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A defect in the retromer accessory protein, SNX27, manifests by infantile myoclonic epilepsy and neurodegeneration

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Abstract

The composition of the neuronal cell surface dictates synaptic plasticity and thereby cognitive development. This remodeling of the synapses is governed by the endocytic network which internalize transmembrane proteins, then sort them back to the cell surface or carry them to the lysosome for degradation. The multi-protein retromer complex is central to this selection, capturing specific transmembrane proteins and remodeling the cell membrane to form isolated cargo-enriched transport carriers. We investigated a consanguineous family with four patients who presented in infancy with intractable myoclonic epilepsy and lack of psychomotor development. Using exome analysis, we identified a homozygous deleterious mutation in SNX27, which encodes sorting nexin 27, a retromer cargo adaptor. In western analysis of patient fibroblasts, the encoded mutant protein was expressed at an undetectable level when compared with a control sample. The patients' presentation and clinical course recapitulate that reported for the SNX27 knock-out mouse. Since the cargo proteins for SNX27-mediated sorting include subunits of ionotropic glutamate receptors and endosome-to-cell surface synaptic insertion of AMPA receptors is severely perturbed in SNX27^{-/-} neurons, it is proposed that at least part of the neurological aberrations observed in the patients is attributed to defective sorting of ionotropic glutamate receptors. SNX27 deficiency is now added to the growing list of neurodegenerative disorders associated with retromer dysfunction.

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Keywords

Myoclonic epilepsy; SNX27; Retromer complex

Introduction

Neural development and the synaptic plasticity required for cognitive development necessitates the dynamic and local remodeling of the protein and lipid content of the neuronal cell surface including millions of highly specialized synaptic connections [1]. Central to controlling the composition of the cell surface is the endocytic network [2]. Here, the specific internalization of transmembrane proteins is balanced by the selective sorting of the internalized proteins and lipids back to the cell surface or to the lysosome for down-regulation and degradation. By regulating the sorting of numerous transmembrane proteins (together termed cargoes), including cell adhesion molecules, signaling receptors and ion channels, the endocytic network regulates the structural and functional remodeling of synapses [1].

A master regulator of protein sorting through the endosomal network is the retromer [3]. Formed from the assembly of Vacuolar Protein Sorting 35 (VPS35) with VPS29 and VPS26 [4, 5], retromer serves to sort transmembrane proteins away from lysosomal-mediated degradation for recycling back to the cell surface, the trans-Golgi network (TGN) or alternatively, to specialized endomembrane compartments [6–9, 5, 10]. Retromer mediates cargo sorting by coordinating two processes: the sequence-dependent capture of specific cargo and the remodeling of membrane to form isolated cargo-enriched transport carriers [3, 11, 12]. Cargo capture can be mediated through direct binding to VPS35 and VPS26 [13–15] or indirectly through retromer's association to cargo adaptors [16], the most notable, to date, being sorting nexin-27 (SNX27) [10]. Membrane remodeling is mediated through the association of retromer with specific SNXBAR proteins that induce the formation of tubular transport carriers [7, 17–20]. Disruption of retromer function is increasingly linked with various neurological conditions (reviewed in [21, 22]). Here, we extend this linkage by reporting the clinical course of patients with congenital deficiency of the retromer cargo adaptor SNX27.

Patients and methods

Patients

Four patients, II-2, II-3, II-4 and II-5, two males and two females were the subjects of this study. They were born to consanguineous, second cousins once removed, Palestinian parents (Fig. 1a). The pregnancies were normal, apart from the pregnancy of patient II-4, where polyhydramnion was noted at mid-gestation. The births were vaginal, at term, and the birth weights were 2800–3000 g, with head circumferences 32–34 cm. Generalized hypotonia was noted soon after birth and startle-like movements appeared at 2 to 4 months of age in all the patients. Physical examination at that stage disclosed normal growth parameters, axial hypotonia with normal peripheral tone, intact deep tendon reflexes and planter response. There were neither dysmorphic features nor organomegaly. Echocardiogram was normal in

patients II-2 and II-4 but patient II-3 had atrial septal defect ostium secundum with mild to moderate left to right shunt, and patient II-5 had ventricular septal defect.

Brain MRI of patient II-2, performed at age 4 months, was normal, but delayed myelination was found in brain MRI of II-4 at 7 months of age (Fig. 1b). The EEG revealed five bursts of generalized poly spike and spike waves of less than 1-s duration, suggestive of myoclonic epilepsy. The seizures were resistant to multiple anti-epileptic drugs, and psychomotor development was markedly delayed; patient II-4, when examined at 16 months of age, had no voluntary movements and was not communicating. At that age, she would turn her head towards light source, but visual fixation was poor; nonetheless, fundoscopic examination was normal. The clinical course was characterized by intractable seizures, failure to thrive and recurrent chest infections with persistent oxygen dependency; three patients, II-2, II-3 and II-5 died at 30, 4 and 12 months, respectively, because of respiratory failure (the clinical and EEG findings are detailed in Table 1). Laboratory investigations including the serum levels of liver transaminases, alkaline phosphatase, ammonia, lactic acid, CPK, amino acids, and transferrin isoelectrical focusing, cerebrospinal fluid (CSF) glucose, lactic acid and amino acids and urinary organic acids, creatine and guanidinoacetate were all normal. The enzymatic activities of the mitochondrial respiratory chain complexes were normal in patient II-3 muscle tissue.

Methods

Whole exome sequencing—Exonic sequences were enriched in the DNA sample of patient II-4 using SureSelect Human All Exon 50Mb v.4 Kit (Agilent Technologies, Santa Clara, California, USA). Sequences were determined using the HiSeq2000 sequencing system with 100-bp paired-end reads (Illumina, San Diego, California, USA). Reads alignment and variant calling and annotation were performed with published human genome build UCSC hg19/GRCh37 as a reference sequence using the DNAnexus software (Palo Alto, California, USA). Parental consent was given for DNA studies.

Western blot—The patient and control fibroblasts were lysed in PBS +2 % TX100 supplemented with protease inhibitor tablets; insoluble debris was cleared by centrifugation, prior to the protein concentration of the cell lysates being measured using spectrometry and normalized between samples. Proteins were then separated using SDS-PAGE electrophoresis and transferred to PVDF membrane using western blotting.

Western blots were probed for β -actin as a load control (anti- β -actin, Sigma-Aldrich A1978 (1:5000 dilution) and for SNX27 and the retromer protein VPS35 (anti-VPS35, Abcam 97545 (1:2000); anti-SNX27, Abcam ab77799 (1:500)). The lower molecular weight section of the blot (10–37 kD) was also probed for the presence of any truncated SNX27. Western blots were visualized using a Licor Odyssey Infrared scanning system using fluorescently labelled secondary antibodies, with band intensities being quantified digitally.

Results

A mutation in the SNX27 gene segregates with disease

The exome analyses of patient II-4 yielded 75.38 million confidently mapped reads (mean coverage X72). Following alignment to the reference genome, 195,791 variants were called. We removed variants which were called less than X8, were off-target, heterozygous, synonymous, MAF>0.1 % in dbSNP132, MAF>1 % at the Hadassah in house database or predicted benign by Mutation Taster software. Seven variants survived this filtering process (Supplementary Table 1), but two were already associated with a different disease in human (LOXHD1 and PLXND1).

We checked four variants by Sanger sequencing for segregation in the family but only the frame shift mutation in the SNX27 gene (SNX27:NM_030918:exon2:c.515_516del:p.H172fs) segregated with the disease (Fig. 1a). The SNX27 gene mutation was not present among 61,486 unrelated individuals (Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: <http://exac.broadinstitute.org>), accessed Jan. 2015).

SNX27 protein levels are undetectable in patient fibroblasts

The human SNX27 gene encodes for a 529-amino acid protein with a molecular weight of 59.6 kDa (Fig. 2a) [23]. SNX27 possesses three recognized functional domains: an amino-terminal PDZ (PSD95, Dlg1, zo-1) domain (residues 41-134); a central PX (phox homology) domain (residues 159-268), which by binding to phosphatidylinositol 3-monophosphate (PtdIns3P) targets the protein to an early endosome compartment [24, 25]; and a carboxy-terminal FERM (4.1/ezrin/radixin/moesin)-like domain (residues 273-526) (Fig. 2a). The identified SNX27 mutation generates a frameshift at histidine 172 and is predicted to encode for a truncated protein comprising the entire PDZ domain and the first β -sheet of the PX domain (Fig. 2b). The consensus sequence for PtdIns3P-binding to PX domains, in the case of SNX27 196R[Y/F]x24Kx12R235 [26], is therefore absent, hence the truncated SNX27, even if stable, will be nonfunctional with regard to the ability to associate with early endosomes.

To examine SNX27 expression, we obtained skin fibroblasts from patient II-4 and probed western blots using an anti-SNX27 monoclonal antibody raised against an amino-terminal fusion of SNX27 (residues 1-267). In patient fibroblasts, we could detect no signal corresponding to full-length SNX27, while a clear signal was visible in fibroblasts obtained from a control patient (Fig. 2c). In a separate set of experiments (Supplementary Figure 1), we established that the epitope for the anti-SNX27 monoclonal antibodies used in our study residues within the region of SNX27 encoded by residues 1-134. The lack of a detectable signal corresponding to the truncated SNX27 mutant predicted from the frameshift at histidine 172 is therefore consistent with the overall conclusion that the identified frameshift results in a SNX27 mutant with dramatically reduced expression.

The ability of SNX27 to control the sorting of captured cargo for endosome-to-plasma membrane recycling is dependent upon its ability to associate with the retromer complex [27, 28, 10]. We therefore also examined the level of expression of the core retromer protein

VPS35 in patient fibroblasts. In the patient fibroblasts, we observed no detectable difference in VPS35 expression (Fig. 2c).

Discussion

In this consanguineous family, four infants presented with hypotonia, myoclonic epilepsy, lack of psychomotor development and early death. A homozygous deleterious mutation in the SNX27 gene was identified in all the affected whose DNA was available. The mutation resulted in a dramatic reduction in SNX27 protein expression, providing a unique opportunity to elucidate the indispensability of this protein for human physiology.

SNX27 is an evolutionary conserved endosome-associated protein that regulates the endosome-to-plasma membrane recycling of hundreds of cell surface proteins [29, 30, 25, 28]. Many of these proteins contain a carboxy-terminal PDZ-binding motif that directly binds to the PDZ domain of SNX27 [31, 28]. In coupling recognition of these PDZ motif containing cargoes with association to retromer and the actin polymerizing WASH complex [27, 28, 10], SNX27 coordinates cargo recognition with the sorting of captured cargo into an endosome-to-plasma membrane recycling pathway. Loss of SNX27 expression generally results in perturbed capture of cargo into the endosome-to-plasma membrane recycling pathway, leading to missorting of the cargo into the endo-lysosomal pathway and a resultant enhanced rate of cargo degradation that correlates with a reduced level of cargo at the cell surface [28, 10].

Cargo proteins for SNX27-mediated sorting include the Menkes disease-associated copper transport ATP7A, the glucose transporter GLUT1, various metal and amino acid transporters, ion channels and numerous signaling receptors [31, 32, 29, 25, 33, 28, 34] that include the β 2-adrenergic receptor [30, 10], and the ionotropic glutamate receptors α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPA-R) and N-methyl-D-aspartate (NMDA) receptor (NMDA-R) [35–38]. With such a central role in controlling the cell surface levels of functionally diverse transmembrane proteins, it is not surprising that SNX27 homozygous knockout mice are born with a non-Mendelian frequency, suggestive of in utero defects in embryonic development, and for those mice that are born, they are weaker and smaller than their littermates, display abnormal behavior and die within 4 weeks of birth [35]. Their brain pathology includes bilateral dilatation of the lateral ventricles, thinning of the cortex and dissociation of the periventricular neuropil, which is abnormally vacuolated [37].

The essential role of SNX27 is underscored by the abnormal behavior observed even in SNX27^{+/-} mice, which exhibit cognitive deficits, and in particular reduced learning and memory [38]. Modulating the number and activity of synaptic AMPA-Rs contributes to the activity-dependent plasticity of the synapse that is argued to contribute to learning and memory (reviewed in [1]). SNX27 binds to the PDZ-binding motifs present in the GluA1 and GluA2 AMPA-R subunits and is enriched in synapses, where its presence is necessary for efficient cell surface expression of these receptors [35–38]. Chemically induced endosome-to-cell surface synaptic insertion of AMPA-R, a mimic of long-term potentiation (LTP), is severely perturbed in SNX27^{-/-} neurons and in rat cortical neurons suppressed for SNX27

expression by short-hairpin RNA [36, 37]. The perturbed cognitive function and perhaps other neurological aberrations observed in SNX27^{-/-} and SNX27^{+/-} mice appear therefore to result at least in part from defective sorting of AMPA-Rs [35–38]. Further highlighting the importance of SNX27 for normal synaptic and cognitive function is evidence that reduced SNX27 expression in Down's syndrome patients contributes to synaptic dysfunction through perturbed glutamate receptor sorting [38].

The infantile onset and the markedly delayed psychomotor development described in the present study is therefore consistent with the observed loss of detectable SNX27 expression. Determining the specific expression level of neuronal receptors and the various other cell surface proteins whose expression is controlled by SNX27 is beyond the scope of this report. Nonetheless, the lack of aberration of glucose metabolism and the normal cerebellum, hair and serum alkaline phosphatase level attest against a clinically significant reduction of GLUT1 and ATP7A, respectively.

SNX27 is part of the retromer complex, which has been previously implicated in adult neurodegenerative diseases. Decreased retromer expression is observed in patients with Alzheimer's disease (AD) [39–41], and this correlates with an increase in amyloidogenic processing of amyloid precursor protein (APP). There is also evidence suggestive of additional retromer accessory proteins as AD risk genes [42]. Mutations in VPS35, VPS26A and the retromer accessory protein DNAJC13 [43–46] have been associated with late-onset autosomal dominant Parkinson's disease (PD) [47–50]. Indeed, the PD-linked VPS35(p.D620N) mutation displays a reduced ability to associate with the actin polymerizing WASH (Wiskott-Aldrich syndrome protein and SCAR Homology) complex [51–53] which leads to altered endosome-to-Golgi transport and autophagosome formation [54, 44, 55, 56]. Perturbed function of the WASH complex is also implicated in hereditary spastic paraplegia [57]. These data point to an important role for retromer and retromer accessory proteins in preventing a range of neurological conditions. The present report therefore further underscores the importance of the retromer complex and retromer accessory proteins for normal brain development in early infancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A- Family pedigree and the SNX27 mutation. The patients are represented by filled symbols. The genotype of individuals whose DNA was available is shown below the symbols. B - T2-weighted brain MRI at age 7 months showing abnormal, symmetric, hyperintense white matter, most prominent around the tip of the frontal horns (arrow).

Figure 2.

Analysis of the effect of the c.515_516del mutation on SNX27 protein expression. (A). Schematic presentation of the domain structure of SNX27. Known direct points of engagement of SNX27 with PDZ-binding motifs (PDZbm) and NPxY motifs present within the intracellular cytosolic domains of cargo proteins, the VPS26 retromer subunit, Ras GTPases and phosphatidylinositol 3-monophosphate (PtdIns3P) are shown. (B). The predicted affect of the SNX27 c.515_516del mutation. (C). Western blotting of control and patient-derived fibroblasts revealing a dramatic reduction in the expression of full length SNX27 and evidence consistent with the lack of a stable SNX27 truncation mutant. Levels of the retromer component, VPS35, are unaffected. Actin is used as a load control.

Table 1

Clinical and EEG findings in the patients.

Patient/gender	Age at presentation (months)	Findings on initial examination	Course	EEG findings
II-2/F	3	Failure to thrive, horizontal nystagmus, axial hypotonia, startle-like movements	Persistent myoclonic seizures, recurrent aspirations, oxygen dependent since 8 months. No milestone acquisition, death at 30 months	Abnormal background activity characterized by high amplitude spikes and sharp waves followed by significant depression of cortical activity for ~10-s duration (suppression-like pattern)
II-3/M	2.5	Failure to thrive, horizontal nystagmus, axial hypotonia, startle-like movements. Atrial septal defect	Persistent myoclonic seizures, recurrent aspirations, no milestone acquisition, death at 4 months	Abnormal background activity characterized by high amplitude spikes and sharp waves followed by significant depression of cortical activity for 10–15-s duration (suppression-like pattern)
II-4/F	4	Mild axial hypotonia, startle-like movements, developmental delay	Myoclonic seizures (improved on Clobazam), recurrent aspirations. At 16 months, no milestones acquisition	Slow background activity at theta-delta range with runs of five electroclinical myoclonic jerks, characterized by generalized spikes and poly spikes of less than 1-s duration
II-5/M	At birth	Mild axial hypotonia, respiratory distress, ventricular septal defect	Myoclonic seizures (10 weeks), recurrent aspirations, no milestone acquisition, Slow background activity at delta range with runs of several electroclinical myoclonic jerks, characterized death at 12 months	Slow background activity at delta range with runs of several electroclinical myoclonic jerks, characterized by generalized spikes and poly spikes of less than 1-s duration.