



RESEARCH ARTICLE

Bioinformatics Analysis of JAZF1 Gene in Broilers with Ascites Syndrome

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Pulmonary vascular remodeling (PVR) is the main characteristic lesion of ascites syndrome (AS) in broilers. JAZF1 plays an important role in PVR, but there is no study on its protein function and structure. In this study, the physical and chemical properties, hydrophilicity/hydrophobicity and transmembrane domain, phosphorylation site and glycosylation site, subcellular localization and signal peptide, secondary and tertiary structure, antigen peptide and conserved domain and phylogenetic relationship of JAZF1 protein were predicted online by bioinformatics tools. The results showed that the number of amino acids of JAZF1 was 243aa, the theoretical isoelectric point was 8.63, the instability index was 58.1, and the average coefficient of hydrophilicity was -0.674. It was found to be a hydrophilic protein having 35 phosphorylation sites and a N-glycosylation site with no transmembrane domain. The protein is expressed in the nucleus, there is no signal peptide distribution in the whole sequence and the secondary structure is mainly composed of random coil and α -helix. There were 7 B cell epitopes, 7 conserved domains and compared with other birds, JAZF1 is 95.61% similar. In summary, from the analysis we came to conclude that the amino acid sequence 64-80aa, 91-108aa, 136-151aa and 179-187aa can be selected as antigen sites and among which 136-151aa may be the best. This study lays a good foundation for follow-up experiments, which then provides powerful conditions for pathological detection of pulmonary vascular remodeling and gene drug therapy of ascites syndrome in broilers.

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INTRODUCTION

Ascites syndrome, also known as broiler pulmonary hypertension syndrome (PHS), is a comprehensive metabolic disease (Ruan, 2019). The distribution of the disease is very wide, and the incidence of the disease has been increasing in recent years, which has brought huge economic losses to the global poultry industry (Wang *et al.*, 2018). Due to the relative hypoxia caused by excessive growth or other factors in broilers, a large number of oxygen free radicals produced under the oxidation barrier of ATP have a peroxidation reaction with lipids on the cell membrane, resulting in vascular endothelial injury and eventually lead to PVR (Wideman

et al., 2013). Many factors have been found to play a key role in the occurrence of PVR, such as forkhead box class O transcription factor 1 (FoxO1), The N-methyl-d-aspartate receptor (NMDAR), extracellular superoxide dismutase (SOD3) and the juxtaposed with another zinc finger gene 1 (JAZF1) (Deng *et al.*, 2017; Dumas *et al.*, 2018; Zelko *et al.*, 2018). Among them, JAZF1 is also known as TAK1-interacting protein27 (TIP27) or zinc finger protein 802 (ZNF802) gene, and it is widely distributed in various tissues, previous literature has asserted that it is involved not only in gluconeogenesis, insulin sensitivity, cell differentiation, lipid metabolism and inflammation (Huang *et al.*, 2019), but also in the negative regulation of vascular smooth muscle cells and

cardiomyocyte cycle and in the development of cardiovascular system (Xie *et al.*, 2009). Moreover, studies have verified that JAZF1-overexpressing (JAZF1-Tg) mice showed cardiac defects, such as high blood pressure, electrocardiogram abnormalities, apoptosis of cardiomyocytes, ventricular non-compaction, and mitochondrial defects, and the expression levels of pro-apoptotic genes were elevated in the hearts (Bae *et al.*, 2011). Furthermore, a study demonstrated that the over-expression of JAZF1 alleviates myocardial I/R injury by enhancing proliferation and angiogenesis of cardiac microvascular endothelial cells (CMECs) and in turn inhibiting apoptosis of CMECs via the activation of the Akt signaling pathway (Shang *et al.*, 2019). Based on the aforementioned content, it is therefore clear that the JAZF1 gene plays an important role in PVR. However, there are still few in-depth studies on the relationship between JAZF1 gene and the occurrence and development of AS in broilers. As a discipline developed on the basis of biology and computer science and mathematics, bioinformatics plays a more and more important role in the research of genome and proteome (Hagen 2000, Sheng 2015). Martínez-Rodrigo *et al.* (2020) used bioinformatics tools to select promising Multiepitope Peptides (HisDTC and AK) from the polyprotein encoded in the HisAK70 DNA, which have been shown to be able to induce the control of visceral leishmaniasis in mice.

In this study, we used bioinformatics methods to analyze the physical and chemical properties, transmembrane domain, signal peptide, secondary and tertiary structure, antigen peptide and conserved domain, etc. of JAZF1 protein, so as to further explore the protein function of JAZF1, to provide favorable reference conditions for the protein expression and antibody preparation of JAZF1, and to lay a foundation for detecting the abnormal expression of JAZF1 gene in the occurrence and development of ascites syndrome in broilers.

MATERIALS AND METHODS

Data sources: Data information of JAZF1 gene was obtained from the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/>): *Gallus gallus* (chicken), NC_006089.5, Gene ID: 420633. The amino acid sequence is:

MTGIAAAASFF	SNACRFGGCG	LHFPTLAEI
EHIEDNHIDT	DPRVLEKQEL	QQPTYVALSY
INRFMTDAAR	REQESLKKKI	QPKLSLTLS
TVSRGNVSTP	PRHSSGSLTP	PVTPPITPSS
SFRSSTPTGS	EYDEEEVDYE	ESDSDESWTT
ESAISSEAIL	SSMCMNGGDE	KPFACPVPGC
KKRYKNVNGI	KYHAKNGHRT	QIRVRKPFKC
RCGKSYKTAQ	GLRHHTINFH	PPVSAEIIRK MQQ.

Physical and chemical property: The amino acid sequence of JAZF1 was submitted to ExPASy server online tool ProtParam module (<https://web.expasy.org/protparam/>) in FASTA format to obtain its physicochemical properties, including the theory of amino acid composition, molecular weight, isoelectric point, instability index, half-life coefficient of amino acid, fat and other physical and chemical properties.

Hydrophobicity/hydrophilicity and transmembrane domain: Hydrophobicity and hydrophilicity analysis have important biological significance for predicting the secondary structure and functional domains of proteins. The amino acid sequence of JAZF1 was submitted to the online tool ExPASy server ProtScale module (<https://web.expasy.org/protscale/>) in FASTA format, and its hydrophilicity and hydrophobicity were analyzed by Kyte-Doolittle algorithm. The transmembrane domain of JAZF1 protein was further analyzed through TMpred (https://embnet.vital-it.ch/software/TMPRED_form.html).

Phosphorylation sites and Glycosylation sites: The amino acid sequence of JAZF1 protein was submitted to the online tools NetPhos and NetNGlyc of DTU Health Tech (<https://services.healthtech.dtu.dk/>) in FASTA format to obtain the phosphorylation sites and glycosylation sites.

Subcellular localization and signal peptide: The amino acid sequence of JAZF1 protein was submitted in FASTA format, the subcellular localization of JAZF1 protein was predicted by online software Predictprotein (<https://www.predictprotein.org/>). The presence and location of signal peptide cleavage sites in the amino acid sequences of JAZF1 protein was predicted by the online tool SignalP (<https://services.healthtech.dtu.dk/service.php?SignalP-5.0>) of DTU Health Tech.

Secondary structure and Tertiary structure: The secondary structure of JAZF1 was predicted by I-TASSER (<https://zhanglab.ccmb.med.umich.edu/I-TASSER>). Then the homology modeling method was used through the SWISS-MODEL (<https://swissmodel.expasy.org>) to predict the tertiary structure, the tertiary structure was obtained using folding recognition method based on the evaluation the I-TASSER.

Antigen peptide and conserved domain: BepiPred 1.0 Server (<http://www.cbs.dtu.dk/services/BepiPred-1.0>) was used to predict the antigenic epitopes of JAZF1, we searched JAZF1 on NCBI to download JAZF1 gene sequences of 10 different species, including chickens, and then compared them with MEME (<http://meme-suite.org/tools/meme>) to get conservative domain analysis.

Overall analysis and phylogenetic analysis: DNASTar was used to carry out the whole analysis of secondary structure, hydrophilicity, flexible regions, antigenic index and surface probability plot etc. After the sequence alignment of JAZF1 on NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), the phylogenetic tree was constructed by MEGA in Neighbor-Joining method.

RESULTS

Physical and chemical property: The JAZF1 protein had 243 amino acids, molecular weight: 27063.48, molecular formula $C_{1176}H_{1859}N_{341}O_{369}S_{12}$, theoretical isoelectric point was 8.63. Among them, there were 27 amino acid residues with negative charge (Asp+Glu) and 31 with positive charge (Arg+Lys), which accounted for 11.1 and 12.8% of the total amino acids, respectively. The instability index was 58.1, and this classifies the protein as unstable,

instability index less than 40 means stable, otherwise means unstable. The half-life was 30 hours in vitro in mammalian reticular erythrocytes, more than 20 hours in yeast in vivo, and more than 10 hours in *Escherichia coli* in vivo. The hydrophobic nature of the protein was predicted by Kyte & Doolittle arithmetic and was -0.674 in average and the aliphatic index was 61.03, which indicated that JAZF1 may be a hydrophilic and lipid soluble protein.

Hydrophobicity/hydrophilicity and transmembrane domain: As shown in Fig. 1a, the highest value of the sixth Ala of the polypeptide is 1.511, and the highest value of the 142nd Ser is -2.656. This means that the sixth Ala has the highest hydrophobicity and the 142nd Ser has the highest hydrophilicity. Overall, hydrophilic amino acids are evenly distributed throughout the peptide chain, that is, the entire peptide chain shows hydrophilicity. The protein can therefore be called a hydrophilic amino acid, which is consistent with the total average hydrophobicity index (-0.674) predicted by ProtParam.

In addition, there was no obvious hydrophobic region in JAZF1, indicating that there was no transmembrane region. As shown in Fig. 1b, TMpred predicted that there was one possible transmembrane region of JAZF1 protein at sequence 1-23 (circled in red), with a score of about 685, in which the inside to outside center was 14 and the outside to inside center was 9.

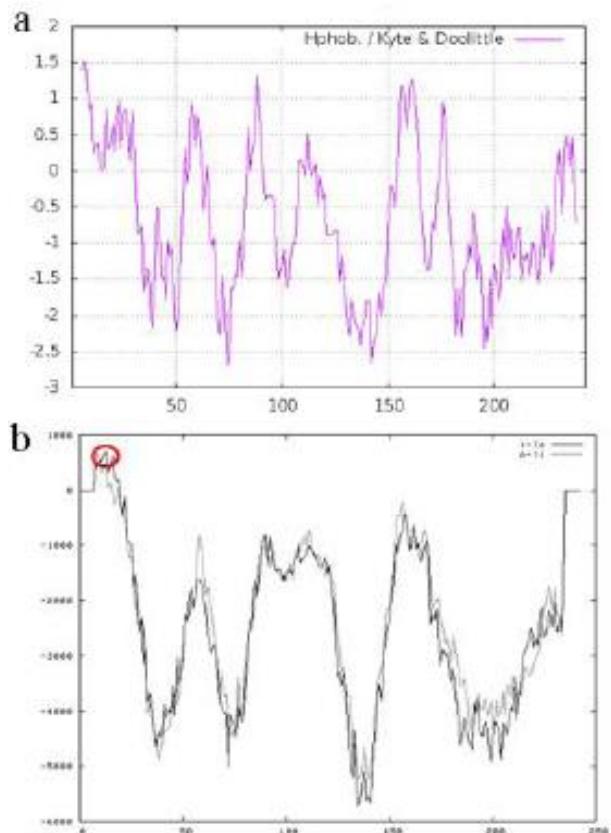


Fig. 1: a: hydrophilicity prediction, positive scores present hydrophobicity and negative scores indicate hydrophilicity, the higher the absolute value, the higher the degree of hydrophilicity. b: transmembrane domain. The solid line indicates from inside to outside, and the dashed line to the contrary, the y axis is score, only scores above 500 are considered significant.

Phosphorylation sites and Glycosylation sites: Phosphorylation is one of the important characteristics of a protein as a gene expression regulatory protein and is closely related to the function of the protein. For JAZF1 protein, a total of 35 phosphorylation sites were assigned including 21 serine phosphorylation sites, i.e., S8, S59, S75, S85, S90, S93, S104, S105, S107, S120, S121, S124, S125, S130, S142, S144, S147, S152, S155, S215 and S234, 9 threonine phosphorylation sites, i.e., T25, T40, T91, T99, T109, T113, T117, T126 and T128; and 5 tyrosine phosphorylation sites, i.e., Y55, Y132, Y139, Y184 and Y216. As shown in Fig. 2a, serine had the most phosphorylation sites, and the predicted score of S130 was the highest, while the predicted score of tyrosine and threonine phosphorylation sites were Y139 (0.990) and T126 (0.855), respectively. As shown in Fig. 2b, there is a n-glycosylation modification site at position 96 (0.7048), NVST, it suggests that this sequence may not contain a signal peptide, since proteins without signal peptides are unlikely to be exposed to the N-glycosylation machinery and thus may not be glycosylated (in vivo) even though they contain potential motifs.

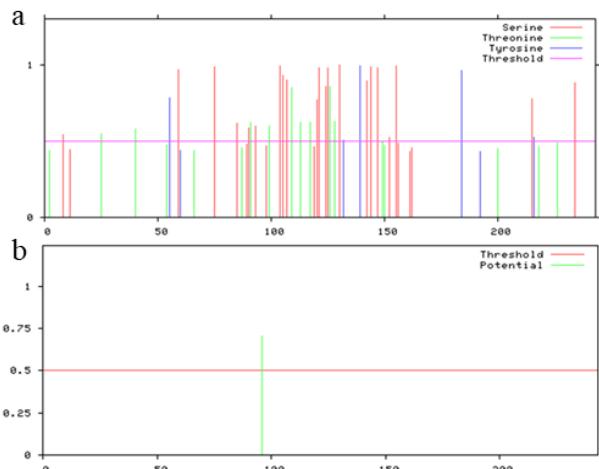


Fig. 2: a: Phosphorylation sites. The red lines represent the serine phosphorylation site, the green lines denote the threonine phosphorylation site, the blue lines denote the tyrosine phosphorylation site, and the purple line represent the threshold, only phosphorylation sites with scores above the threshold of 0.500 were assigned. b: glycosylation site prediction. The green line represents the potential glycosylation site and the red line represents the threshold.

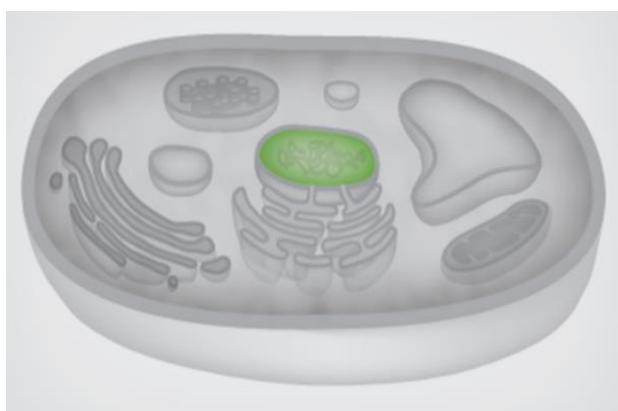


Fig. 3: Subcellular localization prediction. This viewer shows a cell schematic with the predicted subcellular localization compartment highlighted in green.

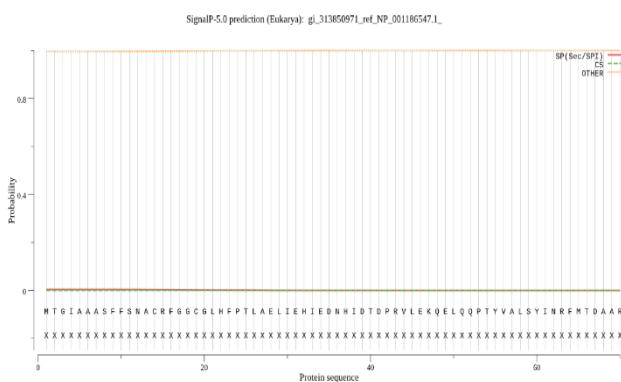


Fig. 4: Signal peptide prediction. The red line represents the signal peptide, the green line denotes the cleavage site, and the orange line represent other.

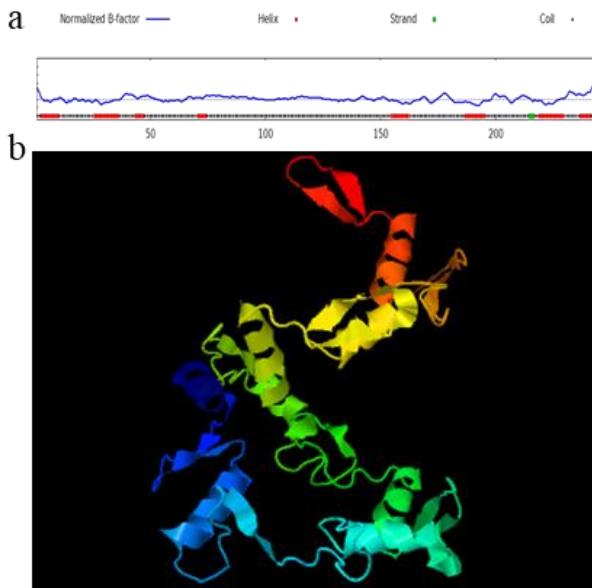


Fig. 5: a: Secondary structure prediction. The red, green and black represent the alpha helix, the extended strand and the coiled regions, respectively. The blue line denote the normalized B-factor, which refers to the normalization of the original b-factor value based on the z-score, the negative value indicates that the residue is relatively stable in the structure. b:Tertiary structure prediction.

Subcellular localization and signal peptide: The prediction of subcellular localization of JAZF1 protein showed in Fig.3 that the protein was most likely located in the nucleus, with a score of 10. The cleavage site of the signal peptide is predicted by the maximum value (Y) of the cleavage point value, and the average value (S) of the signal peptide is used to determine whether it is a secreted protein. If the average value of S is greater than 0.5, it indicates that the protein has a signal peptide, which may be a secretory protein. The results based on the neural network algorithm (Fig. 4) show that the protein has only 0.0037 likelihood of having sec signal peptide, while 0.9963 likelihood of no signal peptide, indicating that the protein may not be a secretory protein, this is consistent with the above inferential results of glycosylation.

Secondary structure and Tertiary structure: The result showed that JAZF1 mostly had coiled regions, with 7 alpha helix regions of 181 amino acids sites, 1 extended strand region of 2 sites and many random coil regions of 60 sites (Fig. 5a).

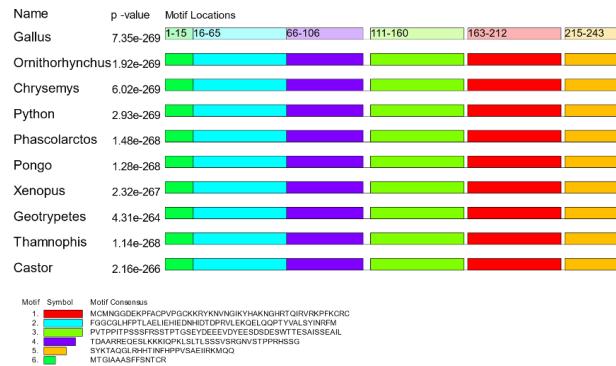


Fig. 6: Conserved domain area prediction. The length and order of the boxes with different colors represents the actual size and position of the conserved motif in the JAZF1 protein sequence.

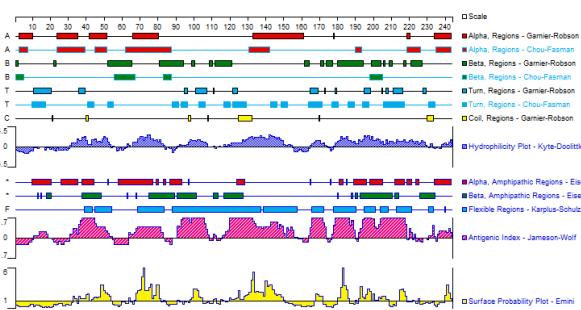


Fig. 7: Overall analysis of JAZF1 protein. From top to bottom are: A: alpha regions; B: beta regions; T: turn regions; C: coil regions. hydrophobicity plot; alpha, beta amphipathic regions and flexible regions; antigenic index; surface probability.

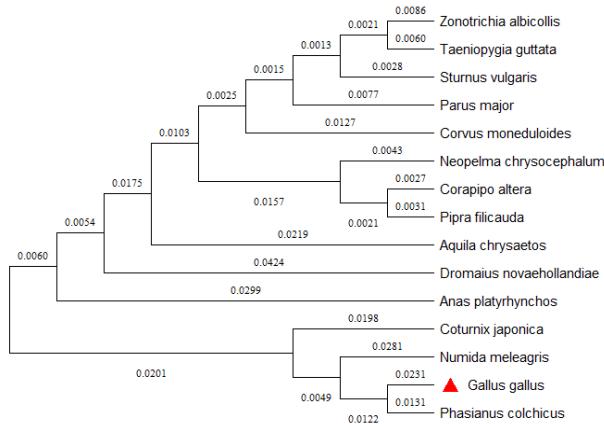


Fig. 8: Construction of JAZF1 gene phylogenetic tree. The number on the branch indicates the length of the branch. The red triangle marks the species in this study: *gallus gallus*.

Through the SWISS-MODEL online website, the homology modeling method was used to predict the tertiary structure of JAZF1 protein. It was found that the template homology was 16.13% and with the GMQE of 0.43, the QMEAN of -5.36, which means the result was unreliable. Then the folding recognition method was used through the I-TASSER online website, and the result is as shown in the Fig. 5b, with the C-score of -0.46, estimated TM-score of 0.65 and estimated RMSD of 6.7 which means it reliable. As can be seen from the Fig. 7, spiral regions were predicted by JAZF1 tertiary structure, which is consistent with the prediction results of the secondary structure above.

Antigen peptide and conserved domain: There were 7 B cell epitopes (critical value 0.35), they were: 36-45aa, 49-50aa, 69-79aa, 92-155aa, 166-185aa, 195-199aa and 215-219aa. As shown in Fig. 6, conservative regions of JAZF1 protein from 10 different species were analyzed, and seven conserved domains were obtained: 1-15aa, 16-65aa, 66-106aa, 111-160aa, 163-212aa, 215-243aa.

Overall analysis and phylogenetic analysis: The Protean software of DNAstar can be used for the prediction of protein secondary structure and antigen epitope (Fig.7). As shown in the secondary structure prediction results, the helix regions were consistent with the prediction results of I-TASSER, while the prediction results of coli were different. Hydrophilic prediction result is consistent with the above for both of them are based on Hphob/Kyte & Doolittle algorithm. Solvent accessibility is an important means to describe the hydrophobicity of proteins, as can be seen from the figure, its accessibility is consistent with the hydrophilic distribution, so it can be considered that the hydrophilic prediction of JAZF1 protein has high reliability. JAZF1 predicted protein antigen areas are mainly distributed in 11-18aa, 31-54aa, 64-82aa, 91-108aa, 116-150aa, 164-173aa, 177-189aa and 236-243aa. As shown in the figure, the JAZF1 protein has many numbers of flexible regions, the largest of which is 88-137aa, and only one site is separated from the next region 139-157aa.

A phylogenetic analysis of 15 different birds' JAZF1 was constructed by the neighbor-joining method using MEGA-X software. As shown in Fig.8, obviously, the phylogenetic tree is grouped in a family-specific way. The JAZF1 sequence of *Gallus gallus* and other birds of the same family has the highest similarity, with the pheasant, reaching 95.61%, and the sequence similarity with other birds is more than 85%.

DISCUSSION

Ascites syndrome in broilers is a global problem that restricts the development of broiler breeding industry because of many inducements and no specific treatment. Previous studies have shown that the expression of JAZF1 is related to cardiovascular endothelial cell proliferation and angiogenesis (Shang *et al.*, 2019). Bioinformatics plays an important role in gene recognition, protein function prediction, protein physiological range determination, protein advanced structure prediction and so on (Whisstock *et al.*, 2003, Calderón-González *et al.*, 2016). In this experiment, basic information of JAZF1 was obtained through bioinformatics analysis, so as to carry out follow-up research on it.

The prediction of antigenic epitopes is generally based on the amino acid sequence, three-dimensional structure and protein hydrophilicity and flexibility of antigens (Ma *et al.*, 2016). Most of the antigenic epitope regions have the characteristics of easy deformation, strong plasticity, high hydrophilicity, high surface accessibility and no formation of alpha helix (Liu *et al.*, 2017). Random coil has a loose structure, easy to deform, and has a large degree of freedom, it is usually located on the surface of protein molecules, which is conducive for chimerism with antibodies and is likely to be an antigen

epitope (Zhao *et al.*, 2018). The proteins have a transmembrane region in the cell membrane, which is inaccessible to the antibody, so this part is often removed when designing the antigen polypeptide chain (Han *et al.*, 2017). The signal peptide refers to the N-terminal amino acid sequence of a newly synthesized polypeptide chain that is used to guide the transmembrane transfer (localization) of proteins (sometimes not necessarily at the N-terminal) (Meyer *et al.*, 2018). Combined with the predicted results, the secondary structure prediction analysis of JAZF1 showed that there were 181 amino acids involved in the formation of irregular crimping, accounting for 74.49%. The instability index of JAZF1 was 58.1, the average hydrophobic (the total average hydrophobicity index) was -0.674, had no obvious transmembrane region and no signal peptide, that is, JAZF1 may belong to unstable hydrophilic non-secretory protein. The instability and hydrophilicity of the protein indicate that it can be used for antibody preparation (Li *et al.*, 2020).

GMQE and QMEAN4 are the scoring criteria of SWISS-MODEL: GMQE credibility range is 0-1, the greater the value is, the better the quality is. The range of QMEAN4 is -4-0, and the closer to 0, the better the matching degree between the tested protein and the template protein (Bordoli *et al.*, 2009). And if the consistency is more than 30%, the result is reliable (Zhang *et al.*, 2005). For the sequences with no homology template or low homology, the homology modeling method cannot be used to predict the protein structure (Moult *et al.*, 2018). SWISS-MODEL results indicated that the GMQE was 0.43, the QMEAN was -5.36 and the template homology was 16.13%, which means the result is unreliable. At this time, the folding recognition method is used, and its main principle is to find the folding mode at the lowest point of energy based on energy calculation (Zhang, 2008; Yang *et al.*, 2015). Finally, a credible tertiary structure is obtained and the predicted tertiary structure of JAZF1 corresponds to the prediction of secondary structure.

Studies have shown that protein phosphorylation and glycosylation play an important role in cellular immunity, protein translation regulation, substance transport and signal pathways (Li *et al.*, 2009; Nakayasu *et al.*, 2009; Yang *et al.*, 2015). If the antibody is used to identify the target protein's post-translational modification sites (such as phosphorylation and glycosylation sites), the antigenic peptide is designed to extend slightly at both ends, while if the antibody is used to recognize the pre-translational protein, the corresponding site needs to be removed (Anderson *et al.*, 2018). JAZF1 protein has been predicted to have multiple phosphorylation sites and a potential N-glycosylation site, which can be used as selection regions for antibody polypeptides. The selected specific protein antibodies are often ineffective because of the variation of alleles in different species, so the selection of polypeptides usually requires strong conservatism (Liu *et al.*, 2017). Through phylogenetic tree analysis and conservative domain prediction with 10 species, we can know that the molecular evolution of JAZF1 is more conservative.

From the predicted results, it can be seen that JAZF1 has many regions with high hydrophilicity, strong

flexibility and multi-surface accessibility, that is, it is inferred that JAZF1 has a good structural basis for the formation of B cell surface antigens. Based on the above analysis, we infer that the following four regions may be selected as antigen sites: 64-80aa, 91-108aa, 136-151aa and 179-187aa, among which 136-151aa may be the best, but the actual antigenic effect still needs further experimental verification.

Conclusions: In this study, homologous modeling methods could not be used to predict the tertiary structure of JAZF1 due to its low homology, and reliable results could be obtained using folding recognition methods. Conservative domain prediction and phylogenetic tree results indicate that the evolution of JAZF1 is conservative. After comprehensive analysis, four candidate antigenic peptide regions of 4-80aa, 91-108aa, 136-151aa and 179-187aa were screened. This paper provides a direction for the study of antibody preparation and protein expression of this protein and lays a foundation for the study of the correlation between JAZF1 and ascites syndrome in broilers.

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Authors contribution: FPG and PL conceived and designed the project, analyzed the results and wrote the original draft. XQG, GL and LL carried out the study and collected important background information. SFC and ZXZ executed the experiment. XLH analyzed the data. VL revised manuscript. GLH and PL performed manuscript review. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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