The insulin gene VNTR: associations and interactions with childhood body fat mass and insulin secretion

Short title: INS VNTR, fat mass and insulin secretion

Barbara Heude¹, Clive J Petry², the ALSPAC study team³, Marcus Pembrey⁴, David B Dunger², Ken K Ong¹,²

¹Medical Research Council Epidemiology Unit, Cambridge, UK
²Department of Paediatrics, University of Cambridge, UK
³Unit of Paediatric and Perinatal Epidemiology, Department of Community Based Medicine, University of Bristol, UK
⁴Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, UK

Correspondence to:
Dr Ken Ong
MRC Epidemiology Unit
Strangeways Research Laboratory, Wort's Causeway
Cambridge CB1 8RN UK
Tel: +44 1223 740 003
Fax: +44 1223 740050
Email: ken.ong@mrc-epid.cam.ac.uk
**Abstract**

**Context** Polymorphism at the insulin gene (INS) VNTR shows variable associations with childhood BMI in different populations.

**Objective** To study INS VNTR associations with body composition and insulin secretion in children.

**Design** Prospective birth cohort study

**Participants** 947 children genotyped for the INS VNTR

**Main outcome measures** Whole body DXA at 9 years to estimate height-corrected fat mass index (FMI), truncal fat index (TFI) and fat-free mass (FFM). Insulin secretion post-oral glucose at 8y.

**Results** Homozygous III/III children had higher BMI (p=0.020), FMI (p=0.015) and TFI (p=0.022) at 9y than class I bearers, but no difference in FFM (p=0.23). Gain in weight SD score between birth to 3y was positively associated with BMI, FMI and TFI in class I bearers, but not in III/III children (p-interaction with genotype = 0.009 to 0.066). INS VNTR genotype was not overall associated with insulin secretion at 8y (p=0.64), but class I bearers showed a stronger positive correlation between insulin secretion and BMI at 8y (regression coefficient ± SE: 0.26±0.05, p<0.0001) than III/III children (-0.10±0.07, p=0.48) (p-interaction=0.003).

**Conclusion** We clarified that the overall association between INS VNTR class III/III genotype and larger BMI in this population relates to fat mass, but not fat-free mass. In contrast, among the sub-group of children who showed rapid infancy weight gain, class I bearers tended to have larger BMI and fat mass than III/III children. This genetic interaction could relate to insulin secretion, which in class I bearers increased more rapidly with overweight and obesity.
Introduction

Hopes of preventing childhood obesity rely not only on effective interventions, but also on the early identification of those children who are at highest risk of becoming overweight or obese. Several studies have recently reported that rapid weight gain during early postnatal life predicts subsequent increased obesity risk during later childhood, and this risk persists in young adults (1-4). We hypothesised that childhood adiposity relates to both genetic factors and rate of early postnatal weight gain.

We recently reported confirmation of association between the insulin gene (INS) variable number of tandem repeat (VNTR) polymorphism and size at birth in the Avon Longitudinal Study of Parents and Children (ALSPAC), both by repeated association in a 2nd ALSPAC sub-group, and by demonstrating association with parental transmitted alleles (5). The class III alleles were recessively associated with larger head circumference at birth, higher cord blood levels of insulin-like growth factor-2, and those associations showed significant interaction with birth order and postnatal rapid weight gain, a marker of previous intra-uterine growth restriction.

In that study of normal representative UK children, we also observed that class III/III children had greater body weight, BMI and waist circumference at age 8 years; however those postnatal associations appeared to be reversed among the 25% of children who showed early postnatal rapid weight gain (5). Similarly, among obese French children the INS VNTR class I alleles, rather than the class III, were associated with increased BMI gains (6). A further study in obese children reported that the INS VNTR was a quantitative trait locus for the insulin response to oral glucose, and those
authors hypothesised that class I alleles could confer greater insulin secretion for the degree of BMI compared to class III alleles (7).

We have now reanalysed our existing INS VNTR genotype data in the ALSPAC children with new phenotype data on body composition at age 9 years, in order to distinguish between genetic associations with fat mass or lean mass. We also aimed to confirm the reported interactive effects of INS VNTR genotype and BMI on insulin secretion.

**Methods**

**Subjects**

As part of the ALSPAC study, previously described (8), the “Children in Focus” and “Control” sub-cohorts were measured at age 3 years, in addition to the whole ALSPAC cohort measurements at birth, 7 years and 9 years, when all children also had a whole body dual X-ray emission absorptiometry (DXA) scan. Analysis of body composition at age 9 years was based on 947 children with full data on DXA parameters, early growth phenotypes and INS VNTR genotype. Analysis of insulin secretion at age 8 years was based on 750 children with complete data. The subjects analysed in this paper did not differ from the whole ALSPAC cohort with respect to childhood growth at age 9 years (data provided to reviewers). Preparation of DNA and genotyping for INS VNTR were previously described (5).

**Body composition**

At age 9.9 (+/- 0.33) years all ALSPAC children were invited to attend for a 3-hour hands-on assessment. Height was measured with shoes and socks removed using a
Harpenden stadiometer (Holtain Ltd, Crymych, Pembs, UK). Weight was measured using a Tanita TBF 305 body fat analyser and weighing scales (Tanita UK Ltd, Yewsley, Middlesex, UK). Total fat (FM), central fat and lean mass (LM) were measured using a Lunar Prodigy DXA scanner (GE Medical Systems Lunar, Madison, WI, USA). The scans were visually inspected and realigned where necessary. Trunk fat mass was estimated using the automatic region of interest that included chest, abdomen and pelvis.

Body composition variables were corrected for differences in height by calculating the fat mass index (fat mass/ height$^2$) and truncal fat mass index (truncal fat mass/ height$^2$) (9).

**Insulin secretion**

At age 8 years (mean ± SD: 8.2 ± 0.1, range 8.0 to 8.5 years), 851 children (750 with INS VNTR genotype data) from the Children in Focus or Control sub-cohorts attended the research clinic to in the morning after an overnight fast (10). Fasting was validated by questionnaire and data were excluded if they were taking oral steroids, or had any current infection. A venous blood sample was taken to measure glucose and insulin levels, before and 30-min after an oral glucose load (1.75 g/kg, maximum 75g) as a drink (Lucozade Energy Original, GlaxoSmithKline PLC, Greenford, Middlesex, UK).

Insulin sensitivity was estimated using the Homeostasis model computer program, kindly provided by Dr Jonathan Levy, University of Oxford, UK (11). Insulin secretion was calculated as the corrected insulin response from 30mins insulin and glucose levels: [Insulin30 / (Glucose30 x (Glucose30 – 3.9))] (mU/mmol$^2$) (12).

Following oral glucose there is a dose-response relationship between glucose and
insulin (12), and this correction for the attained glucose level has been shown to correlate most closely with the first-phase insulin response (13). Similar results were found when insulin secretion was estimated using the insulinogenic index (insulin30 – insulin0) / (glucose30 – glucose0) (14).

Statistical analysis

All data were explored for normality of distribution and log transformed where appropriate. The association between genotype and phenotype was analysed using a linear regression model under both global and recessive models. For the association with DXA, adjustment was performed on age, gender, parity, education level of the mother and change in weight between birth and 3 years.

Interaction between genotype and postnatal weight gain was examined by introducing and testing the interactive term (genotype×weight gain) in the previous models. Adjusted regression coefficients are shown separately for each genotype group.

The association between genotype and insulin secretion at 8 years was examined adjusting for age, gender and BMI at 8 years. The interaction effect between BMI and genotype was analysed introducing and testing the interactive term (genotype×BMI).

Analyses were performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL).
Results

Mean body size and body composition at age 9 years are displayed by genotype in Table 1. Overall, homozygous III/III children had larger body weight, BMI, fat mass index and truncal fat mass index at age 9 years than class I bearers (p=0.01 to 0.02), but there was no difference in fat-free mass (Table 1).

Rate of early postnatal weight gain between birth to 3 years was positively associated with BMI at age 9 years in class I/I and I/III children (p<0.0001), but not in III/III children (p=0.74); and the difference in regression slopes between class I bearers and homozygous III/III children was significant, (p-interaction with genotype=0.009; Table 2, Figure 1). Similarly, rate of weight gain between birth to 3 years was positively associated with fat mass index at age 9 years in class I bearers (p<0.0001), but not in III/III children (p=0.68), (p-interaction=0.064; Table 2).

Mean fasting insulin levels, insulin sensitivity and parameters of insulin secretion at age 8 years are displayed by genotype in Table 3. No differences in mean values were observed between genotypes. However, in class I bearers insulin secretion showed a much closer correlation with BMI at 8 years than in homozygous III/III children (p-interaction=0.003; Table 3; Figure 2). A closer correlation between insulin secretion and insulin sensitivity was also seen in class I bearers (I/I: r= -0.22, p<0.0001; I/III: r= -0.31, p<0.0001) than in homozygous III/III children (III/III: r=0.09, p=0.6) (p-interaction=0.01).
**Discussion**

In this representative cohort of UK children the *INS* VNTR class III/III genotype was associated with increased childhood body fat mass in the overall population. These results do not represent independent confirmation of our previous reported association between *INS* VNTR with BMI at age 8 years in this same group of subjects (5), however they clarify that the association is with body fat mass rather than lean mass.

Furthermore, we provide significant confirmation of two recently reported genetic interactions, which many go some way towards explaining the different *INS* VNTR genotype associations seen in different childhood sub-groups. First, as we previously observed (5), the III/III genotype association with larger size was particularly seen among those children who did not gain weight rapidly during infancy. In contrast, with increasing rate of early postnatal weight gain class I bearers, but not III/III genotype children, had larger BMI and fat mass at age 9 years. Secondly, similar to another previous study (7), we found that in class I bearers insulin secretion increased significantly more rapidly with increasing BMI compared to class III/III children.

In childhood populations obesity risk has been variably associated with either class III or class I *INS* VNTR alleles. Among children with early onset obesity those with the class I allele had greater BMI gains (6), and class I allele have been associated with overweight in a further French childhood growth study (15); in contrast in another study of non-obese girls the III/III homozygotes had greater fat mass by DXA (16). Recent adult population studies the *INS* VNTR showed no overall association with obesity risk (17, 18). Our findings of genetic interaction with early postnatal weight gain on subsequent BMI and body composition could provide a possible explanation for these contrasting reports. Our findings could suggest two separate pathways to
later obesity risk: rapid infant weight gain associated with the *INS* VNTR class I allele, and non-rapid infant weight gain associated with the III/III genotype. Genetic association studies for obesity should consider such potential interactions with early postnatal weight gain.

A further complexity is that effects of *INS* VNTR alleles may differ according to parent-of-origin (19). Among French children with early onset obesity, those who inherited a class I allele from their father (but not from their mother) had a 1.8-fold increased risk of obesity (20). Our studies in this ALSPAC sub-cohort do not have sufficient power to explore parent-of-origin effects (5). Selection of children with extreme early-onset obesity would also provide much greater power to detect the class I association with obesity, however population-based studies are more generalisable and may be more likely to confirm interactive effects with the wider range of early growth trajectories.

Our studies have consistently shown apparent recessive effects of class III alleles on size at birth, childhood growth and IGF2 protein levels at birth (5, 21), and the mean values and regression coefficients reported here in I/I and I/III children were near identical. However, other studies have found apparent dominant effects of class III alleles (7, 22, 23). Recently Rodriguez et al. reported confirmation of associations with a haplotype in IGF2-INS-TH region tagged by *INS* VNTR class I alleles, together with allele A of *IGF2 ApaI*, and allele 9 of *TH01*, with both lower BMI and lower insulin secretion in adult men from two population-based UK cohorts (24, 25). Thus, the genetic associations we observed could possibly be explained by specific
sub-types of INS VNTR allele class, or by linkage disequilibrium with other neighbouring variants (26).

The INS VNTR could directly influence postnatal body size by altering INS or IGF2 transcription in utero (23, 27), or during postnatal life. The increased type 1 diabetes risk associated with class I alleles (22, 26) has been attributed to reduced fetal thymic insulin mRNA expression leading to loss of immune tolerance to insulin (23, 28). In contrast to those studies in the thymus, in transfected pancreatic cell lines and fetal pancreas class I alleles are associated with increased insulin mRNA expression (29)(30). Polymorphism at the INS VNTR minisatellite may alter a transcription promoter binding site (31), and transfection of class I alleles has been reported to influence alternative splicing of INS intron 1 resulting in longer, mature mRNA transcripts and higher proinsulin levels (32). In adults class I allele are associated with pulsatility of beta-cell insulin secretion (33).

Thus, it is possible that the class I allele, could confer a greater responsiveness in insulin secretion to changes in BMI and insulin sensitivity, and therefore a predisposition to storing adipose rather than lean tissue during periods of rapid weight gain, or in obese children (6). This could predispose to future type 2 diabetes, in particular related to the development of insulin resistance. Conversely, relatively lower insulin secretion in class III/III subjects in the face of increasing BMI or insulin resistance could also contribute to increased type 2 diabetes risk (34). Recent large studies in adults have shown no overall influence of INS VNTR genotype on type 2 diabetes risk (35). However, similar to obesity risk, we hypothesise that the
pathogenesis and phenotype of type 2 diabetes could differ according to both early weight gain patterns and INS VNTR genotype.

In conclusion, in these representative children the INS VNTR class III/III genotype was overall associated with increased adiposity. However significant genetic interactions were seen with BMI and insulin secretion that could also support a class I allele predisposition to obesity following rapid infant weight gain. Different INS VNTR genotypes and patterns of early postnatal weight gain could underlie discrete developmental pathways to obesity and type 2 diabetes risks.

Acknowledgments

We are extremely grateful to all the children and parents who took part in the study and to the midwives for their co-operation and help in recruitment. ALSPAC is supported by the Medical Research Council (MRC), the Wellcome Trust, the Department of Health, the Department of the Environment, the European Commission, and many others. DD is supported by the Wellcome Trust and the Juvenile Diabetes Research Foundation.
Table 1: Body size and body composition at age 9 years, stratified by INS VNTR genotype.

<table>
<thead>
<tr>
<th></th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=460</td>
<td>N=420</td>
<td>N=67</td>
<td>p1</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>34.7 (34.1, 35.3)</td>
<td>34.6 (34.0, 35.2)</td>
<td>36.7 (35.1, 38.2)</td>
<td>0.048</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.4 (138.9, 140.0)</td>
<td>139.2 (138.7, 139.8)</td>
<td>140.3 (139.0, 141.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>17.6 (17.3, 17.8)</td>
<td>17.5 (17.3, 17.8)</td>
<td>18.4 (17.6, 18.9)</td>
<td>0.072</td>
</tr>
<tr>
<td>Fat mass index (Kg/m²)</td>
<td>3.78 (3.63, 3.97)</td>
<td>3.67 (3.49, 3.86)</td>
<td>4.35 (3.86, 4.85)</td>
<td>0.077</td>
</tr>
<tr>
<td>Truncal fat index (Kg/m²)</td>
<td>1.45 (1.38, 1.54)</td>
<td>1.40 (1.32, 1.48)</td>
<td>1.68 (1.46, 1.93)</td>
<td>0.065</td>
</tr>
<tr>
<td>Fat free mass (Kg)</td>
<td>24.3 (24.1, 24.1)</td>
<td>24.3 (24.1, 24.6)</td>
<td>24.9 (24.3, 25.6)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Results are Means or Geometric Means (95% CI), and are adjusted for age, gender, mother’s education level, parity and weight change between 0 to 3 years.

p1: Difference between means under the global model (I/I vs. I/III vs. III/III)

p2: Difference between means under the recessive model (III/III vs. I/I and I/III)
Table 2: Correlations between early postnatal weight gain (change in weight SD score from birth to 3 years) and body size and composition at 9 years, stratified by INS VNTR genotype.

<table>
<thead>
<tr>
<th>Correlation with weight gain</th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>p-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3y:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight at 9y (Kg)</td>
<td>2.88 ± 0.29**</td>
<td>3.19 ± 0.29**</td>
<td>1.32 ± 1.12</td>
<td>0.086 0.037</td>
</tr>
<tr>
<td>Height at 9y (cm)</td>
<td>2.24 ± 0.25**</td>
<td>2.19 ± 0.25**</td>
<td>1.94 ± 0.75*</td>
<td>0.90 0.94</td>
</tr>
<tr>
<td>BMI at 9y (Kg/m²)</td>
<td>0.89 ± 0.11**</td>
<td>1.08 ± 0.12**</td>
<td>0.08 ± 0.45</td>
<td>0.025 0.009</td>
</tr>
<tr>
<td>Fat mass index at 9y (Kg/m²)</td>
<td>0.68 ± 0.09**</td>
<td>0.73 ± 0.10**</td>
<td>0.04 ± 0.37</td>
<td>0.049 0.066</td>
</tr>
<tr>
<td>Truncal fat index at 9y (Kg/m²)</td>
<td>0.35 ± 0.05**</td>
<td>0.37 ± 0.05**</td>
<td>0.02 ± 0.19</td>
<td>0.17 0.064</td>
</tr>
<tr>
<td>Fat free mass at 9y (Kg)</td>
<td>1.12 ± 0.12**</td>
<td>1.33 ± 0.12**</td>
<td>0.83 ± 0.40*</td>
<td>0.23 0.31</td>
</tr>
</tbody>
</table>

Regression coefficients are displayed (± SE), adjusted for age, gender, mother’s education level, and parity.

P-values for regression coefficients within each group: *p<0.05; **p<0.0001

P-interaction: p1: Difference between regression coefficients under the global model (I/I vs. I/III vs. III/III); p2: Difference between regression coefficients under the recessive model (III/III vs. I/I and I/III)
**Table 3:** Insulin sensitivity and secretion at 8 years, stratified by *INS* VNTR genotype.

<table>
<thead>
<tr>
<th></th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=349</td>
<td>N=341</td>
<td>N=60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>5.1 (4.8, 5.5)</td>
<td>5.2 (4.9, 5.6)</td>
<td>5.2 (4.4, 6.2)</td>
<td>0.86</td>
<td>0.90</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.9 (4.9, 5.0)</td>
<td>5.0 (4.9, 5.0)</td>
<td>5.0 (4.9, 5.1)</td>
<td>0.66</td>
<td>0.44</td>
</tr>
<tr>
<td>Insulin sensitivity (%HOMA)</td>
<td>189 (177, 201)</td>
<td>185 (173, 197)</td>
<td>183 (156, 213)</td>
<td>0.87</td>
<td>0.82</td>
</tr>
<tr>
<td>Corrected insulin response (mU/mmol²)</td>
<td>1.38 (1.27, 1.51)</td>
<td>1.39 (1.27, 1.52)</td>
<td>1.49 (1.20, 1.84)</td>
<td>0.83</td>
<td>0.55</td>
</tr>
<tr>
<td>Insulinogenic index (mU/mmol)</td>
<td>12.7 (11.7, 13.9)</td>
<td>13.2 (12.0, 14.4)</td>
<td>14.4 (11.6, 17.8)</td>
<td>0.58</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Results are Means or Geometric Means (95% CI), adjusted for age and gender.

p1: Difference between means under the global model (I/I vs. I/III vs. III/III)
p2: Difference between means under the recessive model (III/III vs. I/I and I/III)
Table 4: Correlations between BMI at 8 years and indices of insulin sensitivity and insulin secretion at 8 years, stratified by INS VNTR genotype.

<table>
<thead>
<tr>
<th></th>
<th>I/I</th>
<th>I/III</th>
<th>III/III</th>
<th>p-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlations with BMI at 8y:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>-17.6 ± 2.7**</td>
<td>-13.2 ± 2.4**</td>
<td>-14.2 ± 5.1*</td>
<td>0.72 0.92</td>
</tr>
<tr>
<td>(%HOMA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected insulin response</td>
<td>0.24 ± 0.05**</td>
<td>0.29 ± 0.05**</td>
<td>-0.10 ± 0.07</td>
<td>0.011 0.003</td>
</tr>
<tr>
<td>(mU/mmol²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>1.48 ± 0.29</td>
<td>1.52 ± 0.26</td>
<td>-0.64 ± 0.75</td>
<td>0.015 0.004</td>
</tr>
<tr>
<td>(mU/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Regression coefficients are displayed (± SE), adjusted for age and gender.

P-values for regression coefficients within each group: *p<0.05; **p<0.0001

P-interaction: p1: Difference between regression coefficients under the global model (I/I vs. I/III vs. III/III); p2: Difference between regression coefficients under the recessive model (III/III vs. I/I and I/III)
**Figure 1:** BMI at age 9 years was positively related to early postnatal weight gain (change in weight SD score from birth to 3 years) in *INS VNTR* class I bearers (I/I and I/III: p<0.0001, N=880) but not in III/III children (p=0.74, N=67) (p-interaction with genotype = 0.009).
Figure 2: Insulin secretion at age 8 years (corrected insulin response) was positively related to BMI at age 8 years in INS VNTR class I bearers (I/I and I/III, p<0.0001, N=690) but not in III/III children (p=0.48, N=60) (p-interaction=0.003).
References


