Clinical Case Report

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Diagnosis of hereditary diffuse leukoencephalopathy with neuroaxonal spheroids based on next-generation sequencing in a family

Case report and literature review

Tianji Shi, MD, Jia Li, PhD, Cheng Tan, PhD*, Jiajun Chen, PhD*

Abstract

Rationale: Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids (HDLS) is a rare disease with white matter lesions of the central nervous system, and it usually has autosomal dominant inheritance. Its pathogenesis and causes are complex, and it has obvious clinical and genetic heterogeneities; also, it is classed as a neurodegenerative disease.

Patient concerns: In preliminary clinical work, we identified a family with rapid progressive dementia.

Diagnosis: Within this family, all patients had a *CSF1R* gene c.2696delA mutation (a deletion mutation), and head magnetic resonance imaging showed extensive white matter lesions. We diagnosed these patients with HDLS.

Interventions: The proband was given hormonal treatments and immunoglobulin therapy, and his dementia symptoms have been relieved to a certain extent.

Outcomes: After treatment, the symptoms of dementia were still progressively aggravated. However, the mutation site has not previously been reported.

Lessons: This newly discovered mutation site may provide a new basis for the genetic diagnosis of HDLS disease in clinical work.

Abbreviations: CDR = clinical dementia rating, CSF = cerebrospinal fluid, CSF1R = colony-stimulating factor 1 receptor, HDLS = hereditary diffuse leukoencephalopathy with neuroaxonal spheroids, MMSE = Mini-Mental State Examination, PDGF = platelet-derived growth factor.

Keywords: genetic family trees, HDLS, hereditary diffuse leukoencephalopathy, new mutation site

1. Introduction

Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids (HDLS) is a rare disease involving white matter lesions of the central nervous system, and it is usually inherited in an autosomal dominant fashion. It is a neurodegenerative disease with obvious clinical and genetic heterogeneities, and it has a complex pathogenesis and causes. More than 20 mutation sites related to this disease have been identified in colony-stimulating factor 1 receptor (CSF1R), located on chromosome 5q32.[2] Gene detection and pathological examination of brain tissue are needed for a definitive diagnosis of this disease. In our preliminary clinical work, we identified a family in which the proband and his mother both had rapid progressive dementia and extensive leukoencephalopathy as observed by head magnetic resonance imaging (MRI). We ran gene sequencing on family members and identified a CSF1R gene c.2696delA deletion mutation in the proband, and his mother and son. We thus diagnosed them with HDLS, although this mutation site has not been reported in previous literature. The cases are reported as follows. The case report was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University, and all examinations of the patient were approved by the patient himself. Written informed consent was obtained from the patients. He also provided informed consent for publication of the findings.

2. Case report

2.1. Proband

A 43-year-old male received treatment for 1 year because of progressive mental decline and character changes. The patient was admitted to hospital 1 year before this study, when he reported that his work was not satisfying, and he had memory deterioration, speech reduction, slow responses, character changes, irascibility, and unwillingness to communicate with people; he also expressed that "some people want to hurt me." As

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T.S. and J.L. contributed equally.

All authors declare that there is no conflict interest.

Department of Neurology, China-Japan Union Hospital of Jilin University, Changchun, Jilin Province, China.

* Correspondence: Jiajun Chen, Cheng Tan, Department of Neurology, China-Japan Union Hospital of Jilin University, Changchun, 130033 Jilin Province, China (e-mail: cjj@jlu.edu.cn, tancheng@jlu.edu.cn).

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the patient's condition worsened, he could no longer work. He underwent head MRI and other related examinations in another hospital and was diagnosed with leukoencephalopathy, and he was given symptomatic treatment including hormone and neurotrophic therapies; however, the patient's condition did not improve.

2.2. Physical examination

The proband's blood pressure was 120/80 mm Hg, and his directive force, memory, and calculation ability were poor. His Mini-Mental State Examination (MMSE) score was 18 points (education <6 years) and he had cognitive deficiencies, and his clinical dementia rating (CDR) was 6 points. The patient was conscious, but had reduced speech capabilities. No obvious abnormalities were seen in the cranial nerve through physical examination, the muscular tension of all 4 limbs was normal, muscle force was level 5, no pathological reflexes were observed, and physical examination revealed no obvious abnormalities in residual nervous systems.

2.3. Head MRI scan and diffusion after admission to hospital

In the left basal ganglia and bilateral corona radiata regions, there were multiple patchy long T1 and T2 signal shadows with well-demarcated margins. With FLAIR, there was a slightly high signal, and using DWI there was a patchy, slightly high signal; the regions around the anterior and posterior horns of the lateral ventricle were the most prominent. Using DWI, multiple patchy high signals in the bilateral frontal lobes and the left occipital lobe could be observed with well-demarcated margins. In addition, the genu of the corpus callosum had a slightly lower signal change in disseminated T1WI (Fig. 1). The lesion range was slightly increased compared with images at 3 months before admission.

2.4. Routine test of cerebrospinal fluid (CSF)

In the proband, oligoclonal bands were negative, oligoclonal serum bands were negative, IgG synthesis rate of CSF was within the normal range, and AQP4 antibody was negative. Serum myelin basic protein levels were increased, autoantibody of myelin basic protein was positive, MOG antibody was positive, and blood–brain barrier permeability was elevated. No abnormalities were observed in 2 routine examinations and biochemical tests of CSF.

2.5. Proband's mother

The proband's mother was a 70-year-old female who had received treatment for progressive mental decline and character changes for 10 years. The patient had poor memory ability and had changed from being well-spoken to being taciturn, unsociable, and short-tempered over the course of 10 years before being admitted into hospital. Her condition gradually worsened, and she eventually could not take care of herself.

2.6. Physical examination

The patient's blood pressure was 145/90 mm Hg, and her directive force, memory, and calculation ability were poor. Her MMSE score was 15 points (education <6 years) and she had cognitive deficiencies, and her CDR was 5 points. The patient was conscious and could speak fluently. No obvious abnormalities were observed in the cranial nerve through physical examination, muscular tension of all 4 limbs was normal, muscle force was level 5, no pathological reflexes were observed, and physical examination revealed no obvious abnormalities in residual nervous systems.

2.7. Head MRI scan and diffusion

In the bilateral frontal and parietal lobes, there was interspersed mottling and patchy equal or long T1 signal and slightly long T2 signal with obscure boundaries, and with FLAIR there was a slightly high signal. Laminated long T1 and T2 signal shadows could be observed in the temporal lobe, and there was a high signal using FLAIR; laminated long T1 and T2 signals could be observed within the lesion, and boundaries were obscure. Within the brainstem, body and genu of the corpus callosum, bilateral thalami, basal ganglia, corona radiata, and centrum semiovale, patchy and laminated long T1 and T2 signal shadows could be observed with obscure boundaries, and with FLAIR, there were low or high signal shadows. Within the left basal ganglia region, there were obvious long T1 and T2 signal shadows with arc-shaped signal loops, and where the bilateral lateral ventricles

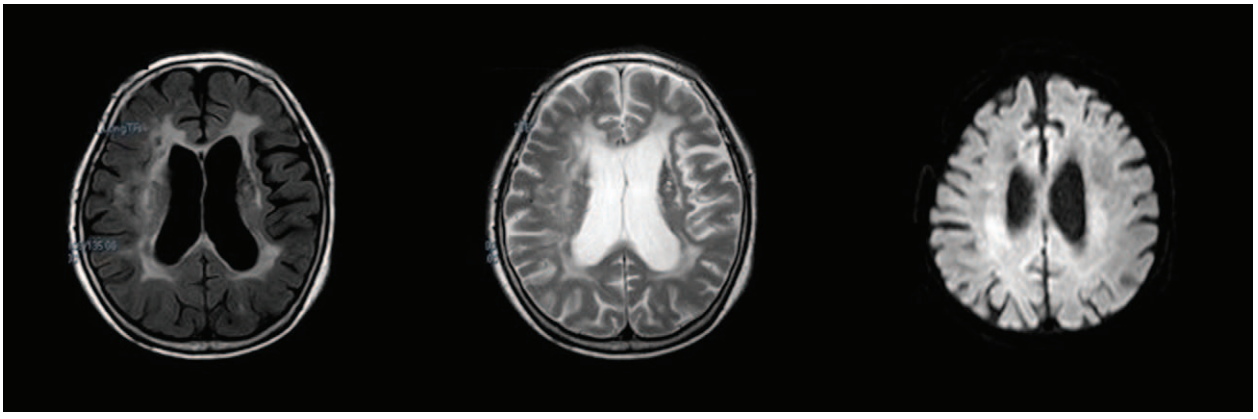

Figure 1. Head MRI scan and diffusion of the proband. MRI = magnetic resonance imaging.

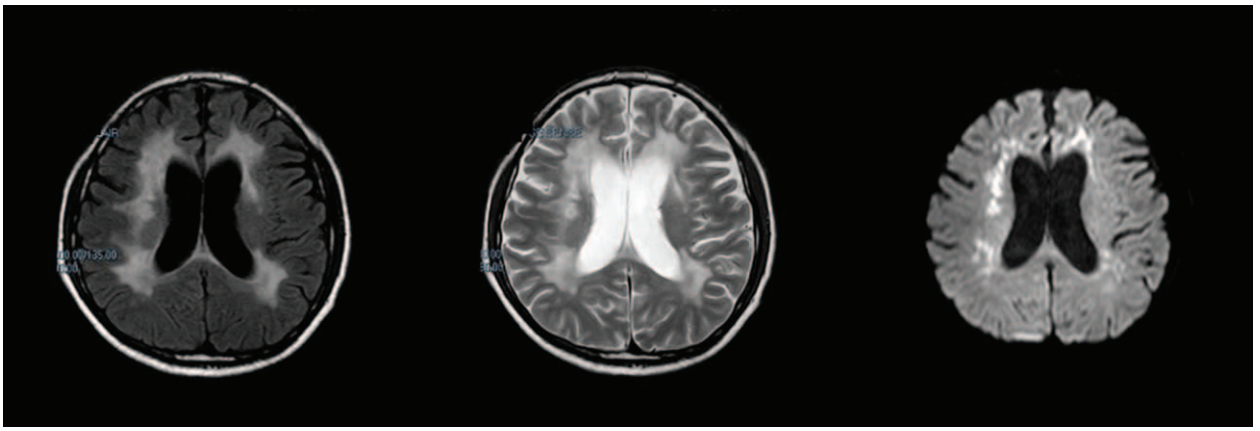

Figure 2. Head MRI scan and diffusion of the proband's mother. MRI = magnetic resonance imaging.

broadened, patchy long T1 and T2 signal shadows could be observed. With FLAIR, there was a high signal with obscure boundaries (Fig. 2).

2.8. Family survey

A family survey was conducted after the informed consent of each family member, and each patient/family member provided informed consent for publication of the case. In the family, there were 12 nonsymptomatic members and 3 symptomatic patients, including 1 male (the proband) and 2 females (as shown in Fig. 3). The elder sister of the proband is deceased, but before her death, she gradually developed character changes and memory loss, and had rapid disease progression that meant that she could not take care of herself after 1 year of developing symptoms; she died 5 years before the current study was conducted. For all 3 symptomatic family members, there were no other clinical manifestations and they were healthy before developing symptoms. All nonsymptomatic family members had no obvious clinical symptoms.

The proband and his mother both had rapidly progressing dementia, and their head MRIs showed extensive leukoencephalopathy; the proband also had a questionable medical history of nervous shock and psychological problems caused by the postnatal environment; however, his symptoms were similar to those of his sister, and his symptoms and imaging manifestations were also similar to those of his mother. Thus, the possibility of a hereditary disease was considered to be high (Fig. 3), and next-generation sequencing was performed in the proband and his mother to detect genes related to dementia.

2.9. The next-generation sequencing

A standard phenol-chloroform extraction method was used to extract genomic DNA from peripheral blood. Next-sequencing was performed by Novogene Bioinformatics Institute. Following the manufacturer's procedures, more than 1.5 μg of genomic DNA from each sampled individual was cut by using a sonicator (Covaris); enriched, hybridized, and captured on the Agilent SureSelect Human All Exon V5; and sequenced using the Illumina HiSeq 2000 sequencer (Illumina Inc., San Diego, CA). Clean reads without adaptors or degraded readings were mapped to the human reference genome (UCSC hg19) using the Burrows-Wheeler alignment (BWA) tool. Single-nucleotide polymorphism (SNP) and insertions/deletions were identified by sequence alignment/map tools and then the identification process was

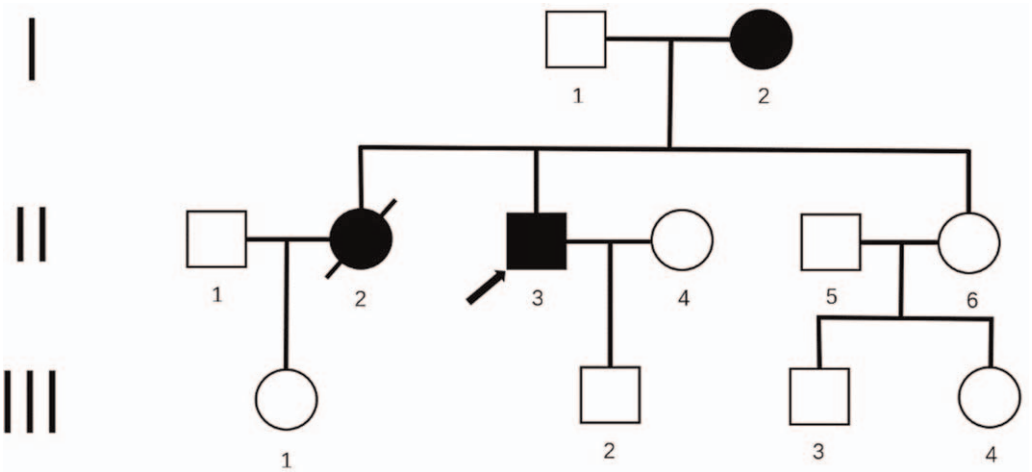

Figure 3. Family tree of a family with hereditary diffuse leukoencephalopathy with neuroaxonal spheroids.

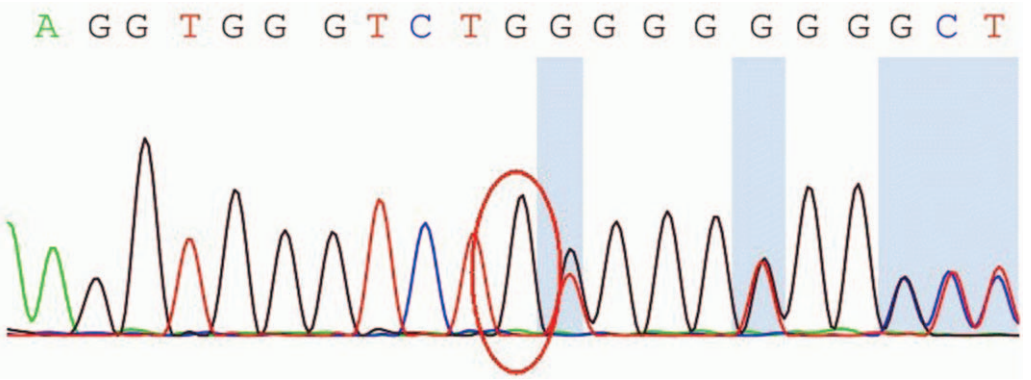

Figure 4. Chr5:149433951 with a heterozygous mutation of c.2696delA.

repeated using Picard tag reading. We screened all variants against the SNP database, 1000 Genomes project, and the outer NHLBI exome sequencing projects (ESP) 6500. Functional prediction was carried out by Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping version 2 (PolyPhen-2). Candidate variants were annotated by the ANNOVAR (Annotate Variation) software.

Direct Sanger sequencing was then performed using an ABI 3500 sequencer (Applied Biosystems, Foster City, CA) to identify the *CSF1R* gene in the family. We revealed that both the proband and his mother had a heterozygous mutation of c.2696delA in the *CSF1R* gene (Fig. 4, Tables 1 and 2), and discovered the existence of a new mutation site—c.2696delA (a deletion mutation)—in *CSF1R* in the patient, which leads to the amino acid change p.His899fs (a frame-shift mutation). After consulting related articles and HGMDpro database, we could find no previous reports of this mutation.

On the basis of the clinical characteristics, and also the results of imaging examinations and gene detection, the proband was diagnosed with HDLS and given steroid pulse therapy and immunoglobulin, which slightly relieved his dementia symptoms. During a follow-up visit 3 months after diagnosis, the patient's condition was observed to have gradually worsened, and the patient currently has difficulty communicating and cannot take care of himself in daily life, or relieve his bowels.

2.10. Ethical statement

Ethics Committee of China-Japan Union Hospital of Jilin University has approved this study (the ethical approval number is 2019040810).

3. Discussion

Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids is a hereditary degenerative disease of the nervous system that mainly manifests as rapidly progressing dementia, and it was reported both clinically and pathologically for the first time in 1984.[2] The major pathological feature of this disease is extensive leukoencephalopathy with neuroaxonal spheroids or demyelination. The main clinical manifestations include progressive cognitive impairments such as aphasia and dementia, and also motor impairments such as dyskinesia, Parkinson disease, and dystaxia because of the influence of the pyramidal tract; some patients may even have epileptic seizures.[3–5] The age of onset is generally between 30 and 50 years, and disease development is rapid: in most cases, the patient dies within a few years, and there are only a few reported cases of patients who have lived with the disease for decades.[2] HDLS is usually autosomal-dominant. *CSF1R* has been determined as a causative gene,[1] and the CSF1R protein that it encodes is a polypeptide containing 972 amino acids, and is a type III tyrosine kinase receptor that belongs

Table 1

Gene detection data and disease information (*CSF1R* gene, AD).

OMIM number	Disease name	Mutation information	Exon number of transcription version	Sequencing depth/heterozygosity/ carrying rate (EXAC ALL)
221820	Familial progressive subcortical gliosis of leukoencephalopathy	c.2696delA chr5-149433951-149433952 p.His899fs	NM_005211 Exon21	133/98 (0.42) hex

Table 2

Verification results of family genetics.

Gene	Mutation information	Patient	Mother of patient	Son of patient
CSF1R	c.2696delA chr5:149433951, p.His899fs	Heterozygous mutation	Heterozygous mutation	Heterozygous mutation

to the platelet-derived growth factor (PDGF) receptor family. This receptor mainly influences the proliferation and differentiation of mononuclear macrophages and neurogliocytes.[6] The *CSF1R* gene is a common causative gene of HDLS, although there are other known causative genes including *EIF2B2* and *POLR3A*.[7] In theory, a pathogenic mutation in just 1 chromosome may result in the disease. In 2012, Rademakers et al confirmed *CSFlR* as a causative gene of HDLS and reported that it is located on chromosome 5q32 and includes 22 exons. However, a later study confirmed that there are, in fact, 24 exons. Studies by Konno et al[8] reported that *CSF1R* mutations may play a role in the pathogenesis of HDLS by causing the dysfunction of microglial phagocytic (or "gitter") cells, no matter what type of *CSF1R* mutation a patient might have or what clinical characteristics or neuroimaging features are observed.

Until now, it has been reported that mutations in more than 60 genes can lead to leukoencephalopathy, among which *NOTCH3*, *EIF2B5*, *AARS2*, and *CSF1R* are the most common genes.[9] In *CSF1R*, more than 50 mutations can lead to disease states, and of these, 21 mutation sites are related to leukoencephalopathy. These include point mutations, missense mutations, and frame-shift mutations. In preliminary clinical work, we identified a rare HDLS family and discovered the existence of a new mutation site —c.2696delA (a deletion mutation)—in *CSF1R* in the patient, which leads to the amino acid change p.His899fs (a frame-shift mutation) and the deletion of *CSF1R* genes related with tumor-like lesions. After consulting related articles and mutation databases, we could find no previous reports of this mutation. This newly discovered mutation site provides a new basis for the genetic diagnosis of HDLS disease in the clinic. Genetic analysis suggested that the family in the current study conformed to the characteristics of autosomal dominant inheritance. The proband, his mother, and his son all carried this mutation, and the symptoms of his elder sister were similar to the symptoms of the proband; she is thus likely to have also suffered from this disease before her death. The proband's son is 15 years old and carries the gene mutation without any clinical symptoms of HDLS; it is considered that he has not yet reached the age of onset, and follow-up observations will continue for this individual.

At present, there are no effective therapeutic drugs for this disease, and symptomatic treatment is the current treatment method. Apart from routine circulation improvement and neurotrophic drug therapy, the proband has also received hormonal treatments and immunoglobulin therapy, and his dementia symptoms have been relieved to a certain extent; however, his continuous deterioration cannot be reversed. We will continue to arrange follow-up treatment for this patient and the other mutation carriers in the family.

In this study, there were the following limitations. First, the family members who underwent gene sequencing were few, and we were unable to perform gene sequencing on the father, brothers, and sisters of the proband, even after concerted efforts. Second, we were unable to perform pathological examination of the proband's brain tissue. Third, we did not further explore the possibly rare inheritance modes of HDLS. A study by Nicholson et al[7] indicated that the parents of individual HDLS patients do not have similar symptoms, and it is considered that the inheritance modes for some patients are unconventional. A final limitation is that we did not investigate the factors related to the age of onset in carriers of the mutated gene. Karle et al reported that a 28-year-old man carrying a *CSF1R* mutation was severely ill, but his 69-year-old father with the same mutation did not have similar symptoms and only showed some nonspecific leukodystrophies by MRI. However, it cannot be excluded that the father may develop symptoms at an older age. According to the latest research, the oldest reported age of HDLS onset is 71 years old.[6] Research into factors that may contribute to age of onset is important because it may allow early intervention in patients who carry or may carry disease genes, and could help in genetic counseling and when there is antenatal diagnosis in families.

Author contributions

Writing – original draft: Tianji Shi.
Writing – review & editing: Jia Li, Cheng Tan, Jiajun Chen.

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