Novel 61-BP Indel of the RIN2 Gene is Significantly Associated with Chicken Growth and Carcass Traits

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Abstract

Background: Ras and Rab interactor 2 (*RIN2*) gene, encoding RAS and Rab interacting protein 2, can interact with GTP-bound Rab5 and participate in early endocytosis. Deletion of *RIN2* may impair Rab5-related endosome signaling, leading to abnormal phenotypes. However, no research has been reported on the functions of *RIN2* related to animal production.

Results: A 61-bp insertion/deletion (indel) in the *RIN2* intron region in this study. The genotype analysis of mutation sites was performed on 550 individuals from 7 different chicken breeds, and it was found that the indel exists in each breed and the local breed chickens are mainly *DD* genotypes.

Correlation analysis of the indel with growth traits and carcass traits of the F_2 population of Xinghua and White Recessive Rock chicken showed that the *RIN2* 61-bp deletion mutation site was significantly correlated with abdominal fat weight, fat width and hatching weight traits ($P < 0.05$). *RIN2* mRNA was expressed in all test tissues, and the expression level of abdominal fat was higher than that in other tissues. In addition, it was further found that the expression level of type *II RIN2* mRNA in abdominal fat was significantly different from that of *ID* type and *DD* type ($P < 0.05$).

Conclusion: The results showed that the mutation was closely related to the abdominal fat-related and hatching weight traits of chickens, which may have certain reference value for molecular marker-assisted selection of chickens.

Background

Ras and Rab interactor 2 *(RIN2*), known as RAS and Rab interacting protein 2, functions as a guanine nucleotide exchange factor (GEF). *RIN2* is shown to interact with Rab5, a small GTPase that participates in early endocytosis [1, 2]. Rab5 is necessary for transport of endocytic vesicles to early endosomes [2]. Deletion of *RIN2* may impair Rab5-related endosome signaling, and may also damage secreted proteins from endoplasmic reticulum to Golgi or Golgi to plasma membrane, leading to collagen fiber structure and observed phenotypic abnormalities [3].

Previous research has shown that *RIN2* syndrome in humans is also called MACS (macrocephaly, alopecia, cutis laxa and scoliosis) syndrome that is a rare hereditary skin disease caused by the loss of the 1-bp homozygote of *RIN2* [3, 4]. A genome-wide selective scan of purebred horses showed that *RIN2* plays similar roles in signal transmission, indicating that the *RIN2* gene is under strong artificial selection in horse racing [5]. Comparative analysis of the genome-wide methylation and transcriptome of the longest muscle in the sheep showed that *RIN2* may be a functional gene that affects meat quality traits [6]. However, no research has been reported on the functions of *RIN2* related to animal production, and the exact functional mechanism in chickens is unclear.

Indel is another form of mutation, and there are a large number of indel polymorphisms in the genome of model organisms [7]. Indel plays an important role in genetic diversity and phenotypic differentiation [8,

9]. Compared with single nucleotide polymorphisms (SNP), indel variants have the advantages of convenient detection and significant effects, and have higher efficiency and wider application prospects [10]. In poultry, the repeat indel in the promoter region of the *CDKN3* gene is significantly related to the economic traits of chickens [11]. The 86-bp indel in the *MLNR* gene downstream region and the two indel variants in the *QPCTL* gene promoter region are both related to chicken growth and carcass traits [12, 7]. An 80-bp indel in *PRLR* was associated with growth traits [13]. A new indel in the promoter region of the *TGFB2* gene is related to body weight at almost all stages [14]. In cattle, a new 19-bp indel in *PLAG1* is related to the growth traits of breed cattle, and a 12-bp indel in *NPM1* was associated with growth traits [15, 16].

In this study, a 61-bp deletion in the intron region of the *RIN2* gene was identified by DNA sequencing and polymerase chain reaction (PCR) analysis. A total of 550 individuals from F_2 resource groups, white feather broilers and local breed chickens, were tested for genotype. The correlations between polymorphisms in F_2 resource populations of Xinghua-White Recessive Rock chicken hybrids and meat quality, growth traits and carcass traits were analyzed. Differences in *RIN2* mRNA levels were investigated in the abdominal fat and breast muscle. The purpose of this study is to clarify the relationship between *RIN2* gene variation and chicken performance traits, and to explore whether *RIN2* gene can be used as a molecular marker for selecting production traits.

Materials And Methods

F_2 resource population

Based on the F_2 resource group (N = 304) with meat quality, growth and carcass traits recorded in this laboratory (Xinghua and White Recessive Rock chicken full sib hybrid F_2 generation, of which Xinghua chicken is a Chinese local slow-growing breed, White Recessive Rock chicken is a fast-growing broiler), and the correlation analysis of *RIN2* was performed. More information on the F_2 population was provided by a previous study [17].

Sample collection

To confirm the distribution of this genotypic variation in chickens of other breeds, genomic DNA samples were collected from a total of 267 healthy individuals. The number of samples contributed by each group was as follows: White Recessive Rock chickens (WRR, n = 41), Wenchang chickens (WC, n = 48), Qingjiaoma Chicken (QJ, n = 48), Lushi chickens (CS, n = 40), Guangxi yellow chickens (GX, n = 46) and Gushi chickens (GS, n = 44).

In addition, in order to detect the expression of *RIN2* mRNA in different tissues, a total of 12 tissues (hearts, liver, spleen, lung, kidney, breast muscle, leg muscle, abdomen fat, jejunum, duodenum, hypothalamus and ovary) were collected from 24-week-old Yellow chickens. Different genotypes of

abdominal fat and breast muscle were taken from four-week-old Yellow chickens to detect the expression of *RIN2* mRNA in different genotypes. All tissues were stored at -80 °C.

Genomic DNA Extraction and PCR

The genomic DNA in the blood was extracted using the DNA extraction kit (Omega, Norcross, America), and the quality of the genome was detected by a spectrophotometer. All primers were designed using online tools provided by NCBI (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Beijing TsingKe Company (Supplementary Appendix Table 1). PCR was performed in a total volume of 10 μL, including 1 μL of DNA, 0.2 μL of each primer, 5 μL of 2 × M5 PCR Mix (Yuexing, Guangzhou, China), and 3.6 μL of water. The cycle parameters are: 95 °C for 3 min, 95 °C for 25 s, 61 °C for 25 s, 72 °C for 15 s, 72 °C extension for 5 min, a total of 34 cycles, and then refrigerated at 4 °C. An aliquot (7 μL) of each reaction was electrophoresed on a 2% agarose gel to determine the genotype.

Table 1
Genetic parameters of 61-bp locus within RIN2 gene in seven chicken breeds.

Breeds	Number	Genotype and Gene frequency					He	Ne	PIC	*P*-value
		II	*ID*	*DD*	*I*	*D*				
F_2	283	0.110	0.350	0.541	0.331	0.735	0.219	1.280	0.195	1.000
WRR	41	0.415	0.390	0.195	0.644	0.442	0.053	1.056	0.051	0.811
WC	48	0.000	0.063	0.938	0.032	0.968	0.476	1.908	0.363	0.249
QJ	48	0.000	0.125	0.875	0.067	0.935	0.061	1.064	0.059	1.000
LS	40	0.050	0.200	0.750	0.224	0.866	0.117	1.133	0.110	0.644
GX	46	0.000	0.109	0.891	0.058	0.944	0.053	1.056	0.051	0.811
GS	44	0.000	0.250	0.750	0.144	0.866	0.103	1.115	0.098	0.697

Note: He represents gene heterozygosity, Ne represents effective allele numbers, Polymorphism information content (*PIC*). F_2 generation resource population(F_2), White Recessive Rock chicken(WRR), Wenchang chicken(WC), Qingjiaoma Chicken(QJ), Lushi chickens(LC), Guangxi Yellow chicken(GX), Gushi chicken(GS), *P*-value of the Hardy-Weinberg equilibrium (*P*-value).

RNA isolation, cDNA synthesis, and qPCR

Use Trizol® reagent to extract total RNA from tissues. Quantitative real-time polymerase chain reaction (qPCR) was used to detect the expression level of *RIN2* mRNA in each tissue. qPCR was performed using the CFX96 system (Bio-Rad, Hercules, CA, USA). The *β-actin* gene was used as an internal control. qPCR conditions were as follows: 95 °C for 5 min, 95 °C for 30 s, 60 °C for 30 s,72 °C for 30 s and a total of 35 cycles. The results were analysed using the $2^{-\Delta\Delta CT}$method [18].

Statistical Analysis

Statistical analysis of all sequence variations and important economic traits related to the F_2 resource group was performed using SPSS Statistics 24 software. The mixed linear models used in the analysis are:

$$\text{Model I}: Y_{ijklm} = \mu + G_i + S_j + H_k + f_l + e_{ijklm}$$

$$\text{Model II}: Y_{ijklm} = \mu + G_i + S_j + H_k + f_l + b(W_{ijklm} - \bar{W}) + e_{ijklm}$$

where Y_{ijklm} is the observed value, μ is the overall average, G_i is the genotype fixed effect, f_l is the family fixed effect, S_j is the gender fixed effect, H_k is the hatching fixed effect, b is the carcass weight regression coefficient, $\bar{W}$ is the average slaughter weight, W_{ijklm} for individual slaughter weight, e_{ijklm} is random error. *P*-value < 0.05 was considered significant, and a Bonferroni's test was performed to control multiple comparisons [19]. Model I is used to assess genotypes related to growth traits, meat quality, and blood biochemical indicators. Considering the effect of body weight on carcass traits, Model II uses carcass weight as a covariate to calculate carcass traits.

Results

Identification of genetic variants correlated with RIN2 expression

Through whole-genome resequencing, a new 61-bp deletion mutation was found downstream of the *RIN2* gene. As shown in Fig. 1, the Indel polymorphism was analyzed by PCR amplification of the region and electrophoresis of the product in a 2.0% agarose gel. Named *II* (646 bp), *ID* (646 bp and 585 bp), and *DD* (585 bp), respectively. The PCR products were sequenced to pinpoint the insertion (Fig. 2).

Genetic parameters of RIN2 among F_2 Resource Populations and Different Varieties

The genotype and allele frequencies, and other genetic parameters associated with the *RIN2* Indel locus were calculated for 550 individuals in the study (Table 1). All seven varieties had a *RIN2* 61-bp deletion mutation, and with the exception of WRR, the allele frequency of *D* was higher than that of *I*. The genotype distribution of different populations is shown in Fig. 3.

By convention, $PIC > 0.5$ is highly polymorphic, $0.25 \leq PIC \leq 0.5$ is moderate polymorphism, and $PIC < 0.25$ is low polymorphism. Except for WC showing moderate polymorphism, the other groups showed low polymorphism. The degree of genetic heterozygosity is 0.053–0.476, and the number of effective alleles is 1.056–1.908.

Differential selection of the 61 bp indel locus

In order to determine whether the differential selection of *RIN2* 61-bp insertion sequence occurred during domestication of chickens, the pairwise fixed index (Fst value) was used to analyze the differentiation between populations. The analysis showed that the *RIN2* 61-bp site deletion type had strong genetic differentiation between WC, QJ, LS, GX, GS, and WRR ($0.2 < Fst < 0.5$; Table 2), indicating that the deletion mutation was in WRR already selected. FST values are usually lower among other breeds.

Table 2
Pairwise fixation index (Fst) of RIN2 gene in various chicken breeds.

Breeds	WRR	WC	QL	LS	GX	GS
WC	0.3977					
QJ	0.3451	0.0055				
LS	0.2236	0.0448	0.0207			
GX	0.3556	0.0033	0.0003	0.0256		
GS	0.2550	0.0312	0.0116	0.0013	0.0154	
F_2	0.0533	0.0427	0.0324	0.0100	0.0338	0.0153

Note: F_2 generation resource population(F_2), White Recessive Rock chicken (WRR), Wenchang chicken(WC), Qingjiaoma Chicken(QJ), Lushi chickens(LC), Guangxi Yellow chicken(GX), Gushi chicken(GS).

Association of the 61-bp indel of the RIN2 gene with chicken carcass traits

In the F_2 population, the 61-bp indel of the *RIN2* gene was significantly associated with slaughter performance. Significant correlations were detected in abdominal fat weight and fat width traits ($P = 0.046$, $P = 0.005$; Table 3). Among them, abdominal fat weight and fat width traits of *DD* genotypes were greater than those in chickens with the *ID* and *II* genotypes. There was no significant difference between *ID* and *II* individuals. The 61-bp indel was not significantly associated with other carcass traits (Supplementary Appendix Table 2).

Table 3
Effect of RIN2 gene polymorphisms on carcass traits in the reciprocal cross F2 population.

Traits	Mean ± SE			*P*-value
	II	*ID*	*DD*	
FW(mm)	10.262 ± 0.678[a]	11.455 ± 0.439[a]	12.539 ± 0.354[b]	0.005
AFW(g)	21.070 ± 3.893[a]	24.757 ± 2.876[a]	29.795 ± 2.492[b]	0.046
SFT(mm)	3.647 ± 0.243	4.090 ± 0.154	4.208 ± 0.123	0.100

Note: fat width(FW), abdominal fat weight(AFW), subcutaneous fat thickness(SFT), Different lowercase letters of the means superscript show significant differences ($P < 0.05$), the same letters show no difference ($P > 0.05$).

Association of the 61-bp indel of the RIN2 gene with chicken growth and meat quality traits

In the F_2 population, the 61-bp indel of the *RIN2* gene was significantly related to hatching weight, with a correlation of $P = 0.027$ (Table 4). Among them, hatching weight traits of *II* genotypes were greater than those in chickens with the *ID* and *DD* genotypes. There was no significant difference between *ID* and *DD* individuals. Other growth traits were not significantly associated with the indel (Supplementary Appendix Table 3). The 61-bp indel in the *RIN2* intron was not significantly associated with meat quality traits (Supplementary Appendix Table 4).

Table 4
Effect of RIN2 polymorphisms on growth traits in the reciprocal cross F2 population.

Traits	Age week	Mean ± SE			*P*-value
		II	*ID*	*DD*	
	0	30.70 ± 0.56[a]	29.82 ± 0.48[b]	29.79 ± 0.46[b]	0.027
	1	60.52 ± 1.73	58.77 ± 1.29	59.50 ± 1.13	0.403
Body weight	2	125.12 ± 3.65	123.66 ± 2.70	122.84 ± 2.34	0.810
(g)	3	213.71 ± 6.57	209.35 ± 4.81	208.37 ± 4.11	0.685
	4	311.02 ± 10.29	308.69 ± 7.52	307.17 ± 6.41	0.930
	5	455.47 ± 15.05	433.91 ± 10.41	430.24 ± 8.82	0.218

Note: Different lowercase letters of the means superscript show significant differences ($P < 0.05$), the same letters show no difference ($P > 0.05$).

Expression of RIN2 in chickens and molecular characterization

Chicken *RIN2* gene is on chromosome 3 (GenBank accession number NC_006090.5),It consists of 20 exons and encodes a protein of 836 amino acids. The expression of *RIN2* mRNA in various tissues of 171-day-old Yellow chickens was studied (Fig. 4), and the results showed that *RIN2* was expressed in all test tissues, with the highest expression level in abdominal fat. The expression levels in heart, liver, lung, kidney, and hypothalamus are moderate. In contrast, the expression levels were lower in spleen, breast muscle, leg muscle, jejunum, and duodenum. The high expression of *RIN2* in abdominal fat suggested that *RIN2* may play a role in the formation of abdominal fat.

Relative Expression of Different Genotypes and of the RIN2 Gene

In abdominal fat, 22-week-old Xinghua bantam chicken type *II RIN2* mRNA expression level was significantly different from *ID* type and *DD* type ($P < 0.05$; Fig. 5a). This indicates that the 61-bp indel site affects the expression of *RIN2* and may affect slaughter traits such as abdominal fat weight. Therefore, it is important to examine the relationship between 61-bp indel sites and growth and slaughter traits in a larger chicken population. There was no significant difference in the expression of *RIN2* mRNA between different genotypes in breast muscle (Fig. 5b).

Discussion

In this study, we speculate that *RIN2* 61-bp indel may have a positive effect on the growth traits of chickens and a negative effect on the abdominal fat weight of chickens. This Indel may be a potential molecular marker for auxiliary selection of good quality broilers.

In animal breeding, the discovery of key genes and molecular mechanisms that affect growth traits is an important step to improve breeding efficiency and speed up the breeding process [20, 21]. In order to improve the selection effect of main traits, traditional selection methods can be complemented by gene-assisted selection or molecular marker-assisted selection (MAS). MAS is an effective way to improve short-term traits [22, 23]. In this study, a new 61-bp deletion mutation was identified on the *RIN2* gene through whole genome resequencing and PCR product sequencing. In the genetic analysis of 550 individuals of 7 varieties, it was found that there was a deletion mutation of *RIN2* 61-bp in all varieties. No functional studies on *RIN2* gene in animal production have been reported.

Hatching weight is the main indicator of chick quality evaluation [24]. Previous studies have shown that there is a positive correlation between the hatching weight of broilers and the weight of slaughter. For every 1 g increase in hatching weight, the slaughter weight increases by 8–13 g [25, 26]. The economic value of high-hatching weight broilers is also generally higher than that of low- hatching weight broilers

The abdomen is an important part of the fat deposition in chickens. Abdominal fat weight is highly related to the total body fat deposition in chickens and can be used as an index for selecting chicken fat deposition. Previous studies have found that excessive fat deposits in modern commercial broiler breeds can waste a lot of feed, while reducing slaughter rates and economic benefits [28, 29]. For consumers, eating broilers that accumulate too much fat may also cause human obesity or cause other diseases [30]. Therefore, the excessive deposition of abdominal fat in chickens has become one of the problems to be solved in the current broiler production.

In this study, we found that *RIN2* gene was expressed in different tissues (Fig. 4), which is consistent with previous reports of widespread *RIN2* expression [31]. In addition, *RIN2* gene is highly expressed in abdominal fat, kidney, and heart, suggesting that it may be related to fat deposition and growth. The *RIN2* 61-bp deletion mutation site was significantly negatively correlated with chicken hatching weight ($P < 0.05$, Table 4). The hatching weight of type *II* individuals was greater than that of *ID* type and *DD* type. During the pre-growth period (1–7 weeks), the weight of the genotype *II* has always been the highest, while the weight of the *DD* genotype has generally been the lowest. Analysis of the gene frequency of 7 different chicken breeds revealed that the 61-bp deletion mutation has been highly selected in WRR (Table 1). We speculate that in terms of growth performance, *RIN2* 61-bp indel type *II* is the dominant genotype, and *DD* type is the inferior genotype. The 61-bp deletion of *RIN2* may have a positive effect on chicken growth traits.

It is worth noting that the expression of *RIN2* gene is highest in abdominal fat tissue. Quantitative analysis of *RIN2* mRNA expression in abdominal fat tissue of different genotypes showed that the expression level of *II* was significantly higher than *ID* and *DD* ($P < 0.05$; Fig. 5a). Correlation analysis found that abdominal fat weight of type *II* individuals was significantly lower than that of *ID* and *DD* individuals ($P < 0.05$, Table 3). Based on the above results, it can be speculated that *RIN2* 61-bp indel is involved in the deposition of fat in the abdomen, which may have a negative effect on the fat traits of chickens, but the specific reason is unknown.

Conclusions

The results of this study indicate that the 61-bp intron of the *RIN2* gene is associated with slaughter and abdominal fatness traits in chickens. *RIN2* mRNA is expressed in all tissues, but the expression level in abdominal fat is higher. Identification of indel related to growth traits, carcass traits, and abdominal fat weight indicates that the indel may be a useful marker in poultry breeding and related QTL identification studies.

Declarations

Ethics approval and consent to participate

All animal experiments performed in this study comply with the requirements of the Institutional Animal Protection and Utilization Committee of South China Agricultural University (approval ID: SCAU # 0014), and the care and use of animals are in compliance with local animal welfare laws.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

These studies were designed by WJL and THR. They conducted experimental analysis and prepared charts. WJL and XQZ analyzed the data and drafted the manuscript. WYL and JYL contributed to the revision of the manuscript. MJX, MQL, DLH, SDL and WL helped to explain the results and revised the final version of the manuscript. All authors read and approved the final manuscript for publication.

References

1. Grosshans BL, Ortiz D, Novick P. Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A. 2006;103(32):11821–7.
2. Saito K, Murai J, Kajiho H, Kontani K, Kurosu H, Katada T. A novel binding protein composed of homophilic tetramer exhibits unique properties for the small GTPase Rab5. J Biol Chem. 2002;277(5):3412–8.
3. Syx D, Malfait F, Van Laer L, Hellemans J, Hermanns-Lê T, Willaert A, Benmansour A, De Paepe A, Verloes A. The RIN2 syndrome: a new autosomal recessive connective tissue disorder caused by deficiency of Ras and Rab interactor 2 (RIN2). Hum Genet. 2010;128(1):79–88.
4. Kameli R, Ashrafi MR, Ehya F, Alizadeh H, Hosseinpour S, Garshasbi M, Tavasoli AR. Leukoencephalopathy in RIN2 syndrome: Novel mutation and expansion of clinical spectrum. Eur J Med Genet. 2020;63(1):103629.
5. Moon S, Lee JW, Shin D, Shin KY, Kim J, Choi IY, Kim J, Kim H. A Genome-wide Scan for Selective ci. 2015;28(11):1525–31.

6. Sandri C, Caccavari F, Valdembri D, Camillo C, Veltel S, Santambrogio M, Lanzetti L, Bussolino F, Ivaska J, Serini G. The R-Ras/RIN2/Rab5 complex controls endothelial cell adhesion and morphogenesis via active integrin endocytosis and Rac signaling. Cell Res. 2012;22(10):1479–501.
7. Ren T, Li W, Liu D, Liang K, Wang X, Li H, Jiang R, Tian Y, Kang X, Li Z. Two insertion/deletion variants in the promoter region of the QPCTL gene are significantly associated with body weight and carcass traits in chickens. Anim Genet. 2019;50(3):279–82.
8. Rao YS, Wang ZF, Chai XW, Wu GZ, Nie QH, Zhang XQ. Indel segregating within introns in the chicken genome are positively correlated with the recombination rates. Hereditas. 2010;147(2):53–7.
9. Yan Y, Yi G, Sun C, Qu L, Yang N. Genome-wide characterization of insertion and deletion variation in chicken using next generation sequencing. PLoS One. 2014;9(8):e104652.
10. Li J, Zhu X, Ma L, Xu H, Cao X, Luo R, Chen H, Sun X, Cai Y, Lan X. Detection of a new 20-bp insertion/deletion (indel) within sheep PRND gene using mathematical expectation (ME) method. Prion. 2017;11(2):143–50.
11. Li W, Liu D, Tang S, Li D, Han R, Tian Y, Li H, Li G, Li W, Liu X, Kang X, Li Z. A multiallelic indel in the promoter region of the Cyclin-dependent kinase inhibitor 3 gene is significantly associated with body weight and carcass traits in chickens. Poult Sci. 2019;98(2):556–65.
12. Liu D, Han R, Wang X, Li W, Tang S, Li W, Wang Y, Jiang R, Yan F, Wang C, Liu X, Kang X, Li Z. A novel 86-bp indel of the motilin receptor gene is significantly associated with growth and carcass traits in Gushi-Anka F_2 reciprocal cross chickens. Br Poult Sci. 2019;60(6):649–58.
13. Liang K, Wang X, Tian X, Geng R, Li W, Jing Z, Han R, Tian Y, Liu X, Kang X, Li Z. Molecular characterization and an 80-bp indel polymorphism within the prolactin receptor (PRLR) gene and its associations with chicken growth and carcass traits. 3 Biotech. 2019;9(8):296.
14. Tang S, Ou J, Sun D, Zhang Y, Xu G, Zhang Y. A novel 62-bp indel mutation in the promoter region of transforming growth factor-beta 2 (TGFB2) gene is associated with body weight in chickens. Anim Genet. 2011;42(1):108–12.
15. Xu W, He H, Zheng L, Xu JW, Lei CZ, Zhang GM, Dang RH, Niu H, Qi XL, Chen H, Huang YZ. Detection of 19-bp deletion within PLAG1 gene and its effect on growth traits in cattle. Gene. 2018;675:144–9.
16. Huang YZ, Zhang EP, Chen H, Wang J, Li ZJ, Huai YT, Ma L, Lan XY, Ren G, Lei CZ, Fang XT, Wang JQ. Novel 12-bp deletion in the coding region of the bovine NPM1 gene affects growth traits. J Appl Genet. 2010;51(2):199–202.
17. Lei MM, Nie QH, Peng X, Zhang DX, Zhang XQ. Single nucleotide polymorphisms of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. Poult Sci. 2005;84(8):1191–8.
18. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101–8.
19. Ren T, Zhou Y, Zhou Y, Tian W, Gu Z, Zhao S, Chen Y, Han R, Liu X, Kang X, Li Z. Identification and association of novel lncRNA pouMU1 gene mutations with chicken performance traits. J Genet.

20. Zhang S, Han RL, Gao ZY, Zhu SK, Tian YD, Sun GR, Kang XT. A novel 31-bp indel in the paired box 7 (PAX7) gene is associated with chicken performance traits. Br Poult Sci. 2014;55(1):31–6.
21. Li ZH, Li H, Zhang H, Wang SZ, Wang QG, Wang YX. Identification of a single nucleotide polymorphism of the insulin-like growth factor binding protein 2 gene and its association with growth and body composition traits in the chicken. J Anim Sci. 2006;84(11):2902–6.
22. Han XL, Xu XW, Liu B. Molecular Characteristics of the Porcine MUSTN1 Gene and its Significant Association with Economic Traits. Journal of Animal Veterinary Advances. 2010;9(18):2351–6.
23. Sodhi SS, Jeong DK, Sharma N, Lee JH, Kim JH, Kim SH. Marker assisted selection-applications and evaluation for commercial poultry breeding. korean journal of poultry science. 2013;40(3):223–34.
24. Nitsan Z, Dunnington E, Siegel P. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. Poult Sci. 1991;70(10):2040–8.
25. Mendes AS, Paixao SJ, Restelatto R, Reffatti R, Possenti JC, De MD. Effects of initial body weight and litter material on broiler production. Revista Brasileira De Cincia Avcola. 2011;13(3):165–70.
26. Singh PM, Nagra S. Effect of day-old chick weight and gender on the performance of commercial broiler. Indian Journal of Animal Sciences. 2006;76(12):1043–6.
27. Leandro NSM, Cunha WCP, Stringhini JH, Cruz CP, Matos MS. Effect of broiler chicken initial weight on performance, carcass yield and economic viability. Revista Brasileira de Zootecnia. 2006;35(6):2314–21.
28. Zhang T, Zhang X, Han K, Zhang G, Wang J, Xie K, Xue Q. Genome-Wide Analysis of lncRNA and mRNA Expression During Differentiation of Abdominal Preadipocytes in the Chicken. G3 (Bethesda). 2017;7(3):953–66.
29. Lingyun Y, Huiyi C, Haijie Y, Shu Z, Zhiyun L. Abdominal fat deposition curve of male AA broilers. Feed Industry. 2015;36(13):25–9.
30. Khatun J, Loh TC, Akit H, Foo HL, Mohamad R. Fatty acid composition, fat deposition, lipogenic gene expression and performance of broiler fed diet supplemented with different sources of oil. Anim Sci J. 2017;88(9):1406–13.
31. Wang Y, Colicelli J. RAS interaction with effector target RIN1. Methods Enzymol. 2001;332:139–51.

Figures

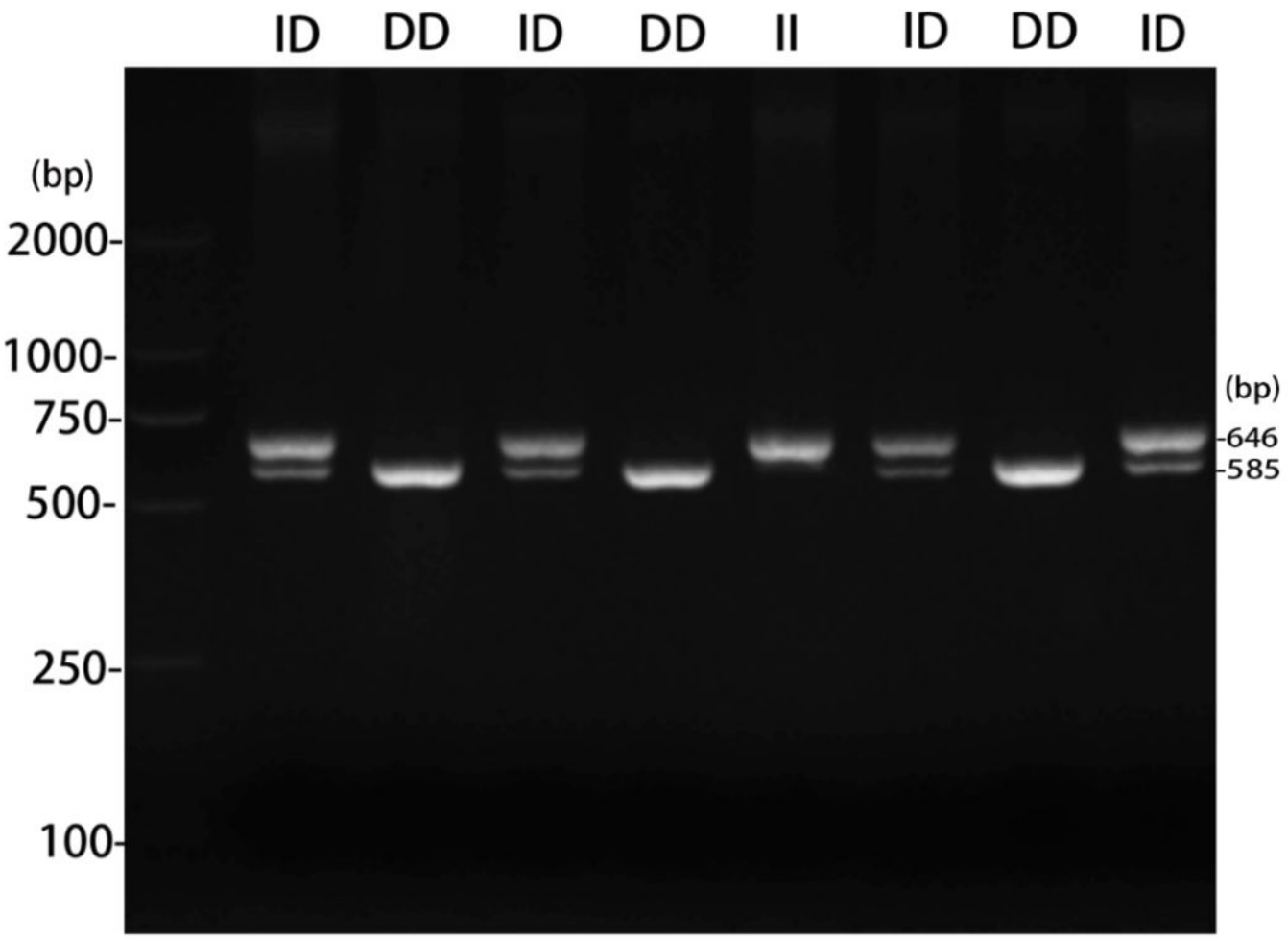

Figure 1

Agarose gel electrophoresis pattern for the RIN2 61-bp indel polymorphism.

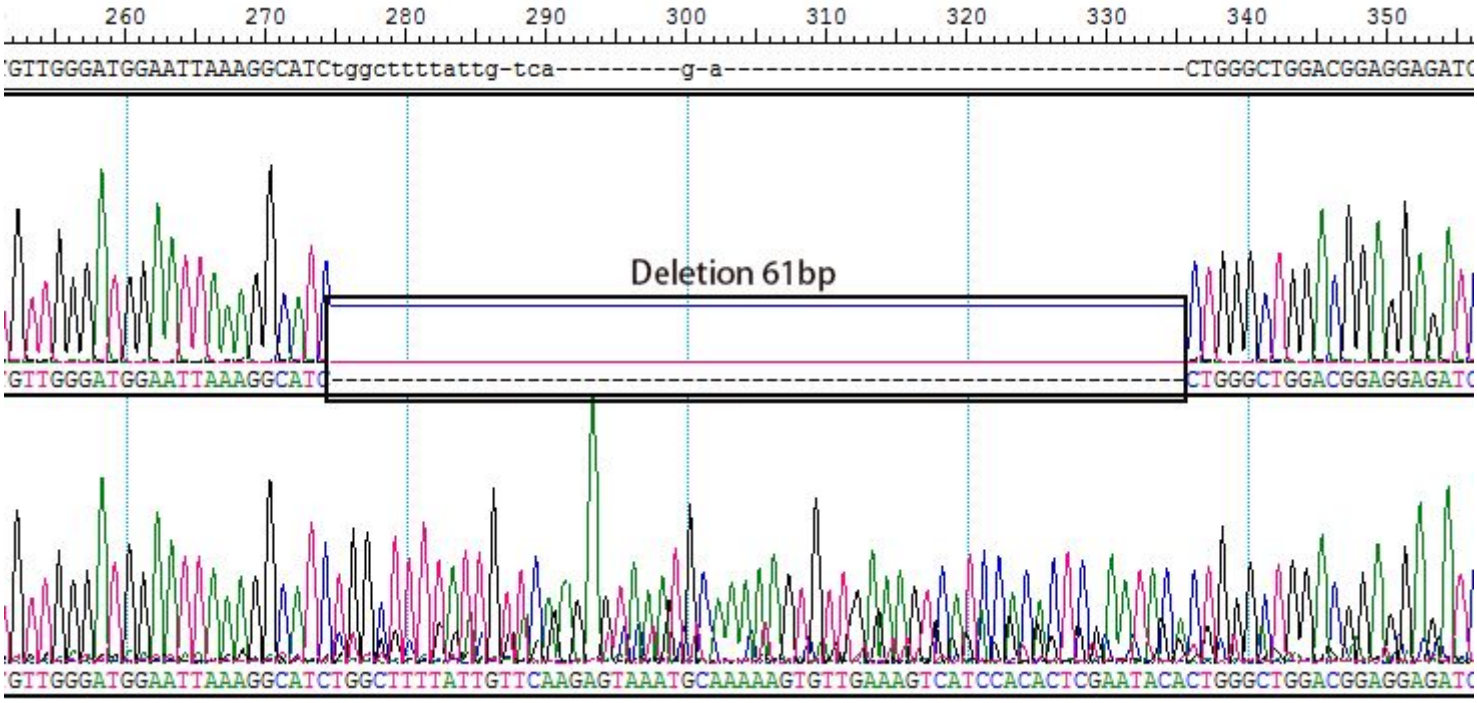

Figure 2

The sequence diagrams of chicken RIN2 61-bp indel locus

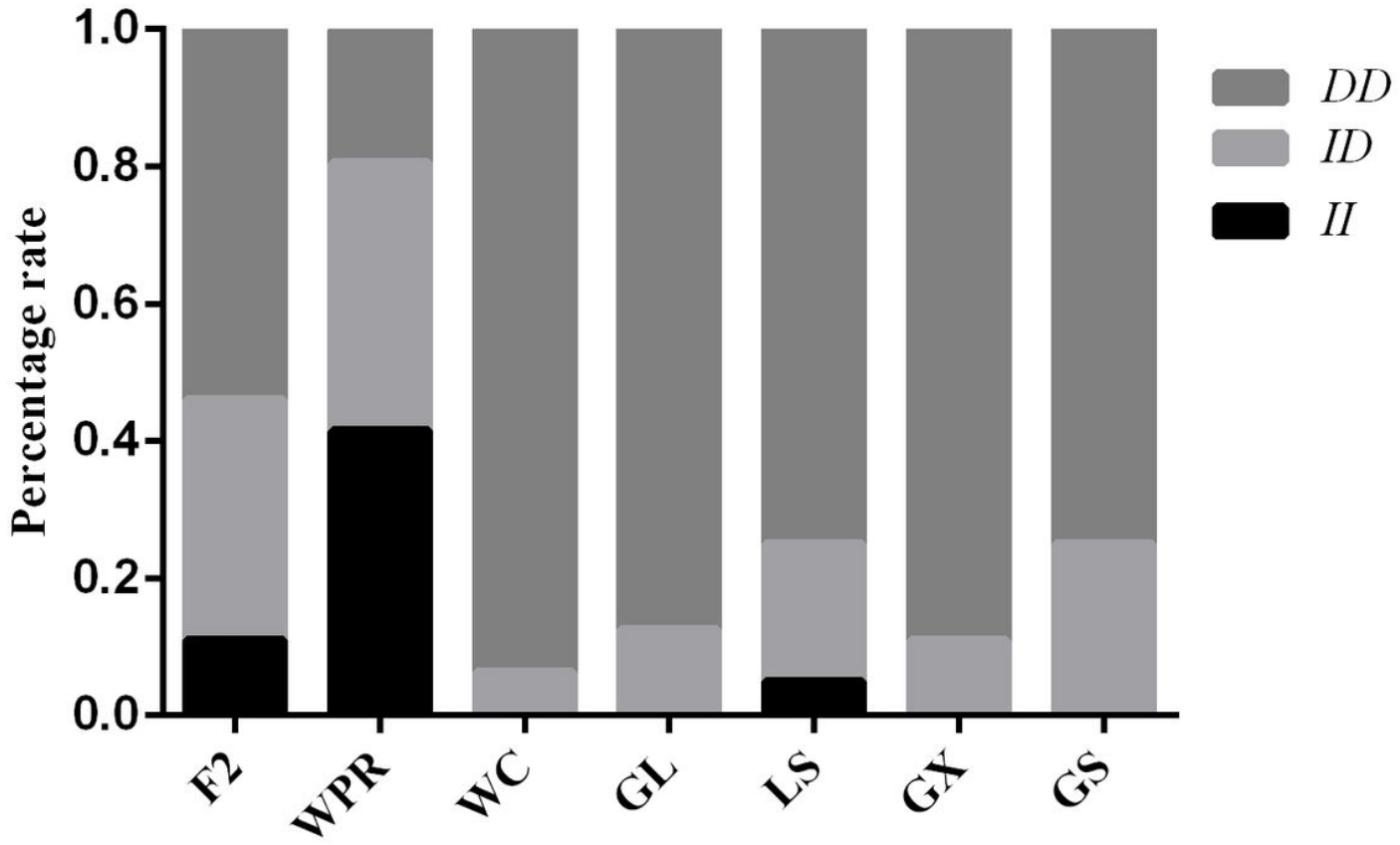

Figure 3

Percentage of different genotypes in different populations.

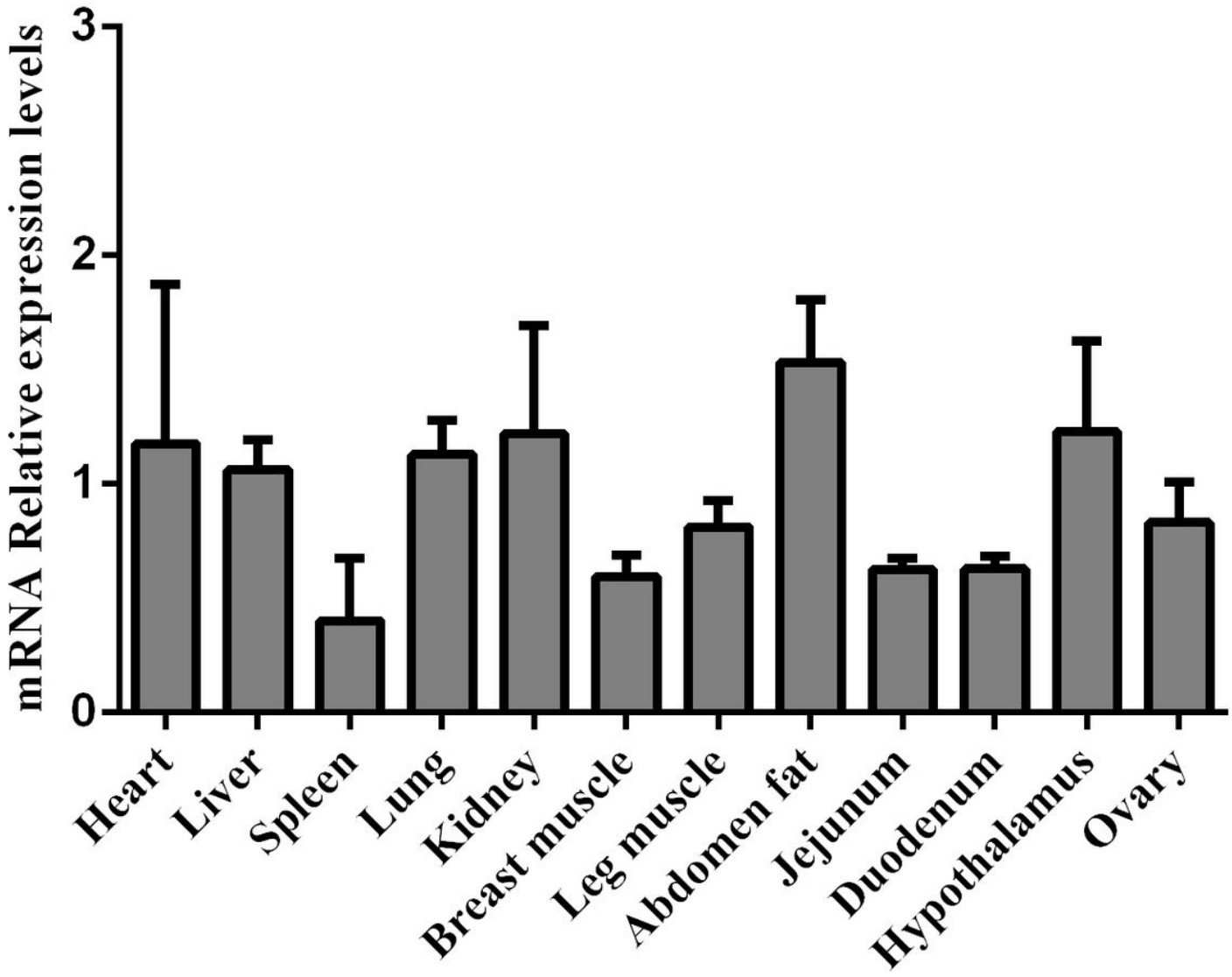

Figure 4

Expression of RIN2 mRNA in different tissues detected by qPCR.

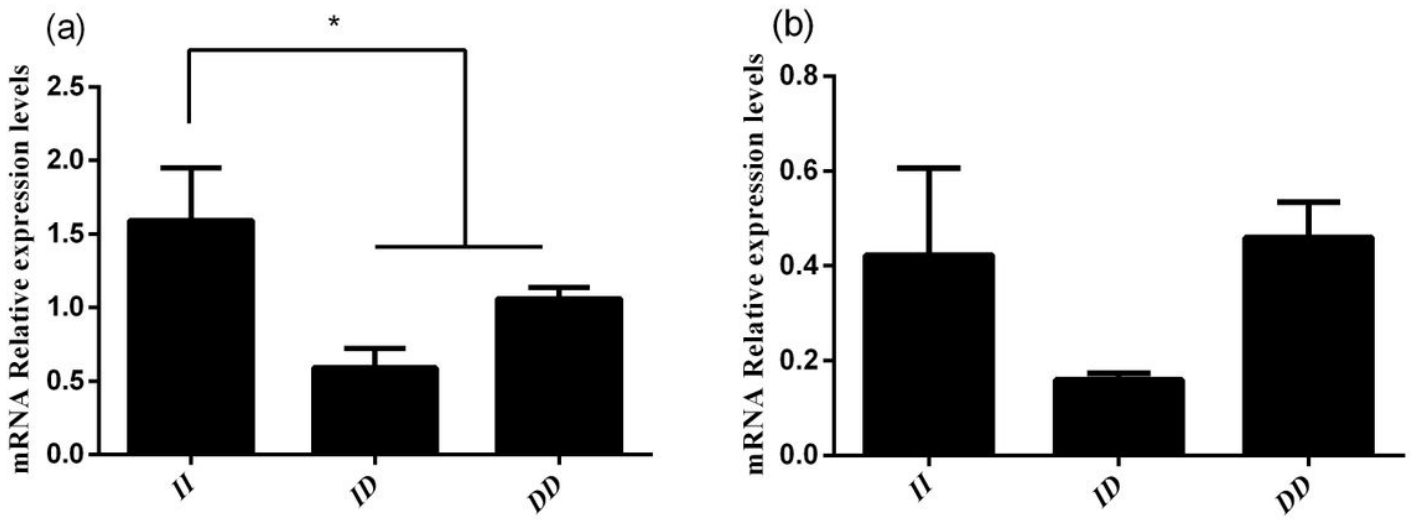

Figure 5

(a)Expression of MLNR mRNA in abdomen fat tissues with different genotypes. (b) Expression of MLNR mRNA in breast muscle tissues with different genotypes. Data represent means ± SD.*P < 0.05

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportinginformation.doc