

# Hepatic Cholesterol Ester Accumulation in Lysosomal Acid Lipase Deficiency: Non-invasive Identification and Treatment Monitoring by Magnetic Resonance

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**Target Audience:** Research scientists and clinicians with an interest in metabolic disorders, Wolman disease, hepatic lipid content and/or hepatic cholesterol ester content, and in vivo <sup>1</sup>H spectroscopy.

**Purpose:** Lysosomal Acid Lipase (LAL) Deficiency is a rare autosomal recessive lysosomal storage disease for which there is no existing therapy. The disease is caused by mutations of the LIPA gene encoding the LAL enzyme and deficiency of this enzyme leads to the accumulation of cholesteryl esters (CE) and triglycerides (TG) in a number of tissues, and causes resultant hepatosplenomegaly and liver dysfunction. Although a single disease, LAL Deficiency presents as a clinical continuum with two major phenotypes: the late onset phenotype is frequently known as Cholesteryl Ester Storage Disease (CESD) and the early onset phenotype is typically referred to as Wolman Disease (1). Wolman's disease patients typically die within the first year of life, whereas patients with the more benign CESD typically live to adulthood but exhibit severe premature liver dysfunction and atherosclerosis. An enzyme replacement therapy for Wolman's disease and CESD is entering clinical trials, providing exogenous active LAL to degrade accumulated cholesterol esters (2,3). Monitoring lipid accumulation in patients has traditionally involved analysis of biopsy samples. This approach is invasive, carries an associated risk to the patient, and samples only a small region of the organ and thus may be prone to sampling errors. Magnetic resonance techniques can provide an alternative to biopsy, offering a non-invasive, safe and repeatable method to measure liver lipid content and composition. We have tested the ability of <sup>1</sup>H MR spectroscopy to provide a non-invasive measure of hepatic cholesterol ester content, and thus provide a method to monitor the efficacy of therapies that target cholesterol ester accumulation. Such a method has a role in both preclinical research studies and clinical trials. We have performed <sup>1</sup>H MR spectroscopy on a previously described (4,5) rat model of LAL Deficiency, and in a cohort of patients with CESD. We hypothesized that a signature of LAL Deficiency would be apparent in liver <sup>1</sup>H spectra, originating from an altered ratio of contributions of CH<sub>2</sub> and CH<sub>3</sub> proton resonances to the <sup>1</sup>H spectrum. We tested this in the preclinical model and then translated our methods to human studies. In addition, we hypothesized that this approach could be used to assess the effects of enzyme replacement therapy, and tested this in the preclinical model to demonstrate a reduction in hepatic CE ester and TG content in sebelipase alfa-treated LAL deficient rats.

**Methods - Preclinical studies:** A LAL deficient rat strain was obtained from NBRP (Japan). Experimental groups comprised liver samples from 8 week old wild type and LAL Deficient rats, and LAL Deficient rats that had previously received four weekly doses of sebelipase alfa enzyme replacement therapy (*i/v*, 3 mg.kg<sup>-1</sup>). Liver samples were also obtained from a diet-induced rat model of non-alcoholic fatty liver disease (NAFLD). Volume-localised hepatic <sup>1</sup>H spectra were acquired from *ex vivo* liver samples on a Varian 7T magnet and spectrometer using a PRESS sequence.

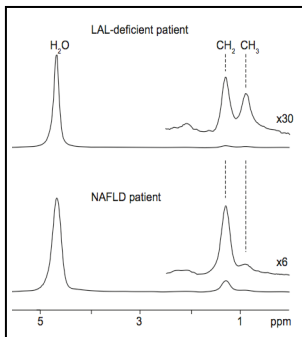
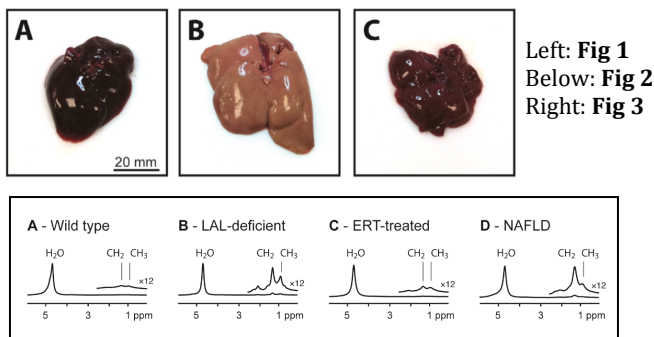
**Human studies:** Study participants were recruited to two cohorts, comprising LAL deficient (n=3) and non-alcoholic fatty liver disease (NAFLD, n=5) patients. Hepatic <sup>1</sup>H spectra were acquired from a 3x3x3 cm<sup>3</sup> voxel using a PRESS pulse sequence on a Philips 3T Achieva scanner.

**Data analysis:** <sup>1</sup>H spectra were analysed to quantify the magnitude of signals arising from water, lipid methylene and lipid methyl protons. A spectral model of lipid and cholesterol resonances was fitted to the experimentally determined ratio of lipid CH<sub>3</sub> to CH<sub>2</sub> protons to determine the ratio of cholesterol to fatty acid moieties in the liver for each subject / sample. Quantitation of cholesterol and fatty acid moiety content was by comparison to the magnitude of the water proton resonance, employing the approach of Longo *et al* (6).

**Results:** Figure 1 shows the physical appearance of wild type (A), LAL deficient (B) and sebelipase alfa-treated LAL deficient (C) rat livers. Accumulation of hepatic lipids are evident as a color change in samples from LAL deficient rats. As previously observed, the ratio of CH<sub>2</sub>:CH<sub>3</sub> proton resonances in NAFLD and control rat and human liver <sup>1</sup>H spectra was approx. 8:1. Variation from this ratio was observed in rat and human LAL deficiency, as observed in the spectra shown in Figures 2 and 3. Analysis of MR data allowed determination of the concentrations of cholesterol and fatty acid moieties from both preclinical and human data, and MR-based measurement of rat liver cholesterol content correlated well with biochemical assay measurements. Table 1 shows the marked difference in cholesterol content between patients with LAL deficiency and patients with NAFLD, consistent with accumulation of CE in LAL deficiency. Preclinical studies demonstrated the efficacy of Sebelipase alfa in reducing both cholesterol and fatty acid moiety content, consistent with cholesterol ester degradation and subsequent clearance.

**Discussion and Conclusions:** We demonstrate an entirely non-invasive method to identify and quantify the hepatic lipid signature associated with a rare genetic cause of fatty liver disease. The approach provides a more favourable alternative to repeated biopsy sampling for diagnosis and disease progression monitoring of patients with LAL deficiency, and we demonstrate ability to monitor efficacy of disease treatment regimes such as enzyme replacement therapy.

**References and Acknowledgements:** This work was supported by Synageva BioPharma and by the Medical Research Council, UK (G0801239). References: (1) Schmitz and Assmann. *In* The Metabolic Basis of Inherited Disease. 6<sup>th</sup> ed. Scriver *et al*. (Eds). McGraw-Hill, New York. 1623-1644. (2) www.clinicaltrials.gov/ct2/show/NCT01488097 (3) www.clinicaltrials.gov/ct2/show/NCT01307098 (4) Yoshida and Kuriyama, Laboratory Animal Science, 40: 486-9 (1990). (5) Nakagawa *et al*. Journal of Lipid Research, 36: 2212-8 (1995). (6) Longo *et al*. JMRI 5:281-285 (1995).



**Table 1**

Volunteer	Phenotype	Cholesterol moiety content (mM)	Cholesterol:fatty acid moiety ratio
1	LAL deficient	11.2	0.24
2	LAL deficient	36.0	0.50
3	LAL deficient	15.1	0.43
4	NAFLD	Not detected	0.00
5	NAFLD	2.1	0.01
6	NAFLD	Not detected	0.00
7	NAFLD	Not detected	0.00
8	NAFLD	9.9	0.02