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Title:
Hepatic expression patterns in psychosocially high-stressed pigs suggest mechanisms following allostatic principles.

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Highlights

- Psychosocial stress altered essential and unessential metabolic pathways
- High-stressed pigs show decreased expression of catechol-O-methyltransferase (COMT)
- mRNA alterations could be summarized with reference to the concept of allostasis

Abstract

Psychosocial challenges are known to introduce cellular and humoral adaptations in various tissues and organs, including parts of the sympatho-adrenal-medullary system and hypothalamic–pituitary–adrenal axis as well as other peripheral tissue being responsive to cortisol and catecholamines. The liver is of particular interest given its vital roles in maintaining homeostasis and health as well as regulating nutrient utilization and overall metabolism. We aimed to evaluate whether and how response to psychosocial stress is reflected by physiological molecular pathways in liver tissue. A pig mixing experiment was conducted to induce psychosocial stress culminating in skin lesions which reflect the involvement in aggressive behavior and fighting. At 27 weeks of age, animals prone to psychosocially low- and high-stress were assigned to mixing groups. Skin lesions were counted before mixing and after slaughter on the carcass. Individual liver samples (n=12) were taken. The isolated RNA was hybridized on Affymetrix GeneChip porcine Genome Arrays. Relative changes of mRNA abundances were estimated via variance analyses. Molecular routes related to tRNA charging, urea cycle, acute phase response, galactose utilization, and steroid receptor signaling were found to be increased in psychosocially high-stressed animals, whereas catecholamine degradation and cholesterol biosynthesis were found to be decreased. In particular, psychosocially high-stressed animals show decreased expression of catechol-O-methyltransferase (COMT) which has been linked to molecular mechanisms regulating aggressiveness and stress response. The expression patterns of high-stressed animals revealed metabolic alterations of key genes related to energy-mobilizing processes at the expense of energy consuming processes. Thus, the coping following psychosocial challenges involves transcriptional alterations in liver tissue which may be summarized with reference to the concept of allostasis, a strategy which is critical for survival.

Keywords: Allostasis: Liver: Microarray: Mixing: Pig: Psychosocial stress
In pigs, the mixing of unfamiliar animals is considered to cause psychosocial stress [1,2], thereby negatively affecting animal welfare [3,4]. It has been reported, that pigs exposed to unfamiliar conspecifics alter their neuroendocrine system [5,6,7] which may culminate in fighting and aggressive behavior [8,9] resulting in injuries as indicated by the ‘skin lesion score’ [10,11]. Hence, multiple effects on production traits, endocrine system and metabolism have been stated. We have previously reported that psychosocial stress induced by mixing unfamiliar animals was indicated by a higher number of carcass skin lesions and higher levels of plasma cortisol at slaughter [2]. Furthermore, gene expression patterns of the adrenal stress response in these pigs were analyzed, revealing altered mRNA abundances for transcripts associated with biosynthesis of steroids, cell growth and cell death [12]. It has been suggested that immune alterations following psychosocial stress are part of evolutionary conserved mechanisms to face potential injuries (e.g. skin lesions) [13]. Consistently, gene expression of porcine peripheral blood mononuclear cells (PBMC) showed adjusted immunological pathways in psychosocially high-stressed females [14]. Experimental designs inducing psychosocial stress indicate that mammals exhibit a broad spectrum of adaptive mechanisms on both the cellular and humoral level. Hence, different tissues and organs have to interact extensively in order to cope with environmental challenges. That can also be described as the concept of allostasis [15] reflecting ‘the ability to achieve stability through change’, a strategy which is critical for survival [16]. Therefore, to adapt to a stressful challenge, a variety of parameters must be orchestrated in different body components, including transcriptional alterations in metabolically active tissues such as in the liver.

To evaluate whether differences in the response to stress introduced by mixing are reflected by physiological molecular pathways that contribute to homeostasis or allostasis we investigated hepatic gene expression in psychosocially high- and low-stressed pigs.

2. Materials and methods

2.1. Animals, balanced mixing, sample collection

A pig mixing experiment was performed when high and low levels of psychosocial stress were induced as described by D’Eath et al. [2]. In brief, the progeny (n=271) derived from a crossbreed including Landrace, Large White, Duroc (sows) and Pietrain (boars) were mixed at approximately 10 weeks of age (Figure 1: MIXING 1). The pigs were assigned to new weight-balanced single-sex groups of eight or ten animals. Immediately before and at 24 hrs after mixing, skin lesions were counted, dividing the body into front (head, neck, shoulder, and front legs), middle (flanks, and back) and rear (rump, hind legs, and tail) sections [10]. The skin lesion score reflects the involvement in aggressive behavior and fighting [10,11] and
was used to distinguish the examined animals as either prone to high-aggression (high skin lesion score) or low-aggression (low skin lesion score). For each mixing group the cut-off criteria for the total lesion score was individually calculated. Thereby, the distribution of skin lesion scores in each group was estimated. Half of the pigs were designated as high aggressiveness (those with front lesions above average) and the remaining half was designated as low aggressiveness (those with front lesions below average), respectively. The first mixing revealed an increased skin lesion score (mean ± SEM) in H animals than in L animals (140.2 ± 69.5 vs. 86.5 ± 30.2, respectively. Pigs remained in the established rearing groups until they reached slaughter weight at approximately 27 weeks of age. Here, the pigs were assigned to mixing groups based on their aggressiveness (Figure 1: MIXING 2), as they were loaded onto a vehicle for a 270 km transport to the abattoir. In detail, single-sex groups were built by mixing four pigs from one rearing group and four pigs from another rearing group. Thus, H pigs were mixed with H pigs resulting in a HH batch, H pigs were mixed with L pigs resulting in a HL batch, or L pigs were mixed with L pigs resulting in a LL batch. Skin lesions were counted before mixing and after slaughter on the carcass, thereby the skin lesion score was calculated (Table 1). In all these batches animals with high and low lesion scores were observed. Apparently, high and low levels of psychosocial stress were induced independently from the initial mixing group. Accordingly, groups of animals with divergent response were built over all batches and termed HS and LS (for high stress/score and low stress/score), respectively. In detail, the second mixing revealed a skin lesion score (mean ± SEM) on HS and LS animals originated from the HH mixing of 181.0 ± 55.6 and, 46.3 ± 18.4 respectively, and a skin lesion score on HS and LS animals originating from LL mixing of 119.7 ± 47.9 and 32.7 ± 6.6, respectively. Liver samples were taken and stored at -80°C until analyses. Based on parameters of different stress levels (skin lesion score, creatine kinase, and cortisol as stated in Table 1) those animals were selected for gene expression profiling which represented extremes within mixing groups (Figure 1: ANALYSIS).

Each sampling group was represented by 4 castrates and 2 females.

2.2 Physiological parameters

The measurements of cortisol levels and creatine kinase activity were previously described [2]. In brief, cortisol levels were measured with the automated analyzer Centaur (Siemens Healthcare Diagnostics S.A.S., Saint Denis, France). The creatine kinase activity was measured with a clinical biochemistry automat (COBAS-MIRA Plus, Roche Diagnostics). Plasma urea nitrogen was analysed with a commercial assay using Fuji DriChem 4000i purchased from scil animal care company GmbH, Viernheim, Germany.

2.3. RNA isolation, target preparation and hybridization
Total tissue RNA was isolated from individual liver samples (n = 12; balanced for mixing group) using Tri-Reagent (Sigma-Aldrich, Taufkirchen, Germany) per manufacturer’s directions. Quantification and purification were performed as previously described [14]. All RNA samples were stored at -80°C until downstream analyses was performed. For the microarray experiments, individual biotin-labeled cRNA samples were hybridized on Affymetrix GeneChip porcine Genome Arrays (Affymetrix, Santa Clara, CA, USA).

2.4. Data analyses
The microarray data were analyzed as previously described [14]. In brief, the arrays passed the appropriate quality control criteria as proposed by Kauffmann et al. (2009) [17]. The data was GC-RMA normalized (Log2) and filtered by MAS5 (present rate > 50% per experimental group), standard deviation (SD > 0.2) and mean (m > 2.5). Relative changes of mRNA abundances were estimated via variance analyses (SAS Institute, Cary, NC, USA), including effects represented by the stress level, sex, slaughter batch, mixing group, and stress level*sex (Vijkl = µ + stress leveli + sexj + slaughter batchk + mixing groupl + (stress level*sex)ij + errorijkl). Due to multiple testing, p-values were converted to a set of q-values [18]. The level of significance was set at p ≤ 0.05. The raw data has been deposited in a MIAME compliant database, the National Center for Biotechnology Information Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) (accession number: GSE49290).

2.5. Pathway analyses
The probe-sets were annotated by Ensembl Susscrofa 9 [19]. Gene lists obtained from the microarray analyses were evaluated with ‘Ingenuity Pathway Analysis’ (IPA release winter 2012, Ingenuity Systems, Redwood City, CA, USA). The level of significance was set at p ≤ 0.05. The significant top five pathways were considered in the discussion.

2.6. Quantitative real-time PCR
Total transcript levels of selected target (ALDH1B1, ALDH9A1, COMT, DHCR24, DHCR7, HMGCR, SQLE, GALP, and IL1R1) and reference genes (RPL10, RPS11) were quantified by real-time qPCR (Table 2) as previously described in detail [14]. In brief, 12 individual liver mRNA samples were analyzed in duplicate on a LightCycler 480 system using LightCycler 480 SYBR Green I Master (Roche, Mannheim, Germany). Data were factorial normalized, the statistical analysis included effects of stress level, sex, slaughter batch, mixing group, and stress level*sex (SAS Institute, Cary, NC). The level of significance was set at p ≤ 0.05.
3. Results

We investigated hepatic gene expression in psychosocially high- and low-stressed pigs. The aim of the current study was to investigate whether emotional responses will be accompanied by distinct physiological molecular paths in liver tissue. The samples used in this study were selected from a larger animal experiment [2]. Six animals with high stress levels (HS) and six animals with low stress levels (LS) were used to create two microarray experimental groups to analyze their transcriptional responses in porcine liver tissue. The animals differed significantly regarding physical and physiological parameters (Table 1). The pigs were characterized by lesion scores as well as by the physiological stress parameters creatine kinase activity and plasma cortisol level, which are known to reflect both damage of muscle fibres due to strenuous physical activity and adrenal responses due to psychosocial stress, respectively. Plasma glucose, plasma lactate, and blood urea nitrogen were found in physiological concentrations and were unaffected by stress level. The initial microarray analyses identified 12,887 expressed probe-sets (~53 % present calls). Further analyses filtered 7,914 probe-sets, representing 5,906 genes [19].

3.1. Transcriptional responses due to different psychosocially induced stress levels

The comparison of liver tissue transcripts derived from HS and LS samples revealed 1,274 different probe-sets (p ≤ 0.05; corresponding q ≤ 0.19) (Table S1). Of these, 694 probe-sets showed increased transcript abundances in HS animals (H > L). Analysis of these differences suggested molecular routes related to tRNA charging, urea cycle, acute phase response, estrogen receptor signaling, and glucocorticoid receptor signaling (Table 3). Additionally, two transcripts associated to galactose degradation showed increased abundances in HS animals: GALE (UDP-galactose-4-epimerase; p = <0.05; Fold change 1.75) and GALK1 (galactokinase 1; p = <0.05; Fold change 2.39). Furthermore, 580 probe-sets showed lowered mRNA abundances in HS animals (HS < LS), related to epoxysqualene biosynthesis, noradrenaline and adrenalin degradation, methylglyoxal degradation, dopamine degradation, and cholesterol biosynthesis (Table 3).

3.2. Alterations in mRNA abundances of selected transcripts

Both microarray and qRT-PCR data were correlated in order to validate the differences in mRNA abundance between the experimental groups. According to Ingenuity pathway analysis no particular pathway was significantly altered (p < 0.05) when transcripts with decreased expression in HS compared to LS samples were investigated (Table 3: mRNA abundance: HS < LS). In order to verify this issue, selected transcripts represented mainly such gene products, which were involved in those pathways. In total, nine transcripts encoding genes associated with catecholamine degradation (ALDH1B1, ALDH9A1, COMT),
cholesterol biosynthesis (DHCR24, DHCR7, HMGCR, SQLE), central nervous system (GALP), and acute phase response (IL1R1) were analyzed (Table 4). The qRT-PCR data reflected the statistical validity of the microarray analyses. The transcripts HMGCR and GALP appeared to be false negatives in the microarray dataset and showed significantly lowered mRNA abundances in HS animals when analyzed by qRT-PCR. The mRNA abundance of the transcript encoding DHCR7 remained unaltered in both MA and qRT-PCR. The differences in mRNA abundances (i.e. Fold change) appeared reproducible in both microarray and qRT-PCR analyses. The correlation coefficients of the data obtained by the different approaches were highly significant and ranged between 0.85 and 0.99, except for IL1R1 ($\rho = 0.57; p = <0.10$).

4. Discussion

In order to gain detailed knowledge about transcriptional responses and mechanisms that might contribute to allostasis we conducted a microarray experiment in porcine liver tissue of animals involved in a mixing experiment [2]. On the transcriptional level we investigated the interrelation between psychosocially induced stress levels and supporting metabolic mechanisms in porcine liver tissue. The differences of cortisol level, CK activity and lesion scores indicate that the experimental groups used different strategies to cope with psychosocial stress induced by aggressive temperament, social dominance, and exogenous stressors associated with the mixing treatment. Hence, the transcriptional alterations found in liver tissue display the basic requirements in terms of allostasis, which enables the organism to respond to psychosocial stress via sophisticated mechanisms covering stress-related, immune and metabolic alterations. In this context, although the liver is seen as subordinated tissue, it has a central metabolic position, thereby processing concomitantly efferent and afferent signals to afford its multiple functions. However, there might be limitations to extrapolate hepatic expression patterns to the organismal level.

4.1. Transcriptional alterations related to stress mediators

Fighting behavior and resulting aggression were shown to impact meat quality parameters [2,20]. In particular, considering all treated animals within our experiment, an increased meat pH at 24 hours appeared when aggressive animals were mixed with unfamiliar mates [2]. Interestingly, due to pre-slaughter stress an increased meat pH is positively correlated with urinary adrenaline and noradrenaline levels at slaughter [21]. Because in our study HS animals showed decreased mRNA abundances of catecholamine degrading enzymes a delay of catecholamine degradation might have occurred which would lead to a prolonged catecholamine appearance. Catecholamines are known as important stress mediators to
conduct signals to tissues and body compartments. In this context, the transcript encoding
catechol-O-methyltransferase (COMT) is of particular interest. The mammalian gene COMT
decodes a key enzyme which contributes to eliminate the catecholamine neurotransmitters,
in particular dopamine. Interestingly, research on the homologous mouse gene COMT1 has
shown its association with aggressiveness and stress response [22,23]. Furthermore, a study
investigating the molecular equivalents of different aggressive phenotypes revealed lowered
mRNA abundances of COMT in aggressive male mice [24]. Accordingly, in our study both
microarray and qRT-PCR data revealed a decreased mRNA abundance of COMT in HS
animals. This observation supports data which link COMT to molecular mechanisms
regulating aggressiveness leading to psychosocial stress [22,25].

Furthermore, HS samples showed increased mRNA abundance of transcripts associated
with estrogen receptor signaling and glucocorticoid receptor signaling. However, the analysis
revealed unaltered mRNA abundances of the particular receptors, NR3A1 and NR3C1,
respectively. Because cortisol levels were found to be numerically increased in HS animals,
a transcriptional response of glucocorticoid receptor associated transcripts was expected.
The large overlap regarding the assigned transcripts in estrogen receptor signaling and
glucocorticoid receptor signaling rather indicate an adjusted crosslink between stress-
response and metabolism than an impact of a particular sexual hormone.

4.2. Transcriptional alterations related to the immune system

As reviewed elsewhere, the adjustment of the immune system is a major concern to cope
with environmental challenges [26]. Regarding this context, we recently reported that female
HS animals alter the expression profiles in peripheral blood mononuclear cells (PBMC),
possibly to retain a prepared immune system [14]. In face of psychosocial stress, our
microarray experiment revealed that porcine liver tissue focused on immunological
components. In particular, transcripts associated with the non-specific acute phase response
showed increased mRNA abundances in HS animals. These alterations may contribute to
establish immune barriers antagonizing potential microorganisms intruding via skin lesions.
Under physiological conditions, the acute phase proteins remain elevated for a minimum of
24 hours after an initial stimulus [27]. Consequently, the revealed hepatic expression pattern
in our study fit to the sampling time point.

4.3. Transcriptional alterations related to metabolism

The microarray experiment revealed increased mRNA abundances of key genes associated
with galactose utilization, possibly to mobilize alternative energy metabolites. Furthermore,
the increased mRNA abundances of transcripts associated with urea cycle may indicate a
higher deamination of amino acids, possibly to gain energy-rich amino acid carbons. In such
energy-mobilizing processes the increased cortisol levels [2] may be involved, highlighting
the potential of cortisol as catabolic hormone [28]. However, these transcriptional alterations
did not appear to be transduced to the metabolite level, at least urea nitrogen in plasma was
unaffected and both blood glucose and lactose maintained in physiological concentrations. In
contrast, hyperglycaemia was observed after mixing unfamiliar pigs [5] and due to
aggressive behavior in male rats [29].
Additionally, the diminished mRNA abundances for cholesterol biosynthesis in HS samples
indicate metabolic alterations at the expense of energy consuming endogenous steroid
biosynthesis. Indeed, the transcriptional clues for a lowered cholesterol biosynthesis are in
line with the known phenomenon that psychosocial stress lead to a suppression of
unnecessary anabolism, including growth, digestion, and reproduction [30]. Thus, these
mRNA profiles indicate a catabolic state, probably driven by superior mechanisms
cumulating in increased energy demand and metabolic costs of psychosocially high-stressed
animals.

The HS animals showed clues for an increased translational activity as indicated by the
pathway tRNA charging. Interestingly, due to the cellular nutritional status the tRNAs
themselves differ in their nucleocytoplasmic distribution while their transcription rate is
independently from metabolic state [31]. In contrast, transcripts encoding tRNA-synthases,
which gene product act as one catalytic principle of the translation machinery, showed
increased mRNA abundances in HS animals, specifically for cysteine, glutamine, glutamic
acid, valine, phenylalanine, asparagine, aspartic acid, tryptophane, threonine, serine, and
proline. Such tRNA-synthases evolved early in evolution and are highly conserved. These
enzymes both interpret the RNA code and catalyze the attachment of specific amino acids to
the tRNAs containing the cognate trinucleotide anticodons. Of cause, in a microarray
approach it appears to be difficult to characterize the metabolic relevance. However, the
character of tRNA charging contributes to anabolic processes in protein metabolisms (e.g.
immunoglobulins, enzymes).

Encoding a neuropeptide, galanin-like peptide (GALP) is mainly expressed in neurons of the
arcuate nucleus of the hypothalamus (ARC) [32]. To date, there is only poor knowledge
about GALP expression in peripheral tissues and its biological function. However, it has been
suggested that GALP is an important mediator at the crossroad between nutritional status,
reproduction and energy balance [32]. Interestingly, in adrenal gland the transcript encoding
GALP showed an increased mRNA abundance in HS animals [12], while in liver tissue a
diminished transcript yield was observed. Thus, the expression of GALP appears to be
tissue-specific in psychosocially high-stressed animals.

5. Conclusions
In order to maintain physiological functions, coping following psychosocial challenges involve a variety of body compartments, including tissues acting in metabolism (liver), endocrine system (adrenal gland [12]), immune system (lymphocytes / PBMC [14]), and neural system (hippocampus [33,34]). These alterations reflect that complex organisms are able to respond to varying environmental conditions in a multidimensional manner, although there are evidences that mixing of animals differing in temperament is likely affecting animal welfare in an undesired way [3,4]. The broad behavioral, phenotypic, and transcriptional alterations observed in our experiment [2,12,14] could be summarized with reference to the concept of allostasis [15,16]. In this context, the brain is seen as key organ which perceives psychosocial stress and produces both behavioral and physiological responses via e.g. hypothalamic-pituitary-adrenal (HPA) axis hormones, catecholamines, and cytokines [35]. Upon corresponding receptors, adaptive effects are produced in various tissues and organs. According to the mRNA pattern in our study, one may speculate that macronutrients (e.g. galactose, amino acids) are redistributed via liver tissue towards body components responsible for e.g. physical exercise and immune function. Further, expression levels of COMT seem to be associated with aggressiveness and stress level and may impact related molecular mechanisms. Notably, these findings reflect the need to distinguish between acute and chronic stressors and other factors like age, social status and genetics. These results complement the findings, that psychosocial stress activates an array of adrenocortical, immunological and neurobiological adaptations [30]. In order to cope with an exogenous stressor, a strong activation of allostatic responses is required, evidently as part of evolutionary inherited mechanisms to face potential injuries during fighting [13]. In this context, liver tissue appeared to be capable to ensure survival by adjusting essential and unessential metabolic paths, possibly orchestrated by superior mechanisms (e.g. in brain and adrenal gland).

Additional material
Supplemental Table S1: Transcripts with higher and lower expression between H and L samples.

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Declaration of interest

The authors have declared that no competing interests exist.

References


### Table 1: Phenotype data of the analyzed animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HS pigs #</th>
<th>LS pigs #</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin lesions §</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Front</td>
<td>72.7 ± 15.7</td>
<td>15.5 ± 4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mid</td>
<td>59.3 ± 17.9</td>
<td>18.3 ± 7.5</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Rear</td>
<td>18.3 ± 11.9</td>
<td>5.7 ± 1.0</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Total</td>
<td>150.3 ± 37.8</td>
<td>39.5 ± 9.3</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Creatine kinase (U/l)</td>
<td>10,635.0 ± 1,703.9</td>
<td>2,126.5 ± 212.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>72.3 ± 4.3</td>
<td>57.9 ± 5.1</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Glucose (nmmol/l)</td>
<td>11.0 ± 1.0</td>
<td>12.9 ± 1.9</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>8.7 ± 2.2</td>
<td>7.6 ± 1.0</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/l)</td>
<td>4.6 ± 0.7</td>
<td>4.4 ± 0.7</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

HS – High stressed; LS – Low stressed; # mean ± SEM; n = 6; balanced for mixing groups; * p-value (2 tailed) of a two sample t-test reflecting differences between animals with high stress level and low stress level; § skin lesions in body sections: front (head, neck, shoulders, and front legs), middle (flanks and back), and rear (rump, hind legs, and tail);
Table 2: Primer used to verify microarray experiments by qPCR.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Sequence 5' - 3' For</th>
<th>Sequence 5' - 3' Rev</th>
<th>size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH1B1</td>
<td>AACGGGATTAGGGCACATTA</td>
<td>CTGTCCACTCCTTCCCTTGA</td>
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<tr>
<td>ALDH9A1</td>
<td>TGGGAACCTGGAGGCAAATC</td>
<td>AGGGGACCCATCTTTGTATC</td>
<td>233</td>
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<tr>
<td>COMT</td>
<td>CAACAGAGGTTGGGTCTCTA</td>
<td>CCCACAGGATTCTCTATTCT</td>
<td>164</td>
</tr>
<tr>
<td>DHCR24</td>
<td>TCCTTAATGATGGGGAGCAC</td>
<td>TACAGAAGCAGCAGCCACAC</td>
<td>126</td>
</tr>
<tr>
<td>DHCR7</td>
<td>GCATGACACTGACTTCTTC</td>
<td>CCCACCTCCTTTTTATTCC</td>
<td>136</td>
</tr>
<tr>
<td>HMGCR</td>
<td>CTGCACCATTGGCATCCATAG</td>
<td>CTTTGCAAGCTCCTTTGAA</td>
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<tr>
<td>SQLE</td>
<td>TGTGAATGTCCCTTGCTCAGG</td>
<td>GCATAGACTGGCAACAGCA</td>
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</tr>
<tr>
<td>GALP</td>
<td>CCGACTGTGCCAGGTTCAC</td>
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<tr>
<td>IL1R1</td>
<td>TATGACGCTGCTGATTGC</td>
<td>GGGAGACATGGGAAAGGT</td>
<td>106</td>
</tr>
<tr>
<td>RPL10 *</td>
<td>CTGTGTTCGTCTTTTCTCC</td>
<td>TCAATCTTTGCTCTTCC</td>
<td>199</td>
</tr>
<tr>
<td>RPS11 *</td>
<td>GAAACTGGCAAGGAAG</td>
<td>TTCGGATAGTGAGGGAG</td>
<td>214</td>
</tr>
</tbody>
</table>

ALDH1B1 - aldehyde dehydrogenase 1 family, member B1; ALDH9A1 - aldehyde dehydrogenase 9 family, member A1; COMT - catechol O-methyltransferase; DHCR24 - 24-dehydrocholesterol reductase; DHCR7 - 7-dehydrocholesterol reductase; HMGCR - HMG-CoA-reductase; SQLE - squalene epoxidase; GALP - galanin-like peptide; IL1R1 - interleukin 1 receptor, type I; RPL10 - ribosomal protein L10; RPS11 - ribosomal protein S11; * reference genes;
Table 3: Top 5 Ingenuity pathways of transcripts with higher and lower expression between HS and LS samples.

<table>
<thead>
<tr>
<th>Canonical pathway</th>
<th>mRNA abundance</th>
<th>p-Value</th>
<th>Number of involved genes</th>
<th>mRNA Abundance</th>
<th>p-Value</th>
<th>Number of involved genes</th>
<th>Metadata of involved genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA charging HS &gt; LS &lt;0.001 10 Gene symbol</td>
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HS – High stressed; LS – Low stressed; P-value: significance of association between dataset and IPA-pathways; Benjamini-Hochberg multiple testing correction;
Table 4: Comparison of microarray and quantitative PCR (qPCR) results for selected transcripts to verify microarray data.

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HS – High stressed; LS – Low stressed; FC - Fold change; # Values were calculated by factorial normalization on RPL10 and RPS11 expression values; ## correlation of normalized expression values was calculated by Spearman;
**Figure 1:** Experimental design used to analyze transcriptional responses due to different levels of psychosocial stress. The origin and composition of the profiled sampling groups is shown regarding their parental genetics, classified and induced aggressive behavior, and resulted stress levels. LR - Landrace; LW – Large White; D – Duroc; P – Pietrain; H – High lesion score; L – Low lesion score; HS – High-stressed; LS – Low-stressed;