An autopsy case report of adult-onset Krabbe disease: Comparison with an infantile-onset case

Miu Sasaki[1]*, Miori Ebata[1]*, Zen-ichi Tanei[1], Yoshitaka Oda[1], Akiko Hamauchi[2,3], Satoshi Tanikawa[1,4], Hirokazu Sugino[1], Yusuke Ishida[1], Takenori Abe[2], Nobutaka Arai[5], Kazuya Sako[2], Shinya Tanaka[1,4]

*Equal contributions

1. Department of Cancer Pathology, Faculty of Medicine, Hokkaido University, Sapporo, Japan

2. Department of Neurology, Nakamura Memorial Hospital, Sapporo, Japan

3. Takeuchi Clinic (Internal Medicine & Neurology), Doushoukai Medical Corporation, Setouchi, Japan

4. Institute for Chemical Reaction Design and Discovery (WPI-ICReDD), Hokkaido University, Sapporo, Japan

5. Laboratory of Neuropathology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Abbreviations:

・Galactocerebrosidase; GALC

・Hematoxylin and Eosin staining; H&E staining

・Klüver–Barrera staining; KB staining

・Magnetic resonance imaging; MRI

・Periodic acid–Schiff; PAS staining

・Triosephosphate isomerase barrel; TIM barrel

Short running title:

An autopsy report of adult-onset Krabbe disease

Correspondence:

Zen-ichi Tanei, M.D., Ph.D.

Department of Cancer Pathology, Faculty of Medicin, Hokkaido University

N15, W7, Kita-ku, Sapporo, 060-8638, Japan

Tel: +81-11-706-7806

Fax: +81-11-706-5902

E-mail: tanei@med.hokudai.ac.jp

Abstract

Krabbe disease is a lysosomal storage disease caused by a deficiency of the galactocerebrosidase (GALC) enzyme, which leads to demyelination of the central and peripheral nervous systems. Almost all patients with Krabbe disease are infants, and this is the first report of adult-onset cases that describe pathological findings. Here, we present two autopsy cases: a 73-year-old female and a 2-year-old male. The adult-onset case developed symptoms in her late thirties and was diagnosed by the identification of GALC D528N and L634S mutations and by T2-weighted magnetic resonance imaging; she had increased signal in the white matter along the pyramidal tract to the bilateral precentral gyrus, as well as from the triangular part to the posterior horn of the lateral ventricle. Microscopically, Klüver–Barrera staining was pale in the white matter of the precentral gyrus and occipito-thalamic radiation, and a few globoid cells were observed. The *GALC* mutations that were identified in the present adult-onset case do not completely inactivate GALC enzyme activity, resulting in focal demyelination of the brain.

Keywords

Krabbe disease, galactocerebrosidase, adult-onset

Introduction

Krabbe disease (globoid cell leukodystrophy), which was identified by Krabbe in 1916 [1], is an autosomal recessive leukodystrophy with a morbidity of one in two hundred thousand people. It is classified as a lysosomal storage disease and is caused by deficiency of the galactocerebrosidase (GALC) enzyme, which leads to demyelination of the central and peripheral nervous systems [2, 3]. Most cases (95%) of Krabbe disease are infantile onset (before the age of 6 months) [4]. Clinical manifestations of the disease include hyperirritability, hypersensitivity, stiffness, episodic fever, hypertonicity, decerebrate posturing, blindness, and unresponsiveness. Most patients die by the age of 2 years. Three other onset types of the disease are recognized: late infantile (6 months to 3 years), juvenile (3 to 8 years), and adult (after 20 years). The manifestations of adult-onset disease include asymmetric limb weakness, spastic gait, poor coordination for balance, and tremors; the symptoms mimic those of motor neuron disease [5, 6]. The disease progresses slowly in some patients, who have a normal life span. This report compares an adult-onset case with an infantile-onset case using both autopsy and pathological findings.

CASE 1

CLINICAL SUMMARY

This was a female autopsy case that was 73 years old. The patient noticed difficulties with lifting her legs in her late thirties. From 56 to 62 years of age, she used crutches to walk and then progressed to a wheelchair. Neurological manifestations at the age of 67 included amyotrophy of the distal muscles in the bilateral limbs (manual muscle test: proximal muscles 2–3, distal muscles 1–2), walking difficulties, jaw jerk reflex, periosteal reflex of the limbs, Babinski reflex were positive, sensation disorder beyond both knees, and axonopathy of the limbs (in a nerve conduction test). On magnetic resonance imaging (MRI; T2-weighted), the cerebral white matter showed increased signals (1) along the pyramidal tract to the bilateral precentral gyrus and (2) from the triangular part to the posterior horn near the lateral ventricle (especially the occipito-thalamic radiation) (Fig. 1A). These findings were unchanged for 13 years until her death. The patient was diagnosed with Krabbe disease because of MRI findings and the identification of GALC mutations (D528N and L634S mutations) when she was 71 years old. She died of aspiration pneumonia aged 73.

PATHOLOGICAL FINDINGS

Macroscopically, a white matter lesion around the central sulcus was not obvious (Fig. 1B). Microscopically, the white matter of the precentral gyrus was pale using Klüver–Barrera (KB) staining (Fig. 1C). In this area, mild astrocytosis and a few multinucleated giant

cells with scant cytoplasm and oval nuclei were identified (Fig. 1D). These multinucleated cells were positive for Periodic acid–Schiff (PAS) stain (Fig. 1E) and CD68 (Supplementary Fig. 1B), and were consistent with globoid cells: the characteristic macrophages of Krabbe disease. There were few T-cells around the vessels. The white matter was strongly positive for neurofilament staining without spheroids; therefore, demyelination is mild (Supplementary Fig. 3A–C). The pyramidal tracts of the midbrain and cervical spinal cord were degenerated and exhibited slight myelin pallor (Supplementary Fig. 1C).

CASE 2

CLINICAL SUMMARY

This was a male autopsy case that was 1 year and 7 months old. The patient had notably delayed milestones of physical development at the ages of 3 and 6 months. At the age of 9 months, enlargement of the subarachnoid space was apparent in computed tomography, early closure of the coronal suture was observed by skull X-ray, and delayed myelination was seen on MRI. Furthermore, his cerebrospinal fluid protein was elevated (110 mg/dL). At the age of 11 months, he lost eye contact and head control, and brain atrophy and demyelination were noted on MRI (Fig. 2A). At 1 year and 4 months of age, truncal opisthotonus and rigid–spastic extremities were noted. Massive myoclonus and brief tonic seizures also appeared. At the age of

1 year and 6 months, he had respiratory failure and apparent weight loss. At 1 year and 7 months of age, he was hospitalized with bacterial pneumonia and died of severe respiratory failure. His lysosomal enzyme activity test revealed that he had low GALC activity (Table 1).

PATHOLOGICAL FINDINGS

Macroscopically, white matter lesions around the internal capsule and cerebral ventricle was noted as light brown and transparent (Fig. 2B). The white matter of the cerebrum and cerebellum was pale in KB staining, but was stained well with Holzer staining, thus suggesting fibrillary gliosis (Fig. 2C). White matter was severely disrupted, and foamy macrophages, globoid cells, and marked astrocytosis were observed. Countless globoid cells had abundant cytoplasm, oval nuclei (Fig. 2D), and PAS-positive inclusions (Fig. 2E). Gliosis was severe with numerous fibrillary astrocytes and gemistocytes (Supplementary Fig. 2B). There was some T-cell infiltration around the vessels. Marked demyelination was observed with no remaining axons in neurofilament staining, but only U-fibers between the gray and the white matter were stained well (Supplementary Fig. 3D–F).

Discussion

Compared with the infantile-onset case, the adult-onset case progressed gradually, and the

symptoms were more focused. A table showing the comparisons between the two cases is included (Table 1). The adult-onset case showed a mild disorder of movement, sensation, neural reflex, amyotrophy, and axonopathy in all four limbs (especially in the inferior limbs). In contrast, the infantile-onset case showed rapid development throughout the whole body and involved a developmental delay of psychomotor performance. In both cases, the activity of GALC was unable to be detected by laboratory tests. Microscopically, the convolutional white matter of the precentral gyrus and occipito-thalamic radiation was demyelinated in the adult-onset case, while all white matter—in both the cerebrum and cerebellum—was affected in the infantile-onset case (Fig. 3). Degeneration of the pyramidal tract throughout the midbrain to the spinal cord was observed in both cases. Additionally, only a few globoid cells with scant cytoplasm and oval nuclei were observed in the adult-onset case; in contrast, these cells were present in large quantities with abundant cytoplasm, oval nuclei, and PAS-positive inclusions in the infantile-onset case. Gliosis and demyelination were mild, and axons remained in the adult-onset case, whereas severe gliosis and demyelination lead to marked axonal degeneration in the infantile-onset case.

In adult-onset cases, demyelination is generally localized in the precentral gyrus and occipito-thalamic radiation. GALC, which is deficient in Krabbe disease, dissolves important components of myelinization in cells, such as galactosylceramide and psychosine [7]. An

accumulation of these substances causes cell damage and leads to the appearance of globoid cells and demyelination. Thus, frequent myelin turnover can be the main lesion of demyelination [8]. The white matter of the precentral gyrus, which was damaged in the present adult-onset case, has neural fibers of the corticospinal tract that travel from Betz cells in the precentral cortex to the lumbo-sacral cord. These are one of the longest and largest neural fibers in the human body [9], and their myelin turnover may be more active than the others. The precentral gyrus might therefore be prone to accumulating substances such as galactosylceramide and psychosine, and thus be more vulnerable to demyelination. White matter lesions of the triangular part of the posterior horn involve a pathway that is related to vision: the occipito-thalamic radiation. Our adult-onset case showed a focal lesion with a few globoid cells in this area, which might have been caused by unknown characteristics of myelin structure or by oligodendrocytes in the occipito-thalamic radiation.

Krabbe disease is caused by mutations in the *GALC* gene that result in degeneration of the GALC protein. GALC consists of 668 amino acids and has three representative domains (the triose-phosphate isomerase [TIM] barrel, β-sandwich domain, and lectin domain) [10]. These domains combine to form a large substrate-binding pocket. Fig. 4 shows the typical mutation sites in the GALC protein that have been found in adult- or infantile-onset patients with Krabbe disease. The mutation sites vary widely but appear to be concentrated in the TIM barrel and

lectin domains. Typical examples of mutations in adult-onset cases include L634S, [I82M+I305V], and G286D [11], while c.683_694delinsCTC (N228_ S232delinsTP), T668P, R220X, 30kDa del, and D528N are more common in infantile-onset cases [11]. In our adult-onset case, L634S and D528N were identified. The L634S substitution occurs in the lectin-binding domain, and has been demonstrated to impair the transport of GALC to lysosomes [12]. Furthermore, the D528N substitution does not result in a complete loss of enzymatic activity in GALC, but its transport into the lysosome is impaired, resulting in a decrease in enzymatic activity [13]. The identified mutations in the present case, L634S and D528N, are characteristic of adult- and infantile-onset cases, respectively; however, the combination of these mutations seems to have resulted in the adult-onset form of the disease. Neither mutation completely inactivates GALC, but they impair its transport to lysosomes, which results in a relatively mild phenotype. Thus, the combination of mutation sites may also be important in determining the type of disease.

In the present report, the adult-onset case exhibited a milder clinical course than the infantile-onset case. Additionally, the demyelinated lesions were limited to the precentral gyrus and triangular area of the posterior horn, and were accompanied by just a few globoid cells. The GALC mutations L634S and D528N do not completely inactivate GALC enzyme activity, thus resulting in focal demyelination of the brain.

Acknowledgments

We thank Mr. Kenji Shishido, Ms. Hiromi Mori, Ms. Miki Iida, Ms. Keiko Kasahara, Ms. Kaori Sudo, Ms. Aiko Matsuda, and Ms. Akemi Ofusa for their technical support. We also thank Bronwen Gardner, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure Statement

None declared.

Ethics Approval

The project was approved by an institutional ethics committee. For human subjects, the investigation was conducted in accordance with the Declaration of Helsinki of 1975.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by M.E., M.S., Z.T., and Y.O. The first draft of the manuscript was written by M.E. and M.S. and revised by Z.T. and S.T. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

References

1 Compston A. A new familial infantile form of diffuse brain-sclerosis. *Brain*. 2013; **136**: 2649-51.
2 Suzuki K, Suzuki Y. Globoid cell leucodystrophy (Krabbe's disease): deficiency of galactocerebroside beta-galactosidase. *Proc Natl Acad Sci U S A*. 1970; **66**: 302-9.
3 Love S, Budka H, Ironside JW, Perry A, Greenfield JG. Greenfield's neuropathology. 9th ed edn: CRC Press 2015; 2 v. Chapter 6.
4 Graziano AC, Cardile V. History, genetic, and recent advances on Krabbe disease. *Gene*. 2015; **555**: 2-13.
5 Henderson RD, MacMillan JC, Bradfield JM. Adult onset Krabbe disease may mimic motor neurone disease. *J Clin Neurosci*. 2003; **10**: 638-39.
6 Krieg SI, Krageloh-Mann I, Groeschel S*, et al.* Natural history of Krabbe disease - a nationwide study in Germany using clinical and MRI data. *Orphanet J Rare Dis*. 2020; **15**: 243.
7 Suzuki K. Evolving perspective of the pathogenesis of globoid cell leukodystrophy (Krabbe disease). *Proceedings of the Japan Academy, Series B*. 2003; **79B**: 1-8.
8 McTigue DM, Tripathi RB. The life, death, and replacement of oligodendrocytes in the adult CNS. *J Neurochem*. 2008; **107**: 1-19.
9 Hall JE, Guyton AC. Textbook of medical physiology. 13th ed edn: Elsevier 2016; xix, 1145 p.
10 Deane JE, Graham SC, Kim NN*, et al.* Insights into Krabbe disease from structures of galactocerebrosidase. *Proc Natl Acad Sci U S A*. 2011; **108**: 15169-73.
11 Hossain MA, Otomo T, Saito S*, et al.* Late-onset Krabbe disease is predominant in Japan and its mutant precursor protein undergoes more effective processing than the infantile-onset form. *Gene*. 2014; **534**: 144-54.
12 Shin D, Feltri ML, Wrabetz L. Altered Trafficking and Processing of GALC Mutants Correlates with Globoid Cell Leukodystrophy Severity. *J Neurosci*. 2016; **36**: 1858-70.
13 Lee WC, Kang D, Causevic E, Herdt AR, Eckman EA, Eckman CB. Molecular characterization of mutations that cause globoid cell leukodystrophy and pharmacological rescue using small molecule chemical chaperones. *J Neurosci*. 2010; **30**: 5489-97.

Figure legends

Fig. 1 Radiological and pathological images of the adult-onset case (Case 1).

(A) Magnetic resonance images (T2-weighted images) 13 years before death showing high intensity in the white matter from the triangular part to the posterior horn in the lateral ventricle (arrows) and along the pyramidal tract to the bilateral precentral gyrus (arrowheads); these findings do not change until death. (B) No remarkable findings around the central sulcus (left) and posterior horn (right) in a coronal section macroscopically. (C) The white matter of the precentral gyrus showing myelin pallor in Klüver–Barrera staining. (D) A few globoid cells with scant cytoplasm and oval nuclei, and mild astrocytosis in the demyelinated lesions. (E) Globoid cell positive for Periodic acid–Schiff staining. Scale bars: 50 mm (B), 10 mm (C), 5 μm (D, E).

Fig. 2 Radiological and pathological images of the infantile-onset case (Case 2).

(A) Magnetic resonance images (T2-weighted images) at the age of 11 months (8 months before death) showing high intensity in the posterior horn of the lateral ventricle (arrows). (B) The white matter around the internal capsule and cerebral ventricle showing brownish and transparent, macroscopically. (C) The white matter around the cerebral ventricle showing myelin pallor in Klüver–Barrera staining and fibrillary gliosis by Holzer staining. (D) Countless globoid cells with abundant cytoplasm and oval nuclei, and marked gliosis. (E) Globoid cell inclusions positive for Periodic acid–Schiff staining.

Scale bars: 50 mm (B, C), 5 μm (D, E).

Fig. 3 Distribution of the lesions.

The demyelinated lesion is colored red, and the secondary degeneration is depicted in blue. The main lesion is localized in the adult-onset case (Case 1) and is widespread in the infantile-onset case (Case 2). Both cases exhibit secondary degeneration of the pyramidal tract.

Fig. 4 Locations of amino acid substitutions in *GALC*.

The specific mutations for adult-onset disease, such as L634S (our case has this mutation) and G286D,

are colored red. In contrast, mutations in blue are common in infantile-onset disease (e.g., c.683_694delinsCTC and T668S).

Tables

Table 1 Summary of the adult-onset case (Case 1) and the infantile-onset case (Case 2)

	Case 1	Case 2
Age of onset	Mid-30s	3 months
Symptoms	Limb weakness Walking difficulty Spastic gait Amyotrophy of distal muscle, especially bilateral limbs Jaw jerk reflex, limbs periosteal reflex, Babinski's reflex All sensation disorder beyond both knees Axonopathy of limbs	Psychomotor retardation Dilation of circumference and subarachnoid cavity Delay of myelination Cerebrospinal fluid protein abnormality Delay of peripheral nerve conduction velocity Opisthotonos of trunk Rigidity and spasm Massive myoclonus Tonic seizure Respiratory disorder
Disease progression	Slow	Rapid
Galactocerebrosidase Reference value 0.75 ± 0.27 (mol/h/mg)	0.08	Trace
***GALC* mutation**	D528N, L634S	Not examined
Lesions	White matter close to precentral gyrus and posterior horn of lateral ventricle (localized) Secondary degeneration of the pyramidal tract	Cerebrum and cerebellar white matter (extensive) Secondary degeneration of the pyramidal tract
Globoid cell	A few (one cell in one slide)	Countless
Number	Scant	Abundant
Cytoplasm	Oval	Oval
PAS-positive inclusions	Few	Rich
CD68 staining	Positive	Positive
Gliosis	Mild	Severe
Demyelination	Mild	Severe
Axon	Remained	Degenerated
Lymphoid cell infiltration	Scant T-cell infiltration around vessels	T-cell infiltration around vessels

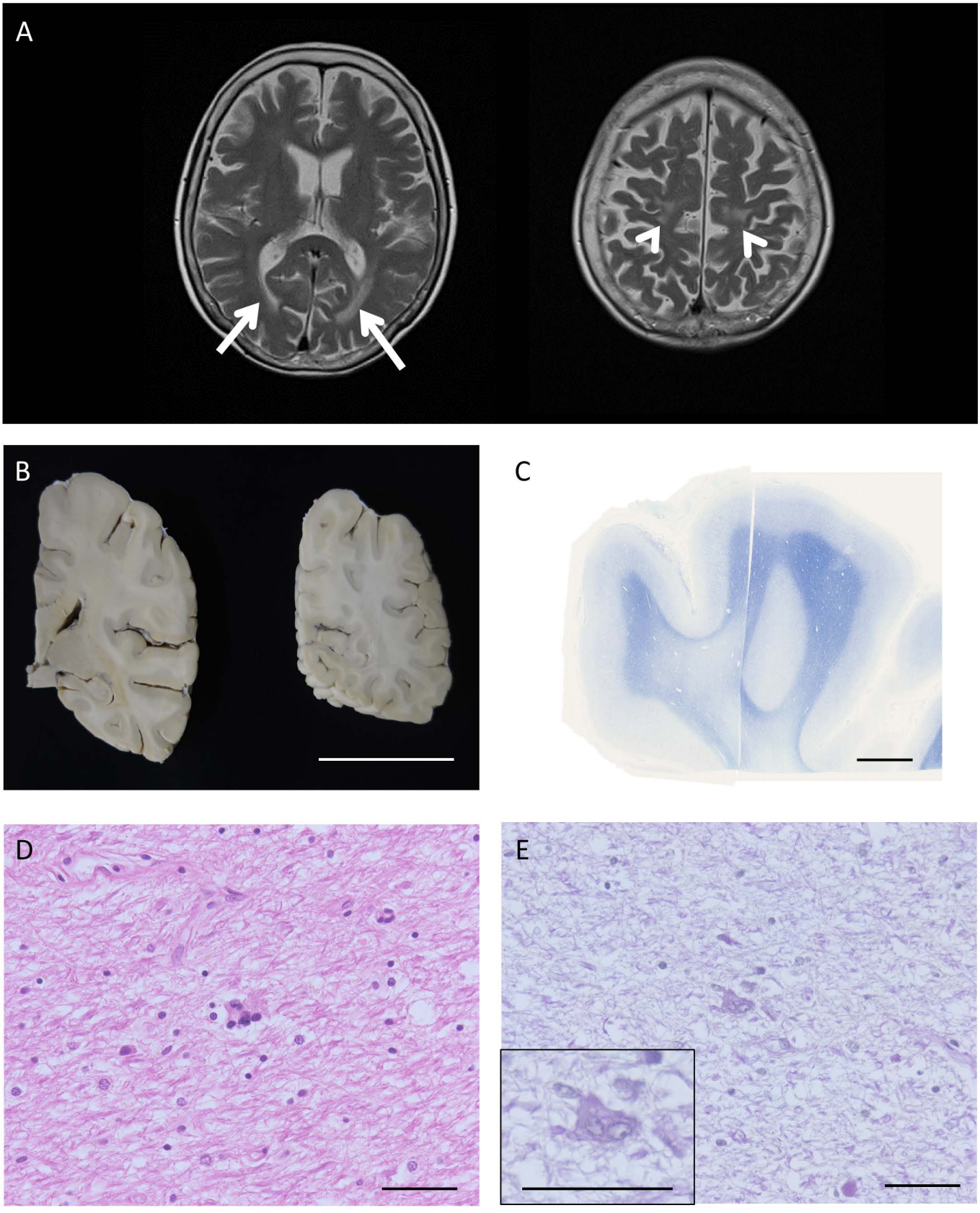

Fig . 1 Radiological and pathological images of adult-onset case (Case 1).

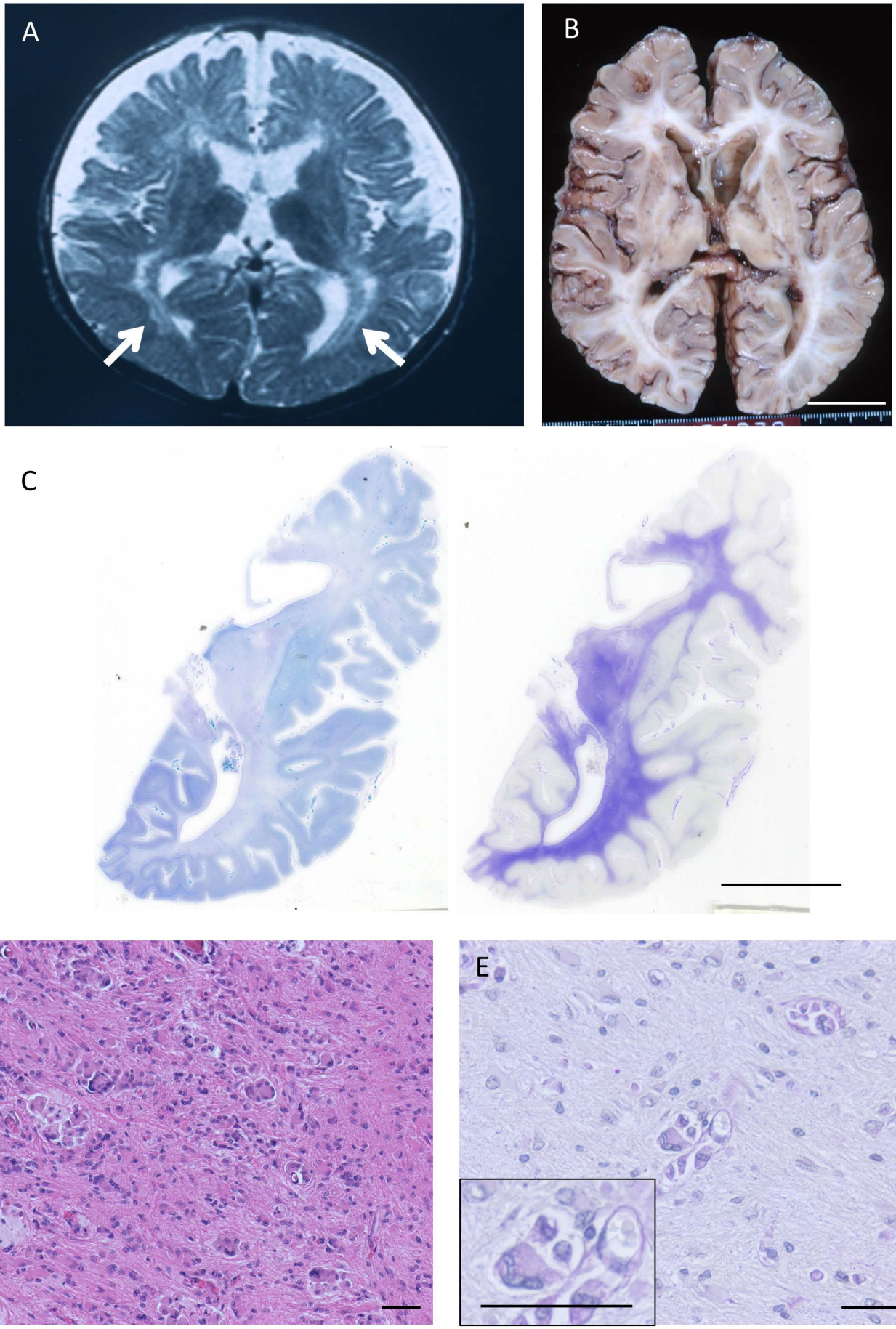

Fig. 2 Radiological and pathological images of infantile-onset case (Case 2) .

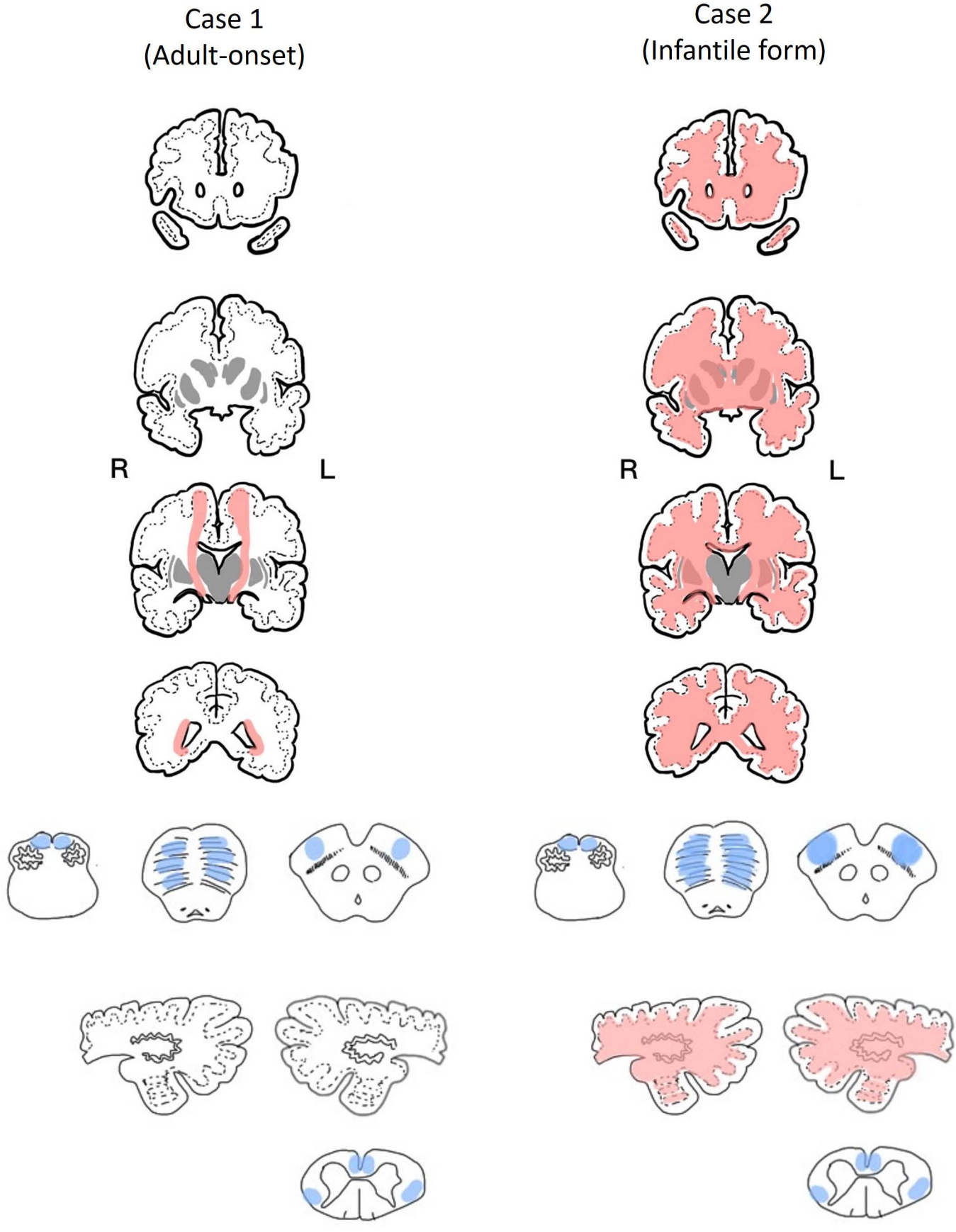

Fig. 3 Distribution of the legions.

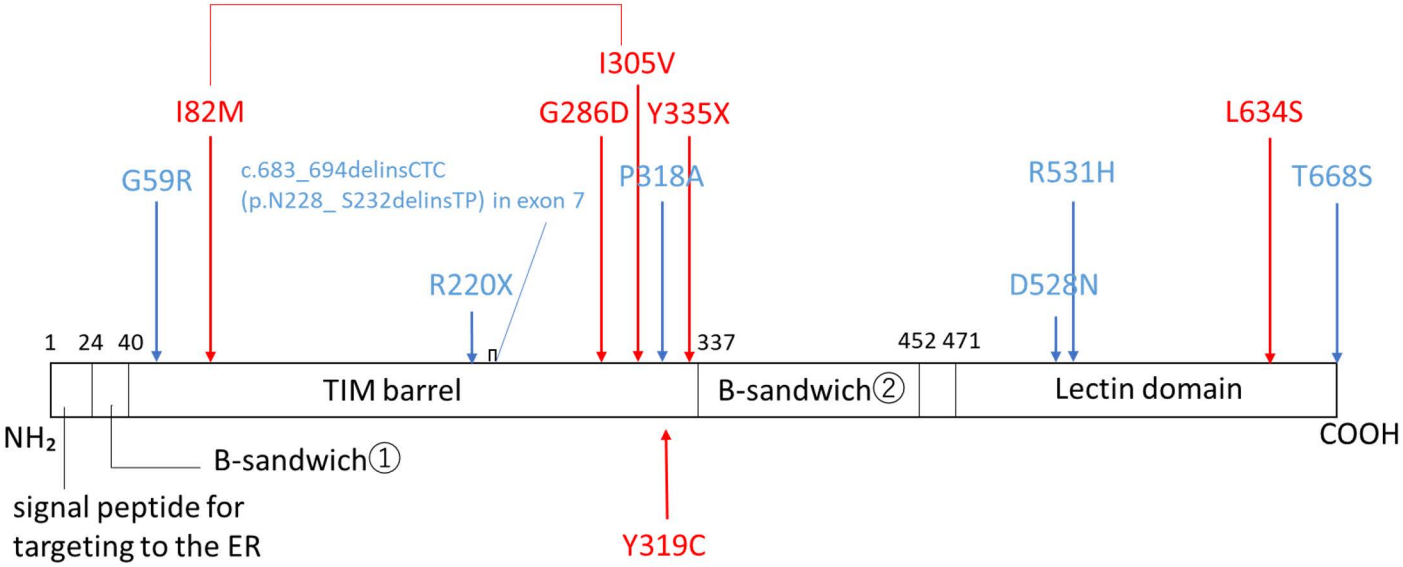

Fig . 4 Locations of amino-acid substitution in GALC.