Identification of newborns with galactosemia based on altered amino acids profile in the metabolic newborn screening

Marcão A[1], Carvalho I[1], Sousa C[1], Fonseca H[1], Rocha H[1], Lopes L[1], Neiva R[1] and Vilarinho L[1]

[1]*Unidade de Rastreio Neonatal Metabolismo e Genética, Departamento de Genética Humana do Instituto Nacional de Saúde Doutor Ricardo Jorge, Porto*
ana.marcao@insa.min-saude.pt

INTRODUCTION

Galactosemia may be due to three distinct enzymatic deficiencies which constitute the Leloir pathway of galactose metabolism. Classic galactosemia (CG, OMIM #230400) is an autosomal recessive disorder caused by deficient activity of galactose-1-phosphate uridyltransferase (GALT), the second enzyme in this pathway and that is responsible for the interconversion of galactose-1-P to glucose-1-P. CG is the most frequent form of galactosemia; clinically, it presents in the neonatal period, with acute manifestations following milk intake, and is often fatal during infancy. Galactose restricted diet, the only currently available therapeutic strategy, can prevent or resolve the acute symptoms, but it is ineffective in preventing long-term complications, even in the cases with early identification and treatment (1, 2).

The non-existence of a long-term effective treatment negatively affects the cost – benefit ratio of galactosemia Newborn Screening (NBS) and it is the main reason why it is only included in a minority of European NBS programs. In spite of this, a significant number of galactosemia cases are identified in programs not including it, due to the differential diagnosis of other pathologies. Also in Portugal this has been happening since the beginning of the Phenylketonuria (PKU) screening in 1979.

With this work, we intend to analyze all cases of CG that come to our knowledge since the inclusion of *tandem* mass spectrometry technology in the Portuguese NBS laboratory, trying to understand the reasons which may dictate normal or abnormal results in the metabolic NBS.

MATERIAL AND METHODS

Since 2004, the newborns are screened in Portugal for metabolic diseases, by *tandem* mass spectrometry. Dried blood spots are collected between the 3rd and the 6th days of life and analyzed for amino acids and acylcarnitines (3). Phenylalanine, tyrosine and methionine are primary markers of four of the 24 metabolic diseases screened (Table 1) and are among the analyzed amino acids. All samples presenting a combination of increased values of phenylalanine plus tyrosine or methionine are selected for enzymatic total galactose determination using the Neonatal Total Galactose Screening Assay from ZenTech. The cases with increased total galactose (N<5 mg/dL) are considered suspect for galactosemia and are urgently reported to the reference metabolic treatment center closer to the newborn' s residence, for clinical evaluation and diagnosis confirmation.

Table 1 – Screened metabolic disorders with phenylalanine, tyrosine or methionine as primary markers

Metabolic disease	Primary markers
Phenylketonuria	Phe (↑) and Tyr (↓)
Tyrosinemia	Tyr (↑)
Classical Homocystinuria	Met (↑)
MAT I/III deficiency	Met (↑)

RESULTS

Since 2004, 1,016,168 newborns were screened for 24 metabolic disorders. During this time, 18 positive cases for CG come to our knowledge (Table 2), but only eight of these, shaded blue in Table 2, presented NBS results suggestive of CG.

Table 2 – NBS and molecular results of patients with galactosemia

Case number	Age at screening (d)	Gal (N<5 mg/dL)	Tyr (N<210 µM)	Phe (N<150 µM)	Met (N<57 µM)	*GALT* gene
1	4	259	663	127	37	not available
2	1	50	1870	324	183	not available
3	5	132	159	74	21	not available
4	5	248	914	123	41	not available
5	not available	350	1072	332	57	not available
6	3	134	96	61	21	not available
7	10	23	101	40	27	not available
8	5	575	213	119	44	not available
9	2	183	163	88	32	not available
10	5	410	801	153	51	not available
11	4	280	266	164	41	not available
12	6	456	965	471	46	p.Q188R/p.Q188R
13	4	66	555	181	55	p.Q188R/p.Q188R
14	not available	121	735	301	71	IVS8 ds +13 A-G/ IVS8 ds +13 A-G
15	4	60	99	60	22	IVS2 as -2 A-G/ p.Y323C
16	3	127	558	138	56	p.Q188R/p.K285N
17	4	84	236	110	18	not available
18	4	36	59	43	21	p.Q188R/p.T350I

DISCUSSION

The Portuguese NBS program does not include CG screening. However, since the beginning of PKU screening in 1979 several cases have been identified due to increased Phe levels and after 2004, with the expanded metabolic screening, due to increased combined levels of Phe, Tyr and Met. The analysis of NBS results of the 18 CG cases that come to our knowledge since 2004, reveals that less than 50% of the cases presented abnormal results. We tried to correlate the abnormal results with the newborn' age at screening, but doesn't seem to exist any correlation. We also tried to correlate these results with the mutation present in the GALT gene, but we don't have enough data.

CONCLUSION

During this period, 2004-2017, 44% of GC cases were identified by NBS.

The possibility of a galactosemia diagnosis must always be considered for newborns presenting compatible clinical signs, even in the presence of a normal metabolic NBS result.

REFERENCES
1 – Pasquali *et al.*, Genetics in Medicine, 2018.
2 – Coelho *et al.*, J Innherit Metab Dis, 2017.
3 - Rashed MS *et al.*, Pediatr Res, 1995.