Biallelic mutations in SNX14 cause a syndromic form of cerebellar atrophy and lysosome-autophagosome dysfunction

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Pediatic-onset ataxias often present clinically as developmental delay and intellectual disability, with prominent cerebellar atrophy as a key neuroradiographic finding. Here we describe a new clinically distinguishable recessive syndrome in 12 families with cerebellar atrophy together with ataxia, coarsened facial features and intellectual disability, due to truncating mutations in the sorting nexin gene SNX14, encoding a ubiquitously expressed modular PX domain–containing sorting factor. We found SNX14 localized to lysosomes and associated with phosphatidylinositol (3,5)-bisphosphate, a key component of late endosomes/lysosomes. Patient-derived cells showed engorged lysosomes and a slower autophagosome clearance rate upon autophagy induction by starvation. Zebrafish morphants for snx14 showed dramatic loss of cerebellar parenchyma, accumulation of autophagosomes and activation of apoptosis. Our results characterize a unique ataxia syndrome due to biallelic SNX14 mutations leading to lysosome-autophagosome dysfunction.

The hereditary cerebellar ataxias are a group of clinical conditions presenting with imbalance, poor coordination and atrophy and/or hypoplasia of the cerebellum, most often with deterioration of neurological function. A common hallmark of the cerebellar ataxias is a progressive cerebellar neurodegeneration due to Purkinje cell loss. A combination of dominant, recessive and X-linked forms of disease, including the spinocerebellar ataxias, Friedreich ataxia and ataxia telangiectasia, contribute to the estimated prevalence of 8.9 per 100,000 (ref. 1). In addition to the dominant trinucleotide-repeat disorders that lead to toxic accumulation of unfolded protein4,5, the recessive forms of disease are associated with inactivating mutations and early-onset presentations. The genes implicated thus far suggest defects in neuronal survival pathways4,5, but knowledge of many mechanisms is still lacking, and most patients elude genetic diagnosis.

Recessive ataxias often show clinical overlap with lysosomal disorders; in fact, many lysosomal diseases such as Niemann-Pick disease, Tay-Sachs disease and I-cell disease show evidence of Purkinje cell loss and clinical features of ataxia, in addition to the well-established

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Figure 1  SNX14 mutations cause a syndromic form of severe cerebellar atrophy and coarsened facial features. (a) Summary of the exome sequencing results from 81 families with cerebellar atrophy. SNX14 mutations accounted for 9.9% of the families, with other genes making individual contributions. (b) Midline sagittal (top) or axial (middle) magnetic resonance imaging (MRI) and facies of affected individuals from representative families. Prominent atrophy of the cerebellum was evidenced by reduced volume and apparent folia (arrows and circles). Facies show prominent forehead, epicanthal folds, long philtrum and full lips. Consent to publish images of the subjects was obtained. (c) SNX14 exons are shown as boxes, with the location of mutations indicated. (d) The location of truncating mutations relative to predicted protein domains. TM, transmembrane; PXA, phox homology associated; RGS, regulator of G protein signaling; PX, phox homology; PXC, sorting nexin, C terminal. (e) ABD-II-2 (p.Arg378*) cerebellum stained with hematoxylin and eosi.
Supplementary Table 1

Supplementary Table 2

Supplementary Table 3

Figure 2 SNX14 localizes to late-endosome/lysosome compartments. (a) RT-PCR expression pattern of human SNX14 showing ubiquitous expression in representative fetal and adult human tissues and the HEK293T cell line. GAPDH was used as a loading control. No template (−) served as a negative control. (b) Cell fractionation of human NPCs. SNX14 was enriched in lysosomal-endosomal compartments (red). ER, endoplasmic reticulum. (c) Immunostaining for LAMP2, EEA1 and GM130 (green) in NPCs expressing DsRed-tagged SNX14. SNX14 overlapped in its localization with the LAMP2 lysosomal marker (arrows). Scale bars, 10 µm. (d) A lipid-binding assay with recombinant SNX14 PX domain on a phosphoinositide-spotted membrane showed preferential binding to PI(3,5)P2 (red) compared with a p40phox PX domain control.

Figure 3 Patient-derived SNX14-mutant NPCs display enlarged lysosomes. (a) Immunoblot of iPSC-derived NPCs for families 468 (p.Arg378*) and 1382 (p.Lys395Argfs*22), with affected (red) and unaffected (black) samples labeled. Affected individuals had undetectable levels of SNX14 protein. GAPDH was used as a loading control. (b) Top, LysoTracker Green DND-26 staining with engorged lysosomes in NPCs derived from affected individuals (arrows). Scale bars, 5 μm. Bottom left, the dot plot shows the relative area for individual LysoTracker-positive lysosomes (n = 223 and 194 lysosomes from the unaffected (U) and affected (A) NPCs of 2 families, respectively). Bottom right, graph bars represent the average number of LysoTracker-positive lysosomes per cell (n = 17 and 18 cells from the unaffected and affected NPCs of 2 families, respectively). Error bars, s.d. ***P < 0.0005; NS, not significant (two-tailed t test).

Overall, patients with SNX14 variants accounted for 10% of the families, making it the single most commonly mutated gene in our cohort. Furthermore, while preparing this manuscript, whole-exome sequencing from an additional consanguineous family with four children showed mutations in genes that fully explained their presentation (Supplementary Table 1), 60% of families showed no obvious candidates and 16% of families displayed putative mutations in a gene or genes not previously implicated in human disease (Fig. 1a).

To identify causative mutations, we focused on family 468, with three similarly affected children and one healthy child, which allowed for parametric linkage analysis, defining a single major locus at chr. 6: 55,153,677–91,988,281 (hg19) (logarithm of odds (LOD) = 2.528) (Supplementary Fig. 1). Alignment of all loci having LOD > 2 with whole-exome sequencing data from two affected individuals highlighted a single c.1132C>T variant in the SNX14 gene predicting a p.Arg378* alteration. Turning our attention to this gene for the remaining patients analyzed by whole-exome sequencing, we identified a total of 16 patients from 8 families with truncating variants throughout the coding region, nearly all in constitutively spliced exons and predicted to cause loss of function (Fig. 1b–d, Supplementary Fig. 2 and Supplementary Table 2). All patients displayed a block of homozygosity on chromosome 6 containing the SNX14 gene (Supplementary Fig. 1), and mutations segregated according to a recessive mode of inheritance. Variants in other genes in these patients were either previously described SNPs in other populations or were of unknown effect (Supplementary Table 3). Four families shared the same p.Arg378* alteration, and analysis confirmed a common 1.5-Mb haplotype, supportive of this alteration representing a founder mutation (Supplementary Fig. 1).
with cerebellar atrophy independently identified a homozygous truncating mutation in SNX14 (Supplementary Fig. 2).

SNX14 encodes a 946-residue protein containing 2 transmembrane domains, a regulator of G protein signaling (RGS) domain predicted to act as a GTPase-activating protein (GAP) and a phox homology (PX) domain predicted to bind phosphatidylinositol lipids and function in intracellular trafficking. Alternative splicing results in transcript variants encoding distinct isoforms. The SNX14 domain predicted to bind phosphatidylinositol lipids and function in intracellular trafficking. Alternative splicing results in transcript variants encoding distinct isoforms. The SNX14 variants identified in patients predicted both early and late truncating events, suggesting loss of function as the disease mechanism (Fig. 1c,d).

Patients showed several common features in addition to age-dependent atrophy of the cerebellum, with evidence of cerebral cortical atrophy in about half of the affected individuals (Table 1 and Supplementary Table 4). One deceased patient studied neuropathologically showed almost complete absence of Purkinje cells. The few Purkinje cells remaining were ectopically located and atrophic, with enlarged apical neurites. Bergmann gliosis was prominent in the depopulated Purkinje cell layer, and neurofilament immunostaining showed radially oriented bundles of distended axons located on the superficial part of the internal granule layer. Forebrain also presented with neuronal loss, although this loss was less severe than in the cerebellum (Fig. 1e and Supplementary Fig. 3).

Most patients with SNX14 mutations presented between birth and 1 year of age with global developmental delay and hypotonia. Seizures developed in half by 2 years and were well controlled with anticonvulsant medication. Nystagmus, difficulty ambulating and upper limb atrophy was present in almost all children, and reduced deep tendon reflexes were present in most children, and sensorineural hearing loss was present in about one-third. Coarse facies features with prominent forehead, epicanthal folds, upturned nares, long philtrum and full lips were seen in all, features approximating those of mucopolysaccharidosis or other lysosomal storage disorders (LSDs) (Fig. 1b and Supplementary Fig. 2b). Likewise, ultrastructural analysis of spinal cord tissue found axonal spheroids filled with membranous structures reminiscent of the cytoplasmic membranous bodies observed in LSDs (Supplementary Fig. 3c). Palpable liver or spleen edge was detected in 5 of 18 patients, but no evidence of abnormal liver, urine or hematological abnormalities was apparent. Urine oligosaccharides showed an abnormal pattern in one affected individual, and two patients showed elevated urinary glycosaminoglycan levels. However, detailed lysosomal enzyme analysis in plasma and leukocytes from two affected individuals proved unremarkable (Supplementary Note). Although whole-exome sequencing was initially required to identify patients with SNX14 mutations, as the clinical presentation was clarified, we were able to predict mutations with 100% accuracy, identifying an additional four patients from three families with homozygous SNX14 mutations (Supplementary Fig. 2 and Supplementary Table 2). These findings suggest that this disorder represents a heretofore unknown, clinically recognizable condition.

SNX14 mRNA showed nearly uniform expression in human fetal and adult tissues (Fig. 2a). Cellular fractionation aimed at distinguishing the major membrane-bound pools of SNX14 protein in wild-type human neural precursor cells identified SNX14 predominantly associated with a lysosomal- rich fraction (Fig. 2b). Overexpression of tagged SNX14 confirmed overlapping localization with lysosomes (Fig. 2c and Supplementary Fig. 4) but not with other endosomal or Golgi markers that were present in the SNX14 fraction, suggesting a role for SNX14 in lysosomal function. Furthermore, a lipid-binding assay with recombinant PX domain from SNX14 showed specific binding with PIP((3,5)P)2, the predominant phosphoinositide associated with lysosomes (Fig. 2d).

To identify lysosomal defects associated with SNX14 mutations, we generated induced pluripotent stem cells (iPSCs) and differentiated them into neural precursor cells (NPCs) through the reprogramming
of fibroblasts from controls and SNX14-mutated patients (from families 468 and 1382)\(^ {17,18}\). As in the fibroblasts from patients, SNX14 protein was absent from patient-derived NPCs (Fig. 3a and Supplementary Fig. 5). Although we noted no difference in reprogramming, differentiation or cellular survival in culture for the control and patient-derived cells (Supplementary Fig. 5), lysosomes appeared increased in size in patient-derived NPCs (Fig. 3b and Supplementary Fig. 6). To quantify this effect, we performed flow cytometry analysis to gate for fluorescent signal upon LysoTracker labeling, which stains intracellular acidic compartments (lysosomes and late endosomes), and we found that about twice as many patient-derived cells as controls fell outside the normalized intensity distribution (Supplementary Fig. 6a).

To assess whether this lysosomal enlargement affected lysosomal activity, we tested NPCs for active cathepsin D (whose levels depend upon both the lysosomal localization of the enzyme and acidification) by its specific binding to pepstatin A conjugated to BODIPY FL\(^ {19}\) and found no obvious differences in the intensity of stained lysosomes for control and patient-derived cells (Supplementary Fig. 6d). However, immunoblot analysis detected a slight but significant (\(P < 0.05\)) reduction in cathepsin D levels in affected NPCs in comparison to unaffected cells (Supplementary Fig. 7c), suggesting that a fraction of lysosomes may be defective for cathepsin D. Although we did not test for defects in other lysosomal enzyme activities in NPCs, our findings are reminiscent of those for LSDs.

Autophagy requires the fusion of lysosomes with autophagosomes, so lysosomal abnormalities could result in autophagic defects such as those observed in LSDs\(^ {6–8}\). To test for potential autophagic defects, we cultured patient-derived NPCs under starvation conditions and then assessed them for the levels of lipidated LC3 (LC3-II), which marks autophagosomes. Although all lines showed increased LC3-II levels upon serum starvation, patient-derived cells showed a more dramatic response, which was reproduced under alternative induction of autophagy through inhibition of the mTOR pathway with rapamycin. Notably, the increased LC3-II levels were restored to basal rates by forced expression of tagged SNX14 in patient-derived cells (Fig. 4a). By LC3 flux analysis under nutrient-deprived conditions, where LC3-II ratios in the presence and absence of lysosomal inhibitors (leupeptin and NH\(_4\)Cl) were calculated\(^ {20}\), we identified slower LC3 flux in patient-derived cells in comparison to controls. This result, together with its specific binding to pepstatin A conjugated to BODIPY FL\(^ {19}\) and found no obvious differences in the intensity of stained lysosomes for control and patient-derived cells (Supplementary Fig. 6d). However, immunoblot analysis detected a slight but significant (\(P < 0.05\)) reduction in cathepsin D levels in affected NPCs in comparison to unaffected cells (Supplementary Fig. 7c), suggesting that a fraction of lysosomes may be defective for cathepsin D. Although we did not test for defects in other lysosomal enzyme activities in NPCs, our findings are reminiscent of those for LSDs.

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with the lack of an observed difference in autophagosome formation (assessed as the increase in LC3-II levels at two time points after inhibition of lysosomal proteolysis; Fig. 4b), suggests that SNX14 mutant neural progenitors are defective in autophagosome clearance. To confirm this finding, we performed electron microscopy and found that patient-derived cells showed autophagosome accumulation (Fig. 4c), consistent with disrupted autophagosome clearance.

We then repeated the cell fractionation analysis upon serum starvation to induce autophagy and observed SNX14 enriched in the most heavily LC3-lipidated fractions (SupplementaryFig. 7a). Furthermore, upon serum starvation, SNX14 showed overlapping immunofluorescence localization with LC3 (SupplementaryFig. 7b), suggesting that at least some fraction of SNX14 associates with autophagic structures, consistent with it having a role in autophagosome clearance.

To further analyze the role of SNX14 in cerebellar function, we established an in vivo zebrafish model, where we found a single snx14 ortholog (NM_001044793) with strong neural expression (SupplementaryFig. 8). Injection with a specific snx14 translation-blocking morpholino resulted in loss of neural tissue volume (Fig. 5). Immunostaining of these embryos for zebrin II, an early Purkinje cell marker, showed significantly reduced cellular area (P < 0.005), an effect that was quantitatively rescued by coinjection with the human SNX14 ortholog (Fig. 5b). Morpholino injection into the Tg(ptf1a:EGFP) zebrafish line, which expresses GFP in the hindbrain21, resulted in an overall reduction in GFP intensity (Fig. 5c), suggesting that SNX14 is required for hindbrain and Purkinje cell generation or survival. To distinguish between these possibilities, we performed staining for activated caspase-3 (involved in apoptosis) and found a dramatic increase in signal throughout the assessed neural tissue. Transmission electron microscopy analysis of neural cells demonstrated accumulation of autophagic structures in snx14 morphants. These data suggest that SNX14 mutations lead to neuronal cell death associated with impaired autophagic degradation.

In summary, we have characterized a cerebellar ataxia syndrome (SCAR17) caused by null mutations in SNX14. Our paper adds to a recent report of cerebellar atrophy with intellectual disability and coarse facies also showing homozygous SNX14 mutations22. Our work, with the addition of a larger cohort, helps identify clinical features that are variable, such as camptodactyly, macrocephaly and DNA damage.

Accession codes. The whole-exome sequencing data from individuals in this study have been deposited in the database of Genotypes and Phenotypes (dbGap) under accession phs000288.v1.p1.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Patient recruitment and phenotyping: M.S.Z., L.-A.G., R.O.R., E.D., A.B.G., R.K.O., M.S.S., M. Azam, L.S., I.G.M., S.-A.H., M. Aglan, G.M.A.-S., S.I., A.E.R., A.A.S., F.M., H.K., A.M., I.B., S.T., I.D., A.D., K.K.V. and J.G.G. Genetic sequencing and interpretation: N.A., V.C., X.W., J.L.S., J.S., E.M.S., B.C., J.-L.C., M.G., S.B.G., P.d.L., and A.D. Cell biology: N.A., V.C., J.-E., M.D.B., S.J.F., G.N., P.S.M.G. and U.M. Zebrafish: B.R., N.A. and X.W. Cell culture: A.E.S. and N.A. Histology: A.B.G. and I.D.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Patient ascertainment. Patients were enrolled and sampled according to standard local practice in approved human subject protocols at the University of California. Patients were recruited from developmental child neurology clinics throughout the Middle East, North Africa and Central Asia presenting with features of neurodevelopmental delay or regression, ataxia, intellectual disability, autism, epilepsy or structural brain malformations between 2004 and 2012. Recruitment was focused in the major population centers of the Middle East, including Morocco, Libya, Egypt, Saudi Arabia, Kuwait, the United Arab Emirates, Oman, Jordan, Pakistan, Turkey and Iran, with consanguinity rates (rates of marriage between first or second cousins) of approximately 50% compared with <1% in the United States and Western Europe. Among the recruited cohort, consanguinity was present for 63% of parents, suggesting some bias in sampling toward those with affected children due to recessive disease. Sampling was performed on both parents and all available genetically informative siblings to include affected and unaffected family members, as well as extended family members if appropriate, upon informed consent approval and consistent with institutional review board (IRB) guidelines. General and neurological examination, clinical records, radiographs, photographs, videos documenting movement and past history were reviewed, and patients were examined by one or more of the authors. All patients presenting with a presumptive diagnosis of cerebellar atrophy, on the basis of the finding of reduced cerebellar volume and excessively prominent interfolial spaces on axial or sagittal sections, were included in the analysis. Patients with MRI showing pronounced pontine atrophy, severe peripheral neuropathy, white matter disease, telangiectasias, retinal blindness or major cortical malformations such as cobblestone lissencephaly were excluded. Patients with evidence of mitochondrial disease, abnormal transferrin isoelectric focusing or lysosomal storage such as mucolipidosis or ceroid were excluded. All patients were excluded as having the common Friedreich ataxia expansion and tested normal for α-fetoprotein and albumin levels. Blood and/or saliva was collected for all consenting, potentially informative family members; DNA was extracted with the Qiagen AutoPure instrument and subjected to quality control measures to determine concentration and purity and to confirm inheritance. The resulting DNA was subjected to subsequent genetic investigation.

Whole-exome sequencing. Whole-exome sequencing was performed on two affected members per family when available or on both parents and the affected individual for singleton cases. Genomic DNA was subjected to Agilent Human All Exon 50 Mb kit library preparation and then paired-end sequencing (2 × 150 bp) on the Illumina HiSeq 2000 instrument. For each patient sample, >96% of the exome was covered by >12x. The Genome Analysis Toolkit (GATK)32 was used for variant identification. We tested for segregating rare structural variants using XHMM33. We then prioritized homozygous variants using custom Python scripts (available upon request) to remove alleles with frequency >0.1% in the sequenced population, not occurring in homoygous intervals of at least 2 cM or linkage intervals with a LOD score of greater than ~2, or without high scores for likely damage to protein function. All variants were prioritized by allele frequency in publically available databases, conservation and predicted effect on protein function and were tested for segregation with disease.

Sanger sequencing. Primers were designed using the Primer3 program and tested for specificity using BLAST software. PCR products were treated with Exonuclease I (Fermentas) and Shrimp Alkaline Phosphatase (USB Corporation) and sequenced using BigDye Terminator Cycle Sequencing Kit with Exonuclease I (Fermentas) and Shrimp Alkaline Phosphatase (USB). Primers were designed using the Primer3 program (GATK)32 was used for variant identification. We tested for segregating rare structural variants using XHMM33. We then prioritized homozygous variants using custom Python scripts (available upon request) to remove alleles with frequency >0.1% in the sequenced population, not occurring in homoygous intervals of at least 2 cM or linkage intervals with a LOD score of greater than ~2, or without high scores for likely damage to protein function. All variants were prioritized by allele frequency in publically available databases, conservation and predicted effect on protein function and were tested for segregation with disease.

Cloning of human SNX14. Human SNX14 was amplified from adult brain cDNA and cloned into the pRedDed2-C1 vector; the sequence was subcloned into the doxycycline-inducible lentiviral pINDUCER20 vector34. For expression of an N-terminal Flag tag, SNX14 was amplified from adult brain cDNA using a 5’ primer containing a sequence encoding the Flag tag and cloned into the pINDUCER20 vector. The sequence encoding the SNX14 PX domain was amplified and cloned into the pGEX-6P-1 vector for purified protein expression.

Human brain histology and oligosaccharide and glycosaminoglycan measurement. Sections were deparaffinized and stained with 0.1% Luxol fast blue, 0.1% Cresyl violet or hematoxylin and eosin. Immunohistochemistry was performed with primary antibodies diluted 1:200 (calbindin: Abcam, ab11426; neurofilament: Pierce, MIC-N18), and signals were visualized with HRP-conjugated secondary antibody (Jackson Labs). Control tissue corresponded to biobank identification number Neuropathologie du Développement_BF-0033-00082/C 2009-935. Oligosaccharide and glycosaminoglycan measurements were performed as described35.

Fibroblast, iPSC and NPC culture. Fibroblasts were isolated from explants of dermal biopsies collected from affected and unaffected volunteers who were previously genotyped and were cultured in MEM ( Gibco) supplemented with 20% FBS (Gibco). iPSCs were generated as previously described36. Briefly, 3 µg of expression plasmid mixture (containing expression plasmids for OCT3, OCT4, SOX2, KLF4, L-MYC and LIN28, and short hairpin RNA (shRNA) targeting TP53) was electroporated into 6 × 10³ cells, cells were trypsinized 7 d afterward and 1.5 × 10³ cells were replated onto 100-mm dishes with a feeder layer consisting of 1.5 × 10³ irradiated CF-1 mouse embryonic fibroblasts (MEFs). The culture medium was replaced the next day with standard hESC/iPSC medium, DMEM:F12 supplemented with 20% KOSR and 20 ng/ml BFGF (Invitrogen), 1× non-essential amino acids and 110 µM 2-mercaptoethanol. Colonies were selected for further cultivation and evaluation. After three passages, iPSCs were transferred to MEF-free plates and grown in mTeSR medium (Stemcell Technologies). NPCs were obtained as previously described37. Briefly, embryoid bodies were formed by mechanical dissociation of cell clusters and plating of cells in suspension in differentiation medium (DMEM:F12 supplemented with 1× N2 ( Gibco, 57502-048), 1 µM dorsomorphin (Tocris) and 2 µM A8301 (Tocris)) where they were kept shacking at 95 rpm for 7 d. The resultant embryoid bodies were plated onto dishes coated with Matrigel ( BD Biosciences) in NBF medium (DMEM:F12 supplemented with 0.5× N2, 0.5× B27 ( Gibco, 57504-044) and 20 ng/ml BFGF). Rosettes were collected after 5–7 d and dissociated with Accutase (Millipore), and the resultant NPCs were plated onto poly(ornithine/laminin) (Sigma) dishes with NPC medium. Medium was replaced every 2 d. Cells were routinely tested for mycoplasma. All experiments were performed with NPCs at passages 5–8.

For the genetic replacement experiments, patient NPCs were transduced with lentivirus expressing Flag- or DsRed-tagged SNX14 (NM_153816) in pINDUCER20 vector34 in the presence of 5 µg/ml polybrene. After 1 week of selection with 200 µg/ml G418, NPCs were treated with 50 ng/ml doxycycline for transgene expression. Bright-field images were acquired on an Olympus IX51 inverted microscope or on an EVO microscope and processed with Photoshop CS5 software (Adobe Systems). For autophagic induction, cells were cultured in EBSS for 1.5–2 h and treated with 200 µM leupeptin and 20 mM NH₄Cl for experiments performed to quantify LC3-II flux and autophagosome formation.

Cell fractionation assays. Cell fractionation was carried out as described previously. Proteins in each fraction were precipitated with methanol-chloroform and resuspended in 60 µl of protein loading buffer, from which 20 µl was processed for immunoblot analysis.

Lipid-binding assays. Lipid blotting was performed essentially as described previously with minor modifications. Briefly, 180, 60 and 20 pmol of lipids were spotted onto PVDF membranes and probed with 0.75 µg of bacterially expressed glutathione S-transferase (GST)-tagged PX domains. Proteins were detected by blotting with an antibody to GST (sc-459, Santa Cruz Biotechnology).

Cellular immunofluorescence and biochemical assays. Cells were fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.05% Triton in PBS or methanol, blocked for 1 h in PBS containing 0.05% Triton and 2% donkey serum, and then incubated with primary antibody (LC3: 1:200 dilution, 0.1% Cresyl violet or hematoxylin and eosin. Immunohistochemistry was performed with primary antibodies diluted 1:200 (calbindin: Abcam, ab11426; neurofilament: Pierce, MIC-N18), and signals were visualized with HRP-conjugated secondary antibody (Jackson Labs). Control tissue corresponded to biobank identification number Neuropathologie du Développement_BF-0033-00082/C 2009-935. Oligosaccharide and glycosaminoglycan measurements were performed as described35.
Adult male and female zebrafish (<18 months old) from wild-type IX51, Leica SP5 or Nikon A2 microscope, and images were processed with Photoshop CS5 software. Cathepsin D activity was assessed with 2 μg/ml Pepstatin A–BODIPY FL for 45 min at 37 °C, and samples were then fixed in 4% paraformaldehyde before imaging.

For immunoblot assays, fibroblasts or NPCs were lysed with ice-cold RIPA buffer supplemented with protease and phosphatase inhibitor cocktails (Roche). Proteins were separated by 10% SDS-PAGE and transferred to PVDF membrane, blocked with 5% milk in 1× TBST and blotted with primary antibody (mouse antibody to SNX14: 1:1,000 dilution, Sigma (SAB130492); rabbit antibody to LC3: 1:5,000 dilution, Novus Biological (NB600-1384); mouse antibody to tubulin: 1:1,000 dilution, Sigma (T6074); mouse antibody to GAPDH: 1:1,000 dilution, Millipore (MAB347); antibody to ribophorin: 1:1,000 dilution, Abcam (ab38451); antibody to p62: 1:1,000 dilution, Progen Biotechnik (GP62-C); antibody to cathepsin D: 1:1,000 dilution, Santa Cruz Biotechnology (C20); antibody to EEA1: 1:500 dilution, BD (610456); antibody to Lamp1: 1:500 dilution, DSHB (H4A3); antibody to GM130: 1:500 dilution, Cell Signaling Technology (2775); antibody to EEA1: 1:500 dilution, BD (610456); antibody to Lamp1: 1:500 dilution, DSHB (H4A3); antibody to GM130: 1:500 dilution, Cell Signaling Technology (2296) overnight at 4 °C. Detection used a peroxidase-coupled antibody to IgG (Pierce) and an enhanced chemiluminescence substrate (Thermo Scientific Pierce ECL). Experiments were replicated three times.

For RT-PCR, total RNA was extracted with the RNeasy Mini kit (Qiagen), and a total of 2 μg of RNA was transcribed to cDNA using SuperScript (Invitrogen) with oligo(dT). Gene-specific primers were used for PCR.

**Flow cytometry for LysoTracker intensity analysis.** NPCs were collected, brought to 1 × 10^5 cells/ml and incubated with 100 nM LysoTracker Green DND-26 for 15 min at 37 °C. Live cells were analyzed for LysoTracker fluorescence intensity levels by first gating on all cell material except small debris in the origin of an FSC (forward scatter) versus SSC (side scatter) dot plot. LysoTracker signals from samples were then compared by dot-plot and histogram analysis.

**Zebrafish in situ hybridization, knockdown and immunofluorescence.** Adult male and female zebrafish (<18 months old) from wild-type (AB Tubingen) and transgenic strains were maintained under standard laboratory conditions. At least three adult pairs were used to generate embryos at 0–5 days post-fertilization (d.p.f.) for each experiment, with embryos from the same pair used both for control and snx14 morpholino injections. No randomization was performed. Translation-blocking antisense morpholino oligonucleotides for snx14 or a scrambled-sequence morpholino was injected into embryos at the one-cell stage. Full-length human wild-type SNX14 mRNA (50 pg) was coinjected with the snx14 morpholino as described. Optic tectum and right eye widths were measured digitally to assess neural affection. Whole-mount *in situ* hybridization was performed on zebrafish embryos at 24 and 48 h.p.f. using *snx14* RNA probes generated by PCR. Experiments followed US National Institutes of Health guidelines and were performed in compliance with the Institutional Animal Care and Use Committee at the University of California San Diego.

**Transmission electron microscopy.** Samples were immersed in modified Karnovsky’s fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.15 M sodium cacodylate buffer, pH 7.4) for at least 4 h, post-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for 1 h and stained en bloc in 2% uranyl acetate for 1 h. Samples were dehydrated in ethanol, embedded in Durcupan epoxy resin (Sigma-Aldrich), sectioned at 50 to 60 nm on a Leica UCT ultramicrotome and picked up on Formvar and carbon-coated copper grids. Sections were stained with 2% uranyl acetate for 5 min and with Sato’s lead stain for 1 min. Grids were viewed using a Tecnai G2 Spirit BioTWIN transmission electron microscope equipped with an Eagle 4k HS digital camera (FEI).

**Statistical analysis.** All experiments were replicated at least twice. Data are expressed as means, with variance as s.e.m. or s.d. For all quantitative measurements, a normal distribution was assumed, and we used the two-tailed Student’s *t* test to perform between-group comparisons. *P* < 0.05 was considered to be indicative of statistical significance. No statistical methods were used to predetermine sample sizes, which were determined empirically from previous experimental experience with similar assays and/or from sizes generally employed in the field. Data collection and analysis were not performed with blinding. The raw values used to generate plots are available as source data.