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Potential role of *Atp5g3* in epigenetic regulation of alcohol preference or obesity from a mouse genomic perspective

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Abstract

The mitochondrial ATP synthase, subunit c, isoform 3 gene (*Atp5g3*) encodes subunit 9, the subunit of the multisubunit enzyme that catalyzes ATP synthesis during oxidative phosphorylation in mitochondria. According to the Ensembl database, *Atp5g3* in mice is located on chromosome 2 between 73746504 and 73749383 bp, within the genomic regions of two sets of quantitative trait loci - alcohol preference and body weight. Both of those traits are more influenced by epigenetic factors than many other traits are. Using currently available phenotype and gene expression profiles from the GeneNetwork database, we obtained correlations between *Atp5g3* and alcoholism- and obesity-relevant phenotypes. The correlation in expression levels between *Atp5g3* and each of its 12 partner genes in the molecular interaction are different in various tissues and genes. Transcriptome mapping indicated that *Atp5g3* is differentially regulated in the hippocampus, cerebellum, and liver. Owing to a lack of known polymorphisms of *Atp5g3* among three relevant mouse strains, C57BL/6J (B6), DBA/2J (D2), and BALB/ cJ, the molecular mechanism for the connection between *Atp5g3* and alcoholism and body weight requires further investigation.

Keywords

Atp5g3; Alcoholism; Epigenetic; Expression; Mouse; Transcriptome

INTRODUCTION

The mitochondrial ATP synthase, subunit c, isoform 3 gene (*Atp5g3*) encodes subunit 9, the subunit of the multisubunit enzyme that catalyzes ATP synthesis during oxidative phosphorylation in mitochondria. Each ATP synthase complex has multiple copies of

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Conflicts of interest

The authors declare no conflict of interest.

subunit 9 in the transmembrane portion of the complex. Although the molecular mechanisms in the mitochondrial membrane ATP synthase are known, its connection to other pathways or human disorders remains unknown.

According to the Ensembl database, the *Atp5g3* gene in mice is located on chromosome 2, between 73746504 and 73749383 bp. Interestingly, two sets of quantitative trait loci (QTLs) have been mapped into this chromosomal region. One is body weight (Mollah and Ishikawa, 2011) and its relevant traits fat distribution (Kobayashi et al., 2010) and obesity (Jerez-Timaure et al., 2004; Farber and Medrano, 2007). The other is alcohol preference (Ruf et al., 2004; Boyle and Gill, 2008). Both alcoholism and obesity are common, late-onset, chronic disorders that are strongly influenced by epigenetic factors (Buscemi and Turchi et al., 2011; Marti and Ordovas, 2011; Slomko et al., 2012). None of the causal genes from these two QTLs has been determined, although some candidate genes have been named.

Atp5g3 is located in the genomic regions of these QTLs but has not been considered a candidate gene. However, based on limited evidence, we hypothesized that *Atp5g3* involves molecular pathways that regulate the alcohol preference QTL. Li et al. (2001) have reported that all three nuclear gene isoforms (*ATP5G1*, *ATP5G2*, and *ATP5G3*) were consistently upregulated, parallel with mitochondrial injury, in the pancreas of alcohol-consuming rats. No additional reports on their potential functions in regulating alcoholism, body weight, or obesity have been published to date, however. The genes that regulate the alcohol and obesity QTLs remain unknown. Rapid progress in genetic technology has yielded results that suggest an important role for epigenetics in the regulation of disorders and physiologic abnormalities; therefore, examinations of the potential role of *Atp5g3* in the regulation of these 2 QTLs are important.

Rapid accumulation of genomic and bioinformatic data in recent years has provided enough resources to evaluate the potential function of the many genes regulating known phenotypes. For example, by October 5, 2011, the GeneNetwork database (http://www.GeneNetwork.org [accessed October 13, 2012]) had accumulated several hundred records for phenotyping (Williams et al., 2001; Chesler et al., 2004). Although more than 70% of those records were for drug/alcohol addiction and behavior, hundreds of records for other phenotypes have been investigated and added into the GeneNetwork database. An enormous advantage of using these data is the availability of gene expression profiles and transcriptome data from 18 tissues, including those of the brain, liver, kidney, lung, spleen, eye, cartilage, hippocampus, cerebellum, striatum, and neocortex and prefrontal cortex. This review exploits genomic and phenotypic resources from GeneNetwork and others sources to evaluate the possibility that *Atp5g3* is an epigenetic factor for the QTLs of alcoholism or body weight and obesity.

MATERIAL AND METHODS

Atp5g3 expression in various tissues in BXD recombinant inbred mice

Using data from GeneNetwork, we first examined the expression level of *Atp5g3* in the tissues of three organs - the hippocampus, cerebellum and liver - that are relevant to alcoholism. As shown in Figure 1, *Atp5g3* is expressed in all of these tissues. The expression of *Atp5g3* in the hippocampus was obtained from a data set from the

Hippocampus Consortium M430v2 (Overall et al., 2009), which provided estimates of messenger RNA expression in the adult hippocampus from 67 BXD recombinant inbred strains. We used the data set GE-NIAAA Cerebellum messenger RNA M430v2 [http://www.genenetwork.org/webqtl/main.py (accessed October 13, 20120)] to examine the expression level of *Atp5g3* in the cerebellum and the data set GSE16780 UCLA Hybrid MDP Liver Affy HT M430A [http://www.genenetwork.org/dbdoc/GSE16780_UCLA_ML0911.html (accessed October 13, 2012)] to examine the expression level of *Atp5g3* in the liver. The relative expression level in the hippocampus and cerebellum was higher than that in the liver. Moreover, variations occurred among mouse strains in the studied tissues. The variation of expression level among mouse strains in the hippocampus and cerebellum was also larger than that in the liver.

RESULTS AND DISCUSSION

correlation between *Atp5g3* expression level and phenotypes in the GeneNetwork database

We examined the correlation between expression levels of *Atp5g3* and records of phenotypes in three tissues. Current data from a limited number of strains suggested potential associations between *Atp5g3* expression and alcohol- and obesity-relevant phenotypes.

1) Phenotypes and the expression of *Atp5g3* in the hippocampus. Our correlation analysis indicated that gene expression in the hippocampus was negatively correlated with ethanol response (1.75 mg/kg *ip*) according to the time to ataxia measured as loss of balance using a dowel test [loss corresponded to blood ethyl alcohol content time 0 (min)] (Kirstein et al., 2002). As shown in Figure 2A, the correlation was R = −0.6795. By contrast, *Atp5g3* expression was positively correlated with long-term ethanol response measured by chronic withdrawal and handling-induced convulsion score (Crabbe, 1998). As shown in Figure 2B, the correlation was R = 0.6039. In addition, the *Atp5g3* expression in the hippocampus was positively correlated to body weight in CXB (BALB/cBy X C57BL/6By) population strains [http://www.genenetwork.org/dbdoc/CXBPublish.html (accessed October 13, 2012); Figure 2C].

2) Phenotypes and expression of *Atp5g3* in the cerebellum. We found that the expression level of *Atp5g3* was highly positively correlated to ethanol preference in males, as measured by residual mean [g/kg body weight (Fernandez et al., 1999)], with an R score as high as 0.8512 (Figure 3A). Surprisingly, *Atp5g3* was also highly positively related to a set of data marked obesity (R = 0.8433; gudrun.brockmann@agrar.huberlin.de; Figure 3B). However, because detailed information on the parameters of obesity in the data set has been unavailable, it is unclear to which obesity score *Atp5g3* was correlated.

3) Phenotypes and expression of *Atp5g3* in the liver. The expression of *Atp5g3* in the liver was negatively correlated with corticosterone plasma levels measured 7 h after ethanol injection (μg/dL; Figure 4A) (Roberts et al., 1995). It was also negatively correlated with locomotor tolerance or sensitization in males (Figure 4B) (Cunningham, 1995). However, expression of *Atp5g3* was highly positively correlated to the handling-induced convulsion

score 7 h after ethanol injection in males and females (U; 4.0 g/kg *ip*; Figure 4C), R = 0.9553 (Philip et al., 2010). The expression of *Atp5g3* was negatively correlated with the obesity-relevant phenotype measured by Brockmann et al. (http://www.genenetwork.org/webqtl/main.py. Contact information: gudrun.brockmann@agrar.huberlin.de) in males (Figure 4D).

SNP information for c57bl/6j (b6) x dba/2j (d2)

To elucidate a possible molecular mechanism of differential expression, we searched the polymorphisms of *Atp5g3* in relevant mouse strains using the Mouse SNP Query Form in the Mouse Genome Informatics database [http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=snpQF (accessed October 12, 2012)]. Our search used the Gene Symbol/Name *Atp5g3*, and the search region included SNPs located within 2 kb up- or downstream of *Atp5g3*. However, currently no polymorphism in *Atp5g3* has been reported immediately up- or downstream among strains C57BL/6J, DBA/2J, and BALB/cJ.

Our second search was conducted using the National Center for Biotechnology Information Single Nucleotide Polymorphism Database. We searched a genomic region similar to that used in the Mouse Genome Informatics database - between 73744504 bp and 73751383 bp. Again, no SNP was identified

Patterns of expression of *Atp5g3* and its partner genes

Owing to the lack of *Atp5g3* polymorphisms and 2000-bp down- and upstream sequences among three relevant mouse strains, the involvement of *Atp5g3* in the regulation of alcoholism appeared not to be solely attributable to *Atp5g3* itself. A regulator or regulation site(s) may be located more distantly, but such a possibility is difficult to predict with current knowledge. Another possibility is that *Atp5g3* interacts with other genes to influence alcoholism. We then considered the interaction of the *Atp5g3* partner genes based on STRING analysis (Figure 5). As shown, *Atp5g3* interacted with 12 other genes. We examined the expression levels of these genes in the hippocampus, cerebellum, and liver. Figure 6 shows the expression patterns in the hippocampus and liver, which varied greatly in the same tissue. One interesting note is that although the expression of *Atp5g3* in the liver showed less variation than that in the hippocampus, its partner genes showed a variation similar to that in the hippocampus.

Gene interaction among *Atp5g3* and its partner genes

We next examined the co-expression of *Atp5g3* and its 12 partner genes in the hippocampus, cerebellum, and liver. The correlation in expression levels between *Atp5g3* and each of those genes was different (Table 1). The co-expression level of the same gene with *Atp5g3* was also different in different tissues. For example, the expression level of *Uqcrfs1* was positively correlated with *Atp5g3* in both the cerebellum and the liver, but it had no correlation or negative correlation with *Atp5g3* in the hippocampus. Furthermore, different probes of the same gene detected different interactions with *Atp5g3*, most likely because of splicing differences. For example, probes from exons 9, 10, and 11 of *Atp5a1* showed expression patterns similar to those of *Atp5g3* in the three tissues, whereas probes from the distal half of the 3′-untranslated region (UTR) and antisense in the 3′-untranslated region

showed expression patterns that differed from those of *Atp5g3* in the hippocampus, cerebellum, and liver. According to the Ensembl database, *Atp5a1* has five transcripts, three of which have protein products. The lengths of transcripts are different and also vary in the 3′ end.

Transcriptome mapping of *Atp5g3* regulation

To investigate whether *Atp5g3* is regulated differently in different tissues, we analyzed the transcriptome map of *Atp5g3* in the hippocampus, cerebellum, and liver. The data showed that the expression levels of *Atp5g3* in the three tissues were regulated differently. Transcriptome maps with 2000 permutation tests showed significant difference among tissues (Figure 7). In the hippocampus, the permutation test indicated that transcriptome loci were significant at a log odds ratio (LOD) of 3.64 and suggestive at LOD 2.27. The expression of *Atp5g3* was regulated mainly by three loci located on chromosomes 4, 5, and 12. In the cerebellum, the permutation test indicated that the significant level was at LOD 3.77 and the suggestive level at LOD 2.32. According to these criteria, two loci on chromosome 8 regulated *Atp5g3* expression: one reached the significance level, whereas the other was at the suggestive level. In the liver, the permutation test indicated that the significant level was LOD 3.60 and the suggestive level was LOD 2.20. One locus on chromosome 1 reached the significance level. In addition, several loci at the suggestive level were detected on chromosomes 1, 9, 12, and 18. The differential regulation of *Atp5g3* expression in various tissues seemed to agree with the fact that *Atp5g3* is correlated with different phenotypes of alcoholism in those tissues.

CONCLUSION

Our current information suggests that *Atp5g3* involves the regulation of alcoholism. However, it is unclear whether the involvement is direct or indirect (through its partner genes). Two findings suggest the involvement of *Atp5g3* in alcoholism regulation. The first finding is the correlation between *Atp5g3* expression and the alcoholism-related phenotypes in three alcoholism-relevant tissues. The second finding is the chromosome location of *Atp5g3*, which is within the fine-mapped region of a QTL for the alcohol preference phenotype. The lack of a known polymorphism between the B6 and D2 strains negates direct involvement of *Atp5g3* in alcoholism, although it cannot be completely ruled out. The fact that *Atp5g3* is a relatively small gene encoding only one transcript with one protein sequence makes the possibility of direct involvement of *Atp5g3* in regulating alcoholism small.

The complexity comes from the interactions between *Atp5g3* and its partner genes. Those interactions are different among not only different genes but also different tissues. Two findings also allow the speculation that *Atp5g3* involves different pathways in different tissues in regulating alcoholism: The first finding is the difference in correlations between *Atp5g3* and its partner genes in different tissues. The second finding is the difference in transcriptome maps that reveal the regulation of *Atp5g3* expression in different tissues. However, these conclusions are purely speculative, not definitive connections between *Atp5g3* and alcoholism and molecular pathways.

In conclusion, the function of *Atp5g3* in the alcoholism pathway needs further investigation. In particular, future studies should focus on the genomic regulation of *Atp5g3* genes and the evidence of its connection to alcoholism. Its role in obesity at present is unknown, although current data suggest that it may be involved. Any breakthrough on either an association with obesity or a potential molecular pathway will greatly improve current knowledge of the function of *Atp5g3* in obesity.

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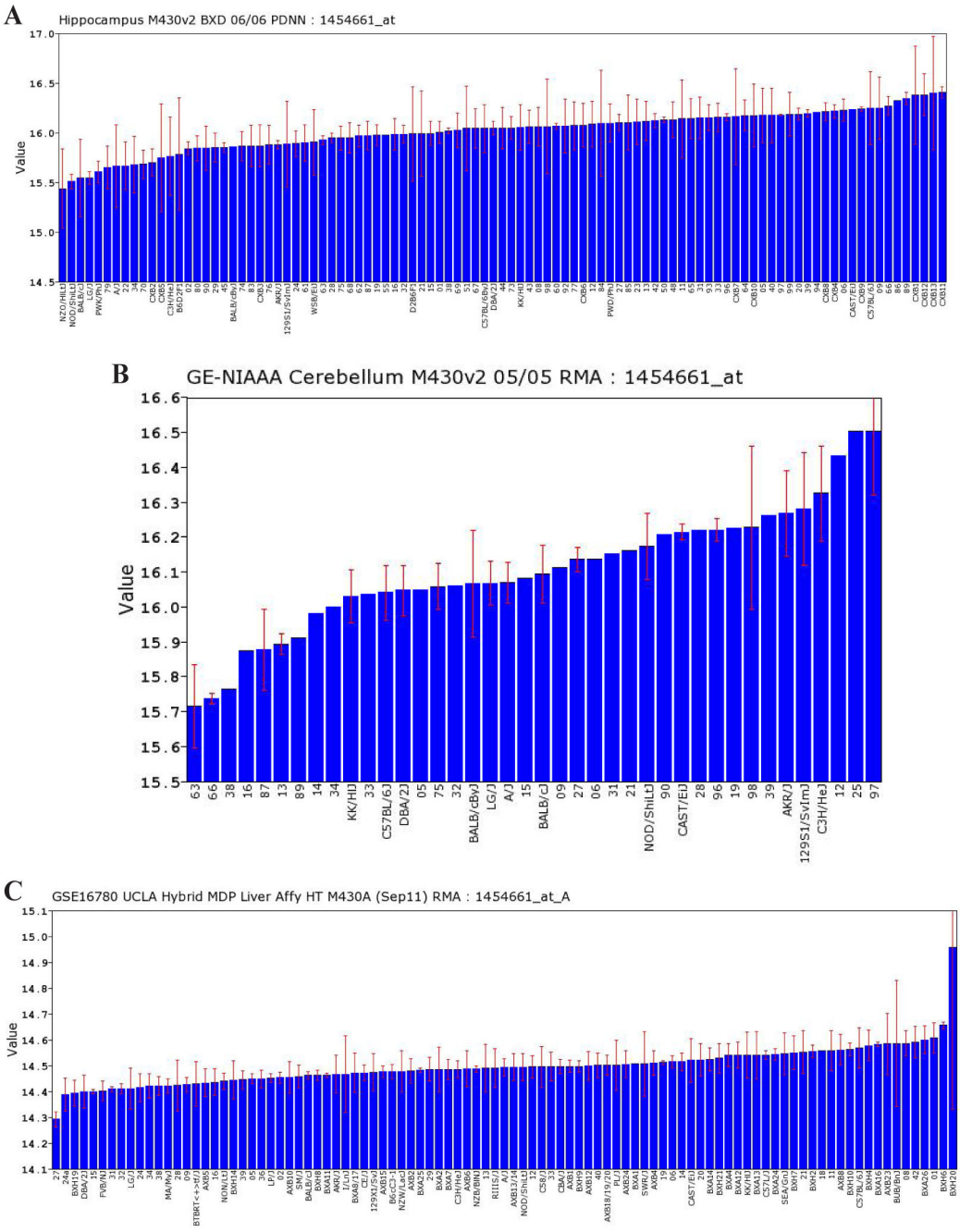

Figure 1.

Bar graphs of expression levels of *Atp5g3* in three different tissues in different strains of mice. Expression levels are relative LOG values as explained in GeneNetwork (http://www.genenetwork.org/webqtl/main.py). **A.** Hippocampus; **B.** cerebellum; **C.** liver.

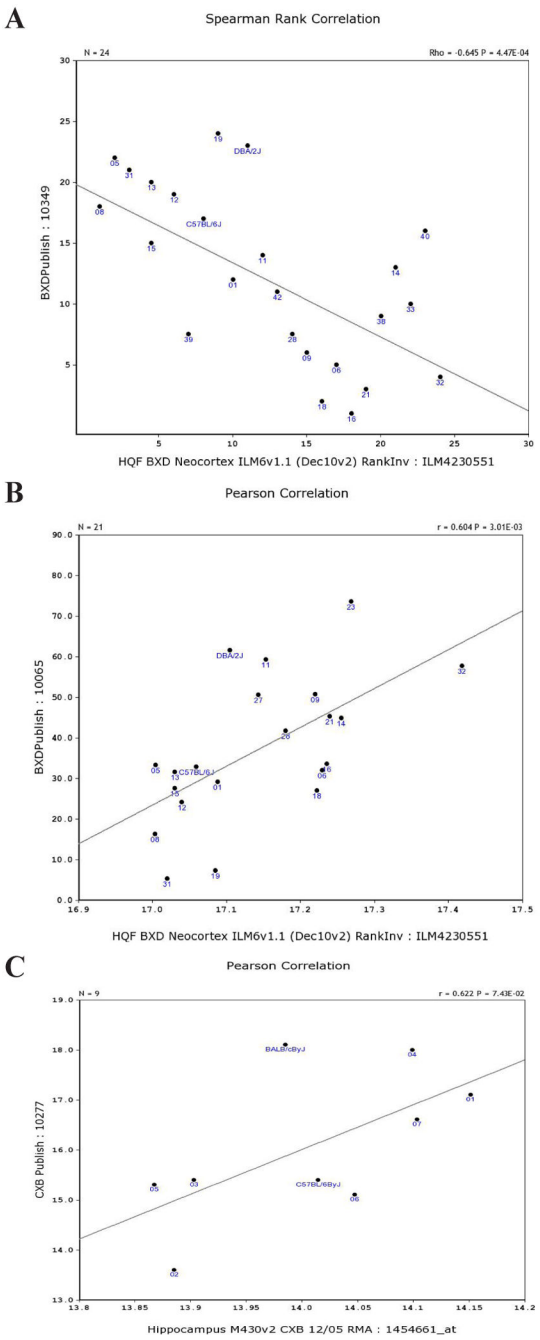

Figure 2.

Correlations between *Atp5g3* expression levels in hippocampus and phenotypes. Spearman rank order correlations were obtained between the *Atp5g3* expression levels and phenotypes in the GeneNetwork database. **A.** Response negatively correlated to ethanol (1.75 mg/kg ip), according to the time to ataxia. **B.** Positive correlation with long-term ethanol response measured by chronic withdrawal. **C.** Positively correlated to body weight.

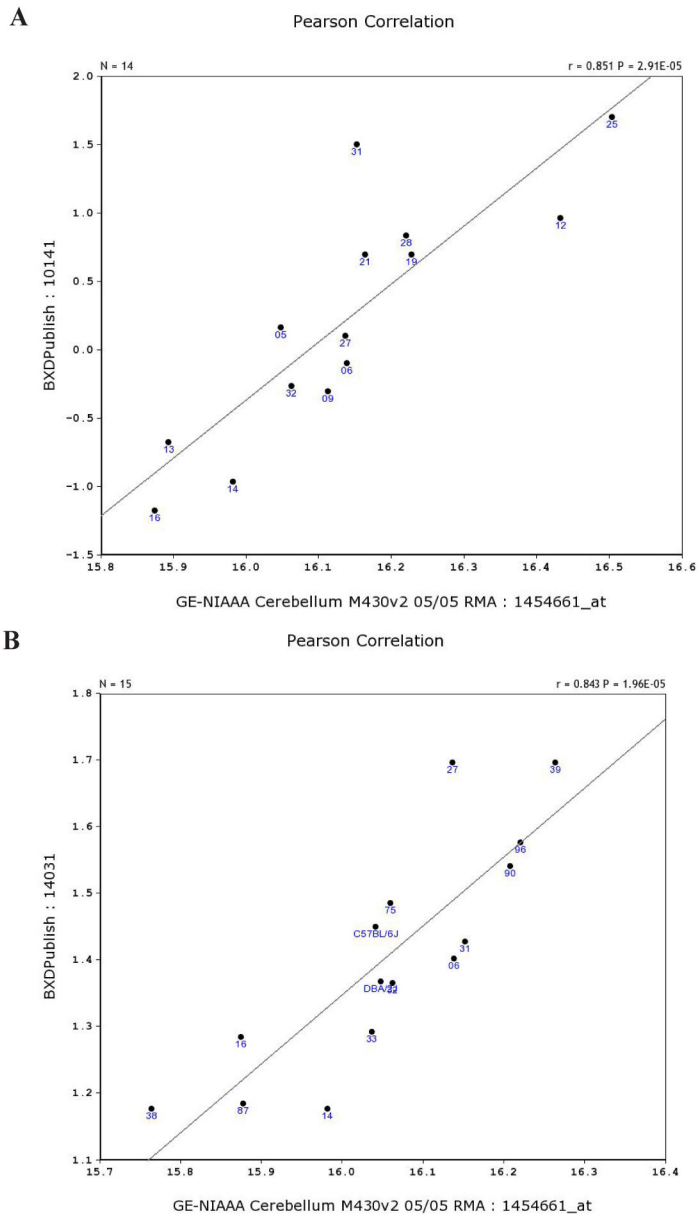

Figure 3.

Correlations between *Atp5g3* expression levels in cerebellum and phenotypes. Spearman rank order correlations were obtained between the *Atp5g3* expression levels and phenotypes in the GeneNetwork database. **A.** Positively correlated to ethanol preference in males. **B.** Positively related to obesity.

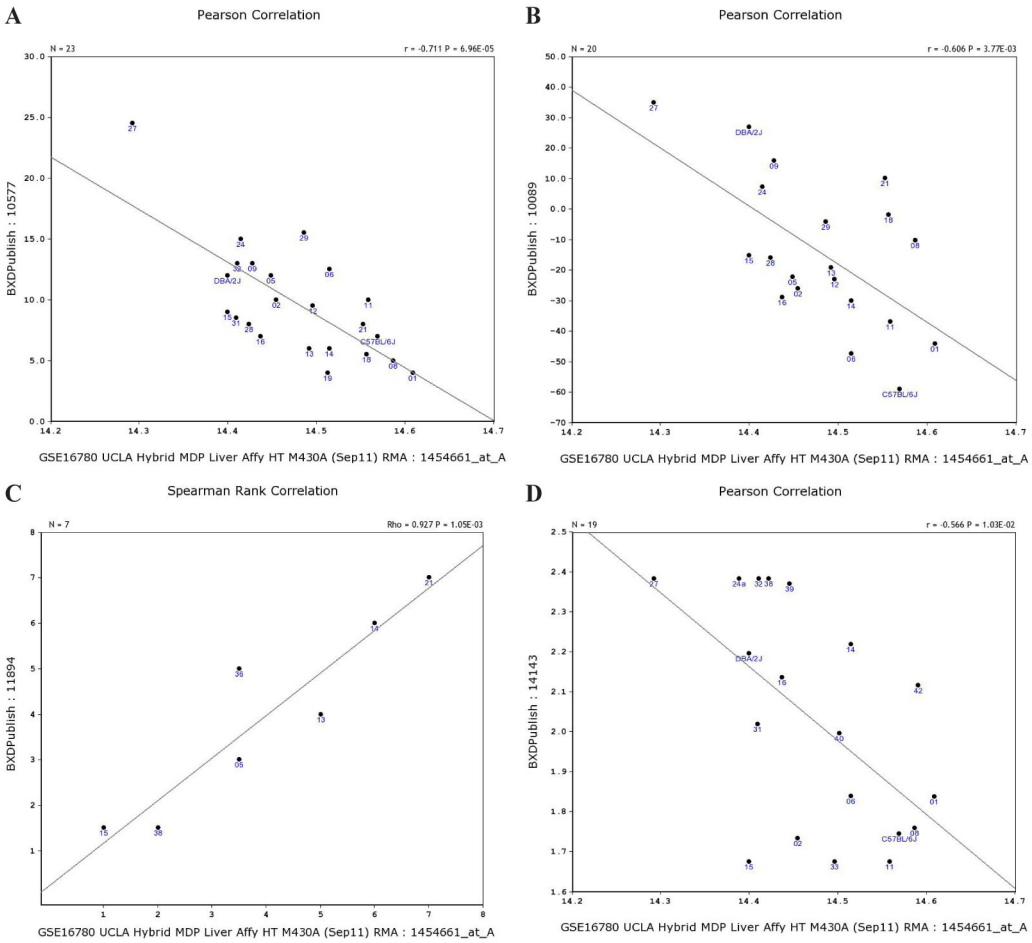

Figure 4.

Correlations between *Atp5g3* expression levels in liver and phenotypes. Spearman rank order correlations were obtained between the *Atp5g3* expression levels and phenotypes in the GeneNetwork database. **A.** Negatively correlated to the corticosterone plasma level measured 7 h after ethanol injection. **B.** Negatively correlated locomotor tolerance or sensitization in males. **C.** Positively correlated to handling-induced convulsion (HIC) score 7 h after ethanol injection. **D.** Negatively correlated to obesity.

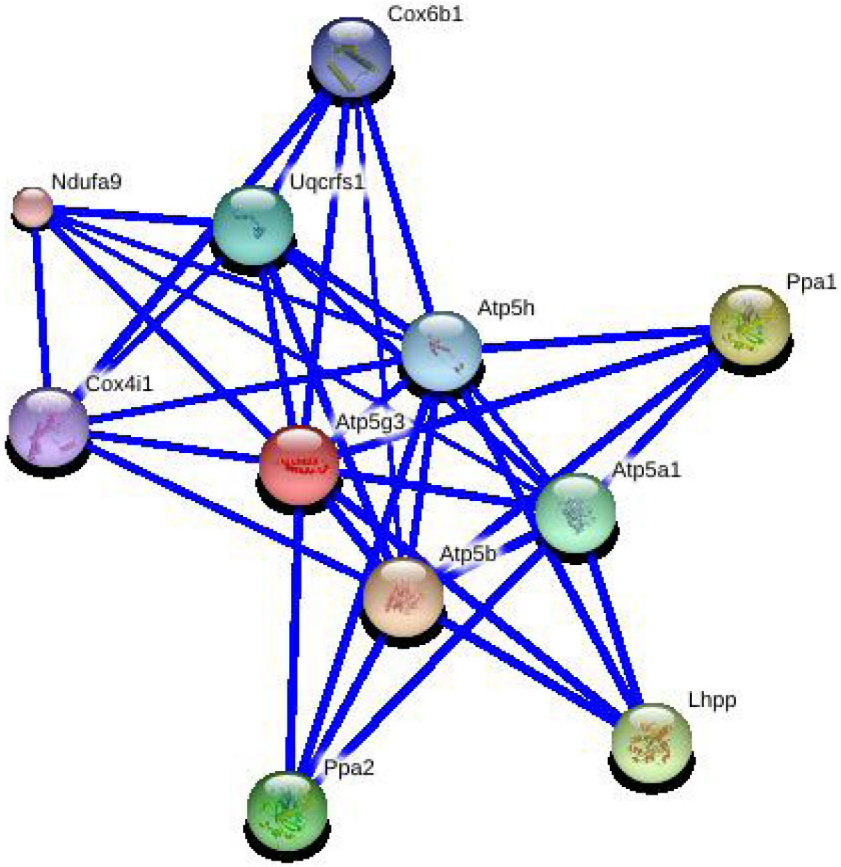

Figure 5.

Interaction partner genes of *Atp5g3* based on STRING analysis (http://string.embl.de/newstring_cgi/show_network_section.pl?input_query_species=auto_detect&network_flavor=confidence&identifier=ENSMUSG00000018770). Associations among *Atp5g3* and its partner genes: atp5a1, atp5b, atp5h, ppa1, ppa2, cox6b1, cox4il, lhpp, ndufa9, or uqcrfs1. Stronger associations are represented by thicker lines.

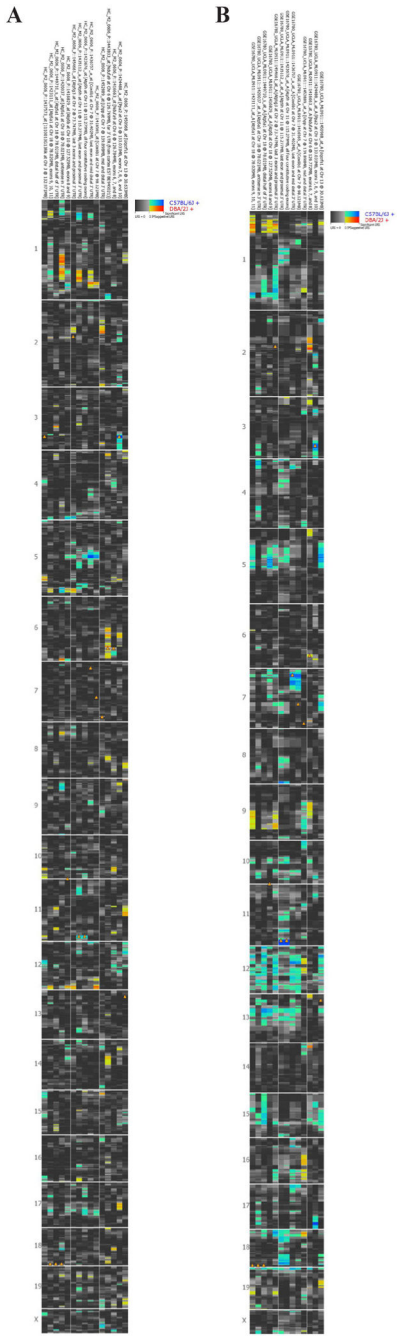

Figure 6.

Heat maps of *Atp5g3* and its partner genes in hippocampus and liver. Left = hippocampus; right = liver.

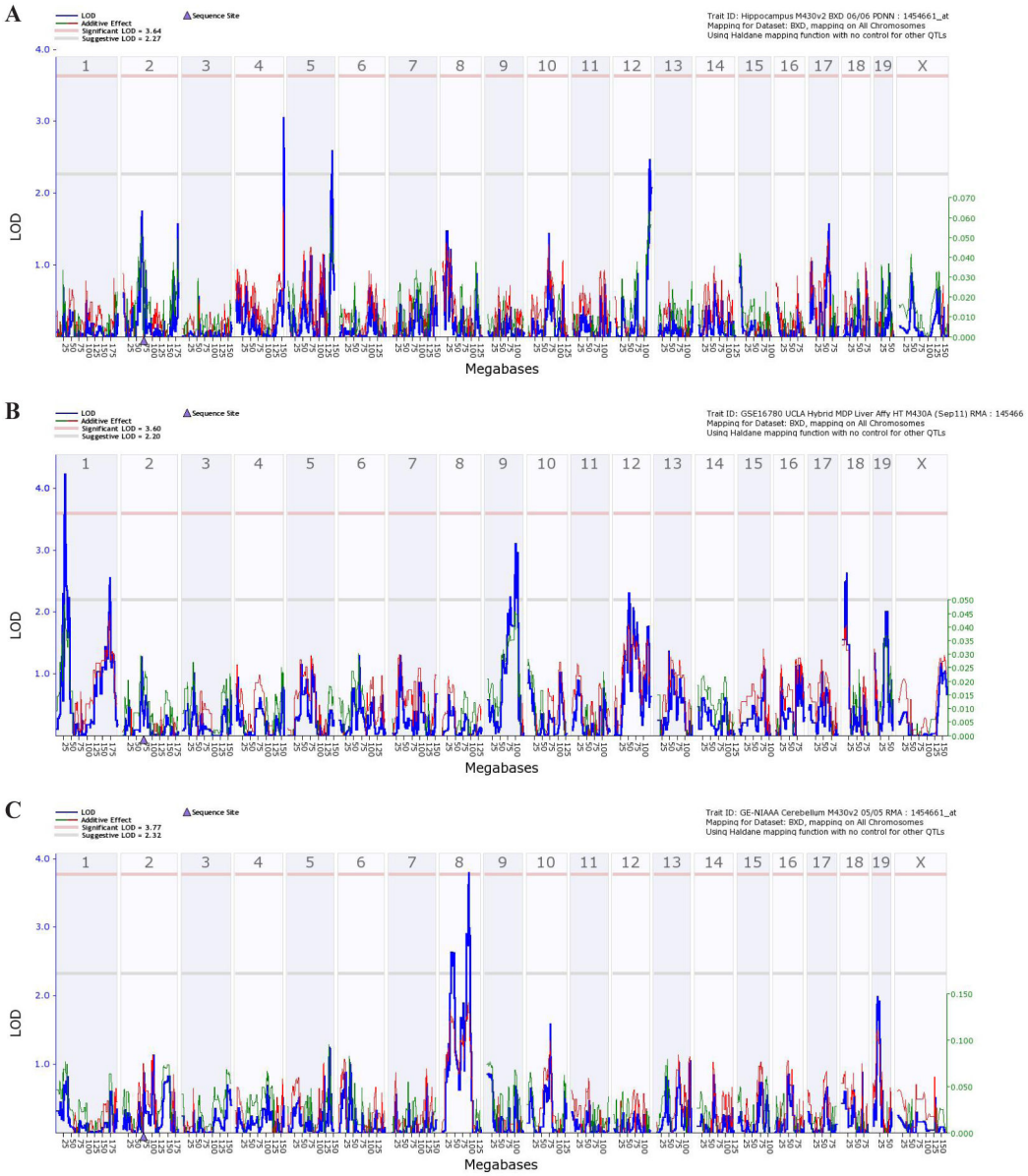

Figure 7.

Transcriptome map of *Atp5g3* in hippocampus, cerebellum, and liver. Transcriptome maps were done with 2000 permutation tests. **A.** Hippocampus; **B.** cerebellum; **C.** liver.

Table 1

Correlation of gene expression between *Atp5g3* and its partners.

Gene/Probes	Hippocampus/72		Cerebellum/30		Liver/32	
	Expression level	R	Expression level	R	Expression level	R
1110013G13Rik/ Ppa2	6.238	−0.361	5.207	−0.545	-	-
Atp5a1 exons 9, 10, 11	15.245	0.717	15.941	0.557	13.693	0.772
Atp5a1 distal half of 3′-UTR	14.769	0.24	14.348	0.61	12.915	0.775
Atp5a1 antisense in 3′-UTR	7.895	−0.315	7.858	0.238	7.234	0.43
Atp5b exons 8 and 9	15.497	0.697	16.144	0.578	14.033	0.557
Atp5g3 last 3 exons and proximal 3′-UTR	16.013	N	16.106	N	14.496	N
Atp5h last exon and proximal 3′-UTR	15.406	0.143	14.342	0.554	13.286	0.41
Atp5h all four constitutive coding exons	14.978	0.385	15.123	0.756	12.973	0.453
Cox6b1 last exon and mid distal 3′-UTR	15.834	0.277	14.89	0.63	14.069	0.638
Cox6b1	15.092	0.377	14.77	0.699	13.724	0.788
Lhpp mid and distal 3′-UTR	10.049	0.19	9.167	0.209	11.193	−0.463
Ndufa9 far 3′-UTR (from cortex EST BE945021)	9.233	−0.276	8.747	−0.147	-	-
Ndufa9 exons 6, 7, and 8	12.656	−0.057	11.929	0.042	12.468	0.478
Ppa2 exons 7, 8, 9, and 10	9.557	−0.384	9.539	−0.156	10.372	−0.027
Uqcrfs1	13.215	−0.065	13.438	0.61	13.075	0.671