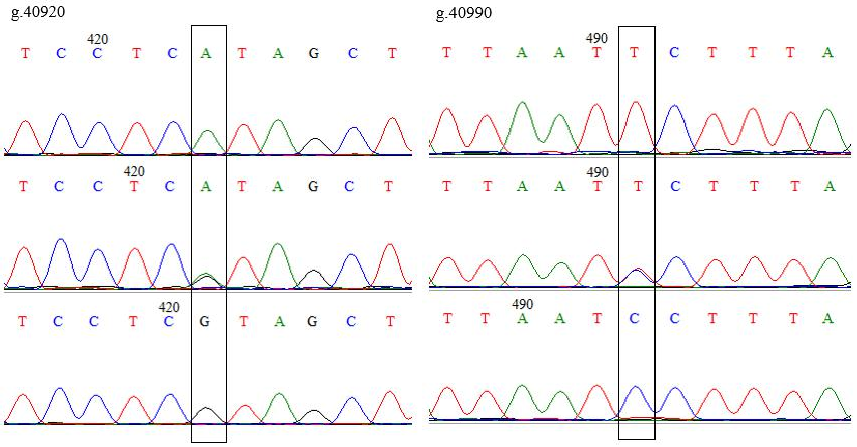
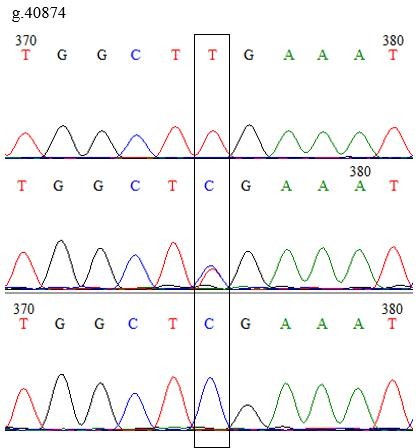
# Effects of genetic variation in the 20th intron of Sansui duck ATP2A2

1. **gene on eggshell quality**
2. **Abstract:**In order to explore the influence of the polymorphism in the 20
3. intron region of the Sansui duck ATP2A2 gene on the eggshell quality,
4. this study used Primer Premier 5 software to design and synthesize a
5. pair of primers in the 20 intron region, two-way direct sequencing and
6. sequence alignment to mine SNPs Sites, SPSS 18.0 software was used to
7. analyze the relationship between SNP sites and eggshell quality of Sansui
8. duck. Three SNP sites were found in the 20 intron region of the
9. ATP2A2 gene: g.40874 T>C, g.40920 G>A and g.40990 T=C, all of which
10. were moderately polymorphic, at the site g.40874 T ＞C and g.40920 G
11. ＞ A both deviated significantly from Hardy-Weinberg equilibrium
12. (P>0.05), position g.40990 T=C accords with Hardy-Weinberg equilibrium
13. (P<0.05), and position g.40874 T ＞ C There is a strong linkage
14. disequilibrium between g.40990 T=C; a total of 4 haplotypes and 9
15. double types were detected at 3 SNP loci; the results of association
16. analysis showed that g.40874 T ＞ C mutation had a significant effect on eggshell
17. strength and eggshell weight. The eggshell strength of CC genotype was significantly higher
18. than that of TC and TT genotypes (P<0.05), the eggshell weight of CC
19. genotype was significantly higher than that of TC genotype (P<0.05),
20. and 40990 T=C mutation had a significant effect on eggshell strength.
21. The eggshell strength of TC genotype was significantly higher than
22. that of the TT genotype (P<0.05). In summary, the g.40874 T>C and
23. g.40990 T=C found in the 20th intron region of the Sansui duck ATP2A2
24. gene may be the marker sites that affect the quality of the eggshell.
25. **Keywords**: Sansui duck; ATP2A2 gene; SNP locus; eggshell quality
26. The ATP2A2 gene is located on chromosome 16, with 21 exons and
27. 20 introns. It encodes sarcoplasmic/endoplasmic reticulum
28. calcium-ATPase 2 (SERCA2). SERCA2 is a p-type cation pump family that
29. regulates Ca2+ transport. Member, involved in calcium ion transport,
30. metabolism, etc., at the same time it plays a major role in the
31. development of the epidermis of organisms.
32. However, it is difficult to identify genes that affect polygenic traits and
33. characterization products [1]. To maintain the genetic diversity of livestock
34. species, it is necessary to fully implement conservation priorities and sustainable
35. management programs, which should be based on comprehensive information
36. about population structure, including the sources of genetic variation between
37. and within varieties [2]. Therefore, ATP2A2 mutation affects Ca2+ signal and
38. affects the formation of cell adhesion and intercellular connection [3].Xia
39. Qianqian et al. [4] showed that most cases of
40. keratosis of hair follicles (Darier’s disease, DD) are caused by mutations
41. in ATP2A2. Zhao et al. [3] showed that the mutation of ATP2A2 changes
42. the calcium signal in keratinocytes and leads to echinolytic dyskeratosis.
43. Detection of mutations in the ATP2A2 gene of a family with keratosis
44. follicularis found that the 1220delAA mutation in the tenth exon of the
45. ATP2A2 gene may be related to the clinical phenotype of patients in this
46. family [5]. So far, at least 250 ATP2A2 mutations have been found and
47. recorded, and the sites related to eggshell quality are relatively rare. The
48. constituent materials of the eggshell are mainly formed by the
49. deposition of eggshell fluid secreted by the eggshell glandular mucosa
50. cells in the uterus. Ca2+ is the main ion in the eggshell fluid and plays an
51. important role in the regulation of eggshell quality. In this study, Sansui
52. duck was used as the test object, with a view to discovering new
53. mutation sites in the ATP2A2 gene sequence that have an impact on
54. eggshell quality.
55. 1 Material method
56. 1.1Used animals and eggshell indicators
57. Twenty 45-week-old Sansui ducks with the same batch of hatchlings,
58. healthy and disease-free were selected from the duck farm of the School
59. of Animal Science of Guizhou University, and the eggs were collected and
60. recorded one by one. According to the egg structure and egg structure in
61. "Poultry Production" [13] The method of quality determination
62. measures eggshell thickness, eggshell strength, egg shape index, egg
63. weight, and eggshell weight. After the eggshell quality was measured,
64. blood was collected from the wing vein of 20 Sansui ducks, stored in an
65. anticoagulated biochemical tube, and the whole blood genomic DNA was
66. extracted one by one according to the operation steps of the DNA rapid
67. extraction kit.
68. 1.2 Main reagents and instruments
69. The whole blood genomic DNA extraction kit was purchased from
70. Shenggong Bioengineering (Shanghai) Co., Ltd.; 2×Taq PCR Master Mix
71. reagent and DL-2000 Marker were purchased from Chongqing Kinco
72. Biotechnology Co., Ltd.
73. The gradient PCR instrument was purchased from Bio-Rad, the
74. United States; the electrophoresis instrument was purchased from
75. Beijing Liuyi Instrument Factory; the chemiluminescence fluorescence
76. automatic analysis imager was purchased from Shanghai Shanfu
77. Scientific Instrument Co., Ltd.; the eggshell strength tester and egg
78. quality analyzer were purchased from Beijing Tianxiang Feiyu Instrument
79. Equipment Co., Ltd.; the nucleic acid concentration detector was
80. purchased from NanoDrop, USA.
81. 1.3 Primer synthesis and PCR amplification
82. Log in to the GenBank database to find the duck ATP2A2(ID:488) gene
83. sequence (NC\_040061.1), and use the software Primer Premier 5 to
84. design and amplify a pair of primers in the 20 intron region, F1:
85. 5'-AGCCAGGAGCCTTAGTGTA -3', R1: 5'-AGAGGGCATTCAAGCGAGT -3 ',
86. the length of the product is 727bp, and the primers are synthesized by
87. Shenggong Bioengineering (Shanghai) Co., Ltd.
88. A total of 20 µL PCR amplification system: RNase-Free Water 7 µL,
89. 2Taq PCR Master Mix 10 µL, upstream primer and downstream primer
90. each 1 µL, DNA template 1 µL; reaction program: 95 ° C
91. pre-denaturation for 5 min; 95 ° C denaturation for 30 s, annealing at
92. 60 °C for 30 s, extension at 72 °C for 30 s, a total of 35 cycles; final
93. extension at 72 ° C for 5 min; storage at 4 °
94. 1.4 SNPs identificationThe
95. PCR products were detected by 1.5% agarose gel electrophoresis, and the
96. PCR products were recovered by the centrifugal column agarose gel DNA
97. recovery kit and sent to Biological Engineering (Shanghai) Co., Ltd. for
98. sequencing. SNP sites were screened and identified by using DNAStar
99. software MegAlign program combined with sequencing peak map.
100. 1.5 Statistics
101. The sequencing results were compared and SNP sites were found by using software Chromas and manual proofreading; SHEsis online
102. software (<http://analysis.bio-x.cn/myAnalysis.php)> analyzes the
103. genotype frequency and alleles of the SNP locus Gene frequency,
104. genotype distribution chi-square value (2), D'value and r2 value of
105. linkage disequilibrium, haplotype; calculate genetic heterozygosity (He),
106. effective allele number (Ne), polymorphic information content ( PIC),
107. SPSS 18.0 software for correlation analysis between SNP locus and
108. eggshell quality.
109. 2 Results and Discussion
110. 2.1 SNP site identification
111. The sequencing results were compared and searched with Chromas
112. software, and 3 SNP loci were found, all of which had 3 genotypes. The
113. results are shown in Figure 1, g.40874 T>C, g.40920 G>A and g.40990
114. T=C are all located on intron 20.



1. Figure 1 SNP locus comparison peak map
2. 2.2 Genetic characteristics of SNP locus
3. The sequencing results of the ATP2A2 gene of Sansui duck are
4. shown in Table 1. It can be seen from the table that the dominant
5. genotype of g.40874 T>C is TT, the genotype frequency is 0.5, the
6. dominant allele is T, the allele frequency is 0.675, and the polymorphism
7. The information content (PIC) is 0.341; the dominant genotype of
8. g.40920 G＞A is GG, the genotype frequency is 0.6, the dominant allele
9. is G, the allele frequency is 0.775, and the PIC is 0.287; g.40990 T= The
10. dominant genotype of C is TC, the genotype frequency is 0.5, the
11. frequency of alleles C and T are both 0.5, and the PIC is 0.375, both of
12. which are moderately polymorphic. The chi-square test values of
13. g.40874 T>C and g.40920 G>A showed significant deviation from
14. Hardy-Weinberg equilibrium (P>0.05), and g.40990 T=C conformed to
15. Hardy-Weinberg equilibrium (P< 0.05).
16. Table 1 Population genetic information of SNP locus in Sansui duck

SNP site Genotype frequency Allele frequency *He Ne PIC χ2*

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 |

1. Note: PIC>0.5 is high polymorphism; 0.5>PIC>0.25 is moderate
2. polymorphism; PIC<0.25 is low polymorphism; χ 2-0.05=5.991, χ

125 2-0.01=9.21.

1. 2.3 Analysis of linkage disequilibrium, haplotype and diploid at SNP loci
2. Linkage disequilibrium analysis was carried out on the three SNP
3. loci of the ATP2A2 gene of Sansui duck. The results are shown in Table 2.
4. It can be seen from the table that the D'value of g.40874 T>C and
5. g.40920 G>A is 1, and the r2 value is 0.14, the D'value of g.40874 T>C
6. and g.40990 T=C is 1, the r2 value is 0.481, the D'value of g.40874 T>C
7. and g.40990 T=C is 1, the r2 value is 0.29. According to the reports of
8. Ardlie et al[5] and Slatkin[6], when |D ʹ | >0.8 and r2 >0.33, there is a
9. strong linkage disequilibrium between SNP sites, and it can be seen that
10. only g.40874 T>C and g. There is a strong linkage disequilibrium between
11. 40990 T=C sites. There are 4 haplotypes at 3 SNP sites: H1, H2, H3 and
12. H4. The combination of 4 haplotypes has detected 9 double types: H1H1,
13. H1H2, H1H3, H1H4, H2H2, H2H3, H2H4, H3H4 and H4H4, of which H1H3
14. and H2H3 have the highest frequency, both at 0.2, followed by H1H1 and
15. H3H4, both at 0.15 (Table 3).

|  |  |  |  |
| --- | --- | --- | --- |
| 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 | -- |

1. Table 2 Analysis of linkage disequilibrium among SNPs loci
2. Note: The upper triangle is the D'value, and the lower triangle is the r2
3. value.
4. Table 3 Haplotype and double-type analysis of SNP locus

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SNPSite |  | g.40874 | g.40920 | g.40990 | frequency |
|  |  | C＞T | G＞A | T＞C |  |
|  | H1(13) | C | G | T | 0.325 |
| Haplotype | H2(10)  H3(10) | T  T | A  G | C  C | 0.225  0.275 |
|  | H4(7) | T | G | T | 0.175 |
|  | 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 |
| Double | 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 |

1. 2.4 Correlation analysis between SNP locus and eggshell quality of Sansui
2. duck
3. The results of the correlation analysis between three SNP loci of
4. ATP2A2 gene and egg quality in Sansui duck are shown in Table 4. From
5. the table, we can see that: g.40874 T＞C mutation has a significant effect
6. on eggshell strength and eggshell weight, and the eggshell of CC
7. genotype The intensity is significantly higher than the TC and TT
8. genotypes (P<0.05). The eggshell weight of the CC genotype is
9. significantly higher than that of the TC genotype (P<0.05). The g.40990
10. T=C mutation has a significant impact on the eggshell strength. TC The
11. eggshell strength of the genotype was significantly higher than that of
12. the TT genotype (P<0.05), and the g.40920 G>A mutation did not have a
13. significant impact on egg quality (P>0.05).
14. Table 4 Correlation analysis between SNP locus and eggshell quality
15. of Sansui duck

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SNP  genotype  site | Eggshell  thickness（mm） | Eggshell strength  （N/cm2） | Egg Shape  Index | Egg weight（g） | Eggshell  weight（g） |
| CC  g.40874  TC  T＞C  TT | 0.428±0.035  0.427±0.023  0.460±0.019 | 41.100±5.299a  38.548±3.469b  37.681±2.902b | 1.372±0.050  1.341±0. 033  1.415±0.028 | 68.667±3.135  69.791±2.053  69.177±1.717 | 6.885±0.295a  6.146±0.193b  6.589±0.162ab |
| AA  g.40920  AG  G＞A  GG | 0.400±0.071  0.473±0.072  0.438±0.052 | 37.800±4.273  38.925±5.225  38.732±6.593 | 1.395±0.078  1.437±0.090  1.355±0.086 | 71.750±1.768  69.283±7.397  70.343±4.089 | 6.530±0.707  6.595±0.532  6.479±0.614 |
| CC | 0.456±0.064 | 37.410±5.356ab | 1.424±0.055 | 70.580±6.411 | 6.782±0.606 |
| g.40990  TC  T=C | 0.442±0.075 | 41.031±5.035a | 1.366±0.117 | 69.481±5.703 | 6.750±0.550 |
| TT | 0.436±0.018 | 34.116±6.730b | 1.373±0.038 | 69.121±3.122 | 6.832±0.556 |

1. Note: Different lowercase letters in the same column indicate
2. significant differences (P<0.05)
3. 3 Discussion
4. ATP2A2 participates in Ca2+ transport, metabolism, etc., and plays an
5. important role in the formation of poultry eggshells. Ca2+ is the most
6. common mineral in poultry, and it is the most critical factor to ensure
7. the normal calcification of eggshells [7]. In this study, three SNP loci were
8. detected in the 20th intron of the ATP2A2 gene, all of which are
9. moderately polymorphic, indicating that these three SNP loci have strong
10. mining potential in the breeding of Changshun green-shell layer hens. [8],
11. only g.40990 T=C mutation in 3 loci meets Hardy-Weinberg equilibrium
12. (P<0.05), and the other 2 loci are significantly deviated from
13. Hardy-Weinberg equilibrium (P>0.05), Mayo et al. [9 ] Pointed out that
14. the Hardy-Weinberg balance is in an infinite population. If individuals
15. mate randomly, without mutation, migration and genetic drift, the
16. genotype and frequency at a locus in the population will remain
17. unchanged from generation to generation. In a state of genetic balance.It
18. shows that g.40874 T>C and g.40920 G>A are affected by mutation,
19. selection, genetic drift and other factors in the randomly mated
20. three-spike duck population. On the contrary, it shows that even in the
21. artificial selection, migration and genetic drift, There is still a dynamic
22. balance at g.40990 T=C during the process of change [10], but it may also
23. be because the number of samples in this test is not large enough that
24. the g.40874 T>C and g.40920 G>A deviate significantly from Hardy
25. -Weinberg balance. There is a strong linkage disequilibrium between
26. g.40874 T>C and g.40990 T=C, which indicates that these two loci tend
27. to be inherited as a whole in the three-spike duck population. A total of
28. 4 haplotypes and 9 double types were detected at 3 SNP loci,
29. theoretically there should be 10 double types, and the other 1 double
30. type may be eliminated during natural selection or artificial selection.
31. Calcium is the main factor that determines the quality of eggshells.
32. Calcium deficiency will cause the thickness and strength of the eggshell
33. to decrease, resulting in the production of soft-shelled eggs,
34. sand-shelled eggs or even shellless eggs, which directly affects the egg
35. production rate and hatchability, especially to the hatching of breeding
36. eggs. Great loss [11]. The strength of the eggshell is an important
37. indicator reflecting the anti-breakage rate of the eggshell, which is
38. directly related to the thickness of the eggshell, the thickness of the
39. eggshell membrane, the mineral content of the eggshell and the protein
40. matrix [12]. The thickness and strength of the eggshell are important
41. quality indicators and economic indicators for eggs. It is generally
42. believed that when the thickness of the eggshell is above 0.35 mm, it has
43. good transportability and preservation [13]. The eggshell thickness data
44. measured in this study are all above 0.35 mm, indicating that the test
45. data is reliable. Previous studies have shown that introns in eukaryotes
46. are also involved in gene splicing. Intron mutations can cause changes in
47. splicing efficiency or accuracy, affect amino acid coding, and indirectly
48. affect animal gene expression, thereby affecting production economic
49. traits [14] . This is consistent with the results of this study. The results of
50. this study show that the g.40874 T＞C and g.40990 T=C mutations in the
51. 20th intron of the ATP2A2 gene have a significant impact on eggshell
52. quality. The CC gene of g.40874 T ＞ C The eggshell strength of the
53. genotype was significantly higher than that of the TC and TT genotypes
54. (P<0.05). The eggshell weight of the CC genotype was significantly higher
55. than that of the TC genotype (P<0.05). g.40990 T=C TC genotype eggs
56. The shell strength was significantly higher than the TT genotype

216 (P<0.05).

# References:

1. [1] **易卜拉希米**，z .，M.R. Mohammadabadi，A.K. Esmailizadeh，A. Khezri and a . Najminori.
2. [2] Mohammadi，a .，M.R. Nassiry，J. Mosafer，M.R. Mohammadabadi and G.E. Sulimova .
3. [3] Li C, Xiao Z, Peng Y, et al. A novel mutation in exon 1 of the ATP2A2
4. gene in four members of one Chinese family with Darier disease. Clinical
5. and Experimental Dermatology, 2020, 45(7): 925-928.
6. [4] Xia Qianqian, Zhao Qing, Sun Lele, et al. Study on ATP2A2 gene
7. mutation in a family of keratosis follicularis and three sporadic
8. patients[J].China Journal of Leprosy and Skin Diseases, 2019, 35(07):

224 385-388.

1. [5] Zhao Q, Fu FH, Fu XA, et al. Three novel mutations of the ATP2A2
2. gene in Chinese patients with Darier disease. 2019, 60(2): e171-e172.
3. [6] Chen Siyuan, Huang Changzheng, Qian Yue, et al. Detection of
4. ATP2A2 gene mutation in a family of follicular keratosis[J]. China Journal
5. of Leprosy and Skin Diseases, 2006(07): 549-551.
6. [7] Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium
7. in the human genome[J]. Nature Reviews Genetics, 2002, 3(4): 299-309.
8. [8]Slatkin M. Linkage disequilibrium-understanding the evolutionary past
9. and mapping the medical future[J]. Nature Reviews Genetics, 2008, 9(6):

234 477-485.

1. [9]Mohamed K, Eva T, Michaela E, et al. Combined Effect of Genotype,
2. Housing System, and Calcium on Performance and Eggshell Quality of
3. Laying Hens. Animals: an open access journal from MDPI, 2020, 10(11):

238 2120

1. [10]Yang Yang, Ma Lin, Liu Qiuyue, et al. Association analysis between
2. LHCGR gene polymorphism and litter size in sheep[J]. Chinese Herbivore

241 Science, 2019, 39(06): 1-6+21.

242 [11]Mayo O. A century of Hardy-Weinberg equilibrium[J]. Twin Research

243 and Human Genetics, 2008, 11(3): 249-256.

1. [12]Ren G, Huang YZ, Wei TB, et al. Linkage disequilibrium and haplotype
2. distribution of the bovine LHX4 gene in relation to growth[J]. Gene, 2014,

246 538(2): 354-360.

1. [13]Jiang Danli, Zhang Zhuoshen, Sun Junfeng, et al. The effect of a small
2. amount of eggshell powder in place of stone powder on laying
3. performance, egg quality and calcium metabolism of laying ducks[J].
4. Guangdong Agricultural Sciences, 2020, 47(03): 117-122.
5. [14]Tan Guanghui, Li Jiezhang, Qin Yuanyu, et al. Correlation between
6. genetic variation of Sansui duck ORAI1 gene and eggshell quality[J].
7. Journal of Fujian Agriculture and Forestry University (Natural Science

254 Edition), 2020, 49(05): 646-651.

1. [15]Luo Yiqiu, Kuang Wei, He Zongliang, et al. Study on the Variation Law
2. of Egg Quality of Jinling White Duck at Different Laying Weeks[J]. Journal
3. of Anhui Agricultural Sciences, 2020, 48(22): 107-109+147.
4. [16]Li Jiezhang, Tan Guanghui, Wu Lei, et al. Identification of SNPs in
5. ITPR1 gene of Sansui duck and its association with eggshell quality
6. traits[J]. Journal of Agricultural Biotechnology, 2020, 28(03): 455-464.