Paediatric Non-Alcoholic Fatty Liver Disease (NAFLD)

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Non-Alcoholic Fatty Liver Disease

Outline of Talk

- Epidemiology
- Natural history
- Risk factors
- Pathogenesis
- Diagnosis
- Therapeutic options

Non-Alcoholic Fatty Liver Disease

- **Emerging as a leading cause of chronic liver disease in children**
  - 3-10% in general paediatric population
  - 80% in obese children
- Mirrors worldwide annual increment in obesity
- National Health Nutritional Examination Survey
  - 17% all children in Western countries are obese

Clin Med 2011,11:176; Scand J Public Health 2008,36:153, Obes Rev 2008,9(Suppl1):78; J Pediatr 2009,155:469; Curr Opin Clin Nutr Metab Care 2011,14:151, Hepatology. 2012;55:2005, World J Hepatol. 2010;2:275, Expert Opin Ther Targets. 2013;17:773,

Non-Alcoholic Fatty Liver Disease

Definition: Hepatic fat infiltration >5% hepatocytes assessed by liver biopsy

In the absence of :

- Viral, autoimmune, metabolic or drug-induced liver disease
- Excessive alcohol intake

Spectrum of disease

- Steatosis
- Steatohepatitis
- Fibrosis / Cirrhosis

Nat Rev Gastroenterol Hepatol 2010,7:195; Adv Anat Pathol 2011,18:294

Non-Alcoholic Fatty Liver Disease

Paediatric NAFLD histopathology has distinct characteristics compared to adults

- ? 2 different phenotypes – adult and paediatric
- ? different pathogenesis

Associated with similar metabolic impairments as in adults

- Insulin resistance
- Hypertension
- Central abdominal obesity

→ Increased risk of Type 2 Diabetes Mellitus, metabolic syndrome, cardiovascular disease

Hepatology 2005;42:64; Hepatology 2006; 44:458, Circulation 2008,118:277; Diabetol Metab Syndr 2009;1:29

Non-Alcoholic Fatty Liver Disease : Histopathology

2 histological patterns of NASH in children

Type 1 : Adult type

- Less common and tends to occur in girls
- Classical histological findings
  - steatosis
  - ballooning → increased risk of disease progression to NASH
  - inflammation
  - fibrosis
- Steatosis in zone 3
- Lobular inflammation, ballooning and peri-sinusoidal fibrosis

World J Gastroenterol 2010;16(42):5286

Non-Alcoholic Fatty Liver Disease : Histopathology

Type 2 : Paediatric type

- More common and tends to occur in boys
- Zone 1 (periportal) steatosis or panacinar steatosis
- Portal inflammation
- Ballooning uncommon
- Portal fibrosis

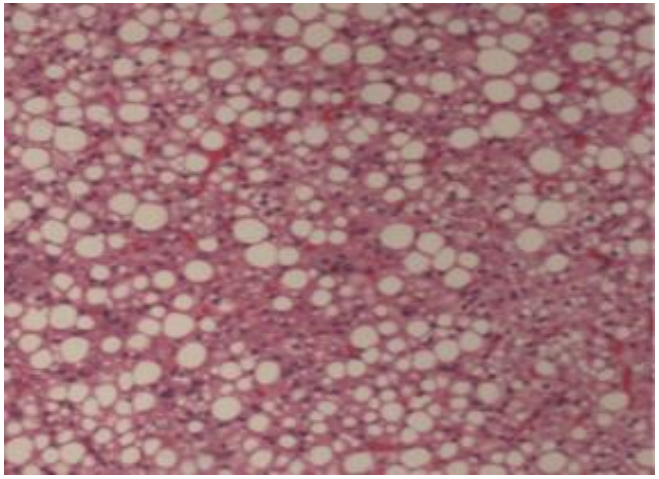
Paediatric NAFLD Histological score

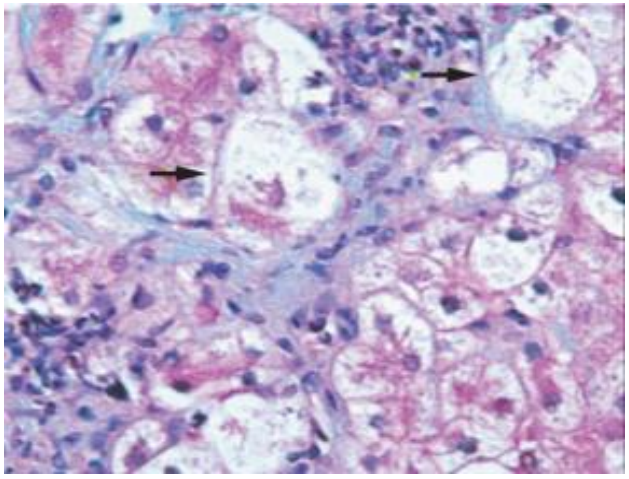
- Steatosis (0-3)
- Ballooning (0-2)
- Lobular inflammation (0-3)
- Portal inflammation (0-2)

World J Gastroenterol 2010;16(42):5286

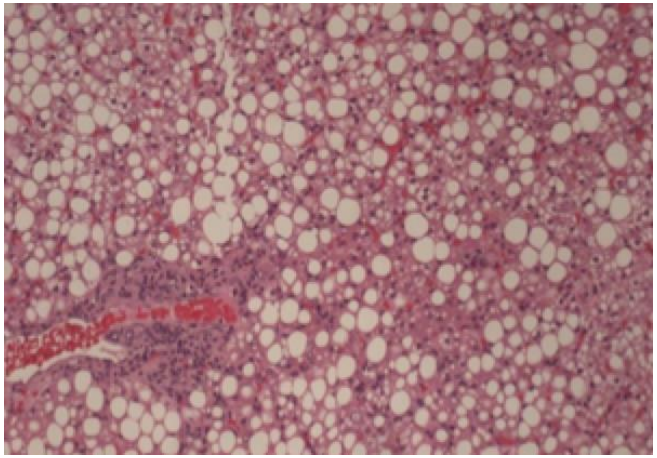
Determine the Severity of NAFLD: Presence of NASH

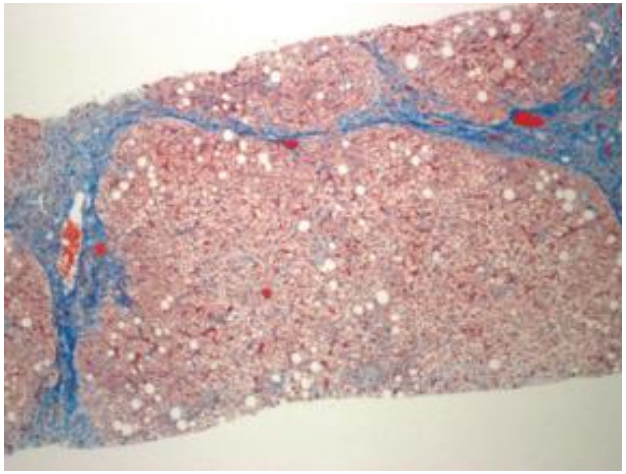
Type 1 NASH (Adult)





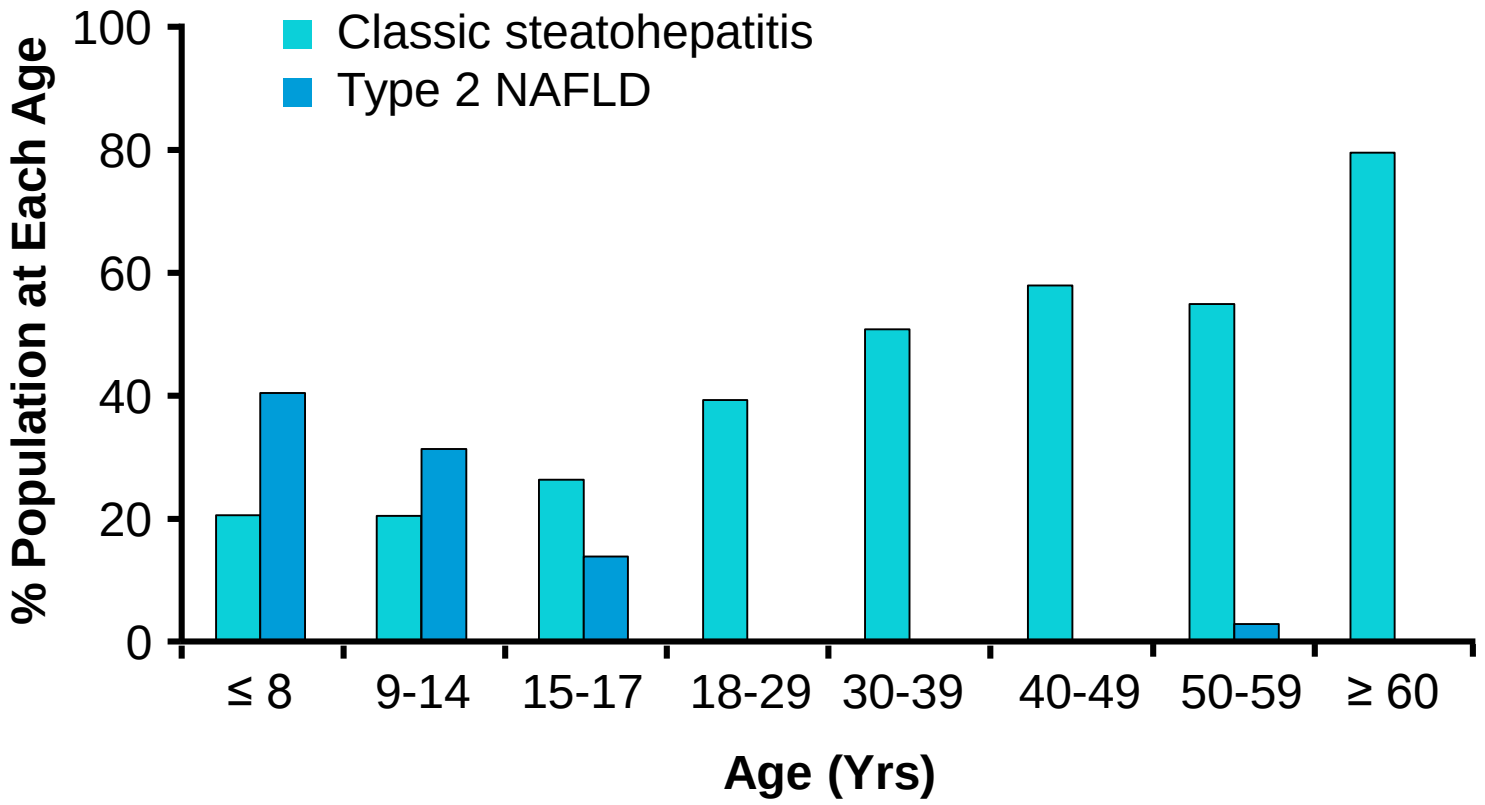
Type 2 NASH (Paediatric)





Mencin A, et al. Nat Rev Gastroenterol Hepatol. 2015.

Inverse Relation of Classic NASH and Type 2 NAFLD With Age



Kleiner DE, et al. AASLD 2006. Abstract 189.

Non-Alcoholic Fatty Liver Disease: Epidemiology

Data from North America, Europe, Asia, South America and Australia

- Paediatric NAFLD prevalence: 3-10 %
  - influenced by population characteristics especially lifestyle habits and diagnostic methods
- Liver biopsy gold standard
  - not feasible to detect disease prevalence
- Screening tests all have diagnostic limitations
  - BMI
  - ALT
  - Ultrasound

JPN J Med 1988.27:142; Am J Clin Nutr 205,82:1046; Front Biosc 2005,10:2306; Nutr Hosp 2010,25:712

Non-Alcoholic Fatty Liver Disease : Epidemiology

NHANES III (USA population-based field report)

- Prevalence of ALT >30 IU/l in adolescents
  - 7.4 % Whites
  - 11.5% Mexican-American
  - 6% Blacks
- ↑ ALT : 12.4% males vs 3.5% females

Similar data from South Korea and Japan

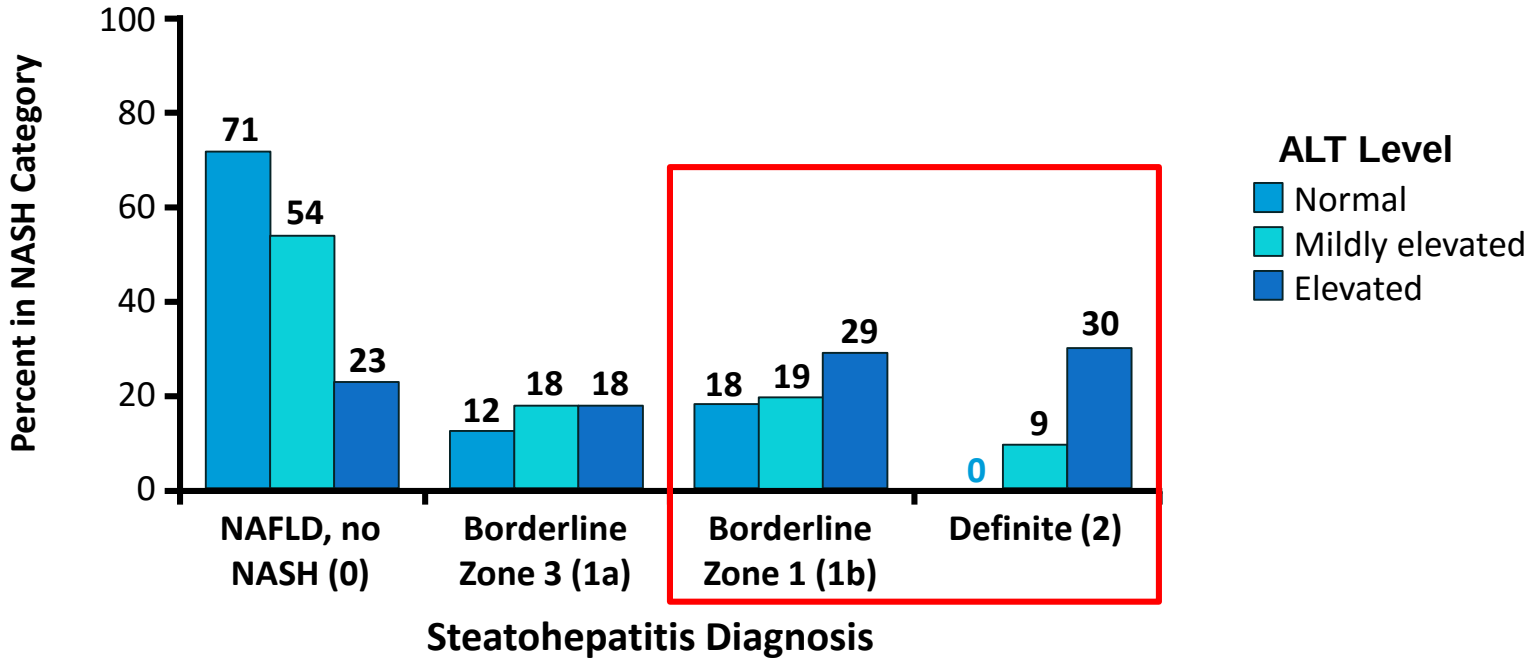
Prevalence of NAFLD in adolescents at least 2.6 - 3.2 %

- Probable underestimate as surrogate markers used for diagnosis

Gastroenterology 2007,133: 1814; Dig Dis Sci 1995,40:2002; Am J Clin Nutr 205,82:1046;

Prevalence of Steatohepatitis based on ALT levels in Children

- Comparison of children with suspected NAFLD and normal or mildly elevated ALT (n = 91) vs children with elevated ALT (n = 392)
- **Plasma ALT may underestimate liver injury in NAFLD**



Molleston JP, et al. J Pediatr. 2014;164:707-713.

Non-Alcoholic Fatty Liver Disease : Natural History

- Natural history and prognosis in children uncertain
  - very little long-term published data
- Documented progression to cirrhosis and hepatocellular carcinoma
  - need for liver transplantation
- **Diagnosed as early as 2 years and cirrhosis as early as 8 years**
- Genetic and environmental factors play a role
  - development and progression of disease
- **Progression to endstage liver disease can occur over 10-20 years**

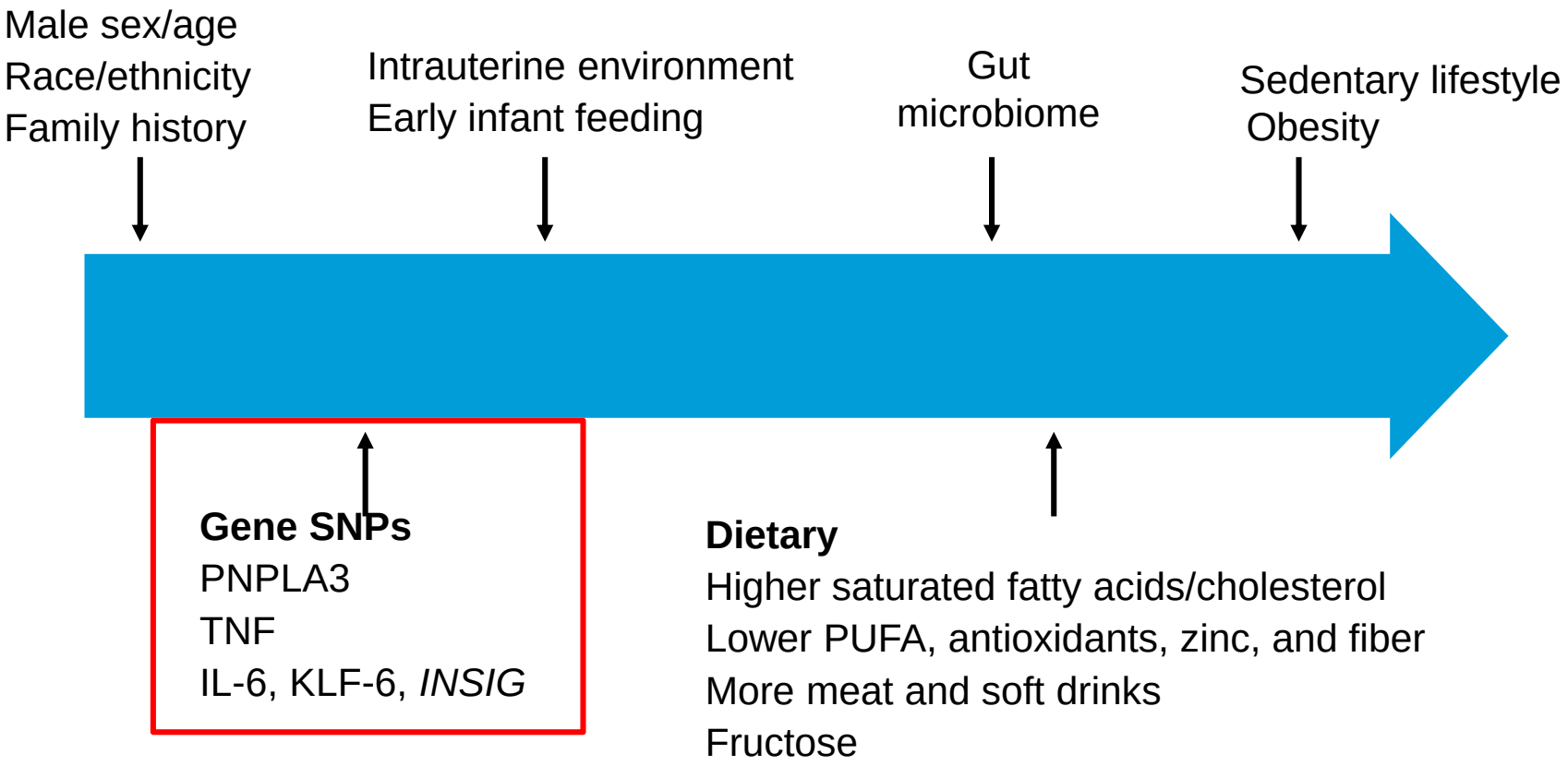
Gut 2009,58:1538; Gut 2009; 58(11):1442, Nat Review Gastroenterol Hepatol 2012,9:152

Non-Alcoholic Fatty Liver Disease : Natural History

- **66 children with NAFLD (mean age 13.9 yrs) - 20 yr follow-up** - total of 409.6 person-yrs follow-up - Retrospective hospital-based cohort study
- Metabolic syndrome present in 19 (29%) at time of NAFLD diagnosis
- **55 (83%) presented with at least one feature of the metabolic syndrome** incl obesity, hypertension, dyslipidaemia, hyperglycaemia
- 13 liver biopsies (5 pts) over a mean of 41.4 mnths
  - progression of fibrosis stage in 4 children
- **Follow-up: 2 died and 2 underwent Liver Tx for decompensated cirrhosis - NAFLD recurred in allografts; ReTx for cirrhosis (1 pt)**
- **Tx free survival significantly shorter in NAFLD cohort** compared to expected survival in general US population - same age and sex (log-rank test, $p<0.00001$), with standardised mortality ratio 13.6

Gut 2009,58:1538

Paediatric NASH: Multiple Risk Factors



Nobili, et al. JAMA Pediatr. 2015;169:170-176.

Non-Alcoholic Fatty Liver Disease : Risk Factors

Obesity main risk factor for paediatric NAFLD

- 80% prevalence in obese children (USA, Europe, Japan)

Population-specific based study in Europe

- 111 paediatric obesity centres (Germany, Austria, Switzerland)
- 16 390 overweight, obese and morbidly obese children
- NAFLD defined by AST and/or ALT >50 ULN
- NAFLD 11% study population
    - M : F = 14.4 : 7.4 %
    - Obese vs extremely obese 9.5 % : 17%

Japan : 219 children (6-12yrs) : NAFLD

- 3% normal weight, 25% overweight and 76% obese children

Dig Dis Sci 1995,40:2002; Pediatrics 2006,118:1388; World J Hepatol 2010,2:275; Dig Dis Sci 1997,42:1428; Int J Obes (Lond) 2010,34:1451; Int J Obes Relat Metab Disord 2004,28;1257; Int J Obes (Lond) 2010,34:1468; J Pediatr Gastro Nutr 2013,56:145

Non-Alcoholic Fatty Liver Disease : Risk Factors

Metabolic syndrome (strong association)

- Insulin resistance
- Type 2 diabetes mellitus
- Hypertriglyceridaemia/hypercholesterolaemia (20-80% children with NAFLD)

NAFLD increases risk of cardiovascular disease in adulthood

- Increased carotid intimal medial thickness (marker of atherosclerosis) in children with NAFLD

→ greater risk of atherosclerosis and future CVS events

Insulin resistance more severe in NASH than in simple steatosis

J Pediatr Gastroenterol Nutr 2005,41:94; Int J Obes (Lond)2008,32:381; Pediatr Cardiol 2013,34:308; Ped Health 2009,3:271; Curr Opin Pediatr 2009,25:529

Non-Alcoholic Fatty Liver Disease : Risk Factors

Age

- Can occur in young children
- More prevalent in adolescents
    - sex hormones and insulin resistance in puberty
    - lifestyle – fast foods and sedentary lifestyle

Gender

- Male : Females: 2:1
    - ? oestrogens are liver protective
    - ? androgens aggravate NASH
    - ? role of alcohol

Aliment Pharmacol Ther 2008,28:13; Pediatrics 2005,15:e561; PLoS One 2011,6:e21178; Hepatology 2009,50:1282; Moll Cell Biochem 2009,324:65; World J Gastroenterol 2004,10:250

Non-Alcoholic Fatty Liver Disease : Risk Factors

Ethnicity

- More common in Hispanic than Caucasian and Afro-American children
- Ethnic differences
  - higher rates of insulin resistance
  - ↑ visceral adiposity at equivalent BMI
- Socio-economic factors
  - type of diet
  - exercise
- Afro-American children - more risk factors for NAFLD
  - Obesity
  - IR
  - Type 2 DM

Gastroenterol 2007,133:1814; Pediatrics 2005,15:e561; J Pediatr Gastroenterol Nutr 2009,49:442; J Pediatr Endocrinol Metab 2010,23:1253

Non-Alcoholic Fatty Liver Disease : Genetic predisposition

Only minority of patients with NAFLD → NASH

- Complex interplay between environmental and genetic factors
- 2 cohort studies and 1 community based study in different ethnicities
  - suggested 35-40% NAFLD patients have genetic predisposition
  (adjusted for age, gender and BMI)
- 1st , 2nd and 3rd generation relatives demonstrate abnormally high fat fractions (MRI) relative to BMI
- **Genes associated with energy balance**
  - adiponutrin/patatin-like phospolipase domain-containing 3 (PNPLA-3)
  - apolipoprotein C3 (APOC3)
- **Genes involved in inflammation, oxidative stress and fibrogenesis**
  - SOD2

Associated with NAFLD and severity of liver injury

Obesity 2009.17:1240; J Lipid Res 2006,47:2799

Genetic Variants Influence Developmental Susceptibility to Paediatric NAFLD and NASH

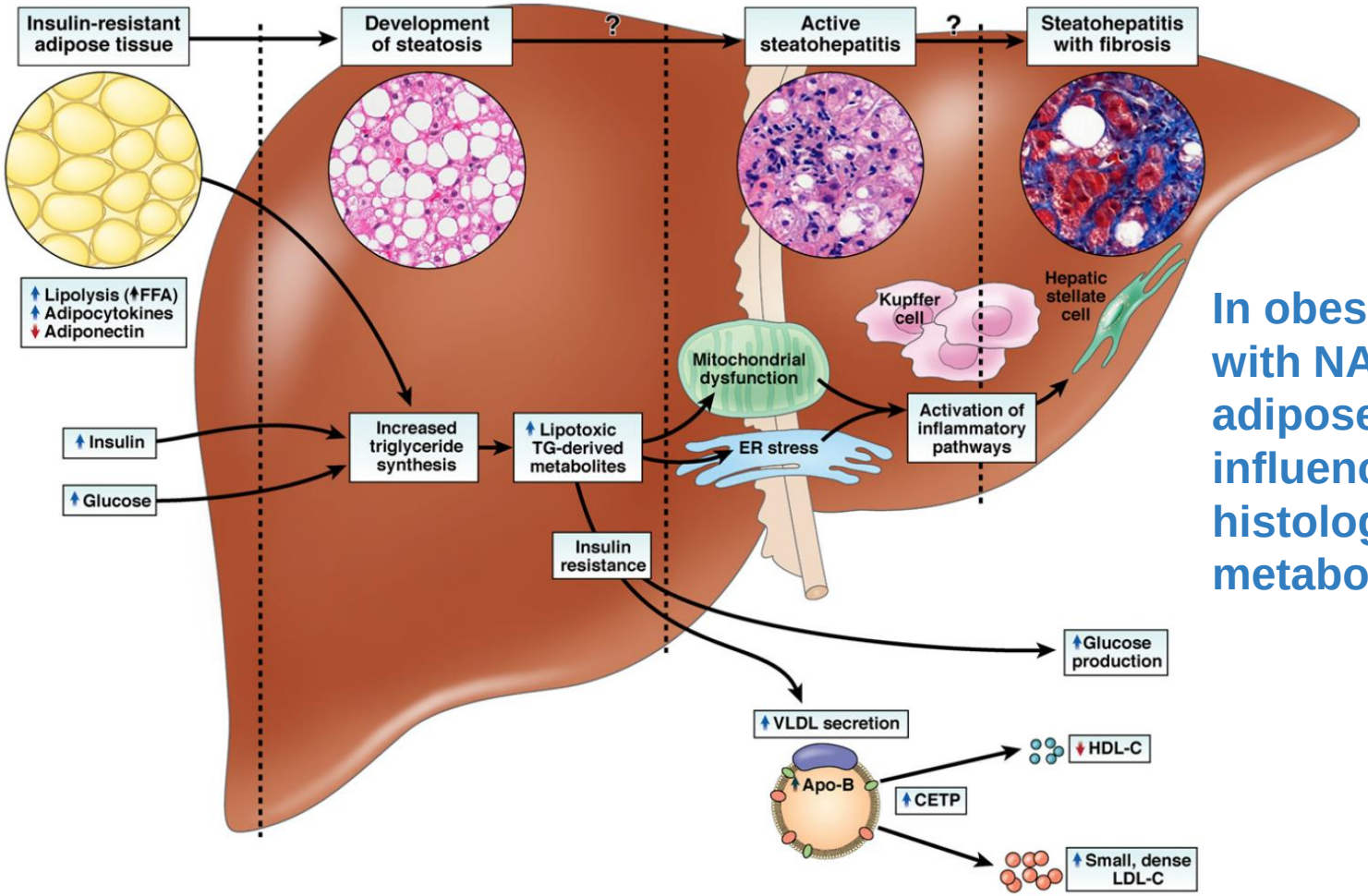
- Analysis of genetic background may identify susceptible children
- Discoveries of pathogenetic mechanisms may lead to therapeutic options
  - **SNPs – altered gene expression or protein function**

Gene	Function	SNP
PNPLA3	Lipid remodeling, lipogenesis	rs738409 C>G
GCKR	Glucose reuptake, lipogenesis	rs738409 C>T
SOD2	Antioxidant response	rs4880 C>T
ENPP1	Insulin signaling	rs1044498 A>C
IRS1	Insulin signaling	rs1801278 G>C
KLF6	Fibrogenesis, glucose metabolism, and lipogenesis	rs3750861 G>A
LPIN1	Lipogenesis, adipogenesis	rs13412852 C>T

Nobili V, et al. J Hepatol. 2013;58:1218-1229.

Non-Alcoholic Fatty Liver Disease : Pathogenesis

Complex interplay: Visceral adipose tissue, Gut, Liver and Immune system in genetically susceptible individuals

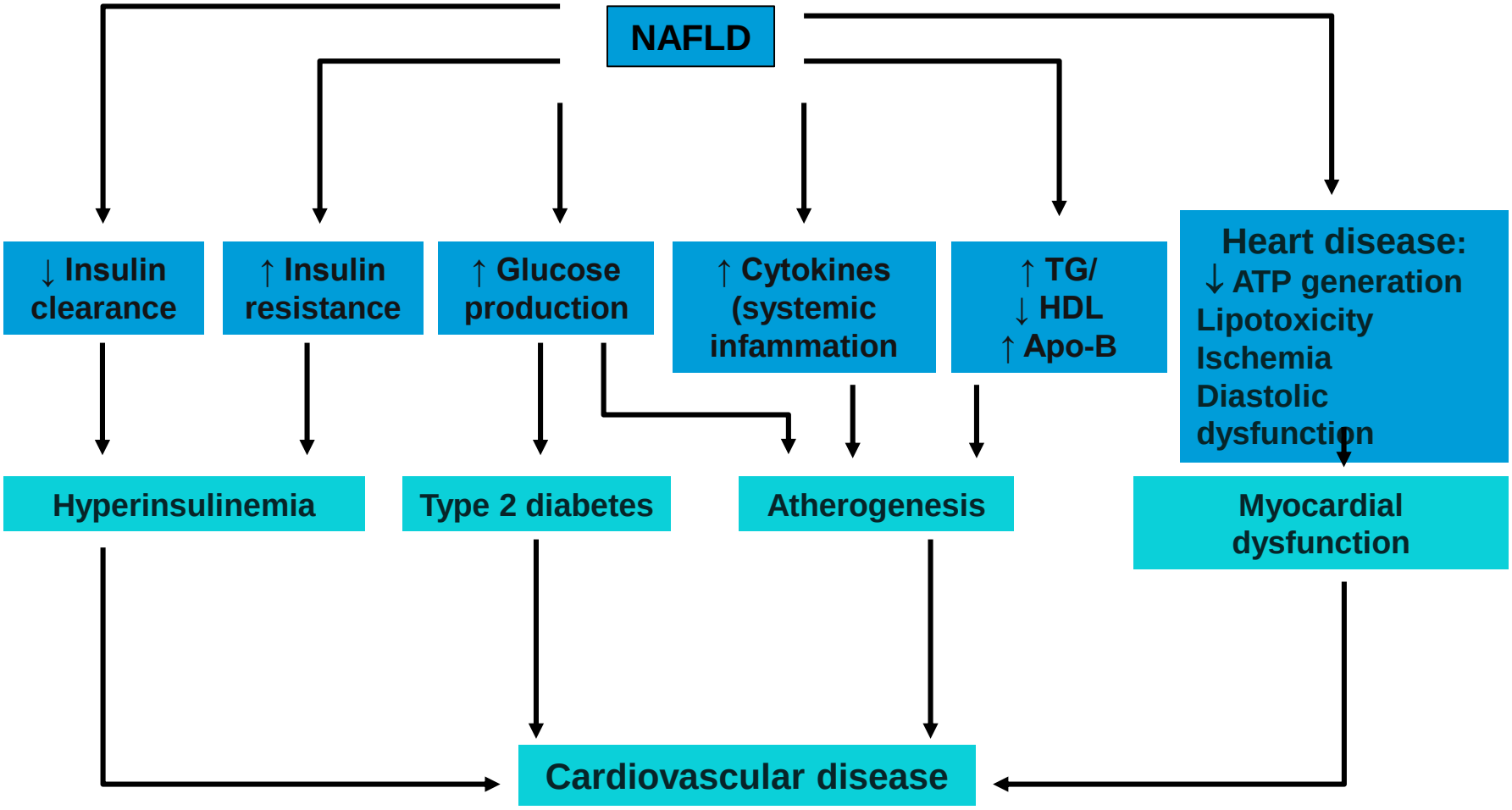


In obese patients with NAFLD, adipose tissue IR influences histologic and metabolic defects

Gastroenterology 2012;142:711-725

Metabolic Consequences of NAFLD

Diagnosis and Management important



Cusi K. Gastroenterology. 2012;142:711-725.

Pediatric NAFLD Clinical Practice Guidelines

Biannual screening (ALT & AST) in children with:

- BMI ≥ 95th percentile
- BMI between 85th and 94th percentile for age & sex with other risk factors:
  - *Metabolic syndrome*
    - ~ Insulin resistance: acanthosis nigricans
    - ~ Type 2 diabetes: Fasting blood glucose, HBA1C, family history
    - ~ Central obesity and other features of metabolic syndrome
  - *Obstructive sleep apnea*
  - *Nocturnal BP on retinal microvasculature - retinopathy*
    - ~ positive relationship between hepatic fibrosis & degree of retinopathy

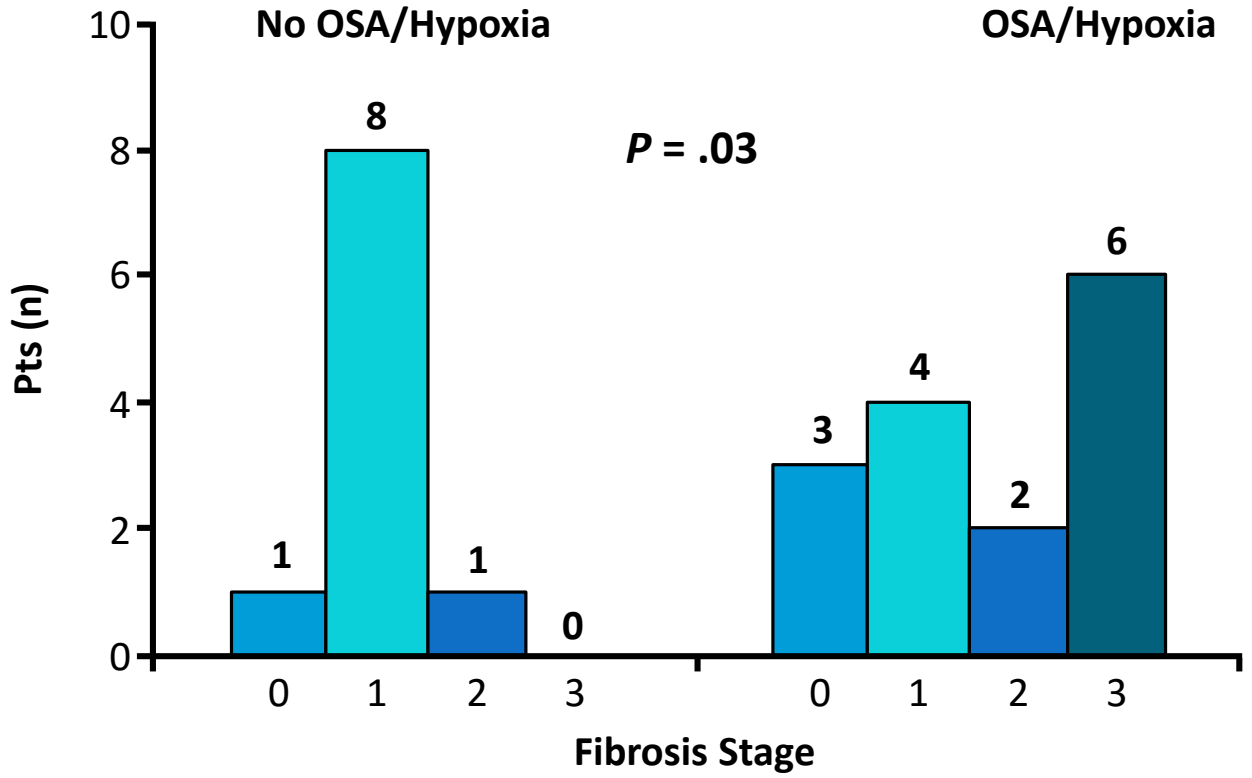
Acanthosis Nigricans : Best Predictor of Insulin Resistance in Paed NAFLD

- ALT 25 IU/L in boys and 22 IU/L in girls

Pediatrics. 2007;120 (suppl 4):S164-S192, Hepatology. 2012;55:2005, J Gastroenterol Hepatol. 2013;28:68, World J Hepatol. 2014;6:33, Hepat Mon. 2014;14:e24635, Obes Rev. 2015;16:393, J Gastroenterol. 2015;50:903

NASH in Children With OSA Often Associated With Worse Liver Fibrosis

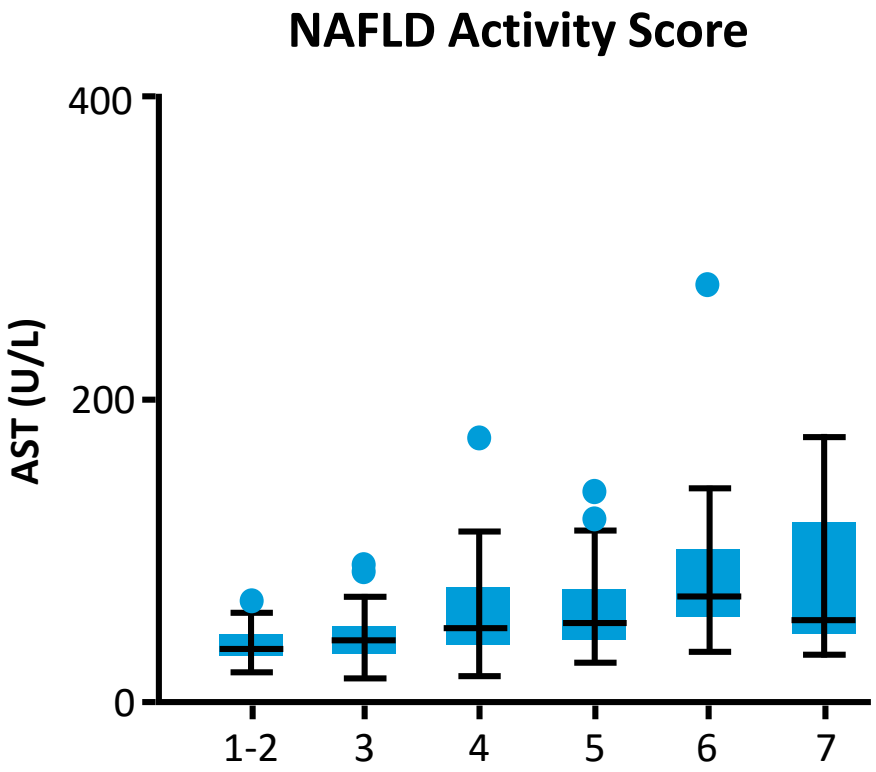
- Prospective study of obese children (N = 25) aged 10-18 yrs with liver biopsy–proven NAFLD

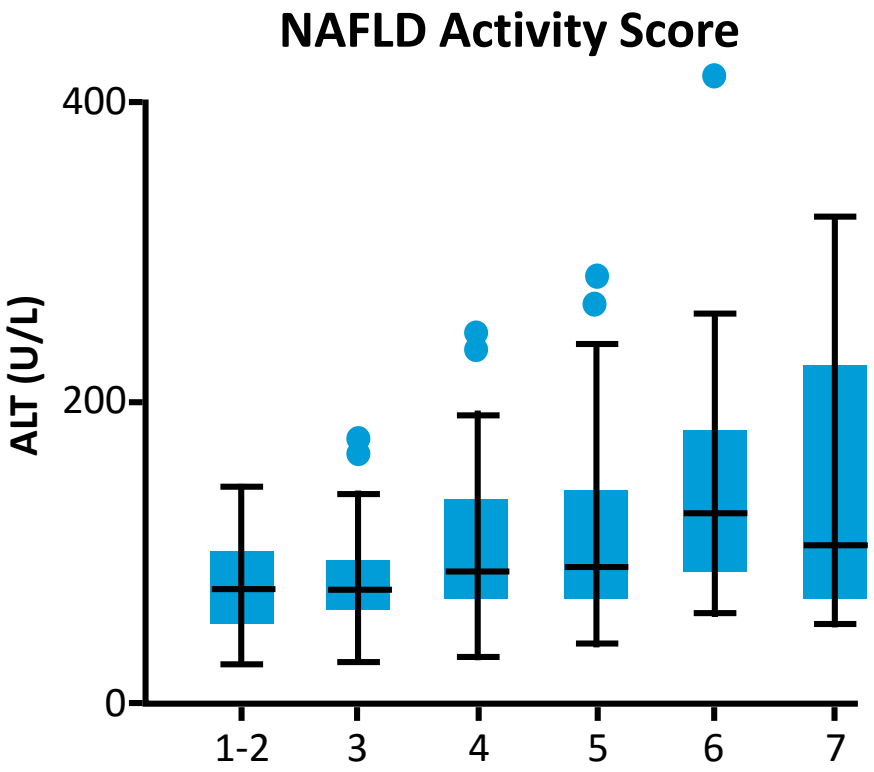


Sundaram SS, et al. J Pediatr. 2014;164:699-706.

Limited Value of AST or ALT to Establish NASH Severity in Children: NAS

- Prospective study of 176 children with NAFLD and available liver biopsies

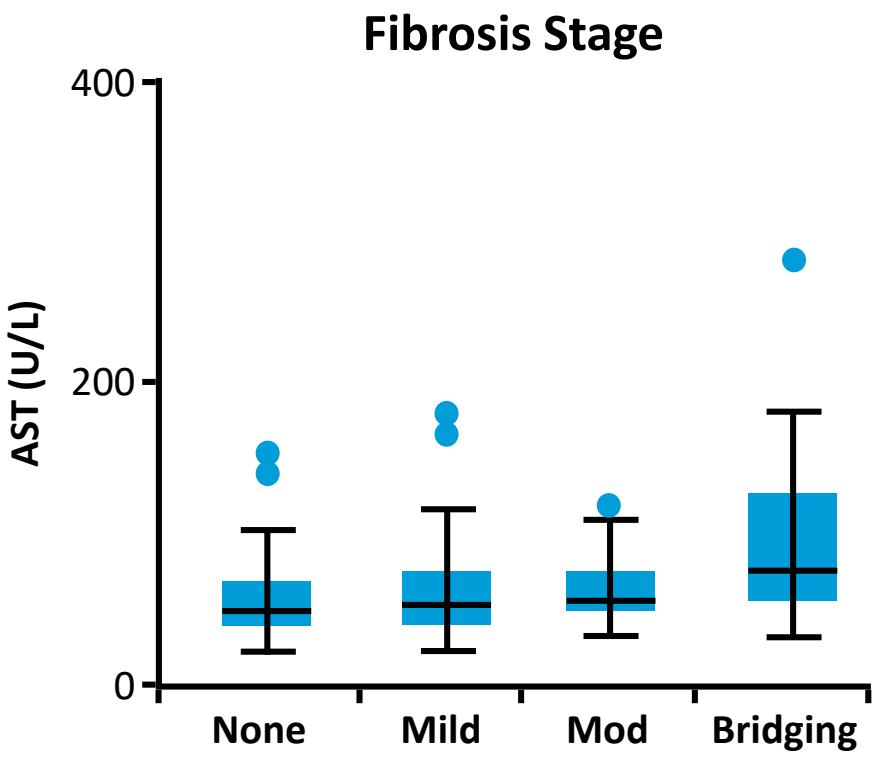


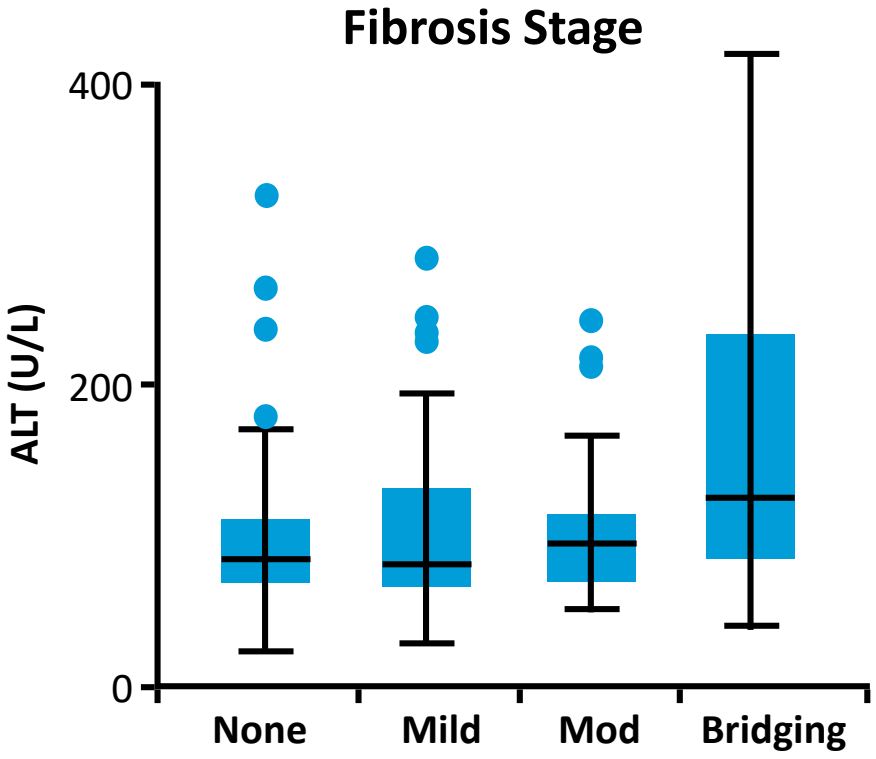


Patton HM, et al. Gastroenterology. 2008;135:1961-1971.

Limited Value of AST or ALT to Establish NASH Severity in Children: Fibrosis Stage

- Prospective study of 176 children with NAFLD and available liver biopsies





Patton HM, et al. Gastroenterology. 2008;135:1961-1971.

Non-Alcoholic Fatty Liver Disease : Diagnosis

- Exclusion of other liver diseases
- Exclusion of other causes of steatosis
- **Liver biopsy is gold standard, but invasive**
  - distinguish between NASH and simple steatosis
  - determine severity of disease
  - exclude AIH, Wilsons disease and metabolic diseases
  - before starting potentially hepatotoxic medication
- **Non-invasive tests**
  - anthropometric parameters: BMI, abdominal circumference
  - biochemistry (LFTs, fasting glucose/insulin/lipid profile), biomarkers
  - imaging/fibroscan/predictive models

    → together with ethnicity, gender, lifestyle and general health

J Pediatr Gastroenterol Nutr 2012,54:700; Dig Dis 2010,28:197

Non-Alcoholic Fatty Liver Disease : Diagnosis

Autoantibodies

- ANF positive in 15.4%
- Anti-smooth muscle antibody positive in 10%
- **Positive autoantibodies associated with higher fibrosis stages**

NEJM 2002,346:1221; Hepatology 2003,37:1286; AM J Gastroenterol 2004,99:1316; Hepatology 2007,46:582

Non-Alcoholic Fatty Liver Disease : Diagnosis

Imaging

Ultrasound

- Diagnostic sensitivity decreases when
  - hepatic steatosis <30%
  - BMI ≥40
- Cannot rule out steatohepatitis or fibrosis
- Overall sensitivity 60-94%, specificity 84-100%

CT Scan

- More specific
- Limitations of radiation exposure

MRI

- Greatest accuracy for determining fat content, but costly

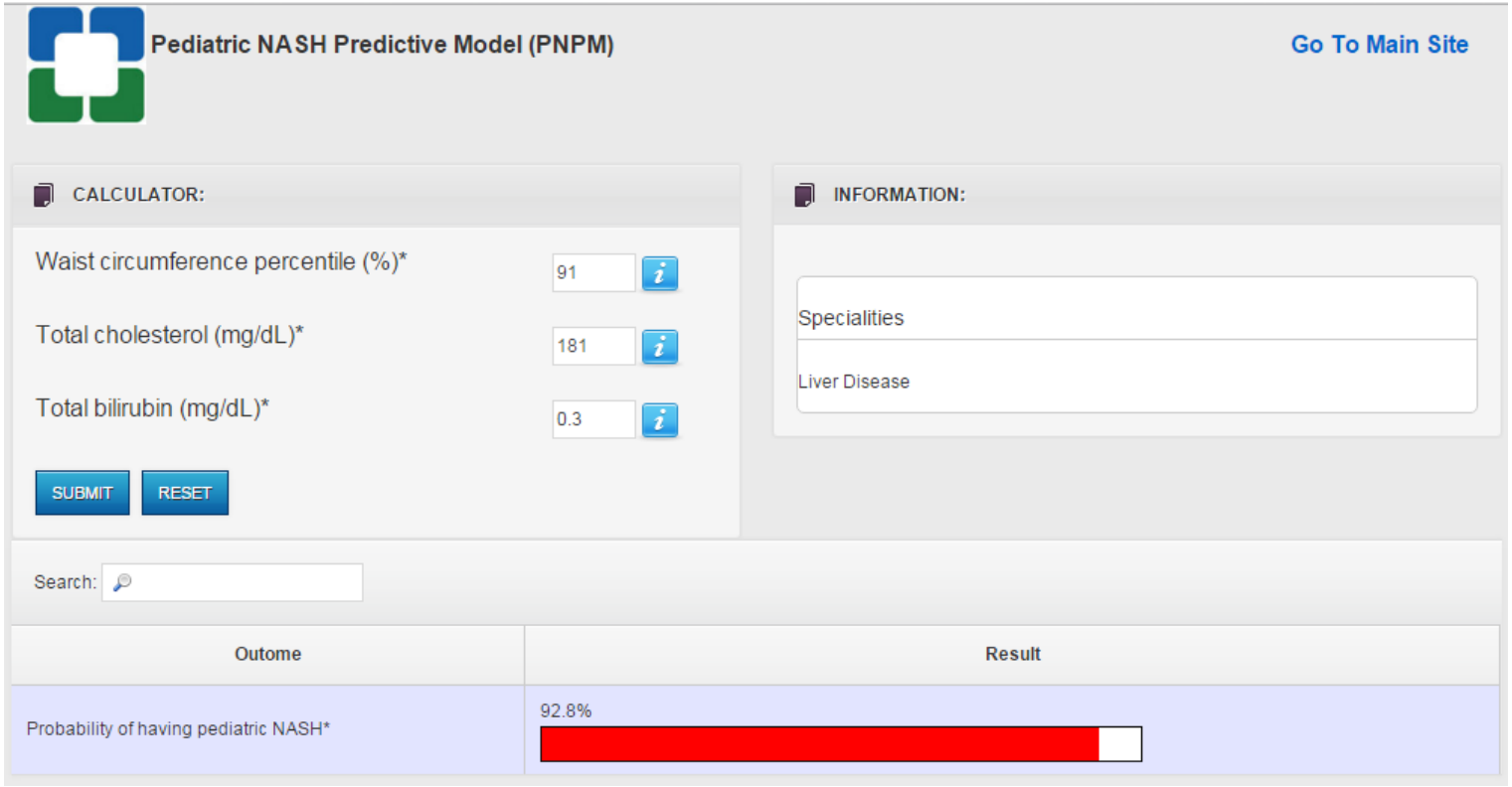
BMC Pediatrics 2013,13:40

Noninvasive Liver Fibrosis Tests in Pediatric NAFLD

Marker	Interpretation	Cost
Transient elastography	▪ TE value 5-7 kPa: F1 ▪ TE value 7-9 kPa: F1-2 ▪ TE value > 9 kPa: F3-4	+++
MR elastography	▪ Liver stiffness value of 2.71 kPa gives sensitivity of 88% and specificity of 85% for ≥ F2 fibrosis	+++

Mansoor S, et al. Curr Gastroenterol Rep. 2015;17:23.

Pediatric NASH Predictive Model

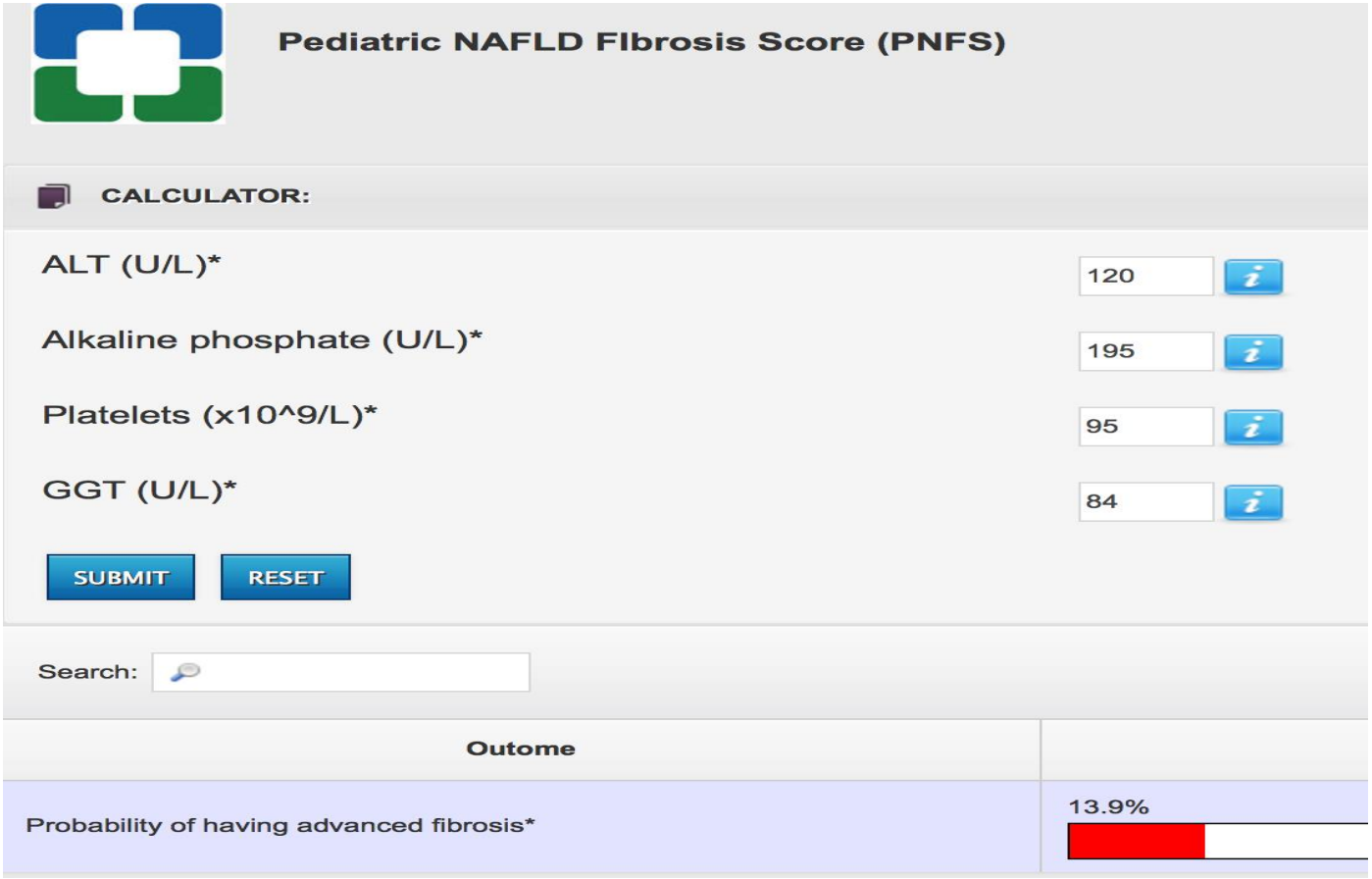


302 children, mean age 12.3 ± 3.1 years, mean BMI percentile of 94.3 ± 6.9, and NASH present in 67%

PNPM AUROC : 0.737

Eng K, et al. Dig Liver Dis. 2014;46:1008-1113. http://www.r-calc.com.

PNFS: Online Calculator

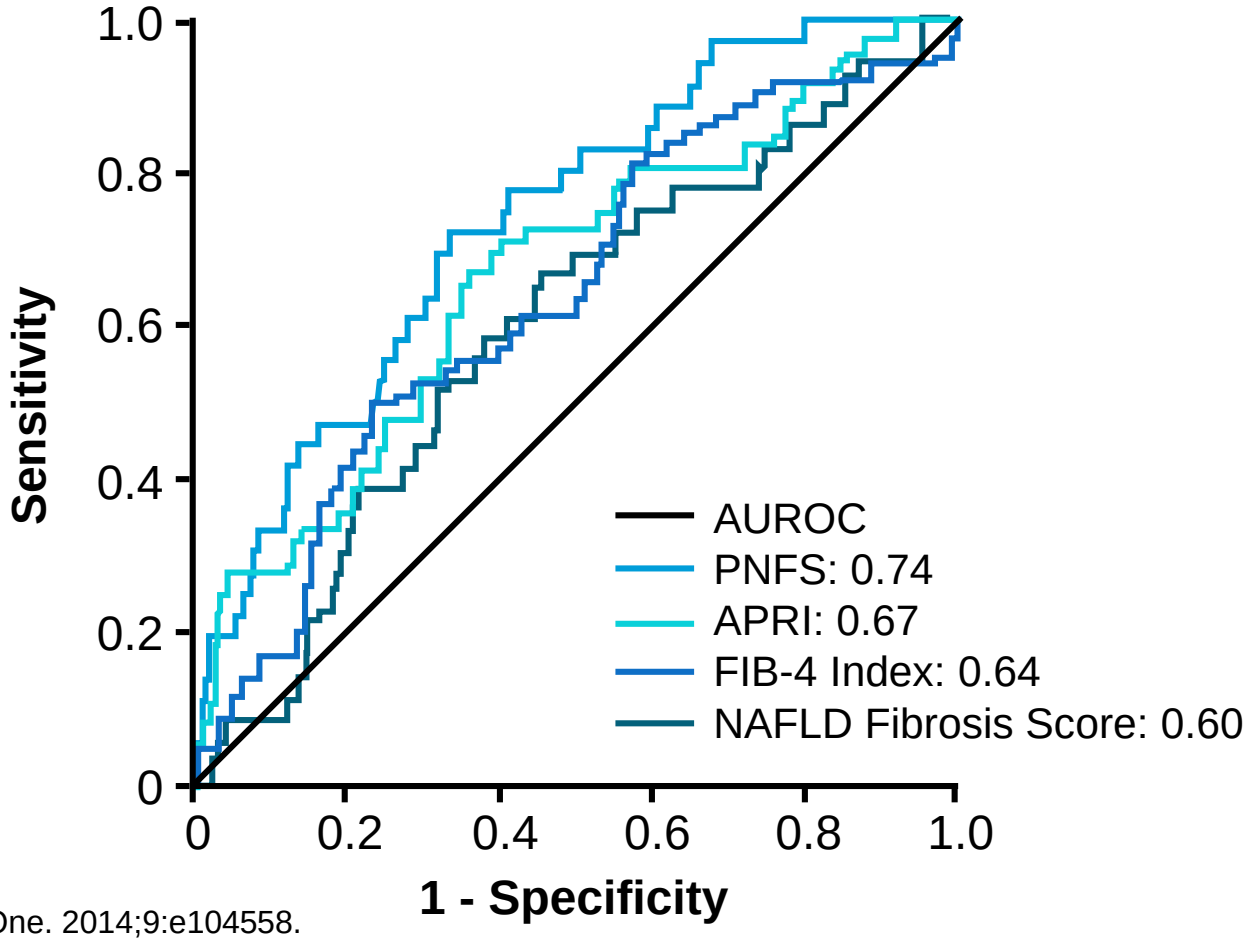


242 children, mean age 12.4± 3.1 years and 15% had advanced fibrosis
AUROC 0.74

Alkhouri N, et al. PLoS One. 2014;9:e104558

Performance of PNFS vs APRI, FIB-4 Index, NAFLD Fibrosis Score

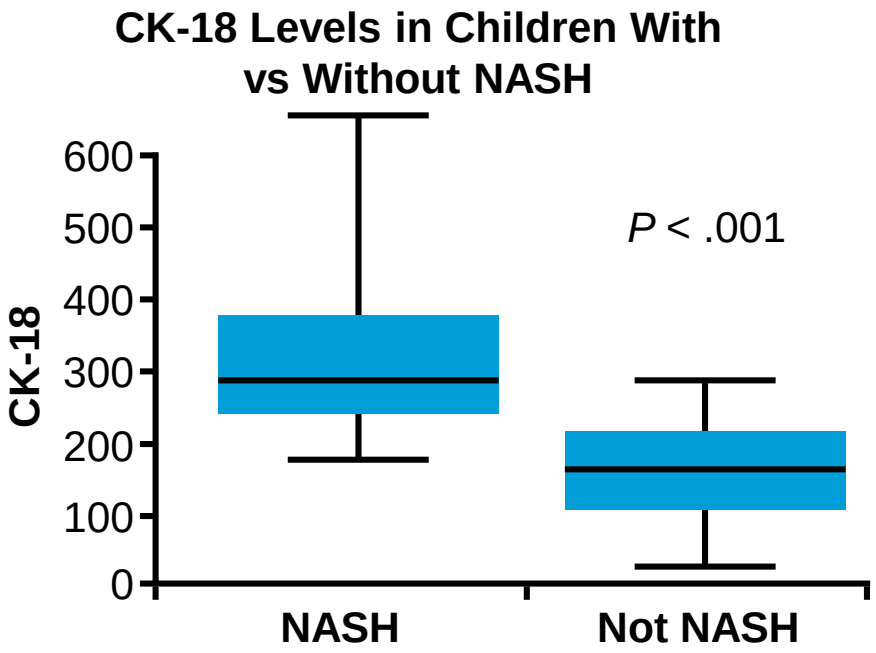
- Single cohort of 242 children in Italy

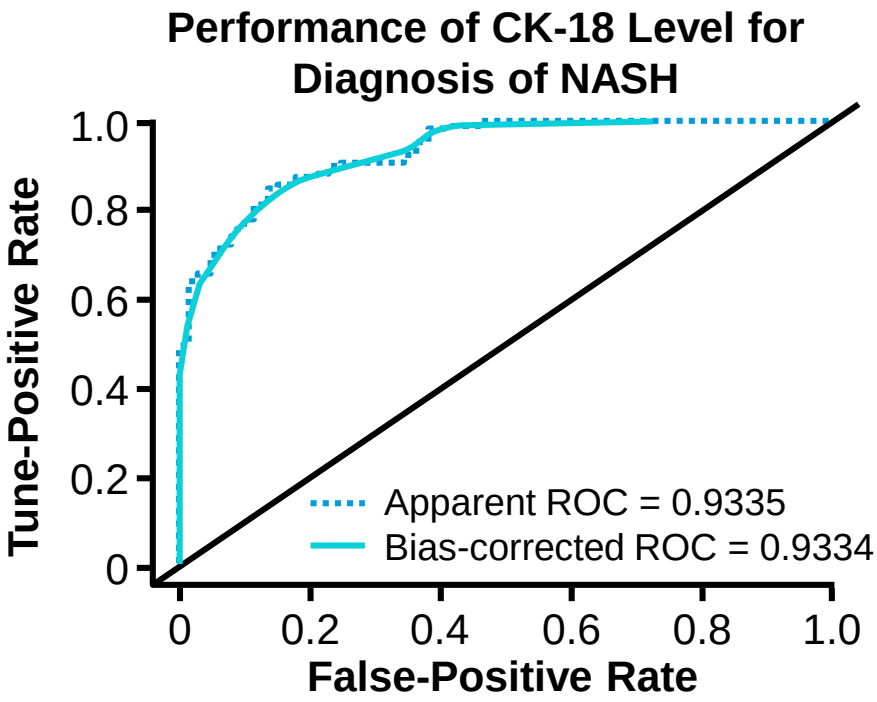


Alkhouri N, et al. PLoS One. 2014;9:e104558.

Biomarkers: CK-18 for Prediction of NASH

Cytokeratin-18 fragments : Apoptosis by-product





Feldstein AE, et al. Am J Gastroenterol. 2013;108:1526-1531.

Non-Alcoholic Fatty Liver Disease : Prevention and Treatment

- Limited knowledge of molecular pathogenesis
  - cross-talk between gut, immune system and liver in genetically susceptible individuals

- Multi-targeted approach
  - diet and lifestyle modification
  - therapeutics: Insulin sensitisers,hepatoprotective agents, antioxidants

- Decrease incidence of known risk factors
  - individual and public health level

- Good pregnancy care
  - prevention of low birth weight infants
  - encourage breast feeding

Lifestyle Recommendations: Paediatric NAFLD

First step : Address obesity

- Weight loss of 10% for those at adult height
  - Weight maintenance otherwise
- Aerobic exercise (play) 60 min/day for 6 days/week at moderate intensity
- Less than 2 hours screen time/day
- Low refined sugar, low trans-fat, low GI fruits and vegetables
- Home-cooked meals with family engagement

Chalasani N, et al. Hepatology. 2012;55:2005-2023.

Non-Alcoholic Fatty Liver Disease : Treatment

Lifestyle modification ± anti-oxidant therapy

- 53 children (5.7-18.8yrs) with biopsy proven NAFLD randomised to

  - lifestyle modification plus anti-oxidant therapy (a-tocopherol 600 IU/day and Vit C 500 mg/day vs lifestyle modification plus placebo for 24 mnths

- Both groups showed improvement in steatosis, lobular inflammation, ballooning and the NAS

- Improvement in ALT, TG, Chol, glucose, insulin and insulin sensitivity

- No additional benefit from anti-oxidant therapy

Hepatology 2008;48:119

Non-Alcoholic Fatty Liver Disease : Treatment

Pharmacotherapy : targeted IR or oxidative stress

TONIC study : Vitamin E

- Multicentre USA study – 8 -17 year olds with NAFLD (N=173)
  - double-blind, placebo-controlled, randomized, multicenter phase II trial
  - compared efficacy of Vitamin E (800 IU/day) vs Metformin (500mg bd) vs placebo over 96 weeks (58 pts in each group)
- No difference in sustained ALT reduction in all 3 groups
  - 50% less than baseline
- Resolution of NASH
  - 58% (Vit E) vs 41% (Metformin) vs 28% (placebo)

JAMA 2011;305:1659

Alternative Pediatric NAFLD Therapeutics

Therapy	Possible Target	Proposed Mechanism
Probiotics/antibiotics	Gut microbiome	Change in TLR, endotoxin nutrient metabolism
Pioglitazone	PPAR-gamma NHR	Hepatic macrophages
Fish oil/omega-3 fatty acids	Anti-inflammatory	Eicosanoid metabolism
Elafibranor	PPAR-alpha/delta receptor	Pleiotropic nuclear hormone transactivator
Hyperimmune colostrum	Endotoxins in gut	Hepatic inflammation reduction
Metformin	AMP-kinase	Reduced insulin resistance

Non-Alcoholic Fatty Liver Disease : Conclusions

- Emerging leading cause of chronic liver disease in children
- Paediatric NAFLD prevalence: 3 -10 %
- Important to exclude other causes of steatosis esp metabolic disorders in young patients
- **Mainstay of treatment is lifestyle modification → sustained weight loss**
  - diet and aerobic exercise
  - address co-morbid conditions: Diabetes Mellitus, hyperlipidaemia, hypertension
- Therapeutics : ? Role of Vitamin E in patients with documented NASH
- Untreated NAFLD
  - can progress to cirrhosis and increased risk of HCC
  - increases risk of cardiovascular disease in adulthood

Non-Alcoholic Fatty Liver Disease : Treatment

Probiotics

- Specific nutrients increase intestinal permeability to bacterial endotoxins
  - activating immune-mediated inflammatory response of liver resident cells (Kupffer cells,hepatocytes, lymphocytes, stellate cells)

    → profibrogenic phenotype

- Animal models - restoring gut microflora

  → protect liver from steatosis and prevents cardiovascular disease

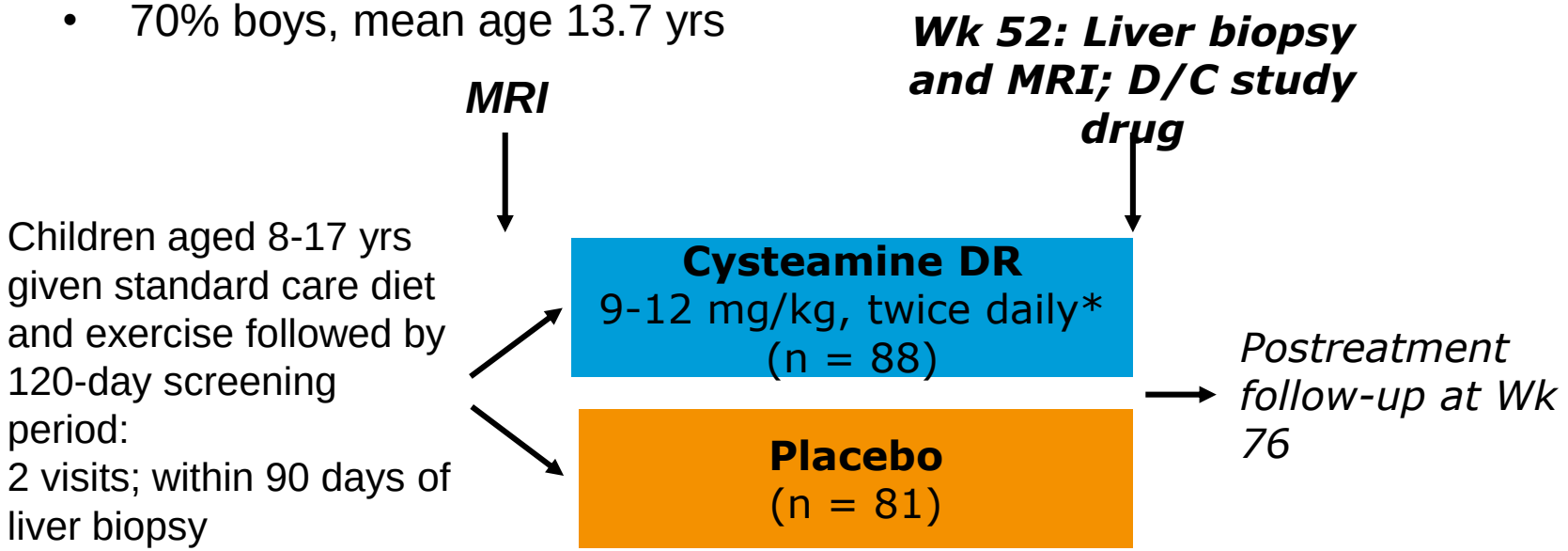
- Animal model- effect of probiotics on intestinal microbiota
  - modulate expression of nuclear receptors
  - correcting IR in liver and adipose tissue
  - protect against steatohepatitis

Ongoing clinical trials

Hepatology 2011,54:1500; PLoS One 2012,7:e45425

CyNCh: Cysteamine DR for Treatment of Paediatric NAFLD

- Randomized, double-blind, placebo-controlled, multicenter phase IIb trial
- Cystine-depleting aminothiol
- 70% boys, mean age 13.7 yrs

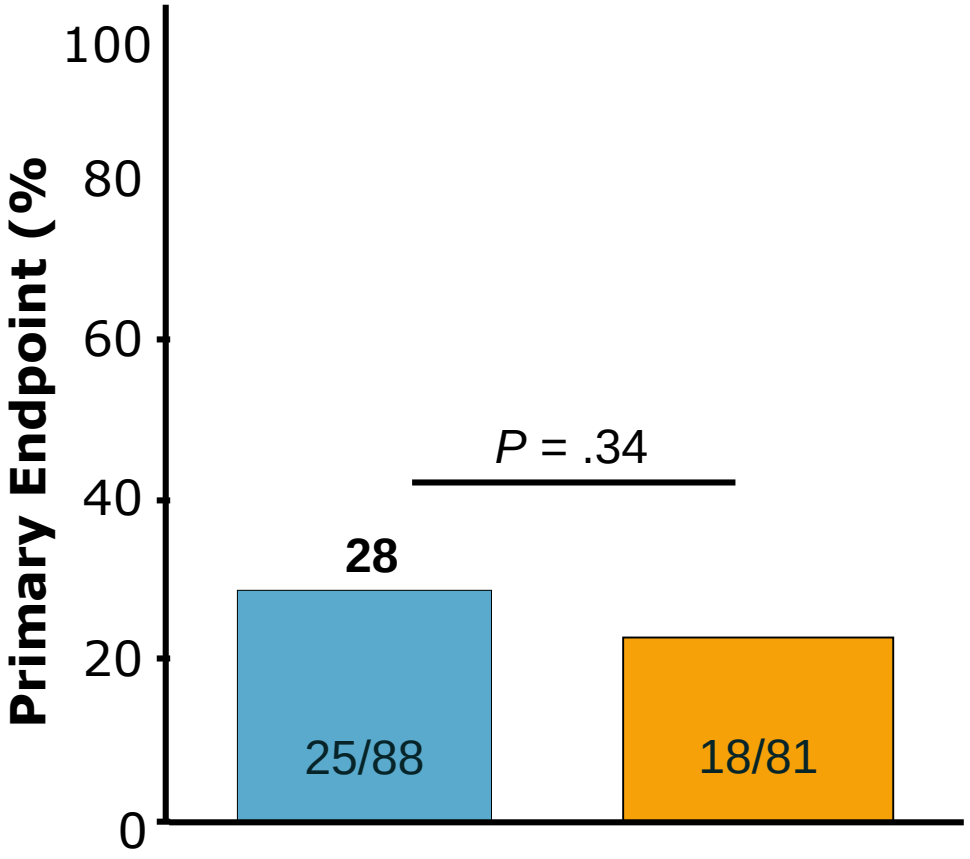


* **Total dose: 300 mg if pt weighed ≤ 65 kg, 375 mg if pt weighed 65-80 kg, 450 mg if pt weighed > 80 kg.**

Schwimmer JB, Lavine JE, et al. AASLD 2015. Abstract LB-31.

Primary Endpoint: Improvement in NAS ≥ 2 Points Without Worsening of Fibrosis

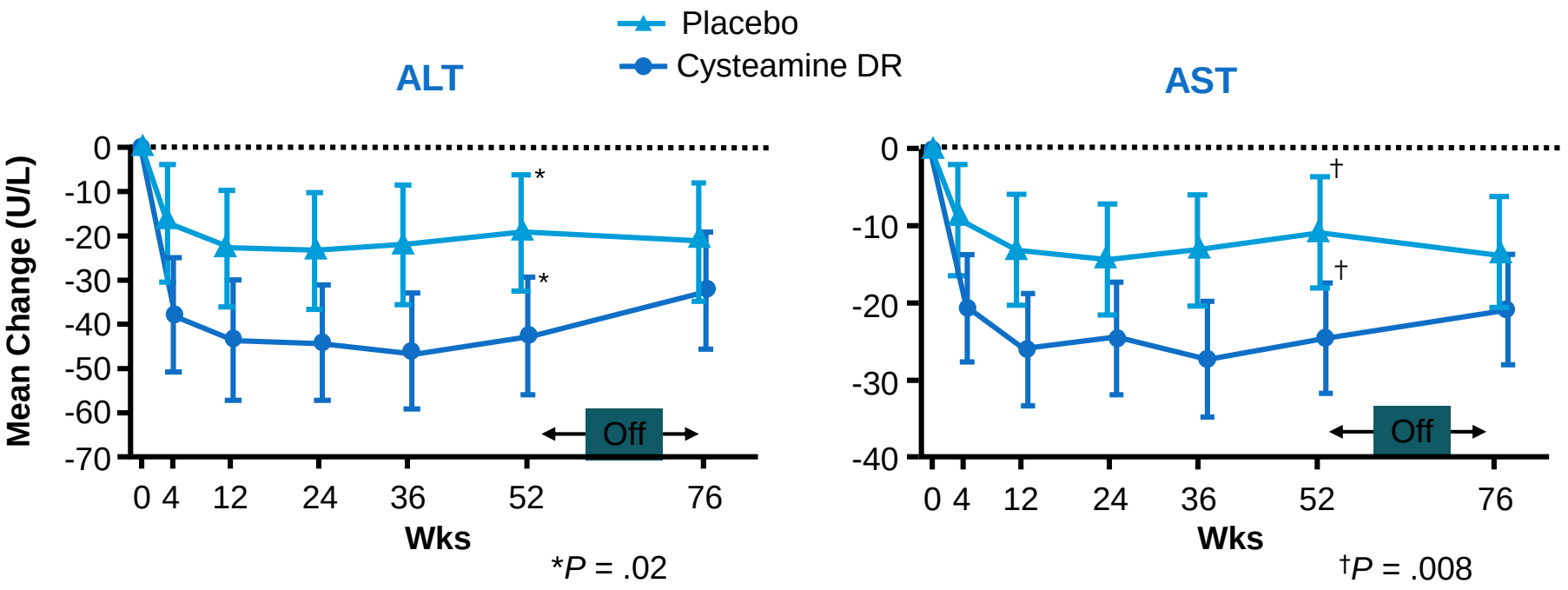
- End of treatment (Wk 52) biopsies in 81% on drug, 93% on placebo (*P* = .03)
- ITT analyses also performed for 4 histologic features including fibrosis, steatosis, ballooning and lobular inflammation
  - Not significant with statistical correction



Schwimmer JB, Lavine JE, et al. AASLD 2015. Abstract LB-31.

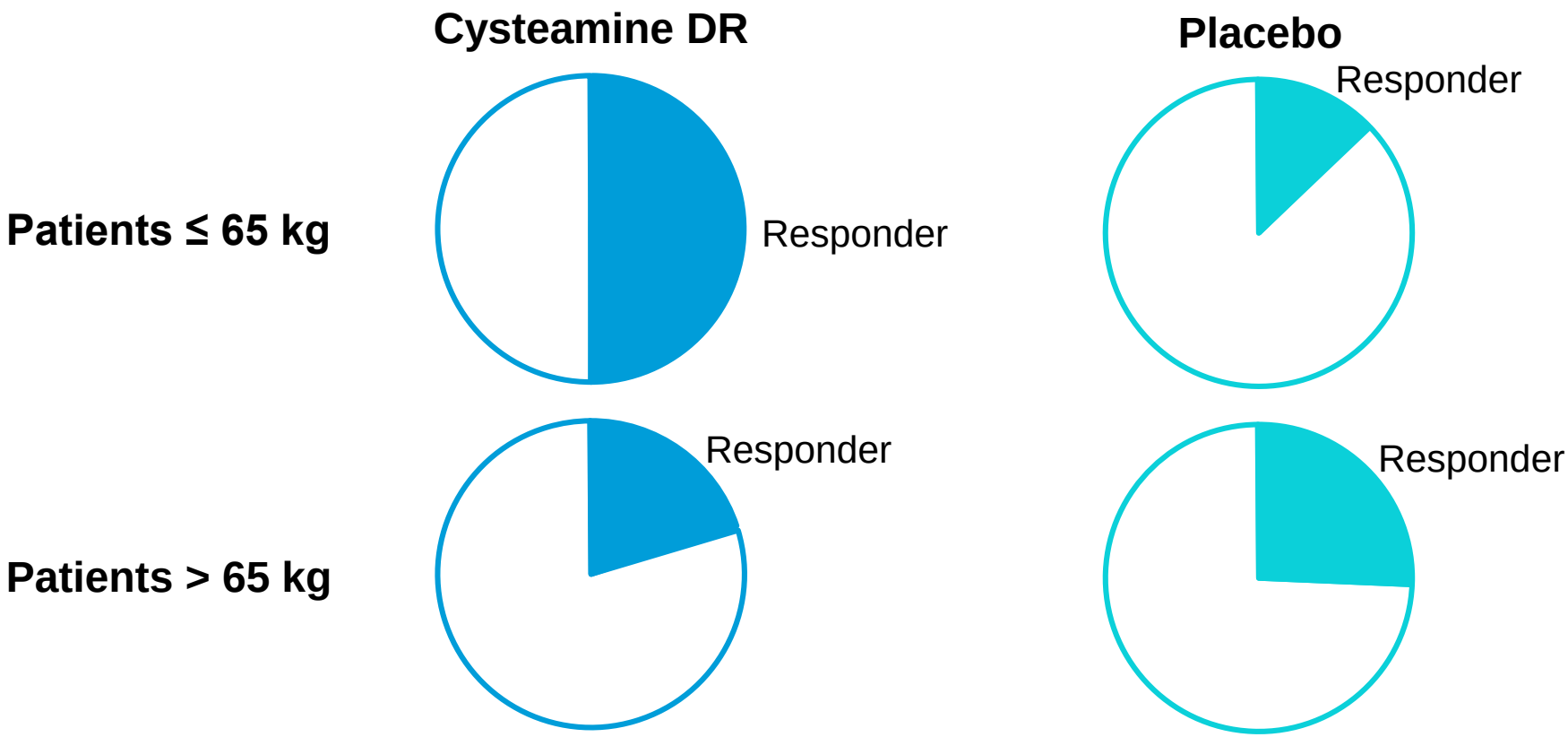
Secondary Outcomes at Wk 52

- No change in serum lipids, cholesterol, insulin sensitivity
- No difference in adverse events



Schwimmer JB, Lavine JE, et al. AASLD 2015. Abstract LB-31.

Improvement in Histology by Weight at Enrollment



Responder: patients with improvement in NAFLD activity score ≥ 2 points without worsening of fibrosis

Schwimmer JB, Lavine JE, et al. AASLD 2015. Abstract LB-31.