CLINICAL STUDY

Insulin gene variable number of tandem repeats is not associated with weight from fetal life until infancy: the Generation R Study

Dennis O Mook-Kanamori^{1,2}, J J Miranda Geelhoed^{1,2}, Eric A P Steegers³, Jacqueline C M Witteman², Albert Hofman², Henriëtte A Moll⁴, Cornelia M van Duijn², Anita C S Hokken-Koelega⁴ and Vincent W V Jaddoe^{1,2,4}

¹The Generation R Study Group, ²Department of Epidemiology and Biostatistics, ³Department of Obstetrics and Gynaecology and ⁴Department of Pediatrics, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands

(Correspondence should be addressed to V W V Jaddoe; Email: v.jaddoe@erasmusmc.nl)

Abstract

Objective: The aim of this study was to examine whether the insulin gene variable number of tandem repeats (*INS* VNTR) is associated with growth patterns in fetal life and infancy.

Design and methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. Fetal growth was assessed by ultrasounds in early, mid-, and late pregnancy. Anthropometry in infancy was assessed at birth and at the ages of 6 weeks, 6 months, and 14 months. DNA for genotyping of the *INS* VNTR promoter region was available in 859 children.

Results: The genotype distribution was I/I 50.8%, I/III 40.0%, and III/III 9.2%. III/III individuals had a shorter gestational age ($P < 0.005$ versus I/I) and a lower birth weight ($P < 0.05$ versus I/I). There were no differences in birth weight after adjusting for gestational age. Class III homozygotes had a smaller abdominal circumference/head circumference (HC) ratio ($P < 0.005$ versus I/I) in mid-pregnancy, but not in late pregnancy. Also, III/III subjects had a relative decrease in HC (SDS) from mid-pregnancy to the age of 14 months ($P < 0.05$ versus I/I). No other differences in pre- and postnatal growth characteristics and patterns were found.

Conclusions: Class III homozygotes were born at an earlier gestational age. No association was found between *INS* VNTR and birth weight adjusted for gestational age. Our data suggest that the III/III genotype may be associated with asymmetrical growth in mid-pregnancy, but not in late pregnancy.

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Introduction

Insulin is the most important fetal growth factor (1). Previous experimental and observational studies have shown that reduced secretion of fetal insulin and insulin-like growth factors is associated with low birth weight (1, 2). Several rare monogenic defects that affect insulin secretion have been shown to be related to altered fetal growth (1, 3). It has been suggested that also more common genetic polymorphisms related to insulin secretion and metabolism may explain part of the differences in birth weight in the normal population (3).

Variation at the insulin gene variable number of tandem repeats (*INS* VNTR) minisatellite has been shown to influence pancreatic insulin gene transcription, both in the fetus and in adulthood (4, 5). The VNTR lies upstream of the imprinted insulin and insulin-like growth factor II genes on chromosome 11p15.5, and has been suggested to influence transcription rate of these genes (5–7). *INS* VNTR has been

suggested as a candidate genetic variant that influences fetal and early postnatal growth in a normal population (8, 9). There are two main classes of VNTR, namely class I (30–44 repeat units) and class III (around 150 repeat units); class II is very rare in the Caucasian population (5). Studies in the human pancreas have suggested that *INS* expression is lower in the VNTR class III homozygous than in the VNTR class I homozygous individuals (5, 10). At birth, *INS* VNTR class III homozygous individuals have been shown to have a larger mean head circumference (HC) (8). Among those subjects without postnatal growth realignment, birth weight and length were also increased (8). However, other cohort studies found a lower birth weight in class III homozygotes or no difference in birth weight at all (9, 11, 12). It has also been suggested that this genotype is involved in childhood obesity and the development of metabolic syndrome (13, 14), polycystic ovary syndrome (15), and type 2 diabetes in adulthood (16, 17). Nevertheless, two large cohort studies were unable to

demonstrate effects of *INS* VNTR on body composition or the risk of metabolic syndrome in adulthood (18, 19).

The inconsistent findings from studies examining the association between *INS* VNTR genotype with birth size may be explained by the fact that size at birth alone is an inappropriate measure for fetal growth. We hypothesized that variants of the *INS* VNTR may be stronger related to longitudinally measured growth in pre- and postnatal life than to one specific growth characteristic such as birth weight. Therefore, we examined in the Generation R Study, a prospective prenatally recruited birth cohort study, the associations of the *INS* VNTR genotype with growth parameters measured in different periods from fetal life until infancy.

Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development, and health from fetal life until young adulthood, and has been described previously in detail (20, 21). In total, the cohort includes 9778 mothers and their children living in Rotterdam, the Netherlands. The vast majority (69%) of all mothers were enrolled during the first trimester of pregnancy (20). Assessments in pregnancy included physical examinations, fetal ultrasounds, biological samples, and questionnaires. These were planned in early (gestational age <18 weeks), mid- (gestational age 18–25 weeks), and late pregnancy (gestational age > 25 weeks) to collect information about fetal growth and its main determinants. Their partners were assessed once during this period. The children were born between April 2002 and January 2006 and from a prenatally recruited birth cohort that is currently being followed until young adulthood. Of all eligible children, 61% participated in the study at birth. Additionally, more detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1232 parents and their children, referred to as the Generation R Focus cohort. This subgroup is completely Caucasian to exclude possible confounding or effect modification by ethnicity. Of all approached women, 80% were enrolled in this subgroup study in late pregnancy (gestational age of 30 weeks). In this subgroup, postnatal examinations were performed at the ages of 6 weeks, 6 months, and 14 months. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants or their parents.

Genotyping *INS* VNTR gene

DNA was collected from cord blood samples at birth. Cord blood for DNA isolation was available in 85% of all children participating in the Focus cohort. Missing cord blood samples were mainly due to logistic constraints at the delivery. PCR was performed to amplify the $-23/HphI$ single nucleotide polymorphism (A/T), which is known to be in almost complete linkage disequilibrium with *INS* VNTR class (22).

The genotype distribution (AA 50.8%, AT 40.0%, and TT 9.2%) was similar to those found in previous studies and the frequency distribution did not deviate from the Hardy–Weinberg equilibrium ($\chi^2 = 1.28$, $P > 0.1$) (8, 11, 19).

Fetal growth and birth characteristics

Fetal ultrasound examinations were carried out in one of the research centers in early, mid-, and late pregnancy. These fetal ultrasounds were used for both establishing gestational age and assessing fetal growth characteristics (23). Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating in early pregnancy (gestational age until 12 weeks and 5 days, crown-rump length smaller than 65 mm) and biparietal diameter for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onward, biparietal diameter larger than 20 mm) (24, 25). Fetal growth measurements used for the present study included HC, abdominal circumference (AC), and femur length (FL) in mid- and late pregnancy, measured to the nearest millimeter using standardized ultrasound procedures (26). AC/HC ratio was calculated, which has been shown to be useful in distinguishing symmetrical from asymmetrical growth (27). Estimated fetal weight was calculated by the Hadlock formula using HC, AC, and FL ($\log_{10} EFW = 1.5662 - 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 (HC)^2 - 0.003685 (AC \times FL)$) (28). Early pregnancy was not included since these fetal ultrasound examinations were primarily performed to establish gestational age. Gestational age-adjusted SDS were constructed for all fetal growth measurements. These were based on reference growth charts from the whole study population.

Postnatal growth characteristics

Date of birth, gender, and birth weight were obtained from community midwife and hospital registries. At the age of 6 weeks, 6 months, and 14 months, anthropometrics were measured without clothes. Weight was measured to the nearest gram using electronic scales. Length was measured in supine position to the nearest millimeter at the ages of 6 weeks and 6 months using a neonatometer, and in upright position at the age of 14 months. HC was measured to the nearest millimeter.

Covariates

Information about maternal age, parity, and weight before pregnancy was obtained by the first questionnaire at enrolment in the study. Maternal height was measured without shoes at our research center, and body mass index (weight/height² (kg/m²)) was calculated. Information on the occurrence of hypertension, pre-eclampsia, gestational diabetes, and labor details (induced or primary caesarean section, spontaneous) was obtained from midwife and obstetrician records.

Population for analysis

In total, 1232 women were enrolled in the Generation R Focus Study. Twin pregnancies ($n=15$) and pregnancies leading to perinatal death ($n=2$) were excluded from the present analysis. Of the remaining 1215 singleton live births, *INS* VNTR genotyping was achieved in 71% ($n=859$) of the subjects. Growth characteristics in mid- and late pregnancy and birth weight were available for all these children. In total, 653 (76%), 653 (76%) and 605 (70%) children participated in the postnatal assessments at the age of 6 weeks, 6 months and 14 months respectively.

Data analysis

First, differences in maternal, fetal and postnatal characteristics between the *INS* VNTR genotypic groups were assessed by independent sample *t*-test or Mann–Whitney *U* test for continuous variables and the χ^2 test for categorical variables. Since the insulin and insulin-like growth factor II regions (*VNTR-INS-IGF2*) are imprinted with their paternally inherited allele being expressed, the I/III heterozygote group can be considered as an indeterminate group, consisting partly of individuals in whom the I allele is expressed and partly of individuals in whom the III allele is expressed. Main interest in our analyses considering etiological associations was on the difference between the I/I and III/III heterozygous subjects. For all analyses, the I/III and III/III genotype groups were both separately compared with the I/I group (reference group). Subsequently, we used multiple linear regression models to assess the associations of *INS* VNTR with gestational age at birth, adjusting for maternal age, parity, hypertension, pre-eclampsia, gestational diabetes, induced labor, and primary caesarean section. To assess the association without the extremes of gestational age or birth weight, we also performed this analysis, excluding those children born prematurely (gestational age < 37 weeks) and small or large for gestational age (-2 SDS or $+2$ SDS respectively). Multiple linear regression models were also performed to study the association between genotype and (estimated) weight and HC and AC/HC ratio at each age cross-sectionally (prenatally: mid- and late pregnancy, at birth; postnatally: 6 weeks, 6 months, and 14 months). These models were adjusted for gender and age, and postnatal data were additionally adjusted for gestational

age at birth. Next, to examine the associations of *INS* VNTR with prospectively measured growth patterns, rather than growth characteristics at one age, we studied the differences in (estimated) weight change (SDS) and HC (SDS) change from mid-pregnancy to 14 months between genotypes using multiple linear regression models. Since it has been suggested that in children from multiparous mothers and/or in children with no postnatal growth realignment, the genetic contribution to birth weight is greater than that in the firstborn, and/or in children with growth realignment, we also examined the associations of *INS* VNTR genotype and birth weight in strata of birth order and growth realignment (6). As described previously, growth realignment was defined as a change, either increase or decrease, in weight, between birth and 14 months, of more than 0.67 SDS ('changers' means, for example, a 3rd to 10th percentile increase, and 'non-changers' means a growth realignment < 0.67 SDS) (8, 17, 29). Finally, using Pearson's χ^2 , we compared the prevalence of catch-up growth between genotypes, where catch-up growth was defined as a positive growth realignment of more than 0.67 SDS.

All effect estimates are presented with their 95% confidence interval (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics for the three genotypes are shown in Tables 1–3. Genotype frequency distribution was I/I 50.8%, I/III 40.0%, and III/III 9.2%. No differences between genotypes were found in maternal characteristics. Gender distribution was similar in the three genotypes. In mid-pregnancy, class III homozygotes had

Table 1 Maternal characteristics according to fetal insulin gene variable number of tandem repeats (VNTR) class genotype.

	I/I ($n=436$)	I/III ($n=344$)	III/III ($n=79$)
Maternal characteristics			
Age (years)	31.7 (4.1)	31.5 (4.3)	31.5 (4.0)
Weight (kg)	68.6 (11.9)	69.1 (13.1)	69.0 (12.3)
Height (cm)	171.0 (6.6)	171.1 (6.4)	170.6 (6.2)
Body mass index (kg/m ²)	23.4 (3.9)	23.6 (4.1)	23.7 (3.9)
Parity (% nulliparous)	59.1%	59.9%	59.0%
Hypertension (%)	5.1%	4.4%	7.7%
Pre-eclampsia (%)	1.2%	1.2%	1.3%
Gestational diabetes (%)	0.2%	1.2%	1.3%
Induced labor (%)	26.2%	24.1%	34.2%
Primary caesarean section (%)	3.1%	3.1%	5.1%

Values are means (SDS) or percentages. Differences were tested using independent sample *t*-test or χ^2 test. Of the total group, data were missing in maternal height before pregnancy ($n=8$), weight and body mass index ($n=146$), parity ($n=12$), hypertension ($n=4$), pre-eclampsia ($n=4$), gestational diabetes ($n=5$), induced labor ($n=35$), and primary caesarean section ($n=42$).

Table 2 Fetal characteristics according to fetal insulin gene variable number of tandem repeats (VNTR) class genotype.

	I/I (n=436)	I/III (n=344)	III/III (n=79)
Fetal characteristics mid-pregnancy			
Gestational age (weeks)	20.6 (1.0)	20.5 (1.0)	20.4 (0.9)
Head circumference (mm)	179 (12.6)	178 (12.8)	178 (11.0)
Abdominal circumference (mm)	157 (13.1)	156 (13.1)	154 (12.0)*
Femur length (mm)	33.1 (3.1)	32.9 (3.1)	32.6 (3.0)
Abdominal/head circumference ratio	0.880 (0.043)	0.880 (0.040)	0.865 (0.035) [†]
Estimated fetal weight (g)	377 (80)	371 (80)	359 (69)
Fetal characteristics late pregnancy			
Gestational age (weeks)	30.4 (1.0)	30.4 (1.0)	30.5 (1.0)
Head circumference (mm)	286 (11.7)	286 (11.7)	287 (13.0)
Abdominal circumference (mm)	265 (15.7)	267 (16.7)	267 (18.0)
Femur length (mm)	57.1 (3.1)	57.4 (2.9)	57.3 (2.8)
Abdominal/head circumference ratio	0.928 (0.042)	0.933 (0.044)	0.931 (0.046)
Estimated fetal weight (g)	1622 (255)	1648 (261)	1648 (267)

Values are means (SDS). Differences were tested using independent sample *t*-test. Of the total group, data were missing in mid-pregnancy gestational age (*n*=10), head circumference (*n*=14), abdominal circumference (*n*=12), femur length (*n*=14), abdominal circumference/head circumference ratio (*n*=36), estimated fetal weight (*n*=16), late pregnancy gestational age (*n*=6), head circumference (*n*=8), abdominal circumference (*n*=10), femur length (*n*=9), abdominal circumference/head circumference ratio (*n*=22), and estimated fetal weight (*n*=10).

**P*<0.05 versus I/I genotype; [†]*P*<0.005 versus I/I genotype.

a smaller AC and a reduced AC/HC compared with class I homozygotes. Subjects with the III/III genotype were born at a significantly shorter gestational age and had a lower birth weight than the I/I individuals. After adjusting for maternal age, parity, hypertension, pre-eclampsia, gestational diabetes, induced labor, and primary caesarean section, gestational age remained

shorter in the homozygous III/III group (differences: −0.39 (95% CI: −0.71, −0.08) weeks versus I/I). Also, when we excluded large and small for gestational age births and preterm births from the analysis, the difference in gestational age was significant (differences: −0.34 (95% CI: −0.62, −0.05) weeks versus I/I). The odds ratio for preterm birth in the III/III group

Table 3 Birth and postnatal characteristics according to insulin gene variable number of tandem repeats (VNTR) class genotype.

	I/I (n=436)	I/III (n=344)	III/III (n=79)
Birth			
Gestational age (weeks)	40.2 (36.7–42.4)	40.2 (37.6–42.4)	39.6 (36.0–42.3)*
Gestational age <37 weeks (%)	3.0%	1.7%	5.1%
Weight (g)	3510 (512)	3591 (478)	3396 (504) [†]
Weight (SDS)	0.01 (0.99)	0.14 (0.98)	−0.01 (0.90)
Weight <−2 SDS (%)	3.2%	2.3%	2.5%
Weight >+2 SDS (%)	2.1%	3.2%	1.3%
Gender (% boy)	50.0%	55.2%	50.6%
Postnatal characteristics 6 weeks			
Age at visit (weeks)	6.9 (4.6–12.7)	6.5 (4.4–10.3)	7.1 (4.2–14.9)
Head circumference (cm)	38.6 (1.4)	38.6 (1.4)	38.5 (1.8)
Weight (g)	4919 (710)	4918 (615)	4817 (802)
Length (cm)	56.7 (2.6)	57.0 (2.4)	56.3 (2.8)
Body mass index (kg/m ²)	15.2 (1.5)	15.1 (1.4)	15.1 (1.4)
Postnatal characteristics 6 months			
Age at visit (weeks)	27.7 (23.3–34.3)	28.4 (23.7–36.4)	28.3 (24.1–35.6)
Head circumference (cm)	43.8 (1.3)	44.0 (1.3)	43.9 (1.7)
Weight (g)	7889 (859)	7998 (897)	7878 (907)
Length (cm)	68.4 (2.6)	69.0 (2.7)	68.6 (2.7)
Body mass index (kg/m ²)	16.8 (1.3)	16.8 (1.3)	16.8 (1.5)
Postnatal characteristics 14 months			
Age at visit (months)	14.7 (13.4–17.5)	14.5 (13.3–16.5)	14.6 (13.5–17.3)
Head circumference (cm)	47.3 (1.4)	47.4 (1.3)	47.0 (1.6)
Weight (g)	10 490 (1074)	10 563 (1053)	10 431 (1181)
Length (cm)	79.1 (2.9)	79.3 (3.0)	78.8 (3.2)
Body mass index (kg/m ²)	16.8 (1.3)	16.8 (1.1)	16.8 (1.2)

Values are means (SDS), medians (95% range) for variables with skewed distribution, or percentages. Differences were tested using independent sample *t*-test, Mann–Whitney *U* test, or χ^2 test. Of the total group, data were missing at 6 weeks head circumference (*n*=242), weight (*n*=232), and length (*n*=235); at 6 months head circumference (*n*=234), weight (*n*=230), and length (*n*=232); and at 14 months head circumference (*n*=293), weight (*n*=262), and length (*n*=270).

**P*<0.005 versus I/I genotype; [†]*P*<0.05 versus I/I genotype.

Figure 1 Values represent differences between genotypes in SDS (95% confidence interval) based on multiple linear regression models, adjusted for (gestational) age and gender. Postnatal estimates are additionally adjusted for gestational age at birth.

versus the I/I group was not significant (1.86 (95% CI: 0.58, 5.94)). No differences in birth weight SDS were found.

Results from the cross-sectional multiple regression analyses of weight, HC, and AC/HC ratio (prenatal) at each age are shown in Figs 1–3. No differences in weight (SDS) were found at any age. In class III homozygous subjects, HC (SDS) tended to be larger in mid-pregnancy and smaller at the age of 14 months compared with class I homozygotes (differences: 0.23 (95% CI: 0.00, 0.46) SDS and -0.25 (95% CI: -0.49 , 0.00) SDS respectively), though these differences were not significant. AC/HC ratio was significantly lower in the III/III subjects than in the I/I individuals in mid-pregnancy (difference: -0.28 (95% CI: -0.47 , -0.09) SDS), but not in late pregnancy.

From mid-pregnancy to 14 months, the III/III individuals had a significant mean decrease in HC SDS compared with the I/I subjects (Table 4). No difference between the genotype groups in weight change were found during any period between mid-pregnancy and the age of 14 months.

The associations between *INS* VNTR genotypes and birth weight, stratified by birth order and weight realignment, are shown in Table 5. Among the firstborn children, subjects with the I/III genotype were significantly heavier than the I/I individuals. Birth weight was similar in the three genotype groups among children born to multiparous mothers. No difference between the three genotypes was found in birth weight

Figure 2 Values represent differences between genotypes in SDS (95% confidence interval) based on multiple linear regression models, adjusted for (gestational) age and gender. Postnatal estimates are additionally adjusted for gestational age at birth.

Figure 3 Values represent differences between genotypes in SDS (95% confidence interval) based on multiple linear regression models, adjusted for gestational age and gender.

among the 'non-changers'. Among the 'changers', however, the I/III subjects had a significantly higher birth weight than the I/I subjects after adjusting for age and gender. And finally, no differences were found between the three genotypes in the prevalence of catch-up growth between birth and the age of 14 months ($P > 0.5$, using Pearson's χ^2).

Discussion

We showed that the III/III individuals of *INS* VNTR had a shorter gestational duration compared with the I/I subjects. No differences were found in birth weight adjusted for gestational age. Class III homozygous subjects had a smaller AC/HC ratio in mid-pregnancy but not in late pregnancy, compared with the I/I individuals. In III/III, we also found a decreased growth rate in HC from mid-pregnancy to 14 months of age. No differences were found for any other growth characteristics or patterns.

To our knowledge, this study is the first prospective cohort that examined the associations between *INS* VNTR and growth in fetal life and infancy. DNA for genotyping was available in 859 Caucasian subjects (71%), and of all genotyped subjects at baseline, about 70–75% participated in the follow-up measurements in infancy. Children who were not genotyped had a shorter gestational age at birth (difference: -0.47 (95% CI: -0.72 , -0.21) weeks, $P < 0.001$) and a lower birth weight (difference: -52.2 (95% CI: -143.7 , 39.6) g, $P = 0.18$). Our effect estimates would be biased if the associations between *INS* VNTR genotype and growth characteristics differ between those with and without complete data. This seems unlikely but cannot be excluded.

The underlying mechanism explaining how *INS* VNTR influences growth remains unclear. In the pancreas, *INS* expression has been found to be lower in the VNTR class III homozygous than in the VNTR

Table 4 Differences in weight change (SDS) and head circumference (SDS) change from mid-pregnancy to 14 months using the I/I genotype as a reference group.

Genotype	Weight change (SDS)	Head circumference change (SDS)
I/I	Reference	Reference
I/III	−0.083 (−0.292, 0.127)	−0.027 (−0.236, 0.181)
III/III	−0.029 (−0.378, 0.320)	−0.443 (−0.799, −0.086)*

Values represent differences in change in SDS (95% confidence interval). Differences were tested using multiple linear regression models.
**P*<0.05 versus I/I genotype.

class I homozygous subjects (5, 10). It has also been hypothesized that the VNTR influences *IGF2*, a neighboring gene on chromosome 11p15.5 (8, 10), though studies have been conflicting. The VNTR class I allele is associated with higher *IGF2* expression in the human placenta (7), while VNTR class III homozygotes have been shown to have higher IGF-II cord blood levels (6). *INS* and *IGF2* are both imprinted genes (30). In the human yolk sac, *INS* has been shown to be exclusively paternally expressed (30), and the imprinting of the *IGF2/H19* region is known to affect growth in syndromes with early growth disorders (31). Therefore, several studies have focused on paternal-specific allele transmission of the VNTR-*INS-IGF2* region in relation to growth and diseases. Paternally derived VNTR class III allele has been shown to be associated with type 2 diabetes (16) and polycystic ovary syndrome (32), while paternally derived VNTR class I allele may be a risk factor for childhood obesity (33). These findings suggest that the greatest phenotypic difference would be between class I and III homozygous individuals, since, by definition, they have inherited an active paternal allele. Other studies however, show no parent-of-origin effect on birth weight or type 2 diabetes (6, 9). Finally, *Igf2* in mice plays an important role in placental development and regulation (34). The VNTR may affect the imprinting of *Igf2* and subsequently impair placental circulation (34).

Several studies have examined the association between *INS* VNTR and growth. In the Avon Longitudinal Study of Parents and Children cohort, Dunger *et al.* was the first to show an association between *INS* VNTR and birth size (8). In those individuals who had no postnatal weight realignment and in children from multiparous mothers, class III genotype was associated with a larger birth size (6, 8). It has been proposed that in these children the maternal uterine factors are less important and the genetic contribution to growth is

amplified (8). On the other hand, in a Pima Indian population, class III genotype was associated with lower birth weight and an increased prevalence of type 2 diabetes (9). Two other studies, one of which was performed in a large Finnish cohort, could not replicate any of the results regarding birth weight, also after stratifying for postnatal realignment (11, 12).

In our study, we found no differences in weight in fetal life, at birth or in infancy. We also did not find associations between patterns in weight gain from fetal life until infancy. Unexpectedly, class III homozygous subjects had a shorter gestational age at birth than class I homozygous subjects. No such association has been described previously. This difference remained significant after adjusting for factors that may explain a shorter gestational period, such as birth weight and pregnancy and delivery complications. Furthermore, this association was still present after excluding all large and small for gestational age births and preterm births, and therefore cannot be explained by outliers or skewed distributions. We have no explanation for this finding. It could be hypothesized that altered intra-uterine growth patterns resulting in earlier maturation or changes in placental function may cause an earlier delivery. However, this association remained significant after adjustment for birth weight. Also, additional adjustment for weight change between 20 weeks and birth did not materially affect our effect estimate (results not shown). On the other hand, this association turned up without a previous hypothesis. Therefore, this finding could be due to chance, and further studies in other population-based cohorts are needed for replication.

Class III genotype subjects also had a considerably smaller AC/HC ratio in mid-pregnancy. We found that class III homozygote subjects tended to have a larger HC and a smaller AC in mid-pregnancy. However, by late pregnancy, HC SDS was decreased and AC SDS was

Table 5 Birth weight per genotype, stratified by birth order and growth realignment.

Genotype	1st pregnancy	2nd + pregnancy	Non-changers	Changers
I/I	3421 (516)	3633 (476)	3498 (413)	3555 (541)
I/III	3512 (490)*	3668 (449)	3510 (421)	3695 (493)*
III/III	3398 (502)	3616 (467)	3462 (524)	3463 (526)

Values represent mean in grams (SDS). Differences were tested using multiple linear regression models, adjusting for gestational age and gender. Change is defined as postnatal growth realignment between birth and 14 months, positive or negative, of more than 0.67 SDS.
**P*<0.05 versus I/I genotype.

increased in these fetuses, resulting in a similar AC/HC ratio as class I/I subjects. AC/HC ratio has been shown to be useful in distinguishing symmetrical from asymmetrical growth (27). Asymmetrical growth with a relatively large HC is also known as brain sparing. Our findings may suggest that brain sparing occurs in early pregnancy in these individuals. On the basis of current literature, we did not hypothesize beforehand to find such an association specifically. Therefore, further studies are necessary to replicate these findings.

Epidemiological studies have demonstrated an inverse relationship between birth weight and the risk of developing type 2 diabetes and cardiovascular disease in adulthood (35, 36). The fetal insulin hypothesis proposes that genetic variants that regulate fetal insulin or sensitivity may lead to both impaired fetal growth and increased morbidity in later life (3). Several studies, however, have suggested that rather than low birth weight *per se* it is postnatal accelerated weight gain in subjects with a small size at birth, that leads to a normal or increasing weight from childhood onward, increasing the risk for adult disease (37–39). Animal models have shown that an altered fetal growth trajectory may also lead to an increased risk of adult morbidity, even when birth weight is normal (40). We only found differences in early fetal growth patterns between *INS* VNTR genotype variants. In our study, postnatal growth characteristics were available until the age of 14 months. Studies with a longer follow-up period are needed to assess whether this genotype is associated with growth patterns in childhood, which are related to the development of type 2 diabetes and cardiovascular disease in adulthood. It has also been suggested that prematurity, regardless of birth weight, can lead to reduced insulin sensitivity and possibly type 2 diabetes in later life (41). Our finding that shows an association between *INS* VNTR and gestational age is in line with the hypothesis that common genetic variations may underlie the association between preterm birth and increased risk of development of type 2 diabetes and cardiovascular disease.

In conclusion, this study demonstrates that *INS* VNTR is not associated with weight from fetal life until infancy. Our data suggest that *INS* VNTR is associated with asymmetrical growth in early and mid-pregnancy, but not in late pregnancy. We found for the first time an association between *INS* VNTR and gestational age at birth. Studies in larger cohorts are necessary to replicate our findings. Also, systematic searches by genome-wide association studies may enable us to obtain a more complete understanding of the functionality of the entire *VNTR-INS-IGF2* region and its relation to growth and morbidity in childhood and later life.

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