January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Polymorphisms and associations of the *RACK1* genes with antibody response to Newcastle disease in KUB chickens

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Ifa Manzila, Puji Lestari, Tike Sartika, et al.

Polymorphisms and Associations of the *RACK1* Genes with Antibody Response to Newcastle Disease in KUB Chickens

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Abstract. *Receptor for Activated C Kinase 1* (*RACK1*) is a gene involved in many biological processes, such as proliferation, cell migration, apoptosis, and innate immune responses. This study aimed to identify *RACK1* gene polymorphisms and their associations with antibody response against Newcastle disease (ND) in *Kampung Unggul Baru* (KUB) chicken. Fifty KUB chickens were used in this study. Polymorphism of *RACK1* gene was identified by PCR analysis and sequencing methods, while ND antibody response was detected by HI test. The results showed that antibody responses were grouped into low (12 of chickens) and high (38 of chickens) categories. Sequencing analyses of *RACK1* gene determined double peaks at nucleotide position 1986 (SNP-1986) and 1993 (SNP-1993) indicating heterozygous genotype and a single peak as homozygous genotype. Both SNP-1986 and SNP-1993 were located in intron 4 of *RACK1* gene. Association analyses showed that two intronic *RACK1* polymorphisms were significantly associated with antibody response in KUB chickens to ND. These SNPs can be used as potential SNP markers for antibody response as parameters for ND resistance in KUB chicken.

INTRODUCTION

Newcastle disease (ND), caused by *Avian paramyxovirus* serotype 1 (APMV-1), is one of the most common and contagious chicken diseases in the world [1]. Since ND was firstly reported in Indonesia in 1926, the disease is prevalent worldwide and causes significant problems in poultry industries in many countries [2, 3]. Nowadays, ND was ranked as the fourth most important disease for poultry species, behind highly pathogenic avian influenza, infectious bronchitis, and lowly pathogenic avian influenza [1].

ND-infected chicken shows severe respiratory distress and nervous disorders signs, decreased egg quality and production, and haemorrhagic intestinal lesions [4]. According to Luo *et al.* [5], there is no effective treatment for ND. However, the use of prophylactic vaccines and maintenance of strict biosecurity measures can reduce the likelihood of outbreaks. However, vaccination is not an adequate control for smallholder farmers who raise local chicken, because of in addition to vaccine instability, it is costly, lack of cold storage, difficulty in correctly administering vaccines, and poor husbandry practices [3, 6, 7]. Hence, there is a need for alternative strategies to control ND. Selective breeding would be an effective complement to vaccination, if genetic variation in resistance and tolerance and/or immune response to ND exist in the population [8].

Previous studies have shown that genetic selection [9] and indirect selection on immune/antibody response traits may be the best strategy [10] to improve resistance of local chicken lines to ND. In addition, the selection for host antibody response can effectively improve disease resistance in chickens [11]. The antibody response is controlled

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by multiple genes, and selection for antibody response parameters may efficiently improve disease resistance [12, 13].

Numerous single-nucleotide polymorphism (SNP) and microsatellite markers, quantitative trait loci (QTLs), and genes associated with immune traits to ND have been reported in several chromosomal regions of chickens (*Gallus gallus*) [5, 12, 14, 15]. Two SNPs associated with antibody response, rs15354805 and rs15355555, are detected in intron 7 and upstream of roundabout, exon guidance receptor, homolog 2 (ROBO2) gene in chromosome 1 [5]. Zhang *et al.* [14] also found 9 SNPs within a narrow region spanning 6.4 kb to 253.4 kb in chromosome 16 containing five candidate genes (*IL4I1*, *CD1b*, *GNB2L1*, *TRIM27*, and *ZNF692*) associated with six immune traits (total serum IgY level, numbers of, and the ratio of heterophils and lymphocytes, and antibody responses against AIV) in Beijing-You chickens. *CD1B* (cluster of differentiation 1B) gene is one of the important genes in antibody response, which plays a role in the presentation of lipid antigens to T helper cells [16]. SNPs analysis showed that *CD1B* was polymorphic in different antibody response of IPB-D1 chickens to ND [17].

In the current study, we analyzed SNPs polymorphism of *Receptor for Activated C Kinase 1* (*RACK1*, syn. *GNB2L1*) gene in indigenous chicken *Kampung Unggul Baru* (KUB). To our knowledge, the SNPs polymorphism information in *RACK1* gene along with its association with ND antibody response in KUB chickens have never been reported. The RACK1 protein plays a pivotal role in a wide range of biological processes through interaction with many protein kinases, transcription factors, and receptors [18], such as protein kinase C [19], Src kinase [20], Janus kinases (JAKs) [21], Hypoxia Inducible Transcription Factor (Hif-1α) [22], signal transducer and activator of transcription (STATs) [23], androgen receptor [24], and NMDA receptor [25].

RACK1, also involved in regulation of the transcription factor NF-κB, plays a pivotal role in innate immune responses to a variety of stimuli [26]. In addition, *RACK1* plays an antiapoptotic role during infectious bursal disease (IBDV) infection via interaction with VDAC2 and VP5 [27]. Apoptosis is generally considered as an important defense mechanism of host response against microbial infection. However, the process of apoptosis occurring in IBDV-infected cells seems to be well controlled by the virus [28]. Interaction of RACK1 with VP5 and VDAC2 form a complex that regulates apoptosis in virus-infected cells, inhibiting apoptosis and enhancing viral replication [27]. The aim of this study was to identify SNPs for the antibody response to ND in KUB chickens. SNP primers obtained from open public database were used to genotyping KUB chickens which are routinely vaccinated.

MATERIALS AND METHODS

This research was carried out in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and Indonesian Research Center for Veterinary Sciences (IVETRI). A total of F_1 population of Indonesian indigenous chicken KUB were used as genetic materials in present study. Individual chickens consisting of 35 male and 67 female were subjected for phenotyping analyse of antibody responses. Then, 50 selected chickens with different antibody response were genotyped and phenotyped. Blood samples were collected from 70-day-old chickens which were maintained with ND vaccination using commercial vaccines in accordance with the instructions.

Haemagglutination Inhibition (HI) Assay

HI tests were used to measure antibody titres for ND. The HI tests were performed on sera in microwell plates according to the OIE Manual of Standard Diagnostic Tests [1]. Antigen from the ND genotype VII (Indonesia/GTT/11) was used in the ND HI test. In HI tests, 25 µl of 2-fold serially-diluted sera were incubated with 4 haemagglutinating units (HAU) of ND genotype VII antigen for 60 min at 37°C. For each well, 50 µl of 1% chicken red blood cell suspension in PBS was added and incubated for 40 min at room temperature. HI antibody titres were calculated as the highest serum dilution able to inhibit haemagglutination completely. The cut-off for a 'positive' titre was set at 2^3 [equivalent to $\log_2 3$ or inhibition at a serum dilution of 1/8] for ND, followed OIE guidelines [1]. Chickens with highest HI antibody titres were reared in an individual cage to generate F_2 population.

Genotyping

Genomic DNA was extracted from the whole blood chicken using DNA Blood Mini Kit (QIAGEN, USA). The DNA was quantified using NanoDrop and stored at -20°C before used. Primers designed from *RACK1* gene of *G. gallus* were used to amplify the sequences between exon 4 and exon 6 of *RACK1* gene of KUB chicken. The

detailed sequences of the primers were as followed, GNB2Li forward (5'-ATTCAGGTCCCACAGCATGG-3') and GNB2Li reverse (5'-GCAGCAACCCCATCATTGTC-3'). PCR was performed in a 20-µl mixture containing 2 µl chicken genomic DNA (10 ng/µl), 10 µl KAPA2G Fast ReadyMix (Kapa Biosystem, USA), 2 µl of each of forward and reverse primers (10µM), and 4 µl sterilized water. The amplification conditions were consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec, and extension at 72°C for 60 sec, and final extension at 60°C for 10 min. PCR products were separated by electrophoresis on 1.5% gel. Gels were stained with GelRed. PCR products were sequenced in both directions by 1st Base in Selangor, Malaysia. The DNA sequences were analyzed using BLAST, Finch TV, and MEGA 7 programs.

RESULTS AND DISCUSSIONS

The chicken *RACK1* gene is located on chromosome 16. Using a pair of GNB2Li specific primers, a 520 bp fragment of the *RACK1* gene was successfully amplified from 50 samples KUB chicken. The example of visualization result of *RACK1* gene amplification (PCR product) was presented in Fig. 1. The thick and clear DNA band indicated the good quality of amplification products. The size of PCR products was as expected (Fig. 1).

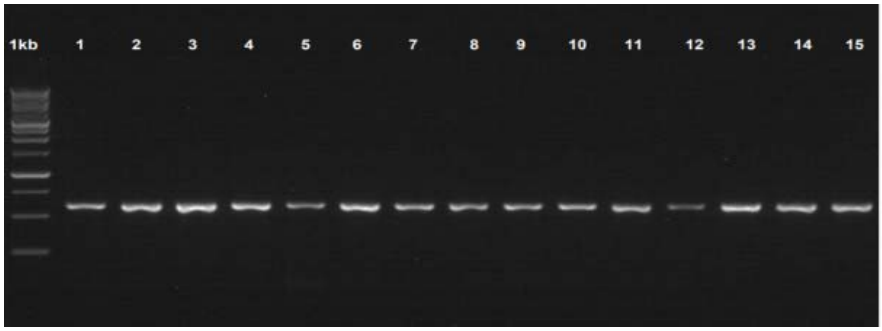

FIGURE 1. The example of visualization result of *RACK1* gene PCR amplification in KUB chickens.

The full-length sequence of *RACK1* gene of *G. gallus* sized 3,252 bp consisted of 8 exons and 7 introns [29] (Fig. 2a). The structure and amplification of *RACK1* gene sequences are presented in Fig. 2. From the 50 sequences it was identified that 2 SNPs were located in intron 4 at nucleotide bases number 1,986 (SNP-1986) and 1,993 (SNP-1993) based on sequences alingment with the reference sequence of NC_006103.5 (www.ncbi.nlm.nih.gov). The sequencing chromatogram showed double peaks at SNP-1986 and SNP-1993 indicating heterozygous genotype and a single peak as homozygous genotype (Fig. 2b). Genotypic analyses were performed for all identified SNPs, as heterozygous or homozygous alleles (Table 1). For SNP-1986 and SNP-1993, all genotypes (CC, CT, and TT) were observed in both low and high antibody titres of ND-vaccinated KUB chickens at 0.01 significant level ($p<0.01$). CT and CC genotypes significantly decreased antibody titre indicating susceptibility of KUB chicken to ND (OR of SNP-198 0.296, 95% CI 0.08–1.04 and OR of SNP-1993 0.769, 95% CI 0.39–1.50; $p \leq 0.050$). In contrast, TT genotype significantly showed the increase in antibody titre of ND-vaccinated KUB.

The antibody titters or level of antibody production is an indicator of antibody response as partial reflection of the potential of an animal to resist pathogen infection (R137). The increasing of antibody response play important role to neutralize, kill, and eliminate invading pathogens from animal body [30]. In addition, antibody response also associated with other economic traits, such as body weight, egg quality, and reproduction [31, 32]. Therefore, the improvement of antibody response is an important goal in poultry production.

Antibody response is a quantitative trait controlled by a number of genes [33]. Molecular identification of antibody response may improve selection for disease resistance in poultry. Previous studies have identified a number of SNP markers associated with antibody response both in genomic regions and specific gene of chicken [5, 12, 14, 15, 17], but it is not in *RACK1* gene. This is the first report for SNP association in *RACK1* gene with antibody response of chickens.

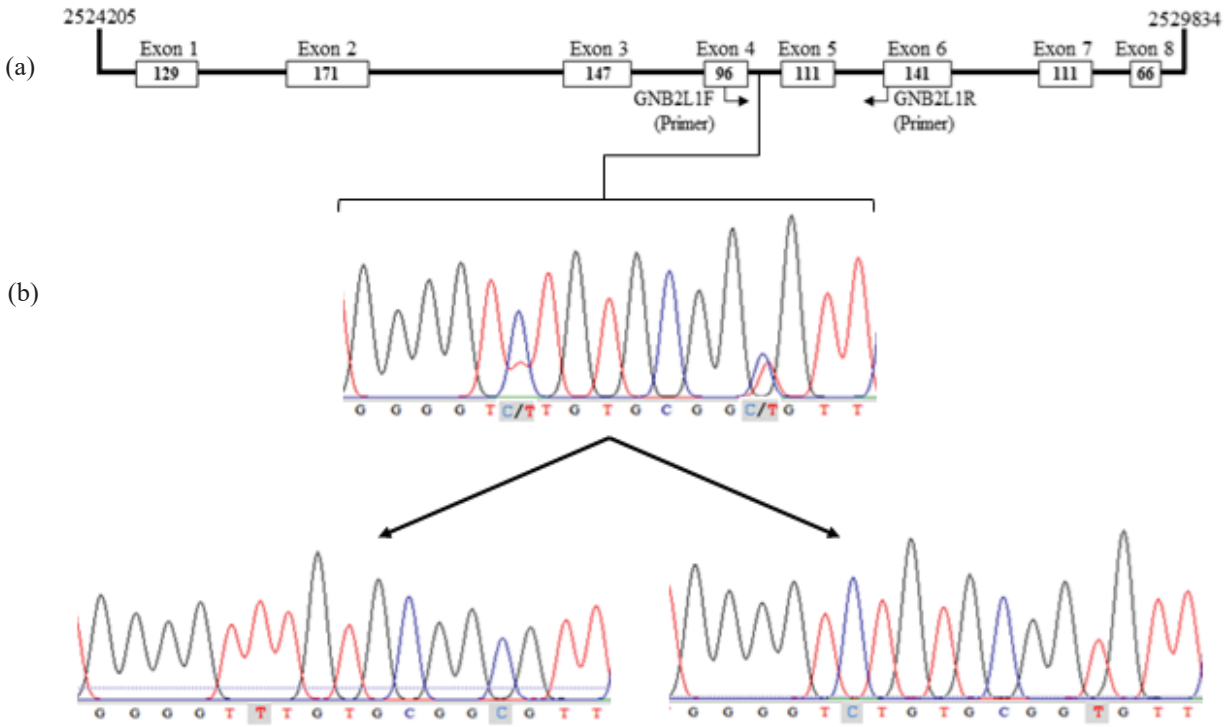

FIGURE 2. (a) Genomic structure of chicken *RACK1* gene in the coding region. The number in each exon was the length of each exon and the number on the top was the location from the first coding exon to the last coding exon (NCBI reference sequences: NC_006103.5, chromosome 16). (b) A representative chromatogram analysis of DNA sequencing assessment of data for sample that reveals a heterozygous mutation corresponding to SNP.

TABLE 1. Statistical analysis of associations between SNP and antibody response of KUB chickens.

Position	SNP	Status of Chickens	N	Genotype				P-value
				Antibody titter	CC	CT	TT	
Intron	SNP-1986	Susceptible	12	<8	8	2	2	0.000141
		Resistance	38	>8	2	27	9	
		OR (95% CI)			16.00 (3.83–66.80)	0.296 (0.08–1.04)	0.89 (0.22–3.61)	
Intron	SNP-1993	Susceptible	12	<8	1	5	6	0.000176
		Resistance	38	>8	11	26	1	
		OR (95% CI)			0.36 (0.03–3.78)	0.769 (0.39–1.50)	24.00 (1.99–289.91)	

It is interesting to note that the SNP-1986 and SNP-1993 for all genotype (CC, CT, and TT) in *RACK1* gene of ND-vaccinated KUB chickens are intronic polymorphisms. Introns contain substantial and multiplicity of functional elements including intron splice enhancers and silencers, trans-splicing elements, and other regulatory elements [34, 35]. Polymorphism in intron can influence expression of the genes conferring susceptibility of host to disease or otherwise modulate the genotype-phenotype relationship [36]. Haralambieva *et al.* [37] reported that two intronic polymorphisms in the *MOG* gene showed significant association with rubella-specific neutralizing antibody response in African-Americans. Intronic polymorphism in *inducible nitric oxide synthase* (*iNOS*) gene of SenSi-1 Agrinak chicken associated with antibody responses to *Salmonella enteritidis* [38]. Therefore, we concluded that intron *RACK1* polymorphism can be used as potential candidate SNP markers for antibody response traits in KUB chickens to ND.

CONCLUSION

Two intronic SNPs in *RACK1* gene were detected in present study and those play an important role in regulating antibody responses following ND vaccination of KUB chickens. These SNPs can be used as selection markers for antibody response traits for ND resistance in KUB chicken.

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