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EDITORIAL

Inherited metabolic disorders beyond the new generation sequencing era: the need for in-depth cellular and molecular phenotyping

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Inherited metabolic disorders (IMD) encompass alterations of one or several biochemical pathways with biochemical, clinical and/or pathophysiological features that have a defined or assumed inherited cause (Ferreira et al. 2021). The phenotypes of IMD expanded in the last years with different clinical expressions for variants in the same gene inducing different clinical presentation in all age ranges. In few cases, altered enzyme activities can be produced by somatic mutations, as is the case with the D-2-hydroxyglutaric aciduria produced by somatic mutations in *isocitrate dehydrogenase 1 (IDH1)* (Bruce-Brand and Govender 2020). The term "disorder" covers any type of alteration, regardless of the cause that leads to the manifestations. It has a wider significance than a disease, which is defined by symptoms related to a defined pathogenesis. Most of IMD are produced by "mutations", e.g., by genetic variants that directly alter the expression and/or the functional properties of enzymes or transporters with subsequent specific metabolic and clinical consequences (Ferreira et al. 2021). The consequences of the pathogenic mutation on clinical manifestations and sensitivity to treatment depend not only on the severity of metabolic alteration but also on other complex factors that include age, diet and physical activity. This is illustrated by the example of *cblC* type of cobalamin IMD due to *MMACHC* pathogenic mutations, in which the spectrum of eye disease depends on metabolic severity and age of onset, regardless of early metabolic treatment (Matmat et al. 2021). The metabolic and cellular phenotyping of IMD is crucial to categorize the functional consequences of mutations in targeted genes (Ferreira et al. 2021). This is illustrated by the article from Forny et al. on cblB-type methylmalonyl aciduria (MMA). This IMD is produced by pathogenic variants in the *MMAB* gene, which cause isolated methylmalonic aciduria of the *cblB* type (Forny et al. 2021). This disease can present with an early onset of severe metabolic acidosis. The authors performed molecular genetics and examined the propionate incorporation activity in *cblB*-type MMA patient fibroblasts. They reported 16 novel pathogenic variants in the *MMAB* gene and provided a clear concordance between the hydroxocobalamin treatment responsiveness and the fibroblast propionate incorporation activity.

Since the revolution of the new generation sequencing (NGS), the use of clinical exome panels or wide exome sequencing (WES) completed by whole genome sequencing in inconclusive cases has allowed to significantly increase the molecular diagnosis of IMD and to identify new genes and new genetic presentations, including digenism (Beaulieu et al. 2014; Fernández-Marmiesse et al. 2018). In this issue, Mergnac et al. assessed the diagnostic yield of a clinical exome panel of the main OMIMM genes as a first-tier genetic test in 128 consecutive pediatric patients addressed to a referral center of IMD in the North-East of France (Mergnac et al. 2021). They found a diagnostic yield of 81% (21/26) for hyperlipidemia, 64% (25/39) for other IMD, and 39% (10/26) for neurological disorders, showing the importance of the clinical and metabolic phenotyping in the bioinformatic analysis of WES (Mergnac et al. 2021). A large part of the diagnostic deadlocks of IMD produced by non-conclusive genome wide sequencing (WGS) corresponds to pathological mechanisms that cannot be identified by current methods because they are linked to developmental anomalies and/or to cell and organ specificities.

Various types of mechanisms may explain the failure of NGS to reach a diagnosis. The causal genes may not be directly involved in the metabolic pathway linked to the diseases and their identification can, therefore, escape

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careful targeted bioinformatics analyses. Indeed, the characterization of the biological phenotype essentially uses the biochemical analysis of intermediate metabolites. It is also based on global cellular approaches which do allow to decipher the dysregulations of the metabolism in connection with the development, the differentiation, the anomalies of intracellular trafficking and the intracellular compartmentation of metabolites. In cases of non-conclusive NGS analyses, there is a great interest to perform in-depth studies in patient cells, including in iPSC progenitors and derived differentiated cells to identify potential underlying genes involved in unexpected cellular mechanisms and/or digenic, oligogenic and / or epigenomic mechanisms. Several type of IMD illustrate this evidence.

The in-depth cellular phenotyping allowed to identify several new Congenital Disorder of Glycosylation (CDG) pathologies affecting intra-vesicular Golgi traffic, divalent cations and pH homeostasis of the Golgi apparatus as well as the synthesis of the glycan precursor at the level of the Endoplasmic Reticulum (ER) (Rymen et al. 2013; Blommaert et al. 2019). The use of cells of patients with CDG sharing such natural mutants allowed to better understand the relation with clinical presentation and the related molecular mechanisms of reticulum and Golgi steps of protein glycosylation. Mutations in the X-linked gene *MAGT1* cause a CDG, with two distinct clinical phenotypes, a primary immunodeficiency (XMEN disorder) versus intellectual and developmental disability. In the present issue, Blommaert et al. revisited the pathophysiological consequences of mutations in *MAGT1* and their consequences in the immunodeficiency of XMEN/MAGT1-CDG patients. They reevaluated the NKG2D protein, which was described in the literature as absent in XMEN patients with Mg2 + homeostasis defects (Chaigne-Delalande et al. 2013; Blommaert et al. 2019). They showed that the NKG2D proper glycosylation depends on MAGT1 and that this glycosylation defect reduces the stability and the NKG2D protein expression in knock out cell lines. Addition of Mg2 + does not restored the expression of NKG2D (Chaigne-Delalande et al. 2013; Blommaert et al. 2019). *SLC10A7* orphan gene is the seventh member of a human sodium/bile acid cotransporter family, known as the SLC10 family. *SLC10A7* is involved in Ca2 + subcellular homeostasis and in proper glycosaminoglycan biosynthesis, especially heparan-sulfate, and N-glycosylation. *SLC10A7* mutations have been identified as responsible for a new CDG likely mediated by glycosaminoglycan defects that lead to skeletal dysplasia with multiple large joints dislocations, short stature and amelogenesis imperfecta. Durin et al. reviewed the current knowledge on the structural and functional properties of this protein, according to altered glycosylation related to *SLC10A7* mutations (Durin et al. 2022).

Other mechanisms may be related to tissue-specific effects and metabolic interactions. For example, the manifestations of IMDs of the cobalamin-dependent remethylation pathway of the one carbon metabolism are closely linked to reticulum stress and decreased the expression of the histone deacetylase SIRT1, with tissue specificities, in in-depth genomic and protein study of cells from patients and transgenic mouse models (Guéant et al. 2022a). Cystathionine beta-synthase is a key heme-protein enzyme of the one carbon metabolism encoded by *CBS* gene. Some IMDs of heme synthesis may produce dramatic hyperhomocysteinemia, with a subsequent decreased activity of CBS that mimics the metabolic presentation of *CBS* pathogenic mutations (Ventura et al. 2020). Over 85% of mutations in CBS deficient patients are missense mutations that produce an inactive mis-folded mutant protein. A review of this issue shows that this mechanism inspired new therapeutic approaches to restoring the function to mutant enzymes. Proteostasis modulators could be used to modulate this network and improve the activity of the mutated CBS (Kruger 2021).

Another type of causal mechanisms of unresolved IMDs is the gene silencing by secondary epimutations. A review of this issue focuses on the epigenetics of two inherited metabolic diseases, epi-*cblC*, an inherited metabolic disorder of cobalamin (vitamin B12) metabolism, and alpha-thalassemia type α-ZF, an inherited disorder of α2-globin synthesis (Guéant et al. 2022b). The new type of cbl IMD named *epi-cblC* was first evidenced in patients from Europe and the United States and further found in Italy and China (Guéant et al. 2018; Guéant et al. 2022b). In both disorders, the epimutation is triggered by an aberrant antisense transcription of flanking genes through the promoter, which produces an H3K36me3 histone mark involved in the recruitment of DNA methyltransferases (Guéant et al. 2022b). Mutations in the *PRDX1* adjacent gene produced the antisense transcription encompassing the *MMACHC*/*CCDC163P* promoter (Guéant et al. 2018; Guéant et al. 2022b). The epimutation in the CpG Island of the *MMACHC* promoter is present in 3 generation and in the sperm of the fathers from cases. The high expression of *PRDX1* in spermatogonia but its nearly undetectable transcription in spermatids and spermatocytes, suggest that the epimutation could be maintained during germline reprogramming despite removal of aberrant transcription (Guéant et al. 2022b). The *PRDX1* splice mutations activate numerous cryptic splice sites and produce antisense readthrough transcripts encompassing not only the bidirectional *MMACHC*/*CCDC163P* promoter but also the neighboring *TESK2* promoter, resulting in impaired expression of both *MMACHC* and *TESK2* genes (Oussalah et al. 2022). The term 'epi-digenism' has been proposed to define this epigenetic disorder that affects two genes (Oussalah et al. 2022). The causal mechanisms of unresolved IMDs may also include mutation on genes encoding transcription factors.

This has been described in *cblC*, the most frequent IMD of cobalamin metabolism, with altered *MMACHC* expression due to mutations in any of the transcription factors HCFC1, THAP11 and ZNF143 that form an interacting complex in *MMACHC* promoter (Michaud et al. 2013; Watkins and Rosenblatt 2022).

In conclusion, the articles of the present issue point out the complexity of the mechanisms and factors that underlie the variability of the clinical manifestations and treatment responsiveness of IMDs. They illustrate the need for in-depth molecular and/or cellular phenotyping in the cases in whom the new generation sequencing genome analyses are inconclusive.

References

Beaulieu CL, Majewski J, Schwartzentruber J et al (2014) FORGE Canada consortium: outcomes of a 2-year national rare-disease gene-discovery project. Am J Hum Genet 94(6):809–817. https://doi.org/10.1016/j.ajhg.2014.05.003

Blommaert E, Péanne R, Cherepanova NA et al (2019) Mutations in MAGT1 lead to a glycosylation disorder with a variable phenotype. Proc Natl Acad Sci USA 116(20):9865–9870. https://doi.org/10.1073/pnas.1817815116

Blommaert E, Cherepanova NA, Staels F, Wilson MP, Gilmore R, Schrijvers R, Jaeken J, Foulquier F, Matthijs G (2022) Lack of NKG2D in MAGT1-deficient patients is caused by hypoglycosylation. Hum Genet. https://doi.org/10.1007/s00439-021-02400-1

Bruce-Brand C, Govender D (2020) Gene of the month: IDH1. J Clin Pathol 73(10):611–615. https://doi.org/10.1136/jclinpath-2020-206813

Chaigne-Delalande B, Li FY, O'Connor GM et al (2013) Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. Science 341(6142):186–191. https://doi.org/10.1126/science.1240094

Durin Z, Dubail J, Layotte A, Legrand D, Cormier-Daire V, Foulquier F (2022) SLC10A7, an orphan member of the SLC10 family involved in congenital disorders of glycosylation. Hum Genet. https://doi.org/10.1007/s00439-021-02420-x

Fernández-Marmiesse A, Gouveia S, Couce ML (2018) NGS technologies as a turning point in rare disease research diagnosis and treatment. Curr Med Chem 25(3):404–432. https://doi.org/10.2174/0929867324666170718101946

Ferreira CR, Rahman S, Keller M, Zschocke J, ICIMD Advisory Group (2021) An international classification of inherited metabolic disorders (ICIMD). J Inherit Metab Dis 44(1):164–177. https://doi.org/10.1002/jimd.12348

Forny P, Plessl T, Frei C, Bürer C, Froese DS, Baumgartner MR (2021) Spectrum and characterization of bi-allelic variants in MMAB causing cblB-type methylmalonic aciduria. Hum Genet. https://doi.org/10.1007/s00439-021-02398-6

Guéant JL, Chéry C, Oussalah A et al (2018) A PRDX1 mutant allele causes a MMACHC secondary epimutation in cblC patients. Nat Commun 9(1):67. https://doi.org/10.1038/s41467-017-02306-5

Guéant JL, Guéant-Rodriguez RM, Kosgei VJ, Coelho D (2022a) Causes and consequences of impaired methionine synthase activity in acquired and inherited disorders of vitamin B12 metabolism. Crit Rev Biochem Mol Biol 57(2):133–155

Guéant JL, Siblini Y, Chéry C et al (2022b) Epimutation in inherited metabolic disorders: the influence of aberrant transcription in adjacent genes. Hum Genet. https://doi.org/10.1007/s00439-021-02414-9

Kruger WD (2021) How to fix a broken protein: restoring function to mutant human cystathionine β-synthase. Hum Genet. https://doi.org/10.1007/s00439-021-02386-w

Matmat K, Guéant-Rodriguez RM, Oussalah A, Wiedemann-Fodé A, Dionisi-Vici C, Coelho D, Guéant JL, Conart JB (2021) Ocular manifestations in patients with inborn errors of intracellular cobalamin metabolism: a systematic review. Hum Genet. https://doi.org/10.1007/s00439-021-02350-8

Mergnac JP, Wiedemann A, Chery C, Ravel JM, Namour F, Guéant JL, Feillet F, Oussalah A (2021) Diagnostic yield of clinical exome sequencing as a first-tier genetic test for the diagnosis of genetic disorders in pediatric patients: results from a referral center study. Hum Genet. https://doi.org/10.1007/s00439-021-02358-0

Michaud J, Praz V, James Faresse N et al (2013) HCFC1 is a common component of active human CpG-island promoters and coincides with ZNF143, THAP11, YY1, and GABP transcription factor occupancy. Genome Res 23(6):907–916. https://doi.org/10.1101/gr.150078.112

Oussalah A, Siblini Y, Hergalant S et al (2022) Epimutations in both the TESK2 and MMACHC promoters in the Epi-cblC inherited disorder of intracellular metabolism of vitamin B12. Clin Epigenetics 14(1):52

Rymen D, Peanne R, Millón MB et al (2013) MAN1B1 deficiency: an unexpected CDG-II. PLoS Genet. 9(12):e1003989. https://doi.org/10.1371/journal.pgen.1003989

Ventura P, Corradini E, Di Pierro E et al (2020) Hyperhomocysteinemia in patients with acute porphyrias: a potentially dangerous metabolic crossroad? Eur J Intern Med 79:101–107. https://doi.org/10.1016/j.ejim.2020.04.002

Watkins D, Rosenblatt DS (2022) Inherited defects of cobalamin metabolism. Vitam Horm 119:355–376. https://doi.org/10.1016/bs.vh.2022.01.010