Identification of LOC338963 and AP3B2 as Potential Biomarkers for Lambert-Eaton Myasthenic Syndrome

Fei Yang
Chinese PLA General Hospital
Feng Jing
Chinese PLA General Hospital
Yang Li
Chinese PLA General Hospital
Shanshan Kong
Chinese PLA General Hospital
Shimin Zhang
Chinese PLA General Hospital
Yunyun Huo
Chinese PLA General Hospital
Xusheng Huang
Chinese PLA General Hospital
Shengyuan Yu (yusy1963@126.com)
Chinese PLA General Hospital https://orcid.org/0000-0001-8933-088X

Research

Keywords: Lamber-Eaton myasthenia syndrome, lncRNA, mRNA, NR_031439, AP3B2

Posted Date: March 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-332560/v1

Abstract

Background: Lambert-Eaton myasthenic syndrome (LEMS) is a rare neuromuscular junction disorder associated with muscle weakness and small-cell lung cancer. Here, we used microarray analysis to identify long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs) that might serve as biomarkers for LEMS.

Methods: Plasma lncRNA and mRNA expression profiles of three patients with paraneoplastic LEMS and three healthy controls were analyzed using Arraystar Human lncRNA Microarray v4.0. Differentially expressed lncRNAs and adjacent mRNAs were analyzed jointly, and candidates were verified in individual samples by quantitative real-time polymerase chain reaction (qRT-PCR). The identified lncRNAs and mRNAs were evaluated in nine patients with paraneoplastic LEMS, eight patients with non-tumor LEMS, and four patients with small cell lung cancer (SCLC).

Results: A total of 320 lncRNAs were differentially expressed in patients with paraneoplastic LEMS compared to healthy controls (fold change >1.5, $P < 0.05$), and nine were further evaluated. One of the identified lncRNAS, LOC338963 (NR_031439), is known to regulated the expression of the mRNA AP3B2, and both were upregulated more than 2-fold in patients with paraneoplastic LEMS compared to healthy controls. Furthermore, qRT-PCR analysis revealed significant upregulation of LOC338963 (NR_031439) and AP3B2 expression in patients with paraneoplastic LEMS compared to those with either non-tumor LEMS (2.37- and 5.06-fold, respectively) or SCLC (4.36- and 14.97-fold, respectively).

Conclusions: Plasma LOC338963 (NR_031439) and AP3B2 were found to be upregulated in LEMS and might be used as diagnostic biomarkers for this disease.

Background

Lambert-Eaton myasthenic syndrome (LEMS) is a rare neuromuscular junction (NMJ) conduction disorder. The pathogenesis of LEMS involves the binding of autoantibodies to voltage-gated calcium channels (VGCCs) of motor nerve endings, which leads to a decrease in the release of acetylcholine, and clinical weakness[1, 2]. Approximately 60% of patients with LEMS have tumors[3], mostly small-cell lung cancer (SCLC), which is a type of lung cancer with neuroendocrine characteristics.

Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that are not translated into proteins[4]. However, they are able to regulate mRNA and proteins synthesis, thus playing important roles in biological regulatory networks[4]. About 90% of the susceptibility genes associated with autoimmune diseases are located in non-coding regions, and approximately 10% are located in lncRNAs[5]. lncRNAs are widely expressed in monocytes, dendritic cells, neutrophils, T cells, B cells, and other immune cells[6, 7]. They are involved in the occurrence and development of autoimmune diseases such as rheumatoid arthritis and Sjögren's syndrome[8-11].

To identify lncRNAs and mRNA that might serve as biomarkers for LEMS, we screened individual plasma samples from patients with paraneoplastic LEMS and healthy controls using a lncRNA/mRNA microarray. We further validated our findings with quantitative real-time polymerase chain reaction (qRT-PCR) analysis of samples obtained from patients with either paraneoplastic LEMS, non-tumor LEMS, or SCLC. From these analyses, we identified the lncRNA LOC338963 (NR_031439) and the mRNA AP3B2 as potential biomarkers for paraneoplastic LEMS.

Methods

Standard protocol approval, registration, and informed consent

The Ethics Committee of PLA General Hospital approved human specimen acquisition (IEC# S2020-073-02). All participants were provided with thorough information before signing their consent forms.

Study population

Three patients with LEMS and advanced-stage SCLC were assigned to the experimental group (LEMS) and three healthy age- and sex-matched volunteers were assigned to the control group (C). The standard clinical manifestations of the LEMS group included[12]: (1) weakness of the proximal limb; (2) decrease or disappearance of tendon reflex; (3) possible autonomic nerve dysfunction; (4) compound action muscle potential (CMAP) amplitude increased by ≥100% in high-frequency repetitive nerve stimulation (HF-RNS) tests (20–50 Hz); and (5) presence of a tumor with consistent tumor stage. The exclusion criteria were as follows: (1) patients and volunteers who had received immunotherapy such as immunoglobulins and glucocorticoid; (2) patients and volunteers who refused to participate or dropped out from the study; and (3) the absence of detailed clinical data.

Blood collection

Blood samples (5 mL) were drawn into EDTA-coated anticoagulant tubes. The anticoagulant blood was added into centrifuge tube and then erythrocyte lysis buffer was added and mixed. The supernatant was removed after centrifugation. RNA samples were prepared after separation, sedimentation, washing and dissolution. The concentration and purity of RNA were determined using ultraviolet absorption spectrophotometry.

Microarray-based lncRNA and mRNA expression profiling

Arraystar Human lncRNA/mRNA Microarray v4.0 testing was performed by KangChen Biotech (Shanghai, China). First, mRNA was purified from total RNA using mRNA-ONLY™ Eukaryotic mRNA Isolation Kit, (Epicenter Inc, USA). Second, each sample was amplified and transcribed into fluorescently labeled complementary RNAs, which were used as probes to hybridize onto the microarray chip. Third, RNA quantity and quality were monitored using NanoDrop ND-1000 (Thermo Inc., USA). Finally, Agilent Feature Extraction software (v11.0.1.1) was used to analyze the acquired array images. GeneSpring GX v12.1 software (Genomax Technologies, Singapore) was used to import files containing the original signal of probes. After quantile normalization, low-quality probes were removed, and lncRNA expression data were obtained. Differential expression of lncRNAs and mRNAs was considered statistically significant at $P < 0.05$ using a cut-off point of 1.5-fold between the two groups.

Gene ontology and pathway analysis

Gene Ontology (GO) enrichment and pathway analysis was performed to observe the functional characteristics of differentially expressed genes. GO was performed using Functional Annotation Tool Database for Annotation, Visualization and Integrated Discovery (DAVID 6.8) (http://david.abcc.ncifcrf.gov/summary.jsp). GO is an international standard classification system of gene function that is divided into three parts: Molecular Function, Biological Process, and Cellular Component. The mRNA differential expression profile data were analyzed using top GO for GO analysis of differential mRNAs to infer their molecular functions. Significant GO terms were identified when $P < 0.05$. Pathway analysis of differentially expressed genes was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg).

Independent validation of differentially expressed plasma lncRNAs and neighboring genes

First, the microarray chip was used to detect the remaining total RNA for cDNA synthesis. Second, a gradient dilution DNA template was prepared in order to draw the standard curve. Third, quantitative real-time polymerase chain reaction (qRT-PCR) was performed for the target genes and housekeeping genes of each sample. Finally, the concentration of target genes and housekeeping genes was calculated according to the gradient diluted standard curve which had been prepared in the second step. The concentration of the target gene in each sample was divided by the concentration of its housekeeping gene, and the corrected relative content of this gene in the sample was obtained.

Expanded sample size validation of differentially expressed plasma lncRNAs and adjacent neighboring genes

For further validation, qRT-PCR was performed for the target and housekeeping genes of nine patients with paraneoplastic LEMS, eight with non-tumor LEMS and four with SCLC (extensive stage).

Results

Clinical characteristics of the study population

The clinical characteristics of the LEMS and control groups are presented in Table 1.

Table 1 Clinical characteristics of patients with LEMS and healthy controls

Subject ID	Age, years	Sex	Cerebellar signs	Presence of a tumor	Treated
LEMS1	56	Male	Negative	SCLC	Negative
LEMS2	55	Male	Negative	SCLC	Negative
LEMS3	54	Male	Negative	SCLC	Negative
N1	52	Male	Negative	Negative	Negative
N2	53	Male	Negative	Negative	Negative
N3	54	Male	Negative	Negative	Negative

LEMS: Lambert-Eaton myasthenic syndrome; N: number, healthy control; SCLC: small-cell lung cancer

Differentially expressed lncRNAs and mRNAs

A total of 34,832 lncRNAs were detected. Among them, 320 lncRNAs were differentially expressed in the LEMS group compared to the control group. The number of differentially upregulated lncRNAs was 197, with ENST00000411554 being the most significantly upregulated at 3.67-fold. The number of differentially downregulated lncRNAs was 123, with ENST00000538934 being the most significantly downregulated (fold change = 4.29).

A total of 168 differentially expressed mRNAs were identified in the LEMS group compared to the control group. The number of differentially upregulated mRNAs was 132, with uc003tgl.2 being the most significantly upregulated (fold change = 3.92). The number of differentially downregulated mRNAs was 36, with the 10.69-fold reduction in NM_004345 being the most significant downregulation (Figure 1). Based on the values of -log10 (P-value) and -log2 (fold change) of differentially expressed lncRNAs and mRNAs, volcano maps were drawn (Figure 2).

Using joint analysis, we have found that 9 different lncRNA and their 11 neighboring mRNAs were differentially expressed in the LEMS group compared to the control group (Table 2). We considered mRNAs within 300 KB upstream or downstream of the lncRNA as their neighboring mRNAs. The results of joint analysis of differentially expressed lincRNAs and neighboring mRNAs are showed in Table 3.

Table 2 Analysis of differentially expressed lincRNAs and neighboring mRNAs between patients with LEMS and healthy controls

lncRNA	Gene symbol	Fold change	P-value	Regulation	Relative position	mRNA	Nearby gene symbol
T357796	G084427	2.02	0.049	Up	Downstream	NM_003289	TPM2
ENST00000427872	RP11-439A17.4	1.97	0.028	Up	Upstream	NM_001017986	FCGR1B
NR_024397	NUTM2A-AS1	1.76	0.041	Up	Upstream	NM_004897	MINPP1
ENST00000452525	AC022201.4	1.64	0.045	Up	Upstream	NM_022173	TIA1
NR_034139	LOC338963	1.63	0.029	Up	Downstream	NM_004644	AP3B2
NR_034139	LOC338963	1.63	0.029	Up	Upstream	NM_001007122	FSD2
ENST00000538934	RP11-807H22.6	4.29	0.032	Down	Downstream	NM_000802	FOLR1
ENST00000538934	RP11-807H22.6	4.29	0.032	Down	Upstream	ENST00000442948	FOLR3
ENST00000422305	RP11-399E6.1	1.76	0.0169	Down	Upstream	NM_001031694	SCMH1
T153963	G035671	1.62	0.045	Down	Downstream	NM_001080466	BTBD17
NR_120479	LOC101930452	1.51	0.050	Down	Downstream	NM_002258	KLRB1

Validation of microarray results by qRT-PCR

Among the mRNAs differentially expressed in LEMS patients, AP3B2 and the protein it synthesizes are known to be abnormally expressed in some nervous system diseases. Therefore, we focused on AP3B2 and its neighboring lncRNA LOC338963 (NR_034139) for further analysis. We used qRT-PCR to validate the microarray data using primers in the University of California, Santa Cruz database that were synthesized by Shanghai Xianjun Biotechnology Co., Ltd (Shanghai, China) (Table 3). LOC338963 (NR_034139) and AP3B2 were upregulated by 2.07- and 2.15-fold, respectively, in the LEMS group compared to the control group (Figure 3).

Table 3 PCR primers for LOC338963 (NR_034139) and AP3B2

Gene name	Primers
LOC338963	F: 5' GGCTACTCGGGGATAAAGACTG 3'
	R: 5' GTCCGTCGCTTCGCTCACT 3'
AP3B2	F: 5' CACCTGTCGGAGTGACCACATT 3'
	R: 5' GTGCCAATCACCATTTTCTCG 3'
b-actin (H)	F: 5' GTGGCCGAGGACTTTGATTG 3'
	R: 5' CCTGTAACAACGCATCTCATATT 3'

F: forward; R: reverse

We further verified the differential expression of LOC338963 (NR_034139) and AP3B2 using qRT-PCR on blood samples taken from patients with either paraneoplastic LEMS (nine patients), non-tumor LEMS (eight), or SCLC (four) (Table 4). LOC338963

(NR_034139) and AP3B2 were significantly upregulated in the paraneoplastic LEMS group compared to both the non-tumor LEMS and SCLC groups (Figure 4). LOC338963 (NR_034139) and AP3B2 were expressed at 2.34- and 5.06-fold, and 4.36- and 14.97-fold higher levels in patients with paraneoplastic LEMS than in the comparative groups, respectively.

Table 4 Clinical characteristics of patients with P-LEMS, N-LEMS and SCLC

	P-LEMS	N-LEMS	SCLC
Number of patients	9	8	4
Age (average ± SD)	65y±10	48y±17	63y±6
Sex (female/male)	5/4	4/4	1/3
Tumor Classification/number	SCLC/4		SCLC/4
	liver cancer/3		
	carcinoma of stomach/1		
	thymic tumor/1		

LEMS: Lambert-Eaton myasthenic syndrome; P-LEMS: paraneoplastic LEMS; N-LEMS: non-tumor LEMS; SCLC: small cell carcinoma of lung

GO and KEGG analysis

GO analysis of differentially expressed mRNAs revealed the following categories: immune system processes, tissue development, cellular defense response, natural killer cell-mediated immunity, and other functional pathways. Figures 5 and 6 show the top ten mRNAs according to the enrichment scores for Molecular Function (blue), Biological Process (red), and Cellular Component (green).

To infer the pathways in which the mRNAs participated, we performed KEGG enrichment analysis (Table 5 and Figure 7-8). The number of downregulated mRNAs was relatively small, so there were fewer enrichment pathways compared to those related to the upregulated mRNAs. KEGG analysis indicated that lncRNAs were mostly related to 12 biological information pathways, mainly focusing on antigen processing and presentation, natural killer cell-mediated cytotoxicity, longevity regulation, oxytocin signaling pathway, primary immune deficiency, glutathione synapse, salt resistance, and endocytosis.

GO analysis of AP3B2 and its interacting proteins showed that the main Biological Process enrichment terms were anterograde synaptic vesicle transport (Enrichment Score =15.80410035) and establishment of synaptic vesicle localization (Enrichment Score =12.86966623). The Cellular Component enrichment terms were AP-type membrane coat adaptor complex (Enrichment Score = 14.29073004) and axon cytoplasm (Enrichment Score = 14.19449914). The Molecular Function enrichment term was transporter activity (Enrichment Score = 1.468521083). KEGG analysis of AP3B2 and its interacting proteins revealed that AP3B2 was enriched into Lysosome pathways (Figure 9).

Table 5 Significantly different KEGG pathways in patients with LEMS versus healthy control

Pathways	Genes	P-value
Antigen processing and presentation	CD8B//KLRC1//KLRC2//KLRC3//KLRD1	0.00
Natural killer cell-mediated cytotoxicity	KLRC1//KLRC2//KLRC3//KLRD1//KLRK1	0.00
Malaria	CD40LG//KLRB1//KLRK1	0.00
Glutamatergic synapse	ADCY1//HOMER3//PLA2G4C//SLC1A7	0.01
Longevity regulatory pathway	ADCY1//IRS1//PRKAB2	0.01
Oxytocin signaling pathway	ADCY1//CD38//PLA2G4C//PRKAB2	0.01
Longevity regulatory pathway	ADCY1//IRS1//PRKAB2	0.02
Primary immunodeficiency	CD40LG//CD8B	0.02
Hematopoietic cell lineage	CD38//CD8B//FCGR1A	0.02
Graft-versus-host disease	KLRC1//KLRD1	0.03
Antifolate resistance	FOLR1//FOLR3//IZUMO1R	1.42
Endocytosis	FOLR1//FOLR3//IZUMO1R	0.01

Discussion

Using microarray-based expression profiling, we identified differentially expressed lncRNA and mRNA (including LOC338963 (NR_031439) and mRNA AP3B2) in LEMS patients. We verified the upregulation of LOC338963 (NR_031439) and mRNA AP3B2 in patients with LEMS using qRT-PCR. Furthermore, joint lncRNA and mRNA analysis showed that AP3B2 was the neighboring mRNA of LOC338963 (NR_031439) lncRNA. Through GO and KEGG pathway enrichment analysis we predicted that AP3B2 might be involved in the pathogenesis of LEMS by participating in the lysosomal pathway. Thus, we have uncovered that plasma LOC338963 (NR_031439) and AP3B2 might be useful as potential biomarkers for LEMS.

More than 70% of the human genome is transcribed and most of the transcribed DNA encodes lncRNAs[13]. Though the function of most lncRNAs have not been determined, many have been shown to play diverse roles in gene transcription and protein regulation. They can control target genes expression at the transcriptional and post-transcriptional level[14]. In fact, lncRNAs play critical roles in nearly all biological systems, including the immune system. Autoimmune diseases refer to heterogeneous disorders caused by immune responses to autoantigens. Increasing data has found that lncRNAs are important regulator of innate and acquired immune response, though the specific mechanisms are still unclear[15, 16]. lncRNAs might regulate the immune system through several mechanisms and participate in human autoimmune disease[17, 18]. Although the clinical features and pathogeneses of each autoimmune diseases are considerably different, lncRNAs have recently been shown to regulate the immune system and might specifically participate in these diseases[18, 19]. Indeed, lncRNAs have been found to play critical roles in the pathogenesis of human autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes mellitus[18]. However, no previous studies have linked lncRNAs with LEMS.

In this study, we used microarray-based expression profiling technology, which can simultaneously detect lncRNAs and mRNAs, to explore RNAs changes. A total of 320 lncRNAs (197 upregulated and 123 downregulated) and 168 mRNAs (132 upregulated and 36 downregulated) were found to be differentially expressed in patients with LEMS compared to healthy controls. To infer the function of lncRNAs, their neighboring mRNAs (< 300 kb) are typically selected for joint analysis[25]. We used this approach to study 9 different lncRNAs and 11 neighboring mRNAs. We predicted that the 11 mRNAs might be associated with the pathogenesis of LEMS. Then GO and KEGG enrichment analysis was performed to further study the function of the differentially expressed mRNAs. GO enrichment analysis revealed the following categories: immune system processes, tissue development, cellular defense response, natural killer cell-mediated immunity, and other regulatory and control functions. KEGG enrichment analysis revealed that the 11 mRNAs may be associated with 12 biological information pathways, mainly focusing on antigen

processing and presentation, natural killer cell-mediated cytotoxicity, longevity regulation, oxytocin signaling pathway, primary immune deficiency, glutathione synapse, salt resistance, and endocytosis. In summary, these analyses indicated that the overall differential mRNA expression in patients with LEMS was mainly related to immune regulation and response.

The main autoimmune attack target of LEMS is P/Q type VGCC[20, 21]. Interestingly, we found that among the proteins encoded by the selected 11 mRNAs, AP3B2 was included. AP3B2 is a heterotrimeric protein complex that is involved in the formation of cypermethrin-encapsulated synaptic vesicles[30]. The encoded subunit binds to cypermethrin and is phosphorylated by casein kinase-like protein, which mediates the assembly of the synaptic vesicle shell. Its related pathways include ataxia mutant proteins and lysosomes. AP3B2 is highly expressed in the nervous system of normal human tissues and is considered to serve neuron-specific functions[31]. The diseases related to AP3B2 include epileptic encephalopathy[32], systemic lupus erythematosus[33], and immune cerebellar ataxia[34]. AP3B2 is also related to gait abnormalities caused by immune-related nervous system damage[36]. Patients with paraneoplastic LEMS are prone to immune-related cerebellar ataxia, which may be accompanied by P/Q or N VGCC antibodies. Due to these similarities between LEMS and AP3B2 related diseases, we predicted that AP3B2 might be related to LEMS. To the best of our knowledge, this is the first study involving AP3B2 and LEMS. Our study establishes the upregulation of AP3B2 in patients with paraneoplastic LEMS, and proposes that AP3B2 may participate in the immune regulation mechanisms of paraneoplastic LEMS.

Then LOC338963 (NR_031439) and its adjacent mRNA AP3B2 were selected for quantitative verification in order to prove our predication. The qRT-PCR results showed that both transcripts were upregulated in LEMS, providing an experimental basis for future studies investigating their specific regulatory mechanisms. In this study, we found AP3B2 was upregulated in LEMS patients with SCLC. Therefore, to further analyze the relationship between LEMS and AP3B2, future research will need to exclude the possibility that the high levels of AP3B2 are caused by SCLC.

By searching the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle) for known correlations between AP3B2 and cancer, we found that expression of AP3B2 in different types of tumors is tissue-specific, which can be seen in neuroblastoma, SCLC, and medulloblastoma. We used the Oncoming database (https://www.oncoming.org/) to analyze differential expression of AP3B2 in clinical cases of SCLC and normal control samples. We screened the data of 11 samples from two institutions (Garber Lung and Bhattacharjee Lung). Abnormal expression of the gene in some SCLC patients was found, with five cases screened by Garber Lung showing up-regulated expression of AP3B2 (fold change = 1.622; P = 0.030). Six patients screened in Bhattacharjee Lung showed downregulated expression of AP3B2 (fold change = -3.957; P = 1.000). These inconsistencies provide reference for follow-up studies. In the current study, we analyzed the expression of LOC338963 (NR_031439) and AP3B2 in patients with paraneoplastic LEMS, non-tumor LEMS and SCLC. We found significant differences in expression levels between paraneoplastic LEMS and both non-tumor LEMS and SCLC. Combined with the quantitative expression results of AP3B2 in paraneoplastic LEMS, we speculate that the expression level of this gene may be related to paraneoplastic LEMS. LOC338963 (NR_031439) may participate in the pathogenesis of paraneoplastic LEMS associated with SCLC by regulating the expression of AP3B2.

Conclusions

In conclusion, our experiments have identified lncRNA LOC338963 (NR_034139) and its adjacent mRNAAP3B2 as potential diagnostic biomarkers for paraneoplastic LEMS. These two genes could also serve as candidates for future studies aimed at investigating the molecular regulatory mechanisms behind paraneoplastic LEMS.

Abbreviations

AP3B2: adaptor protein 3, subunit B2

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

LEMS: Lambert–Eaton myasthenic syndrome

lincRNA: long intergenic non-coding RNA

lncRNA: long non-coding RNA

mRNA: messenger RNA

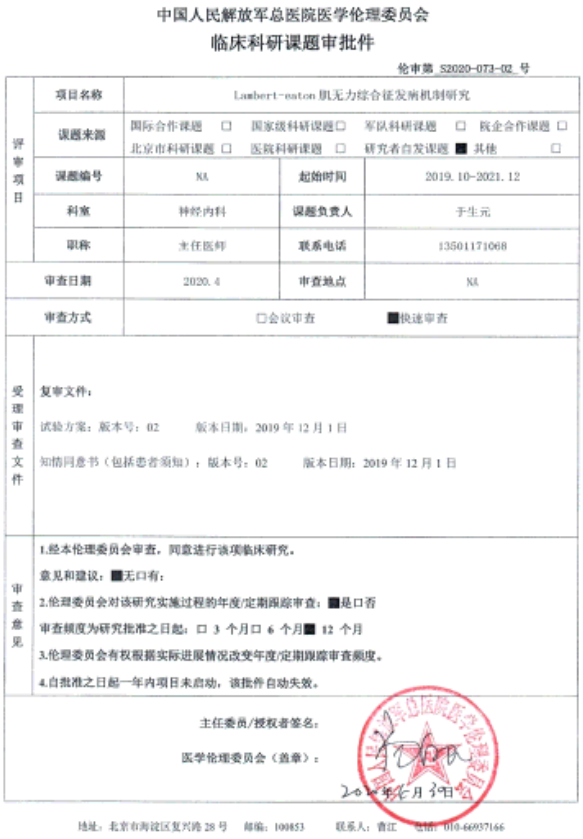
NMJ: neuromuscular junction

qRT-PCR: quantitative real-time polymerase chain reaction

VGCC: voltage-gated calcium channel

Declarations

Ethics approval and consent to participate:



中国人民解放军总医院医学伦理委员会

临床科研课题审批件

伦审第 S2020-073-02 号

评审项目	项目名称	Lambert-eaton 肌无力综合征发病机制研究		
	课题来源	国际合作课题 □ 国家级科研课题□ 军队科研课题 □ 院企合作课题 □ 北京市科研课题 □ 医院科研课题 □ 研究者自发课题 ■ 其他 □		
	课题编号	NA	起始时间	2019. 10-2021. 12
	科室	神经内科	课题负责人	于生元
	职称	主任医师	联系电话	13501171068
审查日期		2020. 4	审查地点	NA
审查方式		□会议审查 ■快速审查		
受理审查文件	复审文件： 试验方案：版本号：02 版本日期：2019 年 12 月 1 日 知情同意书（包括患者须知）：版本号：02 版本日期：2019 年 12 月 1 日			
审查意见	1.经本伦理委员会审查，同意进行该项临床研究。 意见和建议：■无口有： 2.伦理委员会对该研究实施过程的年度/定期跟踪审查：■是口否 审查频度为研究批准之日起：口 3 个月口 6 个月■ 12 个月 3.伦理委员会有权根据实际进展情况改变年度/定期跟踪审查频度。 4.自批准之日起一年内项目未启动，该批件自动失效。			
	主任委员/授权者签名： 医学伦理委员会（盖章）： 2020 年 7 月 9 日			

地址：北京市海淀区复兴路 28 号 邮编：100853 联系人：曹江 电话：010-66937166

This research has been proved by the Ethics Committee of PLA General Hospital.

Consent for publication:

All the patients consented to publication.

Availability of data and materials:

Raw data in this study are not publicly available to preserve the patients' individual privacy under the law of China. But we can provide necessary information with patients' content.

Competing interests:

The authors declare that they have no competing interests.

Funding:

There is no funding support.

Authors' contributions:

Fei Yang designed and drafted the work. Feng Jing substantively revised the work. Fei Yang and Feng Jing contributed equally to this work. Yang Li and Shimin Zhang collected and analyzed the data. Yunyun Huo collected samples and help to conduct the experiment. Xusheng Huang made interpretation of data. Shengyuan Yu is the corresponding author of this work and responsible for the authenticity, reliability and credibility of the content.

Acknowledgements:

All contributors to this work have been listed as authorship.

Authors' information:

References

1. Schoser B, Eymard B, Datt J, et al. Lambert-Eaton myasthenic syndrome (LEMS): a rare autoimmune presynaptic disorder often associated with cancer[J]. J Neurol. 2017;264(9):1854–63.
2. Titulaer MJ, Lang B, Verschuuren JJ. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies[J]. Lancet Neurol. 2011;10(12):1098–107.
3. Wirtz PW, Smallegange TM, Wintzen AR, et al. Differences in clinical features between the Lambert-Eaton myasthenic syndrome with and without cancer: an analysis of 227 published cases[J]. Clinical neurology neurosurgery. 2002;104(4):359–63.
4. Statello L, Guo CJ, Chen LL, et al. Gene regulation by long non-coding RNAs and its biological functions[J]. Nature reviews Molecular cell biology. 2020:1–23.
5. Wu GC, Pan HF, Leng RX, et al. Emerging role of long noncoding RNAs in autoimmune diseases[J]. Autoimmun rev. 2015;14(9):798–805.
6. Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals[J]. Nature. 2009;458(7235):223–7.
7. Carpenter S, Aiello D, Atianand MK, et al. A long noncoding RNA mediates both activation and repression of immune response genes[J]. Science. 2013;341(6147):789–92.
8. Pan F, Zhu L, Lv H, et al. Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating lncRNA MALAT1[J]. Int J Mol Med. 2016;38(5):1507–14.
9. Gualtierotti R, Ingegnoli F, Griffini S, et al. Prothrombotic biomarkers in patients with rheumatoid arthritis: the beneficial effect of IL-6 receptor blockade[J]. Clin Exp Rheumatol. 2016;34(3):451–8.
10. Zhang YY, Huang SH, Zhou HR, et al. Role of HOTAIR in the diagnosis and prognosis of acute leukemia[J]. Oncol Rep. 2016;36(6):3113–22.
11. Jiang SD, Lu J, Deng ZH, et al. Long noncoding RNAs in osteoarthritis[J]. Joint bone spine. 2017;84(5):553–6.
12. Liu Y, Xi J, Zhou L, et al. Clinical characteristics and long term follow-up of Lambert-Eaton myasthenia syndrome in patients with and without small cell lung cancer[J]. Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia. 2019;65:41–5.
13. Djebali S, Davis CA, Merkel A, et al. Landscape of transcription in human cells[J]. Nature. 2012;489(7414):101–8.

14. Dykes IM, Emanueli C. Transcriptional and Post-transcriptional Gene Regulation by Long Non-coding RNA[J]. Genom Proteom Bioinform. 2017;15(3):177–86.
15. Mao AP, Shen J, Zuo Z. Expression and regulation of long noncoding RNAs in TLR4 signaling in mouse macrophages[J]. BMC Genomics. 2015;16(1):45.
16. Hu G, Tang Q, Sharma S, et al. Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation[J]. Nature immunology. 2013;14(11):1190–8.
17. Chen YG, Satpathy AT, Chang HY. Gene regulation in the immune system by long noncoding RNAs[J]. Nature immunology. 2017;18(9):962–72.
18. Lodde V, Murgia G, Simula ER, et al. Long Noncoding RNAs and Circular RNAs in Autoimmune Diseases[J]. Biomolecules. 2020;10(7).
19. Tsokos GC, Rose NR. Immune cell signaling in autoimmune diseases[J]. Clinical immunology (Orlando Fla). 2017;181:1–8.
20. Lang B, Newsom-Davis J, Peers C, et al. The effect of myasthenic syndrome antibody on presynaptic calcium channels in the mouse[J]. J Physiol. 1987;390:257–70.
21. Waterman SA, Lang B, Newsom-Davis J. Effect of Lambert-Eaton myasthenic syndrome antibodies on autonomic neurons in the mouse[J]. Ann Neurol. 1997;42(2):147–56.
22. Takamori M, Iwasa K, Komai K. Antibodies to synthetic peptides of the alpha1A subunit of the voltage-gated calcium channel in Lambert-Eaton myasthenic syndrome[J]. Neurology. 1997;48(5):1261–5.
23. Iwasa K, Takamori M, Komai K, et al. Recombinant calcium channel is recognized by Lambert-Eaton myasthenic syndrome antibodies[J]. Neurology. 2000;54(3):757–9.
24. Han J, Pluhackova K, Böckmann RA. The Multifaceted Role of SNARE Proteins in Membrane Fusion[J]. Frontiers in physiology. 2017;8:5.
25. Cabili MN, Trapnell C, Goff L, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses[J]. Genes Dev. 2011;25(18):1915–27.
26. Maresco DL, Chang E, Theil KS, et al. The three genes of the human FCGR1 gene family encoding Fc gamma RI flank the centromere of chromosome 1 at 1p12 and 1q21[J]. Cytogenet Cell Genet. 1996;73(3):157–63.
27. Kelemen LE. The role of folate receptor alpha in cancer development, progression and treatment: cause, consequence or innocent bystander?[J]. International journal of cancer. 2006;119(2):243–50.
28. Pope S, Artuch R, Heales S, et al. Cerebral folate deficiency: Analytical tests and differential diagnosis[J]. J Inherit Metab Dis. 2019;42(4):655–72.
29. Benson MA, Tinsley CL, Waite AJ, et al. Ryanodine receptors are part of the myospryn complex in cardiac muscle[J]. Scientific reports. 2017;7(1):6312.
30. Chen C, Zou X, Ji C, et al. Characterization of AP3B2_v2, a novel splice variant of human AP3B2[J]. DNA Seq. 2007;18(3):165–8.
31. Narayan RN, McKeon A, Fife TD. Autoimmune Vestibulocerebellar Syndromes[J]. Seminars in neurology. 2020;40(1):97–115.
32. Assoum M, Philippe C, Isidor B, et al. Autosomal-Recessive Mutations in AP3B2, Adaptor-Related Protein Complex 3 Beta 2 Subunit, Cause an Early-Onset Epileptic Encephalopathy with Optic Atrophy[J]. Am J Hum Genet. 2016;99(6):1368–76.
33. Wilbe M, Kozyrev SV, Farias FH, et al. Multiple Changes of Gene Expression and Function Reveal Genomic and Phenotypic Complexity in SLE-like Disease[J]. PLoS Genet. 2015;11(6):e1005248.
34. Jarius S, Wildemann B. 'Medusa head ataxia': the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 3: Anti-Yo/CDR2, anti-Nb/AP3B2, PCA-2, anti-Tr/DNER, other antibodies, diagnostic pitfalls, summary and outlook[J]. J Neuroinflamm. 2015;12:168.
35. Zhu M, Jia L, Li F, et al. Identification of KIAA0513 and Other Hub Genes Associated With Alzheimer Disease Using Weighted Gene Coexpression Network Analysis[J]. Frontiers in genetics. 2020;11:981.
36. Honorat JA, Lopez-Chiriboga AS, Kryzer TJ, et al. Autoimmune gait disturbance accompanying adaptor protein-3B2-IgG[J]. Neurology. 2019;93(10):e954–63.

37. Newell-Litwa K, Salazar G, Smith Y, et al. Roles of BLOC-1 and adaptor protein-3 complexes in cargo sorting to synaptic vesicles[J]. Molecular biology of the cell. 2009;20(5):1441–53.

Figures

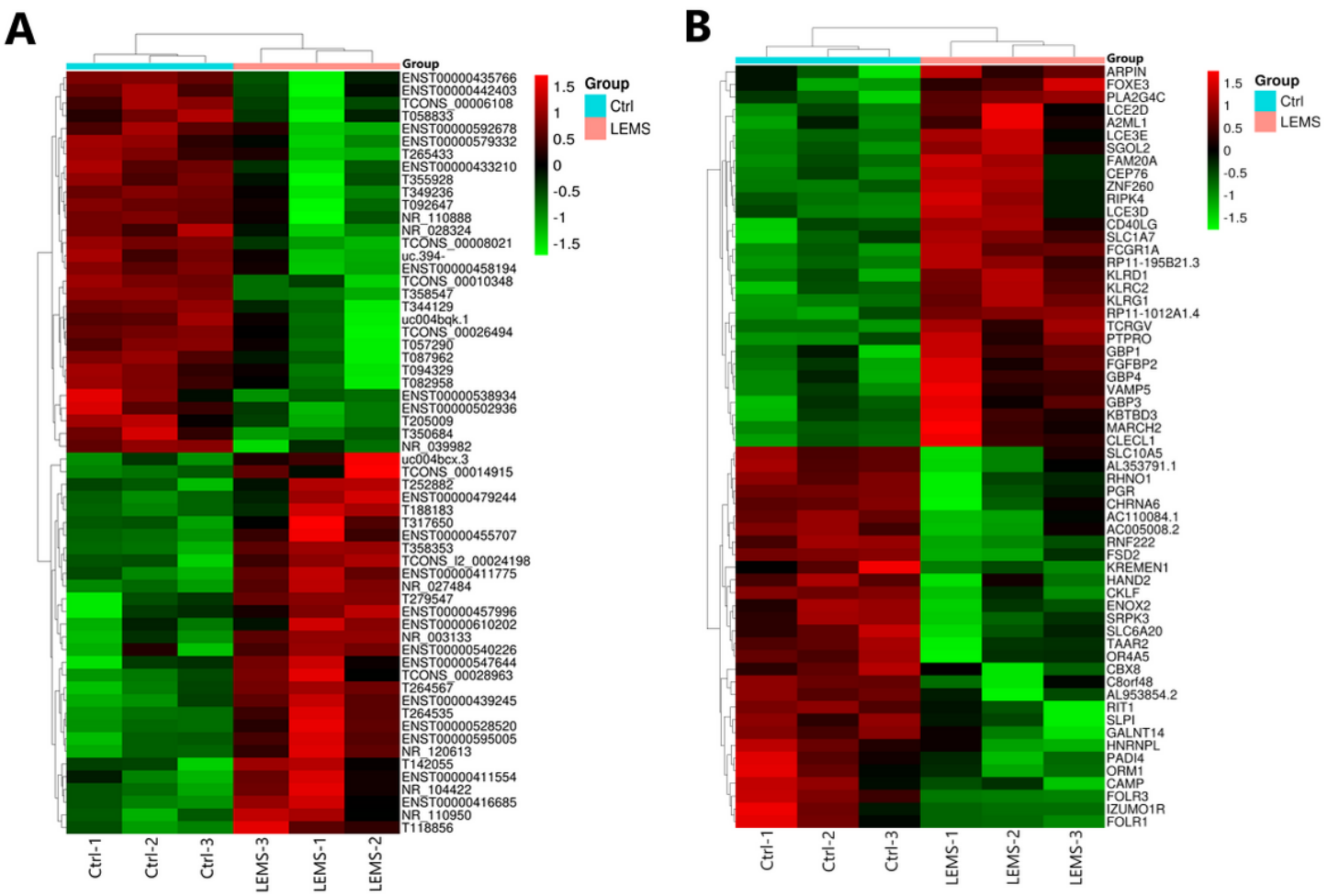


Figure 1

Long non-coding RNA (lncRNA) and messenger RNA (mRNA) expression profiles. Red represents upregulation and green represents downregulation of relative expression level. Hierarchical clustering of (A) lncRNA and (B) mRNA profiles of patients with Lambert–Eaton myasthenic syndrome (LEMS1–LEMS3) and healthy controls (Ctrl1–N3) (fold change ≥ 1.5; $P < 0.05$).

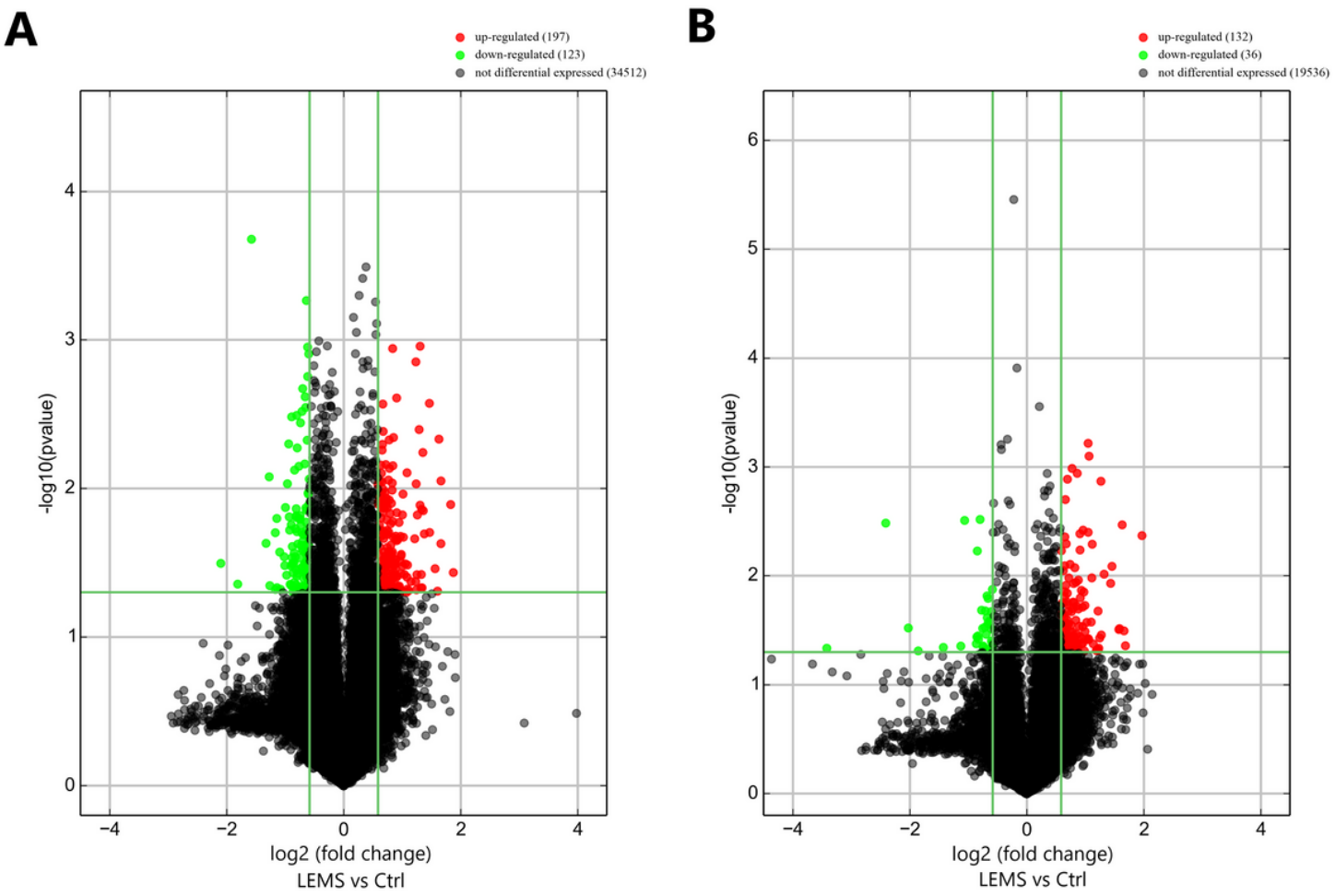


Figure 2

Long non-coding RNA (lncRNA) and messenger RNA (mRNA) differential expression analysis. Volcano plots show the differences between (A) lncRNA and (B) mRNA expression in patients with Lambert–Eaton myasthenic syndrome versus healthy controls. The relative expression level increases from green to red (fold change ≥ 1.5; $P < 0.05$).

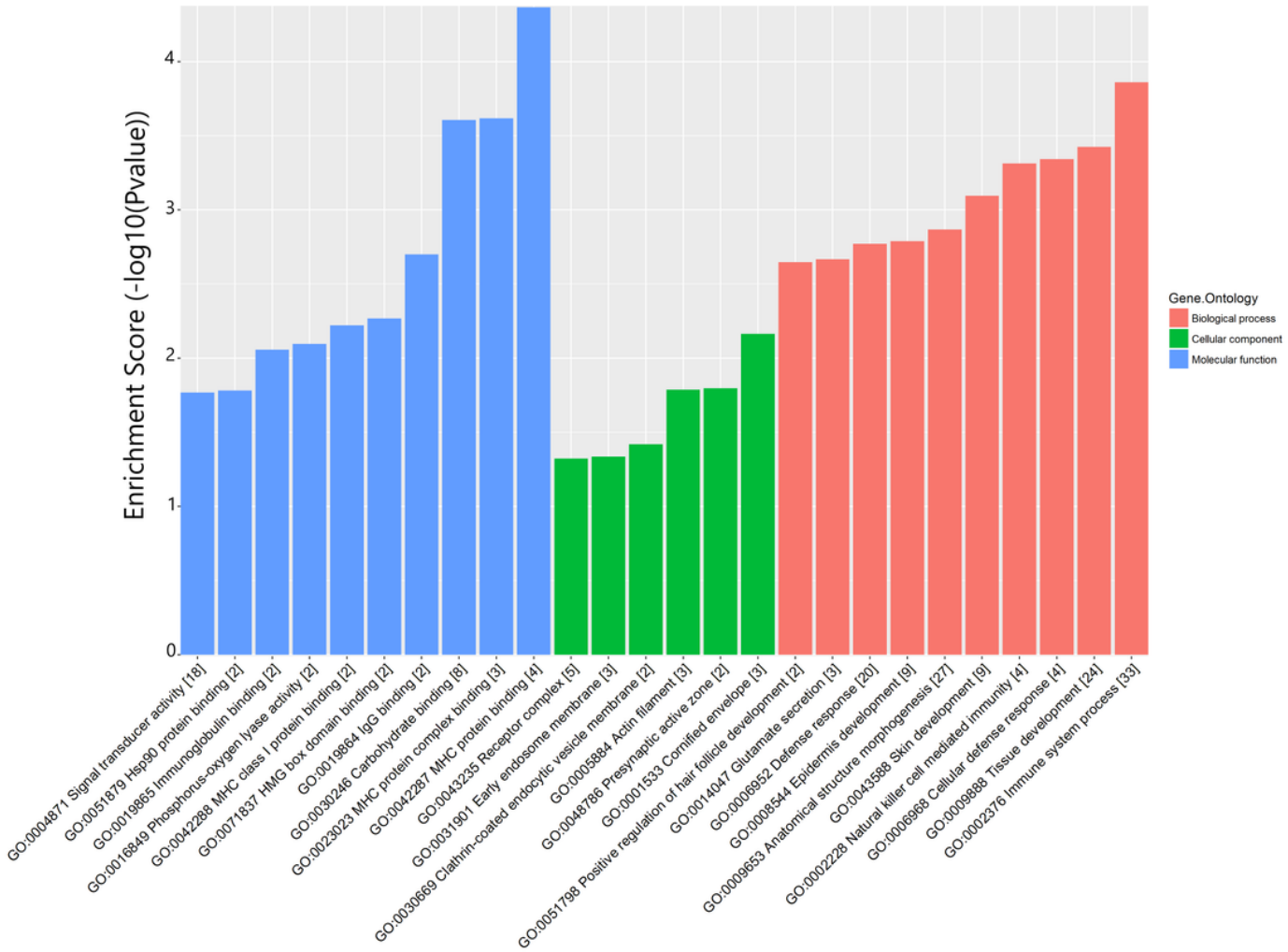


Figure 3

qRT-PCR validation of lncRNA and mRNA microarray analysis. Fold-change in comparison of LEMS group versus C group.

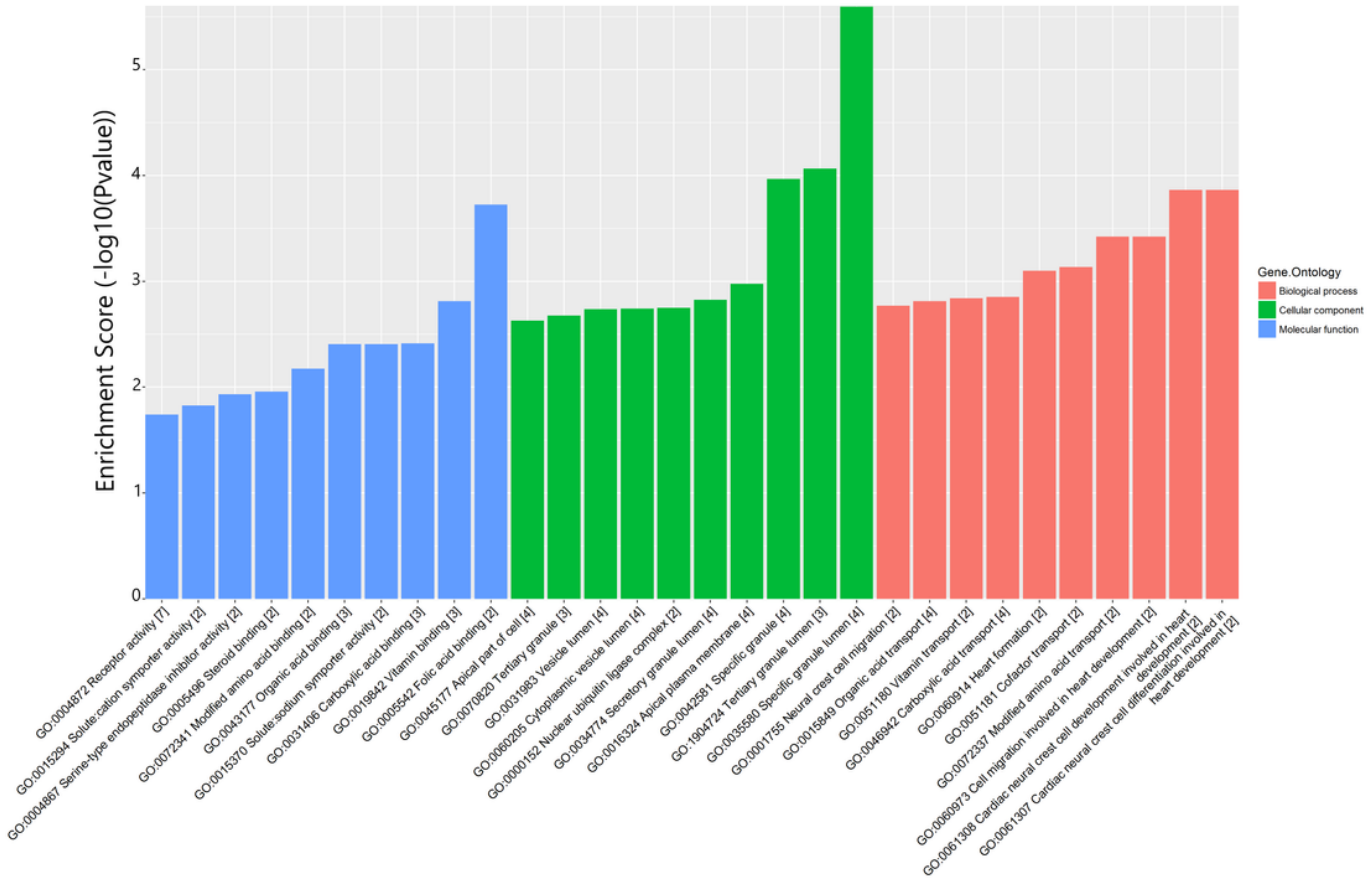


Figure 4

qRT-PCR validation of lncRNA and mRNA microarray analysis. Fold-change in comparison of paraneoplastic LEMS versus non-tumor LEMS and SCLC. *P < .05, **P < .005.

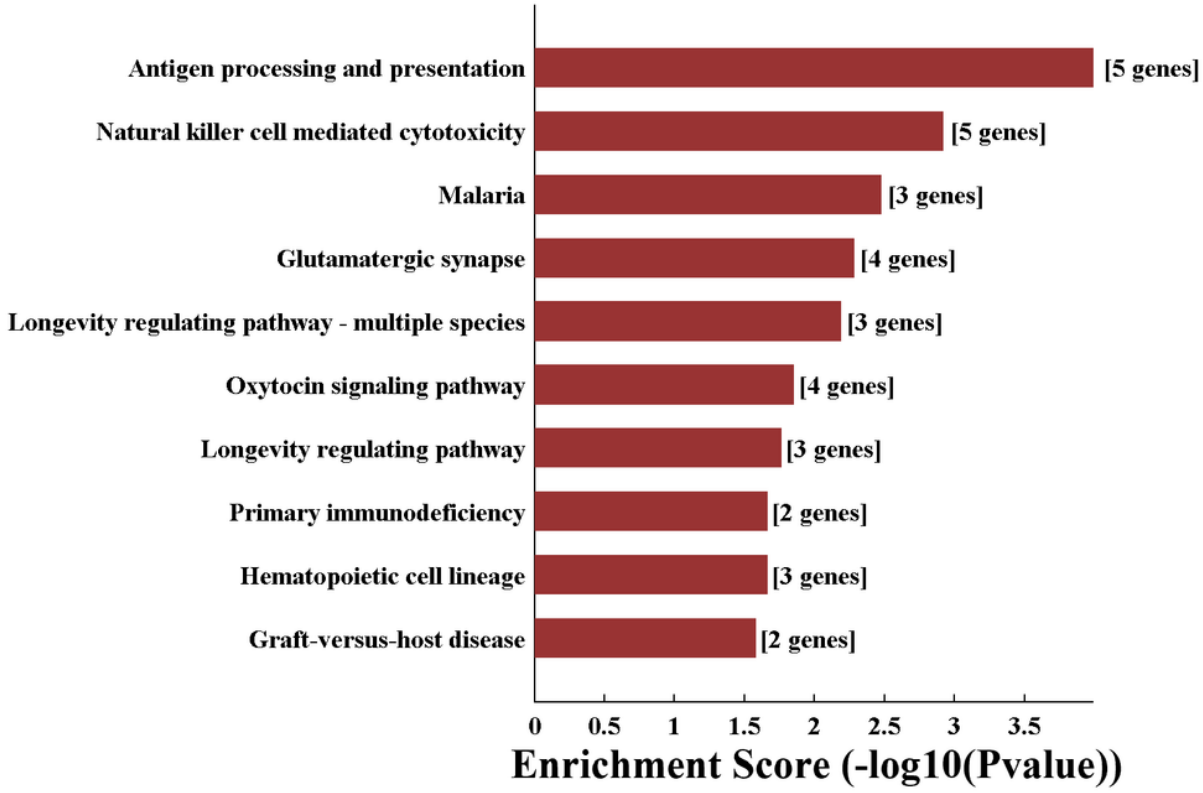


Figure 5

Differentially upregulated messenger RNAs (mRNAs) per Gene Ontology category in patients with Lambert–Eaton myasthenic syndrome compared to healthy controls.

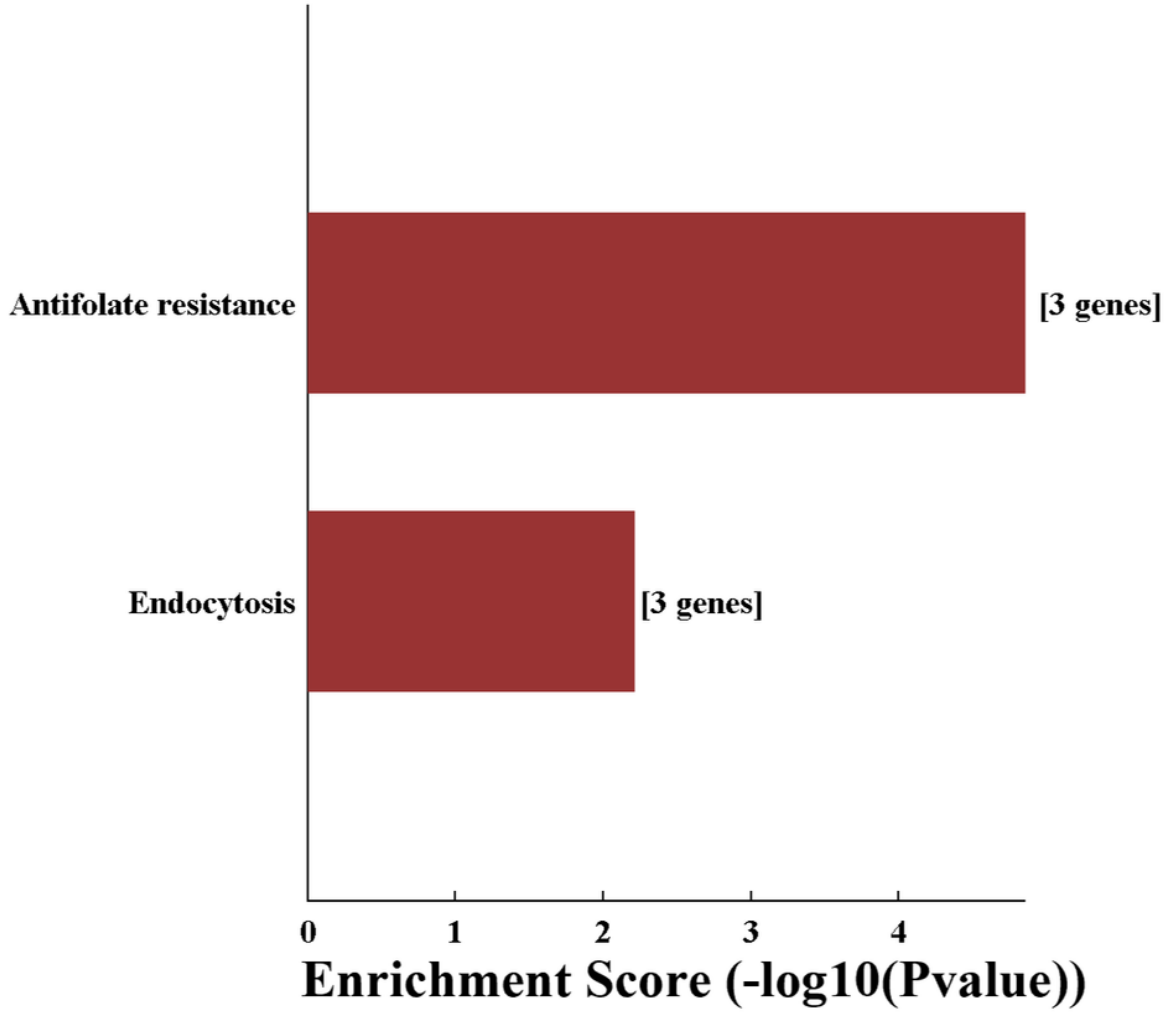


Figure 6

Differentially downregulated messenger RNAs (mRNAs) per Gene Ontology category in patients with Lambert–Eaton myasthenic syndrome compared to healthy controls.

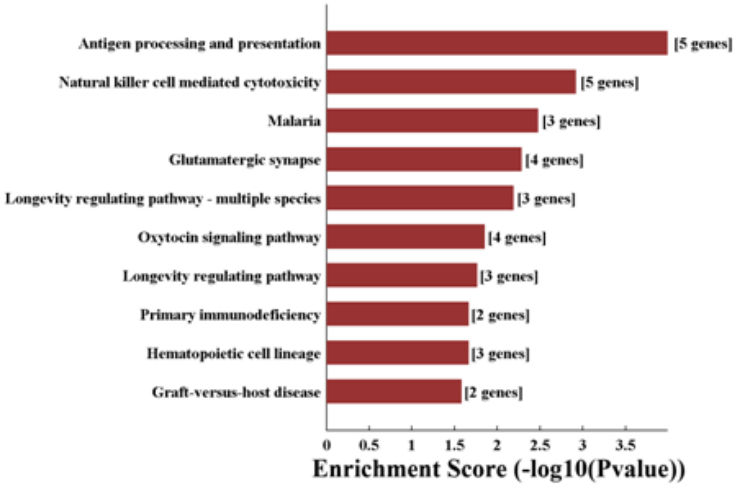


Figure 7

Differentially upregulated messenger RNAs (mRNAs) per Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in patients with Lambert–Eaton myasthenic syndrome compared to healthy controls.

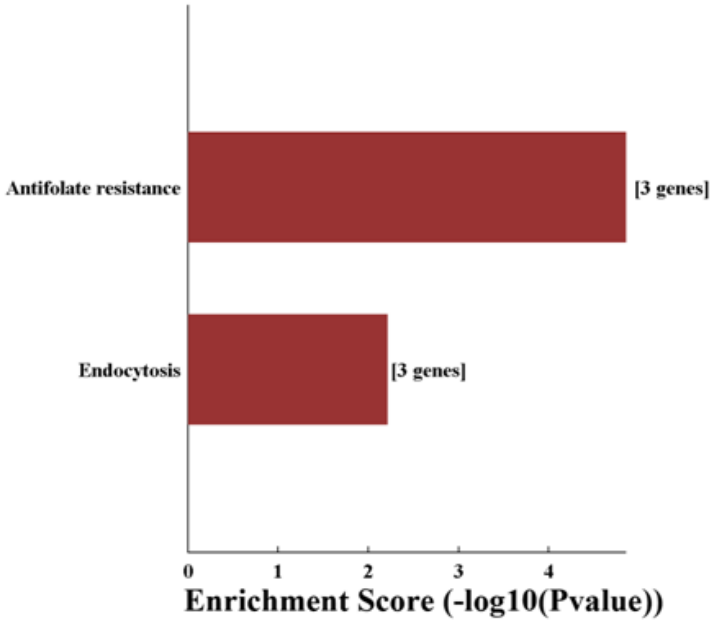


Figure 8

Differentially downregulated messenger RNAs (mRNAs) per Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in patients with Lambert–Eaton myasthenic syndrome compared to healthy controls.

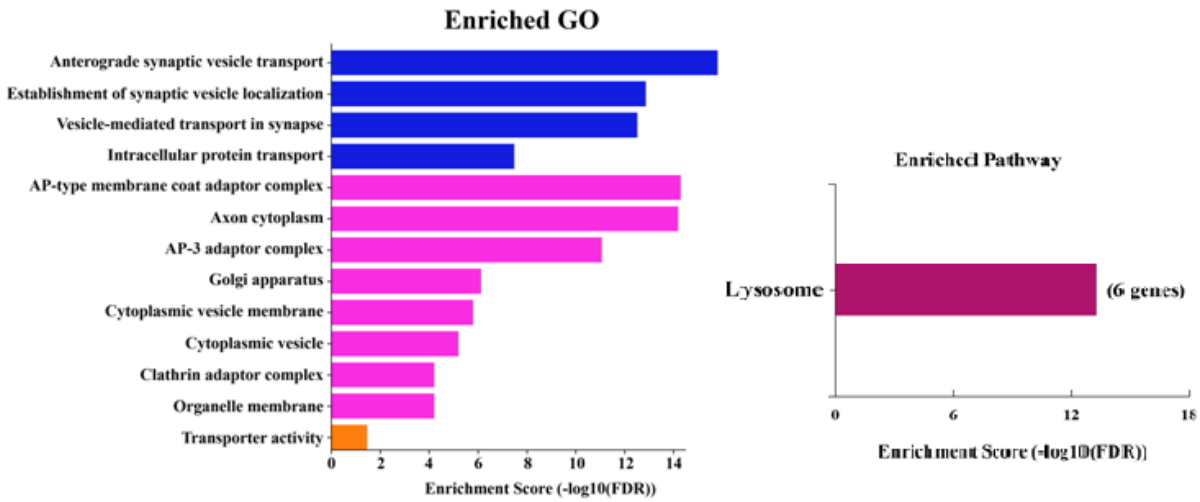


Figure 9

Go and KEEG analysis of AP3B2.