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LIPOPROTEIN LIPASE DEFICIENCY LEADS TO α-SYNUCLEIN AGGREGATION AND UBIQUITIN C-TERMINAL HYDROLASE L1 REDUCTION

H. YANG,[a†] T. ZHOU,[a†] H. WANG,[a] T. LIU,[a] K. UEDA,[b] R. ZHAN,[a] L. ZHAO,[a] Y. TONG,[a] X. TIAN,[a] T. ZHANG,[a] Y. JIN,[a] X. HAN,[c] Z. LI,[d] Y. ZHAO,[d*] X. GUO,[d*] W. XIAO,[d*] D. FAN,[d*] G. LIU[e*] AND D. CHUI[a,d*]

[a] *Neuroscience Research Institute, Peking University Health Science Center, Beijing 100191, China*

[b] *Department of Dementia and Higher Brain Function, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan*

[c] *Diabetes and Obesity Research Center, Sanford/Burnham Medical Research Institute, La Jolla, CA 92037, USA*

[d] *Peking University Third Hospital, Beijing 100191, China*

[e] *Institute of Cardiovascular Science, Health Science Center, Peking University, Beijing 100191, China*

Abstract—We have previously reported that presynaptic dysfunction and cognitive decline have been found in lipoprotein lipase (LPL) deficient mice, but the mechanism remains to be elucidated. Accumulating evidence supported that α-synuclein (α-syn) and ubiquitin C-terminal hydrolase L1 (UCHL1) are required for normal synaptic and cognitive function. In this study, we found that α-syn aggregated and the expression of UCHL1 decreased in the brain of LPL deficient mice. Reduction of UCHL1 was resulted from nuclear retention of DNA cytosine-5-methyltransferase 1 in LPL knockout mice. Reverse changes were found in cultured cells overexpressing LPL. Furthermore, deficiency of LPL increased ubiquitination of α-syn. These results indicated that aggregation of α-syn and reduction of UCHL1 expression in LPL-deficient mice may affect synaptic function.

Key words: lipoprotein lipase, α-synuclein, ubiquitin C-terminal hydrolase L1, DNA cytosine-5-methyltransferase 1.

INTRODUCTION

Lipoprotein lipase (LPL) is involved in the regulation of fatty acid metabolism, and facilitates cellular uptake of lipoproteins, lipids and lipid-soluble vitamins (Wang and Eckel, 2012). LPL is highly expressed in hippocampal neurons (Eckel, 1989; Goldberg, 1996; Preiss-Landl et al., 2002), and involved in the pathogenesis of dementia (Vespa et al., 1999; Scacchi et al., 2004; Gong et al., 2013). Our lab first found that LPL deficient mice displayed memory impairment (Xian et al., 2009) and presynaptic dysfunction, which may due to impaired synaptic vesicle recycling (Liu et al., 2014). However, the exact mechanisms are still to be investigated.

The physiological functions of presynaptic α-synuclein (α-syn) have frequently been linked to synaptic activity and the SNARE complex, the core fusion machinery for vesicle fusion (Chandra et al., 2005; Burré et al., 2010). Ubiquitin C-terminal hydrolase L1 (UCHL1) is required for normal synaptic function and cognition (Gong et al., 2006; Sakurai et al., 2008; Cartier et al., 2009; Chen et al., 2010). Reduction in levels of functional UCHL1 is speculated to contribute to the pathogenesis of neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Setsuie and Wada, 2007; Yasuda et al., 2009; Bilguvar et al., 2013; Mondello et al., 2014). UCHL1 is one of the most abundant proteins in the brain, comprising 1–2% of the total soluble fraction (Wilkinson et al., 1989). DNA methylation in its promoter region regulates UCHL1 expression (Kagara et al., 2008; Zhao et al., 2011). UCHL1 plays an important role in ubiquitin turnover through its C-terminal hydrolytic activity, and has also been suggested to be an ubiquitin ligase, interacting with α-syn (Liu et al., 2002). Since both UCHL1 and α-syn are involved in synaptic activity and cognition, we speculate that deficiency of LPL may affect UCHL1 expression and α-syn aggregation, and eventually result in presynaptic dysfunction and cognitive decline.

In the present study, we found increased aggregation and ubiquitination of α-syn in the brain of LPL deficient mice, as well as decreased expression of UCHL1. UCHL1 reduction was induced by translocation of DNA cytosine-5-methyltransferase 1 (DNMT1) to the nucleus, which caused promoter DNA hypermethylation. These results indicated that LPL deficiency gave rise to increased α-syn aggregation and decreased UCHL1 expression, which may further influence synaptic function.

*Corresponding authors. Address: Peking University Health Science Center, Beijing 100191, China (D. Chui). Tel: +86-1082802920; fax: +86-1082805221.

E-mail address: dchui@bjmu.edu.cn (D. Chui).

† These authors contributed equally to this paper.

Abbreviations: AD, Alzheimer's disease; DMEM, Dulbecco's modified Eagle medium; DNMT1, DNA cytosine-5-methyltransferase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPL, Lipoprotein lipase; PBS, phosphate-buffered saline; PC, Phosphatidylcholine; PCR, polymerase chain reaction; PE, phosphatidylethanolamine; PS, phosphatidylserine; UCHL1, ubiquitin C-terminal hydrolase L1.

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EXPERIMENTAL PROCEDURES

Animals

LPL deficient mice on C57BL/6J background and littermates were used in this study. Since LPL deficient mice died within 48 h after birth, all in vivo experiments were performed in new born LPL deficient mice. Experiments in Fig. 1A were also conducted in adult (12 months) LPL-deficient mice rescued from neonatal death by intramuscular injection of an adenoviral vector coding a human LPL mutant, Ad-LPLS447X, as previously described (Ross et al., 2005; Xian et al., 2009). All procedures were approved by the Animal Care Committee of Peking University Health Science Center in China.

Plasmid construction and transfection

Human LPL cDNA was cloned into the pcDNA3.1-myc/his vector (Invitrogen, Carlsbad, CA, USA) using polymerase chain reaction (PCR) with appropriate pair synthetic oligonucleotide primers (forward: 5′-GCGAATTCATGGA GAGCAAAGCCCTGCT; reverse: 5′-CAGGATCCGCG CCTGACTTCTTATTCAGAG, AuGCT, Beijing, China). The sequence of plasmid was confirmed by DNA sequencing (Sunbiotech, Beijing, China).

Transfection of plasmid into HEK293 cells were carried out using Lipofectamine 2000 (Invitrogen), as previously described (Liu et al., 2013). Briefly, plasmid and transfection reagent (1.5 μL per microgram of DNA) were diluted with Dulbecco's modified Eagle medium (DMEM), mixed gently, and incubated for 20 min at room temperature. The mixture was then added to the medium for transfection. After 5 h, the medium was replaced by DMEM supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA). Cells were harvested 48 h after transfection.

Antibodies

Antibody against α-syn was EQV1, a gift from Dr. Kenji Ueda (Arima et al., 1998). Other primary antibodies included mouse monoclonal anti-ubiquitin (P4D1) antibody (Cell Signaling Technology, Inc., Boston, MA, USA); rabbit monoclonal anti-DNMT1 (D63A6) XP™ antibody (Cell Signaling Technology, Inc.); rabbit polyclonal anti-UCHL1 antibody (Enzo Life Sciences, Inc., Farmingdale, NY, USA); mouse monoclonal anti-β-actin (Sigma–Aldrich, Inc., St. Louis, MO, USA).

Isolation of cytoplasmic and nuclear fractions

Isolation of cytoplasmic and nuclear fractions was performed according to the method described previously (Mori et al., 2002). Nuclear and cytoplasmic fractions were obtained from 1×10^6 cells or 100 mg of mouse brain tissue with the EpiQuik nuclear extraction kit (Epigentek, Farmingdale, NY, USA). Protein concentration was determined by the bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA, USA).

Immunoprecipitation and SDS–PAGE immunoblotting analysis

Immunoprecipitation was performed according to the method described previously (Tanaka et al., 2002). Briefly, mouse brain tissues were washed twice with phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4), then homogenized and incubated on ice for 20 min in ice-chilled lysis buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% NP40 (Sigma–Aldrich), 0.5% sodium deoxycholate (Sigma–Aldrich), 0.1% SDS, and protease inhibitor cocktail (Roche). The homogenates were then centrifuged (20,000*g*, 30 min), and protein concentrations in the supernatants determined using the bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA, USA). For each supernatant, 200 μg were preabsorbed with a protein G-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) for 1 h. The precleared lysates were then incubated at 4 °C overnight with 2 μL α-syn antibody EQV1 (Arima et al., 1998), and the immunocomplexes were precipitated by incubation with protein G-Sepharose at 4 °C early for 2 h. Finally, the samples were washed three times with lysis buffer, washed once with PBS, boiled, and the proteins in the immunoprecipitates were separated using SDS–PAGE and analyzed by immunoblotting with the ubiquitin antibody (1:1000).

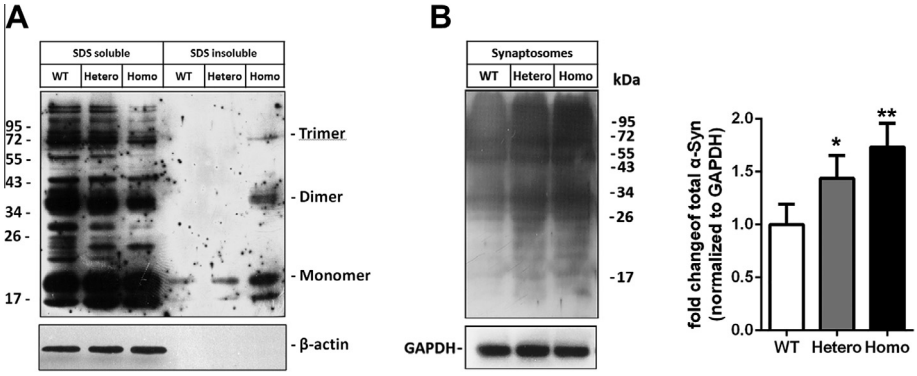

Fig. 1. Aggregation of α-syn in the brain of LPL-deficient mice. α-syn aggregation increased in the brain of LPL-deficient mice. Wild-type, heterozygous and homozygous LPL knockout mouse brain was dissected and its protein was extracted with different detergent: SDS and β-mercaptoethanol, subjected to SDS–PAGE and western blotting (A). Western blotting of α-syn in synaptosomes from wild-type, heterozygous and homozygous LPL knockout mouse brain. Total soluble α-syn (dimer and trimer) in the brain of LPL-deficient mice increase compared with WT ($n = 3$, $^{**}p < 0.01$, One-way ANOVA) (B). WT, Wild-type; Hetero, heterozygous; Homo, homozygous.

Immunofluorescence staining

Immunofluorescence staining was performed according to the method described previously (Yu et al., 2012). Cells on coverslips were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature, permeabilized with 0.3% Triton X-100/PBS for 5 min, and blocked with 10% bovine serum albumin in PBS, then incubated at 4 °C overnight with primary antibodies against DNMT1 (1:100), followed by secondary antibodies Alexa Fluor 488 labeled-goat anti-rabbit IgG (Molecular Probes, Grand Island, NY, USA 1:2000). Cell nuclei were counterstained with Hoechst. Fluorescence images were acquired with a confocal laser scanning microscope (LSM510; Carl Zeiss Co., Oberkochen, Germany), and analyzed with ImageJ (NIH) software.

Biochemical fractionation of neural tissues by graded extraction

SDS-soluble fraction and SDS-insoluble fraction of α-syn biochemical fractionation of cell and neural tissues by graded extraction was performed according to Gallardo et al. (Ihara et al., 2007; Gallardo et al., 2008).

Isolation of brain synaptosomes

Synaptosome isolation was performed according to Dunkley et al. (Dunkley et al., 2008; Liu et al., 2010b). Briefly, the whole-brain lysates were centrifuged at 1000*g* at 4 °C for 10 min after isosmotic homogenization. The supernatants were centrifuged at 17,000*g* at 4 °C for 20 min to obtain the P2 fraction for further Percoll (Pharmacia Biotech, Uppsala, Sweden) gradient separation. In order to obtain more synaptosomes, we collected the fractions between 10% and 23% for western blotting analysis. The final pellets were centrifuged at 17,000*g* at 4 °C for 20 min twice and resuspended with Tris–HCl buffer (pH 7.4) to remove residual Percoll.

mRNA expression analysis

mRNA extraction and reverse-transcription PCR was carried out as previously described (Sun et al., 2012; Liu et al., 2013). Mouse brain tissues or HEK293 cells homogenized in TRIzol (Invitrogen) reagent, and total RNA was isolated using PrimeScript RT-PCR kit (Takara Bio, Otsu, Shiga, Japan) according to the manufacturer's instructions. Reverse transcription was performed with 1 μg of total RNA. TaqMan PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, USA) and pre-designed, pre-optimized primers and probe mix for UCHL1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Threshold cycle numbers (Ct) were determined using the 7500 Real-Time PCR System (Applied Biosystems) and the relative quantities of mRNA per sample were calculated using the ΔΔCt method as described by the manufacturer using GAPDH as the calibrator gene.

DNA methylation analysis

Genomic DNA was extracted from mouse brain using Wizard SV Genomic DNA Purification System according to the manufacturer's instructions (Promega, Madison, WI, USA). DNA concentration and purity were determined based on the absorbance at 260 and 280 nm. A total of 1 mg of genomic DNA from each sample was bisulfite-treated using the EZ-96 DNA methylation kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Sequenom MassARRAY platform (CapitalBio, Beijing, China), which was composed of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, was used to analyze UCHL1 methylation quantitatively (Gen-Bank Accession Number: NM_011670.2). PCR primers were designed using Methprimer (http://www.urogene.org/methprimer/). We used the following primers based on the reverse complementary strands of UCHL1-promoter 1(5′-aggaagagagTAAATAATGGGTATTTGTTAGGGGG-3′ and 5′-cagtaatacgactcactatagggagaaggctTTTC CTCTTTACTTTTAACTTTCAAATACT-3′). Mass spectra were obtained via MassARRAY Compact MALDI-TOF (Sequenom, San Diego, CA, USA) and their methylation ratios were generated using the Epityper software version 1.0 (Sequenom, San Diego, CA, USA).

Shotgun lipidomics analysis of brain lipids

Shotgun lipidomics analysis of lipids was performed as described previously (Cheng et al., 2007; Liu et al., 2010a). Lipids were extracted from each dissected brain tissue by the modified Bligh and Dyer method, as described previously (Cheng et al., 2007). A triple-quadrupole mass spectrometer equipped with a Nanomate device, and the Xcalibur system was used to analyze lipids. All tandem mass spectrometry analyses were automatically acquired by a customized sequence operated under Xcalibur software. Internal standards for quantification of individual molecular species of other lipid classes were added to each brain tissue sample.

Table 1. Phospholipid composition in the brain of LPL-deficient mice (nmol/mg protein)

Phospholipids	Wild type (n = 5)	LPL deficient (n = 5)	p Value
Phosphatidylcholine (PC)	146.96 ± 2.13	173.25 ± 5.03	0.0004***
Phosphatidylethanolamine (PE)	81.14 ± 5.03	95.85 ± 3.57	0.0140*
Phosphatidylserine (PS)	28.92 ± 1.91	33.35 ± 1.80	0.0383*
Phosphatidylglycerol (PG)	1.15 ± 0.06	1.37 ± 0.36	0.3869
Phosphatidylinositol (PI)	8.58 ± 1.28	8.72 ± 0.92	0.8920
Lysophosphatidylcholine (LPC)	4.56 ± 0.37	4.53 ± 0.82	0.9612
Sphingomyelin (SM)	2.81 ± 0.48	3.15 ± 0.56	0.5407

* $p < 0.05$.
*** $p < 0.0001$.

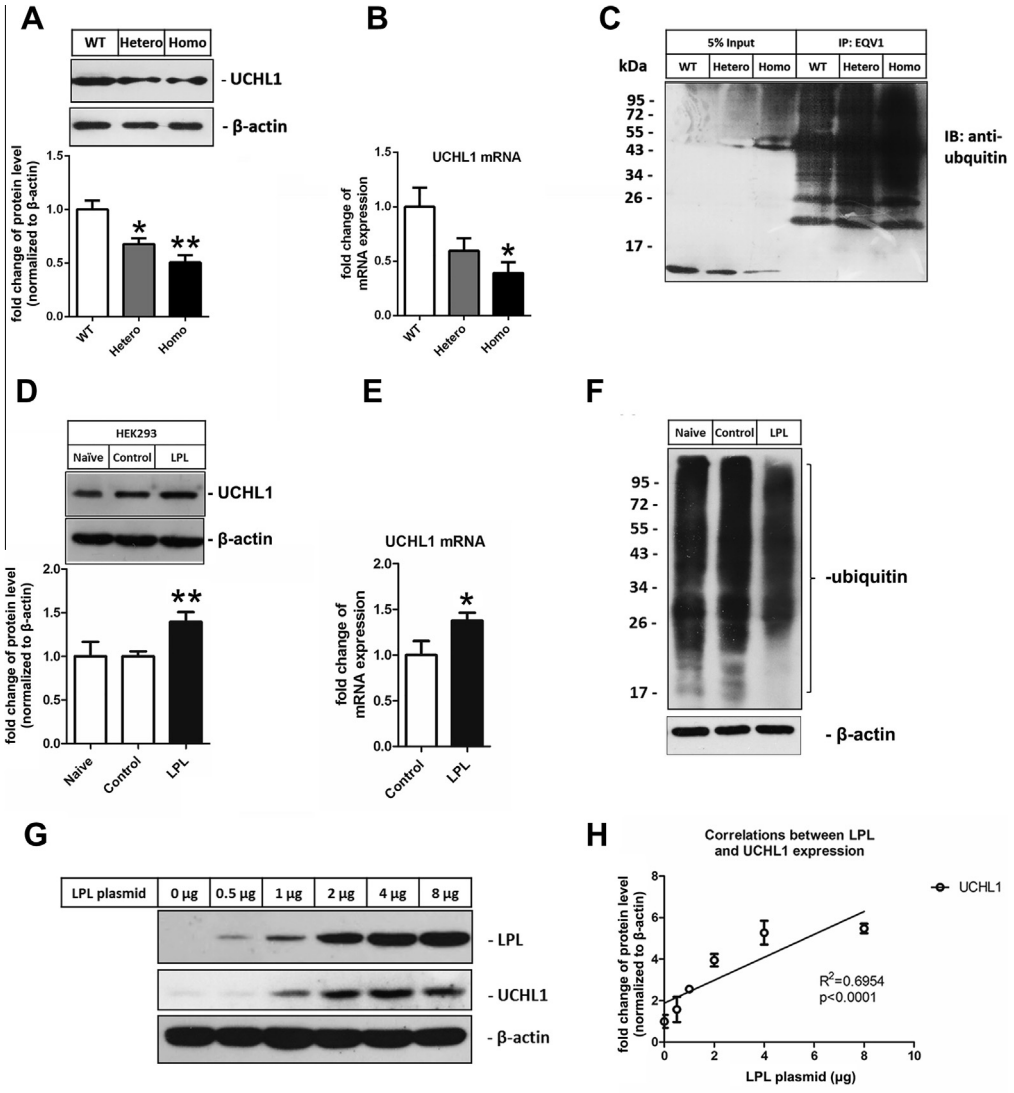

Fig. 2. LPL deficiency reduces UCHL1 expression and results in up-regulation of protein ubiquitination. UCHL1 mRNA and protein levels decreased in LPL-deficient mouse brain (A and B), lead to increased ubiquitination levels, and both total protein and α-syn ubiquitination (C). Elevated UCHL1 expression when overexpressing LPL in HEK293 cells (D and E). Protein ubiquitination level is down regulated by LPL in the HEK293 cells (F, representative ubiquitination blots of 3 independent experiments, plasmid concentration was 2 μg). UCHL1 expression was positively associated with LPL level (G and H). Data in all panels are expressed as mean ± SEM, $n = 3$. $^{*}p < 0.05$; $^{**}p < 0.01$; (One-way ANOVA). WT, Wild-type; Hetero, heterozygous; Homo, homozygous.

Statistical analysis

Statistics were calculated using functions provided in GraphPad Prism 5 for Windows (GraphPad Software Inc., La Jolla, CA, USA). A one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey–Kramer's post hoc tests was performed to compare groups. The significant value was set when $p < 0.05$ (Zhou et al., 2014).

RESULTS

Phospholipid homeostasis disrupted in the brain of LPL deficient mice

To understand the role of LPL in brain lipid metabolism, shotgun lipidomics (Han and Gross, 2005) was used to analyze the molecular species of lipid extracts from the whole brain of neonatal wild-type and LPL-deficient mice. We found that the main membrane lipid components phosphatidylcholine (PC, $p < 0.001$), phosphatidylethanolamine (PE, $p < 0.05$) and phosphatidylserine (PS, $p < 0.05$) were significantly increased in LPL-deficient mice (Table 1, $n = 5$). These results indicate that neuronal LPL is critical for main membrane lipid PC, PE and PS composition homeostasis.

α-syn aggregation in the cerebrum and synaptosomes of LPL-deficient mice

Our recent study showed that impaired synaptic vesicle recycling contributes to the presynaptic dysfunction and plasticity impairment in LPL-deficient neurons (Liu et al., 2014). The physiological functions of presynaptic α-syn have frequently been linked to synaptic activity and the SNARE complex, the core fusion machinery for vesicle fusion (Chandra et al., 2005; Burré et al., 2010). To

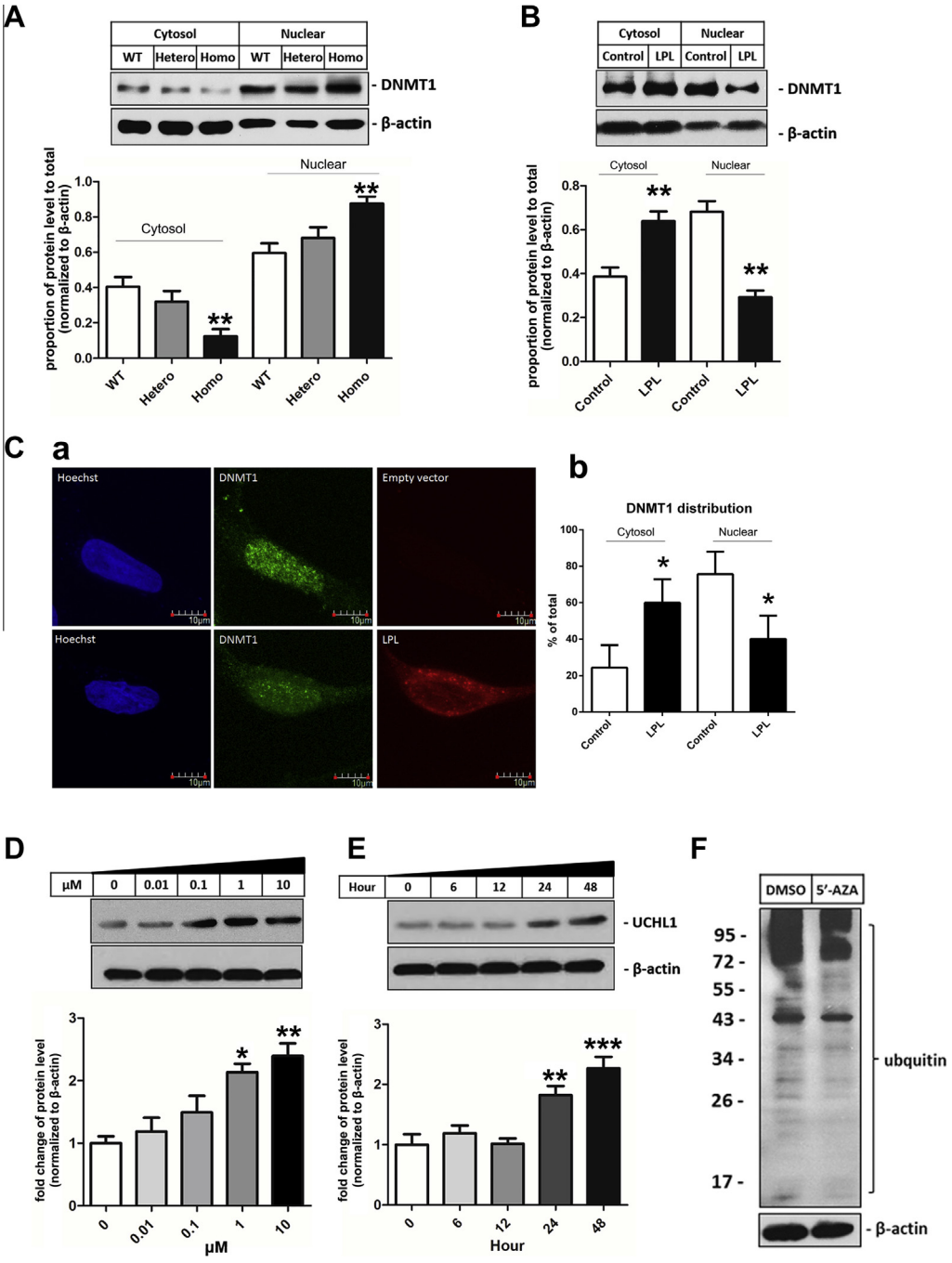

Fig. 3. Misdistribution of DNMT1 leads to UCHL1 expression alternation. Devoid of LPL in mice resulted in nuclear retention of DNMT1 (A). Western blotting and immunofluorescence showed that overexpressing LPL caused cytosolic retention of DNMT1 in the HEK293 cells (B and C). DNA methylation inhibitor 5′-AZA dose and time dependent experiments were conducted in the HEK293 cells. 1 μM 5-Aza and 24 h treatment elevated UCHL1 level in the HEK293 cells (D and E). And consistently, after 1 μM 5-AZA treating the cell 24 h, protein ubiquitination was significantly reduced (F). Data in all panels are expressed as mean ± SEM. $n = 3$, $^{*}p < 0.05$; $^{**}p < 0.01$. (One-way ANOVA.) WT, Wild-type; Hetero, heterozygous; Homo, homozygous.

determine whether the deletion of LPL causes aggregation of α-syn in the brain of LPL-deficient mice, we examined the biochemical status of α-syn in SDS-soluble and SDS-insoluble fractions of the whole brain of both neonatal and adult mice. In each fraction, α-syn was detected mainly as the native form of ~19 kD. This analysis showed an increase in the level of α-syn, especially the putative dimer and trimer, in SDS-insoluble fractions of whole-brains in the LPL-deficient mice (Fig. 1A), indicating aggregation of α-syn in the absence of LPL.

To assess the potential impact of soluble α-syn on presynaptic terminals, synaptosomes were separated by subcellular fractionation from brain homogenates of neonatal mice, followed by immunoblotting analysis.

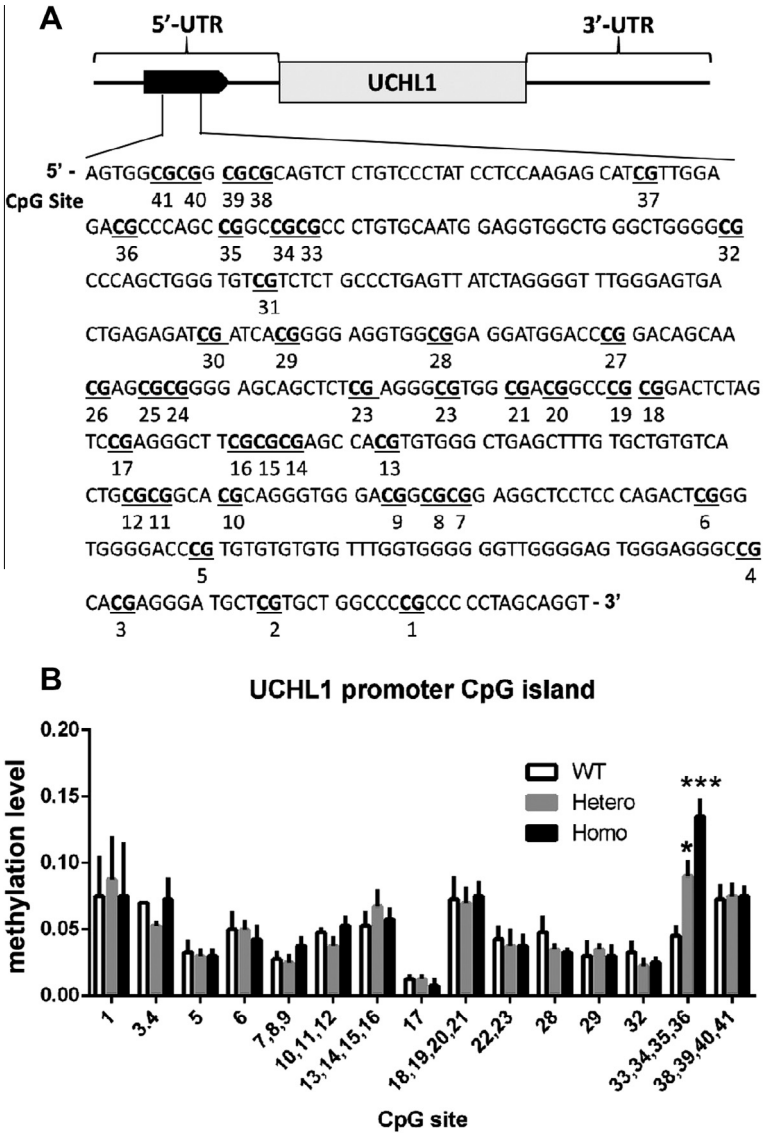

Fig. 4. UCHL1 promoter CpG island is over-methylated in the brain of LPL deficient mice. The methylation levels of CpG sites in UCHL1 promoter regions from mouse brain were compared. Sequenom MassARRAY platform was used for the quantitative methylation analysis (A and B). $n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$ (relative to the respective control, One-way ANOVA). WT, Wild-type; Hetero, heterozygous; Homo, homozygous.

Aggregation of α-syn in the brain synaptosomes of LPL-deficient mice significantly increased compared with WT ($n = 3$, WT: Homo = 1: ($^{**}1.73 \pm 0.17$), $^{**}p < 0.01$) (Fig. 1B).

These results suggest that LPL deficiency promotes α-syn aggregation.

LPL deficiency reduces UCHL1 expression and results in increased protein ubiquitination

Our previous data showed that LPL-deficient mice displayed memory impairment (Xian et al., 2009). In neurons the balance between ubiquitination and de-ubiquitination is critical for synapse function. As a deubiquitinase, UCHL1 is another risk factor of neuro-degenerative disease (Gong et al., 2006; Setsuie and Wada, 2007; Yasuda et al., 2009; Bilguvar et al., 2013). To demonstrate whether LPL deficiency alters UCHL1 expression, we measured both protein and mRNA levels of UCHL1 in the whole brain of neonatal mice. Interestingly, UCHL1 decreased in LPL-deficient mice (Fig. 2A and B). Furthermore, ubiquitination of total protein and α-syn increased in LPL-deficient mice, which may due to decreased UCHL1 expression (Fig. 2C).

In order to examine whether excess LPL will lead to increased UCHL1, we overexpressed LPL in HEK293 cells. Then we detected UCHL1 protein and mRNA levels using western blotting and real-time PCR respectively. The results showed that UCHL1 was up-regulated (Fig. 2 D and E). Also, as a result of this up-regulation, decreased ubiquitination was found (Fig. 2 F). Intriguingly, expression of UCHL1 was positively associated with LPL level (Fig. 2G, H).

Taken together, these findings demonstrate that LPL affects UCHL1 expression. Accordingly, protein ubiquitination changes.

Nuclear retention of DNMT1 contributes to reduction of UCHL1

UCHL1 expression is regulated by DNA methylation of its promoter, and DNA methylation pattern is maintained by DNMT1. So, distribution of DNMT1 in the cytosol and nuclear of neonatal mouse brain was analyzed. We observed that, devoid of LPL resulted in nuclear retention of DNMT1 (Fig. 3 A). Consistently, overexpressing LPL caused cytosolic retention of DNMT1 in HEK293 cells (Fig. 3B, C).

To confirm that DNA methylation of UCHL1 promoter is involved in UCHL1 expression and ubiquitination, we treated HEK293 cells with different concentrations of DNMT1 inhibitor 5′-Aza-2′-Deoxycytidine (5′-AZA, Sigma, St Louis, MO, USA) for different hours. Then, we detected protein levels of UCHL1, as well as ubiquitination. We found that cells treated with 1 μM 5′-AZA for 24 h had a significant increase in UCHL1 (Fig. 3D, E). As a result of this increase, ubiquitination decreased in cells treated with 1 μM 5′-AZA for 24 h (Fig. 3 F). These results suggest that DNMT1 inhibition regulates UCHL1 expression, eventually affecting ubiquitination.

Taken together, LPL deficiency causes nuclear retention of DNMT1, and affects DNA methylation of UCHL1 promoter.

UCHL1 promoter CpG island is over methylated in the brain of LPL deficient mice

To confirm whether LPL deficiency leads to UCHL1 CpG island hypermethylation we detected the methylation status of the UCHL1 CpG island in its promoter using Sequenom MassARRAY platform (Fig. 4A, B). We found that the methylation level of CpG site 33, 34, 35, 36 increased significantly in neonatal LPL-deficient mice, indicated that LPL deficiency results in hypermethylation of UCHL1 promoter CpG island.

DISCUSSION

LPL deficiency results in disturbed phospholipid metabolism

Growing evidence supports that lipid metabolism plays an important role in several neurodegenerative diseases (Ruiperez et al., 2010; de la Monte and Tong, 2014; Leoni and Caccia, 2014). As a key enzyme in lipid metabolism, it has been suggested that LPL is involved in the pathogenesis of dementia (Vespa et al., 1999; Scacchi et al., 2004; Gong et al., 2013). Previous published study shows that LPL is associated with neurite pathology and its levels are markedly reduced in the dentate gyrus of AD brains (Gong et al., 2013). Our recent electrophysiological data also demonstrate that impaired synaptic vesicle recycling contributes to presynaptic dysfunction in LPL-deficient mice (Liu et al., 2014). These results suggest that LPL is required for normal synaptic activity. In the brain of LPL-deficient mice, lipidomics analysis revealed that phospholipids hemostasis had been disrupted (Table 1), which indicated that disturbed lipid composition resulting from LPL deficiency may be one way to cause neurodegeneration.

LPL deficiency promotes α-syn aggregation, contributed to synaptic function

Membrane-associated α-syn is an abundant presynaptic protein, and native α-syn consists of a largely unfold

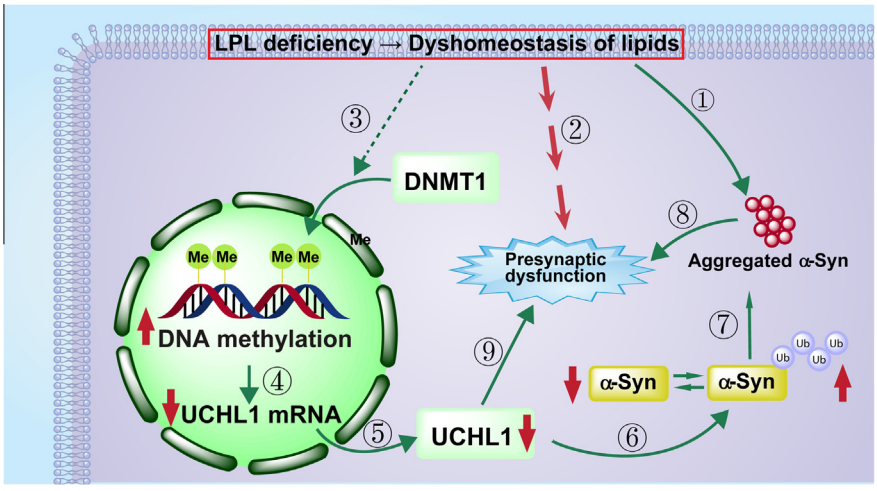

Fig. 5. Possible mechanisms of synaptic dysfunction induced by LPL deficiency – lipid dyshomeostasis, α-syn ubiquitination and decreased UCHL1 expression. ① LPL deficiency disturbs lipid metabolism, resulted in α-syn aggregation. ② Homeostasis of lipid metabolism is essential for normal presynaptic function (Xian et al., 2009; Crupi et al., 2013; Carta et al., 2014). ③ LPL deficiency gives rise to translocation of DNMT1 from cytoplasm to nuclear, and then raises UCHL1 promoter DNA methylation level (Sasaki and Matsui, 2008; Svedruzic, 2011). ④ UCHL1 transcription is inhibited because of DNA hypermethylation of its promoter (Ummanni et al., 2011; Mitsui et al., 2012; Trifa et al., 2013). ⑤ UCHL1 protein decreases. ⑥ Down-regulation of UCHL1 elevates α-syn ubiquitination (Day and Thompson, 2010; Bilguvar et al., 2013). ⑦ Aggregation of α-syn (Engelender, 2008; Cook et al., 2012; Haj-Yahya et al., 2013). ⑧ α-syn aggregation leads to presynaptic dysfunction (Anichtchik et al., 2013; Rockenstein et al., 2014; Spinelli et al., 2014). ⑨ Low level of UCHL1 also affects normal presynaptic function (Ichihara et al., 1995; Gong et al., 2006; Sakurai et al., 2008; Chen et al., 2010).

monomer in the membrane (Fauvet et al., 2012; Burre et al., 2013), formation of oligomeric α-syn due to misfolding and aggregation causes synaptic dysfunction (Volpicelli-Daley et al., 2011). Furthermore, negatively charged phospholipids have been found to accelerate α-syn aggregation under various circumstances (Lee et al., 2002; Jo et al., 2004), function as a soluble SNARE -complex chaperone, the core fusion machinery for vesicle fusion (Burré et al., 2010), indicating that membrane phospholipid composition may be important to stabilize the conformation of α-syn and synaptic plasticity. As phospholipid hemostasis was disrupted in LPL-deficient mice, we further examine its impact on α-syn aggregation, and our results showed that LPL deficiency promotes aggregation of α-syn, which may contribute to synaptic dysfunction.

LPL deficiency reduces UCHL1 expression, which was elevated by overexpressing LPL

In the brain, the balance between ubiquitination and deubiquitination is critical for synapse function (Song and Rape, 2008). As a deubiquitinase, previously published studies have detailed that UCHL1 is involved in the pathogenesis of neurodegenerative diseases (Gong et al., 2006; Setsuie and Wada, 2007; Yasuda et al., 2009; Bilguvar et al., 2013; Mondello et al., 2014). Furthermore, it has been proved that UCHL1 is crucial for normal synaptic function (Gong et al., 2006; Cartier et al., 2009; Chen et al., 2010). In this study, we measured both mRNA and protein levels of UCHL1 in the whole brain of mice, and we found decreased expression of UCHL1 in LPL-deficient mouse brain, which further led to enhanced ubiquitination of both total protein and α-syn, indicating that altered ubiquitination resulting from abnormal UCHL1 expression may contribute to learning and memory impairment found in LPL-deficient mice previously reported by our lab (Xian et al., 2009). In HEK293 cells, which do not express LPL endogenously, we overexpressed LPL and found expression of UCHL1 elevated and ubiquitination reduced, which was consistent with in vivo results. And expression of UCHL1 was positively associated with LPL level. These results suggested that LPL may be involved in modulation of UCHL1 expression. Since the regulation of LPL is tissue specific, a knockdown experiment or overexpress LPL in neuronal cell line would provide additional support.

LPL deficiency leads to UCHL1 promoter DNA hypermethylation

What caused differences in UCHL1 expression level? One possible reason is CpG island hypermethylation in its promoter region (Kagara et al., 2008; Zhao et al., 2011). Since DNMT1 is the enzyme for maintenance of methylation, which preserves the methylation patterns established early in development. DNMT1 is abundantly expressed in the adult brain and constitutes a candidate target to study the mechanisms of aberrant DNA methylation (Liu et al., 2009). So we further examine the distribution pattern of DNMT1 between the cytoplasm and nucleus. Results showed that DNMT1 located much more in the nuclear of LPL-deficient mouse brain, led to up-regulation UCHL1 gene promoter methylation, thus increasing UCHL1 expression while in LPL overexpressed cultured cells, DNMT1 translocated to the cytoplasm. Treatment with DNMT1 inhibitor 5′-AZA increased UCHL1 expression and lessened ubiquitination in both dose-dependent and time-dependent ways, which confirmed the role of DNMT1 in the regulation of UCHL1 expression. We finally analyzed the methylation levels of CpG sites in UCHL1 promoter regions, and results showed that the methylation level of CpG site 33, 34, 35, 36 increased significantly in LPL-deficient mice. These results demonstrated that the expression of UCHL1 might be controlled by distribution of DNMT1 via modulating methylation status of UCHL1 gene promoter. However, the exact relationship between LPL and UCHL1 gene is still to be identified.

LPL-deficient mice without rescue are known to die within 48 h (Ross et al., 2005). We previously found presynaptic dysfunction in LPL-deficient new born mice without rescue (Liu et al., 2014). So in this study, all the in vivo indexes were examined in new born mice without rescue, to provide the molecular mechanism underlying the deficits caused by LPL deficiency. Since adult (12 months, rescued) and new born LPL-deficient mice (without rescue) both displayed presynaptic dysfunction (Xian et al., 2009; Liu et al., 2014), we examined adult LPL-deficient mice and also observed aggregation of α-syn, indicating that there might be similar deficits in adult and new born LPL-deficient mice, which was due to LPL deficiency in the brain.

Altogether, our findings showed that LPL deficiency reduced UCHL1 expression via promoter hypermethylation in the brain of mice. Decrease of UCHL1 resulted in α-syn aggregation. Considering the importance of lipid metabolism in neurodegenerative diseases, α-syn aggregation and decrease of UCHL1 in LPL-deficient mice might be involved in synaptic dysfunction (Fig. 5). Elucidation of the entire functions of LPL in the brain should provide new insights into the molecular pathogenesis for the neurodegenerative disease.

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