# DEVELOPING RECOMBINANT CONGENIC STRAINS (RCS) IN CHICKENS AS A TOOL TO STUDY GENETIC RESISTANCE TO MAREK'S DISEASE (MD)

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## SUMMARY

Nineteen Recombinant Congenic Strains (RCS) are under development by crossing the ADOL inbred lines $6_3$ and $7_2$ that are Marek's disease (MD) resistant and susceptible, respectively. The $F_1$ were backcrossed to line $6_3$ for 2 generations, followed by yearly generations of brother-sister mating. Characterization of these RCS after 2-4 years of sib matings indicates that at least 4 strains are susceptible to MD despite the small portion (~12.5%) of the susceptible line $7_2$ in the genome. The portion of line $7_2$ genome in the susceptible strains was partially tested by genotyping with microsatellite markers linked to 8 regions containing QTL for MD resistance. These susceptible strains provide a valuable resource to study the complex nature of MD resistance by simplifying a multigeneic trait to a series of single gene traits.

**Keywords:** Recombinant congenic strain, genetics, disease resistance, chicken.

## INTRODUCTION

Chickens are an important food source all over the world. Concerted efforts are made by breeders to improve production and reduce losses due to diseases. Marek's disease (MD) is of particular concern to the poultry industry. MD is a lymphoproliferative disease caused by a member of the herpesvirus family, the Marek's disease virus (MDV). Diseased chickens infected by MDV commonly exhibit paralysis, blindness, and visible lymphoid tumors that result in condemnation or reduced egg production of the birds. Since the late 60's, vaccines have been used to control MD. However, the emergence of new strains against which vaccines cannot adquately protect point to the need for additional methods.

Genetic resistance to MD has been known for more than 60 years (Calnek 1985). Although genetic resistance is complex and controlled by several quantitative trait loci (QTL), selection for high levels of resistance can be obtained within relatively few generations (Cole 1968). The best understood mechanism for the involvement of genetic resistance to MD involves the major histocompatibility complex (MHC) or B complex, as it is known in the chicken (Briles *et al.* 1977). Recently, we identified 8 non-MHC QTLs for MD resistance by screening 272 $F_2$ chicks (Vallejo *et al.* 1996). The $F_2$ birds were generated using inbred line $6_3$ (MD resistance) and line $7_2$ (MD susceptible) as parents, and genotyped using microsatellite markers from the East Lansing chicken genomic map (Cheng *et al.* 1995).

To better characterize each non-MHC QTL, Recombinant Congenic Strains (RCS) are being generated using lines $6_3$ and $7_2$ as parents. As proposed by Demant and Hart in 1986, a series of RCS comprises ~20 homozygous strains produced by limited backcrossing (BC) between two parental inbred lines and subsequent brother-sister matings. The result is that each of these strains carries a random ~12.5% (with two BC matings) of the donor genome in the genetic background of the recurrent parent. The major advantage of this approach is the QTL

controlling the complex trait in the donor line are genetically dissected and found individually or in pairs in different RCS. With the QTL sorted into different RCS, each QTL can be fine-mapped using simple crosses as well as characterized for gene function by phenotyping each RCS for various traits. The RCS system has been successfully used to map and characterize genes in mice involved in several traits such as colon tumor susceptibility (Moen *et al.* 1991, 1992; Corina *et al.* 1996) and lung tumor susceptibility (Fijneman *et al.* 1994).

The main objective of this study is to create RCS for MD to verify our QTL identification, fine map the QTL, get clues on the possible gene function of each QTL, and learn more about the complex genetic control of the MD resistance.

## MATERIAL AND METHODS

**Chickens and matings.** ADOL inbred lines $6_3$ and $7_2$ that are MHC identical but MD resistance and susceptible, respectively, were chosen as parents for this study. Pooled semen from 5 line $7_2$ males was used to inseminate 6 line $6_3$ hens to create the $F_1$ population. One $F_1$ male breeder was saved from each of the 6 line $6_3$ hens. The 6 $F_1$ males were each backcrossed to a single line $6_3$ hen to produced a $BC_1$ male breeder. Each of 6 $BC_1$ males was backcrossed again to 4 line $6_3$ hens to produce 10 $BC_2$ chicks per hen. From each of the 24 dam families, a single Recombinant Congenic Strain (6C.7-A - 6C.7-X) is generated by brother-sister mating 1 male to 7 sisters; in 1997, the 4th generation of full-sib mating was conducted.

**Challenge experiments.** In the second generation of brother-sister mating (1995), in addition to repopulating the strains, chicks from 15 RCS and line $7_2$ (control) were challenged using our standard conditions, i.e., 2,000 pfu of JM strain MDV at 1 week of age (interperitoneum). The chickens were observed until they were moribund or up to 10 weeks of age. All the animals were necropsied to score for the presence of MD tumors. In the next two generations of brother-sister mating (1996 and 1997), the 4 highest MD susceptible strains (K, M, P and W) and line $7_2$ (control) were checked again for MD susceptibility following the standard challenge as described above. In 1997, 4 previously untested strains were also included (I, T, V and X).

**Genotyping.** In the third generation of brother-sister mating (1996), DNA was isolated from potential parents of the next generation. The DNA from each individual bird of the four susceptible strains (K, M, P and W) and pooled DNA of the other strains were amplified using the 13 micosatellite markers linked to the 8 MD QTLs (Vallejo *et al.* 1996). The products were loaded on an ABI machine and the alleles scored using the Genescan software.

## RESULTS

After the first generation of brother-sister mating, 24 different RCS were produced. However after the 3rd generation, due to poor reproductivity, only 19 RCS remained. In the 1995 challenge experiment, the majority of the RCS were resistant to the MDV with low percentages of MD (0% to 5.7%) (Bacon *et al.* 1996), whereas in the susceptible line $7_2$ the MD incidence was 74% (Table 1). As shown in Table 1, only 4 of the 15 strains exhibited some susceptibility to MDV: strains K (14%), M (60%), P (7.5%), and W (28.6%). In the 1996 and 1997 challenge experiments, strains M, P, and W continued to exhibit MD susceptibility (10-52%) but strain K was no longer susceptible; line $7_2$ (control) had 90+% MD incidence. In 1997, strain X had a high MD disease incidence at 28.6% while the remaining 3 previously untested strains experienced low MD susceptibility (6.5 - 9.1%).

**Table 1. Marek's disease challenge experiments (1995, 1996, and 1997)**

| Year | | Line K | Line M | Line P | Line W | Line X | Line 7 |
|---|---|---|---|---|---|---|---|
| 1995 | No. of Sires | 5 | 2 | 3 | 1 | ND[1] | pool |
| | No. of Dams | 8 | 2 | 3 | 5 | ND[1] | pool |
| | No. of Chicks | 43 | 5 | 40 | 42 | ND[1] | 57 |
| | No. with MD | 6 | 3 | 3 | 12 | ND[1] | 42 |
| | %MD | **14.0** | **60.0** | **7.5** | **28.6** | ND[1] | **73.7** |
| 1996 | No. of Sires | 1 | 1 | 1 | 1 | ND[1] | pool |
| | No. of Dams | 1 | 4 | 4 | 6 | ND[1] | pool |
| | No. of Chicks | 12 | 29 | 38 | 40 | ND[1] | 31 |
| | No. with MD | 1 | 7 | 4 | 21 | ND[1] | 28 |
| | %MD | **8.0** | **24.0** | **10.0** | **52.0** | ND[1] | **90.0** |
| 1997 | No. of Sires | 1 | 1 | 1 | 1 | 1 | pool |
| | No. of Dams | 7 | 1 | 7 | 9 | 11 | pool |
| | No. of Chicks | 42 | 4 | 32 | 51 | 35 | 14 |
| | No. with MD | 2 | 1 | 7 | 12 | 10 | 14 |
| | %MD | **4.8** | **25.0** | **21.9** | **23.5** | **28.6** | **100** |

[1]ND=Not done.

The genotypes of the 4 susceptible strains using markers linked to MD QTLs are presented in Table 2. In strain K, only 2 QTLs have line $7_2$ alleles with ADL185 already fixed. In strains M and P, DNA markers from 4 QTLs have line $7_2$ alleles that range from 8.3% to 58.3%. In strain W, only 1 marker has a line $7_2$ allele. Overall, the percentage of line $7_2$ MD QTLs in the genomes of strains K, M, P, and W are 8.5%, 10.3%, 12.8%, and 1.9%, respectively.

## DISCUSSION

This is the initial study on the development of RCS in chicken for defining resistance to MD. As expected, the percentage of the donor line $7_2$ genome is ~12.5% on a background of the host line $6_3$.; the low percentage in strain W may be due to chance. At least 4 of the RCS are susceptible to MD confirming our previous result of large effect QTL. To minimize the possibility of losing the MD susceptible phenotype, in the future, progeny from the most susceptible sire-dam combination will be used as parents to repopulate the next generation.

This system will help us to investigate the complex resistance to MD. Studies underway include comparing the expressed genes of the susceptible strains with line $6_3$ by differential display (Liang and Pardee 1992) to increase the density of markers in the MD regions as well as to provide a framework for local comparative maps. Also, the RCS will be characterized for a number of traits that are known or may be different between lines $6_3$ and $7_2$, e.g., antibody levels, lymphocyte ratios, mitogen response, etc., to determine the function of each QTL.

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**Table 2. Percentage of line $7_2$ alleles of the MD QTLs in the susceptible RCS**

| Linkage Group | Marker | Interval (cM) | Recombinant Congenic Strains K | M | P | W |
|---|---|---|---|---|---|---|
| (2n) | | | (18) | (12) | (42) | (24) |
| Ch. 2 | ADL185 | 24 | 100% | 8.3% | 45.2% | 0 |
| | MCW63 | | 0 | 8.3% | 0 | 0 |
| Ch. 3 | ADL131 | 38 | 0 | 0 | 0 | 0 |
| | MCW169 | | 0 | 0 | 0 | 25.1% |
| Ch. 4 | ADL331 | 81 | 0 | 8.3% | 21.4% | 0 |
| | ADL144 | | 0 | 58.3% | 28.6% | 0 |
| Ch. 7 | ADL180 | 76 | 0 | 25.0% | 33.0% | 0 |
| | ADL326 | | 0 | 0 | 0 | 0 |
| Ch. 8 | ADL258 | 23 | 0 | 0 | 0 | 0 |
| | ADL322 | | 0 | 0 | 0 | 0 |
| E16 | ADL240 | -- | 11% | 25.0% | 0 | 0 |
| E27 | ADL289 | -- | 0 | 0 | 38.1% | 0 |
| E41 | ADL149 | -- | 0 | 0 | 0 | 0 |
| Overall | | | **8.5%** | **10.3%** | **12.8%** | **1.9%** |

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