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Correlation analysis between single nucleotide polymorphism of interleukin-1ß gene and interleukin-1 concentration in three chicken breeds

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ABSTRACT

This paper aims to detect single nucleotide polymorphism (SNP) of interleukin-1ß (IL-1ß) gene of chicken and find genetic marker of IL-1 concentration traits. SNPs of IL-1ß gene and IL-1 concentration in blood serum in Rugao (RG), Wenchang (WC), and Anka Chicken (AK) were detected by SSCP-PCR and ELISA, respectively. The results showed that IL-1 concentration in blood serum of AK was extremely significantly higher than that of RG and WC. Four mutations were found in all 3 breeds, G476A, T482C, C1215T and T1507C. The genotype distribution of RG chicken at site nt476 was extremely significantly different from WC and AK, whereas there was no significant difference between WC and AK. Significant correlations were found between C1215T and IL-1 concentration in 3 chickens breeds, showing that IL-1 concentration of TT genotype chicken was significantly higher than that of CC. The results demonstrated that the genotype distributions at SNP sites presented significant difference among three breeds, and the site nt1215 could be a genetic marker of IL-1 concentration traits in RG.

Key words: Chicken, Interleukin-1, Interleukin-1ß gene, SNP

Interleukin-1 (IL-1), one of the first cytokines produced by macrophages, monocytes and dendritic cells, belongs to IL-1 superfamily. IL-1á and IL-1ß, the 2 forms of IL-1, play important roles in inflammatory response when binding to the same cellular receptor. These cytokines increase expression of adhesion factors on endothelial cells to enable transmigration of leukocytes to sites of infection and re-set the hypothalamus thermoregulatory center, leading to an increased body temperature as fever (Morgan *et al.* 2004). IL-1 can increase production of IL-2, IL-6,IL-17 and IFN-γ in T lymphocyte cell. Deepak *et al*. (2007) showed that IL-1 increased the host immunological rejection during the process of transplantation. The retro-orbital fibroblast pretreated with IL-1ß could increase the production of regulated upon activation normal T cell expressed and secreted (RANTES), and the Graves ophthalmopath patients produced higher levels of RANTES compared with the controls (Qin *et al*. 2005). IL-1 increased glutamate uptake in muller cells with a mechanism that increased membrane Na^+/K^+-ATPase localization and required for counteracting the Na+-glutamate co-transportation. IL-1 activates the p38 mitogen-activated protein kinase (MAPK)/capase 11 pathway, which destabilized the actin cytoskeleton allowing Na^+/K^+-ATPase membrane redistribution (Kazuhiko *et al.* 2008). IL-1 also could enhance the sensitivity of nerve in respiratory system (Wu *et al*. 2005, Yu *et al.* 2007).IL-1ß might promote the proliferation of cultured cerebral cortex astrocytes, and could cause significant changes of cell cycle. The percentage of cells in G1 cycle phase was reduced and the percentage of S and G2/M cycle phase was increased (Zhu *et al.* 2005). In addition, IL-1ß could increase prostaglandin (PGE2) release from rat cerebral microvascular endothelial cells (Guo *et al.* 2005). Chicken IL-1ß maps to the 22nd chromosome. The full sequence of 1606 nucleotides contains 6 exons, separated by 5 introns, and encodes a prepropeptide of 267 amino acid residues (Xu *et al.* 2007). rFPVs expressing IL-1ß could significantly increase $CD4^+$ and $CD8^+$T cells proliferations in spleens, and injection of chickens with rFPV expressing IL-1ß promoted protection against challenge with virulent NDV (Shao *et al.* 2008). Furthermore, the expression of IL-1ß may be related with chickens encephalomyelitis (Zhang *et al.* 2006). Generally, as an important cytokine, IL-1 was studied, but studies mostly focused on its biological functions to date. The study on IL-1 gene as a genetic marker only to be found on heart and brain diseases in human, like SNP at

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site nt889 of human IL-1β associated with brain and heart diseases, and a significant increase was found for T allele frequency in brain infarct disease compared with controls (Wei *et al.* 2005), as well as coronary heart disease (Huang *et al.* 2005). This paper is conducted to study SNP in some areas of IL-1ß gene in 3 chicken populations, and analyze the association between polymorphic sites and IL-1 concentration in serum, aiming to detect difference in SNPs of IL-1ß gene between different breeds and explore their possible relations with IL-1 concentration in 2 Chinese indigenous chicken populations and a population from Israel.

MATERIALS AND METHODS

Experimental animals: Random samples of 88 Rugao chicken (RG), 79 Wenchang chicken (WC), and 78 Anka chicken (AK) obtained from Poultry Institute, Chinese Academy of Agricultural Sciences, were used for this experiment. These chickens were reared on a large-scale under the same environment and management conditions. Before the experiment was initiated, the birds were continuously conducted to monitor clinical symptoms and major microbiological surveillance including avian influenza subtype H5N1 and H5N9, infectious bronchitis virus, Newcastle disease virus, mycoplasma synoviae, infectious laryngotracheitis, infectious bronchitis, infectious bursal disease, marek's disease, smallpox, *Eimeria maxima,* pathogenic *Escherichia coli* according to the Chinese criteria GB/T 17999.1-2008 and NY/T 555–2002. Chicks were monitored weekly for clinical signs of disease and morbid chicks were euthanized till to blood sampling day.

IL-1 concentration in blood serum: Blood samples (2 mL) were taken from the wing vein of 3 breeds of chicken at 56-day-old which was away from the 2 weeks of immunization. Serum was harvested at 3 000 rpm for 10 min. And the serum samples were assayed using IL-1 commercial enzyme-linked immunosorbent kit. The absorbance measured at 450 nm is directly proportional to the concentration of IL-1 present. A set of IL-1 standards was used as controls.

DNA extraction: The genomic DNA was extracted according to the procedures (Sambrook *et al.* 1998). DNA was extracted from 100 mL of whole EDTA-blood. Then the mixture solution was made up with 100 mg/mL proteinase K and 80 mg/mL DNase-free pancreatic RNase. After overnight incubation at 37°C, the proteins were removed by phenol and chloroform-isoamyl alcohol extractions and the DNA was precipitated by ethanol.

Primer design: Two primers (P1: forward: 5' CCGAGGA GCAGGGACTTT3', reverse: 5' TTTCTGGCTGGATGAG GG3' and P2: forward: 5' CCGCTTCATCTTCTACCG3', reverse: 5' ATACCTCCACCCCGACAA3') were designed by Primer Premier 5.0 and Oligo 6.0 software according to Red Jungle fowl IL-1ß sequence (GenBank accession number: NC006109). The expected sizes of the amplified fragments were 307 bp and 407 bp, respectively, which contained partial sequences of exon 3, intron 3, exon 4, exon 6 and partial sequences of the 3' end.

PCR-SSCP analysis: A 20 µL PCR system included 200 ng/µL template DNA 1 µL, each primer 1 µL, 2 µL 10×PCR buffer, 20 mM/mL dNTP 0.4 µl, 25 mM/mL $MgCl_2$ 1.2 µL,5 U/µL Taq DNA polymerase 0.2 µL and ddH_2O 13.2 µL. PCR condition was carried out by thermal cycle. Initial denaturation for 10 min at 94°C, was followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 63.3°C for 45s, plus extension at 72°C for 45 s, with a final extension at 72°C for 10 min. The 20 µL of PCR product was mixed with 8 µL loading buffer containing 50 mL formamide deionized, 0.139 g bromophinol blue, 0.139 g xylene cyanole and 5.6 mL glycerol, denatured at 98°C for 15 min and then quickly placed into ice for 15 min. 10 µL of mixture was applied into 10% gel (acrylamide: bisacrylamide=39: 1) electrophoresed in 1×TBE buffer for 12 h at 120 voltage. The gel was stained with sliver according to standard protocol as follows: the gel was washed in 10% ethanol and 0.5% acetic acid for 10 min, followed by incubation for 20 min in 0.1% silver nitrate and finally the gel was washed with 1.5% sodium hydroxide and 0.5% formaldehyde for 8 min, their photographs were visualized by digital camera.

Gene sequencing: Genomic DNA of individuals with variant single strand conformers was used for direct sequencing by a commercial company.

Statistical analysis: The following model was useful for association of each genotype with serum IL-1 concentration in each breed. $Y_{ij}=\mu+G_i+e_{ij}$, where Y_{ij} is phenotypic value of IL-1 concentration, µ is population mean, G_i is the fixed effect of the i^{th} genotype, and e_{ij} is random error effect of each observation. It was analyzed by software SPSS 12.0 using one-way ANOVA.

RESULTS AND DISCUSSION

SNPs in IL-1ß gene: Polyacrylamide gel electrophoresis (PAGE) results of *IL-1ß* gene polymorphisms are presented in Fig. 1 (P1) and 2(P2). Compared with Red Jungle fowl (GTCT/GTCT), 4 SNPs, G476A, T482C, C1215T and T1507C, were found among 3 chicken populations. But, only CC genotype was found at 482 and 1507 site. The SNPs of 476, 482 and 1507 site were located in noncoding region of the gene, whereas C1215T were detected in exon 6. Single nucleotide signals in mutation sites of 476, 482, 1215 and 1507 are presented in Figs 3–6. The mutation of 1215 site did not cause the change of amino acid. Both of them coded the same amino acid of Phe.

Allele frequency and Hardy-Weinberg equilibrium: Agreement of genotype frequencies with the Hardy-Weinberg equilibrium was tested using a chi-square goodness of fit test. In 3 breeds, all genotypes agreed with Hardy-Weinberg equilibrium at nt476 and nt1215 ($P<0.05$) (Table 1).

Comparison of genotype distribution: The genotype

Table 1. Gene frequencies and genotype frequencies of IL-1ß of three chicken breeds

Site	Breeds	Sample size	Genotype frequency			Gene frequency		χ^2
			GG	GA	AA	G	A	
476	RG	88	0.420 (37)	0.477 (42)	0.102 (9)	0.659	0.341	0.11
	WC	79	0.329 (26)	0.354 (28)	0.316 (25)	0.506	0.494	3.72
	AK	78	0.256 (20)	0.423 (33)	0.321 (25)	0.468	0.532	0.93
		CC	CT	TT	C	T		
1215	RG	88	0.523 (46)	0.386 (34)	0.091 (8)	0.716	0.284	0.13
	WC	79	0.696 (55)	0.291 (23)	0.013 (1)	0.842	0.158	0.43
	AK	78	0.628 (49)	0.308 (24)	0.064 (5)	0.782	0.218	0.20

$\chi^2_{0.05(2)}$=5.99, $\chi^2_{0.01(2)}$=9.21.

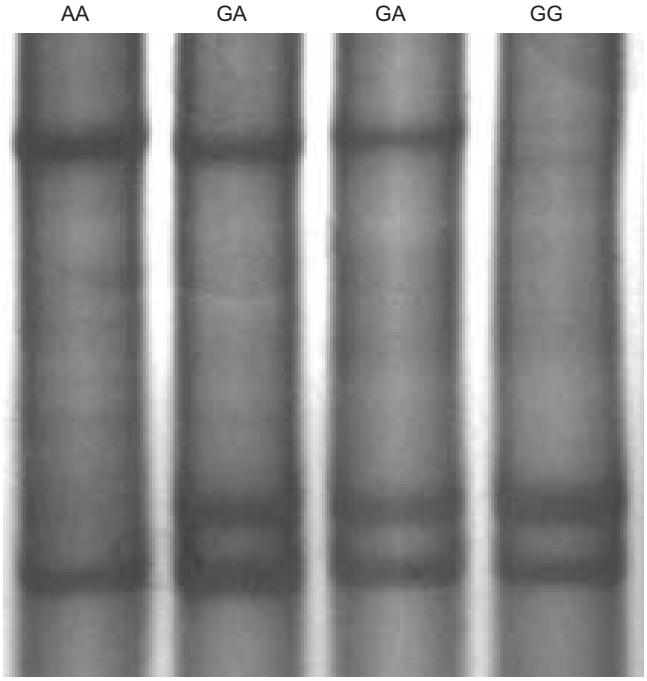

Fig. 1. The SSCP electrophoresis image of P1.

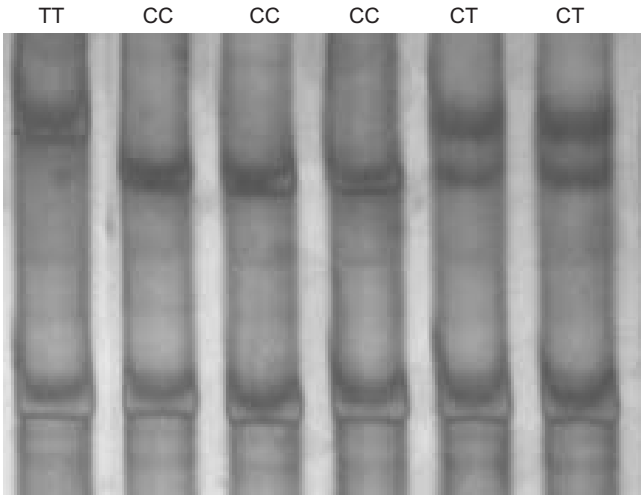

Fig. 2. The SSCP electrophoresis image of P2.

distribution of each SNP site of nt476 and nt1215 between chicken populations were tested by chi-square too. The genotype distribution at nt476 of RG was extremely significantly different from that of WC and AK, P<0.01, while that at site nt1215 of RG was significantly different from that of WC P<0.05 (Table 2).

IL-1 concentration in serum of different populations: In the present study, IL-1 concentration in serum of RG was extremely significantly higher than that of WC (P<0.01) and significantly higher than that of AK (P<0.05), while there was no significant difference between AK and WC (Table 3).

Association of SNPs in IL-1ß gene with IL-1 concentration: Single nucleotide polymorphism at nt1215 was significantly correlated with IL-1 concentration in serum in RG breed (P<0.05), while no relationship was detected in other 2 breeds. But, in all samples of 3 breeds, there are significant differences in IL-1 concentration between 3 genotypes. IL-1 concentration in TT genotype was

Table 2 difference for genotype distribution of IL-1ß gene between different chicken breeds

Site	Breeds	WC	AK
476	RG	11.85**	13.12**
	WC		1.19
1215	RG	7.91*	1.92
	WC		3.03

$\chi^2_{0.05(2)}$=5.99, $\chi^2_{0.01(2)}$=9.21.

Table 3 Comparison of IL-1 concentration in blood serum between 3 chicken breeds

Breeds	Sample size	IL-1 concentration/(pg/ml)
RG	68	517.38 ± 27.92^{B}
WC	60	439.63 ± 27.97^{B}
AK	64	638.52 ± 30.57^{A}

The different letters with the same size mean different level is P<0.01.

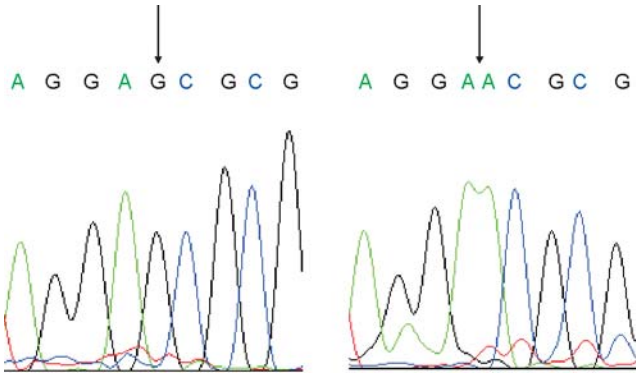

Fig. 3. Sequence of GG and AA genotypes at nt476.

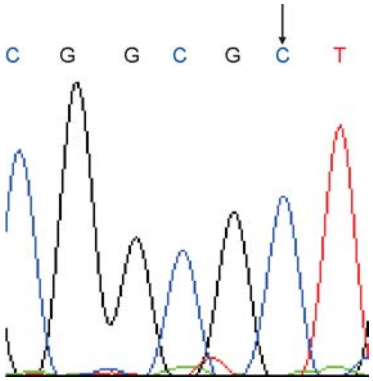

Fig. 4. Sequence of CC genotypes at nt482.

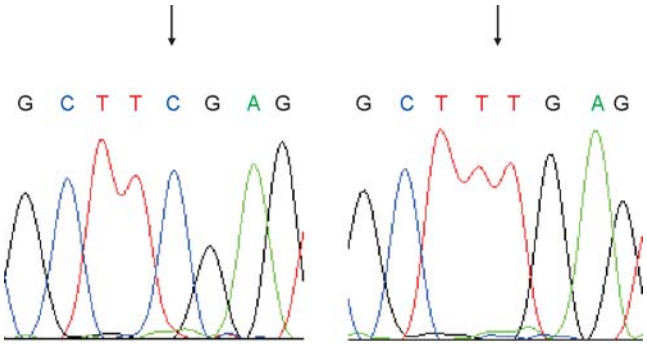

Fig. 5. Sequence of CC and TT genotypes at nt1215.

significantly higher than that in either CC or CT genotype (Table 4). In other breeds and sites, no significant differences were found between genotypes and IL-1 concentration in serum (P<0.05).

SNP difference in IL-1ß gene between breeds: In this study, totally 4 SNP sites related with Red Jungle fowl were found in intron 3, exon 6 and 3'-UTR. But, only homozygous alleles of mutation were found at sites nt482 and nt1507 in all 3 breeds, which suggested that mutation in the access of Red jungle fowl domesticated to domestic fowl, had relation with some adaptive advantage, so wild genotype lost through domestication. Though genotype frequencies at sites nt476

Table 4. Comparison of IL-1 concentration in blood serum between 3 genotype pg/ml

Site	Genotype		
476	GG	GA	AA
	525.26±238.54	512.62±240.34	584.33±255.68
	(67)	(81)	(44)
1215	CC	CT	TT
	516.82±231.47^{B}	533.09±241.34^{B}	681.32±321.00^{A}
	(114)	(65)	(13)

The different letters with the same size mean different level is P<0.01.

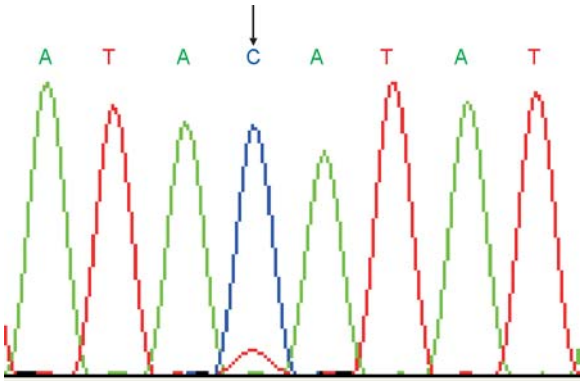

Fig. 6. Sequence of CC genotypes at nt1507.

and nt1215 agreed with the Hardy-Weinberg equilibrium in all 3 breeds, the distributions at both sites presented significant difference among 3 breeds, such as the highest frequency of allele A at site nt476 was found in AK, and the lowest was found in RG. Although RG and WC were both Chinese domestic breeds and AK came from Israel, the genotype distribution of RG at site nt476 was extremely significantly different from WC and AK, whereas there was no significant difference between WC and AK. At site nt1215, RG and WC also showed significant difference in genotype distribution, while no significant difference existed between RG and AK. So we can infer that SNP difference in *IL-1ß* gene between breeds have no relationship with geographical distance. The differences between different breeds may be caused by other factors, such as different origin and evolutionary progress, time of species formation, bottleneck effect at the beginning of domestication, gene flow between different species, crosses between different lines, mixing, and artificial selection.

Comparison of IL-1 concentration between breeds and its associated genetic marker: IL-1 played an important role in the process of immunity and disease, and the concentration of IL-1 expression in sick chickens was higher than that of normal chickens. This paper found that IL-1 concentration in serum of AK was extremely higher than that in RG and WC, while there was no significant difference between RG and WC. Generally, Chinese indigenous chicken breeds have

the advantages in disease resistance than modern commercial breeds or strains, such as AK chicken. Here, it could be 2 causes: (i) RG and WC chicken had stronger disease resistance than AK; (ii) AK chicken in this study was in the sub-clinical stage, and the IL-1 expression increased by the active immune system. We thought that the first cause was credible because AK chicken was very sensitive to the outside stimulation, such as the stress by catching and other stimulation during the blood sampling. By analysis of association of SNPs in *IL-1ß* gene with IL-1 concentration, only the polymorphic site at 1 215 sites in RG breed had relations with IL-1 concentration. As we mentioned in the former, C1215T in RG breed did not bring the change of amino acid, but synonymous mutation could cause secondary structure changing because the structure of protein related with the speed of translation, and suitable codon could accelerate translation (Liu *et al.* 2008). So, the synonymous mutation also could cause the function of protein changed through secondary structure changing. It could be concluded that allele T promoted the expression of *IL-1ß* gene, and increased the IL-1 concentration in serum. Consequently, it resulted in TT individuals had higher IL-1 concentration than that either in CC or CT individuals. Even so, whether this SNP could be a genetic marker of IL-1 concentration and diseases resistance traits need more researches and more samples to verification.

The present study which clearly demonstrated that the SNPs of IL-1ß gene presented significant difference among 3 breeds, Rugao, Wenchang and Anka chicken; and the sera IL-1 concentration involved in immune performances of Anka birds was extremely significantly higher than that of Rugao and Wenchang. Although 4 mutations were found in IL-1ß gene of all 3 breeds, the site nt1215 could be a genetic marker of IL-1 concentration traits in RG.

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