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Testing the silence of mutations: Transcriptomic and behavioral studies of GABA_A receptor $\alpha 1$ and $\alpha 2$ subunit knock-in mice

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Abstract

Knock-in mice were constructed with mutations in the $\alpha 1$ (H₂₇₀, A₂₇₇) and $\alpha 2$ (H₂₇₀, A₂₇₇) subunits of the GABA_A receptor, which resulted in receptors that lacked modulation by ethanol but retained normal responses to GABA *in vitro*. A key question is whether these mutant receptors also function normally *in vivo*. Perturbation of brain function was evaluated by gene expression profiling in the cerebral cortex and by behavioral pharmacology experiments with GABAergic drugs. Analysis of individual transcripts found only six transcripts that were changed in $\alpha 1$ knock-in mice and three in the $\alpha 2$ mutants ($p < 0.05$, corrected for multiple comparisons). Two transcripts that are sensitive to neuronal activity, Arc and Fos, increased about 250% in the $\alpha 2$ mutants, and about 50% in the $\alpha 1$ mutants. Behavioral effects (loss of righting reflex, rotarod) of flurazepam and pentobarbital were not different between $\alpha 2$ mutants and wild-type, but they were enhanced for $\alpha 1$ knock-in mice. These results indicate that introduction of these mutations in the $\alpha 2$ subunit of the GABA_A receptor does not produce marked perturbation of brain function, as measured by gene expression and GABAergic behavioral responses, but the same mutations in the $\alpha 1$ subunit produce more pronounced changes, especially in GABAergic function.

Keywords

GABA_A receptor; alpha1 subunit; alpha2 subunit; microarray; knock-in mutants; flurazepam; pentobarbital; gabaxadol

INTRODUCTION

Null mutant mice have contributed much to our understanding of the role of specific brain genes in behavior and drug actions. This is particularly true for actions of alcohol because the importance of each of the multiple sites of alcohol action for its multiple behaviors can only be evaluated by gene manipulation [6,9]. However, a significant shortcoming of null mutant mice is that extensive compensation may occur in response to the deletion, and the compensation may occur with genes that are distinct from the deletion. For example, deletion of the GABA_A receptor $\alpha 6$ subunit in cerebellar granule cells results in increased

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potassium channel (TASK-1) activity that allows normal functioning of these cells despite the loss or GABAergic inhibition [5]. Because it is likely impossible to predict the changes that the brain may employ to compensate for gene deletion, an unbiased method such as global evaluation of gene expression is most likely to be successful in detecting compensation. Microarray platforms allow simultaneous and accurate measurement of levels of most mRNAs (the transcriptome), and we used this approach to test for transcriptome changes in null mutant mice lacking the $\alpha 1$ subunit of the GABAA receptor and found that deletion of this subunit altered the expression of 326 genes in the cortex of these mice [15]. We recently characterized mutations in the $\alpha 2$ subunit of the GABAA receptor that reduce alcohol and anesthetic modulation of this receptor, introduced these mutations into mice, and used these knock-in mice for behavioral pharmacology [2,18]. One assumption underlying construction and use of knock-in mice is that they will display either no or limited compensation as compared with knock-out mice, which is an advantage over null mutants [2,18]. However, we are not aware of any study that has compared knock-in and knock-out mice for changes in a global measure of brain function, although one study presented gene expression in GABAA receptor knock-in mice after injection of diazepam [12] and another noted memory deficits in GABAA $\alpha 5$ (H101R) knock-in mice due to decreased expression of the $\alpha 5$ subunit [7]. Because of the numerous changes in gene expression detected in our earlier study of $\alpha 1$ null mutant mice [15], the $\alpha 1$ knock-in mice produced by Borghese et al. [4] provide an ideal opportunity to test the hypothesis that the knock-in will be silent in comparison with the null. Our recent studies of $\alpha 2$ knock-in mice [2,18] made studies of compensation important for this additional knock-in.

The goal of this study was to evaluate compensation in GABAA $\alpha 1$ and $\alpha 2$ knock-in mice by measurement of global gene expression in cortex followed by behavioral evaluation of selected GABAergic drugs.

MATERIALS AND METHODS

Animals

Knock-in (homozygous for histidine at 270 and alanine at 277) for $\alpha 1$ and $\alpha 2$ subunits of GABAA-R were generated as described earlier [4,18]. Wild-type (homozygous for serine at 270 and leucine at 277; referred to as SL/SL) and knockin (homozygous for histidine at 270 and alanine at 277; referred to as HA/HA) mice used for these experiments were produced from heterozygous (SL/HA) breeding pairs and maintained on the original mixed genetic background (C57Bl/6J x 129SvJ). Mutant mice were viable and fertile, and no cases of lethality were observed. All mice were genotyped by polymerase chain reaction as was previously described [2,4]. Southern blot analysis of tail DNA was performed as described previously [18].

After weaning, mice were housed in the conventional facility at the University of Texas with *ad libitum* access to rodent chow and water with 12-h light/dark cycles (lights on at 7:00 AM). All mice were between 8 and 12 weeks of age for behavioral and microarray experiments. Littermate wild-type and mutant male mice were used for microarray analyses and for behavioral experiments. Each mouse was used for only one experiment. All experiments were approved by IACUCs and were conducted in accordance with NIH guidelines with regard to the use of animals in research.

RNA Preparation, Microarray, and qRT-PCR Analysis

The cerebral cortex (the entire cortex including striatum, but without hippocampus) was dissected and flash-frozen in liquid N₂ then stored at -80°C . Total RNA was isolated from the cerebral cortex from individual animals (four mice were used for each of the four

groups, giving 16 samples) using the *mirVana* PARIS kit (Ambion/Applied Biosystems, Austin, Texas). After DNase treatment (TURBO DNA-free ; Ambion), RNA concentration and purity were determined by UV spectrometry (Nanodrop; Thermo Scientific, Wilmington, DE) while overall RNA integrity was assessed using a bioanalyzer (Agilent Technologies, Foster City, CA). Aliquots of total RNA were sent to the Keck Microarray Facility (New Haven, CT), where mRNA was amplified and biotin-labeled using the Illumina® Total Prep –96 RNA amplification kit (Ambion). Amplified/labeled RNAs were hybridized to Illumina® MouseRef-8 v2 Expression BeadChips (Illumina, Inc., San Diego, CA) according to manufacturer protocols. Transcript abundance was measured by fluorescent intensity after scanning. A subset of the microarray results was verified using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Genes of interest were selected based on fold change in the microarray experiments and availability of pre-designed assays. Reverse transcriptase reactions were performed on total RNA using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor from Applied Biosystems (Foster City, CA), and control reactions without the RT enzyme were included. Gene expression was measured with qPCR using TaqMan Gene Expression Assays from Applied Biosystems. For both SHLA $\alpha 1$ and SHLA $\alpha 2$, *arc*, *fos*, and *gabaprl2* were assayed along with the endogenous control *gusb*. RNA samples were tested for the presence of genomic DNA by comparing qPCR results from the *gabaprl2* gene expression assay for both RT+ and RT- reactions. The viability of *gusb* as an endogenous control was then verified using all cDNA samples at a concentration 10 ng/ul, and a standard curve was performed for each assay using five dilutions over a 2-log range. Finally, qPCR was performed on all samples with all assays, and the results were analyzed using RQ Manager and DataAssist software from Applied Biosystems. The data were normalized with respect to *gusb*, and delta Ct was calculated. Detailed protocols can be found at <http://www.appliedbiosystems.com/index.cfm>.

Loss of Righting Reflex (LORR)

Sensitivity to flurazepam (225 mg/kg) and pentobarbital (45 mg/kg) was determined using the standard duration of LORR assay in both colonies of mutant mice [17].

Rotarod

Mice were trained on a fixed-speed rotarod (Economet; Columbus Instruments, Columbus, OH; speed of rod, 5.0 rpm), and training was considered complete when mice were able to remain on the rotarod for 60 s. Every 15 min after injection of flurazepam (35 mg/kg) or 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP) (10 mg/kg) each mouse was placed back on the rotarod, and latency to fall was measured until the mouse was able to stay on the rotarod for 60 s.

Drugs

Flurazepam hydrochloride (Sigma-Aldrich, St. Louis, MO) and 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP hydrochloride or gabaxadol, Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline and injected i.p. at a volume of 0.1 ml/10g body weight.

STATISTICAL ANALYSIS

Only genes with a detection p value ≤ 0.05 and a presence on 6 of the 8 arrays used for each group (i.e., $\alpha 1$ wild-types and mutants were one group, $\alpha 2$ wild-types and mutants were the second group) were utilized in the analysis of each tissue data set. Variance stabilization transformation [13] and quantile normalization [3] were used to pre-process the data in Lumi [8]. Expression value outliers were removed using Grubbs' test with a critical value of 1.48.

This resulted in removal of about 1.4% of the data points. Limma [16] was used to fit a linear model for each gene and detect differentially expressed genes by an empirical Bayes method, using a moderated *t* statistic followed by correction for multiple comparisons by the method of Benjamini and Hochberg [1].

Behavioral data are reported as the mean $\pm$ S.E.M. The statistics software program GraphPad Prism (Jandel Scientific, Costa Madre, CA) was used. Analysis of variance (two-way ANOVA) and Student's *t*-test were carried out to evaluate differences between groups.

RESULTS

Gene Expression Comparisons

The Illumina arrays used for these studies have a total of 25,697 oligonucleotide probes; for the $\alpha 1$ arrays, 10,657 probes met our criteria for reliable detection, and 10,855 probes met criteria for the $\alpha 2$ arrays. Expression levels of each gene were compared for wild-type and mutant for the $\alpha 1$ group and for the $\alpha 2$ group, giving uncorrected *p* values and *p* values corrected for multiple comparisons as well as fold change for each gene. These values are given for the 20 most statistically significant genes for $\alpha 1$ (Table 1) and for $\alpha 2$ (Table 2), and all genes with an uncorrected *p* value of <0.05 are given in supplementary Tables 1 and 2. Targeted mutations in GABAA $\alpha 1$ and GABAA $\alpha 2$ resulted in differential expression of 962 and 556 transcripts, respectively, at the uncorrected *p* value of <0.05 . To examine the effects of the genetic mutations on global gene expression, we compared these values with the estimated 5% chance of all expressed transcripts. The number of significantly regulated genes in $\alpha 1$ KI mice significantly exceeded the chance level (chi square = 132, $p < 2E-30$), while the number of significantly regulated genes in $\alpha 2$ KI animals did not (chi square = 0.14, $p = 0.71$), suggesting differential effects of the two mutations on cortical transcriptome. Using a level of significance of $p < 0.05$ (corrected for multiple comparisons), there are only six genes significantly changed in $\alpha 1$ and only three for $\alpha 2$. For the $\alpha 1$ mutants, it is interesting that two GABA-related genes, the gamma subunit and the GABARAP accessory protein of the GABAA receptor are number 7 and 18 in the list, and that expression of both of these genes is increased in mutants compared with wild-type (Table 1). In addition, two genes coding for other GABAA receptor subunits, $\alpha 3$ and delta, are changed at the level of $p < 0.05$ for an uncorrected *p* value (supplementary Table 1). The $\alpha 2$ mutants show regulation of two activity-dependent transcription factors, Arc and Fos, in the top three genes. The level of expression of these two genes was further evaluated by quantitative RT-PCR. For SHLA $\alpha 2$, the increase in Arc was 279% of control ($p < 0.05$) for RT-PCR compared with 250% for the array data, and for Fos the change was 235% ($p < 0.05$) for RT-PCR and 179% for the array. We also checked these two transcripts in SHLA $\alpha 1$ and found that Arc was 157% ($p < 0.05$) and Fos was 154% ($p < 0.05$) of control (compared with 146% and 130% of control by array analysis). Thus, RT-PCR confirmed the large increases in these two transcripts in the $\alpha 2$ samples and also detected smaller, but statistically significant, changes in the $\alpha 1$ samples. We also evaluated Gabarap by RT-PCR and found that this transcript was unchanged in the $\alpha 2$ samples (101% of control) and 140% of control ($p = 0.07$) in the $\alpha 1$ samples.

Sedative/Hypnotic and Motor Ataxic Effects of Drugs

The gene expression results indicate that the mutations introduced into the mice are relatively silent, but the $\alpha 1$ mutation may produce changes in function of synaptic GABAA receptors (which contain the gamma subunit). To test this idea, we studied the behavioral effects of three GABAergic drugs: gabaxadol, an activator (at low concentrations) of extrasynaptic GABAA receptors; flurazepam, a positive allosteric modulator of synaptic GABAA receptors; and pentobarbital, a non-selective GABAergic positive modulator

[10,11] in wild-type and mutant mice. We first compared the ataxic effects of gabaxadol, and we found that acute administration of gabaxadol (10 mg/kg) produced motor ataxia, as measured by the rotarod test, in wild-type and knock-in mice for the $\alpha 1$ as well as the $\alpha 2$ subunits of GABAA-R (Fig. 1a, b). There were no differences between wild-type and mutant mice in recovery from this motor incoordination [$F(8,81) = 14$; $p < 0.001$ dependence on time; for $\alpha 1$ knock-in mice; $F(8,126) = 12$; $p < 0.001$ dependence on time; $F(8,126) = 0.4$; for $\alpha 2$ knock-in mice]. We next tested the ataxic effects of flurazepam. The $\alpha 1$ knock-in mice display a pronounced impairment of rotarod coordination after flurazepam, and recovery from this ataxia was extremely slow compared with wild-type mice [$F(1,90) = 186$; $p < 0.001$ – dependence on genotype; $F(8,90) = 26$; $p < 0.001$ dependence on time; $F(8,90) = 12$; $p < 0.001$ – genotype x time interaction] (Fig. 1c). However, the slow recovery appears not to be solely as a result of sedation. When placed on the rotarod, the $\alpha 1$ knock-in mice demonstrated a strong and persistent body tremor, which interfered with their ability to remain on the rotarod. In contrast, there were no differences in recovery from ataxic effect of flurazepam (35 mg/kg) between wild-type and $\alpha 2$ knock-in mice [$F(9,100) = 25$; $p < 0.001$ dependence on time] (Fig. 1d).

Next, we measured the latency for LORR and duration of LORR for flurazepam and pentobarbital (Fig. 2). The $\alpha 1$ knock-in mice showed increased duration of LORR after administration of flurazepam as well as pentobarbital (Fig. 2a, c). In addition, the latencies for LORR induced by both drugs were reduced for $\alpha 1$ knock-in mice in comparison with wild-type (Fig. 2b, d). However, there were no differences between wild-type and $\alpha 2$ knock-in mice in either duration of LORR (Fig. 2e, i) or latency for LORR (Fig. 10f, g) after administration of flurazepam or pentobarbital.

DISCUSSION

We evaluated possible perturbation of brain function by the introduction of two mutations into the $\alpha 2$ subunit of the GABAA receptor [2,18]. We also included studies of knock-in mice with the same mutations in the $\alpha 1$ subunit, which was previously characterized [4,17]. These mutations in the $\alpha 1$ subunit result in faster deactivation of the receptor, changes in levels of subunit proteins and hyperactivity in the mutant mice, indicating that they are not completely “silent” with respect to GABAergic neurotransmission. We found that complete deletion of the $\alpha 1$ subunit in mice markedly changed brain gene expression [15], and it was of interest to compare the $\alpha 1$ knock-in and knock-out mice.

The $\alpha 2$ knock-in mice showed only three genes with altered expression in the cortex at a level of significance of $p < 0.05$, after correction for multiple comparisons. Arc and Fos showed more than two-fold increases in expression, and we confirmed these changes by quantitative PCR. Using pharmacological probes, the ataxic effects of gabaxadol and flurazepam and the effects of pentobarbital and flurazepam on duration of loss of righting reflex in the $\alpha 2$ knock-in mice were not different from wild-type, which suggests an absence of compensatory changes in GABAergic systems. However, the possibility cannot be completely ruled out that by combining tissue from a large brain area such as a cortex small changes in expression - for example in striatum, where $\alpha 2$ is heavily expressed - might be obscured.

In contrast, the $\alpha 1$ knock-in mice showed changes at both the genomic and behavioral levels that indicate a perturbation of brain GABAergic signaling. Specifically, the level of expression of six genes was changed at $p < 0.05$ (corrected for multiple comparisons). Borghese et al. [4] measured the protein levels of six GABAR subunits in the cortex of wild-type and knock-in mice and found the following (given as percent change from wild-type): $\alpha 1$: -48; $\alpha 2$: 34; $\alpha 3$: 44; $\beta 2$: 31; $\beta 3$: -35; and $\gamma 2$: 37 (all were statistically significant except

for $\alpha 2$). In the present study, we found increased behavioral actions of flurazepam and pentobarbital in the $\alpha 1$ knock-in mice, which is indicative of increased synaptic GABAergic inhibition. This is consistent with the increased levels of $\alpha 2$, $\alpha 3$, $\beta 2$, and $\gamma 2$ subunit protein in the cortex of these mice. In addition, receptors with the $\alpha 2$ subunit display slower kinetics of inactivation than those with the $\alpha 1$ subunit, so increased $\alpha 2$ relative to $\alpha 1$ will increase GABAergic inhibition [14]. Although there are increased behavioral effects of flurazepam, together with the increased levels of subunit proteins, the amount of cortical [3H]-flunitrazepam binding was not different between wild-type and $\alpha 1$ knock-in mice [4]. However, these mice show a reduction of the $\alpha 1$ subunit, and this may balance the increase in other subunits, resulting in no net change in receptor density, but producing receptors with altered subunit composition and kinetic properties.

In summary, this study provides the first transcriptomic analysis of knock-in mice related to ethanol effects and allows us to evaluate the “silence” of the $\alpha 1$ (H₂₇₀, A₂₇₇) and $\alpha 2$ (H₂₇₀, A₂₇₇) mutant subunits of the GABAA receptor using both gene expression and targeted behavioral pharmacology. We found that the $\alpha 2$ knock-in has relatively few differences from wild-type, whereas the same mutations in the $\alpha 1$ subunit produced changes in brain signaling, particularly in the GABAergic system.

Conclusions

The introduction of ethanol-resistant mutations (H₂₇₀, A₂₇₇) in the $\alpha 2$ subunit of the GABAA receptor does not produce marked perturbation of brain function, as measured by gene expression and GABAergic behavioral responses, but the same mutations in the $\alpha 1$ subunit produce more pronounced changes, especially in GABAergic function.

Research highlights

- Ethanol-resistant mutations (H₂₇₀, A₂₇₇) in the $\alpha 1$ subunit of the GABAA-R
- Changes in brain gene expression, including the $\gamma 2$ subunit of GABAA-R
- Modified responses to drugs selective for γ -containing GABAA-R in $\alpha 1$ knockin mice
- This was not seen in $\alpha 2$ knockin mice

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Effects of flurazepam or gabaxadol on motor incoordination in $\alpha 1$ and $\alpha 2$ knock-in mice

Panels A, B. Recovery from motor incoordination measured by time on rotarod before and after gabaxadol (10 mg/kg) in $\alpha 1$ knock-in mice (panel A) and $\alpha 2$ knock-in mice (panel B) ($n = 6-8$ per genotype). Panels C, D. Recovery from motor incoordination measured by time on rotarod before and after flurazepam at 35 g/kg in $\alpha 1$ knock-in mice (panel C) and $\alpha 2$ knock-in mice (panel D) ($n = 6$ per genotype). Values represent mean $\pm$ SEM.

Fig. 2. The duration of loss of righting reflex (LORR) produced by flurazepam or pentobarbital in $\alpha 1$ and $\alpha 2$ knock-in mice

Panel A, E. Duration of LORR (min) following injection of pentobarbital (45 mg/kg) in $\alpha 1$ -knock-in mice (panel A) and $\alpha 2$ -knock-in mice (panel E) ($n = 5-10$ per genotype). Panel B, F. Latency (min) following injection of pentobarbital (45 mg/kg) in $\alpha 1$ knock-in mice (panel B) and $\alpha 2$ knock-in mice (panel F). Panel C, I. Duration of LORR (min) following injection of flurazepam (225 mg/kg) in $\alpha 1$ knock-in mice (panel C) and $\alpha 2$ knock-in mice (panel I) ($n = 6-11$ per genotype). Panel D, G. Latency (min) following injection of pentobarbital flurazepam (225 mg/kg) in $\alpha 1$ knock-in mice (panel D) and $\alpha 2$ knock-in mice (panel G). * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$ – Statistical differences between wild-type and mutant mice (t-Student's test). Values represent mean $\pm$ SEM.

Table 1

Microarray Analysis of $\alpha 1$ Knock-in Mice: The 20 genes With the Most Significant Changes in Expression (Ranked by P Value)

Gene Symbol	Gene Name	Percent of Control	P Value	Adjusted P Value
Mrpl55	mitochondrial ribosomal protein L55	74	6.8E-07	7.3E-03
Acsl6	acyl-CoA synthetase long-chain family member 6	73	4.4E-06	1.6E-02
Armc9	armadillo repeat containing 9	74	5.9E-06	1.6E-02
Tmem160	transmembrane protein 160	77	6.2E-06	1.6E-02
Hspa8	heat shock protein 8	143	1.0E-05	2.2E-02
Per2	period homolog 2 (Drosophila)	130	2.8E-05	4.9E-02
Gabrg2	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2	120	3.6E-05	5.4E-02
Pfkip	phosphofructokinase, platelet	130	6.3E-05	8.4E-02
Arl6ip5	ADP-ribosylation factor-like 6 interacting protein 5	123	8.9E-05	9.3E-02
Tac1	tachykinin 1	128	9.5E-05	9.3E-02
Rab6	RAB6, member RAS oncogene family	127	9.6E-05	9.3E-02
Atp6ap2	ATPase, H ⁺ transporting, lysosomal accessory protein 2	127	1.2E-04	1.4E-01
Dyn11	dynamitin 1-like	119	1.3E-04	1.4E-01
Nol4	nucleolar protein 4	120	1.7E-04	1.4E-01
Pik3r1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	85	2.1E-04	1.5E-01
Tspan3	tetraspanin 3	115	3.4E-04	1.9E-01
Atp6v0d1	ATPase, H ⁺ transporting, lysosomal V0 subunit D1	125	3.6E-04	1.9E-01
Gabarapl2	GABA-A receptor-associated protein-like 2	127	3.7E-04	1.9E-01
Tnfrsf25	tumor necrosis factor receptor superfamily, member 25	119	3.8E-04	1.9E-01
Zfp39	zinc finger protein 39	82	3.9E-04	1.9E-01

Table 2

Microarray Analysis of $\alpha 2$ Knockin Mice: The 20 Genes With the Most Significant Changes in Expression (Ranked by P Value)

Gene Symbol	Gene Name	Percent of Control	P Value	Adjusted P Value
Dusp1	dual specificity phosphatase 1	172	7.03E-07	7.64E-03
Arc	activity regulated cytoskeletal-associated protein	250	7.49E-06	4.06E-02
Fos	FBJ osteosarcoma oncogene	179	1.15E-05	4.17E-02
Sfrs16	splicing factor, arginine/serine-rich 16	69	1.63E-04	4.42E-01
Aco2	aconitase 2, mitochondrial	85	2.58E-04	5.27E-01
Slc38a2	solute carrier family 38, member 2	125	3.47E-04	5.27E-01
Ociad1	OClA domain containing 1	77	3.62E-04	5.27E-01
Csrnp1	cysteine-serine-rich nuclear protein 1	139	3.88E-04	5.27E-01
Bsdc1	BSD domain containing 1	119	6.94E-04	8.05E-01
Cldn5	claudin 5	78	8.22E-04	8.05E-01
Junb	Jun-B oncogene	135	8.72E-04	8.05E-01
Mocs1	molybdenum cofactor synthesis 1	119	8.90E-04	8.05E-01
Zdhhc3	zinc finger, DHHC domain containing 3	85	9.99E-04	8.30E-01
4833439L19Rik	4833439L19Rik	85	1.07E-03	8.30E-01
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	125	1.44E-03	9.42E-01
Crhbp	corticotropin releasing hormone binding protein	119	1.46E-03	9.42E-01
Pknd	paroxysmal nonkinesiolgenic dyskinesia	115	1.55E-03	9.42E-01
Nfkbid	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	114	1.77E-03	9.42E-01
Ly86	lymphocyte antigen 86	86	1.78E-03	9.42E-01
Sox18	SRY-box containing gene 18	84	1.88E-03	9.42E-01