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Two cases of late-onset Argininosuccinic aciduria with normal results at newborn screening

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Introduction

Argininosuccinic aciduria (ASA; OMIM 207900) is an autosomal recessive metabolic disorder caused by argininosuccinate lyase (ASL; MIM 608310) deficiency, and it is the second most frequent urea cycle disorder (UCD), with an estimated frequency of 1:70 000.

The human ASL gene (MIM 608310) is located on chromosome 7q11.21 and comprises 16 exons encoding a 464 amino acids long monomer. The enzyme is functional in a homotetrameric structure and is mainly expressed in the liver, although it can be found in several other tissues (Hu et al. 2013).

The clinical presentation of ASA is very heterogeneous, ranging from asymptomatic to severe hyperammonemic neonatal-onset cases. Complex clinical phenotypes, with neurological deficits and hepatic complications adding to hyperammonemic episodes, are often observed (Nagamani et al. 2012).

Biochemically, ASA is usually characterized by elevation of both citrulline and argininosuccinic acid in plasma and urine, but also at this level heterogeneity is observed, adding to a poor correlation found between residual enzymatic activity and the severity of the clinical phenotype.

ASA newborn screening (NBS) is done in Portugal since 2004, and two cases of late-onset ASA were missed due to normal citrulline and argininosuccinic acid levels at sampling time.

Material and Methods

The Portuguese NBS Program started to include tandem mass spectrometry (ms/ms) analysis for inborn errors of metabolism screening in 2004. Amino acids and acylcarnitines are analyzed as butyl esters (Rashed et al. 1995), using two API 2000 triple quadrupole tandem mass spectrometers (Applied Biosystems, Sciex), and quantified using internal standards from Cambridge Isotope Laboratories, Inc..

ASA is screened according to Table 1. Argininosuccinic acid, citrulline and arginine quantification are performed by multiple reaction monitoring (MRM) experiments as indicated in Table 2.

Approximately nine hundred thousand newborns were already screened by ms/ms, through the analysis of blood samples collected between the 3rd and the 6th days of life on Whatman 903 filter paper.

Positive cases were confirmed by amino acids analysis in urine and molecular analysis, according to standard protocols.

Table 1 – Positive screening criteria for Argininosuccinic aciduria

Primary marker	Secondary markers
Argininosuccinic acid >1.1 µM	Citrulline >50 µM
	Citrulline/ Arginine > 10

Table 2 – ms/ms detection of argininosuccinic acid, citrulline and arginine

Compound	Internal standard	MRM transition (m/z)
Arginine	$^{2}H_4$; 5-^{13}C-Arginine	231→70
Citrulline	$^{2}H_2$-Citrulline	232→113
Argininosuccinic acid	$^{2}H_2$-Citrulline	459→70

Results

Four ASA patients were screened (Table 3): two cases were identified by NBS (patients 1 and 3) and two brothers presented normal results at NBS sampling time (patients 2 and 4).

These brothers presented a late-onset form of ASA and the study of ASL gene revealed homozigosity for R12Q mutation.

Table 3 – Patients characterization and NBS results

Patient	Sex	Actual age	Age at NBS	Age at diagnosis	NBS result	Molecular study
1	F	8y	4d	6d	Cit=186µM (N<50µM) ASA=134,0µM (N<1,1µM) Cit/Arg= 39,0 (N<10)	c.446+1G>A[a] / p.Q286R[b]
2	M	5y	4d	16m	Cit=23µM (N<50µM) ASA=0,90µM (N<1,1µM) Cit/Arg= 1,5 (N<10)	p.R12Q[c] / R12Q
3	F	4y	11d	1m	Cit=34µM (N<50µM) ASA=5,4µM (N<1,1µM) Cit/Arg= 3,7 (N<10)	p.R379C[d] / p.R379C
4	F	8m	3d	3w	Cit= 24µM (N<50) ASA= 0.26µM (N<1.1) Cit/Arg= 2.1 (N<10)	p.R12Q / R12Q

Patients 2 and 4 are brother and sister.
M–male; F-female; y-years; m-months; d-days; w-weeks; [a] Linnebank et al. 2002; [b] Walker et al. 1990; [c]Sampaleanu et al. 2001; [d] Kleijer et al. 2002

Discussion

Patient 2, which is the oldest brother of patient 4, started to be investigated in the first months of age due to developmental delay. Plasmatic amino acids quantification was performed during his first year of live, with a consistent slight increase in citrulline, but only at 16 months the detection of a small amount of argininosuccinic acid in plasma raised the suspicious of a late-onset form of ASA.

This diagnosis was confirmed by identification of R12Q mutation, in homozygosity, in ASL gene. This mutation was reported to be associated with a mild clinical form of the disease and frequently found in late-onset ASA cases (Balmer et al. 2013).

Last June a sister of this patient was born. In spite of the normal NBS biochemical result (Table 3), and due to her brother's history, we recommended the molecular study, which also revealed homozygosity for R12Q mutation. This girl immediately started treatment and to date is clinically well, without any ASA symptoms, and thus reinforcing the importance of early treatment for this disease.

Newborn screening for ASA is widely established although some paradoxal results can be obtained due to the clinical and biochemical ASA heterogeneity: asymptomatic cases can be detected and, on the contrary, late-onset forms with important clinical manifestations observed since the first months of live can be missed due to normal biochemical results.

Conclusions

ASA NBS utility is firmly established due to the benefits that early therapy can bring to the patients. Nevertheless, care must be taken due to the possibility of missing late-onset cases.

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