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MAPPING, CDNA CLONING AND TISSUE EXPRESSION OF THE PORCINE THYROTROPIN-RELEASING HORMONE RECEPTOR GENE

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Thyrotropin-releasing hormone receptor* (TRHR) *is a G-protein-coupled receptor that plays a crucial role in regulating the hypothalamic-pituitary-thyroid axis by conveying the action of the hypothalamic tripeptide TRH, which is the primary central activator of this hormonal cascade. In the present study, the porcine* TRHR (pTRHR) *gene was localized to chromosome 4 by Radiation hybrid mapping. Quantitative trait loci affecting average backfat thickness, daily gain, and carcass and meat quality traits have been mapped to the region containing this gene. Further, the full-length cDNA of* pTRHR *was cloned and sequenced.* pTRHR *contains an open reading frame encoding 398 amino acids and shares 96.2% amino acid identity to human* TRHR. *Real-time quantitative RT-PCR showed that the mRNA of* pTRHR *is expressed in a variety of tissues, with high expression in the brain, hypothalamus, pituitary, testis, and fat tissue. The considerable expression level of* TRHR *mRNA found in fat tissue indicates potential direct action of TRH on lipocyte might exist. Additionally, two alternative spliced transcript variants of* pTRHR *were also isolated in this study. Our data provided basic molecular information which will be useful for further investigation on* pTRHR *gene.

Keywords: Pig; Thyrotropin-releasing hormone receptor; Tissue distribution; *TRHR*

Thyrotropin-releasing hormone (TRH), which is central in regulating the hypothalamic-pituitary-thyroid (HPT) axis, is involved in many important metabolic and physiological processes including gastrointestinal function, thermoregulation, and so on.[1] It initiates its effects by interacting with the TRH receptor (TRHR) in the anterior pituitary to activate intracellular signal transduction pathways and stimulate the synthesis and secretion of thyrotropin and prolactin. The TRHR is a seven transmembrane spanning receptor and belongs to the G protein-coupled receptor superfamily. The number of TRHR expressed on the surface of cells is

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directly related to the magnitude of the TRH response.[2] Further, mutations in the *TRHR* gene may decrease affinity of TRHR for TRH and result in central hypothyroidism,[3] which causes growth retardation, pudginess, and sluggishness. Recently, Liu et al.[4] identified the *TRHR* gene as an important gene for human lean body mass variation in a genome-wide association study. Moreover, leptin, an important circulating factor released by the adipose tissue for the regulation of body fat, exerts its effects by regulating HPT axis directly or indirectly.[5] These suggest that variations of *TRHR* genes in pigs might also have important effects on porcine growth, lean body weight, and fat metabolism, which interests people especially from an economic point of view.

To increase the available genetic information for porcine *TRHR* (*pTRHR*) gene and evaluate whether this gene could be a new candidate gene for growth and carcass traits in pigs, we mapped the *pTRHR* gene and cloned its full-length cDNA in this study. Using real-time quantitative RT-PCR, we also examined the tissue expression pattern of *pTRHR* in adult pigs.

MATERIALS AND METHODS

The porcine radiation hybrid (IMpRH) panel[6] was used for gene mapping. Screening of 118 clones of the panel was done by PCR analysis with the *pTRHR* specific primers which were designed based on sequence AY603095 (*pTRHR* partial CDS, primers are listed in Table 1).

The 3′ rapid amplification of cDNA ends (3RACE; 3′-Full RACE core set, TaKaRa) was employed to obtain C-terminal coding region of the *pTRHR* gene. Total RNA was isolated and purified from the pituitary of a healthy 7-month-old pig using RNeasy Mini columns and on-column RNase-free DNase treatment (Qiagen). Gene specific primers used in 3RACE-PCR are presented in Table 1. The products were sequenced commercially and then their sequences were assembled with known *pTRHR* N-terminal coding region by SeqMan (DNAStar). As a confirmation, full-length cDNA of *pTRHR* was amplified from oligo(dT)-primed cDNA that was synthesized with RevertAid M-MuLV Reverse Transcriptase (Fermentas).

Table 1 List of primers

Purpose	Primer sequence (5′–3′)	Ta[#] (°C)	Amplicon size (bp)
RH mapping	F: TGAAACGGTGACTCTGTGAATGAA R: AACTGGGCTTTGATGGGGTGAC	58	532
3RACE	Outer forward: GGTGTCTTTTATGTTGTGCCAAT Inner forward: TCAACAGCACAGTATCTTCAAGG	55	
Full-length cDNAs	F: TTTCAGAGAAACCTCAAGCCACT R: TCTTTGTCATACATTTTCTTCTACTC	58	1256
qRT-PCR: *pTRHR*	F: ATGTTGTGCCAATGATCCTG R: GGCTGGAGAGAAACGAGTTG	60	266
qRT-PCR: *β-actin*	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	60	114

[#]Ta: annealing temperature.

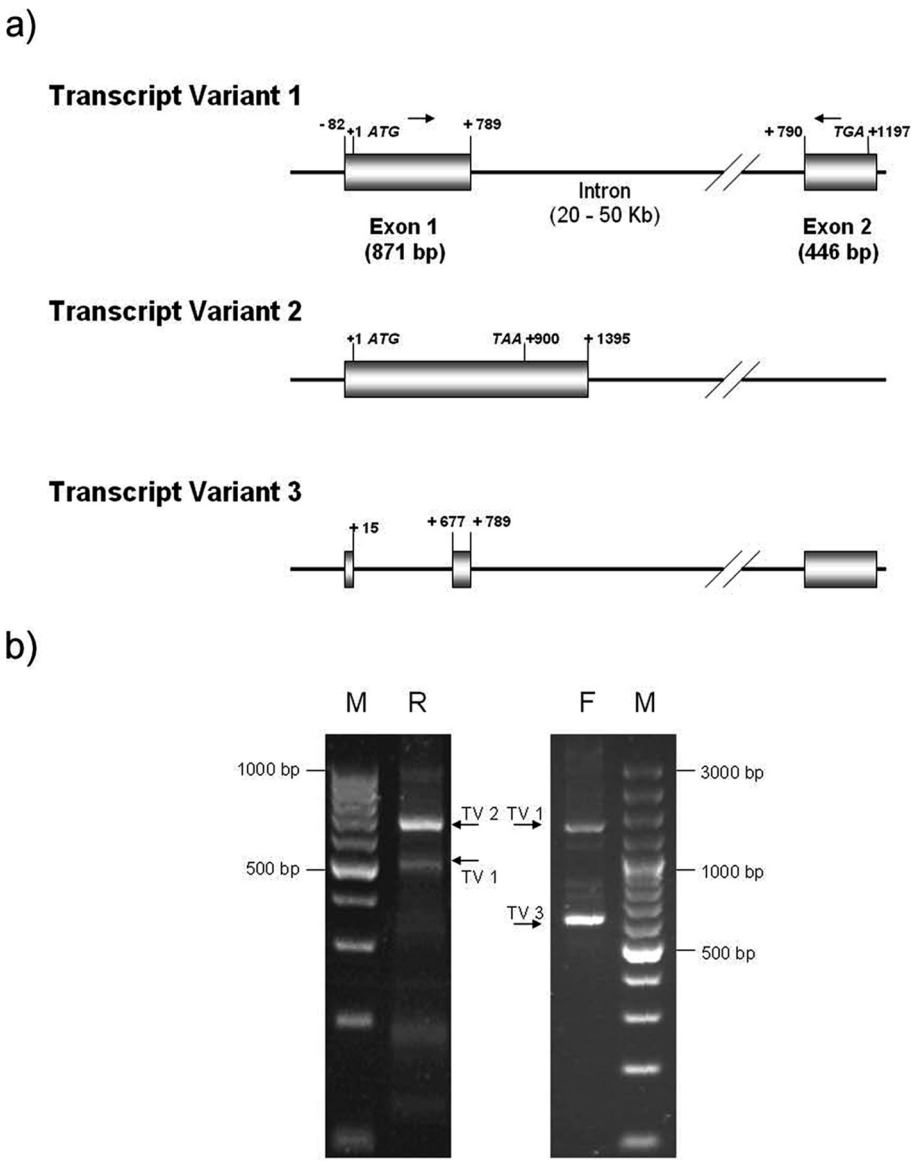

Figure 1 cDNA cloning and tissue expression profile of the *pTRHR*. a) Schematic organization of *pTRHR* mRNA variants. Transcript variant 1 generates the putative functional porcine TRH receptor, the arrows indicate the locations of the primers used in qRT-PCR; transcript variant 2 is the truncated form that retains part of the intron and the transcript variant 3 encompasses an additional 661-bp spliced intron compared to transcript variant 1. b) gel images to show the identification of the transcript variants. lane M: marker, lane R: products of the 3RACE-PCR, lane F: products of the PCR for full-length cDNA, TV: transcript variant. c) Tissue expression of *pTRHR* mRNA in 7-month-old pigs assessed by real-time RT-PCR analysis based on the normalization against *β-actin* expression. Levels of *pTRHR* mRNA in each tissue type are expressed as respective $-\Delta\Delta C_T$ value, where the $\Delta\Delta C_T$ in quested tissue = (C_T of *pTRHR* in quested tissue $- C_T$ of *β-actin* in quested tissue) $-$ (C_T of *pTRHR* in pancreas $- C_T$ of *β-actin* in pancreas).

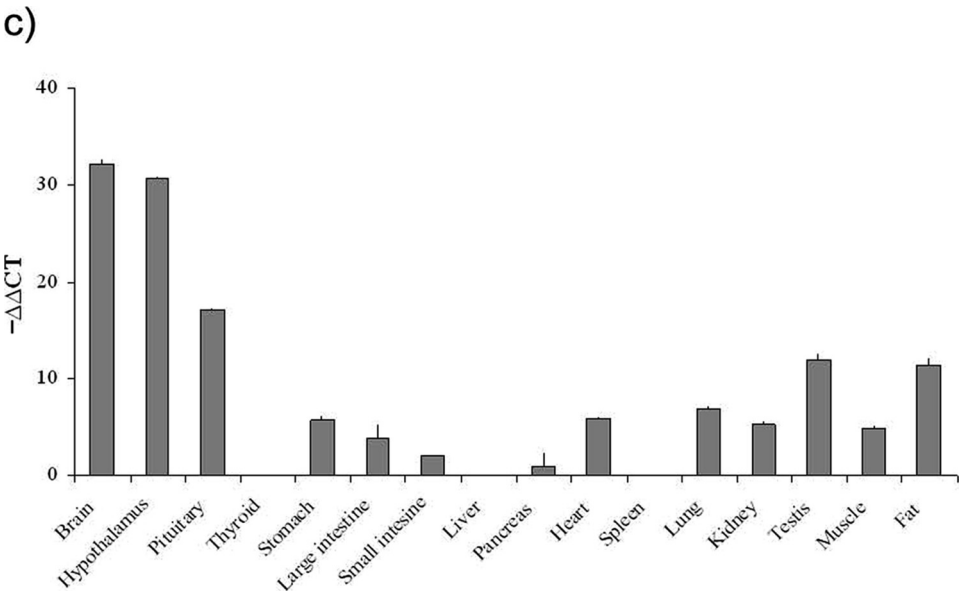

Figure 1 Continued.

Deduced amino acid sequences were compared to the *TRHR* protein sequences of human (NP_003292), cow (NP_776628), sheep (NP_001009407), mouse (NP_038724), chicken (NP_990261), african clawed frog (NP_001079098), and white sucker (AAG31763) by alignment with Clustal W.[7]

Tissue expression pattern of *pTRHR* mRNA was determined by real-time RT-PCR using *β-actin*[8] as the control housekeeping gene (primers are presented in Table 1). The first strand cDNA templates were prepared using total RNA extracted from various tissues of 7-month-old pigs. Real-time PCR was conducted on the StepOnePlus Real-Time PCR System (ABI) with SYBR® *Premix Ex Taq*™ kit (TaKaRa). All samples were analyzed in 2 independent runs, and the PCR products were loaded onto a 1.5% agarose gel for verification.

RESULTS AND DISCUSSION

Sequence analysis of the full-length cDNA encoding *pTRHR* (Transcript variant 1, Genbank accession no. FJ859911) revealed an ORF for a 45.1-kDa protein that comprised 398 amino acids. The deduced amino acid sequence shared 96.2%, 96.7%, 97.5%, 92.9%, 81.3%, 77.8%, and 66.4% identity with human, cow, sheep, mouse, chicken, amphibian, and fish *TRHR*, respectively. Additionally, a transcript variant (Transcript variant 2, Genbank accession no. FJ859912) which was truncated in the nucleotide sequence that encodes the putative third intracellular loop[9–11] and retained a part of the intron was also isolated from 3RACE-PCR (Figure 1). Similar truncated forms of *TRHR* have been reported in chicken but no function was found.[12]

Additionally, another transcript variant (Transcript variant 3, Genbank accession no. GU936497) was amplified from the PCR for full-length cDNA, and sequence analysis demonstrated the existence of a 661-bp alternative spliced intron.

As shown in Figure 1c, expression of *pTRHR* transcript variant 1 was observed in a variety of tissues including the brain, hypothalamus, pituitary, gastrointestinal

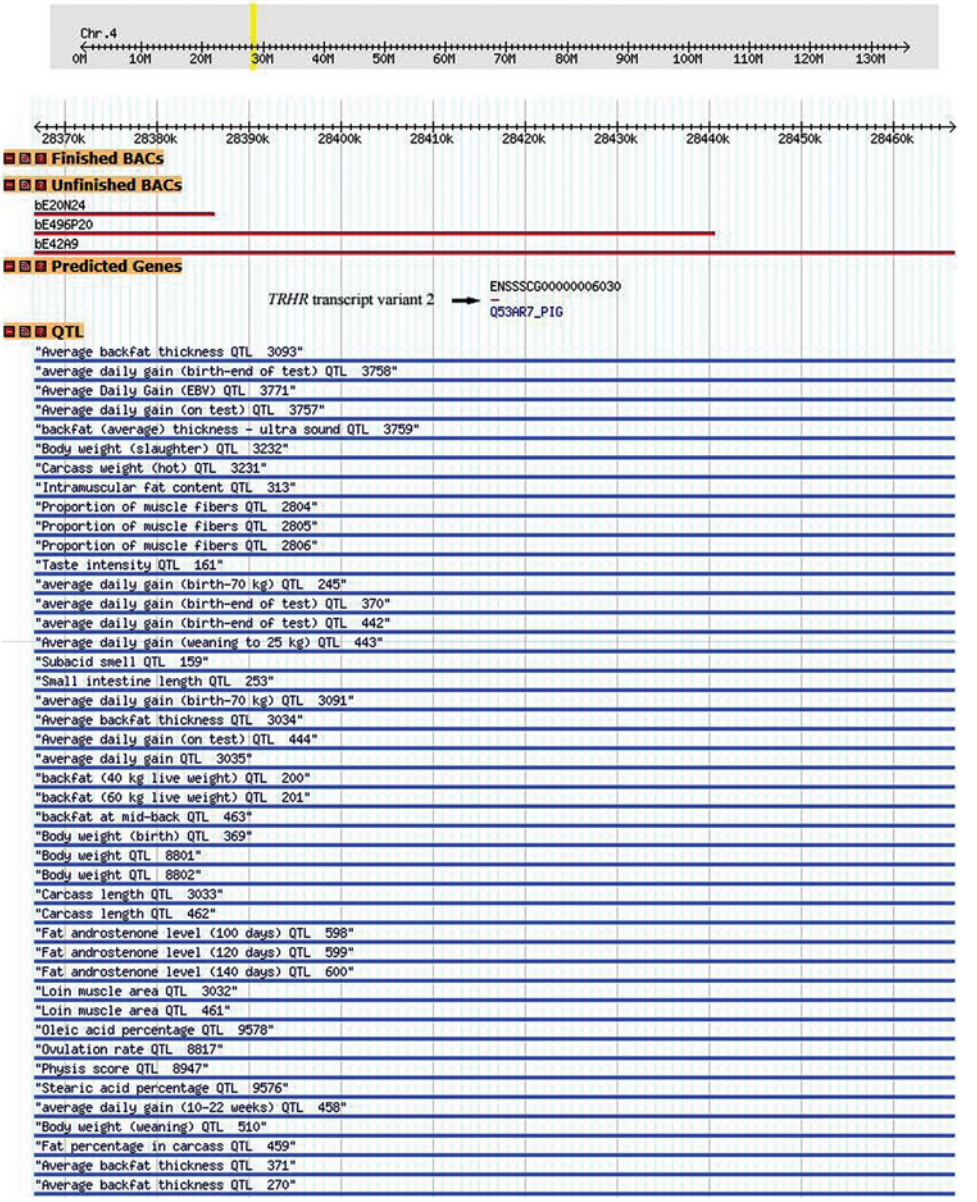

Figure 2 QTLs Mapped to *TRHR* gene on Chromosome 4. QTLs' information is available at http://www.animalgenome.org/cgi-bin/gbrowse/pig/. (Figure available in color online.)

tract, pancreas, heart, lung, kidney, testis, muscle, and fat tissue, but not in the thyroid, spleen, and liver. The distribution and role of *TRHR* has been well studied in brain, pituitary, and testis;[2,13,14] whereas, this is the first evidence identifying *TRHR* mRNA expression in fat tissue as far as we know. The considerable expression level of *TRHR* mRNA found in fat tissue suggests that part of the effects of TRH on fat metabolism might be mediated directly on lipocyte in addition to the classical hormonal cascade of HPT axis.

Two-point analysis (http://imprh.toulouse.inra.fr/) of the RH mapping results revealed that *pTRHR*'s most significantly linked marker was *SW969* on chromosome 4 at a distance of 44 cR (LOD = 9.13). While this paper was in review, *pTRHR* transcript variant 2, which was submitted by us, was utilized for gene annotation of *Sscrofa*9 (http://www.ensembl.org/Sus_scrofa/Info/Index) and was localized to chromosome 4: from 28,416,262 bp to 28,417,059 bp (reverse strand). Quantitative trait loci affecting average backfat thickness, daily gain, and carcass and meat quality traits have been mapped to the region containing the *pTRHR* gene (shown in Figure 2). TRH is well known to be involved in many important physiological processes, such as locomotor activation, food and water intake, gastric acid secretion, gastrointestinal contractility and transit, fat oxidation, and so on.[1] All these processes may have effects on individual's growth and carcass composition, and variations of *pTRHR* gene may exert their effects by adjusting the magnitude of the TRH response in a systemic or tissue-specific manner.

Considering the QTLs mapped to the region containing *pTRHR* gene and its considerable expression level in fat tissue, *pTRHR* could be a functional and positional candidate gene for porcine carcass traits and further exploration on *pTRHR* gene is worthwhile.

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