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Expression of Alcoholism-Relevant Genes in the Liver Are Differently Correlated to Different Parts of the Brain

Lishi Wang,*† Yue Huang,* Yan Jiao,*‡ Hong Chen,§ Yanhong Cao,*¶
Beth Bennett,# Yongjun Wang,** and Weikuan Gu*

*Departments of Orthopaedic Surgery–Campbell Clinic, and Pathology,
University of Tennessee Health Science Center, Memphis, TN, USA
†Department of Basic Medicine, Inner Mongolia Medical University, Huhhot City, Inner Mongolia, China
‡Mudanjiang Medical College, Mudanjiang, People's Republic of China
§The First Hospital of Qiqihaer City, Qiqihaer, China
¶Institute of Kaschin-Beck Disease, Center for Endemic Disease Control,
Centers for Disease Control and Prevention, Harbin Medical University, Harbin, PR China
#School of Pharmacy, University of Colorado Denver, Aurora, CO, USA
**Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

The purpose of this study is to investigate whether expression profiles of alcoholism-relevant genes in different parts of the brain are correlated differently with those in the liver. Four experiments were conducted. First, we used gene expression profiles from five parts of the brain (striatum, prefrontal cortex, nucleus accumbens, hippocampus, and cerebellum) and from liver in a population of recombinant inbred mouse strains to examine the expression association of 10 alcoholism-relevant genes. Second, we conducted the same association analysis between brain structures and the lung. Third, using five randomly selected, nonalcoholism-relevant genes, we conducted the association analysis between brain and liver. Finally, we compared the expression of 10 alcoholism-relevant genes in hippocampus and cerebellum between an alcohol preference strain and a wild-type control. We observed a difference in correlation patterns in expression levels of 10 alcoholism-relevant genes between different parts of the brain with those of liver. We then examined the association of gene expression between alcohol dehydrogenases (*Adh1*, *Adh2*, *Adh5*, and *Adh7*) and different parts of the brain. The results were similar to those of the 10 genes. Then, we found that the association of those genes between brain structures and lung was different from that of liver. Next, we found that the association patterns of five alcoholism-nonrelevant genes were different from those of 10 alcoholism-relevant genes. Finally, we found that the expression level of 10 alcohol-relevant genes is influenced more in hippocampus than in cerebellum in the alcohol preference strain. Our results show that the expression of alcoholism-relevant genes in liver is differently associated with the expression of genes in different parts of the brain. Because different structural changes in different parts of the brain in alcoholism have been reported, it is important to investigate whether those structural differences in the brains of those with alcoholism are due to the difference in the associations of gene expression between genes in liver and in different parts of the brain.

Key words: Alcoholism; Brain structure; Gene expression; Liver

INTRODUCTION

People who have been drinking large amounts of alcohol for long periods of time run the risk of developing serious and persistent changes in the brain (1–4).

The mechanism underlying those changes remains unclear. In particular, several studies on the brain damage of long-term drinkers have been reported recently. Zahr and colleagues (1) examined alcohol-related brain damage (ARBD) from the perspective of Wernicke encephalopathy and Korsakoff syndrome by exploring the clinical presentations, postmortem brain pathology, in vivo MRI findings, and potential molecular mechanisms associated with these conditions. The authors indicated that, based on the results of genomic approaches, multiple pathways may be involved in causing altered neuronal function and structural changes in ARBD. Fortier et al. (2) compared cortical thickness measurements from 31 abstinent individuals with a history of prior alcohol abuse to those of 34 healthy nonalcoholic control participants and concluded that reduction in cortical thickness was a consequence of chronic alcoholism, with the most severe reductions in the frontal and temporal brain regions.

Address correspondence to Weikuan Gu, University of Tennessee Health Science Center, 956 Court Avenue, Memphis, TN 38163, USA.
Tel: +1-901-448-2259; Fax: +1-901-448-6062; E-mail: wgu@uthsc.edu

Chronic alcohol exposure also causes liver damage (5–7). Alcoholic liver disease (ALD) has been used as a phrase to represent hepatic manifestations of alcohol overconsumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis. Despite the fact that alcohol affects both liver and brain (8), the molecular pathways between liver and brain remain elusive.

We assumed some associations in gene expression between brain and liver. We hypothesized that the associations of gene expressions between different parts of the brain and liver were different. To test our hypothesis, we analyzed the expression association of 10 alcoholism-relevant genes between five parts of the brain and the liver in a population of recombinant inbred (RI) mouse strains derived by crossing C57BL/6J (B6) and DBA/2J (D2). Lung was used as a control to investigate whether there is a difference between liver and lung. We then further tested the association of five nonalcoholism-relevant genes between liver and brain structures. The expression levels of 10 alcoholism-relevant genes and five nonalcoholism-relevant genes were further examined in the hippocampus and cerebellum in an alcohol preference mouse model.

MATERIALS AND METHODS

Alcoholism-Relevant and -Nonrelevant Genes

We examined the expression levels of the 10 known relevant genes in alcoholism: alcohol dehydrogenase (*Adh1, Adh5, Adh7*) (9); aldehyde dehydrogenase 2 gene (*Aldh2*); opioid receptor, MU-1 (*Oprm1*) (10); potassium large conductance calcium-activated channel, subfamily M, alpha member 1 (*Kcnma1*) (11); glycogen synthase kinase 3 beta (*Gsk3b*) (11); phosphatase and tensin homolog (*Pten*) (11); neuregulin 3 (*Nrg3*) (12); and finger protein 3 gene (*Phf3*) (11).

We next considered five nonalcoholism-related genes: carbonic anhydrase 3 (*Car3*); ubiquitin-specific protease 12 (*Usp12*); ADAM-like, decysin 1 (*Adamdec1*); Slc4a1 (*Slc4a1*); and bone morphogenetic protein 1 (*Bmp1*). It is impossible to completely rule out the alcoholism relevance of any gene. The definition of nonalcoholism-relevant was synthetically determined by not finding alcoholism-relevant publications of each of those genes in the PubMed database searched on April 20, 2012, using the name of each gene and the key words "alcohol, alcoholism."

Gene Expression Data Were Generated Using the Same Kind of Chip With the Same Platform

Gene expression profiles were found in GeneNetwork (http://www.genenetwork.org/webqtl/main.py) (13). The gene expression profiles of various tissues were generated by Affymetrix chip M430.v2. RNAs for the gene expression profiling were from RI strains derived by crossing C57BL/6J (B6) and DBA/2J (D2) and inbreeding progeny for 20 or more generations (13). We compared expression profiles of some genes from B6 (D2 RI strains in the following tissues: liver, lung, striatum, prefrontal cortex, nucleus accumbens, hippocampus, and cerebellum.

Liver Data Set. Male mice in BXD strains for this study were from The Jackson Laboratory. Mice were between 6 and 10 weeks of age; to ensure adequate acclimatization to a common environment, the mice were maintained until 16 weeks of age [see Li et al. (9) for complete information].

Lung Data Set. Lung expression data set included 47 BXD strains. Total RNA was extracted from the lungs by using RNA STAT-60 (Tel-Test, Inc.). RNA from two to five animals per strain was pooled and used for gene expression analysis. Animals used in this study were between 49 and 93 days of age. All inbred strains were profiled for both sexes; for a given BXD strain, either males or females were used [detailed information in de la Monte et al. (8)].

Striatum Data Set. These April 2005 data [prepared by Dr. Rosen, Beth Israel Deaconess Medical Center (BIDMC), Harvard University] provided estimates of mRNA expression in the striatum (caudate nucleus of the forebrain) of 31 lines of mice including C57BL/6J, DBA/2J, and 29 BXD RI strains. Animals were obtained from The Jackson Laboratory and housed for several weeks at BIDMC until they reached ~2 months of age (detailed information can be found at http://www.genenetwork.org/dbdoc/SA_M2_0405_PC.html).

Prefrontal Cortex Data Set. This BXD data set provided estimates of mRNA expression in the prefrontal cortex following ethanol treatment across 27 BXD RI strains and their B6 and D2 progenitor strains (detailed information can be found at http://www.genenetwork.org/webqtl/main.py?FormID=sharinginfo&GN_AccessionId=136).

Nucleus Accumbens Data Set. The data set was from female mice at 2 months of age. No detailed information is provided in GeneNetwork.

Hippocampus Data Set. The Hippocampus Consortium data set provided estimates of mRNA expression in the adult hippocampus of 67 BXD RI strains. A pool of dissected tissues, typically from six hippocampi and three naive adults of the same strain, sex, and age, was collected in one session and used to generate cRNA samples (detailed information can be found at http://www.genenetwork.org/webqtl/main.py?FormID=sharinginfo&GN_AccessionId=112).

Cerebellum Data Set. This May 2005 data provided estimates of mRNA expression in adult cerebellum of 28 BXD RI strains (detailed information can be found at http://www.genenetwork.org/dbdoc/GCB_M2_0505_M.html).

Association Analysis Was Performed Using Correlation r Value

We analyzed the correlations between the expression levels of each gene in liver and brain tissues. For the expression level of each gene, we collected data of

every probe of every gene. Because the gene expression profiles were generated by the same microarray platform with the same type of chip, the number of probes for each gene in different tissues was the same. The correlation between the expressions of a gene in two tissues, such as liver and nucleus accumbens, was determined by the *r* value, with 1 as the maximum positive value and −1 as the maximum negative correlation. The total association or the positive or negative impact of genes between two tissues was defined as the average of values of *r* (T_r).

$$T_r = \frac{\sum (r_1 \ldots r_n)}{n} \quad (1)$$

To calculate the overall association strength of a gene's expression (either negative or positive) between two tissues, we used a formula to summarize the expression correlation. The strength of correlation (defined as r_a) of a gene between two tissues was defined as the average of absolute values of *r*.

$$r_a = \frac{\sum (|r_1| \ldots |r_n|)}{n} \quad (2)$$

Accordingly, unlike T_r, r_a could only be a positive value between 0 and 1.

Microarray Data from Alcohol Preference Mice

Alcohol preference mouse strain (14) and control animals were produced at the Institute for Behavioral Genetics at the University of Colorado, Boulder (UCB). For this study, animals carrying the full congenic region of interval conferring the D2 phenotype (interval-specific congenic recombinant strains, ISCRS G) were used (14). Tissues were collected at UCB and shipped to the University of Tennessee Health Science Center (UTHSC) at Memphis. Microarray analysis was conducted using RNA from hippocampus and cerebellum of ISCRS mice and wild-type mice. A starting amount of 200 ng of high-quality total RNA, with an RNA integrity score of more than 7, was used to generate cDNA and cRNA with the Illumina TotalPrep RNA Amplification Kit (Ambion, CA). Then, 1.5 µg of sample cRNA was hybridized overnight to the Mouse-6 v1B BeadChip in a multiple-step procedure according to the manufacturer's instructions, and raw data were generated using BeadStudio 2.3.41 (Illumina, San Diego, CA). Data analysis followed our previous procedure (15).

RESULTS

Correlation of Expression Levels of Known Alcoholism-Relevant Genes Between Liver and Different Parts of Brain

We compared the association of gene expression of 10 known alcoholism-relevant genes in organs to brain parts. The overall association T_r values of liver to brain parts were positive (Fig. 1A). Among five different brain structures, the *r* values of each gene between body organs and brain structures varied significantly. Six genes (*Adh7, Aldh2, Kcnma1, Nrg3, Gsk3b*, and *Phf3*) had positive correlation between liver and cerebellum (Fig. 2A). The correlation of four genes (*Adh1, Pten, Adh5*, and *Oprm1*) between liver and cerebellum was negative. The correlation of expression levels between liver and five parts of the brain of two genes (*Oprm1* and *Phf3*) varied greatly from one brain structure to another (Fig. 2A–E).

These data suggest that the correlation of expression levels of alcoholism-relevant genes between liver and brain structures is different.

Overall Impact of Body Organs to Brain Structures

We next focused on the overall impact of those 10 alcoholism-relevant genes on different parts of the brain by analyzing the strength of correlation of gene expression between liver and brain parts. We calculated the r_a values of those 10 genes between liver and five parts of the brain. The highest r_a value was between liver and hippocampus, whereas the lowest r_a values were between liver and striatum (Table 1). The r_a values between liver and cerebellum and between liver and nucleus accumbens were also high. We next examined the effect of alcohol dehydrogenase (*Adh 1, Adh5*, and *Adh7*) and aldehyde dehydrogenase 2 gene (*Aldh2*). The r_a value between liver and hippocampus was still the highest, whereas the r_a value between liver and striatum remained the lowest (Fig. 3). The hippocampus, a brain structure vital to learning and memory, also appears vulnerable to damage from chronic, heavy alcohol consumption (16,17). The striatum seems less affected by alcoholism compared to other parts of the brain.

Correlation of Expression Levels of Known Alcoholism-Relevant Genes Between Lung and Different Parts of Brain

We assumed that the association of gene expression between brain structure and liver was different from that between brain structure and other organs. We next examined the association of the expressions of the same 10 genes between brain structures and the lung. The overall association T_r values of liver and lung to brain parts were positive (Fig. 1A). However, many individual T_r values of genes between liver and brain parts were different from those between lung and brain parts (Fig. 1B).

In cerebellum, six genes (*Adh7, Aldh2, Kcnma1, Nrg3, Oprm1,* and *Phf3*) had positive correlation between both organs and cerebellum (Fig. 2A). However, the positive correlation of *Nrg3* and Phf3 between lung and cerebellum was much higher than that between liver and cerebellum. The correlation of four genes (*Adh1, Pten, Adh5,* and *Gsk3*)

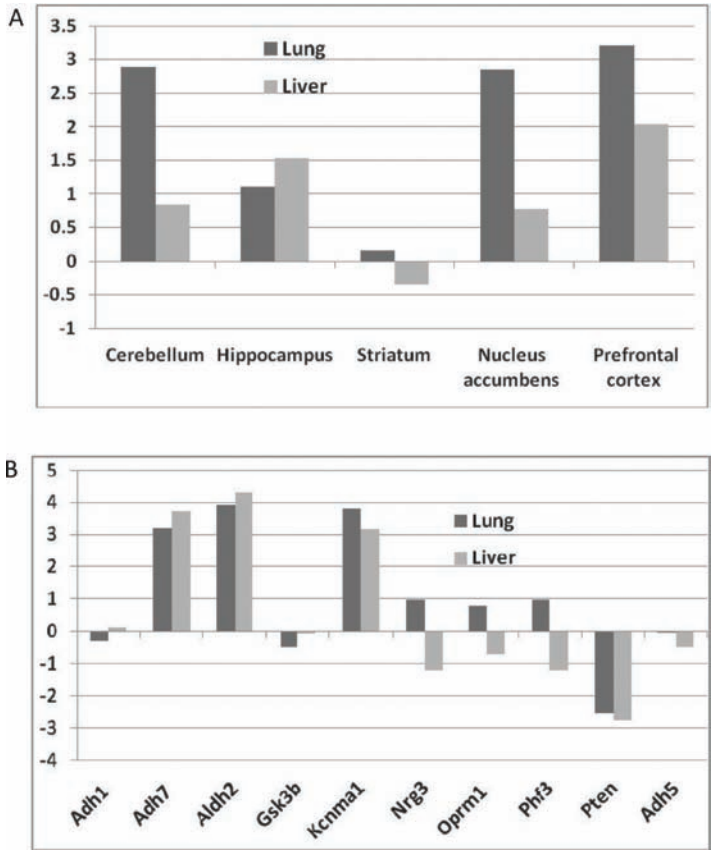

Figure 1. Correlation among gene expressions between body organ (liver, lung) and brain structures. The *y* axis indicates the T_r values between body tissues and brain parts. (A) Correlation of gene expression levels (T) between liver and brain structures and between lung and brain structures. (B) The correlation (T_r) of expression of 10 genes between total brain structure and body organs (liver and lung).

between lung and cerebellum was negative. The expression correlation of *Oprm1* between lung and cerebellum was positive, whereas it was negative between liver and cerebellum. Among the 10 genes, correlation of expression levels of three genes (*Adh7, Aldh2*, and *Kcnma1*) between lung and five parts of the brain was similar to those between liver and five parts of the brain (Fig. 2A–E). The correlation of expression levels between body organs and five parts of the brain of two genes (*Oprm1* and *Phf3*) varied greatly from one brain structure to another (Fig. 2A–E).

These data suggest a significant difference among correlation of expression levels of alcoholism-relevant genes between liver and brain structures than those between lung and brain structure.

Correlation Expression of Genes Known Not to Be Connected to Alcoholism Between Liver and Different Parts of Brain

We then tested the association of expression of five alcoholism-nonrelevant genes between liver and brain structure. The expression of those five alcoholism-nonrelevant genes in liver showed a different correlation to different parts of the brain when compared to those of alcoholism-relevant genes (Table 2). The highest r_a value of genes was between liver and striatum, whereas the

FACING PAGE

Figure 2. T_r values of 10 genes between each of five brain structures and body organs (liver and lung). The *y* axis indicates the T_r values between body tissues and brain parts. (A) T_r values between cerebellum and liver and lung. (B) T_r values between hippocampus and liver and lung. (C) T_r values between striatum and liver and lung. (D) T_r values between nucleus accumbens and liver and lung. (E) T_r values between prefrontal cortex and liver and lung.

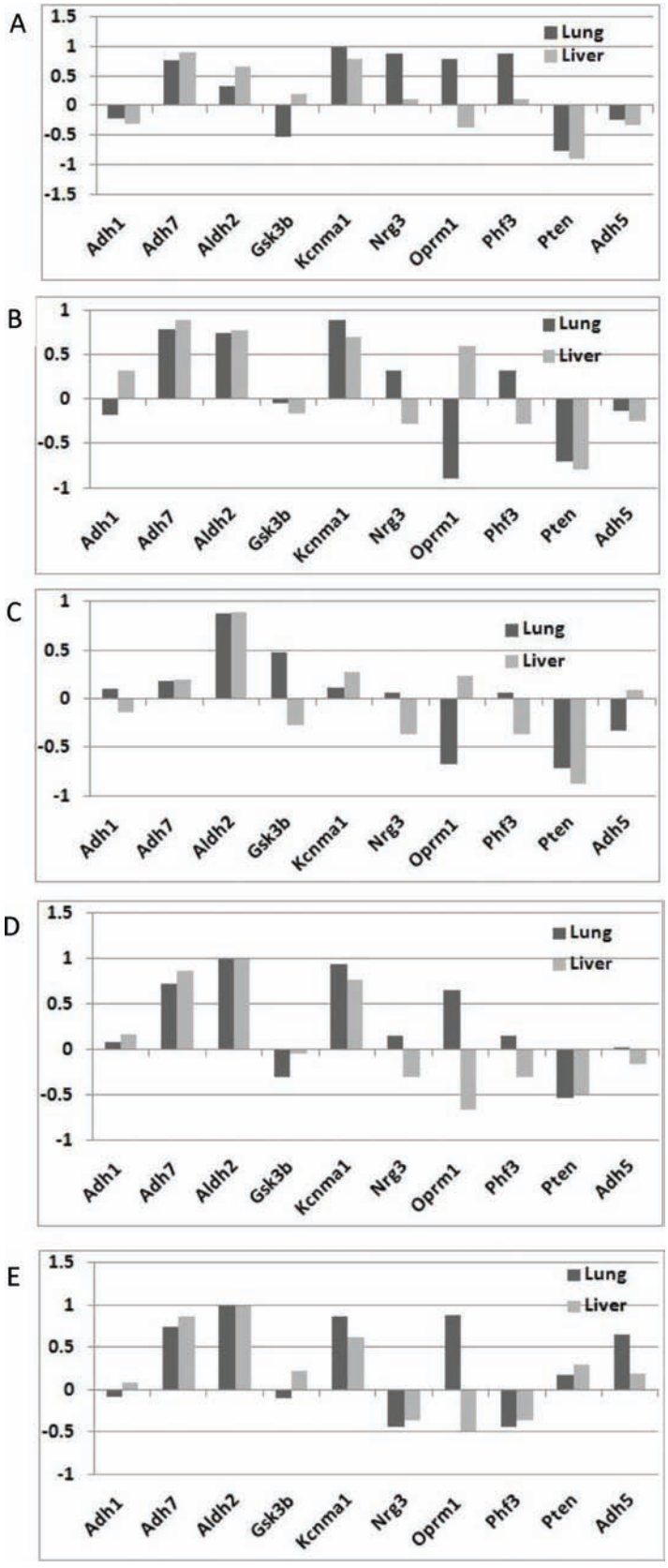
A
B
C
D
E
Lung
Liver
Adh1
Adh7
Aldh2
Gsk3b
Kcnma1
Nrg3
Oprm1
Phf3
Pten
Adh5

Table 1. The r_a Values Between the Liver and Five Parts of the Brain of 10 Alcoholism-Relevant Genes

Gene	Cerebellum	Hippocampus	Striatum	Nucleus Accumbens	Prefrontal Cortex
Adh1	0.297811	0.323259	0.137087	0.157447	0.08104
Adh7	0.897239	0.893602	0.195857	0.872097	0.867744
Aldh2	0.661162	0.766668	0.884187	0.991815	0.991998
Gsk3b	0.189837	0.165886	0.281076	0.054553	0.215643
Kcnma1	0.783562	0.70022	0.276031	0.766056	0.62384
Nrg3	0.116928	0.275103	0.364287	0.306875	0.366945
Oprm1	0.379815	0.600805	0.236335	0.674642	0.483666
Phf3	0.116928	0.275103	0.364287	0.306875	0.366945
Pten	0.905586	0.786375	0.871338	0.493195	0.293631
Adh5	0.33991	0.24827	0.086126	0.171248	0.187285
r_a	0.4688778	0.5035291	0.369661	0.4794805	0.4478738

lowest r_a value was between liver and cerebellum, one of the brain structures most influenced by alcohol.

Expression Levels of Known Alcoholism-Relevant Genes in Hippocampus and Cerebellum in Alcohol Preference Strain

At the time of analysis of the association of gene expression of 10 known alcoholism-relevant genes in organs to brain parts in BXD normal mouse strains, we also obtained the microarray data from the hippocampus and cerebellum from ISCRS strain G (13). We therefore examined whether the expression of those known alcoholism-relevant genes were affected in the ISCRS strain. The results indicated that overall, the expression of most of those genes are affected in ISCRS strain G, and the effect on hippocampus ($p = 0.03059$) is greater than that in cerebellum ($p = 0.05096$) (one-tailed t test, paired samples for the total of 10 genes). A close look at the data reveals that the effect on four genes, *Kcnma1, Gsk3b, Nrg3*, and *Phf3,* in hippocampus (Fig. 4A) is much larger than that in cerebellum (Fig. 4B). The data agree with our early data in BXD mice that among brain parts, the highest r_a value was between liver and hippocampus. We next examined the expression of five alcoholism-nonrelevant genes. Our data indicated that there is no significant difference in hippocampus or cerebellum between ISCRS strain G and wild-type controls ($p = 0.2328$ and 0.3763, respectively).

CONCLUSION

Our analysis indicated that expression levels of alcoholism-relevant genes in liver were differently correlated to different parts of the brain. In particular, the alcoholism-relevant genes were highly correlated with a unique pattern to hippocampus, the part of the brain influenced most by alcohol (16,17). Our data suggest a connection between liver damage and brain damage. Damage to different brain parts may be influenced by alcoholism gene expression, and the changes in expression of alcoholism genes is correlated to those in liver. It is known that alcohol damages liver the most and influences brain structures in the long run. However, knowledge of such a connection between liver and brain, especially the difference in brain

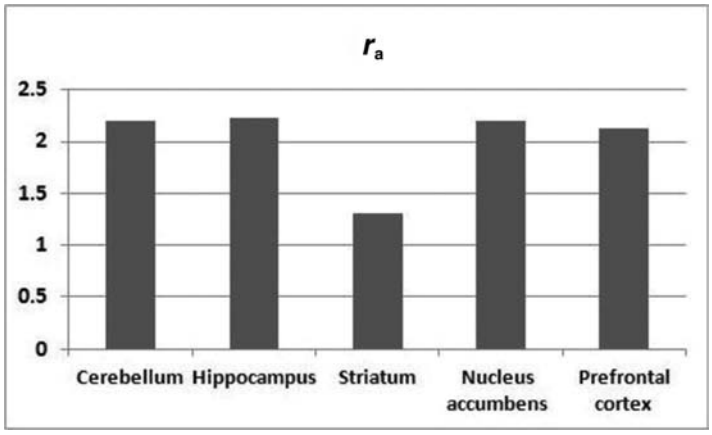

Figure 3. The total r_a values of gene expression in liver and five parts of the brain; the values represent the overall strength of the association of gene expression between liver and a brain structure. The *y* axis indicates the r_a values between body tissues and brain parts.

Table 2. The r_a Values Between the Liver and Five Parts of the Brain of Five Alcoholism-Nonrelevant Genes

Gene	Cerebellum	Hippocampus	Striatum	Nucleus Accumbens	Prefrontal Cortex
Ca3	0.101691	0.701504	0.577154	0.675397	0.641093
Usp12	0.944615	0.973386	0.987839	0.913	0.876006
Adamdec1	0.042178	0.088047	0.087935	0.053011	0.036698
Slc4a1	0.403206	0.386956	0.740329	0.479193	0.340139
Bmp1	0.86051	0.958264	0.886021	0.83	0.856363
r_a	0.47044	0.621631	0.655856	0.59012	0.55006

structures, is not clear. Our data provide preliminary data to bridge the expression of disease genes and genes in particular parts of the brain. Accordingly, we speculate that abnormal expression of genes of chronic abnormalities or abnormal gene expression of body organs can affect specific brain structures. Further testing of our hypothesis will greatly improve our understanding of the molecular basis between brain and liver damage by alcoholism.

Our data also show the difference of impact of liver and lung to different parts of the brain. Our data seemingly suggest that prolonged liver-specific abnormalities may influence the structure of certain parts of the brain, whereas the chronic problem in lung may influence different parts of the brain. Influencing of the structure of certain parts of the brain by chronic disease such as rheumatoid arthritis has been reported (18). It is possible that diseases in different body organs affect different parts of the brain. It is necessary to confirm such a possibility by carrying out future studies using large population sizes and different diseases. The high impact of alcoholism on the 10 alcohol-relevant genes in hippocampus was further confirmed by using alcohol preference mice.

Despite the fact that it is difficult to define a non-alcoholic-relevant gene, our analysis using five genes

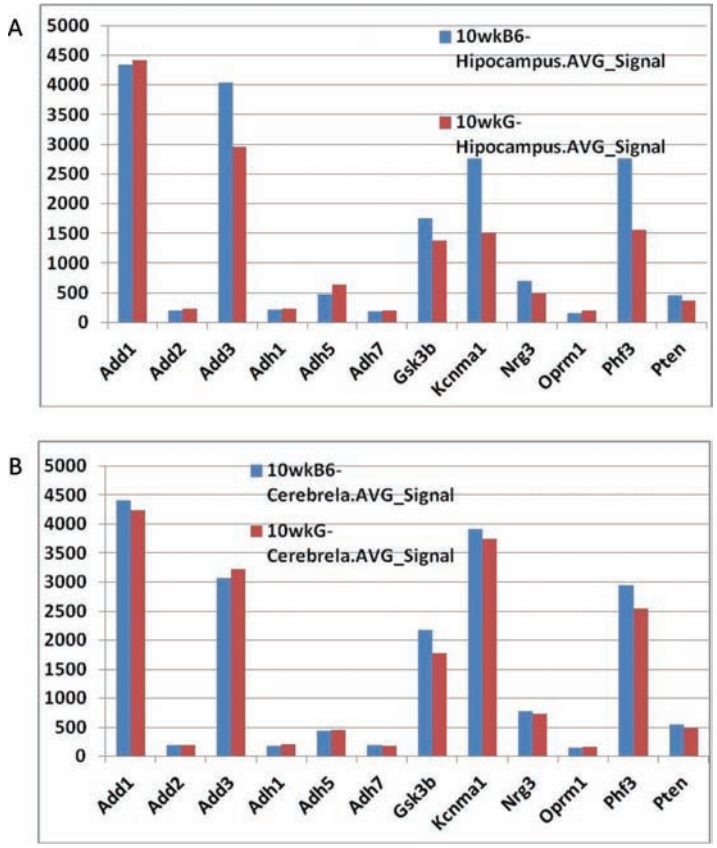

Figure 4. Expression levels of 10 alcoholism-relevant genes between ISCRS strain G and wild-type control, DBA/2. The *y* axis indicates the level of gene expression of a gene. (A) Comparison of gene expression levels between ISCRS strain G and wild-type control in hippocampus. (B). Comparison of gene expression levels between ISCRS strain G and wild-type control in cerebellum.

without knowing the alcohol-relevant function indicated that the correlations of gene expression between liver and brain structures do not agree with the damage to the brain parts from alcohol. Altogether, our data suggest that the morphologic changes in different parts of the brain in alcoholism may be caused by the interaction between alcoholism-relevant genes in liver and in brain. However, we realize that our analysis is at the transcription level; future study on the translational or protein level is necessary.

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REFERENCES

1. Zahr NM, Kaufman KL, Harper CG. Clinical and pathological features of alcohol-related brain damage. Nat Rev Neurol 2011; 7(5):284–294.
2. Fortier CB, Leritz EC, Salat DH, Venne JR, Maksimovskiy AL, Williams V, et al. Reduced cortical thickness in abstinent alcoholics and association with alcoholic behavior. Alcohol Clin Exp Res 2011; 35(12):2193–201.
3. Pitel AL, Chételat G, Le Berre AP, Desgranges B, Eustache F, Beaunieux H. Macrostructural abnormalities in Korsakoff syndrome compared with uncomplicated alcoholism. Neurology 2012; 78(17):1330–1333.
4. Skuja S, Groma V, Smane L. Alcoholism and cellular vulnerability in different brain regions. Ultrastruct Pathol 2012; 36(1):40–47.
5. Wang HJ, Gao B, Zakhari S, Nagy LE. Inflammation in alcoholic liver disease. Annu Rev Nutr 2012; 32:343–368.
6. Rehm J. The risks associated with alcohol use and alcoholism. Alcohol Res Health 2011; 34(2):135–143.
7. Gao B. Hepatoprotective and anti-inflammatory cytokines in alcoholic liver disease. J Gastroenterol Hepatol 2012; 27 Suppl 2:89–93.
8. de la Monte S, Derdak Z, Wands JR. Alcohol, insulin resistance and the liver-brain axis. J Gastroenterol Hepatol 2012; 27 Suppl 2:33–41.
9. Li D, Zhao H, Gelernter J. Further clarification of the contribution of the ADH1C gene to vulnerability of alcoholism and selected liver diseases. Hum Genet 2012; 131(8):1361–1374.
10. Chamorro AJ, Marcos M, Mirón-Canelo JA, Pastor I, González-Sarmiento R, Laso FJ. Association of μ-opioid receptor (OPRM1) gene polymorphism with response to naltrexone in alcohol dependence: A systematic review and meta-analysis. Addict Biol 2012; 17(3):505–512.
11. Wolen AR, Phillips CA, Langston MA, Putman AH, Vorster PJ, Bruce NA, et al. Genetic dissection of acute ethanol responsive gene networks in prefrontal cortex: Functional and mechanistic implications. PLoS One 2012; 7(4):e33575.
12. Zuo L, Zhang CK, Wang F, Li CS, Zhao H, Lu L, et al. A novel, functional and replicable risk gene region for alcohol dependence identified by genome-wide association study. PLoS One 2011; 6(11):e26726.
13. Peirce JL, Lu L, Gu J, Silver LM, Williams RW. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. BMC Genet 2004; 5:7.
14. Bennett B, Beeson M, Gordon L, Carosone-Link P, Johnson TE. Genetic dissection of quantitative trait loci specifying sedative/hypnotic sensitivity to ethanol: Mapping with interval-specific congenic recombinant lines. Alcohol Clin Exp Res 2002; 26:1615–1624.
15. Jiao Y, Zhang J, Yan J, Stuart J, Gibson G, Lu L, et al. Differential gene expression between wild-type and Gulo-deficient mice supplied with vitamin C. Genet Mol Biol 2011; 34(3):386–395.
16. Alfonso-Loeches S, Guerri C. Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. Crit Rev Clin Lab Sci 2011; 48(1):19–47.
17. den Hollander B, Schouw M, Groot P, Huisman H, Caan M, Barkhof F, et al. Preliminary evidence of hippocampal damage in chronic users of ecstasy. J Neurol Neurosurg Psychiatry 2012; 83(1):83–85.
18. Wartolowska K, Hough MG, Jenkinson M, Andersson J, Wordsworth BP, Tracey I. Structural changes of the brain in rheumatoid arthritis. Arthritis Rheum 2012; 64(2):371–379.