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Myoclonic Epilepsy in Gaucher Disease: Genotype-Phenotype Insights from a Rare Patient Subgroup

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ABSTRACT

Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase, presents with a wide spectrum of manifestations. Although Gaucher disease has been divided into three clinical types, patients with atypical presentations continue to be recognized. A careful phenotypic and genotypic assessment of patients with unusual symptoms may help define factors that modify phenotype in this disorder. One such example is a rare subgroup of patients with type 3 Gaucher disease who develop progressive myoclonic epilepsy. We evaluated 16 patients with myoclonic epilepsy, nine of whom were diagnosed by age 4 y with severe visceral involvement and myoclonus, and seven with a more chronic course, who were studied between ages 22 and 40. All of the patients had abnormal horizontal saccadic eye movements. Fourteen different genotypes were encountered, yet there were several shared alleles, including V394L (seen on two alleles), G377S (seen on three alleles), and L444P, N188S, and recombinant alleles (each found on four alleles). V394L, G377S, and N188S are mutations that have previously been associated with non-neuronopathic Gaucher disease. The spectrum of genotypes differed significantly from other patients with type 3 Gaucher disease, where genotypes L444P/L444P and R463C/null allele predominated. Northern blot studies revealed a normal glucocerebrosidase transcript, whereas Western studies showed that the patients studied lacked the processed 56 kD isoform of the enzyme, consistent with neuronopathic Gaucher disease. Brain autopsy samples from two patients demonstrated elevated levels of glucosylsphingosine, a toxic glycolipid, which could contribute to the development of myoclonus. Thus, although there were certain shared mutant alleles found in these patients, both the lack of a shared genotype and the variability in clinical presentations suggest that other modifiers must contribute to this rare phenotype. **(*Pediatr Res* 53: 387–395, 2003)**

Gaucher disease (MIM 230800) is an autosomal, recessively inherited lysosomal storage disorder caused by the deficiency of the enzyme glucocerebrosidase (EC 3.2.1.45). The gene encoding for glucocerebrosidase is located on chromosome 1q21 and, to date, approximately 200 different mutations have been identified in DNA sequence from patients with Gaucher disease (1–5). Patients with Gaucher disease have been divided roughly into three clinical types, based upon the presence and rate of progression of neurologic symptoms. Type 1 Gaucher disease is the most common form and has no associated neurologic symptoms. However, patients with type 1 disease have a wide range of clinical presentations; they may be asymptomatic or have varied ages of onset and severity of symptoms. Type 2 Gaucher disease, the rarest and most severe form, presents with progressive neurologic deterioration and results in fatality *in utero* or within the first 2–3 y of life. Patients with type 3, or chronic neuronopathic Gaucher disease, generally have the onset of symptoms in childhood or in the early adult years, as well as some form of neurologic involvement. Some authors have further divided type 3 Gaucher disease into subtypes 3a, 3b, and 3c based upon the nature of the neurologic manifestations (6, 7), but many patients still do not fit well into any category. In fact, Gaucher disease can be seen as a disorder with a continuum of phenotypes ranging from perinatal lethality to asymptomatic adults, although some forms have brain involvement and others do not.

There has been a wealth of literature detailing the genotypic analysis of populations of patients with Gaucher disease. However, vast genotypic heterogeneity is encountered even among patients who are very similar clinically. Moreover, it has been noted that in some instances, patients with the same genotype

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will not necessarily share the same phenotype (4, 8, 9). Thus, with certain notable exceptions (*i.e.* the association of mutation N370S with non-neuronopathic Gaucher disease), the predictive value of genotyping is somewhat limited.

Our appreciation of the full spectrum of clinical manifestations associated with Gaucher disease has continued to expand. An increasing number of subgroups of patients with Gaucher disease and unique phenotypes have been reported recently. Included among these are patients with parkinsonian symptoms (10, 11), pulmonary hypertension (12), cardiac calcifications and/or fibrosis (13–15), and hydrops fetalis or the collodion baby phenotype (16, 17). Unique phenotypes are especially prevalent among patients classified as having type 3 Gaucher disease. Even 27 y ago, Frederickson and Sloan (18) concluded that, "They undoubtedly represent a heterogeneous group, and . . . under the cover of 'Type 3,' no single certain disorder is implied." An example is patients with type 3 Gaucher disease with slowing of the horizontal saccadic eye movements as their primary neurologic manifestation (19, 20). This form of Gaucher disease, considered by some as type 3b (7), is characterized by severe visceral involvement, including massive hepatosplenomegaly and pancytopenia, severe skeletal involvement and, when untreated, death by adolescence from hepatic or pulmonary complications.

Another specific subtype of neuronopathic Gaucher disease includes patients who develop progressive myoclonic epilepsy. Although this presentation of type 3 Gaucher disease, also referred to as type 3a, was among the first appreciated (7), it is perhaps the least well characterized. Past case reports of patients with Gaucher disease and myoclonic epilepsy have been scattered, and often the myoclonus has not been an emphasized feature. What is remarkable is the variability in age at presentation and the rate of progression of cases. King (21) described a 39-y-old Jewish male with a 22-y history of progressive myoclonic epilepsy who was subsequently found to have Gaucher disease. Winkleman *et al.* (22) documented a clinicopathologic study of a family with Gaucher disease where the proband developed synchronous involuntary jerks at age 34 y, and his sister, also with Gaucher disease, developed myoclonus at age 50 y. Neil *et al.* (23) described a 41-y-old woman with mild intermittent myoclonic jerking who also had a "mask-like" facies. A case report from Japan described a female patient with myoclonic epilepsy that developed in adolescence (24), and another 22-y-old male patient was described from Germany (25). A few of the adult patients have also been found to have Parkinson-like symptoms (11, 23). On the other end of the age spectrum, Grover *et al.* (26) reported two second cousins with Gaucher disease, one with myoclonus and spasticity at age 17 mo and the second with continuous myoclonus at age 8 y. In two other reports from Europe, the myoclonus developed at 16 mo (27) and at 4.5 y (28). Verghese *et al.* (29) described the clinical and neuropathologic findings in a 6-y-old patient who was found to have selective degeneration of the cerebellar dentate nucleus and the dentatorubrothalamic pathway.

Several of the features described in this subgroup of patients, including the horizontal gaze abnormality, progressive dementia, generalized epilepsy, ataxia, and spasticity, are also seen in a group of patients with type 3 Gaucher disease from the Norrbottnian geographic isolate in northern Sweden (30). Although all Norrbottnian patients are believed to share the genotype L444P/L444P, the age and rate of symptom progression is also quite variable in this group. The median age of death for untreated patients is 12 y, the oldest patient described was 47 y, and not all patients have developed neurologic symptoms (31). The Swedish investigators specifically comment that myoclonus was not found in their patients (32).

In the past few years, our understanding of the genetic basis of myoclonic epilepsy has progressed rapidly and many different defects and specific genes and mutations have been identified (33–35). Genetic lesions have been elucidated in recessive disorders with a myoclonic component, such as Unverricht-Lundberg disease, Lafora disease, five forms of ceroid lipofuscinosis, GM_2 gangliosidosis, and sialidosis; in dominant disorders like dentatorubropallidoluysian atrophy (DRPLA); and in a mitochondrial disorder, MERRF (mitochondrial encephalomyopathy and ragged red fibers).

We believe that a focus on patients with atypical presentations of Gaucher disease will enable us to establish the extent to which DNA mutations correlate with specific phenotypes, and will ultimately help in the identification of other factors that contribute to disease manifestations in this disorder. Thus, in this study, we chose to examine the phenotypes, mutations, and proteins encountered in 16 patients with type 3 Gaucher disease associated with progressive myoclonic epilepsy.

MATERIALS AND METHODS

Case reports. The case histories of the 16 patients studied are briefly summarized in Table 1. These included all available patients with Gaucher disease and myoclonic epilepsy referred to the National Institutes of Health between 1981 and 2001, with the exception of two patients with Parkinson-like features described elsewhere (11, 36). Several of the cases have been previously described in the literature. The cell line for patient 3 was purchased from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository. All other patient samples and clinical data were obtained with informed consent under protocols approved by the National Institute of Mental Health and National Institute of Neurological Disorders and Stroke Institute Review Boards. The clinical records and genotypes of 24 children with abnormal saccadic eye movements described previously (20) were also reviewed. Frozen autopsy samples from control individuals were purchased from the Cooperative Human Tissue Network (Philadelphia, PA, U.S.A.).

DNA and RNA preparation. High molecular weight DNA and total RNA were isolated from white blood cells, cultured fibroblast or lymphoblast cell lines, or tissues of affected individuals and normal controls as previously described (37).

Mutation analysis and Southern blots. The patient DNA sequences were initially screened for common mutations, including N370S, L444P, R463C, c.84–85insG, IVS2+1G→A, and c.1263–1317del as previously described (38, 39). To confirm these mutations and to identify others, direct sequencing of amplified PCR fragments was performed using PCR sequencing primers described previously (17). All 11 exons and

Table 1. *Genotypes and phenotypes of 16 patients with Gaucher disease and myoclonic epilepsy*

Patient No.	Sex	Racial or Ethnic Background	Genotype	Age (y)	Age at Diagnosis (y)	Enzyme Therapy	Age at onset of seizures	MRI	Type of Seizures	EEG Findings	Involvement of Horizontal Saccades	Other Neuro Findings	Splenectomy	Other
1	M	Ashkenazi/Sephardic	V394L/Rec*Nci*I*	9	5	Yes	7	WNL	Myoclonic and tonic/clonic	NA	Yes	Cognitive deficits, unsteady gait, halting speech	No	Lung involvement
2	F	Mexican	L444P/F213I	6	4	Yes	5	NA	Myoclonic and tonic/clonic	Multifocal seizure disorder, slow background	Yes	Dysarthria, feeding difficulty, development delay	No	None
3	F	Caucasian	G325R/C342G	3	5/12	No	NA	NA	Myoclonic	NA	NA	None	NA	Death at age 3
4	M	Caucasian	I402F/E326K +G377S	9	2	Yes	7	Bilateral parietoccipital cystic encephalomalacia	Generalized seizures	NA	Yes	Tremor	No	Bone marrow transplantation
5	M	Caucasian	L444P + recomb†/ G202R	4	10/12	Yes	2	WNL	Nocturnal twitching	Epileptiform activity, slow background	Total loss	Developmental delay	No	Gastrostomy, death at age 5
6	M	French	V394L/Rec*Nci*I*	4	1	Yes	3	NA	Myoclonic and tonic/clonic	Photoparoxysmal response	Total loss	Developmental delay	No	Apneic spells, death at age 5
7	M	Mexican	D409H/R131L	6	3	NA	NA	WNL	Myoclonus of head and fingers	NA	Yes	Dystonia, tremor	No	Lung involvement
8	M	Caucasian	G377S/c.1020 delT	14	2	Yes	9	NA	Myoclonic and tonic/clonic	Diffuse irregular background, multifocal independent spikes	Yes	Mental deterioration, wheelchair bound	Partial	Death at age 14
9	F	Australian	L444P/L444P	24	2	No	9	WNL	Myoclonic and tonic/clonic	Slow background, left occipital spikes	Total loss	None	Yes	Severe skeletal involvement
10	M	Caucasian	K157Q/D140H+ E326K	25	14	No	9	WNL	Staring spells, myoclonic, mixed motor seizures, drop attacks	Frequent biphasic or polyphasic spikes, slow background	Yes	Mental deterioration	Yes	Depression
11	M	Caucasian	N188S/RecNciI* (Fusion)	28	7	No	14	Atrophy of cerebellum and brain stem	Myoclonic and tonic/clonic	Slow background, spike waves and polyspike discharges with photosensitivity	Yes	Slow speech, wheelchair bound	Yes	Depression, affected brother
12	M	Turkish	N188S/c.84-85insG	27	14	No	15	WNL	Generalized seizures, myoclonus of eyelids	Paroxysmal rapid spikes, slow background	Yes	None	No	None
13	F	Korean	N188S/Recomb‡ (Fusion)	22	22	No	12	WNL	Myoclonic and tonic/clonic	NA	NA	Unsteady gait, halting speech	No	Two affected sisters
14	F	Caucasian	G377S/Y205C	33	4	Yes	12	Atrophy and old lacunae at basal ganglia	Complete - partial seizures, myoclonus even in sleep	Complex partial seizures	Yes	Can't use hands, mental deterioration	No	Depression
15	F	African American	N188S/RecNciI*	40	38	No	18	NA	Myoclonic and tonic/clonic	Partial seizures	Yes	Cerebellar findings	Yes	None
16	M	Caucasian	c.1263–1317del/ F216y	28	4	No	27	NA	Generalized seizures	Discharges suggestive of petit-mal myoclonic type seizure disorder	Yes	None	Yes	Severe skeletal involvement, renal complications

* RecNciI = The allele carries mutations L444P, A456P and V460V.
† Recomb = The allele carries the pseudogene sequence beginning from the 3′ UTR.
‡ Recomb = The allele carries the pseudogene sequence beginning from intron 4.
WNL, within normal limits; NA, not available.

the surrounding intronic regions were sequenced. In addition, Southern blot analyses using the restriction enzymes *Ssp*I and *Hin*cII and long template PCR were performed to look for any large deletions, recombinations or duplications as previously described (39–41).

RNA analysis. Northern blots were performed using total RNA isolated from cell pellets and hybridized to ^{32}P random prime-labeled glucocerebrosidase and β-actin cDNA as described (39).

Enzyme assay and Western blot. Total protein was extracted from frozen cell pellets of fibroblasts or lymphoblasts. Glucocerebrosidase activity was measured using 4-methyl umbelliferyl β-D-glucopyranoside as a substrate (42). Western blots were performed using 15–30 μg of protein electrophoresed on an 8% SDS-PAGE-Tris glycine gel, transferred, and immunoblotted with a polyclonal antibody to human glucocerebrosidase as previously described (39).

Quantitation of glucosylsphingosine levels. The substrate glucosylsphingosine was measured in brain samples from two subjects, aged 14 and 27 y (patients 8 and 16), obtained at autopsy using HPLC with a 4-fluoro 7- nitrobenzofurazan-generated autofluorescent derivative (43). Levels were compared with those measured in brain samples from seven adults with unrelated diagnoses and from two children, aged 4 mo and 3½ y, obtained at autopsy.

RESULTS

Clinical findings. The 16 cases studied included subjects from a variety of races and ethnic backgrounds from four different continents (Table 1). Consanguinity was not reported in any of the cases. Patients 11 and 13 had other siblings with both Gaucher disease and myoclonus, whereas patient 10 had an adult brother with Gaucher disease who shared the same genotype but who lacked neurologic manifestations (44). Several of the clinical features were shared by all 16 patients and others were more variable.

Clinically, the patients could be subgrouped into two categories based upon whether neurologic findings presented in early childhood or later in adolescence or adulthood. All of the patients tested had a horizontal supranuclear gaze palsy. Patients 1 through 9 were diagnosed with Gaucher disease by age 4 y and seizures developed by age 9. This group generally had significant visceral and/or skeletal involvement and four died in childhood. Cognitive deficits and delayed development were also frequently noted.

The second category included seven adults with myoclonic seizures (patients 10–16). These ranged from age 22 to 40 y at the time of evaluation. In three of these cases, the diagnosis of Gaucher disease was made in early childhood, but neurologic involvement manifested slowly. In fact, in case 16, seizures did not develop until age 27. Of note, in three patients (cases 9, 13, and 15), seizures preceded the diagnosis of Gaucher disease. Among the adult patients, mental deterioration and speech involvement were observed, often accompanied by clinical depression.

Another observation was that almost all of the patients studied had generalized seizures in addition to myoclonus, and other abnormalities were noted on EEG. While most of the subjects evaluated had normal cranial MRI evaluations, atrophy was noted in two adults and one child (case 4) appeared to have bilateral parietoccipital encephalomalacia. Seven of the subjects studied received enzyme replacement therapy. The myoclonus was not reported to improve with therapy.

Genotypic analysis. The genotypes of the 16 patients are listed in Table 1.

Selected exons of patient DNA were initially screened for the four commonly encountered mutations, IVS2+1G→A, c.84–85insG, N370S, and L444P. N370S and IVS2+1G→A were not encountered in this group of patients, whereas the c.84–85insG mutation was present on only one allele. The L444P mutation was found on one allele in two patients, on both alleles in one patient, and as part of a recombinant allele in five others. All 11 exons and splice junctions of the glucocerebrosidase gene were then sequenced in DNA from each patient both to confirm these mutations and to identify the remaining mutant alleles and to establish whether more than one mutation was present on a given allele. All 32 mutant alleles were identified. Table 2 summarizes what is known from the literature regarding the different mutations identified in these patients.

Several rare alleles and genotypes were noted in patients in our sample. Of the 14 different genotypes detected in our sample of 16 patients, the genotype V394L/Rec*Nci*I allele was shared by two patients and the genotype N188S/Rec*Nci*I allele was shared by another two patients. In addition, one patient had the genotype N188S/c.84–85insG. Two patients had the mutation E326K allele as a second mutation on the same allele with another glucocerebrosidase mutation. This substitution may be a polymorphism, for it has also been identified in normal controls (45).

Southern blot analyses using *Ssp*I revealed abnormal bands in three individuals (Fig. 1). Patient 5, with genotype G202R/L444P, had a previously described gene rearrangement on 1q21 that introduced a duplication of the glucocerebrosidase pseudogene and a metaxin fusion gene (46). This resulted in a 17 kb band on *Ssp*I digestion (Fig. 1) and also an extra band on *Hin*cII digestion. An additional band in the *Ssp*I digest of DNA from patient 11 reflected a previously described fusion allele where the site of fusion between the glucocerebrosidase gene and pseudogene occurred between the end of intron 9 and the beginning of exon 10 (39). The extra band observed on Southern blot of DNA from patient 13 resulted from a novel fusion allele, where the site of fusion was established by sequence comparison to be in intron 4. Southern blot analyses using the restriction enzymes *Sst*II and *Hin*cII confirmed these results (data not shown).

Transcriptional studies. Northern blots were prepared using total RNA extracted from the 11 available patient cell lines (patients 1, 2, 5–7, 9–12, 14, and 15) and hybridized to both glucocerebrosidase and β-actin cDNA probes. A transcript with an appropriate size and band intensity was detected in all samples when standardized to β-actin. Quantitative differences in glucocerebrosidase were not found between patient and control samples (data not shown).

Western analyses. Protein extracts were available from 11 of the patients studied (patients 1–3, 5–7, 9, 10, 12, 14, and 15).

Table 2. *What Is Known about These Mutations*

Mutation	Location	No. of Alleles	Pseudogene Derived?	Associated Phenotype	Ref.
Recombinant allele (RecNciI +/− other changes)	Variable	5	Yes	Assumed to be null allele. Recomb/Recomb results in lethal type 2 phenotype.	(17)
L444P	Exon 10	4	Yes	Found in type 1, 2, and 3 patients. L444P/L444P seen mostly in type 3 patients and in Norrbottnian subgroup.	(65)
N188S	Exon 6	4	Yes	Seen in Asian patients with type 1. One homozygote with mild disease.	(4, 52)
G377S	Exon 9	4	No	Previously encountered in types 1 and 3. Three patients with G377S/G377S have type 1.	(55, 66)
V394L	Exon 9	2	No	Previously seen in patients with types 1 and 3, including Ashkenazi patients. No reported homozygotes.	(4, 49, 50)
c.84–85insG	Exon 2	1	No	Presumed to be null allele. No known homozygotes.	(51)
G202R	Exon 6	1	Yes	Found in type 1, 2, and 3 patients. G202R/G202R found in classic type 2.	(17)
R131L	Exon 5	1	No	Found in type 1, 2, and 3 patients. R131L/R131L found in classic type 2.	(17, 67)
F213I	Exon 6	1	Yes	Found in type 1, 2, and 3 patients.	(68)
K157Q	Exon 5	1	No	Found in type 2 and 3 patients.	(44)
c.1263–1317del	Exon 9	1	Yes	Found in type 1, 2, and 3 patients. No known homozygotes.	(38)
D140H	Exon 5	1	No	Unknown	(44)
D409H	Exon 9	1	Yes	Found in type 1, 2, and 3 patients. D409H/D409H associated with a unique type 3 phenotype with cardiac valvular involvement.	(13)
G325R	Exon 8	1	Yes	Unknown	(69)
C342G	Exon 8	1	No	Unknown	(69)
F216Y	Exon 7	1	No	Unknown	(70)
I409F	Exon 9	1	No	Novel mutation	
Y205C	Exon 6	1	No	Novel mutation	
c.1020 delT	Exon 8	1	No	Presumed to be a null allele.	(71)

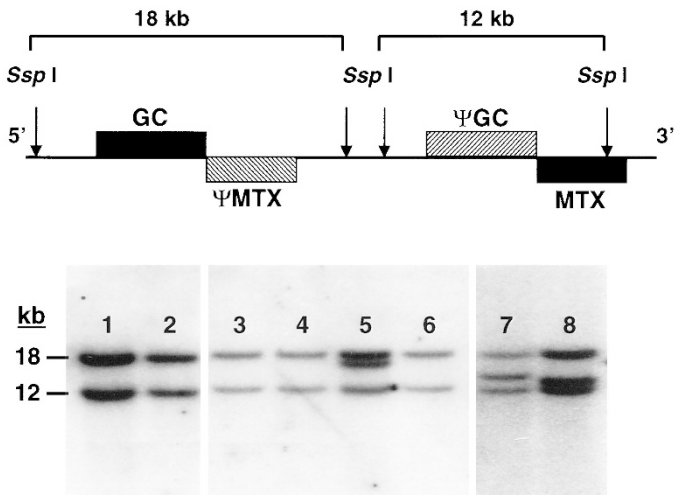

Figure 1. (*A*) Schematic of the glucocerebrosidase gene locus, showing the genes and pseudogenes for glucocerebrosidase and metaxin. The normal *Ssp*I sites are indicated by *arrows*. (*B*) Southern blot of *Ssp*I-digested genomic DNA from patients and normal control probed with a labeled glucocerebrosidase cDNA. The expected 18 and 12 kb bands are observed in the control lanes and in all of the patient samples. *Lanes 1, 3*: normal controls; *lanes 2, 4, and 6*: patients carrying a Rec*Nci*I allele derived from a gene conversion event; *lane 5*: patient 5, showing 17 kb duplication allele; *lanes 7 and 8*: patients 11 and 13, respectively, showing fusion alleles resulting from crossover between the glucocerebrosidase gene and pseudogene.

The residual enzyme activity measured in lymphoblasts or fibroblasts (Table 3) was found to be <5% of control activity in eight patients, but between 7.5% and 26% of control activity in the three others (Table 3). There was no correlation between the level of residual enzyme activity and the age of the patient or severity of symptoms. On Western blots using polyclonal antibody to human glucocerebrosidase, all of the patients evaluated had the 61 kD form of the enzyme, and three showed the 63 kD band (Table 3). As previously reported in other patients with neuronopathic Gaucher disease (47, 48), none of the patients had the 56 kD form, which was clearly shown in samples from controls and from two patients with type 1 Gaucher disease.

Quantitation of glucosylsphingosine in brain samples. Brain autopsy samples were available from two subjects, patients 8 and 16. The glucosylsphingosine levels were measured to be 22 and 32 ng/mg protein, respectively. In contrast, samples from nine pediatric and adult control brains were found to range from 0.04 to 1.2 ng/mg protein with an average value of 0.63 ng/mg protein.

DISCUSSION

The phenotypic analysis of this series of 16 patients with type 3 Gaucher disease substantiates the existence of a subgroup of patients sharing the rare finding of progressive myoclonic epilepsy, yet the wide range of clinical presentations found in these patients demonstrate that this is not a discrete phenotype. There was no characteristic age of onset nor predicted rate of clinical progression. This was also seen in a close evaluation of patients with type 2 Gaucher disease (17, 39) and supports the view of Gaucher disease as having a continuum of manifestations. In fact, patients with type 2 Gaucher disease that live longer also often develop myoclonic seizures (unpublished observation).

Table 3. *Protein Studies*

Patient No.	Genotype	Residual Enzyme Activity (% of Control)	Pattern on Western Blot (Glucocerebrosidase Isoform)	Cell Type Studied
1	V394L/RecNciI	0.3%	61 kD	Lymphoblast
2	L444P/F213I	7.6%	61 kD	Fibroblast
3	G325R/C342G	25.7%	63 kD, 61 kD	Fibroblast
4	I402F/E236K+G377S	NA	NA	NA
5	L444P + Recombinant/G202R	2.8%	61 kD	Fibroblast
6	V394L/RecNciI	2.5%	63 kD, 61 kD	Fibroblast
7	D409H/R131L	3.5%	61 kD	Fibroblast
8	G377S/c.1020 delT	5.7%	NA	Fibroblast
9	L444P/L444P	3.6%	No signal	Fibroblast
10	K157Q/D140H+E236K	2.9%	61 kD	Fibroblast
11	N188S/RecNciI	NA	NA	NA
12	N188S/c.84–85insG	2.0%	NA	Lymphoblast
13	N188S/Recombinant	NA	NA	NA
14	G377S/Y205C	5.2%	61 kD	Fibroblast
15	N188S/RecNciI	24.2%	63 kD, 61 kD	Fibroblast
16	c.1263–1317del/F216Y	NA	NA	NA

The major associated clinical finding shared by all 16 patients was the slowing of the horizontal saccadic eye movements (19). This continues to be a feature common to all patients with type 3 Gaucher disease, independent of the extent of non-neurologic manifestations. This is in contrast to the conclusions of Patterson *et al.* (7), who felt that oculomotor abnormalities were predictive of severe visceral involvement. The other shared clinical finding among these patients was an abnormal EEG, often with generalized seizures.

On the other hand, many clinical features encountered in the group were quite variable, including age; sex; ethnic background; the degree of visceral, skeletal, cognitive, and cerebellar involvement; and MRI findings. There did appear to be two partially distinct subgroups of patients, the first with onset of myoclonus in early childhood associated with significant developmental and/or cognitive delays, severe visceral involvement, and early death. The second group presented later, generally with slower deterioration and less severe systemic manifestations and many were still alive in their 20s and 30s. The existence of this second group with onset of myoclonus at a later age emphasizes the need for careful longitudinal follow-up of all patients with neuronopathic Gaucher disease. Also, the distinction between the two separate groups provides evidence for the contribution of modifying factors other than the deficiency in glucocerebrosidase.

Among the 16 genotypes encountered, few were shared and there was genotypic heterogeneity even among similar patients with the most severe presentations. Thus, in general, the clinical course could not be accurately predicted by genotype. In addition, no transcriptional differences were observed in patients with this phenotype, and the range in residual enzyme activity did not correlate with severity or the age at onset.

Several rare mutant alleles were encountered in this population in a frequency that was greater than expected. Allele V394L was identified in two children, in both cases together with Rec*Nci*I, a recombinant allele that is presumed to be a null mutation. Another group also reported a child with myoclonic epilepsy who died at age 6 y with genotype V394L/recombinant (RecTL) and demonstrated the V394L allele produced small amounts of functional enzyme, which was pH sensitive (29). Mutation V394L has been reported in patients with type 1 and type 3 Gaucher disease and is a rather common mutation among Ashkenazi Jewish patients (4, 49, 50). However, one patient with genotype V394L/L444P has been described with very mild type 1 Gaucher disease (4).

Mutation N188S was another rare mutation found in four adult patients of different ethnic origins, each with onset of seizures at or after age 12 y. Three of these patients had recombinant alleles as the second mutant allele, although the site and mechanism of recombination varied, and the fourth individual carried c.84–85insG, an early frameshift mutation, which is also presumably a null mutation (51). Mutation N188S was first described in Korean and Chinese patients (52) and it was speculated that it could be neuroprotective in this population. One patient with type 1 Gaucher disease was reported to be homozygous for mutation N188S (52, 53). Likewise, mutation G377S, found in two patients in our series, has previously been considered a mild mutation and three Portuguese patients with type 1 disease have been described who are homozygous for G377S (54, 55).

Of note, 18 of 32 mutant alleles encountered in our patient series were pseudogene derived, including recombinant alleles, c.1263–1317 del, N188S, F131I, G202R, G325R, D409H, and L444P. Patient 16 had a known 55 bp deletion that is derived from the pseudogene (38) and patient 5 was found to have another previously described downstream duplication in addition to two glucocerebrosidase point mutations (46). Five patients had sequence changes that would have been identified as mutation Rec*Nci*I, encompassing pseudogene-derived sequence, including mutations L444P, A456P, and V460V. Southern blots and sequencing determined that, in fact, the site and mechanism of recombination differed in the five alleles. Two patients had fusion alleles; in patient 13, the site of fusion appeared to be in intron 4, whereas in patient 11 it was at the end of intron 9 or the beginning of exon 10 (Fig. 1). Close examination of the intronic sequence in patients 1, 6, and 15 showed that the recombinant allele resulted from gene conversion events. In patients 1 and 6, the converted segment in-

cluded a part of intron 11, whereas in patient 15, the intron 11 sequence corresponded to the gene rather than pseudogene, reflecting gene conversion stretches of different sizes.

Two novel mutations were identified in this series: I402F, located in exon 9, and Y205C, located in exon 6, both with mutation G377S on the second allele. Although the two mutations are clearly not "neuroprotective," it is not possible to determine whether the novel mutation, the G377S allele, or both defined the phenotype.

We contrasted the genotypes encountered in patients with myoclonic epilepsy with a second previously described subgroup of patients with type 3 Gaucher disease (4, 20), a series of 24 children with significant visceral and skeletal manifestations who had slowing of the saccadic eye movements as their sole neurologic manifestation (Table 4). Although eight different genotypes were identified, over half of the children were homozygous for point mutation L444P. The second most common mutation found in 10 children was R463C, seen only as a heterozygous mutation together with a null mutation such as Rec*Nci*I, c.84–85insG, or IVS2+1G>A. Actually, only one child carried neither L444P nor R463C. This was a male who died of pulmonary complications at age 4 y, who carried mutation G377S. Notably, mutations N188S and V394L were not found among the type 3 patients without myoclonus.

Thus, generally the genotypes of the patients with myoclonic epilepsy differed significantly from those with horizontal supranuclear gaze palsy without myoclonus. Mutation R463C and homozygosity for L444P were not associated with seizures, with the exception of patient 9. This child was diagnosed with Gaucher disease at age 19 mo, was splenectomized because of severe thrombocytopenia at age 3½ years and had severe skeletal involvement including chronic osteomyelitis and severe kyphosis with pulmonary restriction. Her oculomotor abnormality was identified in early childhood and seizures and an abnormal EEG were reported by age 9 y. At age 18 y, she was described as having jerky episodes involving the upper extremities, and physical examination at age 24 y described a wheelchair-bound woman with cognitive defects and gross myoclonus of the upper extremities. Therefore, although myoclonus was not mentioned at an early age, it later became a prominent feature of her disorder.

This study was conducted to extend the wealth of accumulated information on genotype/phenotype correlation in Gaucher disease by examining a rare subset of patients with progressive myoclonic epilepsy. Of note, no patients carrying mutation N370S were identified. Three glucocerebrosidase point mutations do appear to be associated with this form of the disorder—V394L, N188S, and G377S—although each has also been seen in other types of Gaucher disease. Although it would be premature to suggest that the identification of these mutations is predictive, any patients carrying one of these three mutations without N370S should be carefully evaluated for the development of myoclonic epilepsy. The reason for this apparent genotype/phenotype association is unclear. Although structurally, the protein alterations introduced by any of the mutations do not appear to be dramatic, the complete three-dimensional structure of glucocerebrosidase is not available, and therefore the potential contribution of these changes to protein conformation is unknown. Actually, although the residual enzyme activity did not correlate well with severity, the results of the Western blot studies were more consistent. The identification of multiple molecular forms of glucocerebrosidase has previously been shown to provide a basis for discrimination between the neuronopathic and non-neuronopathic Gaucher phenotypes (47, 48). Normal fibroblasts have two major forms with apparent molecular weight of approximately 63 kD and 56 kD, and a minor form of 61 kD. We confirmed that the smaller processed 56 kD form is not found in neuronopathic Gaucher disease, suggesting that processing is incomplete. It also indicates that although the patients studied had diverse genotypes, there was a shared property at the protein level, which is likely to be the result of post-translational processing, compartmentalization, and/or protein stability.

Table 4. *Genotypes encountered in 24 patients with type 3 Gaucher disease, horizontal supranuclear gaze palsy, and severe visceral involvement without myoclonic epilepsy*

Genotype	No. of Patients
L444P/L444P	13
R463C/Recombinant allele	3
R463C/c.84–85insG	2
R463C/IVS2+1G>A	2
R463C/D399N	1
R463C/Y304X	1
R463C/A176P	1
G377S/A190T	1

Most of this data was previously published in references 4, 8 and 20.

The 35- to 50-fold increase in brain glucosylsphingosine levels observed in two patients in this series at autopsy may also be relevant. Thus far, elevations in brain glucosylsphingosine levels have been detected in all neuronopathic patients studied, with or without myoclonus. Perhaps the potentially neurotoxic effect of this substrate contributes to a vulnerability to myoclonus.

The myoclonus seen in Gaucher disease is cortical in origin and is known to be associated with a marked increase in the amplitude of the somatosensory evoked potential, or "giant potential" indicating a defect of inhibitory input into the cerebral cortex (56, 57). However, because it was recently demonstrated that the amplitude finding of the somatosensory evoked potential is also elevated in type 3 Gaucher patients who do not have myoclonus, this is not necessary predictive of the development of seizures (58) but overt myoclonus in Gaucher disease could be the extreme manifestation of a general cortical pathogenic process.

The finding that specific mutant glucocerebrosidases may be associated with the development of myoclonic epilepsy suggests that the abnormal enzyme might have a modifying role on other proteins involved in epilepsy. Recently, genes for other forms of progressive myoclonic epilepsy have been determined, including MERRF (59), Unverricht-Lundborg (EPM1) (60), neuronal ceroid lipofucinosis (CLN3) (61), and Lafora disease (EPM2) (62). Many of these disorders share clinical features with patients in our series. It may be worthwhile both to screen DNA from our patients for mutations in other genes known to be associated with early onset myoclonic epilepsy,

including cystatin B and laforin, and to screen patients with myoclonic epilepsy for glucocerebrosidase deficiency.

Of note, several different lysosomal storage disorders, including late-onset GM_2 gangliosidosis, GM_1 gangliosidosis type 2, Niemann Pick disease, sialidosis, galactosialidosis, arylsulfatase A pseudodeficiency, and some forms of ceroid lipofuscinosis, have been associated with myoclonic epilepsy (63, 64). It is intriguing to speculate that these disorders could share a common abnormality related to lysosomal targeting or processing that contributes to this rare phenotype. Therefore, although the analysis of mutations encountered in patients with Gaucher disease with myoclonic epilepsy provides some insight into genotype/phenotype correlation in this disorder, these studies reinforce the magnitude of what we still do not understand about the mechanisms of neurologic disease. Our results indicate that the identification of other factors in addition to the point mutations, both environmental and genetic, will ultimately clarify why certain patients with Gaucher disease develop myoclonic epilepsy. Moreover, the insights gained through the study of these patients will likely have implications that will be relevant to understanding both the development of progressive myoclonic epilepsy and the pathogenesis of Gaucher disease.

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