Lysosomal acid lipase deficiency

An under-diagnosed cause of liver dysfunction

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Disclosures

- Support was provided by Synageva BioPharma Corp to develop Lysosomal Acid Lipase testing at ARUP

Learning objectives

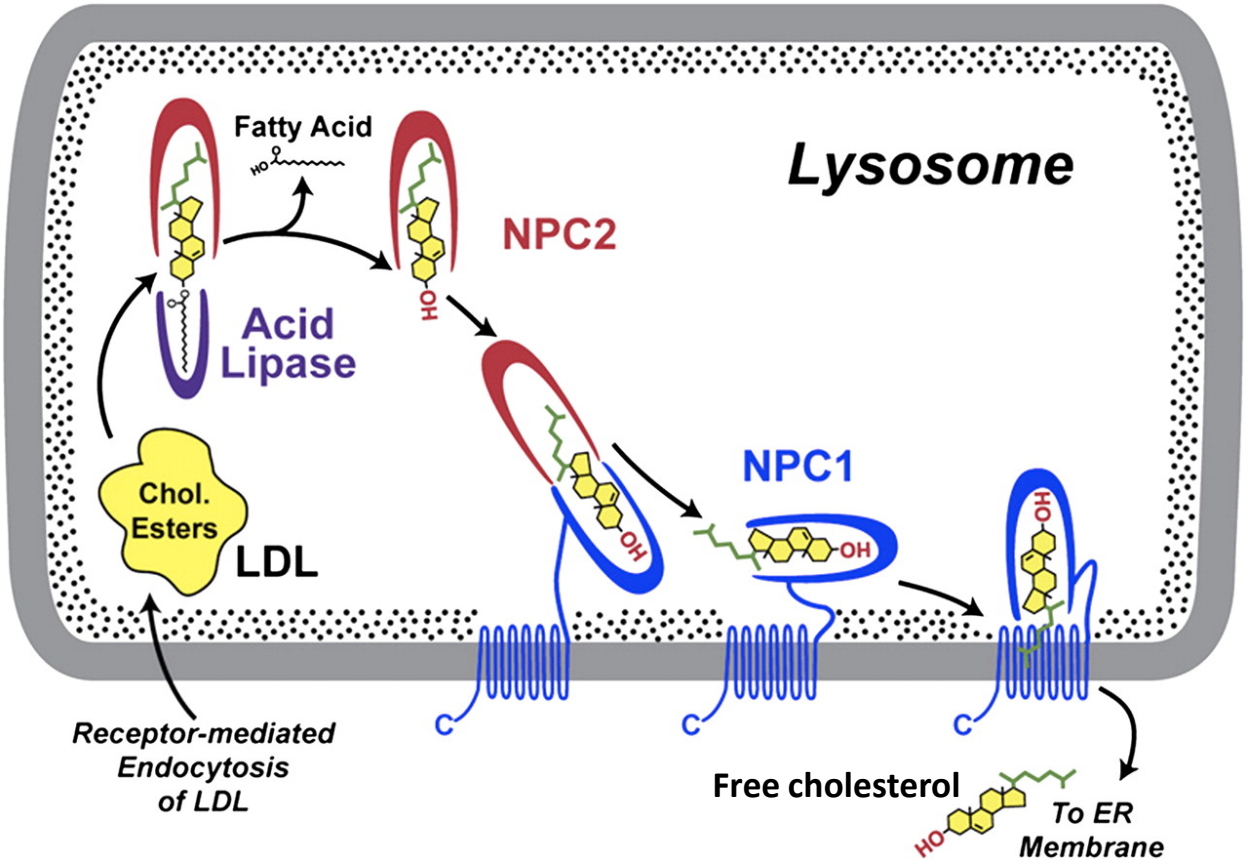
- Explore the role of the lysosomal acid lipase (LAL) on cholesterol homeostasis
- Describe the clinical findings observed in the two phenotype variants of LAL deficiency (LAL-D): Wolman disease (WD)and cholesteryl ester storage disease (CESD)
- Identify the recommended screening criteria and the required investigations to diagnose LAL-D
- Understand the treatment options and the results of clinical trials using recombinant human LAL

Lysosomal acid lipase Deficiency (LAL-D; OMIM #278000)

- Autosomal recessive lysosomal storage disease
  - **Characterized by the accumulation of cholesteryl esters and triglycerides in the late endocytic system**

LAL hydrolyzes cholesterol esters to yield free cholesterol

- Free cholesterol is bound by NPC2, transferred to NPC1, and exported outside the lysosome



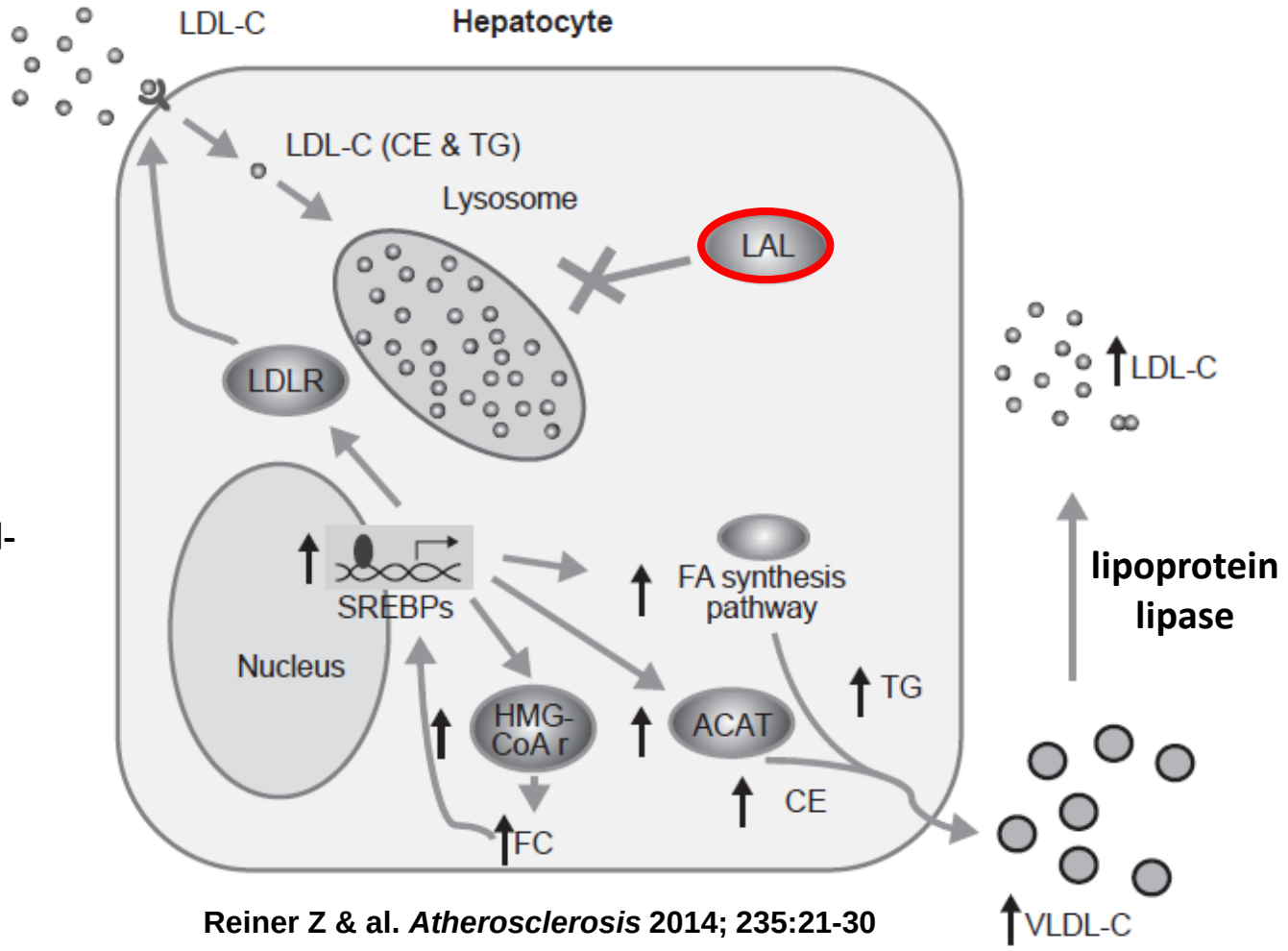
LDL: low-density lipoprotein

NPC: Niemann Pick-protein C

Miller WL & Bose HSJ. *Lipid Res*. 2011; 52:2111-35

Low free cholesterol in the cytosol upregulates cholesterol synthesis and uptake

- Cholesterol homeostasis in LAL-D patients



Reiner Z & al. *Atherosclerosis* 2014; 235:21-30

- **Sterol regulatory element binding proteins (SREBPs)**
- **Hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA r)**
- **Acyl-cholesterol acyltransferase (ACAT)**
- **Free cholesterol (FC)**
- **Triglycerides (TG)**
- **Cholesteryl esters (CE)**

LAL-D Phenotypic variants

- Clinically heterogeneous

Wolman disease (WD)

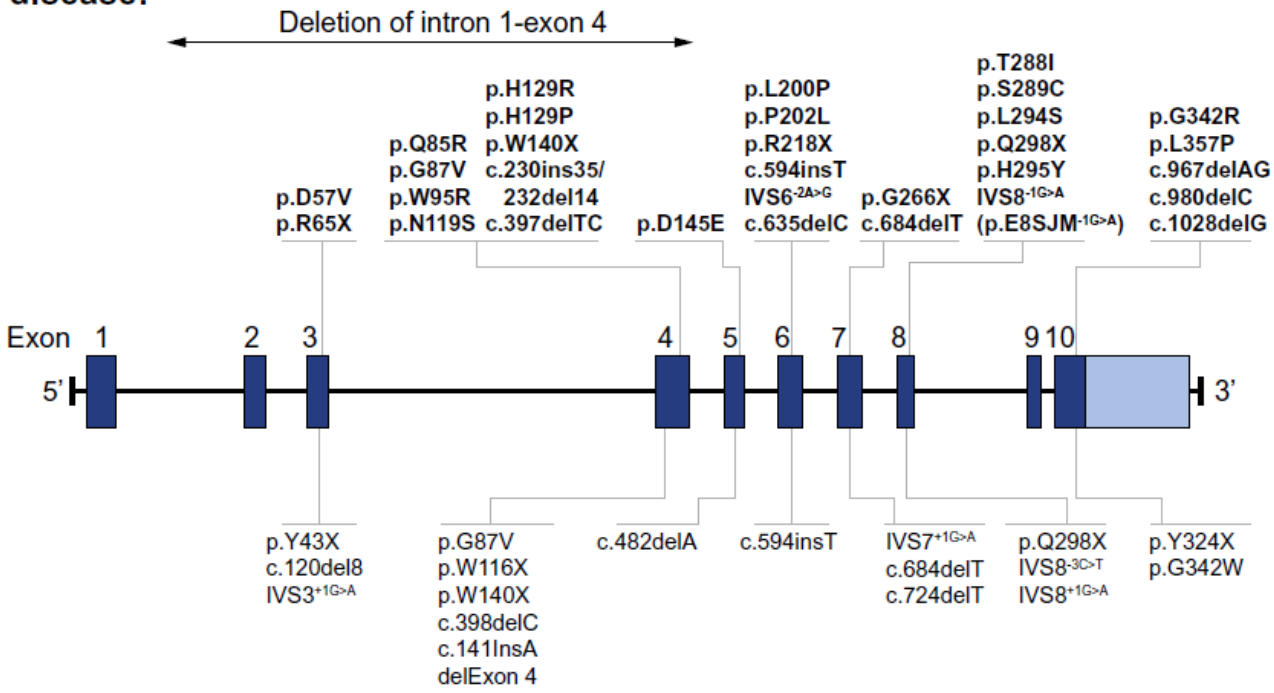
Cholesteryl ester storage disease (CESD)

WD and CESD share the same underlying molecular pathology

LIPA gene

Cholesteryl ester storage disease:

LAL activity
< 10-30%



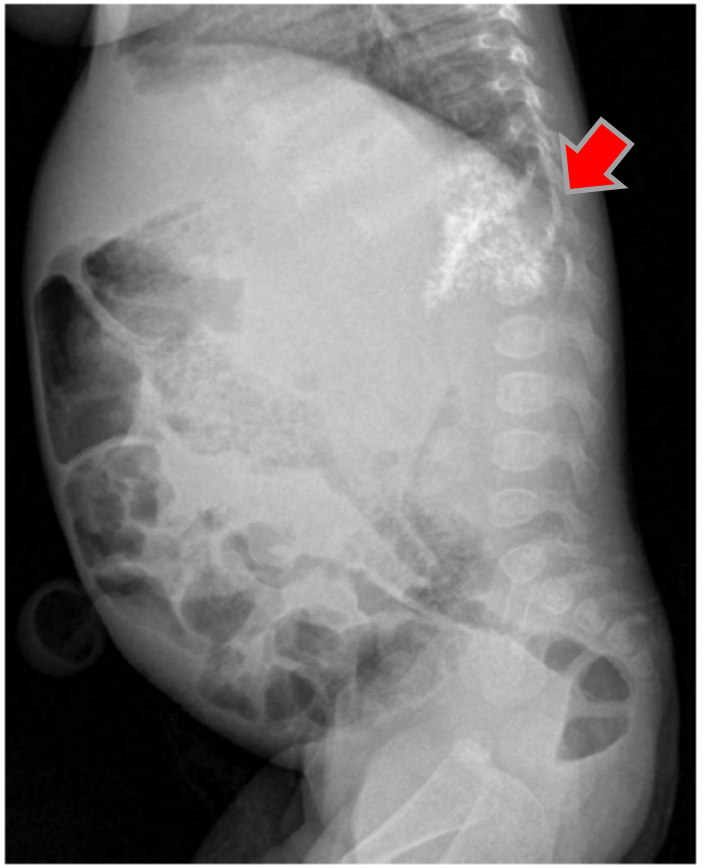
Wolman disease:

LAL activity
< 5%

Bernstein DL & al *J Hepatol*. 2013; 58:1230-43

Wolman disease is the severe, infantile form of LAL-D

- Very rare (< 100 cases published)
- Early onset
- Failure to thrive with vomiting, diarrhea and malabsorption
- Hepatosplenomegaly
- **Bilateral adrenal calcification (in 50% of patients)**
- Uniformly lethal in early infancy (within 1 year of age) due to hepatic and adrenal failure



Lateral radiograph of the abdomen 5-month-old male infant referred for FTT and hepatosplenomegaly

Shenoy P & al *BMJ Case Rep*. 2014

WD can be successfully treated with hematopoietic cell transplantation

- However, survival is poor, mainly due to liver damage
  - only 3/10 published patients survived

Outcomes in published cases of Wolman Disease following HSCT.

Publication (author and year)	Age diagnosis (months)	Age transplant (months)	Outcome (age death months)	Last follow up from transplantation	Early complications (0–3 months after transplantation)	Outcome comments
Krivit W et al. (1992) [2]	6	44	Death (46)	44 days	GVHD Grade 3	
	3	18	Death (20)	50 days	GVHD Grade 3 Renal failure	Splenectomy age 4 months and Liver Tx age 7 months. alveolar macrophage lipid accumulation
	Not stated	3	Death (3)	10 days	Busulfan liver toxicity, severe sepsis; renal & respiratory failure	
	Not stated	Not stated	Death	Not stated	Severe infection	
Krivit W et al. (2000) [1]. Tolar et al. (2009) [3]	Not stated	6.5	Alive	>12 years	GVHD Grade 2 Day 21; severe hyperbilirubinemia week 7	Impaired cognitive function Resolved convulsions GH deficiency Hypothyroidism Adrenal recovery
Stein J et al. (2007) [4]	~2	3	Alive	>4 years	Diarrhea, hypothyroidism, transient hypocalcemia	Growth catch up Hypothyroidism Suspected gonadal failure Adrenal recovery No cognitive impairment
Tolar et al. (2009) [3]	2	4.7	Death (7)	67 days	Sinusoidal obstruction syndrome Hepatorenal & respiratory failure, coagulopathy; sepsis	
	17	1) 19, 20 2) 25	Death (27)	8 months	1) Primary graft failure; 2) GVHD Grade 3, sepsis and liver failure	Diffuse fibrosis of the liver, necrosis of adrenal medulla.
	~2	2.5	Alive	>4 years	GVHD Grade 3	Resolved convulsions Growth catch up Adrenal insufficiency Normal thyroid function
Gramatges MM et al. (2009) [5]	~2	~2.8	Death (5.2)	78 days	HM, ascites, renal failure, sepsis and multi-organ failure	Evidence of peripheral LAL correction

Yanir A & al. *Mol Genet Metab.* 2013; 109(2):224-6.

The clinical spectrum varies greatly in cholesteryl ester storage disease patients

Median age of onset: 5 yrs of age (ranging from birth to 68 yrs)

- Early onset in childhood
  - **Wolman-like manifestations, but better prognosis (survival into childhood or adulthood)**
- Late onset in childhood/adulthood
  - **Gastrointestinal symptoms**
  - **Hepatosplenomegaly/liver dysfunction**
  - **Dyslipidemia progressing to premature atherosclerosis and cardiovascular disease**
  - **Anemia**

Bernstein DL & al *J Hepatol*. 2013; 58:1230-43

LAL-D is a rare cause of dyslipidemia

- Type IIa or type IIb hyperlipidemia
  - ↑ **Total cholesterol**
  - ↑ **Triglycerides**
  - ↑ **LDL-cholesterol**
  - ↓ **HDL-cholesterol**

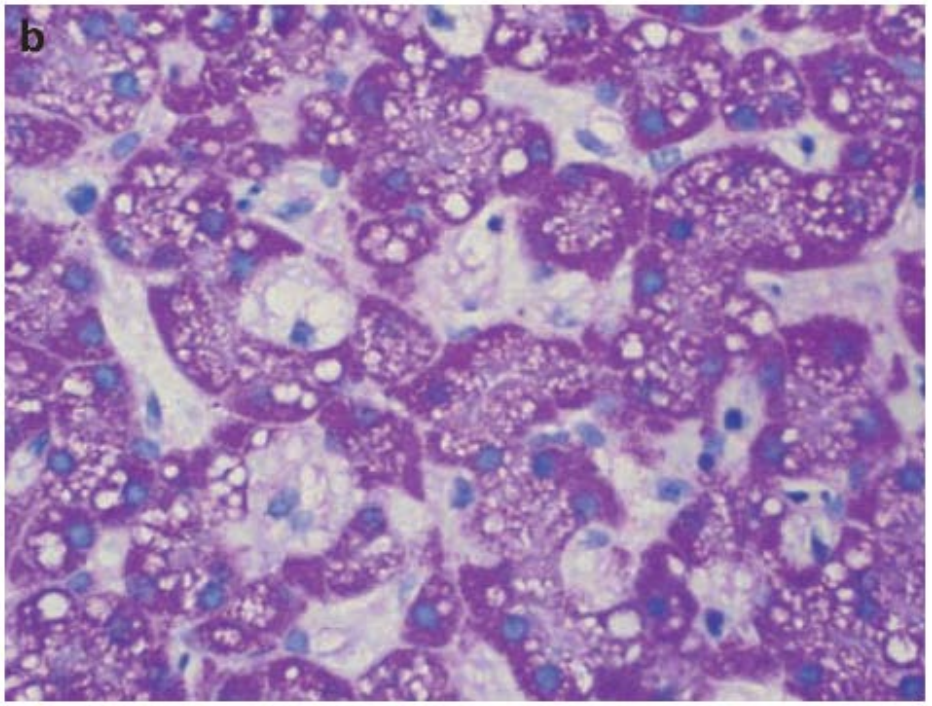
Table 1. Monogenic diseases causing hypercholesterolaemia

Gene	Disease	Prevalence	Metabolic defect
LDLR	FH	1/400 [7]	Lack of functional LDLR, reduced LDLc clearance
ApoB	FDB	1/800 [7]	Disrupted binding LDLR:ApoB, reduced clearance
PCSK9	FH3	1/2500 [8]	Increased LDLR degradation, reduced clearance
LDLRAP1	ARH	1/5 000 000 [8]	Failure to internalize LDLR, reduced clearance
LIPA	CESD or WD	1/130 000 [9▪,10]	Deficient hydrolysis of lipoprotein-bound CE and triglycerides, lysosomal accumulation of CE and TG

Fouchier SW & Defesche JC. *Curr Opin Lipidol*. 2013; 24(4): 322-8

Liver hystopathology in patients with lysosomal acid lipase deficiency

- Microvesicular steatosis
- Enlarged lipid-laden hepatocytes and Kupffer cells



Periodic acid-Schiff (PAS) stained section showing pale-staining Kupffer cells with abundant vacuolated cytoplasm

Tolar J & al. *Bone Marrow Transplant*. 2009; 43(1):21-7

Population screening suggests that CESD may be underdiagnosed

- Population screening indicates high carrier frequency in several ethnic groups for the common CESD mutation E8SJM

- Based on this data, expected disease prevalence is high than currently recognized:

  1:40,000 to 1:300,000 (depending on ethnicity and geographical location)

Muntoni S. & al. *Arterioscler Thromb Vasc Biol.* 2007; 27:1866–68
Scott SA. & al. *Hepatology*. 2013; 58(3): 958–965

Unrecognized condition?

- Due to its similarity with other cardiovascular, liver and metabolic diseases, the differential diagnosis of LAL-D can be challenging
  - **Failing to order diagnostic testing specific for this condition can lead to misdiagnosis**

Recommended screening criteria for LAL-D

- **Liver functional impairment**

- elevated serum transaminase activities

- **Hepatomegaly present**

- May be mild

- **Abnormal liver biopsy**

- Microvesicular steatosis

- **Elevated LDL-C with low HDL-C** (< 50 mg/dL)
- **Normal BMI** ≤ 30 kg/m^2

Bernstein DL & al *J Hepatol*. 2013; 58:1230-43

Investigations to diagnose LAL-D

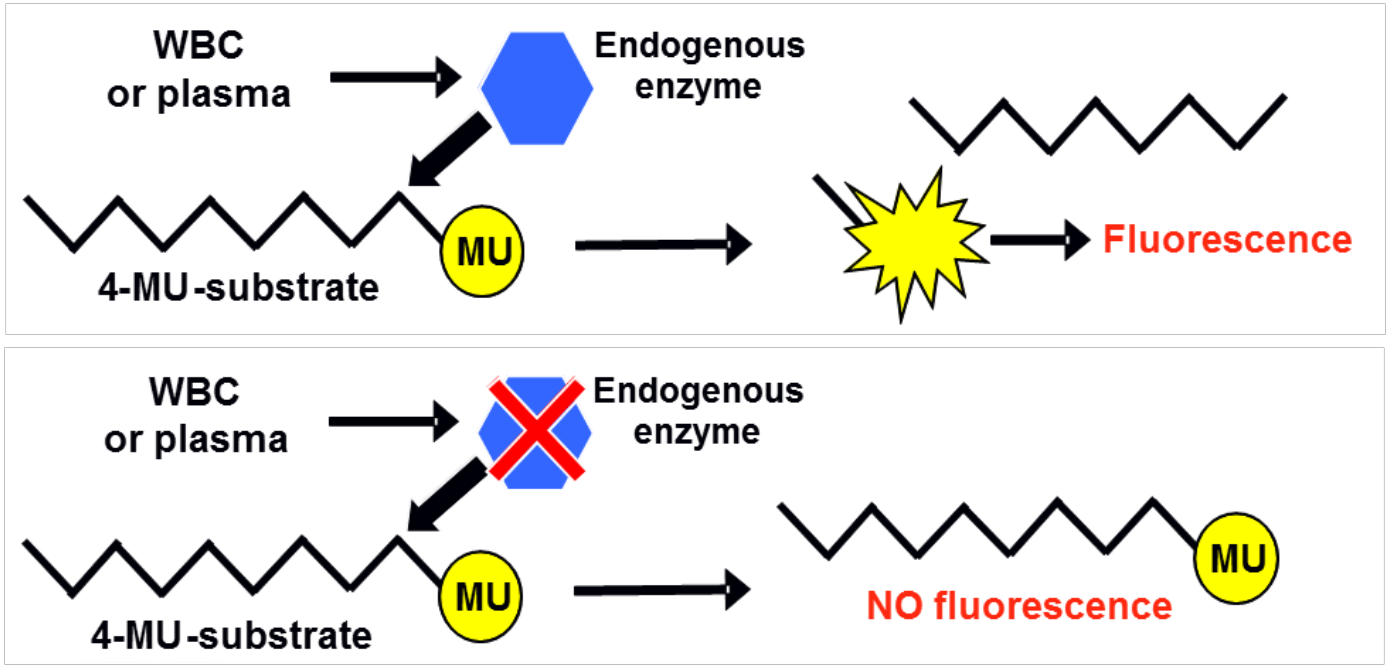
- ✓ **Lysosomal acid lipase enzymatic activity** in cultured fibroblasts, peripheral white cells or liver tissue using synthetic substrates
  - WD < 5% residual activity
  - CESD < 10-30% residual activity

- ✓ ***LIPA* gene testing**
  - Confirm diagnosis
  - Identify specific disease-causing mutations in *at-risk* family members and for prenatal diagnosis

- **Liver biopsy and radiological findings are not considered diagnostic**

Measurement of LAL activity *in vitro*

- Uses enzyme-specific artificial 4-MU-conjugated substrate: 4-methylumbelliferyl palmitate with cardiolipin (LAL activator)

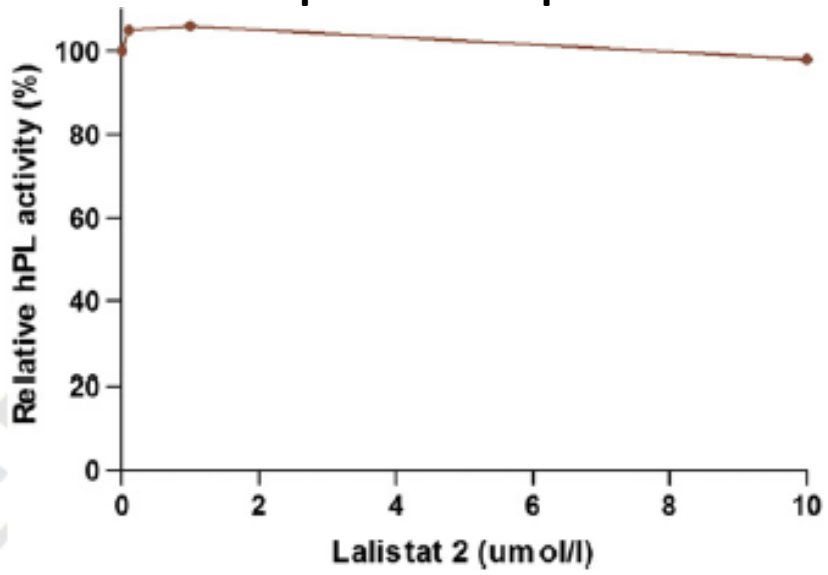


- **Method developed in fibroblasts (Guy et al. 1978)**

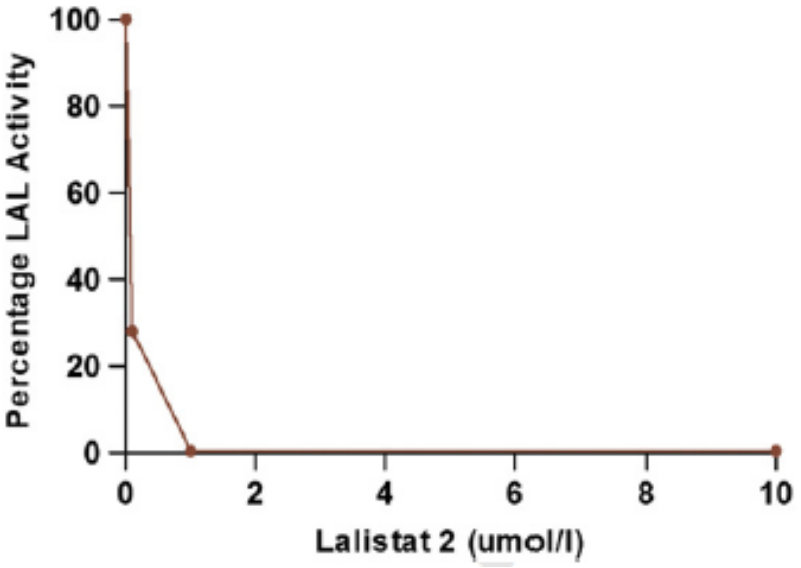
Other lipase interfere with LAL measurement in whole blood

- Highly specific inhibitor LALISTAT2 can be use to determine LAL activity by comparing total lipase activity to lipase activity in the presence of the inhibitor
- The difference can be attributed to LAL enzyme

Lalistat 2 effect on human pancreatic lipase

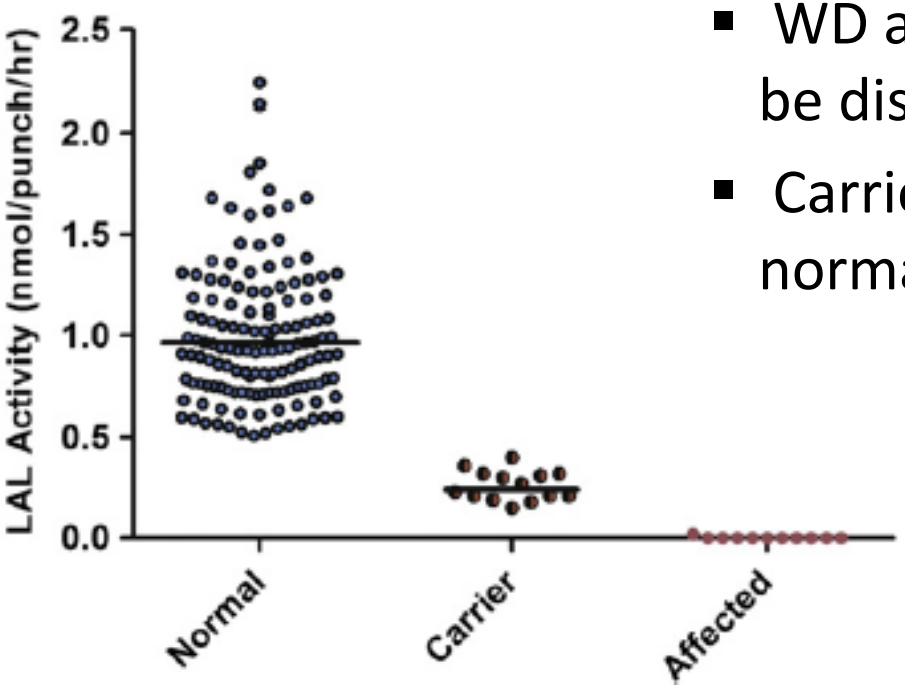


Lalistat 2 effect on human lysosomal acid lipase



Hamilton J & al *Clin Chim Acta*. 2012; 413):1207-10

Using LALISTAT2 shows differentiation between healthy and affected individuals



- WD and CESD variants cannot be discriminated
- Carrier range overlap with normal controls

Hamilton J & al *Clin Chim Acta*. 2012; 413):1207-10

- **Method has been successfully used in dried-blood filter paper (DBFP), leukocytes and fibroblasts (Cavallero et al. 2014)**

Why is it important to identify?

- A recombinant human lysosomal acid lipase is available for enzyme replacement therapy (ERT)
  - Sebelipase alfa; Synageva BioPharma Corp.

- Clinical trials are ongoing in CESD patients

Sebelipase alfa corrects clinically relevant abnormalities in animal models

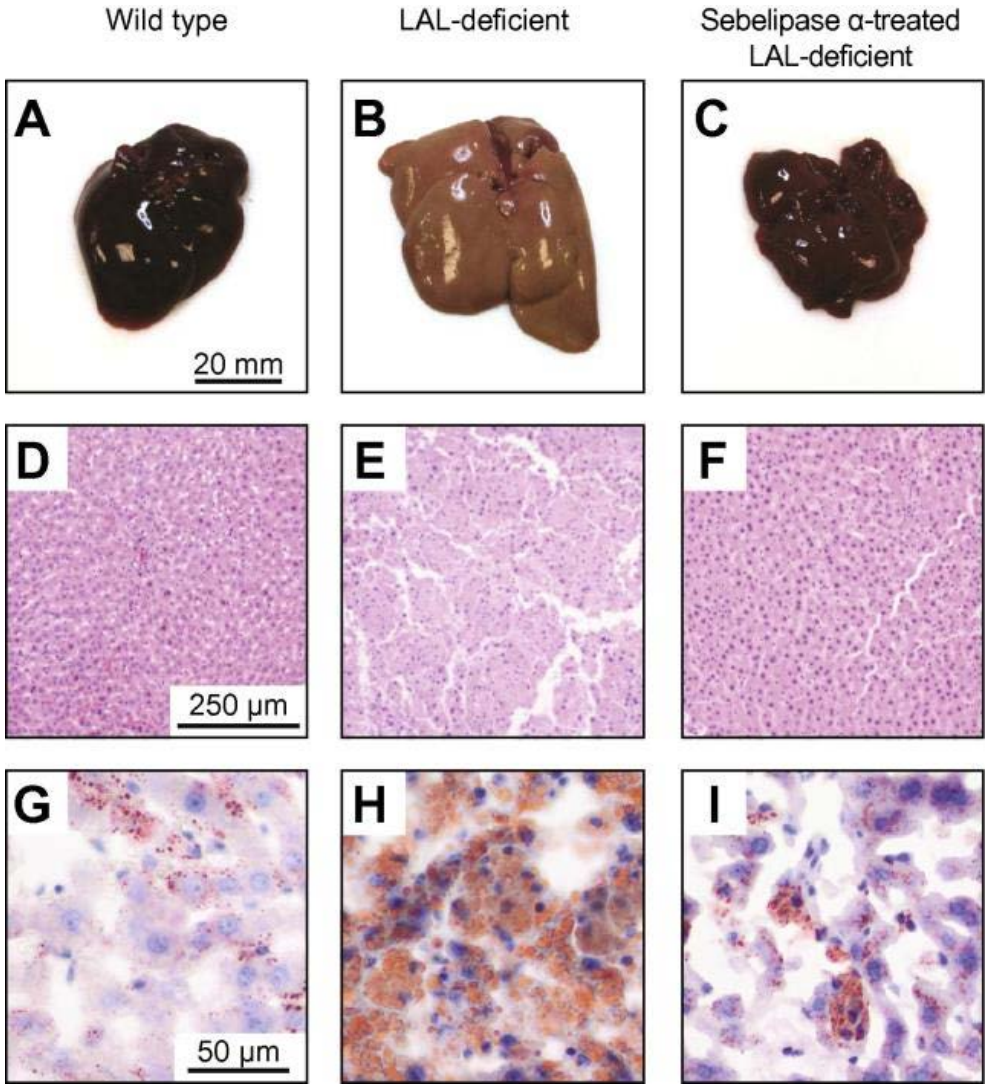
- LAL deficiency rat model
  - Donryu rat strain with a spontaneous deletion
- LAL-null mouse model

- ERT corrects growth failure, hepatosplenomegaly, and transaminase elevations

Thelwall PE & al. *J Hepatol.* 2013; 59(3): 543–9

Liver histopathology in the LAL-D rat model after Sebelipase alfa ERT

- Physical appearance
- Haematoxylin and eosin-stained histological sections
- Oil Red-O stained histological sections



Thelwall PE & al. *J Hepatol.* 2013; 59(3): 543–9

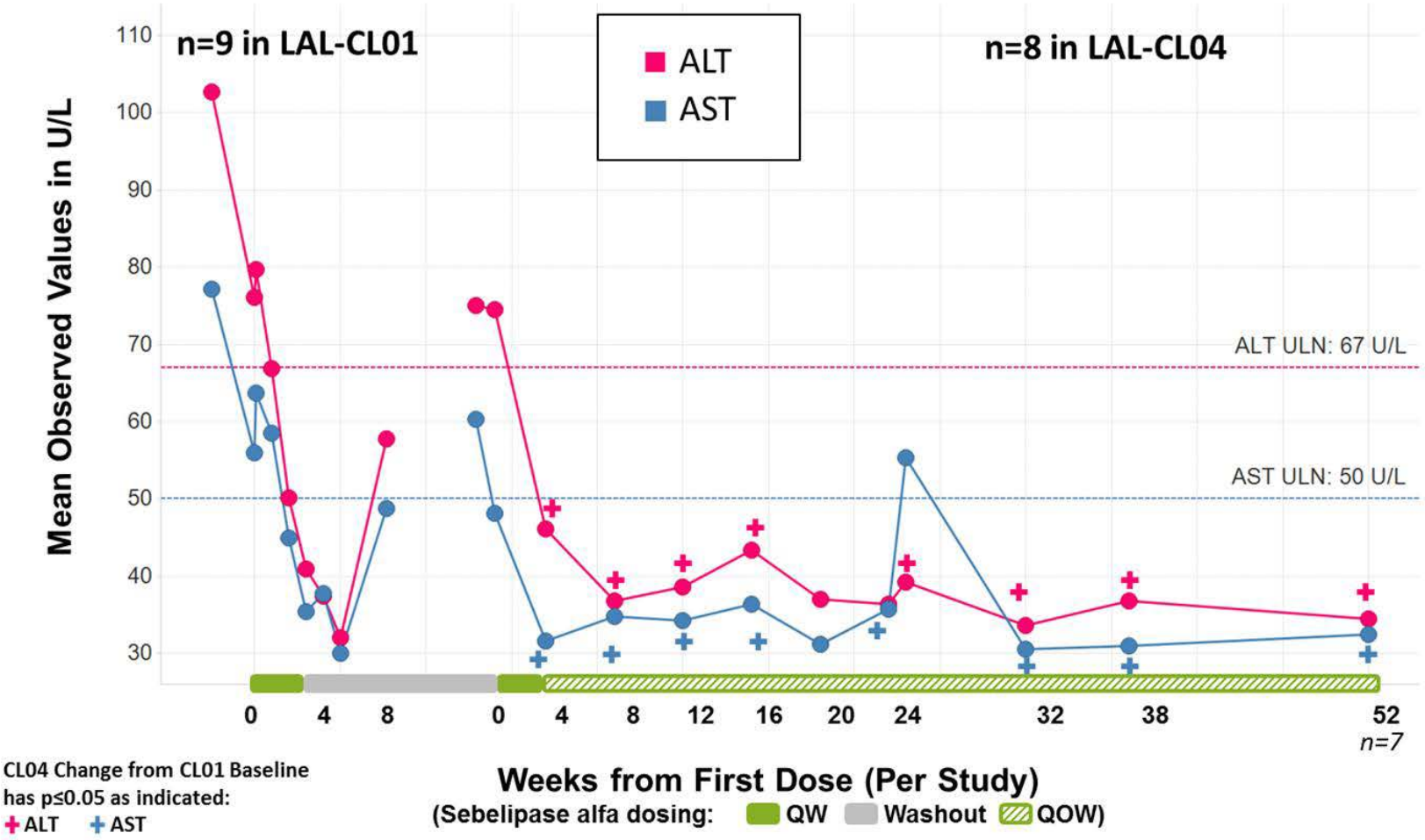
LAL-CL01 clinical trial is the first human study

- A Phase 1/2 open-label, multicenter, dose-escalation study (LAL-CL01) was conducted in 2011 - 2012 across 6 sites in 4 countries to assess safety and the clinical effects
- Patients completing LALCL01 were eligible to enroll in the extension study (LAL-CL04)
- LAL-CL04 is ongoing (published results at 52 ws)
- Long-term Sebelipase alfa dosing is well tolerated and produces sustained results

Balwani M & al. *J Hepatol.* 2013; 58(3):950-7

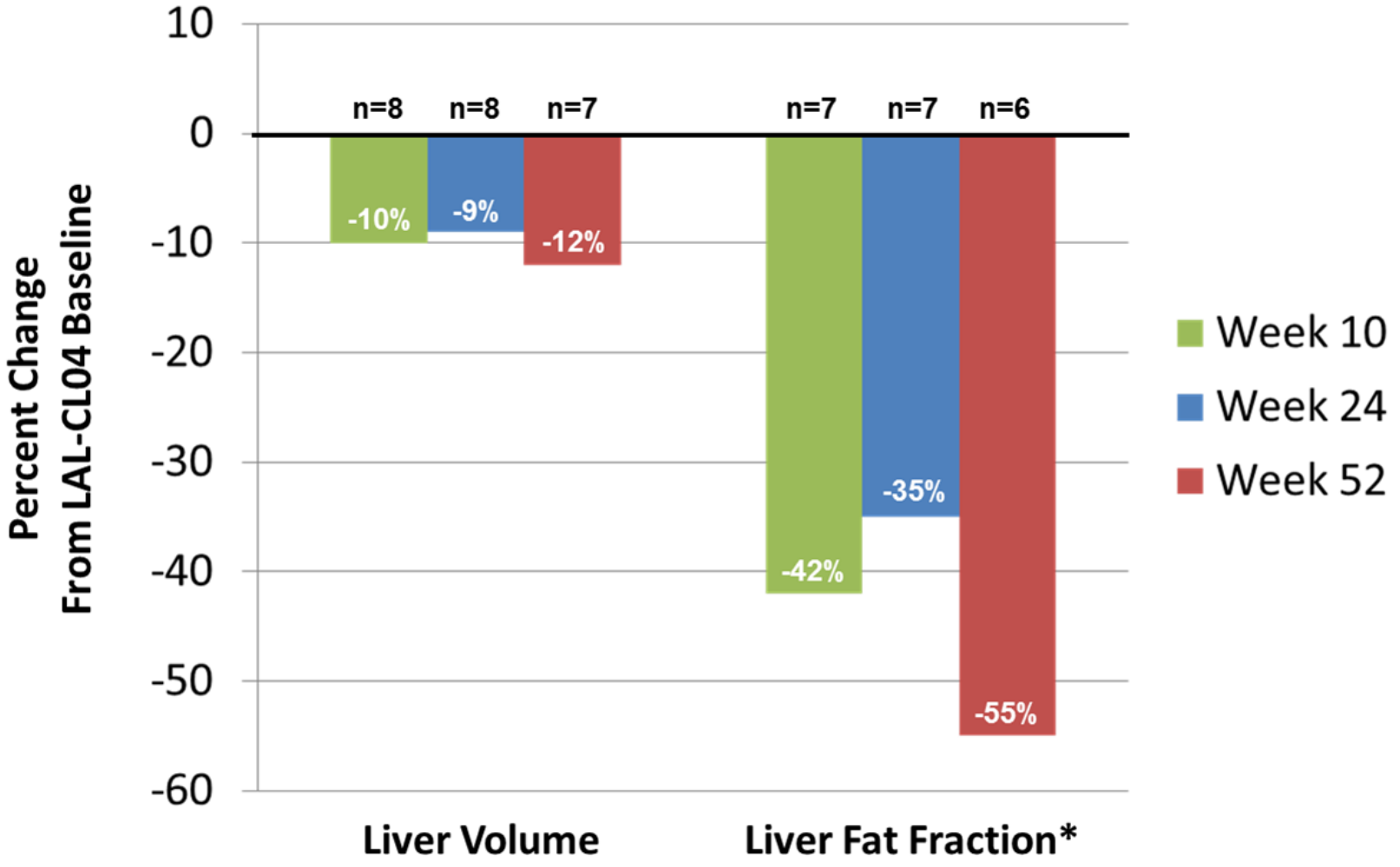
Valayannopoulos V & al. *J Hepatol.* 2014; 61(5): 1135-42

Sebelipase alfa rapidly decreases serum transaminases in CESD patients



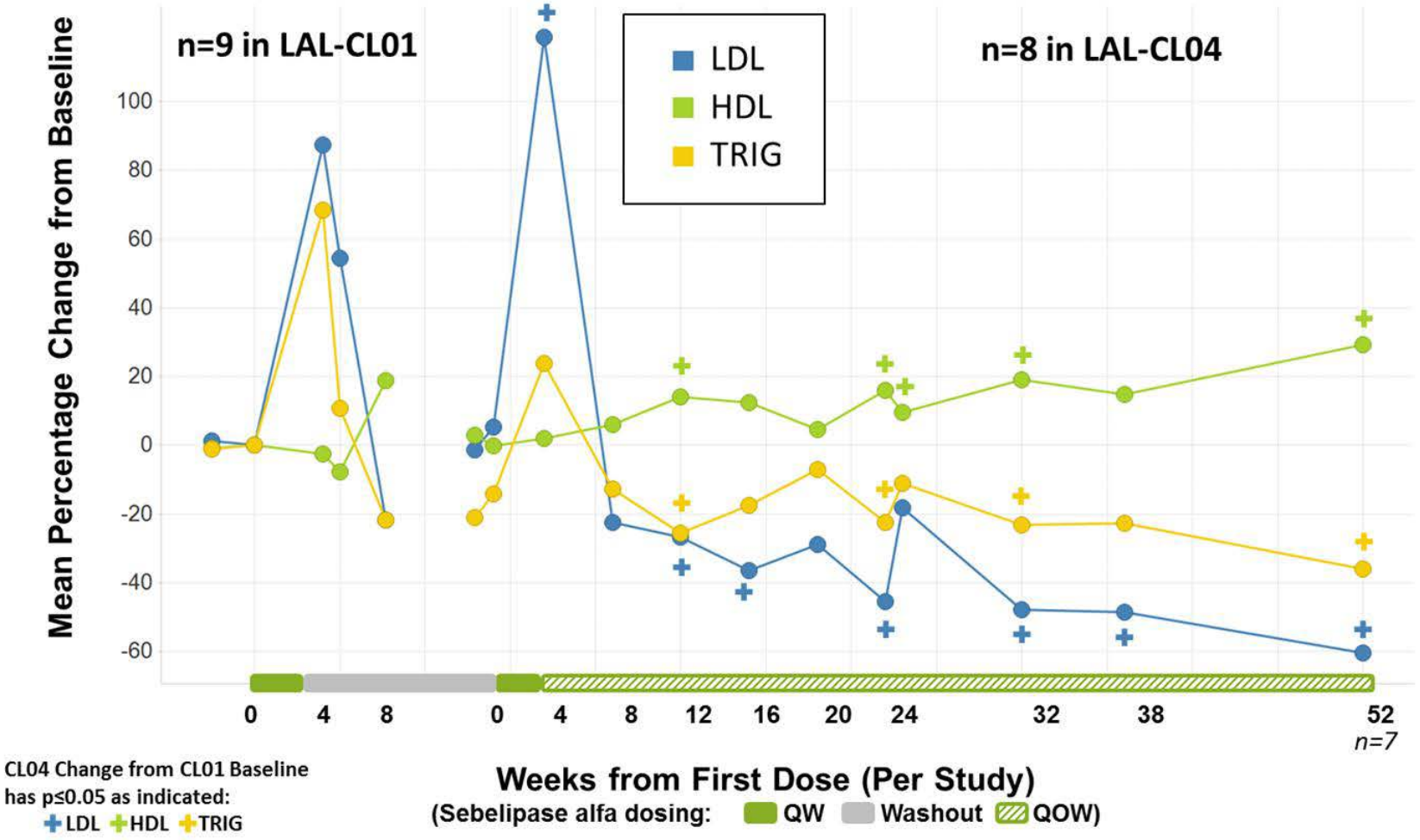
Valayannopoulos V & al. *J Hepatol.* 2014; 61(5): 1135-42

Sebelipase alfa reduces liver volume and hepatic fat fraction



Valayannopoulos V & al. *J Hepatol.* 2014; 61(5): 1135-42

Sebelipase improves the serum lipid profile



Valayannopoulos V & al. *J Hepatol.* 2014; 61(5): 1135-42

Conclusions

- Lysosomal acid lipase (LAL) deficiency results in two phenotypes: severe, early-onset Wolman disease or the less severe cholesteryl ester storage disease (CESD)
- Due to its similarity with other cardiovascular, liver and metabolic diseases, LAL-D may go unrecognized without specific diagnostic testing
- Sebelipase alfa, an investigational enzyme replacement, is well tolerated and very effective in CESD patients

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