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QTL Mapping of Stress Related Gene Expression in a Cross between Domesticated Chickens and Ancestral Red Junglefowl

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Abstract

Domestication of animals is associated with numerous alterations in physiology, morphology, and behavior. Lower reactivity of the hypothalamic-pituitary-adrenal (HPA) axis and reduced fearfulness is seen in most studied domesticates, including chickens. Previously we have shown that the physiological stress response as well as expression levels of hundreds of genes in the hypothalamus and adrenal glands are different between domesticated White Leghorn and the progenitor of modern chickens, the Red Junglefowl. To map genetic loci associated with the transcription levels of genes involved in the physiological stress response, we conducted an eQTL analysis in the F₁₂ generation of an inter-cross between White Leghorn and Red Junglefowl. We selected genes for further studies based on their known function in the regulation of the HPA axis or sympathoadrenal (SA) system, and measured their expression levels in the hypothalamus and the adrenal glands after a brief stress exposure (physical restraint). The expression values were treated as quantitative traits for the eQTL mapping. The plasma levels of corticosterone were also assessed. We analyzed the correlation between gene expression and corticosterone levels and mapped eQTL and their potential effects on corticosterone levels. The effects on gene transcription of a previously found QTL for corticosterone response were also investigated. The expression levels of the *glucocorticosteroid receptor* (*GR*) in the hypothalamus and several genes in the adrenal glands were correlated with the post-stress levels of corticosterone in plasma. We found several cis- and trans-acting eQTL for stress-related genes in both hypothalamus and adrenal. In the hypothalamus, one eQTL for *c-FOS* and one QTL for expression of *GR* were found. In the adrenal tissue, we identified eQTL for the genes *NR0B1*, *RGS4*, *DBH*, *MAOA*, *GRIN1*, *GABRB2*, *GABRB3*, and *HSF1*. None of the found eQTL were significant predictors of corticosterone levels. The previously found QTL for corticosterone was associated with *GR* expression in hypothalamus. Our data suggests that domestication related modification in the

stress response is driven by changes in the transcription levels of several modulators of the HPA and SA systems in hypothalamus and adrenal glands and not by changes in the expression of the steroidogenic genes. The presence of eQTL for *GR* in hypothalamus combined with the negative correlation between *GR* expression and corticosterone response suggests *GR* as a candidate for further functional studies regarding modification of stress response during chicken domestication.

Keywords: animal domestication, stress response, HPA axis, QTL, eQTL

1. Introduction

Domestication of animals is a rapid evolutionary process, which has generated large phenotypic variation that can be used as an efficient model to unravel the genetics of different traits (Jensen, 2006; Albert et al., 2009; Andersson, 2012). For example, aggression and fearfulness are commonly attenuated in domesticates because of the relaxed selection for such traits combined with the active or passive selection against them by humans (Mignon-Grasteau et al., 2005; Jensen, 2006; Jensen, 2010; Wright et al., 2010). Domesticated animals typically show reduced fear of humans, which is heritable and associated with the changes in DNA-methylation and gene expression in the brain (Albert et al., 2008; Kukekova et al., 2011; Béltéky et al., 2016), as well as a modified stress response. Understanding the genetics of these modifications has strong implications for both fundamental stress biology and for practical considerations, since stress is a major welfare problem in modern animal farming (Jensen et al., 2008).

Here, we focus on the chicken, the most common food producing animal in the world (Dawkins et al., 2004). Domesticated chickens originate from the Red Junglefowl native to Southeast Asia, first domesticated approximately 8000 years ago (Fumihito et al., 1994). We have previously compared their stress responses with the domesticated White Leghorn breed, selected mainly for its increased egg laying capacity, and a genetic mapping population based on an intercross between White Leghorn and Red Junglefowl. Similar to other domesticates, White Leghorn shows lower fearfulness and hypothalamic-pituitary-adrenal (HPA) reactivity as compared to Red Junglefowl (Ericsson et al., 2014; Fallahsharoudi et al., 2015). We have shown that the behavioral and physiological differences between the breeds are associated with changes in the transcription levels of hundreds of genes in the adrenal glands and the brain (Nätt et al., 2012; Fallahsharoudi et al., 2015).

To further unravel the genetic architecture of the domestication induced changes in the stress response, we have used quantitative trait locus (QTL) mapping in the present study. We have previously successfully used a technique whereby eQTL and QTL mapping are combined within the same group. This enables each gene within the QTL confidence interval to first be assessed for the presence of an eQTL, and then to assess the correlation between gene expression and the phenotype. Using this technique we have successfully identified putative causal genes for both anxiety behavior (Johnsson et al., 2016) and bone density (Johnsson et al.), as well as a gene for corticosterone response (Fallahsharoudi et al., 2016).

The stress response is a complex process, involving brain neurotransmitters and coordinated central and peripheral processes. Numerous stimuli can initiate the stress response, but regardless of the stimuli, it encompasses the activation of the HPA axis and sympathoadrenal (SA) system (Glaser and Kiecolt-Glaser, 2005). Specific neurons in the paraventricular nucleus of the hypothalamus secrete corticotropin-releasing hormone (CRH) as well as other peptide hormones like arginine vasopressin (AVP) into the hypophyseal portal plexus of veins. The veins transport CRH and AVP into the anterior lobe of the pituitary gland, where they bind to their designated receptors (CRHR1, CRHR2, AVPR1), leading to production and release of adrenocorticotrophic hormone (ACTH) (Herman et al., 2016). Melanocortin Receptor Type 2 (MC2R) in the adrenal cortex is the main target of ACTH. The adrenal glands of birds and rodents respond to ACTH by producing and releasing corticosterone (reviewed by (Payne and Hales, 2004; Stocco et al., 2005)).

The aim of the study was to unravel some of the genetic factors involved in the modification of the stress response induced by domestication of chickens. Furthermore, we aimed to assess downstream as well as trans-acting effects of stress related genes within this intercross.

We used an advanced intercross between domesticated White Leghorn and the ancestral Red Junglefowl to detect eQTL associated with genes, which are known to be involved in the regulation of HPA and SA reactivity. Then we looked at the effects of the identified loci on the HPA axis. We have previously found a significant QTL for stress levels of corticosterone in plasma and reported the putative underlying gene (Fallahsharoudi et al., 2016). In the study presented here, we have gone further to assess 46 candidate genes known to be involved in the regulation of the HPA axis and SA system in adrenal or cortical tissue for possible correlations with corticosterone reaction to stress and for potential eQTL associated with them.

2. Method

2.1. Ethical statement

All experimental protocols were approved by Linköping Council for Ethical Licensing of Animal Experiments, ethical permit no 122-10. Experiments were carried out in accordance with the approved guidelines.

2.2. Animals and Rearing

A total of 232 birds from the 12th generation of an intercross between domesticated White Leghorn and ancestral Red Junglefowl were used for hormone measurements and genotyping. The birds were hatched in 3 batches. The original cross was previously described in detail by (Schütz et al., 2002; Wright et al., 2006b; Johnsson et al., 2016). Briefly, the intercross was generated by crossing one Red Junglefowl male and three White Leghorn females to get 41 F₁ and then 821 F₂ animals. The subsequent generations were kept at population sizes of more than 100 chickens per generation. The animals of the present study were hatched in one batch from 15 families. All birds were pedigree hatched and raised in 4 m² pens with ad libitum

access to food and water. The birds were kept together until sampling at six weeks of age, at which point they were culled.

2.3. Blood Sampling and Tissue collection

For gene expression and eQTL mapping, 86 animals (all from the second batch) were used. The method for stress treatment and blood sampling is previously published (Fallahsharoudi et al., 2015). Briefly, around 500 μ L blood was drained from the wing vein before and after 10 minutes of restraint in a net. The birds were immediately killed with decapitation after the second blood sampling for tissue collection. The entire adrenal glands and the brain region containing thalamus / hypothalamus were dissected and immediately snap frozen in liquid nitrogen. The tissues were kept in -80 °C for further analysis. The blood samples were centrifuged at 2000 g for five minutes to separate the plasma. Plasma samples were kept in -80 °C for further analysis.

2.4. RNA Extraction and Gene Expression

RNA was extracted from the frozen brain and adrenal tissues using AllPrep RNA Mini Kit (Qiagen, Germany) and the potential residual DNA was digested on-column using RNase-Free DNase Set (Qiagen, Germany) during extraction according to manufacturer's instructions. The RNA quality was checked with the Agilent 2100 bioanalyzer and all the RIN values were above 9. The single stranded cDNA was prepared using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, USA) using 2.5 μ g total RNA as template. In hypothalamus, the expression levels of *GR*, *CRH*, *AVP*, *POMC*, *EGR1*, *FOS*, and *FOSL* were measured with Light Cycler 480 (Roche Diagnostics, Basel, Switzerland). Each reaction included 2 μ L water, 1 μ L of each forward and reverse primers (0.5 μ M), 125 pg cDNA diluted in 1 μ L water, and 5 μ L SYBR Green I Master (Roche Diagnostic) as reported previously (Fallahsharoudi et al., 2015). The adrenal gene expression was conducted using

TaqMan Array® Card (Termo Fisher Scientific, USA) with 100 ng cDNA as template for 48 RT reactions. The Real-Time PCR was performed on the Applied Biosystems® Vii™ 7Real-Time PCR system using universal cycling conditions (95 °C/10 min, then 95 °C/15 sec, 60 °C/60 sec for 40 cycles). The corresponding CT values were normalized over *GDP* as housekeeper (Pfaffl, 2001). We measured transcription levels of genes involved in five different pathways. The first group covers the receptors which respond to hypothalamic and pituitary peptides; *MC2R*, *MRAP*, *MRAP2*, *BZRAP1*, *DAX1*, *FDX1*, *AGTRAP*, *RGS2*, *RGS4*, and *RGS18*. The second group of measured genes include *STAR*, *STARD4*, *STARD9*, *CH25H*, *CYP11A1*, *CYP17A1*, *CYP21A2*, *HSD3B2*, and *HSD11B1L*. The third gene group are the adrenal catecholaminergic enzymes including *TH*, *DBH*, *MAOA*, and *MAOB*. Group four consists of *GABA* and glutamate receptors including *GABRB2*, *GABRB3*, *SLC6A1*, *GRIA3*, and *GRIN1*. The last group of measured genes consists of *FOS*, *FOSL*, and several heat shock factors, which respond to a variety of stressors. The list of all genes and their accession numbers is provided (Supplementary Table S1).

2.5. Genotyping and Genetic Map

We used DNeasy Blood & Tissue Kit (Qiagen, Germany) for DNA extraction according to the manufacturer's instructions. We used 739 genetic markers to construct the genetic map. The genotyping was performed by Illumina Golden Gate Assay at NGI Uppsala (SNP&SEQ Technology Platform) in Uppsala sequencing and SNP platform. The GoldenGate genotyping assay uses allele-specific probes that hybridize to the DNA sample. The sequences are extended, ligated, amplified and labeled with different fluorophores for the two alleles. The amplification products also contain unique address sequences that bind to particular beads. The beads are then immobilized on an array and scanned (Shen et al., 2005). We included the fully informative markers from the previous F₈ analyses (Johnsson et al., 2016), and designed another 200 informative markers, based on whole-genome resequencing of the founders

(unpublished data). Because of linkage in the advanced intercross, hundreds rather than thousands of markers are sufficient to cover the genome. Also, a marker is most useful if it is fully informative, i.e. a homozygous difference between the founders of the cross. Therefore, it was most cost-effective to use a custom SNP genotyping assay, rather than a commercially available SNP chip. Total map length was 18500 cM and average spacing was 26 cM. The map construction and QTL mapping was conducted with R/qtl (Broman et al., 2003). The map size of was considerably longer than in previous studies (Groenen et al., 2000; Wright et al., 2006a) where the genetic map in the F₂ generation was around 3000 cM in both cases. Due to accumulation of recombination events in each new generation, map expansion is expected for advanced intercrosses (Darvasi and Soller, 1995). In F₈ of the present intercross the map was already expanded to ~ 9000 cM (Johnsson et al., 2016), and the size of the map in the present study was due to the additional four generations.

2.6. Hormonal Analysis

Hormones were analyzed with Ultra-Performance Convergence Chromatography (UPC2; Waters ACQUITY® UPC2™, Milford, MA) hyphenated with tandem mass spectrometry (XEVO® TQ-S, Milford, MA). The qualitative analysis was performed at 40 °C using an Acquity UPC2 BEH column (100 mm 3.0 mm, 1.7 µm; Waters, Milford, MA, USA). The quantification of steroid hormones was done with multiple reaction monitoring (MRM) coupled with stable isotope dilution mass spectrometry. Duplicate analysis of each sample were performed and the average values were reported (CV <10 %). The detailed methods for hormone analysis are previously published (Ericsson et al., 2014; Fallahsharoudi et al., 2015).

2.7. QTL Analysis

To detect single QTL we examined the data with interval mapping and Haley-Knott regression (Haley and Knott, 1992). The normalized gene expression values were treated as

quantitative traits. In all analyses, batch and sex were included in as fixed effects. A principal component analysis (PCA) of the genotype matrix was performed with R version 3.3.1 (using the function “prcomp”) and 10 PCs with the strongest effects were included in all models as covariates to account for hidden family structure in the advanced intercross (Wu et al., 2011), and the significant PCs were maintained in the final model (Wu et al., 2011). For each trait, we calculated the significant thresholds with 1000 permutations on the full F₁₂ map. A genome-wide 20 % P-value cut off was used for suggestive QTL and P-values at 5 % or less were reported as significant QTL (Center, 1995). The “1.8 drop in LOD” method was used to obtain the 95% confidence intervals (CI) (Manichaikul et al., 2006) and, CIs were extended to the closest marker. The physical location of the closest markers to the 95 % confidence interval for each QTL is reported in the chicken genome (galGal4 genome assembly). To study the correlation between transcription levels and corticosterone response, a regression model with corticosterone response as response variable and transcription levels of each measured genes as explanatory variables were fit (corticosterone response ~ transcription levels + sex + PCs). Corticosterone and transcription levels were continuous variables, while sex consisted of two levels; batch was omitted from analysis because all birds, which were used for gene expression, were in one batch. Ten strongest PCs, which were obtained from the genotype matrix, were included in all models to deal with the relatedness in the advanced intercross. The P-values were adjusted for multiple testing for all regression tests, with FDR correction based on the total number of measured genes in each tissue. We also tested if the eQTL have an effect on corticosterone response (corticosterone response ~ eQTL marker + sex + batch + PCs). Another model was fitted to study the effect of the previously found QTL for corticosterone on gene expression. The model contained the genotype at the QTL marker (for corticosterone response) as fixed factor and normalized transcription levels as dependent variable, as well as sex and family structure (PCs). In the case of eQTL mapping, it is possible

to separate between cis-acting (local) effects and trans-acting (distant) effects. In the case of cis-acting eQTL, only the region immediately adjacent to the gene in question is assessed, thus any detected cis-eQTL are controlled by elements close to the gene itself (and a reduced multiple testing threshold is required for significance as only this local region is assessed for linkage). In the case of trans-acting eQTL, the whole genome is assessed for potential linkage, meaning the controlling element can be situated anywhere in the genome, and the multiple testing threshold is consequently higher.

3. Results

The average levels of baseline corticosterone was 1.2 ng/mL (SD = 0.9), and the average levels of corticosterone after 10 minutes of restraint stress was 6.2 ng/mL (SD = 3.1) (Fig. 1). We first assessed the correlations between transcription levels of a range of genes in hypothalamus and adrenal glands on the plasma levels of corticosterone after a brief session of restraint stress. The results showed that the expression levels of the glucocorticoid receptor (*GR*) in the brain were negatively correlated with corticosterone response. In adrenal glands, expression levels of hydroxysteroid (11-beta) dehydrogenase 2 (*HSD11B2*), dopamine beta-hydroxylase (*DBH*), heat shock transcription factor 1 (*HSF1*), cellular oncogene c-Fos (*c-FOS*), gamma-aminobutyric acid receptor beta-2 (*GABRB2*), and peripheral benzodiazepine receptor (*PBR*) were significantly correlated with corticosterone response (Fig. 2).

Several QTL were detected for the expression levels of the measured genes in hypothalamus and adrenal glands. In hypothalamus, one genome-wide significant trans-eQTL for transcription levels of *GR* was detected on chromosome 3. We also detected one significant trans-eQTL for *c-FOS* (chromosome 5) in hypothalamus (Fig. 3 and Supplementary Table S2). No significant eQTL were found for the expression levels of *AVP*, *CRH*, *POMC*, and *EGR1* in the hypothalamus. In adrenal glands, we identified significant

trans-eQTL for the genes *NR0B1*, *RGS4*, *MAOA*, *GRIN1*, *HSF1*, and *DBH*, and cis-eQTL for the *GABRB2* and *GABRB3* (Fig. 3 and Supplementary Table S2). None of the found eQTL was correlated to plasma levels of corticosterone.

We also investigated the potential effects of a previously reported corticosterone response QTL on chromosome 5 (Fallahsharoudi et al., 2016) on the expression levels of all measured genes. Out of the analyzed genes, this QTL only affected the expression of *GR* in hypothalamus (Fig. 4). The White Leghorn allele was associated with lower levels of *GR* transcription in hypothalamus, which is in agreement with the effect on corticosterone response. *GR* did not have overlapping QTL with any corticosterone QTL.

4. Discussion

We found several correlations between expression levels in the hypothalamus and the adrenal glands and the corticosterone response to an acute stress in an intercross between domesticated White Leghorn and ancestral Red Junglefowl. We also detected several cis- or trans-eQTL for expression of genes that are involved in regulation of the stress system.

The expression of *GR* was negatively associated with corticosterone response. This is in agreement with the fact that glucocorticoid receptors in the brain inhibit HPA activity through negative feedback (Ridder et al., 2005), and *GR* levels are dynamically regulated by glucocorticoids themselves (Herman and Spencer, 1998). Another transcript found to be negatively correlated with the corticosterone response was *HSD11B2* in the adrenal glands. The gene codes for 11-beta-hydroxysteroid dehydrogenase, which in birds is responsible for metabolism and degradation of corticosterone in the adrenal glands (Tanabe et al., 1986; Carsia, 2015).

Furthermore, the adrenal expression of *PBR*, *GABRB2*, and *DBH* were negatively associated with corticosterone response. *PBR* is closely associated with *StAR* in

mitochondrial membranes and affects the transportation of cholesterol across mitochondrial membranes in steroidogenic cells (West et al., 2001). GABA receptors mediate inhibitory effect of GABA in the central and peripheral nervous system (Chen et al., 2005; Kato et al., 2014). Unlike in mammals, the chromaffin and steroidogenic cells are mixed in birds, facilitating a robust synaptic and paracrine interaction between secretion of adrenalin and corticosterone (Nussdorfer et al., 1997; Carsia, 2015). In the case of *GABRB2*, this gene was previously found to influence anxiety-related behavior in the same wild x domestic chicken intercross, and shows links with anxiety behavior in mice (Johnsson et al., 2016).

The adrenal expression level of *HSF1* was positively correlated with corticosterone response. Restraint stress is known to induce rapid expression of various heat shock proteins in adrenal cortex and the effect is mainly mediated through the action of *HSF1* (Blake et al., 1991; Nakai and Morimoto, 1993; Holbrook and Udelsman, 1994). The correlation between expression of *HSF1* and *c-FOS* on one side and corticosterone response on the other may therefore be a consequence of the physiological pathways involved. The models were designed to test the association between expression of each gene independently and corticosterone response. Genetic variation, as well as complex dynamic interactions with environmental factors, and interactions between cells determine the levels of mRNA (Chesler et al., 2005), and hence, expression of functionally similar genes are not independent within individuals. We could not detect a single genetic region that regulate the expression of the genes being studied, and hence were not able to disclose the genetic causes of the correlations.

We did not find any association between the corticosterone response and the expression of *CRH*, *AVP*, and *POMC* in hypothalamus and *CYP11A1* and *HSD3B2* in adrenal glands. This is in accordance with previous studies in rat, mouse and domesticated pigs (Désautés et al., 2002; Solberg et al., 2006), where lowered HPA reactivity was not associated with changes in the mRNA levels of steroidogenic genes. It is worth noting that the

expression of the mentioned early stress genes in hypothalamus is dynamic (Herman et al., 2016) and hence, a time lag between transcription and corticosterone response is expected. We found several loci that affected the transcription levels of genes that are involved in regulation of the HPA axis and SA system (Fig. 3 and Table S1). *NR0B1* (*DAX-1*) is a nuclear receptor transcription factor that functions as global inhibitor of steroid hormone production by suppressing the expression of several genes involved in the steroidogenic pathway (Lalli and Sassone-Corsi, 2003). *RGS4* is a regulator of GPCR and (Romero et al., 2007) and, is involved in modulation of lipolysis in adipose tissue as well as regulation of catecholamine secretion by the adrenal glands (Iankova et al., 2008). Both metabolism and SA system have been modified during domestication or selection for low fearfulness (Agnvall et al., 2015; Elfving et al., 2015).

Some of the eQTL confidence intervals overlapped with the physical location of the respective genes, which suggests that both cis- and trans-regulatory elements affect transcript abundance in our advanced intercross. The only overlapping (where the confidence intervals overlap) eQTL were found for *DBH* and *MAOA*. These genes code for enzymes involved in production and degradation of adrenalin (Sabban and Kvetňanský, 2001). Most studies regarding the genetics architecture of the stress response are performed on model organism such as mice or rat. Better understanding about genetics and physiology of stress responses in non-model animals can, for example, be used in meta-analysis studies for understanding genetic architecture of complex traits across taxa. It may worth nothing that QTL studies are inherently correlational and hence do not provide knowledge about the underlying mechanism, but they provide information which can further be used to characterize the impact of allelic variation on gene function and ultimately phenotype.

In conclusion, our results showed correlations between stress-induced corticosterone levels and expressions of several functionally important genes in hypothalamus

or adrenal. We detected several eQTL for stress related genes and assessed their impact on corticosterone response. Our data suggest that the modified stress response in domesticated chicken is associated with changes in the expression of genes involved in regulation of HPA and SA system, but that the expression levels of steroidogenic genes have not been affected by domestication.

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Author contribution

AF performed the experiments, analysed the data and wrote the paper with input from all other authors. NdK, SJK, AU and JB performed the hormone analyses. MJ, LB and DW assisted in the genetic analysis. PJ conceived, coordinated and supervised the experiment.

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Fig. 1. Corticosterone response to stress. Plasma concentration of corticosterone before (n = 86) and after 10 minutes of restraint stress (n = 82) in the advanced intercross between White Leghorn and Red Junglefowl. Each point represent a value from one individual. The bars represent SEM.

Fig. 2. The relation between normalized gene expression in hypothalamus or adrenal glands and plasma levels of corticosterone after 15 minutes of restraint stress. Gene expression is presented as normalized *delta Ct* values. *Glucocorticoid receptor (GR)* (A) level was measured in hypothalamus. *Hydroxysteroid 11-beta dehydrogenase 2 (HSD11B2)* (B), *dopamine beta-hydroxylase (DBH)* (C), *heat shock transcription factor 1 (HSF1)* (D), *proto-oncogene c-Fos (c-FOS)* (E), *gamma-aminobutyric acid type A receptor beta2 subunit (GABRB2)* (F), and *peripheral-type benzodiazepine receptor (PBR)* (G), were measured in adrenal glands. F- and P-values were obtained by regression analysis between expression of each gene and corticosterone response, and the P-values were adjusted by false discovery rate for the total number genes in each tissue.

Fig. 3. Genomic location of all detected QTL and eQTL. Bars represent QTL confidence intervals obtained by 1.8 LOD drop and, extended to the closest marker for *Proto-oncogene c-Fos (c-FOS)* and *glucocorticoid receptor (GR)* in hypothalamus, and *heat shock transcription factor 1 (HSF1)*, *nuclear receptor subfamily 0 group B member 1 (NR0B1)*, *regulator of G-protein signaling 4 (RGS4)*, *dopamine beta-hydroxylase (DBH)*, *monoamine oxidase A (MAOA)*, *glutamate ionotropic receptor NMDA type subunit 1 (GRIN1)*, *gamma-aminobutyric acid type A receptor beta2 subunit (GABRB2)*, and *gamma-aminobutyric acid type A receptor beta3 subunit (GABRB3)* in adrenal glands. Relevant statistics is provided in Supplementary Table S2. The black bars represent trans-acting eQTL, while the blue bars represent cis-eQTL, i.e. when the physical location of the gene lies within eQTL confidence interval.

Fig. 4. Genotype effect on transcription. Effect of Red Junglefowl, heterozygous (Hz), and White Leghorn genotype of the corticosterone QTL marker (chr5:48303445) on expression levels of *Glucocorticoid receptor (GR)* in hypothalamus. LOD is obtained with interval mapping and Haley-Knott regression.

Supplementary Table S1. The list of measured genes in the adrenal glands and their accession numbers.

Supplementary Table S2. Regression based interval mapping in the 12th generation advanced intercross between White Leghorn and Red Junglefowl with or without interaction with sex. Transcript refers to the name of the gene associated with the QTL. Tissue shows where the gene expression analysis was conducted. Chr shows the number of the chromosome where the QTL was found. LOD and R² (explained variance by QTL) are obtained with interval mapping and Haley-Knott regression. Significant thresholds for each trait were obtained with permutation tests, using 1000 permutations based on the full F₁₂ map for each individual gene expression and model and shown in Sig column. Additive and dominance effect sizes are reported (positive additive values indicate a larger QTL effect size in domestic genotype birds and negative values represent a larger value in wild genotype birds). Lower and Higher CI indicates the physical location of closest markers to the QTL confidence interval (1.8 LOD drop) in the chicken genome (galGal4 genome assembly). Interaction implies if there is a significant genotype by sex interaction. Cis-acting eQTL (when the physical location of the gene is within eQTL confidence interval) are marked with *.