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Genetic and clinical characterization of mainland Chinese patients with sialidosis type 1

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Abstract

Background: Sialidosis type 1 is a rare inherited disorder with a high disability. No genetically confirmed mainland Chinese patient with sialidosis type 1 has been reported. This study evaluated the phenotypes and genotypes of mainland Chinese patients with sialidosis type 1.

Methods: It was a retrospective case series study. Four unrelated patients were enrolled. Comprehensive clinical evaluations and molecular genetic analysis of the *NEU1* gene were performed.

Results: Three out of four patients presented progressive myoclonus epilepsy. The best-corrected visual acuity ranged from 20/2000 to 20/25. Punctate cataracts were found in all of the patients. Distinct macular cherry red spots were observed in three patients by fundoscopy, and a relatively normal fundus was revealed in one patient. Optical coherence tomography (OCT) showed increased reflectivity of the nerve fiber and ganglion cell layers, and fundus autofluorescence (FAF) revealed hyperautofluorescent areas surrounding the fovea in all of the patients. Only superficial retinal vessels can be observed using OCT angiography; the deeper capillary plexus could not be observed. Visual evoked potential revealed varying degrees of decreased amplitude and/or prolonged latency of P100 or P2 waves. The most frequent sequence variant identified was c.544A>G (p.S182G) (NM_000434.3).

Conclusions: Our study first described the ophthalmic and neurologic characteristics of a small cohort of unrelated mainland Chinese patients with sialidosis type 1. We found that c.544A>G (p. S182G) might be a hotspot variant in Chinese patients. The accumulation of metabolic products in the nerve fiber and ganglion cell layers is a characteristic ocular finding that could be sensitively detected by OCT and FAF imaging.

KEYWORDS

FAF, macular cherry red spot, OCT, OCTA, sialidosis type 1

1 | BACKGROUND

Sialidosis (OMIM 256550) is a rare autosomal recessive inherited disorder caused by biallelic pathogenic variants of the *NEU1* gene (OMIM 608272), resulting in isolated deficiency of alpha-N-acetyl neuraminidase (lysosomal sialidase, EC 3.2.1.18). This enzyme deficiency interrupts the normal catabolic pathways, leading to abnormal tissue accumulation as well as urinary excretion of sialylated oligosaccharides (Pshezhetsky et al., 1997). According to the ages of onset and severity, sialidosis has been classified into two types: sialidosis type 1 (normomorphic or mild form) and sialidosis type 2 (dysmorphic or severe form) (Lowden & O'Brien, 1979). Patients with sialidosis type 1 typically present progressive myoclonus epilepsy (PME) in their second or third decade of life, as well as progressive impaired vision and macular cherry red spots. They have no obvious physical defects, and their intelligence is either not impaired or slightly impaired generally. Sialidosis type 2 is more severe and presents earlier with abnormal somatic features, including coarse facial features, hepatosplenomegaly, dysostosis multiplex, developmental delay and mental retardation (Lowden & O'Brien, 1979). The incidence of sialidosis is estimated at one in four million live births in the Caucasian population, and sialidosis type 1 is relatively rare compared to sialidosis type 2 (Meikle, Hopwood, Clague, & Carey, 1999). The incidence in the Chinese population is unknown.

As the lack of understanding of sialidosis by both ophthalmologists and neurologists, many cases cannot be diagnosed correctly. The fundus features are crucial to make the correct diagnosis, however, the cases with detailed ocular examinations are limited reported. To the best of our knowledge, only 45 sialidosis type 1 cases with detailed genetic results have been reported (Table 1), and most of the cases lack the observation of ocular manifestations. None of these patients are from mainland China. It is important to identify the genetic and clinical characteristics of mainland Chinese patients with sialidosis type 1. In addition, we attempt to explore the different measurements and determine parameters with high sensitivity to detect fundus changes. This study is the first report of four unrelated cases from mainland China and will expand the understanding of the genetic and clinical characteristics of Chinese patients with sialidosis type 1.

2 | METHODS

2.1 | Editorial policies and ethical considerations

The study protocol was approved by the Institutional Review Board of Peking Union Medical College Hospital and complies with the principles of the Declaration of Helsinki. A signed informed consent form was obtained from each participant.

2.2 | Subjects

This study was a single-center, retrospective case series study. All of the participating subjects were enrolled on a voluntary basis from the Ophthalmic Genetics Clinic at Peking Union Medical College Hospital, Beijing, China, from 2014 to 2019. The diagnosis of sialidosis was confirmed by molecular genetic testing. According to the clinical characteristics, these patients were classified as type 1.

2.3 | Clinical evaluations

Medical and family histories were documented in detail. All of the patients underwent complete ophthalmic examinations, including best-corrected visual acuity (BCVA) by the Snellen visual acuity test, refractive errors, slit-lamp biomicroscopy and dilated ophthalmoscopy. Moreover, color fundus photographs were obtained using digital fundus cameras (Topcon). Optical coherence tomography (OCT) (Heidelberg Retina Angiograph, HRA; Heidelberg Engineering, and Optovue Inc), optical coherence tomography angiography (OCTA) (Optovue Inc), and fundus autofluorescence (FAF) (Heidelberg Retina Angiograph, HRA; Heidelberg Engineering) images were recorded with standardized set-ups. Visual evoked potential (VEP) was recorded using specialized equipment (RetiPort ERG system; Roland Consult), according to the standard protocols published by the International Society for Clinical Electrophysiology of Vision. It is recommended to perform pattern VEP (P-VEP) first, and flash VEP (F-VEP) was performed if the patient could not cooperate due to poor vision. The amplitude and latency of the waves of VEP were analyzed. The neurological evaluations (including intelligence evaluation) were conducted by a neurologist (Y Huang). Brain magnetic resonance imaging (MRI) and electroencephalography (EEG) were performed according to standard procedures. General blood tests and abdominal B type ultrasonography were performed in all of the patients.

2.4 | Genetic analysis

Venous blood was collected, and DNA was extracted from leukocytes for genetic testing with the QIAamp DNA Blood Midi Kit (Qiagen). All six exons of the *NEU1* gene and adjacent splice sites were amplified with polymerase chain reaction using previously described primer pairs (Sekijima et al., 2013) followed by Sanger sequencing. The resulting

TABLE 1 Characteristics of reported genetically confirmed sialidosis type 1 patients

Reference	Number of cases	Ethnicity	Mutation[a]	Number/type of mutation	Myoclonus	Seizures	Cerebellar Ataxia	Visual impairment	Macular cherry-red spots
Bonten et al. (2000)	6 from 5 families	2 African-American 2 German 1 Greek 1 Dutch	R294S/L231H R294S/G218A G227R/G227R V54M/G378X G328S/Dpl399HY	6/missense 1/nonsense 1/duplication	5/6	4/6	4/6	3/3	4/5
Palmeri et al. (2000) Lukong et al. (2000)	1	Italian	G328S/G328S	1/missense	1/1	1/1	1/1	1/1	1/1
Naganawa et al. (2000)	2 from 2 family	Japanese	V217M/G243R	2/missense	2/2	1/2	1/2	ND	2/2
Itoh et al. (2002)	1	Japanese	P316S/P316S	1/missense	1/1	1/1	1/1	1/1	1/1
Lai et al. (2009)	17 from 12 families	Taiwan	S182G/S182G S182G/A319V S182G/Q55X	2/missense 1/nonsense	17/17	13/17	16/17	14/17	3/17
Ranganath, Sharma, Danda, Nandineni, and Dalal (2012)	1	Indian	R294C/N398Tfs*	1/missense 1/deletion	1/1	ND	1/1	1/1	1/1
Sekijima et al. (2013)	1	Japanese	P80L/D135N	2/missense	1/1	ND	1/1	0/1	1/1
Canafoglia et al. (2014)	6 from 2 families	Italian	S67I/S67I G227R/R305C	3/missense	6/6	1/6	2/6	0/5	0/5
Sobral, Cachulo Mda, Figueira, and Silva (2014)	1	Portugal	D234N/R341X	1/missense 1/nonsense	1/1	ND	1/1	1/1	1/1
Schene et al. (2015)	2 from 1 family	Dutch	H399_Y400dup/E277R	1/duplication 1/missense	1/1	ND	1/1	2/2	2/2
Gowda, Srinivasan, Benakappa, and Benakappa, (2017)	1	Indian	G248C/G248C	1/missense	1/1	1/1	1/1	ND	1/1
Mütze et al. (2017)	1	German	S233R/Y268C	2/missense	1/1	1/1	0/1	1/1	1/1
Aravindhan et al. (2018)	1	Ecuadorian	P210L/P210L	1/missense	1/1	1/1	1/1	1/1	1/1
Gultekin, Bayramov, Karaca, and Acer (2018)	1	Turkish	E209Sfs*94/D310N	1/deletion 1/missense	1/1	1/1	1/1	ND	1/1
Hu et al. (2018), Wang et al. (2017)	1	Taiwan	S182G/Q207X	1/missense 1/nonsense	1/1	1/1	1/1	1/1	1/1
Mohammad, Bruno, Hines, and Atwal (2018)	1	East-Asian	S182G/G227R	2/missense	1/1	1/1	1/1	0/1	0/1
Ahn et al. (2019)	1	Korean	R6Qfs*21/D310N	1/deletion 1/missense	1/1	1/1	1/1	1/1	1/1
Total	45 from 34 families			23 different missense mutations; 4 different nonsense mutations; 3 different deletion mutations; 2 different duplication mutations	43/44 (97.7%)	28/40 (70%)	35/44 (79.5%)	27/37 (73.0%)	22/43 (51.2%)

Abbreviation: ND, no data.

[a]Reference NM_000434.3.

sequences were assembled and analyzed with the SeqMan program (DNASTAR Lasergene Co.) and compared with *NEU1* gene reference sequence (NM_000434.3).

3 | RESULTS

3.1 | Genetic analysis

Genetic test results confirmed the diagnosis of sialidosis in all of the patients. We identified five previously reported disease-causing sequence variants. Three variants were missense variants, which resulted in single amino acid substitutions. Two variants were nonsense, which resulted in premature termination of protein translation. A list of the sequence variants and their predicted effects is provided in Table 2. The sequence variant c.544A>G (p.S182G) in exon 3 of the *NEU1* gene was found in three patients, which was the most frequent sequence variant in this study, and the occurrence frequency was 37.5%. c.239C>T (p.P80L) was found in two patients and was the second most frequent sequence variant. The sequence variant c.403G>A (p.D135N), c.838C>T (p.R280X), and c.1021C>T (p.R341X) was found in one patient. Each of the patients' parents carried one of two sequence variants.

3.2 | Clinical evaluations

Four patients from four unrelated families were included in the study. The clinical features of these patients are summarized in Table 2. Of the four patients, there were two male patients and two female patients. The median age of symptom onset was 10 years (range, 8–12). The median age at first visit was 16.5 years (range, 10–20). The median disease duration was 5.5 years (range, 2–10). Patient 1 was born to a third-degree consanguineous marriage couple, but beyond that, there was no self-reported consanguineous marriage. The prenatal and birth histories were unremarkable in all of

TABLE 2 Clinical and genetic characteristics of four mainland Chinese patients with sialidosis type 1

Patient	P1	P2	P3	P4
Gender	Male	Male	Female	Female
Age at initial visit/years	10	15	18	20
Onset age/years	8	10	12	10
Duration/years	2	5	6	10
Myoclonus	−	+	+	+
Seizures	−	+	+	+
Cerebellar Ataxia	−	+	+	+
Visual impairment	+	+	+	+
BCVA (OD, OS)	20/100, 20/100	20/500, 20/500	20/2000, 20/2000	20/25, 20/32
Nystagmus	−	+	+	−
Punctate cataract	+	+	+	+
Macular cherry-red spots (by fundoscopy)	−	+	+	+
Retinal pathology (by OCT and FAF)	+	+	+	+
VEP	P-VEP: No response was recorded F-VEP: Decreased amplitude of P2	ND	P-VEP and F-VEP: No response was recorded	P-VEP: Prolonged latency and decreased amplitude of P100
Intelligence	Normal	Normal	Normal	Normal
EEG	Normal	Abnormal	Abnormal	Normal
Brain MRI	Normal	Normal	Normal	Normal
Sequence variant[a] (predicted effect)	c.239C>T (p.P80L) c.544A>G (p.S182G)	c.1021C>T (p.R341X) c.544A>G (p.S182G)	c.239C>T (p.P80L) c.544A>G (p.S182G)	c.403G>A (p.D135N) c.838C>T (p.R280X);

Abbreviations: BCVA, best-corrected visual acuity; EEG, electroencephalography; FAF, fundus autofluorescence; F-VEP, flash visual evoked potential; MRI, magnetic resonance imaging; ND, no data; OCT, optical coherence tomography; P-VEP, pattern visual evoked potential; VEP, visual evoked potential.

[a]Reference NM_000434.3.

the patients, as well as the developmental milestones and progress in school. The older brother of patient 1 had a history of progressive myoclonus, seizures, and impaired vision in his second decade of life and died at 21 years old without other diseases. There was no history of seizures, ataxia or other neurologic conditions in other patients' families.

On neurological assessment, 75% (3/4) of patients had a history of progressive myoclonus, initially affecting the lower extremities, progressing to involve the arms and trunk, and evolving to slurred speech and dysphagia in the late stage. A total of 75% (3/4) of patients developed seizures and cerebellar ataxia. All of the patients' intelligence was normal. EEG showed different extents of diffuse paroxysmal features as spike-wave complexes in two patients. The brain MRI was unremarkable in all of the patients.

On ophthalmological assessment, all patients presented with visual impairment. The BCVA of eight eyes from four patients ranged from 2/200 to 20/25. Severe nystagmus was found in two patients. Punctate cataracts were found in all patients by slit-lamp biomicroscopic examination. A relatively normal fundus was revealed in patient 1 on fundus examination, and distinct macular cherry red spots were observed in the other three patients, with pale optic nerve head observed in patient 3. OCT showed increased reflectivity in the retinal nerve fiber layer (RNFL) and ganglion cell layer in all patients. FAF revealed hyperreflective areas surrounding a central hyporeflective fovea in all patients, which corresponded to the hyperreflective areas on OCT (Figure 1). On OCTA, only superficial retinal vessels can be observed; the deeper capillary plexus could not be observed (Figure 2). VEPs were performed in three patients. The results revealed varying degrees of decreased amplitude and/or prolonged latency of P100 or P2 waves (Table 2). Taking all the findings together, the cause of decreased visual acuity was considered to be visual pathway impairment. General blood tests were unremarkable in all patients, and there was no hepatosplenomegaly revealed on abdominal B type ultrasonography.

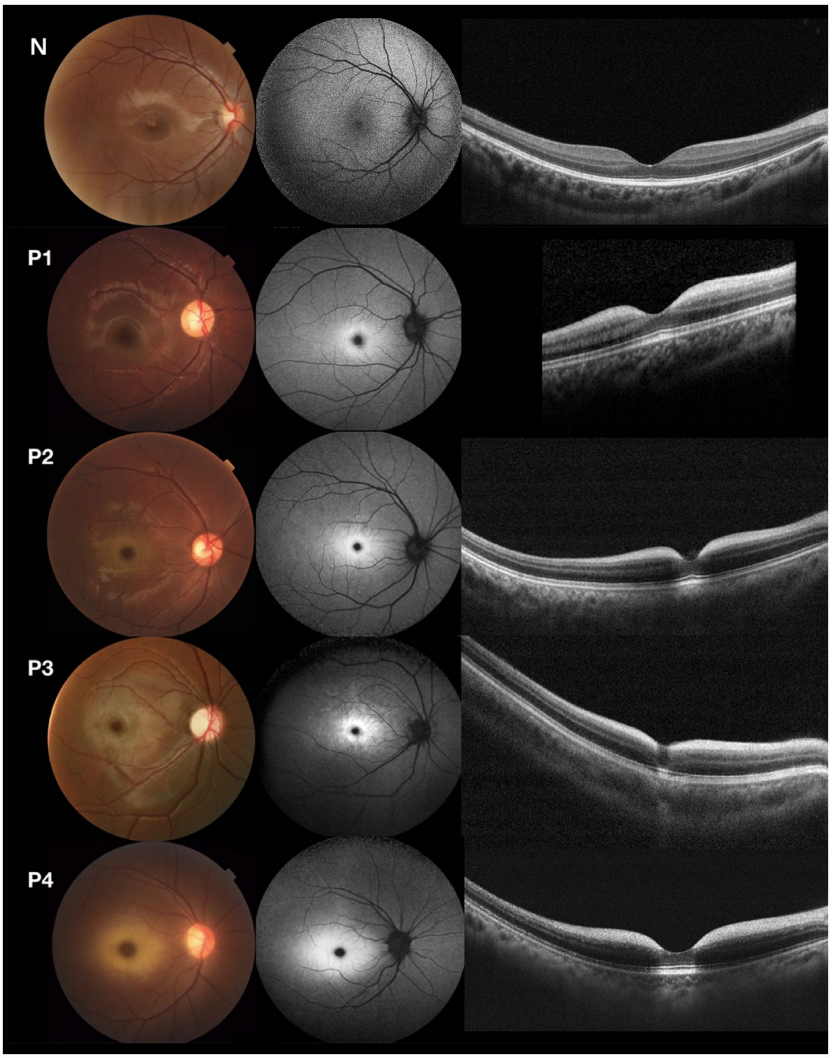

FIGURE 1 Color fundus photographs, fundus autofluorescence (FAF) and optical coherence tomography (OCT) of a healthy person and four patients. They are aged 10 (N), 10 (P1), 15 (P2), 18 (P3), and 20 (P4). P1 had relatively normal fundus in a color fundus photograph, while P2-P4 were found with distinct macular cherry red spots. FAF revealed hyperautofluorescence areas surrounding a central hypoautofluorescent fovea, and OCT showed increased reflectivity of the nerve fiber layer and ganglion cell layer in all four patients

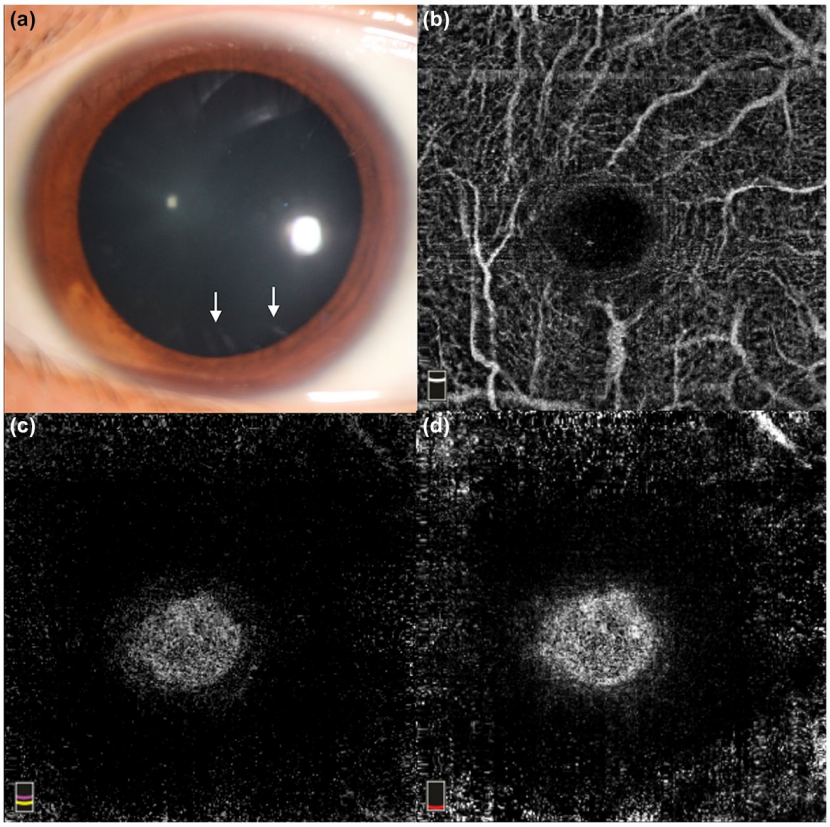

FIGURE 2 The anterior segment photograph and optical coherence tomography angiography (OCTA) of P4. (a) The anterior segment photograph of P4 showed a slight punctate cataract. (b) In the superficial retina layer (between the internal limiting membrane and outer plexiform layer) imaging of en face OCTA, the morphology of large retinal vessels can be displayed with a crude signal, but the capillaries cannot be clearly shown. In other deeper layers (c) outer retina layer and (d) choroid capillary layer), the vascular signals are only displayed at the fovea, and no signals are shown in the parafoveal zone

4 | DISCUSSION

Sialidosis type 1 is a rare lysosomal storage disease resulting in an early morbidity and a high disability. A lack of understanding of this disease makes missed diagnosis and misdiagnosis, which would be avoided by a better understanding and awareness. Due to the distinctive feature of the ocular manifestation, ophthalmologists would help neurologists make the correct diagnosis. This paper focuses on phenotypic and genotypic characterization and is the only study on mainland Chinese patients with sialidosis type 1, which could help clinicians understand the features of mainland Chinese patients with this disease. Understanding the natural history of disease and obtaining sensitive follow-up parameters are also important for rare diseases such as sialidosis. Furthermore, although there is currently no treatment for sialidosis, the recent success of gene therapy on inherited diseases gives hope to patients with sialidosis. In addition to gene replacement, gene editing is also a promising therapeutic strategy that works for specific mutations (Maeder et al., 2019). Defining the disease-causing gene and mutation spectrum are prerequisites for new therapies. Our findings support the above-mentioned information for future treatment consideration.

Macular cherry red spots are a distinctive feature of the ocular manifestation of sialidosis type 1. The appearance of macular cherry red spots is caused by the accumulation of sialyloligosaccharides in the perifoveal ganglion cell layer and RNFL. Consequently, the normal reddish appearance in the foveal pit appears to be cherry red spots. The characteristic features of macular cherry red spots in OCT and FAF are increased reflectivity of the RNFL and ganglion cell layer (Kersten, Roxburgh, Danesh-Meyer, & Hutchinson, 2016; Michalewska et al., 2011; Wang, Lin, & Kao, 2017) and hyperautofluorescence surrounding the foveal pit, respectively. To date, only one case has described the fundus manifestation in FAF (Zou, Wang, & Tian, 2016). In our study, three patients were found with distinct features of macular cherry red spots by fundoscopy, while one patient had a relatively normal fundus. However, increased reflectivity of the RNFL and ganglion cell layer on OCT and hyperautofluorescence surrounding the foveal pit on FAF were observed in all four patients. This demonstrates that OCT and FAF are the parameters with higher sensitivity than fundoscopy to detect macular cherry red spots in sialidosis; therefore, we suggest using fundoscopy combined with OCT and FAF to detect retinal pathology to avoid missed diagnosis. Some studies found that not all patients with sialidosis present with macular cherry red spots (Canafoglia et al., 2014; Khan & Sergi, 2018; Lai et al., 2009). According to the 45 genetically confirmed sialidosis type 1 cases, macular cherry red spots were observed in only 51.2% (22/43) of the patients (Table 1). In our opinion, the low adoption of OCT and FAF might be one of the reasons for the low detection of abnormal fundus features. Based on their noninvasiveness, rapidity, and high sensitivity, we suggest using OCT and FAF for diagnosis and for long-term follow-up.

Fundus examination can help diagnose some neurological diseases and provide more intuitive follow-up methods. PMEs are a group of diseases with symptomatic generalized epilepsies, and sialidosis is one of the five main diseases. Differential diagnoses of PMEs can be difficult for neurologists. However, ocular features, especially the fundus, are different despite similar neurological manifestations (Shahwan, Farrell, & Delanty, 2005). This indicates that ophthalmologic consultation is helpful and detailed ophthalmological examination is necessary for patients with PME, even if they do not complain of visual symptoms. Macular cherry red spots have been described in other inherited metabolic storage diseases, including Sandhoff disease, galactosialidosis, Tay-Sachs disease, metachromatic leukodystrophy, Niemann-Pick disease, GM1 gangliosidosis, GM2 gangliosidosis, Farber's disease, multiple sulfatase deficiency, Gaucher disease, and Wolman disease (Leavitt & Kotagal, 2007). Therefore, a detailed medical history and systemic evaluation should be obtained by both the ophthalmologists and neurologists for the differentiation of patients with macular cherry red spots.

As a new technique, OCTA offers detailed information about the retinal or choroidal capillary blood flow information using the highly efficient split-spectrum amplitude decorrelation angiography algorithm. Currently, there has been no article reporting the manifestation of this disease in OCTA. Although sialidosis is not a retinal vascular disease, the manifestations in OCTA are interesting. Patient 4 underwent OCTA in this study. In the superficial retina layer (between the internal limiting membrane and outer plexiform layer) imaging of en face OCTA, the morphology of large retinal vessels can be displayed; however, because of weak signal, the capillaries cannot be clearly shown, which is considered to be related to the deposit of abnormal substances in the superficial layer of retina. In other capillary plexus, the blood flow signals are only displayed at the fovea, and the dark area of no signals in the parafoveal zone is speculated to be attributed to the masking by metabolic products accumulated in superficial retina (Figure 2). This also provides evidence that abnormal substances are deposited in the superficial layer.

In addition to the macular cherry red spot, punctate cataract and corneal clouding are considered to be characteristic ophthalmic signs in patients with sialidosis (Lowden & O'Brien, 1979), which are speculated to result from the accumulation of abnormal metabolic products as well. In our study, punctate cataracts were found in all four patients. This indicates that punctate cataracts are an important manifestation of the disease, which should not be neglected by ophthalmologists.

To our knowledge, more than 30 different pathogenic variants in the *NEU1* gene have been identified in patients with sialidosis type 1 (www.hgmd.org), including 27 missense/nonsense pathogenic variants, which are the most frequent types (Table 1). Moreover, there are three deletions, and two duplications have been found (Table 1). All of these pathogenic variations can directly affect the active site or the central core of sialidase, leading to folding defects and retention of sialidase in the endoplasmic reticulum/Golgi compartment but may also affect the surface region involved in binding to a multienzyme complex (Lukong et al., 2001). In this study, all four mainland Chinese patients carried compound heterozygous pathogenic variants, and all of the variants found were missense/nonsense, which is consistent with the distribution of the variants types recorded in the Human Genome Mutation Database. The occurrence frequency of the pathogenic variant c.544A>G (p. S182G) in Taiwanese patients was 91.7% according to 18 Taiwanese cases reported previously (Hu, Hung, Chen, & Lee, 2018; Kersten et al., 2016; Lai et al., 2009). Such a high figure suggests that this may be a founder allele that originated in Taiwan, and this pathogenic variant is easily accumulated due to the relatively closed region of Taiwan, which may also explain why this disease has a higher prevalence in Taiwan. In our study, c.544A>G (p. S182G) is still a hotspot variant, which implies that this may be a founder allele of mainland Chinese population as well. Bonten et al. found that the same pathogenic variant mostly occurred in patients of the same race (Bonten et al., 2000), which is coincident with our findings. However, the occurrence frequency of c.544A>G (p.S182G) in our study was far lower than that in the Taiwanese population. All the sequence variants found in our study would impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. S182G is located at the end of the second "Asp-box" motifs and the base in a long flexible surface loop. No obvious structural change in the lysosomal multienzyme complex was observed because of this variant (Lukong et al., 2000), and several studies have shown that most patients carrying this variant present with a mild clinical phenotype (Lai et al., 2009; Lukong et al., 2000). However, the three patients that carried c.544A>G (p.S182G) variant in this study did not present a milder clinical phenotype. Therefore, studies with a larger sample size and protein structure and function are required in the future to elucidate the genotype-phenotype relationship. P80L was found in two patients in this study. The P80 residue is situated in a conserved FRIP motif that is located at the N-terminus of the first strand of the first β-sheet unit (Itoh et al., 2002). The P80L variant causes conformational changes in the main chain that affect the active site that is adjacent to it (Itoh et al., 2002). A transient expression study of lysosomal neuraminidase cDNA revealed that the P80L mutant protein did not show enzymatic activity, indicating that this variant is mostly associated with the severe clinical phenotype (Itoh et al., 2002). The D135 residue is one of the active sites of this enzyme (Itoh et al., 2002), and the D135N variant was predicted to alter the structure and function of the enzyme. The nonsense variant c.1021C>T (p.R341X)

creates a premature termination codon in the NEU1 mRNA open reading frame, leading to a truncated sialidase precursor lacking 74 amino acids (Coutinho et al., 2012). This nonsense variant is thought to cause a severe phenotype, as it results in a truncated protein. However, a more severe phenotype was not observed in patients carrying this variant in previous studies and in our study. We speculated that this may be because the second allele carried by the patients was mild. The other nonsense variant c.838C>T (p.R280X) has been reported by Bonten et al (Bonten et al., 2000), without detailed information about the pathogenicity. In our study, c.838C>T (p.R280X) was indicated as putatively pathogenic by the Polyphen-2 and SIFT programs. Although it has been demonstrated by previous researchers that the genotype-phenotype correlation in sialidosis exists as the severity of the clinical manifestations are paralleled with *NEU1* mutations and the level of residual sialidase activity (Bonten et al., 2000)' (Khan & Sergi, 2018), no clear genotype-phenotype correlation was observed in this study given the small sample size.

Though sialidosis seriously affects the quality of life and even threatens the life of patients, enzyme replacement therapy is not a possible approach to treatment as the recombinant enzyme could not cross the blood-brain barrier and would induce a severe immune response. However, gene therapy using an adeno-associated viral vector to express protective protein cathepsin A (PPCA), the sialidase's auxiliary protein, has been tested successfully in the mouse model of sialidosis type 1 (Bonten et al., 2013). This therapeutic approach is based on the observation that sialidase activity can be increased indirectly by increasing the levels of PPCA in vitro (Bonten, Annunziata, & d'Azzo, 2014). Hence, PPCA–mediated gene therapy may be useful in the treatment of patients carrying *NEU1* missense mutations. Thus, identifying the spectrum of genetic variants is a task that must be performed before proceeding with therapy, even before designing the therapeutic strategy.

5 | CONCLUSIONS

Our study described for the first time the clinical and genetic characteristics of a cohort of mainland Chinese patients with sialidosis type 1. We found that c.544A>G (p.S182G) might be a hotspot variant in Chinese patients. Accumulation of metabolic products in the lens, ganglion cell, and RNFL are characteristic ocular findings, and the latter could be sensitively detected by OCT and FAF. We highly recommend to use OCT and FAF to detect the fundus changes. Our findings have expanded the currently limited knowledge about the spectrum of phenotypes and genotypes in Chinese patients with sialidosis type 1 and suggested the importance of better understanding and awareness of this disease by both the ophthalmologists and neurologists.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

XXH wrote the main manuscript text and prepared Tables 1 and 2, Figures 1 and 2. SJW collected the patient data. MW collected one patient. HL performed OCT, FAF and VEP examinations. YH performed neurological evaluations. RFS conceived the study and collected all of the patients. All authors read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Institutional Review Board of Peking Union Medical College Hospital and complies with the principles of the Declaration of Helsinki. A signed informed consent form was obtained from each participant.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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REFERENCES

Ahn, J. H., Kim, A. R., Lee, C., Kim, N. K. D., Kim, N.-S., Park, W.-Y., … Kim, J. S. (2019). Type 1 sialidosis patient with a novel deletion mutation in the NEU1 gene: Case report and literature review. *The Cerebellum*, *18*(3), 659–664. https://doi.org/10.1007/s12311-019-1005-2

Aravindhan, A., Veerapandiyan, A., Earley, C., Thulasi, V., Kresge, C., & Kornitzer, J. (2018). Child neurology: Type 1 sialidosis due to a novel mutation inNEU1gene. *Neurology*, *90*(13), 622–624. https://doi.org/10.1212/wnl.0000000000005209

Bonten, E. J., Annunziata, I., & d'Azzo, A. (2014). Lysosomal multienzyme complex: Pros and cons of working together. *Cellular and Molecular Life Sciences*, *71*(11), 2017–2032. https://doi.org/10.1007/s00018-013-1538-3

Bonten, E. J., Arts, W. F., Beck, M., Covanis, A., Donati, M. A., Parini, R., … d'Azzo, A. (2000). Novel mutations in lysosomal neuraminidase identify functional domains and determine clinical severity in sialidosis. *Human Molecular Genetics*, *9*(18), 2715–2725. https://doi.org/10.1093/hmg/9.18.2715

Bonten, E. J., Yogalingam, G., Hu, H., Gomero, E., van de Vlekkert, D., & d'Azzo, A. (2013). Chaperone-mediated gene therapy with recombinant AAV-PPCA in a new mouse model of type I sialidosis. *Biochimica Et Biophysica Acta*, *1832*(10), 1784–1792. https://doi.org/10.1016/j.bbadis.2013.06.002

Canafoglia, L., Robbiano, A., Pareyson, D., Panzica, F., Nanetti, L., Giovagnoli, A. R., … Zara, F. (2014). Expanding sialidosis spectrum by genome-wide screening: NEU1 mutations in adult-onset myoclonus. *Neurology*, *82*(22), 2003–2006. https://doi.org/10.1212/wnl.0000000000000482

Coutinho, M. F., Lacerda, L., Macedo-Ribeiro, S., Baptista, E., Ribeiro, H., Prata, M. J., & Alves, S. (2012). Lysosomal multienzymatic complex-related diseases: A genetic study among Portuguese patients. *Clinical Genetics*, *81*(4), 379–393. https://doi.org/10.1111/j.1399-0004.2011.01625.x

Gowda, V. K., Srinivasan, V. M., Benakappa, N., & Benakappa, A. (2017). Sialidosis type 1 with a novel mutation in the neuraminidase-1 (NEU1) gene. *Indian Journal of Pediatrics*, *84*(5), 403–404. https://doi.org/10.1007/s12098-016-2286-9

Gultekin, M., Bayramov, R., Karaca, C., & Acer, N. (2018). Sialidosis type I presenting with a novel mutation and advanced neuroimaging features. *Neurosciences (Riyadh)*, *23*(1), 57–61. https://doi.org/10.17712/nsj.2018.1.20170328

Hu, S.-C., Hung, K.-L., Chen, H.-J., & Lee, W.-T. (2018). Seizure remission and improvement of neurological function in sialidosis with perampanel therapy. *Epilepsy & Behavior Case Reports*, *10*, 32–34. https://doi.org/10.1016/j.ebcr.2018.02.005

Itoh, K., Naganawa, Y., Matsuzawa, F., Aikawa, S., Doi, H., Sasagasako, N., … Sakuraba, H. (2002). Novel missense mutations in the human lysosomal sialidase gene in sialidosis patients and prediction of structural alterations of mutant enzymes. *Journal of Human Genetics*, *47*(1), 29–37. https://doi.org/10.1007/s10038-002-8652-7

Kersten, H. M., Roxburgh, R. H., Danesh-Meyer, H. V., & Hutchinson, D. O. (2016). Optical coherence tomography findings in a patient with type 1 sialidosis. *Journal of Clinical Neuroscience*, *31*, 199–201. https://doi.org/10.1016/j.jocn.2016.02.015

Khan, A., & Sergi, C. (2018). Sialidosis: A review of morphology and molecular biology of a rare pediatric disorder. *Diagnostics (Basel)*, *8*(2), 29. https://doi.org/10.3390/diagnostics8020029

Lai, S.-C., Chen, R.-S., Wu Chou, Y.-H., Chang, H.-C., Kao, L.-Y., Huang, Y.-Z., … Lu, C.-S. (2009). A longitudinal study of Taiwanese sialidosis type 1: An insight into the concept of cherry-red spot myoclonus syndrome. *European Journal of Neurology*, *16*(8), 912–919. https://doi.org/10.1111/j.1468-1331.2009.02622.x

Leavitt, J. A., & Kotagal, S. (2007). The "cherry red" spot. *Pediatric Neurology*, *37*(1), 74–75. https://doi.org/10.1016/j.pediatrneurol.2007.04.011

Lowden, J. A., & O'Brien, J. S. (1979). Sialidosis: A review of human neuraminidase deficiency. *American Journal of Human Genetics*, *31*(1), 1–18.

Lukong, K. E., Elsliger, M. A., Chang, Y., Richard, C., Thomas, G., Carey, W., … Pshezhetsky, A. V. (2000). Characterization of the sialidase molecular defects in sialidosis patients suggests the structural organization of the lysosomal multienzyme complex. *Human Molecular Genetics*, *9*(7), 1075–1085. https://doi.org/10.1093/hmg/9.7.1075

Lukong, K. E., Landry, K., Elsliger, M. A., Chang, Y., Lefrancois, S., Morales, C. R., & Pshezhetsky, A. V. (2001). Mutations in sialidosis impair sialidase binding to the lysosomal multienzyme complex. *Journal of Biological Chemistry*, *276*(20), 17286–17290. https://doi.org/10.1074/jbc.M100460200

Maeder, M. L., Stefanidakis, M., Wilson, C. J., Baral, R., Barrera, L. A., Bounoutas, G. S., … Jiang, H. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nature Medicine*, *25*(2), 229–233. https://doi.org/10.1038/s41591-018-0327-9

Meikle, P. J., Hopwood, J. J., Clague, A. E., & Carey, W. F. (1999). Prevalence of lysosomal storage disorders. *JAMA*, *281*(3), 249–254. https://doi.org/10.1001/jama.281.3.249

Michalewska, Z., Gajos, A., Michalewski, J., Nawrocki, J., Pshezhetsky, A. V., & Bogucki, A. (2011). Spectral optical coherence tomography in a patient with type I sialidosis. *Medical Science Monitor*, *17*(10), CS129–CS131. https://doi.org/10.12659/MSM.881971

Mohammad, A. N., Bruno, K. A., Hines, S., & Atwal, P. S. (2018). Type 1 sialidosis presenting with ataxia, seizures and myoclonus with no visual involvement. *Molecular Genetics and Metabolism Reports*, *15*, 11–14. https://doi.org/10.1016/j.ymgmr.2017.12.005

Mütze, U., Bürger, F., Hoffmann, J., Tegetmeyer, H., Heichel, J., Nickel, P., … Beblo, S. (2017). Multigene panel next generation sequencing in a patient with cherry red macular spot: Identification of two novel mutations in NEU1 gene causing sialidosis type I associated with mild to unspecific biochemical and enzymatic findings. *Molecular Genetics and Metabolism Reports*, *10*, 1–4. https://doi.org/10.1016/j.ymgmr.2016.11.004

Naganawa, Y., Itoh, K., Shimmoto, M., Takiguchi, K., Doi, H., Nishizawa, Y., … Sakuraba, H. (2000). Molecular and structural studies of Japanese patients with sialidosis type 1. *Journal of Human Genetics*, *45*(4), 241–249. https://doi.org/10.1007/s100380070034

Palmeri, S., Villanova, M., Malandrini, A., van Diggelen, O. P., Huijmans, J. G. M., Ceuterick, C., … Guazzi, G. (2000). Type I sialidosis: A clinical, biochemical and neuroradiological study. *European Neurology*, *43*(2), 88–94. https://doi.org/10.1159/000008141

Pshezhetsky, A. V., Richard, C., Michaud, L., Igdoura, S., Wang, S., Elsliger, M.-A., … Potier, M. (1997). Cloning, expression and chromosomal mapping of human lysosomal sialidase and characterization of mutations in sialidosis. *Nature Genetics*, *15*(3), 316–320. https://doi.org/10.1038/ng0397-316

Ranganath, P., Sharma, V., Danda, S., Nandineni, M. R., & Dalal, A. B. (2012). Novel mutations in the neuraminidase-1 (NEU1) gene in two patients of sialidosis in India. *Indian Journal of Medical Research*, *136*(6), 1048–1050.

Schene, I. F., Kalinina Ayuso, V., de Sain-van der Velden, M., van Gassen, K. L. I., Cuppen, I., van Hasselt, P. M., & Visser, G. (2015). Pitfalls in diagnosing neuraminidase deficiency: Psychosomatics and normal sialic acid excretion. In *JIMD reports* (Vol. *25*, pp. 9–13). Berlin: Springer Nature.

Sekijima, Y., Nakamura, K., Kishida, D., Narita, A., Adachi, K., Ohno, K., … Ikeda, S.-I. (2013). Clinical and serial MRI findings of a sialidosis type I patient with a novel missense mutation in the NEU1 gene. *Internal Medicine*, *52*(1), 119–124. https://doi.org/10.2169/internalmedicine.52.8901

Shahwan, A., Farrell, M., & Delanty, N. (2005). Progressive myoclonic epilepsies: A review of genetic and therapeutic aspects. *The Lancet Neurology*, *4*(4), 239–248. https://doi.org/10.1016/s1474-4422(05)70043-0

Sobral, I., Cachulo Mda, L., Figueira, J., & Silva, R. (2014). Sialidosis type I: Ophthalmological findings. *BMJ Case Reports*, *2014*, https://doi.org/10.1136/bcr-2014-205871

Wang, I. H., Lin, T. Y., & Kao, S. T. (2017). Optical coherence tomography features in a case of Type I sialidosis. *Taiwan Journal of Ophthalmology*, *7*(2), 108–111. https://doi.org/10.4103/tjo.tjo_53_17

Zou, W., Wang, X., & Tian, G. (2016). Fundus autofluorescence and optical coherence tomography of a macular cherry-red spot in a case report of sialidosis. *BMC Ophthalmology*, *16*, 30. https://doi.org/10.1186/s12886-016-0201-9

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