Maternal-Fetal Interactions and Birth Order Influence Insulin Variable Number of Tandem Repeats Allele Class Associations with Head Size at Birth and Childhood Weight Gain

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Polymorphism of the insulin gene (INS) variable number of tandem repeats (VNTR; class I or class III alleles) locus has been associated with adult diseases and with birth size. Therefore, this variant is a potential contributory factor to the reported fetal origins of adult disease. In the population-based Avon Longitudinal Study of Pregnancy and Childhood birth cohort, we have confirmed in the present study the association between the INS VNTR III/III genotype and larger head circumference at birth (odds ratio [OR] 1.92, 95% CI 1.23–3.07; \( P = 0.004 \)) and identified an association with higher cord blood IGF-II levels \( (P = 0.05 \) to 0.0001). The genotype association with head circumference was influenced by maternal parity (birth order): the III/III OR for larger head circumference was stronger in second and subsequent pregnancies \( (OR \ 5.0, 95\% \ CI \ 2.2–11.5; \ P = 0.00003) \) than in first pregnancies \( (1.2, 0.6–2.2; \ P = 0.8; \) interaction with birth order, \( P = 0.02) \). During childhood, the III/III genotype remained associated with larger head circumference \( (P = 0.004) \) and was also associated with greater BMI \( (P = 0.03) \), waist circumference \( (P = 0.03) \), and higher fasting insulin levels in girls \( (P = 0.02) \). In addition, there were interactions between INS VNTR genotype and early postnatal weight gain in determining childhood BMI \( (P = 0.001 \) for interaction), weight \( (P = 0.005) \), and waist circumference \( (P = 0.0005) \), such that in the ~25% of children \( (n = 286) \) with rapid early postnatal weight gain, class III genotype–negative children among this group gained weight more rapidly. Our results indicate that complex prenatal and postnatal gene–maternal/fetal interactions influence size at birth and childhood risk factors for adult disease. *Diabetes* 53:1128–1133, 2004

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ALSPAC, Avon Longitudinal Study of Pregnancy and Childhood; VNTR, variable number of tandem repeats.

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cohort (11) with regard to the INS VNTR and head size at birth by analysis of a second group of children from this cohort. In addition, we report an effect of birth order on the INS VNTR–birth size association, with possible implications for postnatal VNTR–early weight gain associations.

RESEARCH DESIGN AND METHODS

ALSPAC is a prospective study of 14,541 pregnancies recruited from all pregnancies in three Bristol-based District Health Authorities with expected dates of delivery between April 1991 and December 1992 (17). All children were measured by the ALSPAC team at birth and at 7 years of age; in addition, children from two ALSPAC subcohorts had further postnatal growth measurements. The collection of blood and/or mouth swabs for DNA fingerprinting (18) was performed at Fortnum’s (18) (a random selection of n = 1,000) and after 6 months of recruitment, were measured every 4 months to age 12 months, and at age 7 years (mean ± SD; age 8.2 ± 0.1 years; range 8.0–8.5). 851 children (750 genotyped) from the Children in Focus or control subcohorts attended the research clinic in the morning after an overnight fast. Fasting was validated by questionnaire, and children were excluded when they were taking oral steroids or had any current infection. A venous blood sample was collected after application of topical anesthetic cream (EMLA cream; AstraZeneca, London, U.K.). Samples were placed immediately onto ice, centrifuged within 30 min, and stored at −70°C until assay. Insulin was measured by enzyme-linked immunosorbent assay using a commercial kit (DSL, London, U.K.). Sensitivity was 0.26 mU/l. Intra-assay coefficients of variation were 4.4 and 5.1% at 10.3 and 35.8 mU/l, respectively, and equivalent interassay coefficients of variation were 8.7 and 2.9%, respectively.

Statistics. Changes in weight SD score between birth and 3 years were calculated, and a gain in weight SD score >0.67 was taken to indicate clinically significant "catch-up" weight gain, as 0.67 SD represents the width of each centile band displayed on standard growth charts (e.g., 2nd to 9th, 9th to 25th, and 25th to 50th centiles). Similarly, a decrease in weight SD score between 0 and 3 years by >0.67 indicated "catch-down" weight gain (11,20). Body weight, BMI, and waist circumference at 7 years and fasting insulin levels at 8 years were log transformed to normal distributions to allow parametric analyses. Differences in quantitative variables between genotypes were examined by ANOVA or t test. Interactions between genotype and postnatal weight gain or maternal parity were examined using logistic regression by centering the interactive term (genotype × weight gain). Odds ratios (ORs) for having larger- versus smaller-than-average head circumference at birth were calculated for genotypes by logistic regression and for allele transmission from parental heterozygotes by 2 × 2 contingency tables. Analyses were performed using SPSS for Windows version 9.0 (SPSS, Chicago, IL). Quantitative transmission distortion tests were calculated using the method of Robinowitz (21). Equality of ORs for allele transmission from mothers and fathers was compared using the "mnhods" routine in the statistical package Stata. Parent-of-origin effects for allele transmission were explored using the methods described by Weinberg et al. (22,23) and Whitaker et al. (24) with and without allowing for parental genotype effects.

RESULTS

Association with birth size. As observed in our initial study of the Children in Focus subcohort (11), in normal, full-term, singleton infants from the second ALSPAC subcohort (control cohort), offspring INS VNTR class III genotype was associated with a 0.5-cm larger mean head circumference at birth than other genotypes (mean ± SD; I/I, 34.8 ± 1.4 cm; n = 189; I/III, 34.8 ± 1.3 cm, n = 184; and III/III, 35.3 ± 0.8 cm, n = 27; ANOVA: I/I vs. I/III vs. III/III, P = 0.04; I vs. III/III, P = 0.01, adjusted for sex and gestational age). However, we found no association with birth weight (I/I, 3,473 ± 436 g; I/III, 3,419 ± 437; and III/III, 3,448 ± 435; ANOVA: P = 0.5) or birth length (I/I, 50.7 ± 1.8 cm; I/III, 50.5 ± 1.8; and III/III, 50.6 ± 1.8; ANOVA P = 0.7). In 353 children with cord blood insulin and INS-II data (15), the III/III genotype was overall weakly associated with higher IGF-II levels (geometric means: I+ vs. III/III 2.63 vs. III/III 2.89 ng/ml; P = 0.05) but not with insulin levels (I+ vs. III/III 3.66 vs. III/III 3.50 mU/l; P = 0.7).

In parent-offspring “tiers” validated by DNA fingerprinting, class III allele transmission from heterozygous parents was associated with larger head circumference at birth (OR for head circumference larger than the mean 1.92, 95% CI 1.23–3.01; 317 informative transmissions; P = 0.004). Similar results were obtained using a quantitative transmission distortion test (P = 0.02, adjusted for sex, gestational age, and parity; P = 0.002, with further adjustment for postnatal weight gain 0–3 years). ORs for class III transmission from mothers (2.36, 1.12–4.96) and fathers (2.35, 1.11–4.99) were statistically significant.
(2.30, 1.10–4.80) were not different ($\chi^2 = 0.002, P = 0.96$). No parent-of-origin effects were detected for allele transmission to larger or smaller head circumference ($P = 0.6–0.7$), using the methods of Weinberg et al. (22,23) based on 344 informative parent-offspring trios, or to head circumference as a quantitative trait ($P = 0.7$) using the method of Whittaker et al. (24).

**Fetal gene × maternal-uterine environment interactions with birth size.** In both cohorts, the III/III genotype association with larger head circumference at birth showed significant interaction with postnatal weight gain 0–3 years ($P = 0.02$ interaction) and was more apparent in nonchangers (difference between mean head circumference in III/III vs. I+ = 0.7 cm) than in “changers” (difference = 0.1 cm). The III/III genotype association with higher cord blood IGF-II levels was also stronger in nonchangers ($P = 0.0001$) (Table 1) than in changers ($P = 0.4$; interaction with postnatal weight gain, $P = 0.003$), but again no genotype associations were seen with birth weight, birth length, or cord blood insulin levels. The III/III genotype association with larger head circumference at birth also showed significant interaction with birth order (interaction $P = 0.02$) (Fig. 1), such that the association was observed in the offspring of mothers’ second and subsequent pregnancies (OR 5.0, 95% CI 2.2–11.5; $P = 0.00003$) but was not evident in first-born children (1.2, 0.6–2.2; $P = 0.8$), who were presumably more restrained with respect to growth in utero.

**Association with childhood size.** In an analysis of the combined data from both subcohorts, the INS VNTR III/III genotype association with larger head circumference persisted at age 7 years ($P = 0.004$) (Table 2), and the III/III genotype was also associated with larger BMI ($P = 0.03$), body weight ($P = 0.02$), and waist circumference ($P = 0.03$) (Table 2). As with size at birth, postnatal size was identical in I/I and I/III children (Table 2). Furthermore, in girls but not in boys, the INS VNTR III/III genotype was associated with higher fasting insulin levels at age 8 years ($P = 0.02$) (Table 3), and parental transmission of class III versus class I alleles was also associated with increased risk of having a fasting insulin level higher than the average (OR 2.9, 95% CI 1.2–6.5; $P = 0.02$). No differences in fasting glucose levels were seen by genotype in girls ($P = 0.4$) or boys ($P = 0.6$, data not shown).

The association of the INS VNTR with postnatal weight gain was complicated by our observation that, within the $\sim 25\%$ of children who showed a postnatal catch-up weight pattern from 0 to 3 years, class I+ genotypes were

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**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>I+ vs. III/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU/l)</td>
<td>Mean 3.7 (2.2–4.9)</td>
<td>3.4 (2.0–4.1)</td>
<td>3.6 (2.0–5.4)</td>
<td>$P = 0.8$</td>
</tr>
<tr>
<td>$n$</td>
<td>64</td>
<td>58</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>Mean 85.6 (67–102)</td>
<td>87.8 (67–99)</td>
<td>110.9 (76–117)</td>
<td>$P = 0.2$</td>
</tr>
<tr>
<td>$n$</td>
<td>64</td>
<td>58</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>IGF-II (ng/ml)</td>
<td>Mean 261 (215–317)</td>
<td>258 (225–308)</td>
<td>375 (278–429)</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td>$n$</td>
<td>64</td>
<td>58</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Data are geometric means (interquartile range), adjusted for sex.
associated with higher BMI at age 7 years than the III/III genotype (Fig. 2). Therefore, there was a significant interaction between early postnatal weight gain 0–3 years and genotype on BMI at 7 years (interaction \( P = 0.001 \)). Similar interactions were seen between early postnatal weight gain and genotype on body weight (interaction \( P = 0.005 \)) and waist circumference at 7 years (interaction \( P = 0.0005 \)). However, the marked genotype–birth order interaction on birth size was no longer evident in relation to BMI at age 7 years (genotype–birth order interaction, \( P = 0.4 \)).

DISCUSSION

In a second ALSPAC subcohort of normal, full-term, singleton infants, which was completely independent of the previous Children in Focus subcohort (11), we have replicated our finding that offspring INS VNTR class III/III genotype was associated with larger head circumference at birth. Furthermore, we have excluded the possibility of confounding as a result of population stratification by showing that parental transmission of class III alleles from heterozygous parents was also associated with larger head circumference at birth. The mechanism underlying the association between the III/III genotype and larger head circumference is unknown but is consistent with our observation of higher cord blood IGF-II levels in III/III+ children. These findings are not in keeping with data from ex vivo studies that have associated the class III allele with lower IGF-II transcription in the placenta (14). However, inconsistencies between transcription studies and in vivo observational studies are not unusual, and it is likely that the underlying biochemistry and mechanisms involved are complex. The effects of the INS VNTR genotype on head size and IGF-II levels at birth in this population-based sample of normal children were confined to III/III homozygotes, with I/I and I/III (I+) infants being indistinguishable. In a two-allele system, the absence of any heterozygote effect of the class III allele on quantitative trait outcomes may be surprising and suggests a nonadditive effect of the class III allele. Alternatively, it is possible that the association of certain INS VNTR haplotypes with body size and other traits may be affected by other functional polymorphisms in close vicinity, for example, in the INS or IGF2 genes, as previous reports have suggested (25).

Consistent with our earlier study (11), these associations were more apparent in nonchangers (i.e., little realignment in postnatal weight centile position); whereas in changers, we postulate that the greater effects of maternal-uterine environment may obscure the genetic association with birth size. In our original report, we also found genotype associations with birth weight and length in nonchangers (11). However, in this second control cohort, we were unable to confirm these findings. This is not unexpected because the weight and length associations with the VNTR were smaller than those for head circumference and the second cohort had fewer subjects. As we have previously suggested, fetal head circumference may be less affected by maternal-uterine “restraint” of fetal growth and may be a more sensitive marker of the fetal genetic growth potential (11).

In support of the changers/nonchangers model of maternal-fetal interaction, we also observed a significant interaction with birth order, and this was independent of maternal age (data not shown). Maternal-uterine restraint of fetal growth is greatest in mothers’ first pregnancies as manifested by smaller size at birth and compensatory postnatal catch-up weight gain (16). In these offspring, we saw no genotype association with head circumference at birth, in contrast to a much clearer effect in subsequent offspring. Earlier observations have also shown that inheritance of birth size seems to be reduced in first pregnancies (26). In this contemporary ALSPAC cohort, maternal-uterine restraint of fetal growth is unlikely to be related to maternal nutrition but rather may be more closely linked to other maternal factors, including mother’s own birth weight and maternal genotype (27,28). The degree of restraint may also be related to father’s height (20) or, in animal cross-breeding studies, to paternal size (29), thus supporting a maternal sensing of fetal growth potential as suggested by Haig’s hypothesis of a between-parent conflict of interests in fetal growth (30). This model of birth-order interaction is consistent with our observation that INS VNTR genetic associations are strongest in nonchangers of postnatal weight centile position, whose fetal

**TABLE 2**

Size at birth and at 7 years by INS VNTR genotype, in ALSPAC children from both subcohorts (Children in Focus and Control)

<table>
<thead>
<tr>
<th>INS VNTR genotype</th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head circumference at birth (cm)</td>
<td>34.9</td>
<td>34.9</td>
<td>35.3†</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3,495</td>
<td>3,489</td>
<td>3,552</td>
</tr>
<tr>
<td>Head circumference at 7 years (cm)</td>
<td>52.5</td>
<td>52.5</td>
<td>52.9†</td>
</tr>
<tr>
<td>Weight at 7 years (kg)</td>
<td>25.4</td>
<td>25.3</td>
<td>26.3*</td>
</tr>
<tr>
<td>BMI at 7 years (kg/m²)</td>
<td>16.1</td>
<td>16.1</td>
<td>16.5*</td>
</tr>
<tr>
<td>Waist circumference at 7 years (cm)</td>
<td>56.4</td>
<td>56.3</td>
<td>57.3*</td>
</tr>
</tbody>
</table>

\( P \) values for III/III vs. the I+ genotype: *\( P < 0.05 \), †\( P < 0.005 \). Adjusted for sex and change in weight SD score 0–3 years; size at birth was also adjusted for gestational age at birth and birth order.

**TABLE 3**

Fasting insulin levels (mU/l) at age 8 years by INS VNTR genotype and sex

<table>
<thead>
<tr>
<th></th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Girls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.1 (3.1–7.7)</td>
<td>5.9 (3.6–9.0)</td>
<td>6.5 (3.8–11.6)</td>
<td>( P = 0.02 )</td>
</tr>
<tr>
<td>( n )</td>
<td>154</td>
<td>164</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.1 (3.0–7.8)</td>
<td>4.8 (2.9–7.1)</td>
<td>4.5 (2.8–6.1)</td>
<td>( P = 0.2 )</td>
</tr>
<tr>
<td>( n )</td>
<td>195</td>
<td>177</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

Data are geometric means (interquartile range). There was significant interaction between genotype and sex on fasting insulin levels (interaction, \( P = 0.012 \)).
Interaction with maternal-uterine environment could make analyses of genetic associations with size at birth difficult to interpret. The III/III genotype association with lower birth weight reported in Pima Indians (31) could possibly relate to other maternal-uterine interactions in this population with a high prevalence of gestational diabetes. Maternal INS VNTR genotype might contribute to variations in maternal glucose levels and could further confound offspring genotype associations with birth size. We found no association between maternal genotype and offspring size at birth (data not shown). However, we did not have data on maternal glucose levels, and gestational diabetes was rare in this normal birth cohort.

The head circumference size advantage of the III/III genotype persisted to age 7 years. We also observed overall III/III genotype associations with higher BMI and waist circumference and, in girls, higher fasting insulin levels. These findings are consistent with class III associations with type 2 diabetes (3–5), central obesity and insulin resistance (6), and polycystic ovary syndrome (7,8) in adults. Le Stunff et al. (9,10) reported higher childhood weight gain, BMI, and insulin levels associated with class I alleles rather than the III/III genotype. Those children had severe obesity (BMI >99th centile), which developed before age 6 years, although no earlier growth data were reported. Class I+ genotypes have also been associated with increased risk of hyperinsulinemic hyperandrogenism in girls who present with precocious pubarche, who show a characteristic sequence of low birth weight, rapid postnatal growth, and increased central obesity (32). Similarly, in our current study, among the subgroup of ~25% (286 of 1,207) ALSPAC children who showed early catch-up growth, class I+ children showed greater postnatal BMI and waist circumference than III/III genotype children (Fig. 2). In ALSPAC and, recently, in other large population studies, rapid early postnatal weight gain has been shown to be a strong predictor for subsequent childhood obesity (20,33,34). The association of class I positivity with larger body size in “catch-up” children could reflect some level of resistance of III/III children to maternal restraint of fetal growth in utero and, thus, less prone than class I+ children to catch-up weight gain after birth. In addition, certain class I haplotypes may have specific effects on early weight gain (25). In contrast, III/III genotype–related disease risks would predictably be more obvious among subjects who showed average early postnatal weight gain, nonchangers, as was observed in our smaller study of impaired glucose tolerance/type 2 diabetes in 50-year-old Hertfordshire men (5).

Studies of populations with type 2 diabetes, polycystic ovary syndrome, or childhood obesity have shown association with paternal-specific INS VNTR allele transmission (4,8,10,35). Although the INS VNTR itself is not transcribed, allelic effects on birth size that are mediated through INS or IGF2 expression might be expected to show parent-of-origin differences; in humans, exclusive paternal expression of INS has been shown in the yolk sac (36) and of IGF2 in the preterm placenta (37). We did not observe any significant parent-of-origin effects, although our study was underpowered to detect a small effect. It is possible that imprinting of IGF2 varies between subjects and between tissues. For example, both alleles are expressed in murine fetal leptomeninges (38). Alternatively, our findings may indicate an unidentified interaction with maternal genotype, the possibility that the INS VNTR directly effects IGF2 imprinting, that the effects on IGF-II levels are consequent to other biochemical changes, or that our results are due to linkage disequilibrium with another functional polymorphism. We also observed higher insulin levels in III/III girls but not in boys. These sex differences may reflect prepubertal changes in body composition in girls or earlier pubertal development even at age 8 years. Even in the girls, this genotype association with insulin levels was not independent of body weight, and we therefore cannot conclude that the INS VNTR directly affects postnatal insulin levels in these children.

FIG. 2. BMI at 7 years by INS VNTR genotype and change in weight SD scores between 0 and 3 years. The III/III genotype association with larger BMI was only apparent in subjects who did not show rapid catch-up early weight gain (P = 0.001). This finding reflected a significant interaction between genotype and early postnatal weight gain on subsequent childhood BMI (interaction P = 0.001). Mean BMI was identical in I/I and I/III subjects. Data are means ± SE.
These observations require confirmation. However, they illustrate that simple paradigms of either genetic or environmental factors are unlikely to fully explain links among birth size, postnatal growth, and adult disease risks. Both size at birth and early postnatal weight gain are determinants of perinatal survival and, thus, are likely subject to strong evolutionary pressures. Similar maternal versus fetal gene interactions are likely to underpin other risk factors for adult disease.

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