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ARTICLE

Value of genetic analysis for confirming inborn errors of metabolism detected through the Spanish neonatal screening program

Rosa Navarrete[1] · Fátima Leal[1] · Ana I. Vega[1] · Ana Morais-López[2] · María Teresa Garcia-Silva[3] · Elena Martín-Hernández[3] · Pilar Quijada-Fraile[3] · Ana Bergua[2] · Inmaculada Vives[4] · Inmaculada García-Jiménez[5] · Raquel Yahyaoui[6] · Consuelo Pedrón-Giner[7] · Amaya Belanger-Quintana[8] · Sinziana Stanescu[8] · Elvira Cañedo[7] · Oscar García-Campos[9] · María Bueno-Delgado[10] · Carmen Delgado-Pecellín[11] · Isidro Vitoria[12] · María Dolores Rausell[13] · Elena Balmaseda[14] · Mari Luz Couce[15] · Lourdes R. Desviat[1] · Begoña Merinero[1] · Pilar Rodríguez-Pombo[1] · Magdalena Ugarte[1] · Celia Pérez-Cerdá[1] · Belén Pérez[1]

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Abstract

The present work describes the value of genetic analysis as a confirmatory measure following the detection of suspected inborn errors of metabolism in the Spanish newborn mass spectrometry screening program. One hundred and forty-one consecutive DNA samples were analyzed by next-generation sequencing using a customized exome sequencing panel. When required, the Illumina extended clinical exome panel was used, as was Sanger sequencing or transcriptional profiling. Biochemical tests were used to confirm the results of the genetic analysis. Using the customized panel, the metabolic disease suspected in 83 newborns (59%) was confirmed. In three further cases, two monoallelic variants were detected for two genes involved in the same biochemical pathway. In the remainder, either a single variant or no variant was identified. Given the persistent absence of biochemical alterations, carrier status was assigned in 39 cases. False positives were recorded for 11. In five cases in which the biochemical pattern was persistently altered, further genetic analysis allowed the detection of two variants affecting the function of *BCAT2, ACSF3*, and *DNAJC12*, as well as a second, deep intronic variant in *ETFDH* or *PTS*. The present results suggest that genetic analysis using extended next-generation sequencing panels can be used as a confirmatory test for suspected inborn errors of metabolism detected in newborn screening programs. Biochemical tests can be very helpful when a diagnosis is unclear. In summary, simultaneous genomic and metabolomic analyses can increase the number of inborn errors of metabolism that can be confirmed following suggestive newborn screening results.

Introduction

The main purpose of newborn screening (NBS) programs is to diagnose genetic disorders early, allowing treatment to begin before symptoms appear. Inborn errors of metabolism (IEM) make up a phenotypically and genetically heterogeneous group of rare disorders resulting from defects in certain metabolic pathways which in many cases cause the accumulation of toxic intermediate metabolites [1, 2]. To date more than 1000 different IEM have been identified.

In the last decade, the use of tandem mass spectrometry (MS/MS) in expanded NBS for IEM has become mandatory in Western countries, such as Spain [3]. The quantification of amino acids and acylcarnitines in dried blood spots (DBS) by MS/MS allows the simultaneous detection of more than 30 metabolic disorders, including those associated with amino acid, organic acid, and fatty acid metabolism. Fast and inexpensive, this technique is almost 100% sensitive for hyperphenylalaninemia (HPA) and medium-chain acyl CoA dehydrogenase deficiency (MCADD), and shows very high sensitivity for early onset organic acidurias, fatty acid oxidation defects, and a number of amino acid disorders. For homocystinuria and tyrosinemia type I,

Shared senior authorship: Magdalena Ugarte, Celia Pérez-Cerdá, Belén Pérez

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✉ Belén Pérez
bperez@cbm.csic.es

Extended author information available on the last page of the article

however, it is not so sensitive [4]. Other disorders detected using different techniques, e.g., galactosemia, biotinidase deficiency, Pompe disease, and mucopolysaccharidosis type I, are now included in some NBS programs, as are some secondary targets which may help in reaching a differential diagnosis [5].

When a metabolic abnormality is detected in DBS analysis, other samples (plasma and urine) are collected for confirmatory biochemical testing—commonly for amino acids, homocysteine, acylcarnitines, and organic acids [6]. In many cases, this may complete a differential diagnosis since certain biomarkers are related to defects in different genes (locus-heterogeneous disorders). In cases of HPA, for example, the analysis of pterins in the urine, and the determination of dihydropteridine reductase (DHPR) activity in DBS, can provide an idea of which gene might be involved [7]. Genetic analysis is currently performed as a definitive test to confirm a diagnosis and to identify the exact gene involved. The identification of a specific variant can reveal the need for a specific treatment, such as the administration of tetrahydrobiopterin (BH4) in phenylketonuria (PKU), or vitamin B_{12} in some cobalamin disorders [7, 8].

Until 5 years ago, the gold standard for making such genetic confirmations was gene-by-gene Sanger sequencing. However, next-generation sequencing (NGS), which is quicker and cheaper, is receiving increasing attention, particularly for locus-heterogeneous disorders [9].

Genetic analysis should be mandatory for disease confirmation and making personalized therapy choices, and can be performed after biochemical detection in NBS programs. The aim of the present work was to assess the value of genetic analysis as the preferred diagnostic method for such confirmation. To that end, 141 cases of newborns with abnormal levels of amino acids/acylcarnitines in DBS, detected by MS/MS, were examined and genetic diagnoses made. Biochemical data were used to validate the genetic results.

Materials and methods

The study was approved by the ethics committee of the *Universidad Autónoma de Madrid*. The legal guardians of the participating infants gave their signed, informed consent for their children to be included in the study.

The study included 141 infants with altered acylcarnitine or amino acid levels—as revealed by DBS at 2 days of life—detected during screening at eight Spanish NBS centers between January 2012 and April 2017. Each center had previously, and independently, determined its internal NBS 99.5 percentile cutoff levels and the criteria for positive screening results. The study sample covers all cases referred from hospitals involved in the management of newborns with positive NBS results. Premature newborns, those with a low birth weight, and those with high plasma bilirubin levels were excluded from the sample by the clinicians involved. Samples (whole blood, plasma, urine, another DBS, or cerebrospinal fluid) for confirmatory biochemical and/or genetic testing were taken in the first 2 weeks of life at the corresponding clinical units before any therapeutic intervention was begun.

High purity DNA or RNA was extracted from whole blood or DBS using the MagNA Pure Compact Kit (Roche Applied Biosciences, Indianapolis, USA) following the manufacturer's instructions. DNA from DBS was also extracted using a Covaris® M220 Focused Ultrasonicator. Two NGS panels were used: PANEL 1—a targeted, customized exome sequencing panel (Table S1) to capture the exome of 119 genes involved in metabolic disorders (Nextera Nature Capture [Illumina, San Diego, CA, USA]) and also the entire sequence of the genes *PAH, ALDOB, OTC, SLC22A5, GLDC*, and *PCCA*; and PANEL 2—an extended panel (Clinical-Exome Sequencing TruSight™ One Gene Panel [Illumina]) that includes all the known (in 2013) disease-associated genes described in the OMIM database (Mendeliome panel). Following sequence enrichment, the reads were aligned with the reference genome hg19. Virtual prioritization of the genes related to NBS biochemical detection was contemplated with both panels. All disease-causing variants reported in the HGMD professional release 2018.4 database, the ClinVar database, and our in-house database, plus all variants with a minor allele frequency (MAF) of less than 1% in the ExAC, EVS, and CSVS databases (CIBERER Spanish Variant Server (http://csvs.babelomics.org/), were taken into account. The prediction of the potential functional effect of variants was made using Alamut Visual software, which combines several predictors. Variants were classified following American College of Medical Genetics guidelines [10, 11] using the application at https://varsome.com. This application uses the conservation score, allelic frequencies (gnomAD, ClinvAr or dbSNP), the identification of changes in an amino acid previously established as affecting function, the location of variants in mutational hot spots and/or critical domains, trans/cis positioning, and segregation studies. New variants refer to changes not included in the HGMD professional release 2018. This software classifies variants under six categories: pathogenic, likely pathogenic, of uncertain significance, likely benign, benign, and disease-associated polymorphisms. Incidental findings for genes unrelated to the clinical/biochemical phenotypes were reported if the guardians so allowed. The libraries generated with either exome panel were sequenced using 250 bp paired-end reads using the Illumina MiSeq or Nextseq500 NGS platforms.

Sanger sequencing of the deep intronic sequence of *PTS*, and of five exonic sequences of *DNAJC12*, was performed as previously described [12, 13]. *ETFDH* transcriptional profiling was performed using SuperScript® VILO (Invitrogen, Carlsbad, CA, USA).

For biochemical confirmatory tests, amino acids, acylcarnitines, and organic acids in biological samples (DBS, plasma, urine or CSF) were analyzed as previously described [14, 15].

Results

PANEL 1 allowed the detection of biallelic variants in 26 different genes in 81 infants. One monoallelic variant in *OTC* (an X-linked disorder), and another in *MAT1A* (hypermethioninemia, a probable dominant disorder), was found in two other infants (Table S2). Overall, four infants had defects in *ACAD8*, eight in *ACADM*, five in *ACADS*, one in *ACADSB*, five in *ACADVL*, one in *ACAT1*, one in ASL, two in *ASS1*, one in CBS, one in *BCKDHA*, one in *CD320*, one in *CPT1A*, one in *ETFB*, three in *GCDH*, one in *HADHA*, two in *IVD*, one in *MAT1A*, three in *MCCC1*, six in *MCCC2*, one in *MMAB*, one in *MMACHC*, two in *MMADHC*, two in *MUT*, one in *OTC*, twenty-five in *PAH*, one in *PCCB*, one in *PRODH*, and one in *SLC22A5*. A diagnosis was reached for 59% (83/141) of the cases examined. Seventy-nine confirmed cases involved a previously described variant affecting function (i.e., recorded in the HGMD professional release 2017.4 database) or a novel loss-of-function variant, e.g., a splicing variant in the canonical splice site, or a small out-of-frame insertion/deletion, or a nonsense variant. The other four infants carried novel missense variations in both alleles (P1, P5, and P54) or a missense variation in the X chromosome (P55, a boy). All variants were predicted to affect protein function (Tables S3, S4, and S5).

In addition, three infants had two monoallelic variants in two different genes involved in the same metabolic pathway (Table S6). To determine the biological significance of these results, segregation analysis was performed for the parents. The results showed that two of these infants, P84 and P86, had the variant in either the paternal or maternal allele, while infant P85 had two novel variants in *ETFDH* and *ETFA*, both inherited from the father. A biochemical analysis of the father's plasma revealed slightly elevated levels of acylcarnitines, similar to those detected in his child.

For the remaining 55 infants with abnormal NBS results, the genetic defect could not be confirmed using PANEL 1. Forty-one infants had only one variant in a candidate gene. To rule out the presence of an unidentified second variant, the data recorded in confirmatory biochemical testing were examined. This revealed that 39 infants with only one identified variant had normal or very mildly abnormal biomarker levels in the first month of life (e.g., 10 infants heterozygous for MCADD [MIM#201450] and 12 for VLCADD [MIM#609575] [Table S7]), a situation that could be compatible with carrier status. One infant with a described variant in *EFTDH* (Table S8-P140), and another with a new variant in *PTS* (Table S8-P141), had persistently abnormal biochemical results and were selected for further study.

For 14 infants, no variants were detected in the exonic regions of the examined genes. Analysis of their biochemical data revealed 10 to have normal biomarkers and one to have low plasma-free carnitine levels (perhaps attributable to a mild nutritional deficiency of the child or its mother) (Table S9). The latter 11 cases might be considered false positives.

The remaining three infants with no variants found using PANEL 1 had persistent biochemical abnormalities on follow-up (Table S8), and were therefore further analyzed, as were infants P140 and P141 (children with one variant in *ETFDH* or *PTS)*. In P140, transcriptional analysis of *ETFDH* was performed and the result showed a potential pseudoexon to be inserted between exons 2 and 3 (r.175_176ins176-635_176-581). Genomic DNA analysis revealed a new variation c.176–636C>G (Table S8). This change created a splice donor site used for the insertion of a new pseudoexon. Genomic analysis of *PTS* by Sanger sequencing of the intronic sequence, where deep intronic variants have been described [12, 16], allowed the identification of a new variant in infant P141 (Table S8) that probably provokes the insertion of a pseudoexon.

Two infants (P137 and P138) were analyzed using PANEL 2 combined with virtual capture based on their biochemical findings (Table S8). These cases had persistent biochemical alterations; their genetic analysis was therefore extended to genes not reported as causing disease or not included in PANEL 1. Variants were detected in *ACSF3* (MIM*614245; #614265) in infant P137, who was referred to the laboratory with isolated methylmalonic aciduria, and in *BCAT2* (MIM*113530) in infant P138, for whom maple syrup urine disease (MSUD) was suspected (Table S8). Malonic acid was detected in subsequent urine organic acid analysis in infant P137. *ACSF3* was not included in the customized panel (PANEL 1) due to the low frequency described at the time of its design. Finally, one infant (P139) with persistent hyperphenylalaninemia (HPA) but no variants in *PAH* or BH4 synthesis-deficient genes were analyzed for variants in *DNAJC12*, a gene recently reported as being associated with this biochemical phenotype (*606060; #617384) [13]. Sanger sequencing was performed since this gene was not covered by PANEL 2, and one variation was detected in homozygosis (Table S8).

Overall, 150 different variants in 37 genes, and 54 novel variants—of which 41 are nucleotide changes (29 likely missense variants, 4 nonsense, and 7 splicing variants) were identified. The remaining new variants were seven small deletions, three insertions/duplications, two duplications, and one large genomic rearrangement. All these variants were classified according to ACMG guidelines. All but four were classified as pathogenic or likely pathogenic (class 1 and class 2 variants) (Table S3–S5).

Confirmatory biochemical test results were used to validate the genetic results (Tables S2, S6, S8, S9), and were consistent for the 91 cases of genetically confirmed amino acid, acylcarnitine, or organic acid profile abnormalities. Almost half of these infants had a mild biochemical phenotype, suggesting a more favorable clinical prognosis. All the children were asymptomatic at the time of testing, although infants P36 and P37 showed drowsiness. The mother of infant P36 was diagnosed with HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) during pregnancy.

Discussion

Recent advances in genomics now allow early diagnoses of IEM to be made, and tailored therapies prescribed, depending on the genetic defects identified. Currently, however, NBS for IEM relies largely on the identification of elevated amino acid and acylcarnitines levels by MS/MS [4, 17]. The gold standard for confirmation is biochemical testing. A genetic analysis may be requested for the identification of the specific genetic disorder, but the present study suggests that in the future, when the turn-around-time of genetic analysis by NGS becomes shorter, it could be used as an alternative, rapid and reliable confirmatory test.

The disease suspected in first tier DBS analysis was confirmed for 83 infants (59%) using the customized panel of 120 genes (PANEL 1). Diagnosis was confirmed by the presence of biallelic variants in recessive disorders, or by one in X-linked or dominant diseases [10, 11]. In addition, the use of PANEL 1 allowed the identification of three infants with two variants (some previously reported as affect function or predicted to be harmful) in two genes involved in the same metabolic pathway. Two of the three infants (P84 and P86) showed only very mild abnormalities in the confirmatory samples. The third (P85) inherited both changes from his healthy father, who also showed slightly increased levels of plasma acylcarnitines, suggesting therapeutic intervention to be unnecessary. However, partial defects in multiple steps of pathways affecting energy metabolism have been described in patients with symptoms [18]. It cannot be ruled out, therefore, that all three infants are at no risk of clinically significant disease; they should be followed up clinically and biochemically and the analysis of other genes possibly undertaken.

The suspected disease could not be confirmed genetically with PANEL 1 in the remaining 55 cases. However, subsequent inspection of the biochemical results (plasma amino acid and acylcarnitine, urine organic acid, and acylglycine analyses, as required) allowed cases of disease to be distinguished from NBS-detected false positives or carrier status. Both the latter were related mainly to mitochondrial fatty acid beta oxidation defects. Twenty-two infants with likely variants affecting the function in one allele in *ACADM* or *ACADVL*, and eight suspected cases of VLCADD with no genetic variation affecting function, were identified. In these cases, the mild or non-elevation of the specific acylcarnitines in the confirmatory plasma samples helped to rule out the presence of variants in the non-analyzed sequences, such as intronic regions and regulatory sequences, etc. Moreover, mild accumulation of long-chain acylcarnitines has been described in healthy children due to the activation of mitochondrial fatty acid oxidation during catabolism in the first days of life [19]. This enhanced catabolism may have been responsible for the exceeded cutoff values for acylcarnitines originally detected in DBS from infants with one or no variants. A revision of the cutoff levels for octanoyl-carnitine (C8) and tetradecenoyl-carnitine (C14:1) would probably improve the genetic diagnosis rate [15]. The results obtained via the combination of the genetic and biochemical analyses show that no genetic analysis is required for borderline acylcarnitine levels.

For those infants showing persistent biochemical alterations, the use of PANEL 2, Sanger sequencing, or transcriptional profile analysis, allowed biallelic variants to be identified in five genes. To date, customized panels have generally been used given the lack of diagnostic coverage of whole-exome capture, but this latter technology has improved considerably in recent years and can be used for the genetic diagnosis of rare diseases [20]. The present results clearly suggest that the use of large panels can increase diagnostic accuracy, identifying genetic defects already reported as involved in disease, as well as novel associated defects. Deeper, whole-exome sequencing or even whole-genome sequencing could be used for the detection of previously described genetic defects associated with specific biochemical conditions, as well as for detecting infrequent and non-described molecular defects. This would also expand our knowledge of classic phenotypic characteristics and provide insight into associations between "old diseases" and "new genes". In fact, if whole-exome sequencing had been used in the present work instead of gene panels, variants in *DNAJC12*, a gene recently associated with HPA [13], would have been detected in infant P139. New genes described in methylmalonic aciduria with

homocystinuria [21–23] would also have been detected. In the future, whole-genome sequencing in combination with daily-updated virtual panels should allow the detection of all variants and genes.

NGS is ready to be used as a clinical diagnostic tool. For DBS or whole blood, sample preparation, sequencing, alignment, and the generation of variant calling files can be performed in 5–7 days. The technology suffers, however, from a bottleneck: the identification of variants which affect function among the thousands of changes detected. An area for future work is to reduce the time for variant interpretation and the increasing of new variants in the database will aid to reduce the time used for variant interpretation. In the present work, most of the infants had one described variant, or a new loss-of-function variant. Four of the present infants (P1; P5; P54; & P55) had two new missense changes, all predicted to affect function. It was helpful to know the severity of biochemical alterations in these infants' confirmatory samples—information that, along with the results of genetic analysis, can help in deciding whether to begin a specific treatment [24]. It would be best of all if testing laboratories could perform both types of analysis, especially when biochemical confirmation is rapid. Sharing all obtained genetic and biochemical information in curated databases, such as the Clinical Genome Resource Database (ClinGen) or the Leiden Open Variation Database (LOVD), would provide curated clues that might reduce the time required to interpret the significance of a variation [25].

Discussion is also required regarding the cost-effectiveness of performing genetic analyses instead of biochemical confirmation. Nobody doubts that the biochemical confirmation of NBS-detected conditions is cheaper than genetic analysis. However, all cases need to be genetically confirmed if personalized therapy and/or genetic counseling are to be provided. Indeed, some therapies are gene-specific, e.g., for hyperphenylalaninemias, and sometimes even variant-specific, e.g., for phenylketonuria and cobalamin disorders. Thus, the trend must be to move toward genetic analysis for the confirmation of conditions detected by MS/MS in NBS screening, with biochemical confirmation called upon to resolve uncertain cases [25].

The main benefit of NBS is the detection of preventable or treatable disorders [4]. Current screening for disorders of the metabolism of amino acids, organic acids, and fatty acids is reliable. However, many metabolic and other treatable disorders detectable in the first days of life, which would benefit from early, tailored treatment (e.g., classical galactosemia, the sixth most common metabolic disorder in the white population), are not included in the majority of NBS programs [26]. Other disorders affecting the metabolism of complex molecules, such as peroxisome and lysosomal disorders, congenital disorders of glycosylation, combined immunodeficiency etc., some of which are treatable, could also be detected by genomic analysis. These could be detected if NGS were used as the diagnosis method in NBS programs, although the pathogenicity of any rare variants detected would need to be determined [24, 27].

As we have indicated above increasing the number of detectable inborn errors of metabolism in the first days of life is a challenge. The use of specific panels for arriving at a diagnosis is attractive since it would reduce the number of variants to be identified, although it might result in misdiagnosis since the number of genes associated with specific biochemical markers is increasing all the time. Whole-exome or whole-genome sequencing might provide a way to increase the number of defects detected in the first days of life, for which action can be taken. However, prioritizing the thousands of variants to distinguish between those that affect function or ultra-rare (in many cases population-specific), and those that are not, is a bottleneck. In addition, new genetic findings such as epimutations [28] or allelic expression imbalances detected in heterozygous individuals [29] require additional biochemical confirmation if misdiagnoses are to be avoided. The simultaneous use of whole-genomic analysis and next-generation metabolic screening (NGMS) has been proposed to increase the number of inborn errors of metabolism detectable [30–32]. However, the need for cost-effectiveness cannot be ignored. Although combined genetic/metabolomic analysis could increase the number of diseases detected, the cost might be difficult for healthcare systems to bear [33].

In summary, a precise diagnosis was provided for a total of 91 of the 141 inspected cases (Figure S1); the remainder were either false positives or the children involved were carriers. The availability of precise biochemical information greatly facilitates the interpretation of genetic data, helping to avoid misdiagnoses. Thus, wherever possible, genetic and metabolomic analyses should go hand in hand to provide the best diagnosis possible.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Pampols T. Inherited metabolic rare disease. Adv Exp Med Biol. 2010;686:397–431.
2. Morava E, Rahman S, Peters V, Baumgartner MR, Patterson M, Zschocke J. Quo vadis: the re-definition of "inborn metabolic diseases". J Inherit Metab Dis. 2015;38:1003–6.
3. Couce ML, Sanchez-Pintos P, Diogo L, Leao-Teles E, Martins E, Santos H, et al. Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency: regional experience and high incidence of carnitine deficiency. Orphanet J Rare Dis. 2013;8:102.
4. Wilcken B. Newborn screening: how are we travelling, and where should we be going? J Inherit Metab Dis. 2011;34:569–74.
5. Martinez-Morillo E, Prieto Garcia B, Alvarez Menendez FV. Challenges for Worldwide Harmonization of Newborn Screening Programs. Clin Chem. 2016;62:689–98.
6. Vilarinho L, Rocha H, Sousa C, Marcao A, Fonseca H, Bogas M, et al. Four years of expanded newborn screening in Portugal with tandem mass spectrometry. J Inherit Metab Dis. 2010;33:Suppl 3: S133–138.
7. Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. Mol Genet Metab. 2011;104:Suppl,S2–9.
8. Fowler B, Leonard JV, Baumgartner MR. Causes of and diagnostic approach to methylmalonic acidurias. J Inherit Metab Dis. 2008;31:350–60.
9. Trujillano D, Ramos MD, Gonzalez J, Tornador C, Sotillo F, Escaramis G, et al. Next generation diagnostics of cystic fibrosis and CFTR-related disorders by targeted multiplex high-coverage resequencing of CFTR. J Med Genet. 2013;50:455–62.
10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
11. Kleinberger J, Maloney KA, Pollin TI, Jeng LJ. An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. Genet Med. 2016;18:1165.
12. Brasil S, Viecelli HM, Meili D, Rassi A, Desviat LR, Perez B, et al. Pseudoexon exclusion by antisense therapy in 6-pyruvoyl-tetrahydropterin synthase deficiency. Hum Mutat. 2011;32:1019–27.
13. Anikster Y, Haack TB, Vilboux T, Pode-Shakked B, Thony B, Shen N, et al. Biallelic Mutations in DNAJC12 Cause Hyperphenylalaninemia, Dystonia, and Intellectual Disability. Am J Hum Genet. 2017;100:257–66.
14. Tondo M, Calpena E, Arriola G, Sanz P, Martorell L, Ormazabal A, et al. Clinical, biochemical, molecular and therapeutic aspects of 2 new cases of 2-aminoadipic semialdehyde synthase deficiency. Mol Genet Metab. 2013;110:231–6.
15. Merinero B, Alcaide P, Martin-Hernandez E, Morais A, Garcia-Silva MT, Quijada-Fraile P, et al. Four Years' Experience in the Diagnosis of Very Long-Chain Acyl-CoA Dehydrogenase Deficiency in Infants Detected in Three Spanish Newborn Screening Centers. JIMD Rep. 2018;39:63–74
16. Meili D, Kralovicova J, Zagalak J, Bonafe L, Fiori L, Blau N, et al. Disease-causing mutations improving the branch site and polypyrimidine tract: pseudoexon activation of LINE-2 and antisense Alu lacking the poly(T)-tail. Hum Mutat. 2009;30:823–31.
17. Wilcken B. Recent advances in newborn screening. J Inherit Metab Dis. 2007;30:129–33.
18. Vockley J, Rinaldo P, Bennett MJ, Matern D, Vladutiu GD. Synergistic heterozygosity: disease resulting from multiple partial defects in one or more metabolic pathways. Mol Genet Metab. 2000;71:10–18.
19. Boneh A, Andresen BS, Gregersen N, Ibrahim M, Tzanakos N, Peters H, et al. VLCAD deficiency: pitfalls in newborn screening and confirmation of diagnosis by mutation analysis. Mol Genet Metab. 2006;88:166–70.
20. Nguyen MT, Charlebois K. The clinical utility of whole-exome sequencing in the context of rare diseases - the changing tides of medical practice. Clin Genet. 2015;88:313–9.
21. Yu HC, Sloan JL, Scharer G, Brebner A, Quintana AM, Achilly NP, et al. An X-linked cobalamin disorder caused by mutations in transcriptional coregulator HCFC1. Am J Hum Genet. 2013;93:506–14.
22. Pupavac M, Watkins D, Petrella F, Fahiminiya S, Janer A, Cheung W, et al. Inborn Error of Cobalamin Metabolism Associated with the Intracellular Accumulation of Transcobalamin-Bound Cobalamin and Mutations in ZNF143, Which Codes for a Transcriptional Activator. Hum Mutat. 2016.
23. Quintana AM, Yu HC, Brebner A, Pupavac M, Geiger EA, Watson A, et al. Mutations in THAP11 cause an inborn error of cobalamin metabolism and developmental abnormalities. Hum Mol Genet. 2017;26:2838–49.
24. Narravula A, Garber KB, Askree SH, Hegde M, Hall PL. Variants of uncertain significance in newborn screening disorders: implications for large-scale genomic sequencing. Genet Med. 2017;19:77–82.
25. Peng G, Shen P, Gandotra N, Le A, Fung E, Jelliffe-Pawlowski L et al. Combining newborn metabolic and DNA analysis for second-tier testing of methylmalonic acidemia. Genet Med. 2018.
26. Varela-Lema L, Paz-Valinas L, Atienza-Merino G, Zubizarreta-Alberdi R, Villares RV, Lopez-Garcia M. Appropriateness of newborn screening for classic galactosaemia: a systematic review. J Inherit Metab Dis. 2016;39:633–49.
27. Pavey AR, Bodian DL, Vilboux T, Khromykh A, Hauser NS, Huddleston K, et al. Utilization of genomic sequencing for population screening of immunodeficiencies in the newborn. Genet Med. 2017;19:1367–75.
28. Gueant JL, Chery C, Oussalah A, Nadaf J, Coelho D, Josse T, et al. APRDX1 mutant allele causes a MMACHC secondary epimutation in cblC patients. Nat Commun. 2018;9:67.
29. Falkenberg KD, Braverman NE, Moser AB, Steinberg SJ, Klouwer FCC, Schluter A, et al. Allelic Expression Imbalance Promoting a Mutant PEX6 Allele Causes Zellweger Spectrum Disorder. Am J Hum Genet. 2017;101:965–76.
30. Wevers RA, Blau N. Think big - think omics. J Inherit Metab Dis. 2018;41:281–3.
31. Del Mar Amador M, Colsch B, Lamari F, Jardel C, Ichou F, Rastetter A, et al. Targeted versus untargeted omics - the CAFSA story. J Inherit Metab Dis. 2018;41:447–56.
32. van Karnebeek CDM, Wortmann SB, Tarailo-Graovac M, Langeveld M, Ferreira CR, van de Kamp JM, et al. The role of the clinician in the multi-omics era: are you ready? J Inherit Metab Dis. 2018;41:571–82.
33. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018.

Affiliations

Rosa Navarrete[1] · Fátima Leal[1] · Ana I. Vega[1] · Ana Morais-López[2] · María Teresa Garcia-Silva[3] · Elena Martín-Hernández[3] · Pilar Quijada-Fraile[3] · Ana Bergua[2] · Inmaculada Vives[4] · Inmaculada García-Jiménez[5] · Raquel Yahyaoui[6] · Consuelo Pedrón-Giner[7] · Amaya Belanger-Quintana[8] · Sinziana Stanescu[8] · Elvira Cañedo[7] · Oscar García-Campos[9] · María Bueno-Delgado[10] · Carmen Delgado-Pecellín[11] · Isidro Vitoria[12] · María Dolores Rausell[13] · Elena Balmaseda[14] · Mari Luz Couce[15] · Lourdes R. Desviat[1] · Begoña Merinero[1] · Pilar Rodríguez-Pombo[1] · Magdalena Ugarte[1] · Celia Pérez-Cerdá[1] · Belén Pérez[1]

[1] Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular, Universidad Autónoma de Madrid, CIBERER, IdiPAZ, Madrid, Spain

[2] Unidad de Nutrición Infantil y Enfermedades Metabólicas, Hospital Universitario La Paz, Madrid, Spain

[3] Unidad de Enfermedades Mitocondriales y Enfermedades Metabólicas Hereditarias, Hospital Universitario 12 de Octubre, Madrid, Spain

[4] Dpto. de Gastroenterología y Nutrición, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain

[5] Unidad de Enfermedades Metabólicas, Hospital Universitario Miguel Servet, Zaragoza, Spain

[6] Laboratorio de Metabolopatías, Hospital Regional Universitario, Instituto de Investigación Biomédica de Málaga, Málaga, Spain

[7] Sección de Gastroenterología y Nutrición, Hospital Universitario Niño Jesús, Madrid, Spain

[8] Unidad de Enfermedades Metabólicas Congénitas, Hospital Universitario Ramón y Cajal, Madrid, Spain

[9] Dpto, de Pediatría, Hospital Virgen de la Salud, Toledo, Spain

[10] Dpto. de Pediatría, Hospital Universitario Virgen del Rocío, Seville, Spain

[11] Laboratorio de Metabolopatías, Hospital Universitario Virgen del Rocío, Seville, Spain

[12] Unidad de Nutrición y Metabolopatías, Hospital Universitario La Fe, Valencia, Spain

[13] Laboratorio de Metabolopatías, Hospital Universitario La Fe, Valencia, Spain

[14] Dpto. de Pediatría, Complejo Hospitalario Universitario de Albacete, Albacete, Spain

[15] Unidad de Enfermedades Metabólicas, Hospital Clínico Universitario de Santiago, Santiago de Compostela, CIBERER, Santiago, Spain