The European-Paediatric NAFLD (EU-PNAFLD) Registry

Longitudinal follow-up of children with non-alcoholic fatty liver disease

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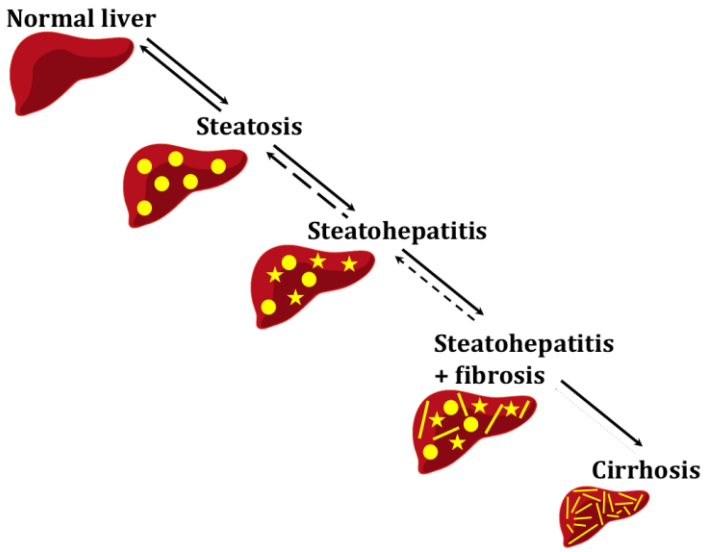
Vision

We plan to develop a Europe-wide clinical research network and registry of children with non-alcoholic fatty liver disease (NAFLD) to facilitate international clinical and translational studies that will ultimately improve the outcome for affected patients. EU-PNAFLD (European Paediatric NALFD Registry) will be a network composed of European centres involved in the care of children with NAFLD, and will include Hepatologists, Endocrinologists, and Scientists supported by relevant international specialists. This collaboration will build on existing infrastructure (local databases and bio-repositories) and align with the adult European NAFLD Registry ("EPoS", Elucidating Pathways of Steatohepatitis) to allow long-term follow-up supported by translational studies. Through an international, well-characterised large-scale cohort, we hope to: facilitate multi-centre clinical trials; extend our understanding of the key disease mechanisms of NAFLD; and establish the natural history of paediatric NAFLD.

Background

Obesity is one of the main public health issues for developed countries[1] with a third of 9-year olds in the UK now overweight or obese[2]. The rise in obesity has been mirrored by an increase in paediatric non-alcoholic fatty liver disease (NAFLD), a term that encompasses a spectrum of disease from steatosis (or non-alcoholic fatty liver (NAFL)) to non-alcoholic steatohepatitis (NASH) and cirrhosis[3].



There are several unanswered questions and important issues in the management of paediatric NAFLD. These include:

1. What is the natural history of the condition? And, what is the prognostic significance of NAFL ('simple steatosis')?
2. What treatments are effective for paediatric NAFLD?
3. How can patients with paediatric NAFLD be non-invasively stratified into high-risk and low-risk groups? Are novel biomarkers or genetics helpful?
4. Do monogenic disorders underlie a subset of patients with paediatric NAFLD?

In adults uncomplicated steatosis often remains a benign condition, whereas steatohepatitis with fibrosis may progress to end-stage liver disease and decompensation[4–8]. However, the natural history of NAFLD in children is much less clear[9] and based on a relatively small number of studies. The first prospective secondary care study of NASH in children demonstrated cirrhosis in one out of 36 children after 10 years follow-up[10]. In 2009, Feldstein *et al* reported a retrospective cohort study that found 2/66 patients required liver transplantation and 3 died (non-liver deaths) within 20 years[11]. In addition to a few selected follow-up studies[12–14], there are also a few small case series of cirrhosis in children or young adults secondary to paediatric NAFLD[15–17].

However, these studies are relatively small in size and are predominantly retrospective in nature, thus limiting their value. There have been calls in the literature for longitudinal research into the natural history of paediatric NAFLD[9,18–20], but as yet such studies have not been established. Such follow-up studies will be important in defining the natural history of such children, as well as allowing for an accurate description of the metabolic complications they may develop[21,22].

Currently, there are no approved pharmacological treatments for paediatric NAFLD[23,24]. Randomised controlled trials have failed to demonstrate any benefit from vitamin E[25], metformin, or cysteamine on histology[26]. Polyunsaturated fatty acids have not been studied with validated outcomes (histology or non-invasive scores)[27]. Therefore, there is an urgent need for new, effective therapies that have been assessed in well-characterised cohorts[28].

Liver biopsy is the gold-standard investigation of paediatric NAFLD and the only fully validated method for assessing the presence of NASH or fibrosis[3,29]. Novel non-invasive

markers (e.g. cytokeratin-18 and cathepsin D, or plasma lipidomics) may replace the need for liver biopsy over the next 5-10 years[30–33].

It is believed that NAFLD is polygenic (plus environmental) in the vast majority of affected patients, however a subset may be caused by rare genetic conditions[34–36]. These may be more likely to present with an abnormal phenotype (e.g. NAFLD with low BMI) but can lead to diagnoses with specific therapeutic options (e.g. lipodystrophy treated with leptin).

Aims

1. Describe natural history of paediatric NAFLD

This observational study aims to describe the natural history of paediatric NAFLD in Europe, providing data on their survival, progression of liver disease and development of metabolic complications. Understanding the burden of metabolic disease in these patients will be important and will focus on aspects such as incidental rate of diabetes, diabetic complications, sleep apnoea, hypertension, and early-onset cardiovascular disease.
This Registry will link with the already established adult EU-NAFLD Registry (PI: Prof. Quentin Anstee, Newcastle, UK).

2. Facilitate translational basic science research

Blood samples taken for genetic and metabolic analysis will allow additional insight into the mechanisms underlying NAFLD. These samples may also be used in future studies related to NAFLD.

3. Backbone study for recruitment into further trials

The study will provide a well characterised cohort of children with NAFLD who could be recruited into translational and interventional studies in the future.

Methods

Overview

- Establish a multi-centre database of UK paediatric NAFLD patients with prospective follow-up and data collection from routine clinic appointments.
- Diagnosis of NAFLD by liver biopsy, or non-invasive investigations
- Prospective follow-up, as dictated by clinical care at individual centres.
- Data will be collected as available, ideally at annual follow-up
- Each centre outside the UK will be responsible for arranging their own ethical approval and study insurance

Participant identification

Retrospective: patient databases at defined institutions (see below) will be searched to screen for patients with biopsy-proven diagnosis of NAFLD between 2012 to-date. Data must be complete and good quality to allow retrospective enrollment.

Prospective: Recruitment of patients from 2017 onwards from liver clinics for children with (invasive or non-invasive) diagnosis of NAFLD.

Exclusion criteria:

- Secondary fatty liver disease (e.g. glycogen storage diseases, Wilson disease, viral hepatitis, drug-related, autoimmune hepatitis, type 1 diabetes mellitus)[1]
- Post-transplant fatty liver
- >20g/day ethanol intake

Inclusion criteria:

- Diagnosis made under 18 years of age.
- Diagnosis of NAFLD spectrum disease (simple steatosis (NAFL), steatosis with abnormal transaminases, NASH ± fibrosis or cirrhosis)
- Diagnosis established by:
    - Radiological evidence of hepatic steatosis (e.g. increased hepatic echogenicity on ultrasound), with
    - Exclusion of secondary causes (negative serological liver screen for HBV/HCV, caeruloplasmin >0.20g/L, no history of excess alcohol consumption, no evidence of iron overload, and no clinically significant alpha-1 antitrypsin (A1AT) phenotype (i.e. SZ, ZZ, SS), with or without
    - Histology (>5% steatosis and histology consistent with paediatric NAFLD)

Definitions:

- NAFL: presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes.
- NAFLD: presence of hepatic steatosis with either abnormal aminotransferases or evidence of hepatocellular injury on histology that doesn't meet criteria for diagnosis of NASH.
- NASH: presence of hepatic steatosis and inflammation on biopsy[2]
    - Type 1 NASH: centrilobular inflammation with hepatocyte injury in the form of ballooning (with Mallory-Denk bodies), with or without fibrosis, and without portal inflammation or fibrosis
    - Type 2 NASH: portal inflammation with or without periportal fibrosis, and absence of ballooning or centrilobular inflammation
    - Overlap NASH: features of both type 1 and type 2 NASH
- Cirrhosis: histological evidence of cirrhosis, radiological evidence strongly suggestive of cirrhosis (e.g. CT or MRI), or intraoperative visualisation of macroscopic cirrhosis.

Institutions involved

- Centres included to date:
    - Birmingham Children's Hospital – Dr. Indra van Mourik & Prof. D. Kelly (DAK)
    - Queen Elizabeth Hospital, Birmingham – Prof. P.N. Newsome & Dr. Matthew J. Armstrong
    - Leeds General Infirmary – Dr. S. Rajwal
    - Addenbrooke's Hospital, Cambridge – Dr. Jake P. Mann (JPM), Dr. G. Noble-Jamieson, Prof. David B. Savage (DBS), & Dr. Matthias Zilbauer (MZ)
    - Bambino Gesu Hospital, Rome – Prof. Valerio Nobili (VN)

[1] NAFLD associated with insulin-resistance syndromes (e.g. lipodystrophy, Bardet-Biedl syndrome) are not to be excluded but will form a specific sub-groups

[2] At this stage, NASH can only be diagnosed on biopsy. However, if a validated method of non-invasive diagnosis becomes available then this can be implemented.

- Newcastle upon Tyne Hospitals NHS Foundation Trust (RVI & Freeman Hospitals), Newcastle – Prof Quentin M. Anstee (QMA)
- Hanover, Germany – Dr. Ulrich Baumann
- Warsaw, Poland – Prof. Piotr Socha (PS)

Data collection

Data will be collected from:

- Electronic and paper patient records
- Patient (and parent) questionnaires at follow-up
- Telephone interviews
- NHS data systems (e.g. HES data, Death registry) for long-term follow-up data of UK-based patients

Data will then be entered into a secure online database hosted by Newcastle University. The database will be an extension of the adult European NAFLD Registry (EPoS Study).

Data collection and entry onto the online database will be performed by local collaborators and research assistants, where available.

Participants who are lost to follow-up after initial diagnosis will remain in the database and can contribute to long-term morbidity/mortality analysis.

If participants are still diagnosed with NAFLD or under the care of a liver clinic when they turn 18-years, they will be invited to join the adult European NAFLD Registry. This would be a separate consent process.

Genetic and metabolic analysis

Participants who consent to having blood taken for genetic and metabolic analysis, samples will be taken when they have their routine blood tests at the liver clinic. Blood may also be taken from parents to facilitate genetic trios analysis.
For DNA: 10ml blood collected into plain tubes, which can be sent without further processing.
For metabolites: 5ml blood collected into serum gel activator tubes, which will be spun and separated at the local laboratory. Serum will be frozen prior to transport.
Blood samples will be sent to the Metabolic Research Laboratories, Biomedical campus, Cambridge, UK, for processing and storage. Samples may also be stored at the National BioResource in Milton Keynes, if necessary.
Ethical approval will also be sought for gut microbiome analysis.

Natural history outcomes:

- *Primary*: Survival (liver- and non-liver related mortality)
- *Secondary*: Transplant-free survival; mortality due to cardiovascular disease; progression of liver histology[3]; development of decompensated liver disease; development of type 2 diabetes mellitus; development of clinical cardiovascular disease; liver disease variables, including: ALT, AST, cholesterol, triglycerides, waist circumference, Fibroscan values
- A sub-analysis of only children with liver biopsies will be performed

[3] Fibrosis staged according to the system proposed by Kleiner *et al.* (2005).

- Data will be analysed in conjunction with natural history data from the adult European NAFLD Registry (EPoS) study

Data will be collected at presentation and at approximately annual-yearly intervals alongside routine care. More frequent follow-up data may be available for those patients under regular review. (**Minimum dataset in bold.**)

- **Name, NHS number (for UK participants), date of birth**, **gender**, post code
- **Date of visit (initial**, or follow-up)
- Demographics (age, gender, ethnicity, country of origin)
- Height, weight and BMI (with centiles[4]), waist circumference, hip circumference, blood pressure
- Method of presentation (abnormal USS, abnormal LFTs, other)
- Self-reported 24-hour food diary
- Alcohol consumption
- Past medical history (diabetes, psychological, other)
- Complications of liver disease, metabolic syndrome or insulin resistance, polycystic ovarian syndrome, sleep apnoea, transplantation, renal impairment, number of hospital admissions in last 6 months
- Family history (obesity, diabetes, ischaemic heart disease, thyroid disease, other)
- Medications
- Clinical evidence of portal hypertension or chronic liver disease
- Liver function tests, to include: AST, ALT, ALP, Bil, GGT, Alb, PT/INR, platelet count
- HbA1c, TSH, lipid profile (ideally fasting), ferritin, CRP, urate, creatinine/eGFR, Immunoglobulins (IgA, IgG, IgM).
- Quantification of insulin resistance (fasting insulin measured at OGTT, HOMA-IR, or ADIPO-IR)
- **On initial visit only: caeruloplasmin, A1AT, serum immunoglobulins, autoantibodies, ferritin, transferrin saturation, viral screen (HBV/HCV)[5]**
- **Ultrasound scan (USS) results: echogenic liver, spleen size[5]**
- **Liver histology: degree and distribution of steatosis, portal/centrilobular inflammation, fibrosis stage[3,5]**
    - Biopsy may have been performed, as clinically indicated, prior to entry the study, but it is not an absolute requirement for prospectively recruited participants
    - Patients may be offered a repeat biopsy within 10-years, according to clinical need
- Diagnosis: simple steatosis / NAFL, NAFLD, NASH, or cirrhosis (and invasive or non-invasive diagnosis)
- Management (new medications, participation in clinical trials of lifestyle change/pharmacological agents)
- Referrals to other specialties (e.g. endocrine & diabetes, cardiology, adult services)

Additional data, if available:

- Bioimpedance
- Non-invasive markers (e.g. ELF, CK-18, NEFA, cathepsin D)
- Fibroscan values

[4] Obesity will be defined as BMI >95% centile for age and sex.

[5] If diagnosis is not established using biopsy, then the full panel of tests for exclusion of secondary causes must be recorded, in addition to ultrasound results.

Research Sample Collection
Participants will be invited to give samples for research purposes that can be used in future translational science studies. Samples to include:

- Blood for DNA / RNA extraction
- Serum & plasma
- Biopsy (liver, adipose, muscle) samples
- Stool samples

Follow-up
Data will be collected as available from follow-up clinics, ideally annually. If patients have been discharged, then follow-up will be via letter/telephone or GP. Total follow-up will continue for up to 10-years in the first instance. This will require interaction between paediatric and adult services. Data will be analysed in conjuction with the adult EU-NAFLD Registry but will not be transferred between the two. If funding allows, data collection will continue as long as patients remain under hepatology follow-up. At 20-years from entry into the trial we will search pseudoanonymised data systems (e.g. HES, GP database, and European-equivalents) to look for cardiovascular and metabolic outcomes. In addition, we may contact participants and their GPs to ask for details if our data is incomplete.
If participants have NAFLD as adults, they may be separately invited to formally join the adult NAFLD Registry.

Statistical analysis
The majority of this analysis will be descriptive so as to define the natural history of the patient group. Survival data from study cohort will be compared to an age- and sex-matched cohort from the national GP database.

Cox multiple variable regression will facilitate survival analysis to assess the primary outcome. This will allow identification of the factors that significantly influence survival. However, due to a lack of published data any power calculation would be entirely speculative.

Ethical approval
Informed consent will be sought prior to starting data collection. National Research Ethics Committee approval will be sought to allow for the collection and analysis of patient data for the purposes of this research study.

Funding
The study is supported by the European Union H2020 programme through the *Elucidating Pathways of Steatohepatitis (EPoS)* consortium grant and an EASL Registry Grant (to Prof Quentin Anstee). Additional support is from the NIHR Cambridge Biomedical Resource Centre, the NIHR Newcastle Biomedical Research Centre and by Wellcome Trust grants to Prof. David B. Savage and Prof. Sir Steve O'Rahilly.

Plan of work: phases & work streams

To manage the implementation of the above protocol, we have split the plan of work into two phases. The direction of the registry will be lead by a steering committee, comprising: VN, DAK, PS, JPM, and QMA. Each Work stream will have a lead clinician responsible for its implementation.

Phase 1 –

Phase 1 has been divided into 2 work streams and will focus on establishing the EU-PNAFLD network, including the set-up of a core database and bio-bank. This phase forms the area of support applied for through the ESPGHAN networking grant.

Work stream 1: *Mapping the natural history of paediatric NAFLD and provide a cohort for trial recruitment*

Lead: QMA, PS

The objective of this work stream is to describe the long-term outcomes of children with NAFLD, including clinical liver events (e.g. HCC, variceal bleeds), subclinical progression (e.g. progression of fibrosis), and metabolic outcomes (e.g. development of type 2 diabetes). The initial goal will be to develop an international, multicenter, registry of children with biopsy-proven NAFLD for a retrospective analysis and which will also facilitate recruitment into interventional trials. Local databases at participating institutions will be used to retrospectively identify potentially eligible participants with a previous biopsy-proven diagnosis of NAFLD-spectrum disease.

The network database will be paediatric-specific extension of the existing adult European NAFLD Registry database and will be managed by the University of Newcastle, UK. Clinical, anthropometric, and biochemical pseudoanonymised data will be uploaded locally.

All participants will be asked give consent to be contacted by other researchers (including those internationally) regarding recruitment into further clinical trials. The EU-PNAFLD Network database will serve as a central point of contact for researchers wishing to recruit a cohort of children with NAFLD.

Work stream 2: *Establishing a bio-bank of samples for translational research*

Lead: DBS, JPM

The objective of work stream 2 is to develop a bio-bank of samples from children with NAFLD that can be used for translational laboratory science project, including those described in Phase 2, below.

We have funding and infrastructure in place for the storage of blood (DNA), serum, stool, and liver biopsy specimens at the Institute of Metabolic Science in Cambridge, UK. Consenting participants will be asked to give an additional sample of blood during their next routine tests, which can be processed by protocolled procedures and sent to Cambridge, UK. Parents of participants will also be invited to give a sample for DNA storage for future genetic trio analysis. Stool samples will be obtained to facilitate microbiome studies.

Phase 2

Phase 2 comprises three work streams, aimed at prospective recruitment/follow-up and translational basic science.

Work streams 3: *Non-invasive biomarkers of paediatric NAFLD*

Lead: VN

Work stream 3 will employ the following key strategies:

I. Validation of existing biomarkers that have previously been assessed in single cohorts only, including: Enhanced Liver Fibrosis (ELF) test, CK-18, cathepsin-D, miR-122.

II. Exploration of novel serum biomarkers, including: targeted and untargeted lipidomic analyses, serum proteome analysis by mass spectrometry, exosomes, intestinal microbiome, and microRNAs.

Work stream 4: *Genetics of paediatric NAFLD*

Leads: JPM, UB

Work stream 4 will focus on three main strategies:

I. Whole exome sequencing of children with suspected monogenic disorders, by selecting children with 'lean' NAFLD, severe insulin resistance, or severe fibrosis, particularly in those with a family history of NAFLD.

II. Assessment of epigenetics in paediatric NAFLD by characterisation of hepatic and circulating methylation profiles, combined with RNA sequencing to

III. Single-cell sequencing of liver biopsy specimens from patients with simple steatosis, NASH, and all stages of fibrosis.

Work stream 5: *Prospective recruitment and follow-up for long-term natural history data*
Lead: DAK

Work stream 5 will be a prospective continuation of Work stream 2, aiming to provide long-term clinical outcome data and recruitment into interventional trials. Prospectively recruited participants will not require a biopsy diagnosis of NAFLD, this is for 4 reasons. Firstly, to include participants who would not meet criteria for biopsy, giving a less biased assessment of natural history of the condition. Secondly, to account for variation in practice between centres; this is necessary to facilitate a multi-centre trial. It will be possible to perform sub-analyses of only participants with a biopsy-diagnosis. Finally, developments in non-invasive assessment of NAFLD may reduce the need for biopsy in the next 5-10 years.

References

1. Bhatt, D. L. *et al.* International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. *JAMA* **295,** 180–9 (2006).
2. Jackson, A. A., Wiseman, M. & Wootton, S. A. Tackling the obesity crisis: how do we 'measure up'? *Arch. Dis. Child.* **99,** 95 (2014).
3. Vajro, P. *et al.* Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J. Pediatr. Gastroenterol. Nutr.* **54,** 700–13 (2012).
4. Ekstedt, M. *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* **44,** 865–73 (2006).
5. Ekstedt, M. *et al.* Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* **61,** 1547–1554 (2015).
6. Bhala, N. *et al.* The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* **54,** 1208–16 (2011).
7. Younossi, Z. M. *et al.* Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64,** 73–84 (2016).
8. Musso, G., Gambino, R., Maurizio, C. & Pagano, G. Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann. Med.* **43,** 617–649 (2011).
9. Nobili, V. *et al.* A 360-degree overview of paediatric NAFLD: Recent insights. *J. Hepatol.* **58,** 1218–1229 (2013).
10. Rashid, M. & Roberts, E. A. Non-alcoholic Steatohepatitis in Children. *J. Pediatr. Gastroenterol. Nutr.* **30,** 48–53 (2000).

11. Feldstein, A. E. *et al.* The natural history of nonalcholic fatty liver disease in children: a follow-up study for up to 20-years. *Gut* **58,** 1538–1544 (2010).
12. Manton, N. D. *et al.* Non-alcoholic steatohepatitis in children and adolescents. *Med. J. Aust.* **173,** 476–479 (2000).
13. Schwimmer, J. B. *et al.* Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* **42,** 641–649 (2005).
14. Nobili, V. *et al.* NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* **44,** 458–65 (2006).
15. Kinugasa, A. *et al.* Fatty liver and its fibrous changes found in simple obesity of children. *J Pediatr Gastroenterol Nutr* **3,** 408–414 (1984).
16. Molleston, J. P., White, F., Teckman, J. & Fitzgerald, J. F. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am. J. Gastroenterol.* **97,** 2460–2462 (2002).
17. Adams, L. A., Feldstein, A., Lindor, K. D. & Angulo, P. Nonalcoholic Fatty Liver Disease among Patients with Hypothalamic and Pituitary Dysfunction. *Hepatology* **39,** 909–914 (2004).
18. Marion, a W., Baker, a J. & Dhawan, a. Fatty liver disease in children. *Arch. Dis. Child.* **89,** 648–52 (2004).
19. Patton, H. M. *et al.* Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J. Pediatr. Gastroenterol. Nutr.* **43,** 413–427 (2006).
20. Roberts, E. a. Pediatric nonalcoholic fatty liver disease (NAFLD): a 'growing' problem? *J. Hepatol.* **46,** 1133–42 (2007).
21. Pacifico, L., Nobili, V., Anania, C., Verdecchia, P. & Chiesa, C. Pediatric nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk. *World J. Gastroenterol.* **17,** 3082–3091 (2011).
22. Nobili, V. *et al.* The potential role of fatty liver in paediatric metabolic syndrome: A distinct phenotype with high metabolic risk? *Pediatric Obesity* **7,** (2012).
23. Vajro, P. *et al.* Therapeutic options in pediatric non alcoholic fatty liver disease: current status and future directions. *Ital J Pediatr* **38,** 55 (2012).
24. Mitchel, E. & Lavine, J. Review article: the management of paediatric nonalcoholic fatty liver disease. *Aliment. Pharmacol. …* **40,** 1155–70 (2014).
25. Lavine, J. E. *et al.* Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* **305,** 1659–68 (2011).
26. Schwimmer, J. B. *et al.* In Children with Nonalcoholic Fatty Liver Disease, Cysteamine Bitartrate Delayed Release Improves Liver Enzymes but does not Reduce Disease Activity Scores. *Gastroenterology* (2016). doi:10.1053/j.gastro.2016.08.027
27. Janczyk, W. *et al.* Omega-3 Fatty acids therapy in children with nonalcoholic Fatty liver disease: a randomized controlled trial. *J. Pediatr.* **166,** 1358–1363 (2015).
28. Rotman, Y. & Sanyal, A. J. Current and upcoming pharmacotherapy for non-alcoholic fatty liver disease. *Gut* gutjnl-2016-312431 (2016). doi:10.1136/gutjnl-2016-312431
29. Manco, M. *et al.* Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. *Int. J. Obes. (Lond).* **32,** 381–7 (2008).
30. Fitzpatrick, E. & Dhawan, A. Noninvasive biomarkers in non-alcoholic fatty liver disease: Current status and a glimpse of the future. *World J. Gastroenterol.* **20,** 10851–10863 (2014).
31. Feldstein, A. E. *et al.* Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *Am. J. Gastroenterol.* **108,** 1526–31 (2013).
32. Mansoor, S., Collyer, E. & Alkhouri, N. A comprehensive review of noninvasive liver

fibrosis tests in pediatric nonalcoholic Fatty liver disease. *Curr. Gastroenterol. Rep.* **17,** 23 (2015).

33. Gorden, D. L. *et al.* Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J. Lipid Res.* **56,** 722–736 (2015).
34. Dongiovanni, P. & Valenti, L. Genetics of nonalcoholic fatty liver disease. *Metabolism* 1–12 (2015). doi:10.1016/j.metabol.2015.08.018
35. Anstee, Q. M. & Day, C. P. The genetics of NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* **10,** 645–55 (2013).
36. Parker, V. E. R. & Semple, R. K. Genetics in endocrinology: genetic forms of severe insulin resistance: what endocrinologists should know. *Eur. J. Endocrinol.* **169,** R71-80 (2013).