

# INHERITED METABOLIC DISORDERS AND NUTRITION

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# INHERITED METABOLIC DISORDERS AND NUTRITION



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# INHERITED METABOLIC DISORDERS AND NUTRITION



## Editorial

**Dear Colleagues,**

We are delighted to announce the launch of the inaugural issue of Inherited Metabolic Disorders and Nutrition (IMDN), the official journal of the Child Nutrition and Metabolism Association. The journal provides a platform for academics to advance scientific research, report rare case studies, and share innovative ideas - all contributing to the growing body of knowledge in our field.

Inherited metabolic disorders represent a critical area of research, with an increasing number of researchers and publications dedicated to this field worldwide. IMDN was established in response to the ongoing need for additional avenues to publish high-quality research in this area. The journal aspires to make a lasting contribution to the fields of paediatric nutrition and inherited metabolic disorders. With the release of the first issue, a strong commitment is renewed to position IMDN as a leading resource within the international scientific community. Over time, we hope that IMDN will become an essential tool for sharing information and experiences related to metabolic disorders and nutrition.

IMDN will be published quarterly and will feature peer-reviewed research articles, case reports, reviews, original studies, and other relevant content. Our editorial board comprises experts from around the globe, and we welcome contributions from our international colleagues.

For more information about IMDN, please visit our website at [www.imdn.org](http://www.imdn.org). I encourage you to read the first issue with great interest, and I extend my sincere thanks to the authors whose work is featured in it. As a newly established journal, IMDN will undoubtedly thrive with your continued support and contributions.

We look forward to receiving your submissions for future issues.

**Best regards,**  
**Nur Arslan**  
**Editor-in-Chief, Inherited Metabolic Disorders and Nutrition (IMDN)**

# Use of Acti-Heart® in Diagnosis, Follow-Up, and Evaluation of Obesity in Childhood and its Relationship to Other Metabolic Parameters

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## Abstract

**Objectives:** In this study we evaluated changes in lifestyle and motivation, obesity diagnosis, diet and exercise treatments in adolescents using Acti-Heart®, as well as effects on body composition, lipid profile, insulin resistance (IR), adipocytokines, basal metabolic rate and daily calorie consumption.

**Materials and Methods:** A total of 14 cases with an age range of 10.1-16.6 years, and puberty stage of III-IV, who were followed up in our department with a diagnosis of exogenous obesity were included. Thirteen children were included as the control group. Body mass index (BMI), waist and hip circumferences, waist/hip ratio, body fat percentage, skin fold thickness measurements were performed, and glucose, insulin, homeostasis model assessment of IR (HOMA-IR), total cholesterol, low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), triglyceride, serum aspartate and alanin aminotransferase (AST and ALT), visfatin, tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and apolipoprotein A (APO A) levels were determined. Calorie consumption was measured using Acti-Heart®.

**Results:** In our patient group, BMI, ALT, glucose, insulin, HOMA-IR, total cholesterol, LDL-C, VLDL-C, triglyceride, visfatin values were high, but TNF-α, IL-10 and APO A levels were low (p<0.05). BMI, waist and hip circumferences, waist/hip ratio, body fat percentage and skin fold thickness decreased in with diet, exercise, and behavioral therapy. HOMA-IR, AST, ALT, VLDL-C, triglyceride, visfatin and hs-C-reactive protein values were also decreased. However, TNF-α, IL-6, and IL-10 levels increased. Daily calorie consumptions measured by Acti-Heart® significantly increased (p=0.004).

**Conclusion:** This is important as it shows increased calorie consumption, parallel changes in body composition, and improvements in metabolic parameters such as decreased VLDL, triglyceride, and IR using Acti-Heart® in obesity treatment monitoring in adolescents. In conclusion, Acti-Heart® is an objective evaluation method for monitoring obesity treatment. The study is planned to be conducted in larger groups of obese children.

**Keywords:** Body Mass Index, Diet, Insulin Resistance, Lipids, Obesity, Visfatin



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## INTRODUCTION

Obesity is a complex, multifactorial disease caused by an imbalance in calorie intake and energy expenditure. The most important cause of obesity is the intake of more energy than consumed. Childhood obesity refers to an unhealthy excess of body fat.<sup>1</sup> The world-wide increase in obesity, which can adversely affect the health of children, appears to be largely influenced by environmental factors, lifestyle and cultural aspects.<sup>2</sup> Childhood obesity has emerged as a global health problem due to its increasing prevalence in both developed and developing countries.<sup>3</sup> Currently, approximately 170 million children worldwide are overweight or obese.<sup>3,4</sup>

Childhood obesity serves as a major contributing factor to the development of several diet-related chronic conditions, including later life conditions such as heart disease, high blood pressure, stroke, type II diabetes, and several types of cancer.<sup>5</sup> Various treatment methods such as appropriate diet preparation, increased physical activity, behavioral modification, pharmacotherapy, and surgical procedures have been employed in obesity treatment.<sup>6-11</sup>

In obesity, the most important source of pro-inflammatory cytokines is macrophages that infiltrate adipose tissue in response to fat cell growth, reduced blood flow, hypoxia, and tissue necrosis. These events collectively create a predisposition to systemic inflammation, a potential triggering factor in the pathogenesis of obesity-related morbidities. Several adipokines including adiponectin, leptin, resistin, visfatin, chemokine, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), IL-6, IL-8, IL-10, plasminogen activator inhibitor 1, monocyte chemoattractant protein-1, and retinol binding protein-4 are involved in insulin resistance (IR) regulation. In healthy obese adults, increased transforming growth factor beta 1 and IL-6 inhibit cell differentiation and the function of adiponectin and leptin. Increased IL-6, IL-1 and TNF- $\alpha$  in obese individuals was associated with progression of several disorders including cardiovascular disease, hypertension and IR. Mortality is associated with increased circulating IL-6, IL-1 $\beta$ , TNF- $\alpha$  and IL-8.<sup>12</sup>

The aim of those treatments is to preserve the body weight for a long time after the suitable body weight is reached, and to prevent weight gain. In this study, we used Acti-Heart® as a monitoring tool of daily calorie consumption in a group of obese adolescents.

## MATERIALS AND METHODS

A total of 14 cases with the age range of 10.1-16.6 years and puberty stage of III-IV, who were followed up at the Department of Pediatric Endocrinology and Metabolic Diseases in the Medical School of Çukurova University with the diagnosis of

exogenous obesity were included in this study. These cases were recently diagnosed and had no known systemic, endocrine or neurological diseases. There were 13 children in the control group. Anthropometric measurements and detailed physical examinations were performed in all subjects.

Venous blood samples from participating cases were collected after a 12-hour overnight fast, before, in the middle of, and after the exercise and diet treatments. Fasting blood glucose, fasting insulin, low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, lipoprotein a, serum aspartate and alanin aminotransferase (AST and ALT), free-T4, thyroid stimulating hormone, insulin-like growth factor 1 (IGF-1), follicle-stimulating hormone, luteinizing hormone, estradiol (in females), testosterone (in males and females), C-reactive protein (CRP) (high sensitive), TNF- $\alpha$ , IL-6, IL-10, adiponectin, and visfatin levels were measured in the blood samples in the central laboratory at the Medical School of Çukurova University.

Serum transaminases were measured using the International Federation of Clinical Chemistry method, and the Roche Modular System/Integra 800 device and kit (Germany). Alanine aminotransferase and aspartate aminotransferase levels  $\geq 40$  IU/L were accepted as abnormal serum aminotransferase values. Serum lipid profile, HDL-C, LDL-C and VLDL-C measurements were performed using enzyme calorimetric methods and Roche modular system/integra 800 device and kit (Germany). Triglyceride levels were measured with GPO/PAP method and Roche modular system/integra 800 device and kit (Germany). Lipoprotein A, Apo A and Apo B were measured using immunoturbidimetric method and Roche modular system/integra 800 device and kit (Germany). Serum glucose was measured with hexokinase method and Roche modular system/integra 800 device and kit (Germany). Serum CRP was measured using nephelometric method and II Dade Behring device and kit. Cytokines, TNF- $\alpha$ , IL-6 and IL-10 were measured using micro-enzyme-linked immunosorbent assay (ELISA) automation system (Biosource kit, Belgium), and Triturus (Spain) micro-ELISA automation system. IGF-1 was measured with chemiluminescence method and immulite 2000 and the kit. Visfatin C-Terminal (Human) was measured in the serum using micro-ELISA method Triturus (France) automatic ELISA device (Human). EIA kit (Phoenix pharmaceuticals, USA) was used for the testing. Normal plasma range for visfatin is 0.1-1000 ng/mL. Adiponectin was studied in the serum by using micro-ELISA method, and Triturus (France) automated ELISA device. BioVendor Human Adiponectin ELISA kit was used for testing. IR and insulin sensitivity indices were calculated.

Electrocardiography (ECG), echocardiography, abdominal ultrasonography, were performed, and arterial intimal thicknesses were measured.

A skinfold caliper was used to determine body fat percentage, and measured values were put in the Yuhasz formula. Direct body fat measurement was performed using the bioelectrical impedance method. Resting metabolism rate was calculated using the calorimetric method.

The children we included in the study were given a motivational, dietary and exercise program for two months.

### Regulation of the Diet Program

Patients and families were first informed about the forms to be filled out. Then, diet records were collected, in which the amounts of main and intermediate meals and beverages for one week were written with the criteria determined by the dietician, (such as tea glass, water glass, tablespoon). From these records, average calorie consumption was calculated by a pediatric dietician, taking into account weekdays and weekend days. Carbohydrate, protein, and fat ratios in the diet were also calculated. After this stage, the required daily energy amount was calculated for each patient according to age, gender, and ideal weight. The ideal weight, normal weight, height standard data, and puberty stages of school-age children were taken into consideration.

For each case, the diet was organized in accordance with the age, socioeconomic, and cultural conditions. Nutrients were selected, and the daily energy intake was organized as 50-55% carbohydrate, 15-20% protein, and 30% fat. In this way, the energy intake of the child was limited and food consumption was balanced. Dietary patterns, caloric intake, and energy distribution were obtained from the patients' seven-day nutritional records. Nutritional mistakes and deficiencies were discussed with the patients. The results of this dietary approach were explained in detail. In patients who were not morbidly obese, a balanced diet suitable for the required weight was given. In morbidly obese patients, short-term energy restriction was applied. The fact that the patients had not completed their growth was taken into consideration. Patient's daily energy intake was reduced by 200-500 calories. Patients were checked by a dietitian before, during, and at the end of the study. During the controls, it was detected that some subjects wanted to get faster results, and tried to consume fewer calories than the calories in the given diet. These patients were interviewed again, and their mistakes were corrected.

### Daily Calorie Expenditure

Acti-Heart® is a light and portable device which records data about heart rate and physical activities at minute intervals via two ECG electrodes placed on the chest. The daily caloric expenditure of the patients was measured before and at the end

of the study with the Acti-Heart® version 2,000.10 (Mini Mitter Company, Inc. USA) device (Figure 1) by entering the age, gender, weight and height data of the patients (covering two days of caloric expenditure on weekdays and weekends). The obtained data, including information about age, gender, weight, and height, were recorded on the computer, and then calculations were performed using a pre-existing program.

It was strongly emphasized to patients they should not perform any activity other than their normal activities during the time the device was worn. Daily calorie intake was calculated using a one-week diet recorded at home. Meal plans were prepared considering the age, socioeconomic conditions, and cultural characteristics of each patient.

A two-month home exercise program, which was based on basal heart rate values, was scheduled. A follow-up chart for exercise pulses, which was calculated for each age-group, was prepared. All measurements were performed at the beginning, the middle, and the end of the treatment.

### Ethical Statements

The study was approved by Çukurova University Faculty of Medicine Ethics Committee (approval number: 2008-15, dated: 03.06.2008). The study was performed in accordance with the ethical rules based on the principles of the Helsinki Declaration. Written informed consent forms were obtained (when appropriate) from the parents and the children.

### Statistical Analysis

Data obtained from this study were analyzed using Statistical Package for Social Sciences for Windows, version 10 (IBM Inc., Armonk, NY, USA). Data were expressed as the mean  $\pm$  standard deviation (SD), median (min.-max.). While evaluating the study data, in addition to descriptive statistical methods (mean  $\pm$  SD), analysis of variance table test was used to compare the obese patient group and the control group. The parameters of the

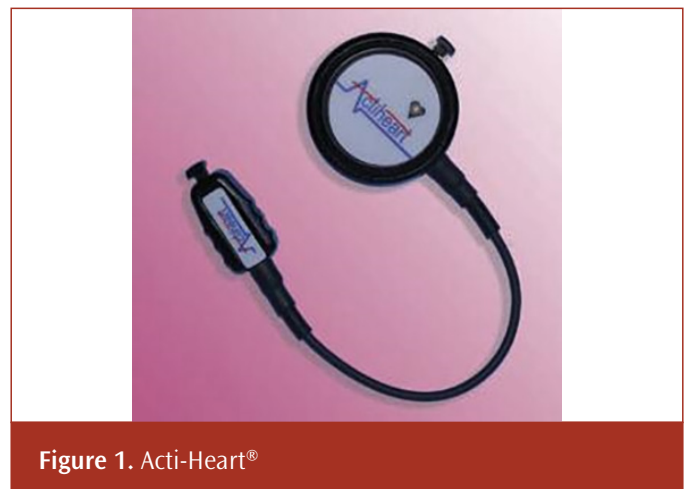


Figure 1. Acti-Heart®

obese patient group that changed with exercise were evaluated with the paired samples t-test. The results were evaluated within the 95% confidence interval and the significance level was  $p < 0.05$ .

## RESULTS

In this study, 27 children (14 were obese, and 13 were healthy children) with the mean age of  $12.92 \pm 1.94$  (range: 10-17) years were included. Of the children, 14 (51.9%) were girls and 13 (48.1%) were boys. Out of the cases, 14 were obese, and 13 were non-obese healthy (control) children. The mean weight of the patient group was  $87.96 \pm 22.31$  kg (min.: 55, max.: 133 kg), and of the control group was  $47.08 \pm 11.54$  kg (min.: 28, max.: 66 kg). There was a statistically significant difference in body weights between patients and controls ( $p = 0.001$ ). The mean BMI in obese children was  $34.35 \pm 5.23$  (ranging from 28 to 45)  $\text{kg/m}^2$ , while it was  $20.02 \pm 2.95$  (ranging from 16 to 27)  $\text{kg/m}^2$  in the control group; there was a statistically significant difference between the groups ( $p = 0.001$ ). Body measurements in the patient group before, in the middle, and at the end of the exercise and diet treatment are displayed in Table 1.

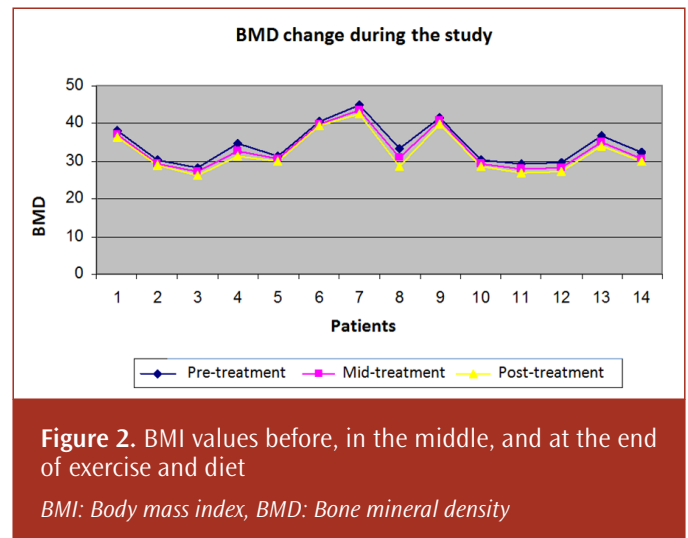
There were statistically significant differences in BMI, skinfold thickness, waist and hip measurement, and waist/hip ratio between pre- and post-treatment measurements. There was a significant difference in the body fat percentage between pre- and post-treatment calculations. The BMI values of the patients before, in the middle, and at the end of exercise and diet are displayed in Figure 2.

There were statistically significant differences in AST, ALT, VLDL-C, triglyceride, and total cholesterol measurements

between pre- and post-treatment measurements (in the same order  $p = 0.015$ ,  $p = 0.029$ ,  $p = 0.015$ ,  $p = 0.013$ ,  $p = 0.028$ ).

When the correlation between obesity and adipokines was investigated, mean TNF- $\alpha$  level was detected as  $1.66 \pm 2.33$   $\text{pg/mL}$  in obese children; it was  $3.62 \pm 2.35$   $\text{pg/mL}$  in the control group. The difference was statistically significant. The mean IL-10 levels were  $0.28 \pm 0.64$   $\text{pg/mL}$  and  $5.61 \pm 8.84$   $\text{pg/mL}$  in obese children and the control group, respectively. The difference was statistically significant. Differences in hs-CRP, IL-6, adiponectin and visfatin values between patient and control groups were not considered significant. The comparison of adipokines between patient and control groups is provided in Table 2.

There was a significant difference in homeostasis model assessment of IR (HOMA-IR) measurements between pre- and



**Figure 2.** BMI values before, in the middle, and at the end of exercise and diet

BMI: Body mass index, BMD: Bone mineral density

**Table 1.** Body measurements in the patient group before, in the middle and at the end of exercise and diet treatment

Variables	Measurements	n	Mean	SD	p-value
BMI ( $\text{kg/m}^2$ )	Pre-treatment	14	34.35	5.23	0.001
	Mid-treatment	14	33.03	5.32	
	Post-treatment	14	32.10	5.35	
Waist (cm)	Pre-treatment	14	105.18	14.66	0.001
	Post-treatment	14	97.75	12.75	
Hip (cm)	Pre-treatment	14	109.61	10.83	0.003
	Post-treatment	14	106.79	10.80	
Waist/Hip ratio	Pre-treatment	14	0.97	0.09	0.029
	Post-treatment	14	0.91	0.08	
Skinfold thickness	Pre-treatment	14	25.43	2.43	0.003
	Post-treatment	14	23.83	2.06	
Body fat percentage (%)	Pre-treatment	14	33.76	11.77	0.007
	Post-treatment	14	31.98	11.49	

The parameters of the obese patient group that changed with treatment were evaluated by paired samples t-test. The significance level of the tests was set as  $p < 0.05$ . SD: Standard deviation, BMI: Body mass index

post-treatment ( $p=0.001$ ). Changes in IR with exercise and diet treatment in the patient group are shown in Table 3 and Figure 3.

When the calorie consumption of the patient group before and after the study, a statistically significant difference was detected between the two measurements ( $p=0.004$ ). There was no statistically significant difference in the basal metabolism rates of the patient group before and after the study ( $p=0.194$ ).

Evaluation of energy consumption with exercise and diet in the patient group. It is shown in Table 4.

### DISCUSSION

Obesity is a chronic energy metabolism disorder, which may ensue from excessive fat accumulation and cause severe problems physically, mentally. There is no obvious cause in the majority of obesity cases. These are defined as simple obesity or exogenous obesity. Obesity has become a public health problem

**Table 2. Comparison of adipokines between patient and control groups**

Variables	Measurements	n	Mean	SD	Median (min.-max.)	p-value
TNF- $\alpha$ (pg/mL)	Obese	14	1.66	2.33	0.50 (0-7)	0.029
	Control	13	3.62	2.03	0 (0-7)	
Hs-CRP (mg/dL)	Obese	14	6.29	3.28	5.82 (3-12)	0.059
	Control	13	9.77	12.75	3.30 (3-13)	
IL-6 (pg/mL)	Obese	14	1.70	1.79	1.25 (0-5)	0.161
	Control	13	1.06	1.08	2 (0-20)	
IL-10 (pg/mL)	Obese	14	0.28	0.64	0 (0-2)	<b>0.033</b>
	Control	13	5.61	8.84	1.80 (0-32)	
Adiponectin ( $\mu$ g/mL)	Obese	14	1.21	1.33	0.60 (0-4)	0.459
	Control	13	1.63	1.59	1.20 (0-7)	
Visfatin (ng/mL)	Obese	14	115.64	19.59	13.15 (3-638)	0.419
	Control	13	64.28	11.83	16.40 (0-403)	

Significance between control and obese group (One-Way ANOVA test). Significance level of the tests was accepted to be  $p<0.05$ . SD: Standard deviation, ANOVA: Analysis of variance, TNF- $\alpha$ : Tumor necrosis factor-alpha, Hs-CRP: High sensitivity-C-reactive protein, IL: Interleukin, min.-max.: Minimum-maximum

**Table 3. Changes in IR in the patient group by exercise and diet treatment**

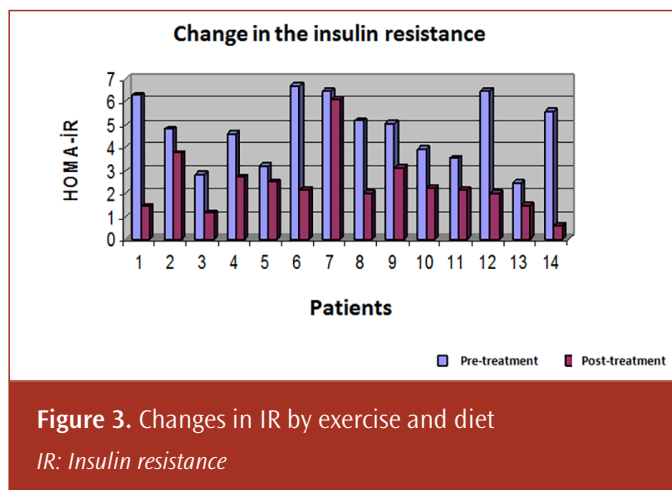
Variables	Measurements	n	Mean	SD	p-value
Glucose (mg/dL)	Pre-treatment	14	84.08	6.66	0.109
	Post-treatment	14	79.38	6.59	
Insulin (mU/L)	Pre-treatment	14	23.25	7.46	0.358
	Post-treatment	14	22.24	7.84	
Glucose/Insulin	Pre-treatment	14	4.22	1.58	0.520
	Post-treatment	14	4.31	1.70	
HOMA-IR	Pre-treatment	14	4.80	1.43	<b>0.001</b>
	Post-treatment	14	2.38	1.33	

The parameters of the obese patient group that changed with treatment were evaluated by paired samples t-test. The significance level of the tests was accepted as  $p<0.05$ . SD: Standard deviation, HOMA-IR: Homeostasis model assessment of insulin resistance

**Table 4. Evaluation of energy consumption in the patient group by exercise and diet**

Variables	Measurements	n	Mean	SD	p-value
Basal metabolism rate	Pre-treatment	14	1675.69	438.21	0.194
	Post-treatment	14	1782.29	508.66	
Daily calorie consumption	Pre-treatment	14	1254.36	491.80	<b>0.004</b>
	Post-treatment	14	1524.71	546.93	

The parameters of the obese patient group that changed with treatment were evaluated by paired samples t-test. The significance level of the tests was accepted as  $p<0.05$ . SD: Standard deviation



**Figure 3.** Changes in IR by exercise and diet

IR: Insulin resistance

all over the world with its increasing prevalence in the last two decades, especially in the Western populations.<sup>13,14</sup> The increase in obesity frequency in children is parallel with that in adults. This is caused by fats, carbohydrates, and fast-foods becoming more prominent among modern dietary habits, and children preferring to watch television and play computer games, rather than performing physical activities.<sup>15,16</sup> Obesity and the severe complications that this pathological condition may lead to have increased the interest in and need for effective, easily applicable, and simpler methods to prevent and treat obesity. Diet, exercise, and behavioral motivation are highly important and effective approaches.<sup>17,18</sup> If exercise treatment is accompanied by diet and behavioral modifications in obese patients, it causes more weight loss than diet alone. Hensrud et al.<sup>19</sup> conducted a two-year study by dividing people into diet only, exercise only, and diet plus exercise groups. In the diet plus exercise group, a mean, 13 kg weight loss was observed among 24 obese women after 1 year. While there was a 6 kg loss in the exercise only group, a 13 kg weight gain was observed in the diet only group.<sup>19</sup>

When the changes in lipid profile in the obese patient group before and after exercise were compared, significant decreases were found in total cholesterol, triglyceride, and VLDL-C levels. There was no statistically significant difference between HDL-C, LDL-C, APO A, APO B, and lipoprotein measurements. In our study, as in the study of Kim et al.,<sup>20</sup> no significant difference was found between the values of HDL-C and LDL-C after diet and exercise treatment. We think that the small number of patients included in the study and the short duration of exercise may have caused this difference. Similar to the study of Kang et al.,<sup>21</sup> no significant changes were found in HDL-C, LDL-C, Apo A, Apo B and lipoprotein A levels with diet and exercise in our study.

When the relationship between obesity and liver function tests was investigated in our study, a statistically significant difference was found in ALT levels in obese children. Similar

to the study of Li et al.,<sup>22</sup> no significant difference was found in AST measurements in our study. When AST and ALT values before and after exercise were compared in the obese patient group, a statistically significant decrease was found between the two measurements. This finding supports the view that exercise improves liver function by regulating energy and lipid metabolism. Jung et al.<sup>23</sup> found significant decreases in TNF- $\alpha$  and IL-6 levels and significant increases in IL-10 and adiponectin levels in obese individuals after a 12 week exercise program.

In the study by Kim et al.,<sup>20</sup> IL-6, TNF- $\alpha$  and hs-CRP levels were found to be significantly elevated in obese individuals, whereas adiponectin levels were found to be significantly lower. While adiponectin levels were found to be low in the same individuals after exercise, no significant difference was found in IL-6, TNF- $\alpha$ , and hs-CRP levels.<sup>20</sup> In our study, TNF- $\alpha$  and IL-6, which are inflammation markers, were found to increase after diet and exercise, contrary to expectations. However, the level of IL-10, an anti-inflammatory cytokine, was found to be lower in obese children compared to the control group, as expected. It increased with exercise. In our study, we attributed the increase in IL-6 and TNF- $\alpha$  to the effect of exercise on muscles, noting that IL-6 and TNF- $\alpha$  are myokines released from muscles during exercise, which demonstrates the effectiveness of exercise by an increase in their levels.<sup>24,25</sup>

In our study, when serum adiponectin levels were examined, no significant difference was found in obese children compared to the control group, nor was there a significant difference in obese children after exercise compared to before exercise. We think that the discordance in adiponectin levels in this study may be related to the insufficient number of patients included in the study, and the short duration of exercise. Furthermore, we suggest that longitudinal studies should be performed in large patient groups in the future to obtain clearer data.

In our study, we did not find a significant difference in serum visfatin levels in obese patients compared to the control group; however, we found a significant decrease in visfatin levels with exercise in obese children. This finding supports the view that obesity reduction may be due to the positive effect of exercise, which has been previously emphasized in the literature.

Crouter et al.<sup>26</sup> performed a study on energy consumption measurement and the reliability of Acti-Heart® in adults. The study was performed on 48 patients (24 males and 24 females; mean age: 35 years). Patients were divided into three groups according to their lifestyles and exercising habits (sedentary time, time at home, and exercising), and concomitant oxygen consumption of patients was measured. Six routine activity programs were scheduled for patients as 10 minutes of physical activity and 1-2 minutes of rest. Heart rate and energy consumption were

measured by Acti-Heart® by estimating heart activity, and the recorded data during all activities were transferred to the computer. In the meantime, energy consumption was measured during each routine activity by using the portable indirect calorimetric method (Cosmed K4b2, Italy). The study is valuable as it has indicated the reliability of measurement by Acti-Heart®.<sup>26</sup> In another study performed by Barreira et al.,<sup>27</sup> energy consumption during short-term physical activity was measured in 34 healthy subjects with the mean age of 21.8 months using Acti-Heart®, and it was reported that there was an increase in daily calorie consumption after the exercise program, which was measured by Acti-Heart®. Changes in daily calorie consumption were measured in obese adolescents before and after a triple treatment program of behavioral motivation, diet, and exercise for one day per week and two days on the weekend. A significant difference was determined in daily calorie consumption of obese patients, before and after treatment ( $p=0.004$ ). Thus, we believe that by using Acti-Heart® we have objectively assessed energy expenditure in the diagnosis, treatment and follow-up of pediatric obesity. Moreover, we think that since the study results showed significant correlations between energy consumption measured before and after treatment by Acti-Heart® and BMI, skin fold thickness, body fat percentage, waist/hip ratio, hepatosteatosis, HOMA-IR, plasma lipid, IL-6, IL-10, TNF- $\alpha$ , hs-CRP, and visfatin levels, further research should explore these correlations in greater detail.

## CONCLUSION

In our study, we showed that energy consumption increases during obesity treatment in adolescence by changing the lifestyle through behavior, diet, and exercise approaches. We used Acti-Heart® to assess calorie consumption in the diagnosis, treatment and follow-up of adolescent obesity. We also showed that Acti-Heart® was an easily applicable, non-invasive, cost-effective (no additional cost other than electrodes) device, and it could gather information about the energy consumption in every part of daily life, including sleeping and sports. Moreover, it was well-correlated with other parameters in obesity treatment follow-up; therefore, it is also an important and practical evaluation method. However, we believe that larger studies should be performed to support our conclusions.

## Ethics

**Ethics Committee Approval:** The study was approved by Çukurova University Faculty of Medicine Ethics Committee (approval number: 2008-15, dated: 03.06.2008).

**Informed Consent:** Written informed consent forms were obtained (when appropriate) from the parents and the children.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: İ.Ö., S.E., A.K.T., B.Y., N.Ö.M., Concept: İ.Ö., E.M., S.E., G.D., A.K.T., B.Y., N.Ö.M., Design: İ.Ö., E.M., S.K., G.K., G.D., N.Ö.M., Data Collection or Processing: İ.Ö., S.K., G.K., K.A., G.D., A.K.T., B.Y., N.Ö.M., Analysis or Interpretation: İ.Ö., E.M., S.K., G.K., S.E., K.A., F.T., F.G., G.D., A.K.T., B.Y., N.Ö.M., Literature Search: İ.Ö., E.M., F.T., F.G., G.D., A.K.T., B.Y., N.Ö.M., Writing: İ.Ö., E.M., N.Ö.M.

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## REFERENCES

1. Umekar S, Joshi A. Obesity and preventive intervention among children: a narrative review. *Cureus*. 2024;16(2):e54520. doi: 10.7759/cureus.54520
2. Kumari S, Shukla S, Acharya S. Childhood obesity: prevalence and prevention in modern society. *Cureus*. 2022;14(11):e31640. doi: 10.7759/cureus.31640
3. Motevalli M, Drenowatz C, Tanous DR, Khan NA, Wirtzner K. Management of childhood obesity-time to shift from generalized to personalized intervention strategies. *Nutrients*. 2021;13(4):1200. doi: 0.3390/nu13041200
4. Lobstein T, Baur L, Uauy R; IASO International Obesity TaskForce. Obesity in children and young people: a crisis in public health. *Obes Rev*. 2004;5(Suppl 1):4-104. doi: 0.3390/nu13041200
5. Khan S, Abbas A, Ali I, Arshad R, Tareen MBK, Shah MI. Prevalence of overweight and obesity and lifestyle assessment among school-going children of Multan, Pakistan. *Isra Med J*. 2019;11(4):230-233. doi: 10.3390/nu13041200
6. Caroli M, Burniat W. Dietary management. In: Burniat W, Cole T, Poskitt E (eds). *Child and Adolescent Obesity*. Cambridge: Cambridge University Press; 2002:282-307. [https://assets.cambridge.org/052165/2375/frontmatter/0521652375\\_frontmatter.pdf](https://assets.cambridge.org/052165/2375/frontmatter/0521652375_frontmatter.pdf)
7. Glover S, Piper CN, Hassan R, Preston G, Wilkinson L, Bowen-Seabrook J, Meyer-Davis B, Williams S. Dietary, physical activity, and lifestyle behaviors of rural African American South Carolina children. *J Natl Med Assoc*. 2011;103(4):300-304. doi: 10.1016/S0027-9684(15)30307-0
8. Rogovik AL, Goldman RD. Pharmacologic treatment of pediatric obesity. *Can Fam Physician*. 2011;57(2):195-197. <https://pubmed.ncbi.nlm.nih.gov/articles/PMC3038814/>
9. Wilson DM, Abrams SH, Aye T, Lee PD, Lenders C, Lustig RH, Osganian SV, Feldman HA; Glaser Pediatric Research Network Obesity Study Group. Metformin extended release treatment of adolescent obesity: a 48-week randomized, double-blind, placebo-controlled trial with 48-week follow-up. *Arch Pediatr Adolesc Med*. 2010;164(2):116-123. doi: 10.1001/archpediatrics.2009.258
10. Friedman JM. Leptin and the regulation of body weight. *Keio J Med*. 2011;60(1):1-9. doi: 10.2302/kjm.60.1
11. Weker H. Simple obesity in children. A study on the role of nutritional factors. *Med Wieku Rozwoj*. 2006;10(1):3-191. <https://pubmed.ncbi.nlm.nih.gov/16733288/>

12. Moghbeli M, Khedmatgozar H, Yadegari M, Avan A, Ferns GA, Ghayour Mobarhan M. Cytokines and the immune response in obesity-related disorders. *Adv Clin Chem*. 2021;101:135-168. doi: 10.1016/bs.acc.2021.01.001
13. Şimşek F, Ulukol B, Berberoğlu M, Başkan Gülnar S, Adıyaman P, Öcal G. Obesity prevalence in a primary school and a high school in Ankara. *J Ankara Univ Fac Med*. 2005;58:163-166. doi: 10.1007/s004310051029
14. Leung AKC, Wong AHC, Hon KL. Childhood obesity: an updated review. *Curr Pediatr Rev*. 2024;20(1):2-26. doi: 10.2174/1573396318666220801093225
15. Troiano RP, Flegal KM, Kuczmarski RJ, Campbell SM, Johnson CL. Overweight prevalence and trends for children and adolescents. The National Health and Nutrition Examination Surveys, 1963 to 1991. *Arch Pediatr Adolesc Med*. 1995;149(10):1085-1091. doi: 10.1016/bs.acc.2021.01.001
16. Zwiauer KF. Prevention and treatment of overweight and obesity in children and adolescents. *Eur J Pediatr*. 2000;159(Suppl 1):56-68. doi: 10.1007/s004310051029
17. Lee JK, Dixon WT, Ling D, Levitt RG, Murphy WA Jr. Fatty infiltration of the liver: demonstration by proton spectroscopic imaging. Preliminary observations. *Radiology*. 1984;153(1):195-201. doi: 10.1148/radiology.153.1.6420842
18. Ali A, Al-Ani O, Al-Ani F. Children's behaviour and childhood obesity. *Pediatr Endocrinol Diabetes Metab*. 2024;30(3):148-158. doi: 10.5114/pedm.2024.142586
19. Hensrud DD, Weinsier RL, Darnell BE, Hunter GR. Relationship of comorbidities of obesity to weight loss and four-year weight maintenance/rebound. *Obes Res*. 1995;3(Suppl 2):217-222. doi: 10.1002/j.1550-8528.1995.tb00156.x
20. Kim ES, Im JA, Kim KC, Park JH, Suh SH, Kang ES, Kim SH, Jekal Y, Lee CW, Yoon YJ, Lee HC, Jeon JY. Improved insulin sensitivity and adiponectin level after exercise training in obese Korean youth. *Obesity (Silver Spring)*. 2007;15(12):3023-3030. doi: 10.1038/oby.2007.360
21. Kang HS, Gutin B, Barbeau P, Owens S, Lemmon CR, Allison J, Litaker MS, Le NA. Physical training improves insulin resistance syndrome markers in obese adolescents. *Med Sci Sports Exerc*. 2002;34(12):1920-1927. doi: 10.1097/00005768-200212000-00010
22. Li TC, Liu CS, Lin CC. The relationship of liver enzyme abnormalities and obesity in Aboriginal children in Taiwan. *J Gastroenterol*. 2004;39(12):1170-1174. doi: 10.1007/s00535-004-1467-x
23. Jung SH, Park HS, Kim KS, et al. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J Nutr Biochem*. 2008;19(6):371-375. doi: 10.1016/j.jnutbio.2007.05.007
24. Pedersen BK, Toft AD. Effects of exercise on lymphocytes and cytokines. *Br J Sports Med*. 2000;34(4):246-251. doi: 10.1136/bjism.34.4.246
25. Berggren JR, Hulver MW, Houmard JA. Fat as an endocrine organ: influence of exercise. *J Appl Physiol (1985)*. 2005;99(2):757-764. doi: 10.1152/japplphysiol.00134.2005
26. Crouter SE, Churilla JR, Bassett DR Jr. Accuracy of the Actiheart for the assessment of energy expenditure in adults. *Eur J Clin Nutr*. 2008;62(6):704-711. doi: 10.1038/sj.ejcn.1602766
27. Barreira TV, Kang M, Caputo JL, Farley RS, Renfrow MS. Validation of the Actiheart monitor for the measurement of physical activity. *Int J Exerc Sci*. 2009;2(1):60-71. [https://www.researchgate.net/publication/28330732\\_Validation\\_of\\_the\\_Actiheart\\_Monitor\\_for\\_the\\_Measurement\\_of\\_Physical\\_Activity](https://www.researchgate.net/publication/28330732_Validation_of_the_Actiheart_Monitor_for_the_Measurement_of_Physical_Activity)

# Analysis of Laboratory and Demographic Data of Late Diagnosed Phenylketonuria Cases

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## Abstract

**Objectives:** In this study, it was aimed to evaluate the clinical, demographic, and laboratory data of the patients we followed up with late diagnosed phenylketonuria (PKU). In addition, the relationship between the age of onset of treatment and neuromotor development will be evaluated.

**Materials and Methods:** In this study, patients diagnosed with PKU and followed in the Pediatric Metabolism Outpatient Clinic were retrospectively examined. Cases diagnosed late were evaluated.

**Results:** We determined that 25 of our patients with PKU received a late diagnosis. Of these patients, 19 had classical PKU, and 6 had a mild PKU phenotype. Eight of our cases were female and 17 were male. The mean age at diagnosis of patients was  $26.3 \pm 38.1$  (range, 2-192) months. The mean age of the patients at the last evaluation was calculated to be  $13.1 \pm 5.3$  (range, 3.8-21.3) years. At the last evaluation, the phenylalanine level of the patients was  $465.3 \pm 275.7$   $\mu\text{mol/L}$  in mild PKU and  $779.1 \pm 449.4$   $\mu\text{mol/L}$  in classical PKU. Fifteen of our cases had global developmental delay, six had moderate developmental delay, and four had mild developmental delay. Twenty-one of our cases received diet therapy, and four of our cases received large neutral amino acid therapy.

**Conclusion:** It causes severe neurological problems in patients who are diagnosed late or undiagnosed. Therefore, close follow-up is important in the diagnosis and treatment phase of the disease. Close follow-up is essential to ensure that patients diagnosed with PKU can receive their treatment in the early period.

**Keywords:** Phenylketonurias, Therapeutics, Nervous System Diseases

## INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive metabolic disease caused by the deficiency of phenylalanine hydroxylase, an enzyme that converts phenylalanine, an essential amino acid, to tyrosine in the liver.<sup>1</sup> The phenylalanine hydroxylase enzyme is encoded by the phenylalanine hydroxylase (PAH) gene, and its cofactor is tetrahydrobiopterin. The gene encoding the PAH enzyme is localized in the q22-q24.<sup>1</sup> band region on the long arm of chromosome 12.

The frequency of PAH deficiency varies according to ethnicity and geographical region. Due to the high rate of consanguineous

marriages in Türkiye, PKU occurs at a frequency of 1/4370. Neonatal PKU screening in our country was first conducted as a pilot study in 1983. It was included in the screening scope nationwide after 1994.<sup>2</sup> In our country, blood samples are taken from babies twice within the scope of neonatal screening. The first blood sample is taken before hospital discharge, and the second is taken one week later. Today, PKU is one of the diseases that is recommended for screening because it is highly prevalent and treatable. Early diagnosis and treatment can prevent irreversible damage in this disease.

In PKU, phenylalanine and its metabolites cause structural brain damage, severe intellectual disability, and psychiatric disorders



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as a result of neurotoxic effects.<sup>3</sup> Early diagnosis and treatment are necessary to prevent or reduce the symptoms of the disease. With the emergence of newborn screening programs, the most serious neuropsychiatric findings of the disease can be prevented. The clinical picture resulting from the *PAH* gene defect varies from mild hyperphenylalaninemia to classical PKU. A phenylalanine-restricted diet is the main treatment method. However, research on the treatment of PKU is continuing, and new treatment options are emerging that can reduce the burden of the difficult and restrictive diet on patients.

Patients with *PAH* deficiency do not have any clinical findings at birth. They usually apply to the hospital when they are 3-4 months old, suggesting these delays prompt the hospital visit. Late-diagnosed patients with classical PKU experience severe intellectual disability, microcephaly, ataxia, autism, convulsions, aggression, eczema-like skin lesions, and behavioural disorders.<sup>4</sup>

In this study, we aimed to evaluate the clinical, demographic, and laboratory data of patients we follow with late-diagnosed PKU. In addition, the relationship between the age of initiation of treatment and neuromotor development will be evaluated.

## MATERIALS AND METHODS

In this study, patients who were followed up in the Pediatric Metabolism Outpatient Clinic between January 2021 and July 2023 and diagnosed with PKU were retrospectively examined. Late-diagnosed cases were evaluated. The patients included in the study were evaluated for age at diagnosis, follow-up period, height, weight, head circumference, gender, history of consanguinity between parents, clinical phenotype, plasma phenylalanine levels at admission, and metabolic control status during treatment and follow-up. These data were obtained from the patients' electronic files in the hospital information management system. Clinical, demographic, and laboratory data of all patients were analysed. *PAH* gene analysis was performed on all our patients, and biallelic variants were detected.

The blood phenylalanine levels of the patients were determined by taking 2 mL of blood in tubes containing ethylenediaminetetraacetic acid and using high-performance liquid chromatography in the Metabolism Laboratory. For mutation screening and genotyping of the patients, exons 1-13 of the *PAH* gene were examined by polymerase chain reaction amplification, followed by DNA sequence analysis. Mutations caused by nucleotide changes, detected by DNA sequence analysis, were identified.

Blood dihydropteridine reductase activity and neopterin, and biopterin analyses were performed to evaluate BH4 metabolism disorders. The normal range for plasma phenylalanine is 60-

120  $\mu\text{mol/L}$ . If phenylalanine levels were  $>1200 \mu\text{mol/L}$  before treatment at the time of diagnosis, it was classified as classical PKU; if phenylalanine levels were between 600-1200  $\mu\text{mol/L}$ , it was classified as mild PKU; and if phenylalanine levels were  $<600 \mu\text{mol/L}$ , it was classified as mild hyperphenylalaninemia. Speech, fine motor, gross motor, personal, and social skills of the patients were evaluated with developmental tests. According to the developmental tests, the patients were defined as having global, moderate, and mild developmental delay.

Each procedure was carried out in accordance with the ethical principles determined by the committee responsible for human experiments and the Declaration of Helsinki. Informed consent was obtained from each patient before participating in the study. Approval for the study was obtained from the University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (approval number: 162, dated: 09.09.2022).

## Statistical Analysis

Descriptive statistics were presented including minimum, maximum, mean  $\pm$  standart deviation, percentage, and frequency values. Categorical data were analyzed with the chi-square test. All data were transferred to the computer, and statistical analysis was performed using Statistical Package for the Social Sciences version 22.0. A p-value of  $<0.05$  was considered significant.

## RESULTS

The highest plasma phenylalanine levels measured at the time of diagnosis were taken as the criterion in determining the phenotypes of the patients. It was determined that 25 of our patients with PKU were diagnosed late. Nineteen of these patients had classical PKU, and 6 had mild PKU. It was determined that 11 (44%) of our cases were excluded from the newborn screening program and applied to our pediatric metabolism clinic due to neuropsychiatric symptoms, and they were diagnosed with PKU. Two of the cases excluded from the newborn screening program were diagnosed due to a history of a sibling with PKU. It was observed that 14 (56%) of our cases were diagnosed through the newborn screening program, but their hospital admission was late.

Eight of our cases were female and 17 were male. The mean age at diagnosis of all patients was  $26.3 \pm 38.1$  months (range, 2-192) months. The mean age at the last evaluation of the patients was calculated as  $13.1 \pm 5.3$  (range, 3.8-21.3) years (Table 1). Thirteen (52%) of the patients were diagnosed and started on treatment before the age of one. There was consanguinity between the parents of 18 of our patients.

**Table 1. Clinical and laboratory data of our late diagnosed PKU cases**

Patient/ gender	Age at diagnosis (month)	Current age (years)	Presentation complaint	Phenylalanine levels at diagnosis (µmol/L)	Phenylalanine levels at last evaluation (µmol/L)	Diagnosis	Neurological status	Treatment	Genotype
1/M	12	11.2	Neonatal screening	1364	320	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ IVS10-11G>A
2/F	60	15.2	Sibling with PKU	1675	1400	Classical PKU	Global developmental delay	Diet	c.1222C>T/ c.1222C>T
3/M	48	12.7	Sibling with PKU	1563	1300	Classical PKU	Global developmental delay	Diet	c.143T>C/ c.143T>C
4/F	42	18.8	Developmental delay, tremor, microcephaly	1680	290	Classical PKU	Global developmental delay	Diet	c.1039C>T/ c.1039C>T
5/M	30	17.7	Developmental delay, autism	820	410	Mild PKU	Global developmental delay	Diet	c.781C>T/ c.781C>T
6/M	7	14.1	Neonatal screening	1828	210	Classical PKU	Mild developmental delay	Diet	c.194T>C/ c.194T>C
7/F	6	11.2	Neonatal screening	1223	620	Classical PKU	Moderate developmental delay	Diet	c.728G>A/ c.728G>A
8/M	6	8.4	Neonatal screening	1320	330	Classical PKU	Global developmental delay	Diet	c.838G>A/ c.838G>A
9/F	13	5.2	Neonatal screening	1625	215	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.168G>T
10/M	8	21.1	Neonatal screening	1466	540	Classical PKU	Global developmental delay	LNAA	IVS10-11G>A/ IVS10-11G>A
11/M	10	11.7	Neonatal screening	917	580	Mild PKU	Moderate developmental delay	Diet	c.143T>C /c.143T>C
12/M	12	10.2	Neonatal screening	650	310	Mild PKU	Global developmental delay	Diet	c.1222C>T/ c.1222C>T
13/M	11	14.9	Neonatal screening	670	335	Mild PKU	Global developmental delay	Diet	c.781C>T/ c.781C>T
14/M	9	13.1	Neonatal screening	1380	943	Classical PKU	Mild developmental delay	Diet	IVS10-11G>A/ IVS10-11G>A
15/M	30	21.3	Epilepsy	743	964	Mild PKU	Mild developmental delay	LNAA	c.782G>A/ c.782G>A
16/F	7	6.1	Neonatal screening	2280	520	Classical PKU	Mild developmental delay	Diet	c.1222C>T/ c.1222C>T
17/F	24	20.1	Epilepsy	1420	850	Classical PKU	Global developmental delay	LNAA	c.143T>C/ c.143T>C
18/M	14	8.3	Developmental delay	866	193	Mild PKU	Global developmental delay	Diet	IVS10-11G>A/ c.782G>A
19/M	24	18.2	Epilepsy	1716	1250	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ c.1222C>T

**Table 1. Continued**

Patient/gender	Age at diagnosis (month)	Current age (years)	Presentation complaint	Phenylalanine levels at diagnosis (µmol/L)	Phenylalanine levels at last evaluation (µmol/L)	Diagnosis	Neurological status	Treatment	Genotype
20/M	24	11.9	Epilepsy	1601	1391	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ c.1222C>T
21/F	4	3.8	Neonatal screening	1341	261	Classical PKU	Moderate developmental delay	Diet	c.1039C>T/ c.1039C>T
22/M	3	5.9	Neonatal screening	1292	719	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.168G>T
23/M	2	7.3	Neonatal screening	1334	1081	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.1039C>T
24/F	192	18.6	Epilepsy, microcephaly, autism, tremor	1409	1420	Classical PKU	Global developmental delay	Diet	c.838G>A/ c.728G>A
25/M	60	20.2	Epilepsy, autism	1480	1144	Classical PKU	Global developmental delay	LNAA	IVS10-11G>A /c.838G>A

The phenylalanine level of the patients at the time of diagnosis was 777.6±107.7 µmol/L in mild PKU and 1526.1±246.8 µmol/L in classical PKU (Table 1). The phenylalanine level of the patients at the final evaluation was 465.3±275.7 µmol/L in mild PKU and 779.1±449.4 µmol/L in classical PKU (Table 1). The most common IVS10-11G>A mutation was found in patients. This allele was followed by the c.1222C>T (p.Arg408Trp) and c.143T>C (p.Leu48Ser) variants, respectively. Fifteen of our cases had global developmental delays, 6 had moderate developmental delays, and 4 had mild developmental delays. When we grouped the patients according to the severity of neurological findings, no statistically significant difference was found between the groups in terms of phenylalanine levels at the time of diagnosis.

In the diet treatment, our patients were given total protein intake 2-2.5 g/kg/day for the first year, 1.1-1.7 g/kg/day for ages 1-11, 1 g/kg/day for ages 12-15, and 0.9 g/kg/day for adults. The phenylalanine content of the diet was set at 130-400 mg/day for the first year, 200-400 mg/day for ages 1-11, 350-800 mg/day for ages 12-15, and 450-1000 mg/day for adults. Diet treatments were organized with natural foods and special formula foods that do not contain phenylalanine. Twenty-one of our cases received diet treatment; and 4 of our cases received large neutral amino acid (LNAA) treatment (Table 1). LNAA supplements were administered to adult patients who did not adhere to dietary treatment.

## DISCUSSION

In our country and the countries in this region (Middle East, West Asia, South Asia, North Africa), the frequency of consanguineous marriages is high. PKU and other autosomal recessive metabolic disorders are more frequently observed in societies where consanguineous marriages are high. In a study conducted in our country in 1993, the frequency of PKU was determined to be 1/4370, and the rate of consanguineous marriage was 21.5%.<sup>5</sup> The frequency of the disease varies considerably worldwide. In Europe, the frequency of PKU is 1/3000-1/30000, with an average of 1/10000 reported.<sup>3,6</sup> In our country, newborn screening for PKU is performed during the neonatal period. The diagnosis is confirmed by the determination of plasma phenylalanine level and *PAH* gene analysis. PKU screening has been performed in our country since 1993. However, in the study conducted by Tezel et al.,<sup>2</sup> the newborn screening rate was 4.7% in 1987, and this rate increased to 95% in 2008. In patients detected through newborn screening, treatment can be started in the second/third week of life.

The cases we present in this study were diagnosed late, and treatment was started after the second month of life. It was determined that 25 of our patients who have PKU and whom we followed up were diagnosed late. Nineteen of these patients

had classical PKU, and six had mild PKU phenotypes. All of our patients were born after the newborn screening program started, but only 56% were screened. 44% of our cases were not screened. In cases where screening could not be done, the diagnosis was made after the onset of neurological and psychiatric symptoms, during the examination. It was determined that our cases included in the screening program were reported late or applied late to the metabolism centre late despite being reported. It was determined that the instances of incomplete screening were generally families who gave birth at home and lived in rural areas.

Neuromotor retardation, microcephaly, and epilepsy can be seen in cases in which the treatment started late. High levels of phenylalanine and its metabolites can cause musty body odour and eczema. Tyrosinase inhibition and low tyrosine levels also cause loss of pigmentation in the skin and hair. In addition, behavioural disorders, aggressive behaviour, depression, and anxiety can be seen.<sup>7-9</sup> Our cases have epilepsy, behavioural disorders, and neuromotor delay. It is thought that cognitive problems seen in PKU are related to prefrontal dopamine depletion.<sup>10</sup> Phenylalanine competes with tyrosine at the blood-brain barrier, but phenylalanine passes through at high phenylalanine levels. CSF tyrosine levels decrease, and adequate dopamine synthesis cannot be achieved.<sup>10</sup> In addition, the negative effect of high phenylalanine levels on glutamate receptor function leads to brain dysfunction in patients with PKU.<sup>11,12</sup> Glushakow et al.<sup>12</sup> have shown that high phenylalanine levels significantly suppress the function of glutamate receptors in excitatory synapses.

The aim of treating PKU is to reduce plasma phenylalanine levels. A multidisciplinary approach is important in the treatment of patients with PKU. Treatment should be started as soon as possible in infants with plasma phenylalanine concentrations above 360  $\mu\text{mol/L}$ . In the treatment of the disease, a protein-restricted diet containing low phenylalanine, sapropterin dihydrochloride, LNAA to a protein-restricted diet, and phenylalanine ammonium lyase enzyme therapy are used.<sup>4,13,14</sup> However, attention should be paid to the protein intake required for optimal growth and development. It is recommended that treatment be continued throughout life. The aim of treatment is to reduce plasma phenylalanine levels, increase natural protein tolerance, maintain normal neuropsychological development, and provide a good quality of life. Twenty-one of our patients receive diet therapy. The other cases receive LNAA therapy. Patients received tyrosine supplements as needed according to their plasma tyrosine levels.

LNAA treatment is not recommended for young children or during pregnancy but is an option for adults who are not in good metabolic control and are not compliant with dietary

therapy.<sup>15</sup> LNAAs (arginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, and valine) compete with phenylalanine at the blood-brain barrier. Therefore, LNAA supplementation can significantly reduce phenylalanine uptake in the brain, in patients.<sup>16</sup> In this study, every patient who received LNAA supplementation was an adult who did not comply with dietary therapy.

### Study Limitations

Our study has some limitations. First, neuroimaging could not be performed in most of our patients. Cooperation with the patients for MRI was not possible due to their neurological impairment. In addition, neuroimaging could not be performed due to the socioeconomic status of some families. Secondly, since our study is retrospective, the patients' treatment interruption status could not be fully evaluated.

In our country, PKU is a treatable metabolic disease that is included in the newborn screening program. It causes severe neurological problems in patients who are diagnosed late, or cannot be diagnosed. Therefore, close monitoring is important during the diagnosis and treatment phase of the disease. Close monitoring is essential to ensure that patients diagnosed with PKU receive their treatment at an early stage.

### Ethics

**Ethics Committee Approval:** Approval for the study was obtained from the University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (approval number: 162, dated: 09.09.2022).

**Informed Consent:** Informed consent was obtained from each patient before participating in the study.

### Footnotes

#### Authorship Contributions

Surgical and Medical Practices: H.B., A.E.B., Concept: H.B., A.E.B., Data Collection or Processing: H.B., Analysis or Interpretation: H.B., A.E.B., Literature Search: H.B., A.E.B., Writing: H.B.

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### REFERENCES

1. Flydal MI, Martinez A. Phenylalanine hydroxylase: function, structure, and regulation. *IUBMB Life*. 2013;65(4):341-349. doi:10.1002/iub.1153
2. Tezel B, Dilli D, Bolat H, Sahman H, Ozbaş S, Acıcan D, Ertek M, Köse MR, Dilmen U. The development and organization of newborn screening programs in Turkey. *J Clin Lab Anal*. 2014;28(1):63-69. doi:10.1002/jcla.21649

3. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet*. 2010;376(9750):1417-1427. doi:10.1016/S0140-6736(10)60961-0
4. Regier DS, Greene CL. Phenylalanine Hydroxylase Deficiency. 2000 Jan 10 [updated 2017 Jan 5]. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1504/>
5. Ozguc M, Ozalp I, Coskun T, Yilmaz E, Erdem H, Ayter S. Mutation analysis in Turkish phenylketonuria patients. *J Med Genet*. 1993;30(2):129-130. doi:10.1136/jmg.30.2.129
6. Loeber JG. Neonatal screening in Europe; the situation in 2004. *J Inherit Metab Dis*. 2007;30(4):430-438. doi:10.1007/s10545-007-0655-3
7. González MJ, Gutiérrez AP, Gassió R, Fusté ME, Vilaseca MA, Campistol J. Neurological complications and behavioral problems in patients with phenylketonuria in a follow-up unit. *Mol Genet Metab*. 2011;104(1-2):73-79. doi:10.1016/j.ymgme.2011.05.018
8. Bilder DA, Kobori JA, Cohen-Pfeffer JL, Johnson EM, Jurecki ER, Grant ML. Neuropsychiatric comorbidities in adults with phenylketonuria: A retrospective cohort study. *Mol Genet Metab*. 2017;121(1):1-8. doi:10.1016/j.ymgme.2017.03.003
9. Leuzzi V, Trasimeni G, Gualdi GF, Antonozzi I. Biochemical, clinical and neuroradiological (MRI) correlations in late-detected phenylketonuria patients. *J Inherit Metab Dis*. 1995;18(5):624-634. doi:10.1007/BF00711594
10. Moritani T, Smoker WR, Sato Y, Numaguchi Y, Westesson PL. Diffusion-weighted imaging of acute excitotoxic brain injury. *AJNR Am J Neuroradiol*. 2005;26(2):216-228. PMID: 15709132
11. Diamond A. Consequences of variations in genes that affect dopamine in prefrontal cortex. *Cereb Cortex*. 2007;17(Suppl 1):i161-i170. doi:10.1093/cercor/bhl165
12. Glushakow AV, Dennis DM, Sumners C, Seubert CN, Martynyuk AE. L-phenylalanine selectively depresses currents at glutamatergic excitatory synapses. *J Neurosci Res*. 2003;72(1):116-124. doi:10.1002/jnr.10558
13. Burgard P, Bremer HJ, Bührdel P, et al. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. *Eur J Pediatr*. 1999;158(1):46-54. doi:10.1007/s004310051005
14. Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Arch Dis Child*. 2007;92(3):213-218. doi:10.1136/adc.2006.103234
15. Singh RH, Rohr F, Frazier D, Cunningham A, Mofidi S, Ogata B, Splett PL, Moseley K, Huntington K, Acosta PB, Vockley J, Van Calcar SC. Recommendations for the nutrition management of phenylalanine hydroxylase deficiency. *Genet Med*. 2014;16(2):121-131. doi:10.1038/gim.2013.179
16. Pietz J, Kreis R, Rupp A, Mayatepek E, Rating D, Boesch C, Bremer HJ. Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. *J Clin Invest*. 1999;103(8):1169-1178. doi:10.1172/JCI61

# A Methylmalonic Acidemia Patient Mimicking Diabetic Ketoacidosis and Long-Term Follow-Up

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## Abstract

Methylmalonic acidemia (MMA) is the most common inherited type of organic acidemia. It has a diverse presentation in older infants without any initial apparent symptoms. MMA sometimes presents with sudden metabolic decompensation, which may mimic common emergencies like diabetic ketoacidosis (DKA); without early recognition, it can be fatal. In this case, we aimed to emphasize that less common diagnoses such as organic acidemia should be kept in mind in infants with severe acidosis and metabolic decompensation, or in patients with an atypical clinical course to prevent serious morbidities and even death. We report a case of MMA in an infant who presented acutely mimicking DKA and underwent long-term surveillance. An 8.5-month-old girl, the first child of an unrelated family, was admitted with complaints of vomiting and hyperglycemia and metabolic acidosis were detected. In her history, complementary feeding started at 7 months, and she had one hospital admission at 7 months due to vomiting, which improved with intravenous fluid therapy. A diagnosis of DKA was made, and appropriate fluid therapy and insulin infusion were started. However, despite achieving normoglycemia, the anion gap (AG) remained high, and metabolic acidosis persisted. Due to ongoing drowsiness and high serum ammonia levels (215 µg/dL), a metabolic disorder was suspected, and peritoneal dialysis was initiated. Tandem mass spectrometry analysis showed markedly elevated C3 propionylcarnitine levels and increased C3/C2 and C3/free carnitine ratios, while urinary organic acid analysis revealed a significant increase in methylmalonic acid excretion, along with a marked rise in 3-hydroxypropionate and methylcitrate. *MUT* gene analysis revealed a homozygous mutation c.360\_361insT (p.K121\*), confirming the diagnosis of MMA. Long-term follow-up has shown a progressive decline in her estimated glomerular filtration rate (eGFR), with even lower levels observed during acidosis attacks. Inborn errors of metabolism, especially organic acidemia, should be suspected in any infant presenting with severe high AG metabolic acidosis. MMA is also associated with chronic tubulointerstitial nephritis and a progressive decline in GFR.

**Keywords:** Methylmalonic Acidemia, Hyperglycemia, Diabetic Ketoacidosis, Glomerular Filtration Rate

## INTRODUCTION

Methylmalonic acidemia (MMA), a form of organic acidemia, occurs due to a defect in the methylmalonyl-CoA mutase (MCM) enzyme, which is responsible for converting methylmalonyl-CoA to succinyl-CoA.<sup>1</sup> A partial or complete deficiency of the cobalamin-dependent MCM enzyme leads to the accumulation of methylmalonyl-CoA, resulting in a significant increase in the excretion of methylmalonic acid (MMA) in both blood and urine.<sup>2</sup> MMA affects around 1 in every 50,000 to 80,000 babies. It is more prevalent in nations with high levels of consanguinity and lack of newborn screening, such as

economically disadvantaged countries.<sup>1</sup> Patients often present between 1 month and 1 year old with symptoms such as poor feeding, vomiting, dehydration, shock, hypoglycemia, hyperammonemia, and high anion gap (AG) metabolic acidosis, which can progress to coma or death if not treated. The mild form of the disease may occur in infancy and childhood.<sup>3</sup> MMA can occur unexpectedly in older infants, mimicking septic shock or diabetic ketoacidosis (DKA) and be potentially lethal if not detected early.<sup>4</sup> We reported a case of MMA in a newborn with severe high AG metabolic acidosis that mimicked DKA, despite no early symptoms.



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## CASE REPORT

An 8.5-month-old female patient, with no prior medical history, presented with a complaint of projectile vomiting including the contents of her meals, for the past four days. There was no associated fever, diarrhea, or abdominal pain. About two days later, respiratory distress developed, her feeding gradually worsened, and she had frequent urination, without a foul odor. There was no history of drug or substance intake. The patient's family history revealed that the parents, who are not related but are from the same village, had their first child with a term birth. She had a history of meconium aspiration and was followed in the neonatal intensive care unit for one week. Complementary feeding started at 7 months, and she had one hospital admission at 7 months due to vomiting, which improved with intravenous fluid therapy. Upon examination, she was found to be dehydrated.

Laboratory tests showed a blood glucose level of 234 mg/dL, and +2 ketones in the urinalysis, and metabolic acidosis (pH: 7.01,  $\text{HCO}_3^-$ : 6.4 mmol/L). A diagnosis of DKA was made, and appropriate fluid therapy and insulin infusion were started. However, despite achieving normoglycemia, the AG remained high (28), and metabolic acidosis (pH: 7.18,  $\text{pCO}_2$ : 20 mmHg,  $\text{HCO}_3^-$ : 10.4 mmol/L) persisted. In addition, insulin, and C-peptide values were also normal. Due to ongoing drowsiness and high serum ammonia levels (215  $\mu\text{g/dL}$ ), a metabolic disorder was suspected, and peritoneal dialysis was initiated, which led to the correction of both the acidosis and hyperammonemia. To clarify the etiology before starting peritoneal dialysis, blood and urine samples were taken for Tandem mass spectrometry (MS), and urinary organic acid analysis, and the patient was referred to our Pediatric Metabolism and Nutrition Department for further investigation and management. On physical examination at the time of admission, body temperature was 36.7 °C; pulse was 118 beats per minute; respiratory rate was 40/min; blood pressure was 95/55 mmHg; body weight was 7400 grams [ $<3$  p, -1.12 standard deviation (SD)]; height was 64 cm ( $<3$  p, -1.96 SD); oxygen saturation was 97%, with no significant findings on systemic examination and no specific odor. Initial laboratory results included hemoglobin: 9.2 g/dL, total leukocyte count: 8240/ $\text{mm}^3$  with 68% neutrophils, platelet count: 251,000/ $\text{mm}^3$ , C-reactive protein: 8 mg/dL, venous blood gas: pH 7.35,  $\text{HCO}_3^-$ : 20.3 mmol/L, AG: 24.5 mmol/L, lactate: 1.2 mmol/L, blood glucose: 75 mg/dL, serum electrolytes: normal, blood urea nitrogen: 7.93 mg/dL, creatinine: 0.4 mg/dL, serum calcium: 7.6 mg/dL, and glomerular filtration rate: 72. Urine analysis showed pH: 5.5; density: 1022; glucose 2+; protein 1+; and ketones 2+. Serum ammonia was 68  $\mu\text{g/dL}$ , lactate was 13 mg/dL, and pyruvate was 0.7 mg/dL; all were normal. Amino acid levels in both urine and blood were normal. In pre-dialysis tests at the external facility, Tandem MS analysis showed markedly

elevated C3 propionylcarnitine levels and increased C3/C2 and C3/free carnitine ratios. Urinary organic acid analysis revealed a significant increase in MMA excretion, along with a marked rise in 3-hydroxypropionate and methylcitrate. Based on these findings, the patient was diagnosed with MMA, and treatment with 100 mg/kg levocarnitine and 1 mg hydroxocobalamin was initiated.

Her feeding was adjusted to a branched-chain amino acid-deficient special formula, and isoleucine powder supplementation was provided upon identifying a low isoleucine level in her blood amino acids. *MUT* gene analysis revealed a homozygous mutation c.360\_361insT (p.K121\*), confirming the diagnosis of MMA. Genetic testing showed that both parents were heterozygous for the same mutation.

After diagnosis, the patient had four episodes of metabolic acidosis, two of which were resistant. At the age of 3, she developed metabolic acidosis, hyperuricemia, and hyperkalemia. In addition to her existing treatment, Shohl's solution, anti-potassium therapy, and allopurinol were started. At the age of 5, the patient experienced a refractory metabolic acidosis and hyperammonemia attack accompanied by delirium, during which antihypertensive medications were added to her treatment. Long-term follow-up has shown a progressive decline in her estimated glomerular filtration rate (eGFR), with even lower levels observed during acidosis attacks. The informed consent of the patient was obtained from her family.

## DISCUSSION

We present a case of an infant with MMA who experienced unexpected decompensation associated with high AG and severe metabolic acidosis without any preceding signs or symptoms. In this report, the infant presented with hyperglycemic DKA with a weak insulin response. Due to the persistence of DKA, an underlying metabolic issue was investigated. Hyperglycemia is a rare but fatal MMA symptom that resembles DKA.<sup>5,6</sup> Although hyperglycemia is an infrequent MMA manifestation,<sup>7</sup> there have been described cases of severe and prolonged metabolic acidosis and hyperglycemia despite substantial insulin doses. Diabetes is the most prevalent cause of DKA, but it responds well to standard treatment; thus, additional causes of acidosis/hyperglycemia should be examined in poor responders.<sup>8</sup>

Organic acidurias (OAs) should be included in the differential diagnosis, especially in countries where national newborn screening is not implemented. Determining the etiology of hyperglycemic DKA is important and can lead to a good outcome.<sup>9</sup> The unusual presentation of our patient, mimicking DKA, reminds us of the wide spectrum of clinical signs of organic acidemia. In infants with severe acidosis and metabolic decompensation, or with atypical clinical course, there should

be a suspicion of a less common diagnosis, such as organic acidemia, to prevent severe morbidities and even death.<sup>10</sup>

Despite significant advancements in treatment, long-term complications such as developmental delay, neurological disorders due to degeneration of the basal ganglia, interstitial nephritis, progressive renal failure, pancreatitis attacks, and cardiomyopathy are commonly observed.<sup>11-13</sup> Impaired kidney function is a well-documented long-term complication of MMA and occurs more frequently than in other organic acidemias. The onset of kidney dysfunction in MMA is related to the molecular subtype. Mut<sup>0</sup> patients are typically affected at an earlier age compared to CblB patients, while CblA and mut-patients may experience kidney issues in later stages of life.<sup>2,14,15</sup> The pathogenesis of kidney damage associated with MMA is not well understood.

Kidney involvement in MMA patients can be both tubular; [proximal renal tubular acidosis (RTAs), impaired urine acidification and concentration ability, and hyporeninemic hypoaldosteronism] and glomerular (chronic interstitial nephritis).<sup>16</sup> Mitochondrial dysfunction appears to play a key role in the pathomechanisms of kidney damage in MMA. In a metabolic acidosis environment, increased ammonia production in the proximal tubule has been suggested as a potential mechanism contributing to the worsening of kidney function.<sup>17</sup> In a rat model, it has been observed that nitrogen nucleophiles, such as ammonia, cause kidney damage and induce chronic tubulointerstitial inflammation through the activation of an alternative complement pathway. Additionally, the activation of the renin-angiotensin system (RAS) plays a role in the pathogenesis of kidney dysfunction associated with metabolic acidosis. This suggests that both toxic metabolites like ammonia, and systemic pathways like RAS, contribute to the kidney damage seen in conditions such as MMA, where metabolic derangements lead to renal complications.<sup>18</sup>

In a study conducted by Şeker Yılmaz et al.<sup>19</sup> from our country, 12 out of 37 isolated MMA patients (32%), were found to have kidney involvement. One patient, despite good metabolic control, exhibited early-onset and rapidly progressing kidney complications, particularly RTA type 4 and stage 3 chronic kidney disease.

In MMA patients, monitoring kidney functions is strongly recommended. Serum creatinine, as a surrogate marker of kidney function, may be misleading, because in MMA patients with protein deficiency, a reduction in muscle mass likely results in an overestimation of GFR. Other kidney function markers, such as serum cystatin C, may better reflect the true eGFR and provide a more accurate assessment of renal function in these patients.<sup>20</sup>

During the follow-up of our patient, we observed that the serum creatinine levels began to rise around the age of one, peaked at the age of five, and then stabilized. During the long-term follow-up, the eGFR, calculated using the Schwartz formula, progressively decreased to a value of 65.66 mL/min/1.73 m<sup>2</sup> at the age of eight. Additionally, during episodes of acidosis, the eGFR was found to be even lower (Table 1).

Blood pressure monitoring should be an integral part of kidney function assessments in patients with conditions like MMA. Hypertension can be a significant complication in these patients and may contribute to the progression of kidney dysfunction, making its early detection and management crucial for preserving renal health.<sup>2</sup> In the case of our patient, during a resistant metabolic acidosis and hyperammonemia episode at the age of five accompanied by a delirium episode, antihypertensive medication was added to the existing treatment.

Combined liver and kidney transplantation appears to be an effective treatment for renal failure in MMA, and it can result in normal kidney function even 10 years after transplantation.<sup>21,22</sup> Kidney transplantation improves kidney function shortly after

**Table 1. Summary of the patient's laboratory values in follow-up**

Age (Year)	BUN (5-18 mg/dL)	Creatinin (0.4-0.6 mg/dL)	eGFR* (mL/min/1.73 m <sup>2</sup> )	Cloride (98-107 mEq/L)	Uric acid (3.4-7 mg/dL)	Urine keton	Urine MMA
1	34	0.25	143	111	3.09	+	-
2	26.85	0.61	76.63	106	9.39	-	-
3	13	0.51	91.66	98	5.3	-	-
4	14	0.53	88.20	104	3.7	-	-
5	45	0.97	54.43	107	8.1	++	-
6	17	0.77	76.071	96	5	-	-
7	19	0.89	72.30	92	4	-	-
8	9.2	0.98	65.66	105	3.4	-	-

BUN: Blood urea nitroge, eGFR: Estimated glomerular filtration rate, MMA: Methylmalonic acid

the transplant, and, in some cases, even years after the procedure (ranging from 1.5 to 14 years). However, it has also been reported that nephropathy and renal failure can recur after kidney transplantation.<sup>23,24</sup> While a few patients show normal kidney function even 15 years after transplantation, some patients develop progressive kidney failure after transplantation. Liver transplantation does not appear to correct a non-functional kidney.<sup>22,25,26</sup>

In countries like Türkiye, where national newborn screening is not implemented, OAs should be included in the differential diagnosis when high AG metabolic acidosis is accompanied by hypo/hyperglycemia. It is especially important to remember that all patients with MMA are at risk of developing kidney failure during the long-term course of the disease.

## Ethics

**Informed Consent:** The informed consent of the patient was obtained from her family.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: G.K.Y., Concept: G.K.Y., Design: M.A.K., G.K.Y., Data Collection or Processing: M.A.K., Analysis or Interpretation: M.A.K., G.K.Y., Literature Search: M.A.K., Writing: G.K.Y.

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## REFERENCES

1. Yu Y, Shuai R, Liang L, Qiu W, Shen L, Wu S, Wei H, Chen Y, Yang C, Xu P, Chen X, Zou H, Feng J, Niu T, Hu H, Ye J, Zhang H, Lu D, Gong Z, Zhan X, Ji W, Gu X, Han L. Different mutations in the *MMUT* gene are associated with the effect of vitamin B12 in a cohort of 266 Chinese patients with mut-type methylmalonic acidemia: A retrospective study. *Mol Genet Genomic Med*. 2021;9(11):e1822. doi: 10.1002/mgg3.1822
2. Forny P, Hörster F, Ballhausen D, Chakrapani A, Chapman KA, Dionisi-Vici C, Dixon M, Grünert SC, Grunewald S, Haliloglu G, Hochuli M, Honzik T, Karall D, Martinelli D, Molema F, Sass JO, Scholl-Bürgi S, Tal G, Williams M, Huemer M, Baumgartner MR. Guidelines for the diagnosis and management of methylmalonic acidemia and propionic acidemia: First revision. *J Inherit Metab Dis*. 2021;44(3):566-592. doi: 10.1002/jimd.12370
3. Zhou X, Cui Y, Han J. Methylmalonic acidemia: Current status and research priorities. *Intractable Rare Dis Res*. 2018;7(2):73-78. doi: 10.5582/iridr.2018.01026
4. Saini N, Malhotra A, Chhabra S, Chhabra S. Methylmalonic acidemia mimicking diabetic ketoacidosis and septic shock in infants. *Indian J Crit Care Med*. 2015;19(3):183-185. doi: 10.4103/0972-5229.152776
5. Clarke JTR. A clinical guide to inherited metabolic diseases. Cambridge: Cambridge University Press; 1996. p. 280.
6. Rezvani I. Defects in metabolism of amino acids. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. *Nelson Textbook of Pediatrics*. 18<sup>th</sup> ed. Philadelphia: Saunders; 2007. p. 547-548.
7. Imen M, Hanene B, Ichraf K, et al. Methylmalonic acidemia and hyperglycemia: an unusual association. *Brain Dev*. 2012;34(2):113-114. doi: 10.1016/j.braindev.2011.07.002
8. Boeckx RL, Hicks JM. Methylmalonic acidemia with the unusual complication of severe hyperglycemia. *Clin Chem*. 1982;28(8):1801-1803. <https://pubmed.ncbi.nlm.nih.gov/7046992/>
9. Guven A, Cebeci N, Dursun A, Aktekin E, Baumgartner M, Fowler B. Methylmalonic acidemia mimicking diabetic ketoacidosis in an infant. *Pediatr Diabetes*. 2012;13(6):e22-e25. doi: 10.1111/j.1399-5448.2011.00784.x
10. Dejkhamron P, Wejapikul K, Unachak K, Sawangareetrakul P, Tanpaiboon P, Wattanasirichaigoon D. Isolated methylmalonic acidemia with unusual presentation mimicking diabetic ketoacidosis. *J Pediatr Endocrinol Metab*. 2016;29(3):373-378. doi: 10.1515/jpem-2015-0228
11. Dionisi-Vici C, Deodato F, Röslinger W, Rhead W, Wilcken B. "Classical" organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. *J Inherit Metab Dis*. 2006;29(2-3):383-389. doi: 10.1007/s10545-006-0278-z
12. Walter JH, Michalski A, Wilson WM, Leonard JV, Barratt TM, Dillon MJ. Chronic renal failure in methylmalonic acidemia. *Eur J Pediatr*. 1989;148(4):344-348. doi: 10.1007/BF00444131
13. Mardach R, Verity MA, Cederbaum SD. Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy. *Mol Genet Metab*. 2005;85(4):286-290. doi: 10.1016/j.ymgme.2005.04.004
14. Cosson MA, Benoist JF, Touati G, Déchaux M, Royer N, Grandin L, Jais JP, Boddaert N, Barbier V, Desguerre I, Campeau PM, Rabier D, Valayannopoulos V, Niaudet P, de Lonlay P. Long-term outcome in methylmalonic aciduria: a series of 30 French patients. *Mol Genet Metab*. 2009;97(3):172-178. doi: 10.1016/j.ymgme.2009.03.006
15. Hörster F, Baumgartner MR, Viardot C, Suormala T, Burgard P, Fowler B, Hoffmann GF, Garbade SF, Kölker S, Baumgartner ER. Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut<sup>q</sup>, mut, cblA, cblB). *Pediatr Res*. 2007;62(2):225-230. doi: 10.1203/PDR.0b013e3180a0325f
16. Alkhunaizi AM, Al-Sanna N. Renal Involvement in Methylmalonic Aciduria. *Kidney Int Rep*. 2017;2(5):956-960. doi: 10.1016/j.ekir.2017.04.007
17. Nath KA, Hostetter MK, Hostetter TH. Pathophysiology of chronic tubulointerstitial disease in rats. Interactions of dietary acid load, ammonia, and complement component C3. *J Clin Invest*. 1985;76(2):667-675. doi: 10.1172/JCI112020
18. Ng HY, Chen HC, Tsai YC, Yang YK, Lee CT. Activation of intrarenal renin-angiotensin system during metabolic acidosis. *Am J Nephrol*. 2011;34(1):55-63. doi: 10.1159/000328742
19. Şeker Yılmaz B, Kor D, Bulut FD, Kilavuz S, Ceylaner S, Önenli Mungan HN. Clinical and molecular findings in 37 Turkish patients with isolated methylmalonic acidemia. *Turk J Med Sci*. 1228-1220:(3)51;2021 doi: 10.3906/sag-2001-72
20. Kruszka PS, Manoli I, Sloan JL, Kopp JB, Venditti CP. Renal growth in isolated methylmalonic acidemia. *Genet Med*. 2013;15(12):990-996. doi: 10.1038/gim.2013.42
21. Duclaux-Loras R, Bacchetta J, Berthiller J, Rivet C, Demède D, Javouhey E, Dubois R, Dijoud F, Lachaux A, Badet L, Boillot O, Cochat P. Pediatric combined liver-kidney transplantation: a single-center experience of 18 cases. *Pediatr Nephrol*. 2016;31(9):1517-1529. doi: 10.1007/s00467-016-3324-6

22. Mc Guire PJ, Lim-Melia E, Diaz GA, Raymond K, Larkin A, Wasserstein MP, Sansaricq C. Combined liver-kidney transplant for the management of methylmalonic aciduria: a case report and review of the literature. *Mol Genet Metab.* 2008;93(1):22-29. doi: 10.1016/j.ymgme.2007.08.119
23. Lubrano R, Bellelli E, Gentile I, Paoli S, Carducci C, Carducci C, Santagata S, Pérez B, Ugarte M, Labriola D, Elli M. Pregnancy in a methylmalonic acidemia patient with kidney transplantation: a case report. *Am J Transplant.* 2013;13(7):1918-1922. doi: 10.1111/ajt.12282
24. Wesół-Kucharska D, Kaczor M, Pajdowska M, Ehmke Vel Emczyńska-Seliga E, Bogdańska A, Kozłowski D, Piekutowska-Abramczuk D, Ciara E, Rokicki D. Clinical picture and treatment effects in 5 patients with Methylmalonic aciduria related to *MMAA* mutations. *Mol Genet Metab Rep.* 22:100559;2020. doi: 10.1016/j.ymgmr.2019.100559
25. Noone D, Riedl M, Atkison P, Avitzur Y, Sharma AP, Filler G, Siriwardena K, Prasad C. Kidney disease and organ transplantation in methylmalonic acidemia. *Pediatr Transplant.* 2019;23(4):e13407. doi: 10.1111/petr.13407
26. Jiang YZ, Sun LY. The value of liver transplantation for methylmalonic acidemia. *Front Pediatr.* 2019;7:87. doi: 10.3389/fped.2019.00087

## An Overview on Selenoproteins and Their Functions

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### Abstract

Selenium, is a vital trace element required by many living organisms. It is mainly incorporated in selenoproteins functioning in boosting immune system, reducing inflammation, decreasing cardiovascular disease and inhibiting cancer progression. Fifty different selenoprotein families have been identified. They fulfill a broad range of physiological roles, notably functioning as antioxidants and participating in thyroid hormone metabolism. They are key regulators of stress responses, metabolism, and immunity. Selenoproteins will may be utilized in the treatment of various pathologies, including cancer, diabetes mellitus, neurodegenerative disorders, and cardiovascular injuries, in the near future. This technical report will provide a general overview of selenoproteins and their functions.

**Keywords:** Cancer, Diabetes Mellitus, Neurodegenerative Disorders

### INTRODUCTION

Selenium (Se), is a vital trace element required by many living organisms. The main sources of Se are bread, cereals, eggs, meat, fish, dairy products, fruits and vegetables. It is an antioxidant and shielding the cells from damage. It is mainly found in selenocysteine (Sec) and incorporated in selenoproteins functioning in boosting immune system, reducing inflammation, decreasing cardiovascular disease and inhibiting cancer progression. Its depletion is accepted as a factor contributing to various pathological conditions, such as cardiovascular disease, neuromuscular disorders, certain cancers, male infertility, and inflammation.<sup>1</sup>

Se, has a very narrow range between beneficial and harmful levels. Deficiency symptoms can appear with daily intake levels below 18 µg, while toxic effects may occur when intake exceeds 400 µg.<sup>2</sup> Considering cancer, plasma Se concentrations below 140 µg/L are linked to a significantly increased risk. On the other hand, levels above 400 µg/L are associated with selenosis, while concentrations exceeding 1000 µg/L indicate acute Se toxicity. The ideal baseline range for Se in plasma is considered to be between 110-135 µg/L, with the production of plasma selenoproteins reaching a plateau around 130 µg/L.<sup>3</sup>

Our understanding of Se's crucial role has significantly deepened since Rotruck and his team identified the first selenoprotein 50 years ago (Figure 1).<sup>4</sup> Though most selenoproteins serve oxidoreductase functions, its importance for immun regulation, thyroid hormon metabolism and etc, its need for proper brain function is undeniable. Studies have revealed that lacking certain selenoproteins in the brain can harm neuronal health and, may trigger neurodegeneration. Additionally, the redox balance maintained by selenoproteins may play a role in modulating neuronal functions such as neurotransmission.<sup>5,6</sup>

### Biosynthesis and Types of Selenoproteins

To date, over 50 different selenoprotein families have been identified. The presence and types of selenoproteins can vary significantly between different species.<sup>5</sup> A majority of Se-containing proteins incorporate the element as the amino acid Sec. Within cells, Sec represents the predominant form of Se and is distinctive because it is encoded by the UGA codon-a codon that normally functions as a stop signal in the standard genetic code (Figure 2).<sup>5</sup> The human selenoproteome is encoded by 25 genes, and it is expected that many more selenoproteins will be found through genome and sequence analysis.



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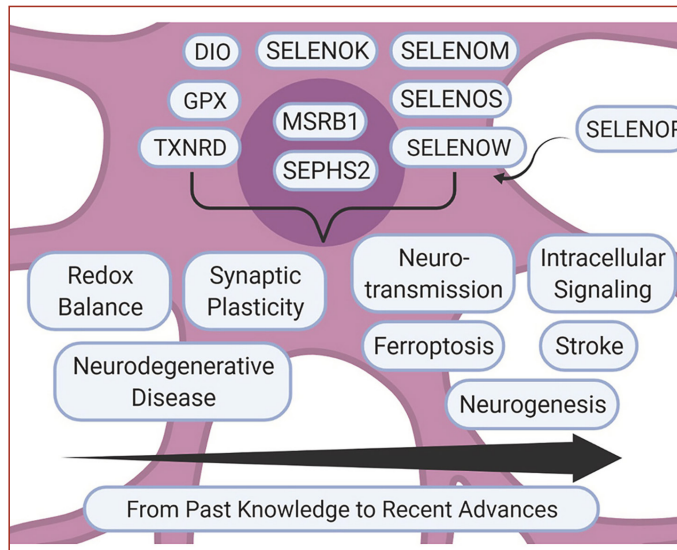
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One key feature common for all selenoproteins is the presence of Sec residues in their sequence. The physiological functions of selenoproteins strictly depend on the presence of Sec, and mutations of Sec to any other amino acid residue leads to enzyme inactivation.<sup>5</sup>



**Figure 1.** Selenoproteins from past to future<sup>4</sup>  
 GPX: Glutathione peroxidase, TXNRD: Thioredoxin reductase, SELENOP: Selenoprotein P

All of the selenoproteins contain one Sec residue, only selenoprotein P (SelP) has 10 Sec residues. Selenoproteins are classified into two large groups in accordance with their Sec location. One of these two groups contains Sec in a site adjacent to the COOH terminal region of the protein, such as selenoproteins S, R, O, I, K, and thioredoxin reductases (TRXRs). The other group has Sec in the NH<sub>2</sub>-terminal region of the protein, such as H, M, N, T, V, W, F (Sep15), selenophosphate synthetase, glutathione peroxidases (GPXs), and deiodinases (DIOs).<sup>7</sup>

The GPXs, TRXRs and DIOs were the first selenoproteins discovered and are the most extensively studied ones. The GPXs are integral to antioxidant glutathione pathways, providing protection from reactive oxygen species (ROS), the TRXRs use NADPH for reduction of thioredoxin in cellular redox pathways and the DIOs cleave iodine-carbon bonds in the metabolism of thyroid hormones.<sup>8</sup>

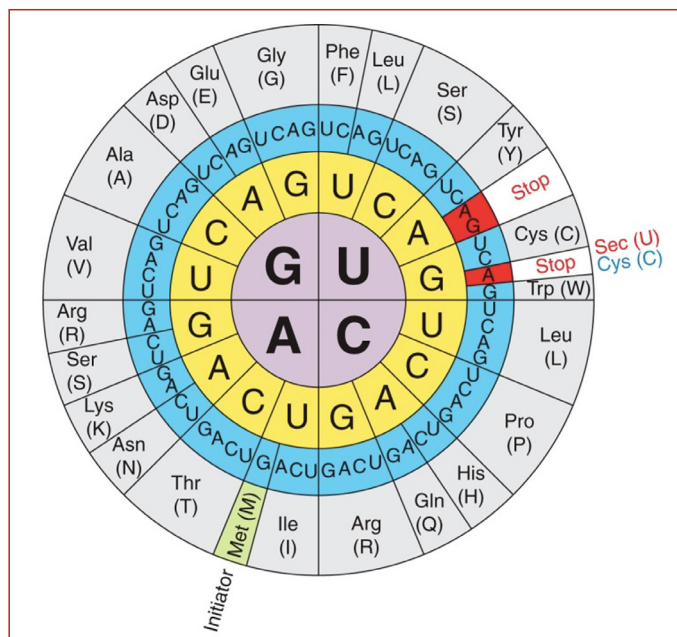
The synthesis of selenoproteins is variably influenced by the availability of Se. Under Se-deficient conditions, the production of certain selenoproteins-such as GPX1, MsrB1, SelW, and SelH-is markedly reduced. These proteins, which are more sensitive to Se levels, are often categorized as stress-responsive selenoproteins. In contrast, another subset of selenoproteins, including TR1 and TR3, shows relatively stable expression regardless of dietary Se intake. These are typically referred to as housekeeping selenoproteins, reflecting their consistent expression to support essential cellular functions.<sup>5</sup>

**Functions of Selenoproteins**

Selenoproteins are key regulators of stress responses, metabolism, and immunity. At least 12 of the known selenoproteins are involved in immune functions and cancer mechanisms. Eleven of the selenoproteins primarily have redox-active function. These selenoproteins have emerged as central regulators of cellular antioxidant capacity and maintenance of redox homeostasis. Other 14 selenoproteins such as F, K, M, N, S, and T encoded within the human genome, have been implicated in endoplasmic reticulum (ER) homeostasis and utilize their oxidative capabilities in protein folding.<sup>3</sup>

The primary functions of selenoproteins include:

- Redox-active functions (antioxidant defense)
- Protects endothelial cells from peroxynitrite damage.
- Reduces the effect of many reactive oxygen species such as hydrogen peroxide and lipid hydroperoxide.
- Protects immune cells from oxidative stress.
- Reduces cytokine release.



**Figure 2.** The genetic code illustrating the dual function of the UGA codon and that Sec is the 21<sup>st</sup> amino acid that is encoded by UGA<sup>7</sup>  
 Sec: Selenocysteine

Regulates many antioxidants.

- Thyroid hormone metabolism
- Immune regulation
- **Protein folding and quality control:** Selenoproteins localized in the ER contribute to proper protein folding and the regulation of cellular stress responses. They also help in the removal of misfolded proteins.
- **Anti-inflammatory and anti-apoptotic functions:** These proteins participate in the suppression of inflammatory pathways and the inhibition of programmed cell death.
- **Regulation of energy metabolism:** Mitochondrial selenoproteins support cellular energy production and metabolic homeostasis through their roles in oxidative phosphorylation and redox regulation.

Other than, SelP and selenoprotein W (SelW), majority of selenoproteins have no known functions. SelW is a small intracellular protein, binds glutathione and function in oxidant defense. SelP is an extracellular glycoprotein and is the most common selenoprotein found in the plasma. It was shown that, patients with high SelP levels (>5.9 mg/L) had significantly lower risk for all-cause mortality and cardiovascular mortality.<sup>9</sup> Plasma concentration of SelP also correlates with protection against diquat liver injury, suggesting that the protein protects against oxidant injury. The disturbance in SelP cellular concentration results in pathophysiological conditions such as insulin resistance, diabetes mellitus type 2, hyperglycemia and pulmonary arterial hypertension.<sup>10</sup>

Selenoprotein N, is expressed in skeletal muscle, heart, lung, and placenta and it controls redox state of the intracellular calcium-release channel [ryanodine receptor (RyR)], and affects Ca<sup>2+</sup> homeostasis. Its encoded by the *SEPN1* gene. Mutations in the *SEPN1* gene, causing the knockdown of selenoprotein N accompanied by recessive gene *RYR1* that encodes RyR1, which are both proteins implicated in calcium homeostasis, cause severe congenital myopathies. In addition to myopathies, these mutations also lead to impaired insulin action in skeletal muscle by decreasing Akt (protein kinase B) phosphorylation and high ER stress. All these facts indicate a correlation between the decrease in glucose tolerance, insulin activity, and increased ER stress in muscles.<sup>5</sup>

Selenoproteins also possess a strong correlation with human cancer. Selenoproteins, enzymes that selectively include the amino acid Sec, make up major classes of antioxidant proteins critical for the protection of cancer cells to elevated ROS. Nearly all GPX and TRXR enzymes fall into this category as a

catalytic Sec is essential for their activities. Selenoprotein gene polymorphisms have been linked to risk of cancer, such as; SelP is associated with the tumorigenesis of colon cancer, whereas Sep15 polymorphisms may increase lung cancer risk. SelK can inhibit cell adhesion and the migration of human gastric cancer cells, besides it is critical in promoting calcium fluxes that induce melanoma progression.<sup>11</sup> Due to the limited research on selenoproteins, the relationship between selenoproteins and cancer has not yet been revealed. Se supplementation do not change all selenoproteins equally, the direct roles of selenoproteins need to be examined to assess whether supplementation is advisable for treatment or prevention of a specific cancers.

## CONCLUSION

In conclusion, selenoproteins, including those containing the amino acid Sec, are intrinsic components of living organisms. They fulfill a broad range of physiological roles, notably functioning as antioxidants and participating in thyroid hormone metabolism. In mammals, the diverse functions of selenoproteins involved in the regulation of energy metabolism, as well as in anti-inflammatory, anti-apoptotic, and anti-ferroptotic responses, require precise spatial and temporal regulation. Innovative research on the medical applications and therapeutic potential of selenoproteins is expected to continue. In the near future, it is anticipated that selenoproteins will undoubtedly be utilized in the treatment of various pathologies, including cancer, diabetes mellitus, neurodegenerative disorders, and cardiovascular injuries.

## Footnotes

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## REFERENCES

1. Elhodaky M, Diamond AM. Selenium-binding protein 1 in human health and disease. *Int J Mol Sci.* 2018;19(11):3437. doi: 10.3390/ijms19113437
2. Chaudière J. Biological and catalytic properties of selenoproteins. *Int J Mol Sci.* 2023;24(12):10109. doi: 10.3390/ijms241210109
3. DeAngelo SL, Györfy B, Koutmos M, Shah YM. Selenoproteins and tRNA-Sec: regulators of cancer redox homeostasis. *Trends Cancer.* 2023;9(12):1006-1018. doi: 10.1016/j.trecan.2023.08.003
4. Nicholson JL, Toh P, Alfulajj N, Berry MJ, Torres DJ. New insights on selenoproteins and neuronal function. *Free Radic Biol Med.* 2022;190:55-61. doi: 10.1016/j.freeradbiomed.2022.07.021
5. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. *Physiol Rev.* 2014;94(3):739-777. doi: 10.1152/physrev.00039.2013
6. Laugwitz L, Buchert R, Olguín P, Estiar MA, Atanasova M, Marsden B, Enssle J, Avilés J, Gutiérrez AG, Candia N, Fabiano M, Morlot S, Peralta S, Groh A, Schillinger C, Kuehn C, Sofan L, Sturm M, Bilal M, Bender B, Tomaselli PJ. EEFSEC deficiency: A selenopathy with early-onset neurodegeneration. *Am J Hum Genet.* 2025;112(1):168-180. doi: 10.1016/j.ajhg.2024.12.001

7. Dogaru CB, Muscurel C, Duță C, Stoian I. "Alphabet" selenoproteins: Their characteristics and physiological roles. *Int J Mol Sci.* 2023;24(21):15992. doi: 10.3390/ijms242115992
8. Bellinger FP, Raman AV, Reeves MA, Berry MJ. Regulation and function of selenoproteins in human disease. *Biochem J.* 2009;422(1):11-22. doi: 10.1042/BJ20090219
9. Al-Mubarak AA, van der Meer P, Bomer N. Selenium, selenoproteins, and heart failure: Current knowledge and future perspective. *Curr Heart Fail Rep.* 2021;18(3):122-131. doi: 10.1007/s11897-021-00511-4
10. Prasad B, Akanksha A, Kaur PS, Gupta S. Understanding selenoproteins: Structural insights, biological functions and transformative applications in therapeutics. *Process Biochem.* 2025;150:148-160. doi: 10.1016/j.procbio.2024.12.028
11. Ye R, Huang J, Wang Z, Chen Y, Dong Y. The role and mechanism of essential selenoproteins for homeostasis. *Antioxidants (Basel).* 2022;11(5):973. doi:10.3390/antiox11050973

# Evaluation of Factors Affecting the Prediction of Risk Factors for Obesity-Related Fatty Liver Disease in Children

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## Abstract

**Objectives:** Childhood fatty liver disease (FLD) has become a major problem due to the dramatic increase in the incidence of obesity. In our country, studies on the factors affecting the risk for FLD are insufficient. In this single-center, retrospective and cross-sectional study, we aimed to evaluate the role of parameters affecting the prediction of the risk of FLD in obese children.

**Materials and Methods:** Ninety-one obese children (48 males, 43 females) aged 12.19±2.88 years were included in the study. Body weight (BW), height, left arm mid-circumference, triceps skinfold thickness (TSF), waist circumference (WC), and hip circumference (HC) were measured. Body mass index (BMI), WC/HC ratio, and WC-to-height ratio were calculated. Obesity was defined as BMI at the 95<sup>th</sup> percentile and above according to age and gender. FLD was diagnosed using ultrasonography.

**Results:** FLD was present in 49.5% of obese children. In obese children with FLD, BW, height, BMI, TSF, TSF- standard deviation scores, WC, and HC measurements were found to be significantly higher than in obese children without FLD ( $p<0.05$ ). In the logistic regression analysis, homeostasis model assessment of insulin resistance (HOMA-IR) score, TSF, and gender were determined as independent variables for FLD, with  $p$ -values and confidence intervals (CIs) identified as follows: for HOMA-IR score ( $p=0.001$ , B: 1.927, 95% CI: 1.323-2.807), for TSF ( $p=0.010$ , B: 1.090, 95% CI: 1.021-1.163), and for gender ( $p=0.008$ , B: 0.215, 95% CI: 0.070-0.666).

**Conclusion:** According to the results of our study, HOMA-IR was identified as the most important factor playing a role in the development and progression of FLD. Male sex is a risk factor for steatosis. Although BMI and WC are the most commonly used measurements, TSF can also be easy indicator for detecting the presence of obesity-related FLD in children.

**Keywords:** Obesity, Non-alcoholic Fatty Liver Disease, Child, Sex Factors, Insulin Resistance, Skinfold Thickness

## INTRODUCTION

Mandatory school closures and protective restrictions during the Coronavirus Disease 2019 pandemic resulted in a considerable drop in physical activity, as well as changes in nutrition and sleep patterns, which raised the rate of obesity in children. Childhood fatty liver disease (FLD), a component of obesity-associated metabolic syndrome, is the most common liver disease characterized by fat accumulation in hepatocytes.<sup>1</sup> The prevalence of FLD was found to be 7.8% in the general population and 34.2% in obese individuals in meta-analysis.<sup>2</sup> In our country, it has been reported that the prevalence of FLD is 23-62% in obese children.<sup>3,4</sup> Although FLD has a benign

prognosis, approximately 3-5% of patients develop non-alcoholic steatohepatitis, which progresses to end-stage liver disease or hepatocellular carcinoma. Therefore, early diagnosis of FLD is crucial for prompt intervention, especially in obese children. Abdominal ultrasonography (USG) and liver function tests are recommended to determine the presence of FLD for all obese children over the age of three.<sup>5</sup> The results of the studies highlighted that some anthropometric indices can be used to predict FLD. However, this issue is still controversial. In this single-center, retrospective, and cross-sectional study, we aimed to evaluate the role of parameters affecting the prediction of the risk of FLD in obese children.



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## MATERIALS AND METHODS

A total of 91 children aged 7-18 years who applied to Eskişehir Osmangazi University Faculty of Medicine, Child Nutrition and Metabolism Outpatient Clinic between January 2020 and February 2021 with complaints of overweight participated in our study. Exogenous obese patients with body mass index (BMI) at or above the 95<sup>th</sup> percentile according to age and gender were included in the study. Those with known systemic or metabolic problems, those using medications that could affect body weight (BW), those with genetic syndromes, and those for whom archive information could not be accessed were excluded from the study. Clinical, anthropometric and biochemical parameters, and hepatobiliary USG data of the patients were recorded retrospectively from the hospital registration system.

Patients' BW, height, left arm mid-circumference (LEMC), triceps skinfold thickness (TSF), waist circumference (WC), and hip circumference (HC) measurements were taken by the same pediatric nurse. With the BMI weight (kg)/height (m<sup>2</sup>) formula, percentile and standard deviation scores (SDSs) of BMI and height were calculated according to national growth charts. LEMC was measured using a non-stretchable tape measure passing through the middle of the distance between the olecranon and acromion, while TSF was measured using a skinfold caliper at the midpoint of the left arm, through a double fold of skin. SDS values for LEMC and TSF were evaluated according to Centers for Disease Control and Prevention references.

The measurement of WC was carried out by marking the point where the lowest rib and midaxillary line intersect the iliac crest on the right side while the child was standing. WC percentile values were calculated according to the percentile values developed by Hatipoglu et al.<sup>6</sup> HC was measured at the widest part of the gluteal area. The WC-to-height ratio (WC/height) ratio was calculated by dividing WC by height. The cut-off point of the WC/height ratio was taken as 0.57. The cut-off point for the WC/HC ratio was determined as  $\geq 0.90$ .<sup>7</sup> According to the USG results, the degrees of steatosis were grouped as none, Grade I, II, and III. Obese patients included in the study were grouped as those with steatosis detected in liver USG (FLD) and those without steatosis (non-FLD).

Patients' age, gender, BW, height, BW for height (BWH), BMI, serum glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), free T4 (fT4), thyroid stimulating hormone, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels, were recorded. The diagnosis of dyslipidemia was made according to the criteria of the TG, TC, and LDL-C levels being at or above the 95<sup>th</sup> percentile or HDL-C levels being below the 5<sup>th</sup> percentile, by comparing

with reference values according to age and gender.<sup>8</sup> The formula for insulin resistance (IR) was calculated using the homeostasis model assessment of IR (HOMA-IR) score:  $\text{glucose} \times \text{insulin} / 405$ .

Demographic, anthropometric, clinical and biochemical parameters were analyzed and compared between the FLD and non-FLD groups.

### Ethical Statements

The study was approved by Eskişehir Osmangazi University Faculty of Medicine Ethics Committee (approval number: 2018-54, dated: 21.09.2018). The study was performed in accordance with the ethical rules based on the principles of the Helsinki Declaration. Written informed consent forms were obtained from the parents and the children (when appropriate).

### Statistical Analysis

IBM SPSS 21 package program was used for analysis of the data. The distribution of the data was analyzed with the Kolmogorov-Smirnov test. For normally distributed variables, differences between groups were compared using the Independent Samples t-test. The Mann-Whitney U test was used for those not showing normal distribution. Logistic regression models were used to predict the presence of FLD using anthropometric parameters. Sensitivity and specificity of anthropometric indices in the suspicion of FLD were calculated by receiver operating characteristic (ROC) curve analysis. Results obtained with  $p < 0.05$  were considered significant.

## RESULTS

Of the 91 exogenously obese patients who participated in the study, 43 were females and 48 were males. The mean age of the patients was  $12.19 \pm 2.88$  years ( $12.81 \pm 3.06$  in females;  $11.63 \pm 2.62$  in males). According to hepatobiliary USG results, 49.5% of the children were found to have FLD (58.3% of the males and 39.5% of the females) (Table 1). The difference between the two genders was not significant ( $p = 0.075$ ). Of those with FLD, 35 had Grade I, 19 had Grade II, and 1 had Grade III hepatosteatorosis. In children with FLD, BW, height, BMI, TSF, TSF-SDS, WC, and HC measurements were statistically significantly higher than in children without FLD (Table 1). WC was above the 97<sup>th</sup> percentile in 88 children and between the 95<sup>th</sup> and 97<sup>th</sup> percentiles in three. The WC/height ratio was  $\geq 0.5$  in all children, and the WC/HC ratio was  $\geq 0.9$  in 52 children. No significant difference was found between those with and without FLD in terms of WC/height ratio and WC/HC ratio ( $p > 0.05$ ).

Dyslipidemia was detected in 34.1% of all patients and in 18 of 45 patients with FLD. No statistically significant difference was found in terms of dyslipidemia between those with and without FLD ( $p = 0.237$ ). ALT, AST, insulin, TG, and HOMA-IR scores were

found to be statistically significantly higher in patients with FLD (Table 2).

With the logistic regression model, HOMA-IR score, TSF, and gender were determined as independent variables for FLD (Table 3). For the detection of FLD, the threshold values determined for TG, TSF, and HOMA-IR were 126 mg/dL, 26.5 mm and 3.35, respectively. HOMA-IR had the highest diagnostic

accuracy with 64.4% sensitivity and 65.2% specificity. For TSF and TG, 62.2% sensitivity and 63% specificity were found (Table 4, Figure 1).

## DISCUSSION

In our study, the rate of FLD was found to be 49.5% (58.3% of males and 39.5% of females). In various studies, the prevalence

**Table 1. Baseline characteristics of children with and without FLD**

Variables	Children with FLD	Children without FLD	p-value
Gender	17 female/28 male	26 female/20 male	0.075*
Weight (kg)	78.55±22.95	66.03±19.40	0.006*
Weight (SD score)	2.95±1.10	2.51±1.06	0.055*
Height (cm)	158.78±14.60	151.42±13.92	0.016*
Height (SD score)	0.94±1.04	0.68±1.34	0.30*
BMI (kg/m <sup>2</sup> )	30.49±5.35	28.05±4.19	0.017*
BMI (SD score)	2.57±0.64	2.36±0.58	0.104*
Mid-upper arm circumference (cm)	32.24±5.16	30.47±4.23	0.078*
Mid-upper arm circumference (SD score)	1.34±0.51	3.21±1.32	0.946**
TSF (cm)	30.20±8.32	25.03±7.85	0.003*
TSF (SD score)	2.02±0.74	2.84±7.74	0.031**
WC	98.44±12.45	90.78±11.76	0.003*
HC	108.25±14.58	101.80±13.41	0.031*
WC/height	0.62±0.057	0.59±0.050	0.062*
WC-to-hip ratio	0.91±0.067	0.89±0.066	0.179*

p<0.05 is statistically significant. \*: Independent Samples t-test, \*\*: Mann-Whitney U test, WC/height: Waist circumference to height ratio, FLD: Fatty liver disease, SD: Standard deviation, BMI: Body mass index, TSF: Triceps skinfold thickness, WC: Waist circumference, HC: Hip circumference

**Table 2. Laboratory values of children with and without FLD**

	Total (n=91)	Children with FLD (n=45)	Children without FLD (n=46)	
Serum levels	Mean ± SD	Mean ± SD/median (IQR)	Mean ± SD/median (IQR)	p-value
Glucose (mg/dL)	83.7±6.55	83.64±7.00	83.76±6.16	0.722*
Insulin (uIU/mL)	19.86±13.73	21.10 (15.70)	13.75 (8.75)	0.00**
ALT (U/L)	23.49±13.91	23.0 (21.5)	16.5 (9.0)	0.002**
AST (U/L)	22.39±8.76	23.0 (8.5)	18.5 (6.5)	0.029**
TC (mg/dL)	161.83±34.11	164.28±33.50	159.43±34.89	0.5*
LDL cholesterol (mg/dL)	105.23±32.13	106.3±30.65	104.18±33.82	0.756*
HDL cholesterol (mg/dL)	44.83±10.29	43.06±8.78	46.46±11.41	0.106*
TG (mg/dL)	138.87±77.662	161.89±87.05	116.36±60.04	0.005*
TSH (uIU/mL)	7.15±37.8	2.63 (1.71)	2.54 (1.16)	0.301**
fT4 (ng/dL)	2.89±15.49	1.17 (0.26)	1.26 (0.27)	0.316**
HOMA-IR score	4.14±3.29	4.1 (3.1)	2.8 (1.58)	0.00**

p<0.05 is statistically significant. \*: Independent Samples t-test, \*\*: Mann-Whitney U test, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TC: Total cholesterol, LDL: Low-density lipoproteins, HDL: High-density lipoproteins, TG: Triglyceride, TSH: Thyroid stimulating hormone, HOMA-IR: Homeostasis model assessment-estimated insulin resistance, FLD: Fatty liver disease, SD: Standard deviation, IQR: Interquartile range, fT4: Free T4

**Table 3. Independent variables in logistic regression analysis**

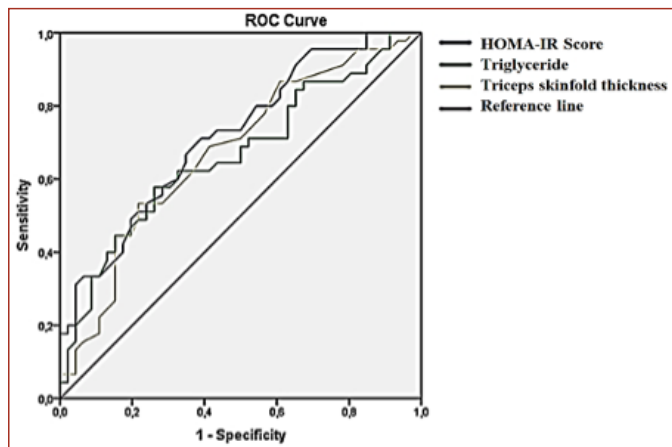
Variables	B parameter equation	Standard error	p-value	Exp. (B) OR (95% CI lower-upper)
Gender	-1.536	0.576	0.008*	0.215 (0.070-0.666)
TSF	0.086	0.033	0.010*	1.090 (1.1021-1.163)
HOMA-IR score	0.656	0.192	0.001*	1.927 (1.323-2.807)
Constant	-4.113	1.183	0.001*	0.016

p<0.05 is statistically significant. \*: Logistic regression, OR: Odds ratio, Exp.: Exponentiated, TSF: Triceps skinfold thickness, HOMA-IR: Homeostasis model assessment of insulin resistance, CI: Confidence interval

**Table 4. Factors effective in estimating FLD with ROC curve**

Risk factors	AUC (OR 95%)	Cut-off	Sensitivity (%)	Specificity (%)	p-value
TG	0.665 (0.553-0.777)	126	62.22	63	0.007
TSF	0.675 (0.564-0.786)	26.5	62.2	63	0.004
HOMA-IR score	0.719 (0.616-0.822)	3.35	64.4	65.2	0.000

p<0.05 is statistically significant. ROC: Receiver operating characteristic, FLD: Fatty liver disease, AUC: Area under the curve, OR: Odds ratio, TG: Triglyceride, TSF: Triceps skinfold thickness, HOMA-IR: Homeostasis model assessment-estimated insulin resistance



**Figure 1. Receiver operating characteristic curve**

HOMA-IR: Homeostasis model assessment of insulin resistance, ROC: Receiver operating characteristic

of FLD among obese children was found to be 12-80%.<sup>9,10</sup> In the study of Özhan et al.,<sup>11</sup> FLD was detected in 60.8% of 332 obese children, while Kirel et al.<sup>3</sup> found it to be 40%.

There are not enough data examining the place of anthropometric measurements in determining the risk factors for FLD in children and adolescents in our country. Commonly used, BMI and WC are strongly associated with FLD in both children and adults. The WC/height ratio has been proposed as an alternative measure that accounts for both longitudinal growth and central adiposity.<sup>7,12</sup> Skinfold thickness measurement, especially TSF, is frequently used to evaluate subcutaneous fat tissue, one of the most important storage areas of body fat.

In our study, BW, height, BMI, TSF, TSF-SDS, WC, and HC measurements were significantly higher in obese children with FLD compared to obese children without FLD. Various studies suggest the use of parameters such as WC, WC/HC ratio, WC/height ratio, and IR to identify obese children at high-risk of FLD.<sup>13</sup> Maffei et al.<sup>14</sup> suggested that the WC/height ratio is an important parameter in predicting FLD. Lin et al.,<sup>15</sup> on the other hand, emphasized the importance of WC in predicting FLD in obese children.

In a study conducted in China with 7229 students, WC/height ratio was reported to be a significant independent risk factor for FLD.<sup>16</sup> In our study, consistent with the literature, WC was above the 97<sup>th</sup> percentile in 96.7% of children and between the 95<sup>th</sup>-97<sup>th</sup> percentile in 3.3% of children. The WC/height ratio was found to be 0.5 and above in all children, and the WC/HC ratio was found to be 0.9 and above in 57.1% of the children. Contrary to the literature, no significant difference was found between those with and without FLD in terms of WC/height ratio and WC/HC ratio. On the other hand, HOMA-IR score, TSF, and gender were determined as independent variables for FLD with the logistic regression model. IR is an important component of the metabolic syndrome and contributes to the pathophysiology of FLD. In our patients with FLD, the HOMA-IR score was statistically significantly higher, and the threshold value determined by the ROC curve was 3.35. Accordingly, the IR rate was 50.54%. IR was identified as the most important factor in the development and progression of FLD, with an odds ratio (OR) of 1.927 and a 95% confidence interval (CI) of 1.323-2.807. TSF was found to be the second most significant factor and an independent

predictor of the presence of FLD, ( $p=0.010$ ,  $OR= 0.090$ , 95% CI: 1.1021-1.163). The threshold value for the detection of FLD was determined as 26.5 mm (for TSF).  $TSF >26.5$  mm was detected in 52.4% of our patients. It has been observed that subcutaneous fat accumulation in the extremity regions of the body is as important as central fat accumulation.

In our study, dyslipidemia was detected in 34.1% of all patients and in 40% of 45 patients with FLD. However, no significant difference was found in terms of dyslipidemia between those with and without FLD. Hypertriglyceridemia is a well-known risk factor for FLD. In our study, only TG levels among lipid parameters were found to be significantly higher in patients with FLD. Another independent risk factor for FLD was found to be sex. Özhan et al.<sup>11</sup> found the prevalence of FLD to be significantly higher in pubertal children, with a prevalence of 67.8% in males compared to 55.0% in females. In our study, similar to the literature, FLD was detected in 58.3% of males and 39.5% of females.

The use of hepatic USG to assess hepatosteatosis is not the most reliable method. The proton density fat fraction, measured by magnetic resonance imaging (MRI-PDFF), provides an accurate, validated marker of hepatic steatosis. The lack of MRI-PDFF measurements is one of the most important limitations of our study. Another limitation of our study is the number of patients. Further studies are needed to increase the power by including more patients in the study.

## CONCLUSION

HOMA-IR was identified as the most important factor playing a role in the development and progression of FLD. Male gender is a risk factor for steatosis. Although BMI and WC are the most commonly used anthropometric measurements, TSF measurement can also be a simple and easy indicator for detecting the presence of obesity-related FLD in children. More studies are needed before it can be used in mass screening.

## Ethics

**Ethics Committee Approval:** The study was approved by Eskişehir Osmangazi University Faculty of Medicine Ethics Committee (approval number: 2018-54, dated: 21.09.2018).

**Informed Consent:** Written informed consent forms were obtained from the parents and the children (when appropriate).

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: G.K.Y., Concept: G.K.Y., Design: G.K.Y., Data Collection or Processing: G.K.Y., A.T.Ç., Analysis or Interpretation: G.K.Y., A.T.Ç., Literature Search: G.K.Y., A.T.Ç., Writing: G.K.Y.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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## REFERENCES

1. Lavine JE, Schwimmer JB. Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis.* 2004;8(3):549-558. doi: 10.1016/j.cld.2004.02.003
2. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PLoS One.* 2015;10(11):e0140908. doi: 10.1371/journal.pone.0140908
3. Kirel B, Şimşek E, Tokar RT, Çolak E. Nonalcoholic fatty liver diseases in obese children and adolescents. *Turk Arch Ped.* 2012;47(3):172-178. doi: 10.4274/tpa.847
4. Gokce S, Atbinici Z, Aycan Z, Çınar HG, Zorlu P. The relationship between pediatric non-alcoholic fatty liver disease and cardiovascular risk factors and increased risk of atherosclerosis in obese children. *Pediatr Cardiol.* 2013;34(3):308-315. doi: 10.1007/s00246-012-0553-x
5. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, Mouzaki M, Sathya P, Schwimmer JB, Sundaram SS, Xanthakos SA. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr.* 2017;64(3):319-334. doi: 10.1097/MPG.0000000000001482
6. Hatipoglu N, Ozturk A, Mazicioglu MM, Kurtoglu S, Seyhan S, Lokoglu F. Waist circumference percentiles for 7- to 17-year-old Turkish children and adolescents. *Eur J Pediatr.* 2008;167(4):383-389. doi: 10.1007/s00431-007-0630-2
7. McCarthy HD, Ashwell M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message: "Keep your waist circumference to less than half your height". *Arch Dis Child.* 2006;30(12):988-992. doi: 10.1136/adc.2005.081696
8. Kliegman RM, St. Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM, editors. *Nelson Textbook of Pediatrics.* 21<sup>st</sup> ed. Philadelphia: Elsevier; 2019.
9. Papandreou D, Rousso I, Mavromichalis I. Update on non-alcoholic fatty liver disease in children. *Clin Nutr.* 2007;26(3):409-415. doi: 10.1016/j.clnu.2007.02.008
10. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics.* 2006;118(4):1388-1393. doi: 10.1542/peds.2006-0902
11. Özhan B, Ersoy B, Özkol M, Kiremitci S, Ergin A. Waist to height ratio: A simple screening tool for nonalcoholic fatty liver disease in obese children. *Turk J Pediatr.* 2016;58(5):518-523. doi: 10.24953/turkjpeds.2016.04.009

12. Hu F. Measurements of adiposity and body composition. New York, NY: Springer; 2008. p. 1-498.
13. Zhang HX, Xu XQ, Fu JF, Lai C, Chen XF. Predicting hepatic steatosis and liver fat content in obese children based on biochemical parameters and anthropometry. *Pediatr Obes*. 2015;10(2):112-117. doi: 10.1111/ijpo.12029
14. Maffei C, Banzato C, Rigotti F, Nobili V, Valandro S, Manfredi R, Morandi A. Biochemical parameters and anthropometry predict NAFLD in obese children. *J Pediatr Gastroenterol Nutr*. 2011;53(5):590-593. doi: 10.1097/MPG.0b013e31822213ff
15. Lin YC, Chang PF, Yeh SJ, Liu K, Chen HC. Risk factors for liver steatosis in obese children and adolescents. *Pediatr Neonatol*. 2010;51(3):149-154. doi: 10.1016/S1875-9572(10)60003-4
16. Zhang X, Wan Y, Zhang S, Lu L, Chen Z, Liu H, Jiang X, Luo K, Cai W. Nonalcoholic fatty liver disease prevalence in urban school-aged children and adolescents from the Yangtze River delta region: A cross-sectional study. *Asia Pac J Clin Nutr*. 2015;24(2):281-288. doi: 10.6133/apjcn.2015.24.2.13

# Biochemical-Clinical Characteristics of Biotin and Possible Evolutionary Insights on the Place of Its in the Origin of life and the Metabolic Organization

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## Abstract

Biotin is a universal molecule that is essential for the metabolism of all life forms. Biotin acts as a coenzyme for carboxylases, playing a crucial role in various metabolic pathways including gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Beyond these functions, biotin also influences gene expression regulation.

The essential role of biotin for life extends back to the beginning of life, and even prebiotic metabolic organizations. This molecule has remained largely unchanged throughout the history of life, indicating its evolutionary conservation. The diversity and conservation of biotin-dependent enzymes highlight their significance in metabolic flexibility and adaptation.

**Keywords:** Biological Evolution, Biotin, Carboxylases, Coenzymes, “Metabolism, Inborn Errors”, Vitamins

## INTRODUCTION

### Biochemical-Clinical Characteristics of Biotin

#### Chemical Properties of Biotin

Biotin is a water-soluble vitamin and a universal molecule required for the metabolism of all living organisms. It is also referred to as vitamin H, vitamin B7, or coenzyme R. Biotin remains stable at room temperature and is not broken down by cooking.<sup>1</sup> The term “biotin” comes from the Ancient Greek words “biotos” (meaning “life”) and “-in” (derived from a common suffix used in chemistry).

Chemically, biotin is a prosthetic group consisting of a valerate side chain attached to a bicyclic ring made up of a ureido and a thiophane ring (Figure 1). The molecule is a combination of a tetrahydroimidazolone ring and a tetrahydrothiophane

ring containing an organosulfur group bearing a valeric acid substituent. Biotin is covalently linked to the protein through a lysine residue.<sup>2</sup>

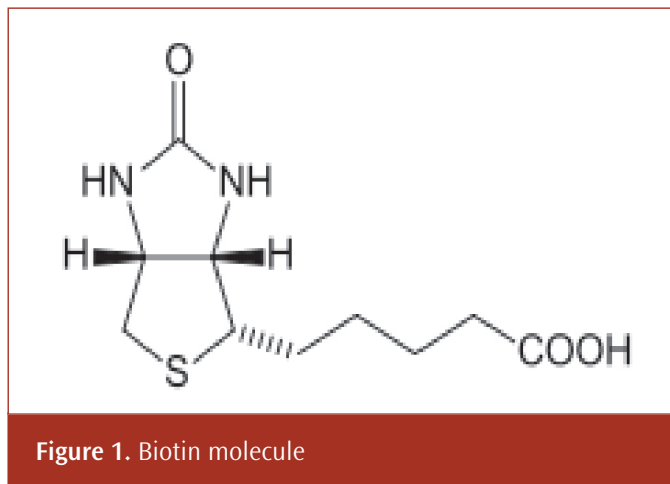


Figure 1. Biotin molecule



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## History of the Discovery of Biotin

The discovery of the biotin molecule and research on its properties are detailed in Table 1.<sup>3</sup>

## Biotin Physiology and Metabolism

Unlike bacteria and plants, higher mammals cannot synthesize biotin endogenously, so they must obtain it from the diet.<sup>4</sup> Biotin taken from foods in the diet is found in free form or bound to proteins. Absorption of biotin from the diet is quite effective, even when taken in large amounts. Therefore, its deficiency is quite rare.<sup>5</sup> Biotin taken with food is optimally utilized through a cyclical system known as the biotin cycle.<sup>6</sup> In this cycle, biotin is used in metabolic pathways and then returned to the circulation to be reused (Figure 2). When biotin is consumed with food, it is broken down into biocytin (biotinyl- $\epsilon$ -lysine) with the assistance of proteolytic enzymes in the digestive tract. In pancreatic and intestinal secretions, biotinidase (BTD) located on the brush borders of the apical surface of enterocytes releases biotin.

With the assistance of the sodium-dependent multivitamin transporter (SMVT) enzyme, free biotin is absorbed from the apical surface of enterocytes as an electroneutral substance.<sup>1</sup> It then enters circulation as an electrogenic solution from the basolateral surface, independently of sodium. Because SMVT is highly sensitive to pantothenic acid and lipoic acid, intake of multivitamins may competitively affect biotin uptake. However, an important way animals acquire biotin is through synthesis by bacteria found in the intestinal microbiota.<sup>7</sup> Unfortunately, the microbial synthesis in the intestines is not sufficient to meet metabolic needs due to the lack of necessary transport proteins.

The normal gut flora and normal intestinal production of biotin in an untreated individual with profound BTD deficiency mean that the affected individual will develop secondary biotin deficiency and multiple carboxylase deficiency.<sup>8</sup> If the biotin produced in the flora were adequate, these individuals would likely not become symptomatic. This fact confirms that biotin synthesized by bacteria in the human intestines is insufficient to meet the requirements in these cases.

Biotin that enters the circulation is then transported to the liver and other peripheral tissue cells with the assistance of SMVT. Reabsorption of biotin from the kidney glomeruli is also facilitated by SMVT. Biotin binds to holocarboxylases, which activate carboxylases in the cell and enable their function. Biotin is subsequently catabolized through two degradation pathways: biotin beta, involving the breakdown of the valeric acid side chain and sulfur oxidation, resulting in the formation of bisnorbiotin, biotin sulfoxide, and other metabolites, which are then excreted in the urine. The remaining biotin is reabsorbed from the kidney glomeruli via SMVT and re-enters the biotin cycle.<sup>9</sup>

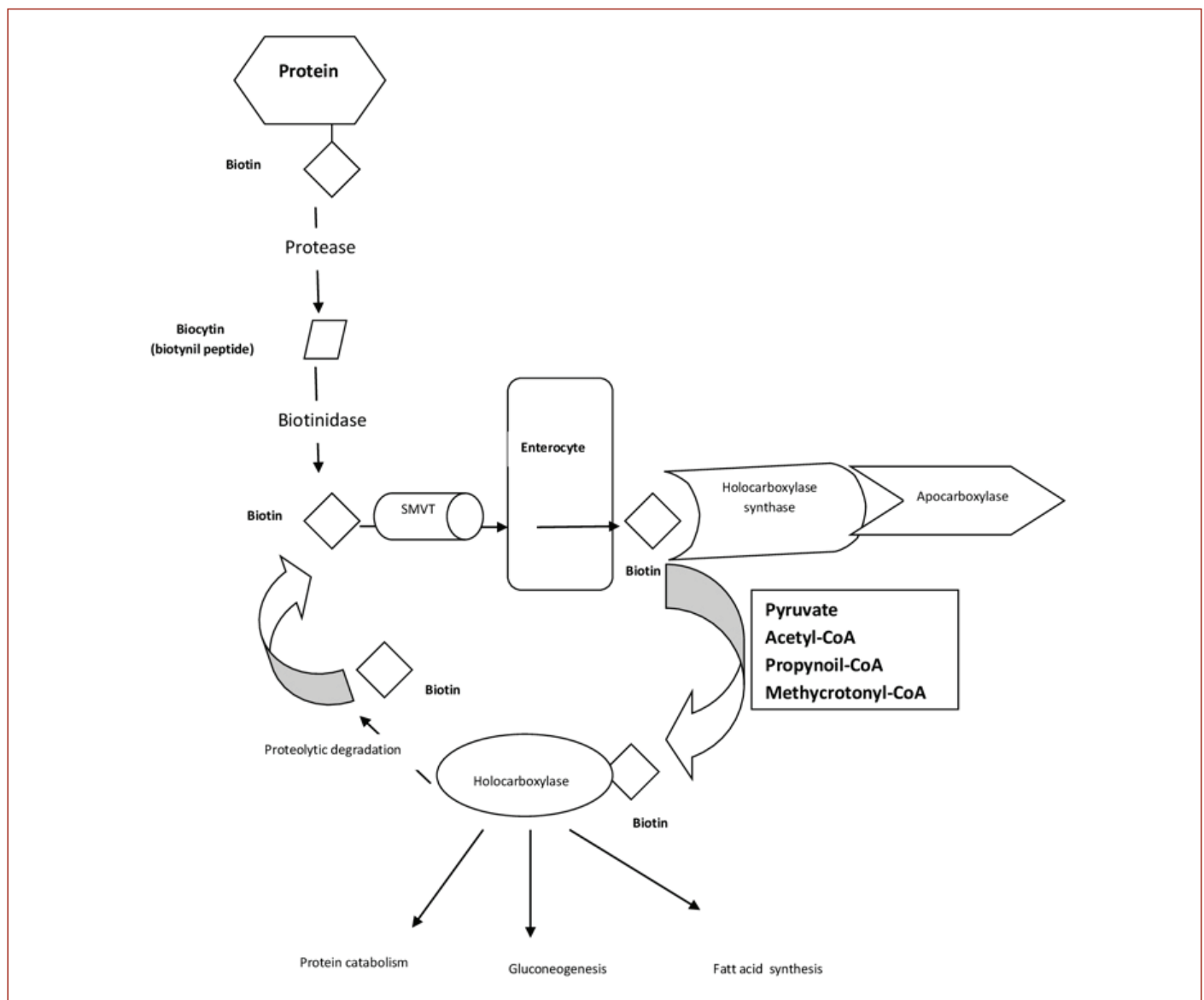
## Biotin's Biological Functions

Biotin serves two roles: as a coenzyme and as a non-coenzyme. Its primary function is to act as a carboxyl carrier in carboxylation reactions. Biotin's main role is to supply CO<sub>2</sub> to carboxylase enzymes, which are found universally in all three branches of life.<sup>10</sup> Carboxylase, decarboxylase, and transcarboxylase enzymes, crucial in various metabolic processes such as branched-chain amino acid catabolism, gluconeogenesis, and fatty acid synthesis, require biotin for their activities. Mammals have four biotin-dependent carboxylases: acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and  $\beta$ -methylcrotonyl-CoA carboxylase.<sup>4</sup> Biotin serves as the universal coenzyme for these enzymes. Biotin serves as a coenzyme responsible for transferring bicarbonate to acetyl-CoA, which is then converted to malonyl-CoA for fatty acid synthesis. Pyruvate carboxylase plays a role in gluconeogenesis, while  $\beta$ -methylcrotonyl-CoA carboxylase catalyzes a step in leucine metabolism. Additionally, propionyl-CoA carboxylase catalyzes a step in gluconeogenesis.

Evidence is accumulating on the important role of biotin as a non-coenzyme. It also plays a role in epigenetic mechanisms, such as biotinylation of histone proteins, stability of nuclear chromatin, and gene expression.<sup>11</sup> Additionally, biotin is effective in cell signaling. More than 2000 biotin-dependent genes have been identified, and there is evidence that bisnorbiotin and biotin catabolites affect gene expression.<sup>12</sup> Research has shown that biotin causes a six-fold downregulation of phosphoenol pyruvate carboxykinase expression.<sup>13</sup>

**Table 1. History of the discovery of biotin**

1898	Discovery of toxicity of raw egg whites (Steinitz)
1916	A diet rich in raw egg whites causes toxic symptoms in dogs, cats, rabbits and humans (WG Bateman)
1927	Egg white damage. Neurotoxicity, alopecia, dermatitis in rats fed only egg whites (M. Boas and H. Parsons)
1936	Isolation of crystallized biotin. Named yeast growth factor (Fritz Kögl and Benno Tönnis)
1939	Discovery of factor responsible for egg white damage. Named vitamin H (P. Gygory, representing Haar und Haut) Named coenzyme R in experiments with yeast and <i>Rhizobium trifolii</i> (West Wilson)
1940	The name biotin was used for the first time. It was discovered that the damage was due to the binding of biotin to avidin (Gygory)
1950	Discovery that biotin plays a role in carboxyl transfer
1968	The synthesis pathways for biotin in bacteria ( <i>Escherichia coli</i> ) were discovered



**Figure 2.** Biotin cycle

SMVT: Sodium-dependent multivitamin transporter

Based on these findings, studies have been conducted on the treatment of diabetic rats with biotin. It has been reported that biotin affects ornithine transcarbamylase activity, and its deficiency, biotin deficiency, is accompanied by hyperammonemia.<sup>14</sup>

### Biotin in the Diet

Biotin is widely found in foods, with absorption levels varying. Offal contains high levels of biotin, while grains contain less. Daily intake recommendations differ based on age, with 5 µg/day recommended for newborns and 35-70 µg/day for adults. No toxicity from nutritional deficiencies or excessive biotin intake has been reported.<sup>15</sup>

Certain conditions can impact daily biotin requirements. These include pregnancy, long-term total parenteral nutrition, severe and prolonged malnutrition, high egg white consumption, chronic alcohol use, use of antiepileptics, use of multiple vitamins containing lipoic acid, gastrectomy, and achlorhydria.<sup>16</sup>

There are many causes of biotin deficiency. It can occur in inborn errors of metabolism, which are rare, including BTD deficiency, carboxylase deficiencies, holocarboxylase synthetase deficiencies, and carrier protein deficiencies.<sup>17</sup> BTD deficiency is a common metabolic disease globally, leading to a decrease in enzyme activity, which results in multiple carboxylase deficiency, resulting in inadequate biotin recycling and utilization.

## Clinical Finding in Biotin Deficiency

The most severe symptoms, including hypotonia, convulsions, developmental delays, ataxia, coma, and hearing loss, can manifest in biotin deficiency.<sup>18</sup> Dermatological symptoms may include alopecia, rashes, periorificial dermatitis, conjunctivitis, thin and brittle hair, and brittle nails. Immunodeficiency symptoms may involve susceptibility to candida infections, low lymphocyte subgroups, and impaired antibody synthesis.<sup>19</sup>

Biotin deficiency may also be linked to lipid metabolism disorders, resulting in high levels of single-chain fatty acids. Additionally, there have been reports suggesting that biotin deficiency could be teratogenic. During pregnancy, biotin deficiency may lead to conditions such as cleft palate-lip, micrognathia, and micromelia.<sup>17</sup>

## Laboratory Findings in Biotin Deficiency

Biotin deficiency can result in keto-lactic acidosis, hyperammonemia, and organic aciduria.<sup>19</sup>

## Diagnosis in Biotin Deficiency

Serum and urine biotin levels are not reliable for diagnosis. Urinary 3-hydroxy isovaleric acid excretion can be helpful for diagnosing metabolic disorders. It is important to note that biotin can interfere with laboratory tests, particularly those using biotin-streptavidin technology for measuring thyroid hormones and vitamin D levels.<sup>20,21</sup>

## Use of Biotin in Diseases

Biotin may be used for experimental purposes or in certain proven cases. Some studies suggest that high doses, such as 300 µg/day, may impact the progression of multiple sclerosis.<sup>22</sup> However, many subsequent studies have shown that biotin has no effect on multiple sclerosis.<sup>23,24</sup> In animal studies, biotin has been shown to increase insulin secretion in diabetic mice.<sup>24</sup> It is also recommended for improving hoof health in animals and hair health and nail health in humans.<sup>25,26</sup>

## Future Perspectives for Biotin-Related Research

Research on fundamental biomolecules such as “coenzymes” and especially “biotin” will lead to promising developments in the future, both in terms of evolutionary biology and in scientific/applied fields.

## Evolutionary Importance

Evolutionary conservation of cofactors: Coenzymes [nicotinamide (NAD)<sup>+</sup>, flavin adenine dinucleotide (FAD), coenzyme A, biotin, etc.] have been used since the earliest stages of life. For example, biotin is essential for the functioning of carboxylase enzymes. The preservation of such molecules from common ancestors to

the present demonstrates that they are among the “universal constants” of biochemical evolution.<sup>27</sup>

Evolution of metabolic pathways: Coenzymes like biotin are involved in key points in carbon metabolism (e.g., gluconeogenesis, fatty acid biosynthesis). Metabolic innovations that increase energy efficiency throughout evolution have often depended on these coenzymes.<sup>2</sup>

Future perspective: New environmental pressures (climate change, artificial feeding environments, space life) will test the metabolic flexibility of organisms. The bioavailability and synthesis of essential coenzymes like biotin may be critical to the adaptive success of species in the future.

## Importance in Scientific and Applied Fields

Biotechnology: Biotin is a unique tool for labeling biomolecules through the “avidin-biotin” system. This system may be more widely used in nanotechnological biosensors, drug delivery systems, and sensitive diagnostic kits in the future.<sup>28</sup> In metabolic engineering, biotin-dependent enzymes can be manipulated to construct artificial biosynthetic pathways.

Medicine and nutrition: The role of biotin deficiency in the nervous system, metabolism, and epigenetics is increasingly being investigated. Its links to neurodegenerative diseases, diabetes, and obesity may become particularly important.<sup>1</sup>

The role of coenzymes (such as NAD<sup>+</sup> and sirtuins) in epigenetic regulation has been demonstrated. Biotin is also known to affect gene expression through histone biotinylation. This could be a target for future epigenetic therapies.<sup>29</sup>

Space biology: Metabolism will be subjected to different stresses during long-term space travel. The stability, synthesis, and recycling of coenzymes may become critical in life support systems.

## Future Scenarios

Synthetic biology: Biotin and similar coenzymes can be used as “modular metabolism tools” in artificial or genetically engineered organisms.

Evolutionary engineering: During laboratory evolution of organisms, selection can be applied to coenzyme metabolism to impart new biochemical properties.<sup>30</sup>

New treatments: Biotin and other coenzymes may be central to both drug development and personalized nutrition approaches in the future.<sup>31</sup>

In the future, biotin may be used as a mitochondrial function supporter or neuroprotective agent.<sup>32</sup>

Plant biotechnology: Biotin is critical for photosynthesis and carbon fixation. Genetic engineering can enhance biotin metabolism to develop drought-resistant, productive crops.<sup>33</sup>

Livestock: Biotin deficiency impairs coat/skin health. In the future, highly bioavailable feed additives may improve both animal welfare and productivity.<sup>34</sup>

Functional foods: Biotin-fortified foods (especially in older adults and metabolic risk groups) may become widespread.<sup>35</sup>

To summarize, coenzymes have a remarkably stable evolutionary role as the “common chemical language” of life. Biotin, in particular, appears poised to remain a strategic molecule in both the evolution of metabolic networks and in biotechnological and medical applications in the near, medium, and long-term.

### **Possible Evolutionary Insights on the Place of Its in the Origin of Life and the Metabolic Organization**

Biotin is a universal coenzyme with an evolutionary origin that dates back to the emergence of life and even before.<sup>36</sup> The role of coenzymes and cofactors in the origin of life which act as catalysts in biochemical reactions, has been largely overlooked until now. Considering the initial conditions of the Earth, the role of coenzymes in the evolution of inorganic molecules into organic molecules, specifically in abiogenesis, is of critical importance. Coenzymes have played a significant role in the evolution of metabolic pathways by extending from proto-metabolism to prebiotic metabolism, and from there to biotic metabolism.<sup>37</sup> The suggestion that amino acid biosynthesis cannot occur without coenzymes and cofactors. Coenzymes emerged on Earth before the evolution of the Last Universal Common Ancestor (LUCA). To gain a comprehensive understanding of the evolution of biotin as a coenzyme, it is necessary to consider the geological evolution of the Earth, the emergence of metabolism, and the emergence of the cell. The emergence of coenzymes, including biotin, is interconnected with the evolution of amino acids, proteins, nucleic acids, metabolism, and the cell, indicating a coordinated evolution.

### **Evolutionary Insights on Origin of Life and the Metabolic Organization**

#### **Geological Chronology**

The observable universe dates back approximately 13.7 billion years. The Earth began to form approximately 4.6 billion years ago with the accumulation of masses that accreted from the solar nebula.<sup>38</sup>

At its beginning, the Earth was quite different from today. There was no atmosphere or water yet. Intense meteor bombardment

and tectonic activity were common. Active volcanic activities, involving magma and lava, produced the electrical energy required for life to emerge through non-enzymatic means. It is suggested that water was carried to Earth by continuous asteroidal bombardment. During this time, Earth was a very hot planet, so the water was in the form of water vapor. Over time, the atmosphere and oceans began to form as a result of changes in the composition of gases. Initially, the atmosphere and oceans were a toxic environment rich in H<sub>2</sub>, N, C, Fe, S, NH<sub>3</sub>, and CH<sub>4</sub>. The atmosphere was primarily rich in N, but over time, O<sub>2</sub> levels increased while N levels decreased. The cycles of N, O<sub>2</sub>, and C formed the biosphere.<sup>39,40</sup>

The continents had not yet formed, and the land mass was a single piece called Pangea. The Moon was formed by the merging of pieces that broke off from Earth when a planet called Theia collided with Earth about 50 million years after Earth's formation.<sup>41,42</sup>

#### **Chronology of Life**

The first proto-cells on Earth evolved approximately 1 billion years after the formation of the Earth, around 3.5-3.6 billion years ago.<sup>43</sup> According to the widely accepted view, the first cells emerged at the base of hydrothermal vents on the ocean floor. There are debates surrounding the definition of life. One of the most well-known definitions of life is as follows: Life is any self-sustaining chemical system capable of Darwinian evolution. Life involves the possession of dynamic information about organized relationships between material entities (Table 2).

Initially, cells emerged as a result of the coordinated development of life characteristics, including compartmentalization (membrane), metabolism, and self-replication (genetics), during millions of years of evolution. Life is an intermediate stage that has gained the ability to renew itself; this is brought about by the chemical kinetic selection resulting from reduction-oxidation (redox) reactions during the global chemical cycle between the most common elements C, H, O, N, S, and Fe found in nature after the formation of the world. Life emerged at each level through the natural evolution of compounds that underwent chemical selection due to the potential for further reproduction, with each simple reaction or event cycle forming a product that activates itself, leading to increasingly complex structures each time.<sup>43</sup>

The question of how life emerged from inorganic molecules under the initial conditions of the Earth, remains a subject of lively scientific interest. There are several significant hypotheses on the subject, including the RNA hypothesis, metabolism hypothesis, and coenzyme hypothesis.<sup>44</sup>

<b>Table 2. Evolution of metabolism and life based on Earth geological periods</b>		
<b>Geological evolution</b>	<b>Evolution of metabolism</b>	<b>Evolution of life</b>
	<b>Hadean period (4.6-3.8 billion years ago)</b>	
<p>Intense meteor bombardment and tectonic activity, lava, magmas</p> <p>Formation of reducing atmosphere</p> <ul style="list-style-type: none"> <li>• H<sub>2</sub>, CO<sub>2</sub>, CH<sub>3</sub>, SO<sub>2</sub>, NH<sub>3</sub></li> </ul> <p>Formation of hydrothermal vents in oceans</p> <ul style="list-style-type: none"> <li>• C, CH<sub>4</sub>, S, NO, Fe</li> </ul>	<p>Prebiotic-protobiotic-abiogenic metabolism</p> <p>C, H, N, S, Fe, cycles (O<sub>2</sub> is very low)</p> <p>Non-enzymatic, anaerobic metabolism</p> <p>Inorganic chemical reactions</p> <p>Primordial soup</p> <p>Chemical reaction rate is very slow</p> <p>Energy source (sun, lightning, ultraviolet, volcanic lava)</p>	<p>Inorganic molecules</p> <p>Coenzyme/cofactors (biotin, RNA, DNA, Ribozyme)</p> <p>Coacervates</p> <p>Chemical synthesis of amino acids</p> <p>RNA world hypothesis/gene first</p> <p>Metabolism hypothesis/metabolism first</p> <p>Coenzyme hypothesis</p>
	<b>Archaean period 3.8-2.5 billion years ago</b>	
<p>In the atmosphere</p> <ul style="list-style-type: none"> <li>• CH<sub>4</sub>, NH<sub>3</sub>, toxic gases, H<sub>2</sub>, CO<sub>2</sub>, CH<sub>3</sub>, SO<sub>2</sub></li> </ul> <p>In the oceans</p> <ul style="list-style-type: none"> <li>• C, Fe, S, NO</li> </ul>	<p>Cellular-biogenic metabolism</p> <p>Anaerobic photosynthesis</p> <p>Chemoautotroph-photoautotroph</p> <p>Autotroph metabolism (capable of creating its energy from inorganic molecules)</p> <p>Heterotroph metabolism (capable of synthesizing its energy from organic structures)</p> <p>Metabolic synthesis of organic molecules aa</p> <p>Emergence of complex metabolic pathways</p> <p>Very fast (discovery of enzymes)</p> <p>Energy source (electrochemical forces-enzyme)</p>	<p>Protocells - evolution of cells</p> <p>Cell membrane</p> <p>Enzymes</p> <p>Evolution of archaea and bacteria</p> <p>Chemical autotrophs (in the ocean)</p> <p>Photosynthetic cyanobacteria (O<sub>2</sub> in the atmosphere)</p> <p>Evolution of the cell Last Universal Common Ancestor 2</p>
	<b>Proterozoic era 2.5 billion-541 million years ago</b>	
<p>Great oxygenation event</p> <p>Increase in O<sub>2</sub> in the atmosphere, decrease in N</p> <p>Decrease in Fe, S, N in the ocean</p>	<p>Anaerobic photosynthesis</p> <p>Chemoautotroph</p> <p>Photoautotroph</p> <p>Nitrogenase, formation of nitrogen cycles</p> <p>Endosymbiosis</p> <p>Interconnection of complex metabolic pathways</p> <p>Krebs cycle</p> <p>Urea cycle</p>	<p>Evolution of eukaryotes</p> <p>Evolution of multicellular life</p> <p>Colonial life</p> <p>Early animals (hydras, sponges)</p> <p>Bilateral animals</p> <p>Chordalians</p> <p>Vertebrates</p>
	<b>Paleozoic era 541-252 million years ago</b>	
<p>Cambrian</p> <p>Ordovician</p> <p>Silurian</p> <p>Devonian</p> <p>Carboniferous</p> <p>Permian</p>		<p>Cambrian explosion</p> <p>Increased biodiversity in vertebrates</p> <p>Plants emerging onto land</p> <p>Tetrapods emerging onto land</p> <p>Evolution of the amniotic egg</p>
	<b>Mesozoic era 252-66 million years ago</b>	
<p>Triassic</p> <p>Jurassic</p> <p>Cretaceous</p>		<p>The emergence of the first mammals</p> <p>dinosaurs</p> <p>The emergence of flowering plants</p>
	<b>Cenozoic era 66 million years ago - present</b>	
<p>Paleogene</p> <p>Neogene</p> <p>Quaternary</p>		<p>Evolution of primates</p> <p>Hominins</p> <p>Hominoids</p> <p>Homo sapiens</p>

### RNA/Gene First Hypothesis

The RNA/Gene First Hypothesis suggests that self-replicating components emerged before metabolism. In the 1960s, Cairn Smith proposed the “clay life model,” which posited that clay may have been the first genetic material capable of replicating itself through crystallization. In this model, clay-based life materials could have utilized organic materials in the environment to create genes.<sup>45</sup>

Another popular model within the gene first approach is the RNA world hypothesis, first introduced by Walter Gilbert. This hypothesis suggests that RNA and DNA appeared before proteins. Initially, RNA molecules had structures that allowed for self-replication, information storage, and enzymatic properties. These RNA structures with enzyme-like properties are known as ribozymes. Evolutionarily, RNA is considered the ancestral molecule of DNA.<sup>46</sup>

Ribose, a sugar found in RNA, is more unstable compared to deoxyribose, a sugar found in DNA that emerged later and is more stable. The first evolved precursor RNA molecule was tRNA. Coenzymes and cofactors, such as biotin, were more commonly associated with RNA rather than proteins.<sup>47</sup>

### Metabolism-First Hypothesis

According to the metabolism first hypothesis, the Earth's conditions initially hosted a series of chemical reactions that created molecules that would eventually give rise to life. The hypothesis was first proposed in 1924 by Russian scientist Alexander I. Oparin, known as the “coacervate theory”.<sup>48</sup> This theory suggested that iron carbides chemically reacted with water vapor, leading to the formation of hydrocarbons, alcohols, aldehydes, and other organic chemicals. These compounds then combined with  $\text{NH}_3$  to form amides, amines, and other  $\text{NH}_3$  compounds, creating colloidal structures known as coacervates, which are protein precursors.

Independent of Oparin, the renowned British scientist JBS Haldane named the environment, where he proposed life began on Earth, the “primordial soup” in 1929. Inspired by these theories, scientists Stanley Miller and Harold Urey demonstrated in 1953 that amino acids could be synthesized from biomolecules by simulating the early Earth conditions, sparking further interest in the hypothesis.<sup>49</sup> The experiment replicated the electrically charged environment with spark discharges by heating a glass bottle containing a mixture of water, methane, hydrogen, and ammonia, and continuously stimulating it with electric sparks.

In the 1980s, Gunter Wächtershäuser reinforced the credibility of the metabolism hypothesis by proposing the “surface metabolism theory. Wächtershäuser demonstrated that protein precursor structures could self-replicate through autotrophic mechanisms on electrically charged iron sulfide mineral surfaces, such as

pyrite, without the need for a cell membrane. This theory suggested that porous rock surfaces in hydrothermal vents in the ocean functioned as protocells without a membrane.<sup>50</sup>

Today, the theory of abiogenesis, the formation of amino acids and proteins from inorganic molecules under Earth's conditions, has been extensively detailed. It is now widely accepted that life did not emerge all at once through a transcendent abstract force but evolved through biochemical processes over billions of years, transforming chemistry into biology.<sup>51</sup>

### Coenzyme Hypothesis

The coenzyme hypothesis suggests that life originated with the help of molecules like biotin, pyridoxal phosphate, thiamine pyrophosphate, adenosine triphosphate (ATP), AGP, siroheme, ferredoxin, ferro sulphure, NAD, FAD, pterin etc. (Figure 3). These molecules are similar to coenzymes and are also simple, catalytically active, self-replicating, and capable of facilitating chemical reactions. They were believed to have settled on the surface of oil droplets in water, altering the surface properties, and allowing for self-replication.<sup>37</sup> The evolution of early systems relied on the collaboration of numerous self-replicating molecules and the development of self-organization among them. Without these molecules, the emergence of organic molecules such as coacervates, amino acids, and proteins would not have been possible.

### The Evolution of Metabolism

Metabolism is a network of processes that repeat and sustain themselves by regulating the flow of matter and energy that gives life to molecular structures. Current metabolic pathways are not simply the sum of their parts. Parts cannot exist independently of the process that creates the whole, as the whole and the parts are the product of a dialectical process of interdependence. Like all other biological phenomena, the current location and state of metabolism depend not only on its current formation, but also on a past that gives rise to different possibilities for the present and future interactions of its parts. As the famous evolutionist Theodosius Dobzhansky said, “Without the light of evolution, nothing in biology has meaning.” No matter how complex metabolic pathways may seem, when the uninterrupted chain where each is connected to the previous is followed, it will be seen that self-organization is at work. The driving force required for the evolution of complex metabolic pathways can be found in the second law of thermodynamics. All systems far from equilibrium are necessarily pushed towards more complex structures as long as there is a flow of energy and substrate (dynamic-kinetic stability), formulated by Nobel Prize-winning scientist Ilya Prigogine, who was inspired by this law.

The origin of metabolism can be traced in two stages: the prebiotic period, when cells had not yet emerged, and the post-cellular biotic period, (Figure 4).

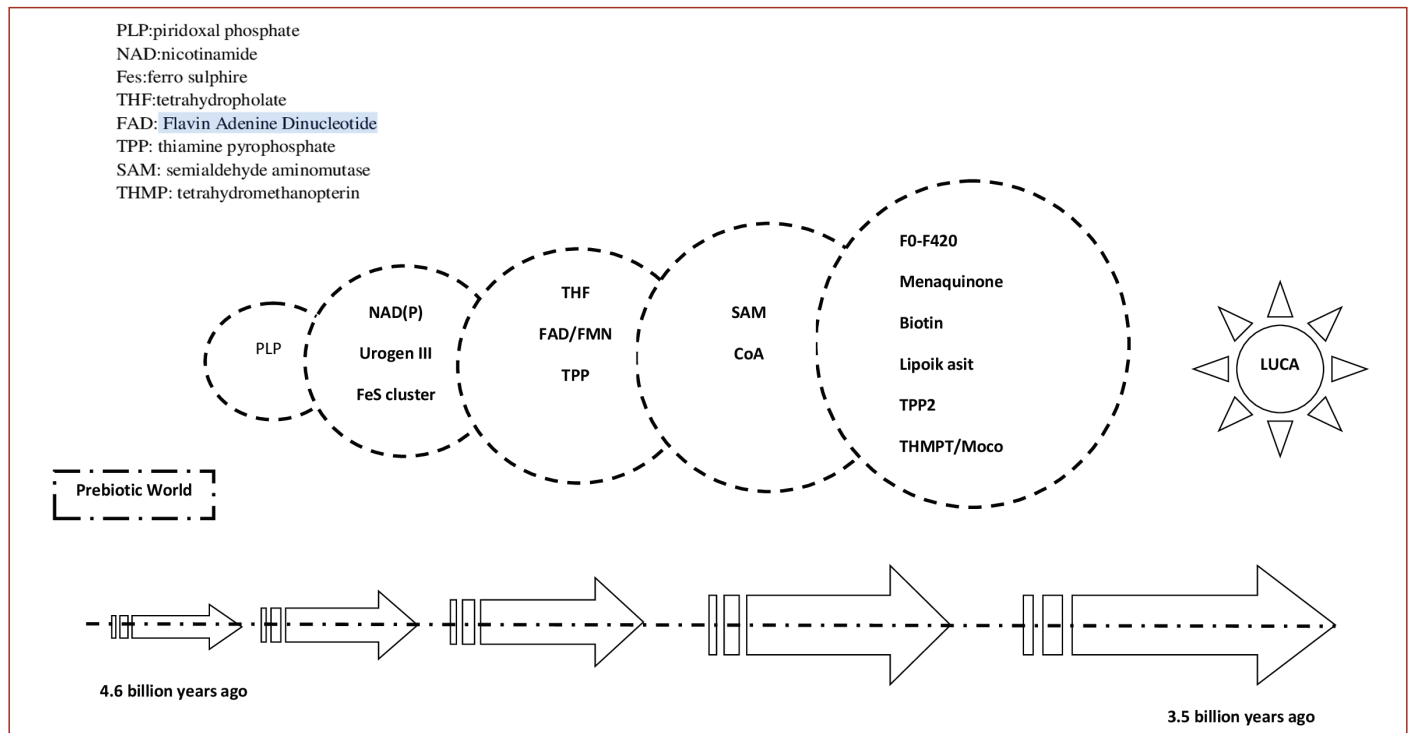


Figure 3. Evolution of coenzymes/cofactors

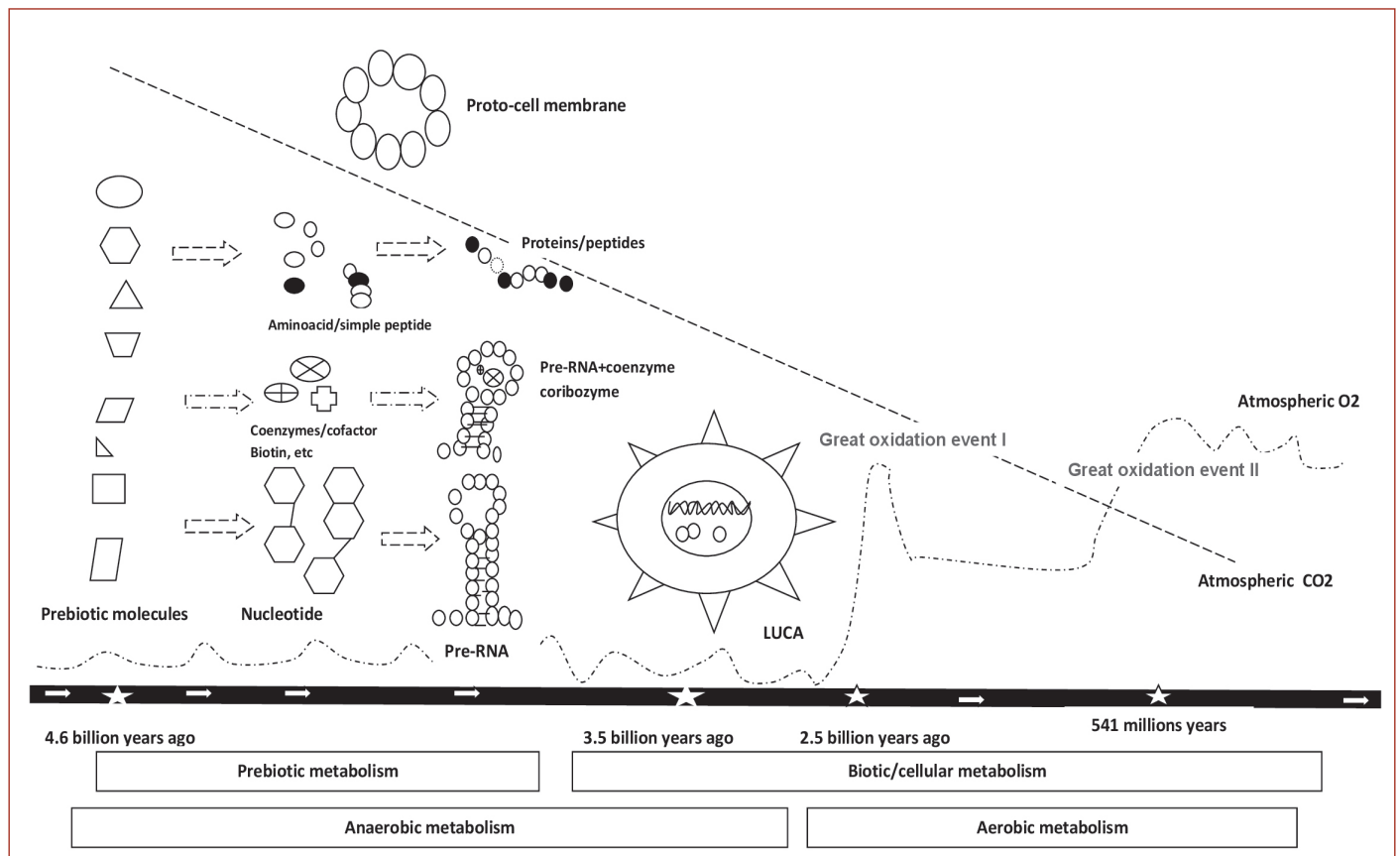


Figure 4. Evolution of life and metabolism

LUCA: Last universal common ancestor

### Prebiotic, Proto-Biotic, Abiotic Metabolism (4.5-3.8 Billion Years Ago)

It was a phase in which organic molecules, derived from inorganic molecules, formed the basis of life, leading to the formation of protocells. This period, known as the Hadean period, was characterized by a reducing atmosphere that eventually gave way to cycles involving carbon, nitrogen, sulfur, iron, and hydrogen.<sup>52</sup> With oxygen not yet dominating the atmosphere, anaerobic metabolism was prevalent. Energy for chemical reactions was derived from sources such as the Sun's ultraviolet rays, lightning, and volcanic lava. Organic molecules were primarily formed at the hydrothermal vents at the ocean floor. Coenzymes, such as amino acids, coacervates, and biotin, were among the substances that emerged during this period, along with precursor molecules for RNA and DNA, whereas NH<sub>3</sub> is not a coenzyme.

### Cellular-Biotic Metabolism (3.8-2.5 Billion Years Ago)

This is the stage when the first cells, LUCA, emerged. Archaea and bacteria evolved. These are cells with autotrophic metabolism that perform anaerobic photosynthesis using Fe and S. Later, cells with heterotrophic metabolism evolved. Thus, cells that can synthesize their energy from organic structures, that is, cells with cellular biotic metabolism, emerged. Complex metabolic pathways were interconnected with each other. During this period, changes in the C, H, O, N, S, P ratios that emerged in the atmosphere and ocean strengthened the evolutionary selection pressures that led to the revelation of cellular heterotrophic metabolism in life forms.<sup>53</sup> Among the pressures that shaped metabolism, the ability to use newly emerged resources (anabolism) and the avoidance of toxic wastes and protection from them were decisive. Metabolism is shaped by the sum of the responses that organisms give to the environment. Therefore, what constitutes metabolism today also requires an understanding of the evolutionary geology that created it.<sup>53,54</sup> The environmental pressures that led to the evolution of cellular metabolism, that is, the transition from autotrophic to heterotrophic metabolism, are as follows:

- Depletion of energy and nutrient source chemicals in the primordial soup.
- Change in the nitrogen cycle (nitrogen crisis). Decrease in reduced nitrogen in the ocean.
- Increase in O<sub>2</sub> in the atmosphere (Great Oxidation Event, GOE). As a result of the pressure to protect from the toxic effects of oxygen and increase the ability to use oxygen, aerobic photosynthetic cells were selected.

- Change in the atmosphere. The need for fixation of atmospheric carbon into organic structures. Here, the evolutionary use of carboxylases and their coenzyme biotin came into play. As a result, the evolution of complex metabolic pathways freed cells from chemical dependence on essential prebiotic molecules.<sup>55</sup>

### Evolution of LUCA

LUCA is a term used to describe the universal cell community that is considered the ancestor of the three main branches in the tree of life: archaea, bacteria, and eukaryotes.<sup>53</sup> It is believed to have evolved approximately between 3.6 and 2.5 billion years ago. Common metabolic pathways found in all cells are thought to have originated from LUCA. Rather than being a single cell, LUCA is considered a community of cells where universally common genetic elements that can be expressed and copied are present. It is believed to have had a genome consisting of 1,000-1,500 ancestral genes.<sup>56</sup> Eukaryotes are thought to have evolved around 2.5 billion years ago as a result of cells with different characteristics combining through endosymbiosis after the increase in atmospheric O<sub>2</sub> (GOE) and the subsequent increase in biodiversity.

### Possible Evolutionary Insights on the Role of Biotin as a Coenzyme in the Origin of Life and Metabolic Organization

#### The Evolutionary Importance of Coenzymes

Coenzymes are organic molecules that play a crucial role in energy conservation by reducing the activation energy needed for biochemical transformations. As a result, they are universal molecules that have been essential in the origin of life and continue to be vital in cellular functions. Coenzymes hold significant evolutionary importance and are believed to be remnants of the prebiotic RNA world, sharing structural similarities with RNA. They are present in all three parallel lineages of molecular and metabolic evolution and can form partnerships with nucleic acids, enzymes, and proteins.<sup>37</sup>

Remarkably, coenzymes have undergone minimal structural changes since the LUCA and have been well-preserved throughout evolution.<sup>37</sup> This preservation allows them to serve as control points in molecular evolution, playing crucial roles in proto-metabolism and current metabolism. Despite their importance, coenzymes are relatively few in number, which limits the scope of molecular evolution.

Genes involved in coenzyme biosynthesis, such as biotin and vitamin C, have been gained and lost in various life forms over time. The evolutionary origins of coenzymes date back even before LUCA, highlighting their critical role in the development and maintenance of life processes.

## The Evolutionary Importance of Biotin

When considering the role of biotin from biochemical and genetic perspectives, this consideration sheds light on the origin and diversity of life. Biotin is a universal molecule that plays an essential role in the evolution of life preserved in the three main branches of life alongside lipoic acid. Biotin has been crucial in the origin of life, serving as a central player in the fixation of biospheric carbon into organic structures and as the key molecule for CO<sub>2</sub> transfer.<sup>2</sup>

Biotinylated enzymes, known as carboxylases, are vital in metabolism across all three branches of life and are dependent on biotin. Evolution is intertwined with biotin, a key coenzyme in basic and intermediate metabolic pathways. Many pathways related to carbon metabolism require biotin, although CO<sub>2</sub> transfer can occur without it. Biotin, along with lipoic acid, evolved relatively late, and both share structural homology.<sup>57</sup>

Before biotin, RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase) played a role in fixing CO<sub>2</sub> from the atmosphere. It is one of the most abundant enzymes on Earth.<sup>58</sup> A mutual and symbiotic relationship has developed between biotin-synthesizing bacteria and plants, highlighting the importance of biotin for metabolic flexibility in most life forms. Species have successfully adapted to this diversity. In addition to its evolutionary roles, biotin also plays a role in gene expression regulation.<sup>10</sup>

Bacteria, plants, and some fungi are capable of synthesizing biotin. A biotin-binding protein binds biotin to carboxylases. This enzyme acts as both a ligase and a transcription factor, indicating an early evolutionary mechanism for regulating biotin metabolism. Mammals, on the other hand, cannot synthesize biotin as they have lost the biosynthesis gene required for it.<sup>59</sup> The biosynthesis of biotin is a costly process, requiring about 19 ATP molecules and at least 6 enzymes to produce just 1 biotin molecule.<sup>2</sup> Therefore, mammals rely on obtaining biotin from their diet and/or intestinal microbiota. This highlights the importance of biotin in symbiotic relationships during evolutionary processes. Acquiring biotin from the environment is more energy-efficient than synthesizing it. The loss of the biosynthesis gene is a well-documented evolutionary phenomenon, driven by energy costs. Genes involved in biosynthesis are often lost when alternative, more cost-effective methods of obtaining essential molecules are available.

There are numerous examples of this evolutionary phenomenon, such as the loss of essential amino acid synthesis genes in eukaryotes with symbiotic life 850-650 million years ago, the loss of the vitamin C synthesis gene after the consumption of plants, 60 million years ago, and the loss of the uricase gene, whereby the pathway evolved for converting fructose from

fruits, into fatty acids for energy gain 25 million years ago.<sup>60-62</sup> Additionally, the loss of 85 genes that facilitated adaptation to aquatic life was observed during the transition from aquatic to terrestrial.<sup>63</sup> The endogenous biosynthesis of vitamin D became less significant with reduced sun exposure following the exodus from Africa 70,000 years ago, making vitamin D essential and it must be obtained externally.<sup>64</sup>

The BTD enzyme is found in everything from prokaryotes to fungi, from arthropods to mammals. The evolution of BTD dates back to the beginning of life. BTD genes in various species have regions containing specific amino acids that are evolutionarily conserved, and these regions show high homology between species.<sup>65</sup>

## Evolution of Biospheric Carbon Fixation

The fixation of inorganic carbon compounds, such as CO<sub>2</sub>, into more reduced organic forms is one of the most fundamental processes of life. This fixation is a prerequisite for life and serves as the starting point of biological evolution, placing the biosphere within geochemistry.<sup>66</sup> Many prokaryotes and all plants have a dominant mechanism, the Calvin-Benson cycle, by which they fix CO<sub>2</sub> into biomass. The diversity in carbon fixation forms the molecular basis of many deep branches on the tree of life, leading to metabolic diversity that traces back to the earliest cells. The evolution and productivity of carbon fixation pathways have also been influenced by changes in oxygen and carbon concentrations throughout geological time.<sup>67</sup>

Acetyl CoA is a critical molecule that plays role in the fixation of atmospheric carbon into organic structures. It serves as the first step in most metabolic pathways, including gluconeogenesis, and acts as the common currency of metabolic processes. Acetyl CoA is the primary building block of carbon-based life forms, marking the beginning of the carbon-based backbone of life.<sup>55</sup> Acetotogens and methanogens utilize acetyl CoA for H<sub>2</sub>-dependent carbon and energy metabolism, forming acetate through the autotrophic fixation of CO<sub>2</sub>.<sup>68</sup>

## Evolution of Biotin-Dependent Carboxylases

Carboxylases are present in all three main branches of life and can be traced back to the LUCA. They exhibit high homology across different species. Biotin-dependent carboxylases have been known to exist since the early stages of life, particularly for pyruvate carboxylation and acetyl CoA carboxylation, highlighting the crucial role of biotin in metabolic processes.<sup>69</sup> Despite the diversity of various life forms, their fundamental functions have remained consistent. They are essential components of biological evolution and metabolism, playing a key role in incorporating atmospheric carbon into organic structures during the evolutionary process. Due to their involvement in CO<sub>2</sub> transport, they are vital in all intermediate stages of metabolism.<sup>70</sup>

The last common ancestor of archaea possessed two biotin-dependent carboxylases, while the last common ancestor of bacteria had three.<sup>71</sup> Eukaryotes likely acquired biotin-dependent carboxylases through symbiotic relationships, such as endosymbioses with mitochondria and plastids, as well as from other unknown bacterial sources. While some bacteria and archaea have evolved the ability to synthesize biotin, organisms like humans must obtain it through their diet, underscoring the evolutionary significance of symbiotic interactions.

It has been proposed that the carboxylase family evolved from small, single-function precursors to generate multifunctional polypeptides through duplication, amplification, and recombination events. Prior to the emergence of carboxylases, RuBisCo played a key role in carbon fixation.<sup>72</sup>

## CONCLUSION

Biotin is a universal molecule that is essential for the metabolism of all life forms. The evolution of biotin provides insight into the origin of life, reaching back to a time before life itself. Biotin acts as a coenzyme for carboxylases, playing a crucial role in various metabolic pathways including gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Beyond these functions, biotin also influences gene expression regulation.

The evolution of biotin dates back to the beginnings of life and even to prebiotic metabolic processes. This molecule has remained largely unchanged throughout the history of life, indicating its evolutionary conservation. The diversity and conservation of biotin-dependent enzymes highlight its significance in metabolic flexibility and adaptation.

For organisms like humans that cannot produce biotin, reliance on intestinal microbiota and dietary sources has emerged as an evolutionary adaptation. Although it is synthesized by bacteria in the gut, mammals have lost the ability to produce biotin internally due to the high cost of biotin biosynthesis and require external sources.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: M.K., Y.K., Concept: M.K., Y.K., Design: M.K., Y.K., Data Collection or Processing: M.K., Y.K., Analysis or Interpretation: M.K., Literature Search: M.K., Y.K., Writing: M.K., Y.K.

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## REFERENCES

- Karachaliou C-E, Livaniou E. Biotin homeostasis and human disorders: recent findings and perspectives. *Int J Mol Sci.* 2024;25(12):6578. doi: 10.3390/ijms25126578
- Sirithanakorn C, Cronan JE. Biotin, a universal and essential cofactor: synthesis, ligation and regulation. *FEMS Microbiol Rev.* 2021;45(4):fuab003. doi: 10.1093/femsre/fuab003
- Lanska DJ. The discovery of niacin, biotin, and pantothenic acid. *Ann Nutr Metab.* 2012;61(3):246-253. doi: 10.1159/000343115
- Tong L. Structure and function of biotin-dependent carboxylases. *Cell Mol Life Sci.* 2013;70(5):863-891. doi: 10.1007/s00018-012-1096-0
- Solvik BS, Strand TA. Biotin: a scoping review for Nordic Nutrition Recommendations 2023. *Food Nutr Res.* 2024;68:10256. doi: 10.29219/fnr.v68.10256
- Heard GS, Grier RE, Weiner DL, McVoy JRS, Wolf B. Biotinidase—A possible mechanism for the recycling of biotin. *Ann N Y Acad Sci.* 1985;447:400. doi: 10.1111/j.1749-6632.1985.tb18458.x
- Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Front Nutr.* 2019;6:48. doi: 10.3389/fnut.2019.00048
- Wolf B. Biotinidase deficiency. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. *GeneReviews*<sup>®</sup> [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. Updated 2023 May 25. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1322/>
- Zempleni J, Wijeratne SS, Hassan YI. Biotin. *Biofactors.* 2009;35(1):36-46. doi: 10.1002/biof.8
- Waldrop GL, Holden HM, St Maurice M. The enzymes of biotin-dependent CO<sub>2</sub> metabolism: what structures reveal about their reaction mechanisms. *Protein Sci.* 2012;21(11):1597-1619. doi: 10.1002/pro.2156
- Rodriguez-Melendez R, Zempleni J. Regulation of gene expression by biotin (review). *J Nutr Biochem.* 2003;14(12):680-690. doi: 10.1016/j.jnutbio.2003.07.001
- Zempleni J. Uptake, localization, and noncarboxylase roles of biotin. *Annu Rev Nutr.* 2005;25:175-196. doi: 10.1146/annurev.nutr.25.121304.131724
- Bowman BB, Selhub J, Rosenberg IH. Intestinal absorption of biotin in the rat. *J Nutr.* 1986;116(7):1266-1271. doi: 10.1093/jn/116.7.1266
- Maeda Y, Kawata S, Inui Y, Fukuda K, Igura T, Matsuzawa Y. Biotin deficiency decreases ornithine transcarbamylase activity and mRNA in rat liver. *J Nutr.* 1996;126(1):61-66. doi: 10.1093/jn/126.1.61
- Jungert A, Ellinger S, Watzl B, Richter M, German Nutrition Society (DGE). Revised D-A-CH reference values for the intake of biotin. *Eur J Nutr.* 2022;61(4):1779-1787. Erratum in: *Eur J Nutr.* 2022;61(4):1789-1790. doi: 10.1007/s00394-022-02824-z
- Dasgupta A. Biotin: Pharmacology, pathophysiology, and assessment of biotin status. In: Dasgupta A, editor. *Biotin and Other Interferences in Immunoassays.* Amsterdam: Elsevier; 2019. p. 17-35. doi: 10.1016/B978-0-12-816429-7.00002-2
- Zempleni J, Hassan YI, Wijeratne SS. Biotin and biotinidase deficiency. *Expert Rev Endocrinol Metab.* 2008;3(6):715-724. doi: 10.1586/17446651.3.6.715
- Genc GA, Sivri-Kalkanoglu HS, Dursun A, Aydin HI, Tokatli A, Sennaroglu L, Belgin E, Wolf B, Coşkun T. Audiologic findings in children with biotinidase deficiency in Türkiye. *Int J Pediatr Otorhinolaryngol.* 2007;71(2):333-339.
- Wolf B. Biotinidase deficiency. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. Updated 2023 May 25. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1322/>

20. Mock DM, Stratton SL, Horvath TD, Bogusiewicz A, Matthews NI, Henrich CL, Dawson AM, Spencer HJ, Owen SN, Boysen G, Moran JH. Urinary excretion of 3-hydroxyisovaleric acid and 3-hydroxyisovaleryl carnitine increases in response to a leucine challenge in marginally biotin-deficient humans. *J Nutr*. 2011;141(11):1925-1930. doi: 10.3945/jn.111.146126
21. Gifford JL, Sadrzadeh SMH, Naugler C. Biotin interference: Underrecognized patient safety risk in laboratory testing. *Can Fam Physician*. 2018;64(5):370.
22. Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, Gout O, Lyon-Caen O, Tourbah A. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. *Mult Scler Relat Disord*. 2015;4(2):159-169. doi: 10.1016/j.msard.2015.01.005
23. Couloume L, Barbin L, Leray E, Wiertlewski S, Le Page E, Kerbrat A, Ory S, Le Port D, Edan G, Laplaud DA, Michel L. High-dose biotin in progressive multiple sclerosis: a prospective study of 178 patients in routine clinical practice. *Mult Scler*. 2020;26(14):1898-1906. doi: 10.1177/1352458519894713
24. Birnbaum G, Stulc J. High dose biotin as treatment for progressive multiple sclerosis. *Mult Scler Relat Disord*. 2017;18:141-143. doi: 10.1016/j.msard.2017.09.030
25. Reddi A, DeAngelis B, Frank O, Lasker N, Baker H. Biotin supplementation improves glucose and insulin tolerances in genetically diabetic KK mice. *Life Sci*. 1988;42(13):1323-1330. doi: 10.1016/0024-3205(88)90226-3
26. Floersheim GL. Behandlung brüchiger Fingernägel mit Biotin [Treatment of brittle fingernails with biotin]. *Z Hautkr*. 1989;64(1):41-48. German.
27. Goldman AD, Kacar B. Cofactors are remnants of life's origin and early evolution. *J Mol Evol*. 2021;89(3):127-133. doi: 10.1007/s00239-020-09988-4
28. Wang S, Hossain MZ, Han T, Shinozuka K, Suzuki T, Kuwana A, Kobayashi H. Avidin-Biotin technology in gold nanoparticle-decorated graphene field effect transistors for detection of biotinylated macromolecules with ultrahigh sensitivity and specificity. *ACS Omega*. 2020;5(46):30037-30046. doi: 10.1021/acsomega.0c04429
29. Jing H, Lin H. Sirtuins in epigenetic regulation. *Chem Rev*. 2015;115(6):2350-2375. doi: 10.1021/cr500457h
30. Bracher JM, de Hulster E, Koster CC, van den Broek M, Daran JG, van Maris AJA, Pronk JT. Laboratory evolution of a biotin-requiring *Saccharomyces cerevisiae* strain for full biotin prototrophy and identification of causal mutations. *Appl Environ Microbiol*. 2017;83(16):e00892-17. doi: 10.1128/AEM.00892-17
31. Yoon J, Grinchuk OV, Kannan S, Ang MJY, Li Z, Tay EXY, Lok KZ, Lee BWL, Chuah YH, Chia K, Tirado Magallanes R, Liu C, Zhao H, Hor JH, Lim JJ, Benoukrat T, Toh TB, Chow EK, Kovalik JP, Ching J, Ng SY, Koh MJ, Liu X, Verma CS, Ong DST. A chemical biology approach reveals a dependency of glioblastoma on biotin distribution. *Sci Adv*. 2021;7(36):eabf6033. doi: 10.1126/sciadv.abf6033
32. Almasi S, Jafarzadeh Shirazi MR, Rezvani MR, Ramezani M, Salehi I, Pegah A, Komaki A. The protective effect of biotin supplementation and swimming training on cognitive impairment and mental symptoms in a rat model of Alzheimer's disease: a behavioral, biochemical, and histological study. *Heliyon*. 2024;10(13):e32299. doi: 10.1016/j.heliyon.2024.e32299
33. Wang Y, Wang M, Ye X, Liu H, Takano T, Tsugama D, Liu S, Bu Y. Biotin plays an important role in *Arabidopsis thaliana* seedlings under carbonate stress. *Plant Sci*. 2020;300:110639. doi: 10.1016/j.plantsci.2020.110639
34. Sun ZW, Fan QH, Wang XX, Guo YM, Wang HJ, Dong X. High dietary biotin levels affect the footpad and hock health of broiler chickens reared at different stocking densities and litter conditions. *J Anim Physiol Anim Nutr (Berl)*. 2017;101(3):521-530. doi: 10.1111/jpn.12465
35. Fekete M, Lehoczki A, Kryczyk-Poprawa A, Zábó V, Varga JT, Bálint M, Fazekas-Pongor V, Csíró T, Ržasa-Duran E, Varga P. Functional foods in modern nutrition science: mechanisms, evidence, and public health implications. *Nutrients*. 2025;17(13):2153. doi: 10.3390/nu17132153
36. Kirschning A. On the evolution of coenzyme biosynthesis. *Nat Prod Rep*. 2022;39:2175-2199.
37. Kirschning A. Coenzymes and their role in the evolution of life. *Angew Chem Int Ed Engl*. 2021;60(12):6242-6269. doi: 10.1002/anie.201914786
38. National Academy of Sciences (US). *Science and creationism: a view from the National Academy of Sciences: second edition*. Washington (DC): National Academies Press (US); 1999. The origin of the universe, earth, and life. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK230211/>
39. Klopogge JTT, Hartman H. Clays and the origin of life: the experiments. *Life (Basel)*. 2022;12(2):259. doi: 10.3390/life12020259
40. Chatterjee S, Yadav S. The coevolution of biomolecules and prebiotic information systems in the origin of life: a visualization model for assembling the first gene. *Life (Basel)*. 2022;12(6):834. doi: 10.3390/life12060834
41. Wegener A, Krause R, Thiede J. *Kontinental-Verschiebungen: Originalnotizen und Literatúrauszüge (Continental drift: the original notes and quotations)*. *Berichte zur Polar- und Meeresforschung*. 2005;516:4. Alfred-Wegener-Institut, Bremerhaven.
42. Canup RM. Simulations of a late lunar-forming impact. *Icarus*. 2004;168:433-456. doi: 10.1016/j.icarus.2003.09.028
43. Cooper GM. *The Cell: A Molecular Approach*. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. The Origin and Evolution of Cells. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK9841/>
44. Zimmermann J, Werner E, Sodei S, Moran J. Pinpointing conditions for a metabolic origin of life: underlying mechanisms and the role of coenzymes. *Accounts Chem Res*. 2024;57(20):3032-3043. doi: 10.1021/acs.accounts.4c00423
45. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, Walter P. *Molecular Biology of the Cell*. 4th edition. New York: Garland Science; 2002. The RNA World and the Origins of Life. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK26876/>
46. Sankaran N. The RNA world at thirty: a look back with its author. *J Mol Evol*. 2016;83(5-6):169-175. doi: 10.1007/s00239-016-9767-3
47. Forterre P, Filée J, Myllykallio H. Origin and evolution of DNA and DNA replication machineries. In: *Madame Curie Bioscience Database [Internet]*. Austin (TX): Landes Bioscience; 2000-2013. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK6360/>
48. Anet FA. The place of metabolism in the origin of life. *Curr Opin Chem Biol*. 2004;8(6):654-659. doi: 10.1016/j.cbpa.2004.10.005
49. Schopf JW. Pioneers of origin of life studies—Darwin, Oparin, Haldane, Miller, Oró—and the oldest known records of life. *Life*. 2024;14(10):1345. doi: 10.3390/life14101345.
50. Altair T, Borges LGF, Galante D, Varela H. Experimental approaches for testing the hypothesis of the emergence of life at submarine alkaline vents. *Life*. 2021;11(8):777. doi: 10.3390/life11080777
51. Freeland S. Undefining life's biochemistry: implications for abiogenesis. *J R Soc Interface*. 2022;19(187):20210814. doi: 10.1098/rsif.2021.0814
52. Kitadai N, Maruyama S. Origins of building blocks of life: a review. *Geosci Front*. 2018;9(4):1117-1153. doi: 10.1016/j.gsf.2017.07.007
53. Weiss MC, Preiner M, Xavier JC, Zimorski V, Martin WF. The last universal common ancestor between ancient Earth chemistry and the onset of genetics. *PLoS Genet*. 2018;14(8):e1007518. doi: 10.1371/journal.pgen.1007518
54. Fani R. The origin and evolution of metabolic pathways: why and how did primordial cells construct metabolic routes? *Evo Edu Outreach*. 2012;5:367-381. doi: 10.1007/s12052-012-0439-5

55. Martin WF. Older than genes: the acetyl CoA pathway and origins. *Front Microbiol.* 2020;11:817. doi: 10.3389/fmicb.2020.00817
56. Moody ERR, Álvarez-Carretero S, Mahendrarajah TA, Clark JW, Betts HC, Dombrowski N, Szánthó LL, Boyle RA, Daines S, Chen X, Lane N, Yang Z, Shields GA, Szöllősi GJ, Spang A, Pisani D, Williams TA, Lenton TM, Donoghue PCJ. The nature of the last universal common ancestor and its impact on the early Earth system. *Nat Ecol Evol.* 2024 Sep;8(9):1654-1666. doi: 10.1038/s41559-024-02461-1
57. Tong L. Structure and function of biotin-dependent carboxylases. *Cell Mol Life Sci.* 2013;70(5):863-891. doi: 10.1007/s00018-012-1096-0
58. Erb TJ, Zarzycki J. A short history of RubisCO: the rise and fall (?) of nature's predominant CO<sub>2</sub>-fixing enzyme. *Curr Opin Biotechnol.* 2018;49:100-107. doi: 10.1016/j.copbio.2017.07.017
59. Feng Y, Zhang H, Cronan JE. Profligate biotin synthesis in  $\alpha$ -proteobacteria - a developing or degenerating regulatory system? *Mol Microbiol.* 2013;88(1):77-92. doi: 10.1111/mmi.12170
60. Payne SH, Loomis WF. Retention and loss of amino acid biosynthetic pathways based on analysis of whole-genome sequences. *Eukaryot Cell.* 2006;5(2):272-276. doi: 10.1128/EC.5.2.272-276.2006
61. Hornung TC, Biesalski HK. Glut-1 explains the evolutionary advantage of the loss of endogenous vitamin C synthesis: the electron transfer hypothesis. *Evol Med Public Health.* 2019;2019(1):221-231. doi: 10.1093/emph/eoz024
62. Karaođlan M, Karaođlan M. The evolution of obesity and the origin of adipose tissue. *Obes Med.* 2024;52:100561. doi: 10.1016/j.obmed.2024.100561
63. Huelsmann M, Hecker N, Springer MS, Gatesy J, Sharma V, Hiller M. Genes lost during the transition from land to water in cetaceans highlight genomic changes associated with aquatic adaptations. *Sci Adv.* 2019;5(9):eaaw6671. doi: 10.1126/sciadv.aaw6671
64. James WPT, Johnson RJ, Speakman JR, Wallace DC, Frühbeck G, Iversen PO, Stover PJ. Nutrition and its role in human evolution. *J Intern Med.* 2019;285(5):533-549. doi: 10.1111/joim.12878
65. Wolf B, Jensen K. Evolutionary conservation of biotinidase: implications for the enzyme's structure and subcellular localization. *Mol Genet Metab.* 2005;86(1-2):44-50. doi: 10.1016/j.ymgme.2005.07.011
66. Braakman R, Smith E. The emergence and early evolution of biological carbon-fixation. *PLoS Comput Biol.* 2012;8(4):e1002455. doi: 10.1371/journal.pcbi.1002455
67. Santos Correa S, Schultz J, Lauersen KJ, Soares Rosado A. Natural carbon fixation and advances in synthetic engineering for redesigning and creating new fixation pathways. *J Adv Res.* 2023;47:75-92. doi: 10.1016/j.jare.2022.07.011
68. Lemaire ON, Jespersen M, Wagner T. CO<sub>2</sub>-fixation strategies in energy extremophiles: what can we learn from acetogens? *Front Microbiol.* 2020;11:486. doi: 10.3389/fmicb.2020.00486
69. Toh H, Kondo H, Tanabe T. Molecular evolution of biotin-dependent carboxylases. *Eur J Biochem.* 1993;215(3):687-696. doi: 10.1111/j.1432-1033.1993.tb18080.x
70. Kroth PG. The biodiversity of carbon assimilation. *J Plant Physiol.* 2015;172:76-81. doi: 10.1016/j.jplph.2014.07.021
71. Lombard J, Moreira D. Early evolution of the biotin-dependent carboxylase family. *BMC Evol Biol.* 2011;11:232. doi: 10.1186/1471-2148-11-232
72. Taylor-Kearney LJ, Wang RZ, Shih PM. Evolution and origins of rubisco. *Curr Biol.* 2024;34(16):R764-R767. doi: 10.1016/j.cub.2024.06.024

# Biobanks and Their Contribution to the Field of Rare Diseases: Current Landscape, Challenges, and Future Directions

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## Abstract

Rare diseases (RDs) are conditions affecting fewer than 1 in 2,000 individuals in the general population. Despite their low individual prevalence, their collective impact poses significant challenges to healthcare systems worldwide. Several factors, such as limited patient numbers, fragmented data collection, and high genotypic and phenotypic heterogeneity, contribute to diagnostic delays. These challenges also hinder research and the development of effective therapeutics, leading to significant clinical, economic, and societal burdens

Biobanks, organized collections of biological samples and their associated data, are essential in addressing these challenges. In this review, we explored key aspects of biobanking for RDs, including operational, ethical, and legal considerations. The need for standardized frameworks and the importance of international collaboration through biobanking networks have been discussed. Future directions, including the integration of artificial intelligence, the implementation of dynamic consent models, and the adoption of decentralized data-sharing approaches, have also been highlighted. We also summarized the functions of biobanks in RD research, including their impact on identifying genetic variants, understanding disease mechanisms, discovering diagnostic markers, and creating personalized therapeutic approaches. By storing high-quality biospecimens and data collected in adherence with ethical and legal requirements, biobanks have been transforming the landscape of diagnosis and treatment, ultimately improving patient outcomes and fostering innovation in precision medicine for RDs.

**Keywords:** Biobank, Biobanking, Rare Diseases

## INTRODUCTION

Rare diseases (RD) have the unique characteristic of affecting a limited percentage of the population, typically fewer than 1 in 2,000 individuals in a given region. They are also defined as “orphan diseases”, since they are neglected conditions with little or no funding or research for treatments due to the high cost of developing them for a limited patient population.<sup>1,2</sup> Nonetheless, since there are collectively between 5,000 and 8,000 diagnosable RDs, they have a significant impact on patients, families, and healthcare systems. These diseases pose diagnostic challenges due to their low prevalence and clinical heterogeneity. As a consequence, patients often experience delayed diagnosis and

increased hospitalization. Moreover, these conditions lead to diverse medical, economic, and psychosocial complications.<sup>3-6</sup> A national survey in Türkiye highlighted key challenges, including limited interdisciplinary cooperation, cost-related obstacles to testing, inadequate insurance coverage, and small patient groups, which impact the validity of the studies. Additionally, limited public and private support, as well as low levels of awareness among healthcare providers regarding the conduct of RD research, complicate these challenges.<sup>7</sup>

Biobanks are organized facilities that collect, process, and store biological samples along with associated data.<sup>8</sup> They provide invaluable support in genetic research, biomarker discovery, and



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the development of precision medicine.<sup>9</sup> Furthermore, their role in fostering partnerships and integrating patient perspectives greatly boosts the efficacy of biobanks, making them an essential part of efforts to improve the understanding and treatment of RDs.<sup>10</sup>

In RD research, where each specimen is highly valuable, biobanks address the challenge of small patient cohorts by pooling limited resources, collecting sufficient numbers of high-quality biospecimens, standardizing sample handling through ethically grounded standard operating procedures (SOPs), and enabling further studies.<sup>11,12</sup> Furthermore, state-of-the-art biobanking processes and integration of clinical data with molecular testing and imaging investigations provide robust genotype-phenotype correlations, facilitating the development of individualized therapeutic approaches.

Thus, the creation of biobanks for RDs enables overcoming problems such as limited sample numbers and increased ethical sensitivities. In this review, we will summarize the current literature on biobanks with a particular focus on their contributions to the field of RD research. The current landscape, limitations, and challenges of RD biobanks will be presented, and their contributions to the diagnosis and treatment of patients with RDs will be discussed.

### Current Challenges in RD Research

Each RD affects only a small number of individuals in any geographic area, which creates even more challenges in obtaining valid clinical data, building large patient cohorts, and conducting statistically significant studies. The wide geographic distribution of patients further complicates the multicenter collaborations required for deep phenotyping and identification of disease-specific biomarkers.<sup>13</sup> The main challenge in RD research comes from the diversity of conditions within these small patient populations. Furthermore, the distribution of patients makes the collection of centralized biological specimens and associated data, as well as the coordination of studies, logistically challenging. Heterogeneity in standardizing protocols across different centers leads to inconsistencies in data collection and patient management.<sup>13,14</sup> Moreover, RDs, by their nature spanning all medical disciplines, make it particularly difficult to collect sufficient data for meaningful analysis.<sup>15</sup> Another major obstacle is the lack of specific diagnostic procedures for many RDs. Challenges include limited awareness among healthcare professionals and prolonged patient suffering due to diagnostic delay.<sup>15</sup> RDs often present with a wide range of symptoms and mimic more common conditions, making early and accurate diagnosis even more difficult. Furthermore, heterogeneity in clinical presentation shows that an absolute diagnostic strategy is often inadequate, and personalized diagnostic protocols must be continuously optimized.<sup>14,16</sup> Additionally, progress is hindered

by inadequate funding, combined with the high cost of advanced diagnostic technologies.

Treatment development is also slowed by several barriers, including the lack of investment by pharmaceutical companies due to high costs and low potential returns, the lack of approved treatments for the majority of RDs, and persistent difficulties despite incentives such as the Orphan Drug Act.<sup>16,17</sup> The absence of specialized infrastructure, including state-of-the-art diagnostic machinery, multidisciplinary clinical teams, and focused research networks, results in limitations in offering an integrated treatment strategy.

### What Are Biobanks and How Do They Work?

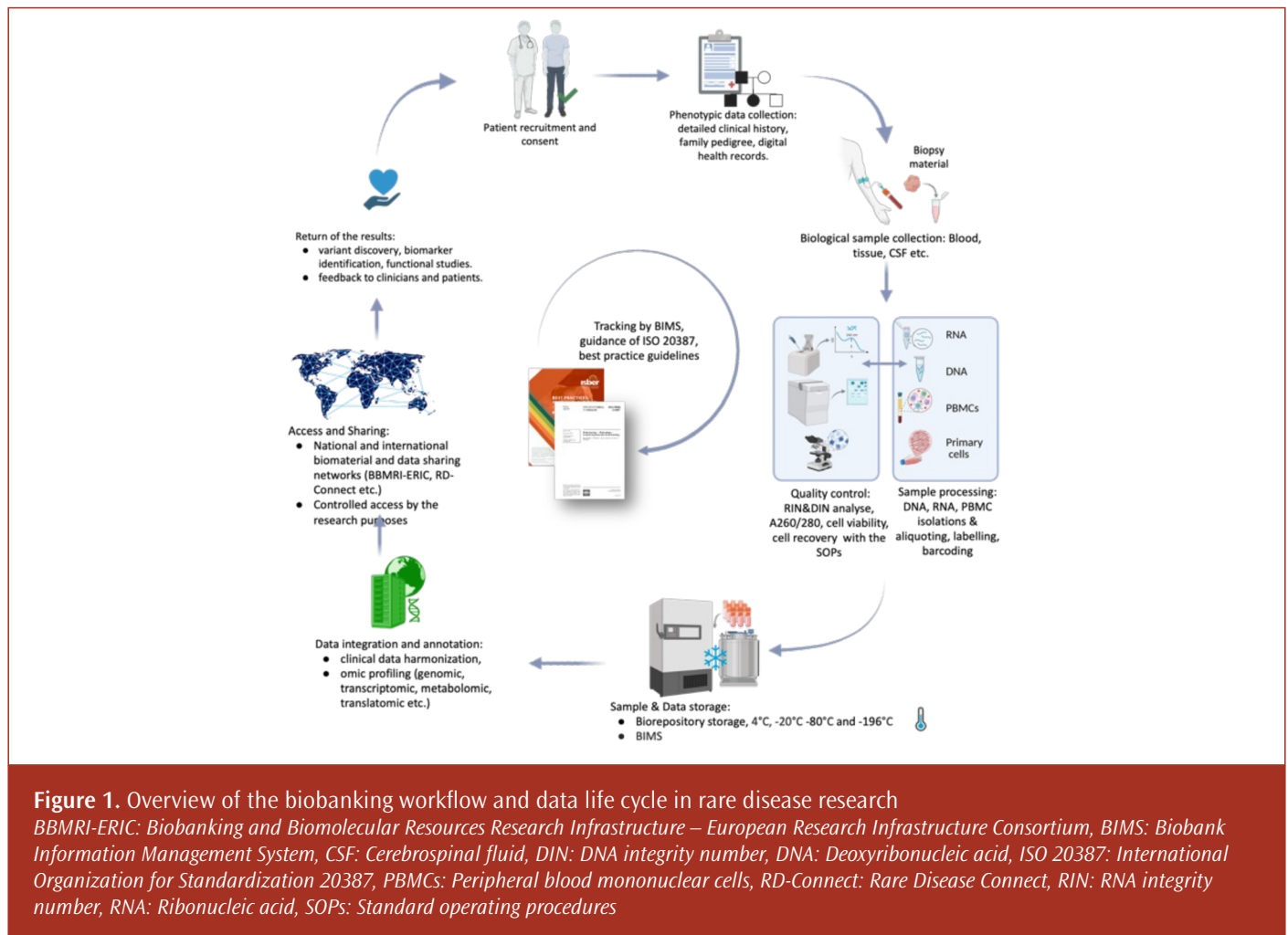
Biobanks are structured storage facilities for biological materials and their related data, constituting significant accumulations of human biological samples, including tissues, blood, cerebrospinal fluid (CSF), breast milk, saliva, urine, and other body fluids. Samples in biobanks are also aligned with health-related and donor-specific details, including medical history, family history, and lifestyle.<sup>18</sup> The earliest examples of “biobanking” emerged in the mid-1990s, as researchers recognized the enormous value in systematically collecting human biological samples for use in future research activities.<sup>11</sup> They have evolved from simple storage warehouses to advanced infrastructures representing a key constituent of modern medical research.<sup>19</sup>

Biobanks are today at the center of translational and clinical research, serving as hub platforms that enable high-throughput investigations to clarify disease pathophysiology and response to treatment. This is achieved by the evolution of biobanks from study-driven sample collections to integrated, well-characterized, high-quality biospecimen collections that ensure sample and data quality, as well as ethical and legal compliance, along with transparent and efficient access procedures.<sup>20</sup>

Disease biobanks are also important for facilitating multicenter and interdisciplinary research, increasing research efficiency and reproducibility, and addressing ethical and legal concerns.

In this context, establishing RD biobanks is vital for developing new treatment strategies and improved diagnostic methods, supporting personalized medicine approaches, and effective preventive strategies and public health policies.<sup>21,22</sup> RD biobanks are also essential for facilitating multicenter and multidisciplinary research, increasing research efficiency and reproducibility, and addressing ethical and legal concerns.<sup>11,12,23</sup>

As defined in Figure 1, the biobanking process begins with patient recruitment and informed consent, followed by the collection of detailed phenotypic data, including clinical history, family pedigree, and digital health records. Informed consent is a cornerstone of biobanking ethics, implemented through



various models, including broad, study-specific, categorical, opt-in, opt-out, and dynamic consent.<sup>24</sup> Since most RDs begin in childhood, involving minors introduces additional ethical concerns, requiring legal guardian consent and re-consent as children reach maturity.<sup>25</sup>

Biological samples (e.g., blood, tissue, CSF) are collected and processed for further studies. Quality control measures (e.g., RIN/DIN analysis, cell viability) are conducted according to SOPs. Samples and associated data are stored in biorepositories at various temperatures and tracked via Biobank Information Management Systems in compliance with with ISO 20387:2018 Biotechnology — Biobanking — General requirements for biobanking standard and biobanking best practice guidelines. Due to the heterogeneous nature of RDs, standardization of samples and data collection, as well as processing, is essential for RD biobanking. Integrated data are annotated and harmonized for downstream analysis, including omics profiling. Finally, controlled access is granted for research purposes through national and international networks (e.g., BBMRI-ERIC, RD-

Connect), and results such as variant discovery and biomarker identification are shared with clinicians and patients.

### Unique Considerations for the RD Biobanks

RD biobanking has several unique features, including operational, ethical, legal, societal, and regulatory aspects.<sup>26</sup> This rarity elevates the scientific importance of each sample, making it valuable and necessitating pre-analytical workflow protocols for collection, processing, storage, and analysis. A multicenter study highlights the critical importance of harmonizing standardized protocols for the collection, processing, and cryopreservation of peripheral blood mononuclear cells across multiple sites in support of large-scale immune phenotyping in neurodevelopmental disorder research.<sup>27</sup>

Equally important, the quality and consistency of biospecimens and associated data are critical in RD research. Harmonized protocols for biospecimen processing and metadata collection are essential to ensure that samples and associated data are both research-ready and comparable across institutions. Consistent

protocols for biospecimen handling, data annotation, and metadata capture ensure high data quality and comparability across institutions. They also facilitate data integration, sharing, and secondary use. This, in turn, maximizes the scientific value of biobank collections in the RD research community. In this context, adherence to the FAIR principles, making data and metadata Findable, Accessible, Interoperable, and Reusable, enhances the utility and longevity of collected resources. It also promotes ethical and effective data sharing, thereby supporting reproducibility and collaboration.<sup>28</sup>

In addition to operational and logistical challenges, ethical issues are another defining feature of RD biobanking. Patients with RDs often face lengthy diagnostic journeys and limited treatment options, and some may take a long time to receive a diagnosis.<sup>1</sup> This may make them more willing to participate in research. However, small patient populations also increase the risk of re-identification, even when anonymization protocols are implemented. For this reason, biobanks implement strict ethical regulations, including informed consent procedures and careful management of data sharing and confidentiality.<sup>29</sup>

Moreover, due to the geographically dispersed and low-prevalence nature of RD populations, international collaboration is often indispensable. No single biobank or country is likely to possess a sufficient number of samples to support statistically robust research. Collaborative infrastructures such as BBMRI-ERIC and global initiatives like RD-Connect and the International RDs Research Consortium (IRDiRC) exemplify the transformative potential of coordinated efforts.<sup>30,31</sup> These platforms facilitate data sharing, ensure interoperability, and promote the harmonization of biobanking practices, elements that are crucial for accelerating research and advancing therapeutic development in the field of RDs.

## Key Contributions of Biobanks to RD Research

Biobanks play a central role in advancing RD research by sharing standardized, high-quality samples and data collections that comply with ethical and legal regulations to enable large-scale studies. The contribution of biobanks in RD research is summarized as (i) facilitating the identification of disease-causing genes and variants, (ii) supporting genomic and multi-omics research to gain insights into disease mechanisms, and (iii) enabling precision medicine and drug discovery efforts (Table 1).

In this regard, extensive cohorts in Biobanks contribute to the development and validation of diagnostic biomarkers,<sup>32</sup> particularly metabolic signatures, as well as diagnostic algorithms and artificial intelligence (AI) models.<sup>33</sup> In addition, biobanks create a platform to re-analyze the sample-associated data, such as whole-genome/exome sequencing, transcriptomics, proteomics, and metabolomics, with state-of-the-art technologies. For example, in a study conducted using optical coherence tomography images and genomic data from the UK Biobank, researchers identified 111 genetic loci and 10 genes associated with photoreceptor cell layer thickness, some of which are linked to rare eye diseases.<sup>34</sup> A notable example of how biobank data can uncover rare variant associations is the study by Liu and Curtis,<sup>35</sup> who analyzed rare loss-of-function and nonsynonymous variants in 470,000 UK Biobank participants and identified three genes, *FLG*, *IL33*, and *PRKCQ*, as significantly associated with childhood asthma risk. Damaging variants in *FLG* and *IL33* were associated with an increased risk, while those in *PRKCQ* appeared protective.<sup>35</sup> These findings demonstrate that large-scale exome sequencing can identify rare coding variants with significant effects on disease susceptibility. This approach provides a valuable framework for advancing RD research by revealing key genetic drivers and underlying pathogenic mechanisms. The storage of longitudinal samples and clinical

**Table 1. Key contributions of biobanks to rare disease research**

Contribution area	Description	Example/application
Genetic variant identification	Enables the discovery of disease-causing genes and mutations	The identification of 420 RDs and their prevalence was analyzed in 23,575 individuals by using data from the UK Biobank <sup>36</sup>
Pathophysiological insights	Supports-omics studies to understand disease mechanisms	Image and genomic data from the UK Biobank have been analyzed to generate novel insights into rare ocular diseases <sup>34</sup>
Therapeutic development	Provides biospecimens for target validation and drug screening	The 145 genes were associated with specific diseases and identified as potential therapeutic targets <sup>37</sup>
Improved diagnostics	Facilitates biomarker identification and validation	A six-gene immune-related prognostic index was identified and validated as a biomarker for predicting prognosis and immunotherapy response in hepatocellular carcinoma <sup>32</sup>
Longitudinal cohort studies	Enables longitudinal sample and data collection	RD-Connect linked biobanks facilitated progression studies in Duchenne muscular dystrophy <sup>31</sup>
Patient stratification	Helps define subtypes and treatment-responsive groups	Genotype-phenotype correlation in rare coding variants related to Childhood Asthma <sup>35</sup>
Collaboration & standardization	Support international research through the systematic processing of samples and data	A rare missense variant in <i>MYBPC3</i> was found to be associated with a significant, 3-fold increase in risk for coagulation defects <sup>38,39</sup>

data across multiple visits with the same high-quality measures enables researchers to investigate disease progression, assess phenotypic variability, and identify potential modifier genes.<sup>31</sup>

Beyond diagnostics, biobank-derived samples and data play a crucial role in advancing therapeutic research for rare and/or undiagnosed diseases. These biological resources allow i) targeted discovery and pathway analysis by supporting the identification of disease-specific therapeutic targets;<sup>37</sup> ii) the development and optimization of treatment strategies, including enzyme replacement therapies, gene therapies, small-molecule drugs, and substrate reduction therapies;<sup>40</sup> and iii) targeted drug screening with patient-derived cell models.

Overall, the multinational/multicenter studies using a standardized biobanking strategy are advancing RD research and innovation.

### Global Initiatives and Best Practices

Globally and nationally coordinated efforts, as well as robust biobanking infrastructures to systematically collect, manage, and share biological samples and associated data, are needed to develop new diagnostic and therapeutic strategies for RDs. To address the challenges posed by rarity and heterogeneity, these infrastructures promote collaboration and develop tools to ensure that data and samples are FAIR. Organizations and initiatives like BBMRI-ERIC, RD-Connect, Orphanet, and EUROSDIS are the key drivers in promoting standardization, collaboration, and accessibility.

BBMRI-ERIC provides a science-based, service-oriented infrastructure across Europe. It supports sample access, legal-ethical consulting, and centralized biobank directories to promote equal access.<sup>41</sup>

RD-Connect, launched under the IRDiRC, integrates genomic data with patient registries, biobanks, and clinical bioinformatics tools. RD-Connect provides researchers with access to harmonized data through Human Phenotype Ontology-based phenotyping and data integration with the European Genome-Phenome Archive,<sup>42</sup> thereby accelerating research collaborations.

Orphanet is a multilingual portal that offers information on RDs and orphan drugs.<sup>43</sup> Its OrphaCode classification standardizes disease coding, facilitating data harmonization and inter-institutional and multidisciplinary collaboration. Through its Orphan Drug Database, it also provides detailed updates on therapies under development or in use. RDs Europe, in collaboration with the Association Française contre les Myopathies, played a key role in establishing the RDs network, Europe's first virtual biobank platform.<sup>44</sup> This platform promotes international access to high-quality biospecimens and associated data, fosters education, and aligns biobanking practices with

patient rights and ethical standards.<sup>45</sup>

To ensure compatibility and high quality across global efforts, standardization frameworks are essential. Minimum Information About Biobank Data Sharing improves data interoperability across biobanks.<sup>46</sup> The International Society for Biological and Environmental Repositories (ISBER) provides best-practice guidelines and tools, such as the Self-Assessment Tool, for operational and ethical quality.<sup>47</sup>

Finally, ISO 20387—Biotechnology—Biobanking—General Requirements for Biobanks, published by the International Organization for Standardization (ISO) in 2018, outlines the requirements for quality management throughout the biological sample lifecycle, from collection to distribution.<sup>48</sup> It mandates traceability, ethical compliance, and continuous quality improvement via SOPs, ensuring the reproducibility and reliability of scientific research.

These global and national initiatives collectively form the backbone of modern, harmonized, and ethical biobanking practices, which are essential for advancing RD research.

### Current Challenges and Gaps in Biobanking for RDs

As outlined throughout this review, the advancement of biobanking has created invaluable opportunities for research on rare and/or undiagnosed diseases, facilitating rapid diagnosis, enhancing understanding of pathophysiology, and enabling the development of personalized treatment strategies. However, several challenges, including sustainability, data harmonization, underrepresentation of diverse populations, limited integration with healthcare systems, and regulatory complexities, such as compliance with the General Data Protection Regulation, need to be overcome. In addition, technical, ethical, and legal barriers to translating high-throughput data into clinical practice further hinder progress. Addressing these gaps is necessary to ensure that biobanks fully support innovation in RD research. The key challenges and suggested strategies in the current literature to overcome them are summarized in Table 2.

Briefly, key aspects for success include encouraging contributions from experts across different countries and disciplines to ensure the applicability of the main principles, technical requirements, and incentive mechanisms.

Beyond establishing technical frameworks, it is essential to raise awareness and foster the willingness of researchers, healthcare professionals, and the public to engage in biobanking through education, training, and extracurricular activities.<sup>50</sup> Additionally, ensuring biobank sustainability requires business planning, operational standardization, and accreditation, stakeholder engagement, and interoperability.<sup>49</sup>

**Table 2. Key challenges and potential solutions in biobanking for rare disease research**

Challenge/gap	Description	Potential solutions
Sustainability and funding models	Long-term financial support is often lacking for biobank maintenance	Develop public-private partnerships, integrate biobanks into national health research strategies, and establish sustainable funding frameworks <sup>49</sup>
Harmonization of data formats and ontologies	Data inconsistency hinders interoperability across biobanks	Promote the adoption of international standards (e.g., MIABIS, FAIR) and invest in harmonization tools and training <sup>30</sup>
Underrepresentation	Some ethnic and geographic groups are underrepresented in biobank datasets	Encourage inclusive sampling strategies, support community engagement, and foster global collaborations <sup>30</sup>
Limited awareness and integration in healthcare systems	Biobanks are often disconnected from clinical workflows	Raise awareness among healthcare professionals, integrate biobanks with electronic health records, and promote translational research links <sup>50</sup>
Compliance with GDPR and ethical regulations	Strict data protection laws may limit data sharing, especially across international borders	Develop robust, informed consent procedures, implement anonymization/pseudonymization techniques, and establish clear data governance frameworks
Lack of risk management strategies	The absence of structured risk mitigation plans can expose biobanks to operational, legal, or reputational threats	Establish risk assessment frameworks, implement ISO-aligned quality management systems (e.g., ISO 20387), and conduct regular audits and contingency planning <sup>48</sup>
Data accessibility and sharing barriers	Institutional and technical barriers limit the flow of data between systems	Establish trusted data-sharing frameworks, APIs, and federated data models <sup>41</sup>
Low data quality and inconsistent collection methods	Poor-quality or incomplete data reduce the usability of research	Standardize data collection protocols and implement quality control mechanisms <sup>48</sup>
Lack of FAIR compliance	Data often fail to meet FAIR principles, limiting its reuse	Develop FAIR-enabling infrastructures, such as metadata standards and open data tools <sup>51</sup>
The legal uncertainty surrounding data ownership and reuse	Uncertainty about who owns or controls the data limits reuse and collaboration	Clarify data ownership in policies, use standardized data use agreements, and promote transparent governance models <sup>52</sup>
Technical, ethical, and legal barriers in integrating omics into practice	Omics data are challenging to standardize, interpret, and implement due to complex legal and technical constraints	Develop ethical frameworks, invest in omics education, utilize AI tools for data analysis, and revise regulatory guidelines to support clinical translation <sup>53</sup>
FAIR: Findable, Accessible, Interoperable, Reusable, GDPR: General Data Protection Regulation, MIABIS: Minimum Information About Biobank Data Sharing ISO 20387: International Standardization Organization 20387		

Building trust among participants, clinicians, and researchers is also crucial for enhancing research impact and maximizing the overall value of biobanking efforts.

### Failed Efforts and Controversies

Despite the growing recognition of their importance, RD biobanks have faced failed efforts and unresolved controversies that limit their effectiveness. Attempts to promote international access and collaboration have frequently fallen short due to researchers' reluctance to share samples, clinicians' protection of their own collections, and complex legal and ethical differences that hinder cross-border cooperation.<sup>54-56</sup> Similarly, while organisations such as ISBER and BBMRI-ERIC have issued harmonisation guidelines, the persistence of variable procedures and quality standards across biobanks demonstrates the incomplete success of standardisation efforts, undermining sample comparability and research reproducibility.<sup>57,58</sup> In the area of public trust, debates continue regarding the impact of commercialisation, cross-border data sharing, and global networking, which in some contexts have made biobanks

appear less transparent or even exploitative.<sup>59,60</sup> Ethical disputes also remain unresolved. Informed consent models continue to provoke controversy, as broad and dynamic consent have not gained universal acceptance. Moreover, issues such as re-consent for adolescents, incidental findings, and the right not to know underline the ethical fragility of current frameworks.<sup>61,62</sup> Finally, sustainability failures are evident in case studies where limited government and clinician support, coupled with poor awareness of rare diseases, have led to obstacles in biobank development, and no long-term funding or strategic planning mechanisms were established to ensure their continued operation.<sup>57,63</sup> Together, these examples illustrate that RD biobanking is not only marked by success stories but also by incomplete initiatives and unresolved controversies that continue to challenge its global integration.

### Future Perspectives

With the rapid evolution of -omics technologies and digital health, biobanking is undergoing a paradigm shift, particularly in the field of RDs, where innovation is not only beneficial

but also necessary. The evolving landscape of biobanking needs significant transformations driven by technological advancements and shifting ethical paradigms. Particularly in the context of RDs, key emerging trends include i) the use of AI, machine learning (ML), deep learning (DL), and big data analytics; ii) federated data sharing models; iii) expansion of longitudinal and real-world data biobanks; iv) promoting patient-centric biobanking; and v) dynamic consent models.

AI and ML provide powerful tools for extracting insights from complex, high-dimensional datasets characteristic of RD research.<sup>64</sup> By integrating multi-omics data (genomics, proteomics, and metabolomics) with clinical and phenotypic information, AI can facilitate pattern recognition, biomarker discovery, and prediction of disease progression in RDs.<sup>53</sup> A DL-based algorithm using convolutional neural networks has been trained on cardiac magnetic resonance imaging data from biobank-derived Fabry disease and hypertrophic cardiomyopathy patients to distinguish between these conditions, achieving high accuracy (area under the curve  $\approx 0.918$ ) in an external single-blind validation study. The same method also automates volumetric assessment of left ventricular function more precisely and more quickly than human experts, aiding in disease monitoring and clinical trial selection.<sup>65</sup> AI-powered algorithms, with the help of ML/DL, can also optimize biospecimen management, improve quality control, and support decision-making in the operations of RD biobanks.<sup>33,65</sup>

As AI continues to revolutionize the interpretation of complex biomedical data, ensuring secure and scalable access to that data becomes equally critical. This is where federated data-sharing models come into play. Rather than centralizing sensitive information, federated models enable secure analysis across distributed datasets, effectively preserving privacy while facilitating large-scale research.<sup>66,67</sup> A disease-specific federated data network enables RD research institutions to retain local control over sensitive patient-level data, thereby enhancing privacy, governance, and transparency, while allowing for harmonized distributed analysis, as seen in the Haematology Outcomes Network in Europe for multiple myeloma and the Federation of Pulmonary Hypertension. These federated networks leverage common data models and strong governance frameworks to conduct collaborative real-world evidence studies across disparate data sources.<sup>68</sup> In RD research, these models are particularly valuable, allowing investigators to overcome data silos, enhance statistical power, and foster international collaboration, all while respecting local governance and data protection regulations.

In the context of RDs, diagnosis often takes time, symptoms vary, and data on disease history are limited. Longitudinal and real-world data biobanks help track disease progression, find

useful biomarkers, and support the use of research in clinical care.<sup>13,27</sup> The Systemic Lupus Erythematosus (SLE) International Collaborating Clinics biobank tracks over 1,800 SLE patients with more than 1,300 DNA samples and over 9,600 serum/plasma specimens spanning more than a decade of follow-up, enabling comprehensive longitudinal biomarker discovery and natural history studies in this rare autoimmune disease.<sup>26</sup>

Patient-centric biobanking in RDs emphasizes the active involvement of patients and advocacy groups in governance, consent processes, and priority-setting to ensure that research aligns with patient needs and values. This approach fosters transparency, trust, and sustained engagement, which are particularly critical in RD communities where patient participation often drives research progress. The Telethon Network of Genetic Biobanks exemplifies this model by integrating patient organizations into advisory roles and policy development, including the drafting of ethical guidelines through dedicated meetings and representation on the advisory board, resulting in formal agreements that centralize RD biospecimens and data while strengthening collaboration.<sup>69</sup>

Dynamic consent models enable ongoing, two-way communication between participants and biobanks, allowing individuals to modify their consent preferences over time.<sup>70</sup> This approach enhances transparency, trust, and engagement, which are crucial factors in RD communities where patients often play a central role in research advocacy. Furthermore, involving patients in biobank governance and research prioritization can help align scientific goals with real-world needs. The Rare UK Diseases of Bone, Joints, and Blood Vessels study is a pioneering initiative that employs digital technologies to create a patient-driven research platform for individuals with rare musculoskeletal diseases. Central to its design is a dynamic consent model, allowing participants to manage their consent preferences over time, thereby enhancing patient autonomy and engagement. The study integrates patient organizations into all stages of development, from study design to data governance, ensuring that research aligns with patient priorities and needs.<sup>71</sup>

## CONCLUSION

Biobanks have emerged as indispensable infrastructures in the landscape of RD research, addressing many of the field's inherent challenges, such as limited patient cohorts, diagnostic delays, and a lack of standardized biological data. By enabling the systematic collection, processing, and sharing of high-quality biospecimens and associated multi-omics and clinical data, biobanks provide a robust foundation for uncovering mechanisms, discovering novel biomarkers, and advancing personalized therapies.

Despite their transformative potential, RD biobanks still face significant challenges, including sustainability, data harmonization, underrepresentation of diverse populations, and complexities in legal and ethical considerations. However, global collaborations, the implementation of standardized biobanking procedures, and technological advancements offer promising solutions. In particular, patient-centric models and dynamic consent frameworks are transforming the way trust, autonomy, and participation are negotiated within research ecosystems.

Altogether, to fully unlock the potential of biobanking in addressing RDs, it is essential to strategically coordinate the efforts of stakeholders, researchers, clinicians, patients, policymakers, and funders. This collaboration is crucial for accelerating advancements in early diagnosis and effective treatment, and ultimately improving the lives of those affected by RDs.

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### Footnotes

### Authorship Contributions

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## REFERENCES

- Adachi T, El-Hattab AW, Jain R, Nogales Crespo KA, Quirland Lazo CI, Scarpa M, Summar M, Wattanasirichaigoon D. Enhancing equitable access to rare disease diagnosis and treatment around the world: a review of evidence, policies, and challenges. *Int J Environ Res Public Health*. 2023;20(6):4732. doi: 10.3390/ijerph20064732
- What is a rare disease? EURORDIS-Rare Diseases Europe. Accessed May 24, 2025. <https://www.eurordis.org/information-support/what-is-a-rare-disease/>
- Baldacci S, Santoro M, Pierini A, Mezzasalma L, Gorini F, Coi A. Healthcare burden of rare diseases: a population-based study in Tuscany (Italy). *Int J Environ Res Public Health*. 2022;19(13):7553. doi: 10.3390/ijerph19137553
- Wang Z, Zhang C, Zhang X, Bian Y, Cao Y. Assessing the impact of long-term storage on the quality and integrity of biological specimens in a reproductive biobank. *Bioeng Transl Med*. 2024;9(6):e10692. doi: 10.1002/btm2.10692
- Willmen L, Völkel L, Willmen T, Deckersbach T, Geyer S, Wagner AD. The economic burden of diagnostic uncertainty on rare disease patients. *BMC Health Serv Res*. 2024;24(1):1388. doi: 10.1186/s12913-024-11763-w
- Buckle N, Doyle O, Kodate N, Kinch M, Somanadhan S. Caregiver-reported economic impacts of pediatric rare diseases—a scoping review. *Healthcare*. 2024;12(24):2578. doi: 10.3390/healthcare12242578
- Durmus S, Yucesan E, Aktug S, Utz B, Caglayan AO, Gencpinar P, Günay C, Oktay Y, Yildirim RN, Yigit A, Ozbek U. Management of rare and undiagnosed diseases: insights from researchers and healthcare professionals in Türkiye. *Front Public Health*. 2025;12:1501942. doi: 10.3389/fpubh.2024.1501942
- Malsagova K, Kopylov A, Stepanov A, Butkova T, Sinitsyna A, Izotov A, Kaysheva A. Biobanks—a platform for scientific and biomedical research. *Diagnostics*. 2020;10(7):485. doi: 10.3390/diagnostics10070485
- Kinkorová J. Biobanks in the era of personalized medicine: objectives, challenges, and innovation: Overview. *EPMA J*. 2015;7(1):4. doi: 10.1186/s13167-016-0053-7
- Garcia M, Downs J, Russell A, Wang W. Impact of biobanks on research outcomes in rare diseases: a systematic review. *Orphanet J Rare Dis*. 2018;13(1):202. doi: 10.1186/s13023-018-0942-z
- Coppola L, Cianflone A, Grimaldi AM, Incoronato M, Bevilacqua P, Messina F, Baselice S, Soricelli A, Mirabelli P, Salvatore M. Biobanking in health care: evolution and future directions. *J Transl Med*. 2019;17(1):172. doi: 10.1186/s12967-019-1922-3
- Paskal W, Paskal AM, Dębski T, Gryziak M, Jaworowski J. Aspects of modern biobank activity – comprehensive review. *Pathol Oncol Res*. 2018;24(4):771-785. doi: 10.1007/s12253-018-0418-4
- Taruscio D, Gahl WA. Rare diseases: challenges and opportunities for research and public health. *Nat Rev Dis Primer*. 2024;10(1):13. doi: 10.1038/s41572-024-00505-1
- Gunes D, Karaca M, Durmus A, Ak B, Aktay Ayaz N, Altinel ZU, Aslanger AD, Atalar F, Balci MC, Bilgin L, Darendeliler F, Demirkol D, Durmaz O, Gedikbasi A, Inan Balci E, Ince EZ, Karadag SG, Keskindemirci G, Nisli K, Ozcetin M, Somer A, Unuvar A, Uysalol M, Yildiz E, Yuruk Yildirim ZN, Demirkol M, Gokcay GF. Challenges in the clinical management of rare diseases and center-based multidisciplinary approach to creating solutions. *Eur J Pediatr*. 2025;184(5):281. doi: 10.1007/s00431-025-06101-z
- Fehr A, Prütz F. Rare diseases: a challenge for medicine and public health. Robert Koch-Institut. Preprint posted online December 13, 2023. doi: 10.25646/11826
- Gittus M, Chong J, Sutton A, Ong ACM, Fotheringham J. Barriers and facilitators to the implementation of guidelines in rare diseases: a systematic review. *Orphanet J Rare Dis*. 2023;18(1):140. doi: 10.1186/s13023-023-02667-9
- Burton A, Castaño A, Bruno M, Riley S, Schumacher J, Sultan MB, See Tai S, Judge DP, Patel JK, Kelly JW. Drug discovery and development in rare diseases: taking a closer look at the Tafamidis story. *Drug Des Devel Ther*. 2021;15:1225-1243. doi: 10.2147/DDDT.S289772
- Annaratone L, De Palma G, Bonizzi G, Sapino A, Botti G, Berrino E, Mannelli C, Arcella P, Di Martino S, Steffan A, Daidone MG, Canzonieri V, Parodi B, Paradiso AV, Barberis M, Marchiò C; Alleanza Contro il Cancro (ACC) Pathology and Biobanking Working Group. Basic principles of biobanking: from biological samples to precision medicine for patients. *Virchows Arch*. 2021;479(2):233-246. doi: 10.1007/s00428-021-03151-0
- De Souza YG, Greenspan JS. Biobanking past, present and future: responsibilities and benefits. *AIDS*. 2013;27(3):303-312. doi: 10.1097/QAD.0b013e32835c1244

20. Korhan P, Tercan Avcı S, Yılmaz Y, Özteker İslakoğlu Y, Atabey N. Role of biobanks for cancer research and precision medicine in hepatocellular carcinoma. *J Gastrointest Cancer*. 2021;52(4):1232-1247. doi: 10.1007/s12029-021-00759-y
21. Olson JE, Bielinski SJ, Ryu E, Winkler EM, Takahashi PY, Pathak J, Cerhan JR. Biobanks and personalized medicine. *Clin Genet*. 2014;86(1):50-55. doi: 10.1111/cge.12370
22. Valenti A, Falcone I, Valenti F, Ricciardi E, Di Martino S, Maccallini MT, Cerro M, Desiderio F, Miso L, Russillo M, Guerrisi A. Biobanks as an indispensable tool in the “Era” of precision medicine: key role in the management of complex diseases, such as melanoma. *J Pers Med*. 2024;14(7):731. doi: 10.3390/jpm14070731
23. Rehm HL. Building biobanks to drive biomedical research and genomically informed care. *JAMA Netw Open*. 2025;8(3):e250925. doi: 10.1001/jamanetworkopen.2025.0925
24. Kinkorová J, Topolčan O, Kučera R. Informed consent in the newly established biobank. *Int J Environ Res Public Health*. 2019;16(20):3943. doi: 10.3390/ijerph16203943
25. van der Velden FJS, Lim E, Gills L, Broadey J, Hayes L, Roberts E, Courtney J, Ball J, Herberg J, Galassini R, Emonts M; DIAMONDS consortium. Biobanking and consenting to research: a qualitative thematic analysis of young people’s perspectives in the North East of England. *BMC Med Ethics*. 2023;24(1):47. doi: 10.1186/s12910-023-00925-w
26. Pellico MR, Day J, Shah M, Yi BY, Saketkoo LA, Lood C, Gupta L. Biorepositories for global rare disease research: a narrative review. *Curr Rheumatol Rep*. 2025;27(1):24. doi: 10.1007/s11926-025-01189-6
27. Cleary S, Teskey G, Mathews C, Crook T, Kaplan AS, Tjernagel K, Gauthier J, Miller M, Hallmayer J, Korvatska E, Bernier R, Vorstman JAS. Assessment of a multisite standardized biospecimen collection protocol for immune phenotyping in neurodevelopmental disorders. *Sci Rep*. 2023;13(1):6971. doi: 10.1038/s41598-023-33380-z
28. Inau ET, Sack J, Waltemath D, Zeleke AA. Initiatives, concepts, and implementation practices of the findable, accessible, interoperable, and reusable data principles in health data stewardship: scoping review. *J Med Internet Res*. 2023;25:e45013. doi: 10.2196/45013
29. International Society for Biological and Environmental Repositories (ISBER). Best Practices: Recommendations for Repositories. 5th ed. ISBER; 2018.
30. Van Karnebeek CDM, O’Donnell-Luria AH, Baynam G, Taruscio D, Groft SC, Austin CP, Boycott KM, Dawkins HJS, Wangler MF, Brownstein CA, Palmer EE, Ellaway C, Bonham J, Adams DR, Ng D, Wright CF, Smedley D, Rehm HL. Leaving no patient behind! Expert recommendation in the use of innovative technologies for diagnosing rare diseases. *Orphanet J Rare Dis*. 2024;19(1):357. doi: 10.1186/s13023-024-03361-0
31. Thompson R, Johnston L, Taruscio D, Monaco L, Bérout C, Mabile L, Shoemaker J, Cederroth H, Kauffmann F, Hansson MG, Ouillade MC, Posada M, Dawkins H, Lochmüller H. RD-Connect: an integrated platform connecting databases, registries, biobanks and clinical bioinformatics for rare disease research. *J Gen Intern Med*. 2014;29(Suppl 3):780-787. doi: 10.1007/s11606-014-2908-8
32. You P, Liu X, Wang M, Zhan Y, Chen L, Chen Y. Development and validation of an immune-related gene-based model for predicting prognosis and immunotherapy outcomes in hepatocellular carcinoma patients. *Sci Rep*. 2025;15(1):6618. doi: 10.1038/s41598-025-90183-0
33. Wojtara M, Rana E, Rahman T, Khanna P, Singh H. Artificial intelligence in rare disease diagnosis and treatment. *Clin Transl Sci*. 2023;16(11):2106-2111. doi: 10.1111/cts.13619
34. Currant H, Fitzgerald TW, Patel PJ, Chua SYL, Marshall H, Pontikos N, Llorente B, Zhao W, Wang W, Petkova M, Carss K, Xie J, Liu Y, Wang Z, Iyengar SK. Sub-cellular level resolution of common genetic variation in the photoreceptor layer identifies continuum between rare disease and common variation. *PLOS Genet*. 2023;19(2):e1010587. doi: 10.1371/journal.pgen.1010587
35. Liu Z, Curtis D. Analysis of rare coding variants in 470,000 UK biobank participants reveals genetic associations with childhood asthma predisposition. *Int J Immunogenet*. Published online May 8, 2025: iji.12714. doi: 10.1111/iji.12714
36. Patrick MT, Bardhi R, Zhou W, Elder JT, Gudjonsson JE, Tsoi LC. Enhanced rare disease mapping for genome-wide genetic association in the UK Biobank. *Genome Med*. 2022;14(1):85. doi: 10.1186/s13073-022-01094-y
37. Ferolito BR, Dashti H, Giambartolomei C, Jiang L, Takeuchi F, Yengo L, Liao C, Bulik-Sullivan BK, Okada Y, Martin AR. Leveraging large-scale biobanks for therapeutic target discovery. Preprint posted online February 12, 2025. doi: 10.1101/2025.02.10.25321487
38. Gallagher CS, Ginsburg GS, Musick A. Biobanking with genetics shapes precision medicine and global health. *Nat Rev Genet*. 2025;26(3):191-202. doi: 10.1038/s41576-024-00794-y
39. Sun BB, Kurki MI, Foley CN, Mechakra A, Chen CY, Marshall E, Wilk JB; Biogen Biobank Team; Chahine M, Chevalier P, Christé G; FinnGen; Palotie A, Daly MJ, Runz H. Genetic associations of protein-coding variants in human disease. *Nature*. 2022;603(7899):95-102. doi: 10.1038/s41586-022-04394-w
40. Bruce IA, Ezgü FS, Kampmann C, Beck M, Giugliani R, Jones SA, Ketteridge D, Link B, Parini R, Reuser AJJ, Scarpa M, Valayannopoulos V, Harmatz P. Addressing the need for patient-friendly medical communications: adaptation of the 2019 recommendations for the management of MPS VI and MPS IVA. *Orphanet J Rare Dis*. 2022;17(1):91. doi: 10.1186/s13023-022-02219-7
41. Reihls R, Proynova R, Maqsood S, Stocker G, Mayrhofer MT, Jagodnik KM, Holub P, Norlin L, Piret C, Cederroth H, van Enckevort D, Licher K, Schueller C, Litton JE. BBMRI-ERIC negotiator: implementing efficient access to biobanks. *Biopreserv Biobank*. 2021;19(5):414-421. doi: 10.1089/bio.2020.0144
42. Lochmüller H, Badowska DM, Thompson R, Knoers NVAM, Aartsma-Rus A, Gut I, Wood L, Harmuth T, Durudas A, Graessner H, Johnston J, Ferlini A, Straub V, Schwartz O. RD-Connect, NeurOmics and EURenOmics: collaborative European initiative for rare diseases. *Eur J Hum Genet*. 2018;26(6):778-785. doi: 10.1038/s41431-018-0115-8
43. Rath A, Olry A, Dhombres F, Brandt MM, Urbero B, Ayme S. Representation of rare diseases in health information systems: The Orphanet approach to serve a wide range of end users. *Hum Mutat*. 2012;33(5):803-808. doi: 10.1002/humu.22078
44. Mora M, Angelini C, Bignami F, Bodin AM, Crimi M, Di Donato JH, Hollak C, Moraes CT, Noakes PG, Santorelli FM, van der Spek PJ, Tavazzi E, Thonhofer M, Lochmüller H. The EuroBioBank Network: 10 years of hands-on experience of collaborative, transnational biobanking for rare diseases. *Eur J Hum Genet*. 2015;23(9):1116-1123. doi: 10.1038/ejhg.2014.272
45. Lochmüller H, Schneiderat P. Biobanking in Rare Disorders. In: Posada De La Paz M, Groft SC, eds. *Rare Diseases Epidemiology*. Vol 686. Springer Netherlands; 2010:105-113. doi: 10.1007/978-90-481-9485-8\_7
46. Norlin L, Fransson MN, Eriksson M, Kurtovic S, Anderberg M, Bruland T, Hansson MG, Litton JE, Merino-Martinez R. A minimum data set for sharing biobank samples, information, and data: MIABIS. *Biopreserv Biobank*. 2012;10(4):343-348. doi: 10.1089/bio.2012.0003
47. Gao D, Grossman G. ISBER corner: ISBER marching forward: a 25-year journey. *Biopreserv Biobank*. 2024;22(6):628-630. doi: 10.1089/bio.2024.0144
48. International Organization for Standardization (ISO). ISO 20387:2018. *Biotechnology — biobanking — general requirements for biobanking*. Published August 2018. Available from: <https://www.iso.org/standard/67888.html>

49. Abdaljalil M, Singer EJ, Yong WH. Sustainability in Biobanking. In: Yong WH, ed. *Biobanking*. Vol 1897. Springer New York; 2019:1-6. doi: 10.1007/978-1-4939-8935-5\_1
50. Karataş M, Azbazar ME, Camkiranlar M, Tercan-Avci S, Atabey N. Biobank education for future physicians: training medical students through student research association networks. *Biopreserv Biobank*. 2024;22(3):217-224. doi: 10.1089/bio.2022.0210
51. Simeon-Dubach D, Kozlakidis Z, Tayal J, Verheij E, Lolkema MP, Silva J, Holub P. Experts speak forum: implementation of the FAIR principles in biobanking needs fair incentives. *Biopreserv Biobank*. 2024;22(6):557-562. doi: 10.1089/bio.2024.0153
52. Edworthy E. Communitarian ethics perspective on UK biobanking: the Newborn Genomes Programme. *J Med Ethics*. Published online May 11, 2025;jme-2025-110846. doi: 10.1136/jme-2025-110846
53. Venturini P, Faria PL, Cordeiro JV. AI and omics technologies in biobanking: Applications and challenges for public health. *Public Health*. 2025;243:105726. doi: 10.1016/j.puhe.2025.105726
54. Fortin S, Pathmasiri S, Grintuch R, Deschênes M. "Access arrangements" for biobanks: a fine line between facilitating and hindering collaboration. *Public Health Genomics*. 2011;14(2):104-114. doi: 10.1159/000309852
55. Colledge F, Elger B, Howard HC. A review of the barriers to sharing in biobanking. *Biopreserv Biobank*. 2013;11(6):339-346. doi: 10.1089/bio.2013.0039
56. Monaco L, Crimi M, Wang CM. The challenge for a European network of biobanks for rare diseases taken up by RD-Connect. *Pathobiology*. 2014;81(5-6):231-236. doi: 10.1159/000358492
57. Tada M, Hirata M, Sasaki M, Mochizuki T, Nakamura Y, Shimizu R, Okazaki Y. The rare disease bank of Japan: establishment, current status and future challenges. *Hum Cell*. 2018;31(3):183-188. doi: 10.1007/s13577-018-0204-3
58. Borisova AL, Pokrovskaya MS, Meshkov AN, Metelskaya VA, Shatalova AM, Drapkina OM. ISO 20387 biobanking standard: Analysis of requirements and experience of implementation. *Klin Lab Diagn*. 2020;65(9):587-592. doi: 10.18821/0869-2084-2020-65-9-587-592
59. Dive L, Critchley C, Otlowski M, Skene L, Slokenberga S, Bialobrzewski A, Kaye J. Public trust and global biobank networks. *BMC Med Ethics*. 2020;21(1):73. doi: 10.1186/s12910-020-00515-0
60. Moodley K, Singh S. "It's all about trust": reflections of researchers on the complexity and controversy surrounding biobanking in South Africa. *BMC Med Ethics*. 2016;17(1):57. doi: 10.1186/s12910-016-0140-2
61. Sadzevičius M, Mizeikis L. Biobanks – legal regulation and ethical challenges. *Vilnius Univ Open Ser*. Published online October 24, 2024:58-78. doi: 10.15388/TMP.2024.3
62. Van Der Velden FJS, Lim E, Gills L, Gill S, McKenzie G, Phelan J, McCarthy M, Pearce MS, Parker M. Biobanking and consenting to research: a qualitative thematic analysis of young people's perspectives in the North East of England. *BMC Med Ethics*. 2023;24(1):47. doi: 10.1186/s12910-023-00925-w
63. Conradie EH, Malherbe H, Hendriksz CJ, Dercksen M, Vorster BC. An overview of benefits and challenges of rare disease biobanking in Africa, focusing on South Africa. *Biopreserv Biobank*. 2021;19(2):143-150. doi: 10.1089/bio.2020.0108
64. Nishat SMH, Shahid Tanweer A, Alshamsi B, Alhammadi M, Ahmed M, Alkaabi N, Alnuaimi N, Alzubaidi R, Altaf T, Murtaza G, Kumar A. Artificial intelligence: a new frontier in rare disease early diagnosis. *Cureus*. Published online February 22, 2025. doi: 10.7759/cureus.79487
65. Germain DP, Gruson D, Malcles M, Garcelon N. Applying artificial intelligence to rare diseases: a literature review highlighting lessons from Fabry disease. *Orphanet J Rare Dis*. 2025;20(1):186. doi: 10.1186/s13023-025-03655-x
66. Cho H, Froelicher D, Chen J, Jaggi M, Soria-Comas J, Kim M, Aslett L, Demirci H, Raghunathan A, Shokri R, Papadimitriou S, Alvim-Gaston M, Lambert SA, Beaulieu-Jones BK. Secure and federated genome-wide association studies for biobank-scale datasets. *Nat Genet*. 2025;57(4):809-814. doi: 10.1038/s41588-025-02109-1
67. Rehm HL, Page AJH, Smith L, Adams JB, Alterovitz G, Babb LJ, Baldridge D, Chasioti D, Church G, Crawford J, Deans Z, Glazer AM, Hansen D, et al. GA4GH: International policies and standards for data sharing across genomic research and healthcare. *Cell Genomics*. 2021;1(2):100029. doi: 10.1016/j.xgen.2021.100029
68. Van Baalen V, Didden E, Rosenberg D, Bardenheuer K, Van Speybroeck M, Brand M. Increase transparency and reproducibility of real-world evidence in rare diseases through disease-specific Federated Data Networks. *Pharmacoepidemiol Drug Saf*. 2024;33(4):e5778. doi: 10.1002/pds.5778
69. Baldo C, Casareto L, Renieri A, Brusco A, Cereda A, Ferlini A, Garavaglia B, Giardino D, Malcovati M, Mottini M, Rizzuti T, Sangiuolo F, Zuffardi O, Taruscio D. The alliance between genetic biobanks and patient organisations: the experience of the Telethon Network of Genetic Biobanks. *Orphanet J Rare Dis*. 2016;11(1):142. doi: 10.1186/s13023-016-0527-7
70. Bruns A, Winkler EC. Dynamic consent: a royal road to research consent? *J Med Ethics*. Published online July 24, 2024;jme-2024-110153. doi:10.1136/jme-2024-110153
71. Teare HJA, Hogg J, Kaye J, Lucchetti AF, Rush E, Turner A, Wilcox R, Parker M, O'Donovan C, van't Hoff W, McDonagh JE, Biggs J, Knight J, Williams R, Fairchild P, Bell J. The RUDY study: using digital technologies to enable a research partnership. *Eur J Hum Genet*. 2017;25(7):816-822. doi: 10.1038/ejhg.2017.57

# The Role of New-Generation Omics Technologies in Diagnosis, Monitoring, and Development of New Treatment Strategies for Inherited Metabolic Diseases

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## Abstract

Omics technologies encompass a suite of high-throughput analytical techniques that enable comprehensive investigation of biological systems at the molecular level. A subset of omics technology, metabolomics, focuses on the comprehensive qualitative, quantitative, relational, spatial, and temporal analysis of metabolites under various conditions and states. Rare diseases are conditions that affect a relatively small number of people. Approximately 6,000-8,000 rare diseases have been identified to date. Inborn errors of metabolism (IEMs) represent a significant portion of rare diseases and have a genetic origin. Although IEMs are genetically based, the traditional one-gene-one-disease model is no longer universally accepted for these disorders. Each IEM presents a unique phenotype, necessitating a personalized approach. Therefore, metabolomics—the global study of small molecules, typically between 50 and 1500 Daltons—is expected to contribute significantly to a better understanding of the pathogenesis and pathophysiology of IEMs. This review discusses the current state of knowledge regarding the diagnosis, monitoring, and development of novel therapeutic strategies for patients with IEMs, based on the latest literature.

**Keywords:** Bioinformatics, Inborn Errors of Metabolism, Metabolomics

## INTRODUCTION

“Omics” refers to the comprehensive study of biological systems. Omics technologies are high-throughput analytical techniques that allow for a deep dive into biological systems at the molecular level.<sup>1</sup> By characterizing and quantifying biological molecules on a large scale, omics techniques enable researchers to identify genetic, transcriptional, protein, and metabolic changes associated with various diseases.<sup>2</sup> Omics science encompasses several sub-disciplines, including genomics, transcriptomics, proteomics, metabolomics, lipidomics, epigenetics, glycomics, and metagenomics. These fields offer a holistic view of biological systems, from DNA to metabolites, allowing researchers to investigate complex interactions between biomolecules and drive advancements in fields such as medicine, agriculture, and environmental science.<sup>3,4</sup> For instance, genomic analysis

can identify genetic predispositions to diseases, enabling early intervention. Proteomics can reveal abnormal protein patterns, leading to the discovery of new biomarkers for disease diagnosis. Ultimately, omics technologies hold the promise of personalized medicine by tailoring treatments to an individual’s unique genetic and molecular profile.<sup>1</sup>

However, metabolomics is at the forefront of omics research most commonly used in medical practice. The metabolome represents the entire collection of small molecules (metabolites) within a biological sample. Metabolomics is the comprehensive study of these metabolites, examining their qualities, quantities, relationships, spatial distribution, and temporal changes under various conditions. In essence, metabolomics provides a snapshot of a cell, tissue, or organism’s metabolic activity at a specific moment.<sup>1</sup> While genetic analysis offers insights into an



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individual's inherent potential, metabolomics complements this by revealing how genetic variations manifest at the phenotypic level. Furthermore, it explores the impact of environmental factors, diet, and lifestyle on metabolic processes, which can significantly influence disease development and progression. By identifying early metabolic changes associated with disease onset, metabolomics facilitates earlier diagnosis and intervention. Combining genetic and metabolomic data allows for the personalization of treatments based on an individual's unique metabolic profile.<sup>5</sup>

### The Role of Omics Technologies in Diagnosing Inherited Metabolic Diseases (IMD)

Over the past two decades, the application of omics technologies to rare and IMD has undergone remarkable evolution. Initially, genomic approaches dominated the field, enabling the identification of novel pathogenic variants and expanding the catalog of IMDs through next-generation sequencing.<sup>6</sup> Subsequently, transcriptomics and proteomics facilitated a deeper understanding of dysregulated molecular networks, highlighting the limitations of the traditional "one gene–one disease" model.<sup>4</sup> With the advent of high-resolution mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, metabolomics has emerged as a powerful complement, bridging the gap between genotype and phenotype by capturing the real-time biochemical consequences of genetic defects.<sup>7</sup>

Recent literature emphasizes the advantages of integrated multi-omics approaches in IMD diagnosis and monitoring. For instance, genomics can identify disease-causing mutations, while metabolomics validates their functional impact through altered biochemical homeostasis.<sup>8</sup> Moreover, multi-omics studies have begun to uncover disease modifiers, reveal compensatory pathways, and identify novel therapeutic targets, shifting the clinical approach from descriptive diagnostics toward precision medicine.<sup>9</sup> This evolution underscores the critical role of omics technologies in shaping both our understanding and the clinical management of IMDs.

Rare diseases are conditions that are infrequently observed in the general population. Approximately 6,000-8,000 rare diseases have been identified to date.<sup>8</sup> In the United States, a rare disease is defined as a condition that affects fewer than 1 in 200,000 people, while in Europe, it is defined as a condition affecting fewer than 50 in 100,000 people. According to the World Health Organization, a rare disease is considered to be a condition that affects fewer than 65 in 100,000 people.<sup>6</sup>

IMDs are genetic disorders that constitute a significant portion of rare diseases. These are complex medical conditions involving human organ systems and resulting from an enzyme defect in biochemical and metabolic pathways affecting protein, fat, and

carbohydrate metabolism, or from impaired organelle function. This basic definition is a dynamic concept that varies as the genetic underpinnings of new diseases are discovered. While the presence of a biomarker was previously a prerequisite for the diagnosis of IMDs, this is no longer the case. On the other hand, the identification of new disease groups related to cellular traffic has highlighted the need for a more holistic approach to the concept of metabolic disease. Another consequence of this holistic approach is the elucidation of the relationships between functional processes within the cell (autophagy, inflammation, oxidative stress, immune dysregulation, energy homeostasis, etc.) and the role of these relationships in determining a patient's diagnosis, treatment, and prognosis. Omics technologies form the foundation of this newly emerging approach and will be the most important component in the medical practice of CMDs in the near future.<sup>8,10</sup>

Rare diseases are those that affect a small portion of the population. IMDs are caused by genetic defects in biochemical pathways. Traditionally categorized into intoxication type, energy metabolism disorders, and complex molecule disorders, metabolic disorders have recently been reclassified by the International Classification of Inherited Metabolic Disorders advisory group, outlining 1450 disorders.<sup>6</sup>

While each IMD has a unique phenotype, the previously accepted one-gene-one-disease model is no longer sufficient to clarify their pathogenesis. Metabolomics, the study of small molecules, offers a comprehensive view of metabolic processes and can aid in understanding the pathogenesis and pathophysiology of IMDs.<sup>7</sup>

A deeper understanding of the biochemical and metabolic mechanisms underlying IMDs highlights the value of metabolomics in clinical practice. For example, in phenylketonuria (PKU), deficiency of phenylalanine hydroxylase impairs the conversion of phenylalanine to tyrosine, leading to the toxic accumulation of phenylalanine and the secondary depletion of neurotransmitter precursors. Metabolomics not only detects elevated phenylalanine, but also identifies secondary metabolites such as phenylpyruvic acid and N-acetylphenylalanine, which reflect broader disturbances in amino acid and neurotransmitter metabolism.<sup>11</sup>

Similarly, in maple syrup urine disease, defective activity of the branched-chain  $\alpha$ -ketoacid dehydrogenase complex prevents degradation of leucine, isoleucine, and valine. This results in the accumulation of branched-chain amino acids and their corresponding ketoacids, which are neurotoxic. Untargeted metabolomics studies have revealed additional downstream perturbations in energy metabolism, mitochondrial function, and neurotransmitter pathways.<sup>12</sup>

In methylmalonic acidemia (MMA), deficiency of methylmalonyl-CoA mutase or defects in cobalamin metabolism disrupt the breakdown of odd-chain fatty acids and certain amino acids, causing accumulation of methylmalonic acid and propionylcarnitine. These metabolites interfere with the tricarboxylic acid (TCA) cycle, impairing ATP production and promoting mitochondrial dysfunction. Metabolomics has been instrumental in uncovering secondary elevations in acylcarnitines, organic acids, and neuroactive steroids that help explain the neurological and ocular complications of MMA.<sup>13</sup>

In lysosomal storage disorders (LSDs), such as Fabry and Gaucher disease, enzyme deficiencies result in the accumulation of undegraded sphingolipids. Metabolomics approaches have identified disease-specific biomarkers like globotriaosylsphingosine (Lyso-Gb3) in Fabry disease and glucosylsphingosine (Lyso-Gb1) in Gaucher disease. These molecules not only serve as diagnostic and monitoring tools but also provide mechanistic insight into how impaired lysosomal clearance disrupts lipid signaling and inflammatory pathways.<sup>14</sup>

Together, these examples illustrate how metabolomics provides a functional window into the biochemical consequences of specific enzyme deficiencies, linking genotype to phenotype and uncovering potential therapeutic targets.

The lack of specific symptoms, delayed diagnosis, and challenges in traditional diagnostic methods have spurred the integration of omics technologies into the diagnosis and management of IMDs. Omics technologies, such as genomics, transcriptomics, and metabolomics, provide a more holistic view of the disease, allowing for earlier diagnosis and tailored treatment. By identifying the underlying genetic and metabolic alterations, these technologies can help to prevent irreversible complications associated with IMDs.<sup>7</sup>

The clinical relevance of omics technologies extends far beyond basic research, offering direct benefits in the care of patients with IMDs.

Metabolomics and other omics approaches enable earlier and more precise diagnosis compared to conventional biochemical methods. Tandem MS-based newborn screening, now standard in many countries, exemplifies how targeted metabolomics can detect dozens of IMDs in a single assay.<sup>14</sup> Furthermore, untargeted metabolomics has identified atypical biochemical signatures that broaden the phenotypic spectrum of known disorders and even reveal novel IMDs.<sup>7</sup>

Once a diagnosis is established, omics approaches facilitate longitudinal monitoring. Targeted metabolite panels, such as phenylalanine in PKU or Lyso-Gb3 in Fabry disease, are widely used to track treatment response and disease burden.

Untargeted metabolomics can also capture broader pathway shifts over time, providing early warning of complications or metabolic decompensation.<sup>15</sup>

Omics data support personalized medicine by linking genetic defects to functional biochemical consequences. For instance, metabolomic profiling has demonstrated how amino acid-restricted diets alter tryptophan and tyrosine metabolism in PKU, informing dietary adjustments.<sup>11</sup> Similarly, in serine biosynthesis defects, metabolomics confirmed the biochemical benefit of serine and glycine supplementation, guiding therapy optimization.<sup>10</sup>

Emerging evidence suggests that omics biomarkers can serve as predictors of long-term outcomes. In Fabry disease, plasma Lyso-Gb3 levels correlate with disease severity and risk of organ complications.<sup>12</sup> In MMA, elevations of secondary metabolites such as neurosteroids have been associated with neurological decline, suggesting potential roles as prognostic indicators.<sup>13</sup>

Taken together, these examples underscore that omics technologies are not merely research tools, but practical instruments for clinical decision-making, from diagnosis through lifelong follow-up. As integration with genomic, proteomic, and transcriptomic data continues to advance, omics-based approaches are poised to transform the standard of care for patients with IMDs.

### Methods Used in Metabolomics Technologies

Unlike genomics and proteomics, where the building blocks (nucleotides and amino acids) are relatively uniform, the metabolome comprises a diverse array of molecules with vastly different chemical structures. This diversity presents a significant challenge for metabolomic analysis, requiring the use of multiple analytical techniques and instruments. Furthermore, the wide range of metabolite concentrations adds complexity to both qualitative and quantitative analyses.<sup>7</sup>

Metabolomic analyses are broadly categorized into two approaches: targeted and untargeted. Targeted metabolomics focuses on measuring specific metabolites, such as those involved in particular biological pathways or associated with certain diseases. While highly sensitive and specific, this approach is limited to the pre-selected metabolites and cannot be used to discover unknown biomarkers.<sup>16</sup>

In contrast, untargeted metabolomics aims to comprehensively analyze all metabolites within a sample, including those that are yet to be identified. This approach provides valuable complementary information to transcriptomics and proteomics. Although the complete characterization of the metabolome remains a challenge due to the diverse nature and varying concentrations of metabolites, advancements in analytical technologies and expanding databases are continuously

improving the sensitivity and specificity of untargeted metabolomics, making it a powerful tool for discovering new biomarkers.<sup>17</sup>

Commonly used techniques for metabolomic analysis include NMR spectroscopy and MS coupled with chromatography (GC-MS and LC-MS). NMR spectroscopy provides valuable information about the structure and dynamics of small molecules without requiring prior separation. However, MS techniques, such as GC-MS and LC-MS, offer significantly higher sensitivity and broader coverage, allowing for more accurate and comprehensive metabolite detection.

The typical metabolomic analysis workflow involves five key steps:

- Sample collection and storage
- Metabolite isolation
- Metabolite analysis using appropriate analytical techniques
- Data filtering and processing
- Biostatistical analysis

Blood and urine samples are the most commonly used sources for metabolomic studies due to their easy accessibility. However, various other biological samples, including tissues and other bodily fluids, can also be analyzed.<sup>16</sup>

### **Omics Technologies in IMD**

While metabolomics provides a functional snapshot of the biochemical consequences of genetic defects, the integration of other omics layers — such as genomics, transcriptomics, and proteomics — is essential to achieve a comprehensive understanding of IMDs.

Genomics remains the foundation for identifying causal variants and expanding the catalog of known IMDs. Next-generation sequencing has revealed hundreds of novel pathogenic mutations and has clarified genotype–phenotype correlations in disorders such as mitochondrial diseases and organic acidemias.<sup>6</sup>

Transcriptomics adds a dynamic layer by showing how gene expression changes under disease or treatment conditions. For example, RNA sequencing has uncovered secondary transcriptional dysregulation in LSDs, highlighting pathways involved in inflammation and autophagy that contribute to disease progression.<sup>4</sup>

Proteomics bridges the gap between gene expression and metabolite profiles, offering insights into enzyme abundance, activity, and post-translational modifications. In fatty acid oxidation disorders, proteomic profiling has identified altered mitochondrial protein networks, complementing metabolomic data and revealing novel therapeutic targets.<sup>5</sup>

Multi-omics integration is increasingly applied in IMDs, allowing for cross-validation of findings across molecular layers. A recent study combined genomics, transcriptomics, and metabolomics to demonstrate the therapeutic effect of sodium phenylbutyrate in combined D,L-2-hydroxyglutaric aciduria, linking metabolite normalization with transcriptional rescue of mitochondrial pathways.<sup>16</sup> Such examples highlight the synergistic power of multi-omics approaches, which not only refine diagnosis but also generate mechanistic insights and guide treatment strategies.

Metabolomics plays a crucial role in diagnosing, monitoring, and managing many IMDs. Newborn screening programs often employ targeted metabolomics, such as measuring blood phenylalanine levels for PKU, using MS. In developed countries, these programs screen for numerous CMDs, including organic acidemias, fatty acid oxidation defects, and even LSDs.<sup>14</sup>

For CMDs requiring dietary restrictions, targeted metabolomics helps assess treatment adherence and effectiveness. It also provides insights into how specific diets impact various metabolic pathways.<sup>11</sup>

Biomarkers identified through metabolomics are valuable tools for diagnosing, monitoring, and predicting treatment responses in CMDs. For example, globotriaosylsphingosine (Lyso-Gb3), a biomarker for Fabry disease, was discovered through metabolomics research. It correlates with disease severity and decreases with effective treatment. Biomarkers can improve patient outcomes by providing crucial information about disease progression, treatment response, and potential complications.

Both targeted and untargeted metabolomics approaches are used to identify biomarkers. While research on untargeted metabolomics in CMDs is ongoing, studies have focused primarily on amino acid metabolism disorders (e.g., PKU, branched-chain amino acid disorders), BH<sub>4</sub> metabolism disorders, and galactosemia.

Untargeted metabolomics studies in PKU have identified potential biomarkers, including phenylpyruvic acid, phenylacetic acid, N-acetylphenylalanine, and N-lactoylphenylalanine. In other studies, metabolomics has revealed changes in indole metabolites in alkaptonuria and tyrosinemia following nitisinone treatment, shedding light on the impact of these metabolites on disease prognosis.<sup>12,15</sup>

Gertsman et al.<sup>18</sup> demonstrated a change in indole metabolites following nitisinone treatment in patients with alkaptonuria and tyrosinemia. This research sheds light on the effects of indole metabolites on prognosis in disorders involving tyrosine metabolism. Norman et al.<sup>19</sup> showed, through metabolomic studies, that alternative pathways are activated in patients with hypertyrosinemia to compensate for elevated tyrosine levels.

The discovery of these alternative pathways will shed light on new treatment options for patients with hypertyrosinemia. Glinton et al.<sup>10</sup> have shown that supplementation with serine and glycine in patients with serine biosynthesis defects balances plasma phospholipid components. In another study conducted in patients with organic aciduria and cobalamin C deficiency, neurosteroids were found to be significantly elevated, and it was suggested that this could shed light on the pathogenesis of accompanying ocular and neurological findings.<sup>13</sup>

Today, targeted metabolomics is used in the routine screening of several CMDs for which specific biomarkers have been identified (Table 1).<sup>20</sup>

Metabolomics research extends beyond diagnosis and monitoring; it holds significant promise for developing novel therapies for inborn errors of metabolism (IEMS).

For example, studies have demonstrated that fibroblast cultures from patients with methylmalonic aciduria exhibit disruptions in the TCA cycle. Notably, treatment with dimethyl oxoglutarate significantly improved these metabolic abnormalities. Similarly, research by Phua et al.<sup>16</sup> showed that sodium phenylbutyrate

effectively reduced elevated levels of 2-ketoglutarate and 2-hydroxyglutarate in fibroblast cultures from patients with combined D,L-2-hydroxyglutaric aciduria, suggesting its potential as a therapeutic agent.

The integration of multiple omics approaches — genomics, transcriptomics, proteomics, and metabolomics — offers significant promise for advancing the diagnosis and management of IMDs. Multi-omics approaches provide complementary information at different molecular levels: while genomics identifies pathogenic variants, transcriptomics captures gene expression changes, proteomics reveals alterations in protein abundance and modification, and metabolomics reflects the real-time biochemical consequences of these disturbances.

Applied together, these layers can enhance diagnostic accuracy. For example, genomic sequencing may identify variants of uncertain significance, but metabolomic or proteomic profiling can determine whether such variants cause measurable biochemical or protein-level abnormalities, thereby clarifying pathogenicity.<sup>21</sup> This integration reduces diagnostic uncertainty and supports earlier interventions.

**Table 1.** Examples of biomarkers used in the diagnosis of several IEMs

IEM type	IEM subtype	Biomarker
Amino acid metabolism disorders	PKU	Phenylalanine (P)
	MSUD	Valine, leucine, isoleucine, allo-isoleucine (P)
	Tyrosinemia	Tyrosine (P) Succinylacetone (B, P, U)
	Homocystinuria	Homocystine (P)
	MMA	Propionyl carnitine (C3) (B, P) Methylmalonic acid (Urine)
	Propionic acidemia	Propionyl carnitine (C3) (B, P) 3-hydroxy-propionic acid (U)
	Isovaleric acidemia	Isovaleryl carnitine (C5) (B, P) Isovaleric acid (U)
	Non-ketotic hyperglycinemia	Glycine (P, CSF)
Fatty acid oxidation disorders	Medium/short-chain acyl-CoA dehydrogenase deficiency (M/SCHAD)	3-hydroxy isobutyryl carnitine (B, P) (C4-OH)
	MCAD	Hexanoyl carnitine (C6) Octanoyl carnitine (C8) Decanoyl carnitine (C10:1)
	VLCAD	Myristoyl carnitine (C14:0) Tetradecanoyl carnitine (C14:1) Tetradecadienoyl carnitine (C14:2)
	Biotinidase deficiency Holocarboxylase synthetase deficiency 3-methylcrotonylCoA carboxylase deficiency 3-hydroxy-3-methylcrotonyl-CoA carboxylase deficiency	3-hydroxy isovaleryl carnitine (C5-OH)

**Table 1.** Continued

IEM type	IEM subtype	Biomarker
Lysosomal storage disease	Gaucher disease	Glycosylsphingosine (P) (Lyso-Gb1) Chitotriosidase (P)
	Fabry disease	Globotriaosylceramide (P) (Lyso-Gb3)
	Pompe disease	Glucotetrasaccharides (Glc4) (U)
	Niemann-Pick type C disease	Lyso-sphingomyeline 509 (P) (Lyso-SM 509)
	Krabbe disease	Galactosylsphingosine/psychosine (P)
	Mucopolysaccharidosis type I (Hurler syndrome )	Dermatan sulfate (U) Heparan sulfate (U)
	Mucopolysaccharidosis type II (Hunter syndrome )	Dermatan sulfate (U) Heparan sulfate (U)
	Mucopolysaccharidosis type III (Sanfilippo type A, B, C, D)	Heparan sulfate (U)
	Mucopolysaccharidosis type IVA (Morquio A syndrome )	Keratan sulfate (U) Chondroitin sulfate (U)
	Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome )	Dermatan sulfate (U)
	Mucopolysaccharidosis type VII (Sly syndrome)	Dermatan sulfate (U) Heparan sulfate (U) Chondroitin sulfate (U)
	Niemann – Pick disease type A-B	Lyso-sphingomyelin (Lyso-SM) Lyso-sphingomyelin – 509 (Lyso-SM-509)
	GM1 gangliosidosis	Lyso-monosialoganglioside (Lyso-GM1)
	GM2 gangliosidoz Tay-Sachs disease Sandhoff disease	Lyso-monosialoganglioside Lyso-GM2
Peroxisomal diseases	Zellweger spectrum disorders	Phytanic acid (S, P) Pipelicolic acid (S, P) Pristanic acid (S, P) Very long chain fatty acids (P)
	Alfa metil KoA racemase deficiency	Phytanic acid (S, P) Pristanic acid (S, P)
	X linked adrenoleukodystrophy	Very long chain fatty acids (P)
	Refsum disease	Phytanic acid (S, P)

IEM: Inborn errors of metabolism, MCAD: Medium-chain acyl-CoA dehydrogenase deficiency, MMA: Methylmalonic acidemia, MSUD: Maple syrup urine disease, PKU: Phenylketonuria, VLCAD: Very long chain acyl-CoA dehydrogenase deficiency

Multi-omics also facilitates personalized medicine by capturing inter-individual variability in disease expression and treatment response. In disorders such as PKU, metabolomics has shown how dietary interventions alter amino acid and neurotransmitter metabolism, while transcriptomic and proteomic studies reveal downstream effects on neuronal and immune pathways.<sup>11</sup>

Such combined insights enable tailored treatment plans based not only on genetic diagnosis but also on functional metabolic profiles.

Moreover, multi-omics data can uncover disease modifiers and therapeutic targets. In mitochondrial disorders, integrated omics analyses have identified compensatory pathways that mitigate

energy deficits, suggesting novel pharmacological interventions. Similarly, a multi-omics study in combined D,L-2-hydroxyglutaric aciduria demonstrated that sodium phenylbutyrate therapy improved both metabolite profiles and transcriptomic signatures of mitochondrial function, providing mechanistic validation for treatment efficacy.<sup>16</sup>

Ultimately, multi-omics approaches shift the paradigm from descriptive diagnostics toward predictive and precision healthcare in IMDs. By combining molecular signatures across different layers, clinicians will be able to stratify patients more accurately, monitor disease trajectories, and optimize individualized therapy. This integration not only enhances our mechanistic understanding but also translates into tangible clinical benefits for patients with rare metabolic disorders.

## CONCLUSION

In conclusion, both targeted and untargeted metabolomics approaches are instrumental in characterizing CMDs, identifying novel biomarkers, and guiding the development of effective diagnostic and monitoring strategies. Furthermore, these studies have shed light on previously unknown metabolites and metabolic pathways, generating valuable hypotheses about disease mechanisms and the impact of treatments. Given the often irreversible consequences of delayed diagnosis and the limited availability of effective treatments for many CMDs, continued investment in metabolomics research is crucial for improving patient outcomes.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: A.O., B.G., A.D., Concept: A.O., B.G., A.D., Design: A.O., B.G., A.D., Data Collection or Processing: A.O., B.G., A.D., Analysis or Interpretation: A.O., B.G., A.D., Literature Search: A.O., B.G., A.D., Writing: A.O., B.G., A.D.

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## REFERENCES

- Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine; Michael CM, Nass SJ, Omenn GS, editors. Evolution of translational omics: Lessons learned and the path forward. Washington (DC): National Academies Press (US); 2012 Mar 23. 2, Omics-Based Clinical Discovery: Science, Technology, and Applications. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK202165/>
- Dai X, Shen L. Advances and trends in omics technology development. *Front Med (Lausanne)*. 2022;9:911861. doi: 10.3389/fmed.2022.911861
- Amer B, Baidoo EEK. Omics-driven biotechnology for industrial applications. *Front Bioeng Biotechnol*. 2021;9:613307. doi: 10.3389/fbioe.2021.613307
- Mohr AE, Ortega-Santos CP, Whisner CM, Klein-Seetharaman J, Jasbi P. Navigating challenges and opportunities in multi-omics integration for personalized healthcare. *Biomedicines*. 2024;12(7):1496. doi: 10.3390/biomedicines12071496
- Weckwerth W. Green systems biology - from single genomes, proteomes and metabolomes to ecosystems research and biotechnology. *J Proteomics*. 2011;75:284-305. doi: 10.1016/j.jpro.2011.07.010
- Ferreira CR, Rahman S, Keller M, Zschocke J; ICIMD Advisory Group. An international classification of inherited metabolic disorders (ICIMD). *J Inherit Metab Dis*. 2021;44:164-177. doi: 10.1002/jimd.12348
- Wurth R, Turgeon C, Stander Z, Oglesbee D. An evaluation of untargeted metabolomics methods to characterize inborn errors of metabolism. *Mol Genet Metab*. 2024;141(1):108115. doi: 10.1016/j.yimgme.2023.108115
- Li L, Zhang Y, Zhou J, Wang J, Wang L. Single-cell metabolomics in rare disease: from technology to disease. *Intractable Rare Dis Res*. 2024;13(2):99-103. doi: 10.5582/irdr.2023.01073
- Glinton KE, Benke PJ, Lines MA, Geraghty MT, Chakraborty P, Al-Dirbashi OY, Jiang Y, Kennedy AD, Grotewiel MS, Sutton VR, Elsea SH, El-Hattab AW. Disturbed phospholipid metabolism in serine biosynthesis defects revealed by metabolomic profiling. *Mol Genet Metab*. 2018;123(3):309-316. doi: 10.1016/j.yimgme.2017.12.009
- Chung CCY; Hong Kong Genome Project; Chu ATW, Chung BHY. Rare disease emerging as a global public health priority. *Front Public Health*. 2022;10:1028545. doi: 10.3389/fpubh.2022.1028545
- Ney DM, Murali SG, Stroup BM, Nair N, Sawin EA, Rohr F, Levy HL. Metabolomic changes demonstrate reduced bioavailability of tyrosine and altered metabolism of tryptophan via the kynurenine pathway with ingestion of medical foods in phenylketonuria. *Mol Genet Metab*. 2017;121:96-103. doi: 10.1016/j.yimgme.2017.04.003
- Ford L, Kennedy AD, Goodman KD, Pappan KL, Evans AM, Miller LAD, Wulff JE, Wiggs BR, Lennon JJ, Elsea S, Toal DR. Precision of a clinical metabolomics profiling platform for use in the identification of inborn errors of metabolism. *J Appl Lab Med*. 2020;5(2):342-356. doi: 10.1093/jalm/jfz026
- Sidorina A, Catesini G, Sacchetti E, Rizzo C, Dionisi-Vici C. Propionic acidemia, methylmalonic acidemia, and cobalamin C deficiency: comparison of untargeted metabolomic profiles. *Metabolites*. 2024;14(8):428. doi: 10.3390/metabo14080428
- Piras D, Locci E, Palmas F, Ferino G, Fanos V, Noto A. Rare disease: a focus on metabolomics. *Exp Opin Orphan Drugs*. 2016;4:1229-1237. doi: 10.1080/21678707.2016.1250592
- van Wegberg AMJ, van der Weerd JC, Engelke UFH, Coene KLM, Jahja R, Bakker SJL, Huijbregts SCJ, Wevers RA, Heiner-Fokkema MR, van Spronsen FJ. The clinical relevance of novel biomarkers as outcome parameter in adults with phenylketonuria. *J Inherit Metab Dis*. 2024;47(4):624-635. doi: 10.1002/jimd.12732
- Phua YL, D'Annibale OM, Karunanidhi A, Mohsen AW, Kirmse B, Dobrowolski SF, Vockley J. A multiomics approach reveals evidence for phenylbutyrate as a potential treatment for combined D,L-2-hydroxyglutaric aciduria. *Mol Genet Metab*. 2024;142(3):108495. doi: 10.1016/j.yimgme.2024.108495
- Bardanzellu F, Fanos V. How could metabolomics change pediatric health? *Ital J Pediatr*. 2020;46:37. doi: 10.1186/s13052-020-00803-x
- Gertsman I, Gangoiti JA, Nyhan WL, Barshop BA. Perturbations of tyrosine metabolism promote the indolepyruvate pathway via tryptophan in host and microbiome. *Mol Genet Metab*. 2015;114(3):431-437. doi: 10.1016/j.yimgme.2015.03.012

19. Norman BP, Davison AS, Hickton B, Ross GA, Milan AM, Hughes AT, Wilson PJM, Sutherland H, Hughes JH, Roberts NB, Bou-Gharios G, Gallagher JA, Ranganath LR. Comprehensive biotransformation analysis of phenylalanine-tyrosine metabolism reveals alternative routes of metabolite clearance in nitisinone-treated alkaptonuria. *Metabolites*. 2022;12(10):927. doi: 10.3390/metabo12100927
20. Fernandes J, Saudubray JM, van den Berghe G, Walter JH, editors. *Inborn Metabolic Diseases: Diagnosis and Treatment*. 5<sup>th</sup> ed. Springer; 2012.
21. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The human serum metabolome. *PLoS One*. 2011;6(2):e16957. doi: 10.1371/journal.pone.0016957

# Expanding the Spectrum of Ethylmalonic Encephalopathy: Mild Phenotype Highlighted by Early Gastrointestinal and Cutaneous Features

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## Abstract

*Ethylmalonic encephalopathy* (EE) is a rare mitochondrial disorder usually presenting with severe early-onset manifestations. This report contributes to the expanding phenotypic spectrum of EE by describing a genetically confirmed case with a clinically mild course, thereby underlining the diagnostic relevance of early gastrointestinal and cutaneous findings.

We describe a 4-year-8-month-old girl diagnosed with EE due to a homozygous *ETHE1* c.3G > T (p.Met1?) mutation. She initially presented with persistent diarrhea beginning in early infancy, followed by livedo reticularis-like rash and progressive gait disturbance. Unlike the classical EE phenotype, the patient exhibited a stable clinical course without encephalopathic crises or rapid neuroregression. Biochemical investigations revealed markedly elevated urinary ethylmalonic acid and mildly increased plasma C4 acylcarnitine. Brain magnetic resonance imaging revealed a small T2/FLAIR hyperintense focus within the left basal ganglia, consistent with a mild and asymmetric pattern. Treatment with oral metronidazole, N-acetylcysteine, riboflavin, coenzyme Q10, carnitine, and supportive supplementation led to transient motor improvement, although petechiae and mild regression persisted during follow-up.

This case reinforces published evidence that the *ETHE1* c.3G > T (p.Met1Ile) mutation is consistently associated with attenuated disease severity. By highlighting the sequence of early gastrointestinal and vascular manifestations preceding neurological decline, this study adds to the literature on genotype-phenotype correlations in EE. Recognition of these non-neurological clues is essential for timely diagnosis, and adjunctive metabolic therapies may contribute to stabilization in atypical, milder presentations.

**Keywords:** Ethylmalonic Encephalopathy, “Metabolic Diseases, Inborn”, Mitochondrial Diseases

## INTRODUCTION

*Ethylmalonic encephalopathy* (EE) is a rare autosomal recessive mitochondrial disorder caused by biallelic pathogenic variants in the *ETHE1* gene, which encodes a mitochondrial sulfur dioxygenase. Loss of function in this enzyme results in toxic accumulation of hydrogen sulfide and its derivatives, leading to multisystemic involvement, particularly affecting the brain, gastrointestinal tract, and microvasculature.<sup>1</sup> Clinically, EE is characterized by a triad of chronic diarrhea, petechial or purpuric rash, and orthostatic or peripheral acrocyanosis, reflecting the prominent vascular component of the disease. In some patients, livedo reticularis-like discoloration may represent a milder or

variant form of acrocyanosis. Alongside these gastrointestinal and cutaneous findings, neurological manifestations such as hypotonia, developmental delay, and later spasticity or dystonia are also common. Elevated levels of ethylmalonic acid in urine and abnormalities in acylcarnitine profiles are typical biochemical hallmarks, while brain imaging often reveals basal ganglia involvement. The disease course is usually aggressive, with most patients experiencing severe encephalopathic crises and early mortality.<sup>2</sup>

In recent years, however, emerging reports have expanded the phenotypic spectrum of EE. While most patients follow the classic severe course, a small subset is described as having milder



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or atypical progressions, such as later onset of neurological decline, longer survival, or even absence of encephalopathic crises.<sup>3,4</sup> These cases indicate that EE is not invariably fulminant, and that early gastrointestinal and vascular skin findings may precede neurological manifestations and serve as key diagnostic clues. Phenotypic variability is also a well-recognized feature of other mitochondrial disorders, where modifier genes and environmental influences have been proposed to shape clinical expression.<sup>5,6</sup> A similar mechanism may also underlie the variability observed in EE.

From a genotypic perspective, specific *ETHE1* variants have repeatedly been associated with milder phenotypes. In particular, the c.3G > T (p.Met1Ile) start-codon mutation has been documented in publications and consistently linked to reduced disease severity.<sup>7,8</sup> Such genotype-phenotype correlations provide essential insights for counseling and clinical management.

Here, we report a female patient with genetically confirmed EE due to homozygous *ETHE1* c.3G>T (p.Met1Ile) mutation, who exhibited the characteristic triad of EE, albeit with a notably attenuated clinical course. This case adds to the expanding understanding of EE's phenotypic variability and emphasizes the diagnostic value of early gastrointestinal and dermatologic findings.

## CASE REPORT

A 4-year-8-month-old girl was referred for evaluation of progressive gait disturbance and recurrent falls. She was born at term by cesarean section, weighing 3750 grams, with no perinatal complications. Her parents are first cousins. The initial symptoms emerged at 2.5 months of age as persistent diarrhea. By 1.5 years, she developed a livedo reticularis-like rash on the limbs, which became more pronounced in cold temperatures (Figure 1), this was consistent with a mild acrocyanotic pattern previously described in the vascular manifestations of EE. Motor development was moderately delayed: she achieved head control at 2 months, sat at 12 months, and walked independently at 18 months. Speech developed between 18 and 24 months. She also had a known egg allergy. Family history was notable for neurodevelopmental delay in a male cousin. Neurological examination revealed hypotonia in the lower extremities and difficulty walking independently over short distances. Orthopedic evaluation demonstrated bilateral flat feet. Brain magnetic resonance imaging (MRI) showed a small T2/FLAIR hyperintense lesion in the left basal ganglia, consistent with a gliotic focus. Electromyography results were normal. Echocardiography revealed a small secundum atrial septal defect.

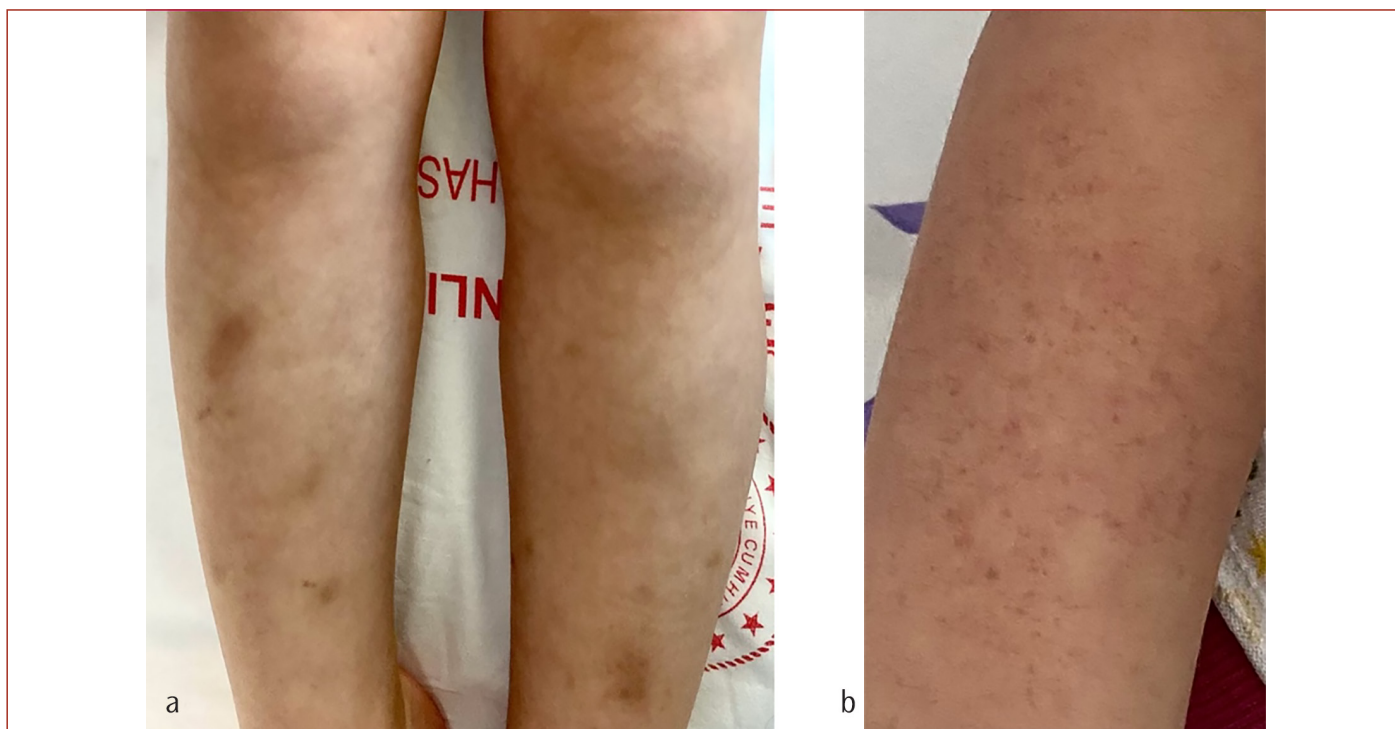


Figure 1. Cutaneous manifestations of ethylmalonic encephalopathy

Biochemical analyses demonstrated consistently elevated serum lactate (3.2 mmol/L; reference <2.2) and markedly increased urinary ethylmalonic acid (up to 554.98 mmol/mol creatinine; reference <17). The acylcarnitine profile revealed a mildly elevated butyryl-carnitine (C4) level of 0.9192  $\mu\text{mol/L}$  (reference range: 0-0.75  $\mu\text{mol/L}$ ). Molecular genetic testing identified a homozygous *ETHE1* c.3G>T (p.Met11Ile) mutation, with both parents found to be heterozygous carriers. Based on these biochemical and genetic findings, the diagnosis of EE was established at the age of 3 years.

Following confirmation of the diagnosis, treatment was initiated with oral metronidazole (30 mg/kg/day) to reduce hydrogen sulfide production, N-acetylcysteine (30 mg/kg/day) to support detoxification pathways, riboflavin (10 mg/kg/day) and coenzyme Q10 (10 mg/kg/day) for mitochondrial support, and L-carnitine (50 mg/kg/day) to enhance energy metabolism. She also received biotin, multivitamin, zinc sulfate, and vitamin D supplementation. Within two months of treatment, she regained the ability to stand with support and crawl, having been previously unable to stand independently. However, by the fourth month, she lost this ability again, while petechiae and vascular skin changes not only persisted but also became more

prominent during follow-up (Figure 1). These cutaneous findings were characterized by non-blanching pinpoint purpura on the forearm, consistent with recurrent microvascular involvement. Differential diagnoses such as vasculitis or connective tissue disease, were considered but excluded based on the chronic course, lack of systemic inflammatory markers, and the presence of biochemical abnormalities specific to EE. The clinical course, developmental milestones, laboratory investigations, and imaging results are summarized in Table 1.

## DISCUSSION

EE is classically described as a severe early-onset disorder characterized by rapidly progressive neurodegeneration, chronic diarrhea, petechiae, and acrocyanosis, with most patients succumbing within the first decade of life.<sup>2</sup> However, accumulating evidence, including recent case reports and cohort analyses, suggests that EE can exhibit a broader clinical spectrum than previously appreciated.<sup>3</sup> The patient described in this report exemplifies an atypically mild phenotype. Despite carrying a homozygous c.3G>T (p.Met11Ile) *ETHE1* mutation predicted to result in complete loss of enzyme function, she demonstrated a protracted clinical course with no acute encephalopathic crises by nearly the age of five years.

**Table 1. Chronological summary of clinical, developmental, biochemical, and imaging findings in the patient**

Age/date	Clinical findings	Developmental milestones	Laboratory results	Imaging/other investigations
2.5 months	Persistent diarrhea	Head control at 2 months	—	—
12 months	-	Sitting achieved	—	—
18 months	Livedo reticularis-like rash on limbs, more pronounced in cold (mild acrocyanotic pattern)	Independent walking achieved	—	—
18-24 months	-	Speech development	—	—
3 y (at diagnosis, treatment started)	Progressive gait disturbance, recurrent falls, petechiae, vascular skin changes	Unable to walk independently (only a few assisted steps)	Serum lactate $\uparrow$ ; Urinary EMA 554.98 mmol/mol Crea (ref <17); Plasma C4 0.9192 $\mu\text{mol/L}$ (ref 0-0.75)	MRI: small T2/FLAIR lesion (basal ganglia); EMG: normal; ECHO: small ASD
3 y 7 m	Good general condition, petechiae + livedo rash, soft systolic murmur	Walking 15-20 m independently	Urinary EMA 171.41 mmol/mol Crea $\uparrow$ ; Plasma C4 0.81 $\mu\text{mol/L}$	—
3 y 10 m	Good general condition with petechiae and livedo rash; mild reduction in walking distance	Independent walking only 8-10 steps	Urinary EMA 186.63 mmol/mol Crea $\uparrow$ ; Plasma C4 0.44 $\mu\text{mol/L}$	—
4 y 3 m	Petechiae and livedo rash; pedal edema; facial telangiectasia	Lost ability to walk independently	Urinary EMA 237.41 mmol/mol Crea $\uparrow$ ; Plasma C4 0.98 $\mu\text{mol/L}$ $\uparrow$	—
4 y 8 m	Persistent diarrhea (2-3 times/day); abdominal pain; petechiae and livedo rash; lower-limb spasticity and hyperreflexia	Stopped walking, only crawling	EMA 85.10 mmol/mol Crea $\uparrow$ ; C4 1.0365 $\mu\text{mol/L}$ $\uparrow$	—

Treatment initiated at age 3 years. Serum lactate <2.2 mmol/L; urinary EMA <17 mmol/mol creatinine; plasma butyryl-carnitine (C4) 0-0.75  $\mu\text{mol/L}$ . ASD: Atrial septal defect, ECHO: Echocardiography, EMA: Ethylmalonic acid, EMG: Electromyography, MRI: Magnetic resonance imaging

This phenotypic heterogeneity has been well documented in recent literature. Kashima et al.,<sup>4</sup> described a child harboring a homozygous *ETHE1* c.586G>A variant who exhibited only mild motor and speech delay, subtle biochemical abnormalities, and a normal MRI, reinforcing the notion that even pathogenic *ETHE1* variants may yield attenuated clinical courses. Similarly, Ersoy et al.,<sup>3</sup> described an 11-year-old boy with the same c.3G>T variant as our patient who did not exhibit cognitive impairment or encephalopathic episodes but suffered from spastic paraparesis and chronic diarrhea. These cases, along with our patient, support the influence of modifier genes or residual enzyme activity in shaping disease severity. Environmental triggers, infection burden, and possible nuclear genetic modifiers have been hypothesized as additional contributors to this clinical variability, although definitive data are lacking.

The clinical progression in our patient parallels that of classical EE, though in a milder and slower course. Diarrhea emerged as the first symptom in early infancy, followed by vascular skin changes around 18 months and progressive gait disturbance in later childhood. This sequence aligns with the pattern proposed in classical EE, albeit in a slower and less aggressive form. Kasapkara et al.<sup>9</sup> have emphasized the diagnostic significance of early gastrointestinal and cutaneous features, for instance, persistent diarrhea and livedo as early signs leading to EE diagnosis in two affected siblings. In our case, the diagnosis was delayed despite the presence of persistent diarrhea and vascular rash in infancy. This delay highlights the lack of awareness about the disease and the non-specific nature of early symptoms, which can be mistaken for common pediatric conditions. Increased recognition of these signs among pediatricians and dermatologists could reduce diagnostic delays.

Cutaneous manifestations, including livedo reticularis-like rash and recurrent petechiae, are frequent but often underrecognized features of EE. These can mimic vasculitis, collagen vascular disorders, or infectious purpura, leading to potential diagnostic delays. In our patient, the chronicity of lesions since infancy, their cold-induced exacerbation, and their coexistence with metabolic hallmarks (ethylmalonic aciduria, elevated C4) supported their attribution to EE. Therefore, careful dermatologic evaluation may provide an early diagnostic clue in atypical or milder phenotypes.

Biochemically, our patient displayed markedly elevated urinary ethylmalonic acid and mildly increased plasma C4 acylcarnitine, consistent with the metabolic fingerprint of EE. Hydrogen sulfide accumulation, resulting from deficient activity of the *ETHE1*-encoded mitochondrial sulfur dioxygenase, leads to secondary inhibition of short-chain acyl-CoA dehydrogenase and cytochrome c oxidase.<sup>10</sup> This pathophysiological mechanism accounts for both the characteristic biochemical abnormalities

and the multisystem clinical features observed in EE. The critical role of the *ETHE1*-encoded mitochondrial sulfur dioxygenase in mitochondrial hydrogen sulfide detoxification has been well established in previous studies.<sup>3,11</sup>

Treatment in EE remains primarily supportive, symptomatic. Our patient received continuous therapy for about two years with metronidazole, N-acetylcysteine, and mitochondrial cofactors such as riboflavin, coenzyme Q10, carnitine, and biotin. These agents aim to reduce hydrogen sulfide accumulation and support oxidative metabolism. Although not curative, they may slow progression and improve quality of life.<sup>12</sup> In our case, transient motor improvements were noted after therapy initiation, consistent with previous reports describing partial clinical responses to metronidazole and N-acetylcysteine therapy.<sup>12,13</sup> However, these improvements were reported by parents and clinical observation rather than standardized scales. The persistence of petechiae and gradual motor regression over time suggest that treatment may have stabilized but did not reverse disease activity. Notably, high-dose intravenous N-acetylcysteine has been reported to improve encephalopathic crises in EE patients,<sup>13</sup> further supporting the therapeutic potential of such metabolic interventions, even in severe presentations.

The literature review by Platt et al.<sup>7</sup> provides additional support for the existence of milder phenotypes. In their cohort of 70 patients, eight cases were classified as mild, characterized by slower neurological progression and lower urinary ethylmalonic acid and C4 acylcarnitine levels compared with classical cases. Among the reported genotypes, the *ETHE1* c.3G>T (p.Met11Ile) variant was one of the most frequent and was exclusively associated with mild presentations, as no patients harboring this variant exhibited a classical or severe phenotype. Importantly, the current age of patients with mild phenotypes was significantly higher, indicating better long-term outcomes. The study also emphasized that symptom onset in milder cases is often dominated by gastrointestinal and vascular manifestations rather than acute neuroregression, further supporting our observations.

In summary, this case contributes to the literature on EE in several important ways. First, it reinforces the genotype-phenotype correlation by demonstrating that the recurrent *ETHE1* c.3G>T (p.Met11Ile) mutation, typically associated with severe disease, can manifest with a mild phenotype. Second, it highlights the diagnostic value of early gastrointestinal and vascular manifestations, which in our patient preceded neurological decline and could help shorten diagnostic delays if recognized. Recognizing these early findings may enable earlier metabolic intervention and prevent irreversible neurological damage. Third, it underscores the risk of misattributing these early non-neurological signs to common pediatric conditions,

emphasizing the need for awareness among pediatricians and dermatologists. Finally, it provides additional evidence that continuous administration of metronidazole, N-acetylcysteine, and mitochondrial cofactors may help stabilize the clinical course, even if they do not reverse disease activity. Taken together, these observations strengthen our understanding of the phenotypic spectrum of EE and offer practical insights for diagnosis and management.

(a) Livedo reticularis-like discoloration on the anterior aspects of both lower limbs, more pronounced in cold temperatures, consistent with a mild acrocyanotic pattern previously described in EE. Differential diagnoses such as vasculitis, connective tissue disorders, and infectious etiologies were considered. However, the persistence of the rash since infancy, absence of systemic inflammatory markers, and concurrent metabolic abnormalities supported attributing it to EE-related microangiopathy.

(b) Petechial lesions on the right forearm observed four months after treatment initiation (age 3 years 4 months). Multiple non-blanching, pinpoint purpuric macules are visible, consistent with recurrent cutaneous microvascular involvement. These findings coincided with loss of previously regained motor abilities, while petechiae and vascular skin changes persisted and became more prominent during follow-up, illustrating the chronic and fluctuating course of the disease.

## Ethics

**Informed Consent:** Informed consent was obtained from the patients' family and/or patients in the study.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: M.Y.Ç., T.Ç., Concept: M.Y.Ç., Design: M.Y.Ç., B.K., E.B., Data Collection or Processing: M.Y.Ç., B.K., E.B., T.Ç., Analysis or Interpretation: M.Y.Ç., Literature Search: M.Y.Ç., Writing: M.Y.Ç.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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## REFERENCES

1. Tiranti V, Diodato D, Lamantea E, Accarino G, Fernandez-Vizarrá E, Mora M, Zeviani M. *ETHE1* mutations are specific to ethylmalonic encephalopathy. *J Med Genet*. 2006;43(4):340-346. doi: 10.1136/jmg.2005.036210
2. Govindaraj P, Vedantham S, Chacko A, Vasudevan A. Child neurology: ethylmalonic encephalopathy. *Neurology*. 2020;94(12):e1336-e1339. doi: 10.1212/WNL.00000000000009144
3. Ersoy M, Tiranti V, Zeviani M. Ethylmalonic encephalopathy: clinical course and therapy response in an uncommon mild case with a severe *ETHE1* mutation. *Mol Genet Metab Rep*. 2020;25:100641. doi: 10.1016/j.ymgmr.2020.100641
4. Kashima DT, Sloan-Heggen CM, Gottlieb-Smith RJ, Barone Pritchard A. An atypically mild case of ethylmalonic encephalopathy with pathogenic *ETHE1* variant. *Am J Med Genet A*. 2023;191(6):1614-1618. doi: 10.1002/ajmg.a.63176
5. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med*. 2003;348(26):2656-2668. doi: 10.1056/NEJMra022567.
6. Chen C, Chen Y, Guan M-X. A peep into mitochondrial disorder: multifaceted from mitochondrial DNA mutations to nuclear gene modulation. *Protein Cell*. 2015;6(12):862-870. doi: 10.1007/s13238-015-0202-5
7. Platt I, Bisgin A, Kilavuz S. Ethylmalonic encephalopathy: a literature review and two new cases of mild phenotype. *Neurol Sci*. 2023;44(11):3827-3852. doi: 10.1007/s10072-023-06904-8
8. Yiş U, Hiz Kurul S, Duman O, Yılmaz C, Dirik E. Importance of acrocyanosis in delayed walking. *J Pediatr Neurosci*. 2015;10(1):80-81. doi: 10.4103/1817-1745.154368
9. Kasapçara ÇS, Güzel-Ozantürk A, Dursun A, Sivri HS, Tokatlı A, Coşkun T. Siblings with ethylmalonic encephalopathy: case report. *Turk J Pediatr*. 2018;60(1):104-107. doi: 10.24953/turkjped.2018.01.018
10. Zafeiriou DI, Augoustidou-Savvopoulou P, Haas D, Lehnert W, Vargiami E, Tsantali C, Sewell AC. Ethylmalonic encephalopathy: clinical and biochemical observations. *Neuropediatrics*. 2007;38(2):78-82. doi: 10.1055/s-2007-984447
11. Luna-Sánchez M, Díaz-Casado ME, Barca E, Tejada MA, Montilla-García Á, Cobos EJ, Escames G, Acuña-Castroviejo D, Quinzii CM. CoQ deficiency causes disruption of mitochondrial sulfide oxidation, a new pathomechanism associated with this syndrome. *EMBO Mol Med*. 2017;9(1):78-95. doi: 10.15252/emmm.201606345
12. Viscomi C, Burlina AB, Dweikat I, Savoirdo M, Lamperti C, Hildebrandt T, Tiranti V, Zeviani M. Combined treatment with oral metronidazole and N-acetylcysteine is effective in ethylmalonic encephalopathy. *Nat Med*. 2010;16(8):869-871. doi: 10.1038/nm.2188
13. Kılıç M, Tokatlı A, Dursun A, Coşkun T, Sivri HS. Successful treatment of a patient with ethylmalonic encephalopathy by intravenous N-acetylcysteine. *Metab Brain Dis*. 2017;32(2):293-296. doi: 10.1007/s11011-016-9928-5