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Neuronal ceroid lipofuscinosis related ER membrane protein CLN8 regulates PP2A activity and ceramide levels

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ABSTRACT

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative lysosomal storage disorders. CLN8 deficiency causes a subtype of NCL, referred to as CLN8 disease. CLN8 is an ER resident protein with unknown function; however, a role in ceramide metabolism has been suggested. In this report, we identified PP2A and its biological inhibitor I2PP2A as interacting proteins of CLN8. PP2A is one of the major serine/threonine phosphatases in cells and governs a wide range of signaling pathways by dephosphorylating critical signaling molecules. We showed that the phosphorylation levels of several substrates of PP2A, namely Akt, S6 kinase, and GSK3β, were decreased in CLN8 disease patient fibroblasts. This reduction can be reversed by inhibiting PP2A phosphatase activity with cantharidin , suggesting a higher PP2A activity in CLN8-deficient cells. Since ceramides are known to bind and influence the activity of PP2A and I2PP2A, we further examined whether ceramide levels in the CLN8-deficient cells were changed. Interestingly, the ceramide levels were reduced by 60% in CLN8 disease patient cells compared to controls. Furthermore, we observed that the conversion of ER-localized NBD-C6-ceramide to glucosylceramide and sphingomyelin in the Golgi apparatus was not affected in CLN8-deficient cells, indicating transport of ceramides from ER to the Golgi apparatus was normal. A model of how CLN8 along with ceramides affects I2PP2A and PP2A binding and activities is proposed.

1. Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a group of neurodegenerative lysosomal disorders that predominantly affect children [1,2]. They lead to severe pathological conditions such as progressive loss of motor neuron functions, loss of vision, mental retardation, epilepsy, ataxia, and eventually premature death. Thirteen genetically distinct subtypes of the NCLs have been identified to date [3]. Intriguingly, these genes encode a variety of unrelated proteins (ceroid-lipofuscinosis neuronal proteins, CLNs) that are localized to various cellular compartments. One of the CLN proteins, CLN8, is an ER resident protein with five predicted transmembrane regions and a C-terminal di-lysine ER retrieval signal [4].

The first hint that the function of CLN8 involves ceramide comes from a conserved domain of ~200 amino acids in CLN8 named TLC. The TLC domain was originally identified in the TRAM, Lag1, and CLN8 proteins [5]. It is postulated that the function of this domain is lipid binding/sensing. The human orthologues of yeast Lag1 is ceramide synthase family protein Lass1–6, later renamed CerS1–6 [6]. Even though the purported ceramide synthase active site is located within the TLC domain, and CLN8 contains two conserved histidine residues essential for the synthase activity, CLN8 does not possess such activity [7]. Decreases in ceramide levels have been observed in CLN8 disease patient cerebral samples and the CLN8 disease *mnd*-mouse model [8,9]. However, how CLN8 is linked to ceramide metabolism is not clear. Besides CLN8, there are also reports suggesting a role for sphingolipid metabolism in other CLN proteins. For instance, CLN3, an *endo*-lysosomal multi-spanning transmembrane protein, has been shown to bind galactosylceramide and this interaction is required for both galactosylceramide and CLN3 trafficking out from the Golgi [10,11]. Altered

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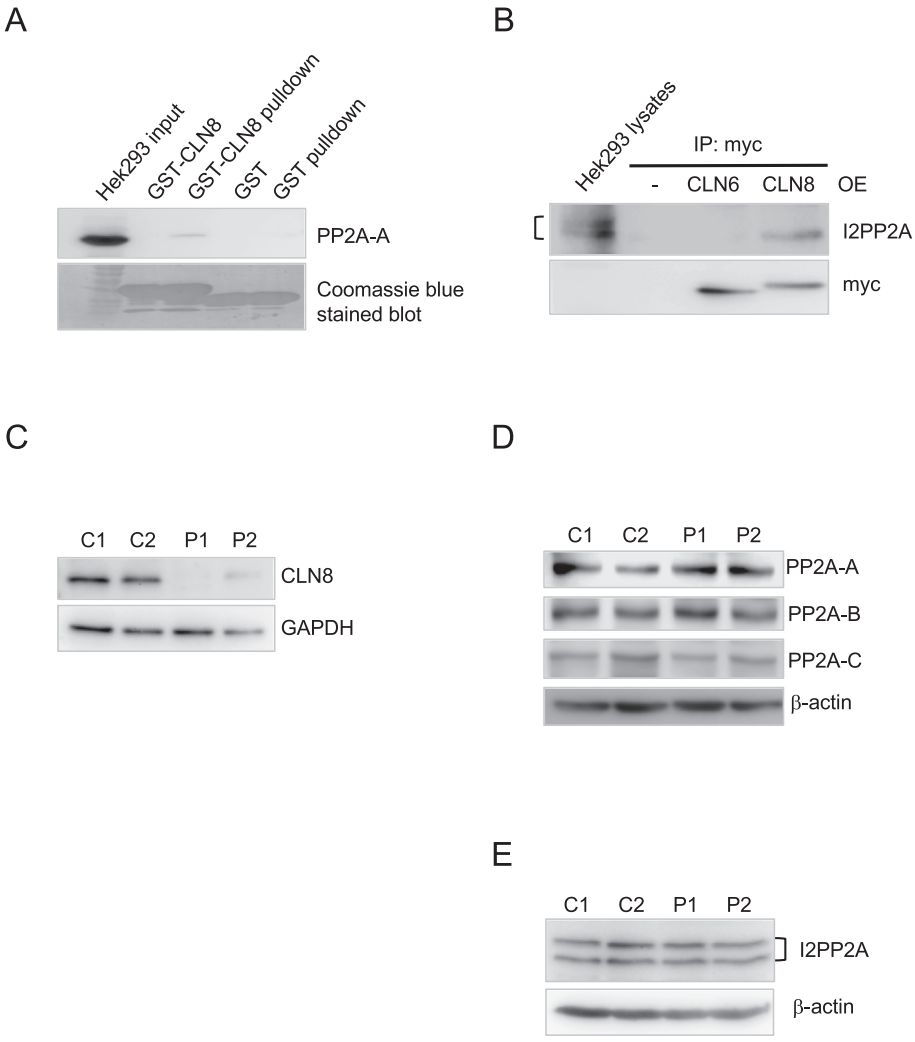

Fig. 1. CLN8 interacts with PP2A-A and I2PP2A. (A) GST pulldown experiments show recombinant GST-CLN8 cytoplasmic tail (246–286), but not GST, interacts with PP2A-A. Hek293 whole lysates were used as prey. Coomassie blue stained blot in A shows GST fusion proteins used in pulldown assays. (B) CLN8, but not CLN6, co-immunoprecipitates with endogenous I2PP2A. Overexpression of CLN6 in lane 3 was used as a negative control. Myc blot shows similar amounts of proteins overexpressed. (C) The CLN8 disease patient fibroblasts (P1 and P2) had low to undetectable levels of CLN8 proteins. C1 and C2 were two healthy control fibroblasts. (D) The protein levels of PP2A subunits, A, B, and C, were similar among control and CLN8 disease patient fibroblasts. (E) The protein levels of I2PP2A were similar among control and CLN8 disease patient fibroblasts. GAPDH and β-actin were used as loading controls. The experiments in (A) and (B) were performed twice. All other experiments were repeated at least three times.

levels of various gangliosides have been shown in a CLN3 disease mouse model [12]. Impaired sphingolipid regulation is implicated in other neurodegenerative disorders as well [13]. For example, increased levels of ceramides and down-regulated sphingosine kinase-1 were found in Alzheimer's disease, Parkinson's disease, and Huntington's disease patients [14–18].

In this report, we show that CLN8 interacts with PP2A and I2PP2A/SET, a biological inhibitor of PP2A. We demonstrate that the phosphorylation levels of several PP2A targets are reduced. Since ceramide binds to I2PP2A and subunit C of PP2A [19,20], ceramide levels and metabolism were also investigated in CLN8-deficient cells. A model of how the CLN8-ceramide-I2PP2A-PP2A axis works is proposed.

2. Materials and methods

2.1. Antibodies

Mouse monoclonal antibodies used in this study were against Myc epitope (9E10, hybridoma cell line from ATCC (CRL 1729)), GAPDH (Developmental Studies Hybridoma Bank (DSHB), 4B7), tubulin (DSHB, 12G10), beta-actin (GenScript, A00702). Rabbit antibodies used in this study were against I2PP2A/SET (GeneTex, GTX 113834), PP2A-A (GeneTex, GTX 102206), CLN8 (Abcam, EPR15048), and the following antibodies were from Cell Signaling Technology: PP2A-B (100C1), PP2A-C (52F8), Akt (C67E7), pS473-Akt (D9E), S6 kinase (49D7), pT389-S6K (108D2), GSK3β (27C10), pS9-GSK3b (D85E12). HRP-conjugated secondary antibodies were purchased from Jackson Laboratory.

2.2. Cell culture

Human healthy skin fibroblasts (C1: GM000498, C2: GM05757), Gaucher disease patient skin fibroblast (GD-P: GM00877), and Niemann Pick type A disease patient fibroblast (NPA-D: GM13205) were purchased from Coriell Institute for Medical Research. The CLN8 disease patient skin fibroblast P1 (homozygous c. 63G > A, p. Trp21X) was received from Massachusetts General Hospital CHGR NCL Disorders Clinical Database and Biorepository, P2 (c.581A > G, p.Gln194Arg; c.66 del G, p. Gly22fs > X78) was received from Batten Disease Registry, Institute for Basic Research, Staten Island, NY. HEK293 cell line was purchased from the American Type Culture Collection (ATCC CRL-1573). Cell culture media and reagents were purchased from Gibco, Hyclone, and Corning. All cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 20 mM HEPES, and gentamicin at 37 °C in a humidified incubator with 5% CO_2. siRNA transfection was performed using the Neon Transfection System (Thermo Fisher). CLN8 siRNA sequence: CAGCUGUCCUCUUCCCUGA (Dharmacon). Cantharidin was purchased from Cayman Chemical.

2.3. Sample preparation for Immunoblotting

Cells grown on culture dishes were scraped and washed once with 1× phosphate buffered saline (PBS), and centrifuged for 3 min at 1,500 ×*g*. Cell pellets were lysed using RIPA lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1%

SDS) supplemented with protease inhibitor cocktail (G-Biosciences) and phosphatase inhibitors (50 mM NaF, 2 mM Na_3VO_4, 10 mM $Na_4P_2O_7$). After incubation for 30 min on ice, extracts were centrifuged at 20,000 ×*g* for 10 min at 4 °C. The supernatant was collected as the whole cell lysates. Protein concentrations were determined by Bradford assay. Samples were separated by SDS-PAGE. Proteins were transferred to PVDF membranes (Millipore) followed by immunoblotting. ECL detection was performed according manufacturer's instruction (Millipore). Membrane imaging employed a G-Box imager from Syngene or C-DiGit imager from Li-COR.

2.4. GST pull down

Bacteria strain Rosetta (DE3) pLysS was used for GST and GST-CLN8 (246–286) fusion protein expression. The recombinant GST fusion protein was purified using glutathione beads (GoldBio). HEK293 cells were lysed in lysis buffer (20 mM Tris HCl pH 7.4, 1 mM EDTA, 150 mM NaCl, 0.5% NP-40, 0.5 mM $MgCl_2$). The supernatants were collected as cell lysates after centrifugation (20,000 ×*g* for 10 min at 4 °C). The cell lysates were pre-cleared with glutathione beads and then incubated with GST or GST-CLN8 (246–286) fusion protein bound beads at 4 °C for 2 h. The GST pulldown samples were run on SDS-PAGE and immunoblotted. For Fig. 1A, after completing immunoblotting analysis, the blot was stained with Coomassie blue.

2.5. Co-immunoprecipitation

The cDNAs encoding CLN8 and CLN6 were amplified from a HeLa cDNA library and the sequences were validated by sequencing. The CLN8 cDNA was cloned into pEGFP-C1 by replacing EGFP with the myc-His tag to create an N-terminal myc-His tag CLN8. CLN6 was cloned into pcDNA3.1/myc-His (-)A to create a C-terminal myc-His tag CLN6. Transient transfection was performed using TransIT-LT1 reagent (Mirus Bio) in HEK293 cells to express myc-His tagged CLN6 and CLN8. Cells were collected 24 h post transfection by scraping. The pellets were lysed in lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 5% glycerol) and cell lysates were collected as mentioned above. The lysates were pre-cleared with Dynabeads protein G (Invitrogen). Myc antibody was loaded onto Dynabeads protein G, mixed with the precleared lysates, and tumbled at 4 °C for 1 h. Co-immunoprecipitated samples were run on SDS-PAGE and immunoblotted.

2.6. Lipid extraction

Lipids were extracted by the method of Bligh and Dyer [21] with slight modifications. Briefly, cells grown on 10 cm plates were trypsinized and pellets were collected. Cell pellets were resuspended in 1.6 ml of water, 2 ml of chloroform, and 4 ml methanol and the mixture was vortexed to get a homogenous solution. 2 ml of chloroform and 2 ml of water were added with additional vortexing, followed by centrifugation at low speed for 10 min to split the solution into three distinct phases. The lower organic phase was collected. 2 ml of chloroform were added to the original tube, followed by vortexing, centrifugation, and removal of the lower layer, three more times. Solvent was evaporated from the combined lower layers under nitrogen; the extracted lipids were dissolved in 1 ml of chloroform for lipidomics by mass spectrometry at Kansas Lipidomics Research Center (KLRC) at Kansas State University.

2.7. Lipid mass spectrometry

Analysis of sphingomyelin, ceramides, and hexosylceramides by electrospray ionization triple quadrupole mass spectrometry were carried out as previously described [22,23].

2.8. NBD-C6-ceramide labeling and thin layer chromatography (TLC)

Cells were incubated with DMEM with 1% BSA containing 1 μM NBD-C6-ceramide complexed to BSA (Thermo Fisher N22651) for 2 h. Cells were then rinsed three times with 1XPBS. DMEM with 10% FBS was added back to cells and chased for various durations. At the end of each time point, cells were scraped with 1 ml of acidic methanol (acetic acid: methanol in 1:50 v/v). Lipid extraction of the cells were carried out as described above. After N_2 evaporation, lipids were dissolved in 100 μl of chloroform: methanol (1:1) solvent and subjected to TLC analysis. The TLC plate (Analtech Uniplate: silica gel matrix) was pre-run with 1% sodium tetraborate in methanol and allowed to dry at room temperature followed by baking the plate at 125 °C for 30 min. The TLC running solvent mixture was chloroform: methanol: water (100: 30: 4). C6-NBD-Ceramide, N-hexanoyl-NBD-glucosylceramide, N-hexanoyl-NBD-sphingomyelin (Matreya LLC) were used as TLC standards. NBD fluorescent signals on TLC plates were imaged using Typhoon imager Trio (GE) and quantified using image J.

2.9. GBA β-glucosylceramidase activity assay

Cells were collected by trypsinization and lysed in citrate-phosphate extraction buffer, pH 5.4. An equal amount of total proteins was used in each assay. A pro-fluorescent substrate 4MU-β-Glc (10 mM) was added to initiate the reaction. After one-hour incubation at 37 °C, the reaction was terminated by addition of stop solution (1 M NaOH, 1 M glycine). Fluorescence was measured at an excitation of 355 nm and an emission of 460 nm using a Victor 3 V plate reader (Perkin Elmer). The assay was performed in triplicate.

3. Results and discussion

Using the cytoplasmic tail (amino-acid 246–286) of CLN8 as bait, we performed a GST pulldown experiment followed by mass spectrometry to identify proteins that interact with CLN8 at the cytosolic face of the ER. From mass spectrometry analysis, we found one set of proteins that was particularly interesting, due to their association with ceramides. These are two subunits of PP2A, PP2A-A and PP2A-B, and a known biological inhibitor of PP2A, I2PP2A/SET. PP2A is one of the major serine/threonine phosphatases in cells. It governs a wide range of signaling pathways by dephosphorylating critical signaling molecules [24]. PP2A is composed of three subunits, a structural A subunit, a regulatory B subunit, and a catalytic C subunit [25]. Intriguingly, both PP2A and I2PP2A can bind ceramide, and the activation of PP2A is stimulated by ceramide [19,20,26]. Independently, we used a co-immunoprecipitation approach followed by mass spectrometry with the full-length CLN8 as the bait and identified I2PP2A as well. We confirmed our finding by GST pull-down and co-immunoprecipitation assays and found the GST-CLN8-cytoplasmic tail and myc-his tagged CLN8 can interact with PP2A-A and I2PP2A at endogenous levels of expression (Fig. 1A and B).

To understand the impact of these interactions in a cellular context, we examined whether presence or absence of CLN8 would affect the cellular levels of PP2A and I2PP2A. Total cell lysates of human skin fibroblasts from healthy controls (C1 and C2) and two CLN8 disease patients (P1 and P2) were analyzed. We first examined the CLN8 protein levels in the patient cells (Fig. 1C), as this has never been shown previously. In P1, there was no detectable level of CLN8, consistent with the genotype of the patient (homozygous c.63G > A, p. Trp21X) resulting in severe truncation of CLN8 protein. On the other hand, in P2, a trace amount of CLN8 protein was detected. This also fits with the genotype of P2 (c.581A > G, p.Gln194Arg; c.66 del G, p. Gly22fs > X78), being one copy point mutation and one copy frame shift leading to severe truncation. Examination of PP2A subunits and I2PP2A indicated similar levels among patient and control cells (Fig. 1D and E).

We then investigated the activity of PP2A in CLN8 disease patient

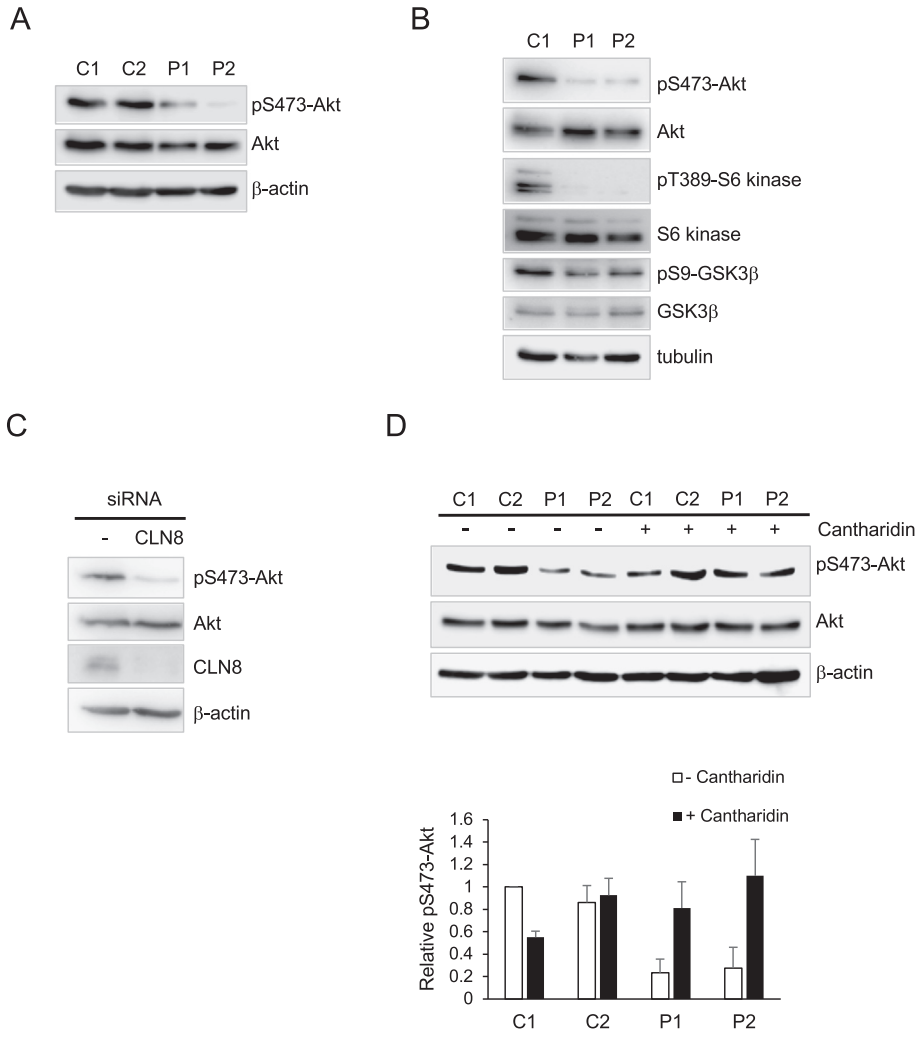

Fig. 2. CLN8-deficient cells exhibit decreased levels of Akt phosphorylation. (A) The basal levels of Akt phosphorylation at Ser473 residue in CLN8 disease patient cells were decreased. (B) The phosphorylation levels of Akt Ser473, S6 kinase Thr389, and GSK3β Ser9 were decreased in CLN8 disease patient cells. (C) Knockdown CLN8 by siRNA reduced the phosphorylation level of pS473-Akt in fibroblasts. (D) Control and CLN8-deficient fibroblasts were treated with cantharidin (25 μM) or DMSO for 1 h before harvesting cell pellets. Total cell lysates were analyzed by immunoblotting to examine phospho-Akt levels. β-Actin and tubulin were used as loading controls. The relative levels of pS473-Akt were normalized with total Akt and quantified from three sets of experiments using Image J. For each set of experiments, C1 no treatment was set as 1. Error bar represents SEM. The experiments in (C) were performed twice. All other experiments were repeated at least three times.

fibroblasts. The phosphorylation levels of Akt, a PP2A substrate, in CLN8 disease patient cells were examined (Fig. 2). Compared to controls, CLN8-deficient cells had substantially lower levels of phosphorylation at Ser473 of Akt (Fig. 2A). Further analysis of other substrates of PP2A showed that phosphorylation levels of S6 kinase-Thr389 and GSK3β-Ser9 were also lower (Fig. 2B). To confirm that the effects on Akt phosphorylation were due to CLN8 deficiency, we performed small interfering RNA knockdown experiments to reduce CLN8 level in control fibroblasts. Cells with reduced levels of CLN8 had decreased pSer473-Akt signals (Fig. 2C), supporting our hypothesis that CLN8 deficiency affected phosphorylation. To investigate whether the effects were specifically due to an increase in PP2A activity or a decrease in upstream kinases, we treated cells with a potent inhibitor of PP2A, cantharidin [27]. Immunoblotting analysis showed that in CLN8-deficient cells treated with cantharidin, the phosphorylation levels of Akt-Ser473 were increased compared to the patient cells treated with DMSO only (Fig. 2D). This result suggests that the reduction in the phosphorylation levels of Akt, GSK-3β, and S6 kinase seen in CLN8 -deficient cells is a consequence of increased PP2A activity. These data suggest that CLN8 protein may have a role in regulating the activation of PP2A.

After observing effects of CLN8 on PP2A activity, and considering the fact that ceramides bind PP2A and I2PP2A, we investigated whether ceramide levels were altered in CLN8-deficient fibroblasts. We performed lipidomic analysis on control and CLN8-deficient patient fibroblasts. In addition, we also included patient fibroblasts from Gaucher disease (GD) and Niemann Pick disease type A (NPA) in our analysis for comparison. Mutations in β-glucosylceramidase gene GBA1 in GD patients cause reduced or non-functional lysosomal glucosylceramidase (GCase) activity [28], leading to accumulation of the substrate glucosylceramides and a reduction of the product ceramides. NPA, on the other hand, is caused by mutations in the lysosomal acid sphingomyelinase gene SMPD1 [29]. Deficiency in acid sphingomyelinase results in sphingomyelin buildup and reduction in ceramide and phosphorylcholine in lysosomes. Ceramides (Cer), sphingomyelin (SM) and dihydro-sphingomyelin (DSM), and hexosyl ceramides (hexCer, ceramides that contain a monosaccharide) were analyzed (Fig. 3). Remarkably, in CLN8-deficient cells, the levels of ceramides were substantially lower compared to healthy controls and were comparable to those of the GD patient (Fig. 3A, Cer). On the other hand, only NPD patient fibroblasts had a significant higher level of SM and DSM compared to controls. No difference in hexCer levels was found in CLN8-deficient cells compared to control cells. The reduction of ceramides was found in all major ceramide species detected (Fig. 3B).

The C-terminal cytoplasmic di-lysine motif of CLN8 is required for ER retrieval from the Golgi apparatus ([4] and our unpublished results). Therefore, we examined if CLN8 was involved in transporting ceramides from the ER to the Golgi, and whether the CLN8 disease patient fibroblasts were deficient in this process. Cells were fed with NBD-C6-ceramide for 2 h. When NBD-C6-ceramide entering the cells, it first arrives at the ER, then is quickly transported to Golgi [30]. We followed the metabolized NBD species by TLC after incubation for different time intervals for up to 10 h. Fluorescent signals from the TLC plates representing different sphingolipid species were shown (Fig. 3C, top). We did not observe differences at the rate of conversion of NBD-ceramide to

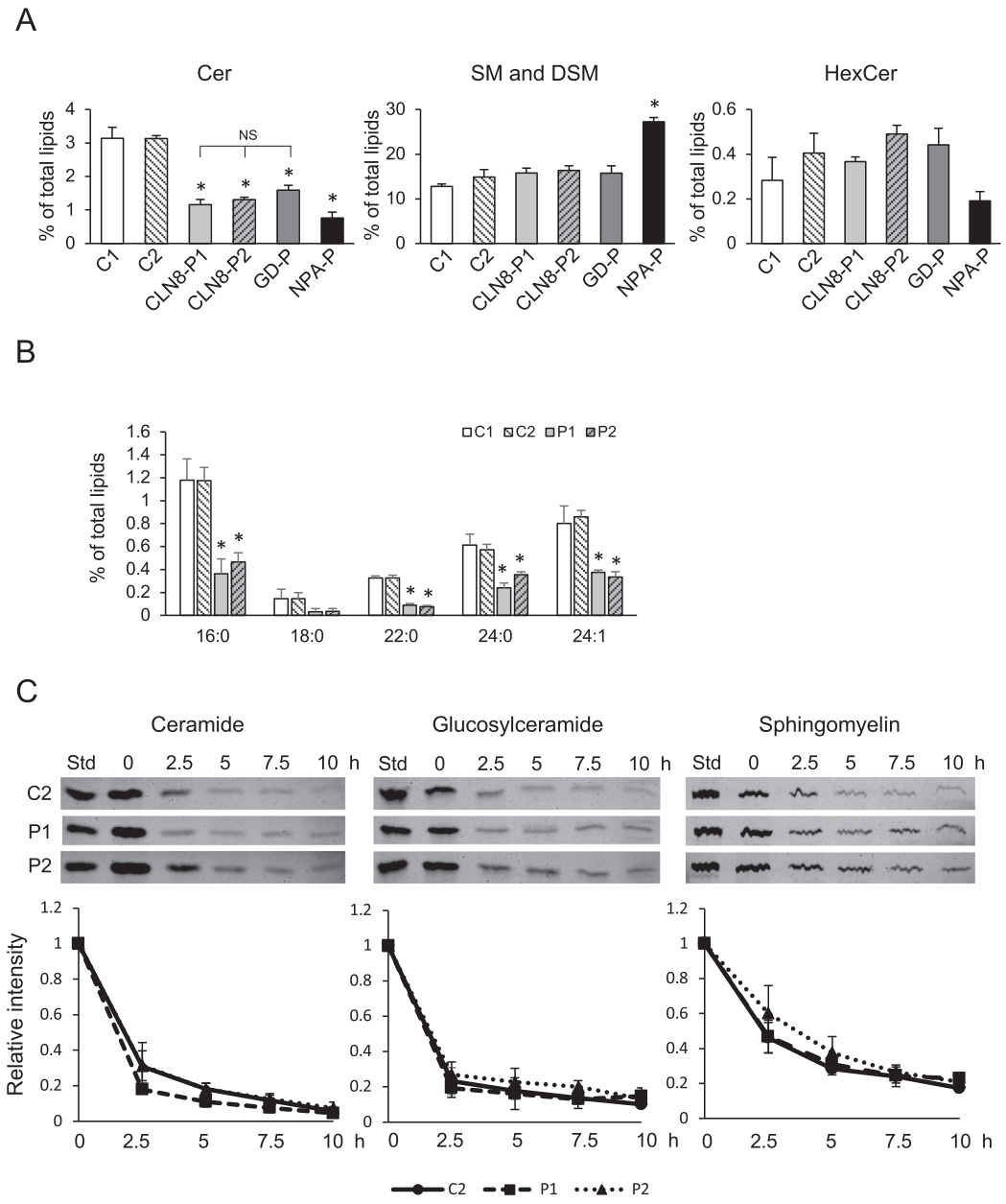

Fig. 3. Sphingolipids analyses of CLN8-deficient fibroblasts. (A) Sphingolipid profiling analyses of total ceramides (Cer), total Sphingomyelin (SM) and dihydrosphingomyelin (DSM), and total hexosyl ceramides (HexCer) showed that the total amount of ceramides in CLN8-deficient cells were reduced, compared to control cells (C1 and C2). GD patient cells (GD-P) and NPA patient cells (NPA-P) were also analyzed as comparison. (B) All major ceramide species detected (Cer-d18:1(16:0), Cer-d18:1(18:0), Cer-d18:1(22:0), Cer-d18:1(24:0), Cer-d18:1(24:1)) were reduced in CLN8 disease patient fibroblasts. In (A) and (B), error bar represents SD. The data were analyzed by one-way ANOVA followed by the Tukey's test. $n = 3$ experiments. * shows that there is a significance difference compared to both C1 and C2. $P < 0.05$. (C) TLC analyses of ceramide metabolism. Cells were incubated with NBD-C6-ceramide complexed to BSA containing media for 2 h and then chased for up to 10 h. Cell pellets were collected at different time points as indicated. The lipid extracts were analyzed by TLC. The NBD-C6-ceramide, NBD-C6-glucosylceramide, and NBD-C6-sphingomyelin were run on the left lane as markers. The relative levels of these three NBD-C6-ceramide metabolites were quantified from three experiments using Image J. The levels of 0 h were set as 1. Error bar represents SEM.

NBD-glucosylceramide or NBD-sphingomyelin between the control and CLN8-deficient fibroblasts (Fig. 3C, bottom). This suggests that transport of ceramides from the ER to the cis-Golgi for glucosylceramide synthesis, as well as from the ER to the trans-Golgi for sphingomyelin synthesis are not delayed in CLN8-deficient cells.

A recent report identified CLN8 as a genetic modifier in Gaucher disease. Patients with milder forms of GD express higher levels of CLN8 [31]. We therefore entertained the idea that CLN8 facilitates the transport of β-GCase to lysosomes. If this were true, in the CLN8 disease patient cells, the β-GCase activity would be reduced. This could also explain the reduced ceramide levels in CLN8-deficient cells. We performed GCase enzyme activity assays and compared control, CLN8 disease patient, and GD patient fibroblasts (Fig. 4A). While the GD patient fibroblasts exhibited substantially reduced levels of GCase activity, CLN8-deficient cells had similar (or even higher) levels of activity as in healthy control cells. This suggests that CLN8 expression influences GD patient cells in another way, rather than providing more GCase to the lysosome. More CLN8 may, for example, increase the level of ceramides and rebalance the sphingolipid homeostasis in GD patients. Further investigation will be needed to comprehend the exact relationship between CLN8 and GCase.

In this report, we showed for the first time that the phosphorylation

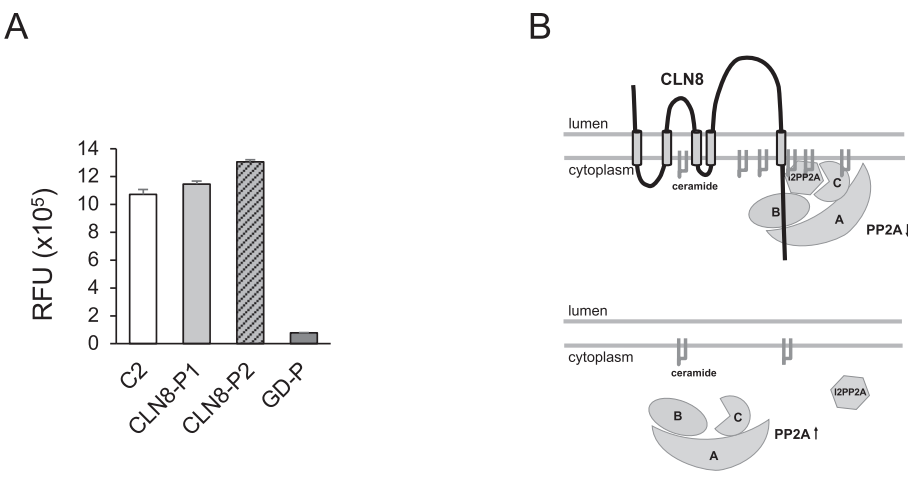

Fig. 4. (A) The β-glucosylceramidase (GCase) activity is normal in CLN8 disease patient fibroblasts. The GCase enzyme activity was analyzed from total cell lysates of control (C2), CLN8 disease (CLN8-P1 and CLN8-P2), and GD (GD-P) cells. Each value represents mean + SD. $n = 3$. (B) A proposed model of how CLN8-ceramide may interact with I2PP2A-PP2A to regulate PP2A activity. Top of figure, increase in ceramide availability in the presence of CLN8, which may potentially recruit I2PP2A and PP2A subunits A and B, and in turn, regulates the PP2A inactivation. Bottom of figure, reduction in ceramide availability in the absence of CLN8. Without the CLN8-ceramide hub at the ER membrane, the recruitment of I2PP2A is reduced, and consequently the PP2A activity increases.

state of Akt is altered in CLN8-deficient cells. This effect can be reversed by inhibiting PP2A activity. We propose that CLN8 along with ceramide provides a platform at the ER membrane to which I2PP2A and PP2A can be recruited. Binding to ceramide activates I2PP2A's ability to inhibit PP2A holoenzyme. When CLN8 is deficient, I2PP2A cannot be recruited properly to the CLN8-ceramide hub and hence is not able to negatively regulate PP2A activity. As a consequence, the enhanced PP2A activity leads to dephosphorylation of Akt (can be direct or indirect) and other PP2A substrates (Fig. 4B). This seems contradictory to the known fact that ceramide binds to and activate PP2A [26]. However, with an additional player I2PP2A as a negative regulator in the CLN8-ceramide-PP2A complex, the regulation of PP2A activity is likely to be more complex. Further studies to dissect the interactions between these proteins will help to elucidate the mechanism by which the PP2A activity is regulated in the CLN8-ceramide hub. PP2A as well as its targeting kinases have been implicated in neuronal development [32–34]. In addition, dysregulation of PP2A activity has been reported in neurodegenerative disorders, such as Alzheimer's disease [35] and Parkinson's disease [36]. It is possible that altered PP2A activity may also contribute to neuronal defects in CLN8 disease.

Several questions remain to be answered. For instance, how does CLN8 affect the ceramide levels in the cell? We have shown that the conversion of ceramide to glucosylceramide or sphingomyelin was not affected in CLN8-deficient cells, nor was the transport of ceramide out from ER to Golgi. The cellular ceramides can be derived from *de novo* synthesis in the ER, or through the salvage pathway in the lysosome by breaking down glucosylceramide and sphingomyelin back to ceramide. Our lipidomic data showed, in spite of lower levels of total ceramides, the sphingomyelin and hexosyl ceramides levels were not changed in CLN8-deficient cells (Fig. 3A). This supports the idea that CLN8 is involved in the salvage pathway. In this pathway, ceramide needs to be recycled from the lysosome to ER. Lipid transfer is one of the major functions of inter-organelle membrane contact sites [37]. It is therefore plausible that CLN8 is present at the ER-lysosome contact sites and regulates this step. Without CLN8, ceramides produced through the salvage pathway cannot be recycled back to ER and are eventually degraded. This then would lead to overall reduction of ceramides in the cell. Supporting this idea, a study has identified Vamp-associated protein A (VAPA) as an interacting protein of CLN8 [38]. VAPA and VAPB are ER resident proteins involving in membrane contact site formation [39]. Further studies are warranted to test this model.

Transparency document

The Transparency document associated with this article can be found, in online version.

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