First report of two Taiwanese siblings with sialidosis type I: A 10-year follow-up study

Chiung-Mei Chen, Szu-Chia Lai, I-Cheng Chen, Kai-Cheng Hsu, Rong-Kuo Lyu, Long-Sun Ro, Hong-Shiu Chang*

Department of Neurology, Chang Gung Memorial Hospital and College of Medicine, Chang-Gung University, 199 Tung Hwa North Road, Taipei, 10591 Taiwan

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Abstract

We report the clinical features, electrophysiological findings and genetic characteristics of the first two Taiwanese siblings ever reported with sialidosis type I. We also provide a 10-year follow-up result. Enzymological analysis revealed a primary sialidase deficit. The back-averaged electroencephalography demonstrated myoclonic jerk-related cortical activities and the somatosensory evoked potential studies revealed giant cortical components. During the 10-year follow-up, the brain magnetic resonance images of the younger brother remained normal, whereas they showed mild cerebellar atrophy in the older sister. Macular cherry red spots were absent in both siblings. However, visual evoked potential revealed progressively prolonged latencies of P100 bilaterally, which was consistent with progressive deterioration of the siblings’ visions. DNA analysis showed that the siblings had a homozygous missense point mutation c.544A→G (Ser182Gly) in the exon 3 of the α-N-acetyl-neuraminidase (NEU1) gene. The mutation is predicted to cause a decreased sialidase activity but the mutant sialidase can still be targeted to the lysosomes, which may correlate with the mild clinical phenotypes and absent cherry red spots in the siblings.

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1. Introduction

Sialidosis, also known as “cherry red spot myoclonus syndrome”, is an autosomal recessive lysosomal storage disease caused by mutations in the NEU1 gene, which leads to deficiency of α-N-acetyl neuraminidase (sialidase) activity [1,2]. Sialidase deficiency disrupts the pathways for degradation of sialylated glycoconjugates, causing their accumulation in the lysosomes and excretion in urine [2]. Sialidosis is subdivided into two main variants with different age of onset and severity. Sialidosis type I has a milder, late-onset, non-dysmorphic presentation and is characterized by visual defects, myoclonus, cherry red macular spots, ataxia and seizures [3,4]. The severe early-onset form, sialidosis type II, is associated with dysostosis multiplex, mental retardation and hepatosplenomegaly [5]. The age of onset and severity of the clinical manifestations correlate with the amount of residual sialidase activity, suggesting the existence of considerable genetic heterogeneity caused by NEU1 mutations [6]. The long-term natural history of genetically proven type 1 sialidosis is unknown, although an Italian case of type 1 sialidosis with a long course illness has been reported [7]. Cherry red spot is a hallmark of type 1 sialidosis and its absence is rare, especially long after onset of the disease. Enzyme assay and genetic analysis are necessary for confirmation of the diagnosis, especially in cases without cherry red spots. Abnormal electroencephalography (EEG) with diffuse paroxysmal features, time-related spikes in back average EEG, prolonged latencies and low amplitudes in visual evoked potential (VEP), and giant potentials in somato-
sory evoked potential (SEP) studies have been shown in type 1 sialidosis [8–10], whereas studies for the long-term outcome of these electrophysiological abnormalities are limited. Characterizing the clinical and electrophysiological features and molecular genetics of the disease is helpful for elucidating pathogenesis, predicting disease prognosis and developing potential treatments. We report herein the long-term clinical features and genetic mutation of the first two siblings with type 1 sialidosis identified in Taiwan.

2. Patients and methods

2.1. Patients and electrophysiological studies

Two siblings with type 1 sialidosis, their older brother and parents were investigated for genetic analysis. The siblings underwent enzymological and a battery of neuro-electrophysiological studies. The younger sibling received EEG, SEP, VEP, motor evoked potentials (MEP) and brainstem auditory evoked potentials (BAEP) at the age of 21, 24 and 29 years. The older sibling received EEG, SEP, MEP and BAEP at the age of 23, 28 and 31 years and performed VEP at the age of 28 and 31 years. The younger sibling had three separate magnetic resonance imaging (MRI) studies at the age of 21, 24 and 31 years. The older sibling had two separate MRI imaging studies at the age of 23 and 28 years. All the examinations were performed after obtaining informed consents.

EEG, SEP, VEP, MEP and BAEP were recorded and analyzed according to the conventional methods [11,12].

Back-averaged EEG was conducted according to the methods described by Shibasaki and Kuroiwa [13]. Briefly, the scalp EEG was recorded from the locations defined according to the standard international 10–20 system. The surface electromyography (EMG) was recorded from the bilateral extensor indices (EI) and anterior tibiales (AT). The bandpass of both the EEG and EMG activities was set between 1.6 and 70 Hz (Galileo NT, EBNeuro). The signals of EEG and EMG were digitalized with a sampling rate of 256 Hz. Focal myoclonic jerks was induced with the patient outstretching the limbs. The peaks of the myoclonus EMG bursts were used as the triggers to average the EMG and EEG activities. A peri-trigger interval was set to 200 ms before and after the triggers to analyze the duration of the EMG bursts and the cortical activity locked to the myoclonic jerks.

2.2. Mutation analysis

Genomic DNA was extracted from the peripheral blood leukocytes using Extraction kits (Stratagene). We screened all six exons including the intron/exon junctions of the NEU1 gene using primers and polymerase chain reactions (PCR) conditions as previously described [14]. PCR products were sequenced directly using the DYEEnamic ET Dye Terminator Kit and MegaBACE Analyzer (Molecular Dynamics, Division of Amersham, Pharmacia Biotech).

3. Results

3.1. Patients

The younger sibling was physically healthy until the age of 14 when he began to experience unsteadiness, incoordination of all four limbs with jerky movements and blurred vision. At the age of 21, due to frequent unexpected falls and two episodes of generalized tonic-clonic seizures, he visited our hospital. Neurological examination revealed normal intelligence. The myoclonic jerks were absent at rest but were provoked by any attempt to move the body or by emotional tension. All four limbs and the trunk were ataxic but speech was normal. The tendon reflexes were brisk and both plantar responses were flexor. Ocular smooth pursuit was saccadic with horizontal nystagmus. Examination of the ocular fundus revealed a normal disc without cherry red spots or retinal degeneration.

The older sibling began with visual disturbance at the age of 17. She visited our clinic when she was 23 due to impaired manual dexterity caused by jerky movements of the arms. Neurological examination revealed normal intelligence. Examination of the eye movements, gait, myoclonus and the ocular fundi was similar to her younger brother’s.

Routine blood tests, brain computerized tomography (CT) scan, single photon emission computerized tomography (SPECT), MRI, routine EEG, MEP, BAEP, SEP and muscle biopsy of each sibling during the first admission were all normal.

3.2. Enzymological analysis

Skin fibroblast neuraminidase activity of both siblings was significantly decreased (0.02 nmol/min/mg protein, normal range: 0.4–2.0 nmol/min/mg protein) and β-galactosidase activity was normal, which confirmed the diagnosis of sialidosis.

3.3. Clinical and image follow-up

The siblings were treated with clonazepam and sodium valproate with moderate improvement in myoclonus. They were followed up for 10 years and visited our clinic every 6 months. They retained normal intelligence without seizures, no cherry red spots, but they developed optic atrophy when the younger brother was 29 years old and when the sister was 31 years old. Although myoclonus gradually became severe, the younger brother remained ambulatory with infrequent falls when he was on medication. The vision of the sister deteriorated rapidly to near blindness within 9 years after disease onset, whereas myoclonus and ataxia...
slowly worsened. Brain MRI of the younger brother 18 years after disease onset was still normal, whereas it revealed mild cerebellar atrophy in the sister 11 years after disease onset.

3.4. EEG, BAEP and MEP follow-up

Follow-up routine EEG, BAEP and MEP studies for each sibling were all normal.

3.5. Back-averaged EEG

Back-averaged EEG was performed on the younger brother at the age of 29 and it showed activity locked to the myoclonic jerks of the four limbs. The most prominent jerk-locked activity was localized to the contralateral fronto-central region from both arms and to the midline fronto-central region from both legs (Fig. 1). It clearly demonstrated that the cortical activity locked to the myoclonic jerks displays complex positive–negative activity before and after the triggers.

3.6. SEP follow-up

The results of two follow-up SEP studies for both siblings were similar. The latencies of components of SEPs in both siblings were normal, but the amplitudes of the cortical evoked responses were increased (amplitude of 12–15 μV, normal value <6 μV). One example of the enlarged cortical SEPs is shown in Fig. 2A.

3.7. VEP follow-up

VEP examinations were carried out on three separate occasions for the younger brother (at 21, 24 and 29 of age, respectively) and two for the older sister (at 28 and 31 of age, respectively) (Table 1). The P100 latencies became progressively prolonged and the waveforms were usually poorly defined as the age advanced (Fig. 2B). The VEP responses eventually became absent bilaterally in the sister and unilaterally in the brother. The degree of VEP abnormality correlated with deterioration of the visual acuity.

| Table 1 |
|----------------------|----------------------|----------------------|
| Occasion | Brother Latency (ms)/age | Sister Latency (ms)/age |
| Right eye | 1st 120.5/21 | 139.2/28 |
| | 2nd 121.5/24 | Absent/31 |
| | 3rd Absent/29 | |
| Left eye | 1st 116.0/21 | 127.2/28 |
| | 2nd 120.6/24 | Absent/31 |
| | 3rd 134.4/29 | |

Normal range of P100 latency (mean ± standard deviation): 99.9±4.4 ms in males, 95.6±4.6 in females.
3.8. NEU1 gene mutation

Genetic analysis revealed a homozygous substitution of A to G at nucleotide 544 (c.544A→G) in exon 3 of the NEU1 gene in the siblings (Fig. 3A) and a heterozygous mutation in both parents (Fig. 3B). This mutation predicts that the serine at amino acid 182 is replaced by a glycine (Ser182Gly), and it was not detected in the older brother of the siblings and 30 other healthy individuals by DNA sequencing (Fig. 3C). It was noted that the Ser182Gly mutation was reported earlier in one Chinese patient, who also presented the milder form disease, sialidosis type 1 [14].

4. Discussion

Lysosomal neuraminidase requires the carboxypeptidase protective protein/cathepsin A (PPCA) for intracellular transport and lysosomal activation [15]. The human lysosomal sialidase is deficient in two genetic disorders: sialidosis, caused by a defect in the NEU1 gene, and galactosidosis, in which the loss of neuraminidase activity is secondary to a deficiency of PPCA[16]. Enzymological analysis of our cases revealed a primary sialidase deficit with intact beta-galactosidase activity diagnostic of sialidosis. The analysis of sialidase mutations in sialidosis has revealed considerable molecular heterogeneity accounting for the diversity of clinical phenotypes[17–19] . On the basis of the intracellular distribution and residual catalytic activity of the mutant neuraminidases, the mutant proteins were assigned to three groups by Bonton et al.: (i) catalytically inactive and not lysosomal, (ii) catalytically inactive, but localized in lysosome, and (iii) catalytically active and localized in lysosome [6]. Genetic analysis of our cases revealed a Ser182Gly mutation. The same mutation was reported earlier in one Chinese patient, who also presented the milder form disease, sialidosis type 1 [14]. Expression of this mutant sialidase in COS-7 cells showed ~ 40% of normal activity, suggesting that the mutant sialidase is still capable of targeting to the lysosomes and form the enzyme complex with PPCA [14]. Thus, the mutant sialidase in our cases may fall into the category of (iii) catalytically active and localized in lysosome, which may explain the milder phenotype. Our report addresses the importance of genetic analysis for sialidosis, which allows delineation of the underlying molecular pathogenesis and prediction of the prognosis.

Cerebellar atrophy is common in type 1 sialidosis. Although both siblings had an ataxic gait, the MRI findings were suggestive of a very slow progression towards cerebellar atrophy. Brain CT can be normal in patients with myoclonic ataxia [9,20], but very few studies have reported the long-term changes of neuroimaging [7]. We demonstrated that brain MRI could remain normal in spite of the disease progressing for a duration as long as 18 years. The giant cortical SEPs and the jerk-locked activity revealed by the back-averaged EEG similar to the previous reports suggest a state of hyperexcitability of the sensorimotor cortex as the origin of the myoclonus in our cases [8–10]. The normal BAEPs and MEPs suggest a relatively intact brainstem auditory pathway and corticospinal tract even 15 years after the disease onset.

A macular cherry red spot is a striking abnormality indicating storage of an abnormal metabolic product by the retinal ganglion cells. Till et al. suggested that macular cherry red spots were present in all adequately described type I patients and all but three patients with type II disease [21]; therefore, proposing that a cherry red spot is a cardinal sign for type 1 sialidosis. To our knowledge, late appearance of cherry red spots was only reported in one patient with type 1 sialidosis, who presented progressive visual acuity impairment starting at the age of 23, and cherry red spots were not found until the age of 40 [7]. It is currently unknown whether cherry red spots will eventually appear in our cases but they are absent even 15 and 14 years after the disease onset in the younger brother and older sister, respectively. The reason for the absence of cherry red spots in our cases is not clear. Interestingly, Rapin et al. reported
that cherry red spots in a patient with type 1 sialidosis faded before she was 20 years old [3]. It may be possible that cherry red spots in our cases had been present early in the course but disappeared when the disease progressed. Alternatively, the absence of cherry red spots in our cases may be due to the mild effect of NEU1 Ser182Gly mutation with relatively high residual NEU1 activity. A Chinese patient with Ser182Gly mutation has been reported by Lukong et al. but whether cherry red spots were present was not described [14]. Further studies are needed to clarify this issue. Despite lack of cherry red spots, the visual system was clinically and electrophysiologically abnormal. Thus, the absence of a cherry red spot does not exclude the diagnoses of sialidosis. The progressively prolonged latencies and ill-defined waveforms of P100 correlated well with the visual deterioration in our cases. This implicates that VEP is a useful indicator of the extent of the disease process and may be used as a tool for assessing future potential enzyme replacement treatment.

Up to now, the Ser182Gly has only been detected in three Chinese patients [14] including the two present cases who had been followed for 10 years and it predicts a milder disease phenotype. Whether this mutation is common in Chinese and associated with absence of cherry red spots warrants further genetic epidemiological studies. In conclusion, these are the first genetically proven Taiwanese siblings with sialidosis type 1 manifesting myoclonic ataxia and progressive visual impairment without cherry red spots.

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References


