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Cloning and SNPs Analysis of OASL Gene in Muscovy Duck

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Abstract

The OASL gene is one of the interferon-stimulating genes whose protein family includes OAS1, OAS2, OAS3 and OASL. 12 Muscovy duck from one pure line was employed for testing. 5'-regulatory region of the Muscovy duck OASL gene was amplified. After sequencing of PCR product, the SNPs were identified and the polymorphism of OASL gene sites could be detected by Popgene and SHEsis. The results showed 11 SNPs (g.313A>G, g.354A>G, g.392T>C, g.423A>G, g.450T>C, g.523G>A, g.528T>C, g.533C>T, g.545A>G, g.604A>T, g.609+>-), consisting 1.3% of total analyzed sites. The mutations were equally distributed which demonstrate low overall heterozygosity and strong linkage disequilibrium. All of the study specified the mutation types and locations on part of the OASL gene, paving the way for the development of gene regulation and the mechanism of antiviral actions of OASL gene and providing related basic dataand information of disease-resistance breeding of poultry.

Keywords

Muscovy Duck, OASL Gene, Single Nucleotide Polymorphism, Bioinformatics Analysis

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1. Introduction

2'-5'-Oligoadenylate synthetase (OAS) was among the first interferon-induced antiviral enzymes to be discovered [1]. Interferon-stimulated genes (ISGs) are a group of gene products that coordinately combat pathogen invasions, in particular viral infections. Several interferon-stimulated genes (ISGs) such as IFN-stimulated protein of OAS function as antiviral effectors and might contribute to virus elimination. In recent years, although a large number of ISGs have been confirmed to have a variety of antiviral activities, only a small part of the biological characteristics of ISGs have been studied clearly. The OAS gene is one of the important ISGs.

Studies have shown that OAS protein can catalyse the synthesis of 2'-5' oligoadenylic acid chains from ATP, activate the endonuclease RNase L to degrade viral RNA, directly block the replication of the virus, and ultimately limit the proliferation of the virus [2-3]. It has been confirmed that OAS1 can resist dengue fever, Japanese encephalitis, *etc*.; OAS2 can effectively inhibit porcine reproductive and respiratory syndrome virus [3], OAS is a large and complex family. At present, related researches mainly focus on rats and humans[4-5], while research on poultry is relatively rare.

This experiment took Muscovy ducks as the research object, using common PCR amplification method combined with the direct sequencing method of PCR products to screen some polymorphic sites of Muscovy duck OASL gene, and conduct bioinformatics analysis on them, providing a basis for disease-resistant genetic breeding basic data and relevant data for future drug development against pathogenic microorganisms.

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2. Materials and Methods

2.1. Ethics Statement

All animal experiments complied with the ethics of Jiangsu Administrative Committee of Laboratory Animals and the guidelines of Jiangsu laboratory animal welfare.

2.2. Sample Collection and Preparation

15 pure-line Muscovy duck were obtained from a high-quality duck farm in Jiangsu province and raised in floor pens under the same standardizedconditions of management and fed with commercial corn soybean diets that met NRC nutrient requirements. Blood samples were collected from 15 individuals. Genomic DNA was obtained by phenol and chloroform (1:1) extraction and stored at −20°C.

2.3. Primer Design, PCR Amplification and Identification of Gene Polymorphism

The OASL genomic sequence (KY775584) was obtained from the National Center for Biotechnology Information (NCBI). One pair of primers (5'-AGAGCCAGGAGGACACCA-3'and 5' -CCACTCAGAGCCAGGACG-3') was designed to amplify5'-regulatory region of the duck OASL gene. The sizeof the product was 842bp. Polymerase chain reaction (PCR) was performed using50ngDNA templates, 10pM of eachprimer, 0.20mM dNTP, 2.5mM $MgCl_2$ and 0.5U Taq DNA polymerase. Thermalcycling began with an initial denaturation step of 95°C for 3 min, followed by 34 cycles of °C for 15s, 53.5°C annealing for 15s, 72°C for 35s and an elongation step at 72°C for 5 min. DNA sequencing was performed using an ABI 3130 genetic analyser (Applied Biosystems, USA). Sequencing variants were detected by visual examination of thesequencing map followed by alignment using DNAMAN 10.0.

2.4. Statistical Analysis

The genotype and allelic frequencies, genotypic numbers, number of different alleles (Na), number of effective alleles (Ne), expected heterozygosity (He) were calculated and the Hardy–Weinberg equilibrium was analysed using the χ^2 test of PopGene32. SHEsis online version (http://analysis2.bio-x.cn/myAnalysis.php) was used to calculate the pairwise linkage disequilibrium and r^2. CpG islands were predicted using online software (http://www.urogene.org/methprimer/index1.html).

3. Results

3.1. Polymorphisms Identification and Detection in 5'-regulatory Region of the Muscovy Duck OASL Gene

A pair of primers was used to amplify and screen singlenucleotide polymorphisms (SNPs) in the 5'-regulatory region of the Muscovy duck OASL gene. PCR amplification of one OASL gene fragment yielded a 842-bp fragment. The polymorphism type and position wereidentified by direct DNA sequencing. Multiple sequencealignment showed that 11 SNPs (g.313A>G, g.354A>G, g.392T>C, g.423A>G, g450T>C, g523G>A, g528T>C, g533C>T, g545A>G, g604A>T and g609AAAAins) were identified in the duck5'-UTR region OASL gene. DNA sequencing maps were shown in Figure 1.

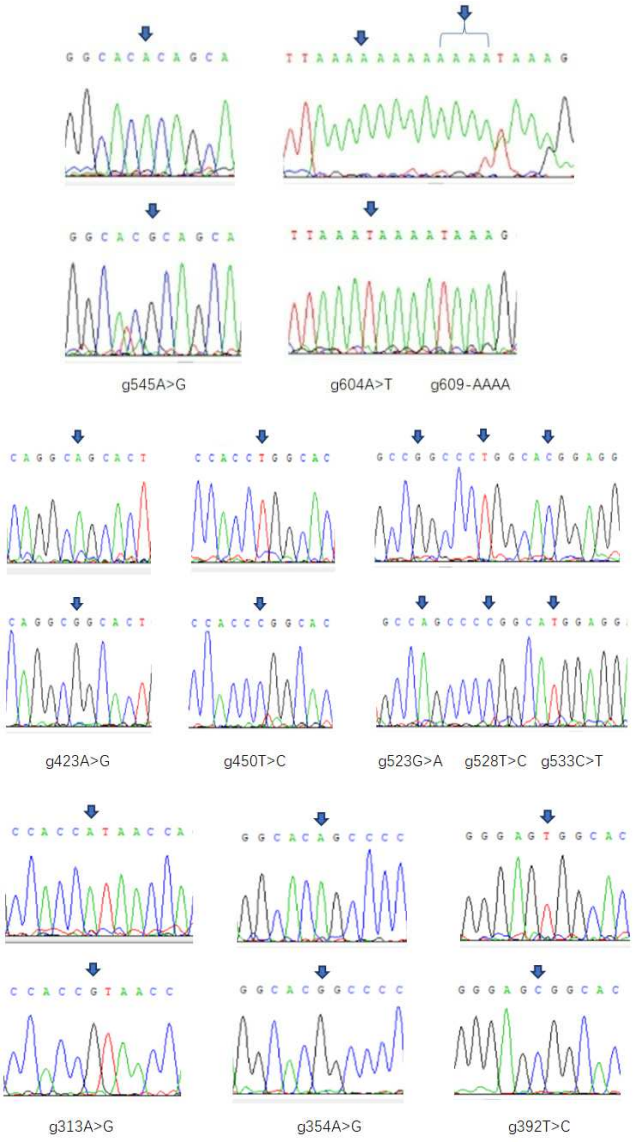

Figure 1. DNA sequencing maps from several DNA templates.

3.2. CpG Island Prediction Analysis

The distribution of OASL gene partial sequence in Muscovy duck population, genotype frequency and allele frequency by PCR sequencing were detected, and CpG island prediction analysis was performed, and genetic heterozygosity (He), effective allele number (Ne), r^2 were alsocalculated. The

linkage disequilibriumof SNPs was analysed.

When CpG nucleotides exist in the genome, part of them is methylated and then deaminated to form thymine, which is easy to be eliminated, and the other part is highly aggregated and inhibits methylation, which exists in the form of "CpG island". The prediction of CpG islands can provide ideas for DNA methylation research and is an important part of studying epigenetics. Enter the full sequence of Muscovy duck OASL gene into the online website to predict the distribution of CpG islands, as shown in Figure 2. The amplified fragment contains a CpG island, the fragment length is 105bp (49-153bp), and the GC content is greater than 70%. After predicting the possibility of CpG islands, further cytological experiments are needed to verify its authenticity.

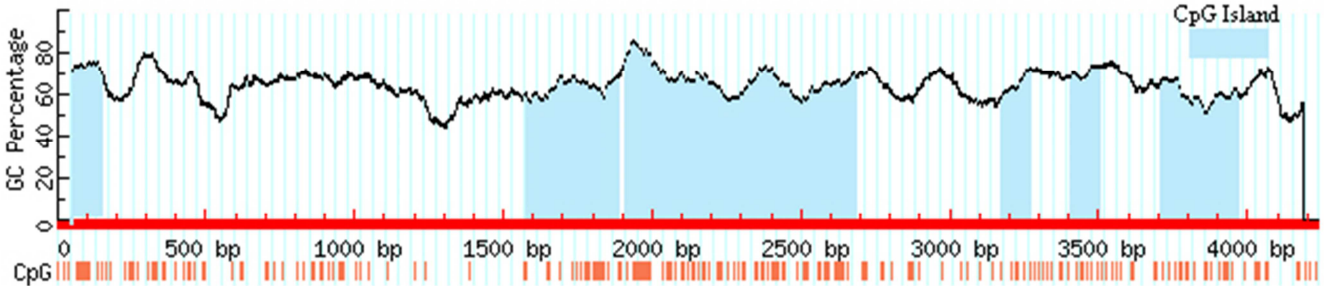

Figure 2. Distribution of CpG islands of Muscovy duck OASL gene.

3.3. Genetic Variation in Muscovy Duck Population

Muscovy Duck population average number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) in the OASL gene are shown in Table 1.

Table 1. Population genetic indexes of 11 polymorphisms in 5'-regulatory region of the Muscovy Duck OASL Gene.

SNPs	Genotype frequency		Allele		Na	Ne	He	Ho
g.313	0.8 (AA)	0.2 (GG)	0.8 (A)	0.2 (G)	2.0	1.47	0.36	0.64
g.354	0.8 (AA)	0.2 (GG)	0.8 (A)	0.2 (G)	2.0	1.47	0.36	0.64
g.392	0.8 (TT)	0.2 (CC)	0.8 (T)	0.2 (C)	2.0	1.47	0.36	0.64
g.423	0.8 (AA)	0.2 (GG)	0.8 (A)	0.2 (G)	2.0	1.47	0.36	0.64
g.450	0.8 (TT)	0.2 (CC)	0.8 (T)	0.2 (C)	2.0	1.47	0.36	0.64
g.523	0.8 (GG)	0.2 (AA)	0.8 (G)	0.2 (A)	2.0	1.47	0.36	0.64
g.528	0.8 (TT)	0.2 (CC)	0.8 (T)	0.2 (C)	2.0	1.47	0.36	0.64
g.533	0.8 (CC)	0.2 (TT)	0.8 (C)	0.2 (T)	2.0	1.47	0.36	0.64
g.545	0.8 (AA)	0.2 (GG)	0.8 (A)	0.2 (G)	2.0	1.47	0.36	0.64
g.604	0.8 (AA)	0.2 (TT)	0.8 (A)	0.2 (T)	2.0	1.47	0.36	0.64
g.609	1.0 (+4bp)	0(-4bp)	1.0 (+4bp)	0(-4bp)	2.0	1.47	0.36	0.64

It can be seen that two genotypes (AA, GG) were found at g.313, g.354, g.423, and g.545, and the dominant allele is A; at g.392, g.450, g. Two genotypes (TT, CC) were found at g.392, g.450, g.528, the dominant allele was T; two genotypes (GG, AA) were found at g.523, and the dominant allele was G; at g.604 A total of two genotypes (AA, TT) were found at the site, the dominant allele was A; One genotype (AA) was found at g.609. Both the effective allele number (Ne) and the degree of heterozygosity (He) can be used as references for population genetic variation, and the degree of variation increases with the increase of the value, so the value of the value directly shows the degree of genetic variation of each individual in the population [6-7]. Among them, the closer Ne is to the absolute number of alleles tested, the more even the alleles are distributed in the population. The test results show that the alleles measured in the sample fragments of Muscovy ducks are more evenly distributed in the population. He represents the frequency of the allele being heterozygous, and its range is between 0 and 1. If He=0, it means no polymorphism; if He=1, it means that there are infinitely many alleles with the same frequency. The test results showed that He=0.36 (<0.5) in the sample population of Muscovy ducks, indicating low heterozygosity.

3.4. Linkage Disequilibrium Analysis of SNP Loci

Linkage disequilibrium (LD) is an important method of population genetics analysis. It refers to the non-random association between alleles of different loci in a population, including the non-random association of two markers [7-9]. In this experiment, SHEsis software was used to analyse the data of Muscovy duck samples, and the results are shown in Figure 3. R^2 =0.99 among g.313, g.392, g.423, g.450, g.523, g.528, g.533, g.545, and g.604, indicating that these sites strong linkage disequilibrium ($0.33<r^2<1$); r^2 =0.16 between g.354 and g.313, g.392, g.423, g.450, g.523, g.528, g.533, g.545, g.604 indicating that there was a linkage disequilibrium

among other loci (0<r2<0.33) in the site except for g.609, and there was no complete linkage disequilibrium between g.609 and the other loci (r^2=0). Most of the strong linkage disequilibrium phenomenon appears, which may be related to the small sample population.

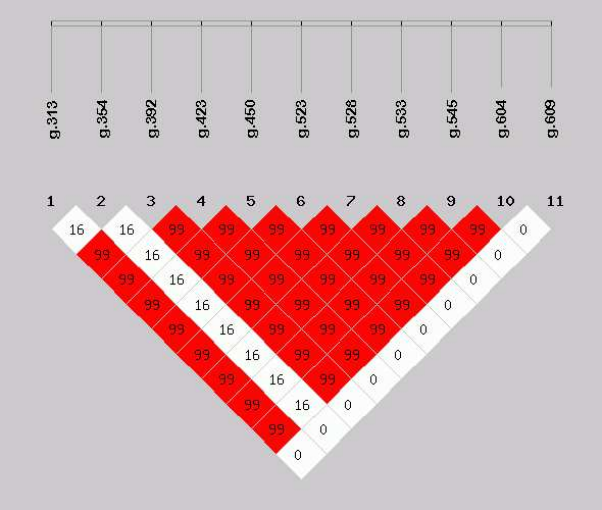

Figure 3. Linkage disequilibrium (LD) plot of the OASL gene in Muscovy ducks. The colour scheme is according to SHEsis r^2 scheme. Numbers in each cell stand for the pairwise r^2 value (%) between the corresponding SNPs.

4. Discussion

The genetic polymorphism analysis of Muscovy ducks can not only reveal its genetic diversity, but also use the obtained polymorphic sites as molecular markers to further analyze the correlation between biological phenotypes and genotypes [10-12], moreover, provide basic data for selection and breeding of fine varieties.

The analysis found that Muscovy ducks OASL gene showed low heterozygosity and high conservation in SNP sites, so the degree of genetic variation was low. Compared with Muscovy ducks, other domestic ducks are generally more polymorphic, which is more conducive to the breeding and reproduction of excellent individuals with high productivity. The polymorphic differences between different breeds of duck flocks may be related to their breeds and economic production uses, and further study of the differences between breeds is needed. In view of the fact that only one breed of duck flock was investigated and analysed in this experiment, the population was incomplete and the sample size was relatively small. To draw a more accurate conclusion, it is necessary to increase the sample size and population size before further analysis.

The avian OAS gene retains the ancient member of the OAS family, namely the OASL gene. OASL lacks oligoadenylate synthase activity, but it can be combined with the C-terminal ubiquitin-like domain with the N-terminal OAS-like domain. Activating the host RIG-I signalling pathway degrades the RNA in the infected cells, so it also has an antiviral effect [2, 7, 13]. This provides hope for OASL's anti-influenza virus, hepatitis C virus and other viral diseases in human medicine, and also suggests the possibility of OASL in the prevention and treatment of poultry viral diseases [14-16].

This experiment took Muscovy ducks as the research object, studied the genetic variation of OASL gene sequence in Muscovy duck populations at the molecular level, clarified the types and locations of some gene mutations, and carried out biological analysis to clarify the functional mechanism of OASL genes and related proteins. The breeding and anti-virus of Muscovy ducks provide reference materials.

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