Association of Bright Liver With the *PNPLA3* I148M Gene Variant in 1-Year-Old Toddlers

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Context: Nonalcoholic fatty liver disease (NAFLD) is being increasingly diagnosed at younger ages, pointing toward an early-life origin.

Objective: To evaluate the frequency and risk factors for bright liver (BL) in 1-year-old toddlers.

Design: Secondary analysis of the 1-year follow-up of the Feeding Study. Exposures were child *PNPLA3* and *TM6SF2* gene variants; child anthropometry at birth and at 1 year of follow-up; child subcutaneous, visceral, and epicardial adipose tissue at 1 year of follow-up; maternal anthropometry at the start and at the end of pregnancy; and maternal red blood cell fatty-acid composition at the third trimester of pregnancy.

Setting: General population.

Participants: Among 505 mother-toddler pairs, 391 children (77%) underwent liver and abdominal ultrasonography at the 1-year follow-up.

Main Outcome: BL as diagnosed by ultrasonography.

Results: Seventeen (4%) of 391 toddlers had BL. Compared with the toddlers with the *PNPLA 3 CC* genotype, the odds (95% CI) of BL were 3.01 (1.05 to 8.64, $P < 0.05$) times higher in those with the *PNAPLA3* CG genotype and 5.37 (1.12 to 25.77, $P < 0.05$) higher in those with the *PNPLA3* CC genotype. We found no association between BL status and *TM6SF2*. Body weight, body mass index, and maternal weight gain during pregnancy were higher in BL^+ than in BL^- children. Visceral adipose tissue was higher but subcutaneous adipose tissue and epicardial adipose tissue were similar in BL^+ and BL^- children.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 14 September 2018. Accepted 9 January 2019.
First Published Online 14 January 2019

Abbreviations: BL, bright liver; BMI, body mass index; EAT, endocardial adipose tissue; IQR, interquartile range; LUS, liver ultrasonography; NAFLD, nonalcoholic fatty liver disease; PMLE, penalized maximum likelihood estimation; *PNPLA3*, patatin-like phospholipase domain-containing protein 3; SAT, subcutaneous adipose tissue; SDS, SD score; *TM6SF2*, transmembrane 6 superfamily 2 human gene; VAT, visceral adipose tissue.

Conclusions: Four percent of the Feeding Study children had BL at 1 year of age. In line with expectations, *PNAPLA3* was already a predictor of BL at this early age. (***J Clin Endocrinol Metab* 104: 2163–2170, 2019)**

The frequency of nonalcoholic fatty liver disease (NAFLD) is increasing in parallel with the epidemic of obesity (1). NAFLD is being increasingly diagnosed at younger ages, pointing toward an early-life origin (2). NAFLD has long been considered the consequence of excess weight (3), even if the paradigm considering NAFLD the hepatic manifestation of the metabolic syndrome is being increasingly challenged (4).

Recent studies have shown that gene variants are involved in the pathogenesis of NAFLD (5, 6). Normal weight and metabolically healthy subjects with such gene variants may develop a disease for which the name "genetic NAFLD" has been proposed (7). The most important gene variants are the patatin-like phospholipase domain-containing protein 3 (PNPLA3 I148M) and transmembrane 6 superfamily member 2 protein (TM6SF2 E167K) mutations, which influence lipid droplet remodeling, lipid secretion from hepatocytes, and retinol metabolism (7, 8). Such mutations may protect against cardiovascular disease while increasing the risk of severe steatosis and steatohepatitis (7, 8). One in three European children carries the PNPLA3 I148M variant and one in three is overweight (7). Thus, many children are at risk for developing "mixed NAFLD" (*i.e.*, NAFLD produced by both genetic and environmental factors) (7). It is also possible that these children may develop more rapidly nonalcoholic steatohepatitis and fibrosis (9).

The developmental origin of health and disease hypothesis postulates that *in utero* exposure to selected stressors contributes to the risk of later disease, including NAFLD (10). Nonhuman primates develop hepatic steatosis following *in utero* exposure to high-fat diets (11, 12). Both low and high birth weight children are overrepresented among young NAFLD patients, suggesting the possibility of metabolic programming of NAFLD (13, 14).

The Feeding Study is a cohort study aimed at investigating the association between the *in utero* exposure to fats and the low-grade inflammatory status and insulin resistance of the offspring at birth (15, 16). The hypothesis that the Feeding Study wanted to test is that low-grade inflammation and insulin resistance predispose the child to develop obesity. The Feeding Study mothers were enrolled during the first trimester of pregnancy when their fatty-acid profile and that of their fetuses was evaluated by analyzing erythrocyte membranes (15, 16). At the 1-year follow-up, liver brightness and subcutaneous (SAT), visceral (VAT), and epicardial (EAT) adipose tissue were measured by ultrasonography. Because an unexpectedly high proportion of 1-year-old toddlers showed bright liver (BL), common variants of the PNPLA3 I148M and TM6SF2 E167K genes were assessed.

The present report describes the frequency of BL in the 1-year-old toddlers of the Feeding Study and its association with selected genetic and environmental factors.

Research Design and Methods

Study design

This paper reports a secondary analysis of the Feeding Study (15, 16), which is a cohort study aimed at investigating the association between the *in utero* exposure to dietary fats and the low-grade inflammatory status and insulin resistance of the offspring at birth (15). The inclusion criteria of the mothers of the Feeding Study were: (1) age ≥18 and ≤35 years; (2) singleton pregnancy; (3) no history of smoking; (4) no history of miscarriage; (5) no history of chronic disease including diabetes; (6) no history of gestational diabetes; and (7) no history of ovulation induction or *in vitro* fertilization. The study was approved by the Ethical Committee of the Bambino Gesù Children's hospital and was performed respecting national and international regulations and the Declaration of Helsinki (2000). All parents gave written informed consent. The main aim of the present secondary analysis was to estimate the frequency of BL at 1-year follow-up and its association with the *PNPLA3* and *TM6SF2* gene variants.

Anthropometric assessment

Weight and length at birth were measured to the nearest 5 g and 0.1 cm, respectively (17, 18). Body weight and length at 1 year of age were measured to the nearest 50 g and 0.5 cm (17). SD scores (SDS) of weight and length at birth were calculated using Italian reference data (18) and those at 1 year using the World Health Organization reference data (19).

Liver ultrasonography

Liver ultrasonography (LUS) was performed by two experienced physicians (G.D.M. and F.P.) using an ACUSON X700 ultrasound system (Siemens Healthcare, Siemens AG, Munich, Germany). VAT thickness was measured using a 1.0- to 5.0-MHz curved array transducer, SAT thickness using a 5.0- to 10.0-MHz linear transducer, and EAT thickness using a 1.0- to 4.0-MHz phased array transducer. To measure VAT and SAT, the transducers were positioned where the xiphoid line meets the waist plane. VAT was measured as the distance between the peritoneal border and the corpus of the lumbar vertebra (2, 20); SAT was measured at the same level of VAT but as the distance between the cutaneous border and the linea alba (2, 20); EAT was measured as the echo-free area extending from the epicardial surface to the parietal pericardium of the right ventricular free wall at its thickest

level using the aortic annulus and the midchordal region as landmarks for the long- and short-axis views, respectively (2, 20). Liver echogenicity was assessed using a 1.0- to 5.0-MHz curved array transducer placed on the subcostal plane along the midclavicular line. BL (0 = no; 1 = yes) was defined on the basis of the difference in echogenicity between the liver and the renal parenchyma, the degree of hepatic penetration of ultrasounds, and the degree of visualization of hepatic vessels (2, 20). Suspected cases of BL were reevaluated within 2 weeks from the first LUS by both physicians in charge of ultrasonography. A definitive diagnosis of BL was reached only when the two physicians agreed on the diagnosis of BL performed at the second LUS.

PLNPLA3 and TMSF2 gene variants

DNA was extracted by the cord blood samples of pregnant women using the QIAamp Blood MiniKit (Qiagen, Hilden, Germany). *PNPLA3* rs738409 C>G and *TM6SF2* rs58542926 C>T single nucleotide polymorphism were genotyped using the TaqMan 5′-nuclease assay (Life Technologies, Foster City, CA). The TaqMan genotyping reactions were performed in a MicroAmp Fast Optical 96 Well Reaction Plate with Barcode (Applied Biosystems, Foster City, CA) using a volume of 25 μL containing 2X TaqMan Universal PCR Master Mix No Amperase UNG (Applied Biosystems), 20X SNP Genotyping Assay, and 10 ng DNA. Reaction plates were run on a 7900 HT Fast instrument (Applied Biosystems) for 10 minutes at 95°C, then for 40 cycles of 15 seconds at 95°C and, last, at 60°C for 1 minute.

Fatty acids

Maternal fatty acids were measured at the third trimester of pregnancy on erythrocyte membranes as described in detail elsewhere (15, 16).

Statistical analysis

Most continuous variables were non-Gaussian distributed and all are reported as median (50th percentile) and interquartile range (IQR; 25th and 75th percentiles). Discrete variables are reported as counts and proportions. Comparisons of continuous variables between BL^+ and BL^- toddlers were performed using median regression (21); those of discrete variables (*PNPLA3* and *TM6SF2*) used logistic regression with Firth's penalized maximum likelihood estimation (PMLE) method (22, 23) as implemented by the user-written Stata command *firthlogit* (24). The maximum likelihood estimation of the logistic model does in fact suffer from a well-known small-sample bias, which is avoided by PMLE (25). A small-sample bias for the current study was expected because of the uneven distribution of the CC, CG, and GG alleles of *PNPLA3* and of the CC, CT, and TT alleles of *TM6SF2* and because the comparison of interest involves a 2 (BL status) × 3 (alleles) contingency table. We used median regression and logistic regression to test the association between BL and known and potential risk factors instead of nonparametric tests (*e.g.*, Wilcoxon-Mann-Whitney and Fisher's exact tests) because we were not interested in *P* values but in effect sizes (26–28). Statistical analysis was performed using Stata 15.1 (Stata Corporation, College Station, TX).

Results

Flow diagram

Eight-hundred forty-seven (85%) mothers continued the study after enrollment; 505 (60%) of them underwent the 1-year follow-up visit (Fig. 1). All the mother-toddler pairs who took part in the 1-year follow-up were invited to undergo LUS; however, mostly because LUS had to be scheduled on special days on which no other examination was performed, some mothers declined the invitation. Thus, only 391 (77%) children underwent LUS at the 1-year follow-up visit.

Comparison of children with and without LUS

Table 1 compares the anthropometric data of the children with and without LUS at birth and at the 1-year follow-up visit. The statistically significant median differences of −0.02 (95% CI, −0.04 to −0.001, $P < 0.05$) years of age at follow-up and of −1.00 (95% CI, −1.87 to −1.13, $P < 0.05$) cm of length at follow-up between LUS^+ *vs* LUS^- children are clearly biologically irrelevant (29). The two samples are quite similar for the all features of interest.

Comparison of children with and without BL

Of the 391 toddlers who underwent LUS, 17 (4%) had BL. The measurements of BL^- and BL^+ toddlers are reported in Table 2. The median gestational age was −1.0 (95% CI, −1.9 to −0.1, $P < 0.05$) months

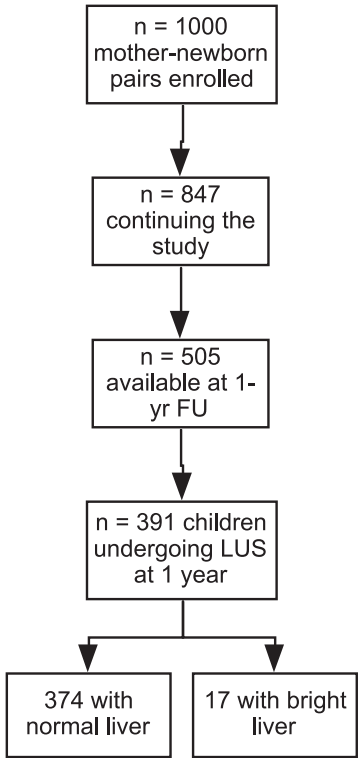

Figure 1. Flow of the mother-newborn and mother-toddler pairs during the Feeding Study. FU, follow-up.

Table 1. Anthropometric Measurements of Feeding Study Toddlers Who Did and Did Not Undergo LUS at the 1-Y Follow-Up

	LUS Not Available		LUS Available	
	N	**Median (IQR) or *n* (%)**	***N***	**Median (IQR) or *n* (%)**
Sex	114		391	
Female		51 (46.4%)		187 (47.8%)
Male		59 (53.6%)		204 (52.2%)
Gestational age, wk	114	39 (38; 40)	391	39 (38; 40)
Duration of breastfeeding, mo	108[a]	3 (0; 11)	391	5 (0; 11)
Weight at birth, kg	114	3.25 (3.00; 3.55)	391	3.30 (2.97; 3.63)
Weight at birth, SDS Bertino	114	0.37 (−0.32; 0.94)	391	0.35 (−0.37; 1.02)
Length at birth, m	114	0.50 (0.49; 0.52)	391	0.50 (0.49; 0.52)
Length at birth, SDS Bertino	114	0.01 (−0.00; 0.02)	391	0.00 (−0.00; 0.01)
Length for gestational age, Bertino	114		391	
Appropriate for gestational age		114 (100.0%)		391 (100.0%)
Age at follow-up visit, y	114	1.04 (1.02; 1.09)	391	1.06 (1.02; 1.13)[b]
Weight at follow-up visit, kg	114	10.00 (9.15; 11.00)	391	10.00 (9.20; 10.70)
Weight at follow-up visit, SDS WHO	114	0.46 (−0.09; 1.27)	391	0.47 (−0.13; 1.11)
Length at follow-up visit, cm	114	77.0 (75.0; 79.0)	391	76.0 (74.0; 78.5)[b]
Length at follow-up visit, SDS WHO	114	0.29 (−0.36; 1.13)	391	0.37 (−0.29; 1.19)
BMI at follow-up visit, kg/m^2	114	17.0 (16.3; 17.9)	391	16.9 (16.0; 18.0)
BMI at follow-up visit, SDS WHO	114	0.48 (−0.20; 0.94)	391	0.28 (−0.34; 0.96)

[a]Not available for 6 children.

[b]$P < 0.05$ (median regression)

Abbreviations: Bertino, per Bertino *et al.* (18); WHO, World Health Organization.

lower in BL$^+$ than in BL$^-$ toddlers. This difference is unlikely to be clinically relevant, however. More interestingly, all toddlers had an appropriate length for their gestational age, which was expected owing to the inclusion criteria for the mothers of the Feeding Study.

At the 1-year follow-up, the median weight was 1.05 (95% CI, 0.44 to 1.64; $P < 0.001$) kg higher in BL$^+$ than in BL$^-$ toddlers; the median weight SDS was 0.67 (95% CI, 0.10 to 1.23; $P < 0.05$) higher in BL$^+$ than in BL$^-$ toddlers; and the median body mass index (BMI) was 1.14 kg/m^2 (95% CI, 0.18 to 2.10; $P < 0.05$) higher in BL$^+$ than in BL$^-$ toddlers. Last, the median VAT was 1.80 (95% CI, 0.05 to 3.35; $P < 0.05$) mm higher in BL$^+$ than in BL$^-$ toddlers. Although all of these estimates have large 95% CI because of the small number of BL$^+$ toddlers, they agree with the available evidence showing that ponderal status and VAT are predictors of fatty liver (2, 30–32).

Comparison of mothers of children with and without BL

The measurements of the mothers of the Feeding Study toddlers with and without BL are given in Table 3. The median maternal weight change was 2.00 (95% CI, 0.15 to 3.85; $P < 0.05$) kg higher in the mothers of BL$^+$ than in those of BL$^-$ toddlers. Not surprisingly, because of the low sample of BL$^+$ children, this estimate has large 95% CI, but it suggests that pregnancy-related factors may be associated with fatty liver at 1 year of age.

Table 3 also reports the main maternal red blood cell fatty acids at the third trimester of pregnancy. These data were available for a subsample of the mothers (BL$^-$, n = 317, 85%; BL$^+$, n = 14, 82%). With the obvious limitation of the low sample size of BL$^+$ toddlers, no biologically relevant difference between the red blood cell fatty acids of the mothers of BL$^+$ and BL$^-$ toddlers could be detected.

Association between BL and *PNPLA3* and *TM6SF2* variants

Compared with toddlers with the *PNPLA* 3 CC genotype (reference group), the odds (95% CI) of BL were 3.01 (1.05 to 8.64, $P < 0.05$) times higher in those with the *PNPLA3* CG genotype and 5.37 (1.12 to 25.77, $P < 0.05$) higher in those with the *PNPLA3* CC genotype (PMLE logistic regression). Compared with the toddlers with the *TM6SF2* CC genotype, the odds (95% CI) of BL were 2.08 (0.62 to 7.00) times higher in those with the *TM6SF2* CT genotype and 2.08 (0.11 to 39.14) higher in those with the *TM6SF2* TT genotype (PMLE logistic regression).

Discussion

We detected BL in 4% of the 1-year-old toddlers of the 391 Feeding Study women who took part in the 1-year follow-up and whose children underwent LUS. The finding of BL in an unexpected proportion of children

Table 2. Anthropometric and Ultrasonographic Measurements of Feeding Study Toddlers at Birth and at 1-Y Follow-Up

	BL^- Toddlers		BL^+ Toddlers	
	N	**Median (IQR) or *n* (%)**	***N***	**Median (IQR) or *n* (%)**
Sex	374		17	
Female		179 (47.9%)		8 (47.1%)
Male		195 (52.1%)		9 (52.9%)
Gestational age, wk	374	39 (38; 40)	17	38 (37; 39)[a]
Duration of breastfeeding, mo	374	5 (0; 11)	17	7 (2; 13)
Weight at birth, kg	374	3.31 (2.99; 3.63)	17	3.20 (2.70; 3.75)
Weight at birth, SDS Bertino	374	0.35 (−0.37; 0.98)	17	0.29 (−0.42; 1.91)
Length at birth, m	374	0.50 (0.49; 0.52)	17	0.50 (0.48; 0.52)
Length at birth, SDS Bertino	374	0.00 (0.00; 0.01)	17	0.00 (0.00; 0.01)
Length for gestational age, Bertino	374		17	
Appropriate for gestational age		374 (100.0%)		17 (100.0%)
Age at follow-up, y	374	1.0 (1.0; 1.1)	17	1.0 (1.0; 1.1)
Weight at follow-up, kg	374	9.95 (9.20; 10.65)	17	11.00 (9.75; 11.40)[b]
Weight at follow-up, SDS WHO	374	0.44 (−0.13; 1.08)	17	1.11 (0.08; 1.84)[a]
Length at follow-up, cm	374	76.0 (74.0; 78.5)	17	76.0 (75.0; 79.0)
Length at follow-up, SDS WHO	374	0.37 (−0.29; 1.15)	17	0.63 (−0.26; 1.71)
BMI at follow-up, kg/m^2	374	16.9 (16.0; 18.0)	17	18.0 (16.6; 19.2)[a]
BMI at follow-up, SDS WHO	374	0.27 (−0.35; 0.94)	17	0.86 (0.07; 1.78)
Subcutaneous adipose tissue at follow-up, mm	373[c]	3.6 (3.1; 3.9)	17	3.7 (3.5; 4.3)
VAT at follow-up, mm	369[d]	45.0 (43.0; 47.0)	16[d]	46.8 (41.7; 47.6)[a]
Epicardial adipose tissue at follow-up, mm	369[d]	3.4 (3.1; 3.6)	16[d]	3.2 (2.8; 3.9)
PNPLA3	374		17	
CC		218 (58.3%)		5 (29.4%)
CG		138 (36.9%)		10 (58.8%)
GG		18 (4.8%)		2 (11.8%)
TM6SF2	374		17	
CC		331 (88.5%)		14 (82.4%)
CT		38 (10.2%)		3 (17.6%)
TT		5 (1.3%)		0 (0.0%)

Abbreviations: SDS Bertino, SDS per Bertino *et al.* (18); WHO, World Health Organization.

[a] $P < 0.05$.

[b] $P < 0.001$ (median regression).

[c] Could not be reproducibly measured in 1 BL^- toddler.

[d] Could not be reproducibly measured in 5 BL^- toddlers and 1 BL^+ toddlers.

prompted us to perform an analysis of *PNPLA3* and *TM6SF2* as potential risk factors for BL in this population. The frequency of the GG, CG, and CC *PNPLA3* alleles in our population is similar to that reported in the white population of the Dallas Heart Study, which showed that *PNPLA3* is a risk factor for fatty liver independently from dyslipidemia and insulin resistance (6).

In line with expectations (2), BL^+ children were heavier and had a higher BMI than BL^- children. As far as fat distribution is concerned, VAT was higher in BL^+ than in BL^- toddlers, but their SAT and EAT were similar. The median difference of 1.8 mm in the VAT of BL^+ and BL^- children may be biologically relevant even if its 95% CIs are large (0.05 to 3.35 mm), partly because of the low number of BL^+ children (n = 17). There is increasing evidence that the inability of SAT to expand may produce an early accumulation of hepatic fat (31, 33). However, we found similar values of SAT and EAT in BL^+ and BL^- children at 1 year of age. Our finding that birth weight and later fatty liver are not associated agrees with the findings of a large cohort study of 17-year-old adolescents from Germany (34) and of a smaller cohort study of 18- to 24-year-old adults from Holland (35).

Interestingly, BL^+ children were born to mothers who had had a greater increase of body weight during pregnancy compared with those of BL^- children. Few and contradictory data are available on the association between maternal weight gain and fatty liver in offspring. The Avon Longitudinal Study of Parents and Children reported that maternal overweight/obesity and gestational diabetes mellitus are associated with hepatic fat content in offspring (36). However, such association disappeared after adjustment for childhood obesity (36). The Exploring Perinatal Outcomes in Children Study found that maternal obesity before pregnancy was associated with an increased hepatic fat fraction in

Table 3. Anthropometric Measurements and Red Blood Cell Fatty-Acid Composition of Feeding Study Mothers Before and During Pregnancy

	Mothers of BL$^-$ Toddlers		Mothers of BL$^+$ Toddlers	
	N	Median (IQR)	*N*	Median (IQR)
Age at child birth, y	374	34.0 (30.0; 38.0)	17	33.0 (29.0; 34.0)
Weight at start of pregnancy, kg	374	60.0 (54.0; 69.0)	17	59.0 (52.0; 65.0)
Weight change during pregnancy, kg	373[a]	13.0 (10.0; 16.0)	16[a]	15.0 (13.0; 17.0)[b]
SFA at third trimester, ng/mL	317	2611 (1367; 4012)	14	2630 (1283; 5152)
SFA at third trimester, % of TFA	317	0.42 (0.38; 0.47)	14	0.43 (0.38; 0.49)
MUFA at third trimester, ng/mL	317	1338 (699; 2139)	14	1144 (712; 1923)
MUFA at third trimester, % of TFA	317	0.22 (0.19; 0.25)	14	0.21 (0.18; 0.23)
PUFA at third trimester, ng/mL	317	1154 (619; 1892)	14	1033 (375; 2481)
PUFA at third trimester, % of TFA	317	0.20 (0.15; 0.24)	14	0.24 (0.14; 0.26)
N-3 fatty acids at third trimester, ng/mL	315[c]	306 (155; 589)	14	331 (119; 513)
N-3 fatty acids at third trimester, % of TFA	315[c]	0.05 (0.03; 0.07)	14	0.05 (0.03; 0.07)
N-6 fatty acids at third trimester, ng/mL	317	697 (255; 1216)	14	655 (151; 1684)
N-6 fatty acids at third trimester, % of TFA	317	0.13 (0.07; 0.16)	14	0.16 (0.08; 0.17)

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, total fatty acids.

[a]Not available for the mother of 1 BL$^+$ toddler and the mother of 1 BL$^-$ toddler.

[b]$P < 0.05$ (median regression).

[c]Could not be reproducibly measured in 2 mothers of BL$^+$ toddlers.

offspring, independent of adiposity (37). In a study of neonates born to obese mothers without gestational diabetes mellitus, intrahepatic fat content was associated with maternal BMI but not with birth weight, suggesting that maternal obesity may be an early determinant of hepatic fat content (38). In the Cardiovascular Risk in Young Finns cohort study, *PNPLA3, TM6SF2,* and low birth weight were predictors of NAFLD at the 31-year follow-up (39).

The current study investigated healthy infants born from healthy mothers after 38 to 42 weeks of gestation. A low number of mothers was obese before pregnancy and all children were appropriate for gestational age. In such a cohort, we cannot properly test the hypothesis that *in utero* development is associated with later fatty liver, because most risk factors for abnormal growth were virtually excluded by the entry criteria applied to the mothers. It is nonetheless of interest that in this healthy population of mothers, weight gain during pregnancy was also associated with BL in the offspring at 1 year. It is likely that *PNPLA3* and maternal weight gain during pregnancy (and other pregnancy-related factors) do interact in determining the risk of fatty liver (39). However, the low number of toddlers with the *PNPLA3* GG allele (18 BL$^-$ and 2 BL$^+$) impeded us from formally testing this hypothesis in the current study. As other researchers have done (39), we wish to emphasize that BMI and its possible interaction with *PNPLA3* are modifiable risk factors, whereas *PNPLA3* is not.

Although this study reports the frequency of fatty liver in 1-year-old children from a general population, it has several limitations. First, of the 1000 mother-newborn pairs enrolled into the Feeding Study, only 505 mothers presented at the 1-year follow-up and only 391 of their children underwent LUS at 1 year; thus, we cannot claim that our data can be generalized to the whole Feeding Study population. Second, we did not evaluate insulin resistance at the 1-year follow-up, even if it is possible that BL$^+$ children at this age are not more insulin-resistant than their BL$^-$ peers, in line with the findings of the Origin of Cardiovascular Disease Study (2). Such assessment is important because there is increasing evidence that hepatic insulin resistance may develop in response to the accumulation of triacylglycerol in the hepatocytes (33). Third, we used LUS to diagnose BL in our children. Although LUS is the only feasible option to diagnose fatty liver in epidemiological studies (30, 32), it is known to offer an acceptable assessment of fatty liver starting from an intrahepatic triglyceride content of 10% only, at least in adults (40). Thus, some cases of BL may have gone undetected in our study, and our estimate of 4% for BL frequency is to be considered conservative. Fourth, the small number of BL$^+$ cases (n = 17, 4%) impeded us from formally evaluating the possibility of a different gene-maternal environment interaction in BL$^+$ *vs* BL$^-$ children.

In conclusion, in the Feeding Study, 4% of 1-year-old toddlers had BL and *PNPLA3* was a predictor of it. Larger cohorts with greater numbers of children with BL are needed to determine whether there is a gene-environment interaction between *PNPLA3* and maternal and child anthropometry.

Acknowledgments

We are very grateful to the parents who participated with their infants and toddlers in the Feeding Study.

Financial Support: The Feeding Study was supported by research grant GR-20102304957 from the Italian Ministry of Health (to M.M.). The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Author Contributions: G.B. and M.M. designed the study, analyzed the data, and drafted, and revised the manuscript. M.M. coordinated the study and got funding. G.D.M., M.F., and F.P. designed and performed data collection. M.M. and M.F. supervised data collection. G.D.M. and F.P. run the clinical study and performed liver ultrasonography. F.S. and V.N. followed the toddlers and performed the clinical assessment. M.F., A.L., and A.C. performed laboratory and genetic tests. B.D. supervised genetic testing. All authors revised and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all the aspects of the work.

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Disclosure Summary: The authors have nothing to disclose.

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