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ARTICLE

Effects of Genetic Variation in the 20th Intron of Sansui Duck ATP2A2 Gene on Eggshell Quality

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ABSTRACT

In order to explore the influence of the polymorphism in the 20intron region of the Sansui duck ATP2A2 gene on the eggshell quality, this study used Primer Premier 5 software to design and synthesize a pair of primers in the 20 intron region, two-way direct sequencing and sequence alignment to mine SNPs Sites, SPSS 18.0 software was used to analyze the relationship between SNP sites and eggshell quality of Sansui duck. Three SNP sites were found in the 20 intron region of the ATP2A2 gene: g.40874 T>C, g.40920 G>A and g.40990 T=C, all of which were moderately polymorphic, at the site g.40874 T>C and g.40920 G>A both deviated significantly from Hardy-Weinberg equilibrium (P>0.05), position g.40990 T=C accords with Hardy-Weinberg equilibrium (P<0.05), and position g.40874 T>C There is a strong linkage disequilibrium between g.40990 T=C; a total of 4 haplotypes and 9 double types were detected at 3 SNP loci; the results of association analysis showed that g.40874 T>C mutation had a significant effect on eggshell strength and eggshell weight. The eggshell strength of CC genotype was significantly higher than that of TC and TT genotypes (P<0.05), the eggshell weight of CC genotype was significantly higher than that of TC genotype (P<0.05), and 40990 T=C mutation had a significant effect on eggshell strength. The eggshell strength of TC genotype was significantly higher than that of the TT genotype (P<0.05). In summary, the g.40874 T>C and g.40990 T=C found in the 20th intron region of the Sansui duck ATP2A2 gene may be the marker sites that affect the quality of the eggshell.

1. Introduction

The ATP2A2 gene is located on chromosome 16, with 21 exons and 20 introns. It encodes sarcoplasmic/endoplasmic reticulum calcium-ATPase 2 (SERCA2). SERCA2 is a p-type cation pump family that regulates Ca^{2+}

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transport. Member, involved in calcium ion transport, metabolism, etc., at the same time it plays a major role in the development of the epidermis of organisms.

Therefore, ATP2A2 mutation affects Ca^{2+} signal and affects the formation of cell adhesion and intercellular connection [1]. Xia Qianqian et al. [2] showed that most cases of keratosis of hair follicles (Darier's disease, DD) are caused by mutations in ATP2A2. Zhao et al. [3] showed that the mutation of ATP2A2 changes the calcium signal in keratinocytes and leads to echinolytic dyskeratosis. Detection of mutations in the ATP2A2 gene of a family with keratosis follicularis found that the 1220delAA mutation in the tenth exon of the ATP2A2 gene may be related to the clinical phenotype of patients in this family [4]. So far, at least 250 ATP2A2 mutations have been found and recorded, and the sites related to eggshell quality are relatively rare. The constituent materials of the eggshell are mainly formed by the deposition of eggshell fluid secreted by the eggshell glandular mucosa cells in the uterus. Ca^{2+} is the main ion in the eggshell fluid and plays an important role in the regulation of eggshell quality. In this study, Sansui duck was used as the test object, with a view to discovering new mutation sites in the ATP2A2 gene sequence that has an impact on eggshell quality.

2. Materials and Method

2.1 Used Animals and Eggshell Indicators

Twenty 45-week-old Sansui ducks with the same batch of hatchlings, healthy and disease-free were selected from the duck farm of the School of Animal Science of Guizhou University, and the eggs were collected and recorded one by one. According to the egg structure and egg structure in "Poultry Production" [13] The method of quality determination measures eggshell thickness, eggshell strength, egg shape index, egg weight, and eggshell weight. After the eggshell quality was measured, blood was collected from the wing vein of 20 Sansui ducks, stored in an anticoagulated biochemical tube, and the whole blood genomic DNA was extracted one by one according to the operation steps of the DNA rapid extraction kit.

2.2 Main Reagents and Instruments

The whole blood genomic DNA extraction kit was purchased from Shenggong Bioengineering (Shanghai) Co., Ltd.; 2×Taq PCR Master Mix reagent and DL-2000 Marker were purchased from Chongqing Kinco Biotechnology Co., Ltd.

The gradient PCR instrument was purchased from Bio-Rad, the United States; the electrophoresis instrument was purchased from Beijing Liuyi Instrument Factory; the chemiluminescence fluorescence automatic analysis imager was purchased from Shanghai Shanfu Scientific Instrument Co., Ltd.; the eggshell strength tester and egg quality analyzer were purchased from Beijing Tianxiang Feiyu Instrument Equipment Co., Ltd.; the nucleic acid concentration detector was purchased from NanoDrop, USA.

2.3 Primer Synthesis and PCR Amplification

Log in to the GenBank database to find the duck ATP2A2 (ID:488) gene sequence (NC_040061.1), and use the software Primer Premier 5 to design and amplify a pair of primers in the 20 intron region, F1: 5'-AGCCAGGAGCCTTAGTGTA -3', R1: 5'-AGAGGGCATTCAAGCGAGT -3 ', the length of the product is 727bp, and the primers are synthesized by Shenggong Bioengineering (Shanghai) Co., Ltd.

A total of 20 μL PCR amplification system: RNase-Free Water 7 μL, 2Taq PCR Master Mix 10 μL, upstream primer and downstream primer each 1 μL, DNA template 1 μL; reaction program: 95 °C pre-denaturation for 5 min; 95 ° C denaturation for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, a total of 35 cycles; final extension at 72 °C for 5 min; storage at 4 °C.

2.4 SNPs Identification

The PCR products were detected by 1.5% agarose gel electrophoresis, and the PCR products were recovered by the centrifugal column agarose gel DNA recovery kit and sent to Biological Engineering (Shanghai) Co., Ltd. for sequencing. SNP sites were screened and identified by using DNAStar software MegAlign program combined with sequencing peak map.

2.5 Statistics

The sequencing results were compared and SNP sites were found by using software Chromas and manual proofreading; SHEs is online software (http://analysis.bio-x.cn/myAnalysis.php) analyzes the genotype frequency and alleles of the SNP locus Gene frequency, genotype distribution chi-square value (2), D'value and r2 value of linkage disequilibrium, haplotype; calculate genetic heterozygosity (He), effective allele number (Ne), polymorphic information content (PIC), SPSS 18.0 software for correlation analysis between SNP locus and eggshell quality.

3. Results and Discussion

3.1 SNP Site Identification

The sequencing results were compared and searched

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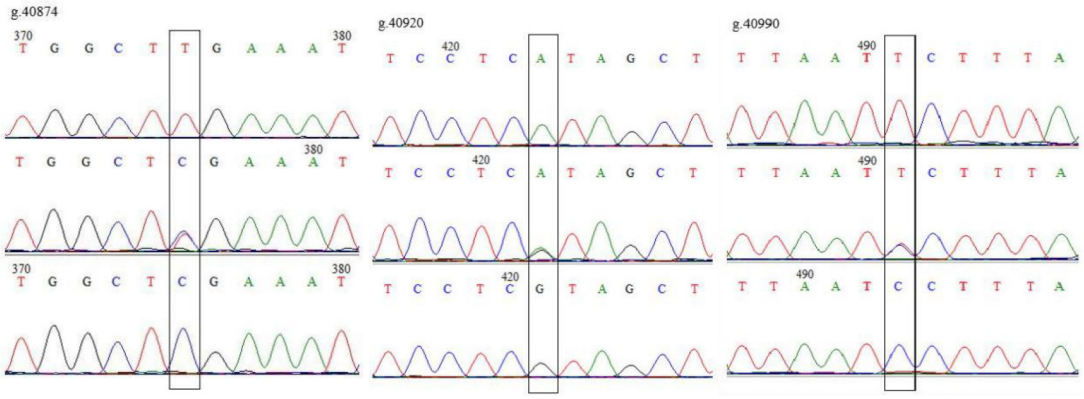

Figure 1. SNP locus comparison peak map

with Chromas software, and 3 SNP loci were found, all of which had 3 genotypes. The results are shown in Figure 1, g.40874 T>C, g.40920 G>A and g.40990 T=C are all located on intron 20.

3.2 Genetic Characteristics of SNP Locus

The sequencing results of the ATP2A2 gene of Sansui duck are shown in Table 1. It can be seen from the table that the dominant genotype of g.40874 T>C is TT, the genotype frequency is 0.5, the dominant allele is T, the allele frequency is 0.675, and the polymorphism information content (PIC) is 0.341; the dominant genotype of g.40920 G > A is GG, the genotype frequency is 0.6, the dominant allele is G, the allele frequency is 0.775, and the PIC is 0.287; g.40990 T= The dominant genotype of C is TC, the genotype frequency is 0.5, the frequency of alleles C and T are both 0.5, and the PIC is 0.375, both of which are moderately polymorphic. The chi-square test values of g.40874 T>C and g.40920 G>A showed significant deviation from Hardy-Weinberg equilibrium (P>0.05), and g.40990 T=C conformed to Hardy-Weinberg equilibrium (P< 0.05).

3.3 Analysis of Linkage Disequilibrium, Haplotype and Diploid at SNP Loci

Linkage disequilibrium analysis was carried out on the three SNP loci of the ATP2A2 gene of Sansui duck. The results are shown in Table 2. It can be seen from the table that the D'value of g.40874 T>C and g.40920 G>A is 1, and the r2 value is 0.14, the D'value of g.40874 T>C and g.40990 T=C is 1, the r2 value is 0.481, the D'value of g.40874 T>C and g.40990 T=C is 1, the r2 value is 0.29. According to the reports of Ardlie et al [5] and Slatkin [6], when |D ′ | >0.8 and r2 >0.33, there is a strong linkage disequilibrium between SNP sites, and it can be seen that only g.40874 T>C and g. There is a strong linkage disequilibrium between 40990 T=C sites. There are 4 haplotypes at 3 SNP sites: H1, H2, H3 and H4. The combination of 4 haplotypes has detected 9 double types: H1H1, H1H2, H1H3, H1H4, H2H2, H2H3, H2H4, H3H4 and H4H4, of which H1H3 and H2H3 have the highest frequency, both at 0.2, followed by H1H1 and

Table 1. Population genetic information of SNP locus in Sansui duck

SNP site	Genotype frequency			Allele frequency		*He*	*Ne*	*PIC*	x^2
g.40874	CC(3)	TC(7)	TT(10)	C(13)	T(27)	0.438	1.779	0.341	0.818
T>C	0.150	0.350	0.500	0.325	0.675				
g.40920 G>A	AA(2) 0.100	AG(6) 0.300	GG(12) 0.600	A(10) 0.225	G(30) 0.775	0.348	1.534	0.287	0.800
g.40990 T=C	CC(5) 0.250	TC(10) 0.500	TT(5) 0.250	C(20) 0.500	T(20) 0.500	0.500	2.000	0.375	0.000

Note:PIC>0.5 is high polymorphism; 0.5>PIC>0.25 is moderate polymorphism; PIC<0.25 is low polymorphism; x^2-0.05=5.991, x^2-0.01=9.21.

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H3H4, both at 0.15 (Table 3).

Table 2. Analysis of linkage disequilibrium among SNPs loci

SNPSite	g.40874 T>C	g.40920 G>A	g.40990 T=C
g.40874 T>C	--	1.000	1.000
g.40920 G>A	0.140	--	1.000
g.40990 T=C	0.481	0.290	--

Note: The upper triangle is the D'value, and the lower triangle is the r2 value.

Table 3. Haplotype and double-type analysis of SNP locus

SNPSite		g.40874	g.40920	g.40990	frequency
		C>T	G>A	T>C	
Haplotype	H1(13)	C	G	T	0.325
	H2(10) H3(10)	T	A	C	0.225
		T	G	C	0.275
	H4(7)	T	G	T	0.175
Double	H1H1(3)	CC	GG	TT	0.150
	H1H2(2)	TC	AG	TC	0.100
	H1H3(4)	TC	GG	TC	0.200
	H1H4(1)	TC	GG	TT	0.050
	H2H2(2)	TT	AA	CC	0.050
	H2H3(3)	TT	AG	CC	0.200
	H2H4(1)	TT	AG	TC	0.050
	H3H4(3)	TT	GG	TC	0.150
	H4H4(1)	TT	GG	TT	0.050

3.4 Correlation analysis between SNP locus and eggshell quality of Sansui duck

The results of the correlation analysis between three SNP loci of ATP2A2 gene and egg quality in Sansui duck are shown in Table 4. From the table, we can see that: g.40874 T > C mutation has a significant effect on eggshell strength and eggshell weight, and the eggshell of CC genotype. The intensity is significantly higher than the TC and TT genotypes (P<0.05). The eggshell weight of the CC genotype is significantly higher than that of the TC genotype (P<0.05). The g.40990 T=C mutation has a significant impact on the eggshell strength. TC The eggshell strength of the genotype was significantly higher than that of the TT genotype (P<0.05), and the g.40920 G>A mutation did not have a significant impact on egg quality (P>0.05).

4. Conclusions

ATP2A2 participates in Ca^{2+} transport, metabolism, etc., and plays an important role in the formation of poultry eggshells. Ca^{2+} is the most common mineral in poultry, and it is the most critical factor to ensure the normal calcification of eggshells [7]. In this study, three SNP loci were detected in the 20th intron of the ATP2A2 gene, all of which are moderately polymorphic, indicating that these three SNP loci have strong mining potential in the breeding of Changshun green-shell layer hens. [8], only g.40990 T=C mutation in 3 loci meets Hardy-Weinberg equilibrium (P<0.05), and the other 2 loci are significantly deviated from Hardy-Weinberg equilibrium (P>0.05), Mayo et al. [9] Pointed out that the Hardy-Weinberg balance is in an infinite population. If individuals mate randomly, without mutation, migration and genetic drift, the genotype and frequency at a locus in the population will remain unchanged from generation to generation. In a state of genetic balance. It shows that g.40874 T>C and g.40920 G>A are affected by mutation, selection, genetic drift and other factors in the randomly mated three-spike duck population. On the contrary, it shows that even in the artificial selection, migration and genetic drift, There is still a dynamic balance at g.40990 T=C during the process of change [10], but it may also be because the number of samples in this test is not large enough that the g.40874 T>C and g.40920 G>A deviate significantly from Hardy-Weinberg balance. There is a strong linkage disequilibrium between g.40874 T>C and g.40990 T=C, which indicates that these two loci tend to be inherited as a whole in the three-spike duck population. A total of 4 haplotypes and 9 double types were detected at 3 SNP loci. Theoretically there should be 10 double types, and the other 1 double type may be eliminated during natural selection or artificial selection.

Calcium is the main factor that determines the quality of eggshells. Calcium deficiency will cause the thickness and strength of the eggshell to decrease, resulting in the production of soft-shelled eggs, sand-shelled eggs or even shellless eggs, which directly affects the egg production rate and hatchability, especially to the hatching of breeding eggs. Great loss [11]. The strength of the egg-

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Table 4. Correlation analysis between SNP locus and eggshell quality of Sansui duck

SNP genotype site	Eggshell thickness(mm)	Eggshell strength (N/cm^2)	Egg Shape Index	Egg weight(g)	Eggshell weight(g)
CC					
g.40874	0.428±0.035	41.100±5.299a	1.372±0.050	68.667±3.135	6.885±0.295a
TC	0.427±0.023	38.548±3.469b	1.341±0. 033	69.791±2.053	6.146±0.193b
T>C	0.460±0.019	37.681±2.902b	1.415±0.028	69.177±1.717	6.589±0.162ab
TT					
AA					
g.40920	0.400±0.071	37.800±4.273	1.395±0.078	71.750±1.768	6.530±0.707
AG	0.473±0.072	38.925±5.225	1.437±0.090	69.283±7.397	6.595±0.532
G>A	0.438±0.052	38.732±6.593	1.355±0.086	70.343±4.089	6.479±0.614
GG					
CC	0.456±0.064	37.410±5.356ab	1.424±0.055	70.580±6.411	6.782±0.606
g.40990					
TC	0.442±0.075	41.031±5.035a	1.366±0.117	69.481±5.703	6.750±0.550
T=C					
TT	0.436±0.018	34.116±6.730b	1.373±0.038	69.121±3.122	6.832±0.556

Note: Different lowercase letters in the same column indicate significant differences (P<0.05)

shell is an important indicator reflecting the anti-breakage rate of the eggshell, which is directly related to the thickness of the eggshell, the thickness of the eggshell membrane, the mineral content of the eggshell and the protein matrix [12]. The thickness and strength of the eggshell are important quality indicators and economic indicators for eggs. It is generally believed that when the thickness of the eggshell is above 0.35 mm, it has good transportability and preservation [13]. The eggshell thickness data measured in this study are all above 0.35 mm, indicating that the test data is reliable. Previous studies have shown that introns in eukaryotes are also involved in gene splicing. Intron mutations can cause changes in splicing efficiency or accuracy, affect amino acid coding, and indirectly affect animal gene expression, thereby affecting production economic traits [14]. This is consistent with the results of this study. The results of this study show that the g.40874 T > C and g.40990 T=C mutations in the 20th intron of the ATP2A2 gene have a significant impact on eggshell quality. The CC gene of g.40874 T > C, the eggshell strength of the genotype was significantly higher than that of the TC and TT genotypes (P<0.05). The eggshell weight of the CC genotype was significantly higher than that of the TC genotype (P<0.05). g.40990 T=C TC genotype eggs. The shell strength was significantly higher than the TT genotype (P<0.05).

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