Genetic assessment of inbred chicken lines indicates genomic signatures of resistance to Marek’s disease

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Abstract
Background: Marek’s disease (MD) is a highly contagious pathogenic and oncogenic disease primarily affecting chickens. However, the mechanisms of genetic resistance for MD are complex and not fully understood. MD-resistant line 63 and MD-susceptible line 7 2 are two highly inbred progenitor lines of White Leghorn. Recombinant Congenic Strains (RCS) were developed from these two lines, which show varied susceptibility to MD.

Results: We investigated genetic structure and genomic signatures across the genome, including the line 63 and line 7 2, six RCSs, and two reciprocally crossed flocks between the lines 63 and 7 2 (F1 63 ×7 2 and F1 7 2 ×6 3) using Affymetrix® Axiom® HD 600 K genotyping array. We observed 18 chickens from RCS lines were specifically clustered into resistance sub-groups distributed around line 63. Additionally, homozygosity analysis was employed to explore potential genetic components related to MD resistance, while runs of homozygosity (ROH) are regions of the genome where the identical haplotypes are inherited from each parent. We found several genes including SIK, SOX1, LIG4, SIK1 and TNFSF13B were contained in ROH region identified in resistant group (line 63 and RCS), and these genes have been reported that are contribute to immunology and survival. Based on FST based population differential analysis, we also identified important genes related to cell death and anti-apoptosis, including AKT1, API5, CDH13, CFDP and USP15, which could be involved in divergent selection during inbreeding process.

Conclusions: Our findings offer valuable insights for understanding the genetic mechanism of resistance to MD and the identified genes could be considered as candidate biomarkers in further evaluation.

Keywords: Genetic structure, Genomic signature, Homozygosity, Marek’s disease, Recombinant Congenic Strains (RCS)

Background
Poultry products are main components of our daily life. The complexity of food safety and quality issues facing the poultry industry are rapidly escalating, and many other critical issues (i.e., bioterrorism, environmental stewardship and global competitiveness) are emerging simultaneously. Marek’s disease (MD) is a T cell lymphoma induced by the widespread and readily transmissible Marek’s disease virus (MDV) [1]. MD is one of the main chronic infectious diseases threatening the poultry worldwide, and interest for its economic importance to the commercial poultry industry.

MDV transforms mainly CD4+ T cells and causes various clinical syndromes in chicken tissues, which include peripheral nerves, gonad, iris, muscle, viscera, and skin [2]. MDV is shed and transmitted between birds via epithelial cells of the feather follicle, dander, chicken house dust, feces and saliva. Vaccines were developed to control the disease but are not sterilizing allowing the virus to replicate and spread. Thus, in the last few decades, field strains of the virus have evolved resulting in new subtypes that the vaccines may not completely control [3]. MDV has proven to be a valuable comparative biomedical model organism for understanding the principles of human disease [4] and MD is a natural model for...
lymphomas overexpressing Hodgkin’s disease antigen [5]. However, there is a shortage of genetic information about the virus host and etiological process leading to MDV uptake, dissemination, latency and tumor formation.

Currently, control strategies for MD predominantly rely on vaccination of chickens. But the vaccination cannot provide complete protection because of the changing nature of the disease itself and evolution of MDV with escalated virulence [4]. Recently, studies using integrating genomic approaches identified genes and molecular markers associated with MD disease resistance [6]. Genetic makers, quantitative trait loci (QTL) and genomics regions in a large reciprocal backcross (BC) were discovered between two partially inbred commercial White Leghorn layer chicken [7] and in an F6 advanced intercross population of commercial layer chickens [8]. Additionally, gene expression using transcript array [9, 10] and allele-specific expression analysis using RNA sequencing [11] have been utilized to understand response to MDV infection and MD genetic resistance.

To determine the genetic components pertaining to disease resistance, the Avian Disease and Oncology Laboratory (ADOL) has successfully developed inbred chicken lines resistant or susceptible to MD as a powerful experimental model for investigating host resistance to this disease, the inbred lines 63 and 72. The percentage of chickens developing a tumor or tumors induced by a partially attenuated vv + strain of MDV differed significantly between the two lines of chickens. Genetic resistance to MD in chickens is commonly evaluated with MD incidence post-MDV challenge, which had been described in previous study [12]. Only 0–3% of line 63 chickens developed tumors 8 wk post infection compared to 99%–100% of line 72 chickens. Importantly, 19 recombinant congenic strains (RCS) have been developed using the inbred lines 63 and 72 as progenitor lines. The tumor incidence differs among RCS either vaccinated or unvaccinated. Thus, the varied resistance among the RCS can provide an ideal model to investigate genomic components that play vital roles in genetic resistance to MD. Although a previous study has demonstrated some genetic variability among the RCSs by microsatellite fingerprinting [2], genetic variations underlying varied susceptibility to MD in the RCS lines remains poorly understood.

In this study, we hypothesized that differential genomic signatures contribute to MD resistance in the RCS. Using ten lines, including line 63 and line 72, two hybrid F1 (F1 63 × 72 and F1 72 × 63) and six RCSs representing various capacity in resistance/susceptibility to MD, we investigated genetic structure in the chicken lines based on Affymetrix® Axiom® Genome-Wide Chicken Genotyping Array (600 K) by the Affymetrix service facility according to the manufacturer’s protocols [14]. Affymetrix adopts a preliminary call rate of 90% that is used by default to pass arrays for further data analysis. Successfully passed arrays were clustered and final genotypes were generated using the Axiom GT1 algorithm. Quality control was assessed in Genotyping Console v4.1.3. All chosen samples genotyped on Affymetrix SNP array met the 99% call rate.

**Methods**

**Experimental population**

In total, 30 chickens without treatments were selected and genotyped in this study. Among them, three were from each of the line 72, line 63, reciprocal cross F1 hybrid 72 × 63, F1 hybrid 63 × 72, and six recombinant congenic strains (RCSs) [C, J, M, N, S and X] [11]. RCSs were developed using line 63 as the parental strain mated to line 72 and then backcrossed to line 63 twice followed by full-sib matings for about 20 generations. Eventually, diverse RCSs were generated and they contain 87.5% of line 63 and 12.5% of line 72 in the genetic background but with different MD resistance (Additional file 1: Figure S1) [3, 13]. In this study, six RCSs above were chosen to check their genetic background and reveal their particular resistant signatures.

**Genotyping and quality control**

Genomic DNA from red blood cells was extracted using the DNeasy Blood & Tissue Mini Kit (QIAGEN). Genotyping for the 30 chickens was performed using the Affymetrix® Axiom® Genome-Wide Chicken Genotyping Array (600 K) by the Affymetrix service facility according to the manufacturer’s protocols [14]. Affymetrix adopts a preliminary call rate of 90% that is used by default to pass arrays for further data analysis. Successfully passed arrays were clustered and final genotypes were generated using the Axiom GT1 algorithm. Quality control was assessed in Genotyping Console v4.1.3. All chosen samples genotyped on Affymetrix SNP array met the 99% call rate.

**Multidimensional scaling and admixture analysis**

Quality control were considered using the following selection criteria: MAF ≥ 0.01 (including SNPs with MAF ≥ 0.01), geno ≥ 0.1 (including only SNPs with a 90% genotyping rate or higher), and P value of x2 test for Hardy-Weinberg equilibrium ≥ 10−6. To avoid linkage distortion for the population structure analysis, SNPs were pruned for linkage disequilibrium (LD) using pair-wise genotype correction (r2 > 0.2) in 50 SNP sliding widows with a step of 10 SNPs across the genome. To look for evidence of population substructure, we carried out Multidimensional Scaling (MDS) analysis using 5,064 LD filtered SNPs among all 30 chickens. Two dimensions in MDS analysis were calculated based on the identity-by-descent (IBD) pairwise distance among all chickens using PLINK, then MDS was plotted using the first-dimension values against the
second-dimension values. To estimate the genetics admixture within 10 lines, STRUCTURE v2.3 was employed using the same LD filtered data set with 5,000 replicates and 2,000 burn-in cycles under the admixture model and correlated allele frequencies [15, 16].

Phylogenetic analysis
Neighbor-joining (NJ) tree was constructed based on the estimation of IBD. As line 72 shows significantly susceptible to MD compared to the RCS, we used line 72 as the outgroup for NJ trees analysis. Pairwise genetic distance (D) between chickens was calculated using PLINK, where $D = 1 - \frac{\text{IBS2} + 0.5\text{IBS1}}{\text{N}}$; IBS2 and IBS1 are the number of loci that share either 2 or 1 alleles identical by state (IBS), respectively, and the N is the number of loci [17, 18]. Then, phylogenetic tree was generated using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). In addition, Reynolds’ distance between pairwise inbred lines was estimated using Arlequin version 3.5 [19].

Differential genomic signature
To investigate genome-wide patterns of genetic polymorphism related to susceptibility and resistance during the inbreeding process, the global $F_{ST}$ was calculated for each single locus using Genepop software across all lines [20], which measured genetic differentiation between subpopulations using the approach previously described by Weir et al. [21]. Genomic signatures, implying divergent selection among inbred lines, can be recognized when $F_{ST}$ values for the adjacent SNPs in a special window size were estimated to exceed the significant threshold [22]. We estimated average $F_{ST}$ values of each nonoverlap region with 50 SNPs windows size. The top 1% regions were considered as candidate regions. Furthermore, we performed gene annotation based on genome assembly (Gallus_gallus-4.0) to identify the candidate genes involved in divergent selection during the inbred process.

Runs of homozygosity
Runs of homozygosity (ROH) are regions of the genome where the identical haplotypes are inherited from each parent. The exploration of ROH can help to identify recessive disease variants and investigate the effects of genome-wide homozygosity on traits of biomedical importance. To investigate homozygosity distribution in six RCS lines as well as the parental line 63 and line 72, we performed ROH analysis with PLINK using a total of 527,021 SNPs [17]. A sliding window of 50 SNPs was used to identify ROH based on filtered SNPs. Here, homozygous segments were defined as more than 50 homozygous SNPs spanning larger than 500 kb. To avoid underestimation of ROHs caused by occasional genotyping error or missing genotype occurring in an otherwise-unbroken homozygous segment, we used the option --homozyg-window-het 1 --homozyg-window-missing 2 in PLINK to allow one heterozygous and two missing calls per window.

Gene Ontology (GO) enrichment analysis
To assess the function of the positive selected genes identified in top 1% windows regions and genes in the ROH segments, we performed gene enrichment annotation and gene functional classification with DAVID (version 6.7) [23]. The default sets were used in DAVID with gene annotations based on Gallus galus, and GOTERM_BP_FAT, GOTERM_CC_FAT, GOTERM_MF_FAT were selected as the functional annotation category.

Results
Summary statistics of SNPs array of QC
The quality of all SNPs in the array met the 99% call rate (Additional file 1: Figure S2). In the present study, a total of 527,021 SNPs in autosome chromosomes were kept for further analysis. Markers with high missing gencall rate (> 0.1), low MAF (< 0.01) and significant deviation from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$) were excluded, leaving a total of 155,216 autosomal SNPs in population genetic structure analysis. Distributions in autosomes for total SNPs and filtered SNPs were presented in Additional file 1: Figures S3 and S4.

Genetic structure and phylogenetic analysis
MDS analysis revealed the first dimension (C1) and the second dimension (C2) separated the lines into three broad non-overlapping clusters, which represent line 72 (susceptibility), line 63 (resistance), RCS lines and F1 (hybrid F1 generation with various level of resistance). Six F1 chickens clustered together, and they were distinguished from both susceptibility and resistance lines. We also observed 18 chickens from RCS lines were specifically clustered into resistance sub-groups distributed around line 63 (Fig. 1a). Moreover, we further investigated genetic structure within 18 RCS chickens using MDS analysis, and observed J lines separated clearly from others, which indicated a differential genomic signature in J (Fig. 1b). Other lines also displayed obviously independent cluster on the MDS plots. Additionally, the results from heatmap and neighbor-joining (NJ) tree were consistent with MDS results (Fig. 2), we also found line 72, next to F1 hybrid lines, were divergent from other lines.

Then, we employed a model-based unsupervised hierarchical clustering across chickens using the program STRUCTURE. The admixture bar plot results were in well agreement with above observations with a clear separation of line 72, F1, line 63 and others, as shown in Fig. 3 (e.g. K = 3). In addition, as K increases to 4, J line was separated from other lines. Interestingly, when K = 10, we found line C, J, N, X were separated from...
line 63 (indicating unique resistance lines). In addition, the neighbor-joining (NJ) tree was constructed based on IBD calculated between unambiguously separated individuals, and line 72 was used as control group (Fig. 4). The results indicated detailed genetic relationships among 30 chickens as shown in Fig. 2. Three groups of inbreed lines corresponding to line 72, line 63, F1 could be clearly distinguished in upper, lower right and lower left, respectively. The results were also consistent with MDS and Admixture results: three individuals from different lines were clustered together at the same branches and individuals from F1 branched in an intermediary position between line 72 and line 63. Most importantly, all RCS lines were closely related to the line 63 MD-resistant lines, which suggesting a higher influence from the genetics background of line

![Fig. 1](image1.png)  
**Fig. 1**  
(a) Multidimensional scaling analysis (MDS) based on genome-wide IBS pairwise distances in a total of 30 chickens.  
(b) Multidimensional scaling analysis based on genome-wide IBS pairwise distances within 18 RCS chickens

![Fig. 2](image2.png)  
**Fig. 2**  
Heatmap and hierarchical clustering tree based on the Reynolds’ distance between 10 inbred lines
In addition, we found chickens from line X, N and chickens from S, M, C, J were readily distinguishable among RCS lines, which suggested that these lines could have their unique genetic characteristics as previously reported [10].

Genomic diverse signatures
To explore the difference of genetic polymorphism related to susceptibility and resistance during the inbreeding process, we carried out genome scan across genome using $F_{ST}$ approach. After splitting the whole genome to 50 SNP windows, we obtained 10,554 raw windows. We detected 106 regions (about top 1%) with diverse signatures. To gain insight of the function enrichment of these regions, we annotated the top 106 candidate regions, and 51 genes were identified to be candidate genes for the resistance to MD. To better understand the biological processes and molecular function involved in MD genetic resistance, we performed GO term analysis using DAVID. We observed the most represented molecular function was arylamine N-acetyltransferase activity (GOTERM_MF_FAT), which included gene PNAT10 (N-acetyltransferase, pineal gland isozyme NAT-10), PNAT3 (N-acetyltransferase, pineal gland isozyme NAT-3), and NAT (N-acetyltransferase, liver isozyme) (Table 1). The most represented biological process was regulation of cell death and anti-apoptosis (GOTERM_BP_FAT). Those genes included AKT1 (v-akt murine thymoma viral oncogene homolog 1), CDH13 (cadherin 13, H-cadherin), CFDP1 (craniofacial development protein 1), API5 (apoptosis inhibitor 5), and ING4 (inhibitor of growth family, member 4). Furthermore, KEGG_PATHWAY analysis identified PNAT10, PNAT3, NAT involved pathway caffeine metabolism and drug metabolism (Additional file 2: Table S1). We also detected many overlaps between the candidate regions with online chicken QTL database (http://www.animalgenome.org/cgi-bin/QTLdb/GG/index). The overlapping positions of each region and QTLs related to MD disease were listed in Additional file 3: Table S2. We observed 19 regions overlapping with known QTL regions, including 9 regions in chr1 [7, 8], 2 regions at chr2 [8, 24], 5 regions in chr5 and 1 region in chr9 [7, 8].

Runs of homozygosity
In this study, we found many loci had been fixed to homozygous. It is impossible to use them to explain the difference of MD resistance. However, the completed differentiations
\( (F_{ST} = 1) \) indicates two different homozygous genotypes existed in populations. To estimate the inbred level across lines, we also calculated the inbreeding coefficient \( (F) \) for all 30 samples using 155,216 high quality SNPs (Additional file 4: Table S3). We found the top inbreeding coefficient \( (F) \) in line 63, which is over 0.98. Next is line 72, nearly 0.95. In contrast, the average of \( F \) among the RCS is 0.88 ± 0.028. Among them, the average of \( F \) values in RCS-S line is up to 0.94, whereas for lines N and X, \( F \) values are both about 0.86, which suggested there existed slight different inbred levels among inbred chicken lines.

To explore the question whether the inbred levels and homozygosity differences across the genome could contribute to the susceptibility/resistance for MD, we conducted homozygosity analysis across all 24 chickens (hybrid F1 lines were not included in ROH analysis). We obtained 2,027 ROH segments, and 1,596 of them were found in RCS lines. The genome wide results from the ROH analysis indicated obviously different ROH distributions across the genome within 24 inbred chicks. We identified 406 ROH segments in line 72 and 206 ROH segments in line 63. We also found ROH

![Fig. 4 Neighbor-joining tree based on genome-wide IBD distances in 30 chickens](image)

### Table 1 Gene Ontology annotations of candidate genes identified using \( F_{ST} \) approach

| GO category | GO term | Gene count | P-value | List of genes |
|-------------|---------|------------|---------|---------------|
| GOMT_BP_FAT | GO:0000905~anti-apoptosis | 4 | 2.40E-04 | AKT1, CDH13, CFDP1, APIS |
| GOMT_BP_FAT | GO:0042981~regulation of apoptosis | 5 | 2.70E-03 | AKT1, CDH13, ING4, CFDP1, APIS |
| GOMT_BP_FAT | GO:0043067~regulation of programmed cell death | 5 | 2.89E-03 | AKT1, CDH13, ING4, CFDP1, APIS |
| GOMT_BP_FAT | GO:0010941~regulation of cell death | 5 | 2.94E-03 | AKT1, CDH13, ING4, CFDP1, APIS |
| GOMT_BP_FAT | GO:0043066~negative regulation of apoptosis | 4 | 3.26E-03 | AKT1, CDH13, CFDP1, APIS |
| GOMT_BP_FAT | GO:0043069~negative regulation of programmed cell death | 4 | 3.44E-03 | AKT1, CDH13, CFDP1, APIS |
| GOMT_BP_FAT | GO:0000548~negative regulation of cell death | 4 | 3.44E-03 | AKT1, CDH13, CFDP1, APIS |
| GOMT_BP_FAT | GO:0004060~arylamine N-acetyltransferase activity | 3 | 5.20E-05 | PNAT10, PNAT3, NAT |
| GOMT_BP_FAT | GO:000880~N-acetyltransferase activity | 3 | 2.12E-03 | PNAT10, PNAT3, NAT |
| GOMT_BP_FAT | GO:0016410~N-acetyltransferase activity | 3 | 2.71E-03 | PNAT10, PNAT3, NAT |
| GOMT_BP_FAT | GO:0016407~acetyltransferase activity | 3 | 3.14E-03 | PNAT10, PNAT3, NAT |
distribution displayed a certain degree of diversity among the six RCS lines, with the longest ROH detected in line 63 and line N, about 90 Mb. However, in line 72, the longest ROH was 44 Mb. The detailed summary statistics for ROH analysis, including ROH number, length and density (nSNP/kb) and SNPs within each ROH segment, was shown in Additional file 5: Table S4.

To compare the ROH distributions among 8 diverse inbred lines, we first merged ROH segments identified from all individuals for each line into nonredundent inbred lines, we finally obtained 58, 71, 64, 65, 63, 58 and 67 ROH regions based on genomics coordinate. We finally obtained 58, 71, 64, 65, 63, 58 and 67 ROH regions in the lines 63, 72, C, J, M, N, S and X, respectively, while the relative more ROH regions were identified on chr1 (10 ROHs) in line 72 and chr2 (8 ROHs) in line L. The most probable reason was both chromosomes cover a larger proportion of genome size as compared with other chromosomes. To facilitate the comparison of difference of ROH regions among lines, genome wide ROH regions were presented in Additional file 5: Table S4. We found some shared ROH regions, both among the RCS lines and between RCS and line 63. The results indicated that ROH region caused by inbreeding may be involved in the different MD resistance. On the other hand, the unique region from line 72 (susceptibility line) comparing other lines may offer some clues of genomic homozygosity region related to susceptibility.

ROH represents regions of genome where the identical haplotype is inherited from each parent. ROH regions could likely increase of recessive deleterious alleles to be co-contributed, and reducing the viability of the organism. To investigate the differential ROH distribution among diverse chickens, we further divided 24 chickens into three groups, representing parental line 63 with resistance, parental susceptibility line 72 group, and RCS group (including line C, J, M, N, S and X), respectively. Comparisons of ROH regions based on genomics coordinates among three groups showed that 98.6% ROH region (902.5/915.0 Mb) were shared by all three groups (Additional file 1: Figure S5).

In our study, two parental inbred line 63 and line 72 are MD-resistant and MD-susceptible respectively, while a Recombinant Congenic Strains (RCS) lines were developed from these two lines showing varying resistance to MD, we observed RCS lines were similar to line 63 based on genetics relation estimation in present study (Figs. 1, 2, 3 and 4). To further explore whether homozygous segments segregated with different resistance to MD diseases, all chickens with varying resistance to MD included RCS lines and line 63 chickens were compared with the line 72 (MD-susceptible). We obtained one region that is uniquely distributed in line 63 with 513 kb length; eight regions (987 kb in size) in line 72 groups, and 47 regions totaling 7,719 kb not in line 72 but in shared by both RCS lines and line 63 (Additional file 6: Table S5).

Subsequently, we observed 56 genes in 47 shared ROH regions, while only one gene was identified within line-specific regions in the line 63. We identified eight unique regions with 13 genes in line 72. Among the 56 genes identified in both line 63 and RCS with different resistance, we found most of them were enriched in regulation of apoptosis and regulation of cell death, this result may indicate their potential association with the resistance of MD disease.

Discussion

We investigated the genetic characteristics of MD resistance and susceptibility using high-density SNP array in inbred chicken lines. Genetic structure indicated differential genomic landscapes during inbreeding process in the past decades. Using genomic signature and homozygosity analysis, we identified a list of genes that may contribute to the MD resistance or susceptibility in the inbred lines.

Previous studies have explored the genetic changes at gene expression level [10], epigenetic regulatory [25, 26], and allele specific expression [11] in inbred chickens. To better understand the genetic mechanism underlying MD-resistance and susceptibility, we employed high density SNP arrays to investigate the genetic structure and relationship in 10 inbred lines. Using MDS analysis, we observed diverse genetic structures among them, which were highly consistent with admixture analysis and phylogenetic analysis. These chicken lines with different MD susceptibility were separated obviously, while RCS lines were clustered together near parental line 63 with similar MD-resistance. Our findings were also consistent with PCA analysis in inbred chicken using expression array [10], these results indicated similar influences on MD-resistance and susceptibility caused by SNPs and gene expression.

Although the limited samples size per line were utilized in the current study, the global FST approach can properly reflect the potential difference among all 30 birds. Allele frequency based method is likely to offer some important clues for searching the candidate genes involved in the MD resistance. Moreover, we found some genes related to disease and immune response. For instance, several genes were significant enriched including arylamine N-acetyltransferase activity (AKT1, CDH13, CFDPI, API5) and cell death and anti-apoptosis (CDH13, ING4, CFDPI and API5). We also detected one region located at 51.5 Mb in chromosome 5 with high selection signal (FST = 0.574986) that contained gene AKT1. AKT1 (protein kinase B, PKB) is a serine/threonine kinase that plays a critical role in regulating cell
survival, insulin signaling, angiogenesis and tumor formation [27–29]. Previous studies in chicken have revealed AKT1 as one of candidate gene for Salmonella response [30]. Moreover, subsequent fine-mapping of heterophil functional response to Salmonella in a highly advanced intercross revealed the position containing both AKT1 and SIVA may work as heterophil function to explain the host-resistance properties [31]. Another gene identified in our analysis was CDH13 was located at 15.6 Mb in chromosome 11. This gene encodes a member of the cadherin superfamily, which is hypermethylated in many types of cancer. Previous studies have showed that CDH13 may be a promising candidate gene for Attention Deficit/Hyperactivity Disorder (ADHD) [32, 33] and plays a central role in the regulation of brain networks [34]. Intriguingly, we found an apoptosis inhibitory protein, coded by gene API5 located at 20 Mb in chromosome. This apoptosis inhibitory protein prevents apoptosis after growth factor deprivation and suppresses the transcription factor E2F1-induced apoptosis, and negatively regulating acinus, a nuclearfactor involved in apoptotic DNA fragmentation. Its depletion enhances the cytotoxic action of the chemotherapeutic drugs [35]. ROH regions can increase the recessive deleterious alleles to be co-contributed, and reduce the viability of the organism. Differential ROH segments across lines may contain potential candidate genes related to MD resistance. In this study, 5 genes (SIK, SOX1, LIG4, SIK1 and TNFSF13B) within ROH region were found in resistant group (line 63 and RCS), and these genes have been previously reported that may be related to immunology and survival. Previous studies demonstrated that SIK1 gene plays critical role in promoting survival of skeletal myocytes using mouse model [36] and involve with regulation of its abundance and stability for myogenesis [37]. Sox1 as a part of the Sox-B1 group of transcriptional regulators in neural progenitor cells is sufficient to induce neuronal lineage commitment [38]. Also, it promotes neuronal cell fate determination and differentiation by integrating multiple independent pathways. In human, the GG genotype of LIG4 was found to be associated with higher IgE levels to Ascaris associated with higher IgE levels to Ascaris [39]. Also, gene TNFSF13B, as one of BAFF receptors, was proved to be a key survival factor during B-cell maturation [40, 41].

Interestingly, we identified one unique ROH region (at 110 Mb in chromosome 2) in line 72, which contains five genes LYN, MOS, PLAG1, TGS1 and TMEM68. Among them, LYN regulates survival and responsiveness of tumor cells by a BCR-ABL1 independent mechanism. In various hematopoietic cells, LYN has emerged as a key enzyme involved in the regulation of cell activation. In these cells, a small amount of LYN is associated with cell surface receptor proteins, including the B cell antigen receptor (BCR) [42], and CD40 [43]. PLAG1 (pleomorphic adenoma gene 1) was found frequently rearranged and activated in human salivary gland pleomorphic adenomas. It encodes a developmentally regulated transcription factor [44]. Ectopic overexpression of PLAG1 has been proposed to play a crucial role in tumorigenesis of salivary gland pleomorphic adenomas. It was reported that PLAG1 can activate the transcription of insulin-like growth factor 2 (IGF2), functioning as a proto-oncogene [45]. PLAG1 is a proto-oncogene whose overexpression is a crucial oncogenic event in salivary gland pleomorphic adenomas (PA), and in carcinoma ex pleomorphic adenoma (CA-ex-PA). Our results provided some important insights for understanding the molecular mechanisms of MD resistance. Moreover, we identified gene USP15 (ubiquitin-specific protease 15) in line 63, which may indicate its potential role for MD resistance. USP15 have been reported that have an essential role for regulation of caspase-3 during Paclitaxel-induced apoptosis [46] and TGF-β pathway in human studies [47].

Conclusions
We investigated the genomic characteristics of inbred chicken lines using high density SNP array by integrating genomic signature, ROH analysis. Our findings revealed that several candidate genes including AKT1, CDH13, CFDP, API5, and USP15 for MD susceptibility. Future studies with large sample size and fine mapping of the genetic variants using powerful association statistics would elucidate the complex genetic mechanisms of resistance/susceptibility to MD.

Additional files

Additional file 1: Figure S1. Histogram plot of MD incidence (%) rate. MD resistance in chickens is generally evaluated with MD incidence (induced gross tumors by MDV) and survival day post MDV challenge, and the extent of resistance is dependent of the virulence of challenge viruses and other factors. Figure S2. Barplot show the call rate for 30 chickens on Affymetrix chicken SNP genotyping array. X axis represent the call rate values, and Y axis represents the chicken individuals. Figure S3. Frequency distribution in autosomes for a total of 527,021 SNPs on the Affymetrix chicken SNP genotyping array. Figure S4. Frequency distribution in autosomes for 155,216 SNPs on the Affymetrix chicken SNP genotyping array. Figure S5. Comparison of ROH regions among three groups, line 63, line 72, lines RCS (C, L, M, N, S, X). The overlap length of ROH was indicated in kb. (DOCX 35 kb)

Additional file 2: Table S1. GO analysis for 51 genes in 106 identified candidate regions. (XLSX 20 kb)

Additional file 3: Table S2. The overlapping positions for 106 identified candidate regions with QTLs related to MD disease. (XLSX 12 kb)

Additional file 4: Table S3. The inbreeding coefficient (F) for all 30 samples using 155,216 high quality SNPs. (XLSX 11 kb)

Additional file 5: Table S4. Summary statistics and ROH distributions among 8 diverse inbred lines including lines 63, 72, C, J, M, N, S and X. (XLSX 31 kb)
Additional file 6: Table S5. Unique and shared homozygous segments across all chickens included RCS lines and line 6, chickens were compared with the line 72. (XLSX 13 kb)

Abbreviations
ADOL: Avian disease and oncology laboratory; BC: Reciprocal backcross; IBD: Identity-by-descent; IBS: Identical by state; LD: Linkage disequilibrium; MD: Marek’s disease; MDS: Multidimensional scaling; MDV: Marek’s disease virus; NJ: Neighbor-joining; QTL: Quantitative trait loci; RCS: Recombinant congenic strains; ROH: Runs of homozygosity

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Availability of data and materials
The Genotype data reported in this article are available upon request for research.

Authors’ contributions
JZS and LYX conceived and designed the experiments. LYX, YHH and YD performed data analyses. HHC, HMZ, and RLT collected samples and generated the SNP genotyping data. LYX, GEL and JZS wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All of the chickens were kept in a pathogen-free facility at the Avian Disease and Oncology Laboratory (ADOL). All animals were approved by the United States Department of Agriculture, Agricultural Research Service, ADOL Animal Care and Use Committee and followed their guidelines (revised April 2005) as well as the Guide for the Care and Use of Laboratory Animals published by Institute for Laboratory Animal Research (ILAR Guide) in 1996 (http://www.nap.edu/openbook.php?record_id=5140) and University of Maryland (R-08-62).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Lee LF, Powell PC, Rennie M, Ross LJ, Payne LN. Nature of genetic resistance to Marek’s disease in chickens. J Natl Cancer Inst. 1981;66:789–96.
2. Bacon LD, Hunt HD, Cheng HH. A review of the development of chicken lines to resolve genes determining resistance to diseases. Poult Sci. 2000;79: 1082–93.
3. Chang S, Dunn JR, Heidari M, Lee LF, Song J, Ernst CW, et al. Genetics and vaccine efficacy: host genetic variation affecting Marek’s disease vaccine efficacy in White Leghorn chickens. Poult Sci. 2010;89:2083–91.
4. Osterrieder N, Kamli JP, Schumacher D, Tischer BK, Trapp S. Marek’s disease virus: from virus to model. Nat Rev Microbiol. 2006;4:283–94.
5. Burgess SC, Young JR, Baaten BJ, Hunt L, Ross LN, Parcell MS, et al. Marek’s disease is a natural model for lymphomas overexpressing Hodgkin’s disease antigen (CD30). Proc Natl Acad Sci U S A. 2004;101:13879–84.
6. Cheng HH, Kaiser P, Lamont SJ. Integrated genomic approaches to enhance genetic resistance in chickens. Annu Rev Anim Biosci. 2013;2:239–60.
7. Heffetz EM, Fulton JE, O’Sullivan NP, Arthur JA, Wang J, Dekkers JC, Soller M. Mapping quantitative trait loci affecting susceptibility to Marek’s disease virus in a backcross population of layer chickens. Genetics. 2007;177:2417–31.
8. Heffetz EM, Fulton JE, O’Sullivan NP, Arthur JA, Cheng H, Wang J, et al. Mapping QTL affecting resistance to Marek’s disease in an F6 advanced intercross population of commercial layer chickens. BMC Genomics. 2009;10:20.
9. Liu HC, Kung HJ, Fulton JE, Morgan RW, Cheng HH. 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. 2001;98:2093–8.
10. Yu Y, Luo J, Mitra A, Chang S, Tian F, Zhang H, et al. Temporal transcriptome changes induced by MDV in Marek’s disease-resistant and -susceptible inbred chickens. BMC Genomics. 2011;12:501.
11. Perumbakkam S, Muir WM, Black-Pyrkosz AK, Okimoto R, Cheng HH. Comparison and contrast of genes and biological pathways responding to Marek’s disease virus infection using allele-specific expression and differential expression in broiler and layer chickens. BMC Genomics. 2013;14:64.
12. Xu L, He Y, Ding Y, Sun G, Carrillo JA, Li Y, et al. Characterization of copy number variation’s potential role in Marek’s disease. Int J Mol Sci. 2017;18:1020.
13. Zhang S, Ding Z, Dunn JR, Lee LF, Heidari M, Song J, et al. A comparative evaluation of the protective efficacy of MD5DeltaVeg and Cl008/8 Resipen against a vv+ strain of Marek’s disease virus infection in a series of recombinant congenic strains of White Leghorn chickens. Avian Dis. 2011;55:384–90.
14. Luo J, Yu Y, Mitra A, Chang S, Zhang H, Liu G, et al. Genome-wide copy number variant analysis in inbred chickens lines with different susceptibility to Marek’s disease. G3. 2013;3:217–23.
15. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.
16. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003;164:1567–87.
17. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–75.
18. Stevens EL, Heckenberg G, Roberson ED, Baughert JD, Downey TJ, Peveiner J. Inference of relationships in population data using identity-by-descent and identity-by-state. PLoS Genet. 2011;7:e1002287.
19. Ecoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010;10:564–74.
20. Rousset F. genepop007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 2008;8:103–6.
21. Weir BS, Hill WG. Estimating F-statistics. Annu Rev Genet. 2002;36:721–50.
22. Akey JM, Zhang G, Zhang K, Jin L, Shriver MD. Investigating a high-density SNP map for signatures of natural selection. Genome Res. 2002;12:1805–14.
23. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, et al. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res. 2007;35:W169–75.
24. Yonas N, Bacon LD, Witter RL, Cheng HH. High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek’s disease. Annu Genet. 1999;50:126–35.
25. Mitra A, Luo J, Zhang H, Cui K, Zhao K, Song J. Marek’s disease virus infection induces widespread differential chromatin marks in inbred chicken lines. BMC Genomics. 2012;13:557.
26. Luo J, Mitra A, Tian F, Chang S, Zhang H, Cui K, et al. Histone methylation analysis and pathway predictions in chickens after MDV infection. PLoS One. 2012;7:e41849.
27. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three acts. Genes Dev. 1999;13:2005–27.
28. Capron JD, Faber AL, Horn C, Donoho PG, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007;448:439–44.
29. Brugge J, Hung MC, Mills GB. A new mutational AKTivation in the PI3K pathway. Cancer Cell. 2007;12:104–7.
30. Fife MS, Salmon N, Hocking PM, Kaiser P. Fine mapping of the chicken salmonellosis resistance locus (SAL1). Anim Genet. 2009;40:871–7.
31. Redmond SB, Chuaamntri P, Andreasean CB, Palic D, Lamont SJ. Genetic control of chicken heterophil function in advanced intercross lines: associations with novel and with known Salmonella resistance loci and a likely mechanism for cell death in extracellular trap production. Immunogenetics. 2011;63:449–58.
32. Franke B, Fanaone SV, Ashenon P, Butelaar J, Bau CH, Ramos-Quiroga JA, et al. AC International Multicentre persistent. The genetics of attention deficit/hyperactivity disorder in adults, a review. Mol Psychiatry. 2012;17:600–87.
33. Jee SH, Sull JW, Lee JE, Shin C, Park J, Kimm H, et al. Adiponectin concentrations: a genome-wide association study. Am J Hum Genet. 2010;87:45–52.
34. Rivero O, Sich S, Popp S, Schmitt A, Franke B, Lesch KP. Impact of the ADHD-susceptibility gene CDH13 on development and function of brain networks. Eur Neuropsychopharmacol. 2013;23:492–507.
35. Morris EJ, Michaud WA, JH, Moon NS, Rocco JW, Dyson NJ. Functional identification of Ap5 as a suppressor of E2F-dependent apoptosis in vivo. PLoS Genet. 2006;2:e196.
36. Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD, Montminy M. SKI is a class II HDAC kinase that promotes survival of skeletal myocytes. Nat Med. 2007;13:597–603.
37. Stewart R, Akhmedov D, Robb C, Leiter C, Berdeaux R. Regulation of SKI abundance and stability is critical for myogenesis. Proc Natl Acad Sci U S A. 2013;110:11–22.
38. Kan L, Irasena N, Zhang Z, Hu M, Zhao LR, Jalali A, et al. Sox1 acts through multiple independent pathways to promote neurogenesis. Dev Biol. 2004;269:580–94.
39. Acevedo N, Mercado D, Vergara C, Sanchez J, Kennedy MW, Jimenez S, et al. Association between total immunoglobulin E and antibody responses to naturally acquired Ascaris lumbricoides infection and polymorphisms of immune system-related IL4, TNFp138 and IRS2 genes. Clin Exp Immunol. 2009;157:282–90.
40. Mackay F, Silveira PA, Brink R. B cells and the BAFF/APRIL axis: fast-forward on autoimmunity and signaling. Curr Opin Immunol. 2007;19:327–36.
41. Ren CL, Morio T, Fu SM, Geha RS. Signal transduction via CD40 involves activation of lyn kinase and phosphatidylinositol-3-kinase, and phosphorylation of phospholipase C gamma 2. J Exp Med. 1994;179:673–80.
42. Van Dyk F, Declercq J, Baem CV, Van de Ven WJ. PLAG1, the prototype of the PLAG gene family: versatility in tumour development (review). Int J Oncol. 2007;30:765–74.
43. Zhao X, Ren W, Yang W, Wang Y, Kong H, Wang L, et al. Wnt pathway is involved in pleomorphic adenomas induced by overexpression of PLAG1 in transgenic mice. Int J Cancer. 2006;118:643–8.
44. Xu M, Takashita M, Okawa K, Tanaka M, Nishi H, Iida K, et al. USP15 plays an essential role for caspase-3 activation during Paclitaxel-induced apoptosis. Biochem Biophys Res Commun. 2009;388:366–71.