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Single nucleotide polymorphisms in chicken *lmbr1* gene were associated with chicken growth and carcass traits

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***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, *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 *lmbr1* were detected from Silky, White Plymouth Rock broilers and their F_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 *lmbr1* gene could be a genetic locus or linked to a major gene significantly affecting these growth and carcass traits in chicken.**

chicken, *lmbr1*, SNPs, shank and claw rate, carcass traits

C7orf2/*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)[1−7]. The level of *lmbr1* transcripts was dramatically misregulated at E12.0 in mouse Hx (Hemimelic extra-toes) mutant[2]. The special deletion of exon 4 and surrounding ≈ 5kb sequence of *c7orf2*/*lmbr1* were detected in human Acheiropodia (ACHP)[3,4].

Human and mouse *c7orf2*/*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 *c7orf2* encompasses about 200 bp of genomic DNA[4]. Mouse *lmbr1* encompasses about 140 kb of genomic DNA. In spite of lacking homology to proteins of known functions, *lmbr1*/LMBR1 is highly conserved among different organisms, and mouse LMBR1L is over 95% identical to human C7ORF2[1,2]. The 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[1,2].

According to Chicken *lmbr1* sequence (GenBank accession No. AY251537 cloned by our group, *lmbr1* polymorphisms and their associations with growth and carcass traits were studied by PCR-SSCP and sequencing methods in F_2 resource population established by Silky × White Plymouth Rock broilers. It was first reported that chicken *lmbr1* gene polymorphisms had

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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 Silkies 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℃ 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℃ and for 15 min at 70℃ in a 20 μL reaction volume, collected by brief centrifugation, and then kept at –20℃.

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℃ for 5 min, 30 cycles of 94℃ for 30 s, 55—60℃ for 30 s and 72℃ for 1 min, followed by 72℃ for 7 min and 4℃ forever.

1.3 Experimental chickens and traits

The F_2 resource population was established by crossing of Silky and White Plymouth Rock broilers, which included P generation, F_1 and F_2 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, F_1 and F_2 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 F_2 chickens and by Intron5P on 337 F_2 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 F_2 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′ CCACCCATACGATTCCAAGG 3	33－499	62℃	467 bp
Primer-C2	F: 5′ TGTTTGTGTTGATGCCCTTTG 3′ R: 5′ CTAATAGGACTGAGATGGATG 3′	356－931	55℃	575 bp
Primer-C3	F: 5′ CTGCTTGGGAGAGGAATTTAG 3′ R: 5′ CACAGTGCTTTCTGATGCCC 3′	854－1466	58℃	613 bp

a) Counted from tranlation start site A of chicken *lmbr1* coding sequence (AB105057).

Table 2 Primers for gene polymorphism detection in F_2 resource population

Primer name	Forward (F) and reverse (R) sequence	Annealing temperature	Size
Exon16L	F: 5′GATTTGATCTGCTTGGAGAC3′ R1: 5′ATGCCTTCAGGAGCTCTTC 3′	58℃	150 bp
Exon16S	F: 5′GATTTGATCTGCTTGGAGAC3′ R2: 5′GTCATGATAGCAAAGAGCAAG 3′	62℃	91 bp
Intron5P	F: 5′TTGTCCTGGTTTATGTCCCTTT 3′ R: 5′GCATGTGCCACTAACACTAAGC 3′	60℃	271 bp

1.5 PCR-SSCP condition

PCR-SSCP condition of Primer Exon16L and Intron5P was as follows: 1 μL PCR products was mixed with 5 μL loading buffer, moment centrifuged and denatured. The denatured PCR products were electrophoresed on non-denaturing polyacrylamide gel (29:1) with 16% concentration for Exon16L and 13% concentration for Intron5P respectively, for 18 h at 140 V at 15℃. Gel was silver stained and SSCP genotypes were judged. SSCP condition of Exon16S was as follows: 3 μL PCR products was mixed with 6 μL loading buffer. The denatured PCR products were run on 20% non-denaturing polyacrylamide gel (29:1) for 25 h at 120 V and 15℃, with other conditions following Exon16L.

The denaturing condition of PCR products was: 98℃ 10 min for denaturing, take the samples out quickly and put them on ice, followed by cooling for 10 min to avoid renaturing prior to electrophoresis. The ingredients of loading buffer consist of 98% formamide, 10 mmol/L EDTA, 0.025% bromophenol blue, 0.025% xylene cyano IFF and 2% glycerin.

1.6 Sequencing methods

Gelose 1.5% was used to reclaim the PCR products. Purified PCR products by GenecleanIII kit were directly sequenced in double strands or clone sequencing. Clone sequencing method was as follows: the purified PCR products were cloned into the pMD-T, transformed to Dh5α; positive clones were identified by X-gal selection, and further identified by PCR amplification. Plasmid DNA was purified from selected clones by the alkaline lysis method and four independent clones of each PCR product were sequenced on PE377 (Perkin-Elmer) DNA sequencer. To confirm the special mutation site of each PCR-SSCP genotype identified by Exon16S, Exon16L was used to amplify the genomic DNA of the corresponding genotype of Exon16S and to conduct sequencing as described above.

1.7 Statistic model and analysis

According to growth-carcass traits and the feature of chicken population, the fixed linear model was as follows: Y = m + g +f + s + b + c + e, where Y is the dependent variable, m is the population mean, g is genotype effect, f = family effect, s is sex effect, b= batch effect; c = crossing effect and e is the random error. Data were subjected to have ANOVA analysis with SAS (V8.2) software package. The data out of range (mean value ±3SD) were eliminated.

2 Results and analysis

2.1 Polymorphisms of coding sequence

With Primer-C1, Primer-C2 and Primer-C3, 6 cSNPs of chicken *lmbr1* were detected from Silky and White Plymouth Rock broilers by RT-PCR (Table 3). Except that two variations (A797G and G1255A) were predicted to cause amino acid changes, others were silent mutations. Sequence comparisons among several species

Table 3 Coding sequence variations of *lmbr1* detected from Silkies and White Plymouth Rock broilers and base conservative among species

	Exon10	Exon16				Exon 17
	A797G	T1254C[a)]	G1255A	A1326G	C1360A	C1387T
Human	A	T	G	A	C	C
Mouse	A	T	G	A	C	C
Rat	A	T	G	A	C	C
Predicted amino acid change	R266Q		G419R			

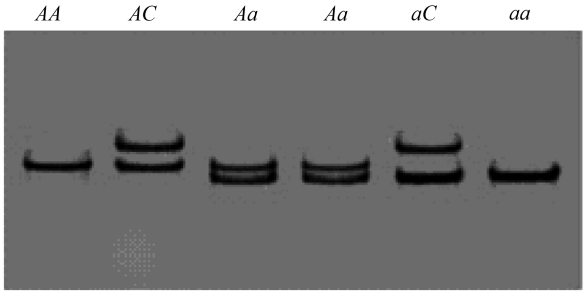
a) Counted from translation start site A of chicken *lmbr1* (AB105057).

show that these variations are conservative among human, mouse and rat *lmbr1* (Table 3). SIFT program was used to predict two potential amino acid variations on gene function (http://blocks.fhcrc.org/ sift/SIFT_BLink_ submit.html). G419R variation was predicted to affect protein function (score = 0.01) and R266Q variation was predicted to be tolerated. Exon 16 is the biggest exon of *lmbr1* (162 bp in length), and includes 4 variations. The frequency of G1255A variation was low, and only one out of eight individuals showed 1255G|A substitution.

2.2 PCR-SSCP genotype of Exon16S and variations analysis

Three PCR-SSCP genotypes were detected by Exon16L from 11 Silkies and 11 White Plymouth Rock broilers of P generation chickens. Four variations were identified from these chickens by sequencing. But the parallelism relationship between PCR-SSCP genotypes and the sequence genotype could not be effectively established, which may be for too many nucleotide variation combinations to be effectively distinguished by no-denaturing polyacrylamide gel.

Exon16S was designed to amplify part segment of Exon16L, only containing T1254C (T/C) and G1255A (G/A) variations. PCR products of Exon16S were used for SSCP analysis and 5 genotypes were detected from resource population. PCR and Clone Sequencing showed that *AA*, *aa* and *Aa* genotypes were caused by T1254C polymorphism, and *aC* and *AC* genotypes were caused by T1254C and G1255A polymorphisms. 1254T was in tight linkage disequilibrium with 1255A and constitutes a TA haplotype (Figure 1). The allele frequency of G1255A variation was low in P generation population, where only 3 variation individuals were found, and all of them were female Silkies of positive-crossing population (Table 4, Figure 2). One 1255A variation dropped in F_1 generation population (Figure 2(c)), so the family sample number containing 1255A variation is little, with only 38 individuals (Figure 2(a) and (b)), and the genotype descending in the three full-sib families abides by linkage genetic law (Figure 2). Only considering T1254C variation, 4 families were in *Aa*×*Aa* genotype mating model in F_1 generation, two families in *aa*×*Aa* model. χ^2 test shows that the genotype segregation rate of F_2 chickens in these 3 families conforms to 1:2:1 and 1:1 rule, respectively.



(a)

Genotype *AA*: 5′-TTGGAGACTT*TG* GAAGGTTT-3′

Genotype *aa*: 5′-TTGGAGACTT*CG* GAAGGTTT-3′

Genotype *Aa*: 5′-TTGGAGACTT*NG* GAAGGTTT-3′

Genotype *AC*: 5′-TTGGAGACTT*TG* GAAGGTTT-3′

5′-TTGGAGACTT*TA* GAAGGTTT-3′

Genotype *aC*: 5′-TTGGAGACTT*CG* GAAGGTTT-3′

5′-TTGGAGACTT*TA* GAAGGTTT-3′

(b)

Figure 1 Five PCR-SSCP genotypes detected by Exon16S and sequencing result of each genotype. (a) Five PCR- SSCP genotypes detected by Exon16S. (b) Sequencing results of each genotype (variations in italics). *AA* genotype contains two 1254T1255G single strands (allele A); *aa* genotype contains two 1254C1255G single strands (allele B); *Aa* genotype contains one 1254T1255G single strand and one 1254C1255G single strand; *AC* genotype contains one 1254T1255A single strand (allele C) and one 1254T1255G single strand; *aC* genotype contains one 1254T1255A single strand and one 1254C1255G single strand.

Table 4 Distribution of genotype detected by Exon16S and Intron5P in P generation and F_1 population

Exon16S				Intron5P			
Genotype	Silky	Broiler [a)]	F_1	genotype	Silky	Broiler[a)]	F_1
AA	23			*BB*	15		
Aa	2		35	*Bb*	1		20
AC	2			*bb*		20	
aC	1		2				
aa		36	2				

a) Here broiler refers in particular to White Plymouth Rock broiler.

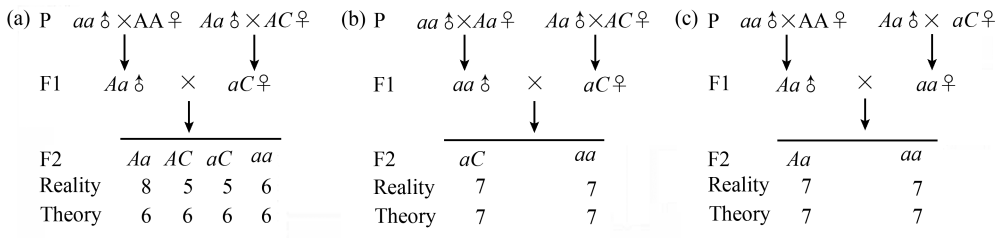


Figure 2 PCR-SSCP genotype descending model involving T1254C and G1255A variations in three full-sib families.

2.3 PCR-SSCP results of Intron5P

One 271 bp fragment was amplified from intron 5 of chicken *lmbr1* by primer Intron5P. Three genotypes were detected from 11 Silkies and 11 White Plymouth Rock broilers, and sequencing results showed that is was caused by C/A variation (Figure 3). Three same PCR-SSCP genotypes were further detected from P generation, F_1 and F_2 chickens of 5 families, only one P generation individual presented heterozygous genotype, while all F_1 individuals were heterozygous (Table 4), and genotype segregation rate of F_2 chickens conforms to 1:2:1 genetic segregation rule.

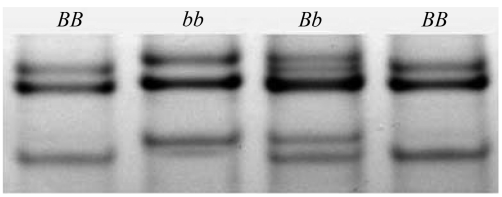
2.4 Genotype frequency, allele frequency and haplotype Stat. in F_2 population

The frequency of allele T and C of T1254C site was near 1:1 in F_2 population, where the frequency of allele A of G1255A site was extremely low (Table 5), which accords with the distribution in natural population (on cDNA level) and P generation population. χ^2 test showed that the allele frequency of T1254C and G1255A polymorphisms in F_2 population did not deviate clearly from Hardy-Weinberg equilibrium ($P = 0.19$).

The distance is about 31kb between A/C and T/C variation detected by Intron5P and Exon16S respectively. The frequency distribution of the haplotypes is shown in Table 6.

2.5 Association analysis of Exon16S PCR-SSCP genotypes with growth and carcass traits

(i) Only T1254C polymorphism being considered. According to the sequencing results, *AC* genotype is homologous TT at T1254C, which can be transformed to *AA* genotype; *aC* genotype is heterozygous at T1254C, which can be transformed to *Aa* genotype (Figure 1). The analysis of variance suggested that T1254C polymorphism was significantly associated with EYR and GR, extremely significantly associated with SCR and SG (Table 7), while not significantly associated with the other analyzed traits.



(a)

Genotype *BB*: 5′-ATGAGCTTT*A* ATTGCATGCT TTC-3′

Genotype *Bb*: 5′-ATGAGCTTT*N* ATTGCATGCT TTC-3′

Genotype *bb*: 5′-ATGAGCTTT*C* ATTGCATGCT TTC-3′

(b)

Figure 3 Three PCR-SSCP genotypes (a) detected by Intron5P and corresponding sequencing results (b), variations are shown in italics.

Table 5 Stat. on Allele frequencies and haplotype frequency of T1254C and G1255A polymorphisms of chicken *lmbr1* exon 16 in F_2 population

Variations	Sequencing genotypes	SSCP genotype of Exon16S	No.	Allele	Allele frequency	Haplotype [a)]	Haplotype frequency
T1254C	T/T	*AA*, *AC*	91	T	0.4664	TG	0.4501
	T/C	*AB*, *BC*	304			CG	0.5336
	C/C	*BB*	126	C	0.5336	TA	0.0163
G1255A(G/A)	G/G	*AA*, *Aa*, *aa*	504	G	0.9837		
	G/A	*AC*, *aC*	17				
	A/A		0	A	0.0163		

a) Base order of haplotype is as follows: T1254C and G1255A of exon16.

Table 6 Genotype and haplotype frequency detected by Exon16S and Intron5P

Intron5P genotype		Exon16S genotype		Haplotype		
SSCP (frequency)	sequencing	SSCP (frequency)	sequencing	type[a)]	number	frequency
BB(26.10%)	A/A	*AA*(17.47%)	T/T	AT	192	0.4211
Bb(56.40%)	A/C	*Aa*(58.35%)	T/C	CT	27	0.0592
bb(17.50%)	C/C	*aa*(24.18%)	C/C	CC	221	0.4846
				AC	16	0.0351

a) Base order of haplotype was as follows: A/C variation detected by Intron5P and T1254C detected by Exon16S.

Table 7 ANOVA results of Exon16S PCR-SSCP genotype (only considering T1254C polymorphism)[a)]

Genotype	Number	EYR[b)](%)	GR[b)] (%)	SCR[b)] (%)	SG[b)] (cm)
AA	85	62.93^{a}	2.21^{b}	6.43^{bB}	4.31^{bB}
Aa	272	63.47^{ab}	2.09^{ab}	6.29^{b}	4.17^{ab}
aa	126	63.83^{b}	2.00^{a}	5.97^{aA}	4.11^{aA}
F value		3.18	3.31	6.35	4.89
P value		0.0424^{*}	0.0374^{*}	0.0019^{**}	0.0079^{**}

a) The LSM difference between different small letters in column gains significant level ($P<0.05$); that between different big letters in column gains extremely significant level ($P<0.01$); that between same letters or no letter in column does not gain significant level; *, ** represent significant statistical model ($P<0.05$) and extremely significant model ($P<0.01$) respectively.

b) Abbreviation: EYR, eviscerated yield rate; GR, gizzard rate; SCR, shank and claw rate; SG, shank girth.

Test of difference of significance on those Least Square Means (LSM) of growth and carcass traits whose linear models gained significant level showed that LSM values of EYR trait follow the order *aa* >*Aa*>*AA*, and the LSM difference value between *aa* and *AA* genotype of EYR was 0.9%, which was 1.42% population mean; for GR, SCR and SG traits, all the LSM values follow the order *aa* < *Aa*< *AA,* and the LSM difference values between *aa* genotype and *AA* genotype were 0.21%, 0.46% and 0.2 cm (Table 7), which were 10.04%, 7.41% and 4.82% population mean of corresponding traits respectively. The lowest SCR, GR and SG value of *aa* genotype can explain its highest EYR value.

(ii) Combination analysis of T1254C and G1255A polymorphisms. Combination analysis of T1254C and G1255A polymorphisms detected by Exon16S showed that the statistic model for SCR and SG also gained extremely significant level; and the SCR and SG of homologous TG haplotype combination were extremely higher than that of homologous CG haplotype (Table 8).

2.6 Association analysis of Intron5P PCR-SSCP genotypes with growth and carcass traits

The analysis of variance showed that Intron5P PCR-SSCP genotype had extremely significant effect on SCR and Liver rate, significant effect on HNW, while no significant effect on other analyzed growth and carcass traits (Table 9). The difference of *BB* and *bb* genotypes was extremely significant for SCR, liver rate and HNW (Table 9).

C/A detected by Intron5P and T1254C variation detected by Exon16S constitute 7 haplotype combinations[8]. It was found that these haplotype combinations also had significant effect on SCR and HNW traits, and the SCR and HNW value of homologous AT/AT haplotype combination had an extremely significant difference from those of homologous CC/CC haplotype combination (Table 10).

Table 8 ANOVA results of haplotype combination detected by Exon16S(combination analysis of T1254C and G1255A)[a)]

Haplotype combination[b)]	Shank and claw rate (SCR)				Shank girth (SG)			
	mean(%)	number	*F* value	*P* value	mean(cm)	number	*F* value	*P* value
TG/TG	6.45^{bB}	81	3.83	0.0045^{**}	4.33^{cdB}	76	4.28	0.0021^{**}
TG/CG	6.27^{bB}	262			4.16^{bA}	248		
TA/CG	6.74^{b}	10			4.52^{d}	8		
TA/TG	6.06^{ab}	4			3.99^{bc}	4		
CG/CG	5.97^{aA}	126			4.11^{abA}	122		

a) The same as in Table 7. b) Base order of haplotype was as follows: T1254C and G1255A of exon16.

Table 9 ANOVA results of Intron5P PCR-SSCP genotype [a)]

Genotype	Shank-claw rate (SCR)		Liver rate		Head-neck weight (HNW)	
	mean(%)	number	mean (%)	number	mean (g)	number
BB	6.46^{B}	58	3.36^{cB}	58	133.2^{A}	58
Bb	6.33	188	3.18^{bA}	187	134.0^{A}	191
bb	6.11^{A}	87	3.01^{aA}	88	144.6^{B}	88
F value	10.95		4.93		4.17	
P value	0.0001**		0.0078**		0.0163*	

a) The same as in Table 7

Table 10 ANOVA results of haplotype combination identified by Exon16S and Intron5P [a)]

Haplotype combination[b)]	Shank-claw rate(SCR)				Head-neck weight(HNW)			
	mean (%)	number	*F* value	*P* value	mean(g)	number	*F* value	*P* value
AT/AT	6.60^{B}	33	2.49	0. 0239*	130.73^{aA}	33	2.53	0.0216*
AT/CT	6.23	17			133.18^{a}	17		
AT/AC	6.92^{B}	9			146.65	9		
AT/CC	6.36^{b}	102			136.90^{a}	102		
CT/CC	6.27	10			143.20	10		
CC/AC	6.36	7			149.20^{b}	7		
CC/CC	6.00^{aA}	50			146.32^{bB}	50		

a) The same as in Table 7. b) Base order of haplotype was as follows: C/A variation detected by Intron5P and T1254C of exon16.

2.7 Association analysis of toe phenotype with growth and carcass traits

F_2 chickens can be divided into four-toed and polydactyl groups by toe phenotype. The association analysis showed that the LSM value of four-toed group for 7 traits was significantly higher than that of the polydactyl group, including eviscerated yield with giblet (EYG), eviscerated yield (EY), breast muscle weight (BMW), head-neck weight (HNW), chest angle, wing weight and eviscerated yield rate (EYR). The LSM value of the four-toed group for shank and claw rate (SCR) was extremely lower than that of the polydactyl group (Table 11).

3 Discussions

In F_2 chickens, the LSM values of four-toed group presented significant difference from that of the polydactyl group in liver weight, eviscerated yield, eviscerated yield with giblet, breast muscle weight, HNW, chest angle, wing weight, EYR traits, and extremely significant difference in SCR traits. Polydactyl is a phenotype variation of chicken[9,10]. The significant association of toe phenotype with these growth and carcass traits gave us a hint that the key gene regulating chicken toe phenotype could be in close linkage disequilibrium with genes controlling these traits, and even controlled by the same gene.

Lmbr1 was the critical candidate gene of human and mouse PPD mutant, playing an important role in limb development. The special deletion of exon 4 and surrounding ≈5kb intron sequence were detected from Human ACHP (deletion of distal limb)[4], and it was found that drastic changes of *lmbr1* expression occur at the same time as the first limb abnormalities seen in Hx mutant mice [2].

Table 11 LSM test of the significance of difference for growth and carcass traits between four-toed and polydactyl groups of F_2 chicken[a)]

Toe phenotype	EYG[b)](g)	EY(g)	BMW(g)	HNW(g)	Chest angle(°)	Wing weight(g)	SCR(%)	EYR(%)
Four-toed (170)	1198.6^{b}	1091.2^{b}	94.8^{b}	137.8^{b}	68.5^{b}	67.9^{b}	5.99^{A}	63.79^{b}
Polydactyl (303)	1151.8^{a}	1043.6^{a}	90.3^{a}	130.8^{a}	67.2^{a}	64.8^{a}	6.35^{B}	63.28^{a}
P value	0.030*	0.017*	0.04*	0.01*	0.013*	0.021*	0.0003**	0.036*

a) The same as in Table 7. b) Abbreviation: EYG, eviscerated yield with giblet ; EY, eviscerated yield; BMW, breast muscle weight ; HNW, head-neck weight; SCR, shank and claw rate; EYR, eviscerated yield rate.

In this experiment, *lmbr1* gene was taken as the candidate gene regulating chicken growth and carcass traits. It was found that T1254C polymorphism located in exon 16 was significantly (or extremely significantly) associated with EYR, GR, SCR and SG; C/A variation located in intron 5 was significantly (or extremely significantly) associated with SCR, liver rate and HNW; and the combination effects of T1254C and C/A variations on SCR and HNW also gain significant level. It demonstrates that chicken *lmbr1* is the main QTL or in tight linkage disequilibrium with these traits. EYR is the important carcass trait, which is the ratio of eviscerated yield with giblet removing heart, liver, head and neck, wing, shank and claw, gizzard, glandular stomach and abdominal fat to live weight. EYR of *aa* genotype was significantly higher than that of *AA* genotype, while GR, SCR and SG of *BB* genotype were all significantly (or extremely significantly) lower than that of *AA* genotype. The lowest LSM value of *aa* genotype in GR, SCR and SG traits can explain its highest LSM value in EYR (Table 7). The results indicate that pure C/C genotype (corresponding to *aa* PCR-SSCP genotype here) at T1254C is the advantageous genotype of these traits, which is promising to be applied to Marker Assistant Selection of these traits in chicken breeding. Further study is needed to confirm the association of *lmbr1* polymorphisms with some growth and carcass traits from bigger/more population.

It was interesting that both SCR and SG traits were related to metatarsus development. In this experiment, T1254C polymorphism showed significant association with SCR and SG traits. Exon 16 polymorphisms, intron 5 polymorphism and their haplotype combinations were all closely associated with SCR, which should be an indirect proof of *lmbr1* affecting chicken limb development. SIFT program predicted that G1255A variation could affect protein function, which suggested that G1255A variation might play a potential important role. Although the LSM value of TA/CG haplotype combination for SCR and SG traits was significantly higher than that of CG/CG haplotype combination (Table 8), it needed to further confirm whether the interaction of T1254C and G1255A polymorphisms had bigger effect, for less individuals with TA/CG haplotype combination involved in the population.

4 Conclusions

Silky is a famous Chinese medicinal breed, while White Plymouth Rock is a fast growth broiler breed. They have obvious difference in many phenotype traits, growth and carcass traits etc. Their F_2 crossing chickens also produce abundant traits separation, which provides a good material for this experiment. Some growth and carcass traits had significant difference between four-toed and polydactylous chickens of F_2 generation. By PCR-SSCP and sequencing methods, T1254C variation of exon 16 and one intron 5 polymorphism of chicken *lmbr1* were detected from F_2 chickens, and it was found that exon 16 polymorphism of chicken *lmbr1* was significantly or extremely significantly associated with EYR, GR, SCR and SG; intron 5 polymorphism was significantly or extremely significantly associated with SCR, liver rate, HNW; haplotype combination of two variations also showed significant or extremely significant association with SCR and HNW, which indicated that *lmbr1* was the main QTL controlling these traits or in close linkage with them. The genotype corresponding to Pure C/C at T1254C should be the advantageous genotype of these traits, which was a potential molecular marker for genotype selection of broiler carcass traits.

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