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REVIEW

Structure, Biochemical Role and Importance of Carboxylase Class Enzymes in Metabolism

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ABSTRACT

Cells, the smallest structures showing the structural and functional characteristics of a living being, obtain their biomass from inorganic carbon. Metabolites involved in central carbon metabolism are utilised within the TCA cycle for numerous biosynthetic purposes, such as synthesizing amino acids and fatty acids. To replenish the intermediates of the TCA, many organisms use anaplerotic reactions, usually involving enzymes such as pyruvate carboxylase, glutamate dehydrogenase, PEP carboxylase, and transaminase reactions. Carboxylases are important in fatty acids, amino acids, carbohydrate metabolism, polyketide biosynthesis, urea utilisation, and other cellular processes. Acetyl-CoA carboxylase, propionyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase, and pyruvate carboxylase are carboxylase group enzymes that have been studied, and their roles in metabolism are well known. As a result of a problem in the production of enzymes involved in metabolism or a situation that prevents them from fulfilling their catalytic activities, abnormal and harmful organic acid metabolites accumulate in the cell. In metabolism, differential diagnoses are important to determine enzyme deficiencies and/or the determination of the catalytic activity of the relevant enzyme. Diagnosis of enzyme deficiencies can be made by genetic, biochemical, imaging, and molecular methods. It should be considered that these enzymes, whose catalytic activities are examined only in a few rare diseases, may be one of the underlying causes of diseases that occur in the metabolic process. Therefore, developing highly accurate, cost-effective, and reproducible methods for analyzing carboxylase group enzymes will greatly benefit patients in terms of the treatment process.

Keywords: Acetyl-CoA carboxylase. Propionyl-CoA carboxylase. 3-methylcrotonyl-CoA carboxylase. Pyruvate carboxylase. Biochemistry. Metabolism.

Karboksilaz Sınıfı Enzimlerin Yapısı, Biyokimyasal Rolü ve Metabolizmadaki Önemi

ÖZET

Organizmalar, tüm hücre biyokütlesini heterotrofik ve ototrofik yollar ile inorganik karbondan (CO_2) elde ederler. Merkezi karbon metabolizmasında yer alan metabolitler amino asitler ve yağ asitlerinin sentezi gibi çok sayıda biyosentetik amaçlar için TCA döngüsünden kullanılır. TCA döngüsünün ara maddelerini yeniden doldurmak için, birçok organizma genellikle piruvat karboksilaz, glutamat dehidrogenaz, PEP karboksilaz, glutamat dehidrogenaz, transaminaz tepkimeleri gibi bir karboksilasyon reaksiyonu kullanan anaplerotik reaksiyonlardan yararlanır. Doğada yaygın olarak bulunan karboksilazlar yağ asitleri, amino asitler, karbohidrat metabolizmasında, poliketid biyosentezinde, üre kullanımında ve diğer hücresel süreçlerde önemli rollere sahiptirler. Asetil-CoA karboksilaz (ACC), propiyonil-CoA karboksilaz (PCC), 3-metilkrotonil-CoA karboksilaz (MCC) ve piruvat karboksilaz (PC) çalışılmış ve metabolizmadaki rolleri bilinen karboksilaz grubu enzimlerdir. Metabolizmada görevli enzimlerin üretilmesinde bir sorun veya katalitik aktivitelerini yerine getiremesine engel bir durumun oluşması sonucunda hücre içersinde anormal ve zararlı organik asit metabolitlerinin birikimi söz konusu olmaktadır. Metabolizmada enzim eksiklikleri ve/veya ilgili enzime ait katalitik aktivite tayinini belirlemek için ayırıcı tanılar önem arz etmektedir. Enzim eksikliklerinde tanı genetik, biyokimyasal, görüntüleme ve moleküler yöntemler ile konulabilmektedir. Sadece bazı nadir hastalıklarda katalitik etkinlikleri incelenen bu enzimlerin metabolik süreçte meydana gelebilecek hastalıkların altında yatan sebeplerden biri olabileceği göz önünde bulundurulmalıdır. Bu nedenle karboksilaz grubu enzimlerin analizlerine yönelik geliştirilecek doğruluğu yüksek, uygun maliyetli ve tekrarlanabilir analiz metodları hastaların tedavi süreci açısından büyük fayda sağlayacaktır.

Anahtar Kelimeler: Asetil-CoA karboksilaz. Propiyonil-CoA karboksilaz. 3-metilkrotonil-CoA karboksilaz. Piruvat karboksilaz. Biyokimya. Metabolizma.

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Inorganic carbon and the importance of the tricarboxylic acid cycle in carbon metabolism

Cells are the smallest structures exhibiting the structural and functional characteristics of a living being andobtain their biomass from inorganic carbon (CO_2)[1]. For biomass synthesis, microorganisms must obtain carbon through autotrophic and heterotrophic pathways[2]. The autotrophic pathway involves carbon dioxide fixation, which enables the conversion of CO_2 into biomass[3]. During this fixation, which takes place in autotrophic pathways, carboxylating enzymes convert CO_2 into acetyl-coenzyme A, pyruvate (PA), or one of the intermediates of the tricarboxylic acid (TCA) cycle (Citric acid cycle, or Krebs cycle), which is a central junction in carbon metabolism[4] (Figure 1). However, some biomolecules synthesised in metabolism can be degraded and converted into biomass by an essential carboxylation step. After the central metabolites are produced in anabolism, they are consumed for the biosynthesis of other molecules, ultimately leading to the depletion of TCA cycle intermediates[5]. To replenish the intermediates of the TCA cycle, many organisms utilise anaplerotic reactions, usually involving a carboxylation process catalyzed by enzymes such as pyruvate carboxylase (PC), or other enzymes like glutamate dehydrogenase, PEP carboxylase, and transaminase reactions[2]. Molecules in the TCA cycle synthesize metabolites such as fatty and amino acids. For the metabolic system's continuity, the TCA cycle's main metabolites must be continuously replenished through anaplerotic reactions[6]. The conversion of PA or phosphoenolpyruvate (PEP) to oxaloacetic acid (OAA) by the enzymes PEP carboxylase and biotin-dependent PC is the best-known anaplerotic reaction[7]. The four-carbon OAA produced is used in protein synthesis to form arginosuccinate, a metabolite of the urea cycle. In addition, OAA is converted to aspartate, which is derived from glutamate and then used to form γ-aminobutyric acid (GABA, which acts as a neurotransmitter. OAA deficiency also leads to a deficiency in NADH production[8]. With glucose deficiency and/or slowing down of the glycolysis pathway in the body, mitochondrial OAA formed in the TCA cycle is converted into PEP and the gluconeogenesis pathway is activated. Inorganic carbon is used to facilitate fatty acid biosynthesis by activating the acetyl-CoA molecule. In order to extend the fatty acid chain, malonyl-CoA is provided through the reaction catalyzed by the acetyl-CoA carboxylase enzyme[9].

The first part of this review aims to provide a comprehensive overview of the different functions of carboxylases in metabolism and the principles underlying the carboxylation reactions involved, to enable the reader to explore the chemical and functional diversity of the carboxylase class of enzymes. The second part presents the methods and challenges of determining the deficiency of carboxylase enzymes, as well as discussions and suggestions for future studies on carboxylases affecting metabolism.

Carboxylase Class Enzymes and Structures

Carboxylases are a group of enzymes that play a critical role in biochemical processes, catalyzing reactions by adding carbon dioxide (CO_2). These enzymes are important in basic biological functions such as energy production, metabolic regulation, and biosynthesis. Understanding the mechanisms of carboxylase enzymes provides deeper insights into cellular processes and contributes to the development of potential therapeutic strategies in medicine and biotechnology. Therefore, the discussion of carboxylase enzymes' structural and functional properties is of foremost importance for both basic biology and applied sciences.

Carboxylases, which were discovered many years ago, are one of the most studied enzymes due to their functions in metabolism[4]. In addition to carbohydrate, fat, and amino acid metabolism, carboxylases involved in urea and polyketide synthesis have important roles in many biochemical processes. PC, MCC, PCC, and ACC are enzymes of the carboxylase group that have been studied, and their roles in metabolism are known[9]. These biotin-dependent carboxylases catalyse carboxylation reactions through two enzymatic activities: a biotin carboxylase (BC), which functions in processing CO_2, and a carboxyl transferase (CT) component that catalyses the transfer of the CO_2 molecule. Biotin-dependent carboxylases contain four domains: the biotin carboxylase domain (BC), the biotin carboxyl carrier protein domain (BCCP), the carboxyltransferase domain (CT), and the domain linking the BC domain and the CT domain (BC-CT). The catalysis mechanism briefly follows: covalently binding to a lysine side chain in the biotin carboxyl carrier protein (BCCP) component via an amide bond, catalyzes CO_2 transfer by acting like an arm that moves back and forth[10].

Carboxylase enzymes are a group that plays a critical role in the regulation of various metabolic pathways in biological systems. These enzymes have structural and functional similarities and differences depending on their specific roles in metabolism. Carboxylases generally carboxylate substrates by adding carbon dioxide (CO_2). The fact that different types of carboxylases have different cofactors and specific substrates is a crucial factor that functionally distinguishes them from each other (Table I). The following table will provide a valuable resource for understanding carboxylase group enzyme functional similarities and differences. The metabolic pathways and target substrates indicated for each enzyme will

Importance of Metabolic Functions of Carboxylases

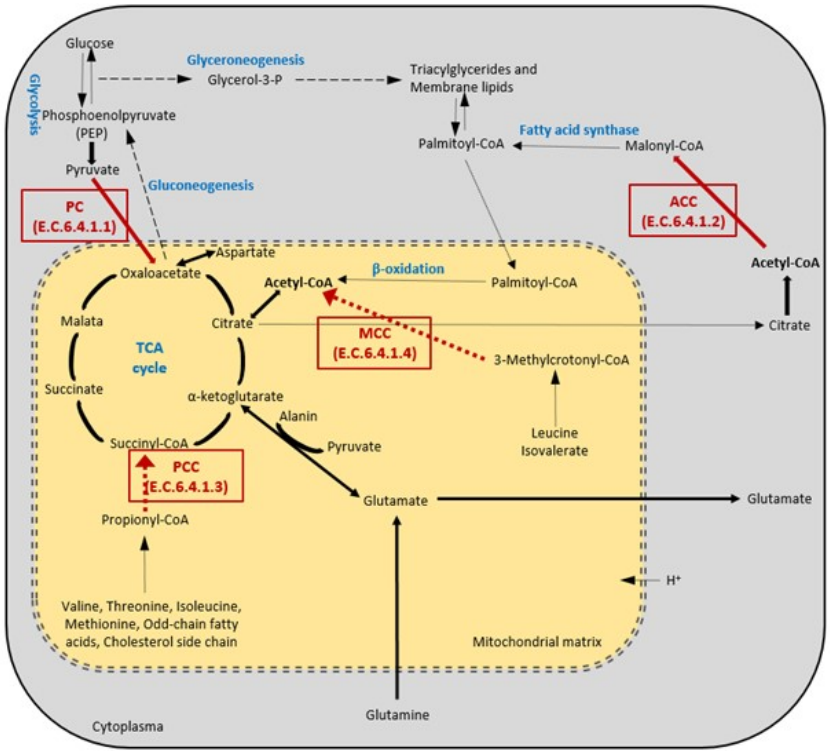

Figure 1:

General appearance of carboxylases in metabolic pathways together with the TCA cycle. Acetyl-CoA carboxylase (ACC) and pyruvate carboxylase (PC) are located in the cytoplasm, while propionyl-CoA carboxylase (PCC) and 3-methylcrotonyl-CoA carboxylase (MCC) are located in the mitochondria.

Table I. Comparative overview table of carboxylase enzymes: structural and functional similarities and differences

Enzyme Name	Cofactor	Metabolic Role	Target Substrate	Associated Metabolic Pathways	Structural Similarities	Functional Differences	References
PC	Biotin	Produces oxaloacetate for gluconeogenesis and energy production	Pyruvate	Gluconeogenesis, Krebs cycle	Biotin-binding domain, multi-domain structure	Plays a crucial role in gluconeogenesis, requires ATP and CO_2.	11-13,63,64
ACC	Biotin	Initiates fatty acid biosynthesis and energy storage	Acetyl-CoA	Fatty acid synthesis, lipid metabolism	Biotin-binding and catalytic domains, dimeric form	Critical role in fatty acid biosynthesis, produces malonyl-CoA.	9,20,22
PCC	Biotin	Regulates the conversion of propionic acid for energy production	Propionyl-CoA	Amino acid metabolism, energy production	Biotin-dependency, monomeric or dimeric structure	Involved in amino acid metabolism, particularly propionic acid metabolism.	4,30,31
MCC	Biotin	Important in isoleucine metabolism	Methylcrotonyl-CoA	Amino acid metabolism, energy production	Biotin-binding and carboxylation features, heteromeric structure	Converts methylcrotonyl-CoA to 3-methylglutamate, linked to genetic disorders.	39-41,65

ACC: Acetyl-CoA carboxylase, PC: pyruvate carboxylase, PCC: propionyl-CoA carboxylase, MCC: 3-methylcrotonyl-CoA carboxylase

provide important benefits in better understanding diseases and following therapeutic developments.

Pyruvate carboxylase (PC) (EC 6.4.1.1.1)

PC is an enzyme composed of four subunits. PC is an essential enzyme with an anaplerotic role, regenerating intermediates of the TCA cycle for the biosynthesis of glucose, fatty acids, amino acids, and other molecules by carboxylating pyruvate to OAA[11] (Figure 2). PA is the end product of glycolysis and an important carbon flux source into the TCA cycle. The mitochondrial PA carrier transports Cytosolic PA into the mitochondrial matrix. Mitochondrial PA can then be converted to OAA by the reaction catalysed by the PC enzyme or to acetyl-CoA by the reaction catalysed by pyruvate dehydrogenase (PDH)[12]. The PC enzyme,

which is localised in the mitochondrial matrix, shows catalytic activity in gluconeogenesis in the liver and kidney, lipogenesis in adipocytes, and the biosynthesis of glutamate, which functions as a neurotransmitter[8]. In starvation, PC and phosphoenolpyruvate carboxykinase activities increase, increasing PA flux through gluconeogenesis. PC, whose activity is allosterically regulated by acetyl-CoA in liver, brain, fat and pancreatic β-cells, increases the production of OAA, which will combine with acetyl-CoA to form citrate[12].

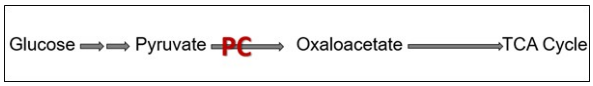

Figure 2:
The reaction catalysed by pyruvate carboxylase enzyme (PC).

As a result of increased PC activity in starvation, the gluconeogenesis pathway is also activated. Thus, glucose is supplied to organs that use only glucose as fuel[8]. Citrate, produced by the combination of OAA and acetyl-CoA in the TCA cycle, is transported to the cytoplasm and is broken down to synthesize fatty acids in lipogenesis. In addition, OAA produced by PC can be converted to malate, catalysed by the enzyme malate dehydrogenase, depending on the concentration of TCA cycle metabolites and the needs of the cell. Thus, high mitochondrial acetyl-CoA levels stimulate the conversion of cytoplasmic PA to OAA to feed the TCA cycle. The acetyl-CoA molecule is the product of the PDH enzyme and the substrate of the PC enzyme. Therefore, the interaction between these two enzymes regulates carbon flow during fasting or satiety. Malate transported to the cytosol is converted to PA in a process catalyzed by pyruvate decarboxylase.

Meanwhile, $NADP^+$ is reduced to NADPH, which is then used to convert oxidised glutathione (GSSG) to reduced glutathione (GSH). The reducing power of NADPH is then utilised for ATP production, which maintains glucose homeostasis by influencing the secretion of insulin, an anabolic hormone[11]. Besides its anaplerotic role in the TCA cycle, PC plays a key role in synthesizing reducing molecules for protection against oxidative stress. GSH, which reduces hydrogen peroxide to water, is one of the most important antioxidants in the body. In addition to its importance in metabolic pathways, PC is closely related to controlling the body's oxidative stress levels[13].

In case of deficiency in the functioning of the PC enzyme, various metabolic disorders occur. In PC deficiency, PA accumulated in plasma is converted to lactate and causes an increase in plasma lactic acid concentration. Due to the decrease in PA concentration, OAA production also decreases. This increases gluconeogenesis and promotes hypoglycaemia. With hypoglycaemia, acetyl-CoA oxidation derived from PA and fatty acids is inhibited, and the concentration of ketone bodies (acetoacetic acid, acetone, and β-hydroxybutyric acid) increases[14]. Since the energy required for brain functions cannot be produced due to PC deficiency, developmental delay, recurrent seizures, neurological disorders, and metabolic acidosis occur in newborns[15]. Deficiency in PC activity is a rare autosomal recessive metabolic disorder in humans. It has been associated with three clinical manifestations: Forms A, B, and C. In Form A, there is chronic, mild to moderate lactic acidemia, psychomotor retardation, and hypotonia, and most patients die within a few years after birth. In Form B, severe lactic acidemia is associated with the most severe symptoms, including hypoglycaemia, hyperammonemia, anorexia, and convulsions. Patients usually die within the first months of life. In Form C, mild lactic acidemia and mild neurological symptoms may occasionally be seen[16]. In recent years, studies investigating the role of PC during tumour development and growth have gained momentum. Many studies have indicated that PC-mediated anaplerosis plays an important role in the growth and survival of cancer cells[8,17,18]. These studies suggest that, in addition to the anaplerotic role of PC in producing OAA in normal cells, it also plays a vital role in supporting the metabolic requirements of cancer cells due to its functions in gluconeogenesis, amino acid, and de novo fatty acid synthesis. Therefore, targeting the PC enzyme may provide additional tools for tumour clearance and/or control[19].

Acetyl-CoA carboxylase (ACC) (E.C. 6.4.1.2)

Acetyl-CoA, the starting compound for the TCA cycle, is an important precursor in lipid biosynthesis and the source of all fatty acid carbons[9] (Figure 3). In the functional structure of ACC, a multidomain polypeptide catalyzes three different activities. The carboxylation of biotin occurs in the BC domain by consuming ATP and bicarbonate first. Then, the carboxyl moiety is transferred to the CT domain via the BCCP domain, which functions as a swinging arm, and subsequently transferred to acetyl-CoA to produce malonyl-CoA[20]. This is the first and decisive step in synthesizing long-chain saturated fatty acids catalysed by fatty acid synthase. The ACC enzyme provides the two-carbon building block for de novo fatty acid biosynthesis and regulates the rate-limiting steps in their conversion to fatty acids[9].

ACC1, a member of the ACC family, is located in the cytosol of the liver, adipose, and other lipogenic tissues. ACC1 converts cytoplasmic acetyl-CoA to malonyl-CoA for lipogenesis. It catalyses the

biosynthesis of palmitate and longer chain fatty acids used in the synthesis of triglycerides (TG) and very-low-density lipoproteins (VLDL)[21] (Figure 1). ACC2, a member of ACCs, is associated with the outer mitochondrial membrane and is most abundant in heart and skeletal muscle mitochondria. Carnitine palmitoyltransferase I (CPT-I) ensures the transport of long-chain fatty acyl-CoAs into mitochondria for β-oxidation. Malonyl-CoA produced by the ACC2 isoform allosterically affects the activity of CPT-I[22]. In mammals, the activity of both ACC1 and ACC2 is stimulated by citrate, inhibited by long-chain saturated acetyl-CoA and inactivated by phosphorylation, particularly by AMP-activated protein kinase and cAMP-dependent protein kinase[23]. Acetyl-CoA is a precursor of the neurotransmitter acetylcholine[24] and positively regulates PC activity[25]. Histone acetylases use acetyl-CoA as a donor of acetyl groups in post-translational acetylation reactions involving histone and non-histone proteins[26].

Figure 3:
The reaction catalysed by the enzyme acetyl-CoA carboxylase (ACC).

In addition to their known roles in metabolism, acetyl-CoA and malonyl-CoA regulate the physiopathological processes of cells by participating in signalling pathways and cellular regulation. ACC activity increases in the presence of excess nutrients and energy, which is stored as fatty acids. ACC activity is suppressed in starvation. In both cases, the regulatory sensor that acts on ACC, whether phosphorylated or dephosphorylated, is the enzyme AMP-activated protein kinase (AMPK)[27]. In addition to phosphorylation, the activity of ACCs can be regulated allosterically by factors such as citrate levels. Fatty acid oxidation disorders are diseases caused by impaired β-oxidation via the carnitine/palmitoyl shuttle pathway or genetically inborn impairment of fatty acid transfer[13]. ACCs play important roles in the interaction of multiple biological processes by facilitating the connection between fatty acid synthesis and other metabolic pathways. Acetyl-CoA deficiency was first recognized by Blom et al[28]. Deficiency of the ACC1 enzyme significantly affects lipid metabolism. A problem in lipid metabolism leads to significant disruptions in various cellular metabolic pathways such as cell motility, cell growth, and cell death. Therefore, it is associated with many diseases such as cancer and metabolic diseases[29].

Propionyl-CoA carboxylase (PCC) (EC 6.4.1.3)

PCC, activated by monovalent cations (K^+, NH_4^+, Cs^+) in the mitochondrial matrix, is an enzyme of the carboxylase class that catalyses the conversion of propionyl-CoA to methylmalonyl-CoA by carboxylation with bicarbonate[30] (Figure 4). The resulting methylmalonyl-CoA is converted to succinyl-CoA and participates in the anaplerotic regeneration of TCA intermediates. PCC is crucial for the catabolism of methionine and β-branched amino acids (Thr, Val, Ile), cholesterol side chain, and fatty acids with an odd number of carbon atoms[4]. The structure of the PCC holoenzyme consists of four layers (α3-β3-β3-α3) with three subunits in each layer. At BCCP in the active site, carboxylation occurs via BC in the presence of MgATP; then, as a result of interaction with CT, the carboxy group is transferred from biotin to the α-carbon of propionyl-CoA, resulting in the synthesis of methylmalonyl-CoA[31]. The participation of methylmalonyl-CoA in the anaplerotic regeneration of TCA intermediates is ensured through succinyl-CoA formed by the catalysis of methylmalonyl-CoA mutase, methylmalonyl-CoA epimerase, and the Cobalamin A and B enzymes.

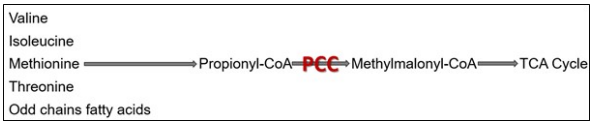

Figure 4:
The reaction catalysed by the enzyme propionyl-CoA carboxylase (PCC).

Since the decrease in the activity of PCC affects the activities of alanine and aspartate aminotransferase enzymes, the concentrations of OAA, malate, and PA formed as a consequence of transamination reactions may also change[32].

Intracellular propionyl-CoA accumulation also inhibits mitochondrial metabolism and reduces citrate, GTP, and ATP[33]. Propionyl-CoA is the end product of the beta oxidation of odd-numbered fatty acids. Inhibition of PCC activation may lead to the accumulation of single-chain fatty acids and affect anabolic reactions[30].

Symptoms of propionic acidemia (PA), which is caused by hereditary deficiencies in PCC activity, first appear in the neonatal period[34]. PA may be caused by mutations in one or both genes encoding PCCA or PCCB, which are propionyl-CoA carboxylase (PCC) subunits, or a deficiency of coenzymes such as biotin[35]. Neurodevelopmental problems, attention deficit-hyperactivity disorder, leukopenia, immunological deficiency, cardiomyopathy, arrhythmia, pancreatitis, and developmental delay are conditions associated with PA[36]. The spectrum of PA

ranges from neonatal-onset disease to late-onset disease[33,34,37,38]. PCC is expressed in the brain and may be important for neurodevelopment, so it is thought that PCC deficiency may be associated with neurological effects, including epilepsy and seizures[30].

Methylcrotonyl-CoA carboxylase (MCC) (EC 6.4.1.4)

MCC carboxylates 3-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA in the leucine catabolic pathway[39] (Figure 5). Leucine is a ketogenic amino acid converted to acetyl-CoA in a six-step pathway, the fourth step of which is catalyzed by MCC. MCC, located in the inner membrane of mitochondria, utilises ATP and bicarbonate in the reaction required for leucine and isovalerate catabolism[40]. MCC, highly expressed in the kidney and liver, is a heterodimeric enzyme composed of α and β subunits in (αβ)6 configuration, with MCCα containing the biotin-carboxylation and biotin transporter domains, and MCCβ containing the carboxyltransferase domain[41].

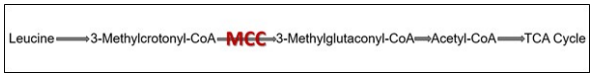

Figure 5:
The reaction catalysed by the enzyme 3-methylcrotonyl-CoA carboxylase (MCC).

Enzyme deficiencies in leucine catabolism lead to hypervalineemia, hyperleucine-isoleucinaemia, maple syrup urine disease, isovaleric acidemia, isolated 3-methylcrotonyl-CoA carboxylase deficiency, methylcrotonylglycinuria, methylglutaconic aciduria, and 3-hydroxy-3-methylglutaric aciduria[42]. In humans, deficiencies in MCC activity are linked to 3-methylcrotonylglycinuria (MCG), one of the most frequently observed inborn errors of metabolism[43]. The clinical manifestations of MCG are highly variable, ranging from asymptomatic individuals to severe cases with neonatal onset that can result in death[44].

Diagnostic Methods in Carboxylase Deficiency

Differential diagnoses are important for determining enzyme deficiencies and the catalytic activity of the related enzyme in metabolism. Genetic, biochemical, imaging, and molecular methods can diagnose enzyme deficiencies. The enzymatic diagnosis of carboxylase enzyme deficiency is made by identifying the deficiency of the relevant carboxylase enzyme in whole blood in a proband with supporting metabolic analyte findings. Genetic mutations of the PC, ACC, MCC, and PCC enzymes cause deficiency of the relevant carboxylase enzyme. Enzyme activity tests performed in fibroblasts are also confirmatory molecular tests used to determine enzyme deficiency. In addition to molecular and genetic tests, biochemical diagnostic tests are performed to detect the relevant enzyme deficiency.

Genetic tests and enzyme activity measurements are important diagnostic tools in carboxylase enzyme deficiencies. Genetic tests are used to detect mutations in the genes encoding carboxylase enzymes. For example, conditions such as PC or PCC deficiency can be determined by gene mutations. Measurement of enzyme activity in fibroblast cell lines (e.g., skin cells) or other biopsy samples is also used to confirm the diagnosis[45]. The table at the end of the chapter summarises the diagnostic methods, with references that may provide additional details for each approach (Table II).

Genetic testing methods may include combining single gene testing, multi-gene panel gene-targeted, multi-gene panels, and comprehensive genomic testing, such as exome and genome sequencing[46]. Gene-targeted testing requires the clinician to identify which gene is or genes are likely to be involved, whereas comprehensive genomic testing does not require such identification. Molecular genetic diagnosis of the enzyme deficiency of interest is established by identification in molecular genetic testing of biallelic PC pathogenic variants in a proband, supported by metabolic analyte findings[47]. In individuals with carboxylase enzyme deficiency, cultured fibroblasts or lymphocyte-based enzyme activity is usually less than 10% of that observed in controls[48].

Although genetic testing is a powerful tool in diagnosing metabolic disorders such as carboxylase enzyme deficiencies, interpretation of variants has many limitations and challenges, such as accessibility, cost, and training[49]. These limitations can make accurate diagnosis difficult and affect the treatment of patients. Variants are a natural part of the genetic makeup of many people. This can make it difficult to determine whether certain variants are associated with the disease clearly[50]. In carboxylase enzyme deficiencies, a single genetic variant may not always be the cause of the entire disease. Multigenic interactions or environmental factors may play a role in the development of the disease, which complicates the interpretation of genetic test results[50]. Genetic tests, especially those that detect genetic mutations, may not be sufficiently accessible in some regions due to transportation, inadequate infrastructure, genetic expertise, and counselling[51]. In particular, advanced genetic analyses and sequence-based tests are often costly, as they require specialised laboratories and high-tech equipment, and repeat studies are often performed in rare diseases. These costs may prevent some patients from receiving these tests and slow down the diagnostic process[52]. On the other hand,

Table II. Diagnostic methods for carboxylase enzyme deficiency

Diagnostic Method	Description	Carboxylase Enzyme Deficiency	References
Clinical Evaluation	Thorough patient history and physical examination to identify symptoms like metabolic acidosis, hypoglycemia, and lethargy.	Identifying clinical signs of metabolic disorders but not definitive.	66,67
Plasma/Serum Acylcarnitine Profile	Measures the levels of acylcarnitines, which can be elevated in carboxylase deficiencies.	Detects accumulation of specific metabolites. Elevated C3 (propionylcarnitine) or C5 (isovalerylcarnitine) levels may suggest carboxylase enzyme defects.	68,69
Plasma or Urine Organic Acids	Measures abnormal metabolites in the plasma or urine, like methylmalonic acid, propionylglycine, or others.	Abnormal results may suggest a defect in propionyl-CoA carboxylase or methylmalonyl-CoA mutase (e.g., Propionic acidemia, Methylmalonic acidemia).	70-72
Enzyme Activity Assay	Direct measurement of enzyme activity from blood, tissue (e.g., fibroblasts, leukocytes), or muscle. The activity of the enzyme of interest from the blood sample can be analysed by colorometric or fluorometric methods, following hydrolysis of the enzyme's natural or artificial substrates, by means of initially defined radioassays. Confirmatory tests are performed in patients with a mean normal enzyme activity below 10%.	Direct confirmation of carboxylase enzyme deficiency by measuring activity. Common assays include propionyl-CoA carboxylase, methylmalonyl-CoA mutase, and pyruvate carboxylase activity.	48,73-75
Genetic Testing (DNA Sequencing)	Genetic analysis identifies mutations in the carboxylase enzyme genes. Verification can be performed by mutational analysis of the gene of the enzyme concerned. Genetic tests for this are performed by single gene testing. Firstly, the enzyme is sequence analysed. In the absence or presence of a single pathogenic variant, gene-targeted deletion/duplication analysis is performed.	Confirmatory for specific enzyme deficiencies (e.g., MUT gene for methylmalonyl-CoA mutase, PCCB for propionyl-CoA carboxylase).	76-79
Cerebrospinal Fluid (CSF) Analysis	CSF analysis identifies organic acid accumulations and neurochemical changes.	Elevated levels of specific metabolites may support the diagnosis of carboxylase deficiencies affecting the central nervous system.	80-82
Biotin (Coenzyme) Response Test	Assessment of response to biotin supplementation in cases of biotin-responsive carboxylase deficiencies.	Biotin-responsive disorders like holocarboxylase synthetase deficiency (HCS) show improvement in enzyme function with biotin treatment.	45,78,83-85

genetic analyses based on large databases may contain some inaccuracies, such as misinterpretation of variants and false negatives or positives[53,54].

Biochemical methods are performed by measuring the concentrations of metabolic products. As a result of a problem in the production of enzymes involved in metabolism or a situation that prevents them from fulfilling their catalytic activities, abnormal and harmful organic acid metabolites accumulate in the cell. Since high concentrations of metabolites are excreted due to organic acidemia, it is possible to detect these metabolites in urine and blood. Organic acidemia can occur at any age, as early as the newborn period[55]. As a result of the accumulation of free organic acids, the body's pH balance is disturbed, and an anion deficit occurs[56]. In the case of deficiency of carboxylase group enzymes, it is extremely important to monitor the patient's organic acids, amino acid profile, free carnitine and acyl carnitine profile, ammonia, lactate, and leukocyte counts[36]. In PCC deficiency, high propionyl carnitine levels determine PA. Propionyl-CoA, propionate, methyl citrate, β-hydroxypropionate, propionylglycine, tiliac acid, and ketone levels are high in patients with PCC enzyme deficiency[30].

In isolated MCC deficiency, urinary levels of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine are generally increased. Plasma levels of 3-hydroxyisovaleryl carnitine increase, while serum levels of free carnitine decrease. In addition, 3-hydroxyisovalerylcarnitine is characteristically found in blood and urine. Many patients develop a severe secondary carnitine deficiency, and urinary levels of 3-methyl citrate, propionic acid, and propionyl carnitine are increased. Molecular analyses in leukocytes or fibroblasts show low or absent 3-MCC activity, while other carboxylase enzymes have normal activity[57]. In PC deficiency, abnormal levels of amino acids, organic acids, glucose, and ammonia constitute the biochemical diagnosis. As a result of a high lactic acid level, the lactate/pyruvate ratio increases, indicating TCA cycle disorders. Lactic

acidosis (serum lactate), lactate, organic acids, and ketone bodies in urine, hyperglycaemia, and ammonia are analyzed to determine PC enzyme activity deficiency[58].

It is well known that accumulated metabolites can lead to neurological symptoms60 in carboxylase enzyme deficiencies. Metabolic disorders, particularly those caused by organic acidemia, can have detrimental effects on the brain. These conditions often lead to neurological findings such as lethargy, seizures, motor disorders, and developmental delay[59]. Due to organic acidemia, symptoms such as metabolic acidosis, hypoglycaemia (low blood sugar), vomiting, dehydration, and liver enlargement may also occur. These symptoms can occur very quickly at the onset of the disease[60]. In diagnosing carboxylase enzyme deficiencies, genetic testing, measuring enzyme activity, detecting biochemical parameters (especially the accumulation of organic acids), and understanding the relationship between neurological findings and enzyme activity are of great importance[61]. These methods can help accurately recognise the disease and treat it early. Disruption of metabolic pathways due to enzyme deficiency, combined with clinical symptoms (neurological and metabolic findings) and biochemical data, confirms the diagnosis[62].

Discussion and Conclusion

Carboxylases are enzymes that play crucial roles in metabolic processes involving the transfer of carboxyl groups. These biotin-dependent enzymes have vital functions in gluconeogenesis, lipogenesis, and amino acid catabolism. In addition, substrates and/or products of ACC, PC, MCC, and PCC carboxylase group enzymes have been shown to affect signal transduction mechanisms. Therefore, deficiency of these enzymes leads to severe metabolic disorders. Carboxylase group enzymes, which are involved in extremely critical steps in metabolism, are not only associated with genetic defects, but are also regarded as molecules that should be monitored regularly due to their effects on the metabolic process molecules that should be monitored regularly. These enzymes, which are usually investigated in rare metabolic disorders, should be considered in metabolic pathologies and their catalytic functions.

Biochemistry and metabolism laboratories analyse the activity of carboxylase enzymes. Metabolic substrate measurement, product quantification, and enzyme activity determination in biological materials are difficult due to matrix interferences and low concentrations. Laboratory accreditation, pre-analytical procedures, methods of analysis, analytical instruments, and qualified technical staff are necessary to overcome these difficulties. On the other hand, it is of utmost importance to develop faster and more sensitive analysis methods and to search for new biomarkers by taking advantage of today's sensitive and advanced technological devices.

Genetic mutations cause carboxylase enzyme deficiencies. Therefore, significant efforts have been made in recent years to repair missing or impaired enzyme functions through gene therapy (e.g., viral vectors or nanoparticle-based delivery systems) and gene editing technologies (e.g., CRISPR-Cas9). Enzyme replacement therapy, one of the most widely used treatment methods, is the external administration of the missing or dysfunctional enzyme to compensate for genetic deficiencies. This approach has been successfully used in the treatment of some metabolic diseases. However, in cases where some enzymes, such as carboxylase enzymes, must function intracellularly, this treatment can become more complex. The deficiency of carboxylase enzymes can disrupt important metabolic pathways in the cell, resulting in the accumulation of organic acids and metabolic acidosis. Therefore, modulation of metabolic pathways may be part of therapeutic strategies.

Research areas such as therapeutic targeting of carboxylase enzyme deficiencies and bioinformatics approaches offer significant opportunities in managing and treating genetic disorders. Research in this area has enormous potential for more effective treatment and faster diagnosis of genetically based diseases. Bioinformatics databases, artificial intelligence (AI), and machine learning can be used to collect data on new and rare mutations and correlate these data with population genetics. These databases will also provide useful data to determine the degree of pathogenicity of mutations and optimise treatment approaches.

Carboxylase enzymes catalyse several biochemical reactions in the body, playing a critical role in cellular energy production and metabolic control processes. Deficiencies of these enzymes can accumulate harmful metabolites in the body and cause serious metabolic disorders such as organic acidemia. Since neurological and metabolic symptoms characterise these disorders, developing accurate diagnosis and treatment methods is very important. Understanding carboxylase enzyme deficiencies and therapeutically targeting these deficiencies may improve patients' quality of life and pave the way for new therapeutic strategies. Furthermore, using advanced research methods such as bioinformatics and genetic analyses may offer critical opportunities to enable more effective management of the functions and deficiencies of these enzymes. The development of biochemical analysis methods and advances in genetics, bioinformatics, and AI will help to make accurate diagnoses, develop individualised treatment strategies, and make treatment processes more effective.

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References

1. 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*. May 2023;47:75–92. doi:10.1016/j.jare.2022.07.011
2. Kornberg HL, Krebs HA. Synthesis of cell constituents from C2-units by a modified tricarboxylic acid cycle. *Nature*. May 18 1957;179(4568):988–91. doi:10.1038/179988a0
3. Tommasi IC. The Biochemistry of Artificial CO2-Fixation Pathways: The Exploitation of Carboxylase Enzymes Alternative to Rubisco. *Catalysts*. 2024;14(10):679.
4. Erb TJ. Carboxylases in natural and synthetic microbial pathways. *Appl Environ Microbiol*. Dec 2011;77(24):8466–77. doi:10.1128/aem.05702-11
5. Santana-Molina C, Williams TA, Snel B, Spang A. Chimeric origins and dynamic evolution of central carbon metabolism in eukaryotes. *Nature Ecology & Evolution*. 2025/04/01 2025;9(4):613–627. doi:10.1038/s41559-025-02648-0
6. Sonnewald U. Glutamate synthesis has to be matched by its degradation - where do all the carbons go? *Journal of Neurochemistry*. Nov 2014;131(4):399–406. doi:10.1111/jnc.12812
7. Inigo M, Deja S, Burgess SC. Ins and Outs of the TCA Cycle: The Central Role of Anaplerosis. *Annu Rev Nutr*. Oct 11 2021;41:19–47. doi:10.1146/annurev-nutr-120420-025558
8. Kiesel VA, Sheeley MP, Coleman MF, et al. Pyruvate carboxylase and cancer progression. *Cancer Metab*. Apr 30 2021;9(1):20. doi:10.1186/s40170-021-00256-7
9. Wang Y, Yu W, Li S, Guo D, He J, Wang Y. Acetyl-CoA Carboxylases and Diseases. *Front Oncol*. 2022;12:836058. doi:10.3389/fonc.2022.836058
10. Shen JJ, Wu WP, Wang KL, et al. Chloroflexus aurantiacus acetyl-CoA carboxylase evolves fused biotin carboxylase and biotin carboxyl carrier protein to complete carboxylation activity. *Mbio*. May 2024;15(5)doi:10.1128/mbio.03414-23
11. Jitrapakdee S, St Maurice M, Rayment I, Cleland WW, Wallace JC, Attwood PV. Structure, mechanism and regulation of pyruvate carboxylase. *Biochem J*. Aug 1 2008;413(3):369–87. doi:10.1042/BJ20080709
12. Gray LR, Tompkins SC, Taylor EB. Regulation of pyruvate metabolism and human disease. *Cellular and Molecular Life Sciences*. 2014/07/01 2014;71(14):2577–2604. doi:10.1007/s00018-013-1539-2
13. Singh M, Elfrink HL, Harms AC, Hankemeier T. Recent developments in the analytical approaches of acyl-CoAs to assess their role in mitochondrial fatty acid oxidation disorders. *Mol Genet Metab*. Oct 20 2023:107711. doi:10.1016/j.ymgme.2023.107711
14. Robinson BH. Lactic acidemia and mitochondrial disease. *Molecular Genetics and Metabolism*. 2006/09/01/ 2006;89(1):3–13. doi:https://doi.org/10.1016/j.ymgme.2006.05.015
15. Demir Köse M, Colak R, Yangin Ergon E, et al. Challenges in the management of an ignored cause of hyperammonemic encephalopathy: pyruvate carboxylase deficiency. *J Pediatr Endocrinol Metab*. Apr 28 2020;33(4):569–574. doi:10.1515/jpem-2019-0307
16. García-Cazorla A, Rabier D, Touati G, et al. Pyruvate carboxylase deficiency: Metabolic characteristics and new neurological aspects. *Annals of Neurology*. 2006;59(1):121–127. doi:https://doi.org/10.1002/ana.20709
17. Liu Y, Liu C, Pan Y, Zhou J, Ju H, Zhang Y. Pyruvate carboxylase promotes malignant transformation of papillary thyroid carcinoma and reduces iodine uptake. *Cell Death Discovery*. 2022/10/20 2022;8(1):423. doi:10.1038/s41420-022-01214-y
18. Sellers K, Fox MP, Bousamra M, 2nd, et al. Pyruvate carboxylase is critical for non-small-cell lung cancer proliferation. *J Clin Invest*. Feb 2015;125(2):687–98. doi:10.1172/jci72873
19. Ngamkham J, Thuwajit C, Thuwajit P, et al. Overexpression of Pyruvate Carboxylase Is Correlated With Colorectal Cancer Progression and Supports Growth of Invasive Colon Cancer HT-29 Cell Line. *Anticancer Research*. 2020;40(11):6285–6293. doi:10.21873/anticanres.14649
20. Hunkeler M, Hagmann A, Stuttfeld E, et al. Structural basis for regulation of human acetyl-CoA carboxylase. *Nature*. 2018/06/01 2018;558(7710):470–474. doi:10.1038/s41586-018-0201-4
21. Chen L, Duan Y, Wei H, et al. Acetyl-CoA carboxylase (ACC) as a therapeutic target for metabolic syndrome and recent developments in ACC1/2 inhibitors. *Expert Opin Investig Drugs*. Oct 2019;28(10):917–930. doi:10.1080/13543784.2019.1657825
22. Abu-Elheiga L, Brinkley WR, Zhong L, Chirala SS, Woldegiorgis G, Wakil SJ. The subcellular localization of acetyl-CoA carboxylase 2. *Proc Natl Acad Sci U S A*. Feb 15 2000;97(4):1444–9. doi:10.1073/pnas.97.4.1444
23. Beaty NB, Lane MD. Kinetics of activation of acetyl-CoA carboxylase by citrate. Relationship to the rate of polymerization of the enzyme. *J Biol Chem*. Nov 10 1983;258(21):13043–50.
24. Szutowicz A, Bielarczyk H, Jankowska-Kulawy A, Pawełczyk T, Ronowska A. Acetyl-CoA the key factor for survival or death of cholinergic neurons in course of neurodegenerative diseases. *Neurochem Res*. Aug 2013;38(8):1523–42. doi:10.1007/s11064-013-1060-x
25. Chai P, Lan P, Li S, et al. Mechanistic insight into allosteric activation of human pyruvate carboxylase by acetyl-CoA. *Mol Cell*. Nov 3 2022;82(21):4116–4130.e6. doi:10.1016/j.molcel.2022.09.033
26. Bradshaw PC. Acetyl-CoA Metabolism and Histone Acetylation in the Regulation of Aging and Lifespan. *Antioxidants (Basel)*. Apr 8 2021;10(4)doi:10.3390/antiox10040572
27. Galic S, Loh K, Murray-Segal L, Steinberg GR, Andrews ZB, Kemp BE. AMPK signaling to acetyl-CoA carboxylase is required for fasting- and cold-induced appetite but not thermogenesis. *Elife*. Feb 13 2018;7doi:10.7554/eLife.32656
28. Blom W, de Muinck Keizer SM, Scholte HR. Acetyl-CoA carboxylase deficiency: an inborn error of de novo fatty acid synthesis. *N Engl J Med*. Aug 20 1981;305(8):465–6. doi:10.1056/NEJM198108203050820
29. Porta F, Maiorana A, Gragnaniello V, et al. Triheptanoin in patients with long-chain fatty acid oxidation disorders: clinical experience in Italy. *Ital J Pediatr*. Oct 7 2024;50(1):204. doi:10.1186/s13052-024-01782-y
30. Wongkittichote P, Ah Mew N, Chapman KA. Propionyl-CoA carboxylase - A review. *Mol Genet Metab*. Dec 2017;122(4):145–152. doi:10.1016/j.ymgme.2017.10.002
31. Vandova GA, O'Brien RV, Lowry B, et al. Heterologous expression of diverse propionyl-CoA carboxylases affects polyketide production in Escherichia coli. *The Journal of Antibiotics*. 2017/07/01 2017;70(7):859–863. doi:10.1038/ja.2017.38

32. Longo N, Price LB, Gappmaier E, et al. Anaplerotic therapy in propionic acidemia. *Mol Genet Metab*. Sep 2017;122(1-2):51–59. doi:10.1016/j.ymgme.2017.07.003
33. AlSayed M, Almasseri Z. Retrospective Study Of The Natural History and Disease Course In 46 Patients With Propionic Aciduria Caused By Propionyl-Coa Carboxylase PCCA and PCCB Mutations. *Molecular Genetics and Metabolism*. 2022/04/01/ 2022;135(4):258–259. doi:https://doi.org/10.1016/j.ymgme.2022.01.016
34. Chloupkova M, Maclean KN, Alkhateeb A, Kraus JP. Propionic acidemia: analysis of mutant propionyl-CoA carboxylase enzymes expressed in Escherichia coli. *Hum Mutat*. Jun 2002;19(6):629–40. doi:10.1002/humu.10085
35. He W, Marchuk H, Koeberl D, Kasumov T, Chen X, Zhang G-F. Fasting alleviates metabolic alterations in mice with propionyl-CoA carboxylase deficiency due to Pcca mutation. *Communications Biology*. 2024/05/29 2024;7(1):659. doi:10.1038/s42003-024-06362-8
36. Baumgartner MR, Hörster F, Dionisi-Vici C, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. *Orphanet Journal of Rare Diseases*. 2014/09/02 2014;9(1):130. doi:10.1186/s13023-014-0130-8
37. Kor D, Seker-Yilmaz B, Bulut FD, et al. Clinical features of 27 Turkish Propionic acidemia patients with 12 novel mutations. *Turk J Pediatr*. 2019;61(3):330–336. doi:10.24953/turkjped.2019.03.003
38. Richard E, Marchuk H, Álvarez M, et al. Metabolic flux analysis in hiPSC-CMs reveals insights into cardiac dysfunction in propionic acidemia Eva Richard. *Res Sq*. Jan 28 2025;doi:10.21203/rs.3.rs-5874705/v1
39. Rodríguez JM, Ruíz-Sala P, Ugarte M, Peñalva MÁ. Fungal Metabolic Model for 3-Methylcrotonyl-CoA Carboxylase Deficiency *. *Journal of Biological Chemistry*. 2004;279(6):4578–4587. doi:10.1074/jbc.M310055200
40. McKean AL, Ke J, Song J, et al. Molecular characterization of the non-biotin-containing subunit of 3-methylcrotonyl-CoA carboxylase. *J Biol Chem*. Feb 25 2000;275(8):5582–90. doi:10.1074/jbc.275.8.5582
41. Hu JJ, Lee JKJ, Liu YT, et al. Discovery, structure, and function of filamentous 3-methylcrotonyl-CoA carboxylase. *Structure*. Jan 2023;31(1):100–+. doi:10.1016/j.str.2022.11.015
42. Manoli I, Venditti CP. Disorders of branched chain amino acid metabolism. *Transl Sci Rare Dis*. Nov 7 2016;1(2):91–110. doi:10.3233/trd-160009
43. Forsyth R, Vockley CW, Edick MJ, et al. Outcomes of cases with 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency - Report from the Inborn Errors of Metabolism Information System. *Mol Genet Metab*. May 2016;118(1):15–20. doi:10.1016/j.ymgme.2016.02.002
44. Sahin S, Yildirim M, Bektas Ö, Kara IS, Ceylan AC, Teber S. Intracranial Calcification Associated with 3-Methylcrotonyl-CoA Carboxylase Deficiency. *Molecular Syndromology*. Oct 2021;12(6):393–398. doi:10.1159/000517272
45. Yilmaz B, Ceylan AC, Gunduz M, et al. Evaluation of clinical, laboratory, and molecular genetic features of patients with biotinidase deficiency. *Eur J Pediatr*. Mar 2024;183(3):1341–1351. doi:10.1007/s00431-023-05376-4
46. Grünert SC, Stucki M, Morscher RJ, et al. 3-methylcrotonyl-CoA carboxylase deficiency: Clinical, biochemical, enzymatic and molecular studies in 88 individuals. *Orphanet Journal of Rare Diseases*. 2012/05/29 2012;7(1):31. doi:10.1186/1750-1172-7-31
47. Shafieipour N, Jafari Khamirani H, Kamal N, Tabei SMB, Dianatpour M, Dastgheib SA. The third patient of ACACA-related acetyl-CoA carboxylase deficiency with seizure and literature review. *European Journal of Medical Genetics*. 2023/04/01/ 2023;66(4):104707. doi:https://doi.org/10.1016/j.ejmg.2023.104707
48. Lasio MLD, Leshinski AC, Ducich NH, et al. Clinical, biochemical and molecular characterization of 12 patients with pyruvate carboxylase deficiency treated with triheptanoin. *Mol Genet Metab*. Jun 2023;139(2):107605. doi:10.1016/j.ymgme.2023.107605
49. Donohue KE, Gooch C, Katz A, Wakelee J, Slavotinek A, Korf BR. Pitfalls and challenges in genetic test interpretation: An exploration of genetic professionals experience with interpretation of results. *Clin Genet*. May 2021;99(5):638–649. doi:10.1111/cge.13917
50. Marin-Valencia I, Roe CR, Pascual JM. Pyruvate carboxylase deficiency: Mechanisms, mimics and anaplerosis. *Molecular Genetics and Metabolism*. 2010/09/01/ 2010;101(1):9–17. doi:https://doi.org/10.1016/j.ymgme.2010.05.004
51. Petersen A. The best experts: The narratives of those who have a genetic condition. *Social Science & Medicine*. 2006/07/01/ 2006;63(1):32–42. doi:https://doi.org/10.1016/j.socscimed.2005.11.068
52. Knoppers BM, Bonilha AE, Laberge A-M, Ahmed A, Newson AJ. Genomic sequencing in newborn screening: balancing consent with the right of the asymptomatic at-risk child to be found. *European Journal of Human Genetics*. 2025/03/01 2025;33(2):182–188. doi:10.1038/s41431-024-01677-w
53. Shahangian S, Chen B. CDC Recommendations: Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders. *Clin Chem*. Dec 2012;58(12):1728. doi:10.1373/clinchem.2012.195362
54. Centers for Disease C, Prevention. Good laboratory practices for biochemical genetic testing and newborn screening for inherited metabolic disorders. *MMWR Recomm Rep*. Apr 6 2012;61(RR-2):1–44.
55. Ramsay J, Morton J, Norris M, Kanungo S. Organic acid disorders. *Ann Transl Med*. Dec 2018;6(24):472. doi:10.21037/atm.2018.12.39
56. Pandey DG, Sharma S. Biochemistry, Anion Gap. *StatPearls*. StatPearls Publishing

57. Cozzolino C, Villani GR, Frisso G, et al. Biochemical and molecular characterization of 3-Methylcrotonylglycinuria in an Italian asymptomatic girl. *Genet Mol Biol*. Apr./Jun 2018;41(2):379–385. doi:10.1590/1678-4685-gmb-2017-0093
58. Habarou F, Brassier A, Rio M, et al. Pyruvate carboxylase deficiency: An underestimated cause of lactic acidosis. *Molecular Genetics and Metabolism Reports*. 2015/03/01/ 2015;2:25–31. doi:https://doi.org/10.1016/j.ymgmr.2014.11.001
59. Emmett M. Review of Clinical Disorders Causing Metabolic Acidosis. *Advances in Chronic Kidney Disease*. 2022/07/01/ 2022;29(4):355–363. doi:https://doi.org/10.1053/j.ackd.2022.07.004
60. Fatima S, Aamir M, Bibi A. Multiple Carboxylase Deficiency Organic Acidemia as a Cause of Infantile Seizures. *Jcpsp-Journal of the College of Physicians and Surgeons Pakistan*. Jan 2021;31(1):95–97. doi:10.29271/jcpsp.2021.01.95
61. Kwon J, D'Aco K. Clinical Neurogenetics Neurologic Presentations of Metabolic Disorders. *Neurologic Clinics*. Nov 2013;31(4):1031–+. doi:10.1016/j.ncl.2013.04.005
62. Ramsay J, Morton J, Norris M, Kanungo S. Organic acid disorders. *Annals of Translational Medicine*. Dec 2018;6(24)472. doi:10.21037/atm.2018.12.39
63. Lao-On U, Attwood PV, Jitrapakdee S. Roles of pyruvate carboxylase in human diseases: from diabetes to cancers and infection. *J Mol Med (Berl)*. Apr 2018;96(3-4):237–247. doi:10.1007/s00109-018-1622-0
64. Kumashiro N, Beddow SA, Vatner DF, et al. Targeting pyruvate carboxylase reduces gluconeogenesis and adiposity and

improves insulin resistance. *Diabetes*. Jul 2013;62(7):2183–94. doi:10.2337/db12-1311

65. Grünert SC, Stucki M, Morscher RJ, et al. 3-methylcrotonyl-CoA carboxylase deficiency: clinical, biochemical, enzymatic and molecular studies in 88 individuals. *Orphanet J Rare Dis*. May 29 2012;7:31. doi:10.1186/1750-1172-7-31
66. Falsaperla R, Sciuto L, La Spina L, Sciuto S, Praticò AD, Ruggieri M. Neonatal seizures as onset of Inborn Errors of Metabolism (IEMs): from diagnosis to treatment. A systematic review. *Metab Brain Dis*. Dec 2021;36(8):2195–2203. doi:10.1007/s11011-021-00798-1
67. Bower A, Imbard A, Benoist J-F, et al. Diagnostic contribution of metabolic workup for neonatal inherited metabolic disorders in the absence of expanded newborn screening. *Scientific Reports*. 2019/10/01 2019;9(1):14098. doi:10.1038/s41598-019-50518-0
68. Rinaldo P, Cowan TM, Matern D. Acylcarnitine profile analysis. *Genet Med*. Feb 2008;10(2):151–6. doi:10.1097/GIM.0b013e3181614289
69. Vargas CR, Ribas GS, da Silva JM, et al. Selective Screening of Fatty Acids Oxidation Defects and Organic Acidemias by Liquid Chromatography/tandem Mass Spectrometry Acylcarnitine Analysis in Brazilian Patients. *Arch Med Res*. Apr 2018;49(3):205–212. doi:10.1016/j.arcmed.2018.08.004
70. Phipps WS, Jones PM, Patel K. Chapter Two - Amino and organic acid analysis: Essential tools in the diagnosis of inborn errors of metabolism. In: Makowski GS, ed. *Advances in Clinical Chemistry*. Elsevier; 2019:59–103.
71. Gallagher RC, Pollard L, Scott AI, Huguenin S, Goodman S, Sun Q. Laboratory analysis of organic acids, 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 2018;20(7):683–691. doi:10.1038/gim.2018.45
72. Patial A, Saini AG, Kaur R, et al. Detection of IEMs by Mass Spectrometry Techniques in High-Risk Children: A Pilot Study. *Indian J Pediatr*. Sep 2022;89(9):885–893. doi:10.1007/s12098-022-04207-y
73. Ling S, Qiu W, Zhang H, et al. Clinical, biochemical, and genetic analysis of 28 Chinese patients with holocarboxylase synthetase deficiency. *Orphanet Journal of Rare Diseases*. 2023/03/08 2023;18(1):48. doi:10.1186/s13023-023-02656-y
74. Destanoglu O, Cansever MS, Isat E, Zubarioglu T, Aktuglu Zeybek AC, Kiykim E. Analysis of Biotinidase Activity in Serum by Digital Imaging Colorimetry Detection. *ACS Omega*. Oct 24 2023;8(42):39796–39806. doi:10.1021/acsomega.3c05759
75. Lobitz S, Frommel C, Brose A, et al. Simultaneous newborn screening for sickle cell disease, biotinidase deficiency, and hereditary tyrosinemia type 1 with an optimized tandem mass spectrometry protocol. *Ann Hematol*. Aug 2022;101(8):1859–1860. doi:10.1007/s00277-022-04811-0
76. Kenneson A, Youngborg L, Singh RH. Genetic testing experiences and genetics knowledge among families with inherited metabolic diseases. *Mol Genet Metab Rep*. Sep 2020;24:100633. doi:10.1016/j.ymgmr.2020.100633
77. Li S, Shi C, Cai Y, et al. Serum differential proteomic profiling of patients with isolated methylmalonic acidemia by iTRAQ. *Front Genet*. 2022;13:765637. doi:10.3389/fgene.2022.765637
78. Gulzar M, Sarani Z, Tariq M, Knerr I. 3-methylcrotonyl-CoA carboxylase deficiency in a child with developmental regression and delay: call for early diagnosis and multidisciplinary approach. *Bmj Case Reports*. Jul 2025;18(7)e262865. doi:10.1136/bcr-2024-262865
79. Funghini S, Tonin R, Malvagia S, et al. High frequency of biotinidase deficiency in Italian population identified by newborn screening. *Mol Genet Metab Rep*. Dec 2020;25:100689. doi:10.1016/j.ymgmr.2020.100689
80. Peters TMA, Engelke UFH, de Boer S, et al. Confirmation of neurometabolic diagnoses using age-dependent cerebrospinal fluid metabolomic profiles. *J Inherit Metab Dis*. Sep 2020;43(5):1112–1120. doi:10.1002/jimd.12253
81. Morris AAM, Leonard JV. Acute presentations of inherited metabolic disorders: investigation and initial management. *Paediatrics and Child Health*. 2015/03/01/ 2015;25(3):97–102. doi:https://doi.org/10.1016/j.paed.2014.10.005
82. Peters T, Engelke U, de Boer S, et al. Confirmation of neurometabolic diagnoses using age-dependent cerebrospinal fluid metabolomic profiles. *Journal of Inherited Metabolic Disease*. Sep 2020;43(5):1112–1120. doi:10.1002/jimd.12253
83. Majid H, Ahmed S, Muneer S, Hamid R, Jafri L, Khan A. Biotin-responsive Multiple Carboxylase Deficiency (MCD). *Jcpsp-Journal of the College of Physicians and Surgeons Pakistan*. Jun 2022;32(6):823–825. doi:10.29271/jcpsp.2022.06.823
84. Karachaliou C, Livaniou E. Biotin Homeostasis and Human Disorders: Recent Findings and Perspectives. *International Journal of Molecular Sciences*. Jun 2024;25(12)6578. doi:10.3390/ijms25126578
85. Sharma R, Kucera CR, Nery CR, Lacbawan FL, Salazar D, Tanpaiboon P. Biotinidase biochemical and molecular analyses: Experience at a large reference laboratory. *Pediatr Int*. Jan–Dec 2024;66(1):e15726. doi:10.1111/ped.15726