Variation in the *ovocalyxin – 32* gene in commercial egg-laying chickens and its relationship with egg production and egg quality traits

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Summary

The quality of eggshells is an important trait for commercial egg production. A number of studies have linked polymorphisms in the gene encoding the eggshell protein *ovocalyxin-32 (OCX32)* with eggshell features including strength and thickness, indicating that variants in the gene may be relevant to the selection of commercial egg-laying poultry lines. In this study (**Fulton *et al.,* 2012**), exons 2-6 of the *OCX32* gene were sequenced in multiple elite commercial egg-laying lines, and SNP detection and analysis carried out using LGC's KASP™ genotyping chemistry to identify multiple polymorphisms. The genotype data was used to identify changes in amino acids, infer novel protein haplotypes, and associate these protein variations with a range of egg traits. The study identified 28 SNPs and 1 SNP/InDel in exons 2-6 of the *OCX32* gene, which encompasses 78% of the gene coding region. SNP analysis data indicated that the poultry lines tested carry 19 different variants of the OCX32 protein. Trait association studies indicated that the variants were linked to different degrees with traits including eggshell colour, early egg weight, albumen height, puncture score, and yolk weight. Selection pressure for some variants over time was also evident in three of the poultry lines, indicating that some polymorphisms in the *OCX32* gene may confer changes in egg traits that are desirable for commercial egg production.

Introduction

Eggshell structure and strength are important traits for commercial egg production, as they can determine whether an egg will be able to withstand handling and transportation, as well as microbial challenge. Numerous studies in the literature have reported relationships between single nucleotide polymorphisms (SNPs) in the *ovacalyxin-32* gene and egg-related traits, including eggshell thickness weight and stiffness, in a number of commercial poultry lines. OCX32 is a 32 kDa matrix protein that is expressed in the avian uterus and isthmus, and incorporated as a component of the outer layers of the eggshell and the shell cuticle. In this study, the researchers used KASP genotyping chemistry from LGC to identify and analyse SNPs spanning exons 2-6 of the *OCX32* gene in eight elite commercial brown and white egg-laying poultry lines, determine amino acid alterations in the protien, and infer exon and protein haplotypes within individual poultry lines.

Trait association studies demonstrated significant effects of *OCX32* polymorphisms on a number of egg characteristics, providing an insight into the selection pressures for certain variants of the *OCX32* gene.

Materials and methods

Sequencing

The study focused on eight elite brown and white eggshell commercial egg-laying lines from three different breeds:

- White Leghorn (white eggshells)
- White Plymouth Rock-derived lines (brown eggshells)
- Rhode Island Red (brown eggshells).

Purified amplicons (exons 2-6) were sequenced by SeqWright, and sequence electopherograms analysed by sequencher 4.9 (Gene Codes Corp.). Sequences were aligned with the Jungle Fowl (JF) genome sequences accessed from UCSC.

SNP detection and genotyping

Initially, SNPs were detected by PCR amplification to yield different products depending on the genotype, followed by gel-based detection. SNP detection was subsequently changed to a more rapid and less expensive method using LGC's KASP chemistry, a

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competitive allele-specific PCR-based fluorescent SNP genotyping system (***see call out box on KASP genotyping chemistry at the end***).

Traits

Both male and female traits were evaluated. Egg production and quality traits assessed included egg weight, shell colour, egg production, albumen height, yolk weight and body weight, during early production and, where possible, during late production.

Statistical analysis

Power-Marker software *(***Liu & Muse, 2005***)* was used for haplotying individuals, for LD analysis to calculate the frequency of haplotypes, and for evaluating phenotypic effects of protein haplotypes (PHTs). Association of PHTs with traits associated with egg production and egg quality was evaluated using a haplotype trend regression (HTR) option of Power-Marker (**Dmitri *et al*., 2002**).

Results

DNA polymorphisms

Exons 2-6 represent 78% of the *OCX32* cDNA, cover 588 bases of exon sequence, and encode 196 amino acids of the OCX32 protein. Within the gene region analysed, 28 SNPs and one SNP/InDel (insertion/deletion) were identified, 15 of which had not previously been described. All but three of the exonic SNPs resulted in a predicted change in protein sequence, and most of the polymorphic sites were found in multiple lines, which suggests that they represent a major fraction of the common polymorphic sites found in commercial white and brown egg lines. Four of the SNPs resulted in amino acid substitutions that may alter OCX32 protein 3D structure or function.

Exon haplotypes

The researchers generated a minimum panel of eight SNPs for genotyping each poultry line. Data from these eight SNPs was used to identify exon amino acid haplotypes in large numbers of individuals from each line. Because all of the SNPs used to identify haplotypes resulted in amino acid changes, the SNPs effectively represent different protein haplotypes. From this data it was found that exon 2 of the *OCX32* gene can present as one of three exon haplotypes, including one that exhibits six SNP variants that always occur together. Exons 3 and 5 each harboured a single SNP, and present two haplotypes each. Exon 4 demonstrated two independent SNPs, and thus four haplotypes, while exon 6 also exhibited four haplotypes, one of which has five SNP variants that always present together.

The combination of SNPs present in the *OCX32* gene resulted in 19 different proteins possible across the eight lines. The keys here is that the approach used in this uncovered far greater diversity than could be identified using single SNP analysis, even within intensively selected lines.

Protein variation and egg traits

Calculated allele frequencies for 13 SNPs, averaged across all generations of both males and females, were additionally evaluated in terms of egg traits. The most common effects were seen for shell colour in all five white egg lines, but not in the brown eggs. Significant effects were found for albumen height in a number of brown and white egg lines. Associations were also found for early and late egg weight in five lines, and there was some support for an association with puncture score in three lines, indicating that *OCX32* may have an impact on these traits as well. In three of the poultry lines there was a substantial change in the level of variation in the *OCX32* gene and its protein among selectively bred poultry, indicating either that there may be a selective advantage for some variants, or perhaps variation itself is advantageous. Further evidence for this was indicated by the finding that there were significant changes in the frequency of some haplotypes in three of the poultry lines over generations, with some variants increasing in frequency, and others decreasing.

Conclusions

The results demonstrate a large potential for variation within the *OCX32* gene, even among a relatively small subset of selectively bred poultry lines. In this study 86% of the variants detected resulted in amino acid changes, with the identification of 19 different OCX32 protein variants within the eight commercial poultry lines tested. Further evaluation of the effects of variation in the *OCX32* gene, and the effects of these variants on egg-production and egg structure or quality traits could potentially help direct future selection strategies for commercial egg-laying poultry lines.

Use of the KASP chemistry for genotyping has facilitated the evaluation of genetic variation across five exons of a gene that has previously been implicated in egg quality. In contrast with other studies, this work identified a large number of SNPs not previously reported, in a far greater number of poultry lines, and thus encompassing a much larger gene pool. The data showed that multiple, complex OCX32 protein haplotypes are found in commercial egg-laying poultry lines that have been selected through intensive breeding.

Key words: eggshell color, eggshell quality, Ovocalyxin-32, protein haplotypes

References

1. Fulton, J.E., Soller, M., Lund, A.R., Arango, J. and Lipkin, E. (2012) Variation in the ovocalyxin-32 gene in commercial eg-laying chickens and its relationship with egg production and egg quality traits. Animal Genetics 43, s1, 102-113.
2. Liu, K. and Muse, S.V. (2005) POWERMAKER: an integrated analysis environment for genetic marker analysis. Bioinformatics 21, 2128-9.
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Set up in 1842 as the Laboratory of the Government Chemist, for more than 100 years LGC has held the unique function of the Government Chemist in the UK. LGC was privatised in 1996 and is now majority-owned by funds managed by Bridgepoint.

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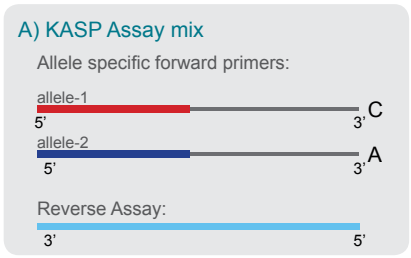
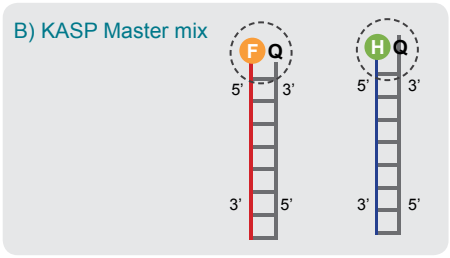
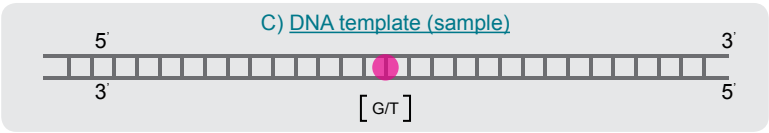
KASP chemistry: How it works

1) Assay components:

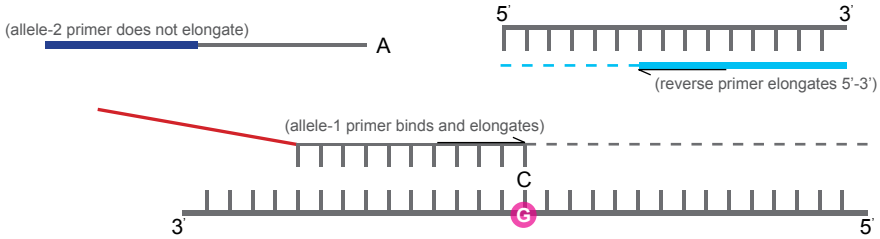
KASP uses three components: test DNA with the SNP of interest; KASP Assay Mix containing two different, allele-specific, competing forward primers with unique tail sequences and one reverse primer; the KASP Master mix containing FRET cassette plus Taq polymerase in an optimised buffer solution.



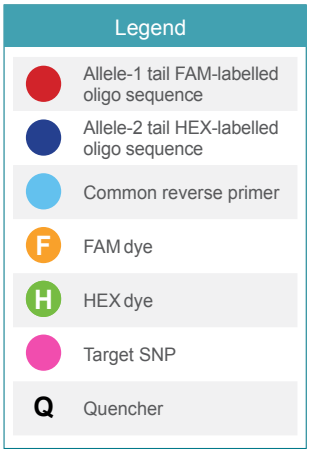




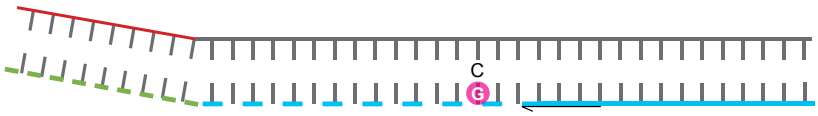
2) Denatured template and annealing components – PCR round 1:



In the first round of PCR, one of the allele-specific primers matches the target SNP and, with the common reverse primer, amplifies the target region.

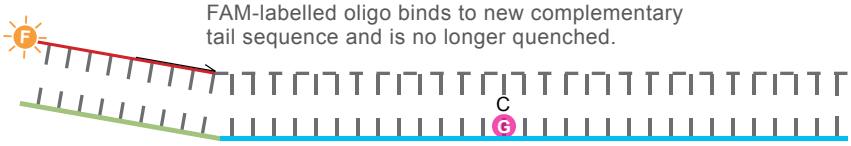


3) Complement of allele-specific tail sequence generated – PCR round 2:

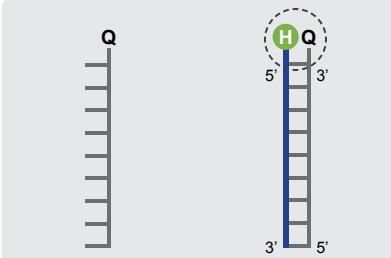


(Reverse primer binds, elongates and makes a complementary copy of the

4) Signal generation – PCR round 3:



In further rounds of PCR, levels of allele- specific tail increase. The fluor labelled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal.



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