In silico analysis of candidate genes associated with humoral innate immune response in chicken

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Abstract

Background: Production and function of natural antibodies (NAbs) constitutes an important mechanism of the humoral innate immunity in vertebrates. The level of NAbs in chicken is heritable and the genetic background has been partly investigated. However, to date the genetic determination of humoral innate immune response in avian species has not been fully described. The goal of this study was to propose a new set of candidate genes with a potential effect on the NAb phenotype for further SNP association study.

Methods: In silico analysis of positional and functional candidate genes covered 14 QTL regions associated with LPS, LTA & KLH NAbs and located on six chromosomes: GGA5, GGA6, GGA9, GGA14, GGA18 and GGAZ. The function of the genes was subsequently determined based on the NCBI, KEGG, Gene Ontology and InnateDB databases.

Results: As a result, the core panel of 38 genes participating in metabolic pathways of innate immune response was proposed. Most of them were assigned to chromosomes: GGA14, GGA5, GGA6 and GGAZ (13, 9, 8 and 5 genes, respectively). These candidate genes encode proteins predicted to play a role in (i) proliferation, differentiation and function of B lymphocytes; (ii) TLR signalling pathway, and (iii) MAP signalling cascade.

Conclusions: Proposed set of candidate genes is recommended to be included in the follow-up studies to model genetic networks of innate humoral immune response in chicken.

Background

Humoral innate immunity in vertebrates that establishes the first barrier against pathogens consists of two basic mechanisms – natural antibodies (NAbs) and complement system. Expanding the knowledge on this field of avian immunology might be of help to overcome the difficulties in poultry industry, struggling constantly with diseases outbreaks eg. Avian Influenza [1]. In chicken, the level of NAbs proved to be heritable [2]. However, the genetic determination of NAbs is not fully described as it lacks information on which genes can be considered as the regulators in the complicated network of NAbs creation and function. This study contributes to the discovery of genetic determination of humoral innate immunity as it lists the proposed positional and functional candidate genes that have the putative impact on the NAb phenotype.

Methods

Chromosomal regions for in silico candidate gene analysis were initially selected based on the location of the QTL associated with the NAb titres directed against LPS (lipopolysaccharide), LTA (lipoteichoic acid) and KLH (keyhole limpet hemocyanine) antigens in chicken. This step was performed based on results from two independent studies, i.e.

• Study 1 – LPS and LTA NAb QTL detection study [3];
• Study 2 – LPS and LTA NAb QTL validation study; KLH NAb detection study (data not published).

Study 2 was carried out within a new chicken reference population, set-up as a F₂ cross between
Table 1 Positional and functional candidate genes associated with innate humoral immune response

| Symbol | ID     | Name                                      | Ch | Metabolic Pathway | Gene Function                                      |
|--------|--------|-------------------------------------------|----|-------------------|---------------------------------------------------|
| BLNK   | 395733 | B cell linker                             | 6  | BCR               | B-cell development                                 |
| CARD11 | 416476 | caspase recruitment domain family, member 11 | 14 | BCR, TCR, NFκB    | NFκB activation                                    |
| CASP7  | 423901 | caspase 7, apoptosis-related cysteine peptidase | 6  | BCR, TNFα         | Apoptosis                                          |
| CAT    | 423600 | Catalase                                  | 5  | NFκB              | Regulation of NFκB activity                        |
| CD59   | 423148 | CD59 molecule, complement regulatory protein | 5  | T cells           | T cell activation, complement system inhibition     |
| CD7    | 417346 | T-cell antigen CD7 precursor              | 18 | T cells           | T cell activation, T and B cell interaction, component of mature T cells |
| CD82   | 423172 | CD82 molecule                             | 5  | NFκB, p53         | Binding of proteins in cell membrane               |
| CIITA  | 427676 | class II, major histocompatibility complex, transactivator | 14 | TLR, MHC         | LRR binding, MHCII transcription activation       |
| CXCL12 | 395180 | chemokine (C-X-C motif) ligand 12         | 6  | IL               | Leukocyte activation, T cell proliferation, chemotaxis |
| FADD   | 423146 | FAS (TNFRSF6)-associated via death domain | 5  | NFκB              | Apoptosis, NFκB cascade activation, early development of T cells |
| FAS    | 395274 | TNF receptor superfamily, member 6        | 6  | TNFα, Fas, B and T cells | Ig production, immune response with (B cells) Homeostasis between B T cells |
| FGF10  | 395432 | fibroblast growth factor 10               | 2  | MAPK              | TLR activation, inflammatory cytokine secretion (with APC) |
| FGF8   | 396313 | fibroblast growth factor B                | 6  | MAPK              | MAPK cascade activation                            |
| FOS    | 396512 | v-fos FBJ murine osteosarcoma viral oncoprotein, transactivator | 5  | TLR, BCR, TCR, MAPK, JNK, IL | Synthesis of AP-1 transcription factor |
| GSS6F  | 771906 | immunoglobulin superfamily, mem. 6        | 14 | B and T cells     | Membrane receptor of T and B cells                 |
| IL20RB | 768437 | interleukin 20 receptor beta              | 14 | Jak-STAT, IL      | T and B cells proliferation and differentiation     |
| IL21R  | 416586 | interleukin 21 receptor                   | 14 | Jak-STAT, IL      | T and B cells proliferation and differentiation     |
| IL31RA | 427140 | interleukin 31 receptor A                 | 14 | MAPK, Jak-STAT, IL | MAPKKK cascade, cytokine and chemokine signal transduction, monocyte and macrophage differentiation |
| IL4R   | 416585 | interleukin 4 receptor                    | 14 | T cells, IL       | Th2 lymphocyte differentiation, cytokine receptor   |
| IL6ST  | 395684 | interleukin 6 signal transducer           | 14 | IL                | Fragment of cytokine receptor complex              |
| IL9R   | 416587 | interleukin 9 receptor                    | 14 | Jak-STAT, IL      | Jak and STAT activation, cytokine receptor         |
| JAK2   | 374199 | Janus kinase 2                           | 14 | Jak-STAT, IL      | Cytokine signalling                                |
| LITAF  | 374125 | lipopolysaccharide induced TNF factor      | 14 | TNFα              | TNFα expression                                   |
| MAPK2K3| 416496 | Mitogen activated protein kinase 3        | 14 | MAPK, TLR, JNK, Fc, p38, TNFα, Jak-STAT, TRAIL | MAPKKK cascade                                   |
| MAPK2K4| 417312 | Mitogen activated protein kinase 4        | 18 | MAPK, TLR, Fas, JNK, Fc, TCR, Jak-STAT, TRAIL | MAP kinase activation, in response to different stimuli, survival signal for T cells |
| MAPK3K1| 427144 | mitogen activated protein kinase 1        | 14 | MAPK, TLR, Fas, JNK, Fc, p38, NFκB, TCR, BCR, INFα, TRAIL, TNFα | Integration of enzyme fosforylation in response to different factors |
| MAPK3K13| 424876| mitogen-activated protein kinase kinase 13 | 9  | MAPK, JNK         | Activation of different MAP kinases                |
| MAPK8IP3| 426986| mitogen-activated protein kinase 8        | 14 | MAPK, JNK         | MAPK and JNK integration                           |
| NFκBIA | 396093 | nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha | 5  | TLR, BCR, TCR, NFκB | NFκB Inhibitor                                   |
| PDCD4  | 374191 | programmed cell death 4 (neoplastic transformation inhibition) | 6  | JNK               | Negative JNK regulation, expression of the gene under control of T cells |
| RAG2   | 423165 | recombination activating gene 2           | 5  | B and T cells     | B and T cells differentiation, gene conversion in Ig |
| RBP4   | 396166 | retinol binding protein 4, plasma         | 6  | B cells           | Activation of Ig secretion                         |
| SOCS1  | 416630 | suppressor of cytokine signalling 1       | 14 | Jak-STAT, IL      | Inhibition of cytokine secretion & Jak-STAT cascade |
| TCF7L2 | 395508 | Transcription factor 7-like 2             | 6  | WNT               | WNT signalling                                   |
commercially selected breed (WL, White Leghorn) and a Polish, unselected native chicken breed (GP, Green-legged Partridge-like). For a candidate gene analysis reported here, the chromosomal regions of interest included QTL associated with LPS and LTA NAb titres that had been detected in study 1 and consecutively validated in study 2 as well as QTL associated with KLH NAb titres that had been detected in study 2. These QTL were located in the following chicken chromosomes: GGA5, GGA6, GGA9, GGA14, GGA18 and GGAZ. The regions of interest were designated based on the physical location of the microsatellite markers flanking the QTLs. The list of candidate genes within the QTL regions was prepared based on NCBI database [4], and gene function was assessed with KEGG [5], InnateDB [6] and Gene Ontology [7]. The genes meeting both the criteria, i.e. location within the QTL regions & function in innate immunity (including signalling pathways and B cell function) were listed in a panel of the candidate genes associated with humoral innate immune response.

Results
The results of the candidate gene analysis are presented in Table 1. Briefly, based on previously described criteria, the total number of 38 candidate genes located on six chromosomes was selected. The highest number of the candidate genes (13 genes) was located on GGA14; 9 genes were found on GGA5 and 8 – on GGA6. Lower number of candidate genes were found on GGAZ (5 genes), on GGA18 (2 genes) and on the GGA9 (1 gene).

It can be summarized that these candidate genes encode proteins predicted to play a role in:

(i) Proliferation, differentiation and function of B lymphocytes, e.g. CXCL12, BLNK, IL21R, RBP4, CD59, TNFRSF13B;

(ii) TLR signalling pathway, e.g. TRAF6, FADD, NfkbIA, CARD11, FAS, FGF8, TGFβ, IL31RA;

(iii) MAP signalling cascade, e.g. MAP2K3, MAP2K4, MAP3K1, MAP3K13, MAPK8IP3.

Discussion
Immune response is a complicated process; encoded by multiple genes organized within the frames of functional networks rather than pathways and regulated by many interactions. However, prior to modelling the most probable genetic network, the information is needed on the genes that can be taken into account and their physiological function.

As mentioned above, the function of the proposed set of candidate genes was associated with three groups of cellular and physiological processes that can hypothetically affect innate humoral immune response in chicken. Briefly, production of antibodies, including NAbs takes place in B cells, stimulated by Th2 cytokines. Therefore, both B and T cells function is a crucial element in antibody release. CXCL12 gene is responsible for B cells proliferation [8]. CXCL12-/- knockout mice produced drastically reduced number of B cells and died during the perinatal period [9]. In turn, BLNK gene affects B cell development, which was completely inhibited in BLNK-/- knockout mouse [10]. Finally, IL21R and RBP4 genes are responsible for maintenance of mature B cells function. Knocked out mice (both IL21R-/- and RBP4-/-) expressed impaired production of antibodies [11,12].

TLR signalling pathway is triggered when molecular patterns (such as LPS or LTA) are recognized. Some of the proposed candidate genes are involved in TLR pathway, just to mention TRAF6 and FADD, as well as genes affecting NfkbB expression and function, such as NfkbB, CARD11, TNFRSF13B and FAS[13-15]. Furthermore, the analysis in silico pointed out a number of genes that activate MAPK cascade, a key signalling pathway initiated by TLR, for example FGF8, TGFβ3 and IL31RA[14]. Additionally, the candidate gene set includes such genes as MAP2K3, MAPK8IP3, MAP3K13, MAP2K4 and MAP3K1, which are the members of MAPK signal transduction pathway [15].

Conclusions
Chicken immune response is one of the major areas recently studied in life science research related to livestock. So far, different approaches have been applied to dissect the genetic bases of avian health traits. Rapid development of technology supporting high-throughput genomic studies provided an excellent tool for fast and efficient genotyping. Still, the accurate gene selection can pose a problem. Therefore, the additional criteria, like validated QTL regions may be of assistance to list the proper genes that can be further on evaluated and
contribute to genetic network modelling of humoral immune response in chicken. For this reason we proposed a panel of candidate genes related to the level of LPS, LTA & KLH NAbs in chicken.

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Authors’ contributions
AS performed the analysis and drafted the manuscript; AW made substantial contributions to acquisition of data; MB participated in the design of the study; MS conceived of the study, participated in its design and coordination and helped to draft the manuscript.

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

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