Elucidating Molecular Networks That Either Affect or Respond to Plasma Cortisol Concentration in Target Tissues of Liver and Muscle

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ABSTRACT Cortisol is a steroid hormone with important roles in regulating immune and metabolic functions and organismal responses to external stimuli are mediated by the glucocorticoid system. Dysregulation of the afferent and efferent axis of glucocorticoid signaling have adverse effects on growth, health status, and well-being. Glucocorticoid secretion and signaling show large interindividual variation that has a considerable genetic component; however, little is known about the underlying genetic variants. Here, we used trait-correlated expression analysis, screening for expression quantitative trait loci (eQTL), genome-wide association (GWA) studies, and causality modeling to identify candidate genes in porcine liver and muscle that affect or respond to plasma cortisol levels. Through trait-correlated expression, we characterized transcript activities in many biological functions in liver and muscle. Candidates from the list of trait-correlated expressed genes were narrowed using only those genes with an eQTL, and these were further prioritized by determining whether their expression was predicted to be related to variation in plasma cortisol levels. Using network edge orienting (NEO), a causality modeling algorithm, 26 of 990 candidates in liver were predicted to affect and 70 to respond to plasma cortisol levels. Of 593 candidates in muscle that were correlated with cortisol levels and were regulated by eQTL, 2 and 25 were predicted as effective and responsive, respectively, to plasma cortisol levels. Comprehensive data integration has helped to elucidate the complex molecular networks contributing to cortisol levels and thus its subsequent metabolic effects. The discrimination of up- and downstream effects of transcripts affecting or responding to plasma cortisol concentrations improves the understanding of the biology of complex traits related to growth, health, and well-being.

CORTISOL, a steroid hormone released in response to stress and a low level of blood glucocorticoids, acts to increase blood sugar through gluconeogenesis; suppress the immune system; and aid in fat, protein, and carbohydrate metabolism. Produced in the adrenal cortex under the control of the hypothalamic–pituitary–adrenal axis and the sympatho-adrenomedullar (SAM) system, cortisol is most important during times of stress, when it modulates many of the acute responses to illness and “resets” metabolism in favor of providing substrates for oxidative metabolism (Andrews and Walker 1999). Stress factors can unbalance metabolic homeostasis, directing the demand for nutrients

and energy for essential physiological processes away from growth (Heetkamp *et al.* 1995). Individual cortisol concentration is variable and depends on sensitivity to stress, which is highly genetically determined (e.g., heritability estimates of 0.40–0.70 in pigs) (Geverink *et al.* 2002; Kadarmideen and Janss 2009). According to the function of cortisol in the maintenance of basal and stress-related homeostasis the adrenocortical steroidogenesis is sensitive to exogenous biotic and abiotic stressors but also to endogenous signals released from peripheral tissues and organs different from the hypothalamic–pituitary–adrenal (HPA) axis or SAM system. Such effective signals are associated with the plasma cortisol concentration that depends on the rate of adrenal steroidogenesis regulated either directly at the level of genes encoding the corresponding enzymes or indirectly by triggering the HPA or SAM systems. Glucocorticoids mainly exert their effects via members of the nuclear receptor superfamily of ligand-dependent transcription factors, primarily the glucocorticoid receptor (GR) and to a lesser extent through the

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mineralocorticoid receptor (MR). The steroid-receptor complex binds to hormone-responsive elements on the chromatin and regulates gene transcription; *i.e.*, genes addressed by this effective mechanism are responsive to cortisol plasma concentration.

Cortisol concentrations in plasma have been shown to be both directly and indirectly related to behavioral, physiological, and metabolic disease in mammals (de Groot *et al.* 2001; Murani *et al.* 2010, 2011). However, the association patterns of gene expression with cortisol concentrations remain little understood.

Pigs, valued as agricultural commodities, are typically raised in intensive production units that likely introduce various stresses to the animals (Black *et al.* 2001). Stress can negatively affect growth, meat quality, and the immune system. Importantly, pigs share both physiological and genomic similarities with humans and therefore provide a more tractable model in which to study genetic determination of behavioral, physiological, and metabolic traits (Bode *et al.* 2010). As stress can also negatively affect human health, the identification of genes related to cortisol concentrations in pigs may enhance the discovery of genes associated with metabolic, immunological, and behavioral traits in humans.

Processes occurring in both the liver and muscle can be influenced by cortisol concentrations. Indeed, the liver is of particular interest given its vital roles in maintaining homeostasis and health as well as regulating nutrient utilization. On the other hand, muscle is a major consumer of energy and is responsive to cortisol in terms of its metabolic and motor activities. Thus, we used expression analyses in liver and muscle to identify genes related to plasma cortisol concentrations to better understand the biological processes and underlying genes and functional networks influenced by this hormone in target tissues.

Functional genomics provide insight into essential cellular functions whose regulation determines a significant proportion of the phenotypic variance. Transcript profiling is a proximal endophenotype affected by genetic variation (Jansen and Nap 2001) and has been used to dissect the genetic architecture of expression regulatory variation in a number of systems (Morley *et al.* 2004; Cheung *et al.* 2005; DeCook *et al.* 2006; Schadt *et al.* 2008; Swanson-Wagner *et al.* 2009; Ponsuksili *et al.* 2010, 2011) and has aided in the identification of quantitative trait loci (QTL) (Bystrykh *et al.* 2005; Hubner *et al.* 2005). Pairwise associations between gene expression traits and other organismal traits can be used to define an undirected traits network. Recently, causality modeling algorithms have been developed by using genetic markers as causal anchors for orienting the edges of a trait network. These algorithms proved effective in establishing causality by integration of gene expression, QTL, and association (Farber *et al.* 2009a,b; Plaisier *et al.* 2009). Using this approach, we determined the biological function of genes in liver and muscle correlated with plasma cortisol concentrations. We then identified candidate genes in these target tissues that affect or respond to plasma cortisol

concentrations by integrating trait-correlated expression analysis, detection of expression quantitative trait loci (eQTL), genome-wide association (GWA) study, and causality modeling. Finally, causal links between SNP, gene expression, and cortisol concentration were generated.

Materials and Methods

Animals, tissue collection, and phenotyping

Animal care and tissue collection procedures followed the guidelines of the German Law of Animal Protection and the experimental protocol was approved by the Animal Care Committee of the Farm Animal Biology (FBN). This study was based on trait measurements, genotyping records, expression profiles, and genome-wide association analyses done with performance-tested pigs from commercial herds of the crossbreed Pietrain $\times$ [German Large White $\times$ German Landrace (female and male castrates (53%))]. Total cortisol secretion was measured in the morning by collection of 50 ml of trunk blood from each pig during exsanguinations at an age of 180 ± 2 days. Blood was collected in a plastic tube containing 1 ml of 0.5 M EDTA, stored on ice until plasma preparation, and then stored long-term at -80° . Plasma cortisol concentrations (total) were determined using commercially available enzyme-linked immunosorbent assay (DRG, Marburg, Germany) in duplicate according to the manufacturer's protocol. The intra- and interassay coefficients of variation were $\leq 7.0\%$ and 9.8% , respectively.

Whole-genome scan and quality control

Illumina bead array technology was used to carry out all genotyping reactions in accordance with the manufacturer's protocol for the SNP Infinium HD assay (<http://www.illumina.com>). Genotyping was performed using the PorcineSNP60 BeadChip (Illumina, San Diego). In brief, 200 ng of DNA was used for genome-wide amplification and subsequent fragmentation. DNA was hybridized to the 62,163 locus-specific 50mers covalently linked to the beads distributed on the surface of the microarray. Single-base extension of oligos on the BeadChip, using captured DNA as a template, was performed, incorporating detectable labels on the BeadChip. Signals of each wavelength were determined using an Illumina iScan that converted the images to intensity data. Intensity data for each SNP were normalized and assigned a cluster position and a resulting genotype with the GenomeStudio software (Illumina); a quality score was generated for each genotype. An overlapping set of 150 (liver) and 207 (muscle) samples was genotyped for 62,163 SNPs, and quality of the data was evaluated. Samples with call rates $<95\%$ were removed. Markers were excluded if they had low minor-allele frequency (MAF) $<5\%$. Deviation from Hardy-Weinberg equilibrium was not considered because a three-way crossbreed pig population was used; deviation from Hardy-Weinberg equilibrium can be expected due to discordant allele frequencies

in the parental breeds. The average call rate for all samples was $99.8\% \pm 0.2\%$.

Whole-genome expression profiling

Gene expression profiling of the liver and *M. longissimus dorsi* samples of pigs was conducted with the same 150 and 207 animals. In brief, total RNA was isolated using TRI Reagent (Sigma, Taufkirchen, Germany) and used for target preparation for microarray hybridization. According to Affymetrix protocols, 500 ng of total RNA was reverse transcribed into cDNA, transcribed into cRNA, and labeled using the Affymetrix One cycle synthesis and labeling kit (Affymetrix, High Wycombe, UK) to prepare antisense biotinylated RNA targets. Liver expression patterns were produced using 150 GeneChip Porcine Genome Arrays (Affymetrix). Quality of hybridization was assessed in all samples, following the manufacturer's recommendations. Data were analyzed with the Affymetrix GCOS 1.1.1 software, using global scaling to a target signal of 500. Data were processed with MAS5.0 to generate cell intensity files (present or absent). Quantitative expression levels of the present transcripts were estimated using the probe logarithmic intensity error (PLIER) algorithm for normalization that was implemented in the Expression Console software (Affymetrix). Normalizations were performed separately per tissue. The microarray data related to all samples were deposited in the Gene Expression Omnibus (GEO) public repository [GEO accession nos. GSE25445 (liver) and GSE32112 (muscle)]. The overlapping set of successfully genotyped samples with available expression data amounted to 150 (liver) and 207 (muscle) individuals.

Correlation between traits and expression levels

Phenotypes and expression levels were adjusted for systematic effects by analysis of variance performed with the procedure "Mixed" of the SAS software package (SAS version 9.1; SAS Institute, Cary, NC) before analyzing their correlation. "Sex" was used as a fixed effect, "sire" and "slaughter day" as random effects, and "carcass weight" as a covariate. In muscle an additional fixed effect of RYR genotype was used. Subsequently, Pearson correlation coefficients were calculated between the residuals of log₂-transformed expression intensities and cortisol concentrations in plasma. Genes that showed correlation at $P \leq 0.05$ were analyzed further.

Pathway analysis and network generation

Based on BLAST comparison of the Affymetrix porcine target sequences with the porcine genome sequence (Ensembl_Sscrofa_10), 20,689 of the 24,123 probe sets on the Affymetrix Porcine GeneChip were localized and annotated. The list of significant trait-correlated transcripts was analyzed, referring to predefined pathways and functional categories of the Ingenuity Pathways Knowledge Base, using Ingenuity Pathways Analysis. Also, canonical pathways were identified from the Ingenuity Pathways Analysis library,

which were most significant to the input data. The significance of the association between the data set and the predefined pathways and functional categories was measured by Fisher's exact test and adjusted using the Benjamini-Hochberg correction (Benjamini and Hochberg 1995).

Population structure analysis

We first computed the genetic similarity matrix between the individuals as identity by descent (IBD) of each pair and visualized the result as a heatmap in Figure 2. A complex multileveled population structure and genetic relatedness are observed in the genetic similarity matrix. The QK mixed model was used to test for association between traits and SNP genotypes from a single SNP at a time while adjusting simultaneously for population structure and family relatedness (Yu *et al.* 2005). A regression testing for linear trend of SNP alleles was performed.

Genome-wide association

For the association analysis, cortisol concentration and log₂-transformed expression levels of individuals were used as traits for whole-genome association analysis with SNPs genotyped by a mixed-model analysis of variance, using JMP Genomics (SAS Institute). "Genotype" and "gender" were used as fixed effects, "relationship matrix" and slaughter day as random effects, and carcass weight as a covariate. In muscle an additional fixed effect of RYR genotype was used. The sequences flanking the SNPs represented on the Illumina SNP chip, which were significantly associated with the level of transcription of any probe set of the Affymetrix expression microarrays, were assigned to the porcine genome sequences (Ensembl_Sscrofa_10). Annotation and localization of SNP sites and probe sets allowed discrimination of *cis*- and *trans*-regulation. We defined an eQTL as *cis* if an associated SNP was located within an area <10 Mb from the probe set/gene. All other eQTL were considered as *trans*. Results are reported at thresholds of $P < 10^{-5}$, corresponding to a genome-wide false discovery rate (FDR) of 11% in both tissues. For the FDR calculation we used the *q*-value package in R (Storey and Tibshirani 2003). In total 80,725,037 *P*-values in liver and 45,625,920 *P*-values from muscle were used to estimate FDR. A significance threshold was used, representing a trade-off between avoidance of false-positive associations while taking into account the likely higher dependence among the expression traits (correlated expression) and among the SNP genotypes (linkage disequilibrium).

Causality modeling using network edge orienting

To infer causal flow between genetic markers, gene expression traits, and cortisol concentration, we utilized the network edge orienting (NEO) package (Aten *et al.* 2008). We used the residuals of gene expression and cortisol concentrations after correction for systematic effects as previously described. Because we were investigating a large amount of expression traits, to avoid the high complexity of the

unknown quantitative traits network, we looked for prediction of pairwise causal relation only between each expression trait and cortisol concentration. On the other hand, concerning the genetic marker selection, we adopted a hybrid method combining manual selection and automatic selection embedded in NEO. For each pair of expression trait and cortisol concentration, a pool of SNPs is constructed using the SNPs found to be significantly associated with the expression level (eQTL) and cortisol concentration. Markers were first divided into two larger groups for each trait with respect to marker assignment consistency to ensure clear separation of two nonoverlapping groups of markers, and the best sets of markers were then picked up using multivariate regression model variable selection with respect to the Akaike Information Criterion (AIC). Before fitting the local structural equation models (SEM) model, the local SEM-based edge-orienting next-best (LEO.NB) orientation score was first evaluated for both directions and proceeded only with the edge direction having a LEO.NB orthogonal causal anchor (LEO.NB.OCA) score >0.3 and a significant P -value <0.05 for the Wald test of path coefficient. The recommended threshold of 0.3 for LEO.NB.OCA is substantially more conservative than the 5% false-positive rate. A local SEM model was therefore fitted for the selected direction of the edge, using the best genetic markers selected, and model probability was then evaluated. The causal model was dropped if a model probability <0.05 was detected. Further evaluation of causality could proceed by inspecting the model-fitting index root mean-square error of approximation (RMSEA) to see whether a smaller value was in agreement. As recommended by the package author, a robustness analysis was also applied, for maximum anchoring SNP number ranging from 1 to 5.

Results

We first determined the distribution of plasma cortisol in our population ($N = 475$) of performance-tested pigs from commercial herds of the crossbreed Pietrain $\times$ (German Large White $\times$ German Landrace); mean ($\pm$ SD) cortisol was $93.9 (\pm 34.6)$ ng/ml (Figure 1). To characterize the architecture of hepatic gene expression influenced by cortisol, a subset of 150 performance-tested pigs was analyzed by Affymetrix Porcine Genome Arrays containing 24,123 probe sets, of which 20,689 probe sets assigned to known genes were applied for transcription profiles. Similarly, gene expression was quantified in *M. longissimus dorsi*, another peripheral target for cortisol, in 207 performance-tested pigs from the same herd (132 of these overlapped with the set used for hepatic expression profiles). MAS5 analysis revealed consistent “present calls” for 10,945 and 11,191 probe sets in liver and muscle, respectively; these probe sets represent genes being “active”. Probe sets with present calls were further analyzed using the PLIER algorithm for normalization that was implemented in the Expression Console

Figure 1 The distribution of plasma cortisol concentrations (nanograms per milliliter) of all 475 individuals with mean $\pm$ SD of 93.9 ± 34.6 ng/ml.

software (Affymetrix) and subsequently logarithmized PLIER values were subjected to association analysis.

The study population ($N = 475$) was also genotyped using the PorcineSNP60K BeadChip (Illumina). SNPs with genotyping call rates $>95\%$ and minor-allele frequencies $>5\%$ were selected for GWA analysis. The population structures in performance-tested pigs from commercial herds were estimated. One approach to using genetic markers to elucidate the relationships between individuals is genome-wide relatedness as calculated from IBD (Oliehoek *et al.* 2006). High IBD was found for paternal half-sibs (Figure 2) and was considered accordingly as a random factor before further analysis.

Identification of gene expression traits correlated with cortisol concentrations

In liver, 10,945 probe sets represented active genes. Analysis of correlation between residuals derived from the mixed-model analysis of cortisol concentrations and gene expression levels revealed 1531 probe sets having significant correlation of their expression with cortisol concentration. The correlation coefficients of expression and cortisol concentrations—adjusted for systematic effects and significant at $P < 0.05$ —ranged between $|0.16\text{--}0.42|$. Cortisol concentrations were positively correlated with 624 probe sets and negatively correlated with 907 probe sets (Supporting Information, Table S1). Similarly, in muscle, of 11,191 probe sets representing active genes, 960 had expression levels correlated with plasma cortisol concentration (Table S2). Of these, 664 probe sets were positively correlated and 296 were negatively correlated, with r ranging between $|0.31\text{--}0.14|$. Cortisol concentrations were most strongly correlated with *ARL4A* in liver ($r = 0.42$, $P = 1.12 \times 10^{-7}$, FDR = 0.0009) and *NFKBIA* in muscle ($r = 0.31$, $P = 6.99 \times 10^{-6}$, FDR = 0.05). Further, 135 probe sets showed correlation with cortisol concentrations and were expressed in both liver and muscle. Indeed, 78 of 135 transcripts had the same directions of correlation between cortisol concentration and expression in these tissues (Table S3).

Functional annotation of liver transcripts correlated to plasma cortisol concentrations

We tested the significant positively and negatively correlated functional candidate genes for enrichment in functional annotation groups, as defined in the Ingenuity Pathways

Figure 2 IBD relationship matrix heat map between individual pigs ($n = 475$), reflecting the population structure among performance-tested pigs from commercial herds. The dendrograms on the bottom and on the side of the heat map are based on the hierarchical clustering of identities by descent (IBDs) assessed from genome-wide genotypes. The genome-wide relatedness was calculated from IBD.

Knowledge Base. In liver, genes represented by probe sets with expression levels that correlated positively with cortisol concentrations were often involved in metabolism of lipids and carbohydrates as well as various macromolecules; genes with negative correlation were generally involved in dynamic cellular processes (Table S4). To further refine the functional annotation of gene sets with cortisol concentrations correlated to expression in liver, their assignment to canonical pathways was explored using the Ingenuity Pathway Knowledge Base. The positively and negatively correlated expression levels of functional candidate genes were uploaded as gene data sets for analysis. Significantly enriched canonical pathways for positively correlated transcripts included cytokine signaling [acute phase response signaling, interleukin (IL)-10, and IL-6], glucocorticoid receptor signaling, amino acid metabolism (glutamate, β -alanine, tryptophan, arginine and proline, and methionine), carbohydrate metabolism (propionate and butanoate), and fatty acid metabolism. Most of the negatively correlated transcripts were categorized in cellular immune response (antigen presentation pathway, interferon signaling, cytotoxic T-lymphocyte-mediated apoptosis of target cells, and IL-4 signaling), and phospholipase C signaling pathways.

Functional annotation of muscle transcripts correlated to plasma cortisol concentrations

Transcripts in muscle positively correlated with cortisol concentrations were categorized as genes involved in protein synthesis and functions of dynamic cellular processes. Tran-

scripts negatively correlated with cortisol concentrations were less often assigned to biological functions, with just 30 genes involved in cell cycle and 44 in cell death (Table S5). Three canonical pathways (NRF2-mediated oxidative stress response, HMGB1 signaling, and IL-17A signaling in fibroblasts) were significantly enriched for genes positively correlated with plasma cortisol. Most of the negatively correlated transcripts belonged to PDGF signaling or an antiproliferative role of TOB in T-cell signaling.

Detection of *cis* and *trans*-eQTL in liver

To characterize the genetic architecture of hepatic gene expression relevant to cortisol concentrations, only correlated expression levels were used for further analysis. eQTL represent variations perturbing the expression of a gene (Rockman and Kruglyak 2006). eQTL of genes for which expression levels are correlated to traits are strong positional and functional candidates. To identify positional and functional candidates regulating cortisol concentrations, we performed an eQTL analysis, using microarray expression profiles of liver and muscle tissue from our population of pigs. Two types of eQTL were distinguished: those that mapped near (<10 Mb from) the gene encoding the transcript (*cis*) and those that mapped elsewhere in the genome (*trans*) (Table 1). eQTL were identified for the 1531 probe sets with trait-correlated expression in liver, with 52,727 SNP markers using the mixed model with systematic adjusting (see *Materials and Methods*) (Figure 3). This analysis identified 6608 eQTL $P < 10^{-5}$ (11% FDR):

Table 1 Numerical summary of the whole-genome association study for eQTL ($P < 10^{-5}$) of genes showing levels of expression correlated with plasma cortisol concentrations in liver and muscle

	Liver	Muscle
No. samples	150	207
Total probe sets	10,945	11,191
No. genes correlated with cortisol concentrations	1,531	960
No. eQTL (gene) ^a	6,608 (990)	4,115 (593)
No. <i>cis</i> -eQTL (gene) ^a	1,019 (116)	2,001 (139)
No. <i>trans</i> -eQTL (gene) ^a	4,873 (927)	1,663 (538)

^a Number of genes whose transcript abundance is regulated by the eQTL.

1019 in *cis* and 4873 in *trans*. The mean negative log values of the P -values of *cis* and *trans*-eQTL were 8.18 and 6.34, respectively. The 4873 *trans*-eQTL and 1019 *cis*-eQTL corresponded to 927 and 116 genes, respectively (Table 1).

Thus, ~64% of genes with expression correlated with plasma cortisol were associated with eQTL; 12% of these eQTL act in *cis* compared to 94% regulated in *trans*. The 6608 SNPs explain 12–64% of the variation in transcript

Figure 3 Manhattan plots displaying the significance of associations of SNP markers and probe sets with trait-associated expression in liver (top) and muscle (bottom).

levels, with, on average, 23% for *cis*-eQTL and 17% for *trans*-eQTL.

Detection of *cis* and *trans*-eQTL in muscle

In muscle, eQTL analysis was performed for 960 probe sets representing genes whose expression correlated with plasma cortisol concentration. We used the same mixed model for analysis of liver data but with the genotype at the SNP c.1843C > T of the porcine ryanodine receptor gene (*RYR1*) as an additional fixed effect because of its known impact on muscle metabolism (Figure 3). A total of 4115 eQTL were identified at $P < 10^{-5}$ (11% FDR), with a mean negative log of the *P*-value of 7.44. We detected 2001 *cis* and 1663 *trans*-eQTL with mean negative log of the *P*-values of 8.62 and 6.02, respectively. These eQTL corresponded to 593 genes: 139 in *cis* and 538 in *trans*. Similar to liver, in muscle 62% of transcripts correlated with cortisol had associated *cis*- or *trans*-eQTL. However, in muscle a higher proportion of *cis*-eQTL, 23%, was found; 91% of the eQTL had *trans* effects. The eQTL associated with the 4115 SNPs explained 9–39% of the variation in transcript levels, with, on average, 15% for *cis*-eQTL and 11% for *trans*-eQTL.

Predicting causal links between genes with eQTL and cortisol concentrations

eQTL of genes showing expression levels correlated with plasma cortisol concentration potentially point either to key drivers of changes in cortisol concentrations or to transcripts responding to cortisol concentrations. However, eQTL and correlation alone are not enough to indicate the direction of the relationship and to support causality. To predict causal relationships between gene expression traits and cortisol concentrations, we used the NEO R software package (Aten *et al.* 2008), which takes SNP genotypes as input into structural equation models that compute local-structure edge-orienting scores (LEO.NB). Here, we used SNPs associated with the expression levels of probe sets (eQTL markers) and SNPs associated with plasma cortisol concentrations (GWA markers) from the same population to predict causal interactions between the two traits, *i.e.*, gene expression and cortisol concentrations.

In total, 6608 markers from eQTL in liver and 4115 markers from eQTL in muscle, at LOD scores >5, as well as 11 significant GWA markers (Table 2), at $P < 10^{-4}$, were used to predict the direction of the relationship of 990 and 593 genes with cortisol concentration-correlated expression that showed eQTL in liver or muscle, respectively. NEO was used to study causal relationships between expression levels of genes and cortisol concentrations, *i.e.*, either gene expression → cortisol (gene expression effects change in cortisol concentration) or cortisol → gene expression (cortisol concentration changes gene expression), to identify the up- and downstream molecular pathways of cortisol. In liver, 26 of 990 genes (Table S6) were predicted as causal (SNP → expression → cortisol ← SNP). Twenty genes predicted as

Table 2 SNPs with genome-wide significant evidence for association with plasma cortisol concentrations ($n = 475$)

SNP	Chromosome	Position	<i>P</i>	% var
MARC0000128	9	20,534,484	1.45E-5	4.0
DIAS0000681	18	41,861,687	2.11E-5	3.8
MARC0001638	9	20,802,172	3.06E-5	3.6
MARC0091789	16	4,169,476	4.75E-5	3.5
H3GA0023370	7	123,200,000	7.70E-5	3.3
H3GA0004371	1	266,700,000	1.05E-4	3.2
ALGA0050699	9	4,713,239	1.33E-4	3.2
MARC0070235	1	12,478,470	1.22E-4	3.1
ASGA0027973	6	21,482,519	2.11E-4	2.9
MARC0052121	Unknown	Unknown	2.58E-4	2.8
MARC0046428	7	85,926,835	3.33E-4	2.7

causal together with their function are presented in Table 3. The most significant prediction of a causal link was for *CD99* (Ssc 3205.1.S1.at), with a LEO.NB.OCA score of 1.07. The three genes *Pla2g10*, *LSR*, and *muCL* had *cis*-eQTL (Table 3). Another 70 genes (Table S7) were predicted to be regulated by cortisol (SNP → cortisol → expression ← SNP). Among these genes being responsive to plasma cortisol concentrations, those annotated were involved in lipid metabolism (*SGK1*, *ABCA3*, *LIPG*, *ACSL1*, *APOC3*, *PCTP*, *TLR4*, *NFKBIA*, *ITGB3*, *RDH11*, and *INSIG1*), transcription regulation (*CCRN4L*, *TAF1A*, *CEBPB*, *EPAS1*, *TEF*, *AKIRIN2*, *CEBPD*, *YY1*, *SREBF2*, and *NFIB*), and cell death (*ASAP3*, *TP53INP1*, *SLC30A10*, *GAS1*, *DBNL*, *GNB1*, *MCTS1*, *CFLAR*, *POLB*, *CYR61*, and *p53RFP*) (Table 4). The most significant responsive gene was *CCRN4L* (Ssc.9136.1.S1.at), with a LEO.NB.OCA score of 2.38. Additionally, *SGK1*, *CEBPB*, and *NFKBIA*—all known to be regulated by cortisol—were predicted as downstream of cortisol. Four genes (*ATP6V1H*, *PCTP*, *DERL1*, and *TAF1A*) of 70 had *cis*-eQTL (Table 4). In contrast, in muscle only 2 of 593 genes were predicted as upstream (*RPS11* and *ARRB2*), and 25 transcripts were predicted as responsive to cortisol (Table S8). Both genes predicted to affect cortisol concentration (*RPS11* and *ARRB2*) had *trans*-eQTL, whereas four genes being responsive to cortisol (*MPDU1*, *TAX1BP3*, *CYBASC3*, and *POLR2G*) revealed *cis*-eQTL (Table 5). The highest LEO.NB.OCA scores (0.854 and 0.804) were for genes *NFKBIA* (Ssc.4759.1.S1.at), *CXCL3* (Ssc.19692.1.S1.at), and *POLR2G* (Ssc_1925_1_S1_at), involved in glucocorticoid receptor signaling. Other responsive genes to plasma cortisol concentrations were involved in inflammatory response (*RGS2*, *F13A1*, *CAPG*, and *HLA-A*) transcription regulation (*SERTAD1*, *ZNF235*, *BHLHE41*, and *RUNX1*), and cellular growth and proliferation (*PSMB2*, *TAX1BP3*, and *CAMK2N1*) (Table 5).

After screening 6608 markers from eQTL analysis in liver, together with 11 markers from a GWA study, the best set of markers serving as causal anchors for orienting the edges of the network related to cortisol concentrations were *DIAS0000681*, *MARC0052121*, and *MARC0000128*. The best set of markers serving as causal anchors from eQTL analysis in muscle, together with markers from the GWA

Table 3 Genes with hepatic expression levels correlated with cortisol concentrations and with eQTL and that were predicted to affect plasma cortisol concentrations

Gene ^a	Chr. (Mb) ^b	R ^{2c}	P-value ^d	LEO.NB.OCA ^e	eQTL -Log10 ^f	eQTL Chr. (Mb) ^g	Category ^h
<i>HDDC2</i>	3 (98.5)	-0.19	2.04E-02	0.39	9.91	1 (39.6)	Catalytic activity
<i>CD99</i>	X (53.1)	-0.21	1.19E-02	1.07	5.78		Cell adhesion
<i>STK4</i>		-0.25	1.81E-03	0.80	5.35	5 (60.2)	Cell death
<i>PREX1</i>	9 (11.7)	-0.29	3.40E-04	0.59	5.53	X (12.9)	Cell death
<i>MAP3K3</i>	12 (15.8)	-0.21	9.55E-03	0.49	6.00	1 (241.0)	Cell death
<i>PMEPA1</i>	17 (64.9)	-0.24	3.42E-03	0.34	5.05	18 (33.6)	Cell death
<i>MPRIIP</i>	13 (19.2)	-0.20	1.46E-02	0.31	5.32	10 (48.5)	Cell death
<i>Pla2g10</i>	3 (29.4)	-0.24	2.97E-03	0.74	5.52	3 (28.6)	Lipid metabolism
<i>LSR</i>	6 (39.1)	-0.23	4.96E-03	0.43	6.06	6 (40.0)	Lipid metabolism
<i>HGSNAT</i>	17 (9.7)	-0.25	2.70E-03	0.60	5.33	9 (151.0)	Lysosomal transport
<i>NAT11</i>	2 (7.2)	-0.26	1.34E-03	0.33	5.67	3 (59.8)	Metabolic process
<i>CGNL1</i>	1 (119.0)	-0.29	2.98E-04	0.48	6.07	3 (28.7)	Motor activity
<i>IDH3A</i>	7 (53.1)	0.18	3.09E-02	0.51	16.23	7 (79.6)	Nucleotide binding
<i>ATP9A</i>	2 (138.0)	-0.16	4.88E-02	0.37	5.21	1 (200.0)	Nucleotide binding
<i>ETFDH</i>	8 (50.9)	0.25	1.90E-03	0.35	5.60	7 (13.3)	Nucleotide binding
<i>muCL</i>	2 (6.1)	-0.23	4.44E-03	0.40	6.18	2 (5.6)	Proteolysis
<i>IKBKAP</i>	1 (262.0)	0.20	1.56E-02	0.53	5.23	15 (49.5)	Transcription regulator
<i>Etv3</i>	4 (98.1)	0.17	3.86E-02	0.51	5.04	11 (24.9)	Transcription regulator
<i>NFRKB</i>	9 (63.3)	-0.27	9.14E-04	0.38	6.53	18 (35.5)	Transcription regulator
<i>TCF21</i>	1 (32.1)	-0.22	7.05E-03	0.32	6.37	16 (35.0)	Transcription regulator

Chr., chromosome.

^a Gene symbol.

^b Map position of the gene.

^c Coefficient of correlation of expression level and cortisol concentration.

^d Statistical significance of correlation.

^e Local SEM-based edge-orienting next-best orthogonal causal anchor score.

^f Statistical significance of eQTL.

^g Map position of eQTL.

^h Functional annotation of gene.

study, were *MARC0001638*, *DIAS0000681*, *ASGA0027973*, and *MARC0091789*. We found linkage disequilibrium between *MARC0000128* and *MARC0001638* with ProbChi = 2.93×10^{-27} . A robustness analysis was also applied, for maximum anchoring SNP number ranging from one to five. According to the robustness evaluation for three or more SNPs anchored to each trait, the LEO.NB.OCA scores are generally higher and more stable (Table S9 and Table S10). An outline of the systems biology approach to dissecting the effects of cortisol concentrations in target organs of liver and muscle is shown in Figure 4.

Discussion

Cortisol is released from the adrenal glands and exerts diverse actions on the immune system, glucose metabolism, and the central nervous system. Few studies have investigated genetic variants in relation to both cortisol secretion and expression pattern in the target organs. In the current study we integrated eQTL and causality modeling to identify candidate genes that affect or respond to cortisol concentrations in the target organs of liver and muscle. The identified candidate genes associated with cortisol concentrations may enhance discovery of genes associated with immune, metabolic, and behavioral traits in humans. Similar studies have identified candidate genes associated with cortisol concentrations that are also causally associated with

depression (Gottesman and Gould 2003; Zobel *et al.* 2010; Velders *et al.* 2011).

Biological function and molecular pathways correlated with cortisol concentrations

The application of global gene expression analyses to studies of cells and tissues has provided a wealth of data relevant to complex traits. Here, ~14% of liver transcripts and 9% of muscle transcripts were found to correlate with plasma cortisol concentrations. Finding statistically significant correlations between a trait and particular genes suggests a biological relationship between them (Ponsuksili *et al.* 2008, 2009, 2011). The potential biological functions and molecular pathways of cortisol-correlated expression differed between liver and muscle. In liver, more genes than expected by chance were identified as involved in lipid and carbohydrate metabolism; in contrast, in muscle, genes encoding various macromolecules related to protein synthesis, cell cycle, and cell death were represented.

Canonical pathway analysis highlighted pathways involving cytokine signaling, including acute phase response signaling, IL-10, and IL-6. The acute phase response (APR) is the body's innate immune defense against infection, inflammation, stress, physical trauma, and pathological conditions such as cardiovascular disease (Ramji *et al.* 1993; Baumann and Gauldie 1994). The liver is the major site for production of acute-phase protein (APP), whose expression is

Table 4 Genes with hepatic expression levels correlated with cortisol concentrations and with eQTL and that were predicted to respond to plasma cortisol concentrations

Gene ^a	Chr. (Mb) ^b	R ^{2c}	P-value ^d	LEO.NB.OCA ^e	eQTL -Log10 ^f	eQTL Chr. (Mb) ^g	Category ^h
<i>EIF4A3</i>	12 (55.3)	0.22	8.71E-03	0.76	6.08	1 (8.0)	ATP binding
<i>ASAP3</i>	X (54.8)	-0.31	1.59E-04	1.00	5.32	12 (37.1)	Cell death
<i>TP53INP1</i>	4 (43.2)	0.29	4.19E-04	0.56	5.09		Cell death
<i>SLC30A10</i>	10 (11.8)	-0.18	2.70E-02	0.51	5.63	5 (64.4)	Cell death
<i>GAS1</i>	11 (42.9)	0.27	1.06E-03	0.42	5.57	9 (66.3)	Cell death
<i>DBNL</i>	18 (52.4)	-0.17	3.85E-02	0.42	5.53		Cell death
<i>GNB1</i>	5 (64.7)	-0.19	2.45E-02	0.38	5.74	6 (118.0)	Cell death
<i>MCTS1</i>	10 (74.0)	-0.17	4.25E-02	0.37	5.46	3 (36.4)	Cell death
<i>CFLAR</i>	15 (112.0)	0.21	1.02E-02	0.36	5.42	6 (22.2)	Cell death
<i>POLB</i>	17 (13.6)	0.25	1.95E-03	0.35	5.00	X (106.0)	Cell death
<i>CYR61</i>	4 (138.0)	0.20	1.74E-02	0.34	5.24	6 (14.1)	Cell death
<i>p53RFP</i>	7 (14.5)	-0.23	4.99E-03	0.31	5.15	2 (81.3)	Cell death
<i>SLC16A10</i>	1 (81.8)	0.29	4.27E-04	0.55	6.83		Ion transport
<i>ATP6V1H</i>	4 (80.6)	0.21	1.19E-02	0.39	5.47	4 (81.5)	Ion transport
<i>SLC25A37</i>	14 (8.7)	-0.27	9.22E-04	0.35	5.33	15 (131.0)	Ion transport
<i>SLC39A5</i>	5 (23.9)	-0.22	7.27E-03	0.34	6.34	1 (2.2)	Ion transport
<i>SLC38A1</i>	5 (78.8)	0.25	2.11E-03	0.31	5.72	1 (174.0)	Ion transport
<i>SGK1</i>	1 (31.8)	0.34	1.98E-05	0.81	5.57		Lipid metabolism
<i>ABCA3</i>	3 (41.5)	-0.22	6.77E-03	0.72	5.87	3 (1.1)	Lipid metabolism
<i>LIPG</i>	1 (103.0)	0.38	2.68E-06	0.65	5.82	2 (134.0)	Lipid metabolism
<i>ACSL1</i>	15 (50.5)	0.21	1.16E-02	0.57	5.27	4 (65.6)	Lipid metabolism
<i>APOC3</i>	9 (50.2)	-0.25	2.55E-03	0.57	5.12	17 (31.5)	Lipid metabolism
<i>PCTP</i>	12 (33.1)	0.28	6.01E-04	0.53	14.82	12 (32.6)	Lipid metabolism
<i>TLR4</i>	1 (270.0)	0.22	7.43E-03	0.50	6.08	3 (43.1)	Lipid metabolism
<i>NFKBIA</i>	7 (69.5)	0.26	1.36E-03	0.48	5.54	10 (63.1)	Lipid metabolism
<i>ITGB3</i>	12 (17.5)	-0.41	2.38E-07	0.44	5.04	9 (119.0)	Lipid metabolism
<i>RDH11</i>	7 (96.9)	0.27	7.78E-04	0.40	6.87	9 (61.7)	Lipid metabolism
<i>INSIG1</i>	18 (3.1)	0.23	4.71E-03	0.37	5.13	1 (111.0)	Lipid metabolism
<i>GNAI2</i>	13 (35.4)	-0.23	4.43E-03	0.52	6.71		Organismal survival
<i>BACE2</i>	13 (209.0)	-0.21	9.32E-03	0.51	5.09	10 (3.3)	Organismal survival
<i>ARG1</i>	1 (34.2)	0.30	2.45E-04	0.40	5.35	1 (175.0)	Organismal survival
<i>GCH1</i>	1 (192.0)	0.22	8.42E-03	0.38	6.52	7 (62.7)	Organismal survival
<i>PPPDE1</i>	10 (20.3)	0.23	5.75E-03	0.60	5.30	1 (237.0)	Pro-apoptosis
<i>PSMG1</i>	13 (207.0)	0.25	2.74E-03	0.62	5.73	11 (15.6)	Proteasome assembly
<i>PAQR3</i>	8 (147.0)	0.21	1.09E-02	0.36	5.30	11 (32.7)	Receptor activity
<i>DERL1</i>	4 (16.5)	0.20	1.47E-02	0.31	22.21	4 (16.5)	Receptor activity
<i>SUP</i>	1 (151.0)	0.33	3.82E-05	0.57	6.08	6 (32.1)	Response to stress
<i>SF3B14a</i>	3 (121.0)	0.28	7.24E-04	0.72	5.77	3 (43.1)	RNA binding
<i>CCRN4L</i>	8 (92.3)	0.40	3.56E-07	2.38	5.44	12 (25.9)	Transcription regulator
<i>TAF1A</i>	10 (13.7)	0.24	3.84E-03	0.72	8.46	10 (13.7)	Transcription regulator
<i>CEBPB</i>	17 (58.4)	0.33	3.51E-05	0.63	5.32		Transcription regulator
<i>EPAS1</i>	3 (98.8)	0.16	4.72E-02	0.58	5.08	15 (97.5)	Transcription regulator
<i>TEF</i>	15 (105.0)	-0.19	1.90E-02	0.37	5.79	3 (77.8)	Transcription regulator
<i>AKIRIN2</i>	1 (60.0)	-0.20	1.54E-02	0.36	5.33	2 (163.0)	Transcription regulator
<i>CEBPD</i>	4 (83.6)	0.31	1.15E-04	0.35	5.92	3 (76.3)	Transcription regulator
<i>YY1</i>	7 (128.0)	0.28	6.06E-04	0.34	6.48	16 (58.6)	Transcription regulator
<i>SREBF2</i>	18 (7.2)	-0.32	9.75E-05	0.32	5.70	3 (125.0)	Transcription regulator
<i>NFIB</i>	1 (218.0)	-0.20	1.51E-02	0.31	6.00	5 (87.8)	Transcription regulator
<i>MRPL27</i>	12 (27.7)	-0.17	4.48E-02	0.57	6.03	3 (36.4)	Translation
<i>MFSD2</i>	6 (88.3)	0.33	3.96E-05	0.43	5.34	2 (134.0)	Transmembrane

^a Gene symbol.

^b Map position of the gene.

^c Coefficient of correlation of expression level and cortisol concentration.

^d Statistical significance of correlation.

^e Local SEM-based edge-orienting next-best orthogonal causal anchor score.

^f Statistical significance of eQTL.

^g Map position of eQTL.

^h Functional annotation of gene.

regulated at the transcriptional level by cytokines (Baumann and Gaudie 1994; Moshage 1997). We identified 18 molecules in liver and 16 molecules in muscle that were posi-

tively correlated to cortisol and are involved in APR signaling. Inflammatory cytokines including IL-1, IL-6, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ)

Table 5 Genes with muscular expression levels correlated with cortisol concentrations and with eQTL and that were predicted to respond to plasma cortisol concentrations

Gene ^a	Chr. (Mb) ^b	R ^{2c}	P-value ^d	LEO.NB.OCA ^e	eQTL -Log10 ^f	eQTL Chr. (Mb) ^g	Category ^h
<i>Avpi1</i>	14 (119.0)	0.19	6.59E-03	0.68	5.88	11 (26.2)	Activation of MAPK activity
<i>MPDU1</i>	12 (55.3)	0.21	2.07E-03	0.75	7.76	12 (55.3)	Carbohydrate metabolism
<i>BACE1</i>	9 (50.6)	-0.16	2.64E-02	0.34	5.58		Cell death
<i>PSMB2</i>	15 (25.3)	0.21	2.00E-03	0.66	5.39	3 (11.0)	Cellular growth and proliferation
<i>TAX1BP3</i>	12 (52.0)	0.20	3.85E-03	0.47	6.86	12 (52.0)	Cellular growth and proliferation
<i>CAMK2N1</i>	6 (71.3)	-0.21	3.15E-03	0.43	5.23	1 (2.8)	Cellular growth and proliferation
<i>PANK3</i>	5 (64.2)	0.28	5.56E-05	0.35	5.99	13 (32.3)	Coenzyme A biosynthetic process
<i>CYBASC3</i>	2 (9.8)	0.19	6.34E-03	0.39	18.40	2 (9.8)	Electron transport chain
<i>NFKBIA</i>	7 (69.5)	0.31	6.99E-06	0.85	5.65	12 (36.1)	Glucocorticoid receptor signaling
<i>CXCL3</i>	8 (73.3)	0.22	1.71E-03	0.80	5.05	13 (12.0)	Glucocorticoid receptor signaling
<i>POLR2G</i>	2 (8.3)	0.15	3.53E-02	0.47	14.37	2 (8.6)	Glucocorticoid receptor signaling
<i>RGS2</i>	10 (2.7)	0.20	4.86E-03	0.63	5.80	13 (32.2)	Inflammatory response
<i>F13A1</i>	7 (3.9)	0.19	7.75E-03	0.49	5.08	13 (32.2)	Inflammatory response
<i>CAPG</i>	3 (61.2)	0.20	3.67E-03	0.37	5.26	10 (13.6)	Inflammatory response
<i>HLA-A</i>		0.15	2.73E-02	0.34	6.04	16 (36.8)	Inflammatory response
<i>LEPROTL1</i>	15 (60.1)	0.19	7.00E-03	0.54	6.13	18 (6.6)	Integral to membrane
<i>C7orf42</i>	3 (16.1)	0.18	8.05E-03	0.34	5.84	5 (21.2)	Integral to membrane
<i>SERTAD1</i>	6 (43.8)	0.29	2.06E-05	0.53	5.66	7 (14.7)	Transcription regulator
<i>ZNF235</i>	2 (64.7)	-0.22	1.27E-03	0.41	5.22	15 (137.0)	Transcription regulator
<i>BHLHE41</i>	5 (49.3)	-0.16	1.84E-02	0.38	5.95	17 (59.4)	Transcription regulator
<i>RUNX1</i>	13 (203.0)	0.15	2.76E-02	0.33	6.38	7 (48.2)	Transcription regulator
<i>PARN</i>		0.23	1.15E-03	0.54	5.14	7 (100.0)	RNA processing
<i>Sfrs16</i>	3 (1.8)	0.18	9.06E-03	0.40	5.34	13 (183.0)	RNA processing

^a Gene symbol.

^b Map position of the gene.

^c Coefficient of correlation of expression level and cortisol concentration.

^d Statistical significance of correlation.

^e Local SEM-based edge-orienting next-best orthogonal causal anchor score.

^f Statistical significance of eQTL.

^g Map position of eQTL.

^h Functional annotation of gene.

are considered to be the mediators of immunological and pathological responses to stress and infection (Kelley *et al.* 1994; Warren *et al.* 1997). In the present study, *FKBP5*, which encodes a member of the immunophilin family, was significantly correlated with cortisol concentrations ($r = 0.23$,

$P = 0.004$, FDR = 0.14) but expression levels were not regulated by an eQTL. *FKBP5* is a cochaperone of HSP90, which is part of a receptor complex that regulates the sensitivity of the GR. Interestingly, a recent report found that an *FKBP5* SNP is associated with a decrease in cortisol and the

Figure 4 Outline of the systems biology approach to dissecting effects of cortisol concentrations in target organs, liver and muscle. Expression levels in liver and muscle that correlated with cortisol concentrations revealed biological functions. These transcripts were integrated with SNP genotypes to map expression QTL. Significant markers from genome-wide association together with eQTL were merged to prioritize genes using network edge orienting. This approach identified gene networks in muscle or liver that affect or respond to cortisol concentrations.

likelihood of depressive symptoms (Velders *et al.* 2011). Indeed, several studies have shown the association between high cortisol concentrations and depression (Vreeburg *et al.* 2009; Velders *et al.* 2011). Associations between inflammatory markers, the immune system, and depression have also been described (Miller *et al.* 2009; Miller 2010). In animals, the elevation of APPs by transportation and weaning stress has been reported (Richards *et al.* 2000; Arthington *et al.* 2003). Weaning stress may induce an APR through increased cortisol production and modulation of inflammatory cytokines (Kim *et al.* 2011). The relationship between metabolic syndrome, oxidative stress, and inflammation has been described (Bastard *et al.* 2006; Holvoet 2008). Similarly, cortisol is a potent glucocorticoid in energy homeostasis in Syrian hamsters, but cortisol alone does not mediate stress-induced increases in food intake or body mass (Solomon *et al.* 2011). Stress hormones released in response to activation of the HPA axis have been shown to affect aspects of the immune system, the metabolic system, and behavior, including depression. Here, we show for the first time the relationship between systemic plasma cortisol concentration and the transcriptome of two tissues/organs, muscle and liver, that both have implications for organismal metabolism. This study points to complex molecular pathways of target molecules of cortisol in liver and muscle.

Whole-genome association analyses for abundance of transcripts with cortisol concentration-correlated expression (eQTL)

We present the first eQTL study of genes influencing or influenced by plasma cortisol concentrations, using whole-genome association analysis in a commercial crossbred population. In the first step we analyzed the correlation between gene expression in liver and muscle and plasma cortisol concentrations, which revealed biologically meaningful relationships. In the second step eQTL were identified for transcripts that showed trait-correlated expression, which informed us about the genomic location of putative regulatory loci. This strategy eliminated several thousand eQTL that are not associated with cortisol concentrations. For the identification of sequence variants that have *cis*- and *trans*-regulatory effects on expression traits, SNPs located within a 10-Mb window at the location of the probe set were defined as *cis*-eQTL. The *trans*-acting eQTL represent transcripts whose abundance is regulated by loci remote from the genomic locus of each of these genes. Our goal was to identify a small number of differentially expressed candidates from the thousands of transcripts correlated with cortisol concentration. The eQTL analysis provides evidence for the presence of genomic variation that affects the abundance of transcripts in relation to plasma cortisol concentrations. In fact, for 65% and 62% of the transcripts whose expression in liver and muscle, respectively, correlated with cortisol concentration, significant eQTL were found. Thus, evidence exists for heritable gene regulation.

Candidate gene screening from eQTL and causality modeling

Recently, causality modeling was successfully applied in a variety of different settings (Farber *et al.* 2009a,b; Plaisier *et al.* 2009; Park *et al.* 2011). With the causality modeling algorithms, we integrated GWA and eQTL markers to identify up- and downstream molecules related to cortisol concentration. In liver, plasma cortisol concentration was anchored by three SNPs (DIAS0000681, SSC18, 41.8 Mb; MARC005212, unknown; and MARC0000128, SSC9, 20.5 Mb) that explained ~3–4% of the variation in cortisol concentrations ($n = 475$). MARC0000128 is located with the gene LOC100627939 (disks large homolog 2-like, *DLG2*). Recently, a genome-wide association study in human revealed significant association of *DLG2* with circulating phospholipid levels that play a crucial function in several diseases, with diverse neurological, psychiatric, and metabolic consequences (Demirkan *et al.* 2012). In the neighborhood ($\pm 10,000$ bp) of the other markers there are no currently annotated genes in the porcine genome. Additionally, 63 known genes were regulated by cortisol and anchored by markers, most of which corresponded to eQTL markers. These eQTL markers explained, on average, >15% of the variation in expression levels. Many genes are regulated by corticosterone (e.g., *SGK1*, *CEBPB*, and *NFKBIA*), some of which were also predicted as downstream of cortisol in this study. For instance, *SGK1*, whose expression is directly affected by corticosterone with an emphasis on metabolism, is involved in cellular functions such as glucose transport (Jeyaraj *et al.* 2007), regulation of voltage-gated channels (Boehmer *et al.* 2008), and apoptotic processes (Schoenebeck *et al.* 2005). Other molecules in our study that were regulated by cortisol with an emphasis effect on lipid or transport metabolism were *ACSL1*, *APOC3*, *PCTP*, *SREBF2*, *INSIG1*, *LIPG*, *NFKBIA*, and *ITGB3*. Ten of 63 known genes (*CEBPB*, *CCRN4L*, *TAF1A*, *EPAS1*, *CEBPD*, *YY1*, *TEF*, *AKIRIN2*, *NFIB*, and *SREBF2*) that were reactive to cortisol have transcription factor activity. *CEBPB* and *CEBPD* are transcription factors belonging to the C/EBP family. These contain a conserved basic leucine zipper motif at the carboxy terminus that is involved in dimerization and DNA binding (Ramji and Foka 2002). Binding sites for the C/EBPs have been identified in the regulatory regions of a large number of APP genes. Further, the main stimulators of APP production are inflammation-associated cytokines such as IL-6, IL-1, tumor necrosis factor- α , and IFN- γ . Three genes (*CEBPB*, *TLR4*, and *AKIRIN2*) downstream of cortisol are involved in IL-6 regulation. Toll-like receptor 4 (*TLR4*), a key member of the TLR family, has been well characterized as inducing inflammatory products of innate immunity and stress response (Eicher *et al.* 2004; O'Loughlin *et al.* 2011; Wang *et al.* 2011).

While most studies have covered genes downstream of cortisol, we identified 24 known genes that are upstream of cortisol concentrations. These 24 genes can be categorized into Gene Ontology (GO) terms of transcription regulator

activity (*TCF21*, *IKBKAP*, *NFRKB*, and *ETV3*), intracellular signaling cascade (*IKBKAP*, *MAP3K3*, *PREX1*, *STK4*, and *PMEPA1*), or KEGG_PATHWAY of MAPK signaling pathway (*PLA2G10*, *MAP3K3*, and *STK4*). The most significant transcript influencing cortisol concentrations was *CD99*. *CD99* is a leukocyte surface glycoprotein and plays a key role in several biological processes, including the regulation of T-cell activation (Pata *et al.* 2011), as well as in pig-to-human xenotransplantation (Schneider *et al.* 2009). Another gene that was identified as contributory for cortisol concentrations is phospholipase A2, group X (*PLA2G10*). *PLA2G10* has been implicated in diverse biological functions. One study showed that lungs from mice deficient in GX sPLA₂ have significantly reduced ovalbumin-induced inflammatory responses, suggesting a role for GX sPLA₂ in asthma (Henderson *et al.* 2007). Additionally, macrophage secretory phospholipase A2 group X enhances anti-inflammatory responses, promotes lipid accumulation, and contributes to aberrant lung pathology (Curfs *et al.* 2008). Recently, a study showed that C57BL/6 mice with targeted deletion of group X secretory phospholipase A₂ (GX KO) have ~80% higher plasma corticosterone concentrations compared with wild-type (WT) mice under both basal and adrenocorticotrophic hormone (ACTH)-induced stress conditions (Shridas *et al.* 2010). Further, GX sPLA₂ negatively regulates corticosterone production by altering the expression of steroidogenic acute regulatory protein (StAR), likely by suppressing LXR-mediated StAR promoter activation. Here, we confirmed that expression levels of *PLA2G10* negatively correlate with cortisol concentrations ($r = -0.243$, $P = 0.003$, FDR = 0.12). Indeed, we found that it is regulated by a *cis*-eQTL (NegLog10 of $P = 5.52$) and that it affects cortisol concentration by causality (LEO.NB.OCA = 0.74).

In muscle, cortisol concentrations were anchored by two significant SNPs (*MARC0001638*, SSC9, 20.8 Mb; *DIAS0000681*, SSC9, 20.5 Mb). The results correspond well with those obtained for liver: Marker *DIAS0000681* was also anchored to cortisol concentration in liver, and *MARC0001638* is in linkage disequilibrium to *MARC0000128*, which anchored cortisol in liver. As expected, most of the causal relationships were “cortisol modulates gene expression.” One of the two transcripts identified in muscle as upstream of cortisol concentrations was *ARRB2*. Recently, a study showed that disruption of β -arrestins (*Arrb1*^{-/-}, *Arrb2*^{-/-}) blocks GR in mice, suggesting that GR is an important downstream effector of the β -arrestin signaling pathway involved in regulation of lung and liver development (Zhang *et al.* 2011). Another study reported that peptidoglycan-induced immune protection in stress-induced changes of cytokine levels appears to require β -arrestin 2, a multifunctional adaptor and signal transducer (Li *et al.* 2011). Here, we show for the first time the up- and downstream effects on cortisol concentrations in muscle. As in liver, many molecules are involved in inflammatory response (*ARRB2*, *CAPG*, *CXCL3*, *HLA-A*, *NFKBIA*, *RGS2*, and *F13A1*) and glucocorticoid receptor signaling (*ARRB2*, *NFKBIA*, *POLR2G*, and *CXCL3*).

Other molecules we identified were noted in the literature as related to stress and therefore indirectly related to cortisol. Polymorphisms in one candidate, *RGS2* (regulator of G-protein signaling 2), are associated with anxious behavior in mice (Leygraf *et al.* 2006) and anxiety in humans (Smoller *et al.* 2008).

Causality modeling has been successfully applied in our study to understand the role of cortisol as either a downstream target or an effector molecule. This approach allowed us to identify two different types of transcripts and associated markers: those influencing expression of individual genes that, in turn, influence cortisol concentrations and, alternately, those influencing cortisol concentrations that, in turn, influence gene expression. We thus identified a list of genes with upstream relationships to plasma cortisol concentrations, as well as a list of genes that are regulated downstream by cortisol concentrations in liver and muscle. Interestingly, the list of our candidate genes in this study did not overlap with that of a previous study by using a comparative, translational systems genetics approach addressing cortisol in mice and pigs (Kadarmideen and Janss 2007). Whereas the previous study of Kadarmideen and Janss (2007) focused on gene expression from mouse INIA brain mRNA, our study aimed to discriminate up- and downstream effects of transcripts affecting or responding to plasma cortisol concentrations in the target tissue of liver and muscle.

In summary, looking at trait-correlated expression patterns in genes in two target tissues of cortisol—liver, the central metabolic organ and source of bioactive molecules, and muscle, the major energy consumer and storage tissue—has helped to elucidate the complex molecular networks contributing to cortisol concentrations in plasma, which, in turn, relates to behavioral, physiological, and metabolic disease in mammals. Many of the candidate genes previously identified in humans and mice have been confirmed here in the porcine model. Moreover, novel candidate genes associated with cortisol concentrations, identified for the first time in a pig model, are valuable for human research due to the genomic and physiological similarities between pigs and humans. The application of this information can facilitate the detection of trait-associated SNPs related to behavioral, physiological, and metabolic traits. The discrimination of up- and downstream effects of transcripts affecting or responding to plasma cortisol concentrations further supports gaining a better understanding of the biology of complex traits.

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Literature Cited

- Andrews, R. C., and R. Walker, 1999 Glucocorticoids and insulin resistance: old hormones, new targets. *Clin. Sci.* 96: 513–523.
- Arthington, J. D., S. D. Eicher, W. E. Kunkle, and F. G. Martin, 2003 Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. *J. Anim. Sci.* 81: 1120–1125.
- Aten, J. E., T. F. Fuller, A. J. Lusis, and S. Horvath, 2008 Using genetic markers to orient the edges in quantitative trait networks: the NEO software. *BMC Syst. Biol.* 2: 34.
- Bastard, J. P., M. Maachi, C. Lagathu, M. J. Kim, M. Caron *et al.*, 2006 Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine Netw.* 17: 4–12.
- Baumann, H., and J. Gauldie, 1994 The acute phase response. *Immunol. Today* 15: 74–80.
- Benjamini, Y., and N. Hochberg, 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Stat. Methodol.* 57: 289–300.
- Black, J. L., L. R. Giles, P. C. Wynn, A. G. Knowles, C. A. Kerr *et al.*, 2001 A review—factors limiting the performance of growing pigs in commercial environments, pp. 9–36 in *Manipulating Pig Production VIII*, edited by P. Cranwell. Australasian Pig Science Association, Werribee, Australia.
- Bode, G., P. Clausen, F. Gervais, J. Loegsted, J. Luft *et al.*, 2010 Steering group of the RETHINK Project, the utility of the minipig as an animal model in regulatory toxicology. *J. Pharmacol. Toxicol. Methods* 62: 196–220.
- Boehmer, C., J. Laufer, S. Jeyaraj, F. Klaus, R. Lindner *et al.*, 2008 Modulation of the voltage-gated potassium channel Kv1.5 by the SGK1 protein kinase involves inhibition of channel ubiquitination. *Cell. Physiol. Biochem.* 22: 591–600.
- Bystrykh, L., E. Weersing, B. Dontje, S. Sutton, M. T. Pletcher *et al.*, 2005 Uncovering regulatory pathways that affect hematopoietic stem cell function using 'genetical genomics'. *Nat. Genet.* 37: 225–232.
- Cheung, V. G., R. S. Spielman, K. G. Ewens, T. M. Weber, M. Morley *et al.*, 2005 Mapping determinants of human gene expression by regional and genome-wide association. *Nature* 437: 1365–1369.
- Curfs, D. M., S. A. Ghesquiere, M. N. Vergouwe, I. van der Made, M. J. Gijbels *et al.*, 2008 Macrophage secretory phospholipase A2 group X enhances anti-inflammatory responses, promotes lipid accumulation, and contributes to aberrant lung pathology. *J. Biol. Chem.* 283: 21640–21648.
- DeCook, R., S. Lall, D. Nettleton, and S. H. Howell, 2006 Genetic regulation of gene expression during shoot development in *Arabidopsis*. *Genetics* 172: 1155–1164.
- de Groot, J., M. A. W. Ruis, J. W. Scholten, J. M. Koolhaas, and W. J. A. Boersma, 2001 Long-term effects of social stress on antiviral immunity in pigs. *Physiol. Behav.* 73: 145–158.
- Demirkan, A., C. M. van Duijn, P. Ugocsai, A. Isaacs, P. P. Pramstaller *et al.*, 2012 Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. *PLoS Genet.* 8: e1002490.
- Eicher, S. D., K. A. McMunn, H. M. Hammon, and S. S. Donkin, 2004 Toll-like receptors 2 and 4, and acute phase cytokine gene expression in dexamethasone and growth hormone treated dairy calves. *Vet. Immunol. Immunopathol.* 98: 115–125.
- Farber, C. R., J. E. Aten, E. A. Farber, V. de Vera, R. Gularte *et al.*, 2009a Genetic dissection of a major mouse obesity QTL (Carfhg2) integration of gene expression and causality modeling. *Physiol. Genomics* 37: 294–302.
- Farber, C. R., A. van Nas, A. Ghazalpour, J. E. Aten, S. Doss *et al.*, 2009b An integrative genetics approach to identify candidate genes regulating BMD: combining linkage, gene expression, and association. *J. Bone Miner. Res.* 24: 105–116.
- Geverink, N. A., W. G. Schouten, G. Gort, and V. M. Wiegant, 2002 Individual differences in behavioral and physiological responses to restraint stress in pigs. *Physiol. Behav.* 77: 451–457.
- Gottesman, I. I., and T. D. Gould, 2003 The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* 160: 636–645.
- Heetkamp, M. J., J. W. Schrama, L. de Jong, J. W. Swinkels, W. G. Schouten *et al.*, 1995 Energy metabolism in young pigs as affected by mixing. *J. Anim. Sci.* 73: 3562–3569.
- Henderson, Jr., W. R., E. Y. Chi, J. G. Bollinger, Y. T. Tien, X. Ye *et al.*, 2007 Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. *J. Exp. Med.* 204: 865–877.
- Holvoet, P., 2008 Relations between metabolic syndrome, oxidative stress and inflammation and cardiovascular disease. *Verh. K. Acad. Geneesk. Belg.* 70: 193–219.
- Hubner, N., C. A. Wallace, H. Zimdahl, E. Petretto, H. Schulz *et al.*, 2005 Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nat. Genet.* 37: 243–253.
- Jansen, R. C., and J. P. Nap, 2001 Genetical genomics: the added value from segregation. *Trends Genet.* 17: 388–391.
- Jeyaraj, S., C. Boehmer, F. Lang, and M. Palmada, 2007 Role of SGK1 kinase in regulating glucose transport via glucose transporter GLUT4. *Biochem. Biophys. Res. Commun.* 356: 629–635.
- Kadarmideen, H. N., and L. L. Janss, 2007 Population and systems genetics analyses of cortisol in pigs divergently selected for stress. *Physiol. Genomics* 29: 57–65.
- Kelley, K. W., R. W. Johnson, and R. Dantzer, 1994 Immunology discovers physiology. *Vet. Immunol. Immunopathol.* 43: 157–165.
- Kim, M. H., J. Y. Yang, S. D. Upadhyaya, H. J. Lee, C. H. Yun *et al.*, 2011 The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. *J. Vet. Sci.* 12: 151–157.
- Leygraf, A., C. Hohoff, C. Freitag, S. A. Willis-Owen, P. Krakowitzky *et al.*, 2006 Rgs 2 gene polymorphisms as modulators of anxiety in humans? *J. Neural Transm.* 113: 1921–1925.
- Li, H., L. Chen, Y. Zhang, G. Lesage, Y. Zhang *et al.*, 2011 Chronic stress promotes lymphocyte reduction through TLR2 mediated PI3K signaling in a β -arrestin 2 dependent manner. *J. Neuroimmunol.* 233: 73–79.
- Miller, A. H., 2010 Depression and immunity: A role for T cells? *Brain Behav. Immun.* 24: 1–8.
- Miller, A. H., V. Maletic, and C. L. Raison, 2009 Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry* 65: 732–741.
- Morley, M., C. M. Molony, T. M. Weber, J. L. Devlin, K. G. Ewens *et al.*, 2004 Genetic analysis of genome-wide variation in human gene expression. *Nature* 430: 743–747.
- Moshage, H., 1997 Cytokines and the hepatic acute phase response. *J. Pathol.* 181: 257–266.
- Murani, E., S. Ponsuksili, R. B. D'Eath, S. P. Turner, E. Kurt *et al.*, 2010 Association of HPA axis-related genetic variation with stress reactivity and aggressive behaviour in pigs. *BMC Genet.* 11: 74.
- Murani, E., S. Ponsuksili, R. B. D'Eath, S. P. Turner, G. Evans *et al.*, 2011 Differential mRNA expression of genes in the porcine adrenal gland associated with psychosocial stress. *J. Mol. Endocrinol.* 46: 165–174.
- Oliehoek, P. A., J. J. Windig, J. A. van Arendonk, and P. Bijma, 2006 Estimating relatedness between individuals in general populations with a focus on their use in conservation programs. *Genetics* 173: 483–496.

- O'Loughlin, A., M. McGee, S. M. Waters, S. Doyle, and B. Earley, 2011 Examination of the bovine leukocyte environment using immunogenetic biomarkers to assess immunocompetence following exposure to weaning stress. *BMC Vet. Res.* 7: 45.
- Park, C. C., G. D. Gale, S. de Jong, A. Ghazalpour, B. J. Bennett *et al.*, 2011 Gene networks associated with conditional fear in mice identified using a systems genetics approach. *BMC Syst. Biol.* 5: 43.
- Pata, S., P. Otáhal, T. Brdička, W. Laopajon, K. Mahasongkram *et al.*, 2011 Association of CD99 short and long forms with MHC class I, MHC class II and tetraspanin CD81 and recruitment into immunological synapses. *BMC Res. Notes.* 4: 293.
- Plaisier, C. L., S. Horvath, A. Huertas-Vazquez, I. Cruz-Bautista, M. F. Herrera *et al.*, 2009 A systems genetics approach implicates USF1, FADS3, and other causal candidate genes for familial combined hyperlipidemia. *PLoS Genet.* 5: e1000642.
- Ponsuksili, S., E. Murani, C. Phatsara, E. Jonas, and C. Walz, 2008 Expression profiling of muscle reveals transcripts differentially expressed in muscle that affect water-holding capacity of pork. *J. Agric. Food Chem.* 56: 10311–10317.
- Ponsuksili, S., E. Murani, C. Phatsara, M. Schwerin, K. Schellander *et al.*, 2009 Porcine muscle sensory attributes associate with major changes in gene networks involving CAPZB, ANKRD1, and CTBP2. *Funct. Integr. Genomics* 9: 455–471.
- Ponsuksili, S., E. Murani, M. Schwerin, K. Schellander, and K. Wimmers, 2010 Identification of expression QTL (eQTL) of genes expressed in porcine *M. longissimus dorsi* and associated with meat quality traits. *BMC Genomics* 11: 572.
- Ponsuksili, S., E. Murani, B. Brand, M. Schwerin, and K. Wimmers, 2011 Integrating expression profiling and whole-genome association for dissection of fat traits in a porcine model. *J. Lipid Res.* 52: 668–678.
- Ramji, D. P., and P. Foka, 2002 CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem. J.* 365: 561–575.
- Ramji, D. P., R. Cortese, and G. Ciliberto, 1993 Regulation of C-reactive protein, haptoglobin and hemopexin gene expression, pp. 365–394 in *Acute Phase Proteins: Molecular Biology, Biochemistry and Clinical Applications*, edited by A. Mackiewicz, I. Kushner, and H. Baumann. CRC–Taylor and Francis, New York, USA.
- Richards, D. F., M. Fernandez, J. Caulfield, and C. M. Hawrylowicz, 2000 Glucocorticoids drive human CD8+ T cell differentiation towards a phenotype with high IL-10 and reduced IL-4, IL-5 and IL-13 production. *Eur. J. Immunol.* 30: 2344–2354.
- Rockman, M. V., and L. Kruglyak, 2006 Genetics of global gene expression. *Nat. Rev. Genet.* 7: 862–872.
- Schadt, E. E., C. Molony, E. Chudin, K. Hao, X. Yang *et al.*, 2008 Mapping the genetic architecture of gene expression in human liver. *PLoS Biol.* 6: e107.
- Schneider, M. K., M. Ghielmetti, D. M. Rhyner, M. A. Antsiferova, and J. D. Seebach, 2009 Source human leukocyte transmigration across Gal α (1,3)Gal-negative porcine endothelium is regulated by human CD18 and CD99. *Transplantation* 87: 491–499.
- Schoenebeck, B., V. Bader, X. R. Zhu, B. Schmitz, H. Lübbert *et al.*, 2005 Sgk1, a cell survival response in neurodegenerative diseases. *Mol. Cell. Neurosci.* 30: 249–264.
- Shridas, P., W. M. Bailey, B. B. Boyanovsky, R. C. Oslund, M. H. Gelb *et al.*, 2010 Group X secretory phospholipase A2 regulates the expression of steroidogenic acute regulatory protein (StAR) in mouse adrenal glands. *J. Biol. Chem.* 285: 20031–20039.
- Smoller, J. W., M. P. Paulus, J. A. Fagerness, S. Purcell, and L. H. Yamaki, 2008 RGS2 influences anxiety-related temperament, personality and brain function. *Arch. Gen. Psychiatry* 65: 298–308.
- Solomon, M. B., R. R. Sakai, S. C. Woods, and M. T. Foster, 2011 Differential effects of glucocorticoids on energy homeostasis in Syrian hamsters. *Am. J. Physiol. Endocrinol. Metab.* 301: E307–E316.
- Storey, J. D., and R. Tibshirani, 2003 Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. USA* 100: 9440–9445.
- Swanson-Wagner, R. A., R. DeCook, Y. Jia, T. Bancroft, T. Ji *et al.*, 2009 Paternal dominance of trans-eQTL influences gene expression patterns in maize hybrids. *Science* 326: 1118–1120.
- Velders, F. P., M. Kuningas, M. Kumari, M. J. Dekker, A. G. Uitterlinden *et al.*, 2011 Genetics of cortisol secretion and depressive symptoms, a candidate gene and genome wide association approach. *Psychoneuroendocrinology* 36: 1053–1061.
- Vreeburg, S. A., B. P. Kruijtzter, J. van Pelt, R. van Dyck, R. H. DeRijk *et al.*, 2009 Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. *Psychoneuroendocrinology* 34: 1109–1120.
- Wang, R. P., Q. Yao, Y. B. Xiao, S. B. Zhu, L. Yang *et al.*, 2011 Toll-like receptor 4/nuclear factor-kappa B pathway is involved in myocardial injury in a rat chronic stress model. *Stress* 14: 567–575.
- Warren, E. J., B. N. Finck, S. Arkins, K. W. Kelley, R. W. Scamurra *et al.*, 1997 Coincidental changes in behavior and plasma cortisol in unrestrained pigs after intracerebroventricular injection of tumor necrosis factor- α . *Endocrinology* 138: 2365–2371.
- Yu, J., G. Pressoir, W. H. Briggs, I. Vroh Bi, M. Yamasaki *et al.*, 2005 A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* 38: 203–208.
- Zhang, M., H. Teng, J. Shi, and Y. Zhang, 2011 Disruption of β -arrestins blocks glucocorticoid receptor and severely retards lung and liver development in mice. *Mech. Dev.* 128: 368–375.
- Zobel, A., A. Schuhmacher, F. Jessen, S. Höfels, O. von Widdern *et al.*, 2010 DNA sequence variants of the FKBP5 gene are associated with unipolar depression. *Int. J. Neuropsychopharmacol.* 13: 649–660.

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GENETICS

Supporting Information

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Elucidating Molecular Networks That Either Affect or Respond to Plasma Cortisol Concentration in Target Tissues of Liver and Muscle

Siriluck Ponsuksili, Yang Du, Eduard Murani, Manfred Schwerin, and Klaus Wimmers

Supporting Tables

Tables S1-S3 and S6-S10 are available for download at
<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143081/-/DC1>.

Table S1 List of probe sets and gene annotations whose expression in liver is correlated with plasma cortisol concentration at $p < 0.05$.

Table S2 List of probe sets and gene annotations whose expression in muscle is correlated with plasma cortisol concentration at $p < 0.05$.

Table S3 List of probe sets and gene annotations whose expression in liver and muscle is correlated with plasma cortisol concentration at $p < 0.05$.

Table S4 Molecular and cellular functions of transcripts with expression levels in liver correlating positively or negatively with plasma cortisol concentrations.

Table S5 Molecular and cellular functions of transcripts with expression levels in muscle correlating positively or negatively with plasma cortisol concentrations.

Table S6 The orthomarker models used for the LEO.NB.OCA marker aggregation method for gene (A) to cortisol concentration (B) edge orientation in the target organ liver

Table S7 The orthomarker models used for the LEO.NB.OCA marker aggregation method for cortisol concentration (A) to expression level (B) edge orientation in the target organ liver

Table S8 The orthomarker models used for the LEO.NB.OCA marker aggregation method for cortisol concentration (A) to gene (B) and from gene (A) to cortisol concentration (B) edge orientation in the target tissue muscle

Table S9 The orthomarker models used for the LEO.NB.OCA marker aggregation method for cortisol concentration (A) to gene (B) and from gene (A) to cortisol concentration (B) edge orientation in the target organ liver

Table S10 The orthomarker models used for the LEO.NB.OCA marker aggregation method for cortisol concentration (A) to gene (B) and from gene (A) to cortisol concentration (B) edge orientation in the target tissue muscle

Table S4 Molecular and cellular functions of transcripts with expression levels in liver correlating positively or negatively with plasma cortisol.

Molecular and cellular functions	No. of genes	B-H multiple testing correction p-value
Positive correlation		
Cell death	130	5.36E-07-4.37E-02
Lipid metabolism	71	1.08E-04-4.15E-02
Small molecule biochemistry	105	1.08E-04-4.46E-02
Carbohydrate metabolism	59	1.28E-04-3.3E-02
Molecular transport	80	1.37E-04-4.46E-02
Negative correlation		
Cell death	190	1.03E-05-9.38E-02
Cellular growth and proliferation	201	1.41E-05-9.38E-02
Cellular movement	108	1.89E-05-9.38E-02
Cellular assembly and organization	108	2.02E-05-9.38E-02
Cellular function and maintenance	101	2.02E-05-9.38E-02

Table S5 Molecular and cellular functions of transcripts with expression levels in muscle correlating positively or negatively with plasma cortisol.

Molecular and cellular functions	No. of genes	B-H multiple testing correction p-value
Positive correlation		
Cellular growth and proliferation	185	2.28E-13-6.81E-03
Cell death	164	1.76E-10-5.94E-03
Protein synthesis	62	1.76E-10-5.29E-10
Cellular movement	106	1.81E-07-7.25E-03
Cell-to-cell signaling and interaction	91	5.43E-07-5.83E-03
Negative correlation		
Cell cycle	30	2.54E-02-1.18E-01
Lipid metabolism	10	2.54E-02-1.18E-01
Small molecule biochemistry	16	2.54E-02-1.18E-01
Cell death	44	3.02E-02-1.18E-01

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Molecular and cellular functions	No. of genes	B-H multiple testing correction p-value
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Lipid metabolism	71	1.08E-04-4.15E-02
Small molecule biochemistry	105	1.08E-04-4.46E-02
Carbohydrate metabolism	59	1.28E-04-3.3E-02
Molecular transport	80	1.37E-04-4.46E-02
Negative correlation		
Cell death	190	1.03E-05-9.38E-02
Cellular growth and proliferation	201	1.41E-05-9.38E-02
Cellular movement	108	1.89E-05-9.38E-02
Cellular assembly and organization	108	2.02E-05-9.38E-02
Cellular function and maintenance	101	2.02E-05-9.38E-02

Table S5 Molecular and cellular functions of transcripts with expression levels in muscle correlating positively or negatively with plasma cortisol.

Molecular and cellular functions	No. of genes	B-H multiple testing correction p-value
Positive correlation		
Cellular growth and proliferation	185	2.28E-13-6.81E-03
Cell death	164	1.76E-10-5.94E-03
Protein synthesis	62	1.76E-10-5.29E-10
Cellular movement	106	1.81E-07-7.25E-03
Cell-to-cell signaling and interaction	91	5.43E-07-5.83E-03
Negative correlation		
Cell cycle	30	2.54E-02-1.18E-01
Lipid metabolism	10	2.54E-02-1.18E-01
Small molecule biochemistry	16	2.54E-02-1.18E-01
Cell death	44	3.02E-02-1.18E-01