Single nucleotide polymorphisms in chicken \textit{lmbr1} gene were associated with chicken growth and carcass traits

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\textit{Lmbr1} is the key candidate gene controlling vertebrate limb development, but its effects on animal growth and carcass traits have never been reported. In this experiment, \textit{lmbr1} was taken as the candidate gene affecting chicken growth and carcass traits. T/C and G/A mutations located in exon 16 and one A/C mutation located in intron 5 of chicken \textit{lmbr1} were detected from Silky, White Plymouth Rock broilers and their F\textsubscript{2} crossing chickens by PCR-SSCP and sequencing methods. The analysis of variance (ANOVA) results suggests that T/C polymorphism of exon 16 had significant association with eviscerated yield rate (EYR), gizzard rate (GR), shank and claw rate (SCR) and shank girth (SG); A/C polymorphism of intron 5 was significantly associated with SCR, liver rate and head-neck weight (HNW), while both sites had no significant association with other growth and carcass traits. These results demonstrate that \textit{lmbr1} gene could be a genetic locus or linked to a major gene significantly affecting these growth and carcass traits in chicken.

\textit{lmbr1}, SNPs, shank and claw rate, carcass traits

\textit{C7orf2}/\textit{lmbr1} is a novel gene of unknown protein function. It is the key candidate gene affecting limb development of vertebrate, located in the key candidate region of human and mouse preaxial polydactyly (PPD)\textsuperscript{[1–7]}. The level of \textit{lmbr1} transcripts was dramatically misregulated at E12.0 in mouse Hx (Hemimelic extra-toes) mutant\textsuperscript{[3]}. The special deletion of exon 4 and surrounding 5kb sequence of \textit{c7orf2}/\textit{lmbr1} were detected in human Acheiropodia (ACHP)\textsuperscript{[3,4]}. Human and mouse \textit{c7orf2}/\textit{lmbr1} is composed of 17 exons, and the transcript sequence encodes a 490-amino-acid open reading frame (ORF). The biggest exon 16 is 162 bp; the least exon 3 is only 40 bp. Human \textit{c7orf2} encompasses about 200 bp of genomic DNA\textsuperscript{[4]}. Mouse \textit{lmbr1} encompasses about 140 kb of genomic DNA. In spite of lacking homology to proteins of known functions, \textit{lmbr1}/LMBR1 is highly conserved among different organisms, and mouse LMBR1L is over 95% identical to human \textit{C7orf2}\textsuperscript{[1,2]}. The \textit{C7orf2}/LMBR1 may be a novel multipass transmembrane protein, predicted to contain nine transmembrane regions, to be an anchoring protein or adhesion molecule and may function as the transporter, or cell surface receptor\textsuperscript{[1,2]}. According to Chicken \textit{lmbr1} sequence (GenBank accession No. AY251537 cloned by our group, \textit{lmbr1} polymorphisms and their associations with growth and carcass traits were studied by PCR-SSCP and sequencing methods in F\textsubscript{2} resource population established by Silky\texttimes{}White Plymouth Rock broilers. It was first reported that chicken \textit{lmbr1} gene polymorphisms had
significant association with some growth and carcass traits. These research results may exert important impact on conducting marker-assistant-selection (MAS) of these traits in chicken breeding program, on uncovering the function of \( lmbr1 \) gene, and on studying its interaction with other genes (as a membrane protein or surface receptor). As a kind of model animal, the research fruit from chicken \( lmbr1 \) can also help to further make clear the function of human \( c7orf2 \).

1 Materials and methods

1.1 Total RNA and cDNA preparation

Total RNA was extracted (Trizol, Invitrogene) from chicken heart tissue of Silkie and White Plymouth Rock broilers and conducted reverse transcription with MMLV reverse transcriptase (Promega) and the oligo(dT)18 primer. The condition of reverse transcription was as follows: the mixture without MMLV was heated to 70°C for 5 min to melt secondary structures of the template, cooled immediately on ice for 2 min to prevent formation of secondary structure. After adding MMLV, the reaction was carried out for 60 min at 37°C and for 15 min at 70°C in a 20 μL reaction volume, collected by brief centrifugation, and then kept at −20°C.

1.2 Primers for single nucleotide polymorphisms of coding region (cSNPs) detection and PCR amplification condition

Three pairs of primers were designed to detect cSNPs of chicken \( lmbr1 \) (Table 1). The RT-PCR reaction condition was 94°C for 5 min, 30 cycles of 94°C for 30 s, 55—60°C for 30 s and 72°C for 1 min, followed by 72°C for 7 min and 4°C forever.

1.3 Experimental chickens and traits

The F2 resource population was established by crossing of Silky and White Plymouth Rock broilers, which included P generation, F1 and F2 chickens. The PCR-SSCP genotypes and corresponding base variations were first identified from 11 White Plymouth Rock broilers and 11 silkies of P generation chickens, and later genotyping was further conducted on other P generation, F1 and F2 chickens, which were randomly selected from those being used in our lab at that time. \( Lmbr1 \) gene polymorphism and its association with growth and carcass traits were detected by Exon16S on 521 F2 chickens and by Intron5P on 337 F2 chickens respectively, including 3 (only one is different between Exon16S and Intron5P) positive-crossing families with broiler being sire and 3 reverse-crossing families with Silky being sire. The analyzed traits include chest angle, shank girth (SG), live weight before slaughter, carcass weight, eviscerated yield with giblet (EYG), eviscerated yield, breast muscle weight (BMW), leg muscle weight, abdominal fat weight, head-neck weight (HNW), shank and claw weight, wing weight, heart weight, liver weight, gizzard weight, glandular stomach weight, small intestine length, carcass rate, eviscerated yield with giblet rate, eviscerated yield rate (EYR), breast muscle rate, leg muscle rate, abdominal fat rate, head and neck rate, shank and claw rate (SCR), wing rate, heart rate, liver rate, gizzard rate (GR), glandular stomach rate. Genomic DNA was extracted from chicken blood by phenol-chloroform extracting methods.

1.4 Primers for polymorphism detection in F2 resource population and PCR reaction condition

According to the cloned sequence (accession No. AY251537) by our laboratory, Exon16L and Exon16S were designed to amplify exon 16 of chicken \( Lmbr1 \). The amplified product of Exon16S was one part of that Exon16L, their forward primer was just the same. According to the sequence from GenBank (accession No. AB092991), primer Intron5P was designed to amplify part fragment of intron 5. The sequence and annealing temperature of primers are listed in Table 2.

| Table 1 Primers for cSNPs detection and PCR reaction condition |
|---|---|---|---|
| Name | Forward (F) and reverse (R) primer | Region \(^a\) | Annealing temperature | Size |
| Primer-C1 | F: 5′ GATCTTCCACAGCCAAGTGC 3′ R: 5′ CCACCATACTGGATTCAAGG 3′ | 33—499 | 62°C | 467 bp |
| Primer-C2 | F: 5′ TGTTTTGTGTTGTAGCTCCTTG 3′ R: 5′ CTATTAGGACGTGATGGAGT 3′ | 356—931 | 55°C | 575 bp |
| Primer-C3 | F: 5′ CTGCTTGGGAGGAGGATTCTAG 3′ R: 5′CACAGGCTCTCTCTTCTAG 3′ | 854—1466 | 58°C | 613 bp |

\(^a\) Counted from translation start site A of chicken \( Lmbr1 \) coding sequence (AB105057).