Diagnosis of inborn errors of metabolism within the expanded newborn screening in the Madrid region

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Abstract

We present the results of our experience in the diagnosis of inborn errors of metabolism (IEM) since the Expanded Newborn Screening was implemented in our Region. Dried blood samples were collected 48 h after birth. Amino acids and acylcarnitines were quantitated by mass spectrometry (MS)/MS. Newborns with alterations were referred to the clinical centers for follow-up. Biochemical and molecular genetic studies for confirmation of a disease were performed. In the period 2011 to 2019, 592,822 children were screened: 902 of them were referred for abnormal results. An IEM was confirmed in 222 (1/2670): aminoacidopathies: 89 hyperphenylalaninemia (HPA) (51 benign HPA, 32 phenylketonuria, 4 DNAJC12 defect, and 2 primapterinuria), 6 hypermethioninemia, 3 tyrosinemia type 1 (TYR-1), 1 TYR-3, 4 maple syrup urine disease (MSUD), 2 branched-chain amino acid transferase 2 deficiency, 2 homocystinuria, 1 cystinuria, 2 ornithine transcarbamylase (OTC) deficiency, 2 citrullinemia type 1 (CTLN1); FAO defects: 43 medium-chain acyl-CoA dehydrogenase deficiency (MCADD), 13 very long-chain acyl-CoA dehydrogenase deficiency, 2 long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, 1 multiple acyl-coA dehydrogenation deficiency, 11 systemic primary carnitine deficiency, 2 carnitine palmitoyltransferase type 2 (CPT-II) deficiency, 1 CPT-I deficiency; organic acidurias: 12 glutaric aciduria type 1 (GA-1), 4 methylmalonic acidemia (MMA), 7 MMA including combined cases with homocystinuria (MMAHC), 6 propionic acidemia (PA), 7 3-methylcrotonyl-CoA carboxylase, 1 3-hydroxy-3-methylglutaryl-CoA lyase deficiency lyase deficiency. Only 19 infants (8.5%) were symptomatic at

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INTRODUCTION

Newborn screening (NBS) started in the early 1960s with Robert Guthrie. In our country, the first NBS program was implemented in Granada in 1968 on the initiative of Prof Federico Mayor Zaragoza. Since then, programs have certainly improved as new technologies have evolved, especially after the introduction of tandem mass spectrometry (MS/MS) in the early 1990s. MS/MS allows the simultaneous quantification of multiple analytes with high sensitivity and specificity, therefore it has been incorporated for inborn errors of metabolism (IEM) screening, providing the capability to detect over 50 conditions. However, countries have different approaches, and the situation varies considerably worldwide. In North America, a specific screening including which minimum IEM is established in all states. However, in the European Union, there is no agreement about which IEM should be screened. Furthermore, the protocols of the different countries vary in numerous aspects, from specimen collection to diagnosis, organization, follow-up, and treatment. In Spain, as in other European Countries such as Belgium, Bosnia-Herzegovina, Germany, Italy, or United Kingdom, the NBS program is carried out under the responsibility of different regions of the country. In 2013, the Spanish National Health System included, as mandatory, screening for seven genetic disorders, including four IEM (phenylketonuria [PKU], medium-chain acyl-CoA dehydrogenase deficiency [MCADD], very long-chain acyl-CoA dehydrogenase deficiency [LCHADD], and glutaric aciduria type 1 [GA-1]). However, in practice, NBS is determined by the policies of individual regions, so there is a lack of uniformity in testing for disorders at birth around the country. In the Community of Madrid, expanded NBS (ENBS) with MS/MS was initiated in 2011, including 17 metabolic disorders. In this article, the results of 9 years’ experience in the diagnosis of IEM, after the implementation of the ENBS in our region, are described. Clinical and demographic data of the patients, along with the biochemical and the molecular characterization are presented.

PATIENTS AND METHODS

Metabolic disorders included in our ENBS program were: benign hyperphenylalaninemia (HPA)/PKU, tyrosinemia type 1 (Tyr-1), maple syrup urine disease (MSUD), MCADD, LCHADD, very long-chain acyl-CoA dehydrogenase deficiency, systemic primary carnitine deficiency (SPCD), 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG-CoA lyase deficiency), GA-1, methylmalonic acidemia (MMA) including combined cases with homocystinuria (MMAHC), propionic acidemia (PA), isovaleric acidemia, and beta-ketothiolase deficiency. Furthermore, the different metabolites analyzed allows the identification of other IEM, such as homocystinuria, tyrosinemia type III, 3-methylcrotonyl-CoA carboxylase deficiency (MCG-3), and some urea cycle defects, which are reported when detected, although not included “per se” in the program.

Dried blood samples were collected 48 h after birth and dried at room temperature. Amino acids, acylcarnitines, and succinylacetone were quantitated by NeoBase non-derivatized MS/MS kit (PerkinElmer, Turku, Finland) from March 2011 to August 2018 and NeoBase 2 non-derivatized MS/MS kit (PerkinElmer) from that moment to December 2019. Tandem mass spectrometer employed was an Acquity TDQ UPLC/MS (Waters, Milford, MA) system. For data acquisition and processing, the applications...
MassLynx (Waters), Neolyx and Specimen Gate MSMS Data Suite (PerkinElmer) were used. Our program analytes and ratios were selected following the Clinical and Laboratory Standards Institute recommendation. Cut-off values were reviewed periodically. When the result for a newborn sample was flagged as above or under the cut-off value (99.5th or 1st percentile), the analysis was repeated in duplicate before it was reported. If the repeat test was slightly outside the normal range, a second sample was requested by the NBS laboratory. On the other hand, if the result was clearly pathological, the patient was referred to one of the four reference clinical centers distributed by demographic criteria (Hospital Universitario 12 de Octubre, Hospital Universitario Ramón y Cajal, Hospital Universitario La Paz, Hospital Infantil Universitario Niño Jesús) for management, counseling, and follow-up. Afterward, serum and urine samples were referred to the biochemical and molecular genetic laboratory (Centro de Diagnóstico de Enfermedades Moleculares), where they performed biochemical studies for confirmation of a disease, including plasma amino acids, acylcarnitines, urinary organic acid profiles, and molecular diagnosis thereafter to confirm the defect. Variants were classified following American College of Medical Genetics guidelines.

In this study, we have included all the patients diagnosed with an IEM, who were referred to the clinical units for an abnormal screening between March 8, 2011 when ENBS was established in the Community of Madrid, and December 31, 2019.

Demographic and clinical conditions of the patients were registered, including sex, gestational age, presence or not of consanguinity, parents’ country of origin, presence or not of symptoms at the time of the NBS result, biochemical and molecular diagnose, and if death occurred due to their pathology.

All these data were statistically analyzed with SPSS Statistics 24.

3 | RESULTS

During the period included, 592,822 children were included in the ENBS program, 902 of them were referred due to an abnormal screening result. An IEM was diagnosed in 1/2670 newborns, a remarkably similar rate was found in other studies.

During the period included, 592,822 children were included in the ENBS program, 902 of them were referred due to an abnormal screening result. An IEM was diagnosed in 1/2670 newborns, a remarkably similar rate was found in other studies.

IEM diagnosis included in the NBS program were 89 HPA (51 benign HPA, 32 classic PKU, 4 DNAJC12

defects, and 2 primapterinuria), 3 Tyr-1, 4 MSUD, 43 MCADD, 2 LCHADD, 13 VLCAD, 11 SPCD, 12 GA-1, 4 MMA, 7 MAMHC (6 cblC, 1 cblD), 6 PA, and 1 HMG-CoA lyase deficiency. No cases of isovaleric acidemia nor beta-ketothiolase deficiency were detected. Other metabolic conditions detected were: 6 hypermethioninemia, 1 tyrosinemia type 3, 2 branched-chain amino acid transferase deficiencies (BCAT-2), 2 homocystinuria (cystathionine beta-synthase deficiencies), 1 cystinuria, 2 ornithine transcarbamylase (OTC) deficiencies, 2 citrullinemia type I (CTLN1), 1 multiple acyl-CoA dehydrogenation deficiency (MADD), 2 carnitine palmitoyltransferase type 2 (CPT-II) deficiencies, 1 CPT 1 deficiency (CPT-I), and 7 MCG-3

Of the total number of cases, 122 neonates were girls (55%). Median time to consultation in the clinical centers was 11 days of life (interquartile range: 8–17), being different according to the different pathologies as shown in Table 1. Nineteen of the patients (8.2%) had consanguineous parents and homozygous variants.

Only 19 infants (8.5%) were symptomatic at the time of the NBS result (1 LCHADD, 5 PA, 1 CPT-II, 1 MMA, 3 MMAHC, 2 MSUD, 2 OTC deficiency, 1 CTLN1, 1 MCADD, 2 TYR-1), displaying different clinical presentations and evolutions (Table 2). Three patients died due to their metabolic disorder, all of them had been detected in a symptomatic phase. One patient with LCHADD and one with OTC deficiency died in the first year of life. The other patient suffered from PA, he died at 3.5 years from a metabolic decompensation. So far, false negative cases have not been identified.

Genetic diagnosis was performed in all biochemically confirmed cases, being conclusive in all of them, except for two patients with HPA: in whom only a variant in one allele in the PAH gene was found and no variants in the DNAJC12 gens were identified. The different genotypes together with the geographical origin of the patients' parents are shown in Table 3. Analyses have detected 27 novel variants (supplementary material, S1 and S2), 18 pathogenic or likely pathogenic; 8 of them are variants with uncertain significance and one is potentially benign. The mutational spectrum included 17 potential missense variants, 3 small exonic insertion/deletions and 7 of them located in intronic sequence, and likely affecting the splicing process.

4 | DISCUSSION

In our population, 26 different IEM have been detected, whereas pathogenic variants have been identified in 32 different genes.

An IEM was diagnosed in 1/2670 newborns, a remarkably similar rate was found in other studies.
**TABLE 1**
Demographic, clinical, and biochemical data of patients diagnosed with an IEM by newborn screening in Madrid. Incidence is only shown for conditions included in the program.

| Biochemical diagnosis                  | MIM     | Gene   | GEN MIM | No. cases | Incidence (range) | Days to clinical referral in asymptomatic patients mean (range) | Number of newborns with clinical symptoms before diagnosis (%) | Biochemical finding (median [range]) |
|---------------------------------------|---------|--------|---------|-----------|-------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------|
| Disorders of amino acid metabolism    |         |        |         |           |                   |                                                               |                                                               |                                      |
| Benign HPA                            | 261 600 | PAH    | 612 349 | 52        | 1/11 400          | 20.5 [7–117]                                                  | 0                                                              | Phe: 172.8 μmol/L [97.5–244.2] Phe/Tyr: 2.1 [1.2–5.6] |
| Classic PKU                           | 261 600 | PAH    | 612 349 | 32        | 1/18 525          | 10.8 [5–41]                                                   | 0                                                              | Phe: 576.8 μmol/L [225–1950] Phe/Tyr: 11.9 [1.8–32.2] |
| DNAJC12 deficiency                    | 261 600 | DNAJC12| 606 060 | 4         | NA                | NA                                                            | 0                                                              | Phe: 162.4 [123.1–265.0] Phe/Tyr: 1.8 [1.6–2.0] |
| Primapterinuria                        | 264 070 | PCBD1  | 126 090 | 2         | 1/296 411         | 12.5 [11–14]                                                  | 0                                                              | Phe: 480, 193.4 μmol/L Phe/Tyr: 193.4, 3.6 |
| GA1                                   | 231 670 | GCDH   | 600 225 | 12        | 1/49 402          | 9.8 [5–30]                                                    | 0                                                              | C5DC: 2.72 μmol/L [0.36–5.17] |
| MCG-3-MCC1D MCC2D                     | 210 200 | MCC1C  | 609 010 | 2         | 1/846 89          | 21.2 [7–58]                                                   | 0                                                              | C5OH: 2.4 μmol/L [0.9–5.8] |
| PA                                    | 606 054 | PCCB   | 232 050 | 6         | 1/98 804          | 9.5 [6–15]                                                    | 5 (83%)                                                        | C3: 11.4 μmol/L [7.5–13.8] C3/C2: 0.9 [0.17–2.03] C3/Met: 0.9 [0.3–1.2] |
| MAT                                   | 250 850 | MAT1A  | 610 550 | 6         | NA                | 18.7 [9–41]                                                   | 0                                                              | Met: 87.3 μmol/L [53–121] |
| TYR-TYRSN1                            | 276 700 | FAH    | 613 871 | 3         | 1/197 607         | 6.7 [6–8]                                                     | 1 (33.3%)                                                      | Tyr: 152.4 μmol/L [107.20–198] SA: 12.69 μmol/L [11.46–13.70] |
| TYRSN3                                | 276 710 | HPD    | 609 695 | 1         | NA                | 13                                                            | 0                                                              | Tyr: 558 mmol/L SA: 0.49 μmol/L |
| MSUD                                  | 248 600 | BCKDHB | 248 611 | 2         | 1/197 607         | 25.5 [8–43]                                                   | 3 (75%)                                                       | Leu + Ile + Hyp: 1182 μmol/L [359–1911] Val: 375 μmol/L [137–616] |
| BCAT-2 deficiency                     | 618 850 | BCAT2  | 113 530 | 2         | NA                | 11 [9–13]                                                     | 0                                                              | Leu + Ile + Hyp: 298, 686 μmol/L Val: 312, 543 μmol/L |
| HC                                    | 236 200 | CBS    | 613 381 | 2         | NA                | 11.5 [10–13]                                                  | 0                                                              | Met: 58.1, 92.0 μmol/L |
| Cystinuria                            | 220 100 | SLC3A1 | 104 614 | 1         | NA                | 21                                                            | 0                                                              | C3/C2: 0.24 μmol/L |
| OTC                                   | 311 250 | OTC    | 300 461 | 1         | NA                | NA                                                            | 1 (100%)                                                      | Cit: 2.5 μmol/L |
| CTLN1                                 | 215 700 | ASS1   | 603 470 | 2         | NA                | 11                                                            | 0                                                              | Cit: 1.55, 1010 μmol/L |
| HMGCLD                                | 246 450 | HMGCL  | 613 898 | 1         | NA                | NA                                                            | 0                                                              | C5OH: 0.8 μmol/L |

(Continues)
TABLE 1 (Continued)

| Biochemical finding | Disorder | MIM | Gene | GEN MIM | No. cases | Incidence | Days to clinical referral in asymptomatic patients mean (range) | Number of newborns with clinical symptoms before diagnosis (%) | Biochemical finding (median [range]) |
|---------------------|----------|-----|------|---------|-----------|-----------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------|
| **Disorders of fatty acid oxidation and transport (FAO)** |          |     |      |         |           |           |                                                              |                                                               |                                   |
| MCADD               | 201 450  | ACADM | 607 008 | 43      | 1/13 787  | 11.3 [4–47] | 1 (2.7%)                                                      | C8: 9.3 μmol/L [0.4–41.2] C8/C10: 10.4 [0.7–15.5]               |                                   |
| VLCAD               | 201 475  | ACADVL | 609 575 | 13      | 1/45 602  | 12.4 [7–26] | 0                                                             | C14:1:2.6 μmol/L [0.6–7.5]                                      |                                   |
| LCHADD              | 609 016  | HADHA | 600 890 | 2       | 1/296 411 | 7          | 1 (50%)                                                      |                                                                | C14:1:0.7, 0.7 μmol/L C16OH: 0.4, 0.8 μmol/L C18:OH: 0.5, 1.1 μmol/L |
| SPCD                | 212 140  | SLC22A5 | 603 377 | 11      | 1/53 893  | 43.2 [8–128] | 0                                                             |                                                                | C0: 3.8 μmol/L [2.9–5.1]                                                |
| CPT II              | 600 649  | CPT2  | 600 650 | 2       | NA        | 20         | 1 (50%)                                                      |                                                                | C12: 0.6, 0.7 μmol/L C14: 1.6, 2.0 μmol/L C16:1:2.5, 2.7 μmol/L C18: 5.9, 5.7 μmol/L |
| MADD                | 231 680  | ETFB  | 130 410 | 1       | 1/592 822 | 5          | 0                                                             |                                                                | C8: 0.6 μmol/L C12: 1.9 μmol/L C14:1:1.7 μmol/L C16:OH: 0.5 μmol/L     |
|                      |          |       |        |         |           |           |                                                              |                                                               |                                   |
| CPT I               | 255 120  | CPT1A | 600 528 | 1       | NA        | 11         | 0                                                             |                                                                | C0: 94.7 μmol/L C16-C18/C0: 160.5                                      |
|                      |          |       |        |         |           |           |                                                              |                                                               |                                   |
| **Disorders of cobalamin metabolism** |          |     |      |         |           |           |                                                              |                                                               |                                   |
| MMAHC               | 277 400  | MMACHC | 609 831 | 6       | 1/84 689  | 20.4 [6–54] | 3 (43%)                                                      |                                                                | C3: 6.8 μmol/L [2.8–9.4] C3/C2: 0.71 [0.22–1.83] C3/Met: 1.07 [0.22–2.21] |
| MMA                 | 251 000  | MMUT  | 609 058 | 2       | 1/148 205 | 30.7 [19–38] | 1 (25%)                                                      |                                                                | C3: 6.30 μmol/L [3.9–9.1] C3/C2: 0.34 [0.24–0.6] C3/Met: 0.39 [0.22–0.72] |
| MMA                 | 251 110  | MMAB  | 607 568 | 2       |           |            |                                                              |                                                               |                                   |

Abbreviations: BCAT-2, branched-chain amino acid transferase 2 deficiency; CPT-I, carnitine palmityltransferase type 1 deficiency; CPT-II, carnitine palmityltransferase type 2 deficiency; CTLN1, citrullinemia type 1; DNAJC12, hyperphenylalaninemia due to DNAJC12 defect; GA-1, glutaric aciduria type 1; HC, homocystinuria; HMG-CLD, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; HPA, hyperphenylalaninemia; Hyp, Hydroxyproline; Ile, Isoleucine; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; Leu, Leucine; MADD, multiple acyl-CoA dehydrogenation deficiency; MAT, hypermethioninemia; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MCG-3:3-methylcrotonyl-CoA carboxylase deficiency; MMA, methylmalonic acidemia; MMAHC, methylmalonic acidemia with homocystinuria; MSUD, maple syrup urinary disease; NA, not applicable; OTC, ornithine transcarbamylase deficiency; PA, propionic acidemia; PKU, phenylketonuria; SPCD, systemic primary carnitine deficiencies; TYRSN-1, tyrosinemia type 1; TYRSN-3, tyrosinemia type 3; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency.
performed in our Country (in the region of Galicia: 1/2060, excluding benign HPA; in the region of Aragon: 1/2573; in the region of Murcia: 1/1884, including Cystic Fibrosis). This rate is also similar in other Western European Countries: Portugal: 1/2396, Germany: 1/2712, or Italy: 1/2000. As in other programs, the conditions with the highest incidence were HPA (1/6587) and MCAD deficiencies (1/13787). A recent systematic review and meta-analysis reported a global worldwide birth prevalence of PAH deficiency of 1/15625, being higher in Europe (1/8771).

The positive predictive value was lower than in other reports in our Country, like in Galicia (76.11%), where they performed second tier tests and collected blood and urine samples simultaneously of all newborns; but comparable to other programs, where second tier tests were also not requested (20%). Nevertheless, the ENBS allowed the diagnosis of other entities different from IEM as vitamin B12 deficiencies, or different maternal diagnoses, which have not been included in this report. These conditions have already been reported in other programs.

Most of the cases benefited from presymptomatic diagnosis. Only 19 patients (8.5%) displayed symptoms before the result of the NBS was available. However, for some fatty acid oxidations disorders, organic acidemias and urea cycle disorders, at least half of the patients presented with clinical symptoms (LCHADD: 1/2, CPT-II: 1/2, PA: 5/6, MMAHC: 3/7, MSUD: 2/4, OTC deficiency 2/2). Only one patient with MCADD, who presented with remarkable high octanoylcarnitine (C8) levels, displayed clinical symptoms (hypoglycemia and hypotonia). He harbored a homozygous pathogenic variant c.985A > G in the ACADM gene, which has been associated with more severe cases and higher levels of neonatal C8 and urinary acylglycines. One of the patients diagnosed with LCHADD had a very early and fatal onset. This disease usually displays immediate complications, but recent data have shown that outcomes can be favorable if early diagnosis and strict dietary regime are initiated. A similar scenario is evident in CPT-II deficiency, with frequent sudden death or severe metabolic decompensation.

For PA and MMA (including MMAHC) several cases presented with neurological deterioration in the first-second weeks of life. It is well known that their clinical course generally starts with an acute metabolic decompensation in the neonatal period, that frequently leads to irreversible neurological damage. However, as in our cases, most of the patients are already symptomatic before NBS results can be available. Furthermore, even when early diagnosis and optimal metabolic control are achieved, disease progression occurs. It has also been postulated that NBS does not have an effect on diagnosis of PA as it is hardly ever asymptomatic. However, as last guidelines stated, we consider necessary to establish a prompt diagnosis in children with suggestive clinical signs and symptoms. On the other hand, for GA-1, as in our series, NBS usually allows the presymptomatic diagnosis, and it has been proven to be effective in preventing the progressive neurological deterioration.

Two of the three patients diagnosed with MSUD were symptomatic, and both have displayed neurological impairment in the follow-up. Indeed, detection of this disorder before the occurrence of severe symptoms has been reported and NBS has demonstrated to avoid neurological deterioration and to clearly improve prognosis of MSUD patients. Diagnosis of OTC by NBS is feasible as low blood citrulline levels can be detected in this condition. Notwithstanding, its inclusion in NBS programs remains controversial as hypocitrullinemia may not be present, especially in late onset forms. Based on our data, OTC should be evaluated and ruled-out when low citrulline levels are detected to diagnose severe cases which can be potentially fatal, as our patient who died on the third day of life. Finally, one patient with Tyr-I also displayed clinical symptoms with an acute liver failure. For this IEM, early diagnosis and treatment has also proven to have a benefit in the natural history although patients seem to remain with several neurological disturbances.

Mean time to clinical referral varied notably among the different IEMs. As shown in Table 1, time was longer for conditions considered “mild” as benign HPA, MCG-3, or SCPOD, where samples are frequently repeated before referral. The routine screening of MCG-3 condition is controversial. In general, authors consider that longer investigations and follow-up are needed to establish its indication. Besides, based on our data, we consider mandatory the accurate evaluation of cases with abnormal C5OH, as our patient with HMG-CoA lyase deficiency presented with similar C5OH values as those with MCG-3, without other biochemical abnormalities. For this condition, novel biomarkers have recently been identified; organic acid analysis in urine is necessary as the second tier-test for confirmation of this disease. Detection of HMG-CoA lyase deficiency is essential as about half of the patients become symptomatic within the neonatal period.

The death rate in our population was 1.3% (3/222), a bit lower than observed in other series. Early mortality data for PA, LCHADD and OTC was 17, 50, and 50%, respectively. It is essential to report also longitudinal outcome data.

In our program, genetic diagnosis not only has confirmed all the biochemical cases (except for two) but also has allowed to detect novel IEM, as HPA due to DNAJC12 pathogenic variants and BCAT-2 deficiency. These new
| Case | IEM | NBS biochemical markers | Genotype | Days of life presenting with symptoms | Health care provider contact prior to NBS result | Clinical manifestations at diagnosis | Biochemical abnormalities | Detoxification measures at diagnosis | Long-term follow-up |
|------|-----|-------------------------|----------|--------------------------------------|-----------------------------------------------|-----------------------------------|--------------------------|-------------------------------------|-----------------|
| 1    | LCHAD | C16:3:0.41 μmol/L | HADHA: c.453 +1G >A (p.?)/c.453 +1G >A (p.?) | 1 | Yes | Acute respiratory distress and poor perfusion of peripheral tissues (dilated cardiomyopathy) | Hypoglycemia, metabolic acidosis, elevated lactate (12.1 mmol/L), hyperammonemia (134 μmol/L), CPK elevation (7.130 U/L) | Glucose 10 mg/kg/min and bicarbonate infusion | Death at 6 months |
| 2    | MCADD | C8: 0.2 μmol/L | ACADM: c.985A >G (p.Lys329Glu)/c.985A >G (p.Lys329Glu) | 2 | No | Hypotonia | Hypoglycemia | No | No clinical alterations or metabolic decompensations |
| 3    | CPT-II | C12:0.7 μmol/L | CPTII: c.1547 T > C (p.Phe516Ser)/c.122_130del19 (p.Pro41_Met43del) | 7 | No | Acute encephalopathy and seizures | Hypoglycemia, hyperammonemia (583 μmol/L) | Glucose 10 mg/kg/min | Autism spectrum disorder |
| 4    | PA | C3: 11 μmol/L | PCCB: c.1218_1231del14ins14 (p.Gly407Argfs*14)/c.1218_1231del14ins14 (p.Gly407Argfs*14) | 2 | Yes | Somnolence | Hypoglycemia, metabolic acidosis, hyperammonemia (480 μmol/L) | Glucose 10 mg/kg/min, arginine, carglumic acid, and ammonia scavengers | Metabolic acidosis, ketosis, anemia, hyperammonemia (239 μmol/L), pancytopenia | Cognitive impairment and epilepsy |
| 5    | PA | C3: 12.2 μmol/L | PCCB: c.1218_1231del14ins14 (p.Gly407Argfs*14)/c.1218_1231del14ins14 (p.Gly407Argfs*14) | 8 | No | Lethargy, hypotonia, and urinary Escherichia coli infection | Metabolic acidosis, ketosis, hyperammonemia (239 μmol/L), pancytopenia | Glucose 10 mg/kg/min, arginine, carglumic acid, and ammonia scavengers | Several episodes of metabolic compensation | Severe–moderate cognitive impairment | Liver transplantation |
| 6    | PA | C3: 12.4 μmol/L | PCCB: c.1173dupT (p.Val392Cysfs*2)/c.1173dupT (p.Val392Cysfs*2) | 5 | Yes | Encephalopathy and vomiting | Anemia, hyperammonemia (805 μmol/L), metabolic acidosis | Hemodialfiltration, carglumic acid, ammonia scavengers, L-arginine, cofactors. | Cognitive impairment |
| 7    | PA | C3: 13.9 μmol/L | PCCB: c.1218_1231del14ins14 (p.Gly407Argfs*14)/c.1218_1231del14ins14 (p.Gly407Argfs*14) | 7 | Yes | Somnolence, poor feeding | Metabolic acidosis, hyperammonemia (585 μmol/L), anemia, thrombocytopenia, neutropenia | Glucose 10 mg/kg/min, arginine, carglumic acid, and sodium phenylbutyrate | Cognitive impairment and epilepsy |
| 8    | PA | C3: 14.36 μmol/L | PCCB: c.1173dupT (p.Val392Cysfs*2)/c.1173dupT (p.Val392Cysfs*2) | 2 | Yes | Somnolence, breathing difficulty | Hypoglycemia, hyperammonemia (723 μmol/L) | Glucose 10 mg/kg/min, arginine, and carglumic acid | Liver transplantation |
| Case | IEM | NBS biochemical markers | Genotype | Days of life presenting with symptoms | Health care provider contact prior to NBS result | Clinical manifestations at diagnosis | Biochemical abnormalities | Detoxification measures at diagnosis | Long-term follow-up Comorbidities |
|------|-----|-------------------------|----------|--------------------------------------|-----------------------------------------------|-----------------------------------|--------------------------|---------------------------------|---------------------------------|
| 9    | MMAHC | C3: 9.5 μmol/L | MMAHC c.271dupA (p.Arg91Lysfs*14)/c.271dupA (p.Arg91Lysfs*14) | 12 | No | Encephalopathy and seizures | Neutropenia | Cobalamin, folinic acid, and betaine | Visual and cognitive impairment |
|      |      | C3/C2: 0.5 | | | | | | | |
|      |      | C5/Met: 1.6 | | | | | | | |
| 10   | MMAHC | C3: 3.80 μmol/L | MMAHC c.271dupA (p.Arg91Lysfs*14)/c.271dupA (p.Arg91Lysfs*14) | 18 | No | Somnolence, poor feeding | Hypoglycemia, anemia, thrombocytopenia | Glucose 10 mg/kg/min | Cognitive impairment, epilepsy and growth restriction |
|      |      | C3/C2: 1.02 | | | | | | | |
|      |      | C5/Met: 0.65 | | | | | | | |
| 11   | MMAHC | C3: 8.72 μmol/L | MMAHC c.271dupA (p.Arg91Lysfs*14)/c.271dupA (p.Arg91Lysfs*14) | 11 | Yes | Cardiomyopathy, hypotonia, seizures, jaundice, eczema, and urinary E. coli infection | Metabolic acidosis | Glucose 10 mg/kg/min | Cognitive impairment, behavioral disorder |
|      |      | C3/C2: 0.52 | | | | | | | |
|      |      | C5/Met: 1.83 | | | | | | | |
| 12   | MMA | C3: 9.17 μmol/L | MMA c.662 T > G (p.Phe221Cys)/c.569G > A (p.Arg190His) | 13 | No | Mild drowsiness | Metabolic acidosis, elevated lactate (7 mmol/L) | Glucose 10 mg/kg/min | Several episodes of metabolic decompensation (acidosis) Normal neurodevelopment |
|      |      | C3/C2: 0.60 C3/ Met: 0.72 | | | | | | | |
| 13   | MSUD | Leu: 1390 μmol/L Val: 616 μmol/L | BCKDHB c.595_596delAG (p.Pro200Ter) | 8 | Yes | Severe encephalopathy (coma), fever, seizures, and facial and perianal eczema | Metabolic acidosis and hyperammonemia | Hemodiafiltration | Cognitive impairment and liver transplantation (March 2018) |
|      |      | | c.595_596delAG (p.Pro200Ter) | | | | | | |
| 14   | MSUD | Leu: 1070 μmol/L Val: 492 μmol/L | DBT c.(51 + 1_52–1)(175 + 1_176–1)/ c.(51 + 1_52–1)(175 + 1_176–1) | 6 | Yes | Somnolence, poor feeding | Ketonuria+++ Mild hyperammonemia (126 μmol/L) | Hemodiafiltration | High leucine levels, with few episodes of metabolic decompensation Central nervous system lesions |
|      |      | | | | | | | | |
| 15   | TRSN1 | SA: 11.46 μmol/L Tyr: 107.2 μmol/L | FAH c.554-1G > T (p.Arg181Glu) | 8 | No | No | Acute hepatic failure: hypoglycemia, coagulopathy | No | Mild cognitive impairment, epilepsy, and attention deficit hyperactivity disorder |
|      |      | | c.554-1G > T (p.Arg181Glu) | | | | | | |
| 16   | TRSN1 | SA: 13.70 μmol/L Tyr: 152 μmol/L | FAH c.G233A (p.Trp97Ter)/c.554-1G > T (p.Arg181Glu) | 16 | No | No | Acute hepatic failure: coagulopathy, thrombocytopenia | No | Asymptomatic |
|      |      | | c.G233A (p.Trp97Ter)/c.554-1G > T (p.Arg181Glu) | | | | | | |
| 17   | OTC | Cit: 2.5 μmol/L | OTC c.928G > A (p.Glu310Lys) | 3 | Yes | Poor general clinical condition, respiratory distress, vomiting, seizures | Hyperammonemia (244 μmol/L), coagulopathy, hypertransaminasemia | Hemodiafiltration, carglumic acid ammonia scavengers, t-arginine, cofactors. | Death in neonatal period |
|      |      | | c.928G > A (p.Glu310Lys) | | | | | | |
| 18   | OTC | Cit: 1.4 μmol/L | OTC c.77G > A (p.Arg26Gln) | 5 | Yes | Somnolence, poor feeding, vomiting | Metabolic acidosis hyperammonemia (1505 μmol/L) | Hemodiafiltration, carglumic acid, and sodium phenylbutyrate | Severe–moderate cognitive impairment |
|      |      | | c.77G > A (p.Arg26Gln) | | | | | | |
| 19   | CTLN1 | Cit: 1010 μmol/L | ASS1 p.Val569Ala (c.206 T > C), p.Glu270Gln (c.808G > C)/p.Arg157His (c.470G > A) | 6 | Yes | Somnolence, decreased urine output, breathing difficulty. | Coagulopathy, hyperammonemia (800 μmol/L) | Hemodiafiltration | Normal neurodevelopment |
|      |      | | p.Val569Ala (c.206 T > C), p.Glu270Gln (c.808G > C)/p.Arg157His (c.470G > A) | | | | | | |

**Abbreviations:** CPT-II, carnitine palmitoyltransferase type 2 deficiency; CTLN1, citrullinemia type 1; IEM, inborn error of metabolism; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MMA, methylmalonic acidemia; MMAHC, methylmalonic acidemia with homocystinuria; MSUD, maple syrup urinary disease; NBS, newborn screening; OTC, ornithine transcarbamylase deficiency; PA, propionic acidemia.
| Genotype | No. cases | Country of origin |
|----------|-----------|------------------|
| c.1208C > T (p.Ala403Val)/c.1208C > T (p.Ala403Val) | 4 | Morocco |
| c.60 + 5G > T /c.158G > A (p.Arg53His) | 2 | Spain |
| c.527G > T (p.Arg176Leu)/c.527G > T (p.Arg176Leu) | 2 | Dominican Republic |
| c.842C > T (p.Pro281Leu)/c.1139C > T (p.Thr380Met) | 2 | Georgia. Spain |
| c.632delC (p.Pro211Hisfs*130)/c.734 T > C (p.Val245Ala) | 1 | Spain |
| c.261C > A (p.Ser87Arg)/c.261C > A (p.Ser87Arg) | 1 | Spain |
| c.441 + 5G > T /c.688G > A (p.Val230Ile) | 1 | Spain |
| c.1243G > A (p.Asp415Asn)/c.441 + 5G > T | 1 | Spain |
| c.165 T > G (p.Phe55Leu)/c.842 + 4A > G | 1 | Romania |
| c.842C > T (p.Pro281Leu)/c.898G > T (p.Ala300Ser) | 2 | Romania. Spain |
| c.734 T > C (p.Val 245Ala)/c.1241A > G (p.Tyr414Cys) | 1 | Spain |
| c.194 T > C (p.Ile65Thr)/c.158G > A (p.Arg53His) | 1 | Spain |
| c.158G > A (p.Arg53His)/c.510-2A > G | 1 | Morocco |
| c.117C > G (p.Phe39Leu)/c.183C > G (p.Asn61Lys) | 1 | Spain |
| c.1045 T > C (p.Tyr414Cys)/c.1139C > T (p.Arg53His) | 1 | Spain |
| c.1243G > A (p.Asp415Asn)/c.158G > A (p.Arg53His) | 1 | Morocco |
| c.688G > A (p.Val230Ile)/c.1241A > G (p.Tyr414Cys) | 1 | Spain |
| c.1262 T > C (p.Ile421Thr)/c.116_118delTCT (p.Phe39del) | 1 | Spain |
| c.261C > A (p.Ser87Arg)/c.754C > T (p.Arg252Trp) | 1 | Spain |
| c.1045 T > C (p.Tyr414Cys)/c.1139C > T (p.Arg53His) | 1 | Spain |
| c.898G > T (p.Ala300Ser)/c.1241A > G (p.Tyr414Cys) | 1 | Spain |
| c.527G > T (p.Arg176Leu)/c.898G > T (p.Ala300Ser) | 1 | Spain |
| c.688G > A (p.Val230Ile)/c.135*2delTAAAG (p.Ter453_Ser454del) | 1 | Spain-Great Britain |
| c.165 T > G (p.Phe55Leu)/c.1066-11G > A (p.Gln355_Tyr356ins3) | 1 | Paraguay |
| c.506G > A (p.Arg169His)/c.842C > T (p.Pro281Leu) | 1 | Georgia |
| c.441 + 5G > T/c.809G > A (p.Arg270Lys) | 1 | Spain |
| c.194 T > C (p.Ile65Thr)/c.688G > A (p.Val230Ile) | 1 | Spain |
| c.1066-11G > A (p.Gln355_Tyr356ins3)/c.1259 G > T (Arg420Met) | 1 | Spain |
| c.510-2A > G/c.158G > A (p.Arg53His) | 1 | Morocco |
| c.898G > T (p.Ala300Ser)/c.441 + 5G > T | 1 | Spain |
| c.165 T > G (p.Phe55Leu)/c.194 T > C (p.Ile65Thr) | 1 | Spain |
| c.827 T > C (p.Met276Thr)/c.1208C > T (p.Ala403Val) | 1 | Spain |
| c.838G > A (p.Glu280Lys)/c.1208C > T (p.Ala403Val) | 1 | Spain |
| c.592_613del22 (p.Tyr198Serfs*136)/c.116_118delTCT (p.Phe39del) | 1 | Spain |
| c.116_118delTCT (p.Phe39del)/c.165 T > G (p.Phe55Leu) | 1 | Spain |
| c.60 + 5G > T/c.529G > A (p.Val177Met) | 1 | Spain |
| c.1241A > G (p.Tyr414Cys)/c.1139C > T (p.Thr380Met) | 1 | Spain |
| c.194 T > C (p.Ile65Thr)/c.1315 + 1G > A | 1 | Spain |
| IEM (No. cases) Gene Genotype | No. cases | Country of origin |
|-------------------------------|-----------|-------------------|
| c.782G > A (p.Arg261Gln)/c.194 T > C (p.Ile65Thr) | 1 | Spain |
| c.1066-11G > A (p.Gln355_Tyr356ins3)/c.805A > C (p.Ile269Leu) | 1 | Spain |
| c.898G > T (p.Ala300Ser)/c.1065 + 3A > C | 1 | Spain |
| c.1208C > T (p.A403V)/c.441 + 5G > T | 1 | Spain |
| c.1066-11 G > A /c.1199 + 17 G > A | 1 | Spain |
| c.746 T > C (p.Leu249Pro)/c.890G > A (p.Arg297His) | 1 | Spain |

**Classic PKU (32) PAH**

| Genotype | No. cases | Country of origin |
|----------|-----------|-------------------|
| c.842C > T (p.Pro281Leu)/c.1162G > A (p.Val388Met) | 2 | Spain |
| c.754C > T (p.Arg252Trp)/c.1066-11G > A (p.Gln355_Tyr356ins3) | 1 | Spain |
| c.185_189delTGACC (Leu62Profs*3)/c.441 + 5G > T (p.Gly148Trpfs*29?) | 1 | Paraguay |
| c.1243G > A (p.Asp415Asn)/c.442-?_509 +?del (p.Gly148Trpfs*29?) | 1 | Colombia |
| c.781C > T (p.Arg261Ter)/c.1223G > A (p.Arg408Gln) | 1 | Spain |
| c.1222C > T (p.Arg408Trp)/c.1222C > T (p.Arg408Trp) | 1 | Romania |
| c.439C > T (p.Pro147Ser)/c.727C > T (p.Arg243Ter) | 1 | Spain |
| c.441 + 5G > T /c.1055delG (p.Gly352Valfs*48) | 1 | Morocco |
| c.204A > T (p.Arg68Ser)/c.136G > A (p.Gly46Ser) | 1 | Cuba |
| c.533A > G (p.Glu178Gly)/c.1222C > T (p.Arg408Trp) | 1 | Romania |
| c.1241A > G (p.Tyr414Cys)/c.1315 + 1G > A | 1 | Spain |
| c.165 T > G (p.Phe55Leu)/c.782G > A (p.Arg261Gln) | 1 | Ecuador/Cuba |
| c.500A > T (p.Asn167Ile)/c.1223G > A (p.Arg408Gln) | 1 | Germany/Spain |
| c.782G > A (p.Arg261Gln)/c.1162G > A (p.Val388Met) | 1 | Spain/Portugal |
| c.782G > A (p.Arg261Gln)/c.842C > T (p.Pro281Leu) | 1 | Spain |
| c.721C > T (p.Arg241Cys)/c.721C > T (p.Arg241Cys) | 1 | Morocco |
| c.439C > T (p.Pro147Ser)/c.727C > T (p.Arg243Ter) | 1 | Spain |
| c.441 + 5G > T /c.782G > A (p.Arg261Gln) | 1 | Spain |
| c.754C > T (p.Arg252Trp)/c.782G > A (p.Arg261Gln) | 1 | Bulgaria |
| c.60 + 5G > T /c.1055delG (p.Gly352Valfs*48) | 1 | Spain |
| c.842C > T (p.Pro281Leu)/c.842C > T (p.Pro281Leu) | 1 | Morocco |
| c.143 C > T (p.Leu48Ser)/c.1222C > T (p.Arg408Trp) | 1 | Romania |
| c.441 + 5G > T /c.1066-11G > A (p.Gln355_Tyr356ins3) | 1 | Spain |
| c.1162G > A (p.Val388Met)/c.1162G > A (p.Val388Met) | 1 | Spain |
| c.561G > C (p.Trp187Cys)/c.1241A > G (p.Tyr414Cys) | 1 | Peru/Spain |
| c.441 + 5G > T /c.1028A > G (p.Tyr343Cys) | 1 | Spain |
| c.781C > T (p.Arg261Ter)/c.1262 T > C (p.Ile421Thr) | 1 | Spain |
| c.1067-11G > A /c.1067-11G > A | 1 | Morocco |
| c.1241A > G (p.Tyr414Cys)/c.1042C > G (p.Leu348Val) | 1 | Spain |
| IEM (No. cases) | Gene | Genotype | No. cases | Country of origin |
|-----------------|------|----------|-----------|-------------------|
| DNAJC12 deficiency (4) | DNAJC12 | c.524G > A (p.Trp175Ter)/c.524G > A (p.Trp175Ter) | 2 | Spain |
| | | c.524G > A (p.Trp175Ter)/c.502 + 1G > C | 1 | Spain |
| | | c.524G > A (p.Trp175Ter)/c.298-2A > C | 1 | Spain |
| Primapterinuria (2) | PCBD1 | c.259G > T (p.Glu87Ter)/c.292C > T(p.Gln98Ter) | 1 | Cape Verde |
| | | c.292C > T(p.Gln98Ter) | 1 | Spain |
| GA-1 (12) | GCDH | c.1198G > A (p.Val400Met)/c.1198G > A (p.Val400Met) | 2 | Spain |
| | | c.1198G > A (p.Val400Met)/c.1240C > T (p.Arg402Trp) | 1 | Spain |
| | | c.1198G > A (p.Val400Met)/c.442G > T (p.Val148Phe) | 1 | Spain |
| | | c.278A > G (p.His93Arg)/c.298-2A > C | 1 | Spain |
| | | c.877G > A (p.Ala293Thr)/c.877G > A (p.Ala293Thr) | 1 | Spain |
| | | c.877G > A (p.Ala293Thr) | 1 | Spain |
| | | c.877G > A (p.Ala293Thr)/c.1198G > A (p.Val400Met) | 1 | Spain |
| | | c.877G > A (p.Ala293Thr)/c.1210G > C (p.Ala404Pro) | 1 | Spain |
| | | c.1210G > C (p.Ala404Pro) | 1 | Spain |
| | | c.946G > A (p.Ala304Thr)/c.1198G > A (p.Val400Met) | 1 | Spain |
| | | c.1198G > A (p.Val400Met) | 1 | Spain |
| | | c.442G > T (p.Val148Phe)/c.442G > T (p.Val148Phe) | 1 | Spain |
| | | c.1423G > A (p.Gly475Arg)/c.1423G > A (p.Gly475Arg) | 1 | Spain |
| | | c.1423G > A (p.Gly475Arg) | 1 | Morocco |
| | | c.1423G > A (p.Gly475Arg)/c.804-14 T > A | 1 | Ecuador |
| | MCCC1 | c.1331G > A (p.Arg444His) /c.1008G > C (pMet336Ileu) | 1 | Morocco |
| | | c.1331G > A (p.Arg444His) | 1 | Spain |
| | | c.1331G > A (p.Arg444His)/c.1331G > A (p.Arg444His) | 1 | Spain |
| | | c.872 (p.Ala291Val)/c.1970 T > C (p.Ile657Thr) | 1 | Spain |
| | MCCC2 | c.1015G > A (p.Val339Met)/c.1635dupT (p.Ser546Ter) | 1 | Spain |
| | | c.1015G > A (p.Val339Met)/c.129 + 3A > G | 1 | Spain |
| | | c.1015G > A (p.Val339Met)/c.641G > C (p.Gly214Ala) | 1 | Spain |
| | | c.1015G > A (p.Val339Met)/c.641G > C (p.Gly214Ala) | 1 | Spain |
| | | c.1423G > A (p.Gly475Arg)/c.1423G > A (p.Gly475Arg) | 1 | Spain |
| | | c.1423G > A (p.Gly475Arg) | 1 | Morocco |
| | | c.804-14 T > A /c.804-14 T > A | 1 | Ecuador |
| PA (5) | PCCB | c.1218_1231del14ins12 (p.Gly407Argfs*14)/c.1218_1231del14ins12 (p.Gly407Argfs*14) | 3 | Spain (2), Spain-Peru (1) |
| | | c.1218_1231del14ins12 (p.Gly407Argfs*14)/c.1173dupT (p.Val392Cysfs*2) | 1 | Spain |
| | MAT1A | c.791G > A (p.Arg264His) | 4 | Spain (3), Argentina (1) |
| | | c.791G > A (p.Arg264His) | 4 | Spain |
| | | c.595C > T (p.Ala259Val) | 1 | Spain |
| | | c.595C > T (p.Ala259Val) | 1 | Spain |
| | | c.770G > A (p.Gly257Glu) | 1 | Spain |
| | TYRSN1 (3) | FAH | c.554-1G > T /c.554-1G > T | 2 | Spain/Morocco |
| | | c.554-1G > T /c.554-1G > T | 2 | Spain |
| | TYRSN3 (1) | HPD | c.778G > A (p.Gly260Arg)/c.1118A > T (p.Glu373Val) | 1 | Ecuador |
| | MSUD (4) | BCKDHB | c.508C > T (p.Arg170Cys) /c.508C > T (p.Arg170Cys) | 1 | Spain |
| | | c.508C > T (p.Arg170Cys) /c.508C > T (p.Arg170Cys) | 1 | Spain |
| | | c.595_596delAG (p.Pro200Ter) /c.595_596delAG | 1 | Spain |
| | | c.595_596delAG (p.Pro200Ter) /c.595_596delAG | 1 | Spain |
| | | c.595_596delAG (p.Pro200Ter) /c.604G > A (p.Ala202Thr) | 1 | Spain-Colombia |
| | BCKDHA | c.370C > T (p.Arg124Trp)/c.743C > T (p.Ala248Val) | 1 | Paraguay |
| | DBT | c.(51 + 1_52–1)_(175 + 1_176–1)/c.(51 + 1_52–1)_(175 + 1_176–1) | 1 | El Salvador |
| IEM (No. cases)   | Gene   | Genotype                                                                 | No. cases | Country of origin        |
|-----------------|--------|--------------------------------------------------------------------------|-----------|--------------------------|
| BCAT-2 deficiency (2) | BCAT2  | c.1154_1160del7ins12 (p.Ala385Valfs*35)/c.1154_1160del7ins12 (p.Ala385Valfs*35) | 1         | Spain                    |
|                 |        | c.762G > C (p.Trp254Cys) /c.923G > A (p.Trp308Ter)                       | 1         | Spain                    |
| HC (2)          | CBS    | c.572C > T (p.Thr191Met) /c.572C > T (p.Thr191Met)                      | 1         | Spain                    |
|                 |        | c.770C > T (p.Thr257Met) /c.803 T > C (p.Leu268Pro)                      | 1         | Netherlands/Spain        |
| Cystinuria (1)  | SLC3A1 | c.797 T > C (p.Phe266Ser)/c.1400 T > C (p.Met467Thr)                     | 1         | Romania                  |
| OTC (2)         | OTC    | c.928G > A (p.Glu310Lys) /c.77G > A (p.Arg26Gln)                        | 1         | Spain                    |
|                 |        |                                                                         |           | Venezuela                |
| CTLN1 (2)       | ASS1   | c.[267 T > C;808G > C] (p.Val69Ala + Glu270Gln) /c.805G > A (p.Val269Met) | 1         | Spain/Peru               |
|                 |        | c.[206 T > C;808G > C] (p.Val69Ala;Glu270Gln) /c.470G > A (p.Arg157His)    | 1         | Spain                    |
| HMGCLD (1)      | HMGCL  | c.109G > T (p.Glu37Ter) /c.785G > A (p.Gly262Glu)                       | 1         | Spain/Argentina          |
| MCADD (43)      | ACADM  | c.985A > G (p.Lys329Glu)/c.985A > G (p.Lys329Glu)                       | 22        | Spain (19). Romania (2)  |
|                 |        |                                                                         |           | Peru (1)                 |
|                 |        | c.985A > G (p.Lys329Glu)/c.638C > A (p.Thr228Asn)                      | 7         | Spain                    |
|                 |        | c.638C > A (p.Thr228Asn)/c.999_1011dup13 (p.Glu338Ter)                  | 2         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.626C > T (p.Pro209Leu)                      | 2         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.351A > C (p.Thr117Thr)                      | 1         | Romania/Colombia         |
|                 |        | c.985A > G (p.Lys329Glu)/c.799G > A (p.Gly267Arg)                      | 1         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.250C > T (p.Leu84phe)                       | 1         | Spain/Canada             |
|                 |        | c.985A > G (p.Lys329Glu)/c.946-2A > C                                | 1         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.609A > C (p.Leu203Phe)                      | 1         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.127G > A (p.Glu43Lys)                       | 1         | Spain                    |
|                 |        | c.985A > G (p.Lys329Glu)/c.599 + 3A > G                              | 1         | Spain/Paraguay           |
|                 |        | c.351A > C (p.Thr117Thr)/c.503A > C (p.Asp168Ala)                      | 1         | Spain                    |
|                 |        | c.338C > A (p.Ala113Asp)/c.940G > C (p.Val314Leu)                      | 1         | Ecuador                  |
|                 |        | c.1247 T > C (p.Ile416Thr)/c.778_782delGAAA (p.Glu260Cysfs*5)         | 1         | Paraguay                 |
| VLCAD (13)      | ACADVL | c.848 T > C (p.Val283Ala)/c.1220G > C (p.Gly407Ala)                     | 2         | Spain                    |
|                 |        | c.848 T > C (p.Val283Ala)/c.685 > T (p.Arg229Term)                     | 2         | Morocco                  |
|                 |        | c.848 T > C (p.Val283Ala)/c.848 T > C (p.Val283Ala)                     | 1         | Spain                    |
|                 |        | c.848 T > C (p.Val283Ala)/c.996delT (p.Ala333Profs*20)                 | 1         | Venezuela                |
|                 |        | c.761G > A (p.Gly254Asp)/c.761G > A (p.Gly254Asp)                      | 1         | Spain                    |
|                 |        | c.520G > A (p.Val174Met)/c.1097G > A (c.1844G > A (p.Arg366His;p.Arg615Gln) | 1         | Spain                    |
|                 |        | c.199A > T (p.Lys67Term)/c.1121A > C (p.His374Pro)                     | 1         | Germany-Spain            |
|                 |        | c.138 + 2 T > C /c.1366C > T (p.Arg456Cys)                             | 1         | Spain                    |
|                 |        | c.1367G > A (p.Arg456His)/c.1678 + 19_1678 + 31del13                   | 1         | Spain                    |
|                 |        | c.1174G > C (p.Val392Leu)/c.1752-2_1755del6                              | 1         | Spain                    |
|                 |        | c.1077G > A (p.Ala359Ala)/c.683 T > C (p.Ile228Thr)                    | 1         | Italy-Spain              |

(Continues)
two entities present with a heterogeneous clinical spectrum,\textsuperscript{52,53} and description of the cases with long-term studies are essential to better understand the natural history of these pathologies. Other recent studies have also established that molecular analyses can increase the number of pathologies from NBS,\textsuperscript{54} and some authors even postulate that whole exome sequencing could be considered as a follow-up test for MS/MS positive individuals, offering an early and accurate definitive diagnosis.\textsuperscript{55,56}

For HPA, as it has been previously described,\textsuperscript{57} molecular characterization was heterogeneous. We have identified close to 60 different pathogenic variants, all of them with a low prevalence. More than 950 variants in the PAH have been identified, being the most

| TABLE 3 (Continued) |
|----------------------|
| IEM (No. cases) | Gene | Genotype | No. cases | Country of origin |
|----------------------|
| LCHADD (2) | HADHA | c.1528G > C (p.Glu510Gln)/c.1915_1918delTATC (p.Tyr639Argfs*4) | 1 | Spain |
| | | c.453 + 1G > A (p.Met106fs)/c.453 + 1G > A (p.Met106fs) | 1 | Ecuador |
| SPCD (11) | SLC22A5 | c.845G > A (p.Arg282Gln)/c.845G > A (p.Arg282Gln) | 1 | Ecuador |
| | | c.845G > A (p.Arg282Gln)/c.1392_1409del18ins2 (p.Val465Thrfs*29) | 1 | Bolivia/Ecuador |
| | | c.806delIT (p.Leu269Hisfs*27)/c.845G > A (p.Arg282Gln) | 1 | Spain-Argentina |
| | | c.760C > T (p.Arg254Ter)/C.1400C > G (p.Ser467Cys) | 1 | China |
| | | c.743 T > C (p.Leu248Pro)/c.806delIT (p.Leu269Hisfs*27) | 1 | Spain |
| | | c.680G > A (p.Arg227His)/c.824 + 1G > T | 1 | Italy-Spain |
| | | c.447C > G (p.Phe149Leu)/c.680G > A (p.Arg227His) | 1 | Spain |
| | | c.419G > A (p.Trp140Ter)/c.845G > A (p.Arg282Gln) | 1 | Peru |
| | | c.364G > T (p.Asp122Tyr)/c.791C > G (p.Trp264Arg) | 1 | Spain |
| | | c.1345 T > G (p.Tyr449Asp)/c.1072 T > A (p.Tyr358Asn) | 1 | Dominican Republic |
| | | c.646G > C (p.Val216Leu)/c.646G > C (p.Val216Leu) | 1 | Morocco |
| CPT II (2) | CPT2 | c.1547 T > C (p.Phe516Ser)/c.122_130del9 (p.Pro41_Met43del) | 1 | Colombia-Spain |
| | | c.587C > T (p.Pro196Leu)/c.587C > T (p.Pro196Leu) | 1 | Spain |
| MADD (1) | ETFB | c.145G > C (p.Ala49Pro)/c.343_345delGAG (p.Glu115del) | 1 | Spain |
| CPT I (1) | CPT1A | c.2125G > A (p.Gly709Arg)/c.1948G > A (p.Gly650Ser) | 1 | Spain |
| MMAHC (7) | MMACHC | c.271dupA (p.Arg91Lysfs*14)/c.271dupA (p.Arg91Lysfs*14) | 4 | Spain (2) |
| | | c.271dupA (p.Arg91Lysfs*14)/c.440G > A (p.Gly147Asp) | 1 | Morocco (2) |
| | | c.271dupA (p.Arg91Lysfs*14)/c.464G > A (p.Gly155Glu) | 1 | Spain |
| MMADHC | MMAC2H | c.748C > T (p.Arg250Ter)/c.748C > T (p.Arg250Ter) | 1 | Spain |
| MMA (4) | MMUT | c.322C > T (p.Arg108Cys)/c.2026G > A (p.Ala676Thr) | 1 | Spain |
| | | c.655A > T (p.Asn219Tyr)/c.2206C > T (p.Leu736Phe) | 1 | Bulgaria |
| | | c.220G > T (p.Glu74Ter)/c.548A > T (p.His183Leu) | 1 | Spain |
| | | c.662 T > G (p.Phe221Cys)/c.569G > A (p.Arg190His) | 1 | Spain |

Abbreviations: BCAT-2, branched-chain amino acid transferase 2 deficiency; CPT-I, carnitine palmitoyltransferase type 1 deficiency; CPT-II, carnitine palmitoyltransferase type 2 deficiency; CTLN1, citrullinemia type 1; GA-1, glutaric aciduria type 1; HC, homocystinuria; HMG-CLD, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; HPA, hyperphenylalaninemia; IEM, inborn error of metabolism; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MADD, multiple acyl-CoA dehydrogenase deficiency; MMAHC, methylmalonic academia with homocystinuria; MSUD, maple syrup urinary disease; OTC, ornithine transcarbamylase deficiency; PA, propionic acidemia; PKU, phenylketonuria; SPCD, systemic primary carnitine deficiencies; TYRSN-1, tyrosinemia type 1; TYRSN-3, tyrosinemia type 3; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency.
The most prevalent variant was the c.1208C > T associated with benign HPA, and the second one the variant c.842C > T, which was not identified in a previous study conducted in our Country.²⁷ However, as NBS has enabled the detection of patients with milder phenotype, the molecular heterogeneity of this defect has increased. Consequently, patients diagnosed within the screening generally show a lower proportion (30-71%) of that common variant.²⁷,⁶¹ In addition, a good correlation between genotype and enzyme function has recently been demonstrated.⁶²

In conclusion, in 9 years, 222 IEM have been detected with a large clinical, biochemical, and molecular heterogeneity. Most of the cases benefited from presymptomatic diagnosis but with quite notable differences among the different disorders and 27 novel variants have been reported.

CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS
Consuelo Pedrón-Giner: Had the original idea and contributed to planning the research design, methods, and preparation of manuscript. Álvaro Martín-Rivada and Laura Palomino Pérez: Contributed to data acquisition, carrying out aspects of the methods and statistical analysis, and writing the draft of the manuscript. Belén Pérez: Performed, reviewed, and discussed data concerning molecular and genetic diagnosis. All authors have been involved in drafting the article and have expressed their agreement to submission.

DATA AVAILABILITY STATEMENT
Data archiving is not mandated but data will be made available on reasonable request.

INFORMED CONSENT
All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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**SUPPORTING INFORMATION**
Additional supporting information may be found in the online version of the article at the publisher’s website.

**Supplementary Material S1** Novel variants detected after the Implementation of Expanded Newborn Screening in Madrid.

| dbSNP: Database for Single Nucleotide Polymorphisms |
| PhiloP |
| GVGD: Grantham Variation - Grantham Deviation |
| SIFT: Scale-invariant feature transform |
| CSVS: Collaborative Spanish Variant Server |
| MAF: Minimum allele frequency |
| GnomAD: Genome Aggregation Database |
| ACMG: American College of Medical Genetics |

**Supplementary Material S2** Novel variants affecting intronic sites detected after the Implementation of Expanded Newborn Screening in Madrid.

| SSF: Splicing Sequences Finder |
| MaxEnt: Maximum Entropy Modeling |
| NNSPLICE: Splice Site Prediction by Neural Network |
| CSVS: Collaborative Spanish Variant Server |
| MAF: Minimum allele frequency |
| GnomAD: Genome Aggregation Database |
| ACMG: American College of Medical Genetics |

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