Rare disease
Differential diagnosis aided by NMR:
Distinction between L- and D-2-hydroxyglutaric acid

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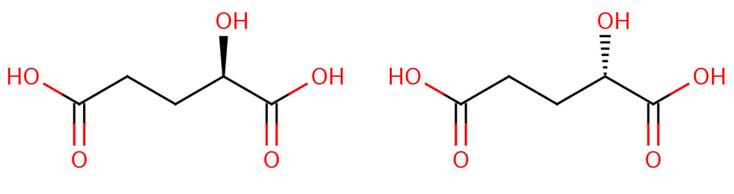
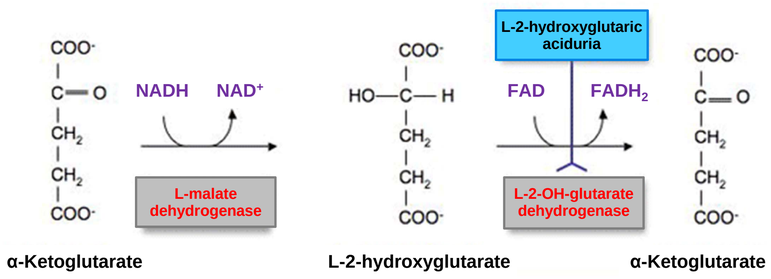
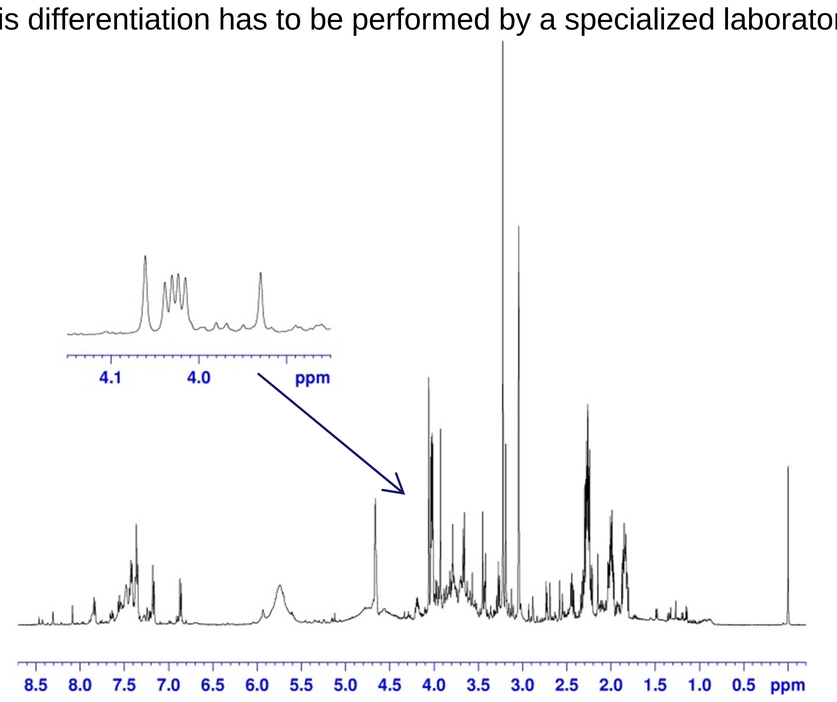
Background: D- or L-2-hydroxyglutaric aciduria are rare clinically variable neurological forms of 2-hydroxyglutaric aciduria characterized biochemically by increased concentrations of D- or L-2-hydroxyglutaric acid in urine, plasma, and cerebrospinal fluid samples. Different enantiomeric forms of 2-hydroxyglutaric acid are related to different diseases. L-2-hydroxyglutaric aciduria (L-2-HGA) [1] is related to the L-enantiomer of 2-hydroxyglutaric acid, while the less common D-2-hydroxyglutaric aciduria (D-2-HGA) [2] is related to the D-enantiomer. The prevalence of this disorder is not known, only 80 cases have been reported to date.





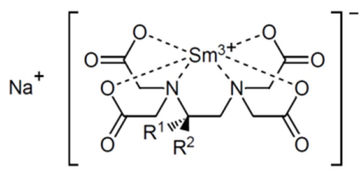
Genetic analysis: For a definite diagnosis, a genetic analysis of affected genes is usually necessary. The affected genes are L2HGDH (in L-2-HGA) or either D2HGDH or IDH2 (in D-2-HGA).

NMR spectrum of urine: The high levels of 2-hydroxyglutaric acid in patient urine result in unambiguous signature resonances in the NMR spectrum (below). Urine organic acid screening does not allow differentiation between L-2-HGA and D-2-HGA. Therefore, this differentiation has to be performed by a specialized laboratory.



Methods and Materials

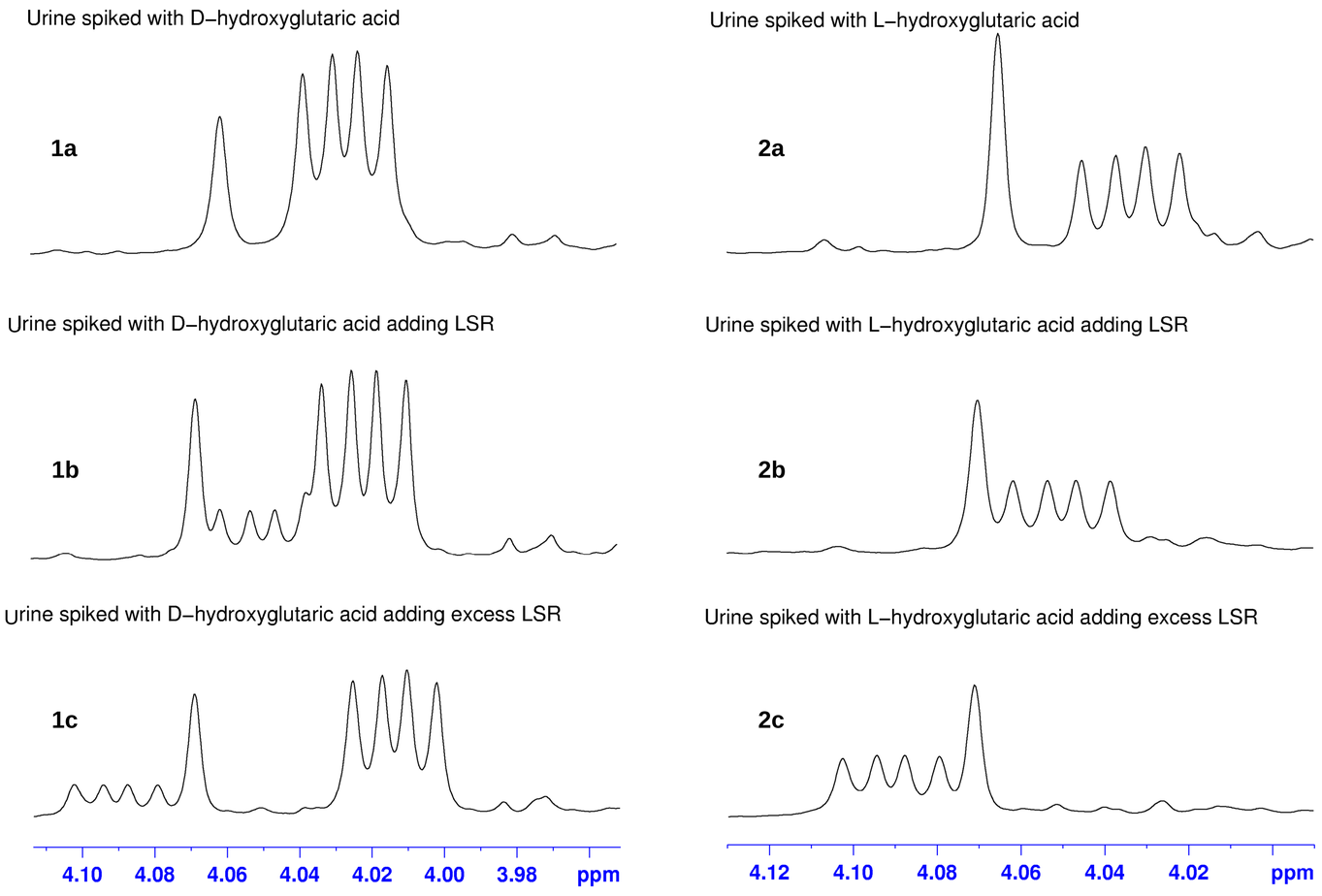
Lanthanide shift reagent (LSR): Enantiomeric forms of a compound give identical NMR spectra. However, the addition of a chiral lanthanide shift reagent (LSR) can result in the formation of complexes that are not mirror images, hence give distinguishable NMR spectra for complexes formed from L- and D-enantiomers of 2-hydroxyglutaric acid [3].



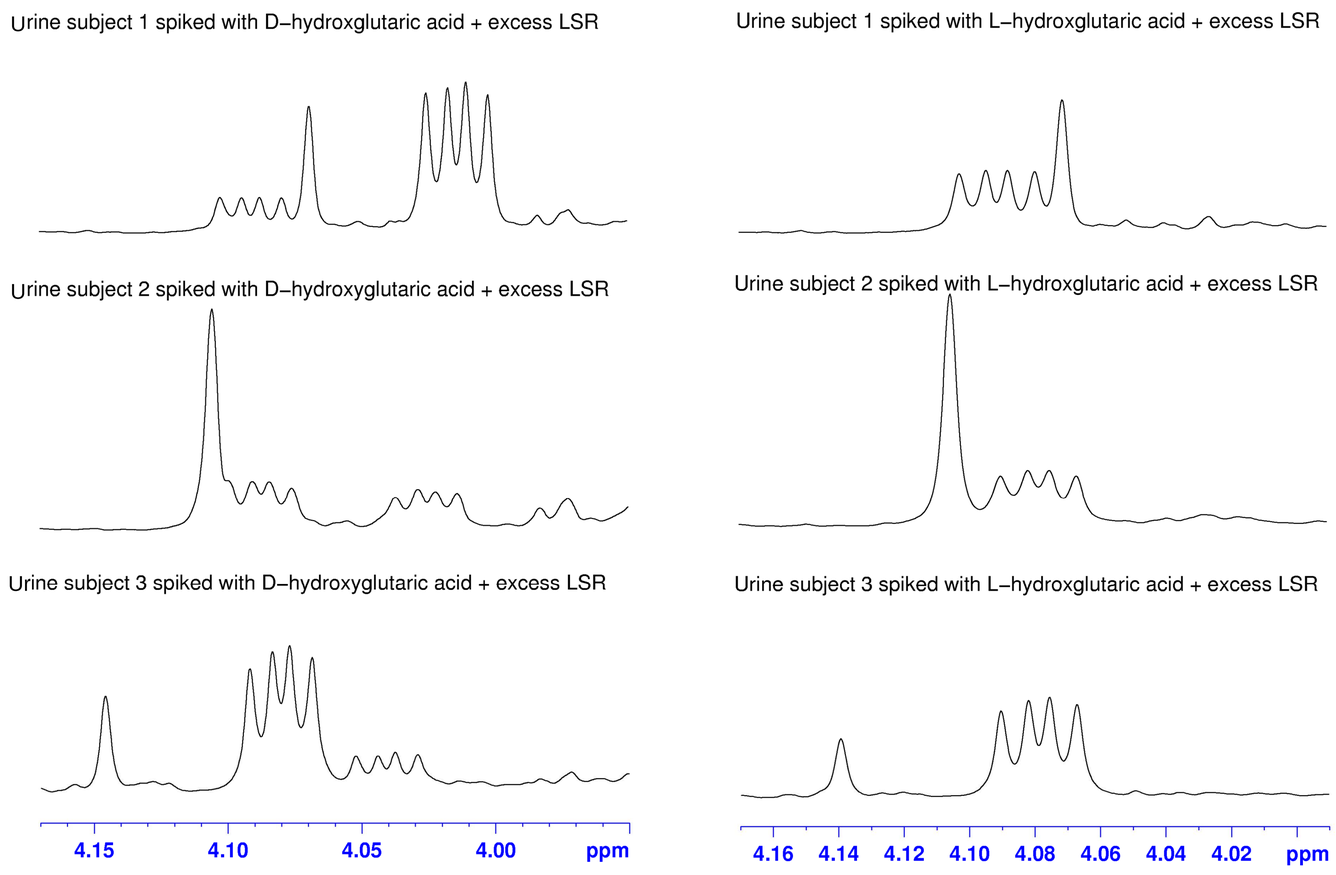
R-enantiomer
of lanthanide shift reagent

Samarium complex as LSR: The samarium complex [(R)-1,2-diaminopropane-*N*,*N*,*N′*,*N′*-tetraaceto]-samarate and the enantiomeric S-form have the described property of being theirselves chiral, hence resulting in different shifts for their complexes with L- and D-hydroxyglutaric acid [3].

Lanthanide shift reagent allows distinction between L- and D-2-hydroxyglutaric acid in NMR spectra of urine: Two test samples of the urine of 2-hydroxyglutaric aciduria patient 1 were prepared by adding ('spiking') D-2-HGA to the sample 1a) and the L-2-HGA to the second test sample 2a). Further addition of both samples with the samarium complex LSR (*R*- or *S-form)* resulting in a shift of the observed quartet, as shown in 1b),c) and 2b),c). Due to the formation of diastereomeric complexes, the shift is different for D- and L-form, resulting in two quartets for the D-2HGA-spiked sample. In the L-2-HGA-spiked sample, there is still only one quartet observable, indicating that the patient's urine contains L-2-hydroxyglutaric acid. The effect becomes more pronounced when the concentration of LSR is increased, 1c) and 2c).



Application to three patients: Our NMR method of L/D-enantiomeric distinction was applied to urine samples from three different patients, of age 1, 5, and 28 years, treated by Prof. Turgay Coşkun and Prof. Ali Dursun, Hacettepe University, Ankara, Turkey. As described above, D- and L-hydroxglutaric acid were added respectively to two different urine samples of each patient which were then treated with the samarium complex LSR. In all three cases, the L-enantiomer was found, confirming a diagnosis of L-2-HGA.



Conclusion: NMR investigations for enantiomeric distinction between L- and D-2-HGA can now be routinely applied in the differential diagnosis of the L- and D-form of 2-hydroxyglutaric aciduria.

In case of all samples, only one signal was observed in the presence of LSR, which indicates that the stereoisomer in the test samples are identical with the added L-2-HGA. As the signals of the D-isomer were not observed, a mixture of stereoisomers can be excluded.

References:

[1] M. Topcu, T. Coşkun, et al. L-2-Hydroxyglutaric aciduria: a report of 29 patients, Turkish J Pediatr 47 (2005) 1
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[3] D. Wal, W. Gradowska, A. Gryff-Keller, Determination of the absolute configuration of 2-hydroxyglutaric acid and 5-oxoproline in urine samples by high-resolution NMR spectroscopy in the presence of chiral lanthanide complexes, J Pharm Biomed Anal 28 (2002) 1061