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# Genetic resilience in chickens against bacterial, viral and protozoal pathogens

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The genome contributes to the uniqueness of an individual breed, and enables distinctive characteristics to be passed from one generation to the next. The allelic heterogeneity of a certain breed results in a different response to a pathogen with different genomic expression. Disease resistance in chicken is a polygenic trait that involves different genes that confer resistance against pathogens. Such resistance also involves major histocompatibility (MHC) molecules, immunoglobulins, cytokines, interleukins, T and B cells, and CD4+ and CD8+ T lymphocytes, which are involved in host protection. The MHC is associated with antigen presentation, antibody production, and cytokine stimulation, which highlight its role in disease resistance. The natural resistance-associated macrophage protein 1 (Nramp-1), interferon (IFN), myxovirus-resistance gene, myeloid differentiation primary response 88 (MyD88), receptor-interacting serine/threonine kinase 2 (RIP2), and heterophile cells are involved in disease resistance and susceptibility of chicken. Studies related to disease resistance genetics, epigenetics, and quantitative trait loci would enable the identification of resistance markers and the development of disease resistance breeds. Microbial infections are responsible for significant outbreaks and have blighted the poultry industry. Breeding disease-resistant chicken strains may be helpful in tackling pathogens and increasing the current understanding on host genetics in the fight against communicable diseases. Advanced technologies, such as the CRISPR/Cas9 system, whole genome sequencing, RNA sequencing, and high-density single nucleotide polymorphism (SNP) genotyping, aid the development of resistant breeds, which would significantly decrease the use of antibiotics and vaccination in poultry. In this review, we aimed to reveal the recent genetic basis of infection and genomic modification that increase resistance against different pathogens in chickens.

## KEYWORDS

chicken MHC, genetics, SNPs, non-coding RNAs, pathogens, infectious diseases, novel technology

## Introduction

The breeding of chicks with polygenic resistance is the top priority of poultry farmers as these chickens may tolerate challenging environments without losing their productivity. The poultry industry is susceptible to bacterial, viral, and protozoal pathogens that cause several infectious diseases and reduce growth yield, productivity, and profit. Prophylactic measures, such as vaccination, antibiotics, disinfectants, and culling, are used to control infections in poultry (1). However, current vaccines lack cross-protection against multiple strains of each virus. Furthermore, the mutagenicity of viruses has led to the emergence of highly virulent strains (2). To counter emerging pathogens, a genetically resistant breed should be developed to prevent outbreaks, enable sustained economic viability, and retain consumer confidence in poultry products. By rearing genetically disease-resistant flocks, a breed that can withstand infectious diseases and pathogens owing to its unique genetic modifications, can be obtained (1, 3, 4).

Many disease-resistant genes, including MHC, chicken interleukin 1beta converting enzyme 1 (Caspase1), inducible nitric oxide synthase, IFN, Nramp-1, myxovirus-resistance gene, and toll-like receptor (TLR) genes, play a role in the active immune system of chickens (4, 5). The immune system varies among different hosts, which exhibit different responses to immune cells, such as T and B cells, antibody production, phagocytosis, and lymphocyte proliferation that protect the host from pathogen damage (3). The communication network of immune cells consists of T and B cell receptors, MHC, antibodies, and cytokines that are involved in antigen processing of the effector cells, and play a pivotal role in resistance and susceptibility against bacterial, viral, and parasitic diseases (3, 5). For instance, the Athens Canadian Random Bred strain, which is the oldest pedigreed meat-type chicken existing since the 1950's, has a more stable immune response and disease-resistant phenotype than modern-day broilers (6).

Based on genomic analysis, phosphoinositide-3-kinase-protein kinase B, Janus kinase/signal transducers and activators of transcription (JAK/STAT), nuclear factor kappa B (NF- $\kappa$ B), IL-1 $\beta$ , and IL-6 mRNA are highly expressed in Athens Canadian Random Bred compared to modern broiler (6). In our previous work, immunoglobulin lambda light chain precursor, Ig-gamma (clone-36 chicken), *P01875*, and *PIT-54* genes were identified to be involved in immune response during embryogenesis (7). In a subsequent study, dietary ellagic acid was found to significantly increase antioxidant and antibacterial activities in layers and improve bird health status (8). Importantly, breeding with new technologies improves poultry productivity and enhances disease resistance traits. For example, the livestock-breeding program produced nematode-resistant sheep (9). Similarly, birds resistant to lymphoid leucosis and Marek's disease (10), mastitis-resistant cattle (11), immunocompetent pigs (12), bird flu-resistant chickens (13), Trypanosoma resistant cows (14),

porcine reproductive and respiratory syndrome virus-resistant pigs (15), and prion protein-resistant sheep and goat (16, 17) have been developed.

As poultry products are globally consumed on a large scale, there has been substantial interest in generating disease-resistant chicken. Here, we aimed to discuss the genetic responses of chickens to bacterial, viral, and protozoal pathogens, and summarize recent advancements in the generation of pathogen-resistant chickens *via* gene expression modulation using the CRISPR/Cas system (clustered regularly interspaced short palindromic repeat/Cas9), RNA interference (RNAi), and viral vectors. Finally, we highlighted some candidate genes that are involved in various biological pathways and may contribute to the resistance of chickens against the diseases.

## Genetic roles in host resistance and susceptibility

The MHC gene is widely evaluated in chickens to identify differences in their resistance and susceptibility to certain pathogens and infectious diseases. MHC class I, II, III, and IV molecules are unique and distinct between species, leading to a differential MHC response among individuals (3). Chickens have few MHC genes with different haplotypes involved in the development of resistance against bacterial, viral, and protozoal pathogens. For instance, MHC haplotype B19 is associated with susceptibility, while B2 and B21 are involved in resistance (18). MHC-dependent resistance and susceptibility rely on peptide-binding specificity. For example, chicken-affected cells expressing MHC-I haplotype, which binds to the Rous sarcoma virus src peptide targeted by cytotoxic CD8+ T cells, are resistant to Rous sarcoma virus (19). In susceptible chickens, the MHC haplotype does not bind with viral peptides, and chickens are infested by the virus. For instance, the MHC class I haplotypes do not bind to the antigenic peptides of Marek's disease virus (MDV), resulting in chickens remaining susceptible (19). The chicken MHC haplotype has a regulatory effect on immune cells resistant to the Rous sarcoma virus and exhibit enhanced natural killer cell activity (20). In a recent study, the MHC haplotypes B15 and B21 homozygotes led to the lowest MDV-induced tumorigenesis and lymphoma formation in VALO specific pathogen-free chickens, demonstrating that MHC conferred resistance to oncogenic herpesviruses (21). Notably, the MHC-peptide complexes engaged T cell receptors (TCRs) that recognize antigens on MHC molecules with the cooperation of CD4+ or CD8+ coreceptors and activate T cells (22). Each T cell has a unique TCR that recognizes and binds with the antigenic peptide on the infected cell surface. The antigen peptides are derived from intracellular pathogens, such as viruses and bacteria, and are displayed at the cell surface by MHC for immune clearance (23). Viruses, such as the avian leucosis virus, have six subgroups, with

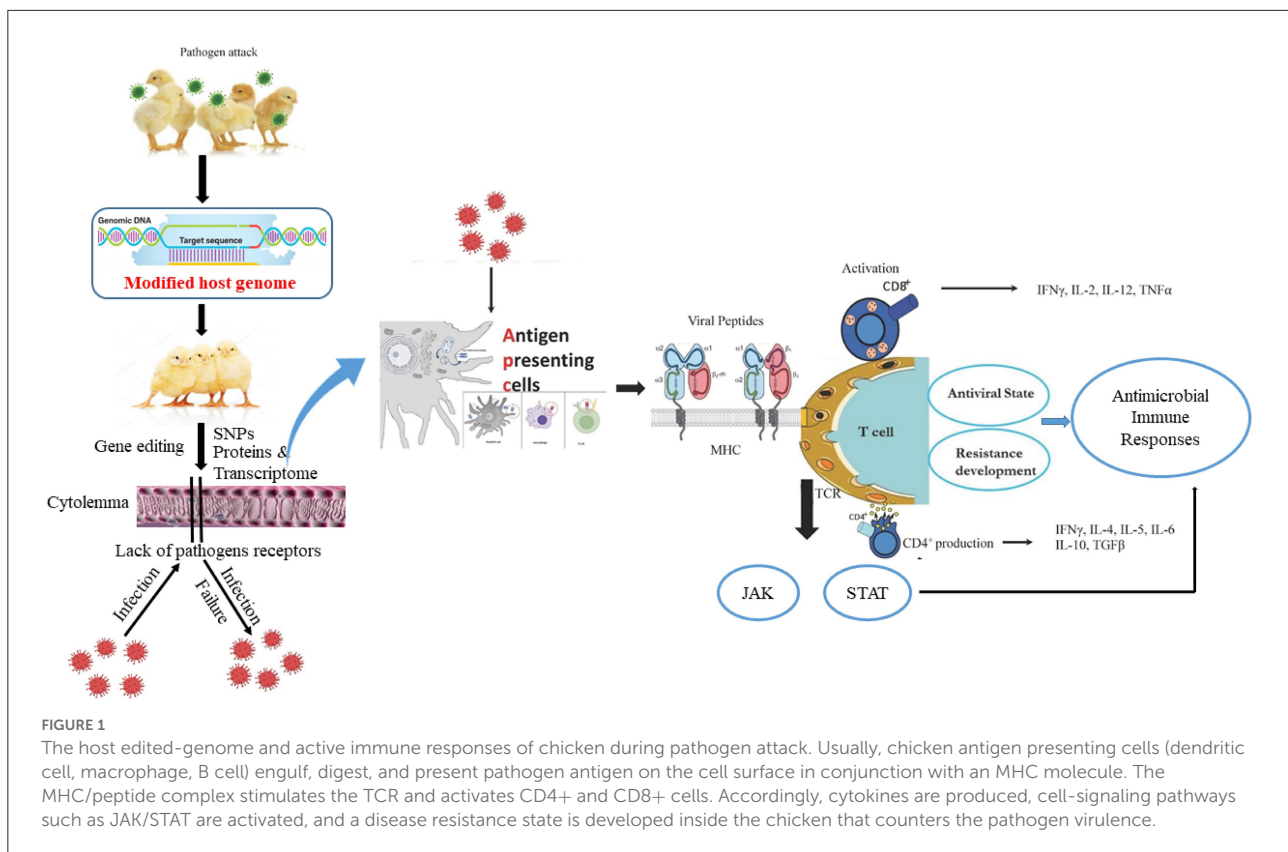
subgroup J causing severe outbreaks in China. The avian leucosis virus subgroup J receptor is a sodium/hydrogen exchanger 1, which is edited on chicken somatic cell lines that are resistant to avian leucosis virus *in vitro* (24). Avian influenza virus replication is facilitated by the acidic leucine-rich nuclear phosphoprotein-32A (ANP32A) protein in chicken and waterfowl. An *in vitro* study revealed that the deletion of a minor region of chicken ANP32A stops the replication of the avian influenza virus (25). Although such studies have increased our understanding of the genetic roles, a functional study of edited ANP32A and sodium/hydrogen exchanger 1 gene has not been performed *in vivo* and edited chicken has hitherto been developed.

Generally, increasing poultry resistance to infectious pathogens *via* gene modification is an ideal approach for the development of transgenic livestock. In particular, resistance to diseases originates from the interplay of numerous genes. For example, the mouse fibroblast cell lines are resistant to influenza virus owing to the autosomal dominant Mx1 allele of the murine Mx gene (26). The introduction of the Mx1 gene in mice lacking the Mx1 allele leads to influenza-resistant mice (27) whereas transfer of the same gene in swine does not result in viral-resistant pigs (28). The overexpression of pathogen anti-receptor proteins blocks viral attachment and penetration, and alters the host receptor genes that prevent viral attachment and enhanced resistance against diseases (29). Transgenic chickens express a recombinant avian leukosis envelope protein, which inhibits the corresponding subgroup of avian leukosis virus attachment (30). Similarly, transgenic sheep express the Maedi-visna virus envelope protein and display resistance *via* the prevention of virus adhesion to host cells (31). Collectively, the observed immune responses of chicken against viral, protozoal, and bacterial agents are pathogen-specific, and are closely linked to expression changes in MHC, Nramp-1, RIP2, MyD88, IFN, interleukin, MX1, TLR4, antibodies, and immune cells that govern antibacterial and antiviral states (Figure 1).

## Genetic resilience and viral pathogens

Viral diseases cause more outbreaks, reduce growth performance and productivity, and cause immunosuppression in poultry (3). Marek's disease, a well-known viral disease, is caused by MDV. Marek's disease is a lymphotropic disease in chickens and the MDV targets all avian species, causing symptoms such as paralysis, loose watery stool, lymphomas, wasting, and immunosuppression. The poultry response to Marek's disease is the activation of MHC molecules and cytokines that give resistance to MDV (22). Other genes that confer resistance to MDV include *GHI*, *SCA2*, *IRG1*, *CD79B*, *PTPN3*, *LY6E*, and *SMO1* (32). Another important virus is influenza, a zoonotic virus that causes avian flu. Genes, such as interferon-inducible transmembrane, a retinoic acid-inducible

gene I, and MX1 gene polymorphisms are reported to be associated with susceptibilities to avian influenza in chickens and ducks (33, 34). Newcastle disease virus widely infects chickens. Newcastle Disease is characterized by ruffled feathers of chicken, and respiratory, neurological, hyperthermia, and listlessness complications in affected chickens (35). Potential genes, such as *IFN $\alpha$* , *IFN- $\gamma$* , *DDX-1*, *MHC-1*, and *IL-6*, were identified in chicken embryos infected with Newcastle disease virus. These important genes have an antiviral function and induce TLR-mediated activation of macrophages and dendritic cells in response to viruses (36). Newcastle disease virus-infected Fayoumis birds were found to have lower expression of EIF2B5, EIF2S3, EIF2B4, and EIF2S3 than Leghorn's infected lines. Such results indicate that different genetic lines display different expression of host translation initiation factor-2 associated genes, which might contribute to their differential resistance to Newcastle disease virus (37). In a study conducted in Ghana, three Ghanaian local chicken ecotype responses to the lentogenic and velogenic strains of Newcastle disease virus assessed. Based on the findings, resistance to Newcastle disease virus was identified to be caused by an individual's chicken genetic makeup and not by the chicken ecotype (38). The genes, MHC-B locus, *LEI0070*, *ADL0146*, *LEI0104*, *ADL0320*, and *ADL0304*, are associated with a direct response of antibody titer against Newcastle disease virus in chickens (39). Wang et al. (40) revealed that the hemoglobin family genes, functional involvement of oxygen transportation and circulation, and cell adhesion molecule signaling pathway play significant roles in disease resistance to AIV infection in chickens. The influenza H5N1 strain was inoculated into genetically resistant and susceptible Ri chicken native to Vietnam. The resistant chicken displayed a group of genes, *MX1*, *TLR3*, *STAT1*, *IRF7*, *IFN*, and cytokines, which are found in H5N1 strain-resistant chickens (41). Avian Leukosis virus infection is highly receptor-specific and the Leukosis virus subtype A uses specific membrane proteins, such as Tva receptors for binding, CAR1 receptors for avian Leukosis virus subtypes B & D attachment, and SEAR receptors for Avian Leukosis virus subtype E, which is encoded by tumor virus genes (42). These chicken breeds express certain receptors on their cell surface, such as Tva and CAR1, and are susceptible to the corresponding avian Leukosis virus subtype (42, 43). Chickens resistant to infectious bronchitis, Newcastle disease, Marek's disease, coccidiosis, and salmonellosis had high production of IFN- $\gamma$ , which validated the enhanced production of Th1 and cytotoxic T cells (44). By examining fowl Adenovirus serotype 4 infection, which causes hepatitis hydropericardium syndrome in poultry, Xiang et al. (45) revealed that the expression levels of IL-6, IL-1 $\beta$ , IFN- $\alpha$ , JAK, and STAT were significantly high after viral infection. In summary, during infection, the host induces changes in gene expression that confer transient or long-lasting protection against pathogens. Exploring why, when, which, and how a host reprograms its genome against infectious pathogens is an



exciting research topic that can reveal the amplitude of virulence and its genetics.

## Genetic resilience and autoimmune diseases

The TCR exhibits polymorphism that creates high diversity and differences in disease response by T cells (38). The TCR diversity is due to gene rearrangement where different segments, including variable (V), diversity (D), and joining (J) segments of the TCR gene, randomly recombine, and genes for the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  chains are formed (46). Although chickens have few V, D, and J genes that limit TCR diversity, TCR heterodimers can be created. For example, the TCR heterodimers of the  $\alpha$  and  $\beta$  chains are the  $\alpha\beta$  T cells distinguished by the V region of the  $\beta$ -chain that causes  $V\beta 1+$  (TCR 2) and  $V\beta 2+$  (TCR 3) with functional multiplicity (47). TCR defects in chicken are associated with susceptibility to autoimmune diseases. In fact, the TCR defects in scleroderma disease cause low CD4+ cells and non-specific T cell response in chicken (48). Moreover, autoimmune thyroiditis disease is prevented by the depletion of CD4+ cells, highlighting the involvement of the TCR  $V\beta 1$  gene (49). Coccidiosis-resistant chicken lines have a high number of

CD4+ cells whereas susceptible chickens have a high number of CD8+ cells (50). Moreover, a low number of CD8+ was detected in turkeys infected with Newcastle disease virus, *Pasteurella multocida*, and *Erysipelothrix rhusiopathiae* (50) whereas a high number of CD8+ cells was found in amyloidosis-resistant chickens compared to susceptible chickens (51). Altogether, the amount of CD4+ and CD8+ in resistant or susceptible birds does not align with a particular disease or pathogen in poultry, which might be due to the polymorphism of CD8+ and CD4+. CD4+ cells exhibit resistance toward non-intracellular while CD8+ cells exhibit toward intracellular pathogens that direct differential immune responses against a pathogen (52, 53). In conjunction with cellular immunity, humoral immunity plays a very key role in resistance to diseases. Immunoglobulin genes produce antibodies, and chickens with high antibody production display resistance against microbes, such as *Mycoplasma gallisepticum*, *Escherichia coli*, Newcastle disease virus, and *Salmonella enteritidis* relative to low antibody producers (54, 55). Chicks that are high antibody producers have numerous CD4+ and type II helper T lymphocytes (Th2), whereas low antibody producers have numerous CD8+ cells and type I helper T lymphocytes (Th1) that improve their resistance against pathogens (56, 57). The Th1 cytokines include IFN- $\gamma$ , IL-2, and IL-12 whereas the Th2 cytokines include IL-4, IL-5, IL-6, and IL-10, which stimulate cell-mediated and



antibody responses, respectively (58). Altogether, variations exist in cellular and humoral immune responses in different chicken breeds, and high expression of cytokines leads to a higher immunocompetence of the host.

## Genetic resilience and bacterial pathogens

Bacterial invasiveness in chickens depends on the species, severity, and virulence of the pathogen. The predominant bacterial pathogens affecting poultry are *Escherichia coli*, *Campylobacter jejuni*, *Clostridium perfringens*, *Mycoplasma*, and *Salmonella spp.* In contrast, *Erysipelothrix rhusiopathiae*, *Gallibacterium anatis*, *Pasteurella multocida*, *Riemerella anatipestifer*, *Avibacterium paragallinarum*, *Ornitobacterium rhinotracheale*, and *Bordetella avium* are infrequently detected (59, 60). The most devastating bacteria in terms of yield in the poultry industry belong to the genus, *Salmonella*, and include the species, *S. enterica* and *S. bongori*, that easily infect the newly hatched chicks and cause a decline in growth, egg production, and hatchability in chickens (61). To counter salmonellosis, prophylactic measures, such as antibiotics, vaccination, and disease management, are insufficient in poultry flock surveillance (62). Accordingly, the importance of resistant-chickens has increased, and the development of disease-resistant traits through genetic improvement has become more significant. Chicken MHC I, MHC II, Nramp-1, heterophils, IFN  $\gamma$ , and interleukins are involved in *Salmonella*-specific antibody responses and lead to resistance to salmonellosis (63). In a previous study, heterophils from chicken resistant to *S. enteritidis* had a higher level of cytokine mRNA than heterophils isolated from susceptible chickens (64). The mRNA level of interleukins and IFN  $\gamma$  increased in resistant chicks relative to that in chickens susceptible to salmonellosis (65). IFN  $\gamma$  plays a significant role in the eradication of *Salmonella* carriers and persistence state (66). The genes, *Nramp-1* and *Nramp-2*, are the macrophage proteins expressed in heterophils and leukocytes that facilitate *S. enteritidis* phagocytosis in resistant chicks (67). Other genes, such as transforming growth factor B4 (*TGFB4*) and *Sal1*, are involved in controlling *Salmonella* and other bacterial loads in the spleen, and have been linked to increasing genetic resistance against *S. enteritidis* (68). In a recent study, Beijing-You and Cobb chicks were orally challenged with *S. typhimurium*, which revealed robust responses of natural killer-cell-mediated-cytotoxicity, phagosomes, cytokines, MHC, and antibody production in Beijing-You chicken, ultimately indicating the greater resistance of Beijing-You breed to *S. typhimurium* (69). The chicken RIP2 pathway plays a significant role in resistance against avian pathogenic *E. coli* infection. *E. coli* infection promotes RIP2 expression and inhibits cell viability, whereas

knockdown of RIP2 restores cell viability and represses the apoptosis of chicken HD11 cells. Nuclear factor I B increases the expression of RIP2, reduces cell viability, and accelerates *E. coli*-induced apoptosis, confirming that RIP2 supported *E. coli* proliferation in chicken cells (70). *Mycoplasma gallisepticum* infects the lungs of chickens and causes chronic respiratory disease. Glycyrrhizic acid is a herb that has anti-inflammatory, anti-microbial, and antioxidant activities and inhibits *M. gallisepticum* infection by suppressing the expression of matrix metalloproteinases through the JNK (c-Jun N-terminal kinase) and p38 pathways and inhibiting the expression of virulence genes of *M. gallisepticum* (71). *Campylobacter jejuni* infections are prevalent in poultry and colonize the intestine of birds. The bird's response to *C. jejuni* is similar to *Salmonella* infection, and high expression levels of cytokines, T and B cells, and antibodies are detected in *C. jejuni*-resistant birds relative to susceptible birds (72), except quantitative trait loci localization, which is located in different chromosomes (73). Breeder selection of traits that correlate with enhanced resistance against pathogens is highly desirable, and can be determined *via* extensive immunogenetics research. Therefore, screening host genome for disease-resistance genes and pathways in chickens can pave the way for the development of immunocompetent chickens.

## Genetic resilience and protozoans

The next important etiological agents that cause infectious diseases in chicken are protozoal parasites, including *Eimeria tenella*, *Ascaridia galli*, and *Histomonas meleagridis*. The protozoan, *H. meleagridis*, causes blackhead disease or histomoniasis (74); *E. tenella* causes coccidiosis in chickens (75); and *A. galli* infects chickens and turkeys and causes stunted growth and enteritis (76). Pathogen-specific immune responses occur against parasitic infections in chicken. For instance, the myeloid leukemia factor 2 gene help in resistance to *Eimeria* (77), and the *IFNG* gene is associated with *Ascaridia* resistance in poultry (78). Moreover, the MHC haplotypes protect the jungle fowl from coccidian (79) and chicken lines from *Ascaridia* infections (80). Other genes, such as TGF $\beta$  2-TGF $\beta$  4, Caspase-1, inhibitor of apoptosis protein1, prosaposin, inducible nitric oxide production, IL-2, immunoglobulin light chain, and tumor necrosis factor-related apoptosis-inducing ligand, have been relatively less explored in protozoan resistance, but can improve the disease resistance traits in poultry.

Yang et al. (81) discovered that butyrate, forskolin, and lactose compounds synergistically increase the expression of multiple host defense peptides, improve the survival of chickens, and reduce the colonization of *Eimeria maxima* and *Clostridium perfringens*. A list of candidate genes in poultry that exhibit important functional activities in animals, but have not been explored for disease resistance in chickens, is provided in Table 1.

TABLE 1 The key genes are involved in the infectious diseases of chickens.

Gene ID	Gene symbol	Gene name	Access code	Study type	Function	Location	Reference
396241	TF	Transferrin	NM_205304.2	<i>In vitro</i>	Iron-binding glycoprotein and involved in anti-microbial activities, against Marek's disease	chromosome: 9	(7, 82)
416928	IgLL1	Immunoglobulin lambda-like polypeptide 1	NM_001278545.1	<i>In vivo</i>	Antibacterial properties against <i>Streptococcus mutans</i>	chromosome: 15	(83)
100543636	LOC100543636	Ovoinhibitor	XM_010719004.3	<i>In vitro</i>	Antibacterial activities during embryo developments	chromosome: 15	(7, 84)
424533	VTG2	Vitellogenin 2	NM_001031276.2	<i>In vivo</i>	Transfer of nutrients for developing embryo and reduce intestinal oxidative stress	chromosome: 8	(7, 85)
418974	VMO1	Vitelline membrane outer layer 1	NM_001167761.2	<i>In vivo</i>	Diagnostic marker of ovarian cancer in hen	chromosome: 1	(86)
395364	PIT54	PIT54 protein	NM_207180.2	<i>In vivo</i>	Hemoglobin-binding protein of plasma in chicken which has antioxidant activity	chromosome: 31	(87)
420897	OVALY	Ovalbumin-related protein Y	NM_001031001.1	<i>In vitro and In vivo</i>	Ovalbumin has antioxidant and radical scavenging activities	chromosome: 2	(88, 89)
396393	EX-FABP	Extracellular fatty acid-binding protein	NM_205422.2	<i>In vitro</i>	Function as an antibacterial siderophore binding lipocalin	chromosome: 17	(90)
395722	CLU	Clusterin	NM_001396177.1	<i>In vivo</i>	Serve as a marker for follicular atresia and involve in developmental phases of follicles	chromosome: 3	(91)
396384	IRF1	Interferon regulatory factor 1	NM_205415.2	<i>In vitro</i>	Inhibits the replication of avian influenza virus and Newcastle disease virus	chromosome: 13	(92)
769014	TLR2	Toll like receptor 2	NM_001161650.3	<i>In vivo</i>	Immunity and resistance to bacterial infection	chromosome: 4	(93)
395764	CASP1	Caspase 1	XM_015295935.4	<i>In vitro and In vivo</i>	Involved in apoptosis, necrosis, mitophagy, and autophagy	chromosome: 19	(94–96)
418300	ZYX	Zyxin	NM_001004386.2	<i>In vivo</i>	Zyxin is a candidate gene potentially associated with increased resistance to experimental avian coccidiosis.	chromosome: 1	(97)
396260	AVD	Avidin	NM_205320.2	<i>In vitro</i>	Antimicrobial activity	chromosome: Z	(98, 99)
418812	ACOD1	Aconitate decarboxylase 1	NM_001030821.2	<i>In vivo</i>	Antimicrobial activity	chromosome: 1	(100)

(Continued)

TABLE 1 (Continued)

Gene ID	Gene symbol	Gene name	Access code	Study type	Function	Location	Reference
374125	LITAF	Lipopolysaccharide induced TNF factor	NM_204267.2	<i>In vitro</i> and <i>In vivo</i>	Initiates the activation of caspases and kinase protein signaling of the cell death pathway and has antimicrobial activity	chromosome: 14	(101)
395283	TRAIL-LIKE	TNF-related apoptosis inducing ligand-like	XM_015278184.4	<i>In vitro</i> and <i>In vivo</i>	It declines the autoimmune response by suppressing cell cycle progression.	chromosome: 4	(102)
420963	PTPN3	Protein tyrosine phosphatase, non-receptor type 3	XM_419047.8	<i>In vivo</i>	Involved in immune suppression disease.	chromosome: 2	(103)
378897	THY1	Thy-1 cell surface antigen	NM_204381.3	<i>In vitro</i>	Involved in chicken Marek disease.	chromosome: 24	(104)
768688	SMOC1	SPARC related modular calcium binding 1	XM_015287582.4	<i>In vivo</i>	Enhanced endothelial cell proliferation and angiogenesis.	chromosome: 5	(105)
422993	LOC422993	Interferon-induced transmembrane protein 3-like	NM_001350059.2	<i>In vivo</i>	Highly expressed in response to avian Tembusu virus infection	chromosome: 5	(106)

## SNP-dependent resistance and susceptibility in chickens

SNP is the nucleotide sequence variation that occurs at a single position in DNA fragments and is extensively used as a molecular marker in genetic studies. The roles of SNPs are largely associated with production traits in chicken. SNPs have been detected in follicle-stimulating hormone, prolactin receptor, dopamine receptor 2, low-density lipoprotein receptor-related protein, and luteinizing hormone receptors, with characteristic changes in duck and chicken (107, 108). For instance, the follicle-stimulating hormone regulates reproductive activities in birds, and the SNP detected in the follicle-stimulating hormone is linked to the reproductive traits of chickens (109). Two key SNPs, A227G and C320T, were identified in the Muscovy duck follicle-stimulating hormone gene that improve egg production traits (110). Ye et al. (111) revealed two SNPs in the insulin-like growth factor 2 gene and 11 SNPs in dopamine receptor 2 that are linked with egg-laying traits (111). In a recent study, the polymorphism of the DMA gene, a member of the non-classical MHC class II gene, was associated with disease resistance traits in broiler chickens. Four SNPs linked to seven haplotype formations were found,

with haplotypes 1 and 5 associated with high immunoglobulin yolk concentration and ND antibody level, respectively (112). The SNPs detected in the carboxypeptidase Q and leucine-rich repeat transmembrane neuronal 4 gene regions resulted in a decrease in pulmonary hypertension syndrome and greater innate ascites resistance in chicken offspring (113). Mountford et al. (2) correlated SNPs with resistance and susceptibility to MDV, infectious bursal disease virus, avian influenza virus, and infectious bronchitis virus. These researchers detected 10 SNPs that were involved in the resistance to MDV and 8 SNPs associated with the susceptibility to infectious bursal disease virus. Recently, IL10R $\beta$  SNP resulted in an R318K amino acid substitution that was involved in the enhanced regulation of the type III interferon pathway that reduced bursal damage in infectious bursal disease virus-infected birds (114). A previous study revealed the same SNP involvement in increased susceptibility to MDV (115). Thus, IFN response can vary for viruses owing to viral mutagenicity and strain diversity. As a result, viruses can block the IFN responses. Nramp-1, Sal-1, and Tnc are the genes involved in resistance to *Mycobacterium*, *Salmonella*, and *Leishmania* infections (116). In chicken, Nramp-1 polymorphism is correlated with susceptibility to salmonellosis. Frequent sequence variations were detected in this gene that conferred resistance differences in chicken (4, 117). MyD88

polymorphism is associated with *S. pullorum* susceptibility in chickens and has a favorable effect on vulnerability to *S. pullorum* infection (118). These recently identified SNPs are associated with disease-resistance genotypes that can help in the identification of new genes and their roles in eradicating infectious diseases.

## Non-coding RNA resistance in chicken

Non-coding RNAs are biological molecules involved in epigenetic regulation and disease resistance (119). There are different classes of non-coding RNAs, such as circular RNAs, small interfering RNAs, long non-coding RNAs (lncRNAs), microRNAs, and transfer RNAs, that play important roles in avian immunity and cell development. Among these classes of RNAs, lncRNAs, circular RNAs, and microRNAs are called regulatory RNAs that mediate gene expression in different hosts (120). lncRNAs are longer than 200 nucleotides and are known as signaling molecules that interact with mRNA, miRNA, DNA, and proteins, thereby regulating various processes, such as apoptosis, tumor cell invasion, RNA transcription, and host resistance to pathogen infection (121, 122). Based on recent studies, lncRNAs regulate vitamins A and D during bacterial and fungal infections and activate immune responses during chicken leukemia virus infection (123). Specialized lncRNAs that reduce the production of inflammatory cytokines, such as IL-6, IL-8, and TNF- $\alpha$ , were identified in response to *E. tenella* infection in chickens (124). An important ERL lncRNA acts as an antisense transcript of MDV carcinogen, is expressed during the lytic and lysogenic phases of viral infection, and inhibits the expression of MDV miRNAs (125, 126). The lncRNA, GLAMD3, cis-regulates the gga-miR-223 expression that targets IGF1R (insulin-like growth factor 1 receptor), which regulates Marek's disease lymphoma (127). Another important lncRNA, linc-stab1, regulates the Marek's disease resistance gene, SATB1, which is also involved in cell-mediated immunity for termination of MDV-infected cells (128).

Several studies revealed the roles of circRNAs in avian leukosis virus infection. Furthermore, differentially-expressed circRNAs were detected in infected organs. circRNAs are involved in T and B-cell activation (129), and Jak-STAT pathway regulation (130). In contrast to lncRNAs and circRNAs, the expression profile and functional mechanism of miRNAs are well-characterized in disease resistance in chickens. In fact, differentially expressed miRNAs have a significant effect on oncogenicity (131); the regulation of MAPK, Jak/STAT, and Wnt pathways (132); and suppression of chronic myeloid leukemia caused by avian leukosis virus in chicken (133). In conclusion, non-coding RNAs regulate disease resistance traits,

interact with host and pathogen genes, and help to control infectious diseases.

## Modern technology and development of disease-resistant chicken

Gene-editing techniques, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), pronuclear injection, sperm-mediated gene transfer, somatic cell nuclear transfer, recombinases, transposons, viral vectors, and CRISPR/Cas9 systems, are novel molecular tools that are efficiently used in mice, cattle, sheep, and goat. For instance, transgenic mice, rabbits, pigs, and sheep were engineered by microinjection of the target DNA into the fertilized embryo (134, 135); lentiviral vectors and embryonic stem cells were used to produce germline transgenic birds (136, 137); and successful knock-out in chickens were achieved by homologous recombination in primordial germ cells (138). In zinc-finger nucleases and transcription activator-like effector nucleases techniques, the proteins bind to the target DNA sequence for modification, whereas the CRISPR requires a guide RNA to recognize the target DNA fragments. Further, the endonuclease enzyme performs a target-specific cut (139). Since the introduction of the CRISPR/Cas9 system in genome editing, substantial progress has been made in the use of the CRISPR/Cas9 technology in chickens. A CRISPR/Cas9-mediated chicken was engineered in 2015 (139) and ovomucoid gene-targeted chickens and knocked-in of human interferon beta into the chicken ovalbumin gene were edited successfully (140, 141). The emerging viral strains of avian leukosis and MDV are highly pathogenic. Further, existing vaccines and antiviral drugs are becoming less effective. Thus, novel antiviral strategies are needed. For instance, through CRISPR/Cas9, the avian leukosis virus subgroup J receptor sodium/hydrogen exchanger type 1 is mutated, which protects the chicken line from avian leukosis virus subgroup J. Subgroup J prototype strain replication is also impaired in mutated birds (142). Resistance was found to develop in chicken cells against avian leukosis virus subgroup J by creating tryptophan mutations at position 38 (143). In another study, genetic resistance to avian leukosis virus subgroups A, C, J was induced by creating frame-shift mutations in *tva* (tumor virus locus A gene), *tvb*, and *tvj* genes (144). Koslová et al. (143) and Hellmich et al. (144) produced ALV-J-resistant chicken lines *via* precise gene editing of chicken sodium/hydrogen exchanger 1. A recent study revealed that transgenic chickens constitutively express Cas9 and guide RNAs specific to the immediate early infected-cell polypeptide-4 (gICP4) of MDV upon challenge with MDV, and exhibit reduced replication compared to wild-type chickens (145). These examples highlight the use of the CRISPR/Cas9 system to edit genes of interest and engineer chicken flocks that



exhibit resistance characteristics to viral infection (146). Lately, CRISPR/Cas9 has been used to develop transgenic animals. Accordingly, transgenic animals are generated *via* the targeted placement of *Streptococcus pyogenes* Cas9 at the ROSA26 locus and endogenous pseudo attP site in pigs and chickens, respectively. Transgenic chickens and pigs constitutively express Cas9. Cas9 was confirmed in pigs and chicken for different target genes in many cell types with the *S. pyogenes* Cas9 platform for *in vitro* and *in vivo* genome editing in livestock species (147). Similarly, different computational and bioinformatics approaches can be used to design synthetic RNA duplexes that would target the mRNA sites of viral, bacterial, and protozoal pathogens. For example, synthetic RNA duplexes that target specific domains of viral genes can inhibit viral replication (148). Techniques, such as RNA interference technology, have strong applications in the development of transgenic poultry that is resistant to microbial infections. RNA interference is the method of choice, where RNA molecules inhibit gene expression by targeting specific mRNA. Similarly, a lentiviral vector containing influenza-specific RNA hairpin rendered the cells refractory to viral infection and inhibited influenza virus replication in mice (149). These results provide evidence and scope for the development of pathogen-resistant poultry flocks *via* the transgenic expression of gene-specific RNA. In an earlier study, a recombinant plasmid with synthetic RNA duplex gene was constructed and transferred into Madin-Darby Canine Kidney cells. The study revealed that the transfected cell lines were resistant to the avian influenza virus (150). This landmark experiment provided the breakthrough for transgenic chicken development and resistance to influenza virus. RNA-Sequencing is another advanced technique that reveals the poultry genome responses to different stresses and diseases. The development of a disease-resistant chick through traditional breeding is a difficult and labor-intensive task, while the use of gene-editing technology and production traits is time-saving and profitable (151). With the development of next-generation sequencing technology, interest in whole-genome sequencing as an alternative to SNP chips for genotyping has increased as it allows the capture of a wide range of variations. For instance, a genome-wide association study and quantitative trait loci mapping identified candidate genes for egg production in ducks (152). These tools would help in the editing of the chicken genome and fulfill the dire need for disease-resistance breeds in poultry.

## Applications of chicken-genomics in biomedical research

Chickens are widely used in developmental research owing to their easy rearing, fecundity, growth rates, and genetic variations, thereby advancing the field of biomedical research. The chicken model has been used to evaluate cancer

metastasis, test chemotherapy agents, tissue morphogenesis, and angiogenesis, and perform toxicology studies. The egg is an important source of protein and contains phosvitin, which protects against oxidative stress-induced DNA damage in human leukocytes (153), and ovotransferrin, which is used as growth inhibitor for cancer cell lines (154). Avian-derived cell lines are used for viral culture and are helpful in vaccine and recombinant protein production (155). Chicken has also been used as a xenotransplantation model for human stem cells (156), human multiple myeloma xenograft (157), and the production of human antibodies (158). The Omni Chicken by Ligand Pharmaceuticals Inc. is a worldwide unique platform used to produce human monoclonal antibodies from chickens (159). Oishi et al. (141) integrated human interferon beta (hIFN- $\beta$ ) into the chicken ovalbumin locus and produced hIFN- $\beta$  in egg white. Notably, antibodies produced from humanized chickens and antibodies produced in chicken eggs represent significant industrial applications. Accordingly, chicken is an attractive developmental model for biomedical research.

## Conclusion

This review summarized the disease-resistance genes in poultry and provided an outlook of advanced technologies that can be used to engineer disease-resistance characteristics in poultry. The poultry industry is one of the fastest growing sectors of livestock for meat and egg production; however, this industry is threatened by different pathogens, which lead to substantial economic losses. Vaccination, antibiotics, culling, and disease management techniques are frequently employed in flocks to control disease outbreak; however, the success rate is nominal. Genetic resistance is a promising alternative method to augment prophylactic measures. Genetic resistance can be acquired through genetic breeding and genetic modification. Breeding chickens with disease-resistant strains can increase flock resistance; however, the genome modification process can underpin a characteristic of interest and assimilate into offspring to improve immune responses. Currently, genome editing technologies are driving desirable phenotypic traits, as genetic modifications are meeting enhanced production goals in the poultry industry, and engineering elite chicken for breeders. Further studies are required to effectively determine the roles of candidate genes in generating an ideal disease-resistant chicken.

## Future prospective

Next-generation sequencing of chicken-genome and pathogens helps in the understanding of host-pathogen interactions, natural variations, and the discovery of new QTLs that may be associated with disease-resistance and susceptibility traits in poultry. The use of lentiviral vectors is very efficient for gene delivery in animals and poultry compared

to homologous recombination of embryonic and somatic cells. Other alternatives for embryonic stem cells include RNAi and ZFNs technologies, which may be used for gene targeting and disruption in animals. The amplified genomic information of poultry and the advent of more sophisticated transgenic tools would result in resistance against pathogens. By investigating the genomics of chickens, new genes with divergent characteristics may lead to enhanced chicken yield. The use of other bird species with similar and unique characteristics will also advance avian research.

## Author contributions

HG, IK, and YL contributed to conceptualization. GH contributed to the methodology. IK and SR contributed to software. YL and HG contributed to validation and original draft. NK and SR contributed to investigation. YL contributed to resources and funding acquisition. GH, IK, HW, and NK contributed to the final draft and editing. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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