the 3′-UTR. Analysis of about 800 bp in the 5′-flanking region of the THRSPα gene shows a TATA box that is 39 bp upstream of the transcription start site.

Shotgun sequences from two different clones were found that correspond to the THRSPβ gene (GenBank GI no. 253911732 and 298831363), whereas two sequences (GenBank GI no. 253911843 and 298831453), derived from the other end of these two clones, aligned to the THRSPα genomic contig. The average size of the shotgun sequencing clones was 4.2 kb; therefore, the chicken THRSP paralogs are closely linked. The alignment of these shotgun sequences with THRSP paralogs indicates that both genes are transcribed in the same direction. In silico chromosomal walking revealed a synteny group of chicken orthologs of human genes, hypothetical protein MGC2376 (GenBank accession no. NP_0576984) and NDUFC2 (GenBank accession no. AAW07323) in the flanking region of the THRSP paralogs. Furthermore, a PCR analysis demonstrates the presence of THRSPα, THRSPβ, NDUFC2 and an additional gene glucosyltransferase (ALG8; GenBank accession no. NP_0576984) in two previously identified (Carre et al., 2001) THRSPα-positive BAC clones (65J23 and 94A1) (data not shown). Therefore, this synteny group contains the THRSPα, THRSPβ, NDUFC2, ALG8 and MGC2376 genes.

3.4. Expression of THRSP mRNA in chicken tissues

The expression of the chicken THRSP genes was examined by qRT-PCR using two primers (32F/93R) (Table 1) that are common to both THRSPα and THRSPβ (Fig. 1A,B). Among 11 tissues examined, liver had the highest expression level of THRSP mRNA, with fat, thymus and ovary expressing lower amounts (Fig. 1C). The chicken THRSP genes appear to be predominantly expressed in lipogenic tissue. Direct measurement of THRSPβ was not possible by TaqMan analysis (qRT-PCR) because the unique region in THRSPβ cDNA is very GC-rich. Therefore, we used an indirect method to examine THRSPβ expression in liver and fat tissue (Fig. 1B, C and D). First, we obtained the total THRSP mRNA level by using 32F/93R primer pairs; then, the THRSPα mRNA was determined using the specific DeletionF/DeletionR primer set. The relative abundance of THRSPα was calculated from the difference between total THRSP and specific THRSPα mRNA levels (Fig. 1B, C and D). The relative abundance of THRSPα and THRSPβ was examined in liver and abdominal fat of 5-week-old broiler chickens, where the abundance of THRSPα was 2- to 3-times greater than that of THRSPβ, respectively (Fig. 3B). We have previously found a dramatic increase in chicken total THRSP mRNA levels in liver of 1-day-old chicks when compared to late embryos (e16, e18 and e20) (Cogburn et al., 2003a). Therefore, we examined whether the expression of THRSPα and THRSPβ was differentially regulated during this period. A dramatic increase of 13- to 20-fold was detected in THRSPα and THRSPβ (Fig. 3C) mRNA levels (respectively) at 1-day post-hatching. Since the expression

Fig. 1. cDNA and predicted protein sequences of chicken THRSPα (A) and THRSPβ (B). Primer sequences used for PCR are indicated by the bold underlined letters. The predicted leucine zipper motif is shown in bold and the poly(A) signal is underlined. The boxes represent the VNTR region; the shaded portion indicates the missing nt and aa residues in the deletion alleles (a2 and b2). The sequence of the 5′-UTR and 3′-UTR (exon 2) of THRSPα is shown in lower case letters. The asterisk shows the stop codon. The junction between exons 1 and 2 in THRSPα is indicated by the inverted solid triangle. (C) Alignment of chicken THRSPα and THRSPβ protein sequences using the blastp program. Three functional domains of the putative THRSPα and THRSPβ proteins are shown in bold letters. The positive aa substitutions are shown by the + sign. The α-VNTR and β-VNTR polymorphisms are shown by the shaded boxes.
A. Conserved domains of THRSP Family

B. Phylogenetic Relationships of THRSP (Spot 14) Protein Family
of THRSP responds rapidly to nutritional factors, we also examined whether prolonged fasting or re-feeding (Beccavin et al., 2001) would differentially regulate expression of the hepatic THRSP paralogs. THRSPα and THRSPβ mRNA levels are down-regulated after a 48-h fast and up-regulated at 4 h after re-feeding (Fig. 3D), although the re-feeding response of THRSPβ was slightly higher (8-fold increase) than that of THRSPα (5-fold increase). Therefore, the transcription of THRSPα and THRSPβ appears to be controlled by both developmental and nutritional factors.

3.5. THRSP polymorphisms and association of THRSPα alleles with fat traits

The Spot 14 polymorphisms were discovered after detailed examination of EST and shotgun sequence assemblies. Both THRSP paralogs are polymorphic which involve VNTRs (ATAGATGGC in THRSPα and ACGCCG in THRSPβ) located near the leucine zipper motif (Fig. 1A,B,C). These polymorphisms result in either the insertion or deletion of the 9-bp (three aa) in the THRSPα gene and the insertion or deletion of either 6 or 12 bp (two or four aa) in THRSPβ. The 9- and 6-bp polymorphisms have been verified by PCR analysis of broiler, Leghorn and Fayoumi strains (Fig. 4).

The IGCRP (Deeb and Lamont, 2002) was genotyped for THRSPα to determine if the polymorphism is associated with deposition of abdominal fat in chickens. In the broiler/C2 Leghorn cross, THRSPα1 homozygotes present a higher fat content than heterozygotes and THRSPα2 homozygotes [Fat (P < 0.048) and %Fat (P < 0.02)] (Table 2). Variation of these two alleles accounts for a 3.3-g mean difference in abdominal fat weight between the two homozygous THRSPα genotypes. However, there was no associ-

Fig. 2. Protein sequence alignment of the Spot 14 family members (A): Thyroid hormone-responsive Spot 14 protein (THRSP), gastrulation specific protein G12 and the hypothetical protein, STRAIT11499. Protein sequences for chicken [c] THRSPα (UD CAP3 Contig_8452.1) and THRSPβ (UD CAP3 Contig_8452.2), human [h] THRSP (AAH31989), mouse [m] THRSP (Q62264), rat [r] THRSP (P04143) and zebrafish [z] (TC192887) THRSP were aligned using ClustalW with default parameters and BLOSUM62 scoring matrix. This alignment includes two structurally related proteins: gastrulation-specific protein G12 from zebrafish (P47805) and an apparently duplicated G12 protein (zTC194742) found in TIGR (http://www.tigr.org/tdb/tgi/zgi/) which show a high degree of structural similarity to the hypothetical human protein hSTRAIT11499 (AAH19332), mSTRAIT11499 (Q9CQ20) and cSTRAIT11499 (derived from UD CAP3 Contig_22252.1). Identical aa residues are shown in black, similar (positive) aa residues are shown in gray and the hyphens denote gaps. A dendrogram showing the phylogenetic relationship among three Spot 14 family members (B): THRSP, the gastrulation-specific protein G12 and hypothetical protein STRAIT11499. The phylogenetic tree was created using the ClustalW program with default settings and the BLOSUM62 scoring matrix.
that the flanking either human or mouse orthologs. It is interesting to note duplicated gene usually shows a faster rate of evolution mutations that could lead to altered function. One copy of a THRSP to mammalian STRAIT11499 group, while zebrafish G12 and TC194742 belong to the chicken family among mammals, birds and fishes shows that the population. The phylogenetic analysis of the polymorphic. Alignment of 22 ESTs and chicken genomic common process in genome evolution (Tatusov et al., 1997), that a duplication of the THRSP gene appeared after the first hydrophobic domain are required for homodimerization of THRSP (Cunningham et al., 1997). The middle hydrophobic domain could mediate the interaction of THRSP with other factors.

Expression of the murine THRSP has been extensively studied in liver and adipose tissue, where nutritional and hormonal factors intricately regulate its expression (Clarke et al., 1990; Jump et al., 1994; Liu and Towle, 1994). The mammalian THRSP promoter contains multiple elements that respond to thyroid hormone (TRE) (Liu and Towle, 1994), carbohydrates (ChoRE) (Koo and Towle, 2000) and sterols (sterol response element, SRE), particularly SREBP-1c (Jump et al., 2001). In chickens, T3 treatment up-regulates hepatic expression of THRSP mRNA (Wang et al., 2002) The expression of THRSP paralogs is also dramatically increased in liver of newly hatched chicks and of re-fed chickens following a 48-h fast. Thus, the expression of THRSPa and THRSPb appears to be controlled by similar cis-elements that respond to developmental, hormonal and nutritional factors, as has been described for mammals.

We have consistently found that THRSP has the same expression profile of functionally related genes involved in fat deposition in the chicken (Cogburn et al., 2003a,b). In humans, lipogenic breast cancer is associated with amplification of the THRSP gene and enhanced long-chain fatty acid synthesis (Moncur et al., 1998). Furthermore, the only evidence that THRSP is directly involved in transcription of metabolic genes is its interaction with COUP-TF1 in the

<table>
<thead>
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<th>Table 2</th>
<th>Association of chicken THRSPa alleles with fat traits in the Iowa Growth and Composition Resource Population (IGCRP)</th>
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<tr>
<td></td>
<td>( \text{Fat (g)} )</td>
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<tr>
<td>Whole population</td>
<td>N=180</td>
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<tr>
<td>Fat (g)</td>
<td>49.33</td>
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<td>Broiler &gt; Leghorn</td>
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<td>%Fat</td>
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The values represent least square means. The number (N) of birds per population (or cross) and genotype is indicated.
control of L-PK (a glycolytic enzyme) (Cunningham et al., 1997, 1998; Compe et al., 2001). Although, the exact mechanism by which THRSP exerts its action on transcription of lipogenic enzymes has not yet been established, there is considerable support for a role in regulation of the lipogenic pathway in birds and mammals.

In the present study, we have demonstrated the association of the THRSP\(\alpha\) locus with abdominal fat traits in a broiler × Leghorn cross, but not in the broiler × Fayoumi cross. This discrepancy could be explained by a large difference in genetic background between the Leghorn and Fayoumi breeds. The polymorphism in THRSP\(\alpha\) explains about 6% of the variation in abdominal fat, which correlates well with the estimate of about 21 polymorphs that control expression of the %Fat trait in this population (Deeb and Lamont, 2002). It is particularly interesting that polymorphisms in THRSP\(\alpha\) and THRSP\(\beta\) involve aspartic acid near the leucine zipper motif, which is critical for homodimerization and transcriptional regulation of lipogenic enzymes (Cunningham et al., 1997). The duplicated THRSP\(\alpha\) and THRSP\(\beta\) genes, and the interaction of their haplotypes could add additional complexity to dimerization of this putative transcriptional activator in chickens. It could be much more complicated to determine the effect of each individual allele if the two THRSP isoforms act differently in controlling fat deposition. Clearly, the duplicated chicken THRSP genes (THRSP\(\alpha\) and THRSP\(\beta\) paralogs) are worthy of further study as potential candidate genes involved in crucial metabolic processes and for genotyping of these alleles in other resource populations and in elite lines of commercial broiler breeders.

5. Conclusions

We have discovered duplicated paralogs of Spot 14 in the chicken, THRSP\(\alpha\) and THRSP\(\beta\). The duplicated THRSP genes were identified by sequence analysis of contigs assembled from our chicken EST collection and those in public databases. The transcription of THRSP\(\alpha\) and THRSP\(\beta\) mRNA in liver is controlled by developmental, hormonal and nutritional factors. A computational analysis of THRSP proteins has revealed three highly conserved domains in two structurally related proteins from the THRSP family (THRSP and STRAIT11499) across a number of vertebrates (chicken, zebrafish, rat, mouse and human). Furthermore, an analysis of the genomic sequence has revealed a synteny group, composed of THRSP and its flanking genes [NADH dehydrogenase (NDUF\(C\)2), glucosyltransferase (ALG8) and MGC2376], that is conserved among chickens, humans, mice and rats. The chicken THRSP paralogs are located on Chr1q41–44 near QTL for fatness. Polymorphic alleles involving VNTR were found in the coding region of the chicken THRSP\(\alpha\) and THRSP\(\beta\) genes. Our study shows that the THRSP\(\alpha\) locus is associated with abdominal fat traits in a broiler × Leghorn resource population. These observations support a role of THRSP in control of lipogenesis and expression of fat traits in the domestic chicken.

Note added in proof

The sequences reported in this paper have been deposited into Genbank under the following accession numbers: AY568628 (THRSP\(\alpha\)1), AY568629 (THRSP\(\alpha\)2), AY568630 (THRSP\(\beta\)1), AY568631 (THRSP\(\beta\)2), AY568632 (STRAIT11499) and AY568633 (NDUF\(C\)2).

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