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Minimal sex differences in gene expression in the rat superior olivary complex

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ABSTRACT

A critical issue in large-scale gene expression analysis is the impact of sexually dimorphic genes, which may confound the results when sampling across sexes. Here, we assessed, for the first time, sex differences at the transcriptome level in the auditory brainstem. To this end, microarray experiments covering the whole rat genome were performed in the superior olivary complex (SOC) of 16day-old Sprague-Dawley rats. Sexually dimorphic genes were identified using two criteria: $a \ge 2$ -fold change and a P value < 0.05. Only 12 out of 41,374 probes (0.03%) showed sexually dimorphic expression. For comparison, pituitaries from 60-day-old female and male rats were analyzed, as this gland is known to display many sex-specific features. Indeed, almost 40 times more probes, i.e. 460 (1.1%), displayed sexual dimorphism. Quantitative RT-PCR confirmed 47 out of 48 microarray results from both tissues. Taking microarray and gRT-PCR data together, the expression of six genes (Prl, Eif2s3y, Gnrhr, Pomc, Ddx3y, Akr1c6) was higher in the male SOC, whereas two genes were upregulated in the female SOC (LOC302172, Xist). Four of these genes are sex-chromosome linked (Eif2s3y, Ddx3y, LOC302172, Xist). In summary, our data indicate only minor and negligible sex-specific differences in gene expression within the SOC at P16.

Keywords: auditory system, superior olivary complex, transcriptome, sexual dimorphism

LIST OF ABBREVATIONS

- CT <u>cycle threshold</u>
- E primer <u>e</u>fficiency
- F <u>f</u>emale
- fc <u>f</u>old <u>c</u>hange
- qRT-PCR <u>quantitative reverse transcription-polymerase chain reaction</u>

MA

- M <u>m</u>ale
- SOC <u>superior olivary complex</u>

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INTRODUCTION

The mammalian auditory brainstem comprises the first processing centers of acoustic information and subserves various functions, such as sound localization and the generation of auditory space maps (Grothe, 2003; Konishi, 2003). Its main constituents are the cochlear nuclear complex, the superior olivary complex (SOC), and the inferior colliculus (Smith and Spirou, 2002). A major task lying ahead is the dissection of the molecular repertoire involved in auditory processing. Recently, the advent of modern genomics technologies has fostered the application of large-scale gene expression analysis in neurobiology (Blackshaw and Livesey, 2002). This has sparked various transcriptomics studies (Cho et al., 2002; Friedland et al., 2006; Harris et al., 2005; Holt et al., 2005; Koehl et al., 2004; Tadros et al., 2007) and proteomics studies (Becker et al., 2008; Nothwang et al., 2003) in the auditory brainstem. Likely, these reports represent but the spearhead of further investigations into the molecular mechanisms underlying the development and function of the various centers.

An increasing body of data demonstrates sexual dimorphisms at the anatomical, functional, and transcriptional level in the nervous system (Cahill, 2006; Dewing et al., 2003; Yang et al., 2006). Hence, a major concern in largescale gene expression studies is that sexually dimorphic genes may confound the data set if both sexes are sampled together. Sexual dimorphisms have also been reported for the auditory system. Well-established differences in humans include higher hearing sensitivity and increased likeliness of spontaneous

otoacoustic emissions (McFadden, 1993), an estimated 13% shorter cochlea (Don et al., 1993) and higher transient-evoked otoacoustic emission levels (Berninger, 2007) in females compared to males. Females show also shorter latencies and higher amplitudes of auditory brainstem responses (Hultcrantz et al., 2006; Sininger et al., 1998), as well as higher temporal-order perception thresholds (Fink et al., 2005; Szymaszek et al., 2006). Finally, hearing loss occurs at a more rapidly rate in men than in women (Pearson et al., 1995).

Here, we addressed the question of whether sexual dimorphisms, so far mainly found at the functional level, can be extended to the transcriptional level in the auditory brainstem. To this end, we compared the gene expression profile of the rat SOC in P16 males and females. This juvenile age is often used in functional SOC studies, as most properties resemble the mature situation (Ehrlich et al., 1999; Srinivasan et al., 2004; Klug and Trussell, 2006; Song and Kaczmarek, 2006, Smith et al., 2000). To validate our approach, we also analyzed the adult pituitary gland, which plays a central role in sexual development and displays anatomical (MacMaster et al., 2007) and transcriptional (Nishida et al., 2005; Zhan and Desiderio, 2003) sexual dimorphisms.

MATERIAL AND METHODS

Tissue preparation

Total RNA for microarray experiments and quantitative RT-PCR (qRT-PCR) was isolated from female and male Sprague-Dawley rats at postnatal day (P) 16 for the SOC and at P60 for the pituitary gland. Rats were deeply anesthetized with 7% chloralhydrate (1 ml/100 g) and decapitated. In order to prevent apoptotic signaling and degradation of mRNA, brains were rapidly removed and dissected in a chilled solution (4 °C) containing (mM): 25 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 260 D-glucose, 2 sodium pyruvate, 3 myoinositol and 1 kynurenic acid, at pH 7.4, when gassed 30 min with 95% O₂ and 5% CO₂. Coronal slices (300-µm thickness), containing the SOC, were cut with a vibratome (Leica VT 100 S, Leica, Nussloch, Germany). The SOC areas of both sides were then manually excised from the slices (Koehl et al., 2004). The entire procedure lasted approximately 30 min. To collect the pituitary, the brain was removed from the skull and the pituitary was dissected. Tissue was stored in RNAlater (Ambion, Darmstadt, Germany) at -80 ℃. Extraction of total RNA was performed using the RNeasy lipid tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Prior to isolation, the tissue of a single animal was macerated in 1 ml Qiazol using a homogenizer (Miccra D-8, Roth, Karlsruhe, Germany) at 23,500 rpm for 15 s. RNA integrity and purity were determined using the 2100 Bioanalyzer (Agilent, Böblingen, Germany).

Microarray experiments

A total of 12 rats were used in the microarray studies. Three biological replicates and three technical dye swap experiments were performed per tissue. Fluorescently-labeled cRNA was synthesized with the Agilent low RNA input linear amplification kit, according to the manufacturer's instructions. A total of 500 ng total RNA was used as a starting amount. The yield and incorporation of the dye was determined using a Nanodrop D-1000 UV-Vis spectrophotometer (Peglab, Erlangen, Germany). The specific activity of the samples was higher than 7 pmol Cy3 or Cy5 per µg RNA. Labeled cRNA probes (1,000 ng) were fragmented and hybridized to rat whole genome 60mer oligonucleotide microarrays designed from Agilent (1x44K, containing 41,374 probes). After washing steps, the chips were immediately scanned with an Agilent microarray scanner. The extraction of the microarray data and a local background subtraction were done with Agilent feature extraction software (v8.1). The data normalization and statistics were performed by in-house software packages and algorithms of the Fraunhofer ITWM, Kaiserslautern. Furthermore, the intensitydependent error of the dyes was reduced by a lowess-transformation with smoothing parameter of 0.2. As no assumption was made concerning the distribution type of the data, the non-parametric fisher-pitman-test was used to identify differentially expressed genes.

Quantitative RT-PCR

Quantitative RT-PCR was performed to validate the results of the microarray experiments. For the pituitary, RNA from three animals of same sex used for microarray analysis was pooled and proceeded for qRT-PCR. In case of the

SOC, two different kinds of RNA pools were used, due to the small sample amount. They either consisted of samples from the three animals also applied to the microarray or were derived from additional four animals. No difference was observed between the two pools in the qRT-PCR experiments. Total RNA was reverse transcribed using 200 ng random hexamers, 500 ng oligo $(dT)_{18}$ primers and 1 µl (200 units) superscript II (Invitrogen, Karlsruhe, Germany). Primer pairs were designed to amplify the spotted 60mer sequences of the array. Primer efficiency (E) was determined by measuring serial dilutions of cDNA in triplicate. Efficiency was calculated according to the equation:

E = 10 ^(-1/slope) (Pfaffl, 2001). Primer sets for the transcripts are listed in Table 1. The gene peptidylprolyl isomerase A (*Ppia*) served as the reference in all experiments (Feroze-Merzoug et al., 2002). Quantitative RT-PCR was performed on a MyiQ thermal cycler (Bio-Rad, Munich, Germany). Reactions contained 1 µl cDNA template, 0.5 µl 20 pM forward and reverse primer, 5.5 µl RNase free water, and 12.5 µl master mix (absolute SYBR green fluorescein, Thermo scientific, Schwerte, Germany). Thermal cycling conditions were as follows: 15 min 95 °C activation, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. After a final step of 72 °C for 5 min, a melt curve analysis was performed. Conditions were 80 cycles with a stepwise temperature rise of 0.5 °C, each for 30 s, starting at 55 °C. Samples for each transcript were run in triplicate, and a negative control was run in each experiment and for each primer set. The cycle threshold (CT) of each transcript was averaged for each triplicate and for the reference gene *Ppia*. Each experiment was run a total of three times. These

runs represented technical replicates for the pituitary and both technical and biological replicates for the SOC. For statistical analysis, the mean CT value of *Ppia* was subtracted from the mean CT of each transcript in each run. These Δ CT values were tested for normal distribution behavior and a one-tailed student paired t-test was performed between males and females.

The regulation factor of each run was calculated as previously described (Pfaffl, 2001). The fold change of the triplicate experiment was calculated as follows: The assumption of normally distributed CT values leads to a log-normal distribution for the resulting fold-changes: Given the normally distributed random variables of the CT values c_{11} , c_{12} , c_{21} and c_{22} and the qRT-PCR efficiencies E_1 and E_2 , the fold-change (fc) is defined as:

$$fc = \frac{E_1^{c_{11}-c_{12}}}{E_2^{c_{21}-c_{22}}} = e^{c_{11}lnE_1-c_{12}lnE_1-c_{21}lnE_2+c_{22}lnE_2}$$

The linear combination

$$c = c_{11} ln E_1 - c_{12} ln E_1 - c_{21} ln E_2 + c_{22} ln E_2$$

is again normally distributed and, therefore, fc is log-normally distributed whose mean μ_{ln} and corresponding standard deviation σ_{ln} can be estimated by the arithmetic mean μ and the standard deviation σ of the random variable c using the following formulas:

$$\mu_{lm} = e^{\mu + \frac{\sigma^2}{2}} \qquad \sigma_{lm} = \sqrt{e^{2\mu + \sigma^2} (e^{\sigma^2} - 1)}$$

Animals

All protocols were in accordance with the German Animal Protection law and were approved by the responsible animal care and use committee (Landesuntersuchungsamt, Rhineland Palatinate, Germany). Protocols also

inal.

RESULTS

RNA quality and quantity

To determine the existence and the degree of sexual dimorphisms at the transcriptional level in the SOC and the pituitary, RNA was isolated from the SOC of P16 male (M) and female (F) rats and from the pituitary of P60 male and female rats. The quality of the isolated RNA population is a critical issue in microarray analysis. To assess the quality of the isolated RNA from both tissues, an aliquot of the samples was analyzed on a picochip using the Agilent 2100 Bioanalyzer. The electropherograms demonstrated a high integrity of the RNA with the ratio of 28S/18S ribosomal peak area ranging from 1.49 to 1.70. The A_{260/A280} ratio was > 1.9. Representative examples are illustrated in Fig. 1. These data implied a sufficient RNA quality to proceed with. The average amount of RNA obtained from both SOCs of a single animal was 2.2 μ g, and for the pituitary 41.8 μ g.

Sexual dimorphism in the pituitary

To determine the capacity of our microarray technique in assessing sexual dimorphisms in gene expression, we first analyzed RNA from the adult pituitary (P60), a known sexually dimorphic organ (MacMaster et al., 2007; Nishida et al., 2005; Zhan and Desiderio, 2003). At this age, sexual dimorphisms are well established in this organ, rendering it a promising tissue for evaluation. Differentially labeled cRNAs obtained from males and females were hybridized together on an Agilent microarray containing 41,374 probes for known genes,

predicted genes, or expressed sequence tags. Across the entire transcriptome, scatter plot analysis revealed sex-specific differences in gene expression (Fig. 2A). Several spots, representing mean log intensity hybridization signals, showed a clear dislocation from the solid line on which the female RNA level equals the male RNA level. Maximal changes in expression level were an 18.24-fold upregulation in males and a 29.07-fold upregulation in females (Suppl. Tables 2, 3). The overall determination correlation for the pituitary was $R^2 = 0.976$. Statistics revealed a significant sexual dimorphism for 460 probes (P < 0.05) with a \geq 2-fold change (Suppl. Tables 2, 3). This amounts to a total of 1.1% probes. In the female pituitary, 352 probes (0.85%) showed increased hybridization signals, whereas 108 probes (0.26%) revealed an upregulation in males. The considerably higher amount of upregulated transcripts in the female is in agreement with a proteomics study in the human pituitary, in which five out of seven sexually dimorphic proteins were more abundant in females (Zhan and Desiderio, 2003).

To validate the microarray data, we next performed qRT-PCR experiments on a total of 24 exemplary genes. They were selected such that they covered probes displaying sexual dimorphisms to various extents in either direction (Tables 2, 4). They included two previously reported sexually dimorphic genes in the pituitary (*Gal, Prl;* Nishida et al., 2005). Furthermore, several genes with no sexual dimorphism (Suppl. Table 1), as well as all but one gene with sexdependent expression in the SOC, were investigated (Table 4). Finally, the probes covered the entire range of signal intensities on the array. Quantitative RT-PCR confirmed the microarray data for all 24 genes. Among them were 17

genes displaying a statistically significant sexual dimorphism on the microarray (*Spp1, Chga, Vsnl, Kcc4, Spin2b, Bdnf, Drd4, Gal, Grik1, Pdlim3, Pvalb, Prl, Eifs3y, Gnrhr, Pomc2, Ddx3y,* and *Xist*). The direction of changed expression level was identical to the one observed in the microarray experiments. Comparing microarray data with qRT-PCR data, many sexually dimorphic genes (*Chga, Kcc4, Bdnf, Drd4, Gnrhr* and *Pomc2*) showed a similar fold change in both types of experiments. Other genes (*Spin2b, Gal, Grik1, Pvalb, Eif2s3y, Xist, and Ddx3y*) showed higher fold changes by qRT-PCR. Among them, the three sex-chromosome-linked genes *Eif2s3y, Xist, and Ddx3y* displayed the largest difference between both types of experiments (Table 4; also see discussion). Taking microarray and qRT-PCR together, *Eif2s3y and Xist* displayed the strongest sexual dimorphism. From these experiments, we concluded that our microarray technology is well suited to study sexual dimorphism in tissues.

Sexual dimorphism in the SOC

In order to assess sex-specific gene expression in the SOC, RNA was isolated from the SOC of female and male P16 rats. We chose this age, as for technical reasons this "juvenile" stage is often used for investigations of the mature SOC and, therefore, highly relevant to many studies. Especially electrophysiological patch-clamp studies are rendered more complicated at older ages due to a developmental enrichment of glial cells. Across the entire transcriptome, RNA expression strongly correlated between male and female, indicated by a very high determination coefficient ($R^2 = 0.994$), which was

considerably higher than that observed in the pituitary ($R^2 = 0.976$; Fig. 2B,C). In the scatterplots, overall majority of spots were located onto or close to the solid line, indicating similar expression levels between both sexes. Indeed, only 12 oligomers displayed a significant sexual dimorphism, corresponding to 0.03% of all interrogated probes (Table 3). Overall, the SOC displayed almost 40 times fewer sexual dimorphisms in gene expression than the pituitary. Seven probes, corresponding to six different genes, showed upregulation in males: prolactin (Prl), pro-opiomelanocortin (Pomc2), the eukaryotic translation initiation factor gamma (*Eif2s3y*), the gonadotropin releasing hormone receptor (Gnrhr), the RNA helicase Dby (Ddx3y), and the aldo-ketolase reductase member 6 (Akr1c6). Their changes ranged from 2.15-fold for Akr1c6 to 57.55fold for Prl, with the two probe sets for Pomc showing similar changes (3.42-fold and 3.67-fold). Five probe sets, corresponding to three different genes, were significantly upregulated in females: Xist (3 probes), a gene selectively expressed from one of the two X-chromosomes in females (Brown et al., 1991). Rt1-ce16, encoding an antigen of the class I MHC heavy chain, and LOC302172, encoding a protein similar to the synaptonemal complex protein 3. They displayed 2.44-fold (Rt1-ce16) to 19.01-fold changes (Xist) compared to males. Interestingly, two Xist probes showed a more than 17-fold change, whereas the third Xist probe displayed only a 2.63-fold change. This difference likely reflects the existence of Xist isoforms, consistent with previous findings on alternative splice products (Ma and Strauss, 2005). Four of the 9 sexually dimorphic genes in the SOC are located on the sex chromosomes: Eif2s3y and Ddx3y are Y-chromosome linked, and Xist and LOC302172 are X-chromosome

linked (Table 3).

In order to validate the microarray data, qRT-PCR experiments were performed for 24 probes. These included 8 out of the 9 genes displaying sexual dimorphism in the SOC (Table 4). *LOC302172* could not be analyzed, because four different primer pairs failed to amplify the probe with efficiency sufficient for qRT-PCR. An additional 16 genes that we analyzed by qRT-PCR displayed no sexual dimorphism in the SOC, albeit 11 of them were differentially expressed in the pituitary (Table 2, Suppl. Tables 1-3).

The results of the qRT-PCR experiments confirmed for 7 of the 8 probes the sexually dimorphic expression in the SOC as well as the direction of change (Table 4). The only exception was Rt-ce16, which showed a nonsignificant 1.22fold change in gRT-PCR compared to a significant 2.44-fold change in the microarray experiments. Nevertheless, the direction of the change was the same, i.e. a higher expression in females. Thus, combining microarray and gRT-PCR data, the following 8 genes showed sexual dimorphism in the SOC: Prl, Pomc2, Eif2s3y, Gnrhr, Ddx3y, Akr1c6, Xist, and LOC302172. Similar to the pituitary, qRT-PCR for the three sex-chromosomal genes Ddx3y, Eif2s3y, and Xist indicated a much higer sexual dimorphism (a 16,164-fold change for *Eif2s3y*, a 409-fold change for *Xist*, and a 246-fold change for *Ddx3y*) compared to the microarray experiments (17.81, 19.01, and 2.37-fold changes for *Eif2s3y*, Xist, and Ddx3y, respectively). This is likely related to their highly selective expression in either of the two sexes (see discussion). Prl, Eif2s3y, Xist, and Gnrhr demonstrated a strong sexual dimorphism, whereas Pomc2 and Akr1c6 exhibited a weak sexual dimorphism in both types of experiments.

Finally, we compared microarray data of the sexually dimorphic genes identified in the SOC in both microarray and qRT-PCR experiments with the pituitary (Table 4). Except for *Akr1c6*, these genes were differentially expressed in the pituitary as well. Moreover, the direction of their sexual dimorphism was identical for five of them. The only exception was *Prl*, which was upregulated in the male SOC, whereas it was upregulated in the female pituitary.

Taken together, these data demonstrate only minimal sex-dependent differences in gene expression in the SOC at P16. Furthermore, the few sexual dimorphisms are unlikely to be correlated with specific auditory function, as the majority of them also occur in the pituitary.

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DISCUSSION

In this study, we investigated sex differences in gene expression in the rat SOC and, for comparison, in the pituitary gland. The main conclusion is that sexual dimorphisms at the transcriptional level are negligible in the SOC at P16. This conclusion is based on our finding that only 12 of the 41,374 interrogated probes (0.03%) showed significant differences between males and females (\geq 2-fold change) on the microarray (Table 3). This small number was even lowered to 11 probes by qRT-PCR, corresponding to 8 different genes (*Prl, Eif2s3y, Gnrhr, Pomc, Ddx3y, Akr1c6, LOC302172 and Xist*), as *Rt-ce-16* could not be confirmed (Table 4). In contrast, in the adult pituitary, 460 probes (1.1%) showed a sexual dimorphism (Suppl. Tables 2, 3), of which a selected set of 17 samples could be confirmed by qRT-PCR (Tables 2, 4). Altogether, qRT-PCR confirmed 47 out of 48 microarray results, lending strong support to the microarray data.

Our analysis was performed in P16 animals, shortly after hearing onset. We used this age in order to match the age often used in functional studies aiming at characterizing the mature SOC (e.g. Smith et al., 2000). At later stages, many investigations, such as electrophysiological experiments in slices, are rendered difficult. This would have impaired subsequent functional analysis of sexually dimorphic genes with potential impact on SOC physiology. We hence preferred to analyze P16 animals instead of older ages. We consider it unlikely that in young-adult animals, aged P30-P60, significantly more sexually dimorphic genes would have been detected. Transcriptome analysis, for

instance, revealed only minor changes in gene expression in the SOC between P16 and P25 (unpublished data). Indeed, samples from P60 male and female animals have already been pooled in a recent transcriptome study in the SOC (Koehl et al., 2004).

An extrapolation of our data towards much older animals, however, may be unwarranted. A recent analysis of hearing loss in C57BL/6 mice showed that at P100, high frequency hearing loss was greater in females than in males (Henry, 2002). It is noteworthy that this study was performed in a mouse model for presbyacusis and analyzed peripheral effects. It will be of interest to perform a detailed time course study on sex differences in such a model system in the central auditory system. This would provide further insight into sexually dimorphic genes in the auditory brainstem and whether it rises with age.

We defined sexually dimorphic genes on the microarray as those displaying $a \ge 2$ -fold change. The threshold was set so because similar thresholds were applied in many microarray experiments (Chaudhuri, 2005; Gomez-Ospina et al., 2006; Mirnics et al., 2000). Remarkably, the inclusion of genes with $a \ge 1.8$ -fold change in the SOC would have increased the number of significantly sexually dimorphic genes only marginally from 12 (0.03%) to 16 (0.04%). So far, 0.03% is the lowest value observed for sexually dimorphic genes with a regulation factor of ≥ 2 -fold in the nervous system. A microarray analysis of the total brain in an adult mouse identified 0.13% (Yang et al., 2006) and at embryonic stage E10.5, i.e. prior to gonadal differentiation, 0.1% (Dewing et al., 2003) sexually dimorphic transcripts. Thus, the number of sexually dimorphic

genes in the SOC is even below the number observed before sexual differentiation when analyzing the entire nervous system.

We consider it highly unlikely that the very low proportion of sexually dimorphic genes in the SOC is caused by the microarray platforms used in the present study. Three (*Eif2s3y*, *Xist*, *Ddx3y*) of the sexually dimorphic genes in the SOC were also detected in the mouse embryonic nervous system, and two of them showed similar regulation factors. For Eif2s3y, we observed a regulation factor of 17.79 and for Xist, regulation factors of 17.80 and 19.01, whereas in the embryonic tissue, the regulation factors for the two genes were 9.0 and 18.5, respectively (Dewing et al., 2003). Only Ddx3y had a lower regulation factor in the SOC (2.37) compared to the embryonic tissue (10.0). In addition, in our parallel analysis of the pituitary, performed with the same technique, 1.1% of the probe sets showed a sexual dimorphism. This number is almost 40 times higher than in the SOC and also 10 times higher than obtained in the other two studies of the nervous system (Dewing et al., 2003; Yang et al., 2006). Finally, our analysis identified several genes previously reported to be sexually dimorphic, such as Ddx3y, Xist (Dewing et al., 2003; Yang et al., 2006), Gal, Prl, (Nishida et al., 2005; Zhan and Desiderio, 2003), and Gnrhr (Moles et al., 2007). This demonstrates the capability of our approach to detect biological differences and rules out that the low number in the SOC reflects a sensitivity problem. Furthermore, qRT-PCR analysis confirmed the absence of sexually dimorphic genes for 16 SOC probes, albeit 11 of them displayed sexual dimorphism in the pituitary. In total, 24 probes were analyzed in both tissues by qRT-PCR. This included probes with sexual dimorphism restricted to

one of the two tissues analyzed as well as several probes displaying no sexual dimorphism in both tissues. These data confirmed 47 microarray results. Only one data point, *Rt-ce-16* in the SOC, could not be confirmed. Notably, the qRT-PCR data reduced, and did not increase, the number of sexually dimorphic genes detected by microarray in the SOC. Together, these validation data demonstrate the reliability of our microarray platform.

Another explanation for the low rate of sexually dimorphic genes may be our choice to analyze the entire SOC. This auditory center is a composite region of different nuclei, with the major ones being the lateral superior olive, the medial superior olive, and the medial nucleus of the trapezoid body. Sex differences specific to any of the individual nuclei might thus have been masked by probing the SOC in toto. Currently available RNA amplification techniques afford the analysis of few cells by microarray analysis. However, these techniques are still challenging and time consuming. Indeed, long lasting tissue preparation often leads to degraded RNA from the SOC (unpublished data), and analysis at the single-cell level would thus have impeded the required RNA quality control measurements. Furthermore, such an analysis would have required a considerable extension of the study to different nuclei and even to different neuronal subtypes within a given nucleus. This, however, was beyond the scope of our investigation. Finally, we consider heterogenous sexual dimorphism within the SOC conceptually unlikely, as the main nuclei share a common function, i.e. processing acoustic cues for sound localization (Grothe, 2003).

The sexual dimorphism of the 8 genes identified in the SOC ranged from a 2.15-fold change for Akr1c6 to a 57.55-fold change for Prl. Except for Prl, the fold changes observed by qRT-PCR were higher than those observed by microarray analysis (Table 4). Differences in fold changes obtained by these two methods for the same gene have often been observed and can be attributed to different efficiencies of reverse transcription when preparing the samples for microarray or for the PCR, non-specific or cross hybridization of labeled targets to the microarray probes or amplification biases in the gRT-PCR (Morey et al., 2006). Furthermore, different normalization procedures are used in both technologies (Morey et al., 2006). Finally, in cases with a low or even absent gene expression, the qRT-PCR technique, which exponentially amplifies the original template, may yield a considerably higher difference than the hybridization-based microarray technology. This explanation is supported by our finding that the highest disagreements were observed for the probes Xist, *Eif2s3y*, and *Ddx3y*, that are only expressed in one sex (Brown et al., 1991; Xu et al., 2002).

None of the 8 genes displaying sexual dimorphism in the SOC represents a strong candidate for previously reported sex differences in audition. Five of these 8 genes had a similar sexually dimorphic expression in the pituitary, which is an endocrine organ. Three of them, *Xist*, *Ddx3y*, and *Eif2s3y*, are sex-chromosome linked and their sexually dimorphic expression in the brain has been reported previously (Dewing et al., 2003; Nishida et al., 2005; Xu et al., 2002; Yang et al., 2006). A fourth gene (*LOC302172*) is also located on the X-chromosome. Its higher expression in females indicates that it represents one of

the 15-20% of X-chromosomal genes escaping X-inactivation (Carrel and Willard, 2005). The reported function of these four genes is furthermore outside neurophysiological processes. Xist forms a non-coding RNA which coats one X chromosome in females in order to inactivate it (Brown et al., 1991). Ddx3yencodes an RNA helicase essential for spermatogenesis (Rosner and Rinkevich, 2007). *Eif2s3y* encodes a translation factor (Ehrmann et al., 1998), and LOC302172 likely represents a protein involved in chromosomal pairing (Cromie and Smith, 2007). The remaining four genes with sexual dimorphism in the SOC are related to hormones, which often demonstrate sexual dimorphism. Prl, showing the highest change in the SOC (57.55-fold) according to microarray analysis, mainly promotes lactation in the mammary gland (Pang and Hartmann, 2007). Our finding of a higher expression of *Prl* in males is likely associated with additional functions of this gene. The gonadotropin releasing hormone receptor (Gnrhr) is involved in regulation of reproduction (Kah et al., 2007), and Akr1c6 is likely involved in steroid metabolism (Vergnes et al., 2003). Finally, *Pomc* has multiple functions, such as stimulation of cortisol release from adrenal glands (Kempna and Fluck, 2008) and pigmentation of the skin (Schallreuter et al., 2007).

For all 8 genes, the relevance of their sexually dimorphic expression in the SOC is hence difficult to perceive despite the fact that some of them have established roles in the nervous system. *Pomc*, for instance, acts as an endogenous opiate (Vrinten et al., 2001). However, the presence of most sexual dimorphisms observed in the SOC in other tissues, including the pituitary, indicates that they represent the minimum of sex differences in a tissue caused

by the very nature of the sex chromosomes and general hormonal differences between the sexes. Thus, previously reported auditory sex differences likely have their foundation outside the auditory brainstem. Furthermore, several of them might represent uniquely human aspects of hearing, as they have not been reported from non-human species (McFadden, 1993).

Taken together, our data indicate that sexual dimorphisms in gene expression are minimal and negligible in the SOC of P16 rats. We therefore conclude that functional genomics, anatomical, morphological, and electrophysiological studies in the SOC at around this age do not require the separation of male and female animals. This will ease further studies in all those fields, as pooling data from both sexes will be possible. To extend this finding to other ages and to other auditory centers, however, further studies have to be performed.

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FIGURES

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FIGURE LEGENDS

Fig. 1. Electropherograms of RNA quality assessed by a 2100 Bioanalyzer. (A) RNA from male SOC tissue (SOC-M). (B) RNA from female SOC tissue (SOC-F). (C) RNA from male pituitary gland (Pituitary-M). (D) RNA from female pituitary gland (Pituitary-F). Bands in the gel-like images and peaks in the electropherograms indicate 18S and 28S ribosomal RNAs. Ratios (r) of 28S/18S ribosomal peak areas are given in the upper right corner of each panel and range from 1.52 to 1.69. The data demonstrate that the RNA was of sufficient quality to allow subsequent microarray analysis.

Fig. 2. Scatterplots showing mean log values of six microarray experiments. Each scatterplot represents 41,374 oligomers. The abscissa and ordinate values depict the signal in females and males, respectively. (A) pituitary. (B) SOC. (C) overlay of pituitary and SOC. The correlation coefficient R² was 0.994 for the SOC and 0.976 for the pituitary, illustrating a higher degree of sex differences in gene expression in the pituitary compared to the SOC.

TABLES

Table 1

Primer sequences for qRT-PCR

Primer	Sequence	Efficiency (%)	Amplicon size (bp)	
<i>rnAkr1c6</i> -for <i>rnAkr1c6</i> -rev	5`-AGAACTTCCAGGTGTTTGACTTTG 5`-ACTGCTTCTTAGCCACCCCAC	85.6	219	
<i>rnBcl2l1</i> -for <i>rnBcl2l1</i> -rev	5`-GCTGACACCAGTGAGTGCCTT 5`-CGCATTCTCCCCTATTCCAG	96.7	143	2
<i>rnBdnf-</i> for <i>rnBdnf-</i> rev	5`-GAATCCCCTGTTTTTGGAAGAC 5`-AGATTGTGATGGGGCTCCTTT	94.5	184	
<i>rnChga-</i> for <i>rnChga-</i> rev	5`-CACAGAAGGCAGTGAGAGAGGTC 5`-TCCGACTGACCATCATCATCTT	101.5	228	
<i>rnCobll1</i> -for <i>rnCobll1</i> -rev	5`-TACAGGATGGAAAGACACAAACAG 5`-TGAAGGGTGAACGATGACGG	102.2	145	
<i>rnDdx3y</i> -for <i>rnDdx3y</i> -rev	5`-GCTTATGAACACCACTACAAGGGAA 5`-ATAGCCACCTCCACCAAATCC	96.1	183	
<i>rnDrd4</i> -for <i>rnDrd4</i> -rev	5`-CAACCCCATCATCTACACCATC 5`-GCACACAGGCTTGGAACCC	100.1	122	
<i>rnEif2s3y</i> -for <i>rnEif2s3y</i> -rev	5`-CCTGATAGATCTTACTGATAAGCTGTG 5`-CAAGCCAACAATAGATGATGAATG	96.3	235	
rnFoxm1 -for rnFoxm1 -rev	5`-CCAATCATGCCAGGGAGTCTA 5`-GCTCCATCACCCCTCACTCA	97.5	221	
<i>rnGal-</i> for <i>rnGal-</i> rev	5`-GTCCTGAGACCACACCCACT 5`-TCAGCATCAAAGCAGAGAACA	102.4	121	
<i>rnGdf11</i> -for <i>rnGdf11</i> -rev	5`-GACCGCAGGGGGGGGG 5`-TTGCTTTGTGGCTGGCGAA	97.0	155	
<i>rnGnrhr</i> -for <i>rnGnrhr</i> -rev	5`-CAGCAGAGAACTACATGGAATTGC 5`-GGTACCAAACATCAGGCTGTTG	101.3	113	
<i>rnGrik1</i> -for <i>rnGrik1</i> -rev	5`-CAAACCAAGATAGAATATGGGGC 5`-CCATCAGCAGTGCGTAGTCG	105.2	184	
<i>rnKcc4-</i> for <i>rnKcc4-</i> rev	5`-GCATCTCTGGCTTCCTCCG 5`-CCTCTGGCTGCCTAACAAAGTC	103.2	117	
<i>rnPdlim3-</i> for <i>rnPdlim3-</i> rev	5`-CGGCGGATGATAAGGAGGA 5`-GGATGGGCGTGTAACAGATG	107.7	274	
<i>rnPld2-</i> for <i>rnPld2-</i> rev	5`-CACAGATAGCCACAGAAGCAGG 5`-GATGGGAGAGCAACCTAATACCTT	97.5	118	
<i>rnPomc2-</i> for <i>rnPomc2-</i> rev	5`-CCTGATAGATCTTACTGATAAGCTGTG 5`-CAAGCCAACAATAGATGATGAATG	102.1	150	
<i>rnPpia-</i> for <i>rnPpia-</i> rev	5`-AGCACTGGGGAGAAAGGATT 5`-GACCCAAAACGCTCCATG	95.0	323	
<i>rnPrI</i> -for <i>rnPrI</i> -rev	5`-CCCTGCAAGGAGTTGATGAAG 5`-GGACAATTTGGCACCTCAGG	100.2	120	
rnPvalb-for rnPvalb-rev	5`-GTTCAGTCTTTGTGCCTTTCCT 5`-TGAGTTTCGACCTAGTTATTCCTCA	102.4	121	
rnRt-ce16-for rnRt-ce16-rev	5`-GTGGTGGTGCCTCTTGGG 5`-CCACCTGTGTTTCTCCTCCTC	97.5	221	
<i>rnSpin2b</i> -for <i>rnSpin2b</i> -rev	5`-CAGCAGTCACAGCAGCCCTA 5`-CAGCATCCAGGCTCTGTTCC	98.6	198	
rnSpp1 -for rnSpp1 -rev	5`-CAGCCATGAGGACAAGCTAGTC 5`-CCACTGAACTGAGAAACAAGCA	99.0	209	
rnVsnl1 -for rnVsnl1 -rev	5`-TCCAGAAAGGTGGCAATAGATGT 5`-CTGGATGACAAGTTGGAAAGTG	96.8	206	
<i>rnXist</i> -for <i>rnXist</i> -rev	5`-CTCATTGCCTGGCTCAGAGAC 5`-AGTACACTTTGGCTTACATCCTTTAA	100.9	138	

Table 2

A CH

Microarray and qRT-PCR data showing fold changes for genes with sexual dimorphism in the pituitary (P60), but not in the SOC (P16)

Gene symbol	Microarray (Microarray (M vs F) ^a		qRT-PCR $(\mu_{LN}$ +/- $\delta_{LN})^b$	
Spp1 (pituitary)	8.66	**	15.18 ± 0.97	***	
Spp1 (SOC)	1.21	n.s.	1.15 ± 0.15	n.s.	
Chga (pituitary)	3.80	**	4.84 ± 0.26	**	
Chga (SOC)	1.05	n.s.	-1.07 ± 0.21	n.s.	
Vsnl1 (pituitary)	3.39	**	10.88 ± 1.04	**	
Vsnl1 (SOC)	-1.09	n.s.	1.17 ± 0.15	n.s.	
Kcc4 (pituitary)	2.35	**	4.31 ± 1.29	**	
Kcc4 (SOC)	1.20	n.s.	-1.31 ± 0.44	n.s.	
Spin2b (pituitary)	2.91	**	6.93 ± 0.93	*	
Spin2b (SOC)	-1.09	n.s.	-1.24 ± 0.37	n.s.	
Bdnf (pituitary)	-3.13	**	-2.14 ± 0.19	*	
Bdnf (SOC)	1.05	n.s.	-1.67 ± 0.56	n.s.	
Drd4 (pituitary)	-4.06	**	-6.32 ± 1.23	**	
Drd4 (SOC)	-1.22	n.s.	1.18 ± 0.40	n.s.	
Gal (pituitary)	-6.96	**	-26.53 ± 1.44	***	
Gal (SOC)	1.14	n.s.	1.32 ± 0.28	n.s.	
Grik1 (pituitary)	-9.13	**	-33.74 ± 3.28	*	
Grik1 (SOC)	-1.26	n.s.	-1.04 ± 0.18	n.s.	
Pdlim3 (pituitary)	-9.88	**	-8.99 ± 1.70	**	
Pdlim3 (SOC)	-1.12	n.s.	-1.15 ± 0.38	n.s.	
Pvalb (pituitary)	-12.74	**	-48.04 ± 7.45	**	
Pvalb (SOC)	1.07	n.s.	1.24 ± 0.27	n.s.	

 a + = M > F, - = F $\,$ > M; b see material and methods; n.s. = not significant; * = $\,$ P < 0.05; ** = P < 0.01; *** = P < 0.001
Table 3 Sexually dimorphic transcripts in the SOC (fold change ≥ 2 : P-value < 0.05)

Gene symbol	Gene name 60mer Oligosequence	M vs F ^a	Chromosome
Prl	prolactin GCAGGGATTCCCACAAGGTTGACAATTATCTCAAGTTCCTGAGGTGCCAAATTGTCCA	57.55	17p11
Eif2s3y	elF2 gamma CAAGCCAACAATAGATGATGAATGAATGAATGATACATTTAGGTGAAACCAGAAATGCTTT(17.79	Y
Gnrhr	gonadotropin releasing hormone receptor TGCTGCTCATCCAGTCATGGCTGGGGGCCCGTGCAGTTTCTCAGCAGGATCTTTACCA.	6.20	10q32.1
Pomc	pro-opiomelanocortin (beta endorphin) CGCCCCTGGTGACGCTCTTCAAGAACGCCATCATCAAGAACGCGCACAAGAAGGGC(CTAGCCTCTTAGAGTTACCTGTGTTAGGAAATAAAACCTTTCAGATTTCACAGTCGGC	3.67 3.42	6q14
Ddx3y	ATP-dependent RNA helicase Dby AACTCCCAGGCAGTTGACTGGTGGGGGCAATTTGAATTTGTGCCTAAATCGTCATTTCA	2.37	Y
Akr1c6	aldo-keto reductase family 1, member C-6 CATTTTTGGAAGAATATTGAACATGAGCTGCTAAACATTATGGAGACTTTCCTCCTTCC	2.15	17q12.3
Rt1-ce16 ^b	MHC class I heavy chain RT1n antigen CCGACTCCAACATGGAAACCTATGTCATTTATGTCGTCCTCGGAGCTGTGGCCATCA1	-2.44	20
LOC302172 ^{n.a}	similar to synaptonemal complex protein 3 CTTGAACCTGAGTTGATGTAATTCATATTATCTCAGACACATGTCTTACCAATTTGACT	-2.61	Xq36
Xist	X inactive specific transcript AATATAGGGTCAGAGTCATGTAATCCCAGAGCATGGATATCAATGAGGGAACAGAAA(GTACTCAAAAGAACAAAGACATTTAAAGGATGTAAGCCAAAGTGTACTTTACCTCAGT/ GTTTGCACCCCGTAGTATTCATTTACAAGGAATGAGATAATGCTTAACGCTTAATTTAA	-2.63 -17.80 -19.01	Xq31

a + = M > F, - = F > M; b sexual dimorphism could not be confirmed by qRT-PCR; n.d. = not determined by qRT-PCR (unsufficient primer e

A

Table 4

ACCEP

Genes identified by microarray to be sexually dimorphic in the SOC (P16) and their qRT-PCR data, as well as the corresponding data in the pituitary (P60)

Gene symbol	Microarray ((M vs F) ^a	qRT-PCR (μ_{LN} +/-	${\delta_{LN}})^b$
Prl (SOC)	57.55	*	51.43 ± 3.65	***
Prl (pituitary)	-1.45	*	-5.78 ± 0.17	***
Eif2s3y (SOC)	17.79	**	16,164 ± 8,693	***
Eif2s3y (pituitary)	18.59	**	37,112 ± 13,586	***
Gnrhr (SOC)	6.20	*	45.22 ± 0.85	***
Gnrhr (pituitary)	1.91	*	1.95 ± 0.40	***
Pomc2 (SOC)	3.67	*	4.38 ± 0.53	**
Pomc2 (pituitary)	1.92	*	2.53 ± 0.26	**
Ddx3y (SOC)	2.37	**	246 ± 90	***
<i>Ddx3y</i> (pituitary)	2.49	**	$6,792 \pm 2,000$	***
Akr1c6 (SOC)	2.15	*	3.95 ± 1.41	**
Akr1c6 (pituitary)	1.14	n.s.	-1.42 ± 0.73	n.s.
Rt-ce16 (SOC) ^c	-2.44	**	-1.22 ± 0.13	n.s.
Rt-ce16 (pituitary)	-1.42	n.s.	-1.26 ± 0.05	n.s.
LOC302172 (SOC)	-2.61	**	n.d.	-
LOC302172 (pituitary)	-1.07	n.s.	n.d.	-
Xist (SOC)	-19.01	**	-409 ± 50	***
Xist (pituitary)	-19.94	**	-310 ± 122	***

^a + = M > F, - = F > M; ^b see material and methods; ^c sexual dimorphism could not be confirmed by qRT-PCR; n.d. = not determined by qRT-PCR (unsufficient primer efficiency); * = P < 0.05; ** = P < 0.01; *** = P < 0.001

SUPPLEMENTARY TABLES

Supplementary Table 1

Microarray and gRT-PCR data on genes not to be sexually dimorphic in both the SOC (P16) and the pituitary (P60)

Gene symbol	Microarray (M vs F) ^a	qRT-PCR $(\mu_{LN} + - \delta_{LN})^{b}$
Cobll1 (SOC) Cobll1 (pituitary)	1.14 n.s. 1.09 n.s.	-1.14 ± 0.38 n.s 2.43 ± 0.30 n.s
<i>Pld2</i> (SOC) <i>Pld2</i> (pituitary)	1.08 n.s. 1.10 n.s.	-1.16 ± 0.39 n.s 1.59 ± 0.56 n.s
<i>Bcl2l1</i> (SOC) <i>Bcl2l1</i> (pituitary)	1.03 n.s. 1.00 n.s.	1.07 ± 0.36 n.s 1.31 ± 0.12 n.s
<i>Gdf11</i> (SOC) <i>Gdf11</i> (pituitary)	-1.10 n.s. -1.02 n.s.	-1.09 ± 0.37 n.s 1.12 ± 0.06 n.s
<i>Foxm1</i> (SOC) <i>Foxm1</i> (pituitary)	-1.15 n.s. 1.15 n.s.	1.29 ± 0.43 n.s 1.42 ± 0.10 n.s
	CO M.	
	CD M.	
St.P	CD M.	
OFR	- C M	
offer		

Supplementary Table 2 Upregulated transcripts in the female pituitary (fold change \ge 2; P-Value < 0.05)

No.	Gene name 60mer Oligosequence	Fold change F > M	P-Value
1	calbindin 3 CGACACCACCTACTGATTGAATCCTATCCAATCCCAAAGATCTAGCTGTGAGAGCAAGAT	29.07	0.0011
2	unknown GTTTGCACCCCGTAGTATTCATTTACAAGGAATGAGATAATGCTTAACGCTTAATTTAAC	24.27	0.0011
3	cocaine and amphetamine regulated transcript TAGTTAATTTTGGCAGATGACATCATCATCCCGGAAAACAAATCACCCCAAAGCAACACAA	22.83	0.0303
4	unknown GTACTCAAAAGAACAAAGACATTTAAAGGATGTAAGCCAAAGTGTACTTTACCTCAGTAG	19.76	0.0011
5	peptidyl arginine deiminase, type II ATGGAGTCTGAGGAGTGTCTGGGGGGAAGAGCCAAAATAAAGAGCAATAAAATAAGCCGTC	16.16	0.0249
6	parvalbumin CTGAGGAATAACTAGGTCGAAGGACTCAAATGACACTCTATCAATTGCTTTTGACTTTGC	12.74	0.0097
7	cytochrome P450, subfamily 24 ATATAAAGACATGAGGTACCCAATTTAGTATGGAACTTGTCCTGAAATGTAATCATAGGG	12.17	0.0249
8	nerve growth factor, gamma CAGCATCACACCTGACGGATTGGAATTAAGTGATGATCTCCAGTGTGTGAACATCGATCT	12.11	0.0011
9	calpain 8 TGAGCTAACTGGGCAGATCCCAGGGTTCAGCAGAAGGAAAAGAATCAATTAAAGTTGTGG	10.79	0.0022
10	PDZ and LIM domain 3 GCAATGGTGACATTTAAAGCAATAACGTTGTAATACCATGCTCTACGGTTTCCATCTGTT	9.82	0.0065
11	unknown CCCACAGATCTGAACAGTTTTCACCATAAAGTACTAGAGTAAAGGAAGTCATGTGACCGA	9.71	0.0206
12	unknown CCCCCCAATGTCAAGTTTTTACCCTGCATGTACTGGAGTATTTATT	9.46	0.0184
13	unknown TTTTGTTTCTTTTATGGACTGCAGTGTAAACAAACCCATCCGACCAACTCCACTTCCGGG	9.27	0.0011
14	glutamate receptor, ionotropic, kainate 1 CACCTATGAGAAAATGTGGGCTTTCATGAGCAGTAGACAGCAGAGCGCACTGGTTAAAAA	9.13	0.0011
15	unknown AGCACCACGTCCCTGGGGATGGCTGAGAACGTGGCGCCCTCAGAAGCGACCCCCGAGCCC	8.66	0.027
16	kallikrein CAGGCATCTACACCAAACTTATTAAGTTCACCTCCTGGATAAAAGAAGTTATGAAGAAAA	8.27	0.001
17	spinal cord expression protein 4 ACATTTGACATGGGTTAGTCAGATTGACCAGTATGGAAATTTGCTTTTGTAGTTAGT	8.24	0.001
18	galanin AGTCATTCTAGGCTAAAAAGAATCTTCCGCCAACTCCTCAAGCCAACACTTTGTTCTCTG	6.96	0.001
19	fibroblast growth factor 13 TTGCATGGAAGAAAGTTGGAATCTTGGCATAGAGTTGCATGATATGTAAGATTTGTGCAT	6.68	0.0022
20	kallikrein ACAAAACCCCTTATATCAGAATTACCAGATGATCTCCAGTGTGTGAACATCGATCTTCTG	6.61	0.001
21	unknown TGGATGCCGCTGTTAGTATTAACAGAACAAAGCCCATAGTGTTGGGAAGGTTAATAAATT	5.92	0.001
22	unknown GTGGTGAACCCAATAAGCCATCAGTCTACACCAAACTTATAAAGTTCACCTCCTGGATAA	5.68	0.001
23	wingless-related MMTV integration site 4 ATACGGATGAGGACCTGGTGTACCTGGAGCCAAGTCCAGACTTCTGTGAGCAGGACATGC	5.36	0.001
24	natriuretic peptide precursor type C GGACGGTTTTTAAAGTGACTGACAAAACCAGCTAGCTGTAAAAACATTGCTGTTTGTAAA	5.31	0.028
25	kallikrein TGAGTGGGAATTCCCTGATGATCTCCAGTGTGTGAACATCCACCTACTGTCTAATGAGAA	5.22	0.001
26	unknown AGTGAAGAAGAAAACAGAAAAAGAAGTTACCTTGTATTATGTATTTTACTACACTTTTC	5.05	0.0022
27	unknown GCTTCCAGTCAGGAGTAACTTATTCCCCAGTGAATGTAGGAGACATTGGTCCAGACAATA	4.80	0.022
28	VGF nerve growth factor inducible CTTCTGTTGTAAATACCCCTCACGGAGGAAATAGTTTTGCTAAGAAATAAAAGTGACTAT	4.68	0.001
29	similar to cDNA sequence AY358078 CTTTATATCTATGATGAGTGGGACCACAGGCTGCATGTCGAATTGCCAGTCCTTCAATCT	4.55	0.001
30	unknown TCCAGCTGAGAGACCACCCTTTCAACAGTTGGTTCTGGAAGGCTGGGGGCCAGGCGTTATA	4.49	0.001
31	unknown TCATAGAGAATAACCATTGGAAACTCATTAATGACCTGGACACTATCATAGAAAACACTC	4.45	0.045
32	unknown TGGCCAGTGACAAGTCACGGGACCGCTTCTCGCGCGAACAGTTCAAGCTGGGCGTCAAGT	4.40	0.0022
33	similar to RIKEN cDNA 1700001E04 CAACATTGGAAAGACTTTGTGACAAAGTCTGTAATATCATTTATACTCAGTGTTGGATGG	4.39	0.0011
34	kallikrein 7 CAAAACCAACATGCCAGCCATCTACACCAAACTTATTAAGTTCACCTCCTGGATAAAAGA	4.39	0.001
35	unknown GAAATATTTCCTTGACTTCAAGAAGAAAGATAAAGACCAGCAACATCCGGACCCAGCATC	4.37	0.001
36	Jun dimerization protein 1 GCTTCACGAGGAACATGAGAGTCTGGAGCAGGAGAACTCTGTGCTGCGCAGGGAGATCGC	4.33	0.0130
37	similar to spermatogenesis associated glutamate (E)-rich protein 4d ACCCCAGTAAGAGAGCTTCCTAAAGAATAAGTTCCGTTCTCAGGAGTCCCTGATGACTAA	4.24	0.0011

38 similar to Discs, large homolog 5 TTGACGTCAACAAGAAAGATAAAGACCAGCAACGTCCAGACCCAGCATCATCTGAGCTTA 4.18 0.0011 potassium channel, subfamily K, member 1 ATGTGAAGAAAGACAAGGATGAAGACCAAGTTCACATCATGGAGCATGACCAACTGTCCT 39 4.17 0.0011 unknown GCTAAGAAGGAAAAGGAGAGGCTGATTAAAGAGCTGCAGCTCATTACCAAGGAGAGAAAT 4.12 0.0011 40 41 4.10 0.0011 unknown TGGCTGCATGTCAAATTGCCAGTCCTTCAATCTGAACATGAGATGAGAATGATGGCTATG 42 dentin matrix protein 1 TTAAATATTTAGTATGGAAAGGCATTCTCAAACGAGACGGCACAATGGGATAACTTGGAT 4.09 0.0195 43 Rho GTPase activating protein 24 (predicted) TGGATCTACGTGTAGAGGGTAGTTTCTAAAATGTCGGTAAGAGGTAGAAGCATATATCTC 4.06 0.0011 similar to aspartyl beta-hydroxylase; calsequestrin-binding protein TGATCACGGAGCACTATGGGCCCACAAACATCCGAATCCGGTGCCACCTAGGTCTGAAGA 44 4 06 0 0195 4.05 0.0011 45 dopamine receptor 4 TCATCTACACCATCTTCAATGCCGAGTTTCGAAGTGTCTTCCGCAAGACTCTTCGTCTCC similar to spermatogenesis associated glutamate (E)-rich protein 4d CGAGGAGAGAAATGACCTGAGAGATCGCCTGAGGTTTCTGACAGAGAGATCTATGAAAAA 4.05 0.0011 46 fatty acid binding protein 5, epidermal GACTTTTCATCATAGACACTTTACCCGAAACCCATGTCAGACCGTTGGTTTACCCAGGAT 47 4.04 0.0195 similar to RIKEN cDNA 4930555G01 0.0011 48 4.01 CCAGAAGGGTTTCATGGAGATCAGTTCATACATTGGAAAGACTTTGAGACAAAGTCTGTA growth associated protein 43 GCAAATGTGCCAATTAGCGTAACTTAAGGCTGTGAGGCTCCTTTTTCAATCTGAATATTA 49 4.00 0.0011 chorionic somatomammotropin hormone 2 AAGTTGACAATTTTCTCAAGGTCTTGAAATGCCGCGATATTTATAACAACAACTGCTGAG 50 4.00 0.0011 0.0011 51 3.97 unknown CATGAAGGAAAAGGAGAAGCTGATTAAAGAGCTGCAGCTCATTACCGAGGAGAGAAATGA unknown TATCGCCTGAGGTTTCTGACAGAGAGAGTCCAAGAACAACAGATTTGAGTACATTGAACAG 0.0011 52 3.93 53 3.88 0.0011 3.87 0.0011 54 unknowi CTGAGGTTTCTGACAGAGAGAGCCCATGAAGAACAGGTCACACTTCAGGCCAAATCCATAT 55 unknown GAAATATTTTCCTTGACTTCAAGAAGAAAGATAAAGACCAGCAACATCCGGACCCAGCATC 3.87 0.0028 56 Jun dimerization protein 2 3.85 0.0026 GCTTCACGAGGAACATGAGAGTCTGGAGCAGGAGAACTCTGTGCTGCGCAGGGAGATCGC 57 3.82 0.0025 similar to spermatogenesis associated glutamate (E)-rich protein 4d ACCCCAGTAAGAGAGCTTCCTAAAGAATAAGTTCCGTTCTCAGGAGTCCCTGATGACTAA 58 similar to Discs, large homolog 6 TTGACGTCAACAAGAAAGATAAAGACCAGCAACGTCCAGACCCAGCATCATCTGAGCTTA 3.80 0.0023 potassium channel, subfamily K, member 2 ATGTGAAGAAAGACAAGGATGAAGACCAAGTTCACATCATGGAGCATGACCAACTGTCCT 3.78 0.0021 59 0.0020 60 unknown 3.76 GCTAAGAAGGAAAAGGAGAGGCTGATTAAAGAGCTGCAGCTCATTACCAAGGAGAGAAAAT 61 3.74 0.0018 unknown TGGCTGCATGTCAAATTGCCAGTCCTTCAATCTGAACATGAGATGAGAATGATGGCTATG 62 dentin matrix protein 2 TTAAATATTTAGTATGGAAAGGCATTCTCAAACGAGACGGCACAATGGGATAACTTGGAT 3.72 0.0016 The GTPase activating protein 24 (predicted) TGGATCTACGTGTAGAGGGTAGTAGTATCTAAAATGTCGGTAAGAGGTAGAAGCATATATCTC 3.70 0.0015 63 similar to aspartyl beta-hydroxylase; calsequestrin-binding protein TGATCACGGAGCACTATGGGCCCACAAACATCCGAATCCGGTGCCACCTAGGTCTGAAGA 64 3.68 0.0013 dopamine receptor 5 TCATCTACACCATCTTCAATGCCGAGTTTCGAAGTGTCTTCCGCAAGACTCTTCGTCTCC 3.65 0.0011 65 similar to spermatogenesis associated glutamate (E)-rich protein 4d CGAGGAGAGAAATGACCTGAGAGATCGCCTGAGGTTTCTGACAGAGAGATCTATGAAAAA 66 3.63 0.0010 fatty acid binding protein 5, epidermal GACTTTTCATCATAGACACTTTACCCGAAACCCATGTCAGACCGTTGGTTTACCCAGGAT 3.61 0.0008 similar to RIKEN cDNA 4930555G02 CCAGAAGGGTTTCATGGAGATCAGTTCATACATTGGAAAGACTTTGAGACAAAGTCTGTA 68 3.59 0.0006 growth associated protein 44 GCAAATGTGCCCAATTAGCGTAACTTAAGGCTGTGAGGCTCCTTTTTCAATCTGAATATTA 69 3.57 0.0005 chorionic somatomammotropin hormone 3 AAGTTGACAATTTTCTCAAGGTCTTGAAATGCCGCGATATTTATAACAACAACTGCTGAG 70 3.55 0.0003 unknown CATGAAGGAAAAGGAGAAGCTGATTAAAGAGCTGCAGCTCATTACCGAGGAGAGAAATGA 71 0.0003 3.53 72 0.0003 unknown 3.51 TATCGCCTGAGGTTTCTGACAGAGAGAGATCCAAGAACAACAGATTTGAGTACATTGAACAG 0.0003 73 3.48 74 unknown CTGAGGTTTCTGACAGAGAGATCCATGAAGAACAGGTCACACTTCAGGCCAAATCCATAT 3.46 0.0001

75 3.62 0.0011 unknowi 3.62 0.0011 76 unknown TGCACGTGTAAATTTGTACTTGTCCTGATGCGTCATCCAAAAGGGTTTCATGGAGATCAG similar to phospholipase C-like 2 AAATCCTGGATCTTTATATCTATGATGAGTGGGACCACAGGCTGCATGTCGAATTGACAG 3.59 0.0011 77

67

78	unknown GGTAGTGAGCGCTTCCTATAACTCACCAATTGGGCTCTATTCAACTAGCAATATCCGAGA	3.58	0.0152
79	unknown GTACTTTTCCTGATGGGTCATCCCAAAGGGTTTCATGGAGATCAGTTCACAACATTGGAA	3.58	0.0011
80	unknown TTGGTTTTGGTAGGGAGGCATCATCCCCAAAATGTCATCATTAAGCAAGAAGAGTGTCTAA	3.53	0.0011
81	unknown AGTACAAGAGAGTTGGAATTGGACACACACCAGGAAGAGAGCTTCCTGAAGAATAAGTTG	3.51	0.0011
82	similar to cDNA sequence AY358078 GTCATCCAGAAGAGTTTCATGGAGATCAGTTCACAATATTGGAAATGTCTTTGTGACAAAG	3.51	0.0011
83		3.50	0.0011
84	unknown	3.49	0.0011
85	TGATAAGGATATAGACTGTATATATAGACTACCTGCATCTGGTTACTACCAAGTAAAGTG similar to hypothetical protein FLJ13448	3.49	0.0368
86	AAACCACATTTGGTAAAGGCCTCTTGTACTGATGGGAAACTTTTCAATCATCTGGAGACT	3.48	0.0011
87	GATCCATGAAAAATAGGCCACACTTCAGGCCAAATCCTTATTATGAAGACCTGGAGAGAA unknown	3.47	0.0011
88	CGCCAGAGCAACAGAATCAAGGTTTTGGTAGGAAGGCATCATCCCAAAATGTCATCAGTA similar to SPEER 2	3.46	0.0011
89	TCCATGAGAACAACCATAAGCTGAAGAAGGAGATGACCTTCTCTAGAAACCTGCTCAACC unknown	3.44	0.0011
90	GAAATATTTGGTTGACTTCAACAAGAAAGATAAAGACCAGCAACGTCTCAGAAAGTGCAA similar to spermatogenesis associated glutamate (E)-rich protein 4d	3.44	0.0011
91	TATTTGGTTGACTTCAACAAGAAAGATAAAGACCCAGCATCATCTGGTCTCAGAAAGTGC uterine sensitization-associated gene 1 protein	3.42	0.0054
92		3.41	0.0011
	TACAAAAGTCATTATTTACAAAAATCTGTACACACATTTGAAAAACTCACAAAATTGTCA		0.0011
93	UNKNOWN TAGAGATGGAGAAACACTGAAATCCATGAGAAACAACCATAAGCTGCAGAAGGAGATTACCT	3.40	
94	similar to RIKEN cDNA 4930555G01 TATCTATGATGAGTGGGACCACAGGCTGGATGTCGAATTGCCAGTACTTCAATCCGAATA	3.40	0.0011
95	similar to putative pheromone receptor (Go-VN5) GAATGAACATCTGTGACCCCAGAGCCAAGCAACAGCAGTTTCATGAGAAACGTTCTCTAA	3.37	0.0011
96	unknown CGGGTAAATTTGTACTTGTCCTGATGGGTCATCCAGAAGAGTTTCATGGAGATCAGTTCA	3.35	0.0011
97	similar to cDNA sequence AY358078 CTTTATCAAATCCTGGATCTTTATATCTATGATGAGTGGGACCACAGGCTGCACGTCGAA	3.34	0.0011
98	unknown AAGGTTTTGGTAGGAAGGCATCATCCGAAAGTGTCATCAGCAAGCA	3.33	0.0011
99	unknown AGCATCATCTGGTCTCAGAAAGTGCAAGAGAGCTGGAATTGGACAAATCCCAGTAAGAGA	3.33	0.0022
100	unknown CACGTTCCTTGAAACTTAAACCAATTTCAAACTCACTCATTTGGTTCTACCCATATCTTC	3.32	0.0173
101	Spetex-2D protein CTAAGATGACCAACTGGATAAGTGATGCCATGGAGAAGTACAAGGAGCTCATGCAAGAGA	3.32	0.0011
102	leucine-rich repeat-containing G protein-coupled receptor 7 GCATGTTTTACAGTGTTCATCAAAGCACCATAACAGCACCGAAATACAGAAGCAGGTGA	3.32	0.0011
103	unknown CTTCACAACTTAGAGATGGAGAACACTGAGGTCCATGAGAACAACCATAAGCTGAAGAAG	3.31	0.0011
104	unknown	3.31	0.0011
105	CAATCTGAACATGAGATGAGAATGATGGCTATGCAAATGATGACCAACTCAATAAGTGAT similar to RIKEN cDNA 1700001E04 CTGTACTTTCACCTCAGCTCTCTCCAACAAGTGTGCACATTTTGAGGGACTCAGATAAGT	3.31	0.0011
106	cytochrome P450, subfamily 24	3.29	0.0076
107	CCCAAGTGTGCCATTTACAACTCGGACCCTTGACAAACCAACGGTTCTGGGTGAATACGC olfactomedin-like 3 (predicted)	3.28	0.0011
108	ATGAGAAATACGATATGGTGACAGACTGTAGCTACACAATCTCTCAGGTGAGGTCAATGA hypothetical LOC363354	3.28	0.0011
109	TGACTGCAACAAGAAAGATAAAGACCATCAACGGCCAGAACCAGCATTATCAGGTAGGGA Spetex-2D protein	3.28	0.0011
110	GCATAAGATAACCAAGTGGATAAGTGATGCCATGGAGAAGTACAAGGAGCTCATGCAAGA hypothetical LOC287855	3.28	0.0011
111	CTGATGACAAACATCCTGAATGAAAACACCACTTGAGAGACAACTTGGGGGGACCGCATTT unknown	3.27	0.0097
	TTAAGTGTAGTCTTCTTCCCATGAATCACCGTTAGCATAGACTGCAACAATGGAATACGC	3.27	0.0011
		3.26	0.0011
		3.26	0.0249
	ACTAAAAGGTGTTGTGAGTACTCGGGGTAAGCTTTTGAAAGCTGAGGTAACTAATCATGG		
	unknown AGGTCCATGAGAACAACCATAAGCTGAAGAAGGAGATTACCTTCTCTAGGCCACACTTCA	3.25	0.0011
116	unknown GGCTATGCAAATGATGACCAACTCAATAAGTGATGCCATGGAGAGGTACAAGGAGCACAT	3.25	0.0011

117 unknown 3.24 0.0011 ATCTATGAAGAACAGGCCACACTTCAGGCCAAATCCATATTATGAAGACCTGGAGAGAAAT

118	unknown	3.23	0.0011
119	CATACAGTAGCATCATGTATTCATGGTCATTTTGAAGAACTACTTAGATTAACCTGACCT similar to RIKEN cDNA 1700001E04	3.23	0.0011
	AACAGCAGGTCTAAAATTCTCCAGCAGAAACTTGAACATGACACAGGCCAGGACAAGTCT similar to spermatogenesis associated glutamate (E)-rich protein 4d	3.23	0.0011
121	GAGGTCCATGAGAACAACCATAAGCTGAAGAAGGAGATTACCTTCTCTAGAAATCTGCTC similar to protocadherin X long isoform AGGCTAAGATTAGGACAACCTGATTCTCCTCTCAATGTTGTAAATGTCAATCTCTTTG	3.22	0.0011
122		3.20	0.0011
123	ATP-binding cassette, sub-family G (WHITE), member 2 ACAGCACCTGTGTTAACAGCTATACAATATGTACTGGTAATGACCTACTTGATAAATCAGG	3.20	0.0076
124	similar to RIKEN cDNA 4930555G01 TATCTATGATGATGGGACCACAGGCTGGATGTTGAATTGCCAGTCCTTCAATCTGACA	3.19	0.0011
125	similar to RIKEN cDNA 1700026D08 (predicted) ACCGCTTTCGGTATCTGAGACAGACGCTATGTCCACTATGGTGACAAAGTGATCCTTGTGAA	3.19	0.0249
126		3.18	0.0011
127	hypothetical LOC291888 TGGTTGACTTCAACAGGAAAGATAAAGACCATCATCGGCCAGAACCAGCATTATCAGGTC	3.18	0.0011
128	unknown	3.18	0.0011
129		3.17	0.0011
130	CAGGAGTCCCTGATGACAAACATCCTGAATGGTAACAAAAACAGCACTTGAGAGACAACT similar to L-lactate dehydrogenase A chain (LDH-A) (LDH muscle subunit) (LDH-M) GATCTATGTGGCTTGGAAGAACAGCAGCTTCCCCAAAAAACAGTTATTGGAAGTGGTTGC	3.15	0.0065
131	hypothetical protein LOC363306 ACTITATCAAATCCTGGATCTTTATATCTATGATGAGTGGGACCACAGGCTGCATGTAG	3.15	0.0011
132	crystallin, lamda 1 TTTGTAACCATTTTCTAACCCCTTTCTGAGTGTCCTGTAACCTTCACAATAAAGCAATCA	3.14	0.0271
133	hypothetical LOC363366 TGACTGCAACAAGAAAGATAAAGACCATCAACGGCCAGAACCAGCATTATCAGCTGAGGT	3.14	0.0011
134		3.13	0.0011
135	similar to spermatogenesis associated glutamate (E)-rich protein 4d TGGTTGACTTCAACAAGAAAGATAAAGACCACCAACGTCCAGACCCAGCATCATCTGGTA	3.13	0.0011
136		3.13	0.0011
137	hypothetical LOC363369 TAAGCTGAAGAAGGAGATTACCTTCTCTAGAAACCTGCTCAGCCAGC	3.13	0.0011
138	brain derived neurotrophic factor CTGCTATATGCTAACTTTTTGCAGCTTCATTCTGAGAGAGCGTTAGTCAAACAAA	3.13	0.0022
139	Spetex-2B protein TAAGTGATGCCATGGAGAAGTACAAGGAGGCTCACACAAGAGAATAATTCCTACCGCATCA	3.12	0.0011
140	similar to CDNA sequence AY358078 GGTCCTATAATAGTTTTGGTAGGAAGGCATCATCCCAAAGTGTCATAAGTCAGCAAGTGG	3.12	0.0011
141		3.11	0.0011
142		3.11	0.0216
143		3.11	0.0011
144	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4 (predicted) CTTCCCTAGTAGCACACGGTTÄAGAACTTGTTCCAGCTAATGATTTGGCTGAAAATTTTCC	3.11	0.0281
145	similar to Integrin alpha-6 precursor (VLA-6) (CD49f) CCTGATGACAAACATCTTGAATGAAAACACCACCTTGAGAGACAACTTGGGGGGACTGCCT	3.10	0.0011
146		3.10	0.0011
147		3.09	0.0022
148	type I keratin KA38 AGGAATGAGCTGACGGAAATGAAACGTACGCTCCAAACCTTGGAGATTGAACTACAGTCT	3.07	0.0011
149		3.06	0.0011
150	similar to L-lactate dehydrogenase A chain (LDH-A) ATCACTCTGAACGTTGTCAAATACAGTCCACAGTGCAAGCTGCTCATCGTCTCAAATCCA	3.06	0.0184
151	unknown	3.04	0.0011
152		3.04	0.0227
153		3.04	0.0152
154	GACACCATGGCCAAAGAGACGAGTATAATCGGAGTCTCTCTC	3.04	0.0011
155		3.02	0.0011
156	TTTATATACCACTAAGCCAGCCTGACTGATTGAAGTCTTACTTTCTTCTCAGAAAACAGC unknown GTAAATTCCAGAGCGCATGAACTTTATCAAATCCTCGACTTTATATTTGTGATGATGATGGG	3.00	0.0011
	GTAAATTCGAGAGGGATGAACTTTATCAAATCCTGGACTTTTATATTTGTGATGATTGGG		

157 unknown TTCTGTCTAAAGAGATTCTGTGTCCAGTATCGTTTTGTGGAACTATTTTGATTCTGCTGT

2.99

0.0011

158 unknown TTTGTACCTGAATAAAGCATATTTTGCACTTGTAAATGAGAAATCTGTATGTGGGCTCTG 2.98 0.0065 similar to Kinesin family member 18A CAAACGCATTCGACAGGATAATTCAAGTGTTAAGCTCATCCGAGAAAACAGACTGAGAGT 2.97 0.0011 159 procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha 1 polypeptide ACTGGATGTTTCCACGGCAGAGGAATTACAGGTAGCAAATTATGGAGTTGGAGGACAGTA 2.96 0.0011 160 activating transcription factor 3 TCTGTGAAATCCTCAGTGTTCAATCCAGACTCAGTAGTATATTACAGTTTTCTGTAAGAG 161 2.94 0.0303 2.93 0.0011 162 163 unknown ACCTTCTCTAGAAATCACCACTTAAGAGACAACTTGGGCGACCGCCTTACATTATGTGTG 2.93 0.0011 guanylate cyclase activator 2b CATAGCAAGACAATATGGATGCAGAGCCGCCATATTTGGTCCCCAGGCAGCTGCACCGGA 164 2 92 0 0054 0.0119 2.91 165 unknown AATATCTTCAAGTTCATCATTCCGACATTGTGAAGTACAGTCTACAGTGCAAGCTGCTCA 2.91 0.0260 166 unknown GCCAAAGTTAAGTTTAATGTGAACCGGGTGGACAACATGATCATTCAGTCTATAAGCCTT 167 2.88 0.0097 unknown GGAAGGAGGTTTTCTGTTAGATGGAAATATGTGTTCTGCTATGAATGTTAGTAACTGAGT 2.87 0.0097 168 unknown CCACAACATAACTCTTGCGGATATGGACAGGGTATTGAGTGTCTATAACGGCATCCCAAT 0.0011 169 2.87 unknowr 170 2.85 0.0152 CAAGCTGCTCATTGTCTCAAATCCAGTAGATATCCTGACCTACATGGCTTGGAATATCAG similar to L-lactate dehydrogenase A chain (LDH-A) GGTCTCTATGTAATCAACGATGACGTCTTCCTTAGTGTCCCGTGTATCCTGGGACAAAAT 0.0227 2.85 171 unknown AAACAGCACCTGAGAGTCAAGTTGGGAGACCGCTTTTCATTATGTGTGCTAGAGGAGAAA 0.0011 172 2.83 slit homolog 1 (Drosophila) GTTGCATCCGGAATCTATACATCAACAACGAACTGCAGGACTTCACCAAGACACAGATGA 173 2.83 0.0011 2.81 0.0011 174 unknow AACTITATCAAATCCTGGACCTTTATATCTATGATGAGTGGGACCACAGCATCAGGCACT melanoma cell adhesion molecule GAATGGAACCCTGATCCTCAGACAGTAGTGAGCACCTTGAATGTCCTTGTGACCCCAGAG 2.80 0.0011 175 similar to Seizure 6-like protein precursor TCATGTACTCCCATCCCTACAGCCAGATCACTGTGGAAACTGAGTTCGACAACCCCATCT 176 2.80 0.0011 2.79 0.0292 177 unknown CAAGCCCTGCAATTCCCAGGGCCAGATTCAAGGCAATAATTAGGAGGATAGGGGCAAAGG 178 unknown TTGAGGCTTCATCATGACCCAGTCTGTTTCAACTTCAGGAACACTGACTCAGACAGTTGT 2.78 0.0032 unknown AAATTGTTCCGTATCTGTATTTATCATTGCATTTCAGATGGGAACTAGAAAACTGGACAG 2.77 0.0011 179 0.0011 180 unknown 2.77 AAGTTGCTTTCTCAGGAGTCCCTGATGACCAACATCCTGAATGGTAACAAAAACACACTT similar to SPEER 2 GCTAGAGGAGAAACACCAATACGTCTGTGCTTCTACATGTTCGTTAAGAATATGCTTATG 2.76 0.0011 181 182 2.75 0.0011 AAGGATACAAACTGTATATATTGACTACCTGCATCTGGATACTATTGATGTATTCTGGTG unknown TTGGTTTTGGTAGGAGGGCATCATCCCCAAATGTCATCAGAAGGCATCATCCCAAAATGT 2.74 0.0011 183 184 unknown CTATGCTTTACCTGTGGGAAACATGGTCATTTGACACATATTGTGAACAAGGCATCTCC 2.74 0.0184 unknown ATGGACGCATTATTGACACTTCTCTGACCAGAGATCCTCTGGTCATAGAACTTGGCCAAA 2.73 0.0011 185 0.0227 186 2.72 unknown ATCACACAATGCAAAACTGTCTAACTAAAGGACAGGAAGAAGAGTTTAGGTGTTTCAGAA schlafen 2 (predicted) ATTTTTTAACAAGACCGAATTTCAGTATGAGGAAACTTTCTTCTTACCAGGTCCAGGTA 187 2.71 0.0011 solute carrier family 6 (neurotransmitter transporter, glycine), member 5 GTCTCACGTGAACAATACTGTTCACGAATAAAGATGTTTCCTAGTCAGGTCCTTATATTT 188 2.70 0.0054 189 2.70 0.0054 similar to spermatogenesis associated glutamate (E)-rich protein 4d TTCGTCCTCTGAGTTAATTTGTCATTTCTTAGAGACCCTAAATTTATATACCACTAAGCC 2.69 0.0011 190 unknown TATTCTATCATTTGGGGGCCAGGCCACACTTCAGGCCAAATCCATATTATGAAGACCTGGA 0.0011 191 2.69 nudix (nucleoside diphosphate linked moiety X)-type motif 7 (predicted) TCGAGACCGAATTTGATCTCCATGACCTGATACCATCTTGTGAGAAGACCTTTCTTCATA 0.0011 192 2.68 0.0032 2.68 193 unknown GAAAGTGTTGATCAAGGAGAAACCACCACGGTTTTGGTAGGAAGGCATCATCCCAAAAT CD44 antigen CCACATGCTTCTGAGAGATTCCCCCAAAGGTGACGCTATTTATCTTTAGTAAGCTATTTAT 2.68 0.0011 194 plakophilin 2 (predicted) CGGAATCTTTCTCTGCAGAATGAAATTGCTAAAGAAACTCTACCAGATTTGGTTTCTATA 195 2 67 0.0476 olfactory receptor 1451 (predicted) GCCTGCGCAGACACCCAAAGCCTTTGAGTTCTTCATGTACATCTGCTGCATCCTGATGCTC 2.67 0.0011 196

 197
 hypothetical LOC363336
 2.67
 0.0011

 TTTCGTCTCAAAGTAATGATGGACAAAGACAGGACTTCTGGGCAATGTCAAATGCTGGGA
 2.67
 0.0011

198	unknown ACATCTTGCTTGTGCACTGAACCAGAAATATTCCGTTGATTTCAACAAGAAAGA	2.65	0.0011
199	similar to spermatogenesis associated glutamate (E)-rich protein 4d GTGCTAGAGGAGAAACAGCAATACGTCTGTGCTTCTAAATGTTCGTTAAGAATAGCTTTT	2.64	0.0011
200	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3 (predicted) CAGTGTACATGATCTATTGGAAGTTCCGGAAAACGTTTGACATTGAAAATGACACATTCC	2.64	0.0011
201	catechol-O-methyltransferase AAGTCAGCAAACTGTAAATGTCCAGACGCCAAATAACAGCAGAGGGCTCAGAGACAGTACT	2.63	0.0011
202	unknown ATGTGTGCAAGAGGAGAAACAGCAATACATCTGTGCTTCTAAATGTTTGTT	2.62	0.0011
203	unknown AGGAAACCCTACAGGAAAATGGAATGGAAGACCCACCTGTCTCTTTGCCTAAAACCAAGA	2.61	0.0303
204	hypothetical LOC363354 GTGCACTGAACCAGAAATATTTGGTTGACTTCAACAAGAAAGA	2.61	0.0011
205	ATP-binding cassette, sub-family C (CFR/MRP), member 3 AAATTCTGCAAATTGCCTTACAGACTAGCCATACTTAACAGTGGAATGAGGAAGTGGGTC	2.61	0.0032
206		2.60	0.0011
207		2.60	0.0011
208	vitronectin	2.59	0.0011
209	TTCAGAGCGTCTATTTCTTCTCTGGAGACAAGTACTACCGAGTTAACCTTAGAACACGGC unknown	2.59	0.0011
210	GTGCTAGAGGAGAAACAGCAATACGTCTGTGCTTCTAAATGTTCGTTAAGAATATGCTTT unknown	2.59	0.0216
211	CAGCTTACATTTCCATTCCATTATTACAAGTGGTGAAAAACAAGAATTGCAAGTAGCATG corticotropin releasing hormone binding protein	2.58	0.0292
	AGATGAAAATTAGÖTGCGACAATGÖTGTGGTGAGGATGGTCTCCAGTGGAAAACACATGA	2.58	0.0011
		2.57	0.0011
	ATCAAATCCTGGACCTTTATATCTATGATGATTGGGACTACAGCAACACGCACTTCCAGC		
	unknown AAATATTCTCTCAGATGAATCCAGATATAACTGCTAAGTCTAAATTGGTCCTTCAAGGTC	2.56	0.0216
215	unknown AAGGAGGTACATCTTCATTGTGCACTGAACCAGAAATATTTGTTGACTTTAACAAGAACG	2.56	0.0011
216	growth arrest and DNA-damage-inducible 45 beta (predicted) AACCAGTGGGTCCCCTATATCTCTCTGGAGGGAACGCTGAGGCCCACTCTAAACACCTAA	2.55	0.0303
217	unknown TGTGTGCAAGAGGAGAAACAGCAATACATCTATGCTTCTAAATATTCCTAAATGTTCTTG	2.55	0.0011
218	similar to L-lactate dehydrogenase A chain (LDH-A) GTGGCTTGGAAAATCAGTGGCGTTCTCAAAAGCAGAGTTATTGGAAGAGAGTATGGTGAC	2.55	0.0184
219	cell growth regulator with EF hand domain 1 ATCAACCCGGTGATCCTGGTAGTAGACATGGTGCTTGAGACTCAGGACCTGGATGGA	2.53	0.0011
220	amiloride-sensitive cation channel 1, neuronal (degenerin) ATTGGTGCTAGTCTCCTCACAATACTAGAGCTCTTTGATTATATTTATGAGCTGATCAAA	2.53	0.0162
221	Rho GTPase activating protein 18 (predicted) AAGGAGAAGTTTTCCTGTATGAGATTGGAGGAAATATTGGGGAACGTTGCCTTGATGACG	2.52	0.0011
222	unknown CCAGAAATATTTGGTTGACTTCAACAAGAAAGATAAAGATAAAGATCAATGGCCAGAACC	2.52	0.0011
223	unknown	2.52	0.0011
224	AAGTTGCTTTCTCACGAGTCCCTGATGACAAACATCCTGAACGGTAACAAAACATCACT apolipoprotein A-IV	2.50	0.0011
225	TCTGTCACGCTGTTCCCAAGCACTTCTCGTACCAGCTTGAGGACACATGTCCTGTGGGTG unknown	2.50	0.0011
226	TATGCAAATGATGACCAGTTCAAACATCAGGCACTCCCAGCTTCTGCGTGAACAAACTCA unknown	2.48	0.0303
227	CAGTCTGTACAAACGCTTACAAAAATCATAACTGTGAACTGACTTAAGACCAGAGTTTAC similar to spermatogenesis associated glutamate (E)-rich protein 4d	2.47	0.0011
	AAGAACAGGAGATGACCAGCAACCTCCAGACCCAGCAAAATGTGTGTCCAAAGGATATAT similar to hypothetical protein	2.46	0.0022
0	TGGGTTCCTTCTTGTGAACATGAAAATCAGTGGCATGATCCCAGAGAGCCTGGCCGACGC hypothetical LOC307706	2.45	0.0011
	UNING AGG COMONTOWN COMPACT ACT ACT ACT ACT ACT ACT ACT ACT ACT	2.44	0.0022
	CCTCCTTCCACACTGAATTGCTTACTAGTTTGAAGAAGCTATTGGAAGTGGGCATTTTGT		
	similar to CD69 antigen (p60, early T-cell activation antigen) CTACTTCTCCCGAGGAGCCTAGAGACTGGAATACAGGACGGCAGTACTGCCATACCCACGA	2.43	0.0173
232	RT1 class II, locus Bb CAGCTGTGACAGTTGTGAAATACCCTAGCTTCTGATAACAGAATGAGTTACTTCTTCCCA	2.42	0.0065
233	unknown TACTACTAATGCACTTCCGCTTTCCTTAAGCAAGTTGATGTTAGCGAATGCCATCTTTGT	2.41	0.0011
234	myelocytomatosis viral oncogene homolog (avian) AACTTGGACTTCAAAAAATGCATGCTCAAAGCCTAACCTCACAACCTTGGCTGGGGGCTTT	2.41	0.0281
235	SLIT and NTRK-like family, member 5 (predicted) CAAACAGGATCTCTGTAAGACAGAACTACTATTGTTACAGTCTCATATGTATCCCAGCAC	2.41	0.0011
236	hypothetical LOC317026 GAAGAGGATAACCTTCCTCAAACCTTCCTGGAAGTCAGAGCACTGGCTATGGACTTCCCT	2.41	0.0011
237	hypothetical LOC302243	2.40	0.0011

237 hypothetical LOC302243 AACATTGAACATCGTGAGAAATTTCAGGAGCTCAAGAAGGAGATTAACTTCTATCGGTAA 2.40

2	238	similar to RIKEN cDNA 6720458F09 gene TTAACCATGTCATATGTCATACCTGACATTACCTAGAGGAGCAATAAATGTTTTACCCCA	2.40	0.0292
2	239	unknown AACGTGAAATTAAAGGAGAAAGAGGTCATGTCATTACTGCACAACTTAGACACAAAGAAC	2.39	0.0011
2	240	hypothetical LOC363365 CTACTAGAGGAACCACAAGAAGAGGACCACCTTCCTCAAACCTTCCTGGAAGTCAGAGTA	2.39	0.0011
2	241	dermatopontin (predicted) GATGACTGACTATGACTGTGAATTCGAAAACGTTTAGATTCGCCACGTACAGAAGTCCGG	2.39	0.0043
2	242	unknown TTAGTTTAAGACTGTATGTACTGTATCATAGTAGGACCTATGTATCTCATCGCTGTGATG	2.39	0.0238
2	243	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5 AGGCTTTTGAAAATGACTACATTTGAATCATCAAACAATAACTCAGGGAAAGAAGTTGCC	2.38	0.0087
2	244	RT1 class lb, locus Aw2 CATTGCAGCTCTGGTGACTGTTGTGAGGAAGAGGAGAAACACAGGTGGAAAAGTAGGAGT	2.37	0.0097
2	245	unknown AAAGATAAAGACGAACAGGAGAAAAACCAGCAACCTCCAGACCCAGCAACATCTGAGAGTT	2.36	0.0011
2	246	unknown TTAACTGCAGGTCCCATCCCTGCCTTTAATAGGTCAGCCAGTAAAAGAAACCCAGCCACG	2.36	0.0011
2	247	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7 CCTTTGCAGATCAGATTTTTGGAATATTCAACTGGACAATTCCATTAGCAGTTGCATTAT	2.35	0.0011
2	248	hypothetical LOC367576 CGCAGAAAAACACGTTTCTGAAGCTGAAGAAGATCCAATATGTAGAATTCAGAAGGTGTC	2.35	0.0011
2	249	macrophage scavenger receptor 2 (predicted) TGTGGCTTATCCTTCAAGAGGACTCGAAACAATCTGCTCTGAGATATTGTCCTAACAGCT	2.34	0.0130
2	250	unknown AGGCAGCTGCTTTGCCATCTCCTCCTGCTGTGGATGTACAACAGGAGCATGGGACAAAGC	2.34	0.0011
2	251	unknown CCGAATCAGGCAATCCCAGCTCCTACATGAACAAGCTCAATTGAAGAACAATATACAGAT	2.34	0.0011
2	252	unknown TCTGACCCAGAGGGTCTTCATTCCTATCACCAGTGGGAATATATAT	2.34	0.0011
2	253	polo-like kinase 3 (Drosophila) CTCATGCTGTTCAGTGACGGCACTGTCCAGGTGAACTTCTACGGGGACCATACGAAGCTG	2.34	0.0260
2	254	unknown AGCTTTTGTGCCTACTCAATTTTGTCTGGCAATTACATTTTCCATACAGGTTGTGTCTA	2.34	0.0281
2	255	hypothetical LOC363369 AGAGACATCTTGAGTGTGCACTGAACCAGAAATATTTGGTTGACTTCAGCAAGAAAGA	2.33	0.0011
2	256	hypothetical LOC307706 AGCAGGAGAGCAAGGAGTTACAGCGATATTTGTTTGAGTTGAACCCCGAATGATGAAGATG	2.33	0.0011
2	257	unknown CAGAACCAAAGAGAAAGCAACTGTATTTACTTTGGTGAACCATCAATTATGGGTTTTACTA	2.33	0.0011
2	258	hypothetical LOC307706 ATGAAAACTTGAAGATAAAGGAGAAAGAGGTCATGTCAT	2.32	0.0011
2	259	Arganano Tity acid-binding protein, epidermal (E-FABP) ACGATAACAAGAAAACTGAAGGACGACGACGAAGATGGTGGTGGAATGTACCATGAACAAT	2.32	0.0271
2	260		2.32	0.0011
2	261	acidic ribosomal phosphoprotein P0 CTTAAGATCATCCAACTITTGGATGACTACCCAAAATGCTTCATTGTGGGAGCAGACAAT	2.31	0.0011
2	262	similar to Serine/threonine protein phosphatase PP1-beta catalytic subunit	2.30	0.0011
2	263	ATGTGTGCTAGAGGAGAAACAGCAATACATCTGTGCTTCTAAATGTTAAGAATATGCTGT hypothetical LOC307706 AGAACATTGAACATTGTGAGAAATTTCAGGAGCTCAAGAAGGAGATTCACTTCTATCGTG	2.30	0.0011
2	264	similar to leucine rich repeat containing 10	2.29	0.0249
2	265	TCCTAGTGGGTGAGGGCGCCGTCGAGCGCATGGCTGAGCGTGACGAACCCATTCCGCGGC unknown CATGGTTTTGTATAAACAAGACCTTCTAAATGAGGTAACAACTACCTTTGAACTTGTGAG	2.29	0.0011
2	266	unknown	2.29	0.0065
2	267		2.29	0.0011
2	268	TAGCTACCATGTTTTGTTGGGCCGAAACAATCTATCTGAAGATGAACCCTTTGCTCAGTA	2.28	0.0379
2	269		2.27	0.0011
2	270		2.27	0.0011
2	271		2.27	0.0011
2	272	CTATGGAGTTATTCCATGTTGAATTTCTTTTTGGAAGCAAAGCTGTCTGAACTATTACTG	2.26	0.0011
2	273	TTCCTGACAGAGAGATCCATGAAGAACAGAGAGAGATTCAGAACCTGACCCTGAGGATTGA similar to Myosin IXb (Unconventional myosin-9b)	2.26	0.0011
2	274	CTGTGAACACAGGTTTCTGTTGGAGAGAGCAGGCCTGTGCAGGCCCAGAACCTAGGCCATAA apolipoprotein A-II	2.25	0.0032
2	275	TTTGGTTCGGAGACAGGCAGCGGAGACGGATGTGCAGACCCTGTTCAGCCAGTATCTTCA	2.25	0.0206
2	276	TACCCGAAGATGATGACCTGAATACAGACACAGCTAATGTACATGGAAATAGTGCTGGCC unknown	2.25	0.0292
2	277	CTTTGGTCTCATCACGGGGTTGAAAACATCAGATCTACTCTGAGTGACTACTGTAGAAAT hypoxia inducible factor 1, alpha subunit	2.25	0.0238
		CCCTTGCTCTTTGTAGTTGGGTCTAACACTAACTGTACTGTTTTGTTATATCAAATAAAC		

278	unknown CACTCACTAGTCAGTCTACCTCACATTTTGTTTATGATTCTAAGTTGTCTTAAGCTGTGT	2.24	0.0227
279	hypothetical LOC307706 AACTTGAAGATAAAGGAGAAAGAGGTCATGTCATTACTGCACAACTTAGACACAAAGAAC	2.24	0.0011
280	unknown AGTCTTTCCCACTCACTGGATTCAATTAGATATTAAAAGGCTGCTTTTCAGCCTCGTGCC	2.24	0.0043
281	stathmin-like 4 TTGCAGAGAAACGAGAGCATGAGCGTGAGGTAATCCAGAAAGCTATCGAGGAAAACAACA	2.24	0.0054
282	integral type I protein AGCTGAAGACCTTAATAGTCGAGTGTCTTACTGGTCTGTAGGAGAGACTATTGCCCTGTT	2.24	0.0022
283		2.23	0.0011
284	gap junction membrane channel protein beta 2 CAACAGGGGACACTTCTTCCTGCCAAGAATGTCGTTGGGAAGCCATTCTGTAACAATAAA	2.23	0.0011
285	unknown TCTCATTTTGGCATACAAGTCCACAGTATGCCTACATTCTCAATGATGACTACTTCTGGG	2.23	0.0216
286	unknown GTGGACTGCATTTTCCTCCTTTACTTTGATTTCTTTGGATTGAATAGACCGTACTTTCAC	2.21	0.0011
287	growth arrest and DNA-damage-inducible 45 gamma (predicted) AGCGAGGCAGGTGTGACTCAGCAAGCAGCCTTCAGTGAAAGGAGGGGAAAGGCAAGGCAA	2.20	0.0206
288	putative pheromone receptor (Go-VN1) AAAGACAGCATAGGAGTAAAAATTCTGAAACATAGCAGTCAAGACAAACATTGGCCTAGC	2.20	0.0011
289	neurogenic differentiation 4 (predicted) GACTTGGAAAAATCCTACAACTTCATGCCACATTATACCTCTGCAAGTGTAAGTTCAGGG	2.20	0.0249
290	unknown TGCATTTTAAAGGGATAGCATTTGTTGATAATCAATTAGAATTTTGATAAAATTCACCAG	2.20	0.0032
291	villin 2 CATGCTTACCCTGTTAGCATTCATTGTTGGACTGATACACCTAATGATCTTCTATAGAGA	2.19	0.0336
292	unknown CATCAACTATTACGACGTGAACGCAGCCAACATAGGTTGGAACGGTTCCACCTTCGCTTG	2.19	0.0011
293	unknown AAGTCGTTTTTCTAAGCCATAGGTTCACTCACACAGCCAAACGATCAGCACCAACAGCAA	2.18	0.0011
294	unknown CAGCTAAGCCTTCCATGTACTTCATCAAGTTTGATGGCGACATCCACATCCACGTCTATA	2.18	0.0184
295	similar to Laminin alpha-4 chain precursor TTAGAGACTCCAACGTGGTTCAGTTGGATGTAGATTCAGAGGTGAACCATGTAGTTGGAC	2.18	0.0011
296	unknown GTTATGTAAGAGATTCCCACTCTGGTTGACGATTGGATTTATAGTTTTGAGGTTGCATTC	2.17	0.0011
297	Angiotensinogen ATCTACGAGCGGGACTCAGGTGCGCTGCACTTTCTGGGCAGAGTGGATAACCCCCCAAAAT	2.17	0.0011
298	unknown TTATCTTTCTTGTTGAAGTCAACCAAATATTTCTGGTTCAGTGCACAATCAAGATGTACC	2.17	0.0011
299	zinc finger protein 618 (predicted) CACAGCTCAGGAGGATGACCCGCTGGGCAAGAATGAAGTATACGATTACCTTCAGGAACC	2.16	0.0011
300	similar to CD69 antigen (p60, early T-cell activation antigen) TGGGTGAGGCAGGCAGGAACAGCTTCCCCCATGCGTTGGAGAGAACAGCCCGGAGCTGGA	2.16	0.0184
301	unknown GCTGCACTTGCCCTCGCGTCTTCAGAAAACAGCAGTAGTACTCCAGAGGAGTGTGAGGAG	2.16	0.0292
302	unknown TCGTGAATCACGCAAGCTTTTATCAGACTTTCAGCACATTGGGAGCATCAGAGTGACTGA	2.15	0.0303
303	hypoxia inducible factor 1, alpha subunit TTTTTTGGCCTATGGAATTGTTAAGCCTGGATCATGATGCTGTTGATCTTATAATGATTC	2.15	0.0108
304	spermidine synthase CTCAGTTGGATTGGACCAAGGAGCTTCCAAGCTGTCTGGGACCACCAGTCCTGGACCAAA	2.15	0.0173
305	cyclin G1 TGGAGTTAACAGAAGGAGTAGAATGTATTCAGAAACATTCCAAGATAAGTGGCCCGAGATC	2.14	0.0043
306	unknown AGAAGGTCTTCATACTATGGATTTGGCCTGAAATTTTGCCTGGCCCCAAATGATAGAATA	2.13	0.0011
307	SPARC-like 1 (mast9, hevin) CCCAACAAGGACAAGCATATCACCTTGAAGGAATGGGGCCACTGCTTTGGAATTAAAGAA	2.12	0.0011
308	unknown TAGACAACCCAAAGCTACATTTAAAAGACCATAAAGATTCTATTAGAAAGTTCGTGGACC	2.12	0.0032
309		2.12	0.0184
310	aldehyde dehydrogenase family 3, member A1 GAAGGACTCTCACCTCACTCCTCCAAATTCCACTGTTTGCTGGGCACAGAAATCAATAAA	2.12	0.0011
311	guanine nucleotide binding protein, alpha inhibiting 1 TAAACGTTGCACAGACTATTTTAGTACCATGATTTGTATACAGGCTTTTGATTCATAGGG	2.12	0.0011
312	procollagen, type XVI, alpha 1 (predicted) CGCACTGAGAGGAAGGACTCTACTAGGAATAAATGGCCAAAGCTTACAGGACTCTGACAG	2.12	0.0011
313	epidermal growth factor receptor TGGTCTTCCAACTGTGAAGATTCCACTGAAAGGTATCCATCGAGAACATTGTCCTTTTGG	2.12	0.0130
314		2.11	0.0011
315	reticulocalbin 3, EF-hand calcium binding domain GGTTGGGAGGGAGTTGCGCAATGCCACCTATGGCCATTATGAGCCCGGGGGGGG	2.11	0.0054
316	unknown AGAGGTCATTTCATTACTGCACAACTTAGACACAAAGAACATTGAACATCGTGAGAAATT	2.11	0.0011
317	myosin, heavy polypeptide 14 (predicted) TGCTCAAGGACCACTACCGAAAGCTGGTGCTACAGGTAGAGACCCTCACCACAGAGCTGT	2.11	0.0108

318 unknowr 2.10 0.0130 319 unknown GATGGATTGCAGATCAGAATTGGATCAAGAAGTATCAATACCACAAGGCTTACCTCATCC 2.10 0.0022 unknown CTGAGCGGTCATTTCCTAGCTAGTACTTAAGATCCAGTCAGAAAGCTCTTGGAGAAGCTA 2.09 0.0011 320 321 2.09 0.0054 unknow AAAGATGCTTCAGTCATCCAAAAGATTGGACTCCAGTATACTTCAGGCCAGGAAAGAAGC protein tyrosine phosphatase, receptor type, V CTGGAGCAGTATATCTACCTCTACAACTGTCTGAACAGCGCACTGCTGAACGGGCTGCCC 2.09 0.0011 322 cytidine 5'-triphosphate synthase (predicted) AACCCTGTTCCAGACCAAGAACTCAGTCATGAGGAAACTCTATGGAGACACAGACTACTT 323 2.09 0.0227 324 unknown 2 08 0.0043 ACGAATGCATTGTGAAGACCATTCCCAATGAACTCTATTGAATGTCTAATACACAGGTAT 325 ATPase, H+ transporting, V1 subunit G, isoform 3 (predicted) GATATGCTCTGTTAGGATGAATTGTCCACCGAAGTTGCCTACACGCTTTCATGTCACGTA 2.08 0.0455 unknown CCGAAGGGACTGGTTTCTTAAACCGTGGAACAGAATGTTTAAAAGAAGATAAATCTAGCC 2.08 0.0238 326 327 signal-induced proliferation-associated 1 like 1 AGTCTGCTCATATTGGACAGTGCACAATACAACAACTGCAGATCAACAATCATTATCTGC 2.08 0.0238 coatomer protein complex, subunit zeta 2 (predicted) CATTTTTGGCGGGCATGACTATTGTCTACAAGAGCAGTATTGACATCTTCCTATATGTGG 2.07 0.0011 328 329 2.06 0.0011 unknown ACCAAGCAATAATGCACATTTCTGACTGCTTACGTCAAGCGGACACCAACACTTCGCAAA unknow 330 2.06 0.0336 CAAGGTTGGCAATTAGAAAAGACAAAAACAGCTTGAATCATGAAACAAAGAGGCATCCAG ATP-binding cassette, sub-family C (CFTR/MRP), member 3 CTTGGACAAAGGAGTAGTAGCTGAATTTGATTCTCCAGTAAACCTCATTGCAGCTGGAGG 2.06 0.0097 331 phospholipase C-like 1 TTTGGGGGAGCCTAGAGAAGATAATCATTGAAATTTTGCAAATATAAACATCCTCTACAG 0.0032 332 2.06 protein tyrosine phosphatase, receptor type, U CAACTGCGTGAAGACCCTCTGTTCCCGGCGGGTCAACATGATCCAGACCGAGGAACAATA 333 2.05 0.0043 potassium inwardly-rectifying channel, subfamily J, member 8 TGCTGAGTTCTGGTGATCGAAGTATCGGACCTGTCGCCAATTCAGGGCAGGAACACAACA 2.05 0.0011 334 335 unknown CTGTATCGTCAGTGGATACAAGGATCCTGCTTGGTCTGTGGAGACTGAATACACCTTCCC 2.04 0.0011 similar to Acidic ribosomal phosphoprotein P0 GAGGTACCATTGAAATTCTGAGTGATATGCAGCAGGTGTTTGACAATAGCAGCATTTATA 336 2.04 0.0011 337 2.04 0.0011 unknown CAAGCTGTTTATTAGAGTTTTGCAACAGAGTTCTTGTTTGAAGACTCTAAAGACTACTTG 338 ADP-ribosylation factor-like 4 TGGGAAGCCAAAAAAGGCTAGTAATTGACCAGAAAACAATTTTGTGGAAATTTGACCTGA 2.03 0.0011 amyloid beta (A4) precursor protein TTCGGACATGATTCAGGCTTCGAAGTCCGCCATCAAAAACTGGTGTTCTTTGCAGAAGAT 2.03 0.0022 339 340 unknown 2.03 0.0043 AGGTTGATGCTGAAAAGGAACAGACAATAGAAAGTGTATCTTCCCATAATTGGAGGTCAA 341 unknown ACTGCCAGATTGTACAATTTTATGTGTATTTTCAAAGCTCATGATATGAGGGTCACTAAG 2.02 0.0065 342 natriuretic peptide receptor 2 GATGGGCAATGGTCACTGTGTCTCCACTTGACAGAAACAGACGTCATATGAGATGGAAAA 2.02 0.0043 unknown 2.02 343 0.0011 AGATAGGTAAGGATACATCTCTATAGTTGTTGATTTACCTTGAAACAGTTATGGGGAAAC 344 unknown AATGTTGAGAATGCTATTAGTCCATGTAGGAGTGATTCTGAAATTGAGTTGTTTTTGCAG 2.02 0.0260 unknown ACTACGTGAAGGAGCTAGGAGTAGGGCTGGCTCTTATGGGTGCCATGGCCAAACCAGACT 2.02 0.0206 345 346 2.02 0.0011 unknown GTTTCAGAACCAAGATCGAAACCAGGATGGCAAGATCACAGCTGAAGAACTCAAGCTGAA 347 mesoderm development candiate 2 (predicted) 2.01 0.0303 TATGCCTGGGAGATCAAGGACTTTTTGGTĆAATCAAGACAGGTGTGCTGAAGTCACTCTA similar to RIKEN cDNA 1700026D08 (predicted) CAGCTGCGCTTGGAATATGAAGGCTTCCCAGTCAGGGCAAATGAAAAGATTGTCATCTAT 2.01 0.0184 348 hypothetical LOC363325 349 2.01 0.0011 TTCTCCCCAAAGCAAGATCCCTGTCTTATCTGACAGGTATGTGGGCTTAGGGATGGGA solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 GCCCTGCTACTGCGAATATAAAACGTGAAGTTTGTTTCTAAATGCAAACCACTCCTGACC 2.01 0.0292 350 hypothetical LOC363325 ACAGAGAGGGACCAGAGCTTCTCCCCAAAGCAAGATCCCTGTCTTATCTGACAGGTATGT 0.0011 351 2.00 similar to Eso3 protein 352 2.00 0.0336 CTGATCTGTTACCTGGTACAAGACAAGACAGAGAGAGCATTTCATGCTTTTGGGGGATCCAGT

Supplementary Table 3 Upregulated transcripts in the male pituitary (fold change \geq 2; P-Value < 0.05)

No.	Gene name 60mer Oligosequence	Fold change M > F	P-Value	
1	unknown CAAGCCAACAATAGATGATGAATGAAATGATACATTTAGGTGAAACCAGAAATGCTTTGC	18.24	0.0011	
2	secreted phosphoprotein 1 TAAGAGTAAGGAAGATGATAGGTATCTGAAATTCCGCATTTCTCATGAATTAGAGAGTTC	8.66	0.0011	
3	tachykinin 1 AAAGCACAGTGTTGATGGAGTTGTACAAGTTTGCCAGCGATGCAAGTCTCCCAAAGACAGA	6.92	0.0011	
4	follicle - stimulating hormone subunit beta TCTGCTTTTCAGAGCCATCAGACTTTTAAAGACTCCAGCATGATTGCAAGCGAAAGGTCT	4.53	0.0011	
5	chloride channel Kb AATGAAGACACCAATGCGTTCTGTCACATCGGGACCCTTGGTCCTTCTAGGGTTGTCTGC	4.46	0.0011	Þ
6	syndecan 1 TTGCTTTTGGCAAAACGCTACTTAATCCAATGGGTTCTGTACAGTAGATTTTGCAGATGT	4.45	0.0011	
7	SEC14-like 3 (S. cerevisiae) GCTTCTCCCAGATGAGGGCATGCAGAAGTACGATGAGGAGCTCACCCCTATCTAGGTGGA	4.42	0.0054	
8	visinin-like 1 CAATGTTCATAGGGGTCAGCTAGTGATGACTTACTAAATCTACCCTTACACACATCTTTG	4.27	0.0011	
9	unknown AATTTGTACTCTCGATACTATTAACTGGTAATAGTGTAATGCCACAGTAATACTGTTCCG	3.91	0.0411	
10	chromogranin A AACTCCTGACTATTAAGATATTCCAGATAAAATTTATTGAAGGAAG	3.80	0.0011	
11	unknown ACGTTAAGCCCACTCTACCAGAAGTTAAATACTTAGCTGGTACTGACTG	3.79	0.0011	
12	potassium large conductance calcium-activated channel, subfamily M, beta member 4 AAGAACTCGGAAAGTGTCATGAACTGGCAACAGTATTGGAAAGATGAGATCGGTTCCCAG	3.51	0.0011	
13	visinin-like 1 CCTCACGCCAGAACAGCGAGTGGACAAGATTTTCAGCAAGATGGATAAGAACAAAGATGA	3.39	0.0011	
14	potassium channel, subfamily K, member 2 TTCTTGAAGGCTGATGTGGGGATGTACCACGCATAAACTGTGAACTGGATGGA	3.31	0.0011	
15	chitinase, acidic ACTAATGTTCTTCACGAAATTTCTGCCTTCTCCCCTTAATCCTCACCAACGTAGCTAGC	3.11	0.0054	
16	esterase 2 TTTCTGGACTGAGCTCTTGGCTAAGAATCCACCTCAGACAGA	3.04	0.0011	
17	receptor (calcitonin) activity modifying protein 1 CTCTGAGGAAGACTGCACAGATGTGTTTGTGGATGCATAGTTTGTGATTAAACGAGCGTT	3.00	0.0011	
18		2.96	0.0032	
19	Serine protease inhibitor	2.91	0.0022	
20	TTCTTTATGGGCAAAGTCACTAACCCCATGTGATCTGAAGCTCCCCAAAATCTGACAATT WAP four-disulfide core domain 1	2.87	0.0011	
21	AAGGGACAACAGAGGCACTTTCCATGAAGTGGAGACTGGCTGCCTTTGTGGGGGCCTTTCC chromogranin A CACGGCAGCATCCAGTTCTCACTTCTATTCAGGCTACAAGAAGATCCAGAAAGATGATGA	2.85	0.0011	
22	unknown	2.77	0.0011	
23		2.76	0.0011	
24	TCGTAAGAGGAGTAAGTCTAGAGCCAGTAAAAACTACTTAAAAAGGGTTTACGGAAGAGG	2.71	0.0011	
25	TGGTTTCACTTGTATTGTTCTATAATCCAGGCAATAAATGCTAATTAGCAATGCTCAAGG myosin heavy chain, polypeptide 6	2.71	0.0011	
26	ACAAGCTGCGGGCCAAGAAGCCGTGACATTGGCGCCCAAGCAGAAAATGCACGATGAGGAAAT myosin, heavy polypeptide 7, cardiac muscle, beta	2.66	0.0011	
27	GCCAAGAGCCGTGACATTGGCGCCCAAGGGCCTGAATGAA	2.58	0.0249	
28	TCTCTGATGACTCTAGCTTGTGTCTAGCAAGGACAGAAACTGAGCTCCCAACAGCACTGG unknown	2.56	0.0011	
29	TCAGGTGTTGTCTGGAGTAAACGTGCACTTCTCATTAAGCTTCATGTACAAATGGAGTAC carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9 (predicted)	2.54	0.0011	
30	TGGGÅAAAGAAGTTGTTTTGGCATTGTAGCTTATACTGTGGAAGCAATAACCTAGCCAGC cysteine rich protein 61	2.50	0.0011	
31	TTGAATGTTTTTATTTATCAAAATGTAGCTTTCGGGGAGGGA	2.50	0.0011	
32	ACGCTGCTATACTCTGGAAATGTCTGTAATTGCAGTATGTCTGATGGAATAAATCATATG unknown	2.49	0.0011	
33	AACTCCCAGGCAGTTGACTGGTGGGGGCAATTTGAATTTGTGCCTAAATCGTCATTTCATA similar to mKIAA0704 protein	2.47	0.0022	
34	TCGTTTTAGACCAGATCAAAGGCTTCTAGAAAGAAGGAAACATAGAGGAGGCTGAGGTGC phospholipase A2, group VII (predicted)	2.46	0.0011	
35	TGGTAAATGTACACTGTACTATAAGATGGTGATGAATGCCTGGCCTAAGATTCCCCCCCC	2.45	0.0108	
36	ACACGTGGATAGCCTGACTTGGATTÁCAAAAGGAGATCTGCTAGACATATACAGCTGTAA sterol-C4-methyl oxidase-like	2.45	0.0011	
37	AGGTGGTCTĆGGAGCTTTGTGAATTCTCTATCTGCACCTGTCAGAGCTGGTGAAATAAAT	2.44	0.0054	
38	TCCCAAAATGTGATTCCCAAACATGTCAAGGCCCAAGTATTCCGGACAGTTGATGGTAGA fibrinogen-like 2	2.43	0.0032	
	GCTTTTTAAAAAAGAAAAAGAAAAAGAAGATTTTGATATTAT			

39

40

41

2.42 unknown CAACGCTGGTTTCTAAATATTTTTGTATTGTGTACATTCTGTATATTTTTGTTGTAACGT 2.42 unknown TTAGATAGCTTGTACAGCCATGAGTAGAGTCCATGCGTACTGATGTATTAGATAGCTCGT 2.41 42 cadherin 1 TTGCTGTACTCACATAATTTTGGAAGCAAATGATGACTGCAATCAACTGTGAGAACTGTT 2.40 43 similar to cofactor required for Sp1 transcriptional activation, subunit 2, 150kDa 2.40

43	SIMILAR TO COTACTOR REQUIRED TO SPT TRANSCRIPTIONAL ACTIVATION, SUDUNIT 2, 150KDA	2.40	0.0011
44	early growth response 1 TTIGTATGCTATGAACATGAAGTTCATTATTTTGTGGTTTTATTTTACTTCGTACTTGTG	2.39	0.0216
45	NTE-related protein AGGTCTCCCCCCTGTCTCCAGGTCTCCCCCTGTCTCCAGGTCTCCCCCCTGTCTCCAGG	2.38	0.0054
46	unknown AAGGCTTAGGGGATCTGCTTTCGCCTGATAACCCTGGTTGTTTGCATTTCACAAATCTGT	2.36	0.0022
47	unknown AGCCCTGACCTCCAGCTATTAAACGTGTCACTAGACTCTTGCAGGGTTCTCTGGCTCCTA	2.35	0.0011
48	unknown AAATATGCAAACTTGTAACTGGGATGATAAAAGGCTTAGGGGATCTGCTTTCGCCTGATA	2.35	0.0011
49	unknown AGGGTCTAACCTATCTTCCCTGCACTGGGCAGAGGACAGGCTGGGAAGCCTGTTTAGTCA	2.33	0.0011
50	RAB3B, member RAS oncogene family CAGGCCTTCGAGCGTCTGGTGGACGCCATCTGCGATAAGATGTCTGACTCAATGGACACA	2.32	0.0011
51	similar to Tescalcin GCCCATTGACACTACCCTGGGTGAGGAACAAGTGGAGCTGTCTCGGAAGGAGAAGCTGAA	2.31	0.0011
52	unknown AAATGGAATGTGACGGTACTTTTACAAAAGAGAGAAAAATGTTATTTTTACTGTTTGAAG	2.30	0.0011
53	unknown TTCTAAAAGGTTGAAAATGTATATTTTGTTGCTTAAATGTGTCTTTGCAGAAATTGACAA	2.27	0.0011
54	unknown AGGGACAGCTCTTTAGGAAAAAGGAAAAAACCTTAAATAGTGAATAAACAACTACAACCAC	2.26	0.0011
55	nuclear receptor subfamily 3, group C, member 2 AGGTGGAGAAGGTCTACATTAGTCTTTTTGTATTAGTGAAGTTACTGGCTCCTCATGTGT	2.26	0.0011
56	interferon-induced protein 44 (predicted) AAAGGAGAGACTTTATCATGCCTTAATAATGAAGTCATCAACAAAGTAGTGAGAACAGAG	2.25	0.0054
57	glypican 3 CCACGTGTTATGTTTTCGAAAATCAAATGGTATCTTTATGAGGAAGGTAAATTTTAGTGG	2.25	0.0011
58	unknown TTTTTCCCAGACAACATAGAAGCGGAAGCTGTAACTGACATTGGCTATTTGAGAAGCACC	2.24	0.0292
59	glutathione S-transferase, alpha 4 (predicted) ACGAGAGAACAATATGAGAAGTTGCAAAAGGATGGATGCCTGCTTTTTGGCCAAGTCCCA	2.23	0.0227
60	carboxylesterase 3 CCACAGAAGATTTTGTAAAGACATAACACTTCTTGTCTTTGAGACTATAACATCACATGG	2.22	0.0011
61	unknown CCTAGTTATTTAGGTTGTCTATAAGACCGCAGTTAAAGTTGGATTGGAATACTGGTATTT	2.22	0.0011
62	RAB3B, member RAS oncogene family GAAGACAGTTTACCGTCATGAGAAGCGTGTGAAGCTGCAGATATGGGACACGGCAGGGCA	2.21	0.0011
63	unknown GTGATGGTTTTATTAGCAGCCAATTAGCGTTGGAAAAACTGGAGATACAACATGCCTTCC	2.21	0.0011
64	interferon-induced protein 44 (predicted) GATAGCCTGACTTGGATTACAAAAGGAGATCTGCTAGACATATACAGCTGCAATCCTGTG	2.21	0.0152
65	NEL-like 1 (chicken) AATGGCTGGATCTCCTTGTACAACCTGCAAATGCAAGAATGGGAGAGTCTGCTGCTGCTGT	2.21	0.0011
66	unknown GGTCTGCCAAAACAAACAAGGGACTCGGAAGGAGAGTGGAAAGGATACAAAAGAGAAATA	2.19	0.0022
67	gap junction membrane channel protein beta 6 AAGTGAGACCCTCTCTAAAATTAGCCTTTCCACCGGTGGCGAACAGTGTAAGCACAAATA	2.19	0.0032
68	neuronatin GGTTGCTATTGTGGTAGTCGCTAATTGTACTAGTTTACGTGTGCATTAGTTGTGTCTCCC	2.18	0.0032
69	unknown TCCTGTGAGTTACTAAGGATGGTTTATTTTCGAGAACCACATCAGATAGGGAATGAGTTA	2.17	0.0108
70	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 CTGTATGTTTTGAAAAGAATAATTTAACGTTTGGGTTGCCAGGAAGGGGGGCTTTCCCAGA	2.16	0.0011
71	delta sleep inducing peptide, immunoreactor ATGGAGTCCTGTGGTAGACAAGAAGAGTAAATAGTTTTGATCCCTTCGGGGCCTGGAAAA	2.16	0.0022
72		2.16	0.0011
73	similar to Serine/threonine-protein kinase SNK (predicted) TCTGGCCATGCTCTTTAGCAATGGGACAGTGCAGGTCAGTTCCAAAACCTCCCCAGTCCA	2.16	0.0141
74	unknown ACCGTATGTGATAAATCAGAGTATAATGTTTATGGTAATGAAATTAAAAGCTTCGTATAT	2.15	0.0011
75	neurotensin (predicted) ATAAAGAGAAAAATCCCTTATATTCTGAAACGGCAGCTCTATGAGAATAAACCCAGAAGG	2.15	0.0011
76	sulforansferase family 1A, phenol-preferring, member 1 GTGGATTCCATTGTTCACCACACATCTTTCCAGAAAATGAAAGAGAACTGCATGACTAAC	2.14	0.0011
77	syndecan 2 TGTGTCTGAATCCTTAATGGCCCTTAATTTCTGTCCAATTATAGCCGTAATTCACCTTATT	2.14	0.0011
78	esterase 2 TCTACTCTCTCGAAGGCAGAATGAATGAATGAGAAAATGGCCAGTTCTTCTTGAAGAGGCTTCAG	2.14	0.0032

0.0022

0.0011

0.0141

0.0022

0.0011

79 stathmin 1 TCCCTGACAAATATTCTAGAAGCTGATGTAGGAACCGTATAGGTAGATCCAGACCGTGAG 2.13 0.0011 80 unknown ACCGGTTACTTCCCAGTGTTTGAAAAGATTTTAAAAGACCACGGAGAGGCATTTCTTGTT 2.13 0.0184 interleukin 6 receptor TAGTGCTCATTAAGGAACATTGTCAGTTTTGTGAACATATGCTCAGATGGAGATCTTGTT 2.13 0.0011 81 similar to Stathmin (Phosphoprotein p19) (Oncoprotein 18) TGCCAAGCTGGAGTGTATACAAGAGAAGGACAAGCACGTTGAAGAGGTGCGGAAGAACAA 82 2.12 0.0011 glutamyl aminopeptidase TAATATACTCAGGGATTTCTGCTAAGTGTACTTTATAGGGATGTCTTTACAAACTGAAGG 0.0011 83 2.11 84 K-Cl cotransporter KCC4 GTTTCTTCAGACTTTGTTAGGCAGCCAGAGGTAGGCTTGCCAAGTAACTGCTGCCCGGTG 2.11 0.0011 85 unknown 2 10 0.0022 TGGGACCCCTTGTCACTGCCTATAGTGGCAAGCACTTGGACCTTGGTGCCTCTGAGAGAT 2.10 0.0011 86 synaptotagmin-like 4 TTTCACTGAAGTTTGAGCAGAAAACACAGACTCTGGTCATCCATGTCAAGGAGTGCCACC retinol binding protein 4, plasma CTCCCTTCTCTAGGTGGACATTAAAACCATCGTCCAAATACATGGGAATGCCTGAATCCA 2.09 0.0011 87 88 gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis) GAGCTGAACGACTGAAAAGAGATTCCTCGCCATATTGAATATCATCTACATTGTGTATTT 2.08 0.0022 0.0032 89 2.08 unknown ATGGTAACCTGAACGTCACCTACTTGCACAGAGAGAGATGGAAAATGTGTGGAGAAATTCT 0.0011 90 2.07 unknown TGAAAAGCCTAGAAATTAGGTCCTCTTAAAGTGCAATATTATTTAATCTCAGAATCGGGC 91 unknow 2.07 0.0011 GCCGTAAAAGTGTAAATTGCATGTGTGGGCATAATTACCGAACCTCATTGCCATGAGGTA low density lipoprotein receptor-related protein 2 TTGTTGCTCGTATTTTGAGTACCCATTGTAATTACTTTGATTAGAAATTAAAAAGCTACT 0.0011 92 2.07 sodium channel, voltage-gated, type 6, alpha polypeptide GTATTTCTTGCAACTTGAAGAAAATGACCCTTCAAACAATAGGGGTAGTGAGGAAGCAGA 2.07 0.0011 93 stathmin 1 GCTCCAGAAAGCCATTGAGGATAACAACAACTTCAGCAAAATGGCAGAGGAGAAACTGAC 94 2.06 0.0011 95 2.06 0.0011 96 unknown AAATCAACTCTGTTATATCCTAAAGGACTTCTGTCTTTTATATTCAGGATAATAAAGACT 2.05 0.0032 lipoma HMGIC fusion partner-like protein 4 GGCTGCATCACCTGCTTCGCTCTTTTCTTCTGCAACACCGCCACTGTCTACAAGATC 97 2.04 0.0173 amphoterin induced gene 2 GTGTTAGCATTCTTTAAAAATAGAACCTTTTAACTTACTAGAGCCAAAGTTGAGCTGAGC 98 2.04 0.0011 99 adenylate cyclase 2 CTCACAGCCTAGGACCAGTTTTGTACCAAACTCATCTGATGTTTTGATGCCATTTGTCAA 2.04 0.0011 solute carrier family 4 (anion exchanger), member 8 TGGCAAGAACAACAGCTTCAGATGTGACCCTTCTGAGATTAATATATCAGATGAAATGCC 2.04 0.0043 100 carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9 (predicted) GATGCCAATTACTTTCTACAGTTGATTGGTGCTCCAAAAGAGTTGAAATTTCCAAACTTT 0.0011 101 2.03 desmoplakin (predicted) TTCCCGTAGTGGGTCTCGAAGAGGAAGCTTCGATGCGACCGGGAATTCCTCCTACTCCTA 2.03 0.0011 102 103 2.03 0.0022 supervillin (predicted) AAACATCTCAATTTCTCATACCCATTGTAAACATTACACACGTCATTTTGTGACACAGGA 2.01 0.0011 104 105 unknown AACTAGAAAATCCTTAACAAAAAGAATTTAAGCTAAGAACCCCGAAACCAAACGAGCTAC 2.01 0.0184 phosphodiesterase 3B TTTTAAAAGTGTTTAACAATGAAGGAACTTTATTCTTTAGTCAAAACTGTTATTTTATT 2.01 0.0054 106 type II keratin Kb40 AGAGCAGGATGTCTGGAGATTGTCCCAGTGCAATCAGCATCTCGGTGACCGGCAACTCTA 107 2.00 0.0022 GNAS complex locus AACAGCAATCAGAAAACGCATCGATATCTTGATAATCCGCTGTACCAAAAGTTGGCATCT 108 2.00 0.0011



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