

Associations between BMI and the *FTO* Gene Are Age Dependent: Results from the GINI and LISA Birth Cohort Studies up to Age 6 Years

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Keywords

FTO · BMI · Longitudinal · GINI study · LISA study · Birth cohort · Kompetenznetz Adipositas · NGFNplus · MCHEALTH

Summary

Objective: The association between polymorphisms in intron 1 of the fat mass and obesity associated gene (*FTO*) and obesity-related traits is one of the most robust associations reported for complex traits and is established both in adults and children. However, little is known about the longitudinal dynamics of these polymorphisms on body mass index (BMI), overweight, and obesity. **Methods:** This study is based on the 2,732 full-term neonates of the German GINI-plus and LISA-plus birth cohorts, for whom genotyping data on the

FTO variants rs1558902 (T>A) or rs9935401 (G>A) were available. Children were followed from birth up to age 6 years. Up to 9 anthropometric measurements of BMI were obtained. Fractional-Polynomial-Generalized-Estimation-Equation modeling was used to assess developmental trends and their potential dependence on genotype status. **Results:** We observed no evidence for BMI differences between genotypes of both variants for the first 3 years of life. However, from age 3 years onwards, we noted a higher BMI for the homozygous minor alleles carriers in comparison to the other two genotype groups. However, evidence for statistical significance was reached from the age of 4 years onwards. **Conclusions:** This is one of the first studies investigating in detail the development of BMI depending on *FTO* genotype between birth and the age of 6 years in a birth cohort not selected for the phenotype studied. We observed that the association between BMI and *FTO* genotype evolves gradually and becomes descriptively detectable from the age of 3 years onwards.

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**For list of study group members see acknowledgement section

Introduction

The association between single nucleotide polymorphisms (SNPs) in intron 1 of the fat mass and obesity associated gene (*FTO*) and obesity-related traits has been robustly reported in both adults and children [1–6]. However, little is known about the longitudinal relationship between these *FTO* polymorphisms and body mass index (BMI) and overweight/obesity development. Although some longitudinal studies are published based on populations of adults or children [7–15], only some have more than 2 or 3 measurements within the same person [8, 10, 13–15], and only 2 studies examined weight dynamics from birth up to adulthood [10, 15]. A longitudinal study in 1,629 Danish men indicated that the influence of intron 1 *FTO* polymorphisms on body weight is not stable over the life span [10], and a recent British cohort study suggested that these life course variations may start from the age 2 years onwards [15]. In addition, a British study of 3,582 twin pairs showed that the genetic influence (heritability) of the *FTO* intron 1 variance on BMI became progressively stronger in children aged between 4 and 11 years [8]. Thus, despite these longitudinal findings, confirmatory studies are needed to support these observations regarding the longitudinal dynamics of the *FTO* variants on BMI development. Due to the critical periods for growth and metabolic programming towards obesity risk, investigations in childhood and adolescence should be the main focus [16–18]. A closer look regarding the genetic influence during childhood between birth and school age may therefore give further insight into growth dynamics, later obesity risks, and the extent and time of expression of the genetic influence. In addition, as another study reports that the influence of the *FTO* gene on fat mass develops already within the first 2 weeks of life [12], it is still unclear whether these associations are already manifest at birth or strengthen gradually with increasing age. Thus, the aim of the present study is to investigate further, whether associations between *FTO* polymorphisms and BMI and obesity development are already manifest at birth or strengthen gradually during childhood.

Material and Methods

Study Design and Population

Data from two ongoing German birth cohorts of healthy full-term neonates (gestational age ≥ 37 weeks) born between 1995 and 1999 in Munich, Wesel, Bad Honnef, and Leipzig were combined for longitudinal analyses of growth. The GINI-plus (German Infant Nutritional Intervention) study is an ongoing birth cohort, initiated to prospectively investigate the influence of nutrition intervention during infancy plus air pollution and genetics on allergy development. Between September 1995 and July 1998, a total of 5,991 healthy full-term newborns were recruited in obstetric clinics in Munich and Wesel. The cohort is composed of an intervention ($n = 2,252$) and a non-intervention group ($n = 3,739$). Group assignment has been based on family history of allergy. The intervention comprised nutritional advice promoting breastfeeding for at least 4

months and a randomized trial on the effect of hydrolyzed formula vs. conventional cow-milk formula in preventing allergies. Details on study design are described elsewhere [19–21]. The LISA-plus study is an ongoing population-based birth cohort study of unselected infants, designed to assess ‘Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood’. Between November 1997 and January 1999, $n = 3,097$ healthy full-term newborns were recruited from 14 obstetrical clinics in Munich, Leipzig, Wesel, and Bad Honnef. Details on study design are published elsewhere [22, 23]. Both birth cohort studies share identical standard operating procedures for the 6-year follow-up and very similar study protocols. Scheduled timing of follow-ups for questionnaires were at 0, 6, 12, 24, 36, 48, 60, and 72 months in the GINI-plus study and at 0, 6, 12, 18, 24, 48, and 72 months in the LISA-plus study. For both studies, approval by the respective local Ethics Committees (Bavarian General Medical Council, University of Leipzig, Medical Council of North-Rhine-Westphalia) and written consent from the families of participants were obtained.

At the 6-year follow-up, all parents of the children still participating in the birth cohorts ($n = 6,081$) were contacted and asked to consent to their child’s blood sampling and genotyping. About half of these targeted children actually participated ($n = 2,897$). Genotyping for the SNPs rs1558902 and rs9935401 was successfully conducted in $n = 2,851$ and $n = 2,839$ infants, respectively. Missing values in weight and length or age of measurements left $n = 2,738$ and $n = 2,728$ infants. As all analyses were adjusted for the dichotomous coded variable ‘any maternal smoking during pregnancy’ (with further missing values), the final analyses population comprised $n = 2,732$ and $n = 2,722$ infants, respectively, at birth. Note that the final analysis population for rs1558902 comprises 1,006 children from the LISA study (37%) and 1,726 children from the GINI study (63%). Among the latter, 867 children (50%) participated in the interventional part of the GINI study within the first 16 weeks of life as described above.

Measurements

BMI, Overweight, and Obesity

Anthropometric measurements of weight and length were obtained by questionnaire reported results of the recorded preventive medical check-ups in the well-baby check-up books (‘U-Untersuchungen’). These are repeated physical examinations of the child conducted by a pediatrician at birth, at day 3–10, week 4–6, month 3–4, 6–7, 10–12, 21–24, 43–48, and at the 60–64th months of life (designated time schedule) to monitor physical growth and indications of adverse health outcomes. Due to variation in age of actual weight and length measurement, data are available for almost every month in the first 2 years of life and in due detail for the remaining years up to the age of 6 years. BMI is defined as weight in kilogram divided by squared length in meters. Note that we did not model the influence of the two *FTO* variants on overweight and obesity longitudinally by dichotomizing our sample using the cut-offs for overweight and obesity (90th and 97th percentile) following the recommendation of the German obesity society (www.adipositas-gesellschaft.de/daten/Leitlinie-AGA-S3-2009.pdf) as dichotomization of a quantitative trait using population-based data in general [24] and power considerations indicated that this is of limited value. Power calculations assuming relative risks of 1.5 and 2 and 1.25 and 1.5 for outcomes obesity and overweight between the genotypes revealed a maximum comparisonwise power of 69 and 65% at 2 years of age and only 46 and 45% at the age of 5–6 years using the genetic power calculator software [25], with $\alpha = 0.05$ for the HapMap-based minor allele frequency estimates of the two SNPs.

Adjustment for Maternal Smoking during Pregnancy

Mothers’ questionnaire-based reports (shortly after birth) of any maternal smoking during pregnancy (indicator coded as 1 = yes vs. 0 = no) was used to adjust for the known influence of maternal smoke on birth weight

Table 1. Characteristics of the analysis population^a

	Boys (n = 1,419)		Girls (n = 1,313)		Total (n = 2,732)	
	mean or %	SD or 95% CI	mean or %	SD or 95% CI	mean or %	SD or 95% CI
Weight at birth, g	3,541	462	3,393	449	3,470	462
Length at birth, cm	52.4	2.5	51.5	2.4	52.0	2.5
BMI at birth, kg/m ²	12.9	1.2	12.8	1.2	12.8	1.2
% Overweight at birth ^b	3.6 (51/1,419)	2.6–4.6	4.5 (59/1,313)	3.4–5.6	4.0 (110/2,732)	3.3–4.8
% Obese at birth ^c	0.9 (13/1,419)	0.4–1.4	1.1 (14/1,313)	0.5–1.6	1.0 (27/2,732)	0.6–1.4
% Any maternal smoking during pregnancy	14.1 (197/1,419)	12.1–15.7	13.6 (179/1,313)	11.8–15.5	13.8 (376/2,732)	12.5–15.1

^aAnalysis population comprises all infants with information at SNPs rs1558902 or rs9935401, BMI at birth and maternal smoking status during pregnancy.

^bDefined as ≥ 90 th percentile of BMI at birth according to new age and gender-specific WHO-Child-Growth Standards [42–44].

^cDefined as ≥ 97 th percentile of BMI at birth according to new age and gender-specific WHO-Child-Growth Standards [42–44].

Table 2. Characteristics of two intron 1 *FTO* variants (rs1558902 and rs9935401)

dbSNP, Build 129	Position, bp	Possible functional region	Alleles (major/minor), 1/2	Number of subjects with genotype ^a			Genotyping success rate, %
				11	12	22	
rs1558902	51,196,264	chrom 16: intron 1	T/A	945	1,300	487	98.4
rs9935401	52,374,339	chrom 16: intron 1	G/A	969	1,316	437	98.0

^aGenotype frequencies are based on all available genotyping information among the analysis population (see table 1), i.e. all infants with information on genotype at SNPs rs1558902 or rs9935401, BMI at birth, and maternal smoking status during pregnancy.

and later growth [26, 27]. As maternal smoking during pregnancy is strongly associated with socio-economic status and education, adjustment was restricted to mothers' smoking status. [28]

Genotyping

The *FTO* gene was tagged by two SNPs (rs1558902, rs9935401). Genomic DNA of GINI and LISA participants was extracted from blood leukocytes using the Puregene™ DNA Isolation Kit (Gentra Systems, Big Lake, MN, USA) according to the manufacturer's recommendations. Genotyping was done with MALDI TOF MS using iPLEX™ Gold chemistry (Sequenom, San Diego, CA, USA). We chose rs1558902 and rs9935401 and not the previously published *FTO*-SNP rs9939609 as both tagged SNPs are in high linkage disequilibrium (LD) with rs9939609. According to HapMap LD, rs9939609 shows r^2 and D' of 0.934 and 0.966 with rs1558902 and 1.0 and 1.0 with rs9935401, respectively. Discordance in 10% routine duplicates was $<0.5\%$.

Statistical Analysis

Generalized estimation equation (GEE) models were used to account for correlations between repeated measurements over time and to assess the potential influence of the *FTO*-SNP genotype for rs1558902 and rs9935401 on the development of BMI, overweight, and obesity in children from birth up to 6 years. Genotypes were coded as additive (i.e. 0 = no minor allele, 1 = 1 minor allele, 2 = 2 minor alleles). We chose additive coding as most previous studies did so and because testing for deviation from an additive genetic model did not support a dominant or recessive model of association with the outcome BMI. The test was performed by including an indicator variable for the heterozygous genotype in addition to the additive coded SNP in a regression model for the outcome BMI. This test was performed at each of the 9 measurement occasions sepa-

rately. All reported models were adjusted for the child's gender and the mother's smoking status during pregnancy. For more flexibility with regard to the modeling of the development charts for the continuous outcome BMI, we used fractional polynomial (FP) models proposed by Royston, Altman, Sauerbrei and others [29–31]. These FP models require less parameters to be estimated as compared to traditional higher order polynomial fits; consequently, they are frequently more robust. The best-fitting functional form of the variables age, *FTO* genotype and interaction of age and *FTO* genotype were identified by the 'mfp' module of the statistical software Stata version 9.2. (up to second degree FP and with potential transformation powers $p = \{\text{of } -2, -1, 0.5, 0 = \ln(x), 1, 2, 3\}$ within GEE models for which an exchangeable working correlation was used [32].

Before potential transformation of variables 'SNP' and 'AGE' (and their interaction) by FP, the models can be written down as:

$g(\text{outcome}) = \beta_0 + \beta_1 \text{SNP} + \beta_2 \text{AGE} + \beta_3 \text{SNP} \times \text{AGE} + \beta_4 \text{boy} + \beta_5 \text{maternal smoke}$, where link function $g(\cdot)$ is identity for BMI. Note that the FP software requires that all variables used for FP transformation are greater than zero. Thus '1' was added to the variables 'AGE' and 'SNP' and to the interaction 'AGE' \times 'SNP'.

We assessed statistical significant differences among genotype trajectories at certain ages by adapting an approach from Singer et al. ([33], chapter 5.4.) In brief, for each age of interest, a cubic GEE model with all the variables of the original model was estimated, however, with age centered at 24, 30, 36, 39, 42, 45, 48, 51, 54, 57, 60, and 66 months, respectively. By this proceeding all terms involving centered age are zero, because the age of interest is subtracted from the original age. Thus the regression coefficients of the two indicator-coded variables of the genotypes with no or one risk allele (reference is A/A genotype) are the estimates of the difference in trajectories between these genotypes and the genotype with two risk alleles at that particular age, and can thus be evaluated for-

Table 3. Best fitting fractional polynomial GEE model for the association of rs9935401 and BMI development from birth to age 6 years

Variable ^a	β	SE	95% CI ^b		p value
<i>FTO</i> -SNP: rs9935401					
FP1: $\log_e(\text{SNP}) + c1$	1.001	0.046	0.91	1.09	< 0.001
Age, months					
FP2: $(\text{age}/10)^{-2} + c2$	-0.462	0.005	-0.47	-0.45	< 0.001
$(\text{age}/10)^{-2} \times \log_e(\text{age}/10) + c3$	-0.171	0.002	-0.18	-0.17	< 0.001
Interaction SNP \times age					
FP2: $\log_e(\text{SNP} \times \text{age}/100) + c4$	-1.005	0.015	-1.03	-0.98	< 0.001
$(\text{SNP} \times \text{age}/100)^3 + c5$	0.035	0.009	0.02	0.05	< 0.001
Boy versus girl	0.308	0.036	0.24	0.38	< 0.001
Maternal smoking during pregnancy	0.168	0.052	0.07	0.27	< 0.001
Intercept	16.213	0.028	16.16	16.27	< 0.001

^aGender is coded as boy = 1 vs. girl = 0; any reported maternal smoking during pregnancy is coded as 1 and no smoking as 0; due to requirements of fractional polynomial software (see statistical methods section), the SNP is coded as G/G = 1, G/A = 2, A/A = 3, and AGE is coded in months plus 1. The following constants were incorporated in the FP by the estimation procedure of the software: c1 = -0.5899246656; c2 = -0.2994074434; c3 = -0.1805351951; c4 = 1.109227723; c5 = -0.0358761278.

^b95% confidence interval around estimate of regression coefficient β .

Table 4. Best fitting fractional polynomial GEE model for the association of rs1558902 and BMI development from birth to age 6 years^a

Variable	β	SE	95% CI ^b		p value
<i>FTO</i> -SNP: rs1558902					
FP1: $\log_e(\text{SNP}) + c1$	0.998	0.045	0.91–1.09		< 0.001
Age, months					
FP2: $(\text{age}/10)^{-2} + c2$	-0.462	0.005	-0.47 to -0.45		< 0.001
$(\text{age}/10)^{-2} \log_e(\text{age}/10) + c3$	-0.171	0.002	-0.18 to -0.17		< 0.001
Interaction SNP \times age					
FP2: $\log_e(\text{SNP} \times \text{age}/100) + c4$	-1.007	0.015	-1.04 to -0.98		< 0.001
$(\text{SNP} \times \text{age}/100)^3 + c5$	0.033	0.008	0.02–0.05		< 0.001
Boy versus girl	0.316	0.036	0.25–0.39		< 0.001
Maternal smoking during pregnancy	0.166	0.052	0.06–0.27		0.001
Intercept	16.204	0.028	16.15–16.26		< 0.001

^aGender is coded as boy = 1 versus girl = 0, any reported maternal smoking during pregnancy is coded as 1 and no smoking as 0, and due to requirements of fractional polynomial software (see statistical methods section), the SNP is coded as T/T = 1, T/A = 2, A/A = 3 and AGE is coded in months plus 1. The following constants were incorporated in the FP by the estimation procedure of the software: c1 = -0.6051436599; c2 = -0.2994851627; c3 = -0.1805431932; c4 = 1.094088163; c5 = -0.0375431445.

^b95% confidence interval around estimate of regression coefficient β .

mally. All analyses were done using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) or Stata version 9.2. (StataCorp LP, College Station, Texas, USA). For evaluation of evidence against the respective null hypotheses, standard errors (SE), exact p values for those > 0.001, and confidence intervals (95% CI) for the estimates and main results are reported following the proposal by Sterne and Davey Smith [34].

Results

Characteristics of the analyzed population at birth are listed in table 1. Information regarding position, functional region, genotyping frequencies, and success rate for the two analyzed

FTO SNPs are listed in table 2. As all results below were very similar for rs9935401, which is in strong linkage disequilibrium (estimated $r^2 = 0.94$) with rs1558902, we decided to focus on rs1558902. The results for rs9935401 are summarized in table 3.

Development of BMI

Regression coefficients from the best fitting fractional polynomial model for the influence of the genotypes of SNP rs1558902 on BMI from birth up to age 6 years are shown in table 4. The resulting trajectories in BMI over age from birth up to 6 years for the 3 genotypes are displayed in figure 1. For

Fig. 1. BMI development from birth to the age of 6 years stratified by *FTO* genotype status (rs1558902) modeled by fractional polynomial GEEs adjusted for gender and maternal smoking during pregnancy. Genotype: — T/T; — T/A; — A/A; ●●● 95% prediction interval around the BMI trend for the known obesity risk genotype A/A of rs1558902.

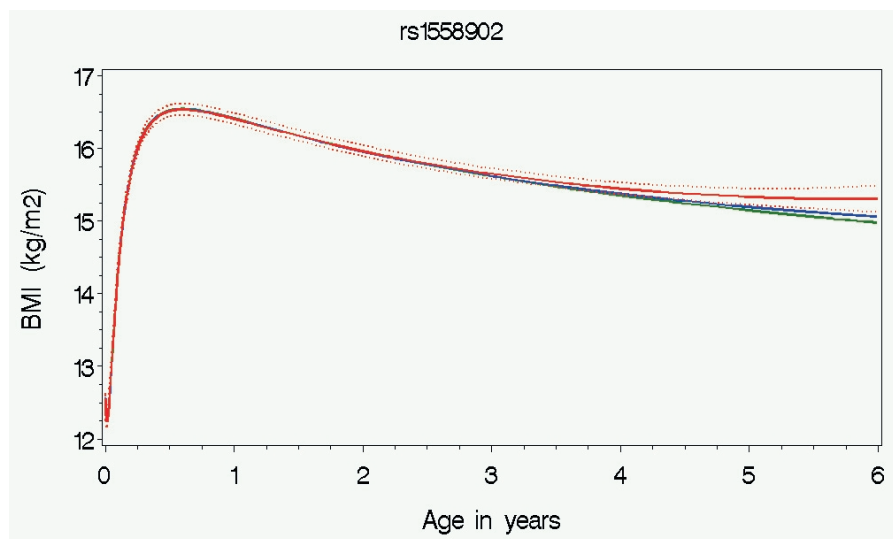
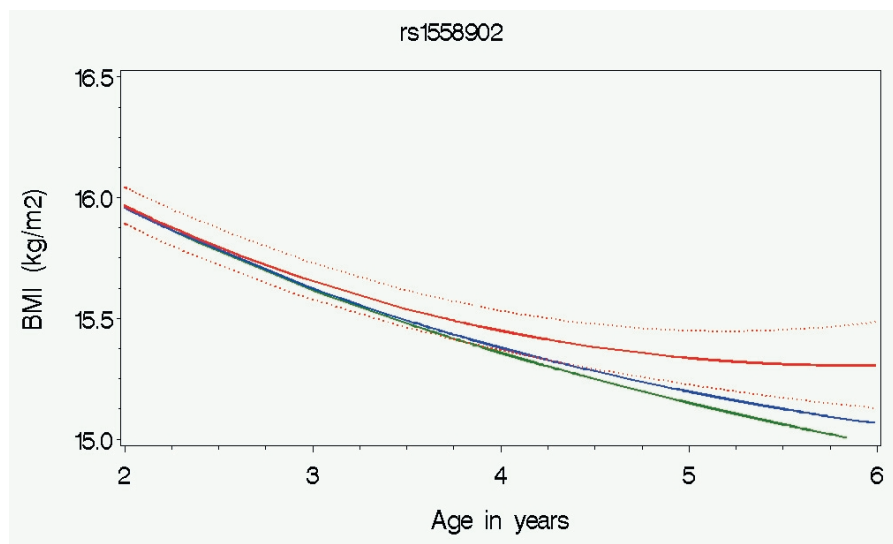


Fig. 2. BMI development from age 2 to 6 years stratified by *FTO* genotype status (rs1558902) modeled by fractional polynomial GEEs adjusted for gender and maternal smoking during pregnancy. Genotype: — T/T; — T/A; — A/A; ●●● 95% prediction interval around the BMI trend for the known obesity risk genotype A/A of rs1558902.



a closer inspection of the differences between the genotype's trajectories, figure 2 shows an enlarged version of figure 1 restricted to ages from 2 years onwards.

As indicated by the 95% CI and the very small p values of the regression coefficients for the *FTO* SNP and its interaction with the variable 'AGE', there is strong evidence against the null hypotheses of no influence of the genotypes on BMI, which varied longitudinally. As outlined by the trends for the 3 genotype status strata, the 3 BMI trajectories start to differ after the age of 3 years. This drift is most obvious for the A/A genotype carriers. However, although a tendency for divergence is already detectable from 3 years onwards, statistical significance in trajectories between carriers of no versus carriers of two risk alleles are seen only from month 48 onwards (data not shown). Note that all analyses were adjusted for child's gender and maternal smoking during pregnancy. Similar results (data not shown) were found in sensitivity analyses

stratified by the child's gender and in analyses omitting the mother's smoking status. Moreover, sensitivity analyses excluding 867 children from the interventional part of the GINI study did not substantially change the results reported for the full sample (data not shown).

Discussion

We analyzed developmental aspects of the common intron 1 variants rs1558902 and rs9935401 of the *FTO* gene from birth up to the age of 6 years in a population-based birth cohort of 2,732 full-term infants. To our knowledge, this is one of the first reports based on frequently repeated anthropometric measures (2–9 measurements, average 8.7) in the same children. We show that the BMI trajectories vary by genotype status, and that these differences become observable by an-

thropometric measures from the age of 3 years onwards. However, significance was reached by the age of 4 years and later. This drift is most obvious for the A/A genotype carriers and could potentially indicate or contribute to an earlier 'adiposity rebound' [35] for this genotype than for the two other genotypes. However, data beyond the 6-year follow-up are needed to confirm this. In addition, our modeling indicated that the change of the BMI trajectories varies by genotype status.

Our observations match those made by Haworth et al. [8] who report a progressively larger heritability estimate of BMI over time and an age-dependent stronger association signal for the common variant rs9939609 of *FTO* and BMI in 3,582 twin-pairs at ages 4, 7, 10, and 11 years. That the genetic influence is likely smaller at birth is also supported by an early study on variations of birth weights in twins, in which a heritability of only 40% was reported [36]. Moreover, our results are consistent with the reports that demonstrate no association of *FTO* and BMI at birth, but do show an association at age 7+ [1–3, 9, 10]. Note that none of these studies have reported data on BMI or obesity-related traits for children younger than 6 years. Finally, similar results have also been reported by Jess et al. [10] who show in 1,629 Danish men that the *FTO* SNP rs9939609 is associated with BMI over the life span (birth, 7, 10, 13, 20, 35, 46 years) with varying strengths (no effect at birth), with the strongest effect observed at the age of 7 years. In addition, a recent study from Hardy et al. [15] analyzing the association between *FTO* variant rs9939609 and BMI from 2 to 53 years of age, with anthropometric measurements at age 2, 4, 6, 7, 15, 20, 26, 36, 43, and 53 years, also supports our claim that BMI varies longitudinally with *FTO* genotype from early childhood (i.e. from 4 years) onwards, and that this association strengthens with age.

Shifting to measures of fat mass other than BMI, the data of Lopez-Bermejo et al. [12] for 234 full-term neonates indicate, however, that an association between the *FTO* SNP rs9939609 and fat mass accretion (as measured by serum visfatin) might be present as early as 2 week of age. One possible explanation is the known inaccuracy of BMI as a proxy marker for fat mass [37]. In particular, fat mass-related effects of the *FTO* genotype that cause observable changes of BMI may indeed go undetected at earlier ages (younger than 3 years). Thus, with regard to the anthropometric measure BMI, we interpret our result as evidence of a gradually emerging impact of the *FTO* genotype on BMI. A recent epidemiological study measuring food intake in children and showing that enjoyment of food is enhanced and satiety responsiveness is reduced for carriers of two copies of the risk allele, suggests that the effect of *FTO* on BMI works via appetite control [38]. This could also be a biologically plausible explanation why the effect of *FTO* on BMI is not immediately seen at birth in our study. If *FTO* affects BMI development via appetite control, its effect would start with enteral feeding, and it may take

some time to accumulate excessive weight. However, relatively little is known about the biological meaning of the *FTO* finding (see Fischer et al. [39] for an exception, whose results of an animal model indicate that *FTO* is functionally involved in energy homeostasis by control of energy expenditure; another recent reference is Church et al. [40]). Like other epidemiological knowledge, our observation might nevertheless be of importance to resolve some of the open questions regarding the biology in humans, once more is known from animal models, for example.

Other limitations of the present study are the population which is not enriched for overweight or obese children, and that the follow-up is limited to the age of 6 years. While the first constraint leads to a lack of power to detect associations with overweight and obesity, the second constraint will be the subject of subsequent analyses as both birth cohorts are ongoing. A further limitation is that written consent to genotyping was given only by half the parents of the children still in follow-up at 6 years. However, participation in blood sampling for genotyping was independent from child's BMI at the 6-year follow-up. Therefore, we consider it unlikely that the sample has a relevant selection bias. As another limitation, further adjustments in addition to 'gender' and 'maternal smoking during pregnancy' would certainly be of interest with the most obvious candidates being the mother's BMI or obesity status at the beginning of pregnancy. As information on this variable is available for only a small number of individuals ($n = 985$), we decided against this proceeding for obvious reasons related to lack of power. Again referring to the ongoing sampling, we may be able to address such questions later.

In addition, we performed several sensitivity analyses. We accounted for the problem of increasing variability of BMI with age as discussed by Cole [41] by adjusting for BMI at birth, at 2 years of age, or both. The potential bias resulting from the fact that about a third ($n = 867$) of the studied population ($n = 2,732$) took part in a nutritional intervention within the first 4 months of life, was investigated by excluding this group of infants in sensitivity analyses. Additional secondary analyses were performed for the outcome BMI-SDS (derived from new gender- and age-specific growth curve standards of the WHO (www.who.int/childgrowth/en/ and www.who.int/growthref/en/ for children 5 years and older). Finally, we also explored the effect of the necessary adding of a constant to the age variable as FP models require variables > 0 by evaluating models with different constants (0.1, 0.5, 1.0). All results of these sensitivity analyses were in line with the results of the original FP models and resulted in similar estimates (data not shown).

In conclusion, this is (to our knowledge) one of the first papers analyzing developmental aspects of common intron 1 variants of the best validated obesity gene *FTO* from birth up to the age of 6 years with 2–9 (average 8.7) repeated measurements in a large population-based birth cohort of 2,732 full-term infants. We show that the association between BMI and

the *FTO* variants develops gradually and is observable at the age of 3 years or later and reaches statistical significance from age 4 years onwards. At younger ages, BMI might not be the optimal measure to establish associations with obesity-related genetic variants. This drift is most obvious for the A/A genotype carriers and could potentially indicate an earlier ‘adiposity rebound’ for this genotype than for the two other genotypes. However, data beyond the 6-year follow-up are needed to confirm this. One might speculate whether a potential reason for this gradual evolvement could be that *FTO* affects BMI development via appetite control as the results from a recent study in children suggest. However, functional studies about the biological meaning of the *FTO* finding are still very rare. Like other epidemiological knowledge, our observation might nevertheless be of importance to resolve some of the open questions regarding weight regulation biology in humans, in particular to back up some of the findings based on e.g. animal research.

Funding

The GINI Intervention study was funded for 3 years by grants of the Federal Ministry for Education, Science, Research and Technology (Grant No. 01 EE 9401–4), the 6-year follow-up of the GINI-plus study was partly funded by the Federal Ministry of Environment (IUF, FKZ 20462296). The LISA-plus study was funded by grants of the Federal Ministry for Education, Science, Research and Technology (Grant No. 01 EG 9705/2 and 01EG9732) and the 6-year follow-up of the LISA-plus study was partly funded by the Federal Ministry of Environment (IUF, FKS 20462296). This work was supported by the ‘Kompetenznetz Adipositas (Competence Network Obesity)’ and NGFN^{plus} funded by the Federal Ministry of Education and Research (FKZ: 01GI0826, 01GI0823, 01GS0820, 01KU0903). Personal and financial support by the Munich Center of Health Sciences (MCHEALTH) which contributed to this research is gratefully acknowledged.

Acknowledgements

We thank the families for participation in the studies, the obstetric units for allowing recruitment, the GINI and LISA study teams for excellent work, and several funding agencies listed above.

References

- 1 Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoecur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougneres P, Kovacs P, Marre M, Balkau B, Cauchi S, Chevre JC, Froguel P: Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet* 2007;39:724–726.
- 2 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JRB, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJF, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CNA, Doney ASF, Morris AD, Smith GD, The Wellcome Trust Case Control Consortium, Hattersley AT, McCarthy MI: A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;316:889–894.
- 3 Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, Grallert H, Illig T, Wichmann HE, Rief W, Schafer H, Hebebrand J: Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (*FTO*) variants. *PLoS ONE* 2007;2:e1361.
- 4 Price RA, Li WD, Zhao H: *FTO* gene SNPs associated with extreme obesity in cases, controls and extremely discordant sister pairs. *BMC Med Genet* 2008;9:4.

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Disclosure

The authors declared no conflict of interest.

- 5 Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orru M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR: Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007;3:e115.
- 6 Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Rocaasecca RM, Sanna S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O'Rahilly S, Purmann C, Rees MG, Ridderstrale M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimalaswaran KS, Vollenweider P, Waeber G, Wallace C, Watanabe RM, Waterworth DM, Watkins N, Witteman JC, Zeggini E, Zhai G, Zillikens MC, Altschuler D, Caulfield MJ, Chanock SJ, Farooqi IS, Ferrucci L, Guralnik JM, Hattersley AT, Hu FB, Jarvelin MR, Laakso M, Mooser V, Ong KK, Ouwehand WH, Salomaa V, Samani NJ, Spector TD, Tuomi T, Tuomilehto J, Uda M, Uitterlinden AG, Wareham NJ, Deloukas P, Frayling TM, Groop LC, Hayes RB, Hunter DJ, Mohlke KL, Peltonen L, Schlessinger D, Strachan DP, Wichmann HE, McCarthy MI, Boehnke M, Barroso I, Abecasis GR, Hirschhorn JN: Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009;41:25–34.
- 7 Haupt A, Thamer C, Machann J, Kirchhoff K, Stefan N, Tschritter O, Machicao F, Schick F, Haring HU, Fritsche A: Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. *Obesity* 2008;16:1969–1972.
- 8 Haworth CMA, Carnell S, Meaburn EL, Davis OSP, Plomin R, Wardle J: Increasing heritability of BMI and stronger associations with the FTO gene over childhood. *Obesity* 2008;16:2663–2668.
- 9 Hunt SC, Stone S, Xin Y, Scherer CA, Magness CL, Iadonato SP, Hopkins PN, Adams TD: Association of the FTO gene with BMI. *Obesity* 2008;16:902–904.
- 10 Jess T, Zimmermann E, Kring SII, Berentzen T, Holst C, Toubro S, Astrup A, Hansen T, Pedersen O, Sorensen TIA: Impact on weight dynamics and general growth of the common FTO rs9939609: a longitudinal Danish cohort study. *Int J Obes* 2008;32:1388–1394.
- 11 Lawlor DA, Timpson NJ, Harbord RM, Leary S, Ness A, McCarthy MI, Frayling TM, Hattersley AT, Smith GD: Exploring the developmental overnutrition hypothesis using parental-offspring associations and FTO as an instrumental variable. *PLoS Medicine* 2008;5:e33.
- 12 Lopez-Bermejo A, Petry CJ, Diaz M, Sebastiani G, de Zegher F, Dunger DB, Ibanez L: The association between the FTO gene and fat mass in humans develops by the postnatal age of two weeks. *J Clin Endocrinol Metab* 2008;93:1501–1505.
- 13 Marville AF, Lange LA, Qin L, Adair LS, Mohlke KL: Association of FTO with obesity-related traits in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) cohort. *Diabetes* 2008;57:1987–1991.
- 14 Qi L, Kang K, Zhang C, van Dam RM, Kraft P, Hunter D, Lee CH, Hu FB: Fat mass- and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test. *Diabetes* 2008;57:3145–3151.
- 15 Hardy R, Wills AK, Wong A, Elks CE, Wareham NJ, Loos RJF, Kuh D, Ong KK: Life course variations in the associations between FTO and MC4R gene variants and body size. *Hum Mol Genet* 2009; ddp504.
- 16 Dietz WH: Periods of risk in childhood for the development of adult obesity – what do we need to learn? *J Nutr* 1997;127:1884S–1886S.
- 17 Koletzko B: Long-term consequences of early feeding on later obesity risk. *Nestle Nutr Workshop Ser Pediatr Program* 2006;1–18.
- 18 Lucas A, Fewtrell MS, Cole TJ: Fetal origins of adult disease – the hypothesis revisited. *BMJ* 1999;319:245–249.
- 19 Filipiak B, Zutavern A, Koletzko S, von Berg A, Brockow I, Grubl A, Berdel D, Reinhardt D, Bauer CP, Wichmann HE, Heinrich J: Solid food introduction in relation to eczema: results from a four-year prospective birth cohort study. *J Pediatr* 2007;151:352–358.
- 20 Von Berg A, Koletzko S, Filipiak-Pittroff B, Laubereau B, Grubl A, Wichmann HE, Bauer CP, Reinhardt D, Berdel D: Certain hydrolyzed formulas reduce the incidence of atopic dermatitis but not that of asthma: three-year results of the German Infant Nutritional Intervention Study. *J Allergy Clin Immunol* 2007;119:718–725.
- 21 Rzehak P, Sausenthaler S, Koletzko S, Reinhardt D, von Berg A, Kramer U, Berdel D, Bollrath C, Grubl A, Bauer CP, Wichmann HE, Heinrich J: Short- and long-term effects of feeding hydrolyzed protein infant formulas on growth at ≤ 6 y of age: results from the German Infant Nutritional Intervention Study. *Am J Clin Nutr* 2009;89:1846–1856.
- 22 Chen CM, Rzehak P, Zutavern A, Fahlbusch B, Bischof W, Herbarth O, Borte M, Lehmann I, Behrendt H, Kramer U, Wichmann HE, Heinrich J: Longitudinal study on cat allergen exposure and the development of allergy in young children. *J Allergy Clin Immunol* 2007;119:1148–1155.
- 23 Zutavern A, Rzehak P, Brockow I, Schaaf B, Bollrath C, von Berg A, Link E, Kraemer U, Borte M, Herbarth O, Wichmann HE, Heinrich J: Day care in relation to respiratory-tract and gastrointestinal infections in a German birth cohort study. *Acta Paediatr* 2007.
- 24 Royston P, Altman DG, Sauerbrei W: Dichotomizing continuous predictors in multiple regression: a bad idea. *Stat Med* 2006;25:127–141.
- 25 Purcell S, Cherny SS, Sham PC: Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19:149–150.
- 26 Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB: Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000;320:967–971.
- 27 Triche EW, Hossain N: Environmental factors implicated in the causation of adverse pregnancy outcome. *Semin Perinatol* 2007;31:240–242.
- 28 Heinrich J, Popescu MA, Wjst M, Goldstein IF, Wichmann HE: Atopy in children and parental social class. *Am J Public Health* 1998;88:1319–1324.
- 29 Greenland S: Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 1995;6:356–365.
- 30 Royston P, Altman DG: Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling (with discussion). *Appl Stat* 1994;43:429–467.
- 31 Sauerbrei W, Royston P, Binder H: Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat Med* 2007;26:5512–5528.
- 32 Sauerbrei W, Meier-Hirmer C, Benner A, Royston P: Multivariable regression model building by using fractional polynomials: description of SAS, STATA and R programs. *Comput Stat Data Anal* 2006;50:3464–3485.
- 33 Singer JD, Willett JB. *Applied Longitudinal Data Analysis. Modeling Change and Event Occurrence*. Oxford New York, NY, Oxford University Press, 2003.
- 34 Sterne JA, Davey SG: Sifting the evidence – what's wrong with significance tests? *BMJ* 2001;322:226–231.
- 35 Rolland-Cachera MF, Deheeger M, Maillot M, Bellisle F: Early adiposity rebound: causes and consequences for obesity in children and adults. *Int J Obes (Lond)* 2006;30(suppl 4):S11–S17.
- 36 Vlietinck R, Derom R, Neale MC, Maes H, van Loon H, Derom C, Thiery M: Genetic and environmental variation in the birth weight of twins. *Behav Genet* 1989;19:151–161.
- 37 Schaefer F, Georgi M, Wuhl E, Scharer K: Body mass index and percentage fat mass in healthy German schoolchildren and adolescents. *Int J Obes Relat Metab Disord* 1998;22:461–469.
- 38 Wardle J, Carnell S, Haworth CMA, Farooqi IS, O'Rahilly S, Plomin R: Obesity associated genetic variation in FTO is associated with diminished satiety. *J Clin Endocrinol Metab* 2008;93:3640–3643.
- 39 Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, Ruther U: Inactivation of the FTO gene protects from obesity. *Nature* 2009;458:894–898.
- 40 Church C, Lee S, Bagg EA, McTaggart JS, Deacon R, Gerken T, Lee A, Moir L, Mecinovic J, Quwailid MM, Schofield CJ, Ashcroft FM, Cox RD: A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. *PLoS Genet* 2009;5:e1000599.
- 41 Cole TJ: Modeling postnatal exposures and their interactions with birth size. *J Nutr* 2004;134:201–204.
- 42 Butte NF, Garza C, de Onis M: Evaluation of the feasibility of international growth standards for school-aged children and adolescents. *J Nutr* 2007;137:153–157.
- 43 de Onis M, Garza C, Onyango AW, Borghi E: Comparison of the WHO child growth standards and the CDC 2000 growth charts. *J Nutr* 2007;137:144–148.
- 44 Ziegler EE, Nelson SE: The new WHO growth standards. *Ann Nestlé (Engl)* 2007;65:109–115.