The Role of Genetic Predisposition, Programing During Fetal Life, Family Conditions, and Post-natal Diet in the Development of Pediatric Fatty Liver Disease

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Objective To evaluate, in patients with nonalcoholic fatty liver disease (NAFLD), the role of lifetime exposures associated with genetic predisposition, family history (parental obesity, economic income), programming during fetal life (gestational age, birthweight), being breastfed or not, and later biomarkers of dietary habits and lifestyle in the development of fibrosis.

Study design In total, 182 children with overweight/obesity diagnosed with NAFLD proven by biopsy results were enrolled in our study and evaluated for liver fibrosis. We estimated prevalence ORs of fibrosis according to genetics, parental obesity, occupational socioeconomic status (SES), birth weight, breastfeeding, fructose intake (indicator of junk food consumption), and vitamin D status (inflammatory indicator) using logistic regression models, adjusted for age and children's body mass.

Results One hundred thirty-seven patients (75.3%) had liver fibrosis, and 45 patients (24.7%) did not have liver fibrosis. The ORs of fibrosis were significant ($P < .05$) for patatin like phospholipase domain-containing 3-GG genotype (OR 2.1), parental obesity (OR 2.9), not being breastfed (OR 3.1), vitamin D status (<20 mg/dL) (OR 1.24), and fructose consumption (OR 1.6 per 1 g/day increase), whereas a high SES maternal occupation was inversely associated with fibrosis (OR 0.30).

Conclusions Our results show independent roles of the patatin like phospholipase domain-containing 3 gene, parental obesity, maternal SES, and postnatal diet and lifestyle in the development of progressive liver disease secondary to NAFLD. *(J Pediatr 2019;211:72-7)*.

The epidemic of obesity and nonalcoholic fatty liver disease (NAFLD) in children of the Western world is attributed to numerous factors influencing the course of the disease. Accordingly, genetic predisposition, parental conditions and lifestyle, and epigenetic influences as well as the fetal programming phase (including duration and intrauterine growth rate) may be considered as primary factors in the development of NAFLD up to the most advanced stages of fibrosis.[1,2] Within genetic predisposition, the I148 M patatin like phospholipase domain-containing 3 (PNPLA3) polymorphism is indeed strongly associated with the severity of steatosis and fibrosis and the presence of nonalcoholic steatohepatitis.[3] After birth, breastfeeding is considered a preventive measure against the development of metabolic syndrome and/or its individual components even in young adulthood. It is actually associated with a lower risk of developing obesity and NAFLD and nonalcoholic steatohepatitis (NASH).[4,5] A healthy lifestyle, confounded by the environment surrounding breastfeeding, could also play a positive role.[6] Formula feeding in Western countries is linked to lower socioeconomic status and poorer academic/academic outcomes in parents and less economically rewarding types of occupation.[7] Following the introduction of solids, demographic and environmental factors may also drive the type of food selection.[8] The use of so-called junk foods is widespread in the poorer classes of the social structure at any age because they are cheaper and easily available, providing extra-needed calories with low nutritional value, and are associated with NAFLD.[9,10] Finally, suboptimal circulating levels of vitamin D have been linked to the development of NAFLD/NASH.[11] Although vitamin D status is important because it is associated with markers of oxidative stress and inflammation in the pediatric population,[12] it

BMI	Body mass index
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NAS	NAFLD activity score
PNPLA3	Patatin like phospholipase domain-containing 3
SES	Socioeconomic status
SGA	Small for gestational age

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The authors declare no conflicts of interest.

0022-3476/$ - see front matter.
https://doi.org/10.1016/j.jpeds.2019.04.018

can in turn be linked to other dietary and environmental conditions potentially associated with NAFLD/NASH.[13]

A better understanding of the independent and cumulative role of these components for health outcomes is necessary to plan preventive programs that reach the most economically advantageous and feasible preventive approaches.

The objective of this cross-sectional, retrospective study is to evaluate the independent role of main variables in the development of progressive liver disease following fatty liver. Genetics, obesity, and parental work, gestational age and birth weight, breastfeeding, and fructose consumption, as an indicator of junk food consumption, together with vitamin D status, have been tested for independent associations with fibrosis as a primary outcome and the presence of NASH as a secondary end point, in a white pediatric population affected by fatty liver and obesity.[5,14-17]

Methods

Children consecutively diagnosed with NAFLD proven by biopsy results were included from a population of 339 white patients with obesity admitted at the Hepato-Metabolic Unit of Bambino Gesù Children's Hospital in Rome, Italy, from June 2014 to December 2016. Diagnosis of NAFLD was established by excluding viral infections (hepatitis A-B-C, cytomegalovirus, and Epstein-Barr virus), alcohol intake, and use of drugs (ie, prednisone, valproate), and autoimmune or congenital metabolic liver diseases, alpha-1-antitrypsin deficiency, or Wilson disease. Inclusion criteria for the study were a diagnosis of NAFLD proven by biopsy results, absence of specific dietary or any other therapeutic treatment, comorbidities, and the availability of complete familial, maternal, and neonatal anamnesis data.

NASH as secondary outcome was coded according to NAFLD activity score (NAS), defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2), ranging from 0 to 8, and calculated for all patients. A score point of NAS ≥5 was required for the diagnosis of NASH.[17] Liver fibrosis is not included as a component of this activity score.

The degree of fibrosis was scored according to the NASH Clinical Research Network (0 = absence of fibrosis; 1 = perisinusoidal or portal fibrosis; 2 = perisinusoidal and portal/periportal fibrosis; 3 = septal or bridging fibrosis; 4 = cirrhosis).[18]

The study protocol was approved by the Ethical Committee of Bambino Gesù Children's Hospital, and written informed consent was obtained from the parents of the children. For frequencies of exposure approximately 30%-50% in the NAFLD group, this study could a priori detect ORs above 2.4 with a 95% CI level and a power of 80%.

Liver Biopsy

Liver biopsy was performed using an automatic core biopsy device (Biopince; Amedic, Sweden) with an 18G needle, under general anesthesia and ultrasound guidance. An experienced liver pathologist reviewed all the liver biopsy specimens to confirm the diagnosis.[18]

Anthropometric Measures

All patients underwent anthropomorphic measurements (weight, height, and waist circumference) using standardized methods, and body mass index (BMI) was calculated as weight (kg)/height $(m)^2$ together with z score.[19]

Obstetric Anamnesis

Obstetrical data were derived from clinical charts displayed by mothers. Accordingly, the history of maternal diabetes has been directly assessed on the basis of the screening provided in the region at the 24th week of gestational age.

Neonatal, Breastfeeding, and Family History

Neonatal data were collected from clinical charts for all the included subjects. Birthweight was approximated at ±5 g. Breastfeeding and its duration in months (up to 2 feedings at least per day) were obtained from the same source and by parents. Formula-fed infants had to be breastfed for more than 50% of feedings after the first 2 weeks. Gestational age (term, ≥37 weeks of gestational age, otherwise preterm) and birthweight adequacy (<10th percentile, small for gestational age [SGA], ≥10th to ≤90th percentile, appropriate for gestational age, >90th percentile, large for gestational age) were recorded. The occupation of either parent was coded as proxy of socioeconomic status (SES) as follows: 0 = which comprises the 8 and 7 categories of Istituto Nazionale di Statistica (ISTAT [National Institute of Statistics]) classification (lowest level), 1 = the 5 + 6 + 9 categories of ISTAT and, 2 = the 1 + 2 + 3 + 4 categories (highest level).[20,21] Parental obesity (at least 1 parent with BMI >30 kg/m^2 before pregnancy) was recorded at diagnosis of NASH.

Genotyping

The mutated PNPLA3-I148M variant, attached to the surface of lipid droplets, may reduce the cleavage of triglycerides leading to lipid retention in hepatocytes lipid droplet, so inducing the onset of hepatic steatosis. Genotyping for PNPLA3 I148M gene variant (rs738409) was carried out at first diagnosis of liver involvement using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, California). Commercial genotyping assays were available for the following rs738409 (cat. C_7241_10). An assay was created by Applied Biosystems for rs12979860. The genotyping call was done with SDS software v 1.3.0 (ABI Prism 7500, Foster City, California).

Assessment of Dietary Fructose Consumption

A food frequency questionnaire and the portion size (atlas of Scotti-Bassani[22,23]) were administered to all patients as previously reported. The questionnaire included breakfast, morning snack, lunch, afternoon snack, and dinner, and investigated the intake of specific foods and portions.[16,22] The food composition database described in Livelli di

Assunzione di Riferimento di Nutrienti (IV edition), published by the National Italian Institute of Food Research and Nutrition, and UK charts were used to assess the intake of fructose per day.[23,24] The study design conformed to the ethical guidelines of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008) and the Institutional Review Board of the "Bambino Gesù" Children's Hospital, Rome, Italy, approved the study.

Blood Biochemistry

Fasting blood was drawn for total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, uric acid, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl-transpeptidase, international normalized ratio, fasting plasma glucose, and insulin in all patients. Moreover, oral glucose tolerance tests were performed, according to World Health Organization recommendations. The homeostatic model assessment of insulin resistance score was used for estimating insulin resistance. A cut-off value of >2.5 was considered as index of insulin resistance.[25] In all patients, serum 25-hydroxyvitamin D (vitamin D) concentration was measured by radioimmunoassay (IDS Immunodiagnostics, IDS Limited, Tyne and Wear, United Kingdom). The deficit of vitamin D was definite with values <20 ng/mL.[26,27]

Statistical Analyses

Data are reported as mean ± SD for normal continuous variables, median and IQR ranges for not normally distributed continuous variables, and frequencies for categorical variables. Differences between groups were evaluated with the *t* test for variables normally distributed or with the Mann-Whitney test if variables were not-normally distributed. For categorical variable, differences between groups were assessed with the χ^2 test. Moreover, the attributable risk of each factor was estimated using the method proposed by Bruzzi.[28] The attributable risk is calculated by the difference between the incidence in the exposed and the incidence of the nonexposed individuals and represents the impact of any single factor. Subsequently, we estimated prevalence ORs of fibrosis and NASH (NAS ≥5) according to various factors by means of logistic regression model including terms for age, children's BMI, PNPLA3 status (GG/CC or CG); parental obesity (yes/no), parental occupational SES, birth age (small/appropriate for gestational age) having been breastfed (no/yes), daily fructose intake (g/day), and vitamin D status (lower than or ≥20 ng/mL. We also fitted a logistic regression model for cumulative probability (hazards regression) for fibrosis in our children population. For frequencies of exposure approximately 30%-50% in the NAFL group, this study could a priori detect ORs above 2.4 with a 95% CI level and a power of 80%. All analyses were performed using MedCalc Software v 12.7 (Ostend, Belgium) and STATA software v 14 (College Station, Texas).

Results

The study included 182 consecutive children with NAFLD, mean age 10.1 years (5-12 years), 102 male children (56%), who underwent liver biopsy. Among them, 137 (75.3%) had liver fibrosis (with 39 patients [21.4%] with F2 or F3). As a secondary outcome of the study, 96 (52.7%) had NAS ≥5. Within this group, 54.4% (99) of children had at least 1 parent with obesity, 24.7% (45) were born preterm, 37.3% (68) were SGA, and 32.9% (60) were breastfed (average duration 8, range 4-12, months). At starting pregnancy, 26% were mothers with obesity, but, among these at-risk mothers, none were diagnosed with gestational diabetes. Out of the 339 patients screened at origin, 95 were excluded from the study (34 for therapeutic treatments [such as nonsteroidal anti-inflammatory drugs (NSAIDs), metformin, and steroids] and 24 for co-presence of other diseases [4 with Wilson disease, 7 with autoimmune diseases, 3 with celiac disease, 10 with genetic obesity], and 37 dropped out of the therapeutic program). Of the remaining 244 subjects, only 182 were eligible for liver biopsy, according to the Hepatology Committee of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition.[29] All the remaining subjects presented with the complete required anamnesis data, recorded at diagnosis.

Table I and **Table II** (available at www.jpeds.com) report the subdivision of demographic, anthropometric, and biochemical characteristics in 3 groups (F0, F1, and F ≥2, respectively). Compared with F0 children, those with fibrosis ≥2 have higher trends for the biochemical variables connected with the metabolic syndrome as well as lower levels of vitamin D. PNPLA3-GG variants were significantly different between the F0 and F2 groups ($P = .02$). Patients with F ≥2 showed a prevalence of parental, especially maternal, obesity compared with those without fibrosis (53% vs 33.4%), as well as being SGA, preterm, and formula-fed ($P < .05$) (**Table II**). No major differences in energy intakes and macronutrient distribution were found among groups (data not shown).

The regression analysis with F ≥2 and F <2 as the binary outcome (**Table III**) shows that PNPLA3-GG genotype, suboptimal levels of circulating vitamin D, parental obesity, and type of feeding (either not being breastfed and fructose consumption) stand as predisposing factors, whereas a high score for maternal occupation stands as protective factor.

Association Between Fibrosis Degree and Variables Associated with NAFLD

Table IV (available at www.jpeds.com) reports the anthropometrics and biochemical difference between the 2 groups (NAS ≥5 vs NAS <5), respectively. Those with NASH (NAS ≥5), compared with not-NASH, show the same trends as for F ≥ 2 vs F = 0 (**Table I**). Consistent with these findings, in NAS ≥5 the PNPLA3-GG is more expressed compared with NAS <5 (35.4% vs 12.8%) (**Table IV**). Also, in this analysis no differences in energy

Table I. Anthropometric, biochemical and demographic characteristics in Fibrosis degree

Characteristics	F0 (45)	F1 (98)	F ≥2 (39)	F0 vs F2
Male/Female	24/21	60/38	21/18	
Age (y)	10.1 (0.9)	9.8 (1.5)	9.9 (1.1)	.55
Weight, Kg (mean ± SD)	72.4 (12.3)	73.6 (14.8)	82.8 (7.3)	.09
BMI, Kg/mq (mean ± SD)	26.6 (4.1)	27 (4)	29.1 (3.3)	.04
WC, cm (mean ± SD)	81.6 (10.8)	80.5 (14)	88.4 (8.1)	.03
z-BMI (mean ± SD)	1.84 (0.6)	1.98 (0.5)	2.21 (0.7)	.04
AST, UI/L (median; IQR)*	33 (21-88)	36 (18-102)	37.3 (19-92)	.01
ALT, UI/L (median; IQR)*	44.5 (19-93)	49 (12-70)	48.7 (21-133)	.04
Uric Acid, mg/dl (mean ± SD)	5.8 (1.2)	6.3 (1.2)	6.6 (0.9)	.03
Total Cholesterol, mg/dl (median; IQR)*	153.7 (102-221)	166 (108-266)	161 (111-210)	.44
LDL Cholesterol, mg/dl (median; IQR)*	88.9 (77-122)	105 (51-124)	99 (78-144)	.10
HDL cholesterol, mg/dl (median; IQR)*	48 (21-55)	40 (21-85)	41 (20-65)	.23
Triglycerides, mg/dl (median; IQR)*	124 (80-166)	132 (49-225)	143 (75-248)	.04
Fasting plasma glucose, mg/dl (median; IQR)*	79 (69-103)	78 (60-104)	89 (62-90)	.84
Plasma gluc-120'(median; IQR)*	115 (99-123)	114 (88-148)	112 (98-135)	.55
Basal Insulin, mU/L (median; IQR)*	25 (15-46)	28 (14-48)	31 (16-45)	.04
Insulin -120' (median; IQR)*	136 (99-334)	175 (72-332)	197 (110-340)	.05
HOMA-IR (mean± SD)	4.7 (1.6)	5.3 (1.6)	5.9 (1.6)	.03
Vitamin D, mg/dl (mean± SD)	23.2 (2.5)	17.8 (3.9)	15.3 (3.2)	.0001
Fructose, g/day (mean± SD)	65.7 (28)	70.2 (24)	78.5 (30.4)	.001

BMI, body mass index; *WC*, waist circumference; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *HDL*, high-density lipoprotein cholesterol; *LDL*, low-density lipoprotein cholesterol; *HOMA-IR*, homeostasis model assessment of insulin resistance.
*Chi-squared test. F>2, all patients with fibrosis > or = 2.

and macronutrient intakes are found between the 2 groups (data not shown).

The distribution of parental occupation in the 3 groups of fibrosis and NASH is showed in **Table V** (available at www.jpeds.com).

The regression analysis with NAS <5/NAS ≥5 as the binary outcome (**Table VI**) consistently shows that PNPLA3-GG genotype, deficiency of vitamin D, parental obesity, birthweight (SGA vs appropriate for gestational age condition), and either early and later type of feeding (not being breastfed and fructose consumption) stand as predisposing factors, and a high level of maternal occupation stands as protective factor.

Table VII shows the attributable risk for the factors found to be associated in bivariate analyses for fibrosis of ≥2. Finally, predictive algorithms may be derived (**Figure** and **Appendix I**; available at www.jpeds.com). For instance, a clinical history of NAFLD, a male child of approximately 10 years, born with a genetic pattern in either heterozygosity or homozygosis for PNPLA3, with vitamin D deficiency, with at least 1 parent with obesity, low maternal work, SGA at birth, not breastfed, and with a high daily consumption of fructose, may show a scale that indicates a score of 9, the maximum of the attributable risk, equivalent to an expected higher predicted probability of 0.31 of development of fibrosis through a life course of 10 years.

Table III. Prevalence ORs of high fibrosis degree (F ≥2 vs F <2) according to gestational and environmental variables

Variables	OR (95% CIs)	*P*
PNPLA3-GG	2.1 (1.3-4.4)	.01
Parental obesity	2.95 (1.0-4.9)	.048
Maternal occupational SES (high vs low)	0.30 (0.11-0.79)	.01
Paternal occupational SES (high vs low)	2.8 (1.6-3.1)	.88
SGA vs AGA	1.3 (0.8-2.8)	.09
Not breastfed	3.1 (1.5-4.8)	.03
Dietary fructose, g/d	1.4 (1.1-3.4)	.002
Vitamin D (<20 mg/dL)	1.24 (1.1-1.4)	.01

AGA, appropriate for gestational age.

Discussion

The reported observations, based on liver histology from 182 patients followed in a monocentric setting, should be interpreted through a holistic approach consistent with a life-course increase of the sum of the risk, as supported by the World Health Organization.[30-32] Accordingly, the sum of factors expressing genetic pattern, family history and socio-economic factors, pregnancy and intrauterine development, type of feedings (early type, breastfeeding or not, and later feeding, with fructose consumption as indicator), and circulating vitamin D status may express either the individual and the cumulative risk of developing liver fibrosis and more complicated stages of disease within patients followed for NAFLD. As expected, bio-indicators of developing metabolic syndrome and a less favorable lifestyle are worst in patients with fibrosis ≥2 and NASH (NAS ≥5), respectively. Univariate associations as well as the analysis of attributable risk show independent roles of most indicators, with 2 factors without any significant association (father's occupation and pregnancy duration, respectively) and 1 factor (birthweight) showing a mild trend. The final logistic model, including also birthweight for the biologic plausibility,[22] with NASH and/or fibrosis >2 as outcome variables, indicates genetics with family history, birthweight, dietary indicators (not being breastfed and daily fructose consumption later on), and a

Table VI. Prevalence ORs of NAS ≥5 according to gestational and environmental variables

Variables	OR (95% CIs)*	P
PNPLA3-GG	2.81 (1.5-5.1)	.0006
Parental obesity	4.45 (1.9-7.6)	.006
Maternal occupational SES (high vs low)	0.38 (0.2-0.7)	.001
Paternal occupational SES (high vs low)	1.31 (0.7-2.3)	.31
SGA vs AGA	2.96 (1.1-5)	.01
Not breastfed	2.65 (1.1-6.1)	.02
Dietary fructose, g/d	1.7 (1.2-3.2)	.03
Vitamin D (<20 mg/dL)	2.17 (1.5-2.97)	.001

*Adjusted for age and BMI.

poor vitamin D status (dietary and environmental bio-indicator) as predisposing factors, whereas higher levels of maternal occupation (SES indicator) stands as an independent protective factor. In the final cumulative analysis, those with the worst condition in these variables have approximately 30% higher risk of developing fibrosis and/or more complicated liver disease from NAFLD.

The practical meaning of our observations is represented by the reinforcement of general recommendations on pregnancy, balanced dietary habits, and a proactive lifestyle in the pediatric ages, at a population level, when disorders are still not diagnosed, within a comprehensive approach toward the primary prevention of chronic noncommunicable diseases. Indeed, a secondary prevention may be managed with stringent recommendations on dietary intakes, regular exercise, and more controlled lifestyle patterns,[33,34] when well-established lifestyle habits are difficult to change. Our data may have a highly representative translational value, showing the independent interconnections of the better defined risk factors of development of chronic, noncommunicable liver disease.

Our retrospective study has 2 major limitations. First, the sample size may be limited, when compared with the number of variables, yet limited to "proxies" of specific categories (parental status, pregnancy outcome, socioeconomic indicators, environmental, and dietary indicators). This may have resulted in some discrepancies (for instance, the lack of significance for the condition of being "preterm" and/or "SGA," because of unavoidable collinearity using analytical models), although the included numbers could allow for deriving a statistical meaning. On the other hand, we have kept the advantage of analyzing homogeneous data from a unique center with a widespread clinical experience in Italy.

Genetics, parental conditions, environmental, and diet-related patterns may all be part of a large bio-ecological system with relevant health implications[35,36] in which the family environment where children grow up plays an even more important role. In Italy, the rate of pediatric poverty increased from 4.9% in 2008 to 10.9% in 2015, in line with the prevalence increase in obesity and overweight, today at 9.3% and 22.5%, respectively, of the Italian pediatric population.[36] Further studies are needed to investigate the possibility of multistep interventions on unfavorable health outcomes. Given the complex context in which noncommunicable disorders are evolving not just in rich, but also transition countries, we believe that only full-spectrum interventions might reverse this trend. ■

Table VII. Attributable fraction of fibrosis in children with NAFLD undergoing liver biopsy according to selected factors

Factors	Fibrosis attributable fraction (%)
PNPLA3-GG	26
Parental obesity	48
Low maternal occupational SES	37
SGA	11
Not breastfed	48
Daily fructose (1 g/d increase)	18
Deficit of vitamin D	11

We thank Dr Negri Eva of the Department of Biomedical and Clinical Sciences Milan University, for her valuable and constructive suggestions during the statistical planning and development of this research work. Her willingness to give her time so generously is much appreciated.

Submitted for publication Dec 3, 2018; last revision received Mar 8, 2019; accepted Apr 9, 2019.

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Appendix

Cumulative probability formula

$$\text{CumPr}(10; X) = 1 - S(10, \text{Age})^{\exp(\text{Pr ed Lin}(X))}$$

X = risk factors.

S = survival function obtained with a linear B-spline in hazard models.

PredLin (X) = function obtained summarized the all risk factor coefficient (SE) * Age and sex.

Coefficients

Risk factors	Coefficient (SE)	Hazard ratio (95 % CI)
Sugar	0.30 (0.57)	1.1 (0.98-1.2)
PNPLA3	0.61 (0.40)	1.3 (0.54-2.2)
SGA	0.42 (0.62)	1.5 (0.99-5.8)
Vitamin D	0.29 (0.16)	1.15 (1.1-1.29)
Family obesity	0.34 (0.5)	1.8 (0.93-2.21)
Not breastfeeding	0.57 (0.05)	1.4 (0.61-2.2)
Low maternal job	0.33 (0.34)	1.5 (0.79-3.12)
Sex (male)	0.20 (0.5)	1.2 (0.91-2.17)

For the score, based on age and sex, all risk factors are considered (1 = yes or 0 = no), individuals can be assigned a score between 0 and 9.

Factors	Score
Sugar >30 g/d	Yes = 1, No = 0
PNPLA3- GG	Yes = 1, No = 0
SGA	Yes = 1, No = 0
Vitamin D z 20 mg/dL	Yes = 1, No = 0
Family obesity	Yes = 1, No = 0
Not breastfeeding	Yes = 1, No = 0
Low maternal job	Yes = 1, No = 0
Sex (male)	Yes = 1, No = 0
Age >10 y	Yes = 1, No = 0

Score	Cumulation prob hazard	Survival at 10 y
2	0.012	0.98
3	0.016	0.97
4	0.022	0.93
5	0.092	0.91
6	0.187	0.88
7	0.231	0.83
8	0.283	0.81
9	0.311	0.76

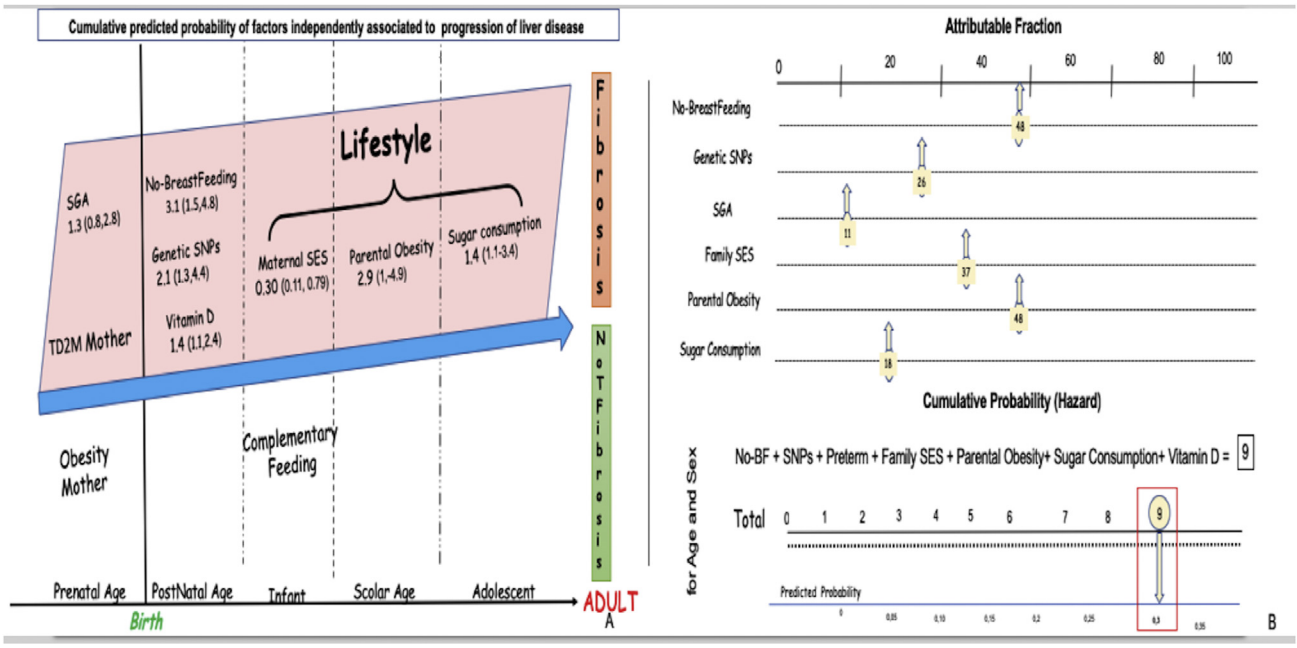

Figure. Cumulative predicted probability of factors independently associated to progression of liver disease.

Table II. Demographic and genetics characteristics in fibrosis degree

Characteristics	F0 (45)	F1 (98)	F ≥2 (39)	F0 vs F2
PNPLA3-CC (%)*	35 (77.8%)	26 (26.5%)	2 (5.2%)	0.09
PNPLA3-CG*	8 (17.7%)	49 (50%)	17 (43.5%)	0.89
PNPLA3-GG*	2 (4.5%)	23 (23.5%)	20 (51.3.%)	0.02
Birth weight, g (median; IQR)	3032 (1200-4100)	3085 (1150-4100)	2800 (1800-3990)	0.05
Gestational age (median; IQR)	37.9 (32-40)	38 (31-40)	37 (34-41)	0.03
SGA (%)*	9 (20)	42 (42.8)	17 (43.5)	0.01
Preterm (%)*	9 (20)	22 (22.4)	14 (35.9)	0.04
Breast feeding (%)*	24 (53.3)	25 (25.5)	11 (28.2)	0.04
Parental obesity (%)*	19 (42)	52 (53.1)	28 (71.7)	0.05
Mother	6 (31.6)	28 (53.8)	15 (53.6)	0.05
Father	6 (31.6)	11 (21.1)	7 (25)	0.53
Mother and father	7 (38.8)	13 (25)	6 (21.4)	0.10

*χ^2 test. F >2, all patients with fibrosis ≥2.

Table IV. Anthropometric, biochemical and demographic characteristics in NAS >5 (NASH) and NAS <5 children

Characteristics	NAS <5 (86)	NAS ≥5 (96)	P
Male/Female	37/49	60/36	
Age (y)	9.7 (1.3)	10.1 (1.3)	.10
Weight, Kg (mean ± SD)	70.7 (10.3)	79.7 (3.9)	.69
BMI, Kg/mq (mean ± SD)	27.1 (4.5)	27.7 (3.5)	.73
WC, cm (mean ± SD)	83.4 (9.9)	82.8 (13.6)	.36
z-BMI (mean ± SD)	1.91 (0.6)	2.1 (0.9)	.66
AST, UI/L (median; IQR)	25 (12-41)	41 (19-97)	.001
ALT, UI/L (median; IQR)	36 (12-70)	54.6 (21-144)	.002
Uric Acid, mg/dl (mean ± SD)	6.1 (1)	6.7 (0.9)	.01
Total Cholesterol, mg/dl (median; IQR)	157 (90-221)	167 (116-264)	.16
LDL Cholesterol, mg/dl (median; IQR)	99 (64-171)	102 (67-173)	.40
HDL cholesterol, mg/dl (median; IQR)	43 (27-88)	40 (20-68)	.64
Triglycerides, mg/dl (median; IQR)	124 (74-178)	141 (79-248)	.04
Fasting plasma glucose, mg/dl (median; IQR)	75 (64-103)	82.5 (69-103)	.01
Plasma gluc-120'(median; IQR)	110 (86-143)	116 (86-149)	.06
Basal Insulin, mU/L (median; IQR)	24 (11-42)	29.6 (16-49)	.04
Insulin -120' (median; IQR)	144 (54-230)	192 (78-342)	.01
HOMA-IR (mean± SD)	4.7 (1.6)	5.8 (1.7)	.001
Vitamin D (mean± SD)	20.4 (4.8)	16.3 (3.6)	.01
Fructose, g/day (mean± SD)	56.4 (20.8)	65.9 (26)	.001
Demographic characteristics			
PNPLA3 (n%)			
CC	43 (50)	20 (20.8)	.03
CG	32 (37.2)	42 (43.7)	.18
GG	11 (12.8)	34 (35.4)	.04
Birth Weight,gr (median; IQR)	3180 (1200-4100)	2860 (2100-3990)	.05
Gestational Age (median; IQR)	38 (30-42)	37.5 (34-41)	.10
SGA, small gestational age (%)*	30 (34.8)	38 (39.5)	.41
Preterm (%)*	10 (11.6)	35 (36.4)	.04
Breast Feeding (%)*	38 (44.1)	22 (22.9)	.04
Parental Obesity (%)*	36 (41.8)	63 (65.6)	.05
Mother Obesity	19 (52.7)	30 (47.6)	.33
Father Obesity	11 (30.6)	13 (20.6)	.10
Mother and Father Obesity	6 (16.7)	20 (31.8)	.04

BMI = body mass index; *WC*= waist circumference; *AST* = aspartate aminotransferase; *ALT* = alanine aminotransferase; *HDL*= high-density lipoprotein cholesterol; *LDL* = low-density lipoprotein cholesterol; *HOMA-IR* = homeostasis model assessment of insulin resistance.
*Chi-squared test.

Table V. Job ISTAT categories of parents

1A

	Mother's group			
	F0 (45)	**F1 (98)**	**F ≥2 (39)**	***P* (F0 vs F ≥2)**
0	8.8%	36.7%	20.5%	.04
1	68.8%	48.9%	53.8%	.22
2	22.2%	14.2 %	25.7%	.79
	Father's group			
0	26.6%	30 .6%	30.7%	.24
1	66.7%	59.2%	56.4%	.82
2	6.7%	10.2%	12.9%	.10

1B

	Mother's group		
	NAS <5 (86)	**NAS >5 (96)**	***P***
0	18.6%	33.4%	.06
1	65.2%	45.8%	.39
2	16.2%	20.8%	.63
	Father's group		
0	26.7%	32.3%	.85
1	61.6%	59.4%	.79
2	11.7%	8.3%	.48

0 = which comprises the 8 and 7 categories of ISTAT classification, 1 = the 5 + 6 + 9 categories of ISTAT and, 2 = the 1 + 2 + 3 + 4 categories.
1 – Legislators and high directors; 2 - Intellectual, scientific and high-specialized professions; 3 - Technical professions; 4 - Executive professions in office work; 5 - Qualified professions in commercial activities and services; 6 - Artisans, specialized workers and farmers; 7 - Plant conductors, fixed and mobile machinery workers and vehicle drivers; 8 - Professions not qualified; 9 - Armed forces.