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CASE REPORT

A Japanese Adult Case of Guanidinoacetate Methyltransferase Deficiency

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Abstract Guanidinoacetate methyltransferase (GAMT) deficiency is a rare disorder of creatine synthesis resulting in cerebral creatine depletion. We present a 38-year-old patient, the first Japanese case of GAMT deficiency. Developmental delay started after a few months of age with a marked delay in language, which resulted in severe intellectual deficit. She showed hyperactivity and trichotillomania from childhood. Epileptic seizures appeared at 18 months and she had multiple types of seizures including epileptic spasms, brief tonic seizures, atypical absences, complex partial seizures with secondary generalization, and "drop" seizures. They have been refractory to multiple antiepileptic drugs. Although there have been no involuntary movements, magnetic resonance imaging revealed T2 hyperintense lesions in bilateral globus pallidi. Motor regression started around 30 years of age and the patient is now able to walk for only short periods. Very low serum creatinine levels measured by enzymatic method raised a suspicion of GAMT deficiency, which was confirmed by proton magnetic resonance spectroscopy and urinary guanidinoacetate assay. *GAMT* gene analysis revealed that the patient is a compound heterozygote of c.578A>G, p.Gln193Arg and splice site mutation, c.391G>C, p.Gly131Arg, neither of which have been reported in the literature. We also identified two aberrant splice products from the patient's cDNA analysis. The patient was recently started on supplementation of high-dose creatine and ornithine, the effects of which are currently under evaluation. Although rare, patients with developmental delay, epilepsy, behavioral problems, and movement disorders should be vigorously screened for GAMT deficiency, as it is a treatable disorder.

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Introduction

Guanidinoacetate methyltransferase (GAMT; OMIM 601240) deficiency is a rare autosomal recessive disorder of creatine synthesis resulting in cerebral creatine depletion (Stöckler et al. 1994, 1996b). Guanidinoacetate (GAA) accumulates in body fluids. Symptoms of GAMT deficiency usually emerge after a few months of life, such as intellectual disability, speech delay, autistic behaviors, epileptic seizures, and involuntary movements (Mercimek-Mahmutoglu et al. 2006). Making a diagnosis of GAMT deficiency is challenging; nonetheless, early diagnosis is crucial because this disorder is treatable (Stöckler et al. 1996a). Only approximately 80 cases have been reported to date, mostly from Europe and the Middle East. Here we report on the first Japanese patient with GAMT deficiency with two novel gene mutations.

Case Report

The patient, a 38-year-old female with intractable epilepsy and severe mental retardation, was born at full term with a birth weight of 3,260 g. There were no pre- or perinatal complications. She is the third of four children of Japanese non-consanguineous healthy parents. The first child, a boy, started having epileptic seizures after 1 year of age with unknown cause and died at 28 years of age at an institution for the mentally handicapped. The other two children have been healthy.

Although the patient showed a social smile by 3 months and head control by 4 months of age, her development has been delayed since then. She sat alone at 14 months, walked alone at around 20 months, and became able to take the stairs one step at a time with support around 5 years of age. She has spoken no meaningful words and gained little language comprehension. Her medical chart at 7 years of age described her as speechless, unable to follow verbal commands, but able to run and walk up the stairs one step at a time without support. She showed no involuntary movements. She was hyperactive and had trichotillomania. Neuropsychological assessment at 7 years 7 months by analytic test for development in infancy and childhood (Enjoji and Yanai 1961) demonstrated her developmental quotient was 14. Around 30 years of age, she was unable to walk for a long time but was able to take the stairs with support. At 32 years of age, she was no longer able to run. Currently, at 38 years of age, the patient has severe intellectual deficit with no speech or language comprehension. She still has trichotillomania. Her transport is mostly by wheelchair, although she is able to walk slowly for short periods. Her muscle tone is normal and there are no involuntary movements.

The onset of epilepsy was at around 18 months of age, characterized by epileptic spasms and brief tonic seizures. At 2 years of age, atypical absences appeared. Despite therapy with multiple antiepileptic drugs, the patient continued to have these seizures until 15 years of age, when her seizures were suppressed by valproic acid and clonazepam. When they recurred at 20 years of age, her seizures were characterized by consciousness impairment with head and body version to left followed by generalized tonic-clonic convulsions lasting up to 1 minute, suggesting complex partial seizures with secondary generalization. At around 23 years, brief "drop" seizures occurring in clusters started. She has continued to have these seizures since then, although she has been tried on multiple antiepileptic drugs including phenobarbital, valproic acid, clonazepam, phenytoin, clobazam, topiramate, lamotrigine, and levetiracetam.

Electroencephalograms (EEGs) at 2–12 years of age showed a slow background activity, generalized 1.5–2.5 Hz slow spike-wave bursts and some multifocal spikes, consistent with Lennox-Gastaut syndrome. EEGs after adolescence showed multifocal spike-waves with anterior head predominance and intermittent generalized slow spike-waves. The most recent EEG at 38 years of age demonstrated background slowing and no spikes during wakefulness but intermittent focal polyspikes and polyspike-waves over bilateral anterior and left posterior head regions during sleep.

Laboratory blood tests demonstrated low levels of serum creatinine (5–7 μmol/L by enzymatic method; normal range 40–71 μmol/L). Subsequent tests using enzymatic methods demonstrated serum creatine levels were below detection limit (normal range 23–92 μmol/L). Proton magnetic resonance spectroscopy (^{1}H-MRS) demonstrated absent creatine peak (Fig. 1a). Brain magnetic resonance imaging (MRI) demonstrated T2 high-intensity lesions in globus pallidi (Fig. 1b). Analysis of urinary creatine metabolites by weak-acid ion chromatography (Wada et al. 2012) demonstrated elevated GAA (548.53, 782.52 mmol/mol creatinine; normal 3–78 mmol/mol creatine (Almeida et al. 2004)) and creatine below detection limit. These findings suggested GAMT deficiency.

Genomic DNA analysis of the *GAMT* gene (Suppl. Table 1) showed a compound heterozygosity for two novel point mutations, an exonic splicing mutation c.391G>C located at the last nucleotide of exon 3 and a missense mutation c.578A>G, p.Gln193Arg in exon 6 (Fig. 2a). Analysis of cDNA revealed two aberrantly spliced transcription products at the allele of splicing mutation (Fig. 2b, c). One transcript had the complete exon 3 (64-bp) deletion by exon skipping and the other transcript was aberrantly spliced at exon 2 involving intron 2 insertion (44-bp) followed by exon 3 skipping, resulting in a 20-bp deletion. Both transcripts are expected to result in frame shift and premature termination of p.Val110Glyfs*30 and p.Ile111Profs*73, respectively. A novel A to G transition on exon 6 (c.578A>G) results in the replacement of arginine by glutamine at position 193 (p.Gln193Arg). This missense variation was not found in 100 control alleles. Glutamine193 is highly conserved in evolution (Fig. 2d), suggesting this mutation represents a pathogenic mutation.

This patient was recently started on supplementation of high-dose creatine and ornithine, and its effects are currently under evaluation.

Discussion

We reported on the first Japanese case of an adult patient with GAMT deficiency. Cases have been reported mostly from Europe and the Middle East (Mercimek-Mahmutoglu et al. 2006).

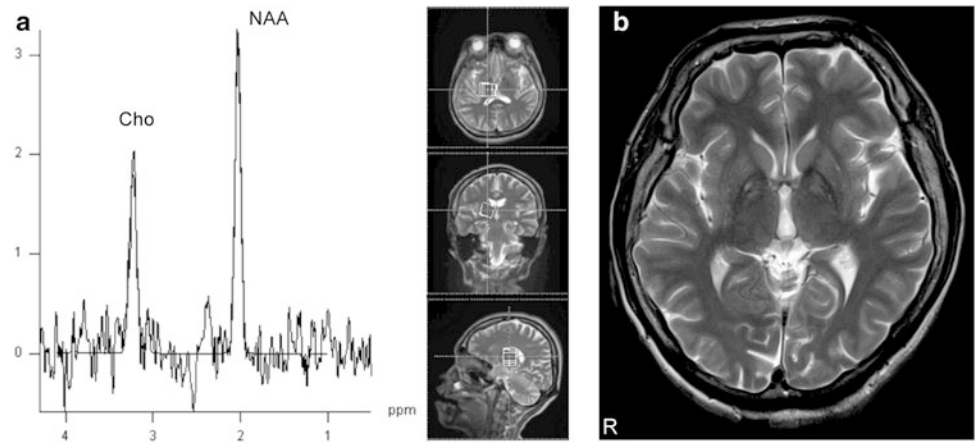

Fig. 1 MR spectroscopy and MRI from the patient with GAMT deficiency. (**a**) ^{1}H-MRS at the right basal ganglia demonstrates absence of creatine peak. (**b**) T2-weighted brain MRI shows high-intensity lesions in bilateral globus pallidi. *Cho* choline; *NAA* N-acetylaspartate

Compared with cases in the literature, our patient showed similar MRI findings and clinical course, with severe intellectual deficit, intractable epilepsy, behavioral problems, but she lacked involuntary movements. Although no definite progression of symptoms was seen during adolescence and young adulthood, motor regression slowly started around 30 years of age. This suggests GAMT deficiency can be slowly progressive if untreated.

Onset of symptoms in GAMT deficiency is from a few months to young childhood (Longo et al. 2011). Intellectual disability is seen in all cases and is severe (IQ < 35) in the majority, especially with profound speech disturbance (Mercimek-Mahmutoglu et al. 2006). Epilepsy is the second most frequent symptom, intractable in most cases, and partially responsive to antiepileptic drugs in two thirds (Leuzzi et al. 2013). Various types of seizures, such as generalized tonic-clonic seizures, absences, myoclonic seizures, myoclonic-astatic seizures, and partial seizures with secondary generalization, have been reported (Leuzzi et al. 2013). Involuntary movements, behavioral problems, and abnormal MRI signals in globus pallidi are seen in some cases. Adult cases that help to understand the natural history of GAMT deficiency are scarce (Schulze et al. 2003; Caldeira Araújo et al. 2005). Progression of neurological deficits, such as paraparesis, hypertonia, and rigidity, has been reported in some cases (Caldeira Araújo et al. 2005).

GAMT gene analysis revealed a compound heterozygosity of two novel mutations: c.391G>C splice donor site of exon 3 and c.578A>G, p.Gln193Arg in exon 6. The former led to two abnormal transcripts lacking exon 3, resulting in a premature stop codon. Reverse transcription polymerase chain reaction detected a higher expression level of the allele with the c.578A>G mutation, which implies the degradation of mRNA from the allele with the splice site mutation by nonsense-mediated mRNA decay (Fig. 2b). Gln193Arg substitution by the latter mutation is presumed to destabilize the tertiary structure of GAMT (Komoto et al. 2002) by increasing the bulkiness and changing the neutral to a positively charged residue, as Gln193 is situated in the middle of α-helix and protrudes into this enzyme.

Making a diagnosis of GAMT deficiency is challenging, because of its nonspecific symptoms and limited access or capacity of ^{1}H-MRS. GAA assay may not be readily available. While not as specific as GAA, measurement of creatinine is helpful, as creatinine can be low in GAMT deficiency (Verhoeven et al. 2000). It should be warned that creatinine may also be low in patients with decreased muscle volume. Another caveat is that creatinine measurement by Jaffé method is not as sensitive in detecting GAMT deficiency as the enzymatic method or high-performance liquid chromatography (Verhoeven et al. 2000). Our patient showed low levels of serum creatinine as determined by enzymatic method, which directed us to the diagnosis of GAMT deficiency. The assay of creatine and creatinine is also important to detect creatine transporter 1 deficiency, another type of cerebral creatine deficiency, as the urinary creatine/creatinine ratio is elevated in this disorder (Salomons et al. 2003; Verhoeven et al. 2005). GAA is a more sensitive marker than creatine and creatinine in GAMT deficiency and arginine: glycine amidinotransferase deficiency, the other type of cerebral creatine deficiency (Verhoeven et al. 2005). Therefore, blood and urine tests of creatinine, creatine and GAA should be a part of the workup for developmental delay and/or epilepsy with unknown cause, if creatine and GAA measurements are available.

Early diagnosis is crucial to achieve a favorable outcome in GAMT deficiency. Ideally, treatment should be initiated as early as possible before the creatine pool supplied from maternal body during gestation becomes

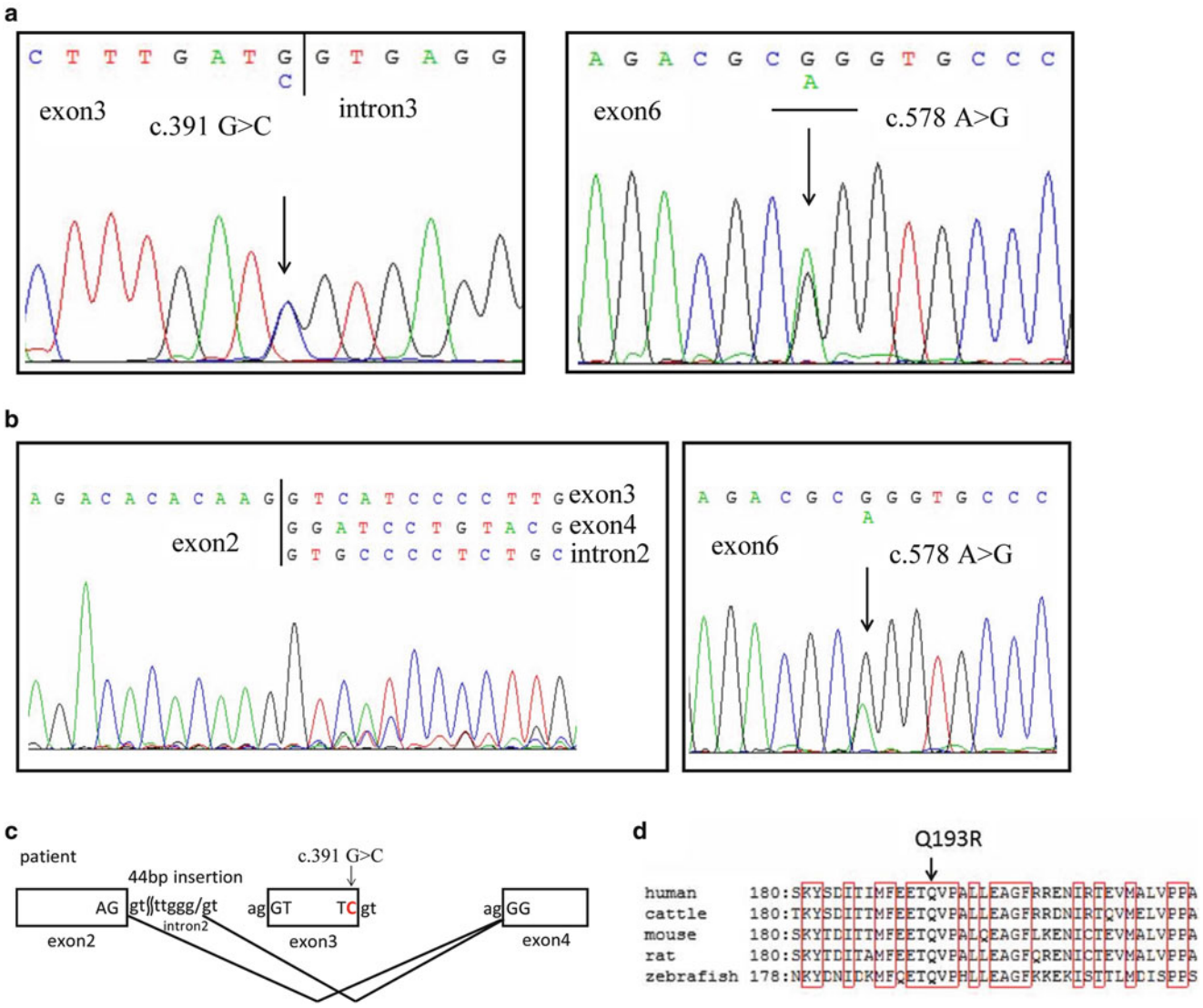

Fig. 2 Genetic analysis of the mutation in *GAMT*. (**a**) Chromatogram of genomic DNA analysis in a patient shows the heterozygote of c.391G>C (*left*) and c.578A>G (*right*). (**b**) cDNA analysis in the patient shows two aberrantly spliced transcription products (*left*) and c.578A>G (*right*). (**c**) c.391G>C mutation causes two aberrant splicing products: one with complete exon 3 (64-bp) skipping and the other involving intron 2 insertion (44-bp) followed by exon 3 skipping. (**d**) Aligned GAMT amino acid sequence of the patient with several other animals, revealing Gln193 is highly conserved among species

depleted and clinical symptoms appear. Presymptomatic treatment has been shown to be successful in achieving normal development (Schulze et al. 2006; El-Gharbawy et al. 2013). Even when diagnosed later, creatine supplementation with reduction of GAA by arginine restriction and ornithine supplementation can alleviate symptoms and prevent further progression of the disease (Schulze et al. 2001). GAMT deficiency is a good candidate for neonatal mass screening. Elevated GAA levels in neonatal blood (Schulze et al. 2006; El-Gharbawy et al. 2013) and amniotic fluid (Cheillan et al. 2006) have been reported, and validity of these tests needs to be elucidated.

In conclusion, we presented a 38-year-old patient, the first Japanese case of GAMT deficiency with two novel gene mutations. We should always include this disorder on the list of differential diagnoses when seeing patients with neurological symptoms such as intellectual disability, epilepsy, behavioral problems, and involuntary movements, since GAMT deficiency is a treatable disorder.

Take-Home Message

A 38-year-old patient, the first Japanese case of guanidinoacetate methyltransferase deficiency with two novel gene

mutations (splice site mutation and missense mutation) was reported.

Compliance with Ethics Guidelines

Contributions of Individual Authors

Tomoyuki Akiyama, Hitoshi Osaka, Hiroko Shimbo, and Tomoshi Nakajiri: Drafting/revising the manuscript for content, analysis, and interpretation of data

Katsuhiro Kobayashi, Makio Oka, Fumika Endoh, and Harumi Yoshinaga: Drafting/revising the manuscript for content

Guarantor for the Article

Tomoyuki Akiyama

Details of Funding

None

Details of Ethics Approval

This study was approved by the ethics board at Kanagawa Children's Medical Center.

Conflict of Interest

Tomoyuki Akiyama, Hitoshi Osaka, Hiroko Shimbo, Tomoshi Nakajiri, Katsuhiro Kobayashi, Makio Oka, Fumika Endoh, and Harumi Yoshinaga declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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