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Tuiskula-Haavisto, M., Honkatukia, M., Dunn, I.C., Bain, M.M., De Koning, D.J., Preisinger, R., Schmutz, M., Arango, J., Fischer, D. and Vilkki, J. (2018) Validated quantitative trait loci for eggshell quality in experimental and commercial laying hens. *Animal Genetics*, 49(4), pp. 329-333. (doi:10.1111/age.12671)

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This is the peer-reviewed version of the following article: Tuiskula-Haavisto, M., Honkatukia, M., Dunn, I.C., Bain, M.M., De Koning, D.J., Preisinger, R., Schmutz, M., Arango, J., Fischer, D. and Vilkki, J. (2018) Validated quantitative trait loci for eggshell quality in experimental and commercial laying hens. *Animal Genetics*, 49(4), pp. 329-333, which has been published in final form at 10.1111/age.12671. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

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Deposited on: 19 July 2018

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Validated QTL for egg shell quality in experimental and commercial laying hens

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Summary

Compromised egg shell quality causes considerable economic losses for the egg industry. Breeding for improved egg shell quality has been very challenging. Egg shell quality is a trait that would greatly benefit from marker assisted selection, which would allow the selection of sires for their direct contribution to the trait and would also allow implementation of measurements integrating a number of shell parameters that are difficult to measure. In this study we selected the most promising autosomal quantitative trait loci (QTL) affecting egg shell quality on chromosomes 2, 3, 6, and 14 from earlier experiments and we extended the F_2 population to include 1599 F_2 females. The study was repeated on two commercial populations, Lohmann Tierzucht RIR line (692 females) and a Hy-Line WPR line (290 progeny tested males). We analyzed the selected autosomal QTL regions on the three populations with SNP markers at 4-13 SNPs/Mb density. QTL for egg shell quality were replicated on all studied regions in the F_2. New QTL were detected for egg shell color on chromosomes 3 and 6. Marker associations to egg shell quality traits were validated in the tested commercial lines on chromosomes 2, 3, and 6, thus paving the way for marker assisted selection for improved egg shell quality.

Keywords: chicken, eggshell quality, quantitative trait locus, SNP marker, association.

An intact, high quality shell is an effective barrier against pathogen infections of the egg (Denagamage et al. 2015). Compromised egg shell quality causes considerable economic losses for the egg industry (Hamilton et al. 1979). Losses can be reduced by nutrition, disease control, good management practices and the use of genetic lines with superior quality eggshell traits. Improving shell quality by genetic selection, however, can be challenging (Cordts et al. 2002). The trait is sex-limited and for males, the breeding value estimated from the full-sibs' performance and other female relatives has to be used and there is a relatively low correlation between eggs which get damaged and the shell strength measurements used as selection traits (Cordts et al. 2002). Egg shell quality would therefore benefit from marker assisted- or genomic selection, to select sires for their direct contribution to the trait and would allow implementation of measurements that are difficult to measure (Dunn et al. 2009). Previous studies have indicated 136 quantitative trait loci (QTL) affecting different egg shell quality traits (Chicken QTL database, 2016 (http://www.animalgenome.org/cgi-bin/QTLdb/GG/oview?qc=1&srchtr=egg shell&qtype=QTL), but have not pinpointed the specific markers that could be used for selection. Differential gene expression and protein profiling have revealed candidate biological markers for eggshell quality (Brionne et al. 2014; Fulton et al. 2012; Marie et al. 2015; Takahashi et al. 2010), however, the objective of this study was to identify genetic markers for selection to improve eggshell quality to increase food safety and reduce waste and economic losses.

We selected the most promising autosomal QTL regions affecting egg shell quality on chromosomes 2, 3, 6, 14 from our earlier experiment (Tuiskula-Haavisto et al. 2011). On chromosome 2 the traits associated with the QTL were deformation (DE) and breaking force (BF), on chromosome 3 breaking force, on chromosome 6 deformation and shell weight, and on chromosome 14 deformation. The F_2 population was extended to comprise 1599 females, including the 668 F_2 hens from the first study, with the phenotypes measured described in Tuiskula-Haavisto et al. (2011). The F_2-population was initially created by reciprocal crossing of Rhode Island Red

(RIR) and White Plymouth Rock (WPR) lines from Lohmann Tierzucht GmbH to generate the F_1 generation, from which 20 males and 120 females were crossed to produce the F_2. The study was replicated on two commercial populations with similar egg quality phenotypic recordings. Material from the Lohmann RIR consisted of 692 females from a generation 10 years after the P-generation and Hy-line WPR line material included 290 progeny tested males. Detailed information on the phenotypes and their variability in the populations is given in Supporting information Table S1.

The traits measured in the Lohmann RIR line were: eggshell dynamic stiffness (DS) which depends on the acoustic properties of the egg (Bain et al. 2006), total shell thickness (in mm), shape index, breaking force (in N), shell color (SC), and cuticle deposition (CC) measured by specific staining intensity (Bain et al. 2013). Shell thickness was measured by scanning electron microscopy as previously described (Dunn et al. 2005; Dunn et al. 2009; Dunn et al. 2012). Shell color was measured as part of the cuticle measurement using reflectance at 650 nm before staining (Bain et al. 2013).

The phenotypic data of Hy-line WPR line consisted of estimated breeding values calculated from measurements from the breeding program at beginning, early and late laying period (26 and 42 weeks of age) for egg color and speckles (small areas of darker pigmentation) on the shell and puncture score for egg shell strength.

Preparation of DNA was described previously for the F_2, WPR (Honkatukia et al. 2013) and the RIR line (Dunn et al. 2004). A set of 845 SNPs were selected from the associated regions on chromosomes 2, 3, 6 and 14 and genotyped by Illumina BeadXpress at Luke (Natural Resources Institute Finland) and Roslin. Genotyping of the RIR line was carried out by Kbiosciences (Hoddesdon, Herts, UK.). The SNP's positions are according to Galgal4 (http://jul2016.archive.ensembl.org/Gallus_gallus/Info/Index).

A total of 468 informative SNP markers were analysed in the F_2 population using Linkage Disequilibrium and Linkage Analysis (LDLA) with the GridQTL software (Hernandez-Sanchez et

al. 2009). The regions containing the most significant SNPs in the F_2 were then analyzed in the two commercial lines and are shown in Figure 1.

The RIR line data (phenotypic records) was analysed with a linear model by fitting hatch/house (h), and tier (t) as fixed effects, and the marker genotypes (g), together with sires (s) and error (e) as random effects to the responses (y), as $y_{ijkl} = s_i + h_j + t_k + g_l + e_{ijkl}$. The WPR data (estimated breeding values) was analysed with a linear model by fitting year (a) as a fixed effect, and the marker genotypes (g), together with sires (s) and error (e) as random effects to the responses (y), as $y_{ijk} = s_i + a_j + g_k + e_{ijk}$. Linear models were fitted by REML (Genstat v13), followed by approximate Student t-tests to assess marker effects. The additive effect of each marker was estimated as half the difference between homozygote mean values and the dominance effect as the difference between the heterozygote mean value and the average of the homozygote mean values. Additive effects and the variances explained for associated markers are shown in Table 1.

A multiple testing adjustment was omitted, as the loci in this study are based on preselected QTL regions. A nominal significance threshold of 0.05 was used to indicate replication of QTL in the extended F_2 and validation of QTL in the commercial lines. A nominal significance threshold of 0.001 was used to indicate discovery of new QTL. All results with nominal p-values <0.05 are shown in Figure 1 and in Supporting information Table S2.

The QTL affecting egg shell breaking force and/or deformation were replicated in the F_2 on all studied regions, although the trait was not always at the same age or precisely identical. On chromosome 2, two QTL for deformation (DE50 and DE) were detected. The phenotypic variances explained by these QTL were of the same magnitude as in the original scan (Tuiskula-Haavisto et al. 2011) - for DE50 5.7 % of the phenotypic variance and for DE 1.9 %. On chromosome 3, the traits BF35, DE, DE35 and DE40 show QTL within the same interval at Mb 79.3- 80.2. On chromosome 6, a QTL for BF35 was detected. On chromosome 14, three QTL affecting BF40, DE40, and DE50 were detected.

Also some new QTL (p<0.001) were detected. In the F_2, QTL affecting shell weight and shell color were detected on chromosome 6. The new SC QTL explains 4 % of the total phenotypic variance. On chromosome 3, in the WPR two SNP markers Mb had significant effects on early shell color and early speckles; rs14381923 and rs14383250. In addition, a single marker on chromosome 14, a synonymous variation in *periplakin* (*PPL*) gene, showed potential association to shell color in both RIR (p<0.05) and WPR (p<0.01). Although shell color was not the intended target of this study, the QTL are interesting because the color of the egg shell is an important quality parameter and has an influence on consumers' preference (Samiullah et al. 2015).

Many shell quality QTL were validated for similar or correlated traits in the RIR and WPR pure lines within or very close to the regions detected from the F_2. On chromosome 2, in the RIR two SNP markers rs14230405 and rs14230514 were associated with breaking strength and shape index, dynamic stiffness, effective thickness, static stiffness and total thickness. In WPR the marker rs16093617 was associated with puncture score and speckles late. On chromosome 3, three markers analyzed in the RIR line showed association with cuticle coverage (difference in reflectance at 650 nm after-before staining). In addition, rs15407105 was also positively associated with dynamic stiffness. Three markers had effect on puncture score in the WPR: rs16298113 (Mb 70.7), rs15403174 (Mb 78.5) and rs14383958 (Mb 79.1). On chromosome 6, rs14581786 was associated with dynamic stiffness and cuticle coverage in the RIR.

The validated egg shell strength QTL areas include genes/proteins that are differentially expressed in the shell gland when the egg shell is forming. These genes include *desmoglein 2* (*DSG2*) and *transthyretin* (*TTR*) (Marie et al. 2015; Sun et al. 2013) on chromosome 2, and *filamin A interacting protein 1* (*FILIP1*) (Brionne et al.2014) and *collagen type XII alpha 1 chain* (*COL12A1)* (Zhang et al. 2015), on chromosome 3. Desmogleins are calcium- binding components involved in cell-cell adhesion (Klessner et al. 2009). The associated SNP rs16093617 in WPR, is a missense mutation in exon 13 of *DSG2*, but it is estimated to be tolerated (0.25) by SIFT analysis.

Transthyretin is involved in calcium metabolism through its association with thyroid hormone transport, and therefore a candidate gene for egg shell quality. *Type XII collagen* is involved in the regulation of bone formation (Izu et al. 2011) but it is not one of the principal collagens in shell formation. On chromosome 3, two SNPs associated with cuticle coverage in RIR, locate within gene introns, rs15406314 in *IMPG1* (*interphotoreceptor matrix proteoglycan 1*) and rs15407105 in *FILIP1*.

To conclude, a subset of the original QTL effects were validated in new, independent material. This is an important finding for the use of associated markers in selection - these QTL are actually segregating within commercial lines and not just in an experimental population created from different lines.

So by combining data from a number of sources it is possible to get a clearer concept of genome regions which are important to eggshell quality traits which can underpin greater understanding of egg shell formation and importantly its improvement. Although, it remains to be shown if SNPs in these genes are causative for variation in egg shell quality, and could be useful as markers for selection, this data will, increase confidence in areas to target.

Acknowledgements

This work was supported by the European Commission through FP6 Project FOOD-CT-2006-016250 “SABRE”.

References

Bain M.M., MacLeod N., Thomson R. & Hancock J.W. (2006). Microcracks in eggs. Poultry Science 85, 2001-8.

Bain M.M., McDade K., Burchmore R., Law A., Wilson P.W., Schmutz M., Preisinger R. & Dunn I.C. (2013) Enhancing the egg's natural defence against bacterial penetration by increasing

cuticle deposition. Animal Genetics 44, 661-8.

Brionne A., Nys Y., Hennequet-Antier C. & Gautron J. (2014) Hen uterine gene expression profiling during eggshell formation reveals putative proteins involved in the supply of minerals or in the shell mineralization process. BMC Genomics 15, 220.

Cordts C., Schmutz M. & Preisinger R. (2002) New alternatives for improving egg shell stability through breeding. Lohmann Information 26, 1-4.

Denagamage T., Jayarao B., Patterson P., Wallner-Pendleton E. & Kariyawasam S. (2015) Risk factors associated with *Salmonella* in laying hen farms: Systematic review of observational studies. Avian Diseases 59, 291-302.

Dunn I.C., Bain M., Edmond A., Wilson P.W., Joseph N., Solomon S., De Ketelaere B., De Baerdemaeker J., Schmutz M., Preisinger R. & Waddington D. (2005) Heritability and genetic correlation of measurements derived from acoustic resonance frequency analysis; a novel method of determining eggshell quality in domestic hens. British Poultry Science 46, 280-6.

Dunn I.C., Joseph N.T., Bain M., Edmond A., Wilson P.W., Milona P., Nys Y., Gautron J, Schmutz M, Preisinger R. & Waddington D. (2009) Polymorphisms in eggshell organic matrix genes are associated with eggshell quality measurements in pedigree Rhode Island Red hens. Animal Genetics 40, 110-4.

Dunn I.C., Rodriguez-Navarro A.B., Mcdade K., Schmutz M., Preisinger R., Waddington D., Wilson P.W. & Bain M.M. (20121) Genetic variation in eggshell crystal size and orientation is large and these traits are correlated with shell thickness and are associated with eggshell matrix protein markers. Animal Genetics 43, 410-8.

Fulton J.E., Soller M., Lund, A.R., Arango J. & Lipkin E. (2012) Variation in the *ovocalyxin-32* gene in commercial egg-laying chickens and its relationship with egg production and egg quality traits. Animal Genetics 43, 102-13.

Hamilton R.M.G., Hollands K.G., Voisey P.W. & Grunder A.A. (1979) Relationship between egg shell quality and shell breakage and factors that affect shell breakage in the field - a review. World's Poultry Science Journal 35, 177-90.

Hernandez-Sanchez J., Grunchec J.A. & Knott S. (2009) A web application to perform linkage disequilibrium and linkage analyses on a computational grid. Bioinformatics 25, 1377-83.

Honkatukia M., Tuiskula-Haavisto M., Arango J., Tabell J., Schmutz M., Preisinger R. &Vilkki J. (2013) QTL mapping of egg albumen quality in egg layers. Genetics, Selection, Evolution 45, 31.

Izu Y., Sun M., Zwolanek D., Veit G., Williams V., Cha B., Jepsen K.J., Koch M. & Birk D.E. (2011) Type XII collagen regulates osteoblast polarity and communication during bone formation. Journal of Cell Biology 193,1115–30.

Klessner J.L., Desai B.V., Amargo E.V., Getsios S. & Green K.J. (2009) EGFR and ADAMs cooperate to regulate shedding and endocytic trafficking of the desmosomal cadherin desmoglein 2. Molecular Biology of the Cell 20, 328-37.

Marie P., Labas V., Brionne A., Harichaux G., Hennequet-Antier C., Nys Y. & Gautron J. (2015) Quantitative proteomics and bioinformatic analysis provide new insight into protein function during avian eggshell biomineralization. Journal of Proteomics 113, 178-93.

Samiullah S., Roberts J.R. & Chousalkar K. (2015) Eggshell color in brown-egg laying hens - a review. Poultry Science 94, 2566-75.

Sun C., Xu G. & Yang N. (2013) Differential label-free quantitative proteomic analysis of avian eggshell matrix and uterine fluid proteins associated with eggshell mechanical property. Proteomics 13, 3523-36.

Takahashi H., Sasaki O., Nirasawa K. & Furukawa T. (2010) Association between *ovocalyxin-32* gene haplotypes and eggshell quality traits in an F2 intercross between two chicken lines

divergently selected for eggshell strength. Animal Genetics 41, 541-44.

Tuiskula-Haavisto M., Honkatukia M., Preisinger R., Schmutz M., De Koning D.J., Wei W.H. & Vilkki J. (2011) Quantitative trait loci affecting eggshell traits in an F(2) population. Animal Genetics 42, 293-9.

Zhang Q., Zhu F., Liu L., Zheng C.W., Wang de H., Hou Z.C. & Ning Z.H. (2015) Integrating transcriptome and genome re-sequencing data to identify key genes and mutations affecting chicken eggshell qualities. PLoS One 10, e0125890.

Figure legend

Figure1. The analysed regions on chromosomes 2, 3, 6 and 14 (GGA2, GGA3, GGA6, GGA14). The location on each chromosome is indicated as Mb on the bar on the left side. Positions of potential candidate genes are indicated on this bar. The regions analysed in each population (F_2, RIR, WPR) are shown as bars, where associations ($p<0.05$) are indicated by colored marks (green, egg shell quality; blue, egg shell color; red, egg shell weight). The number of analysed markers within each population (per region on GGA2, GGA3, GGA6, GGA14, respectively) were: F_2 468 (124, 89, 85,170), RIR 9 (3, 3, 1, 2), WPR 51 (6, 24, 1, 20). The phenotypes included in each trait category are: Egg shell quality: breaking force and deformation at different ages, dynamic stiffness, static stiffness, effective thickness, total thickness, shape index, puncture score, cuticle coverage; Egg shell colour: shell color at different ages, speckles at different ages, SCL_45; Egg shell weight: shell weight.

Table 1. Additive effects and variances explained for the associated markers in commercial lines.

The trait abbreviations stand for: DE, BF indicate deformation or breaking force mean or at specific weeks of age SW: shell weight; DS: dynamic stiffness; ET: effective thickness; SI: shape index; SS: static stiffness; TT: total thickness;, PS: puncture score; SPe: speckles early; SPl speckles late; SC3:

shell color of the three first eggs; SCe: shell color early, SCl: shell color late, CCa: reflectance at 650 nM after staining; CCb: reflectance at 650 nM before staining, CCd;cuticle coverage (difference in reflectance at 650 nM after - before staining)

Supporting Information

Table S1. The variances of the measured egg quality traits in the analysed populations. a) F2, b) RIR, c) WPR.

Table S2. Significant associations with egg shell traits by region (flanking SNPs) in the F_2 population or associated marker in Lohmann RIR or Hy-line WPR line. p<0.05*, P<0.01**, P<0.001*** For trait abbreviations and descriptions, see table S1.