Performance and Molecular Findings of the 1st Newborn Screening for Pompe disease and MPS-I in Spain

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Background:

Early detection of Pompe disease and mucopolysaccharidosis type I (MPS-I) enables timely therapeutic intervention, preventing irreversible organ damage. However, pseudodeficiencies and variants of uncertain significance may complicate screening workflows. We report the analytical performance, molecular results, and clinical correlation from the 1st official newborn screening program for these diseases in Spain.

Methods:

Enzymatic activity of acid α-glucosidase (GAA) and α-L-iduronidase (IDUA) was measured on dried blood spots using the NeoLSD™ MSMS kit intended for the quantitative measurement of the activity of the enzymes ABG, ASM, GAA, GALC, GLA and IDUA. (Figure 1).
A screening result is considered positive when the corresponding enzyme activity falls below the 5th percentile of the reference population distribution and at least three of the ratios with the other enzymes (i.e. GAA/GLA and IDUA/GLA as showed in results tables 1 and 2). As 2nd tier for MPS-I, glycosaminoglycan (GAG) quantification dermatan, heparan, and chondroitin/dermatan sulfate (DS, HS, CS/DS) was performed when IDUA activity was low by MS/MS spect. Infants with abnormal enzymatic results underwent molecular analysis in both cases (GAA or IDUA low activity) (Figure 2). The confirmatory analysis was performed by Illumina Genetic Analysis (Figure 3). From September 2024 to December 2025 we analyzed a total of **20,384 newborns**. (Figure 4).

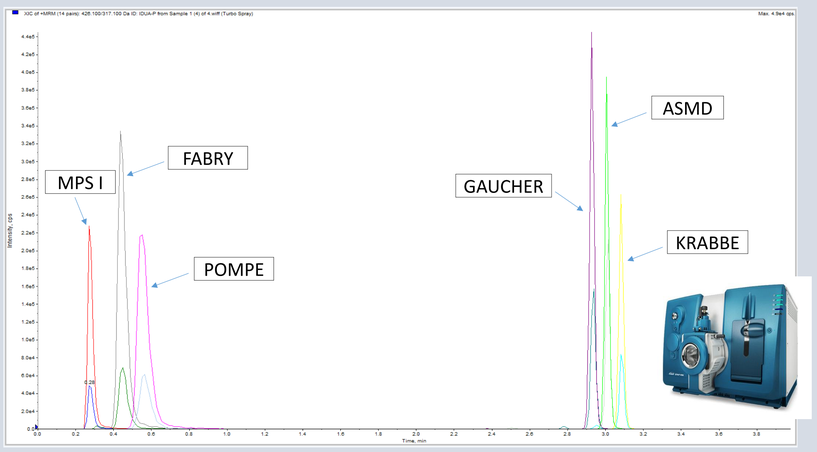

Figure 1. Enzyme spectrum of the MS/MS kit

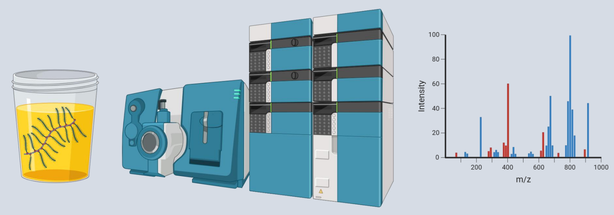

Figure 2. Mass spectrometry analysis method for urine samples for fractional determination of GAGs, using methanolysis method.

Figure 3. Genetic test, confirmed by Illumina Genetic Analysis

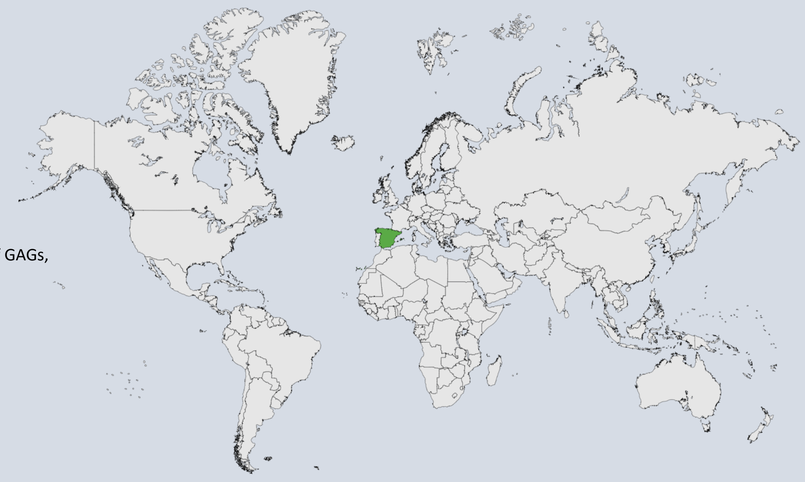

Figure 4. 1st year Neonatal Screening Spain (Galicia) 20,384 newborns

Results:

Pompe disease: Four infants showed low GAA activity. All cases were genetically confirmed, resulting in a **positive predictive value (PPV) of 100%**. The estimated prevalence was **1:5,096**. The common late-onset variant **c.-32-13T>G** was identified in multiple cases, including compound heterozygotes. Clinical follow-up showed cardiologic stability in most infants; however, previous reports have associated this genotype with earlier motor involvement and persistent hyperCKemia. Only one case presented light hyperCKemia.
MPS-I: Nine newborns (0.0004%) showed low IDUA activity. Eight had normal GAGs levels; among these, seven were classified as pseudodeficiencies and one as carrier. One infant (prevalence **1:20,384**) presented with elevated GAGs and pathogenic variants (Glu404* / Pro533Arg) and has already undergone transplantation. When GAG analysis was not included, the PPV was 14.3% reflecting a high frequency of pseudodeficiency (**1:2,912**). Incorporation of GAG quantification increased, both PPV and specificity to 100%.

POMPE	*SEX*	*PREGNANCY*	*GENE GAA*	CK UI/L	GAA µmol/L/h	GAA /GLA	GAA /ASM	GAA /GALC	GAA /GBA	GAA /IDUA
				42-232	*>2.6*	*>0.2*	*>0.2*	*>0.4*	*>0.2*	*>0.5*
4 CASES	Male	Full-term	c.277G>A (p.Ala93Thr) c.-32-13T>G (p.?)	**59**	**2.3**	0.4	0.2	1.6	0.1	0.2
	Female	Preterm dichorionic diamniotic twin pregnancy at 32 weeks	c.-32-13T>G in homozygous	**139**	**1.2**	0.0	0.1	0.1	0.1	0.1
	Male	Full-term	c.-32-13T>G in homozygous	**273**	**1.1**	0.1	0.1	0.4	0.1	0.2
	Male	Full-term	Pending	**222**	**0,8**	0,0	0,0	5,6	14,1	16,6
NO PSEUDODEFICIENCIES. NO CARRIERS										

Table 1.Results of the 4 cases detected as positive in the GAA analysis, where the ratios of other enzymes are used as a double control, the ratios used have been GAA/GLA, GAA/ASM, GAA/GALC, GAA/GBA, GAA/IDUA, at least 3 ratios below the reference values are needed to confirm the sample as a possible positive case, the blood samples were confirmed by genetic tests

MPS-I	*SEX*	*PARENTS ORIGIN*	*GENE IDUA*	IDUA µmol/L/h	IDUA /GBA	IDUA /GLA	IDUA /GALC	IDUA /GAA	IDUA /ASM	DS	HS	CS/DS
				>2.0	*>0.2*	*>0.1*	*>0.3*	*>0.3*	*>0.2*	*<30*	*<15*	*>1*
1 CASE	male	Venezuela	c.[1210G>T, c.1598C>G] p.[Glu404*, Pro533Arg]	**0.15**	0.01	0.01	0.04	0.01	0.01	**120.5**	**417.0**	**0.5**
7 PSEUDO-DEFICIENCIES	female	Spain	His82Glu; Ser269Cys	**1.12**	0.07	0.10	0.28	0.17	0.18	12.3	7.9	4.1
	male	Uruguay	His82Glu; Ser269Cys	**0.94**	0.12	0.13	0.43	0.14	0.24	7.7	8.3	3.6
	male	Cape Verde	Val322Glu; T99Ileu	**0.77**	0.15	0.02	0.03	0.04	0.11	21.4	12.7	2.8
	female	Cape Verde	Val322Glu; Ala79Thr	**0.72**	0.09	0.04	0.08	0.18	0.09	26.6	13.1	1.1
	female	Brasil	Pro357Leu; Ser586Phe	**0.55**	0.05	0.07	0.21	0.10	0.10	13.6	5.8	1.6
	male	Morocco	Pending	**0,38**	0,02	0,03	0,18	0,04	0,04	16,6	3	1,5
	female	Colombia	Pending	**0,68**	0,06	0,09	0,32	0,09	0,07	8,3	3,4	4,3
1 CARRIER	male	United States	c.1205G>A (p.Trp402*) in heterozygosity	**0,64**	0,15	0,13	0,35	0,10	0,06	16,4	2,7	1,5

Table 2. Results of the 9 cases detected with low activity in the IDUA analysis. One case was determined to be positive, where the ratios of other enzymes were used as a double control. The ratios used were IDUA/GBA, IDUA/GLA, IDUA/GALC, IDUA/GAA, and IDUA/ASM. This was confirmed with genetic studies and GAG analysis. Seven cases of pseudodeficiency were identified, with ratios of other enzymes below reference values. Genetic studies of the analyzed cases showed mutations described as pseudodeficiencies, but with normal GAG values. These cases are not considered MPS I. One final case was confirmed as a carrier due to low enzyme activity values, carrying a heterozygous MPS I mutation, but with normal GAG values.

Conclusions:

Newborn screening for Pompe disease demonstrated excellent diagnostic performance, with high specificity and reliable molecular confirmation. For MPS-I, pseudodeficiencies remain a major challenge, but combined enzymatic and GAG testing eliminates false positives and improves accuracy. These data support the integration of second-tier GAG analysis and reinforce the clinical value of early detection strategies.