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Effects of genetic and environmental factors and gene-environment interaction on expression variations of genes related to stroke in rat brain

Yuande Tan[1], Myriam Fornage[2]

[1]College of Life Science, Hunan Normal University, Changsha, China;
[2]The University of Texas Health Science Center at Houston, Brown Foundation Institute of Molecular Medicine, Houston, USA.
E-mail: tanyuande@gmail.com

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ABSTRACT

To determine if genetic and environmental (dietary) factors and gene-environment interaction impact on the expression variations of genes related to stroke, we conducted microarray experiments using two homozygous rat strains SHRSR and SHRSP fed with high and low dietary salt levels. We obtained expression data of 8779 genes and performed the ranking analysis of microarray data. The results show that the genetic difference for stroke in rat brain has a strong effect on expression variations of genes. At false discovery rate (FDR) ≤ 5%, 534 genes were found to be differentially expressed between the genotypes resistant and prone to stroke, among which 304 genes were up-regulated in the resistant genotype and down-regulated in the prone genotype and 230 were down-regulated in the former and up-regulated in the latter. In addition, 365 were functional genes for transcription and translation, receptors (in particular, neurotransmitter receptor), channels of ions, transportation, metabolism and enzymes, and functional and structural proteins. Some of these genes are pivotal genes that cause stroke. However, dietary salt levels and GE interaction do not strongly impact on the expression variations of these genes detected on arrays.

Keywords: Rat; Ischemia Stroke; Microarray; Differential Expression; Genotype; Environment Factor; GE-Interaction

1. INTRODUCTION

Stroke is a major cause of severe disability and the third leading cause of death in the world. Stroke occurrence is a complex biological process involving obstruction of blood flow in a major cerebral vessel which leads to deregulation of genes whose expression promotes ischemic neuronal death and subsequent neurological dysfunction [1-3]. The development of stroke in an individual is influenced by a number of cardiovascular risk factors including genetic predispositions, hypertension, smoking, diabetes mellitus [4] as well as by dietary salt. The importance of genetic factor for stroke etiology has been documented by several rare monogenic diseases such CADASIL (cerebral autosomal dominant ateriopathy with subcortical infarcts and leukoencenphalopathy) and the genetic behavior of some genes for stroke [5-10]. However, the genetic basis of stroke is quite complex and the genes that are relevant to strokes have continuously being discovered. Some genetic factors including stroke-prediposing loci (QTLs) have been identified in the regions on rat chromosomes 1, 3, 4 and 5 [11,12]. In recent years, single nucleotide polymorphisms (SNPs) as an important and conservative genetic variations have widely been used to monitor human genetic diseases. For instance, G-50T in genes cytochrome P450 2J2 (CYP2J2) [4], G860A in a soluble epoxide hydrolase (EPHX2) [4], and plasma IL6 and CRP levels [13] were found to be significantly associated with risk of ischemic stroke.

Global gene expression profiles are becoming an important and necessary tool for exploring etiological mechanism of stroke. Several microarray experiments [14-22] have been used to demonstrate gene expression change in the postischemic rat subjected to ischemic stroke, hemorrhagic stroke, sham surgeries, hypoxia, and insulin-induced hypoglycemia. The blood genomic expression profiles of genes in human stroke have been obtained from the pilot studies [23,24]. But all these studies concentrated on gene-expression change in time

series after stroke while the associations between the stroke risk factors and expression variations of genes still remain unclear. Detection of effect of genetic backgrounds of stroke on differential expressions of genes is significant for illustrating mechanism of which stroke occurs, in particular, for finding functional genes participating in stroke.

In addition, since dietary factors play an important role in the onset of stroke in rat [25-28], to investigate if dietary factors regulate significantly the expressions of genes may be helpful for understanding stroke etiology. It is also deserved to ascertain if interaction between genetic and environmental (dietary) factors significantly contributes to expression variations of genes. In order to investigate effects of stroke genetic and dietary factors and their interactions on expression variations of genes, we employed a two-by-two design to conduct microarray experiments. Unlike the conventional two-by-two design that yields frequency data for association between two factors, here our two-by-two design is utilized to obtain large-scale continuous data of expression variations of genes in two ways: genetic and environmental (dietary) factors. Actually, for the continuous data, two-by-two design may be viewed as a simple two-factor design in which the data may be analyzed by two-way analysis of variance (ANOVA). For example, Kerr, *et al.* [29] and Black and Doerge [30] provided ANOVA models to account for multiple-factor microarray data. In two-by-two design, each factor has only two levels, and hence, ANOVA analysis is square of conventional t-statistic for each gene. ANOVA analysis of two-class data therefore is equivalent to the conventional two-tail t-test. In microarray experiments, however, since sample sizes are extremely small compared to conventional experiments but number of genes detected is huge, there would be a lot of chances to generate fudge effects in t-tests [31,32], that is, some of the t-values would be falsely inflated by standard errors smaller than 1. Except for significance analysis of microarray (SAM) [32] and ranking analysis of microarray (RAM) [31], all existing methods for *t*-tests do not consider the fudge effects. But SAM has a low power to identify genes differentially expressed, so we here choose RAM for our microarray data.

In this paper, our focus is on genetic and environmental factors and GE interaction related to stroke producing effects on differential expression of genes in rat. In another paper, we will use ranking analysis of correlation coefficients (RAC) [33], a large-scale correlation analysis method, to ascertain coexpression or coregulation of these differential expressed genes, furthermore, classify them into different functional groups, and build coexpression/coregulation network within groups.

2. MATERIALS AND METHODS

2.1. Animal Model and Two-by-Two Experimental Design

The experiments were performed on male stroke-resistant SHR/N (CRiv) (SHRSR) and stroke-prone SHR/A3 (Heid) (SHRSP) rats from a breeding colony maintained by the investigators as previously described [34]. Both rat strains SHRSR and SHRSP are inbred and homozygous, as a result of brother-sister mating over many generations. Almost all loci are the same in these two rat strains except those relevant to stroke. There are distinct gender differences in the establishment of hypertension and in stroke mortality rate in the SHRSP. Blood pressure is higher and rises more quickly and the incidence of stroke mortality is accelerated in males as compared to females. The cerebral cortex is the predilection site of cerebrovascular lesions in the SHRSP where rate of stroke occurrence is over 70%. Age-matched male rats from each strain (12 SHRSP rats and 12 SHRSR rats) were fed with a standard rat chow and water ad libitum until age 8 weeks. Subsequently, animals from each strain were randomized to one of 2 dietary regimens (N = 6 in each strain-diet group): a "stroke-permissive diet" high in sodium (HS) (0.63% potassium, 0.37% sodium) and 1% NaCl drinking solution; a "stroke-protective diet" low in sodium and high in potassium (LS) (1.3% potassium, 0.37% sodium) and regular drinking water. All animals were housed at 23˚C on a 12-hour light-dark cycle. The SHRSP rat strain in the HS environment showed stroke symptoms and died at 12 weeks of age. The stroke symptoms are defined as severe lethargy, loss of balance, poor grooming, convulsive rhythmic movement of the forelimbs, immobility, and kangaroo-like posture [35]. The brain tissues were collected for RNA extraction and subsequent microarray analysis. The study protocols were approved by the Animal Care Committee of the University of Texas-Houston. Thus, HS-SHRSPs, LS-SHRSPs, HS-SHRSRs, and LS-SHRSRs were tabbed by two-by-two tables.

2.2. Brain Tissues Collection and RNA Isolation

The brain was quickly removed, weighted, cut, and then transferred to an ice cold brain matrix block in two 2 mm coronal slices that was incubated at 37˚C for 30 min with 2% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.9% normal saline according to a modified protocol [36]. Then, the tissue slices were transiently immersed in a phosphate-buffered solution with 10% formalin and examined. The cortical tissue from the remaining slices was dissected and total RNA was isolated using the method of Chomczynski and Sacchi [37], washed in ethanol, resuspended in RNase-free water, and quantified by

spectrophotometric determination of optical density at 260 nm.

2.3. Microarray Experiment

Microarray analysis was performed as described by Lockhart, *et al.* [38]. Briefly, 10 µg total RNA extracted from each of the 24 rats was used to synthesize cDNA, which was then used as a template to generate biotinylated cRNA. cRNA was fragmented and hybridized to a Test 2 chip to verify quality and quantity of the samples. Each sample was then hybridized to a RGU34A array (Affymetrix, Santa Clara, CA) that contains 7779 full length cDNA and 1000 ESTs. After hybridization, each array was washed and scanned, and fluorescence values were measured and normalized using the Affymetrix Microarray Suite v.5.0 software.

2.4. Statistical Methods

For convenience, inbred animals SHRSR with genotype resistant to stroke are denoted by G^+ and SHRSP with genotype prone to stroke by G^-. The animals with G^+ and G^- exposed to the high and low levels of salt are separately labeled by E^+ and E^-. Thus animals are grouped into four groups (G^+E^+, G^+E^-, G^-E^+, G^-E^-) and each group has n individuals for statistical analysis [39]. Assume that genome-wide expression data of N genes are obtained from microarray experiments, each of which can be summarized by a 2 × 2 table. For example, the kth 2 × 2 table may be similar to **Table 1**. It is worth noting that the data in **Table 1** are continuous variables instead of categorical ones.

Let x be an expression value of gene g, which can be original or transformed. We here assume a linear model for x as

$$x_{gijk} = \mu_g + G_{gi} + E_{gj} + \mathbf{I}_{gij} + e_{gijk} \quad (1)$$

where μ_g is the mean of expression values for gene g (g = 1, 2, ⋯, N), G_{gi}, the effect of the ith genotype averaged over all environmental factor levels, E_{gj}, the effect of the jth environmental factor level over all genotypes, $\mathbf{I}_{gij}$, effect of GE interaction between genotype i and level j of environmental factor, and e_{gijk}, the special expression noise of observation k (k = 1, 2, ⋯, n) in genotype i and at level j of the environmental factor where both genotypes and environmental factor levels are dichotomous variables with i = 1 for G^- and i = 2 for G^+, j = 1 for E^- and j = 2 for E^+. For such a 2 × 2 experimental design, the estimates of μ_g, G_{gi}, E_{gj} and $\mathbf{I}_{gij}$ are respectively given by

$$\hat{G}_{gi} = (\bar{x}_{gi} - \bar{x}_g), \quad (2)$$

$$\hat{E}_{gj} = (\bar{x}_{gj} - \bar{x}_g), \quad (3)$$

$$\hat{\mathbf{I}}_{gij} = \bar{x}_{gij} - \bar{x}_g - \hat{G}_{gi} - \hat{E}_{gj} \quad (4)$$

where

$$\bar{x}_g = \frac{1}{4n}\sum_{k=1}^{n}\sum_{i=1}^{2}\sum_{j=1}^{2} x_{gijk}, \quad \bar{x}_{gij} = \frac{1}{n}\sum_{k=1}^{n} x_{gijk},$$

$$\bar{x}_{gi} = \frac{1}{2n}\sum_{j=1}^{2}\sum_{k=1}^{n} x_{gijk}, \quad \bar{x}_{gj} = \frac{1}{2n}\sum_{i=1}^{2}\sum_{k=1}^{n} x_{gijk}$$

Note that $\bar{x}_g$ is an estimate of μ_g. Thus, the following three sets of equalities $G_{g1} = G_{g2} = 0$, $E_{g1} = E_{g2} = 0$, and $\mathbf{I}_{g11} = \mathbf{I}_{g12} = \mathbf{I}_{g21} = \mathbf{I}_{g22} = 0$ correspond, respectively, to the three null hypotheses: no genetic effects, no environmental effects, and no GE interaction effects on expression variations of gene g. In the case of microarray data, sample sizes are extremely small but number of genes detected on arrays is huge, therefore, there would be a lot of chances to generate a fudge effect in traditional t-tests [31,32], that is, some of the t-values would be falsely inflated by standard deviation smaller than 1 due to $d_g > \sigma_g < 1$ where d_g is difference between two means and $\sigma_g = \sqrt{\sigma_{1g}^2 / n_1 + \sigma_{2g}^2 / n_2}$ for gene g. To remove the fudge effects, we have developed a modified t-statistic [31]. In the current notation, we used T-statistics to test for the above three hypotheses. Appendix A shows that the T-statistic is an extension of the traditional t-statistic and reduced to the traditional t-statistics when $A(c_g) = 0$. Associated with the T-statistic, we can perform a ranking analysis of microarray (RAM) [31]. RAM is based on comparisons between a set of ranked T statistics and a set of ranked Z values (a set of ranked estimated null T-statistics) yielded by a "randomly splitting" approach instead of a "permutation" approach and a two-simulation strategy for estimating the proportion of genes identified by chance, *i.e.*, the false discovery rate (FDR) [31]. RAM is powerful to identify genes of differential expressions, especially, between small samples.

Table 1. Two-by-two design for studying genetic, environmental, and gene-environment interaction effects on expression of genes related to rat stroke.

Exposure	Genotypes	
	G^-	G^+
E^-	(G^-E^-)	(G^+E^-)
E^+	(G^-E^+)	(G^+E^+)

3. RESULTS

3.1. Effects of Genetic and Environmental Factors (Dietary Salt) for Stroke and GE Interaction on Gene-Expression Variations

Figure 1 shows the observed linear T-Z dots with respect to the contribution of genetic factors for stroke in

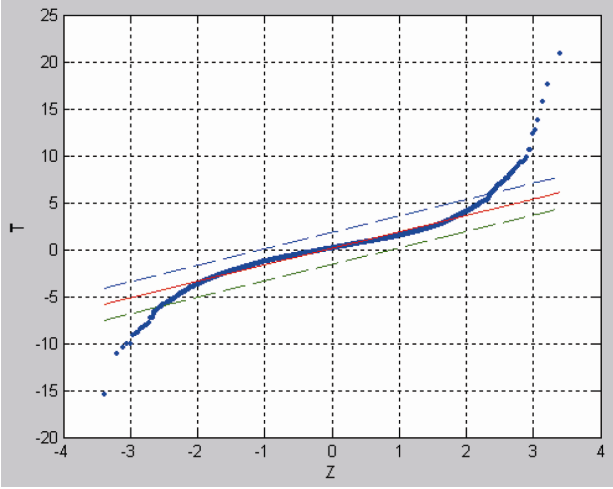

Figure 1 Linear plot of genetic effects on expression variations of genes. The T- and Z-values were obtained from the real microarray data set of 8799 genes. The blue linear dots are the ranked T-Z dots and red linear dots, the ranked Z-Z dots. The T-Z dots violently deviate from the Z-Z dots at two sides. Two break lines represent a given pair of thresholds Δ and –Δ.

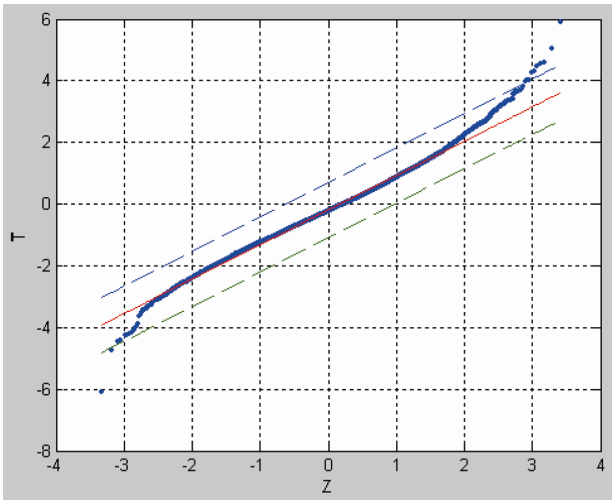

Figure 2. Linear plot of environmental (salt) effects on expression variations of genes. The T- and Z-values were obtained from the real microarray data set of 8799 genes. The blue linear dots are the ranked T-Z dots and red linear dots, the ranked Z-Z dots. The T-Z dots slightly deviate from the Z-Z dots at two sides. Two break lines represent a given pair of thresholds Δ and –Δ.

rat brain to the expression variations of genes. Two tails rat brain to the expression variations of genes. Two tails of the linear T-Z dots remarkably deviate from the linear Z-Z dots (red line) at $|Z| > 2$, indicating that the genetic difference between two genotypes with respect to stroke strongly altered expression regulations of a bulk of genes. But the environmental factors (salt) and GE interaction show a quite weak effect on the expression variations of genes (**Figures 2** and **3**).

3.2. Genes Differentially Expressed between Genotypes Resistant and Prone to Stroke

Table 2 offers the numbers of the genes called differential expressions between genotypes with respect to stroke in the rat brain at a set of given threshold levels and controls of false discovery rates (FDR) [31,39]. In **Table 2**, we found 534, 375, and 311 cDNAs displaying differential expressions between genotypes resistant and prone to stroke in the rat brain at FDR ≤ 5%, 1%, and 0.5%, respectively. Here we chose these 534 cDNAs at FDR ≤ 5 %. Of which 304 genes were up-regulated and 230 were down-regulated. In addition, 169 cDNAs were the expressed sequence tags (ESTs) (supplemental **Table 2**) and 341 of the remainders have been recognized as different functional genes in the rat brain or cerebral cortex due to replicates of some cDNAs and were sorted to several major functional groups: 1) Transcription and translation regulations, 2) Receptors, 3) Channels of ions, 4) Transporters, 5) Metabolisms and enzymes, and 6) functional and structural proteins (supplemental **Table 1**).

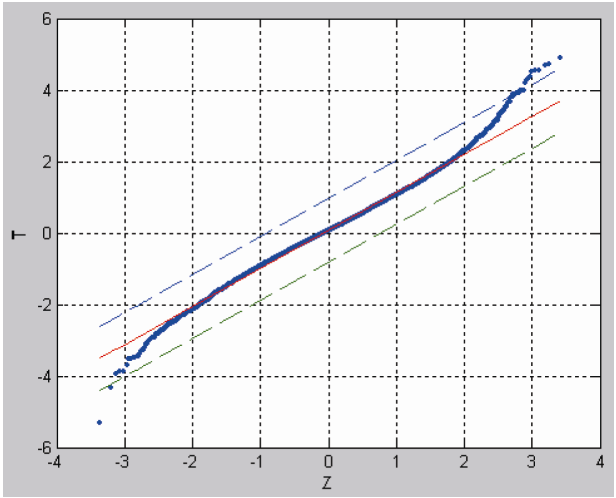

Figure 3. Linear plot of GE interaction effects on expression variations of genes. The T- and Z-values were obtained from the real microarray data set of 8799 genes. The blue linear dots are the ranked T-Z dots and red linear dots, the ranked Z-Z dots. The T-Z dots slightly deviate from Z-Z dots in two sides. Two break lines represent a given pair of thresholds Δ and –Δ.

A remarkable genetic effect is that some of genes for transcript factors, translation factors, receptors, channels, and transportation show strongly differential expressions between two genotypes of stroke (**Figure 4**). **Figure 4** shows that there are significantly more genes for transcript factors, receptors, and channels in up-regulation than in down-regulation. But in translation, 14 of the 17

Table 2. Number of genes whose expression are impacted by genetic effects identified by RAM and estimated FDR at a given threshold.

Threshold	Number of positive genes	Number of false positive genes	Estimated FDR
0.274734934	4311	2874	0.667
0.391600220	3053	2034	0.666
0.436597059	2736	1791	0.655
0.444170987	2698	1758	0.652
0.490108147	2433	1549	0.637
0.529096989	2240	1384	0.618
0.576847353	2058	1213	0.589
0.601159520	1967	1131	0.575
0.650760219	1824	979	0.537
0.684624193	1714	883	0.515
0.754544671	1504	704	0.468
0.763511830	1477	685	0.464
0.837328665	1291	532	0.412
0.875781020	1232	472	0.383
0.935709478	1123	382	0.340
0.966811203	1081	343	0.317
1.031635965	973	272	0.280
1.077064276	932	228	0.245
1.174369276	831	154	0.185
1.187233769	813	145	0.178
1.297105088	713	88	0.123
1.357611212	665	69	0.104
1.457567137	585	45	0.077
1.512749689	567	33	0.058
1.572320626	534	24	0.045
1.593312976	531	21	0.040
1.614943053	520	18	0.035
1.637260305	513	15	0.029
1.708927672	478	11	0.023
1.818484265	427	7	0.016
1.849113170	411	6	0.015
1.951549098	375	3	0.008
2.076044015	323	2	0.006
2.124648736	311	1	0.003
2.237727633	282	0	0.000

genes coding for ribosomal proteins (5 small subunits and 7 large subunits, one P subunit, and one 40 kDa subunit) were up-regulated in genotype SHRSP and down-regulated in genotype SHRSR. Two genes coding for translation factors, *i.e.*, elongin A and protein synthesis initiation factor 4AII were down-regulated in SHRSHP.

29 genes for receptors were found to be differentially expressed between these two genotypes with respect to stroke in our data. The highlight is the genes for recaptors that respond to γ-aminobutyric acid (GABA), one of the most important neurotransmitters, in the brain. GABA-B receptors 1 and 2 are expressed in the nerve fibers at early development stage. The nerve fibers are covered by myelin sheath. Stroke, an acute neurological event leading to death of neural tissue, is involved in subcortical infarcts and leukoencephalopathy that destructs the myelin sheaths. Co-activation of GABA-A and GABA-B receptors results in neuroprotection during in vitro ischemia, which is possibly due to the fact that co-activation of GABA-A and GABA-B receptors could strongly increase activation of Akt (or protein kinase B) and inhibit activation of apoptosis signal-regulating kinase 1 (ASK1) by phosphorylation of serine 83 of ASK1 [40]. Therefore, expression variations of GABA receptor genes in these two genotypes of being resistant and prone to stroke may be associated with stroke in etiology.

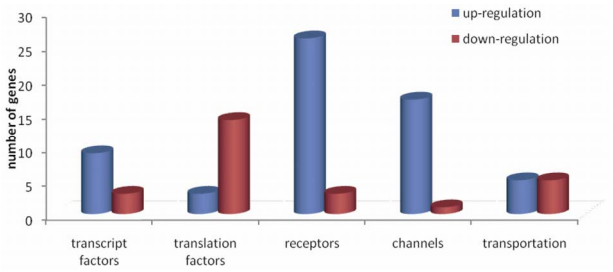

Figure 4. Number of differential expressed genes for transcript factors, translation factors, receptors, channel, and transportation. Up-regulations and down-regulations are defined in the genotype resistant to stroke.

Similar situation also happened in expression of the gene for glutamate receptor, a prominent neurotransmitter receptor. It is interesting that 26 genes for receptors displayed lower expression levels in SHRSP than in SHRSR. More interestingly, except for the gene for protein kinase C-regulated chloride channel, all 17 ionic channel genes were down-regulated in SHRSP. It is worth noting that the genes for glutamate transporter and glutamate/aspartate transporter were differentially expressed between these two genotypes. The glutamate transporters might increase the susceptibility of tissue to the consequences of insults that lead to a collapse of the electrochemical gradients required for a normal function [41]. Excitotoxicity may be an important pathophysiological mechanism of which Purkinje cell would be died after ischemia.

The glutamate transporter can remove glutamate from the extracellular fluid in the brain, suggesting that glutamate transporters may play a critical role in protecting Purkinje cells from ischemia-induced damage**.** Among 10 genes for transporters showing significant change in expression, 4 genes are responsible for glutamate or glu-

tamate/aspartate transport. Yamsashita, *et al* also found that glutamate/aspartate transporter (GLAST) is abundantly expressed in the cerebellar cortex [42].

Another big genetic effect on gene-expression variations was also found in those coding for enzymes controlling metabolisms. In our microarray data, 88 genes found to be differentially expressed between SHRSP and SHRSR encode enzymes that respectively participate in phosphate metabolism, oxidization and reduction, energy metabolism, carbohydrate metabolism, lipid metabolism, amino acid and nucleotide acid metabolism, sterol metabolism, neurotransmitter, extracellular and intracellular signaling, and so on. **Figure 5** shows that these 88 genes mostly work in phosphate metabolism, oxidization and reduction, extracellular and intracellular signaling, and carbohydrate metabolism. But here our focus is on a pivotal enzyme, *i.e*., Casein Kinase II (CKII) because CKII may play a crucial role in IFN-[gamma] signaling relevant to change in gene-expression in macrophage during atherosclerosis [43]. In particular, nuclear factor-κB (NFκB), a transcription factor, is activated after cerebral ischemia. NFκB activation leads to the expressions of many inflammatory genes involved in the pathogenesis of stroke [44]. NFκB is in general sequestered in the cytoplasm via interaction with specific inhibitory proteins (IκBα) [45]. Phosphorylation by CKII of serine/threonine in the C-terminus influences IκBα stability [46-50] and promotes deg- radation of IκBα via calpain [45]. Our results (supple- mental **Table 1**) show that the genes for CKII alpha subunit and calpain small subunit were significantly up-regulated in SHRSP but down-regulated in SHRSR. This indicates that expressions of CKII and calpain genes are suppressed in SHRSR so that IκBα is active and stable. The active IκBα inhibits NFκB. As a result, the expressions of many inflammatory genes dealing with the pathogenesis of stroke are closed in SHRSR. Therefore, CKII and calpain may be viewed as biomarkers for early diagnosis of stroke.

In addition to those above, 166 genes for functional and structural proteins such as binding proteins, membrane proteins, microtubule and skeletal proteins, glycoproteins, nervous system-associated protein, cell signal-associated protein, cell growth and cell division-associated proteins, immunological system-associated proteins, cell adhesion proteins, tumor-associated proteins, *etc* also were found to have strong expression variations. Among these genes coding for proteins, the most are those for nervous system-associated proteins. Most of the genes for glycoproteins, nervous system-associated protein, cell signal-associated protein, and cell division-associated proteins were down-regulated in genotype SHRSP compared to genotype SHRSR (**Figure 6**). Here it is especially worth noting that the genes for cathepsin S and NaPi-2 were significantly higher in genotype SHRSP than in genotype SHRSR while the calpastatin was significantly down-regulated in genotype SHRSP (supplemental **Table 1**). Cathepsin S is a prominent protein, a novel biomarker relevant to atherogenesis [51-53]. It is well known that stroke occurs when a blood clot forms and blocks blood flow in an artery damaged by atherosclerosis. Excess cathepsin S would produce a potential deleterious effect on the arterial wall because cathepsin S forms a plausible molecular link between enlarged fat mass and atherosclerosis [53]**.** Atherosclerosis complicated by plaque rupture or thrombosis could be a major factor causing potential lethal acute coronary syndromes and stroke [54]. In addition**,** type 2 sodium phosphate cotransporter (NaPi-2) beta has been demonstrated to be associated with hypertension and obesity [55-57]**.** This is why cathepsin S and NaPi-2 were up-regulated in genotype SHRSP but down-regulated in SHRSR. But calpastatin is a calpain-specific inhibitor

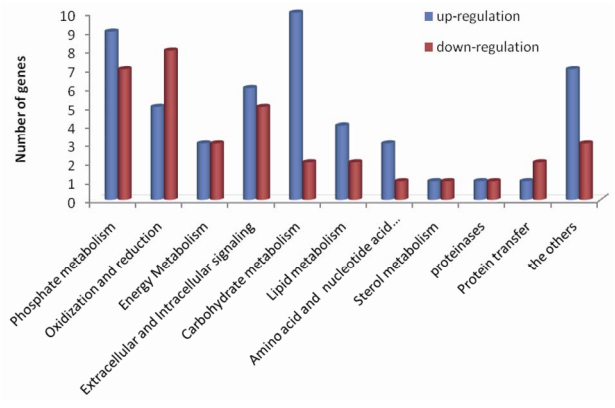

Figure 5. Number of differential expressed genes participating in metabolisms. Up-regulations and down-regulations of genes are defined in the genotype resistant to stroke.

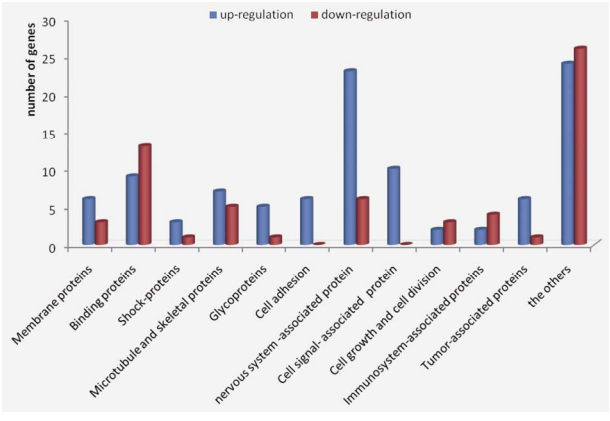

Figure 6. Number of differential expressed genes for proteins. Up-regulations and down-regulations of genes are defined in the genotype resistant to stroke.

[58]. As mentioned above, calpain aids CKII to degrade IκBα. Hence, in presence of calpastatin, IκBα has a higher activity level. The higher active IκBα inhibits NFκB.

3.3. Genes Differentially Expressed between Two Salt Levels

The role of environmental factors is an exterior effect in stroke. Therefore, compared to the genetic effects, as seen in **Figure 2**, the environmental effect on expression variations of genes is very weak. The numbers of genes differentially expressed between two different dietary salt levels at a set of given thresholds are listed in **Table 3**. As expected in **Figure 2**, merely 22 genes were found to have differential expressions between two dietary salt levels: high in sodium (HS) and low in sodium (LS) at FDR ≤ 5%. Among them, 10 are EST, 9 are functional genes for connexin 40, carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase, NF1-B2, prolyl 4-hydroxylase alpha subunit, RhoA, syntaxin B, and taurine transporter, respectively, and 4 are unkown sequences (supplemental **Table 1**).

3.4. Genes Differentially Expressed Due to Interaction between Genotypes and Environmental Factor (Dietary Salt)

As such, contribution of interaction (GE) between genotypes and environmental factor (dietary salt) to expression variations of genes is also pretty small (**Figure 3**). The numbers of the genes differential expressed due to GE interaction at a set of threshold levels were listed in **Table 4**. 25 genes were found to have the interesting change in expression at FDR ≤ 5%. Among them, 11 are EST, 12 are functional genes coding for synuclein 1, synaptojanin, flk protein, metabotropic glutamate receptor 3, neurexin III-alpha, carboxypeptidase D precursor (Cpd), electrogenic Na^+ bicarbonate cotransporter (NBC), and ET-B endothelin receptor, GST Yc1, respectively, and 3 are unknown sequences (Supplemental **Table 1**). 23 of these 25 genes were down-regulated in the genotype resistant to stroke and the low salt environment or in the genotype prone to stroke and the high salt environment but up-regulated in the genotype resistant to stroke and the high salt environment or in the genotype prone to stroke and the low salt environment.

Table 3. Number of genes whose expression variations were impacted by environmental factors (dietary salt) identified by RAM and estimated FDR at a given threshold.

Threshold	Number of positive genes	Number of false positive genes	Estimated FDR
0.23054	4452	2968	0.667
0.307166	774	398	0.514
0.341366	485	292	0.602
0.378962	338	198	0.586
0.386128	301	165	0.548
0.409155	266	145	0.545
0.467169	168	86	0.512
0.478735	157	71	0.452
0.484306	147	65	0.442
0.510608	142	62	0.437
0.56461	114	46	0.404
0.579948	101	35	0.346
0.666147	62	20	0.322
0.686774	57	15	0.263
0.789565	36	9	0.250
0.92422	24	3	0.125
1.07455	22	1	0.045
1.323427	7	0	0
1.480786	4	0	0
1.546755	3	0	0

4. DISCUSSION

From **Figures 1**, **2** and **3**, it can be seen that the effects of these factors for stroke on the expression variations of genes were well displayed by deviation of the T-distribution from the Z-distribution at two sides. As expected, the genetic difference between genotypes results in the significant expression variations of genes related to stroke (**Figure 1**). At FDR ≤ 5%, we found 341 functional genes differentially expressed between the genotypes SHRSP and SHRSR. Unlike the environmentally

Table 4. Number of genes whose expression variations were impacted by GE identified by RAM and estimated FDR at a given threshold.

Threshold	Number of positive genes	Number of false positive genes	Estimated FDR
0.04302	7487	5729	0.765
0.125184	839	642	0.765
0.176549	511	385	0.753
0.186523	453	303	0.667
0.290752	263	175	0.665
0.390944	160	71	0.444
0.394555	158	58	0.367
0.536208	99	35	0.355
0.597688	77	16	0.208
0.621986	73	13	0.178
0.631139	69	11	0.159
0.685069	55	7	0.127
0.849769	38	4	0.105
0.876529	35	2	0.057
1.015051	25	1	0.040
1.071766	24	1	0.041
1.345698	8	0	0

differential expressions of the genes, the genetically differential expressions of the genes are resulted from change in structure of these genes or from altering regulation elements of an operon system. Compare to SHRSP, SHRSR genetically strengthens (up-regulate) expressions of the genes associated with rat resistant to stroke and weakens (or down-regulate) expressions of the genes making rat prone to stroke. Our data show that these genes work in transcription and translation regulations, ion and molecule transportations including channels and transporters, metabolisms, nervous system, and functional and structural proteins. Since stroke, which mostly occur in the cortical region of the brain, is a complex neurological event, the genes working for the nervous system, including receptors, neurotransmissions, binding proteins, neurons, synapses, etc, were strongly regulated by some other key genes for stroke. Mutations would change expressions of these genes working in the nervous system. For example, as mentioned above, 8 genes for the GABA receptors (GABA-A and -B receptors) showed higher expression levels in SHRSR than in SHRSP. The GABA-B receptors potentially play an important role in the inflammatory response and neutrophil-dependent ischemia-reperfusion injury such as stroke [59]. Another example is that the TrkB receptor, a high-affinity receptor of two neurotrophins (brain-derived neurotrophic factor and neurotrophin 4/5), is important for neuronal growth and differentiation and regulation of synaptic transmission and for prevention from neuronal damage after ischemia [60,61]. In our microarray data genes for the full-length (FL) and the truncated TrKB showed differential expressions between SHRSR and SHRSP even though their differential expression direction is just opposite (supplemental **Table 1**).

In addition, the genetic difference for stroke between two rat strains also alters expressions of some critical genes involved in stroke. For instance, CKII plays potentially important roles in specific neural functions and is significantly associated with change in expressions of many inflammatory genes involved in stroke. The expression difference of CKII gene between SHRSR and SHRSP causes differential expressions of a set of relevant genes.

Fornage, *et al.* [34] used TagMan assay to measure the relative expression levels of 7 functional genes encoding atrial natriuretic peptide (Anp), the neurotrophin receptor protein tyrosine kinase (TrkB, a truncated form), casein kinase 2 (CKII), complexin 2 (Cplx2), stearoyl CoA desaturase 2 (Scd2), glycerol-3-phosophate acyltransterase (Gpan), and inositol 1,4,5-triphosphate receptor (Itpr1). They found that these 7 genes were significantly differentially expressed between genotypes SHRSR and SHRSP with $p < 0.05$. In our current microarray data, these 7 genes were also found to have significant expression change between these two genotypes at FDR < 0.3%. Furthermore, our microarray data were well agreeable with the TaqMan data for the direction of change in expressions between these two genotypes. In addition, Tropea, *et al.* [62] also found that the genes encoding glutamate receptor (GluR-A) and GABA receptor had significantly expression change between two mice treated by respective dark rearing and monocular deprivation. We performed RAC for these 534 genes detected to be differentially expressed between two genotypes and the other 481 genes that were not identified to be differently expressed and found that there were a lot of strongly positive and negative correlation expressions between these differentially expressed 534 genes, while all the 481 genes without differential expression were not significantly correlated in expression variations (the results will be shown elsewhere).

The differentially expressed genes may be classified into different functional groups because genes in a functional group possibly have the same or similar expression pattern. The similar expression pattern may be measured by correlated expressions, including co-expressions and co-regulations of gene-expressions. By using correlation of gene-expressions, one can build clusters or networks of functional genes related to stroke and find associations between functional genes and build gene pathways for a global insight into a pathogenesis of stroke. These valuable and interesting studies will be given elsewhere.

The role of dietary factors in occurrence of stroke has been well documented. For example, when feeding a diet low in potassium and high in sodium, the SHRSP strain developed a rapid onset of stroke [25,26], while potassium supplementation remarkably reduced the incidence of stroke and delayed its onset [63]. In addition, high dietary potassium intake was significantly associated with a reduced risk to stroke [64]. However, our current data did not show that the dietary salt has a strong regulation effect on expression variations of genes in vivo. But it might play an important role in metabolic and physiological processes for stroke. We also found that the interaction between genetic difference and dietary salt for stroke has a weak contribution to expression variations of genes.

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Supplemental Materials

Supplemental **Table 1**: 341 functional genes differentially expressed between two genotypes SHRSR and SHRSP, 8 functional genes differentially expressed between high salt and low salt and 11 genes differentially expressed due to GE interaction. Supplemental **Table 2**: 169, 4, and 3 ESTs differentially expressed due to genetic, environmental, and GE interaction effects, separately. ”

Supplemental **Table 1**. Functional genes found to have significantly differential expressions by RAM at FDR ≤ 0.05.

Genetic effects for stroke in rat brain				
Gene ID	Gene notation	Prone	Resistant	T-value
	Transcription and translation			
	Transcript factor and regulators			
L42855	RNA polymerase II transcription factor SIII p18 subunit	234.083	–234.083	–5.454
AF031657	Zinc finger protein 94 (Zfp94)	–32.325	32.325	3.752
AF052042	Zinc finger protein Y1 (RLZF-Y)	49.258	–49.258	–3.738
U41164	Cys2/His2 zinc finger protein (rKr1)	–206.252	206.252	15.78
U67082	KRAB-zinc finger protein KZF-1	–20.306	20.306	4.111
X12744	c-erb-A thyroid hormone receptor	–169.979	169.979	3.976
D14046	DNA topoisomerase IIB	–20.331	20.331	4.045
AF036959	nuclear serine/threonine protein kinase	–58.142	58.142	4.083
M65148	rATF2	–36.535	36.535	4.548
D26307	jun-D gene	241.619	–241.619	–15.401
M84716	putative v-fos transformation effector protein (Fte-1)	235.642	–235.642	–3.684
U61405	aryl hydrocarbon receptor nuclear translocator 2 (ARNT2)	–49.4	49.4	4.562
U09228	New England Deaconess E-box binding factor	–94.215	94.215	4.752
	Translation factors and ribosomal protein			
L46816	elongin A	–22.565	22.565	4.543
U64705cds	protein synthesis initiation factor 4AII	–372.002	372.002	4.522
X83747	5S rRNA gene	68.804	–68.804	–7.259
M89646	ribosomal protein S24	84.14	–84.14	–5.805
X06423	ribosomal protein S8	455.608	–455.608	5.442
X57529cds	ribosomal protein S18	298.652	298.652	–3.979
X58465	ribosomal protein S5	245.819	–245.819	–4.64
X59375	ribosomal protein S27	37.665	–37.665	–3.574
M17419	ribosomal protein L5	384.702	–384.702	–4.453
X78167	ribosomal protein L15	147.944	–147.944	–3.554
X78327	ribosomal protein L13	264.454	–264.454	–7.126
X62145cds	ribosomal protein L8	672.379	–672.379	–8.816
X62166cds	ribosomal protein L3	241.604	–241.604	–4.127
X06483cds	ribosomal protein L32	332.167	–332.167	–5.067
X55153	ribosomal protein P2	292.629	–292.629	–4.896
D25224	40 kDa ribosomal protein	153.731	–153.731	–3.745
X60212	ASI mammalian equivalent of bacterial large ribosomal subunit protein L22	–1367.33	1367.325	13.748
	Receptors			
AB016160	GABAB receptor 1c	–375.954	375.954	4.597

AB016161UTR#1	GABAB receptor 1d	–375.954	375.954	4.597
AF058795	GABA-B receptor gb2	385.231	–385.231	–3.622
L08490cds	GABA-A receptor alpha-1 subunit	–200.477	200.477	6.777
L08491cds	GABA-A receptor alpha-2 subunit	–42.858	42.858	3.774
L08493cds	GABA-A receptor alpha-4 subunit	–59.423	59.423	4.818
L08497cds	GABA-A receptor gamma-2 subunit	–144.552	144.552	3.686
X15467cds	GABA(A) receptor beta-2 subunit	–33.492	33.492	4.57
X15468cds	GABA(A) receptor beta-3 subunit	–39.298	39.298	6.074
L09653	transforming growth factor-b type II receptor	–26.788	26.788	3.54
L19112	heparin-binding fibroblast growth factor receptor 2	–35.744	35.744	4.911
D28498	Flt-1 tyrosine kinase receptor	–48.652	48.652	3.676
L19341	activin type I receptor	–38.242	38.242	4.348
L27487	calcitonin receptor-like receptor (CRLR)	–17.877	17.877	4.011
L31622	nicotinic acetylcholine receptor beta 2 subunit	–39.371	39.371	4.856
M64699	inositol 145-trisphosphate receptor (IP-3-R)	–95.504	95.504	8.663
M77184	parathyroid hormone receptor	–34.298	34.298	4.616
S39221	NMDA receptor	–89.133	89.133	4.177
U21871	outer mitochondrial membrane receptor rTOM20	205.529	–205.529	–4.44
U38653	olfactory inositol 145-trisphosphate receptor (InsP3R) alternatively spliced variant	–134.683	134.683	10.547
U79031	Alpha-2D adrenergic receptor	–14.206	14.206	3.942
AF071014	alpha-1D adrenergic receptor	–50.375	50.375	3.708
U87306	transmembrane receptor Unc5H2	–130.031	130.031	4.307
D14908	PACAP receptor	–37.054	37.054	3.638
M36418	glutamate receptor (GluR-A)	–115.765	115.765	7.397
U11419	glutamate receptor subunit	–105.019	105.019	4.847
S68284	S1 progestin receptor form B	–33.294	33.294	4.343
S64044	progesterone receptor steroid-binding domain	–26.452	26.452	3.971
X97121	NTR2 receptor	294.258	–294.258	–6.335
	Channel			
M59211	potassium channel Kv3.2b	–16.429	16.429	4.404
M27159cds	potassium channel-Kv2	–41.379	41.379	7.229
AF091247	potassium channel (KCNQ3)	–98.731	98.731	5.995
M81783	K+ channel	–63.573	63.573	4.469
M84203	K+ channel protein (KSHIIIA3)	–32.598	32.598	4.066
X70662	K+ channel protein beta subunit	–93.221	93.221	6.27
U37147	sodium channel beta 2 subunit (SCNB2) gene	–56.073	56.073	9.165
Z36944	putative chloride channel	–45.958	45.958	3.496
U27558	brain-specific inwardly rectifying K+ channel 1	–60.713	60.713	4.059
Z67744	CLC-7 chloride channel protein	–25.602	25.602	3.472
AF048828	voltage dependent anion channel (RVDAC1)	–99.319	99.319	5.013
AF049239	voltage-gated sodium channel rPN4	–74.358	74.358	6.928
D38101	rCACN4A L-type voltage-dependent calcium channel alpha 1 subunit	–43.992	43.992	4.918

D17521	protein kinase C-regulated chloride channel	125.692	–125.692	–5.216
L39018	sodium channel protein 6 (SCP6)	–104.696	104.696	4.592
AF021923	potassium-dependent sodium-calcium exchanger (NCKX2)	–91.338	91.338	5.179
X76724	RCK beta2	–93.646	93.646	4.92
X76452cds	ATPase isoform 4 calcium-pumping	–65.59	65.59	7.027
	Transportation			
S59158	glutamate transporter	439.546	–439.546	–10.514
S75687	glutamate/aspartate transporter	–195.281	195.281	4.825
X63744	glutamate/aspartate transporter protein	23.71	–23.71	–3.952
S82233	rBSC2 = Na-K-Cl cotransporter homolog	–31.594	31.594	4.381
U15098	GluT and GluT-R glutamate transporter	–43.744	43.744	5.19
U75395UTR#1	furosemide-sensitive K-Cl cotransporter	31.923	–31.923	–5.004
U89529	fatty acid transport	132.927	–132.927	–4.963
AB015432	LAT1 (L-type amino acid transporter 1)	227.856	–227.856	–8.148
D13962	neuron glucose transporter	–130.55	130.55	6.329
M15882	clathrin light chain (LCA1)	–122.792	122.792	3.55
	Metabolism and enzymes			
	Phosphate metabolism			
M89945	farnesyl diphosphate synthase	96.021	–96.021	–5.884
U28938	protein tyrosine phosphatase D30	41.208	–41.208	–4.588
U36771	glycerol 3-phosphate acyltransferase nuclear gene encoding mitochondrial protein	–24.71	24.71	3.755
U36772	glycerol-3-phosphate acyltransferase nuclear gene encoding mitochondrial protein	–30.313	30.313	4.578
U36773	glycerol-3-phosphate acyltransferase nuclear gene encoding mitochondrial protein	–175.954	175.954	7.066
U55192	inositol polyphosphate 5 phosphatase Ship (SHIP)	45.587	–45.588	–6.486
U73458	protein tyrosine phosphatase (PTPNE6)	–25.885	25.885	7.108
X12355	phosphoinositide-specific phospholipase C form-I (PI-PLC I)	165.656	–165.656	–4.498
X94185	RNMKP3 dual specificity phosphatase MKP-3	78.738	–78.738	–5.876
Y12635	vacuolar adenosine triphosphatase subunit B	–215.946	215.946	5.968
D14419	PP2A BRa B regulatory subunit of protein phosphatase 2A	–36.3	36.3	3.894
D38261	BRgamma B-regulatory subunit of protein phosphatase 2A	–104.346	104.346	3.515
M27726	phosphorylase (B-GP1)	–66.765	66.765	3.864
M23591#2	catalytic protein phosphatase 2A-beta	228.944	–228.944	–3.916
D83538	230kDa phosphatidylinositol 4-kinase	–35.392	35.392	2.266
	Oxidization and reduction			
S45812	monoamine oxidase A	65.823	–65.823	–4.234
U75927UTR#1	cytochrome oxidase subunit VIIa	41.571	–41.571	–4.632
L48209	cytochrome c oxidase subunit VIII (COX-VIII)	386.258	–386.258	–4.105
D00688	monoamine oxidase A	115.533	–115.533	–4.675
X60328	cytosolic epoxide hydrolase	49.658	–49.658	–5.367
AA686031	NGF-treated similar to NADH-ubiquinone oxidoreductase 75 kDa subunit	–34.798	34.798	3.78

S81448	type I 5 alpha-reductase	–52.048	52.048	3.688
M29249cds	3-hydroxy-3-methylglutaryl coenzyme A reductase	–22.027	22.027	3.695
U60063	aldehyde dehydrogenase	23.792	–23.792	–3.517
AF001898	aldehyde dehydrogenase (ALDH)	134.763	–134.763	–10
X97772	D-3-phosphoglycerate dehydrogenase	46.502	–46.502	–3.913
U64451	short-branched chain acyl-CoA dehydrogenase precursor	–34.629	34.629	3.718
E03428cds	peptidylglycin-alpha-amidating monooxygenase	–255.258	255.258	4.885
	Energy Metabolism			
D84450	Na+ K+-ATPase beta-3 subunit	164.085	–164.085	–4.705
D90048exon	Na+ K+-ATPase (EC 3.6.1.3) beta2 subunit	–67.304	67.304	4.455
D90049exon#1-2	Na+ K+-ATPase (EC 3.6.1.3) alpha2 subunit	–387.342	387.342	10.57
M74494	sodium/potassium ATPase alpha-1 subunit truncated isoform	–722.437	722.438	8.373
D50696	proteasomal ATPase (S4)	68.333	–68.333	–5.248
X56133	F1-ATPase alpha subunit (EC 3.6.1.34)	124.333	–124.333	–3.66
U15408	plasma membrane Ca2+-ATPase isoform 4 and alternatively spliced variations	–98.173	98.173	7.509
	Extracellular and Intracellular signaling			
D64045	phosphatidylinositol 3-kinase p85 alpha subunit	10.219	–10.219	–3.738
X53428cds	glycogen synthase kinase 3 beta (EC 2.7.1.37)	50.821	–50.821	–5.437
U42627	dual-specificity protein tyrosine phosphatase (rVH6)	64.627	–64.627	–6.088
M25350	cAMP phosphodiesterase (PDE4)	–30.869	30.869	5.297
Z36276	GK II cGMP dependent protein kinase II	41.292	–41.292	–5.598
X07286cds	protein kinase C alpha	–25.513	25.513	4.173
M64300	extracellular signal-related kinase (ERK2)	–88.298	88.298	4.187
AF068261	pancreatic serine threonine kinase	–37.533	37.533	3.625
AJ000557	RNJAK2 Janus protein tyrosine kinase 2 JAK2	–25.952	25.952	5.331
L15618	**casein kinase II alpha** subunit (CK2)	111.242	–111.242	–6.057
M96159	adenylyl cyclase type V	–44.754	44.754	4.219
	Carbohydrate metabolism			
U27319exon	type I hexokinase (HKI)	–129.292	129.292	4.522
J04526	brain hexokinase	–383.367	383.367	6.856
M54926	lactate dehydrogenase A	191.99	–191.99	–3.665
D21869	PKF-M (phosphofructokinase-M)	–217.973	217.973	6.708
X89383	SNF1-related kinase	–47.942	47.942	6.312
D10852	N-acetylglucosaminyltransferase III	–50.229	50.229	3.922
D49434	ARSB arylsulfatase B	–59.842	59.842	4.292
D89340	dipeptidyl peptidase	60.481	–60.481	–5.147
L27075	ATP-citrate lyase	–95.488	95.488	7.533
L02615	cAMP-dependent protein kinase inhibitor (PKI)	–47.113	47.113	3.991
D10770	beta isoform of catalytic subunit of cAMP-dependent protein kinase	–209.046	209.046	7.777
U75932	cAMP-dependent protein kinase type I regulatory subunit	–183.675	183.675	5.152
	Lipid metabolism			
U08976	Wistar peroxisomal enoyl hydratase-like protein (PXEL)	62.96	–62.96	–6.325

U67995	stearyl-CoA desaturase 2	–433.358	433.358	4.194
AF036761	stearoyl-CoA desaturase 2	–1419.63	1419.633	12.336
S75730	stearoyl-CoA desaturase 2 SCD2 homolog	–367.006	367.006	9.267
X05341	3-oxoacyl-CoA thiolase	–87.423	87.423	5.111
E12286cds	GM2 activator protein	12.392	–12.392	–3.793
	Amino acid and nucleotide acid metabolism			
U35774	cytosolic branch chain aminotransferase	–749.29	749.29	7.912
L34821	succinate-semialdehyde dehydrogenase (SSADH)	–41.54	41.54	4.973
D26073	phosphoribosylpyrophosphate synthetase-associated protein (39kDa)	67.235	–67.235	–4.348
	Neurotransmitter			
M93257	cathechol-O-methyltransferase	69.431	–69.431	–4.271
X02610	non-neuronal enolase (NNE) (alpha-alpha enolase 2-phospho-D-glycerate hydrolase EC 4.2.1.11)	–451.39	451.39	3.074
M55291	neural receptor protein-tyrosine kinase (trkB) (FL)	304.527	–304.527	–8.829
M55293	neural receptor protein-tyrosine kinase (trkB) (short)	–49.388	49.388	5.896
	Sterol metabolism			
U17697	lanosterol 14-alpha-demethylase	115.121	–115.121	–4.903
D45252	23-oxidosqualene: lanosterol cyclase	–43.848	43.848	3.936
	proteinases			
U27201	tissue inhibitor of metalloproteinase 3 (TIMP-3)	–43.229	43.229	3.814
U38379	gamma-glutamyl hydrolase precursor	32.988	–32.988	–4.052
	Protein transfer			
X73653	tau protein kinase I	68.044	–68.044	–6.602
U86635	glutathione s-transferase M5	120.1	–120.1	–3.987
AF084205	serine/threonine protein kinase TAO1	–74.75	74.75	3.623
	The others			
Z48444	disintegrin-metalloprotease	–52.638	52.637	6.193
M13707	protein kinase C type I	112.571	–112.571	–4.183
E01789cds	Protein kinase C type-II	–169.185	169.185	4.561
K03486	protein kinase C type III	–295.848	295.848	3.502
E07296cds	N-acetylglucosamine transferase-I (brain)	–41.748	41.748	4.17
L19998	minoxidil sulfotransferase	125.008	–125.008	–5.916
L13406	calcium/calmodulin-dependent protein kinase II delta subunit (brain)	–24.379	24.379	4.142
L05557cds	plasma membrane calcium ATPase isoform 2	–43.725	43.725	3.659
D30041	RAC protein kinase beta	–50.008	50.008	5.205
M81225	farnesyl transferase alpha subunit	45.469	–45.469	–3.674
Protein				
	Membrane proteins			
X53565	trans-Golgi network integral membrane protein TGN38	–71.646	71.646	8.695
AF102853	membrane-associated guanylate kinase-interacting protein 1 Maguin-1	–61.667	61.667	3.883
M24104	vesicle associated membrane protein(VAMP-1)	–169.092	169.092	3.698
D13623	p34 protein	69.16	–69.16	–3.889
AB016425	occludin	–35.948	35.948	4.087

U31367	myelin protein MVP17	225.469	–225.469	–4.242
L18889	calnexin	–111.698	111.698	3.926
U27767	reversibly glycosylated polypeptide 4(RGP4)	–221.242	221.242	3.772
D28111	myelin-associated oligodendrocytic basic protein (MOBP)	469.748	–469.748	–9.107
	Binding proteins			
M69055	insulin-like growth factor binding protein (rIGFBP-6)	45.185	–45.185	–4.194
U02096	acid binding protein	61.238	–61.238	–4.051
S83025	TSH receptor suppressor element-binding protein-1	301.702	–301.702	–6.346
S69874	cutaneous fatty acid-binding protein (C-FABP)	122.942	–122.942	–6.337
U39875	EF-hand Ca^{2+}-binding protein p22	–57.144	57.144	5.125
AF090306	retinoblastoma binding protein	99.437	–99.438	–3.724
X13167cds	NF-1 like DNA-binding protein	–30.185	30.185	4.489
AF053768	brain specific cortactin-binding protein CBP90	–17.725	17.725	3.852
D13125	neural visinin-like Ca^{2+}-binding protein type 2 (NVP-2)	–94.375	94.375	5.185
D13309	DNA-binding protein B	160.867	–160.867	–4.92
M12672	guanine nucleotide-binding protein G-i alpha subunit	–107.513	107.513	4.01
M14050	immunoglobulin heavy chain binding protein (BiP)	178.44	–178.44	–3.741
L27663	DNA binding protein (Brn-2)	14.6	–14.6	–3.555
D14819	calcium-binding protein P23k beta	–89.675	89.675	4.891
L10326	alternatively spliced GTP-binding protein alpha subunit	282.581	–282.581	–7.238
L19698	GTP-binding protein (ral A)	34.673	–34.673	–4.669
M64986	amphoterin	62.135	–62.135	–4.356
L12380	ADP-ribosylation factor 1	–292.769	292.769	4.657
L12382	ADP-ribosylation factor 3	–190.792	190.792	5.941
AB000362	cold inducible RNA binding protein (CIRP)	68.896	–68.896	–3.631
X13933	calmodulin (pRCM1) (a calcium-binding protein)	472.477	–472.477	–3.965
AF019043	dynamin-like protein (DLP1) a large GTP-binding protein	–72.504	72.504	5.145
	Shock-proteins			
S81917	34 kDa DnaJ-hsp40 heat shock-chaperone protein	–81.517	81.517	6.626
S75280	heat shock protein precursor	–38.175	38.175	3.7
S45392	heat shock protein 90	646.619	–646.619	–3.743
AJ002967	utrophin	–50.177	50.177	4.153
	Microtubule and skeletal proteins			
S74265	high molecular weight microtubule-associated protein(HMW MAP2)	–20.917	20.917	4.551
U25264	skeletal muscle selenoprotein W (SelW)	254.646	–254.646	–3.824
J00692	skeletal muscle alpha-actin	77.365	–77.365	–8.101
X53455cds	microtubule-associated protein 2	–67.748	67.748	4.63
X66840cds	microtubule associated protein 1A (partial)	–20.808	20.808	3.475
AF035953	kinesin-related protein KRP4 (KRP4)	–36.773	36.773	4.588
D88461	N-WASP	–53.617	53.617	4.436
U59241	E-tropomodulin	–52.869	52.869	4.021
S77900	myosin regulatory light chain isoform C	30.39	–30.39	–3.746

AJ000485	cytoplasmic linker proteins CLIP-115 protein	45.527	–45.527	–3.783
X62952	vimentin	75.221	–75.221	–3.977
U15766#1	nonmuscle myosin heavy chain-B fragment II	–40.644	40.644	4.194
	Glycoproteins			
M99485	myelin/oligodendrocyte glycoprotein (MOG)	63.856	–63.856	–3.506
X02002	thy-1 gene for cell-surface glycoprotein	–557.267	557.267	20.92
X07648cds	amyloidogenic glycoprotein (rAG)	–444.925	444.925	4.226
X99337	RNGP55 glycoprotein 55	–267.369	267.369	4.572
X99338	RNGP56 glycoprotein 65	–254.163	254.163	4.228
D10587	85kDa sialoglycoprotein (LGP85)	–31.871	31.871	4.611
	nervous system-associated protein			
Y16563	brain-specific synapse-associated protein	–25.227	25.227	4.512
Y08981	synaptonemal complex lateral element protein	9.256	–9.256	–3.786
U56261	SNAP-25a	–121.554	121.554	5.507
AB003991	SNAP-25A	–236.8	236.8	5.942
AB003992	SNAP-25B	–236.8	236.8	5.942
D32249	neurodegeneration associated protein 1	574.427	–574.427	–6.016
U33553	neuroglycan C precursor	384.708	–384.708	–4.548
X16623cds	neuraxin	–75.504	75.504	7.341
AF060879	neurocan	–106.413	106.413	4.138
L10362	synaptic vesicle protein 2B (SV2B)	–224.181	224.181	9.267
M64488	synaptotagmin II	–11.073	11.073	3.545
L27421	neuronal calcium sensor (NCS-1)	–33.075	33.075	3.921
M27812	synapsin Ia	–346.852	346.852	3.45
U20105	synaptotagmin VI	–21.094	21.094	3.881
U14398	synaptotagmin IV homolog	–141.652	141.652	4.313
AF000423	synaptotagmin XI.	–157.29	157.29	6.632
AF007836	rab3 effector (RIM)	–22.688	22.688	3.762
S65091	cAMP-regulated phosphoprotein	135.383	–135.383	–6.047
S73007	synuclein SYN1	–248.146	248.146	4.063
U39320	cysteine string protein	–51.579	51.579	6.221
X77934	RNWAPLP2 (Wistar) amyloid precursor-like protein 2	–168.815	168.815	3.714
Y08355	PKC-zeta-interacting protein	–581.092	581.092	5.502
Y13413	Fe65L2 protein	–111.915	111.915	4.706
M31176#2	gastrin-releasing peptide	36.546	–36.546	–3.848
AF091834	N-ethylmaleimide sensitive factor NSF (phosphorylation of NSF by PKC)	–205.527	205.527	4.191
U01022	Huntington's disease	–27.44	27.44	5.351
U35099	complexin II (related to Huntington disease)	–174.902	174.902	7.925
Y17048	caldendrin	133.208	–133.208	–4.055
X78689	RNEHK1 ehk-1	–31.852	31.852	3.744
	Cell signal-associated protein			
AB011544	TUBBY protein	–37.306	37.306	4.655

AF055065	signal regulatory protein alpha	–78.685	78.685	6.927
D44481	CRK-II	–40.298	40.298	4.428
AF023621	sortilin	–149.46	149.46	7.603
AF081196	calcium and DAG-regulated guanine nucleotide exchange factor II	–159.973	159.973	4.234
D14425	calcineurin B	–312.738	312.737	7.18
AJ003148	RNAJ3148 GAS-7 protein	–109.26	109.26	8.053
U50842	ubiquitin ligase (Nedd4) protein	–115.719	115.719	4.823
U49049	chapsyn-110	–21.508	21.508	5.745
X80290	pituitary adenylate cyclase activating peptide	–51.6	51.6	5.056
	Cell growth and cell division			
AF083330	kinesin-like protein KIF3C (KIF3C)	–148.24	148.24	8.059
D38629	adenomatosis polyposis coli (APC) protein	–51.337	51.338	3.54
L26268	anti-proliferative factor (BTG1)	90.512	–90.512	–6.308
D16308	cyclin D2	127.925	–127.925	–7.945
X62322	epithelin 1 and 2	–0.769	0.769	0.042
	Immunosystem-associated proteins			
L10336	guanine nucleotide-releasing protein (mss4)	–47.744	47.744	5.329
AF060819	ras guanyl releasing protein (rasGRP)	–106.413	106.413	4.138
AF036548	response gene to complement 32 (RGC-32)	23.581	–23.581	–3.918
U49062	heat sle antigen CD24	83.394	–83.394	–5.509
X54640	the OX47 antigen	242.831	–242.831	–3.929
M58404	thymosin beta-10 (testis-specific)	193.348	–193.348	–4.213
	Cell adhesion			
U81037	ankyrin binding cell adhesion molecule (NrCAM)	–145.677	145.677	5.063
M88709	cell adhesion-like	–186.229	186.229	3.961
U65916	ankyrin membrane binding domain	–37.515	37.515	8.653
AB004276	protocadherin 4	–152.015	152.015	4.879
U83230	l-Afadin	–20.998	20.998	3.555
S58528	integrin alpha v subunit	–20.865	20.865	3.877
	Tumor-associated proteins			
X12535cds	ras-related protein p23	–184.023	184.023	7.176
X13905cds	ras-related rab1B protein	–102.883	102.883	5.705
L19304	tumor suppressor fragment 2 of 6	–81.4	81.4	7.522
L19306	tumor suppressor fragment 4 of 6	–69.252	69.252	4.743
D89863	M-Ras	–56.265	56.265	4.035
AF015911	NAC-1 protein (NAC-1) linked to ovarian cancer recurrence	–29.006	29.006	3.794
M91235	VL30 element	52.354	–52.354	–4.13
	The other proteins			
M81687	core protein (HSPG)	–32.6	32.6	3.579
U07619	tissue factor protein	15.644	–15.644	–4.19
U20181	iron-regulatory protein 2 (IRP2)	–23.352	23.352	4.003
U37142	brevican core protein	100.385	–100.385	–4.032

V01543	fragment isolated from the brain and coding for brain specific peptide	–86.646	86.646	3.995
X01118	gamma atrial natriuretic peptide precursor (gamma-γANP)	30.504	–30.504	–5.358
E00698cds	gamma atrium natriuretic polypeptide gamma-γANP	46.577	–46.577	–7.296
X96394	multidrug resistance protein	–38.102	38.102	4.624
U61729	proline rich protein	39.804	–39.804	–4.279
X57405	homologue of Drosophila notch protein	–54.158	54.158	3.928
AF053362	death effector domain-containing protein DEFT	26.854	–26.854	–3.77
U77918	spermatogenic cell/sperm-associated Tat-binding protein homolog Sata	76.302	–76.302	–5.379
AF020212	DLP1 splice variant 2 (DLP1)	–40.938	40.938	5.057
AF087697	dlg 3	–58.725	58.725	3.933
AF095741	MG87	80.017	–80.017	–3.914
V01217	cytoplasmic beta-actin	–517.133	517.133	4.073
D00092	70 kd mitochondrial autoantigen	–38.969	38.969	3.611
AJ007291	RNO7291 CAP1 gene	208.46	–208.46	–5.285
D30804	proteasome subunit RC6-1	–5.644	5.644	0.172
D45247	proteasome subunit RCX	–82.225	82.225	1.542
K01934#2	hepatic product spot 14	34.698	–34.698	–4.442
K02816	unidentified expressed in embryo and tumor	–236.781	236.781	4.875
L02915	RATSOM fragment	–41.888	41.888	6.031
L03201	**cathepsin S**	439.281	–439.281	–5.23
L14462	R-esp1	322.681	–322.681	–4.06
L21192	GAP-43	217.45	–217.45	–4.111
AA684963	RPCAU48	134.917	–134.917	–4.781
AB003515	GEF-2	133.3	–133.3	–4.544
AB006451	Tim23	132.988	–132.988	–5.285
AB008908	FHF-4b	–20.794	20.794	4.27
AB013454	**type 2 sodium phosphate cotransporter NaPi-2 beta**	36.871	–36.871	–5.444
M34176	adaptin	–141.508	141.508	4.957
M83679	RAB15	–37.377	37.377	4.02
M87634	BF-1	90.588	–90.588	–4.551
S70011	tricarboxylate carrier	44.098	–44.098	–3.671
S75019	turgor protein homolog	42.773	–42.773	–3.833
U15138	LIC-2 dynein light intermediate chain 53/55	382.858	–382.858	–4.316
U47312	R2 cerebellum DDRT-T-PCR LIARCD-3	–70.275	70.275	4.388
U53859	**calpain** small subunit (css1)	315.635	–315.635	–4.154
Y13591	**calpastatin** (a **calpain**-specific inhibitor)	–6.879	6.879	4.014
U94189	Duo	–29.715	29.715	6.779
X05300	ribophorin I	–63.44	63.44	3.449
X05472cds#3	2.4 kb repeat DNA right terminal region	39.817	–39.817	–5.063
X52140	integrin alpha-1	–22.483	22.483	3.974
X52772cds	p65	–164.415	164.415	8.833
X52817cds	C1-13 gene product	–549.125	549.125	3.517

X82445	C15	80.196	–80.196	–5.542
X74401	(GDP dissociation inhibitor 2) GDI beta	–72.648	72.648	3.635
X74402	(GDP dissociation inhibitor 1) GDI alpha	–377.185	377.185	8.079
Z34004	growth hormone-releasing hormone alternate	–86.606	86.606	5.154
	Environmental effects for stroke in rat brain			
Gene ID	Gene notation	Low salt	High salt	T-value
M96601	taurine transporter	–39.423	39.423	4.317
AF022136	connexin 40 (GJA5)	19.531	–19.531	–4.268
X78949	prolyl 4-hydroxylase alpha subunit	49.721	–49.721	–4.405
M95735	syntaxin B	296.838	–296.838	–4.479
U53505s	type II iodothyronine deiodinase	22.735	–22.735	–4.209
D84477	RhoA	86.992	–86.992	–6.09
AB012231	NF1-B2	–87.129	87.129	4.456
AF037071	carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase	–145.683	145.683	4.597
	Genetic-Environmental interaction for stroke in rat brain			
Gene ID	Gene notation	LSSP, HSSR	LSSR, HSSP	T-value
S65355	nonselective-type endothelin receptor	–38.51	38.51	3.929
D64061	annexin V-binding protein (ABP-7)	–148.86	148.86	4.195
U45479	synaptojanin	–214.742	214.742	4.301
AF007758	synuclein 1	–233.569	233.569	3.79
X13412cds	flk protein	–18.106	18.106	3.8
L14851	neurexin III-alpha gene	–100.21	100.21	4.531
AF004017	Na+ bicarbonate cotransporter (NBC)	–65.935	65.935	4.559
X57764	ET-B endothelin receptor	–50.192	50.192	4.692
U62897	carboxypeptidase D precursor (Cpd)	–73.869	73.869	4.721
M92076	metabotropic glutamate receptor 3	–185.475	185.475	4.901
X78848cds	RNGSTYC1F GST Yc1	154.285	–154.285	–4.334

Supplemental **Table 2.** ESTs found to have significantly differential expressions by RAM at FDR ≤ 0.05.

Genetic effects for stroke in rat brain				
Gene ID	Expressed sequence tag	Prone	Resistant	T-value
rc_AA799406	EST188903	173.358	–173.358	–5.893
rc_AA799538	EST189035	–0.892	0.892	0.259
rc_AA799576	EST189073	47.631	–47.631	–3.585
rc_AA799599	EST189096	128.765	–128.765	–4.717
rc_AA799621	EST189118	–25.621	25.621	4.007
rc_AA799721	EST189218	–39.392	39.392	3.459
rc_AA799755	EST189252	6.452	–6.452	–3.823
rc_AA799786	EST189283	–13.406	13.406	3.687
rc_AA800015	EST189512	101.158	–101.158	–3.96
rc_AA800034	EST189531	102.317	–102.317	–3.532
rc_AA800170	EST189667	–70.846	70.846	4.246

rc_AA800198	EST189695	91.712	–91.713	–3.705
rc_AA800535	EST190032	–36.675	36.675	3.463
rc_AA800784	EST190281	42.215	–42.215	–4.606
rc_AA800908	EST190405	–14.529	14.529	3.573
rc_AA818072	UI-R-A0-ag-b-06-0-UI.s2	81.819	–81.819	–3.96
rc_AA818888	UI-R-A0-av-c-08-0-UI.s1	279.373	–279.373	–3.708
rc_AA849038	EST191800	450.656	–450.656	–4.609
rc_AA849648	EST192415	46.796	–46.796	–4.079
rc_AA849648	EST192415	46.796	–46.796	–4.079
rc_AA849722	EST192489	125.521	–125.521	–3.549
rc_AA852004	EST194773	–311.069	311.069	3.777
rc_AA859372	UI-R-E0-bt-a-03-0-UI.s1	21.508	–21.508	–4.561
rc_AA859520	UI-R-E0-br-b-02-0-UI.s1	84.873	–84.873	–5.748
rc_AA859633	UI-R-E0-bs-h-09-0-UI.s1	–57.373	57.373	5.532
rc_AA859663	UI-R-E0-bs-c-07-0-UI.s1	110.933	–110.933	–4.617
rc_AA859665	UI-R-E0-bs-c-09-0-UI.s1	–59.358	59.358	3.475
rc_AA859688	UI-R-E0-bx-e-09-0-UI.s1	–134.154	134.154	3.65
rc_AA859829	UI-R-E0-cc-f-12-0-UI.s1	–112.177	112.177	4.193
rc_AA859837	UI-R-E0-cc-g-09-0-UI.s1	–288.188	288.188	7.272
rc_AA859837	UI-R-E0-cc-g-09-0-UI.s1	–288.188	288.188	7.272
rc_AA859848	UI-R-E0-cc-h-10-0-UI.	26.071	–26.071	–4.346
rc_AA859877	UI-R-E0-cc-c-04-0-UI.s1	224.473	–224.473	–3.716
rc_AA859990	UI-R-E0-ca-a-08-0-UI.s1	157.642	–157.642	–8.206
rc_AA866237	UI-R-A0-bg-f-12-0-UI.s1	40.165	–40.165	–11.117
rc_AA874856	UI-R-E0-cg-h-11-0-UI.s1	–16.44	16.44	5.08
rc_AA874873	UI-R-E0-ci-d-11-0-UI.s1	–59.525	59.525	3.519
rc_AA874918	UI-R-E0-ck-g-08-0-UI.s1	–23.206	23.206	5.16
rc_AA874934	UI-R-E0-ci-c-05-0-UI.s1	313.988	–313.988	–9.047
rc_AA875054	UI-R-E0-cb-e-04-0-UI.s1	–158.067	158.067	17.55
rc_AA875105	UI-R-E0-cf-h-06-0-UI.s1	–25.24	25.24	3.756
rc_AA875135	UI-R-E0-bu-f-01-0-UI.s2	–22.981	22.981	3.749
rc_AA875225	UI-R-E0-cq-a-06-0-UI.s1	–63.581	63.581	0.51
rc_AA875275	UI-R-E0-ce-c-01-0-UI.s1	–25.106	25.106	5.258
rc_AA875427	UI-R-E0-cs-f-11-0-UI.s1	86.154	–86.154	–5.631
rc_AA875427	UI-R-E0-cs-f-11-0-UI.s1	86.154	–86.154	–5.631
rc_AA875470	UI-R-E0-cp-c-12-0-UI.s1	–183.688	183.688	3.829
rc_AA875506	UI-R-E0-ct-c-05-0-UI.s1	–74.846	74.846	12.647
rc_AA875577	UI-R-E0-cm-c-10-0-UI.s1	99.771	–99.771	–3.737
rc_AA891049	EST194852	84.856	–84.856	–5.772
rc_AA891302	EST195105	16.606	–16.606	–3.758
rc_AA891311	EST195114	15.308	–15.308	–3.581
rc_AA891595	EST195398	–36.196	36.196	5.339

rc_AA891666	EST195469	104.517	–104.517	–4.073
rc_AA891729	EST195532	500.083	–500.083	–5.682
rc_AA891740	EST195543	–73.5	73.5	4.687
rc_AA891742	EST195545	–38.715	38.715	5.191
rc_AA891785	EST195588	–4.871	4.871	0.285
rc_AA891800	EST195603	–7.783	7.783	0.717
rc_AA891810	EST195613	–125.629	125.629	3.795
rc_AA891818	EST195621	–69.681	69.681	4.168
rc_AA891890	EST195693	–26.223	26.223	4.413
rc_AA891911	EST195714	–23.404	23.404	3.78
rc_AA891920	EST195723	15.777	–15.777	–4.464
rc_AA891949	EST195752	40.694	–40.694	–3.814
rc_AA892012	EST195815	–62.613	62.613	3.945
rc_AA892297	EST196100	50.64	–50.64	–4.875
rc_AA892310	EST196113	–81.129	81.129	3.673
rc_AA892325	EST196128	26.917	–26.917	–3.534
rc_AA892339	EST196142	20.683	–20.683	–3.828
rc_AA892376	EST196179	81.869	–81.869	–3.651
rc_AA892378	EST196181	168.796	–168.796	–6.312
rc_AA892570	EST196373	–108.971	108.971	4.349
rc_AA892582	EST196385	243.565	–243.565	–3.642
rc_AA892798	EST196601	–26.538	26.538	4.344
rc_AA892801	EST196604	–590.127	590.127	5.166
rc_AA892801	EST196604	–590.127	590.127	5.166
rc_AA892813	EST196616	14.998	–14.998	–4.039
rc_AA892851	EST196654	35.777	–35.777	–4.049
rc_AA892895	EST196698	–341.698	341.698	3.614
rc_AA892895	EST196698	–341.698	341.698	3.614
rc_AA892918	EST196721	78.99	–78.99	–3.957
rc_AA893043	EST196846	11.035	–11.035	–4.26
rc_AA893172	EST196975	46.271	–46.271	–7.257
rc_AA893206	EST197009	–37.717	37.717	4.722
rc_AA893857	EST197660	–19.871	19.871	5.195
rc_AA893870	EST197673	34.294	–34.294	–5.568
rc_AA894119	EST197922	–36.665	36.665	3.785
rc_AA894148	EST197951	25.363	–25.363	–3.968
rc_AA894317	EST198120	–198.2	198.2	3.715
rc_AA899106	UI-R-E0-cw-d-04-0-UI.s1	137.733	–137.733	–5.317
rc_AA924925	UI-R-A1-eg-d-06-0-UI.s1	229.085	–229.085	–10.088
rc_AA925495	UI-R-A1-ep-c-04-0-UI.s1	11.731	–11.731	–3.525
rc_AA925506	UI-R-A1-ep-d-03-0-UI.s1	–79.448	79.448	5.077
rc_AA925762	UI-R-A1-ep-g-08-0-UI.s1	33.254	–33.254	–3.873

rc_AA925887	UI-R-A1-eo-h-06-0-UI.s1	–20.552	20.552	4.13
rc_AA946040	EST201539	139.458	–139.458	–5.187
rc_AA946313	EST201812	119.421	–119.421	–4.632
rc_AA946439	EST201938	–50.002	50.002	8.464
rc_AA956930	UI-R-E1-fl-a-11-0-UI.s1	–29.996	29.996	3.626
rc_AA956941	UI-R-E1-fl-c-10-0-UI.s1	35.215	–35.215	–4.96
rc_AA957777	UI-R-E1-fv-f-08-0-UI.s1	98.269	–98.269	–5.608
rc_AA957961	UI-R-E1-fz-g-08-0-UI.s1	–51.963	51.963	6.959
rc_AA963674	UI-R-E1-gg-h-01-0-UI.s1	–617.158	617.158	3.626
rc_AA963682	UI-R-E1-gg-h-11-0-UI.s1	28.265	–28.265	–5.223
rc_AA996484	UI-R-C0-hi-h-10-0-UI.s1	–65.765	65.765	3.628
rc_AA997367	UI-R-C0-hl-d-02-0-UI.s1	–31.423	31.423	4.105
rc_AA998683	UI-R-C0-ig-h-06-0-UI.s1	–103.6	103.6	1.671
rc_AI008852	EST203303	47.133	–47.133	–5.554
rc_AI012805	EST207256	300.475	–300.475	–6.671
rc_AI013472	EST208147	111.158	–111.158	–6.1
rc_AI013627	EST208302	155.006	–155.006	–4.062
rc_AI014087	EST207642	256.046	–256.046	–4.601
rc_AI029183	UI-R-C0-iv-h-08-0-UI.s1	–39.973	39.973	3.938
rc_AI044508	UI-R-C1-kc-a-07-0-UI.s1	–114.175	114.175	7.203
rc_AI044517	UI-R-C1-kc-b-10-0-UI.s1	–55.152	55.152	6.194
rc_AI044716	UI-R-C1-ki-a-09-0-UI.s1	36.3	–36.3	–3.858
rc_AI058393	UI-R-C1-kx-c-12-0-UI.s1	–123.498	123.498	3.79
rc_AI058601	UI-R-C1-kv-h-10-0-UI.s1	–44.135	44.135	3.681
rc_AI070521	UI-R-Y0-lv-f-09-0-UI.s1	100.425	–100.425	–6.923
rc_AI071507	UI-R-C2-nc-g-02-0-UI.s1	–19.15	19.15	3.735
rc_AI072089	UI-R-C2-nf-d-09-0-UI.s1	42.902	–42.902	–5.489
rc_AI073164	UI-R-Y0-mi-e-03-0-UI.s1	–87.256	87.256	3.521
rc_AI101103	EST210392	–484.602	484.602	4.081
rc_AI103236	EST212525	142.031	–142.031	–4.016
rc_AI104012	EST213301	24.904	–24.904	–3.634
rc_AI104035	EST213324	533.519	–533.519	–5.991
rc_AI104399	EST213688	487.592	–487.592	–3.652
rc_AI104500	EST213789	–31.413	31.413	3.467
rc_AI104513	EST213802	25.035	–25.035	–4.046
rc_AI105076	EST214365	–48.475	48.475	5.096
rc_AI105463	EST214752	–38.646	38.646	3.836
rc_AI137862	UI-R-C0-ik-g-07-0-UI.s1	–205.906	205.906	3.962
rc_AI145044	UI-R-BT0-pt-a-03-0-UI.s1	174.692	–174.692	–4.792
rc_AI145444	UI-R-BT0-pv-c-12-0-UI.s1	–15.123	15.123	4.733
rc_AI145494	UI-R-BT0-qf-f-12-0-UI.s1	–77.698	77.698	6.099
rc_AI170268	EST216194	198.048	–198.048	–5.21

rc_AI170613	EST216547	193.254	–193.254	–5.073
rc_AI171844	EST217831	269.933	–269.933	–4.5
rc_AI172097	EST218092	34.271	–34.271	–3.602
rc_AI172162	EST218157	262.413	–262.412	–5.996
rc_AI175900	EST219472	–81.635	81.635	4.146
rc_AI176460	EST220045	24.296	–24.296	–4.874
rc_AI177096	EST220703	62.14	–62.14	–4.168
rc_AI179399	EST223101	16.315	–16.315	–3.738
rc_AI228738	EST225433	–394.217	394.217	3.809
rc_AI228850	EST225545	–101.344	101.344	4.271
rc_AI229497	EST226192	144.385	–144.385	–4.771
rc_AI230572	EST227267	12.604	–12.604	–4.771
rc_AI231213	EST227901	126.613	–126.613	–8.349
rc_AI231354	EST228042	–107.173	107.173	6.339
rc_AI231445	EST228133	–79.029	79.029	6.748
rc_AI232096	EST228784	32.133	–32.133	–3.686
rc_AI232194	EST228882	–25.577	25.577	3.554
rc_AI232256	EST228944	–26.481	26.481	3.644
rc_AI236721	EST233283	–13.515	13.515	3.849
rc_AI237576	EST234138	–38.842	38.842	6.849
rx02409 3	sequence []	–22.883	22.883	3.879
rx01268 3	sequence []	–207.946	207.946	7.748
rx04826 3	sequence []	–272.36	272.36	3.438
rx04485 3	sequence []	38.325	–38.325	–5.887
rx00364 3	sequence []	–148.348	148.348	3.869
rx05007 3	sequence []	–13.146	13.146	3.541
rx04757 3	sequence []	–26.315	26.315	4.264
rx02055 3	sequence []	33.294	–33.294	–3.614
rx01635 3	sequence []	–31.179	31.179	3.608
rx01030 3	sequence []	28.467	–28.467	–4.879
rc_H32977	EST108553	85.158	–85.158	–4.815
rc_H33426	EST109414	34.346	–34.346	–4.422

Environmental effects for stroke in rat brain

Gene ID	Expressed sequence tag	Low salt	High salt	T-value
rc_AA892817	EST196620	46.233	–46.233	–3.958
rc_AA875263	UI-R-E0-ce-a-08-0-UI.s1	73.427	–73.427	–4.096
rc_AI105374	EST214663	47.902	–47.902	–4.26
rc_AA891998	EST195801	32.26	–32.26	–4.755
rc_AA858621	UI-R-E0-bq-b-10-0-UI.s1	–330.613	330.613	3.971
rc_AA893939	EST197742	–77.875	77.875	4.004
rc_AA799340	EST188837	–215.35	215.35	4.019
rc_AI176456	EST220041	–366.979	366.979	4.028

rc_AA859627	UI-R-E0-bs-h-03-0-UI.s1	–15.579	15.579	4.267
rc_AA893065	EST196868	–246.806	246.806	5.056
rz00769 3	Unknown	–29.769	29.769	5.909
rx01287 3	Unknown	122.506	–122.506	–4.01
rx02839 3	Unknown	21.352	–21.352	–4.19
rx01185 3	Unknown	–15.431	15.431	4.56
Genetic-Environmental effects for stroke in rat brain				
Gene ID	Expressed sequence tag	LSSP,HSSR	LSSR,HSSP	T-value
rc_AA859869	UI-R-E0-cc-b-08-0-UI.s1	–81.396	81.396	3.759
rc_AA858621	UI-R-E0-bq-b-10-0-UI.s1	–697.848	697.848	3.977
rc_AA875659	*UI-R-E0-ct-h-07-0-UI.s1*	–218.225	218.225	3.903
rc_AA800029	EST189526	–239.983	239.983	4.523
rc_AA891069	EST194872	–182.685	182.685	3.97
rc_H31692	EST106007	–93.073	93.073	3.878
rc_AI013194	EST207869	–271.792	271.792	3.885
rc_H31610	EST105814	–319.533	319.533	3.897
rc_AA894321	EST198124	–36.919	36.919	4.272
rc_AA799607	EST189104	–96.388	96.388	3.988
rc_AA893853	EST197656	–67.677	67.677	3.913
rx05078 3	unknown	–305.14	305.14	4.368
rx01187 3	unknown	–305.14	305.14	3.879
rx04752 3	unknown	23.421	–23.421	–5.32

Appendix A

$$T(G_g)=\frac{\bar{x}(G_g^+)-\bar{x}(G_g^-)}{\sqrt{A(G_g)+\dfrac{\sigma^2(G_g^+)}{n(G_g^+)}+\dfrac{\sigma^2(G_g^-)}{n(G_g^-)}}}=\frac{D(G_g)}{\delta(G_g)}, \quad \text{(A1)}$$

$$T(E_g)=\frac{\bar{x}(E_g^+)-\bar{x}(E_g^-)}{\sqrt{A(E_g)+\dfrac{\sigma^2(E_g^+)}{n(E_g^+)}+\dfrac{\sigma^2(E_g^-)}{n(E_g^-)}}}=\frac{D(E_g)}{\delta(E_g)}, \quad \text{(A2)}$$

$$T(\mathrm{I}_g)=\frac{\bar{x}(G_g^1E_g^1)-\bar{x}(G_g^2E_g^2)}{\sqrt{A(\mathrm{I}_g)+\dfrac{\sigma^2(G_g^1E_g^1)}{n(G_g^1E_{E_g}^1)}+\dfrac{\sigma^2(G_g^2E_g^2)}{n(G_g^2E_g^2)}}}=\frac{D(\mathrm{I}_g)}{\delta(\mathrm{I}_g)} \quad \text{(A3)}$$

in the case of two unequal sample variances or

$$T(G_g)=\frac{\bar{x}(G_g^+)-\bar{x}(G_g^-)}{\sqrt{A(G_g)+\dfrac{[n(G_g^+)-1]\sigma^2(G_g^+)+[n(G_g^-)-1]\sigma^2(G_g^-)}{n(G_g^+)+n(G_g^-)-2}\left(\dfrac{1}{n(G_g^+)}+\dfrac{1}{n(G_g^-)}\right)}}=\frac{D(G_g)}{\delta(G_g)} \quad \text{(A4)}$$

$$T(E_g)=\frac{\bar{x}(E_g^+)-\bar{x}(E_g^-)}{\sqrt{A(E_g)+\dfrac{[n(E_g^+)-1]\sigma^2(E_g^+)+[n(E_g^-)-1]\sigma^2(E_g^-)}{n(E_g^+)+n(E_g^-)-2}\left(\dfrac{1}{n(E_g^+)}+\dfrac{1}{n(E_g^-)}\right)}}=\frac{D(E_g)}{\delta(E_g)} \quad \text{(A5)}$$

$$T(\mathrm{I}_g)=\frac{\bar{x}(G_g^1E_g^1)-\bar{x}(G_g^2E_g^2)}{\sqrt{A(I_g)+\dfrac{[n(G_g^1E_g^1)-1]\sigma^2(G_g^1E_g^1)+[n(G_g^2E_g^2)-1]\sigma^2(G_g^2E_g^2)}{n(G_g^1E_g^1)+n(G_g^2E_g^2)-2}\left[\dfrac{1}{n(G_g^1E_g^1)}+\dfrac{1}{n(G_g^2E_g^2)}\right]}}=\frac{D(\mathrm{I}_g)}{\delta(\mathrm{I}_g)} \quad \text{(A6)}$$

in the case of two equal sample variances but unequal sample sizes where $D(G_g)=\bar{x}(G_g^+)-\bar{x}(G_g^-)$, $D(E_g)=\bar{x}(E_g^+)-\bar{x}(E_g^-)$, $D(\mathrm{I}_g)=\bar{x}(G_g^1E_g^1)-\bar{x}(G_g^2E_g^2)$ where $(G_g^1E_g^1)=(G_g^+E_g^+,G_g^-E_g^-)$ and $(G_g^2E_g^2)=(G_g^+E_g^-,G_g^-E_g^+)$. Note that $n(G_g^+)=n(G_g^-)=n(E_g^+)=n(E_g^-)=n(G_g^1E_g^1)=n(G_g^2E_g^2)=n$ if there are not missing data in microarray dataset. For $C_g=G_g$, E_g or I_g, $A(c_g)$ is defined as

$$A(C_g)=\begin{cases}1, & \text{if } D(C_g)>\sigma(C_g)<1\\ 0 & \text{otherwise}\end{cases} \quad \text{(A7)}$$

