



Data in Brief

Gene expression profiling reveals a possible role for somatostatin in the innate immune response of the liver

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ABSTRACT

Somatostatin is a neuropeptide hormone that inhibits pituitary growth hormone (GH) release. Using microarray analysis of gene expression in the livers of wildtype control and somatostatin knockout mice, we have previously identified a panel of genes whose GH-dependent and sexually dimorphic expression patterns are significantly altered by the absence of somatostatin (1). Here, we provide methodological and analytical details of that study, the raw data of which is deposited in the Gene Expression Omnibus as data set GSE56520. In addition, we performed further gene ontology analysis of the data and found that the differential expression of a second subset of genes in the livers of somatostatin-knockout mice versus wildtype controls is likely independent of GH signaling and involved in the innate immune response.

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Specifications

Organism/cell line/tissue	Mus musculus
Sex	Male and female
Sequencer or array type	Affymetrix GeneChip Mouse 430 2.0 Array
Data format	CEL files
Experimental factors	Somatostatin-knockout and wildtype control liver mRNA, three biological replicates per sex and genotype
Experimental features	Identify gene expression changes in the liver of mice that lack somatostatin.
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

All data can be found at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56520>.

2. Experimental design

Somatostatin (SST) is a highly evolutionarily conserved peptide hormone that is expressed in many tissues. Its most studied function is as an inhibitor of endocrine and exocrine secretions [2]. Indeed, it was first discovered as an inhibitor of growth hormone (GH) release from the pituitary gland [3]. Sex-specific properties of pulsatile GH secretion induce well-characterized sexually dimorphic gene expression patterns in the mouse liver [4]. To test the involvement of SST in this phenomenon, we generated *Sst*-knockout (KO) mice and performed microarray gene expression profiling on RNA isolated from the liver of knockouts and wildtype controls of both sexes. We confirmed that SST is essential

for the sexually dimorphic hepatic expression of a large panel of genes [1]. We also found a non-overlapping subset of differentially expressed genes, discussed here, that appear to be regulated independently of sexually dimorphic GH signaling.

3. Animals

The generation of somatostatin (*Sst*)-knockout mice has been described previously [5]. The mutant allele was backcrossed onto the C57BL/6J background for 14 generations and heterozygous breeding pairs were used to produce the experimental mice for this study. All mice were housed in a temperature- and light-controlled murine-specific pathogen free environment (72 ± 2 F, lights on 7:00 am to 7:00 pm) with free access to standard laboratory chow and water. All animal studies were approved by the IACUC at Oregon Health and Science University. Experimental knockout and wildtype control mice (12 total, 3 biological replicates per sex and genotype) were euthanized by cervical dislocation between 9:00 am and 12:00 pm at age 16 weeks. Liver tissue was immediately dissected, frozen on dry ice and stored at −80 °C until RNA extraction.

As controls for a separate experiment, the female mice of both genotypes had received a single subcutaneous injection of 10 µL sterile sesame oil at postnatal day 1, and had an empty 1 cm long piece of medical grade silastic tubing (Dow Corning, 0.0635" inner diameter) implanted subcutaneously between the shoulder blades under anesthesia with 2% Avertin at age 8 weeks. Male mice of both genotypes underwent 2 sham gonadectomy surgeries at postnatal day 1 and at 8 weeks of age under general anesthesia either by hypothermia or 2% Avertin, respectively. For both surgeries, a small incision was made

over the lower abdomen and the gonads were visualized, then the skin incision was closed with either medical grade super glue (neonates) or stainless steel wound clips (adults). During the second surgery, an empty piece of silastic tubing was inserted between the shoulder blades as described for the females. We have no reason to believe that these interventions, performed on both genotypes of mice, would result in altered hepatic gene expression patterns 8 weeks later.

4. RNA isolation

100 mg of liver from each mouse was homogenized in 1 mL of TRIzol reagent (Invitrogen) for 60 s using a rotor–stator mechanical homogenizer. All subsequent centrifugation steps were carried out for 10 min at 12,000 rpm and 4 °C. First, samples were centrifuged and the upper phase was transferred to a new tube with addition of 200 μ L chloroform. Following an additional centrifugation, the aqueous (upper) phase was carefully removed and the RNA precipitated with 500 μ L isopropanol. Samples were centrifuged again and a pellet containing RNA was obtained and washed first with 1 mL of 4 M LiCl and then with 70% EtOH. The pellet was allowed to air dry and then the RNA was resuspended in 100 μ L of diethylpyrocarbonate (DEPC)-treated H₂O. RNA was quantified using a spectrophotometer. Concentrations were between 420 and 1200 ng/ μ L with OD 260:280 values ranging from 1.94 to 2.06 and OD 260:230 values from 1.81 to 2.24. Further quality control was performed using Agilent 2100 BioAnalyzer chips and the RNA Integrity Numbers (RINs) ranged from 7.4–7.9. All samples are listed in Table 1.

5. Microarray

RNA samples were submitted to the University of Michigan Microarray Core Facility, where 250 ng total RNA per sample was used to synthesize cDNA, generate biotin-modified amplified RNA and prepare the aRNA for hybridization utilizing 3' IVT Express Kits (Affymetrix). 16 μ g of aRNA per sample were then hybridized to GeneChip Mouse Genome 430 2.0 Arrays (Affymetrix) for 16 h at 45 °C in Hyb Oven 640 (Affymetrix). Washing and staining of the GeneChips was performed according to the manufacturer's protocol using Fluidics Station 450 (Affymetrix) and GeneChips were scanned using the 3000 7G GeneChip Scanner with Autoloader (Affymetrix).

6. Quality control and data analysis

The distributions of the perfect match (PM) probes for each chip were compared. The distributions of each chip were similar (Fig. 1A). RNA degradation was examined and all samples were determined to be adequate (Fig. 1B). A probe-level model was fitted and standard error (SE) estimates from each gene on each array are shown in a boxplot summary (Fig. 1C). Sample 2 (Mouse 58, wild-type female replicate #2) showed a slightly elevated SE compared to the other

11 samples. Log₂-transformed expression values for each gene were calculated using robust multi-array average (RMA) [6]. A principal components analysis (PCA) was performed and the first two principal components plotted. Biological replicates from each group clustered together, but there was a clear separation between the 4 groups (Fig. 1D). Linear models specifically designed for microarray analysis [7] were fitted to the data and samples were weighted based on a gene-by-gene algorithm designed to down-weight chips that are deemed less reproducible, such as sample 2 [8]. The specific contrasts of male wildtype versus male Sst-KO and female wildtype versus female Sst-KO were computed. Probe-sets with a log₂ fold change ≥ 1 and an adjusted *P*-value of ≤ 0.05 were selected. *P*-values were adjusted for multiple comparisons using false discovery rate [9]. Analyses were conducted in the R statistical environment implementing the affy [10], affyPLM, and limma [11] packages. Gene Ontology (GO) analysis was performed using DAVID [12,13].

7. Results

376 annotated genes/cDNAs and 29 non-annotated sequences that showed no sexual dimorphism in wildtype mice were found to be differentially regulated in the liver of one or rarely both sexes of Sst-KO compared to WT mice (Supplemental Table 1). Of these, 126 were down-regulated and 279 were up-regulated in Sst-KO mice. Some of the more highly differentially expressed genes in this subset have been reported to be sexually dimorphic in other publications [14–17], and therefore may belong in the group of genes regulated by GH, which was the topic of our previously published article [1]. Regardless, using the DAVID tool to functionally cluster all 405 of these differentially regulated sequences by similarly annotated gene ontology (GO) biological process terms, we found that six of the seven significantly enriched annotation clusters were related to the immune system and included terms such as defense response, inflammatory response, MHC protein complex, cell chemotaxis and leukocyte migration (Supplemental Table 2). The remaining cluster was related to carbohydrate metabolism.

8. Discussion

Our study was not designed to differentiate between direct and indirect effects of the absence of SST on gene expression in the liver, although there are limited reports of SST receptor expression on hepatocytes [18]. Accumulating evidence has indicated that it is likely not hepatocytes that account for the bulk of SST receptor expression in the liver, but rather hepatic stellate cells (HSCs), which particularly begin to express SST-receptors after activation by various signals [19]. Because HSCs have recently been identified as an important component of the innate immune system [20], it is tempting to speculate that the enriched clusters of immune response genes that we found to be differentially expressed in the liver of Sst-KO mice are being expressed

Table 1
Samples used in this analysis and deposited to GEO as GSE56520.

Sample number	Mouse ID	Genotype	Sex	RNA concentration (ng/ μ L)	RIN	File name	GEO sample number
1	57	WT	F	778.1	7.9	Low_057(Mouse430_2).CEL	GSM1363209
2	58	WT	F	734.7	7.7	Low_058(Mouse430_2).CEL	GSM1363210
3	72	WT	M	895.5	7.6	Low_072(Mouse430_2).CEL	GSM1363211
4	87	KO	M	881.4	7.4	Low_087(Mouse430_2).CEL	GSM1363212
5	89	WT	M	823.4	7.6	Low_089(Mouse430_2).CEL	GSM1363213
6	95	WT	F	815.1	7.9	Low_095(Mouse430_2).CEL	GSM1363214
7	106	WT	M	1197.0	7.5	Low_106(Mouse430_2).CEL	GSM1363215
8	126	KO	M	783.6	7.4	Low_126(Mouse430_2).CEL	GSM1363216
9	171	KO	M	768.0	7.7	Low_171(Mouse430_2).CEL	GSM1363217
10	204	KO	F	672.9	7.9	Low_204(Mouse430_2).CEL	GSM1363218
11	258	KO	F	765.1	7.5	Low_258(Mouse430_2).CEL	GSM1363219
12	299	KO	F	427.8	7.6	Low_299(Mouse430_2).CEL	GSM1363220

WT: wildtype; KO: knockout; RIN: RNA Integrity Number.

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