2-Hydroxyglutaric acidurias (2-HGA) are inborn errors of metabolism defined by increased physiological 2-hydroxyglutarate concentrations observed in patient's body fluids like urine, plasma and cerebrospinal fluid, expressing neurological impairments which manifest at young age. Originally, two classes were identified based on the chirality of the accumulating metabolite: D-2-hydroxyglutaric aciduria (D-2-HGA) and L-2-hydroxyglutaric aciduria (L-2-HGA) [1,2]. As understanding of these disorders progressed, a third variant was described and defined as combined D-2- and L-2-hydroxyglutaric aciduria (D,L-2-HGA) [3].

The identification of mutations in the gene L2HGDH encoding L-2-hydroxyglutarate dehydrogenase (L-2-HGDH) revealed the metabolic defect in L-2-HGA [4,5]. Enzymatic confirmation of impaired L-2-HGDH catalytic activity was performed in patient’s fibroblast, lymphoblast and lymphocyte lysates with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Chapter 2). This enzyme assay, based on the conversion of stable isotope labeled L-2-hydroxyglutarate (L-2-HG) to stable isotope labeled 2-ketoglutarate (2-KG), provided a functional link between the metabolic and genetic features of this disorder.

Two anecdotic L-2-HGA case reports described promising results with improved clinical signs and a decrease in urinary L-2-HG concentrations upon oral supplementation of FAD or riboflavin (a precursor of FAD) [6,7]. It is hypothesized that the enzyme’s catalytic activity of FAD-dependent L-2-HGDH can be restored by increasing physiological FAD concentrations. The effect of increasing catalytic activity with increasing FAD concentrations was previously shown in a purified enzyme extract of rat liver [4]. In a cohort of eleven L-2-HGA patients only one patient lymphoblast cell lines showed residual L-2-HGDH activity (20% of mean control activity) (Chapter 2). Rescuing the enzyme activity with increased physiological FAD concentration is a potential therapeutic strategy which needs further research.

Homogeneous genetic, enzymatic and biochemical characteristics were found in 24 out of 50 D-2-HGA patients (Chapter 3). They carried two mutations in the gene D2HGDH and impaired D-2-hydroxyglutarate dehydrogenase (D-2-HGDH) catalytic activity was detected. This group of patients was denoted D-2-HGA type I, an autosomal recessive form of D-2-HGA. These patients had significantly lower D-2-hydroxyglutarate (D-2-HG) accumulation in urine, plasma and cerebrospinal fluid compared to D-2-HGA patients without D2HGDH mutations.
A novel cause of D-2-HGA was discovered by the identification of de novo heterozygous germline mutations in isocitrate dehydrogenase 2 (IDH2) (Chapter 4). These patients had no mutations in D2HGDH, had normal D-2-HGDH activity and were subsequently denoted D-2-HGA type II, an autosomal dominant form of D-2-HGA. The altered amino acid residue Arg-140 in IDH2-mutants disabled the enzyme’s normal ability to convert reversibly isocitrate to 2-KG and conferred on the enzyme a new function: the ability to convert 2-KG to D-2-HG. Significantly higher physiological D-2-HG concentrations were observed in D-2-HGA type II compared to D-2-HGA type I. Several non-related D-2-HGA patients had no mutations in D2HGDH and IDH2, the defect in these patients remains unresolved.

The presence of IDH2-mutant gain-of-function activity in D-2-HGA type II was confirmed with a functional assay performed in patient lymphoblast lysates (Chapter 5). Stable isotope labeled 2-KG is converted to D-2-HG which is detected by LC-MS/MS. Eight fold increased D-2-HG production was detected in D-2-HGA type II compared to controls and D-2-HGA type I. The enzyme D-2-HGDH functions normally in D-2-HGA type II, and apparently D-2-HGDH’s capacity is insufficient to convert all D-2-HG produced by the IDH2-mutant. The IDH2-mutant assay was applied for therapeutic exploration by screening endogenous metabolites closely related to 2-KG, D-2-HG or isocitrate for their inhibiting potential. Oxaloacetate appeared to be the most potent competitive inhibitor for decreasing D-2-HG production.

The recognition of D-2-HGA type I and D-2-HGA type II as distinct inborn errors of metabolism was proceeded with the description of the phenotype distilled from data retrieved by international clinical questionnaires (Chapter 6). D-2-HGA type I and type II share the biochemical hallmark of D-2-HG accumulation in body fluids, which correlate probably with the clinical features as developmental delay, hypotonia and seizures observed in both disorders. Cardiomyopathy is exclusively found in D-2-HGA type II, supposedly related to decreased (mitochondrial) NADPH and 2-KG levels.

The disease causing IDH gain-of-function mechanism in D-2-HGA type II is found frequently in neoplastic disorders [8,9]. Until now, cancer was not reported in D-2-HGA type I or type II, therefore we do currently not consider D-2-HG an “onco-metabolite” which was suggested by Dang et al. In contrast, increased risk of brain tumors is found in L-2-HGA suggesting carcinogenic properties for L-2-HG. Nevertheless, the discovery of an identical mechanism causing D-2-HG accumulation in D-2-HGA and cancer link these diseases and may provide further insights in pathophysiology.
D-2-HGA and L-2-HGA have very different clinical presentations, pointing towards different pathophysiologic consequences of D-2-HG and L-2-HG accumulation in human. The poorly understood pathophysiologic mechanisms of these disorders need to be explored more intensively to create opportunities for the development of therapeutic strategies.

The (genetic) defect in D,L-2-HGA is still unknown, but homogeneous clinical and metabolic findings in five cases showed similarity suggesting the presence of one disease causing mechanism for this severe neonatal epileptic encephalopathy. Exome sequencing is considered to be a potential strategy for continuing research to find the defect.

**Final conclusion**

In this Thesis it is recognized that D-2-HGA consists of at least two entities: D-2-HGA type I and D-2-HGA type II. This extended the definition to four distinct neurometabolic disorders encompassing the majority of diagnosed 2-HGA patients: L-2-HGA, D-2-HGA type I, D-2-HGA type II en D,L-2-HGA (Figure 1). Recently, IDH1 gain-of-function mutations were identified in patients with combined Metaphyseal Chondromatosis and D-2-HGA (MC-HGA) [10], whereas a few patients remain unidentified with a miscellaneous phenotype of unknown origin. The proceedings in metabolic, enzymatic, genetic and clinical studies described in this Thesis elucidated D-2-HGA I, D-2-HGA II and L-2-HGA in further detail, and exploration of therapeutic interventions in D-2-HGA type II has been successfully initiated. Resolving the pathophysiological mechanisms in 2-HGA may help to find a proper therapy for these diseases or protect from the adverse effects of D-2-HG and L-2-HG accumulations.

![Figure 1: Total 2-hydroxyglutaric aciduria distribution (n=276)](image-url)
References


