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SHORT COMMUNICATION

Genome scan linkage analysis identifies quantitative trait loci affecting serum clinical–chemical traits in Korean native chicken

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Abstract Alterations in robustness- and health-related traits lead to physiological changes, such as changes in the serum clinical chemical parameters in individuals. Therefore, clinical–chemical traits can be used as biomarkers to examine the health status of chickens. The aim of the present study was to detect the quantitative trait loci (QTLs) influencing eight clinical–chemical traits (glucose, total protein, creatinine, high-density lipoprotein cholesterol, total cholesterol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and α-amylase) in an F_1 nuclear families comprising 83 F_0 founders and 585 F_1 progeny of Korean native chickens. Genotypic data on 135 DNA markers representing 26 autosomes have been generated for this resource pedigree. The total length of the map was 2729.4 cM. We used a multipoint variance component linkage approach to identify QTLs for the traits. A significant QTL affecting serum α-amylase levels was identified on chicken chromosome (GGA) 7 [logarithm of odds (LOD) = 3.02, P value = 1.92×10^{-4}]. Additionally, we detected several suggestive linkage signals for the levels of total cholesterol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and creatinine on GGA 4, 12, 13, and 15. In this study, serum α-amylase levels related significant QTL was mapped on GGA7 and cholesterol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and creatinine traits related suggestive QTLs were detected on GGA4, 12, 13 and 15, respectively. Further verification and fine mapping of these identified QTLs can provide valuable information for understanding the variations of clinical chemical trait in chickens.

Keywords Korean native chicken · Quantitative trait loci · Clinical–chemical trait · Microsatellite marker

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Introduction

Genetic enhancement of robustness- and health-related traits has become a growing concern in the poultry industry, since the current chicken production system has to withstand climate changes and intensification of housing conditions to provide healthy and fresh meat to consumers. Moreover, increasing the ability to adapt to changing environments by enhancing the robustness may result in desired productivity [1]. Therefore, elucidation of the genetic structure that can impart robustness to chickens may aid in providing genetic information to optimize the production system. Clinical–chemical traits can be used as essential parameters to diagnose the robustness and health

status of animals and humans. Normally, changes in serum clinical–chemical levels are assessed in order to determine the diseases status and physiological conditions. Several studies have been conducted in chickens to identify quantitative trait loci (QTLs) for serum clinical–chemical traits [2–4]. However, the number of studies conducted is still limited compared to QTL studies conducted on production traits.

Not only is the chicken an economically important animal species in the poultry industry, but it is also an important animal model for biomedical research such as embryology, immunology, and neurology. In addition, the chicken is becoming a cardinal vertebrate model for the genetic dissection of complex quantitative phenotypic traits, such as serum clinical–chemical traits, due to the release of a high-quality draft genome sequence at 6.6 coverage [5] and a genetic map comprising approximately 2.8 million single nucleotide polymorphisms (SNP) markers [6] together with long history of analysis of quantitative traits [7].

The aim of the present study was to identify the QTL that influences clinical–chemical traits through variance component linkage analyses using F_1 nuclear families of Korean native chicken (KNC).

Materials and methods

Ethics statement

Animal care facilities and all experimental procedures fulfilled the standards established by the Committee for Accreditation of Laboratory Animal Care at National Institute of Animal Science (NIAS) in Korea (No. 2012-C-037).

Experimental animals

A two-generation resource pedigree comprising 88 F_0 founders and 597 F_1 progeny was generated and managed. Clinical–chemical traits were measured on 585 F_1 outbred Korean native chickens (282 males and 303 females) comprising 68 nuclear families ranging in size from 3 to 20 chickens. These two-generation families consisted of 83 founders (15 sires and 68 dams) that were not included for phenotype measurements and their F_1 progeny which were divided into five lines classified by plumage colors (110 gray–brown, 88 black, 135 red–brown, 122 white and 130 yellow–brown). All animals were raised under standard indoor conditions at the experimental facilities of the National Institute of Animal Science (NIAS), Cheonan, Republic of Korea.

Phenotypic analysis

Following a fast of at least 10 h (range 10–21 h), 5 ml of blood was sampled at slaughter. The F_1 chickens were slaughtered at an average age of 144 days (range 143–146 days), and the blood samples were collected from the brachial vein. The serum was separated and stored at −70 °C at least 1 month before phenotypic analysis. The serum samples were used for analysis of eight clinical–chemical traits [i.e. glucose (Glu), total protein (T-Pro), creatinine (Cre), high-density lipoprotein cholesterol (HDL-c), total cholesterol (T-Cho), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and α-amylase (Amy)] using the SPOT-CHEMTMEZ-SP4430 (ARKRAY, Japan).

Genotypic analysis

A total of 127 informative microsatellite (MS) and eight SNP markers covering the 26 autosomes were amplified using polymerase chain reaction (PCR) in the F_1 resource pedigree. The map order and genetic map distance were computed using the build and fixed options in the CRIMAP program version 2.4 [8]. The total autosomal map length was 2729.4 cM with an average spacing of 20.2 cM on the autosomes [9]. The sex-average autosomal linkage map was used for further variance component QTL analysis. MS markers on the Z-chromosome were not considered in the analysis.

Statistical and QTL analysis

Prior to QTL analysis, we obtained basic descriptive statistics and validated normal distribution of phenotype data. When putative outliers were observed, we excluded them, based on the ascertainment of normality, using the Ryan-Joiner test in the Minitab software (Minitab, USA). The phenotypic values were transformed by natural logarithm [i.e., creatinine (Cre), glutamic-oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), α-amylase (Amy)] or inverse normalization [i.e., glucose (Glu), total protein (T-Pro)] for reducing kurtosis, as necessary. Heritabilities of each trait were estimated with the following mixed-effects model by maximum likelihood implemented in the SOLAR program [10]:

$$y = Xa + bc + Zu + e$$

where y is a vector of the phenotypic values for serum clinical–chemical traits; a is a vector of fixed effects including sex, batch, and fasting time; c is a vector of slaughter weight; b is the regression coefficient; u is a vector of additive polygenic effects; e is a vector of

Table 1 Descriptive statistics and maximum likelihood estimates of heritablities (h^2) for clinical–chemical traits in Korean native chickens

Trait	Abbr.	N	Mean	SD	Min.	Max.	h^2 (%)
Glucose (mg/dl)[a]	Glu	585 (0)	0.0016	0.99	−2.93	2.93	10.93
Total protein (g/dl)[a]	T-Pro	583 (2)	0.0026	0.99	−2.93	2.93	10.94
Creatinine (mg/dl)[b]	Cre	585 (0)	−0.22	0.32	−1.20	0.74	26.32
High-density lipoprotein cholesterol (mg/dl)	HDL-c	585 (0)	96.18	30.14	26	150	33.79
Total cholesterol (mg/dl)	T-Cho	583 (2)	134.58	36.88	50	231	30.26
Glutamic oxaloacetic transaminase (IU/l)[b]	GOT	583 (2)	5.40	0.23	4.82	6.42	9.20
Glutamic pyruvic transaminase (IU/l)[b]	GPT	585 (0)	2.92	0.42	2.30	4.17	32.53
Amylase (IU/l)[b]	Amy	576 (9)	5.37	0.31	4.44	6.36	35.76

For no. of chickens (N), values in parentheses are the no. of chickens omitted according to ascertainment of normality

[a] Data transformed using inverse normalization due to kurtosis

[b] Data transformed using natural logarithm due to skewness

Table 2 Quantitative trait loci for clinical–chemical traits in KNC

Trait	Chromosome	Position (cM)	LOD[a]	*P* value	Closest marker (cM)[b]	Flanking marker[c]
Amy	4	61	2.05[d]	0.0021	*MCW0295* (58.9)	*ADL0203-LEI0094*
Amy	7	100	3.02[e]	1.9×10^{-4}	*MCW0316* (99.3)	*ROS0019-ADL0169*
Amy	8	40	2.75[d]	0.0004	*MCW0160* (38.4)	*MCW0275-ADL0278*
Amy	11	18	1.92[d]	0.003	*ADL0123* (8.7)	*MCW0097-MCW0230*
Amy	24	0	1.89[a]	0.0032	*MCW0301* (0.0)	*MCW0301-LEI0069*
Cre	15	34	1.36[d]	0.012	*MCW0080* (42.7)	*LEI0120-MCW0080*
GOT	12	52	1.25[d]	0.016	*MCW0198* (53.6)	*ADL0372-MCW0198*
GPT	13	2	1.50[d]	0.009	*LEI0251* (0.0)	*LEI0251-MCW0104*
T-Cho	4	9	2.27[d]	0.0012	*ADL0317* (10.2)	*ADL0255-ADL0203*

[a] LOD is test statistic for the quantitative trait locus (QTL) and level of significance

[b] Closest marker to QTL and its position

[c] Markers are flanking markers for QTL support intervals estimated by the 1-LOD drop method

[d] Chromosome-wide 5 % suggestive significance

[e] Experiment-wide 5 % significance

residual environmental effects; X, Z are incidence matrices for a and u, respectively. The mean and variance for the residual environmental effect of individuals were assumed to be: $e \sim N(0, I\sigma_e^2)$, where I is the identity matrix and σ_e^2 is the residual variance. The mean and variance for additive polygenic effects can be defined as: $u \sim N(0, A\sigma_a^2)$, where A is the additive genetic relationship matrix computed from the F_1 pedigree in this study and σ_a^2 is the additive polygenic variance.

The QTL analysis was performed using a variance component based program [10], SOLAR, for outbred nuclear F_1 resource pedigree. The identical-by-decent (IBD) matrix was calculated based on the marker and pedigree information. Using this IBD matrix, the logarithm of the odds (LOD) score to test presence of QTL was computed at a 1-cM interval across the genome for each F_1 chicken. The IBD matrix is incorporated to a linear mixed model to model a QTL as follows:

$$y = Xa + bc + Zu + Wq + e$$

where y is a vector of the phenotypic values for serum clinical–chemical traits; a is a vector of fixed effects including sex, batch, and fasting time; c is a vector of slaughter weight; b is the regression coefficient; u is a vector of residual additive polygenic effects; q is a vector of additive QTL effect; e is a vector of residual environmental effects; X, Z, W are incidence matrices for a, u, and q, respectively. The mean and variance for residual additive polygenic effects can be defined as: $u \sim N(0, A\sigma_a^2)$, where A is the additive genetic relationship matrix computed from the F_1 pedigree in this study and σ_a^2 is the residual additive polygenic variance. The mean and variance for additive QTL effects can be defined as: $q \sim N(0, G\sigma_q^2)$, where G is the IBD matrix and σ_q^2 is QTL effect variance.

Thresholds for evaluating significance of linkage were calculated by a numerical method [11]. The threshold

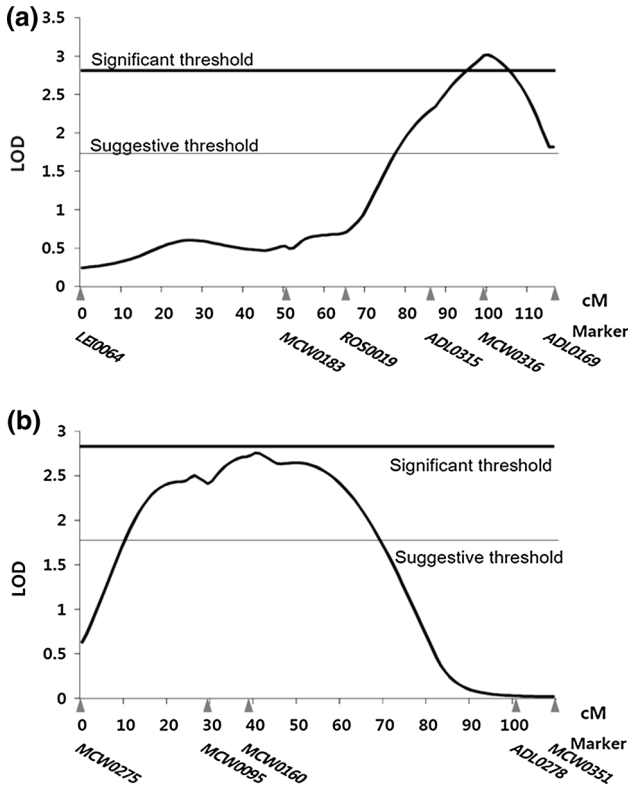

Fig. 1 Test statistic profiles for serum α-amylase levels on GGA7 (**a**) and GGA8 (**b**). Marker map with distances between markers in Kosambi cM is given on the *x-axis*. The *y-axis* represents the LOD score testing the hypothesis of a QTL in a given genomic position. *Horizontal lines* represent the significant (i.e., 5 % experiment-wide significance) and suggestive (i.e. 5 % chromosome-wide significance) thresholds

levels established by this method vary as a function of the trait studied and the chromosome length. A 5 % experiment-wide threshold for the significant linkage was employed. A suggestive linkage was employed using a 5 % chromosome-wide threshold. Unless otherwise mentioned, the *P* values are nominal. The 1-LOD drop method was used to estimate the support intervals (SIs) for the identified QTL [12].

Results and discussion

The descriptive statistics of the phenotypic data given in Table 1 together with heritabilities of each trait, estimated by the SOLAR program. The heritabilities ranged from 9.2 % (GOT) to 35.8 % (Amy).

A total of five QTLs affecting Amy were identified from the genome scan linkage analysis. The most significant QTL was detected on GGA7 with an experiment-wide significant threshold of <5 % (LOD = 3.02, *P* value = 1.9×10^{-4}; Table 2; Fig. 1). A comparable linkage was also detected on GGA8 (LOD = 2.75, *P* value = 0.0004; Table 2; Fig. 1). In addition, three additional suggestive QTLs affecting Amy were revealed on GGA4, GGA11, and GGA24, respectively (Table 2). To our knowledge, fasting serum Amy levels were never investigated in previous QTL studies in chickens. Interestingly, *AMY1A* was located in the QTL region for the Amy on GGA8, and in the orthologus region on human chromosome 1. Amylases are digestive enzymes that hydrolyze 1,4-α-glucosidic bonds into oligosaccharides and polysaccharides, and thus, catalyze the initial step in glycogen and dietary starch digestion. A cluster of diverse amylase genes in the human genome are expressed at high levels in the pancreas and salivary gland. *AMY1A* encodes an Amy isoenzyme produced by the salivary gland. We found that *AMY2A*, encoding an Amy isoenzyme secreted by the pancreas, was also located on GGA8. However, the genetic map in our study did not include the *AMY2A* locus. Thus, further studies using the map with the *AMY2A* locus are necessary to examine the quantitative effects of the *AMY2A* gene on serum Amy levels in KNC. Normally, high serum levels of Amy are associated with acute pancreatitis. Moreover, Nakajima et al. [13] reported that low levels of serum amylase are associated with increased risk of metabolic syndrome, and diabetes.

We also detected suggestive QTLs affecting serum levels of GOT and GPT on GGA 12 and 13, respectively (Table 1). GOT and GPT can be used as useful serum parameter to detect and monitor liver cell damage. Linkage signals for Cre, used as an indicator of renal function, was identified on GGA 15. We identified a QTL affecting T-Cho on GGA 4. Serum T-Cho levels are used as a strong indicator for coronary artery disease. In this QTL region for T-Cho, forkhead box O4 (*FOXO4*) is located. Forkhead transcription factor proteins play an important role in metabolism and development [14]. Especially, *FOXO4* has a potential effect on the regulation of cholesterol biosynthesis [15]. Thus, we suggest that *FOXO4* can be a positional candidate gene for T-Cho in chickens. For Glu, T-Pro, and HDL-c, we could not detect any significant or suggestive linkage signals. In this study, the mean genetic distance between adjacent markers mapped to each autosomal linkage group was 20.2 cM; Regarding the chromosomes containing the QTLs for clinical–chemical traits, there were four gaps greater than 50 cM. If the marker density is low, we cannot exclude a possibility that a QTL turns up arbitrarily. We mentioned the closest marker of most likely position of each QTL in Table 2. Therefore, one of the prioritized topic for further study is to fill the gaps with more genetic markers.

In conclusion, we performed a genome scan linkage analysis to identify QTLs for clinical–chemical traits using a purebred F_1 nuclear pedigree of KNC. A total of nine QTLs were detected (five Amy, one Cre, one GOT, one

GPT, and one T-Cho). The QTLs identified in this study need to be further researched in follow-up fine-mapping studies before finally ascertaining the accurate position and nature of these QTLs.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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