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QTLs mapped by selective DNA pooling explain substantial proportion of phenotypic and genetic variation in Marek's Disease mortality in chicken layer lines

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Summary

Marek's disease (MD) has a major effect on poultry health, production and welfare. Intensive use of vaccines is driving the virus to increasing virulence. New, more virulent strains continue to emerge, calling for other control means, such as selective breeding. In the present study, we estimated the effects of MD QTLs mapped by selective DNA pooling in 8 chicken layer lines selected for MD resistance, and estimated the QTL contribution to the within-line phenotypic and genetic variation. The absolute effects of the QTLs were appreciable, substantially contributing to the phenotypic variation, and in some lines accounting for up to 100% of the genetic variation of sire progeny test for MD mortality.

Keywords: Marek's disease, Selective DNA pooling, GWAS, allele effect, genetic variation

Introduction

Marek's disease (MD) is a major disease affecting poultry health, production and welfare, with estimated annual global losses of US $1 billion (Kennedy *et al.*, 2017). MD virus (MDV) is a highly contagious α-herpesvirus associated with T-cell lymphomas. Intensive use of vaccines is driving the virus to increase virulence, and new, more virulent strains continue to emerge (Davison & Nair, 2005; Gimeno, 2008; Witter, 1997). Therefore, other means of controlling the disease are of interest, such as selective breeding for enhanced genetic resistance to MDV. Identification of genetic markers for MD resistance will increase accuracy, reduce costs and allow selection with less reliance on routine challenge.

MD resistance QTLs were mapped in line crosses (Heifetz *et al.*, 2007; McElroy *et al.*, 2005; Vallejo *et al.*, 1998; Yonash *et al.*, 1999), but usually at low resolution and small effect, suggesting resistance is controlled by a large number of genes. Genes in the MHC locus are long known to contribute to MD resistance (Kaufman, 2000; Lamont, 1989; Pazderka *et al.*, 1975). Only a small number of additional causative genes have been identified, including GH1 (Liu *et al.*, 2001a; Yonash *et al.*, 1999), SCYC1 (Liu *et al.*, 2001b), SCA2 (Liu *et al.*, 2003), VDR (Praslickova *et al.*, 2008), and IRG1 (Smith *et al.*, 2011).

In the present study, we used selective DNA pooling and a high-density SNP array in a GWAS to map QTLs affecting chicken MD mortality in eight layer pure-lines selected for enhanced resistance to MD (Lipkin *et al.*, 2017), and here we estimate allele effects and QTL contribution to the within-line phenotypic and genetic variation in MD mortality.

Material and methods

Populations and genotypes

Males from eight lines were used, each line sampled across 15 generations, with known MHC genotype, and progeny tests for MD mortality following challenge (Lipkin *et al.*, 2017). Males with progeny presenting high or low MD mortality were selected within-generations and within-lines for the phenotypic tails of the populations, taking MHC genotype into account. All pools were genotyped by Affymetrix 600K chicken SNP array. Autosomal markers were used in the analysis.

Statistical analyses

Following Lipkin *et al.* (2016), frequencies of SNP alleles were estimated based on intensities. P-values of the frequency difference between tails were calculated based on empirical SE within tails, assuming no QTL effect within tails. QTLs were identified within lines by moving average of -LogP, using a threshold of -LogP = 2.0 (P = 0.01) to declare a QTL, and Log Drop 1 to define QTL regions (QTLRs). QTLRs were validated by individual genotyping of selected SNPs from the QTLRs, and there was good correspondence of significance between individual genotyping and pool results.

Following Darvasi & Soller (1994), allele effects (δ, where 2δ is the difference between alternative homozygotes) was calculated based on the estimated phenotypic difference between the two alternative homozygote groups across both tails, corrected for the selection of the phenotypic tails.

At each QTLR, the mean δ of the three markers having the largest δ were used to estimate the allele effect of that QTL. From this the contribution of an individual QTL to the genetic component (VarG) of population phenotypic variation (VarP), calculated as $VarQ = 2pq\delta^2$ (Mosig *et al.*, 2001), where p and q are the mean frequencies of the two SNPs alleles taken across the same three top markers. The proportional contribution of the QTL to VarP and VarG, were calculated as VarQ/VarP and VarQ/VarG, respectively. VarG was calculated as h^2VarP, where h^2 is the heritability of the challenge test, estimated as h^2=0.13 (Wolc *et al.*, 2013). The proportional contributions of all QTLs were summed within each line, to give total proportion of phenotypic and genetic variation explained by the mapped QTLRs.

Results

Table 1 shows individual QTL effects and proportional contributions to VarP and VarG, by lines. QTL were not found for Line 8. Across all other seven lines, mean of individual QTL allele effects (δ) averaged 2.3% MD-mortality in the progeny test. Line means ranged from 1.1% to 3.7%. Thus, the absolute effect of these individual QTLRs is appreciable - the difference between alternative homozygotes averaged 4.6% daughter mortality. VarP estimated within lines and then pooled across lines was 219% (Table 2), VarG estimate was 68.8%. Individual QTL contribution to the population *phenotypic* and *genotypic* variation (cP and cG) averaged 1.2% and 9.3%, respectively (Table 1).

Table 1. Individual QTLs within lines: effects (δ) and proportional contributions to the phenotypic and genetic variation of MD mortality (cP and cG).

Line	QTL	δ	cP	cG	Line	QTL	δ	cP	cG	Line	QTL	δ	cP	cG
1	1	2.058	0.007	0.056	2	8	2.805	0.013	0.102	4	1	3.171	0.018	0.141
1	2	2.744	0.017	0.131	2	9	2.922	0.014	0.111	4	2	3.658	0.025	0.192
1	3	2.186	0.011	0.088	2	10	2.383	0.009	0.070	4	3	2.466	0.011	0.083
1	4	1.948	0.009	0.069	2	11	2.464	0.007	0.051	4	4	3.127	0.010	0.081
1	5	2.342	0.013	0.100	2	12	3.110	0.016	0.126	5	1	2.144	0.008	0.063
1	6	1.666	0.006	0.050	2	13	2.302	0.009	0.068	5	2	2.007	0.007	0.053
1	7	2.057	0.009	0.071	2	14	2.681	0.011	0.086	5	3	3.194	0.018	0.138
1	8	2.330	0.012	0.096	2	15	2.249	0.008	0.065	6	1	1.761	0.007	0.057
1	9	2.206	0.008	0.061	3	1	2.802	0.031	0.240	6	2	1.922	0.009	0.067
1	10	2.272	0.012	0.092	3	2	2.066	0.017	0.133	7	1	1.420	0.008	0.062
1	11	2.345	0.012	0.094	3	3	2.411	0.023	0.177	7	2	1.261	0.007	0.050
2	1	2.408	0.010	0.075	3	4	2.221	0.014	0.107	7	3	1.317	0.008	0.065
2	2	2.263	0.004	0.034	3	5	1.965	0.015	0.116	7	4	1.145	0.006	0.046
2	3	2.440	0.009	0.072	3	6	2.302	0.021	0.162	7	5	1.384	0.009	0.069
2	4	2.604	0.011	0.085	3	7	2.065	0.013	0.102	7	6	1.387	0.009	0.065
2	5	2.998	0.015	0.115	3	8	1.800	0.013	0.097	7	7	1.551	0.007	0.056
2	6	2.913	0.012	0.093	3	9	1.897	0.011	0.082		Avg	2.273	0.012	0.093
2	7	2.796	0.013	0.101	3	10	2.237	0.020	0.153					

QTLs explained an average of 8.9% and 68.8% of the *phenotypic* and *genotypic* variation within lines, respectively; from 1.6% in Line 6 to 17.8% in Line 3 for VarP, and from 12.5% in Line 6 to 137% in Line 3 for VarG (Table 2).

Table 2. Summary phenotypic and genetic variance explained by QTLs by lines.

Line		QTLs		
no.	Var	QTLs	VarP	VarG
1	208.8	11	0.118	0.909
2	296.1	15	0.163	1.252
3	121.5	10	0.178	1.369
4	268.4	4	0.065	0.496
5	281.9	3	0.033	0.253
6	207.0	2	0.016	0.125
7	102.2	7	0.054	0.413
8	266.2	0		
Avg	219.0	6.5	0.089	0.688

Discussion

These results accord well with Wolc *et al.*, (2013). Using individual genotypes of Illumina 42K array and Bayesian variable selection models, they reported that genetic markers explained about 11% of the *phenotypic* variation. The present results and Wolc *et al.* (2013)

imply that most of the phenotypic variation is affected by non-genotypic factors.

QTLs found in 7 of the 8 lines (Lipkin *et al.*, 2017), contributed substantial proportion to the *genetic* variation. In fact, in Lines 2 and 3 they summed up above the heritability estimate of Wolc *et al.* (2013). This excess above 100% could be due to chance variation, over-estimation of marker effects, under-estimation of Wolc *et al.* (2013) heritability in the present study populations, and so on.

Taken as they stand, the results of the present study indicate that a large proportion of standing genetic variation in MD mortality can be linked to genetic markers in GWAS.

List of References

Darvasi, A. & M. Soller, 1994. Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus. Genetics. 138(4): 1365-1373.

Davison, F. & V. Nair, 2005. Use of Marek's disease vaccines: could they be driving the virus to increasing virulence? Expert Rev Vaccines. 4(1): 77-88.

Gimeno, I.M., 2008. Marek's disease vaccines: a solution for today but a worry for tomorrow? Vaccine. 26(Suppl 3): C31-41.

Heifetz, E.M, J.E. Fulton, N.P. O'Sullivan, J.A. Arthur, H. Cheng, J. Wang, M. Soller, J.C.M. Dekkers, 2007. Mapping QTL affecting resistance to Marek's disease in an F6 advanced intercross population of commercial layer chickens. BMC Genomics 10: 20.

Heifetz, E.M., J.E. Fulton, N.P. O'Sullivan, J.A. Arthur, J. Wang, J.C.M. Dekkers, M. Soller, 2007. Mapping quantitative trait loci affecting susceptibility to Marek's disease virus in a backcross population of layer chickens. Genetics 177: 2417–2431.

Kaufman, J., 2000. The simple chicken major histocompatibility complex: life and death in the face of pathogens and vaccines. Philos Trans R Soc Lond B Biol Sci. 355(1400): 1077-1084.

Kennedy, D.A., C. Cairns, M.J. Jones, A.S. Bell, R.M. Salathé, S.J. Baigent, V.K. Nair, P.A. Dunn, A. F. Read, 2017. Industry-Wide Surveillance of Marek's Disease Virus on Commercial Poultry Farms. Avian Dis. 61(2): 153-164.

Lamont, S.J., 1989. The chicken major histocompatibility complex in disease resistance and poultry breeding. J Dairy Sci. 72(5): 1328-1333.

Lipkin, E., J. Smith, D. Burt, M. Soller, J. E. Fulton, 2017. Mapping QTLs affecting Marek's disease by selective DNA pooling in eight lines across 15 generations. Proc. 36th Conf. ISAG. Talk 54, Poster MT62.

Lipkin, E., M.G. Strillacci, H. Eitam, M. Yishay, F. Schiavini, M. Soller, A. Bagnato, A. Shabtay, 2016. The use of kosher phenotyping for mapping QTL affecting susceptibility to Bovine Respiratory Disease. Plos One, 11(4): e0153423.

Liu, H.C., H.J. Kung, J.E. Fulton, R.W. Morgan, H.H. Cheng, 2001a. Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. Proc Natl Acad Sci U S A. 98(16): 9203-9208.

Liu, H.C., H.H. Cheng, V. Tirunagaru, L. Sofer, J. Burnside, 2001b. A strategy to identify positional candidate genes conferring Marek's disease resistance by integrating DNA microarrays and genetic mapping. Anim Genet. 32(6): 351-359.

Liu, H.C., M. Niikura, J.E. Fulton, H.H. Cheng, 2003. Identification of chicken lymphocyte antigen 6 complex, locus E (LY6E, alias SCA2) as a putative Marek's disease resistance gene via a virus-host protein interaction screen. Cytogenet Genome Res. 102(1-4): 304-308.

McElroy, J.P., J.C.M. Dekkers, J.E. Fulton, N.P. O'Sullivan, M. Soller, E. Lipkin, W. Zhang,

K.J. Koehler, S.J. Lamont, H.H. Cheng, 2005. Microsatellite markers associated with resistance to Marek's disease in commercial layer chickens. Poult Sci. 84: 1678–1688.
Mosig M.O., E. Lipkin, G. Khutoreskaya, E. Tchourzyna, M. Soller, A. Friedmann. 2001. A whole genome scan for QTL affecting milk protein percent in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using a modified false discovery rate criterion. Genetics 157: 1983-1698.
Pazderka, F., B.M. Longenecker, G.R.J. Law, H.A. Stone, R.F. Ruth, 1975. Histocompatibility of chicken populations selected for resistance to Marek's disease. Immunogenetics 2: 93-100.
Praslickova, D., S. Sharif, A. Sarson, M.F. Abdul-Careem,D. Zadworny, A. Kulenkamp, G. Ansah, U. Kuhnlein, 2008. Association of a marker in the vitamin D receptor gene with Marek's disease resistance in poultry. Poult Sci. 87(6): 1112-1119.
Smith J., J.R. Sadeyen, I.R. Paton, P.M. Hocking, N, Salmon, M. Fife, V. Nair, D.W. Burt, P. Kaiser, 2011. Systems analysis of immune responses in Marek's disease virus-infected chickens identifies a gene involved in susceptibility and highlights a possible novel pathogenicity mechanism. J Virol. 85(21): 11146-11158.
Vallejo, R.L., L.D. Bacon, H.C. Liu, R.L. Witter, M.A. Groenen, J. Hillel, H.H. Cheng, 1998. Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens. Genetics 148: 349–360.
Witter, R.L., 1997. Increased virulence of Marek's disease virus field isolates. Avian Dis. 41(1): 149-163.
Wolc, A., J. Arango, T. Jankowski, P. Settar, J.E. Fulton, N.P., O'Sullivan, R. Fernando, D.J. Garrick, J.C. Dekkers, 2013. Genome-wide association study for Marek's disease mortality in layer chickens. Avian Dis. 57(2 Suppl): 395-400.
Yonash, N., L.D. Bacon, R.L. Witter, H.H. Cheng, 1999. High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Anim. Genet. 30: 126–135.