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INTRODUCTION

In Western countries, mental retardation (MR) affects about 3% of the general population. For the majority of the cases of inherited MR, the genetic causes are not yet elucidated.

Accurate diagnosis is important because it has implications regarding treatment (in potentially treatable disorders), prognosis, genetic counseling of families.

Patients with creatine deficiency disorders (CDD) may present with MR/developmental delay as well as expressive speech and language delay, autism and epilepsy. They represent a group of treatable inborn errors of creatine biosynthesis (L-arginine-glycine amidinotransferase - AGAT and guanidinoacetate methyltransferase - GAMT) and transport (SLC6A8, CT1) across the blood brain barrier.

PATIENTS AND METHODS

A cohort of 6,761 children and young adults with MR were studied for defects in creatine metabolism. We started with the determination of guanidinoacetate (GAA) and creatine in urine samples by GC-MS-SIM.

DNA mutation analysis was performed in all suspected cases (with abnormal levels of GAA and creatine).

In 2007 the Newborn Screening (NBS) lab performed a pilot study in order to evaluate the variability GAA values in our newborn population and the feasibility of screening GAMT deficiency.

RESULTS

Urine biochemical analysis revealed eight cases compatible with GAMT deficiency (Table 1) and 15 patients suggestive of a defect in SLC6A8 (Table 2). All GAMT deficient patients show the same mutation which suggests a founder effect in our population. SLC6A8 deficiency patients revealed a large spectrum of mutations.

The NBS analysis of GAA revealed an average of 1.19 μ moles/L (standard deviation: 0.62) in Portuguese population.

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Table 1: Biochemical and molecular data of GAMT deficient patients.

Patient	Age (years)	Gender	GAA (μ mol/mmol crn)	Cr (μ mol/L)	Mutation analysis	Protein
GAMT-1	16	Male	827 \uparrow	456	c.59G>C	p.Trp20Ser
GAMT-2	20	Male	406 \uparrow	366	c.59G>C	p. Trp20Ser
GAMT-3	21	Male	423 \uparrow	337	c.59G>C	p. Trp20Ser
GAMT-4	19	Male	546 \uparrow	462	c.59G>C	p. Trp20Ser
GAMT-5	15	Male	1230 \uparrow	78	c.59G>C	p. Trp20Ser
GAMT-6	9	Female	1064 \uparrow	110	c.59G>C	p. Trp20Ser
GAMT-7	12	Female	911 \uparrow	129	c.59G>C/c.521G>A	p. Trp20Ser.Trp174X
GAMT-8	6	Male	1480 \uparrow	206	c.59G>C	p. Trp20Ser

Controls: 5-11y: GAA: 18-130; Cr: 146-8560; >12y: GAA: 18-130; Cr: 142-5952

Table 2: Biochemical and molecular data of SLC6A8 deficient patients.

Patient	Age (years)	Cr (μ mol/L)	Cr/Crn	Mutation analysis	Protein
SLC6A8-1	4	12 337	5.87	c.IVS11+1G>A, intron 11	(*)
SLC6A8-2	8	19 684	3.45	c.1261G>C, exon 9	p.Gly421Arg (*)
SLC6A8-3	3	4680	4.25	c.1A>G, exon 1	p.Met1? (*)
SLC6A8-4	12	11 889	2.90	c.1169C>T, exon 8	p.Pro390Leu (*)
SLC6A8-5	4	5922	4.93	c.1222_1224delTTC, exon 8	p.Phe408del (N)
SLC6A8-6	2	5371	3.58	c.1432dupG, exão 10	p.Ala478GlyfsX24 (*)
SLC6A8-7	2	10 092	2.80	c.884_885delCT, exon 5	p.Pro295ArgfsX169 (*)
SLC6A8-8	15	4932	2.24	c.1456C>T, exon 10	p.Gln476X (*)
SLC6A8-9	15	5891	1.84	c.1456C>T, exon 10	p.Gln476X (*)
SLC6A8-10	6	6098	2.26	c.1661C>T, exon 12	p.Pro554Leu (?)
SLC6A8-11	2	20 475	9.31	c.986G>T, exon 6	p.Ser329Ile (N)
SLC6A8-12	5	27 807	7.72	c.321_323delCCT, exon 2	p.Phe107del
SLC6A8-13	4	30 996	6.20	c.1299_1309del, exon 9	p.Pro434LeufsX27 (*)
SLC6A8-14	6	4009	2.00	c.355G>T, exon 2	p.Gly119Cys (?)
SLC6A8-15	4	10 590	2.71	No mutation found	(?)

(*) - carrier mother; (N) - Non carrier mother (?) - DNA analysis of the mother not available
Controls: <4y: Cr: 140-7910; Cr/Crn: 0.04-1.51; 5-11y: Cr: 146-8560; Cr/Crn:0.04-1.07; >12y: Cr: 142-5952; Cr/Crn: 0.04-0.56

DISCUSSION

So far, 23 patients with CDD were identified in our laboratory (1:294). We believe these defects are still underdiagnosed, so the possibility should be considered in all children affected by unexplained MR, seizures, and speech delay. SLC6A8 defect should also be considered in males with MR and negative fragile-X testing.

Therapeutic intervention has been effective in moderating the biochemical abnormalities reported in GAMT deficient patients and has had a positive impact on some symptoms.

Case studies reporting excellent neurodevelopmental outcomes in infants treated from birth stress the importance of early diagnosis and intervention for this disorder. Accordingly, GAMT deficiency has long been considered a promising candidate for inclusion in NBS programs.

Our pilot study was stopped due the high false GAA positives rates and the inability to detect true positive cases. These findings are in agreement with other NBS pilot studies carried out in countries such as USA, Austria, Canada and Italy.

A new approach to GAMT screening is needed which includes cut-offs, algorithms and second-tier test.

There is still no evident effective treatment for SLC6A8 deficiency, it is however important to female siblings with intellectual disability.