

Altered Gamma-Aminobutyric Acid Type B Receptor Subunit 1 Receptor Splicing In Alcoholics

Supplemental Information

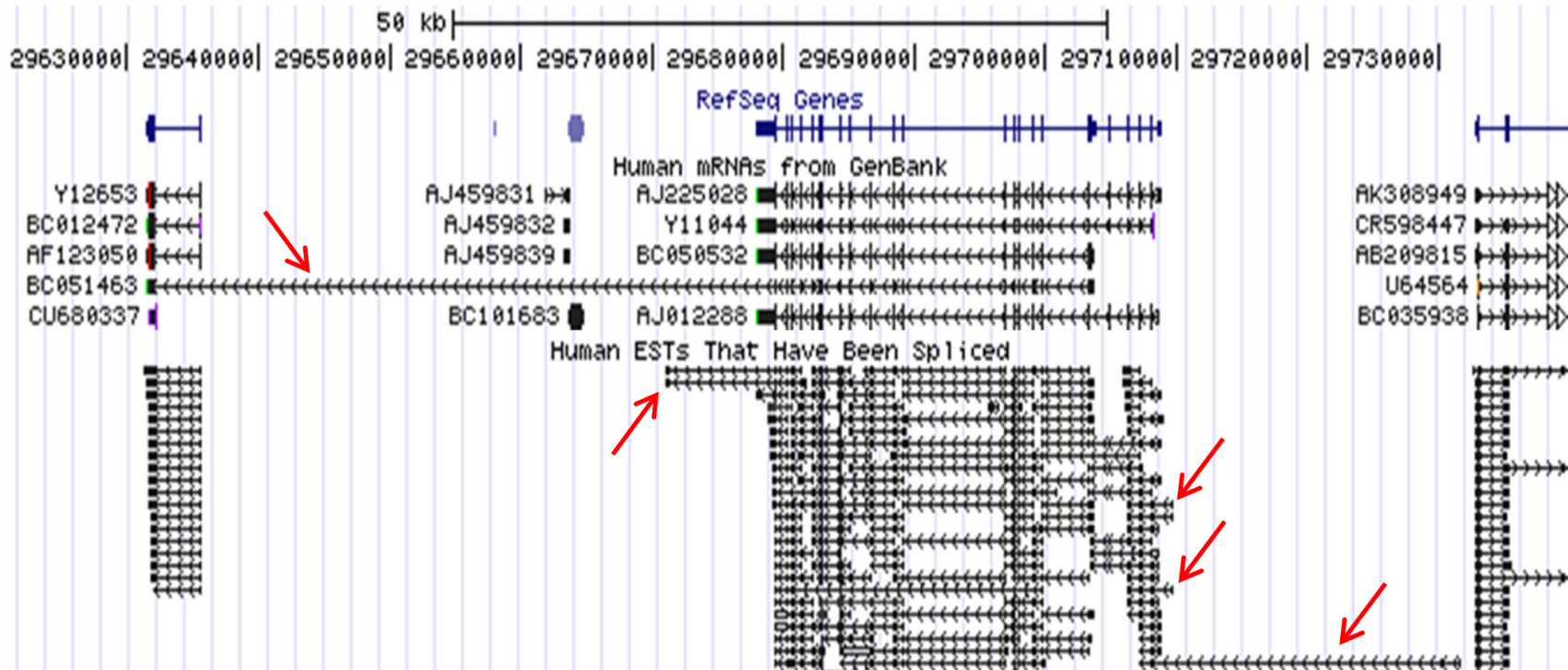


Figure S1. GABAB1 gene boundary based on UCSC genome browser database. From two databases of the UCSC genome browser (human mRNAs from GeneBank and human ESTs that have been spliced), we estimated a minimum GABAB1 gene length of 100,266 bp (chr6: 29,631,387 - 29,731,652). From top to bottom the tracks include RefSeq Genes, human mRNAs from GeneBank, and human ESTs that have been spliced. GABAB1 gene is at the center of RefSeq Genes tract, and its 5' end exons are located to the right side of the tract. Red arrows point to clones that showed extended GABAB1 gene length from the two UCSC genome browser databases. GABAB1, gamma-aminobutyric acid type B receptor subunit 1; UCSC, University of California, Santa Cruz; EST, expressed sequence tag.

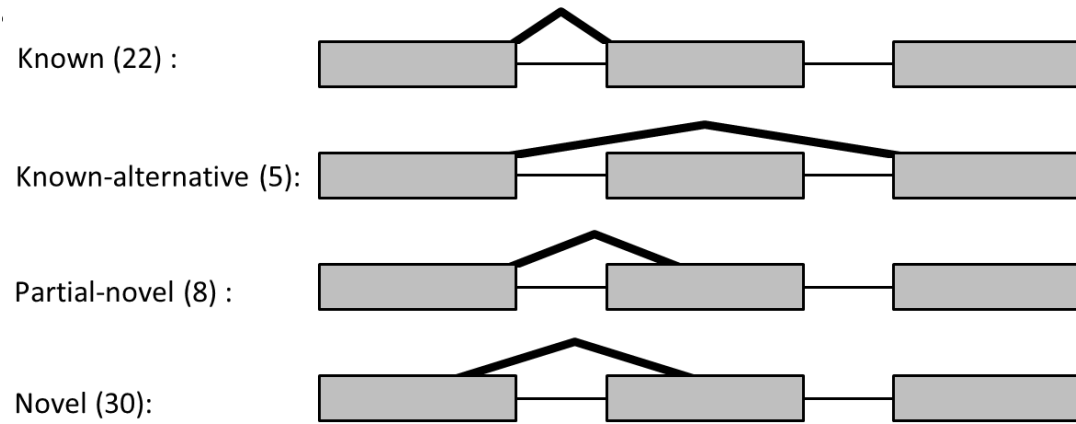


Figure S2. Four types of GABAB1 splicing junctions. The 65 splice junctions were one of four types (known, known-alternative, partial-novel, and novel splice junctions). “Known” represents the known splice junctions from the RefSeq Genes model. “Known-alternative” represents splice junctions that have different splicing site combinations of the known splice junctions. “Partial-novel” represents splice junctions that have only one known splicing site and an unknown splicing site. “Novel” represents junctions that do not have any known splicing sites. The numbers in parentheses are counts of each type. The known splice junctions are about one third of the other GABAB1 splice junctions. GABAB1, gamma-aminobutyric acid type B receptor subunit 1.

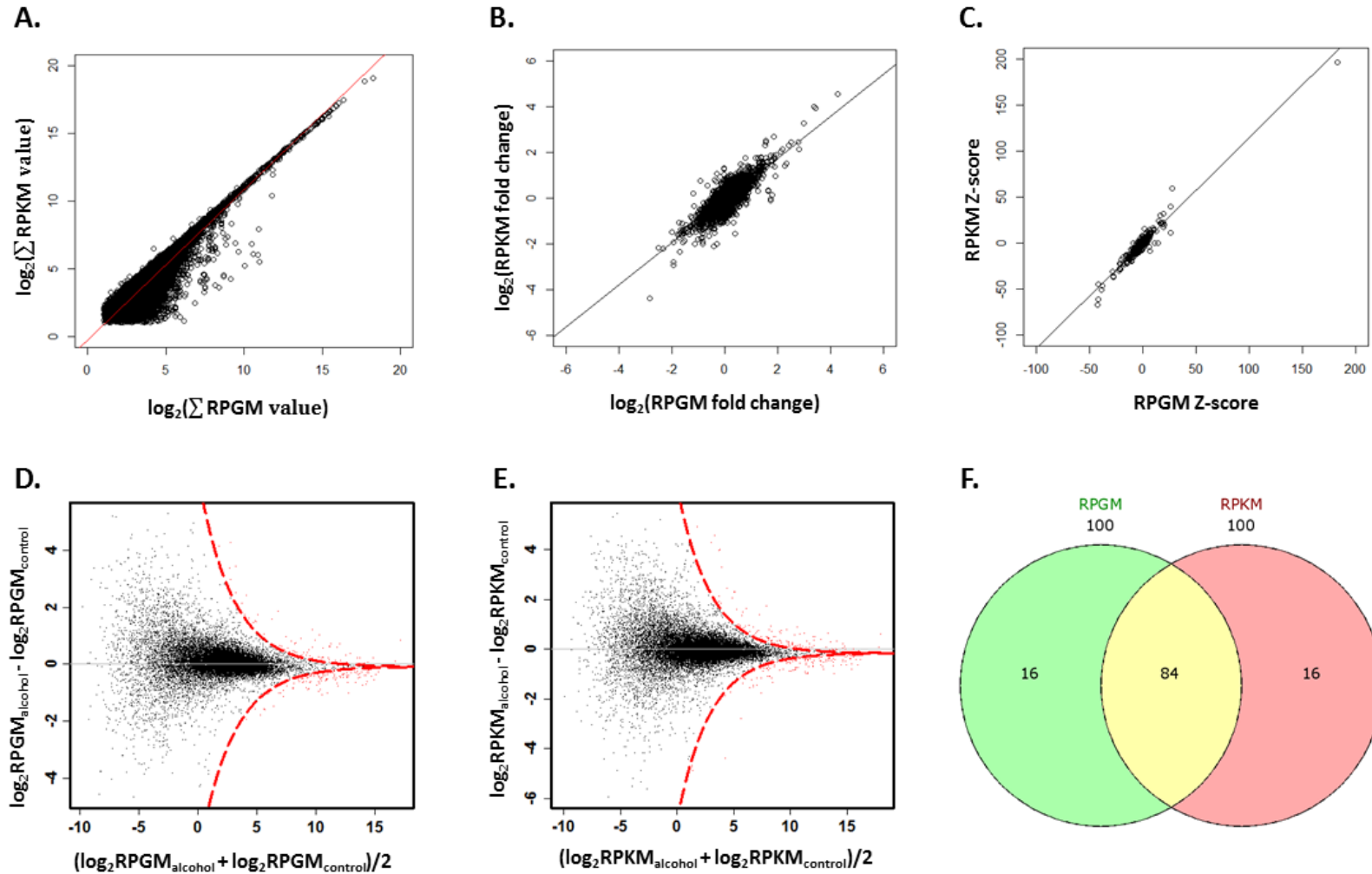
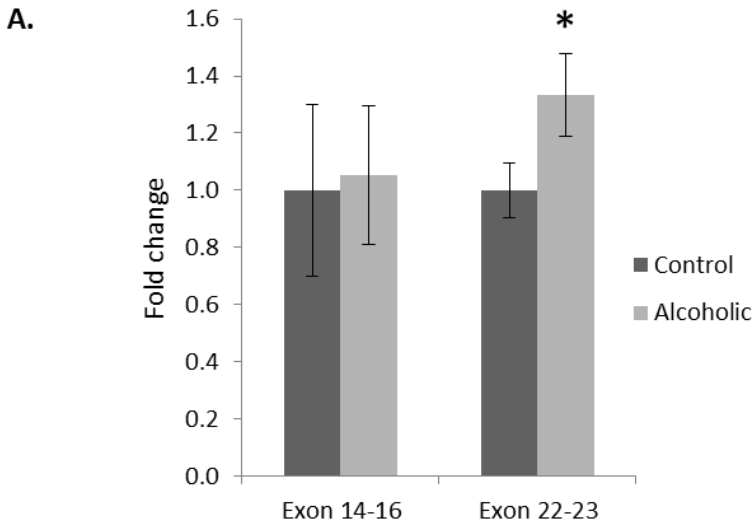
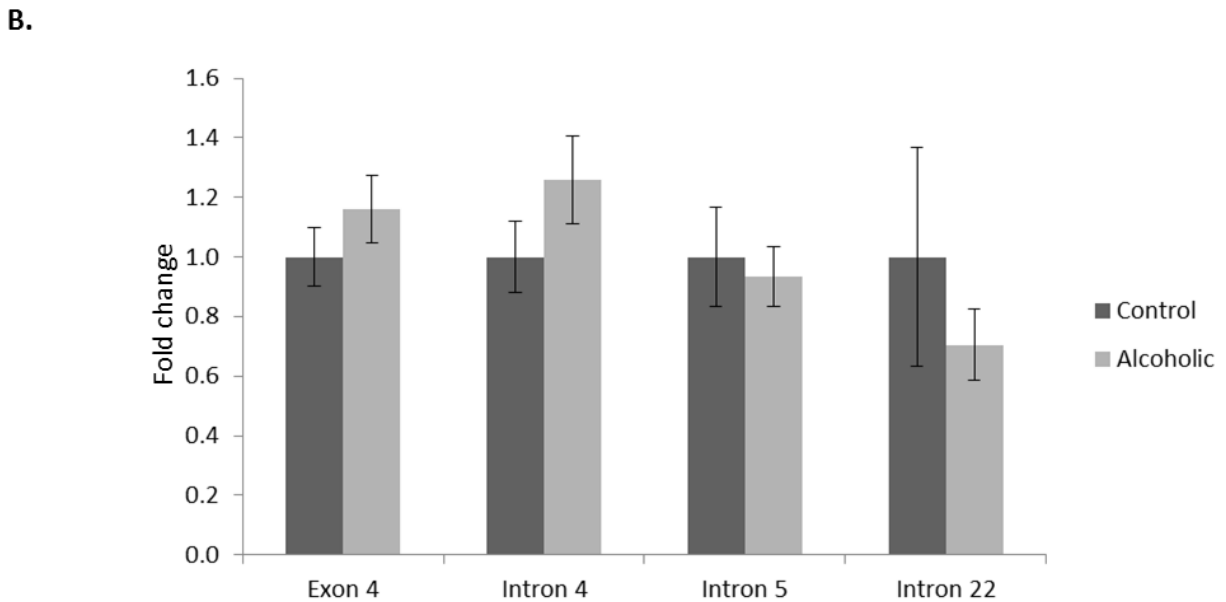


Figure S3. Expression comparison of genome wide transcripts in alcoholic brains using RPGM and RPKM values. (A) Expression level of RPGM and RPKM values are compared. X- and Y- axes represents \log_2 transformed sum of RPGM and RPKM values, respectively. (B) RPGM and RPKM fold change were compared. All fold change values are \log_2 transformed. X- axis is for RPGM fold change, and Y-axis is for RPKM fold change. (C) Z-scores of RPGM and RPKM values are compared. X- and Y- axes are Z-scores from DEGseq using RPGM and RPKM, respectively. (D) Genome wide transcript expression changes were compared

in alcoholic brains using RPGM. From RPGM values of all transcripts based on the RefSeq Genes model, we compared transcript expression levels between alcoholic and control groups. X-axis represents the averages of \log_2 RPGM values for alcoholic and control groups. Y-axis is for differences between the values. **(E)** Based on RPKM, genome wide transcripts expression changes were also compared in alcoholic samples. Like RPGM calculation, RPKM values were calculated for all transcripts based on the RefSeq Genes model, and their expression changes in alcoholic brains were examined. X-axis is for the averages of \log_2 RPKM values for alcoholic and control groups, and Y-axis represents differences between the values. Both RPGM and RPKM data showed similar patterns from MA plot analyses using DEGseq package. The significantly changed transcripts were 0.74% and 0.99% at A and B, respectively (p -values < 0.001). Red dots represent expression changes in alcoholic group, and the red dotted lines are p -values < 0.001 . **(F)** The top 100 significantly changed transcripts were compared between RPGM and RPKM. RPGM, reads per kilobase of gene model per million mapped reads; RPKM, reads per kilobase of exon model per million mapped reads.



Fold change	1.05	1.33
p-value	0.4486	0.0309



Fold change	1.16	1.26	0.93	0.71
p-value	0.1433	0.0903	0.3740	0.2419

Figure S4. Quantitative real-time PCR data for GABAB1 splicing junctions and exons/introns. (A) Quantitative real-time PCR was performed on GABAB1 splice junctions found from RNA-seq. The increase in RPJM expression of exons 22-23 and no change in

expression of exons 14-16 was similar to our RNA-seq data. **(B)** To validate RPGM data for exons and introns, we did further quantitative real-time PCR validation. As seen in our RNA-seq data, exon 4, intron 4, intron 5, and intron 22 showed no expression changes. One-tailed unpaired Student's *t*-tests were performed between alcoholic and control groups. Data are expressed as the mean \pm SEM. PCR, polymerase chain reaction; GABAB1, gamma-aminobutyric acid type B receptor subunit 1; RPJM, reads per kilobase of splice junction model per million uniquely mapped reads; RPGM, reads per kilobase of gene model per million mapped reads.

Table S1. Sample information.

Sample	Age	Gender	Brain pH	RIN	PMI (hr)	Classification (DSMIV for Alcoholics)
cSFC209	37	Male	5.25	6.1	11.0	Control
cSFC228	59	Male	6.56	6.9	20.0	Control
cSFC232	48	Male	6.71	8.7	17.0	Control
cSFC257	58	Male	6.46	7.6	12.0	Control
cSFC295	34	Male	6.73	6.5	20.5	Control
cSFC329	48	Male	6.73	7.3	24.0	Control
cSFC339	56	Male	6.76	6.5	37.0	Control
cSFC366	69	Male	6.60	7.2	16.0	Control
cSFC377	78	Male	6.20	7.4	6.5	Control
cSFC442	63	Male	6.94	7.0	24.0	Control
cSFC451	73	Male	6.80	6.3	48.0	Control
cSFC459	64	Male	6.94	8.3	9.5	Control
cSFC463	73	Male	6.82	5.7	51.0	Control
cSFC474	41	Male	6.96	7.2	48.0	Control
cSFC477	52	Female	6.21	2.3	11.0	Control
aSFC204	46	Female	6.50	6.4	27.0	Alcoholic
aSFC210	51	Male	5.58	7.4	27.0	Alcoholic
aSFC217	79	Male	6.34	7.5	48.0	Alcoholic
aSFC234	39	Male	6.56	8.3	24.0	Alcoholic
aSFC260	70	Male	6.34	5.4	19.0	Alcoholic
aSFC303	67	Male	6.40	6.2	48.0	Alcoholic
aSFC313	70	Male	6.82	6.4	62.0	Alcoholic
aSFC315	52	Male	6.78	5.8	45.5	Alcoholic
aSFC327	59	Female	6.63	7.5	22.0	Alcoholic
aSFC330	53	Male	6.75	5.4	57.0	Alcoholic
aSFC332	41	Male	6.70	6.4	54.0	Alcoholic
aSFC351	42	Male	6.50	5.8	41.0	Alcoholic
aSFC430	43	Male	6.29	5.2	29.0	Alcoholic
aSFC476	60	Male	6.48	7.3	16.5	Alcoholic

PMI, post-mortem interval; RIN, RNA integrity number.

Table S2. Mapping statistics of gene specific library and whole transcriptome library.

	Gene specific library	Whole transcriptome library
Mapped reads	2,542,338	2,314,525
GABAB1 specific reads	37,739	249
GABAB1 specific splice junction containing reads	1,076	18

GABAB1, gamma-aminobutyric acid type B receptor subunit 1.

Table S3. Mapping statistics of whole transcriptome libraries for 15 control and 14 alcoholic human subjects.

Sample	cSFC209	cSFC228	cSFC232	cSFC257	cSFC295	cSFC329	cSFC339	cSFC366	cSFC377	cSFC442	cSFC451	cSFC459	cSFC463	cSFC474	cSFC477
Raw reads	33,094,843	35,343,212	18,581,068	38,888,111	36,224,909	83,654,681	36,663,941	22,449,165	11,911,746	32,833,007	35,882,930	40,277,699	48,561,631	25,737,53	34,263,844
Quality filter	26,632,535	24,225,746	15,668,505	32,749,659	28,681,117	70,114,358	29,285,931	17,992,194	10,075,364	26,096,695	28,305,869	33,316,350	40,821,155	21,519,617	25,889,478
	80.47%	68.54%	84.33%	84.22%	79.18%	83.81%	79.88%	80.15%	84.58%	79.48%	78.88%	82.72%	84.06%	83.61%	75.56%
ncRNA and adaptor filter (a)	18,267,859	12,356,032	11,638,800	24,390,013	17,750,765	57,245,003	21,713,129	10,383,532	6,886,818	17,876,790	21,322,125	25,701,722	24,003,408	16,197,374	10,611,480
	55.20%	34.96%	62.64%	62.72%	49.00%	68.43%	59.22%	46.25%	57.82%	54.45%	59.42%	63.81%	49.43%	62.93%	30.97%
Unique mapped reads (b)	7,866,682	4,165,394	4,867,740	10,981,783	7,290,526	27,434,260	8,444,624	4,349,063	3,087,588	7,190,915	7,682,991	12,082,894	11,115,208	6,969,258	4,992,016
	23.77%	11.79%	26.20%	28.24%	20.13%	32.79%	23.03%	19.37%	25.92%	21.90%	21.41%	30.00%	22.89%	27.08%	14.57%
(b)/(a)	43.06%	33.71%	41.82%	45.03%	41.07%	47.92%	38.89%	41.88%	44.83%	40.22%	36.03%	47.01%	46.31%	43.03%	47.04%

Sample	aSFC204	aSFC210	aSFC217	aSFC234	aSFC260	aSFC303	aSFC313	aSFC315	aSFC327	aSFC330	aSFC332	aSFC351	aSFC430	aSFC476
Raw reads	27,821,439	26,047,363	14,566,308	21,984,169	126,588,851	36,030,714	52,006,412	34,642,762	90,749,179	32,384,057	35,020,905	38,226,079	29,939,449	25,688,439
Quality filter	21,406,081	21,840,200	12,404,367	18,367,384	99,273,661	27,099,685	37,856,724	27,081,823	75,869,348	26,756,321	27,865,908	25,866,170	23,554,695	21,218,728
	76.94%	83.85%	85.16%	83.55%	78.42%	75.21%	72.79%	78.17%	83.60%	82.62%	79.57%	67.67%	78.67%	82.60%
ncRNA and adaptor filter (a)	13,609,570	14,751,500	9,144,836	12,710,443	71,410,465	12,952,198	24,532,333	18,495,269	45,739,200	19,094,716	17,724,874	19,449,128	17,347,506	15,598,453
	48.92%	56.63%	62.78%	57.82%	56.41%	35.95%	47.17%	53.39%	50.40%	58.96%	50.61%	50.88%	57.94%	60.72%
Unique mapped reads (b)	5,364,440	6,617,476	4,022,523	5,315,184	32,701,183	5,051,370	4,546,158	6,862,433	12,282,055	8,108,307	7,473,312	6,860,571	7,612,834	7,027,911
	19.28%	25.41%	27.62%	24.18%	25.83%	14.02%	8.74%	19.81%	13.53%	25.04%	21.34%	17.95%	25.43%	27.36%
(b)/(a)	39.42%	44.86%	43.99%	41.82%	45.79%	39.00%	18.53%	37.10%	26.85%	42.46%	42.16%	35.27%	43.88%	45.06%

Under the read numbers, ratios were calculated from raw reads. (b)/(a) represented the ratio of unique mapped reads (b) to ncRNA and adaptor filter (a).

Table S4. GABAB1 exon and intron expression changes in alcoholic brains.

Exon / intron	Ctl RPGM	Alc RPGM	Fold change	<i>p</i>-value	q-value (Benjamini <i>et al.</i> (1))
exon 7	21.04	32.55	1.47	9.39E-08	5.38E-06
exon 8	29.97	43.33	1.38	2.30E-07	1.18E-05
exon 10	85.77	71.20	0.79	2.33E-08	1.55E-06
exon 11	21.23	28.21	1.27	1.68E-03	2.15E-02
exon 12	46.58	55.94	1.14	9.58E-03	8.28E-02
exon 14	76.44	105.48	1.31	2.99E-12	4.55E-10
exon 15	62.25	86.05	1.32	2.30E-10	2.43E-08
exon 16	34.81	53.71	1.47	9.02E-12	1.24E-09
exon 17	6.63	4.37	0.63	3.72E-03	4.02E-02
exon 19	25.44	32.64	1.22	3.76E-03	4.06E-02
exon 22	30.74	37.44	1.16	1.96E-02	1.41E-01

Ctl RPGM and Alc RPGM are the RPGM means of control group and alcoholic group, respectively.

GABAB1, gamma-aminobutyric acid type B receptor subunit 1; RPGM, reads per kilobase of gene model per million mapped reads.

Table S5. GABAB1 splice junction changes in alcoholic brains.

Location	Type	Exon / intron	Ctl RPJM	Alc RPJM	Fold change	<i>p</i> -value	q-value (Benjamini <i>et al.</i> (1))	q-value (Storey <i>et al.</i> (2))
chr6:29,699,781- 29,703,241	Known	exon 6 - exon 7	5.72	10.88	1.71	5.42E-05	7.27E-04	3.67E-04
chr6:29,699,232- 29,699,645	Known	exon 7 - exon 8	5.69	9.64	1.52	2.20E-03	1.39E-02	7.03E-03
chr6:29,697,574- 29,697,859	Known	exon 9 - exon 10	20.71	16.38	0.71	6.90E-05	8.86E-04	4.48E-04
chr6:29,697,049- 29,697,507	Known	exon 10 - exon 11	9.17	7.32	0.72	9.83E-03	4.26E-02	2.15E-02
chr6:29,689,242- 29,696,856	Known	exon 11 - exon 12	16.76	25.42	1.36	1.76E-04	1.91E-03	9.65E-04
chr6:29,686,758- 29,688,306	Known	exon 13 - exon 14	36.87	61.99	1.51	2.31E-14	2.73E-12	1.38E-12
chr6:29,684,490- 29,684,984	Known	exon 15 - exon 16	15.45	22.22	1.29	3.31E-03	1.91E-02	9.64E-03
chr6:29,682,975- 29,684,356	Known	exon 16 - exon 17	0.75	0.16	0.19	8.63E-03	3.88E-02	1.96E-02
chr6:29,680,745- 29,681,324	Known	exon 20 - exon 21	0.09	0.72	6.89	8.20E-03	3.73E-02	1.89E-02
chr6:29,679,473- 29,680,249	Known	exon 22 - exon 23	1.73	4.64	2.40	7.35E-05	9.34E-04	4.72E-04
chr6:29,679,072- 29,679,179	Novel	exon 23	0.60	0.18	0.27	4.13E-02	1.20E-01	6.05E-02

Ctl RPJM and Alc RPJM are the RPJM means of control group and alcoholic group, respectively.

GABAB1, gamma-aminobutyric acid type B receptor subunit 1; RPJM, reads per kilobase of splice junction model per million uniquely mapped reads.

Table S6. Known splicing junction change of GABAB signaling pathway genes.

Gene name	Gene length (bp)	Number of known splicing junction change	Number of known splicing junction change. / gene length (Kb)
GABAB1	30,958	10	0.323
GABAB2	420,810	11	0.026
KCNJ3(GIRK1)	157,922	1	0.006
KCNJ5(GIRK4)	26,639	0	0.000
GNAI1	84,586	2	0.024
GNB1	105,767	7	0.066

Supplemental References

1. Benjamini Y, Hochberg Y (1995): Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met.* 57:289-300.
2. Storey JD (2003): The positive false discovery rate: A Bayesian interpretation and the q-value. *Ann Stat.* 31:2013-2035.