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Refined Localization of the *O* Gene for Blue Egg Phenotype on Chicken Chromosome 1

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Abstract: The blue egg is caused by a dominant mutation (O) and preliminary assigned to the interval of 1.8 Mb from 67.29, 6.991-69.1, 40.571 bp on chromosome 1. In order to refine the localization of the *O* gene, linkage analysis was performed between 6 markers in the mapping interval and the O locus using 149 F_2 offspring from an intercross between DongXiang blue-shelled and DongXiang brown-shelled chickens.

Key words: Chicken, blue egg, mapping, linkage, association, China

INTRODUCTION

In domestic fowl, blue egg is caused by an autosomal dominant mutation (O) and behaves as normal Mendelian inheritance (Punnett, 1933). The *O* gene is localized on the proximal end of the short (p) are of chromosome 1 and shows close linkage with Pea comb (P) (Bruckner and Hutt, 1939; Zartman, 1973; Bitgood *et al*., 1980). Due to the use of different segregation group, variant map distances of from 0.6-6 cM between P and O were also suggested by several research groups (Bruckner and Hutt, 1939; Bitgood *et al*., 1980, 1983, 2000; Bitgood, 1985; Crawford, 1986).

In addition, a map distance of 1.8 cM between the *O* gene and chicken endogenous viral element 1 (ev1) was also reported by Bartlett *et al*. (1996). Although, single marker analyses have demonstrated the presence of *O* gene near these markers it is not the most powerful means for positional cloning of the *O* gene using these map distance information. Therefore, Wang firstly performed a interval mapping to the *O* gene using 4 molecular markers based 98 F_2 individuals and suggested that the *O* gene was most likely to be assigned to the interval of 1.8 Mb from 67.29, 6.991-69, 140.571 bp on chromosome 1.

In this study, a more accurate genetic mapping of the O locus was performed by linkage and association analysis with 6 markers in the 1.8 Mb region. We show that the ss244244378 is closely linked to the O locus and display strongest association with blue egg among 6 markers. The findings could lead to the identification of the gene and mutation responsible for the blue egg phenotype.

MATERIALS AND METHODS

All hens were from a local breed protection farm in DongXiang town, JiangXi province of China. It is noteworthy that not all females produce blue eggs in this population where minor females laying brown-shelled eggs are found. Now, >90 brown-shelled and blue-shelled hens from the DongXiang population were used in the association study.

A three-generation pedigree was used for linkage analyses between O and 6 markers. The pedigree was generated after crossing five blue-shelled males with genotypes of O/O with 20 brown-shelled females with genotypes of o/o (o indicates the recessive allele causing brown egg phenotype at blue egg locus).

Genotypes of the five male founders at blue egg locus have been conformed to be dominant homozygote (O/O) by test-crossing with the White Leghorn females. Ten males and 46 females from the F_1 generation were intercrossed. Finally, 149 F_2 females with shell color records were used for linkage analysis.

The positions, primer sequences and genotyping method of these markers were shown in Table 1. The rs13879271, ss244255171 and rs14839665 genotypes were determined by Restriction Fragment Length olymorphism (RFLP) with appropriate restriction enzymes (New England Biolabs), subjected to electrophoresis on 2% agarose gels and visualized after an ethidium bromide staining. The ss244244378 genotype was analyzed through Single-Strand Conformation Polymorphism (SSCP). In brief, 2 μL of PCR product were mixed with 8 μL of denaturing buffer consisting of 98% (v/v) formamide

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Table 1: Primers, positions and genotyping methods for 6 markers used in fine mapping to the *O* gene

Marker ID[a]	Poly-morphism	Position at chr1[b]	Primer (5-3)	Genotyping method
rs13879271	A>C	67308926	F: TAGCAATCTGCCACCCAC	PCR-RFLP,
			R: TGCCACCT ACTGACAAAG	HinfI
ss244255169	+>80 bp	67401270-	F: CTTCTGCTCCTCACCTCA	12% PAGE gel
	del	67401349	R: ACGGGACAAGTCTTTCTTAC	electrophoresis
ss244244378	+>13 bp	67419892-	F: ATCTATAAAGGAGCAAGG	PCR-SSCP
	del	67419904	R: ATGAGGGTAAGAGGACAC	
ss244255171	T>G	68170663	F: GGTGTTTCTGTCGGGTAT	PCR-RFLP,
			R:TTTCAGGCTGCTTCATTT	DraI
HM461745	(TCTG) n	68680338-	F: TTTCTGTATTGCCTTGCA	12% PAGE gel
		68680375	R: CATTTGTTCCGTTCCACT	electrophoresis
rs14839665	T>C	69060495	F: CCAGGCAGGGCACATACA	PCR-RFLP,
			R: GTGCTGGTTTCATCAAGGT	PstI

[a]The markers data reported in this study have been submitted to the NCBI GenBank and dbSNP database (http://www.ncbi.nlm.nih.gov/snp/); [b]Chromosome locations of markers are given according to the May 2006 chicken (*Gallus gallus*) v2.1 draft assembly (http://genome.ucsc.edu)

deionized (Amresco) and 2% (v/v) 0.5 M pH = 8 EDTA. Samples were heat denatured at 98°C for 10 min, snap-cooled in ice bath for 10 min.

Finally, samples were resolved on 12% nondenaturing polyacrylamide gels at 150 V for 15 h and visualized after sliver staining. The ss244255169 and HM461745 genotypes were determined by running 12% non-denatured PAGE gel. Using the 50 bp DNA Step Ladder (Promega) as the standard, the length of microsatellite alleles were calculated by Quantity One v 4.26 (Bio-Rad).

The information content for each marker was calculated using the Web-based QTL Express software. The TWO-POINT option in CRI-MAP v2.4 was used to calculate the recombination fractions between loci and corresponding LOD-scores (Green *et al*., 1990). A LOD score value >3 was set as a criterion for significant linkage. Allelic association of 6 markers with blue egg was tested using Chi square test. The Odds Ratios (OR) with 95% Confidence Intervals (CIs) were calculated to assess the strength of association between alleles and blue egg.

RESULTS AND DISCUSSION

Average information content of 6 markers was 0.80 in this pedigree (Table 2). The results of the two-point analysis involving the *O* gene are shown in Table 2. The recombination rates between 6 markers and O revealed that the *O* gene is proximal to the ss244244378. When blue egg phenotype is selected in the DongXiang blue-shelled chickens breeding, it is conceivable that the closer a marker is to the O locus, the stronger it exhibits association with blue egg due to strong linkage disequilibrium. Thus, the joint association study can provide additional evidence for the mapping result. Results showed that allelic association of 6 markers with blue egg was in line with their linkage relationship with the O locus (Table 3). The +allele of the ss244244378 displayed the strongest association with blue egg with the highest OR score of 104.4 (95% CI 59.8-182.2; $p<10^{-5}$) among 6 markers.

Table 2: Two-point analysis between the O and 6 markers

Marker ID	Information content	Recombination fraction	LOD
rs13879271	0.84	0.14	3.53
ss244255169	0.76	0.07	4.41
ss244244378	0.96	0.02	13.51
ss244255171	0.74	0.12	5.74
HM461745	0.72	0.14	5.40
rs14839665	0.76	0.19	2.51

Physiologically, it is well established that the biliverdin is responsible for blue egg coloration and deposited on the shell by the shell gland. Kennedy and Vevers (1973) suggested that the *O* gene is likely to be a member of a specific enzyme system which was involved in oxidation of heme to biliverdin. Heme Oxygenase-1 (HO-1) is the rate-limiting enzyme in the catabolism of heme into biliverdin (Liu *et al*., 2005) whereby it is a plausible candidate gene for blue egg. In previous study, we showed that blue-shelled chickens indeed expressed more HO-1 transcript and protein than brown-shelled chicken in shell gland. However, HO-1 which is located on chr1:54136215-54141675 in chicken is far away from the studied region harboring the *O* gene (chr1: 67308926-69060495). Taken together, we propose the following model for explaining the molecular mechanism behind blue egg phenotype. HO-1 indeed plays an important role in blue egg formation but it is not the *O* gene. It could act as a downstream gene regulated by the *O* gene involving in formation of blue egg.

A databases search (http://genome.ucsc.edu/) for the studied region revealed that the ss244244378 is proximal to three genes, 104.3 kb downstream of SLCO1C1 (solute carrier organic anion transporter family, member 1C1), 2.7 kb upstream of IAPP (islet amyloid polypeptide) and

Table 3: Tests of allelic association with blue egg

	Sample size (n)		Allele frequency (assoc. allele)				
Markers	Brown egg	Blue egg	Brown egg (%)	Blue egg (%)	p (χ^2)	OR	95% CI
rs13879271	115	107	44.5 (A)	93.5 (A)	$<10^{-5}$	17.9	9.7-33.20
ss244255169	97	93	40.2 (+)	94.0 (+)	$<10^{-5}$	23.3	11.7-46.30
ss244244378	126	202	10.7 (+)	92.6 (+)	$<10^{-5}$	104.4	59.8-182.2
ss244255171	95	93	35.3 (T)	83.3 (T)	$<10^{-5}$	9.1	5.6-15.00
HM461745	95	93	12.6 (188[a])	19.1 (188[a])	0.18	1.6	0.9-2.900
rs14839665	97	93	37.1 (T)	52.2 (T)	0.003	1.8	1.2-2.800

[a]The numbers represent fragment size

55.2 kb upstream of RECQL (RecQ protein-like (DNA helicase Q1-like)). The SLCO1C1 encodes a member of the organic anion transporter family. It, as a transmembrane receptor plays an important role in uptake of thyroid hormones (Visser *et al*., 2008). The protein encoded by RECQL is a member of the RecQ DNA helicase family. DNA helicases are needed in maintaining genome stability and various processes of DNA metabolism including DNA replication, mismatch repair, recombination and transcription (Zhang and Xi, 2002; Wu and Brosh, 2010). The IAPP, also known as amylin is a 37 amino acid peptide of the calcitonin gene family (Macfarlane *et al*., 2000). Huang *et al*. (2007) suggested that toxic oligomers of IAPP induce Endoplasmic Reticulum (ER) stress which plays a role in development of type 2 diatebes (Huang *et al*., 2007). In another study, ER stress was found to stimulate HO-1 gene expression (Liu *et al*., 2005). Although, the precise regulatory mechanism between these genes and HO-1 is still not well understood, it is very necessary further to analyze association of these gene polymorphisms and expression traits with blue egg based on recent mapping results.

CONCLUSION

Results showed the ss244244378 (recombination rate = 0.02, LOD = 13.51) was closest to the *O* gene compared to the other 5 markers (recombination rate = 0.07-0.19, LOD = 2.51-13.51). The joint association study further confirmed the result due to the ss244244378 exhibiting the strongest association with blue egg (OR 104.4; $p<10^{-5}$) in contrast to the other 5 markers (OR 1.6-23.3, $p<0.18\text{-}10^{-5}$). These results further narrow down the localization of the *O* gene and suggest three genes (SLCO1C1, RECQL and IAPP) near the ss244244378 being very plausible candidate genes for blue egg phenotype.

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