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Differential gene expression profiling on the muscle of acetylcholinesterase knockout mice: A preliminary analysis

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ABSTRACT

Acetylcholinesterase (AChE) (EC. 3.1.1.7) is the acetylcholine-hydrolyzing enzyme that plays an essential role on cholinergic neurotransmission at the synapses of the brain and at the neuromuscular junctions. In order to gain insight into the molecular mechanisms of neuromuscular dysfunction associated with AChE deficiency, we have compared the RNA expression profiles of the muscles of AChE knockout mice with those of the wild-type siblings. Total RNA from the leg muscle of the mice of the wild-type and the AChE nullizygous mice were subjected to microarray analyses with Affymetrix GeneChip® Mouse Gene 1.0 ST Array. The pair-wise comparison of gene expression levels of the 28,853 mRNA transcripts showed that 303 genes were either up- or down-regulated by more than 2.0 folds in the AChE knockout mice. The interaction study of these differentially regulated genes indicated that some of these genes are clustered in biological functions that are related to lipid metabolism and the skeletal–muscular functions.

1. Introduction

Acetylcholinesterase (AChE) (EC. 3.1.1.7) is the acetylcholine-hydrolyzing enzyme that plays an essential role on cholinergic neuromuscular transmission in the brain and at the neuromuscular junctions (for review see [1]). Abnormal expression and localization of AChE have been implicated in the etiology of neurodegenerative diseases such as Alzheimer's disease [2]. The AChE knockout (KO) mice provide a valuable animal model to study the role of this enzyme in muscle function [3,4]. Previous work demonstrated that the AChE KO mice were alive but exhibited body tremor and impaired body movement, indicating a severe muscular dysfunction [5]. In order to gain insight into the molecular mechanisms of neuromuscular dysfunction due to AChE deficiency, we have compared the RNA expression profiles of the muscle of the AChE knockout mice with those of the muscle of the wild-type siblings.

2. Materials and methods

2.1. AChE knockout animals and microarray gene chips

AChE knockout mice (129/Sv) were kindly donated by Dr. Oksana Lockridge of University of Nebraska (NE, USA) [6]. Both the homozygous and the nullizygous AChE mice were obtained by mating with the heterozygote AChE mice in-house at the Animal House of the Chinese University of Hong Kong. The mice were kept under controlled temperature and humidity conditions, with a 12/12 h day and night cycle. The GeneChip® Mouse Gene 1.0 ST Array was purchased from Affymetrix (Affymetrix, USA).

2.2. Sample preparation

Mice were sacrificed according to animal ethics regulations. Tissues from three animals were collected for each group (homozygous wild-type and AChE nullizygous mice). Tissues dissected from leg muscles were extracted with trizol reagent according to the manufacturer's protocol (Invitrogen). An aliquot (300 ng) of RNA was used for the preparation of targets for GeneChip Gene 1.0 ST arrays according to the GeneChip Whole Transcript (WT) Sense Target Labeling Assay manual.

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Table 1
A list of 37 gene transcripts that are up-regulated by >3-fold in the muscle of AChE KO mice.

Gene bank ID	Gene symbol	Gene name	Fold change
NR_001463	Xist	Inactive X-specific transcripts	8.12
NM_025288	Stfa3	Stefin A3	7.33
NM_011978	Slc27a2	Solute carrier family 27 (fatty acid transporter)	7.06
ENSMUST00000052168	Otudi	OTU domain containing 1	6.76
NM_177369	Myh8	Myosin, heavy polypeptide 8, skeletal muscle, perinatal	6.41
NM_133690	Atp1b4	ATPase (Na^+)/(K^+) transporting, beta 4 polypeptide	5.85
NM_009999	Cyp2b10	Cytochrome P450, family 2, subfamily b, polypeptide 10	5.26
NM_009463	Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier)	4.57
NM_007703	Elovl3	Elongation of very long chain fatty acids	4.56
NM_011769	Zim1	Zinc finger, imprinted 1	4.52
NM_173869	Stfa2l1	Stefin A2 like 1	4.45
NM_007669	Cdknia	Cyclin-dependent kinase inhibitor 1A (P21)	4.40
NM_029662	Mfsd2	Major facilitator superfamily domain containing 2	4.34
NM_008106	Opnimw	Opsin 1 (cone pigments)	4.33
NM_007702	Cidea	Cell death-inducing DNA fragmentation factor, alpha subunit	4.15
NM_008817	Peg3	Paternally expressed 3	4.13
NM_007469	Apod	Apolipoprotein C-I	4.10
NM_013749	Tnfrsf12a	Tumor necrosis factor receptor superfamily, member 12a	4.07
NM_181748	Gpr120	G protein-coupled receptor 120	3.95
NM_028133	Egln3	EGL nine homolog 3 (*C. elegans*)	3.76
NM_030700	Maged2	Melanoma antigen, family D, 2	3.63
NM_001082543	Stfal	Stefin A1	3.58
NM_007498	Atf3	Activating transcription factor 3	3.50
NM_212444	Gyk	Glycerol kinase	3.43
NM_001082545	Stfa2	Stefin A2	3.42
NM_175540	Eda2r	Ectodysplasin A2 isoform receptor	3.42
NM_032002	Nrg4	Neuregulin 4	3.40
NM_011044	Pck1	Phosphoenolpyruvate carboxykinase 1	3.20
NM_033037	Cdo1	Cysteine dioxygenase 1, cytosolic	3.19
NM_001081212	Irs2	Insulin receptor substrate 2	3.16
NM_201375	Kng2	Kininogen 2	3.16
NM_001013370	Sesni	Sestrin 1	3.13
NM_153170	Slc36a2	Solute carrier family 36 (proton/amino acid symporter)	3.06
NM_008904	Ppargda	Peroxisome proliferative activated receptor, gamma, coa	3.06
NM_023737	Ehhadh	Enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A	3.05
NM_026346	Fbxo32	F-box protein 32	3.03
NM_025540	Sin	Sarcolipin	3.03

Table 2
A list of 20 gene transcripts that are down-regulated by >3-fold in the muscle of AChE KO mice.

Gene bank ID	Gene symbol	Gene name	Fold change
NM_009599	Ache	Acetylcholinesterase	−5.74
NM_011419	Jaridid	Jumonji, AT rich interactive domain 1D (Rbp2 like)	−5.50
NM_009220	Sstyl	Spermiogenesis specific transcript on the Y 1	−4.71
NM_027356	Ufspi	UFM1-specific peptidase 1	−4.61
ENSMUST00000057428	Mylk4	Myosin light chain kinase family, member 4	−4.18
NM_029803	Ifi27	Interferon, alpha-inducible protein 27	−4.11
BC119816	Zmynd17	Zinc finger, MYND domain containing 17	−4.08
XM_001473625	LOC100040187	mCG1037230	−3.74
XR_032509	LOC100041587	Hypothetical protein LOC100041587	−3.69
NM_030255	Apobec3	Apolipoprotein B editing complex 3	−3.49
NM_001081456	Plcd4	Phospholipase C, delta 4	−3.40
NR_003522	Abhdi	Abhydrolase domain containing 1	−3.39
NM_024291	Ky	Kyphoscoliosis peptidase	−3.34
NM_011253	Rbmy1a1	RNA binding motif protein, Y chromosome, family 1,	−3.28
ENSMUST00000115482	Ssty2	Spermiogenesis specific transcript on the Y 2	−3.24
NM_008134	Glycami	Glycosylation dependent cell adhesion molecule 1	−3.11
ENSMUST00000023246	Ly6g	Lymphocyte antigen 6 complex, locus G	−3.06
NM_012008	Ddx3y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	−3.05
NM_176920	Lrtml	Leucine-rich repeats and transmembrane domains 1	−3.03
NM_016974	Dbp	D site albumin promoter binding protein	−3.00

2.3. Data collection and data analysis

The images were scanned by Affymetrix GeneChip Command Console (AGCC) and analyzed with the Affymetrix GeneChip Expression Console. Raw expression values from the Affymetrix mouse gene 1.0 ST chip were analyzed and normalized using Partek Genomics Suite 6.4 (Partek Incorporated).

3. Results and discussion

The pair-wise comparison of expression values for all of the 28,853 mRNA transcripts in the mouse genome was carried out with the dataset generated by microarray analysis. Fig. 1A shows the pie-chart distribution of the 28,550 mRNA transcripts which showed more than 2-fold deviations from wild-type animals. There