Polymorphism of DMA (DM α Chain) gene in IPB-D2 chicken

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Abstract. The DM gene group is a non-classical MHC Class II gene group that plays an important role in the process of presenting antigens by producing DM protein. The DMA gene, which is a group of DM genes, has an α chain that helps present exogenous antigens to CD4 + via MHC class II molecules. IPB-D2 chickens are the result of selection from IPB-D1 chickens which have disease-resistant trait. The aim of this study was to determine genetic polymorphism of DMA genes in IPB-D2 chickens. In this study, blood samples were collected from 21 weeks old chickens that are raised in the field laboratory of the Faculty of Animal Science IPB University. The PCR was used to amplify 601 bp fragments for DMA gen locus. SNP identification used direct sequencing method and analyzed using MegaX and Bioedit. The results found 4 SNPs, namely base position c.2328 G>A on exon 3, c.2612 G>A, c.2686 G>A, and c.2710 G>A on exon 4. All SNPs are polymorphic and in Hardy-Weinberg equilibrium. The highest allele frequency for each SNP is allele A (0.5745; 0.5851; 0.9362), except at SNP c.2612 G>A (0.4575).

1. Introduction

The DM gene group is a non-classical MHC class II gene group that plays an important role in the process of presenting antigen peptides by producing DM protein [1]. There are 3 genes in the DM gene group, namely DMA, DMB1, and DMB2. DMA, DMB1 and DMB2 genes are polymorphic and involved in antigen processing [2]. Chazara [3] stated that there were 28 non-synonymous single nucleotide polymorphisms (SNPs) on the B-DMA protein (4 SNPs in exon 3 of DMA gene) which formed 9 amino acid variations in the DMA gene. The DMA gene in addition to being expressed in the spleen, the DMA gene was also expressed in other tissues and cells such as liver, kidney, small intestine, brain and T cells [3].

IPB-D2 chickens are the result of selection from IPB-D1 chickens which have disease-resistant trait. The selection of IPB-D2 chickens was based on several immunocompetences related to standard-conforming immune properties such as ND antibody titer and total IgY concentration. ND antibody titer values with vaccination ≥ 3 log 2 HI units are categorized as protective antibody titers [4] while total IgY concentrations ≥ 9.55 mgmL⁻¹ are categorized as high IgY concentrations [5]. IPB-D2 chickens were selected based on total IgY concentration ≥ 9.55 mgmL⁻¹ and ND antibody titer ≥ 3 log 2 HI units.
DMA gene can be used as a candidates for marker assisted selection of disease resistance in IPB-D2 chickens. The aim of this study was to identify the diversity of DMA genes in IPB-D2 chickens.

2. Material and method

2.1. Material
A total of 47 samples were genotyped for DMA gene. The chickens used for this study was IPB-D2 chicken aged 21 weeks. DNA extraction was collected from the jugular vein as much as ± 5 mL of each individual with EDTA as an anticoagulant. Genomic DNA was extracted from whole blood by phenol/chloroform extraction method. The DMA genotypes were analyzed using direct sequencing method. The primer used for DMA gene fragment amplifications based on the ENSEMBL (ENSGALG00000000158): Forward 5’- CAT TCC CAC CGA TGT GTC -3, Reverse5’- CTG CTG TCT CCA TTG TTC -3.

DNA amplification (PCR) was carried out in thermocycler machine (Applied Biosystem 9700). Cycles applied were predenaturation 95°C 1 minute, denaturation 95°C 15 second, annealing 56°C 15 second, extension 72°C 10 second and final extension 72°C 1 minute. DNA amplification reagents were 12.5 µL 2x MyTaq HS Red Mix, 11.7 Nuclease Free Water, 0.25 µL forward primer, 0.25 µL reverse primer and 0.3 µL DNA samples. The DMA genotypes were done by 1st Base Laboratory, Selangor Malaysia. SNP data analysis was performed using Bioedit and MegaX.

2.2. Genotype and allele frequencies
Genotype and allele frequencies are calculated based on Nei and Kumar [6]:

Allele frequencies \( X_i = \frac{(2N_{ii}+N_{ij})}{2N} \);

Genotype frequencies \( X_{ii} = \frac{N_{ii}}{N} \), \( X_{ij} = \frac{N_{ij}}{N} \), \( X_{jj} = \frac{N_{jj}}{N} \)

\( X_i \) = allele frequency
\( X_{ii} \) = genotype frequency ii
\( X_{ij} \) = genotype frequency ij
\( X_{jj} \) = genotype frequency jj
\( N_{ii} \) = number of samples with genotype ij
\( N_{ij} \) = number of samples with genotype ij
\( N \) = number of sample

2.3. Hardy-Weinberg Equilibrium
Hardy-Weinberg equilibrium are calculated based on chi square test \((x^2)\) [7]:

\[ X^2 = \sum_{i=1}^{n} \frac{(O-E)^2}{E} \]

\( X^2 \) = chi square test
\( O \) = observation value of ii genotype
\( E \) = expected value of ii genotype

2.4. Heterozygosity
Genetic diversity can be identified by estimating the frequency of expected heterozygosity \((He)\) and observed heterozygosity \((Ho)\) [6]:

\[ Ho = \sum_{ij}^n \frac{n_{ij}}{N} \]

\[ He = 1 - \sum_{i=1}^{q} X_i^2 \]

\( Ho \) = observed heterozygosity
\( He \) = expected heterozygosity
\( n_{ij} \) = number of heterozygous individuals
\( N \) = number of individuals observed
\( X_i \) = allele frequencies
\( q \) = number of alleles.
3. Results and discussion

3.1. Amplification of DMA gene
Amplification of the DMA gene has been successfully carried out 601 bp fragment. The electrophoresis profile fragment of DMA gene obtained from primer pair are shown in figure 1.

![Figure 1](image_url)

Figure 1. Gel picture of DMA gene fragment amplified by primer pair. M (marker), 1-6 (sample).

3.2. Polymorphism identification
The polymorphism of DMA gene are identified using direct sequencing method. Sequencing analysis result shown 4 new mutation at base position c.2328 G>A on exon 3, c.2612 G>A, c.2686 G>A, and c.2710 G>A on exon 4. The four SNPs are classified as a substitution mutation. Substitution mutation are divided into transitions and transversions [8]. Base mutations c.2328 G>A, c.2612 G>A, c.2686 G>A, and c.2710 G>A indicates transition mutation. Transition are substitution mutations between A and G (purines) or between C or T (pyrimidines). The visualization of partial mutations are shown in figure 2.

![Figure 2](image_url)

Figure 2. Partial sequences DMA gene (a) has no mutation (b) transition mutation.

Substitution mutation devided into two classes. Synonymous mutation if it changes a codon into another that specifies the same amino acid as the original codon otherwise it is nonsynonymous. Based on the results, it was found 3 types of non-synonymous mutations and 1 type of synonymous mutation. A synonymous mutation may, for instance, create a new splicing site or oblirate an exiting one, thus turning an exonic sequence into an intron or vice versa and causing a different polypeptide to be produced [8]. The changes in amino acid are shown in table 1.
Table 1. Amino acids changes.

| SNPs Position | Amino Acid |   |
|---------------|------------|---|
| c.2328 G>A    | Valine     | > Isoleucine |
| c.2612 G>A    | Alanine    | > Alanine    |
| c.2686 G>A    | Arginine   | > Glutamine  |
| c.2710 G>A    | Arginine   | > Lysine     |

3.3. Allele frequency, genotype frequency and Hardy-Weinberg equilibrium

Populations with high genetic diversity are more likely to be disease resistant. Genetic diversity can be seen from the allele frequency, genotype frequency and heterozygosity. In a population it is said to be polymorphic if there are 2 or more allele with a relative frequency more than 0.01 (1%) [6]. The Hardy-Weinberg equilibrium known through the chi-square test \((x^2)\) [7]. The allele frequency, genotype frequency and Hardy-Weinberg equilibrium are shown in table 2.

Table 2. The allele frequency, genotype frequency and Hardy-Weinberg equilibrium.

| Gene  | SNPs       | Genotype frequency | Allele frequency | \(x^2\) | Ho   | He   |
|-------|------------|--------------------|------------------|--------|------|------|
| DMA   | c.2328 G>A | 0.5319 0.0851      | 0.3829 0.5745    | 0.4255 | 0.6852 | 0.0851 | 0.4942 |
|       | c.2612 G>A | 0.3829 0.1489      | 0.4680 0.4575    | 0.5426 | 0.4945 | 0.1489 | 0.5017 |
|       | c.2686 G>A | 0.5744 0.0212      | 0.4042 0.5851    | 0.4149 | 0.9150 | 0.0213 | 0.4907 |
|       | c.2710 G>A | 0.9361 0           | 0.0638 0.9362    | 0.0638 | 1     | 0.0000 | 0.1208 |

\(x^2(0.05;1) : 3.84\)

Reduction in heterozygosity is caused by changes in allele frequency from one generation to the next. Hardy-Weinberg equilibrium will occur if any one generation as long as mating is random [7]. Based on the allele frequency c.2328 G>A, c.2612 G>A, c.2686 G>A and c.2710 G>A are polymorphic with the highest frequency is A allele expect c.2612 G > A. SNP is categorized as polymorphic if the allele frequency is ≤0.99 in large populations and ≤0.95 in small populations [7] and all SNPs are polymorphic. All SNPs has 2 homozygous genotypes and 1 heterozygous genotype, except for SNP c.2710 G>A which only has 2 homozygous genotypes.

Heterozygosity indicates levels of genetic diversity in a population. Heterozygosity ranges between zero and one. Ho is the observed proportion of the heterozygotes and He is the expected proportion of heterozygotes [7]. All SNPs has lower Ho than He. A lower Ho value than He indicates inbreeding. High genetic diversity has a heterozygosity value of more than 0.50 [7]. The difference in Ho and He values can be used as an indicator of the balance of the genotype in a population.

The Hardy-Weinberg equilibrium can be seen from the chi-square test \((x^2)\) test. A population is said to be in a balanced state if the \(x^2\) value is smaller than the \(x^2\) table. A population is in Hardy-Weinberg equilibrium if there is random mating, no mutation, large population size, no natural selection and no immigration [7]. Based on table 2, all SNPs are in Hardy-Weinberg equilibrium with \(P<0.05\).

4. Conclusion

The result of the study found 4 SNPs of DMA gene in IPB-D2 chicken, namely c.2328 G>A on exon 3, c.2612 G>A, c.2686 G>A, and c.2710 G>A on exon 4. All SNPs are polymorphic and in Hardy-Weinberg equilibrium.

Acknowledgment

This research was supported by Research Grant “Program Hibah PMDSU”, Ministry of Research, Technology and Higher Education, Indonesia.
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