WOULD YOU FIGURE IT OUT? DIFFERENTIAL DIAGNOSES: BEYOND THE USUAL

Summary of Presentations from the Synageva Symposium, held at the International Liver Congress™ 2015, the 50th Annual Meeting of the European Association for the Study of the Liver, Vienna, Austria, 23rd April 2015

Chairperson

Vlad Ratziu[1]

Speakers

Lauren Johansen,[2] Christophe Moreno,[3] Ali Canbay,[4] Mark Bechter[5]

1. Hôpital La Pitié-Salpêtrière, Paris, France
2. Birmingham Children's Hospital, Birmingham, UK
3. CUB Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium
4. Essen University Hospital, Essen, Germany
5. Synageva BioPharma, Lexington, Massachusetts, USA

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MEETING SUMMARY

The Synageva BioPharma-sponsored symposium discussed the differential diagnoses for liver diseases that may be under-recognised in clinical settings, with a focus on lysosomal acid lipase deficiency (LAL D). LAL D is a lysosomal storage disorder caused by deficient activity of the lysosomal acid lipase enzyme, resulting in the accumulation of cholesteryl esters and triglycerides throughout the body, predominantly in the liver, spleen, gastrointestinal tract, and blood vessel walls. LAL D is a progressive, multisystem disease with early mortality and significant morbidity that is characterised by hepatic dysfunction and dyslipidaemia. Evidence suggests that LAL D may be substantially underdiagnosed or misdiagnosed, which is critical given that disease progression can be unpredictable, with liver failure and/or accelerated atherosclerosis potentially contributing to early mortality. However, a definitive diagnosis of LAL D can be made using a LAL enzyme-based biochemical test, thereby allowing for active monitoring of patients to reduce the potential for disease complications. To raise awareness of LAL D, this symposium, chaired by Prof Vlad Ratziu, centered on the presentation of patient cases by Dr Lauren Johansen, Prof Christophe Moreno, and Prof Ali Canbay, who discussed the path to diagnosing LAL D in children and adults. In addition, Dr Mark Bechter of Synageva BioPharma provided an overview of current data from an ongoing Phase III clinical trial assessing the efficacy and safety of sebelipase alfa, a recombinant LAL replacement therapy, in children and adults with LAL D.

Keywords: Lysosomal acid lipase deficiency, lysosomal storage disease, microvesicular steatosis, dyslipidaemia, elevated serum transaminases, enzyme replacement therapy, hepatomegaly, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis.

Overview of Lysosomal Acid Lipase Deficiency

Professor Vlad Ratziu

Prof Ratziu opened the symposium by stating that, although this meeting consists primarily of adult hepatologists, lysosomal acid lipase deficiency (LAL D) is a disease of interest across paediatric and adult specialties. Although LAL D has been historically described as Wolman's disease in infants and cholesteryl ester storage disease in children and adults,[1-3] it is now recognised as a single disease that presents across an age continuum. To this end, Prof Ratziu emphasised that the purpose of the symposium was to raise awareness about the clinical presentation of LAL D and its diagnosis, particularly in adults. Indeed, LAL D can present at any age, and currently many adults with 'silent' disease are being diagnosed.

Clinical Presentation and Diagnosis of LAL D

Professor Ali Canbay and Doctor Mark Bechter

Clinical presentation

LAL D is an autosomal recessive disease resulting from homozygous or compound heterozygous mutations of the *LIPA* gene that markedly reduce or eliminate the activity of the LAL enzyme.[4] Because LAL is important for the lysosomal degradation of cholesteryl esters and triglycerides, these products accumulate in the lysosomes across multiple body tissues, primarily the liver, spleen, gastrointestinal (GI) tract, and blood vessel walls, producing progressive organ damage (Figure 1).[5,6]

Presenting across an age continuum, LAL D is a progressive, multisystem disease with early mortality and significant morbidity affecting infants, children, and adults.[4,5] LAL D is rapidly progressive and fatal in infants, who present with growth failure along with prominent hepatic and GI manifestations, but LAL D in children and adults may have a more variable progression and complex disease presentation, including a combination of dyslipidaemia (elevated low-density lipoprotein [LDL] cholesterol and decreased high-density lipoprotein [HDL] cholesterol) and hepatic dysfunction. In particular, the latter may manifest as hepatomegaly, persistently elevated serum transaminases, microvesicular steatosis (MVS) on liver biopsy, and a high frequency of fibrosis and cirrhosis. Chronic dyslipidaemia may also lead to early atherosclerosis (AS) and increased risk of myocardial infarction, coronary heart disease, stroke, and death.[4,5,7]

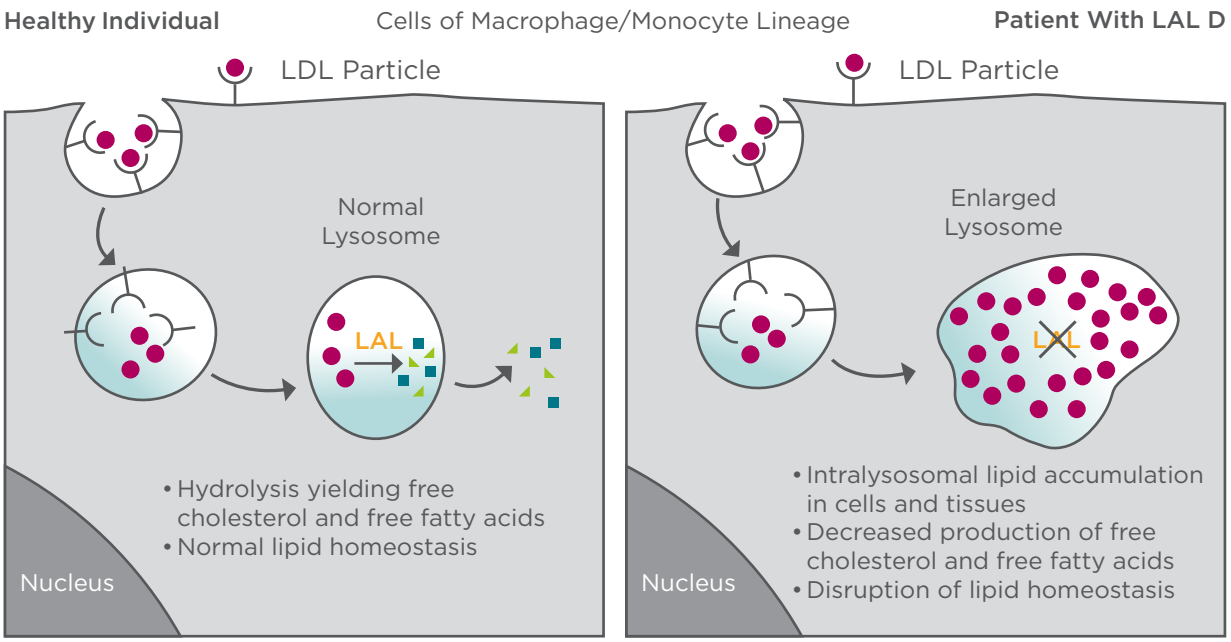

Figure 1: Lysosomal acid lipase plays a key role in lysosomal degradation of cholesteryl esters and triglycerides.

LDL: low-density lipoprotein; LAL D: lysosomal acid lipase deficiency.

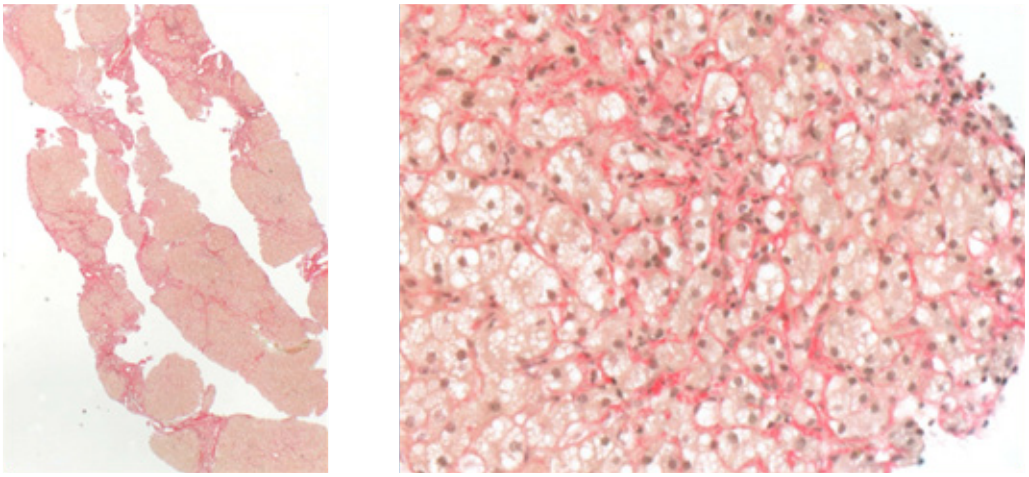

Figure 2: Liver biopsy with haematoxylin/van Gieson stain showing periportal fibrosis as typically seen in lysosomal storage diseases.

The prevalence of disease is difficult to determine due to misdiagnosis and potential underdiagnosis, with estimates ranging between 1:40,000 and 1:300,000.[3,6,8,9] That a small number of cases of LAL D have been reported in the literature suggests that LAL D may be substantially underdiagnosed or misdiagnosed.[7] Given that LAL D shares clinical presentation with other cardiovascular, liver, and metabolic diseases, LAL D might not be readily recognised in clinical practice and many patients may not be accurately diagnosed. This is critical given that the disease may be 'silent' and disease progression can be unpredictable, with liver failure and/or accelerated AS potentially contributing to early mortality.[4,6]

Diagnosis

To date, over 40 loss-of-function *LIPA* mutations have been identified in patients with LAL D, with the most common splice mutation occurring in exon 8 (E8SJM-1).[5] Because the *LIPA* gene is responsible for production of LAL, mutations result in little to no production of LAL. LAL D can be definitively diagnosed using a LAL enzyme-based biochemical test to assess enzyme levels in the peripheral blood of patients with a suspicion of LAL D, with subsequent genetic assays useful from a clinical research perspective.[5,10] LAL enzyme activity levels do not predict disease severity or progression, and there are no genotype/phenotype correlations reported for the presentation of LAL D in children and adults.[5]

LAL D is currently managed with dietary modifications (low-fat diet), lipid-lowering medications, liver transplantation, and haematopoietic stem cell transplantation.[4] There are currently no disease-specific treatments approved for LAL D that address the underlying cause of disease, but studies of potential treatments are ongoing. Existing supportive approaches can help manage disease symptoms; however, they do not treat the underlying cause or alter the course of disease. Early diagnosis is critical to allow for active monitoring of patients to reduce the potential of disease complications. Given the high potential for misdiagnosis or underdiagnosis of LAL D, efforts are increasing to raise awareness of LAL D and make it part of the differential diagnosis for patients with liver dysfunction and/or dyslipidaemia.[5,10]

Keep An Open Mind

Doctor Lauren Johansen

Doctor Johansen discussed a case involving a 7-year-old girl who initially presented to a general practitioner with fatigue, anaemia, and abnormal liver function tests (alanine aminotransferase [ALT]: 166 IU/l; aspartate aminotransferase [AST]: 201 IU/l; gamma-glutamyltransferase [GGT]: 123 IU/l). Investigations at a tertiary center revealed hepatosplenomegaly, prolonged prothrombin time (15 seconds), raised immunoglobulin G (IgG), and a positive antinuclear antibody (ANA) test. Liver biopsy demonstrated chronic hepatitis with moderate fibrosis and fatty change. The steatosis prompted metabolic investigations, which excluded

Wilson's disease (WD). There was also villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis on duodenal biopsy, in keeping with a diagnosis of coeliac disease (CoD).

The patient's symptomatology improved with a gluten-free diet, but her transaminases did not fully normalise. Treating physicians theorised that the patient may have dual pathology, with autoimmune hepatitis (AIH) — as indicated by raised IgG and positive ANA — and CoD. This prompted the initiation of corticosteroids and then azathioprine; however, the patient's persistent lack of response and subsequent development of neutropaenia prompted further evaluation. At 9 years of age, she had a normal body mass index (BMI), but presented with elevated ALT, AST, and GGT despite being compliant with therapy. Magnetic resonance cholangiopancreatography demonstrated that the spleen remained enlarged at 12 cm, but there was no evidence of intra or extra-hepatic bile duct dilatation. Subsequent blood work revealed a profile consistent with dyslipidaemia (elevated total cholesterol [normal range]: 9.9 mmol/l [2.8-4.8], low HDL: 0.9 mmol/l [>0.9], and elevated triglycerides: 1.84 mmol/l [0.38-1.38]). Dr Johansen stressed that dyslipidaemia in conjunction with ongoing signs of inflammation and the development of cirrhosis on liver biopsy (Figure 2) raised the suspicion of LAL D.

LAL D diagnosis was confirmed through the LAL enzyme-based biochemical blood test (BBT), which showed that the patient had low LAL activity (<0.01 nmol/punch/hour [normal 0.07-0.50]). The patient has subsequently received investigational treatment for LAL D in a clinical trial and demonstrated improvement with normalisation of ALT over a 3-month period. She will be re-evaluated for CoD to confirm the dual diagnosis. Given that it took approximately 2 years to arrive at the correct diagnosis of LAL D, Dr Johansen emphasised the following key learning points:

- A lipid profile is mandatory in paediatric patients with liver disease
- It is critical to revisit the initial diagnosis if the patient's profile does not fit the disease
- Do not discontinue diagnostic exploration even if the patient appears to have some response to treatment
- LAL D can now be easily diagnosed with a BBT

This case uniquely highlights that there may be more than one underlying cause for a patient's symptoms, making the process of diagnosing LAL D perhaps complex but not complicated. Dr Johansen's concluding statements further suggest LAL D should be part of the differential diagnosis in paediatric patients with:

- Liver disease, including unexplained persistently raised liver transaminases and/or unexplained hepatomegaly, cryptogenic cirrhosis, and those with predominantly MVS or mixed macro/MVS on biopsy
- Lipid abnormalities, including dyslipidaemia (LDL ≥4.1 mmol/l, HDL ≤1 mmol/l in males or ≤1.3 mmol/l in females), or presumed diagnosis of familial hypercholesterolaemia, and those with an unclear family history or lacking the common genetic mutations LDLR/APO B/PCSK9

Overweight and Elevated Liver Enzymes: Not Always NAFLD!

Professor Christophe Moreno

Prof Moreno described a case of a 33-year-old woman with elevated liver enzymes who was referred by her general practitioner to the outpatient clinic at Erasme Hospital. She was suspected of having Epstein-Barr virus (EBV) infection with current symptoms of fatigue and recent gastroenteritis. The patient had a medical history of hypercholesterolaemia and urticaria; in addition, she did not drink alcohol but smoked two packets of cigarettes per month and was currently taking simvastatin 20 mg, and ibuprofen and ebastine as needed.

A physical examination revealed that the patient was overweight but with normal blood pressure (BP) (weight: 80 kg, height: 170 cm, BMI: 27.7 kg/m^2, BP: 110/60 mmHg). Outside of reddish maculae on her lower extremities, the physical examination was normal. The patient's laboratory workup indicated normal haematologic, renal, and thyroid functioning, no evidence of inflammation, a fasting blood sugar of 86 mg/dl, and ongoing elevated transaminases (AST: 36 U/l; ALT: 58 U/l; GGT: 42 U/l; alkaline phosphatase: 52 U/l; bilirubin total: 20.5 µmol/l); an ultrasound revealed moderate steatosis as well as an ovarian cyst.

Based on these findings, Prof Moreno queried whether the attendees would suspect

non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), EBV reactivation, AIH, or another diagnosis. The treating physicians conducted a fibroscan, more extensive blood workup, and a liver biopsy. The fibroscan revealed no significant liver fibrosis, but hepatic ultrasound was indicative of severe steatosis.[11,12] Further, laboratory evaluation showed hypertriglyceridaemia (1.77 mmol/l) and reduced HDL cholesterol (<1.30 mmol/l), which in conjunction with the patient's central obesity (waist circumference: 94 cm) was indicative of metabolic syndrome. Having excluded WD and other fatty liver diseases, NAFLD/NASH was proposed as the likely diagnosis. Of note, IgG levels were elevated and ANAs were positive (1/160).

After 3 months during which the patient stopped taking statin therapy and modified her diet, a follow-up liver biopsy revealed signs of chronic hepatitis of unknown origin, septal fibrosis, rare hepatocyte ballooning, MVS, and slight lobular inflammation (Figure 3). At this point, AIH was excluded[13,14] and an alternative cause was suspected, with the patient's condition considered borderline NASH. Investigators suspected alternative causes of MVS (Reye's syndrome, medication use [valproate, antiretroviral medicines], acute fatty liver of pregnancy, HELLP syndrome, and inborn errors of metabolism [e.g. LAL D]).[15]

At this point, LAL D was raised as a differential diagnosis. The LAL enzyme-based BBT confirmed that there were undetectable LAL activity levels (<0.02 nmol/punch/hour; reference range: 0.37-2.30), making this the first adult case of LAL D to be officially diagnosed in Belgium. Based on this patient case, Prof Moreno concluded that LAL D is not only a paediatric disease, but should be considered in young adult patients — even those who are overweight — based on their family history and in the presence of dyslipidaemia and MVS.

Lysosomal Acid Lipase Deficiency — Differential Diagnosis Versus NASH or NAFLD

Professor Ali Canbay

Prof Canbay introduced his presentation by discussing common causes of steatosis and steatohepatitis, which include alcohol-induced steatosis, NAFLD/NASH, and chemotherapy-induced steatosis, as well as CoD, Gaucher's disease, and LAL D. He then focussed on LAL D as part of the differential diagnosis versus NASH or NAFLD. As obesity rates increase — approximately 50% of the European population is now considered overweight (BMI >25)[16,17] — NAFLD/NASH has become the primary cause of liver disease in Western countries, and the prevalence has doubled worldwide even though other chronic liver diseases have been stable or decreasing.[18] NAFLD is primarily defined by macrovesicular steatosis; NASH is a subgroup of NAFLD characterised by liver cell injury, inflammation, and the possible presence of fibrosis in addition to steatohepatitis.[18]

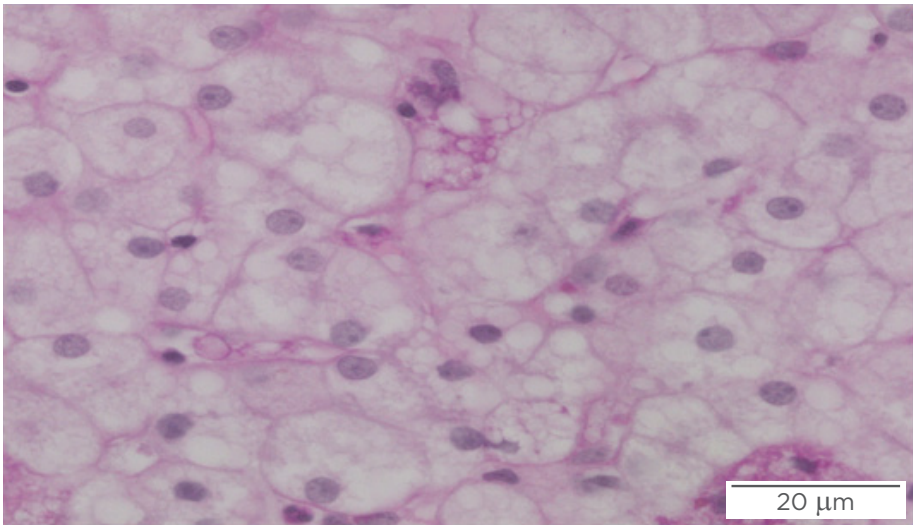

Figure 3: Liver biopsy showing signs of chronic hepatitis of unknown origin, septal fibrosis, rare hepatocyte ballooning, microvesicular steatosis, and slight lobular inflammation.

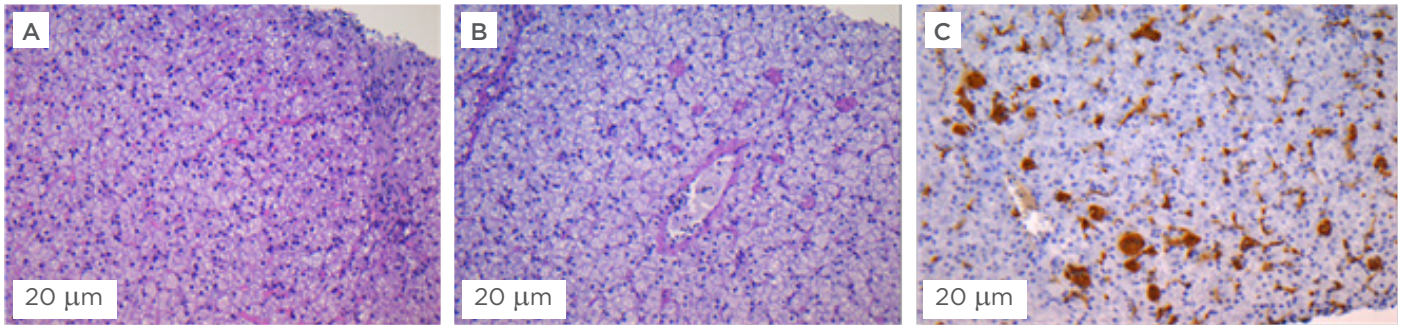

Figure 4: Liver biopsy showing A) periportal fibrosis and microvesicular steatosis, B) D-PAS-positive Kupffer cells, and C) intrahepatic CD68-positive cells.

Generally, NAFLD/NASH does not cause specific symptoms: patients potentially experience fatigue, malaise, and abdominal discomfort; however, accurately diagnosing NAFLD/NASH is critical given that disease progression to cirrhosis/liver failure and hepatocellular carcinoma can occur.[15,18,19]

Other less common diseases such as LAL D should be in the differential diagnosis for NAFLD/NASH. To illustrate this, Prof Canbay described a case of a 39-year-old woman with a normal weight (BMI: 24) who presented with hepatomegaly and splenomegaly of unknown origin as well as thrombocytopaenia and a suspicion of portal hypertension. Following a liver biopsy, periportal fibrosis with 20% MVS and Kupffer cells with positive lysosomes were identified as well as intrahepatic CD68-positive cells (Figure 4).

Dr Canbay stated that these histologic findings raised a suspicion of LAL D and prompted treating physicians to assess LAL and chitotriosidase activity levels, the latter of which is elevated in various lysosomal storage disorders. Tests returned with practically undetectable LAL enzyme activity levels (<0.01 nmol/punch/hour; reference range: 0.10-2.0) and elevated chitotriosidase activity in plasma (12.3 nmol/min/ml; reference range: <1.5). These findings provided the definitive diagnosis of LAL D. Genetic testing identified homozygote mutation G934->A in exon 8 of the *LIPA* gene.

Guidelines currently recommend that a diagnosis of NAFLD requires the presence of steatosis (with the exception of cirrhotic disease) and no other causes for secondary hepatic steatosis.[15,20] Based on this patient case, Prof Canbay urged that it is critical to revisit NAFLD/NASH in all patients whose symptoms may not exactly match this diagnosis. When evaluating a patient with suspected NAFLD, it is essential that all aetiologies of steatosis be considered, and any patient with MVS should be evaluated for LAL D using the biochemical enzyme-based assay.

He further indicated that although clinical and biochemical abnormalities associated with LAL D are subtle and may not trigger medical concerns, LAL D is progressive and unpredictable with the potential for rapid clinical decline.[4,7] He stated that because diagnosis requires only a simple enzymatic BBT, it will be important to identify LAL D patients so that medical follow-up can be tailored to known risks.[5,10]

Silent Fibrosis and Cirrhosis — Acid Lipase Replacement Investigating Safety and Efficacy (ARISE) Trial

Doctor Mark Bechter

LAL D is currently managed through dietary modifications (low-fat diet), lipid-lowering medications, liver transplantation, and haematopoietic stem cell transplantation.[4] Although these supportive approaches can help to manage disease symptoms, they do not treat the underlying cause or notably alter disease progression. Sebelipase alfa (SA) is an enzyme replacement therapy currently in clinical development. It is an exogenous replacement of the deficient LAL enzyme with a recombinant human enzyme that catalyses the hydrolysis of cholesteryl esters and triglycerides. In pre-clinical studies and studies in patients with LAL D, SA has been shown to improve markers of liver damage and lipid abnormalities.[21-23] In a Phase I/II open-label, dose-escalation trial to evaluate the

long-term safety of SA in adults with LAL D, patients (n=8) received intravenous infusions of SA for up to 104 weeks. Improvements in serum transaminases and lipid levels were sustained through the entire 104-week treatment period. Further, SA was not associated with drug-related serious adverse events (SAEs), although one patient had one unrelated SAE (cholecystitis and cholelithiasis) and continued on the study. Most infusion-associated reactions were mild and primarily gastrointestinal, and there was no evidence of anti-drug antibodies in patients tested to date in this study.[21]

At this symposium, Dr Bechter presented current data from an ongoing Phase III randomised, placebo-controlled clinical trial that is assessing the efficacy and safety of intravenous infusions of SA in children and adults with LAL D.[24,25] In the Acid Lipase Replacement Investigating Safety and Efficacy (ARISE) trial, the study population includes patients with LAL D who were a minimum of 4 years of age with ALT levels ≥1.5 times the upper limit of normal and who could be taking a stable dose of lipid-lowering medications. Patients who had undergone a previous liver or haematopoietic stem cell transplant, or who had severe hepatic dysfunction (Child-Pugh Class C) were excluded. The primary endpoint for ARISE is ALT normalisation; secondary endpoints include LDL cholesterol reduction, non-HDL cholesterol reduction, AST normalisation, triglyceride reduction, HDL cholesterol increase, liver fat content reduction, improvement in steatosis, and liver volume reduction.

In summary, it will be critical to increase our understanding of LAL D. In addition to ongoing studies of new therapies for LAL D, there is currently a LAL D registry available for collecting longitudinal data.[25]

REFERENCES

1. Abramov A et al. Generalized xanthomatosis with calcified adrenals. AMA J Dis Child. 1956;91(3):282-6.

2. Fredrickson DS et al. Lipolytic activity of post-heparin plasma in hyperglyceridemia. J Lipid Res. 1963;4: 24-33.

3. Muntoni S et al. Prevalence of cholesteryl ester storage disease. Arterioscler Thromb Vasc Biol. 2007;27(8):1866-8.

4. Reiner Ž et al. Lysosomal acid lipase deficiency--an under-recognized cause of dyslipidaemia and liver dysfunction. Atherosclerosis. 2014;235(1):21-30.

5. Bernstein DL et al. Cholesteryl ester storage disease: review of the findings in 135 reported patients with an underdiagnosed disease. J Hepatol. 2013;58(6):1230-43.

6. Grabowski GA et al. Lysosomal acid lipase deficiencies: the Wolman disease/ cholesteryl ester storage disease spectrum. 2012. Available at: http:// ommbid.mhmedical.com/content.aspx?bookid=474&sectionid=45374143. Last accessed: May 29, 2015.

7. Jones S et al. Rapidly progressive disease course in the natural history of infants with lysosomal acid lipase deficiency. Poster 113. Lysosomal Disease Network WORLD Symposium, 11-13 February 2014.

8. Scott SA et al. Frequency of the cholesteryl ester storage disease common LIPA E8SJM mutation (c.894G>A) in various racial and ethnic groups. Hepatology. 2013;58(3):958-65.

9. Stitziel NO et al. Exome sequencing and directed clinical phenotyping diagnose cholesterol ester storage disease presenting as autosomal recessive hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2013;33(12):2909-14.

10. Hamilton J et al. A new method for the measurement of lysosomal acid lipase in dried blood spots using the inhibitor Lalistat 2. Clin Chim Acta. 2012;413(15-16):1207-10.

11. de Lédinghen V et al. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. J Hepatol. 2014;60(5):1026-31.

12. Wong VW et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51(2):454-62.

13. Alvarez F et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J Hepatol. 1999;30(2):222-7.

14. Hennes EM et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48(1):169-76.

15. Chalasani N et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005-23.

16. Blachier M et al. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol. 2013;58(3):593-608.

17. Organisation for Economic Co-operation and Development (OECD). Obesity Update 2014. 2014. Available at: http://www.oecd.org/els/health-systems/Obesity-Update-2014.pdf. Last accessed: 3 June 2015.

18. World Gastroenterology Organisation. World Gastroenterology Organisation Global Guidelines: Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. 2012. Available at: http://www.worldgastroenterology.org/assets/export/userfiles/2012_NASH%20and%20NAFLD_Final_long.pdf. Last accessed: 29 May 2015.

19. Ertle J et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer. 2011;128(10): 2436-43.

20. Ratziu V et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol. 2010;53(2):372-84.

21. Balwani M et al. Clinical effect and safety profile of recombinant human lysosomal acid lipase in patients with cholesteryl ester storage disease. Hepatology. 2013;58(3):950-7.

22. Thelwall PE et al. Hepatic cholesteryl ester accumulation in lysosomal acid lipase deficiency: non-invasive identification

and treatment monitoring by magnetic resonance. J Hepatol. 2013;59(3):543-9.

23. Valayannopoulos V et al. Sebelipase alfa over 52 weeks reduces serum transaminases, liver volume and improves serum lipids in patients with lysosomal acid lipase deficiency. J Hepatol. 2014;61(5):1135-42.

24. Synageva BioPharma Corporation. A multicenter study of SBC-102 (sebelipase alfa) in patients with lysosomal acid lipase deficiency/ARISE (acid lipase replacement investigating safety and efficacy). NCT01757184. https://clinicaltrials.gov/ct2/show/NCT01757184.

25. Synageva BioPharma Corporation. Identification of undiagnosed lysosomal acid lipase deficiency. NCT01716728. https://clinicaltrials.gov/ct2/show/NCT01716728.