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Clinical report

# *NGLY1* mutation causes neuromotor impairment, intellectual disability, and neuropathy

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ABSTRACT

N-glycanase 1 (*NGLY1*) is a conserved enzyme that is responsible for the deglycosylation of misfolded N-glycosylated proteins in the cytoplasm prior to their proteasome-mediated degradation. Disruption of this degradation process has been associated with various neurologic diseases including amyotrophic lateral sclerosis and Parkinson's disease. Here, we describe two siblings with neuromotor impairment, apparent intellectual disability, corneal opacities, and neuropathy who were found to possess a novel homozygous frame-shift mutation due to a 4 base pair deletion in *NGLY1* (c.1533_1536delTCAA, p.Asn511LysfsX51). We hypothesize that this mutation likely limits the capability of neuronal cells to respond to stress due to accumulation of misfolded proteins, thereby impairing their survival and resulting in progressive loss of neurological function.



## 1. Introduction

*NGLY1* (ENSG00000151092) encodes the cytoplasmic peptide N-glycanase 1 which is responsible for the deglycosylation of N-linked glycoproteins and glycopeptides in the cytosol of eukaryotic cells [Hirsch et al., 2003; Suzuki, 2007]. This deglycosylation has been shown to have a pivotal role in the proteasome-mediated degradation of misfolded proteins of the endoplasmic reticulum (ER). This process, termed ER-associated degradation (ERAD), is an essential quality control mechanism for glycoproteins and other secretory proteins that undergo translation and post-translational modification within the ER [Chakrabarti et al., 2011]. Disruption of the ERAD mechanism has been previously associated with a number of diseases including amyotrophic lateral sclerosis, Parkinson's disease, and diabetes [Chakrabarti et al., 2011]. Recently, studies have begun to link N-glycanase and *NGLY1* to neurodevelopment and neurologic disorders. In humans, a compound heterozygous mutation in *NGLY1* has been reported in a 3-year-old boy who presented with developmental delay, multifocal epilepsy, involuntary movements, absent tears, and abnormal liver function [Need et al., 2012]. More recently, Enns et al. reported 8 patients with loss-of-function (LoF) mutations in *NGLY1* [Enns et al., 2014]. Here, we present 2 siblings, who presented with global developmental delay, apparent intellectual disability, corneal opacities and severe neuropathy; both found to possess a novel homozygous frame-shift mutation resulting from a 4 base pair deletion in *NGLY1*.

## 2. Clinical report

The index case (NG1278-1) was a full term male who was delivered via Caesarean section due to a nuchal cord (Fig. 1A, Table 1). The

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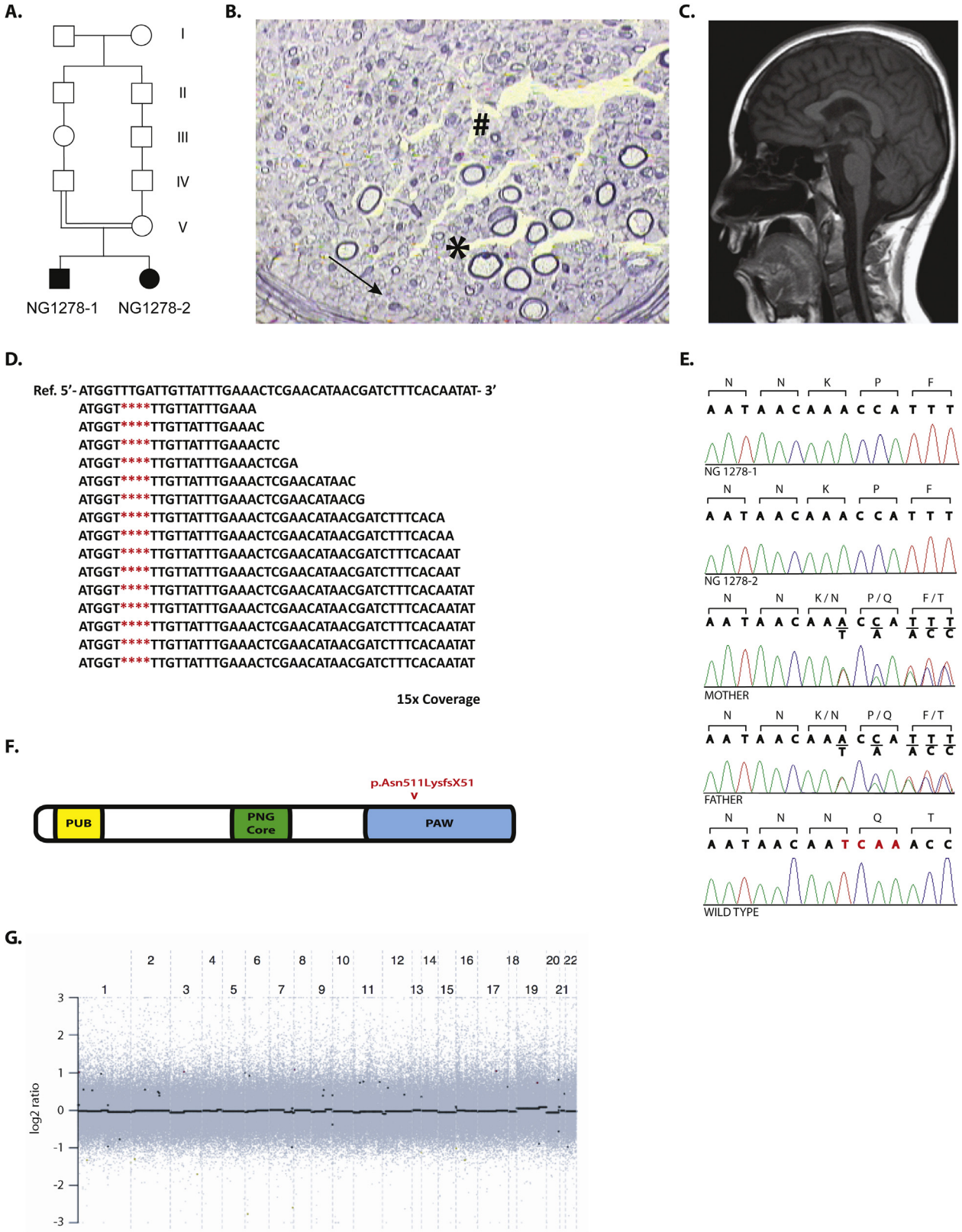


**Fig. 1.** **A) Pedigree of Family NG1278**: Pedigree of family NG1278 demonstrating the affected siblings. Note the consanguinity of the parents. **B) Sural Nerve Biopsy of Patient NG1278-1:** 40× magnification image of a representative section of sural nerve tissue obtained via biopsy from patient NG1278-1. There is general axonal loss and decreased myelination; # indicates the marked decrease in small myelinated fibers; * indicates the segmentally decreased number of large diameter myelinated fibers; non-myelinated fibers also demonstrate a slight decrease in numbers, but appear relatively spared (arrow). **C) Brain MRI of Patient NG1278-1:** Sagittal T1 weighted brain MRI demonstrating a thin spinal cord. No cortical abnormalities were noted. **D) Sequence Alignment:** Whole-exome sequencing results for patient NG1278-1 demonstrating the homozygous 4 base pair deletion in *NGLY1* — delTTGA. The top line represents the wild-type reference sequence. The subsequent lines below the reference lines depict the results from exome sequencing. Each line represents a distinct coverage read. Coverage achieved for this region was 15×. **E) Sanger Sequencing Results:** Chromatographs obtained via Sanger sequencing analysis of the two

procedure was uncomplicated and the child's development appeared normal at 6 months of age at which time he displayed appropriate motor control of his head and neck and was able to sit with minimal support. Soon after 6 months of age, however, the child developed neurological symptoms, losing motor control of his neck and head, becoming hypotonic, and having difficulty swallowing. A physical examination at that time documented these findings and also revealed hepatomegaly. Laboratory tests were notable for increased transaminase levels (approximately 3 times the upper limit of normal); these elevations were transient, persisting for the next 6 months but then resolving spontaneously. At 1 year of age, he appeared normocephalic but head circumference was never documented. No seizures were ever reported and EEG's performed at 1 and 2 years of age were normal. At the age of 3, he displayed apathy with limited interaction; he never developed the ability to walk or speak. Ptosis and areflexia were present. Pain insensitivity was noted by the parents and confirmed via lack of reaction to pinprick. Hypolacrima and severe corneal opacities were identified and the patient underwent tarsorrhaphy. The previously documented hepatomegaly was not detected at this time, however a unilateral undescended testical was documented. Electromyography (EMG) revealed neurogenic involvement with more pronounced impairment in the lower extremities. Occasional fibrillation potentials were detected and suggestive of anterior horn involvement. Initial nerve conduction studies indicated a polyneuropathy with significantly decreased sensory nerve conduction velocities and diminished motor nerve action potentials. Nerve conduction velocities of the peroneal nerves were delayed at 20–30 m/s. Around 7 years of age, paucity of sweating was noted and heat sensitivity and hyperthermia became problematic especially during the summer months. He performed limited purposeful hand movements and atrophy of the intrinsic hand muscles was noted. A sural nerve biopsy was performed at 8 years of age and revealed significant loss of large and small myelinated fibers, slight loss of unmyelinated fibers, and diffuse axonal loss (Fig. 1B). At age 11, he experienced a femur fracture, possibly due to a minor trauma in his osteoporotic state from lack of weight bearing. Lack of pain associated with the fracture was noted. Repeat neurological examination at 16 years of age demonstrated strabismus and persistence of hypotonia, difficulty swallowing, areflexia, decreased pain sensation, and anhidrosis. He developed scoliosis and contractures in the ankles without spasticity. Routine blood studies including neurometabolic assays, complete metabolic panel, complete blood count, and thyroid function tests were all within normal limits. An MRI of the brain and spine did not demonstrate any cortical abnormalities; however, a thin cervical cord was noted (Fig. 1C). The patient died at age 16 secondary to respiratory difficulties and recurrent respiratory infections (Supplementary Table 1).

The index case's 9-year-old sister (NG1278-2) displayed similar symptoms (Table 1, Supplementary Table 1). She was brought to medical attention at 10 months of age with developmental delay and was found to be hypotonic with diminished reflexes. Her head circumference was within normal limits for age and a brain MRI that was performed at 1 year of age demonstrated no abnormalities. Like her brother, she also exhibited significantly diminished pain sensation. Nerve conduction studies of the peroneal nerves at 14 months of age demonstrated a marked decrease in sensory nerve conduction and mild motor nerve conduction deficits. General examination was remarkable for corneal opacities, hypertelorism and a transverse palmar crease on her right hand. At 3 years of age, she developed seizures. She remained severely developmentally delayed, lacking any language development. She was always restless, displayed a hyperkinetic movement disorder similar to akathisia, and had difficulty swallowing. EEG examination revealed generalized discharges with polyspikes and paroxysmal fast activities. Response to antiepileptics could not be assessed properly due to compliance issues. At the age of 9 years, she could only crawl and sit, but could not walk or speak.

## 3. Whole genome genotyping and whole-exome sequencing

We initially performed whole genome genotyping and determined the inbreeding coefficient for the index case (NG1278-1) to be 0.01, consistent with a birth from a consanguineous union (Supplementary Materials and Methods). We identified the homozygous genomic segments (>2.5 centiMorgan each) of the index case (Supplementary Table 2) and focused on the discovery of potential disease causing mutation within these regions using whole-exome capture and sequencing of the germ line DNA obtained from the index case (Supplementary Table 3) [Bilguvar et al., 2010]. Percentage of all bases with 10× coverage was 94% and 20× coverage was 89%. Variant analysis identified only 2 novel homozygous mutations located within the aforementioned regions of homozygosity. The first was a missense mutation (c.1382G>A, p.Arg461His) affecting the *TIE1* (tyrosine kinase with immunoglobulin-like and EGF-like domains 1) (ENSG00000066056) gene. However, this variant did not segregate with the disease phenotype and was found to be heterozygous in his affected sibling (NG1278-2) (Supplementary Fig. 1). The second homozygous variant was a putative LoF frame-shift mutation affecting the *NGLY1* on chromosome 3p24.2 (Fig. 1D, Supplementary Table 4). The mutation was a homozygous 4 base pair deletion (ENST00000280700.5: c.1533_1536delTCAA) within the PAW domain of the *NGLY1* gene, resulting in premature termination (ENSP00000280700.5:p.Asn511LysfsX51). This mutation segregated in the expected pattern in the family with the affected sister being homozygous and both unaffected parents being heterozygous (Fig. 1E). This *NGLY1* mutation (Fig. 1F) has neither been previously reported in the dbSNP, NHLBI GO ESP Exome Variant Server, or 1000 Genomes databases, nor has it been observed within a cohort of 3000 subjects with non-neurological diseases who were whole-exome sequenced at Yale School of Medicine. In addition, copy number variation (CNV) analysis, based on exome sequencing of the index case, demonstrated no disease causing large-scale amplifications, deletions, or loss of heterozygosity (aside from aforementioned inherited region of homozygosity) within the coding regions of the entire genome (Fig. 1G). These findings provide strong genetic evidence that the identified *NGLY1* variant is the disease causing mutation in this family.

## 4. Discussion

*NGLY1* is located on 3p24.2, has 12 exons, and encodes the 654-amino acid long protein N-glycanase 1 which is fundamental for the deglycosylation of cytoplasmic proteins [Suzuki et al., 2003]. *NGLY1* plays an important role in protein quality control, cellular maintenance, and cellular response to stress via the ERAD

patients and their parents. Sanger sequencing of wild-type control DNA was also performed. Note that the mutation identified via whole-exome sequencing was confirmed as being homozygous versus heterozygous, in the two affected siblings versus their parents, respectively. The bases outlined in red in the wild-type sequence indicate the mutated base pairs. **F) Schematic Representation of N-glycanase 1:** Figure shows the functional and conserved domains. Arrow head indicates location of the identified mutation. **G) CNV Analysis of NG1278-1:** The log ratio comparing NG1278-1 and control sequence depths of coverage for each exon are depicted as gray dots. The black lines demonstrate regions of segmented copy neutral events, green lines are segmented deletion events and red lines are amplification events.

**Table 1**
Comparison of clinical findings with reported patients and previously published patients with *NGLY1* mutations. (ND: Not documented, NP: Not performed).

| Feature | | Need et al., 2012 | Enns et al. (2014) | Present report |
|---|---|---|---|---|
| Number of cases | | 1 | 8 | 2 |
| Ethnic origin | | Caucasian | Caucasian | Caucasian |
| Parental consanguinity | | ND | 1/8 | 2/2 |
| Age of onset | | ND | Early infancy | Early infancy |
| Neuromotor developmental delay | | Yes | 8/8 | 2/2 |
| Hypotonia | | ND | 8/8 | 2/2 |
| Movement abnormalities | | Yes | 8/8 | 1/2 |
| Prenatal | IUGR | ND | 5/8 | 0/2 |
| | Second trimester low uE3 | ND | 3/8 | 0/2 |
| Head and neck | Hearing abnormalities | ND | 2/5 | 0/2 |
| | Alacrima/ hypolacrima | Yes | 7/8 | 2/2 |
| | Corneal ulcerations/ scarring | ND | 4/8 | 2/2 |
| | Strabismus | ND | 4/8 | 1/2 |
| Skeletal | Scoliosis | ND | 4/8 | 1/2 |
| | Fractures due to osteoporosis | ND | 1/8 | 1/2 |
| | Small hands/feet | ND | 4/8 | 0/2 |
| GIS | Feeding difficulties | ND | 1/1 | 2/2 |
| | Storage or vacuoles in hepatocytes | Yes | 5/6 | NP |
| | Hepatic fibrosis | ND | 2/6 | 0/2 |
| Neurologic | Peripheral neuropathy | ND | 3/3 | 2/2 |
| | Seizures | Yes | 4/8 | 1/2 |
| | Ocular apraxia | ND | 4/8 | 0/2 |
| | Diminished reflexes | ND | 6/8 | 2/2 |
| | Speech impairment | ND | 8/8 | 2/2 |
| | Pain sensation | ND | ND | Decreased |
| Others | Anhydrosis | ND | 0/8 | 2/2 |
| | Dysmorphic features | ND | 4/8 | 1/2 |
| | Undescended testis | ND | 0/8 | 1/1 |
| | Neonatal jaundice | ND | 4/8 | 0/2 |
| | Elevated liver enzymes | ND | 6/7 | 1/2 |
| | Elevated alpha fetoprotein | ND | 3/5 | 0/2 |
| | Raised blood lactate | ND | 4/6 | 0/2 |
| | Died within two decades | ND | 2/8 | 1/8 |
| Investigations | Abnormal EEG | ND | 7/8 | 1/2 |
| | Abnormal nerve conduction velocities/Type | ND | 3/8/Mixt type axonal loss and demyelination; sensory more than motor | 2/2 |
| | EMG abnormalities | ND | 2/8 | 1/1 |
| | Nerve biopsy | NP | 1/8 | 1/2 |
| Central nerve system abnormality | | ND | 8/8 | 1/2 |

pathway. LoF mutations in *NGLY1* likely lead to the accumulation of misfolded proteins.

Recently Enns et al. reported 8 patients with three compound heterozygous and five same homozygous LoF mutations in *NGLY1*. As in our two patients, all eight of these cases had neurodevelopmental delay, a movement disorder, and hypotonia (Table 1). However peripheral neuropathy, which was a pronounced feature in our cases, was only documented in three of these patients. While our cases again clearly possess some similar clinical characteristics with Enns et al.'s patients, such as seizures, elevated liver enzymes, diminished reflexes, scoliosis, strabismus and others, our cases illustrate additional phenotypic manifestations which can be related to LoF of NGLY1. These additional findings include difficulty swallowing, transient hepatomegaly, anhydrosis, undescended testis, and pain insensitivity as well as histologically confirmed axonal loss and decreased myelination. This further illustrated phenotypic heterogeneity in patients with LoF mutations in *NGLY1*. Of note, this heterogeneity exists not only between patients from different families but also between patients who harbor the identical mutation in *NGLY1*.

As a differential diagnosis, the constellation of symptoms observed in our patients is reminiscent of those found in patients with hereditary sensory and autonomic neuropathies (HSAN) [Auer-Grumbach et al., 2006; Dyck, 1993]. However, the precise phenotype exhibited by our patients fail to fit one of the previously described subtypes of HSAN (Supplementary Table 1) [Dyck, 1993]. Further, human congenital glycosylation disorders (CDGs), which include more than 100 disorders, have a large clinical spectrum affecting almost each organ system [Freeze, 2013; Freeze et al., 2014; Morava et al., 2009]. While the clinical findings of these children may indicate the inclusion of the synthetic CDGs in the differential diagnosis, the potential diagnosis of a deglycosylation defect – such as those caused by *NGLY1* mutations – should now also be included.

There are a number of potential mechanisms by which impaired *NGLY1* function could be deleterious. A previous study demonstrated that impaired PNG1 function, an ortholog to NGLY1, results in improper innervation of target organs via peripheral axons in *Caenorhabditis elegans* [Habibi-Babadi et al., 2010]. This suggests that impaired axonal guidance may account for a subset of the clinical findings observed in these patients. Another possibility is that loss of *NGLY1* results in loss of the myelinated nerve fibers-as we and others observed via nerve biopsy. This may be a direct effect of neurotoxic accumulation of misfolded proteins due to impaired ERAD function or secondary to inhibited myelin production via the ER [Lin and Popko, 2009; Ron and Walter, 2007]. Indeed, Need et al. and Enns et al. reported an accumulation of unidentified substance in cytoplasm of the hepatocytes in patients with LoF mutations in *NGLY1* – a similar pathophysiological mechanism may play a role in neuronal dysfunction.

Recent advances in next generation genomic sequencing technologies have revolutionized our ability to elucidate the genetic classification of hereditary disorders, including those affecting the nervous system. We describe 2 siblings with novel homozygous loss-of-function mutation in *NGLY1* who develop an early-onset neurodegenerative syndrome with phenotypic heterogeneity.

## Conflict of interest

We report no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2014.08.008.

## References

Auer-Grumbach M, Mauko B, Auer-Grumbach P, Pieber TR. Molecular genetics of hereditary sensory neuropathies. Neuromol Med 2006;8:147–58.

Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, Tatli B, et al. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. Nature 2010;467:207–10.
Chakrabarti A, Chen AW, Varner JD. A review of the mammalian unfolded protein response. Biotechnol Bioeng 2011;108:2777–93.
Dyck P. Neuronal atrophy and degeneration predominantly affecting peripheral sensory and autonomic neurons. In: Dyck P, Thomas PK, Griffin JW, Low PA, editors. Peripheral neuropathy. Philadelphia: WB Saunders; 1993. pp. 1065–93.
Enns GM, Shashi V, Bainbridge M, Gambello MJ, Zahir FR, Bast T, et al. Mutations in NGLY1 cause an inherited disorder of the endoplasmic reticulum-associated degradation pathway. Genet Med 2014;16(10):751–8.
Freeze HH. Understanding human glycosylation disorders: biochemistry leads the charge. J Biol Chem 2013;288:6936–45.
Freeze HH, Chong JX, Bamshad MJ, Ng BG. Solving glycosylation disorders: fundamental approaches reveal complicated pathways. Am J Hum Genet 2014;94:161–75.
Habibi-Babadi N, Su A, de Carvalho CE, Colavita A. The N-glycanase png-1 acts to limit axon branching during organ formation in *Caenorhabditis elegans*. J Neurosci 2010;30:1766–76.
Hirsch C, Blom D, Ploegh HL. A role for N-glycanase in the cytosolic turnover of glycoproteins. EMBO J 2003;22:1036–46.
Lin W, Popko B. Endoplasmic reticulum stress in disorders of myelinating cells. Nat Neurosci 2009;12:379–85.
Morava E, Wosik HN, Sykut-Cegielska J, Adamowicz M, Guillard M, Wevers RA, Lefeber DJ, Cruysberg JR. Ophthalmological abnormalities in children with congenital disorders of glycosylation type I. Br J Ophthalmol 2009;93:350–4.
Need AC, Shashi V, Hitomi Y, Schoch K, Shianna KV, McDonald MT, Meisler MH, Goldstein DB. Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 2012;49:353–61.
Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007;8:519–29.
Suzuki T. Cytoplasmic peptide:N-glycanase and catabolic pathway for free N-glycans in the cytosol. Semin Cell Dev Biol 2007;18:762–9.
Suzuki T, Kwofie MA, Lennarz WJ. Ngly1, a mouse gene encoding a deglycosylating enzyme implicated in proteasomal degradation: expression, genomic organization, and chromosomal mapping. Biochem Biophys Res Commun 2003;304:326–32.