# Survey of Major Histocompatibility Complex Class II Haplotypes in Four Turkey Lines Using Restriction Fragment Length Polymorphism Analysis with Nonradioactive DNA Detection

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**ABSTRACT** Four turkey lines were typed for MHC Class II haplotypes with restriction fragment length polymorphism (RFLP) analysis using a nonradioactive probe made from a chicken genomic clone of MHC Class II genes. The RFLP analysis detected 18 new patterns in the populations. There were three new haplotypes that had a frequency of about 10% or more in a population, whereas the rest appeared only once. The haplotype frequencies were significantly different in the E line, selected only for increased egg production, and the F line, selected only for increased body weight, compared with their respective randombred control lines. The shift of haplotype frequencies in the two selected lines seemed to be in opposite directions. One, but not the same, haplotype predominated in the selected lines, with about 50% of total haplotypes. Fewer haplotypes were frequent in the selected lines, whereas the frequencies in the control lines were relatively widely distributed, with the most frequent haplotype being below 35%. The frequency of homozygotes of the Class II haplotypes was the highest in the F line.

(*Key words*: major histocompatibility complex, Class II haplotypes, egg production, growth, turkey)

1995 Poultry Science 74:1067–1073

## INTRODUCTION

The MHC is a gene family encoding a group of glycoproteins involved in some important aspects of the immune response, such as antigen presentation and self recognition (Guillemot *et al.*, 1988a). The chicken MHC was originally described as a blood group system (Briles *et al.*, 1950). Three classes, I, II, and IV, of the chicken MHC have been discovered (reviewed by Dietert *et al.*, 1991). The Class I antigens are distributed on all nucleated cell membranes, and they present endogenously synthesized antigen to cytotoxic T cells. Unlike the Class I antigens, the Class II antigens are expressed mainly on antigen-presenting cells, such as B cells and macrophages, and they present internally processed antigens, which originate exogenously, to T helper cells (reviewed by Guillemot *et al.*, 1988a; Dietert *et al.*, 1991). The antigen presentation to T helper cells is a critical initiating step in inducing an immune response. The Class IV antigens are unique proteins in avian species with unknown functions. They were first found

Received for publication July 18, 1994.
Accepted for publication March 8, 1995.
[1]Salaries and research support provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Manuscript Number 3-94.
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on red blood cells (Pink *et al.*, 1977) and recently detected in chicken intestinal epithelium (Miller *et al.*, 1990).

The MHC Class I and II loci were found to be multigenic and highly polymorphic. Three MHC Class II genes and six Class I genes were cloned from one chicken (Guillemot *et al.*, 1988b; Xu *et al.*, 1989). Many polymorphisms were identified with different methods, such as serological and molecular biological methods. The serological method was most frequently used and over 30 haplotypes were discovered with hemagglutination tests (reviewed by Dietert *et al.*, 1991). With cloned MHC genes available, genotyping with restriction fragment length polymorphism (RFLP) analysis was demonstrated to be a powerful method for the identification of MHC polymorphisms (Andersson *et al.*, 1987; Hala *et al.*, 1988; Miller *et al.*, 1988; Chausse *et al.*, 1989; Chen and Lamont, 1992; Emara *et al.*, 1992). The RFLP analysis can type each MHC class specifically by using different probes. The RFLP method distinguished the polymorphisms typed by the serological method and even additional polymorphisms that the serological method did not detect (Chausse *et al.*, 1989).

Four haplotypes of turkey MHC Class II genes were discovered in two randombred control lines by Emara *et al.* (1992) using RFLP analysis. The haplotypes were characterized to be in linkage disequilibrium with allelic specificities by mixed lymphocyte reaction, graft-versus-host reaction, and skin graft reaction (Emara *et al.*, 1993). The purpose of the present experiment was to identify additional haplotypes and to determine the frequencies of the MHC Class II haplotypes in selected and randombred control turkey lines.

## MATERIALS AND METHODS

### Turkey Lines

All individuals in four turkey lines maintained at the Ohio Agricultural Research and Development Center were used for MHC Class II genotyping. The RBC1 was a randombred control population derived from a wide genetic background (McCartney, 1964). The RBC2 was another randombred control line established from the crossing of two commercial lines of turkeys with high body weight (Nestor *et al.*, 1969). The randombred control populations were maintained with 36 parental pairs mated at random except that full-sib matings were avoided to reduce inbreeding. The E line was started from the RBC1 line by selection for increased egg production based on the total egg number in certain reproductive periods (Nestor, 1980). The F line was another selected line derived from the RBC2 line by selecting for increased 16-wk body weight by mass selection (Nestor, 1977). Seventy-two parental pairs in the E line and 36 males and 72 females (1 sire mated to 2 dams) in the F line were used for reproducing the selected lines. The 18 best families (4 males and 4 females per family) in the E line were selected to produce offspring for the next generation, and the top 36 males and 72 females in the F line were chosen. The mating system in the selected lines was the same as in the randombred control lines. The E and F lines had been selected for 36 and 25 generations, respectively.

### DNA Isolation

Turkey blood (.5 mL) was collected from the brachial vein of each individual (180 sires and 216 dams) in the lines with EDTA used as anticoagulant. The method used for the DNA isolation was reported by Emara *et al.* (1992). Some steps of the procedure were modified for scale-down preparation and simplification. The frozen blood samples were thawed in a 37 C water bath, then microcentrifuged for 15 s. The pellets were washed with low-salt buffer (10 m*M* NaCl; 10 m*M* EDTA) twice and PBS (pH 7.2) once. Twenty microliters of the pellets were resuspended in 20 $\mu$L of PBS, then 1 mL of cell lysis buffer (50 m*M* Tris-HCl, pH 8.0; .5% SDS; 100 m*M* EDTA) was added to the tubes. Ribonuclease A[3] was added to a final concentration of 100 $\mu$g/mL, and the mixtures were incubated for 30 min followed by addition of protease K[3] at final concentra-

[3]AMRESCO, Solon, OH 44139.

tion of 100 μg/mL and incubation at 55 C overnight. After digestion, the samples were extracted once with Tris-EDTA (TE) saturated phenol and 49:1 chloroform isoamylalcohol. The DNA was precipitated with 2 vol of 95% ethanol and .1 vol of 4 *M* NaCl. The DNA pellets were washed with 70% ethanol, dried, and suspended in double-distilled $H_2O$ for at least 24 h.

### Probe Preparation

A 2.3-kb fragment of a genomic clone of a chicken MHC Class II β gene (Xu *et al.*, 1989) was purified by gel electrophoresis and elution (Maniatis *et al.*, 1986). The fragment was used as a DNA template for probe labeling with a Genius 1 DNA labeling and detection kit.[4] A 100-μL scale-up reaction was prepared according to the manufacturer's instructions (Boehringer Mannheim, 1992).

### DNA Digestion and Southern Blot Analysis

Approximately 10 μg of DNA from each sample was digested with 20 U of *Pvu*II restriction enzyme for over 3 h in 30 μL total sample volume. The digested samples were separated on a .8% gel for 30 h electrophoresis at 35 V. The gels were submersed in a gel denature buffer (1.5 *M* NaCl, .5 *M* NaOH) for 30 min, and a neutralization buffer (1.5 *M* NaCl, 1.0 *M* Tris-Cl, pH 7.4) for 30 min. Then the DNA was blotted from the gels to nylon membranes[4] with 10 × SSC (1 SSC = .15 *M* NaCl, .015 *M* sodium citrate) using a Posiblot Pressure Blotter[5] for 2 h. The membranes were baked at 80 C for 1 h. Prehybridization, hybridization, and color development were based on the procedures described in Genius System User's Guide, except that hybridization was conducted without formamide (Maniatis *et al.*, 1986). Hybridization temperature was 65 C, and the final concentration of the probe was 10 ng/mL.

[4]Boehringer Mannheim, Indianapolis, IN 46250.
[5]Stratagene, La Jolla, CA 92037.

### Statistical Analysis

The SAS® software package (SAS Institute, 1988) was used for chi-square analysis to test the differences in the frequencies (percentages) of the MHC haplotypes among the lines and between the sexes. Haplotypes appearing less than five times in the four lines were pooled.

## RESULTS

In addition to the four types discovered previously, 18 new RFLP patterns were identified with the same restriction enzyme, *Pvu*II (Figure 1). Because the patterns were not known to be from homozygous individuals, the new haplotypes have not yet been determined. However, through examining the patterns within families from offspring with new patterns and in the entire populations, there were probably three new frequent haplotypes (Table 1; Figure 2) occurring more than 15 times in the four turkey lines and some new rare types appearing less than 5 times. There was a totally new band (4.8 kb) in Haplotypes X and Y. Haplotype Z was a new combination pattern shared with some of Haplotypes B and D. The hypothesized haplotypes were used as types for a chi-square test.

The haplotype frequencies were significantly different between the line comparisons, RBC1 vs RBC2, RBC1 vs E, RBC2 vs F, and E vs F ($P < .01$) (Table 2). Compared with its control (RBC1), Haplotypes A, B, and X were greatly increased in the E line, especially Haplotype X, which was almost 15 times greater in frequency. On the other hand, Haplotypes C, D, Y, and Z were eliminated or almost eliminated in the E line. There were also significant changes in haplotypic frequencies in the F line relative to its control (RBC2). Frequencies of Haplotypes C and D increased greatly, and frequencies of Haplotypes A, B, and Z decreased in the F line. Differences in predominant haplotypes in the two selected lines were evident. In the E line, Haplotype B was predominate with almost 50% of the total haplotypes, whereas Haplotype D in the F line had a similar percentage. The frequencies of the common haplotypes in the two

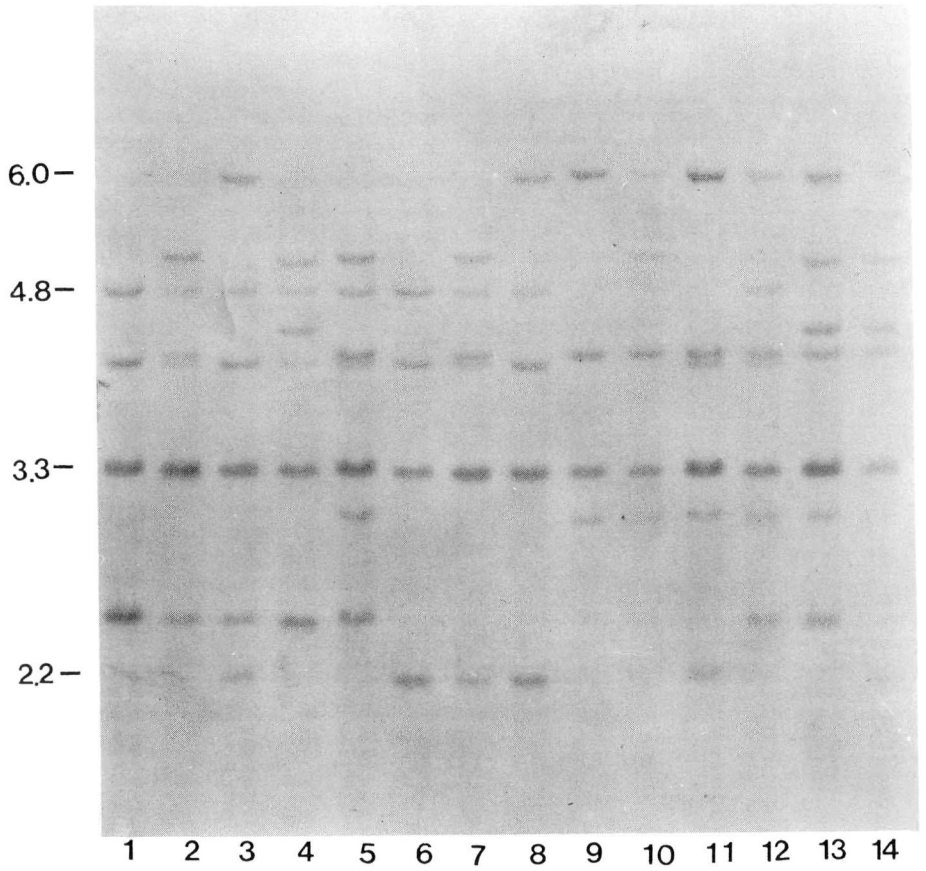


**FIGURE 1.** Southern blot analysis of *Pvu*II-digested turkey DNA hybridized with a nonradioactive probe made from a chicken MHC Class II genomic clone. Lane 1 = Haplotype YY, 2 = AY, 3 = BY, 4 = CY, 5 = DY, 6 = XX, 7 = AX, 8 = BX, 9 = ZZ, 10 = DZ, 11 = BZ, 12 = CZ, 13 = AZ, and 14 = one of the rare patterns.

selected lines changed in opposite directions. Unlike the selected lines, the frequencies in the control lines were more widely distributed among the haplotypes.

**TABLE 1. DNA *Pvu*II restriction fragment lengths (kilobases) of turkey MHC Class II haplotypes**

| RFLP haplotype patterns | | | | | | |
|---|---|---|---|---|---|---|
| A[1] | B[1] | C[1] | D[1] | X | Y | Z |
| 5.1 | 6.0 | 5.0 | 5.1 | 4.8 | 4.8 | 6.0 |
| 4.1 | 4.2 | 4.5 | 4.3 | 4.2 | 4.2 | 4.3 |
| 3.3 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 |
| | 2.2 | 2.6 | 3.1 | 2.2 | 2.6 | 3.1 |

[1]The haplotypes have been reported by Emara *et al.* (1992).

They shared the three same frequent Haplotypes (A, B, and D), whose frequencies ranged from about 10 to 34%.

There was a significant difference in the percentages of heterozygotes and homozygotes between the F line and its control line, but no differences were found in the other comparisons (Table 3). Homozygous frequency regarding the MHC Class II haplotypes was the highest in the F line. The haplotype frequencies were not different between the sexes (Table 4).

## DISCUSSION

In a previous study conducted by Emara *et al.* (1992), the turkey MHC Class II genes, like those of other species, were found to be conserved. The RFLP analysis

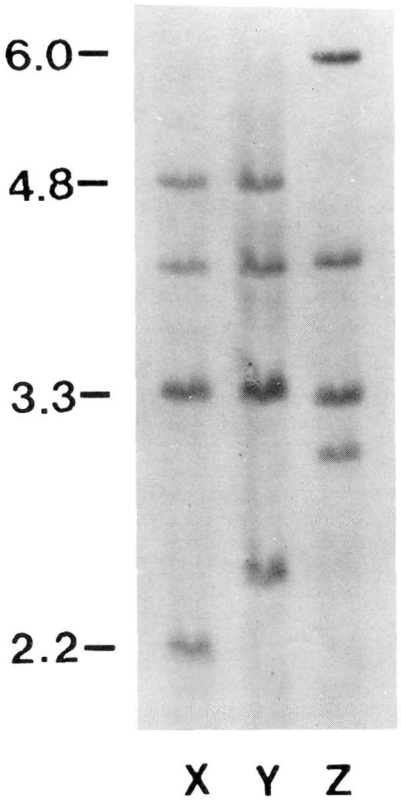


FIGURE 2. Southern blot analysis of *Pvu*II-digested turkey DNA hybridized with a nonradioactive probe made from a chicken MHC Class II genomic clone. Lane X = Haplotype X; Lane Y = Haplotype Y; Lane Z = Haplotype Z.

with a probe made from a chicken genomic clone of the MHC Class II genes detected two haplotypes in *Bam*HI-digested genomic DNA from turkeys derived from the two randombred control lines by full-sib matings for four generations. Four haplotypes were detected in *Pvu*II-digested DNA from those turkeys. The four haplotypes were further characterized to be associated with allelic specificity (Emara *et al.*, 1993). Because using *Pvu*II revealed more polymorphisms in the RFLP analysis, that restriction enzyme was used in the present haplotype survey.

Recently, another MHC-like locus, designated as *Rfp-Y*, was discovered in chickens using RFLP analysis by Briles *et al.* (1993). However, data obtained from the current experiment based on information limited to two generations did not provide evidence for the additional *Rfp-Y* locus. The restriction fragments segregated in a single-locus codominate, Mendelian manner in the previous study (Emara *et al.*, 1992) and in the present experiment. Further investigation using large family sizes and more restriction enzymes is needed to reveal the *Rfp-Y* locus, if present, in turkeys.

In the E and F lines, the changes of haplotype frequencies appeared to go in opposite directions. The frequency

**TABLE 2. Haplotype frequencies of the MHC Class II in the four turkey lines**

| Haplotypes | Lines[1,2] RBC1 | E | RBC2 | F |
|---|---|---|---|---|
| | (%) | | | |
| A | 13.9 | 29.9 | 34.0 | 29.6 |
| B | 31.3 | 47.9 | 24.3 | 10.2 |
| C | 9.0 | 0 | 1.4 | 8.8 |
| D | 31.9 | .4 | 10.4 | 46.8 |
| X | 1.4 | 20.8 | 0 | .9 |
| Y | 9.7 | 0 | 0 | 0 |
| Z | 2.8 | 0 | 24.3 | 2.8 |
| R[3] | 0 | 1.0 | 5.6 | .9 |

[1]RBC1 = randombred control population (36 males, 36 females); E = subline of RBC1 selected for egg production (72 males, 72 females); RBC2 = randombred control population (36 males, 36 females); and F = subline of RBC2 selected for 16-wk body weight (36 males, 72 females).

[2]Haplotype frequencies between four lines (RBC1 vs RBC2, RBC1 vs E, RBC2 vs F, and E vs F) were significantly different ($P < .01$).

[3]R = pooled rare haplotypes.

**TABLE 3. Heterozygote and homozygote frequencies of the MHC Class II haplotypes in the four turkey lines**

| Zygosity | Lines[1,2] RBC1 | E | RBC2 | F |
|---|---|---|---|---|
| | (%) | | | |
| Homozygote | 37.5 | 31.9 | 33.3 | 45.4 |
| Heterozygote | 62.5 | 68.1 | 66.7 | 54.6 |

[1]RBC1 = randombred control population; E = subline of RBC1 selected for egg production; RBC2 = randombred control population; and F = subline of RBC2 selected for 16-wk body weight.

[2]Haplotype zygosities were significantly different in the four lines ($P < .05$).

**TABLE 4. The haplotype frequencies of the MHC Class II in the male and female turkeys of the four lines**

| Haplotypes | Sexes[1] | |
|---|---|---|
| | Males | Females |
| | (%) | |
| A | 27.4 | 27.8 |
| B | 31.4 | 29.4 |
| C | 3.1 | 5.2 |
| D | 17.2 | 22.8 |
| X | 8.9 | 7.5 |
| Y | 3.1 | .9 |
| Z | 7.2 | 4.6 |
| R[2] | 1.7 | 1.8 |

[1]No significant difference between the sexes ($P > .05$).

[2]R = pooled rare haplotypes.

changes may be due to MHC associations with growth and egg production. In long-term selection, a negative phenotypic association between growth and egg production was detected in the F and E lines relative to their control lines (Nestor, 1985), and there was evidence indicating that MHC haplotype associations with egg production and growth do exist (reviewed by Bacon, 1987; Dietert *et al.*, 1991; Plachy *et al.*, 1992). Recently, Abplanalp *et al.* (1992) used inbred congenic Leghorns different in MHC haplotypes to prove the associations. They found significant differences between the haplotypes in egg production and body weight. A similar result was also reported in mice (Simpson *et al.*, 1982), in which individuals in the sublines selected for small body size were predominately *H-2*$^b$ haplotype and for large body size *H-2*$^g$, whereas the control stock were *H-2*$^b$, *H-2*$^g$, or both. Some genes controlling growth and reproduction in rats have also been linked to the MHC (Kunz *et al.*, 1980).

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