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Prospective Phenotyping of NGLY1-CDDG, the First Congenital Disorder of Deglycosylation

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Abstract

Purpose: The cytosolic enzyme N-glycanase 1, encoded by *NGLY1*, catalyzes cleavage of the beta-aspartyl glycosylamine bond of N-linked glycoproteins, releasing intact N-glycans from proteins bound for degradation. In this study, we describe the clinical spectrum of NGLY1 deficiency (NGLY1-CDDG).

Methods: Prospective natural history protocol

Results: In 12 individuals ages 2 to 21 years with confirmed, biallelic, pathogenic *NGLY1* mutations, we identified previously unreported clinical features, including optic atrophy and retinal pigmentary changes/cone dystrophy, delayed bone age, joint hypermobility, and lower than predicted resting energy expenditure. Novel laboratory findings include low CSF total protein and albumin, and unusually high antibody titers towards rubella and/or rubeola following vaccination. We also confirmed and further quantified previously reported findings noting that decreased tear production, transient transaminitis, small feet, a complex hyperkinetic movement disorder, and varying degrees of global developmental delay with relatively preserved socialization are the most consistent features.

Conclusion: Our prospective phenotyping expands the clinical spectrum of NGLY1-CDDG, offers prognostic information, and provides baseline data for evaluating therapeutic interventions.

Keywords

NGLY1; Deglycosylation; glycosylation; natural history; NGLY1-CDDG; phenotype; congenital disorder of glycosylation; N-linked glycosylation; human

INTRODUCTION

Congenital disorders of glycosylation (CDGs) are a group of inborn errors characterized by abnormalities in the process of glycosylation of biomolecules[1–3]. While more than 100 different CDGs have been reported since the first description by Jaeken et al. in 1980[4–7], only one congenital disorder of de-glycosylation has been described. NGLY1 deficiency (OMIM: 610661 and 615273), or NGLY1-CDDG, was first reported in 2012 by Need et al., who, through exome sequencing, identified biallelic mutations in the *NGLY1* gene as the cause of disease in one child[8].

NGLY1 encodes N-glycanase 1, an enzyme involved in the cytosolic degradation of misfolded glycoproteins and other glycoproteins bound for degradation[9]. In the index case, a 3-year-old male inherited a maternal c.1891delC (p.Q631S NM_018297.3) variant and a paternal c.1201A>T (p.R401* NM_018297.3) variant. NGLY1 protein expression in leukocytes was reduced in the parents and undetectable in the proband compared to controls. Transferrin isoelectric focusing and N-glycan profiling were normal, accounting for the difficulty in ascertaining other affected individuals[8]. However, a social media campaign identified seven additional affected individuals[10], and in 2014 Enns et al. published a retrospective chart review highlighting the most common findings in the eight known NGLY1-CDDG patients [11]. At the same time, we initiated a natural history study of the disorder at the National Institutes of Health (NIH) Clinical Center (http://clinicaltrials.gov,

trial NCT00369421 and trial NCT02089789); this report documents the results of comprehensive, prospective, clinical, molecular, radiologic, and laboratory investigations performed on 12 affected individuals

PATIENTS AND METHODS

Patients

The families were enrolled in NIH protocol #76-HG-0238 “Diagnosis and Treatment of Patients with Inborn Errors of Metabolism or Other Genetic Disorders” (http://clinicaltrials.gov, trial NCT00369421), and #14-HG-0071 “Clinical and Basic Investigations into Known and Suspected Congenital Disorders of Glycosylation” (http://clinicaltrials.gov, trial NCT02089789), approved by the National Human Genome Research Institute’s Institutional Review Board. The parents gave written informed consent for their children and dependents. Consents to publish full face and body photographs and videos were also obtained. Medical records were collected and reviewed, and each subject was admitted to the NIH Clinical Center for a 4–15 day evaluation.

Clinical Studies

Clinical studies were designed to detail the phenotypic features of NGLY1-CDDG. Blood, urine, cerebral spinal fluid (CSF), lymphoblasts, and primary dermal fibroblasts were collected, analyzed, and stored. Studies included brain magnetic resonance imaging and spectroscopy (MRI and MRS, supplementary methods), routine and overnight electroencephalograms (EEGs) with a limited montage performed during a sleep study, electromyogram (EMG, supplementary methods) and nerve conduction studies (NCS, supplementary methods), indirect calorimetry, awake and sedated eye examination with Schirmer II testing, optical coherence tomography scans and electroretinography, behavioral determination of pure tone thresholds, tympanometry, distortion product otoacoustic emissions, auditory brainstem evoked potentials (ABR), quantitative sweat analysis autonomic testing (QSWEAT, supplementary methods), gastric aspiration, swallow study, skeletal survey, bone age, dual X-ray absorptiometry (DEXA), abdominal ultrasound, vibration controlled transient elastography (Fibroscan)[12], echocardiogram, and electrocardiogram. Consultations included clinical neurology, audiology, nutrition, ophthalmology, hepatology, growth, puberty and hormonal studies, allergy and immunology, genetic counseling, physiatry, and speech, occupational, and physical therapy. Eleven individuals underwent developmental psychological evaluations, consisting of at least the Vineland Adaptive Behavior Scales, 2nd edition[13]. Cognitive function was assessed with testing specific for age and developmental level that provided either an intelligence quotient (IQ) or developmental quotient (DQ) score. In addition, the Nijmegen pediatric CDG rating scale, a measure of clinical disease progression developed for CDG, was applied to all affected individuals younger than 18 years[14].

RESULTS

Molecular Findings

Twelve individuals from ten families with confirmed biallelic mutations in *NGLY1* were admitted to the NIH Clinical Center. Subjects 3 and 11 are siblings, and subjects 7 and 8 are siblings. Six individuals (#2, 3, 6, 9, 11, and 12) were included in previous clinical publications[11,15,16]. All individuals (six female; six male) were white and ranged from 2.5–21.3 years of age. We identified 13 different mutations: five missense, five nonsense, two splice site, and one frameshift mutation (Table 1). The most common mutation was c.1201A>T (p.R401*), occurring in seven alleles. The mutations were widely dispersed along the gene with no obvious hotspot[17]. Only four of the mutations lay within the catalytic domain (Figure 1). Family histories are detailed in the Supplementary Materials and Methods.

Dysmorphology

Most affected individuals had hypotonic facies, and the features of older individuals reflected their low weight, with thin facies, hollowed cheeks, and visible zygomatic arches. Eye measurements, performed on 10 subjects, varied broadly and revealed no consistent abnormality (Figure 2).

Growth

Ten of twelve subjects were born at term; two were born at 36 and 34 weeks, respectively. In the majority of individuals, birth weight, length, and occipital-frontal circumference (OFC) were appropriate for gestational age. Individuals grew poorly after mid-childhood, with weight affected more than height. Acquired microcephaly was documented in the four oldest subjects (Supplementary Figure S1). The total foot length was < 3_{rd} percentile in all 12 individuals.

Nijmegen Scores

The 11 individuals under age 18 years were evaluated using the Nijmegen Pediatric CDG Severity scale[14]. Total Nijmegen scores ranged from 9 (mild) to 52 (severe), with a mean of 28 ± 4 (SEM); the median was 32 (Table 1, Supplementary Table S1). Based on definitions by Achouitar et al[14], three individuals scored in the mild range, two in the moderate range, and six in the severe range. All five individuals carrying the common mutation c.1201A>T were either moderately or severely impaired; those carrying at least one copy had a higher mean score (36) than those without this mutation (20.5) (p=0.0184). The sibling pair (individuals 7 and 8) with a private cryptic splice site mutation (c.930C>T) and a private nonsense mutation (c.622C>T) both exhibited relatively mild impairment in all domains. All other mutations were private, precluding additional genotype-phenotype correlations. There was no significant difference in disease severity of males compared to females (data not shown).

Development

All twelve subjects had at least some developmental delay or intellectual disability, with a broad range of severity (Table 1 and S1). IQ was below average (n=2) or in the range of intellectual disability (n=9) for the entire group; seven individuals had profound intellectual disability. The two individuals with IQ below average were verbally fluent; the remainder were nonverbal (n=7) or used only single words (n=1) or phrase speech (n=1).

On the Vineland Adaptive Behavior Scales, Second Edition[13], composite scores ranged from 24 to 98 with a mean of 51 (SEM = 7) (Table 1 and S1). There was a consistent profile characterized by relatively strong Socialization scores, followed by Communication, and Daily Living Skills (Supplementary Figure S2). Motor deficits were also reflected in impaired Daily Living Skills scores. Vineland scores were higher than cognitive scores for all subjects. We found no cross-sectional relationship between age and Vineland Adaptive Behavior Composite.

Neurologic Phenotype

Seven of twelve subjects had clinical seizures, and one had subclinical seizures recognized on previous EEG. Details regarding age of onset, seizure type and frequency, medications, and EEG findings are noted in Supplementary Table S2. On overnight EEG, only one individual (#6) had active seizures recorded, but seven had multifocal epileptiform activity. There were no age or genotype differences between individuals having seizures and those without. In fact, in each sibling pair, one had seizures and the other did not.

All twelve individuals exhibited hyperkinetic movement disorders that included choreiform, athetoid, dystonic, myoclonic, action tremor, and dysmetric movements and were more severe in the younger individuals (Supplementary Movie S1).

Brain MRI and MRS

Eleven individuals underwent MRI and MRS of the brain. Clinical assessment of the images was not striking (Figure 3). Delayed myelination was present in three of the four youngest individuals, but all the older individuals had complete myelination. Six of nine individuals had qualitatively-evident cerebral atrophy that ranged from slight to moderate. Four individuals (#1, #2, #6, #10) also had slight cerebellar atrophy. The atrophy tended to be greater in the older individuals ($p=0.17$, Supplementary Figure S3), and in one teenager (#11) follow-up imaging showed atrophy measurably worse after a 20-month interval (net loss of 34 cm_3 relative to expected). Increased atrophy correlated with worsening of all functional measurements (Supplementary Figure S3), including IQ or DQ ($p<0.03$), Vineland assessments ($p<0.03$), and Nijmegen scores ($p=0.01$). Brain volume also directly correlated with CSF levels of 5-HIAA ($p=0.03$), tetrahydrobiopterin ($p=0.02$), and 5-HVA ($p=0.06$) (Supplementary Figure S3).

Compared to the reference population, N-acetylaspartylglutamate + N-acetylaspartate (NAA) was lower than normal in the left centrum semiovale (LCSO) ($p=0.004$), the midline parietal grey matter (PGM) ($p=0.02$), and superior cerebellar vermis (SVERM) ($p<0.0001$). There was a deficit of glutamine + glutamate + gamma-aminobutyric acid (Glx) in the PGM

(p=0.03), LCSO (p=0.01), and pons (p=0.0002). Choline was higher than expected for age only in the LCSO (p=0.0097), and myo-inositol was higher than expected for age in the pons (p=0.002). Multiple correlations between these MRS-measured metabolites and age, functional assessments, brain volume, and neurotransmitters in the CSF were found (Supplementary Figure S3). The general trend showed that the differences noted above became more pronounced with increasing age, worsening function, and lower brain volume (Supplementary Figure S4). MRS metabolite measurements did not correlate with total CSF protein, CSF albumin, or CSF/serum albumin ratio. There was a weak correlation (p=0.09) between atrophy and total CSF protein, but not CSF albumin or CSF/serum albumin ratio.

CSF Laboratory Results

Nine subjects underwent lumbar puncture. CSF total protein and albumin concentrations, as well as the CSF/serum albumin ratios, were low in nearly all individuals (Supplementary Table S3). There was no correlation between age and CSF protein or albumin levels. In the two oldest subjects, CSF 5-hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA) were decreased, suggesting neuronal loss. Neopterin levels were normal but decreased with age, and CSF tetrahydrobiopterin (BH4) levels were below the lower limit of normal in all but one subject tested; however, there was no correlation with age. CSF 5-HIAA, HVA, and BH4 levels strongly and directly correlated with brain atrophy. CSF lactate and amino acid levels were essentially normal (Supplementary Table S3). CSF leucocyte counts (0–4; normal 0–5), glucose concentrations (54–72 mg/dL; normal 40–70), 3-O-methyldopa concentrations (12–28 nM; normal <150) and 5-methyltetrahydrofolate concentrations (45–85 nmol/L; normal 40–150) were unremarkable (data not shown).

NCS, EMG, and QSWEAT Testing

Nerve conduction studies were performed in 11 individuals (Supplementary Table S4). The predominant finding was an axonal sensorimotor polyneuropathy (n=8) with additional demyelinative features (n=6). Results of neurophysiological testing demonstrated a length-dependent, progressive loss of sensory and motor axons and sympathetic nerve function, apparently static abnormalities in myelination of peripheral nerves, and possible motor neuron degeneration. A single individual (#6) had a repeat study at one year, showing progression of the neuropathy. On needle EMG, neurogenic findings were noted in 9 subjects with varying degrees of acute and chronic changes consistent with the NCS results.

QSWEATs, performed in 11 individuals, were abnormal in the same 8 individuals who had axonal sensorimotor neuropathies. The distal lower extremity QSWEAT was more frequently absent (7/11) compared to the forearm (1/11), consistent with a length dependent neuropathy. There was a trend to greater severity of neuropathy in the older individuals, with an inverse correlation between tibial motor amplitude and age ($p = 0.002$, $r_2 = 0.799$); the amplitude decreased by −0.3 mV per year (normal, >+2.5mV per year).

Ophthalmology

Awake and sedated ophthalmic examinations were performed on 11 study participants (Supplementary Table S5). Lagophthalmous, ptosis, exotropia and/or esotropia, corneal neovascularization, pannus formation or scarring, op tic nerve pallor or atrophy, retinal

pigmentary changes including pigment granularity and pigmentary retinopathy or pigmentary changes, blonde or poorly pigmented retinal periphery, and refractive errors were observed (Figure 4). All individuals had evidence of hypo- or complete alacrima on Schirmer II testing (Supplementary Table S5). Tear production did not correlate with age. One participant underwent sedated electroretinography and was diagnosed with cone dystrophy; the photopic responses were reduced to ~50% of the lower limit of normal for age.

Audiology

Audiologic assessments, including otoacoustic emissions and/or cochlear microphonics to evaluate for the functional integrity of the inner ear, and auditory brainstem response (ABRs), were conducted on 11 subjects. Behavioral hearing thresholds could be established reliably in only three subjects and all had normal hearing sensitivity. Tympanometry was unremarkable. Most remarkable was the dyssynchronous and/or absent transmission through the auditory brainstem and/or 8_{th} nerve in most individuals. The severity of the transmission abnormality through the auditory brainstem increased with increasing age, and the eighth nerve was bilaterally involved the 4 oldest individuals (Supplementary Table S6).

Clinical Feeding and Swallowing Assessments

Clinical feeding and modified barium swallow assessments were performed on 11 subjects. Oral motor deficits, including persistent nutritive suckle swallow and poor oral bolus formation, were present in 10 of 11. Premature spillage and pharyngeal swallow response delays remained the primary deficits of all subjects. No aspiration or laryngeal penetration for any textures were observed in the swallow studies. Developmental delays in m astication were evident and characterized by munching patterns without matured rotary chewing of solid textures. Generalized weakness of the lips and tongue was observed in all individuals during cranial nerve assessments. Dystonic movements of the tongue and persistent oral reflexes of suckling and suck/swallows were seen in the majority of individuals.

Cardiorespiratory Status

Echocardiograms were unremarkable (n=12). Electrocardiograms showed heart rates in the low 100s in all subjects (n=12), with two individuals having QTcB >440 ms, but a normal QTcF. Sleep studies (n=9) identified evidence of obstructive sleep apnea (n=2), central sleep apnea (n=1), and combined obstructive and central sleep apnea (n=2). Five individuals had frequent periodic limb movements. There were no associations between age and either sleep apnea or frequent periodic limb movements.

Gastrointestinal and Nutritional Findings

Gastric pH was assessed after H2 blockers and osmotic pump inhibitors had been discontinued for 5 days. Gastric pH was appropriately acidic in all individuals tested except one, whose pH was 7.25. Ten of twelve individuals had some degree of constipation. Abdominal ultrasounds were normal in six of 12 individuals. Abnormalities in abdominal ultrasound included splenomegaly, steatosis, coarse or inhomogeneous liver texture, or hepatomegaly. No hydronephrosis, polycystic kidneys, horseshoe kidneys, kidney calculi,

nephrocalcinosis, or perinephric fluid were seen. Three of twelve had Fibroscan scores above 7 kPa, the upper limit of normal, indicating possible liver fibrosis. Resting energy expenditure was close to 100% of expected in three individuals, but ranged from 51% to 82% of predicted in the other 9 (data not shown).

Laboratory values reflecting gastrointestinal and hepatic function were essentially normal at the time of the NIH evaluation (Supplementary Table S7). However, chart review revealed elevations in aspartate aminotransferase (AST) and/or alanine transaminase (ALT) in all eight subjects that had these measured in their first two years of life. One individual had undergone liver transplantation for presumed hepatocellular carcinoma (further details in Supplementary Materials and Methods). These transaminase levels normalized around age 4 (Supplementary Figure S5). Circulating proteins were normal or borderline low in all individuals (Supplementary Table S7). Total cholesterol, LDL, and triglycerides were also low (Supplementary Table S7). In the two subjects with the lowest cholesterol levels, high density lipoprotein, low density lipoprotein, and very low density lipoprotein particle number and sizes were normal (data not shown).

Hematologic, Endocrine, Immunologic, and Biochemical Phenotype

Hematologic profiles showed unremarkable complete blood counts (Supplementary Table S8) with the exception of one subject with lymphoblasts, subsequently diagnosed with acute lymphocytic leukemia (supplementary results). Coagulation studies showed normal prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR), but low protein C activity (n=6), Factor II activity (n=1), Factor IX (n=2), Factor XI (n=2), and fibrinogen (n=5) (Supplementary Table S8). On average, all factors except for Antithrombin III were lower in individuals with the c.1201A>T mutation; for Factor II, Factor IX, and Factor XI, the differences were significant at $p<0.05$ using the two sample t-test.

Detailed laboratory analyses of endocrine (Supplementary Table S9) and immune (Supplementary Table S10) function were performed. The most striking finding was that seven of 11 individuals tested exhibited out of range elevations in antibody titers towards rubella and/or rubeola following MMR vaccination. Biochemical markers known to be abnormal in mitochondropathies and hypoglycosylation disorders showed inconsistent results (Supplementary Tables S11 and S12).

Musculoskeletal Findings

Bone age was delayed in eight of the 11 subjects tested without any consistent abnormalities of the endocrine system: the somatotropic axis and thyroid function were normal in all studied individuals. Femoral bone density was low in all nine individuals who underwent DEXA scanning (mean, SEM z-scores for 8 patients < 21 years adjacent to the growth plate = −3, 0.4; metaphysis-diaphysis = −2.2, 0.6, and diaphysis = −1.8, 0.5). All subjects had joint hypermobility except the older ones, who had contractures in both small and large joints. Complete skeletal surveys were performed on 11 individuals; abnormalities included coxa valga (n=11), scoliosis (n=6), growth arrest lines or metaphyseal banding without palmidronate treatment (n=4), dislocations or subluxations involving the hips and shoulder

joints (n=3), and sclerosis of the phalanges or tarsal bones (n=2). Findings noted only once included flexion deformity of the fingers, hallux valgus, sclerotic lesion of the distal femur, thinning of the fibulas, prominent apophyseal bone formation in the ileac crests and ischia, ulnar bowing, expansion of the cortexes in the proximal and middle phalanges in digits 2–4, widened phalanges, and shortened and widened metatarsals.

DISCUSSION:

Summary of Novel Findings

Our prospective investigations into NGLY1-CDDG revealed several new discoveries associated with this disorder. These included low CSF total protein and albumin, optic atrophy and retinal pigmentary changes/cone dystrophy, poor weight gain starting in mid-childhood, lower than predicted resting energy expenditure, central and obstructive sleep apnea, delayed bone age, joint hypermobility, progressive brain atrophy, abnormalities in brain MRS-measured metabolites, various abnormalities on skeletal survey, high antibody titers after rubella and rubeola vaccination, and low levels of several pro- and anti-coagulation factors. Echocardiograms, electrocardiograms, and CSF lactate levels were normal.

We also confirmed and further detailed the global developmental delay, movement disorder, frequent seizure disorder, hypotonia, hypolacrima or alacrima, corneal disease, ptosis, lagophthalmous, strabismus, peripheral neuropathy and occasional diminished reflexes, hypohidrosis, auditory brainstem response abnormalities, abnormal brain imaging, scoliosis, acquired microcephaly, small hands and feet, dysmorphic features, constipation, elevated liver enzymes present only early in childhood, osteopenia, and hypocholesterolemia associated with NGLY1-CDDG. Compared with previously reported biopsy findings, our transient elastography results showed minimal if any fibrosis. In addition, we could not confirm abnormal storage material in the three liver biopsy specimens we analyzed, nor did we observe ocular apraxia in our individuals, despite performing detailed ophthalmologic evaluations that involved some subjects previously reported to have ocular apraxia[11].

Based on our findings, we recommend considering NGLY1-CDDG in the differential diagnosis of any patient with the tetrad of developmental delay/intellectual disability, hyperkinetic movement disorder, hypolacrima, and a history of elevated transaminases during early childhood.

Therapeutic Implications

Several specific phenotypic findings have therapeutic implications. The auditory neural pathway dysfunction without peripheral hearing loss resembles that observed in auditory neuropathy. Persons with auditory neuropathy experience difficulty hearing in the presence of background noise and benefit from quiet listening environments[21]. If hypohidrosis is detected, preventative measures (hydration, ventilation, etc.) against situations that cause dangerous core body hyperthermia can be taken. It is important to aggressively manage hypo-lacrima with artificial tears and bland ointment to prevent secondary complications that can impact vision. The finding of disordered mastication for solids but a functional

ability to swallow suggests the need for oral motor and swallowing therapies for all subjects to facilitate better control and chewing maturation.

Motor Skills scores were low and resembled the Daily Living Skills scores, reflecting the significant motor involvement required for daily tasks. This suggests that if motor deficits and the hyperkinetic movement disorder of NGLY1-CDDG could be treated effectively, quality of life could be significantly improved. Contributors to the movement disorder that could be targeted include myoclonic seizures, neurotransmitter deficiency, and/or peripheral neuropathy manifesting as sensory ataxia.

Hypotheses Generated for Pathophysiology of Clinical Findings

Our detailed assessment of the phenotypic features has also led to several hypotheses regarding underlying pathophysiology. The strong correlation between brain atrophy on MRI and functional assessments suggests that loss of neurons contributes to the functional impairment. The atrophy also correlated with CSF metabolites (BH4, 5-HIAA, HVA), which are known to be lower when there is damage to neurotransmitter producing neurons[22–24]. This suggests that these biochemical abnormalities may be secondary to brain atrophy.

Results from MRS showed that as functional impairment worsened and age increased, NAA decreased, while choline, myo-inositol and creatine increased. Additionally, creatine and myoinositol were inversely correlated, and NAA directly correlated, with neurotransmitter levels (5-HIAA, 5-HVA, 3-OMD, and neopterin, Supplementary Figure S3). NAA may be found exclusively in neurons and declines as neurons become unhealthy or die[25,26]. Elevations in choline could be consistent with a relative abundance of glial cells secondary to neuronal loss[26]. Myo-inositol is a glia-specific marker that is elevated by gliosis or inflammation[27,28]; in our cohort we do not have any evidence of inflammation. Creatine levels are relatively higher in glial cells compared to neurons[29]. Taken together, the MRS findings suggest that there is a relative abundance of glial cells compared to neurons in the brain of NGLY1-CDDG individuals, possibly due to loss of neurons, and that the degree of this imbalance contributes significantly to the severity of the phenotype.

The impaired sweat response, largely affecting distal responses, is consistent with a small fiber neuropathy rather than a central etiology or generalized cholinergic dysfunction. Albumin CSF/serum quotient, or Q_{Alb}, is a marker of blood-CSF barrier dysfunction, and its value is inversely correlated to the CSF turnover rate[30]. There was no significant change in the Q_{Alb} ratio (data not shown), arguing against increased turnover as a possible etiology. Regardless of the mechanism, we propose that decreased CSF protein and albumin concentrations represent a novel diagnostic marker for this disorder.

Individuals with NGLY1-CDDG and those with N-linked glycosylation disorders share the phenotypic features of low cholesterol, hepatopathy, peripheral neuropathy, retinal and optic nerve abnormalities, seizures, developmental delay with socialization as a relative strength, and delayed bone age[18]. NGLY1-CDDG also overlaps with O-linked glycosylation disorders with respect to hypolacrima[19]. There may be a pathogenic relationship; N-glycanase 1 catalyzes the cleavage of the amide bond between the proximal N-acetylglucosamine residue of glycans and the asparagine residue of the protein[9], so NGLY1-CDDG could impair

glycan recycling and lead to hypoglycosylation. Indeed, the individuals with NGLY1-CDDG exhibited subtle abnormalities in transferrin and ApoC-III glycosylation[20].

Another hypothesis for the etiology of the NGLY1-CDDG phenotype is based on the fact that misfolded glycoproteins are processed through the endoplasmic reticulum associated degradation pathway (ERAD) and then retrotranslocated into the cytoplasm. There, N-glycanase is the first step in further degradation of these molecules, making ERAD dysfunction a possible pathophysiologic contributor especially given evidence of ER-stress in mouse embryonic fibroblasts[31]. However, preliminary experiments found no impairment or enhancement of standard ERAD marker expression under normal conditions in NGLY1-CDDG patient fibroblasts (H.H. Freeze, personal communication, April 2016).

Age Progression of Disease and Implications for Future Therapeutic Trials

Our study, which documents cross-sectional findings and covers a large range of ages, indicates that NGLY1-CDDG is a progressive disorder. The Nijmegen severity scores and Vineland scores indicated worsening function with age. Also progressive were the peripheral neuropathy, auditory neural dysfunction, scoliosis, inability to maintain weight, brain atrophy on MRI, abnormalities in brain metabolites measured on MRS, and CSF neurotransmitter levels.

However, other aspects of N-glycanase deficiency appear static or even regressive. Hypolacrima occurred at all ages, and the hepatopathy and hyperkinetic movement disorder appeared to improve with age. These observations provide a guide to determine reliable outcome measures for efficacy in future therapeutic trials. Effective outcome measures would derive from findings that are progressive, such as brain volume, choline levels on MRS, nerve conduction velocities and amplitudes, and auditory brainstem evoked responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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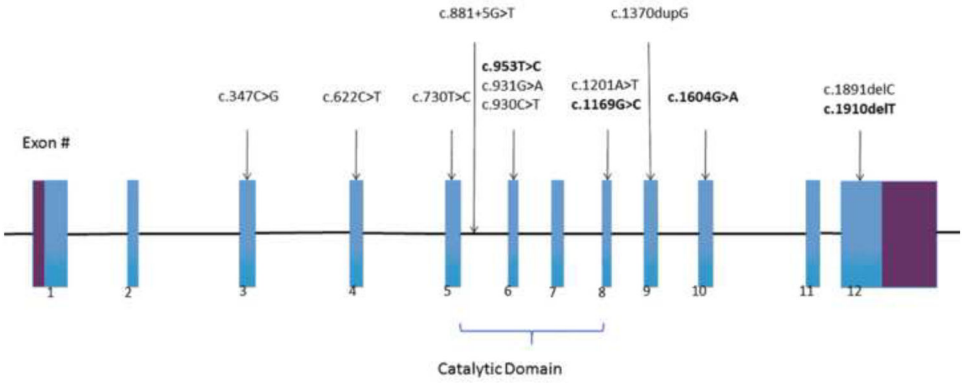

Figure 1: Distribution of *NGLY1* mutations.

Shaded bars are exons. Previously unreported mutations are in bold. Mutations previously published and not in this paper include c.1624C>T (exon 11), c.1205_1207delGAA (exon 8) and c.1533_1536delTCAA (exon 10). Four mutations lie within the catalytic domain (amino acid residues 292–353);, i.e., c.730T>C, c.953T>C, c.931G>A, and c.930C>T. The accession numbers for the NM_018297.3:c.931G>A, NM_018297.3:c.1604G>A, NM_018297.3:c.622C>T, NM_018297.3:c.347C>G, NM_018297.3:c.1169G>C, NM_018297.3:c.730T>C, NM_018297.3:c.1910delT, NM_018297.3:c.930C>T, NM_018297.3:c.881+5G>T sequences reported in this paper are ClinVar: SCV000259179, SCV000259180, SCV000259181, SCV000259182, SCV000259183, SCV000259184, SCV000259185, SCV000259186, and SCV000259187 respectively.

Figure 2: Facial features of NGLY1-CDDG.

Individuals with NGLY1-CDDG are arranged from youngest (top left) to oldest (bottom right). Facial features include upturned nasal tip, hypotonic facies, ptosis, brachycephaly, thinned facies, hollowed cheeks, and visible zygomatic arches. Interpupillary distance ranged from the 13–95 centiles with the mean at the 66th centile, and SEM of 8. Horizontal fissure length ranged from 3–70 centiles with mean at the 20th centile, and SEM of 1. Inner canthal distance ranged from 3–100 centiles with the mean at the 59th centile and SEM of 10. Outer canthal distance ranged from 6–81 centile with mean at the 43 centile and SEM of 7. Canthal index ranged from −2 to +2 SD from the normal mean.

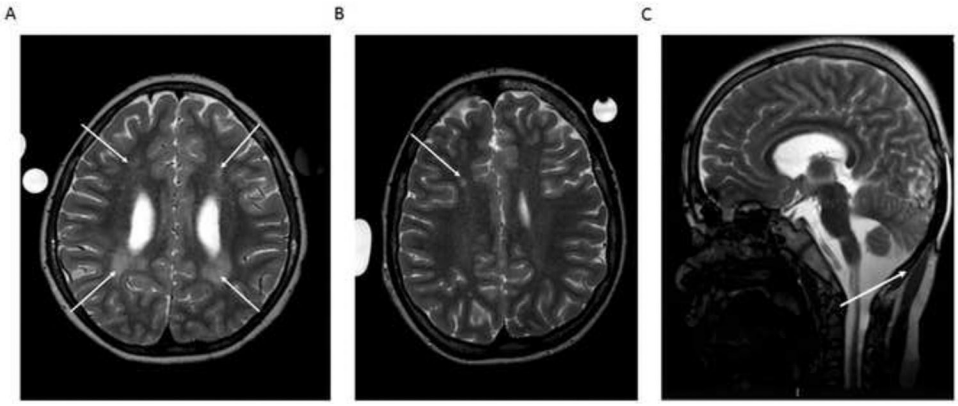

Figure 3: Brain MRI findings.

White matter lesions were seen in two of eleven individuals. A shows multiple lesions in the periventricular white matter, some of which were confluent. B shows a single lesion in the periventricular white matter. Both A and B illustrate cerebral atrophy and were performed using T2-weighting. Sulci are slightly prominent in both A and B, and prominent ventricles are visible in A. C illustrates high position of the cerebellar tonsils, and large foramen of Magendie and cisterna magna.

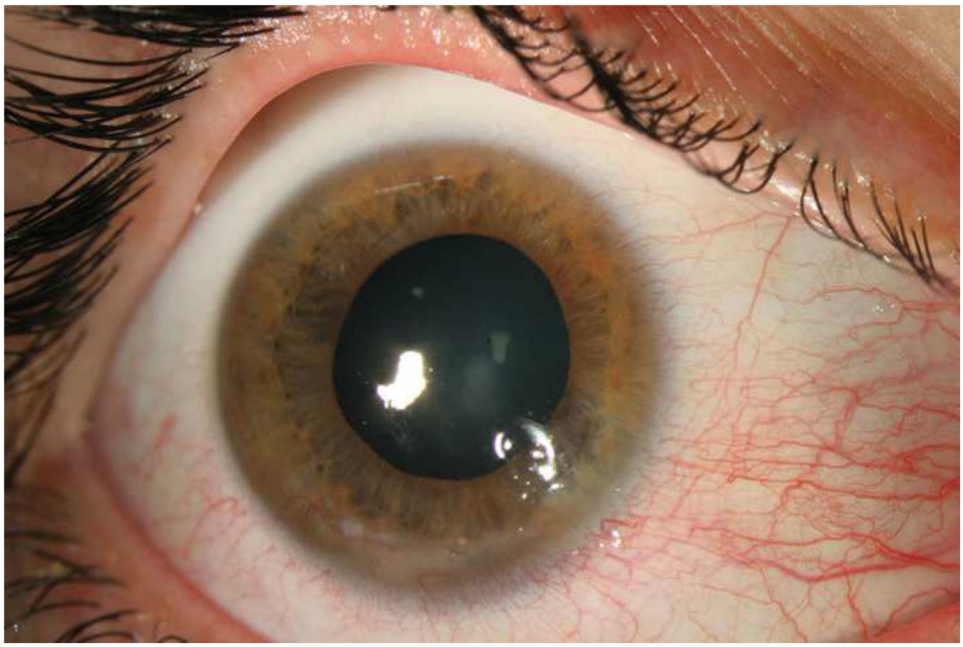

Figure 4: Eye findings.

Right eye close-up showing conjunctival injection, limbal neovascularization, and corneal scarring in an NGLY1 patient. Findings caused by severe dry eyes and lagophthalmous.

Table 1.

Molecular Characteristics and Severity Scores for Individuals with NGLY1-CDDG

Patient #	Age (y) / Sex	Allele #1[a]		Allele #2[a]		Functional Scores		
		Mutation (NM_018297.3)	Protein	Mutation (NM_018297.3)	Protein	Nijmegen[b]	IQ or DQ	Vineland[c]
1	3 / M	c.953T>C	p.L318P	c.1169G>C	p.R390P	14	ND	62
2	4 / M	c.1201A>T	p.R401*	c.1201A>T	p.R401*	33	25	52
3	4 / F	c.1201A>T	p.R401*	c.1201A>T	p.R401*	34	5	40
4	5 / F	c.931G>A	p.E311K	c.730T>C	p.W244R	33	ND	ND
5	6 / M	c.1604G>A	p.W535*	c.1910delT	p.L637*	25	8	43
6	7 / M	c.1891delC	p.Q631S	c.1201A>T	p.R401*	36	5	37
7	8 / F	c.622C>T	p.Q208*	c.930C>T	p.G310G (splice site)	10	74	98
8	10 / M	c.622C>T	p.Q208*	c.930C>T	p.G310G (splice site)	9	81	94
9	16 / M	c.347C>G	p.S116*	c.881+5G>T	IVS5+5G>T	32	2	28
10	17 / F	c.1201A>T	p.R401*	c.1201A>T	p.R401*	25	16	42
11	18 / F	c.1201A>T	p.R401*	c.1201A>T	p.R401*	52	2	24
12	21 / F	c.1370dupG	p.R458Kfs*14	c.1370dupG	p.R458Kfs*14	ND	ND	37
Mean	9	-	-	-	-	28	23	51
SEM	2	-	-	-	-	4	10	7

Abbreviations: IQ, intellectual quotient; DQ, developmental quotient; ND, not determined; M, male; F, female.

[a]The following list specifies each mutation and its associated dbSNP ID and clinVar ID in the format (mutation, dbSNP ID, clinVar ID): (c1201A>T, rs201337954, 65632), (c.931G>A, rs201791209, none), (c.1891delC, rs587776982, 65631), (c.622C>T, rs200561967, none), and (c.1370dupG, rs587777265, 131954). If a particular mutation does not have both a dbSNP ID and a clinVar ID, it is not listed. Gray shading indicates alleles that fall within the transglutaminase catalytic domain.

[b]Nijmegen Pediatric CDG Rating Scale total score: Mild 0–14, Moderate 15–25, Severe ≥26.

[c]Vineland Adaptive Behavior Scales composite score; in a normal population the mean is 100 and standard deviation is 15.