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Expanded clinical and molecular spectrum of guanidinoacetate methyltransferase (GAMT) deficiency

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ABSTRACT

Guanidinoacetate methyltransferase (GAMT) deficiency is a disorder of creatine biosynthesis, characterized by excessive amounts of guanidinoacetate in body fluids, deficiency of creatine in the brain, and presence of mutations in the *GAMT* gene. We present here 8 new patients with GAMT deficiency along with their clinical, biochemical and molecular data. The age at diagnosis of our patients ranges from 0 to 14 years. The age of onset of seizures usually ranges from infancy to 3 years. However, one of our patients developed seizures at age 5; progressing to myoclonic epilepsy at age 8 years and another patient has not developed seizures at age 17 years. Five novel mutations were identified: c.37ins26 (p.G13PfsX38), c.403G>T (p.D135Y), c.507_521dup15 (p.C169_S173dup), c.402C>G (p.Y134X) and c.610_611delAGinsGAA (p.R204EfsX63). Six patients had the c.327G>A (last nucleotide of exon 2) splice-site mutation which suggests that this is one of the most common mutations in the *GAMT* gene, second only to the known Portuguese founder mutation, c.59G>C (p.W20S). Our data suggests that the clinical presentation can be variable and the diagnosis may be overlooked due to unawareness of this disorder. Therefore, *GAMT* deficiency should be considered in the differential diagnosis of progressive myoclonic epilepsy as well as in unexplained developmental delay or regression with dystonia, even if the patient has no history of seizures. As more patients are reported, the prevalence of GAMT deficiency will become known and guidelines for prenatal diagnosis, newborn screening, presymptomatic testing and treatment, will need to be formulated.

Introduction

Creatine deficiency syndromes include three inborn errors of metabolism, involving three proteins—AGAT deficiency (arginine: glycine amidinotransferase [MIM 602360]), GAMT deficiency (*S*-adenosyl-L-methionine: *N*-guanidinoacetate methyltransferase [MIM 601240]) and the creatine transporter defect [MIM 300036] [1] (Fig. 1). Among these disorders, GAMT deficiency was the first condition to be described in 1994 by Stockler et al. [2]. The index patient had global developmental delay which became evident in the first year of life, with an abnormal dyskinetic movement pattern, failing head control and irregular eye movements. Over the years, 29 patients have been reported with age at diagnosis ranging from birth to 29 years [3,4]. This is an autosomal recessive disorder characterized by decreased concentrations of creatine in the brain, cerebrospinal fluid, plasma and urine and by an accumulation of guanidinoacetate (GAA) in body fluids.

The clinical features of GAMT deficiency are variable and include developmental delay, muscular hypotonia, weakness, progressive extrapyramidal signs and symptoms, mental retardation, epilepsy and autistic or self-aggressive behavior [5]. Although the clinical phenotype is complex, this disorder is classified into severe, intermediate and mild [6]. In a review by Mercimek-Mahmutoglu et al. [4], the most common clinical manifestation was found to be intellectual disability, followed by epilepsy.

Determination of creatine and GAA in urine or plasma is a simple and fast screening test for the diagnosis of GAMT deficiency. GAA concentration is 2–200 times higher in urine and 10–20 times the upper limit of normal in the plasma [7].

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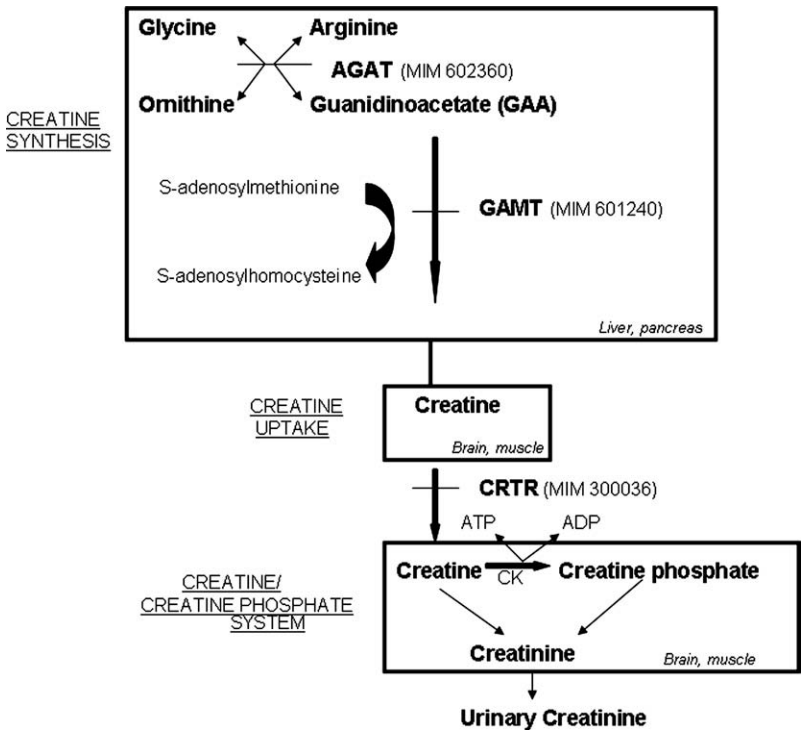

Fig. 1. Creatine metabolism pathway [14].

Magnetic Resonance Spectroscopy (MRS) is another sensitive and noninvasive method for the detection of creatine deficiency and GAA accumulation in the brain. Abnormal MRI signals in the basal ganglia, particularly the globi pallidi are characteristic for this disorder.

Mutation analysis of the *GAMT* gene is also now available and is the confirmatory test for a diagnosis of GAMT deficiency. The *GAMT* gene is located on chromosome 19p13.3 and consists of 6 exons. Fifteen different types of mutations including nonsense, splice-site as well as small deletions and insertions have been reported so far. Ten out of the 29 patients known to have this disorder are of Portuguese origin and in 17 out of the 20 Portuguese alleles, the c.59G>C; (p.W20S) mutation was found [3].

We present here the clinical, biochemical and molecular data on eight additional, non-Portuguese patients with GAMT deficiency. Given the paucity of data on patients with GAMT deficiency we aim to further this knowledge with these 8 patients.

Materials and methods

Patients and DNA

Patients were initially diagnosed at various centers in the US and Canada based on their biochemical analysis of low creatine and elevated GAA. Blood samples were sent to one of three laboratories, Baylor Medical Genetics Laboratories, Houston, TX, USA, Department of Clinical Chemistry, Metabolic Unit, De Boclelaan 1117, HV Amsterdam, The Netherlands and the Kennedy Krieger Institute, Baltimore, MD, USA. Genomic DNA was extracted according to standard protocols. The respective physicians in different centers in the US and Canada were then contacted and clinical, biochemical and radiological data were collected after informed consent was obtained from the families of the respective patients, in accordance with the respective Institutional Review Boards.

Biochemical analysis

Creatine and guanidinoacetate analyses were performed using selected mass transitions on a Micromass Quatro tandem mass spectroscopy or by gas chromatography/MS after toluene extraction, derivatization and quantitation by chemical ionization with selective ion monitoring.

Molecular analysis

GAMT gene sequence-specific oligonucleotide primers for the *GAMT* gene, which were linked to the M13 universal primer sequences at the 5′-ends, were designed to amplify the six coding exons including at least 50 intronic nucleotides flanking both ends of each exon. PCR products were generated using FastStart DNA polymerase (Roche, Indianapolis, IN) and purified using ExcelaPure 96-well UF PCR purification plates (Edge BioSystems, Gaithersburg, MD). Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing kit (version 3.1, Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI3730XL automated DNA sequencer with the Sequencing Analysis Software v5.1.1 (Applied Biosystems, Foster City, CA, USA). The sequencing results were compared to the GenBank *GAMT* sequence (NT_011255) by using the Mutation Surveyor version 2.61. For patient number 6, 7 and 8, mutation analysis was performed by Dr. G. Salomons and Prof. C. Jakobs at VU University Medical Center, Metabolic Unit, Amsterdam, The Netherlands.

Results

Seven affected patients and one newborn sibling of an affected patient were seen in different centers in the US and Canada. All patients were products of non-consanguineous relationships and came to clinical attention at various ages ranging from 0 months to 14 years (Table. 1).

Table 1
Clinical features with age at diagnosis in our patient cohort.

Patient	Patient/ethnicity	Age at diagnosis/ gender	Developmental delay	Seizures	EEG findings	Hypotonia	Ataxia	Other
1	Irish/Scottish/German	8 year/F	+	+	Diffuse epileptic encephalo pathy	+	+	Regression; myoclonic epilepsy; speech delay; autistic features; dysphagia; poor weight gain
2	English	6 year/F	+	+	Report not available	+	+	Speech delay
3	English/Scottish/ German	13 year/M	+	+	Generalized epileptiform activity	–	–	Speech delay
4	Scottish/English/ German/Irish/ Italian/French	2 year/M	+	–	Not done	+	–	Speech delay; poor weight gain
5	French Canadian	3 year/M	+	+	Slow dysrythmia	+	–	Speech delay
6	Italian/English/ German/Polish[a]	6 year/F	+	–	Not done	+	+	Speech delay; autistic features
7	Italian/English/ German/Polish[a]	0 year/M	+	–	Not done	+	–	Treated since DOL#8
8	Egyptian	14 year/M	+	–	Not done	+	+	Regression; autistic features; dysphagia; no speech; poor weight gain; dystonia

[a] Patients 6 and 7 are siblings.

All of our patients had moderate to severe developmental delay with complete absence to minimal speech development. Two patients had regression of skills. In Patient 1, the onset of speech regression with personality changes occurred 10 months prior to her diagnosis of GAMT deficiency at the age of 7.5 years. Her seizures had increased in frequency at that time. In Patient 8, worsening of symptoms occurred at the age of 14 years with his gait becoming so unsteady as to require him to be wheelchair bound. At this time his intellectual and communication skills regressed and the worsening of his clinical status led to the final diagnosis of GAMT deficiency. Seizures developed in 4/8 patients.

Characteristic findings seen on Brain MRI in GAMT deficient patients are bilateral hyperintensities of the globi pallidi and/or delayed myelination. As shown in Table 2, 3 out of 7 of our affected patients had normal brain imaging by MRI. The abnormalities in the remainder 4 patients are described in Table 2. Patient 1 had a Chiari I malformation which is likely an incidental finding and not associated with the diagnosis of GAMT deficiency. On the other hand, MRS is a noninvasive tool to study various components of energy metabolism in the brain. MRS of the brain of patients with GAMT deficiency revealed a strongly reduced creatine peak with a new broad peak at 3.8 ppm which corresponds to GAA [2]. MRS findings on our patients are also described in Table 2. These exams were done at the time of diagnosis of GAMT deficiency before treatment was initiated. All of the seven affected patients who had a brain MRS, showed typical decreases in their creatine peak with four of them also showing a typical GAA peak.

Although clinical manifestations of GAMT deficiency are heterogeneous, specific laboratory parameters have been clearly established. Decreased levels of creatine in plasma and urine and especially in the cerebrospinal fluid are characteristic [8]. Table 3 demonstrates the low levels of serum creatine and the high levels of GAA in our patients, measured at the time of diagnosis as expected in patients with GAMT deficiency. Creatine and GAA values, after treatment taken as outlined in Table 3, are also listed. The analysis of GAA in plasma is superior to its analysis in urine due to the variability of urine creatinine in subjects with GAMT deficiency [9]. Mutation analysis of the *GAMT* gene revealed five novel mutations (Table 2). Most of the mutations identified are loss-of-function mutations which lead to truncated protein.

Patient 1 had onset of seizures at a later age of 5 years which gradually progressed to myoclonic epilepsy at the age of 8 years. She was on multiple anti-convulsants without much improvement. After treatment was initiated with creatine and L-ornithine supplementation in addition to arginine restriction, the myoclonic epilepsy resolved and she began to develop language. Patient 3 developed only one episode of seizure at the age of 12 years at the time of diagnosis of GAMT deficiency. He was then placed on the standard therapeutic regimen of L-ornithine and creatine and has had no seizures since (Table 3).

Discussion

GAMT deficient patients have a non-specific phenotype with a variable age of onset. Only a small number of patients have been reported in the literature thus far, arguing for a low incidence of GAMT deficiency. However, the diagnosis may be overlooked because of unawareness of this disorder amongst clinicians. In our series, developmental delay was the first clinical manifestation that was noted. In particular, speech delay was noted in 7 out of 8 patients. Patient 7 is now 11 months old and has not acquired speech yet. These are usually the first symptoms that bring the patients to clinical attention.

Seizures usually develop from the age of 10 months to 3 years in GAMT deficient patients [3]. These include myoclonic, generalized tonic–clonic, sporadic partial complex seizures, head nodding and drop attacks. In severe cases, the seizures are refractory to anti-convulsant treatment. Of interest, Patient 1 in our cohort developed seizures at the age of 5 years, progressing to myoclonic epilepsy at the age of 8 years. She had a clinical presentation reminiscent of the myoclonic epilepsy of Unverricht and Lundborg (MIM 254800). This is an autosomal recessive disorder caused by mutations in the cystatin B gene. Onset of this disorder occurs between 6 and 13 years of age with convulsions. Myoclonus begins 1–5 years later. Signs of cerebellar ataxia occur later in the course. Motor impairment and mental deterioration as well as dysarthria are also observed. In addition, GAMT deficiency needs to be considered in the differential diagnosis of additional disorders that may present with myoclonic epilepsy including neuronal ceroid lipofuscinosis type A, sialidosis type 1, Gaucher disease type III, Lafora disease and MERRF syndrome.

Seizures have commonly been included in the clinical spectrum of GAMT deficiency; however, it is interesting to note that 3 out of our 7 affected patients had not developed seizures at the time of their diagnosis. Of particular note is Patient 8, who was diagnosed at a later age of 14 years due to his developmental delay and regression as well as dystonia, but had no history of seizures. In

Table 2
Biochemical, radiological and molecular data.

Patient	Age at diagnosis /gender	S.Cr (before treatment)	S.Cr (after treatment)	S.GAA (before treatment)	S.GAA (after treatment)	MRI findings	MRS findings	Genotype	
								Allele 1	Allele2
1	8 year/F	2.8	875.3	9.1	2.8	Normal; Chiari I malformation	Decreased creatine peak with elevated GAA peak	c.327G>A[a]	c.327G>A[a]
2	6 year/F	6	447	16.6	9.8	Normal	Small creatine peak with evidence of GAA peak	c.403G>T (p.D135Y)	c.507_521dup15 (p.C169_S173dup)
3	13 year/M	20.3	429.3	13.9	6.4	Normal	Absent creatine peak	c.327G>A[a]	c.297_c.309dup13 (p.P104PfsX27)
4	10 month/M	1.2	1173.1	14.3	3.7	Bright globus pallidi on diffusion weighted imaging with normal T1/T2	Decreased creatine peak	c.327G>A[a]	c.522G>A (p.W174X)
5	3y/M	6	101.3	8.52	5.85	2 mm T2 hypersignal in pons	Decreased creatine peak and elevated GAA peak	c.327G>A[a]	c.36_c.37ins26 (p.G13PfsX38)
6^	6 year/F	44.3	402	27.6	6.5	Small corpus callosum, hyperintense globus pallidus, delayed myelination	Absence of creatine peak	c.327G>A[a]	c.327G>A[a]
7[b]	0 year/M	33.1	161	14.7	8.89	Not done	Not done	c.327G>A[a]	c.327G>A[a]
8	14 year/M	4.2	42	33.3	8.63	Normal	Decreased creatine peak with slight elevation of GAA peak	c.402C>G (p.Y134X)	c.610_611delAGinsGAA (p.R204EfsX63)

Normal values: serum creatine: 34–130 uM/L; serum GAA: 0.7–5.4 uM/L.
[a] Last base of exon, splice-site mutation.
[b] Patients 6 and 7 are siblings.

addition, patient 3 had only one episode of seizures at the age of 12 years. This finding underscores the importance of considering a diagnosis of GAMT deficiency even without the presence of a history of seizures.

Although characteristic brain MRI findings are known to be associated with GAMT deficiency, they are not diagnostic in some cases. Initially, the MRI may be normal [9,10] As reported by Schulze et al. [11], brain MRI was normal in a male patient diagnosed with GAMT deficiency at the age of 26 years. Although their patient had developed the full blown spectrum of clinical symptoms of GAMT deficiency, his normal MRI argued against establishing a correlation of brain MRI findings to either duration or severity of the disease. Our findings also corroborate this observation.

Confirmation of the diagnosis of GAMT deficiency by molecular testing has become available in the recent years. The most frequent mutation observed in our patient cohort is the c.327G>A. This appears to be a pan-ethnic mutation. This finding corroborates the data shown by Mercimek et al. [4]. In their analysis of 27 patients, they found 4 patients from four unrelated families of various ethnic origins with this mutation. In our cohort, six/seven affected unrelated families carried this splice-site mutation with two families being homozygous. We have calculated allele frequencies in Table 4. As expected, the frequency of the splice-site mutation, c.327G>A is approximately 30%. In addition, the majority of mutations known to occur in GAMT deficient patients are null mutations (65%), thus leading to loss-of-function. The most common missense mutation is the Portuguese common mutation, p. W20S [3].

Fig. 2 depicts the spectrum of *GAMT* mutations reported in the literature along with the five novel mutations from our study. It has been difficult to establish a clear genotype–phenotype correlation as the clinical phenotype is complex and additional genetic or epigenetic factors might add to the final determination of the clinical

Table 3
Treatment regimens and duration along with changes in clinical features secondary to the treatment.

Patient	Age at start of treatment	Duration of treatment	Creatine (mg/kg/day)	Ornithine (mg/kg/day)	Arginine restriction	Change in clinical features
1	8 year	1 year	800	800	Yes	Improved attention span, no seizures, follows commands, speaks two word sentences
2	7 year	3 year	260	No	No	Decrease in seizure activity, no jerky movements, more attentive, speaks single words
3	12 year	1 year	600	200	Yes	Improvement in fine motor skills, increase in vocabulary
4	10 month	21 month	348	217	Yes	Improved tone, more alert, speaks two word sentences
5	3 year	1 year	400	Not known	Yes	No seizures, speaks few words
6	2.5 year	4.5 year	600	800	No	Improved motor skills, started walking, improved tone, improved autistic features
7	DOL 8	11 month	600	600	No	Central hypotonia, developmental delay persists
8	14 year	2.5 year	130	200	Yes	Regression halted, improved alertness and weight gain, speaks few words

Table 4
Frequency of alleles in the *GAMT* gene.

Mutations	Frequency (%)
c.36_c.37ins26[a]	1 (1.66)
c.59G>C (p.W20S)	13 (21.66)
c.59_64insG	2 (3.33)
c.152A>C (p.H51P)	1 (1.66)
c.160G>C (p.A54P)	2 (3.33)
c.297_309dup13[a]	2 (3.33)
g.1637_1787del151	2 (3.33)
c.327G>A[a] (splice-site)	19 (31.66)
c.402C>G (p.Y134X)[a]	1 (1.66)
c.403G>T (p.D135Y)[a]	1 (1.66)
c.486_491insG	3 (5)
c.486_491delG	2 (3.33)
c.506G>A (p.C169Y)	2 (3.33)
c.507_521dup15(p.C169_S173dup)[a]	1 (1.66)
c.521G>A (p.W174X)	2 (3.33)
c.521_526insG	1 (1.66)
c.522G>A[a] (p.W174X)	1 (1.66)
c.571-3C>G	1 (1.66)
c.590T>C (p.L197P)	2 (3.33)
c.610_611delAGinsGAA (p.R204EfsX63)[a]	1 (1.66)
Total	60 (100)

Frequency of null mutations: 65%; Frequency of missense mutations: 35%.

[a] Mutations found in our patients.

phenotype. However, the severity of the clinical phenotype does not seem to correspond to the type of mutation. As more patients are reported, it may become possible to predict the phenotype by means of mutational analysis.

Treatment of GAMT deficiency aims at increasing creatine and decreasing GAA levels in the central nervous system by oral supplementation with creatine monohydrate and by dietary restriction of arginine. Moreover, low dose L-ornithine supplementation (100 mg/kg/day) prevents shortage of arginine to the urea cycle while higher doses (800 mg/kg/day) may have an additional effect by further decreasing GAA levels due to feedback inhibition of AGAT [4]. Therapy can be monitored by determination of creatine and GAA concentrations in urine and plasma and by brain MRS studies. Follow-up of our patients after treatment revealed improvement of symptoms, although none of the patients demonstrated a complete reversal of symptoms (Table 3). An interesting observation is that after initial dramatic improvements in the clinical condition, most of the patients reach a plateau wherein no further improvement is noticeable. However, it would be difficult to extrapolate firm conclusions from our data since it was collected retrospectively and the therapy in these patients was not followed as part of a standardized longitudinal study using the same regimen for creatine and L-ornithine supplementation and protein restriction.

Presymptomatic treatment of a neonate diagnosed with GAMT deficiency at birth resulted in normal development with no manifestations of GAMT deficiency [12]. However, in our Patient 7, who was diagnosed at birth and treated from DOL 8, developmental delay was noticed at 6 months of age. He was treated on a dose of 400 mg/kg/day of creatine and L-ornithine [4], however it is possible that this dosage was not adequate for him or that the parents were non-compliant. Moreover, he was not on arginine restriction which could also explain the failure of his treatment. Since arginine is the rate-limiting substrate for GAA synthesis, it is essential to restrict the intake of arginine through diet as well [13]. In comparing the phenotypes of our two siblings with those described by Schulze et al. [12], it appears that our Patient 6 had a more severe phenotype with basal ganglia changes compared to the affected brother of the presymptomatic neonate described by Schulze et al. Their 6 years old boy was categorized as a having a milder phenotype {he is Patient 9 in Mercimek-Mahmutoglu's paper [4]}. In addition, our siblings had very high amounts of GAA levels compared to other patients in our cohort (Table 2). Thus, it is likely that the severe underlying phenotype has led to the unsatisfactory outcome in Patient 7. It is also possible that there could be another etiology for his developmental delay which has not been evaluated yet.

Given the irreversibility of intellectual dysfunction that develops with creatine depletion in the brain, early or presymptomatic initiation of treatment may be warranted and guidelines for prenatal diagnosis, newborn screening and presymptomatic testing need to be formulated. Bodamer et al. have described the use of isotope dilution electrospray tandem mass spectrometry (ES-MS/MS) assay for the simultaneous measurement of GAA and creatine concentrations from filter paper blood spots, which would allow for high-throughput of samples, thus making this a likely assay for neonatal screening [8]. However, more studies are required to establish whether this technique would be specific and sensitive for newborn screening. Positive test results should always be followed-up and the diagnosis confirmed with molecular testing.

With the addition of our 8 patients to the 29 patients reported in the literature, we aim to further characterize this seemingly rare albeit under diagnosed disorder. It is important to consider GAMT deficiency in patients with non-specific developmental delay with seizures, ataxia and extrapyramidal signs and to exclude this disorder in patients presenting with progressive myoclonic epilepsy. In addition, as evidenced in our cohort of patients, the presence of seizures should not be a requisite for suspecting or testing for GAMT deficiency. An awareness of this disorder is essential since diagnosis can be easily made with biochemical tests and confirmed by sequencing of the *GAMT* gene. This will also help initiate early treatment to limit the cognitive impairment observed in this condition.

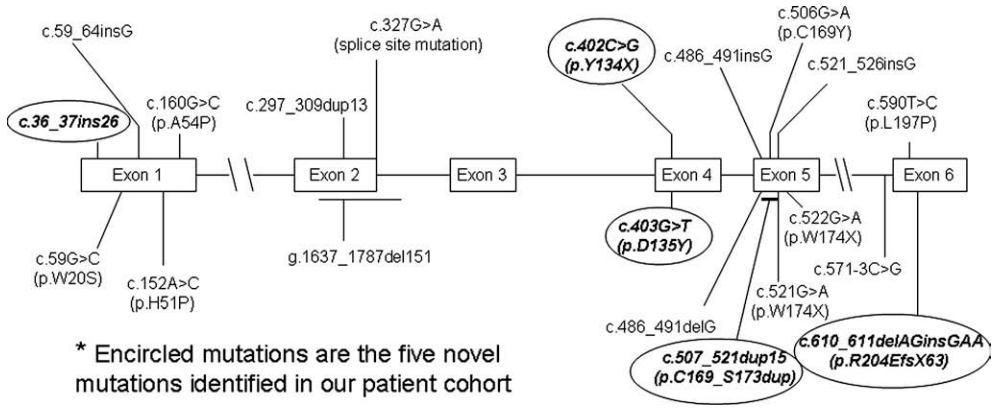

Fig. 2. Showing the five novel mutations in the GAMT gene found in our patients with the known existing mutations reported in the literature [3,4].

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