Insulin gene VNTR genotype is associated with insulin sensitivity and secretion in infancy

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Summary

AIMS We have previously demonstrated that insulin sensitivity and secretion at age 1 year was in part related to variation in weight and height gain during infancy. In order to determine whether genetic variation at the insulin gene could also influence these associations, we have studied the relationship between insulin gene variable number of tandem repeat (*INS* VNTR) genotypes, insulin secretion and early postnatal growth.

METHODS We assessed fasting and dynamic insulin secretion in 99 healthy infants at age 1 year, using a short intravenous glucose tolerance test (sIVGTT). Infants were genotyped at the *–23 Hph*I locus, as a surrogate marker for *INS* VNTR allele classes I and III. Anthropometric data were recorded at birth and at 1 year. Data are shown as median (interquartile range).

RESULTS Fasting insulin levels were higher in III/III infants ($n = 9$) than in I/I infants [$n = 55$; 27·4 (17·6–75·6) pmol/l *vs.* 18·1 (10·3–25·2) pmol/l; $P < 0·05$]. Insulin secretion during the sIVGTT, as estimated by the serum insulin area under the curve, was also higher in III/III infants [2417 (891–4041) pmol min/l *vs.* 1208 (592–2284) pmol min/l; $P < 0·05$]. Fasting and postload plasma glucose levels were similar in both groups. Analysis of covariance showed that genotype differences in fasting insulin sensitivity and insulin secretion were independent of size at birth, postnatal growth velocity and current body mass index.

CONCLUSIONS Significant associations between *INS* VNTR genotype and both insulin sensitivity and secretion were apparent in infancy; these might interact with childhood appetite and nutrition to impact the development of childhood obesity and insulin resistance.

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Interindividual variability in growth rates during infancy is dependent on both genetic and environmental factors. This variability is not only relevant in determining adult body size, but may also have an impact on morbidity later in life (Ong *et al*., 2000). However, the biological determinants of variation in postnatal growth rates are not completely understood. As well as GH and IGF-I, insulin may continue to act as a growth factor in early infancy, as it does during the last trimester of pregnancy (Kjos & Buchanan, 1999). A correlation has been found between insulin secretion and longitudinal growth velocity at the age of 6 months (Colle *et al*., 1976). Moreover, our observations in a prospective cohort of infants born small for gestational age (SGA) indicate that compensatory ('catch-up') growth during the first year is correlated with subsequent basal and postglucose insulin plasma levels at 1 year (Soto *et al*., 2003).

Differences in insulin secretion during infancy might be genetically determined. Polymorphisms have been described in a number of genes potentially involved in the control of insulin synthesis and/or release. In this regard, the insulin gene (*INS*) variable number of tandem repeat (VNTR) on chromosome 11p15·5 is particularly interesting. At this locus, class I alleles confer susceptibility to type 1 diabetes (Bennett *et al*., 1995), while class III alleles have been linked with type 2 diabetes risk, as well as hyperinsulinaemic polycystic ovary syndrome and insulin resistance (Bennett & Todd, 1996; Ong *et al*., 1999; Michelmore *et al*., 2001). Moreover, the homozygous class III/III genotype is associated with a larger size at birth (Dunger *et al*., 1998).

Whether these associations are related to differences in insulin secretion remains to be confirmed. The *INS* VNTR lies upstream to *INS* and *IGF2*, and *in vitro* studies have shown that it has regulatory influences on the expression of both genes (Kennedy *et al*., 1995; Vafiadis *et al*., 1996, 2001). However, studies assessing the effect of *INS* VNTR variation on beta cell function *in vivo* have been difficult to interpret, as they have had to deal with a number of potentially confounding factors, such as birth size, age, pubertal status and adiposity (Cocozza *et al*., 1988; Ahmed *et al*., 1999; Le Stunff *et al*., 2000).

We have hypothesized that *INS* VNTR variation may underlie the observed correlation between insulin secretion and early postnatal growth. To address this, we have studied the effect of *INS* VNTR genotype on growth rates and basal and postglucose insulin levels in a prospective cohort of 1-year-old infants.

Table 1 Body size at birth and 1 year according to by *INS* VNTR genotype

	I/I	I/III	III/III
n	55	35	9
Sex (M : F)	20 : 35	11 : 24	3 : 6
Gestational age (weeks)	38 (38–39)	39 (38–40)	40 (39–40)
Birthweight (g)	2640 (2430–2800)	2580 (2470–2890)	2710 (2355–3320)
Birthlength (cm)	47·0 (46·0–49·1)	47·0 (44·6–48·0)	48·4 (45·7–50·0)
Ponderal Index at birth (g/cm^3)	2·57 (2·42–2·69)	2·62 (2·41–2·72)	2·44 (2·33–2·69)
Head circumference at birth (cm)	33·0 (32·3–33·5)	32·5 (32·0–34·0)	32·5 (32·5–34·5)
Weight at 1 year (kg)	8·97 (8·26–9·93)	8·98 (8·45–9·84)	9·11 (8·10–10·8)
Length at 1 year (cm)	73·0 (71·9–75·0)	72·0 (71·0–74·5)	73·5 (71·5–76·5)
Head circumference at 1 year (cm)	45·5 (44·8–46·5)	45·2 (44·2–46·0)	44·6 (44·0–47·1)
BMI 1 year (kg/m^2)	17·01 (15·98–18·10)	17·26 (15·68–18·19)	17·37 (15·70–18·68)
Weight increment during the first year (SDS)	0·89 (–0·01–1·76)	0·89 (0·27–1·80)	0·60 (–0·20–1·63)
Length increment during the first year (SDS)	0·38 (–0·34–1·28)	0·46 (0·00–1·10)	0·29 (–0·42–1·08)

Values are medians (interquartile range).

Methods

Subjects

Our study group consisted of a prospective cohort of SGA (birthweight below 10th percentile) and AGA (appropriate for gestational age, birthweight above 10th percentile) infants born at full-term. They were delivered between July 1999 and October 2000 at two public hospitals (San Borja-Arriarán and Sótero del Río) in Santiago, Chile, serving medium- and low-income populations with a rather homogeneous Caucasian–Mongoloid admixture (Cifuentes *et al*., 1988). Protocols and consent forms were approved by the respective institutional review boards, according to the principles of the Declaration of Helsinki.

During the recruitment period, approximately 20 000 children were born at these two hospitals. According to their records, 8% of births (approximately 1600) corresponded to SGA infants, of which 75% were delivered at full-term. All infants included in this study were born on weekdays. Therefore, our sample of SGA newborns was selected among 860 live births. Infants showing evidence for malformations or genetic disorders were excluded.

Forty-eight hours after birth, parents were invited by a staff neonatologist to participate in a yearly follow-up of insulin sensitivity and secretion during the first 3 years of life. A total of 136 SGA newborns were enrolled. For each of them, parents of one AGA infant, born on the same day and hospital, were invited to participate in the study. Thirty-four AGA newborns were recruited.

Thereafter, a staff nurse contacted parents every 3 months in order to remind them about the first-year appointment. Fifty-nine (43·4%) SGA and 11 (35·2%) AGA parents either could not be localized or declined to have their children studied. Therefore, 99 infants (77 SGA and 22 AGA) attended the initial assessment at 1 year of age. All of them had been breast fed for at least 4 months.

Anthropomethric measurements at birth and at 1 year, including weight, crown-heel length and head circumference were recorded, and standard deviation scores (SDS) were calculated using local normative data (Juez *et al*., 1989). Weight and length gains during the first year of life are expressed as the increment in SDS between birth and age 1 year. Selected clinical data from these infants are shown in Table 1.

Short intravenous glucose tolerance test

Basal and first phase insulin release were studied using a short intravenous glucose tolerance test (sIVGTT; Allen *et al*., 1993). After an overnight fast, two venous accesses were established on opposite sides of the body. One of them was used for the administration of 0·5 g/kg 25% glucose over 3 min, sampling being carried out through the contralateral cannula at times –5, 0, 1, 3, 5 and 10 min (where time 0 represents the start of glucose infusion). Blood glucose levels were determined using a commercial glucometer (Accutrend Sensor Comfort, Roche Diagnostics Inc., Basel, Switzerland, correlation coefficient with reference method in our hands is 0·986). Samples were subsequently centrifuged at 3500 g for 10 min and serum stored at –20 °C until further processing. An additional 3-ml sample was collected into EDTA containing tubes for genomic DNA isolation using the 'salting out' method (Lahiri & Nurnberger, 1991).

Serum insulin was measured using a commercial radioimmunoassay (RIA) from Immunotech (Marseille, France). The intra- and interassay coefficients of variation (CV) were 3·8%, and 4·7%, respectively. Cross-reactivities for this RIA are declared by the manufacturer as follows: 68% against des-64–65-proinsulin, 55% against proinsulin and 50% against des-31–32-proinsulin.

Basal insulin was calculated as the average of insulin levels at times –5 and 0 min. Fasting insulin sensitivity was estimated

Table 2 Insulin sensitivity and secretion at 1 year during an IVGTT, according to *INS* VNTR genotype

	I/I	I/III	III/III
n	55	35	9
Fasting glucose (mmol/l)	4·8 (4·6–5·1)	4·9 (4·4–5·6)	4·8 (4·5–5·2)
Fasting insulin (pmol/l)	18·1 (10·3–25·2)	16·0 (10·1–39·9)	27·4* (17·6–75·6)
HOMA IR	0·54 (0·32–0·83)	0·52 (0·29–1·28)	0·90* (0·48–2·33)
AUC glucose (mmol min/l)	197·1 (187·5–210·9)	195·3 (179·2–210·6)	190·5 (183·0–203·8)
FPIR (pmol/l)	163·2 (82·6–332·0)	212·5 (98·6–433·3)	496·6* (143·8–758·0)
AUC insulin (pmol min/l)	1208 (592–2284)	1573 (631–3013)	2417* (891–4041)
Peak insulin (pmol/l)	226·4 (108·3–382·9)	288·2 (120·1–451·4)	483·4* (154·9–792·8)

*$P < 0{\cdot}05$, I/I *vs.* III/III, adjusted for sex and BMI. Values are medians (interquartile range).

using the homeostatic model assessment (HOMA) index (Huang *et al*., 2002). First-phase insulin release (FPIR) was estimated using the sum of insulin levels at times 1 and 3, the incremental area under the curve (AUC) over 10 min as well as peak insulin levels reached during the sIVGTT (Allen *et al*., 1993).

Genotyping

At the *INS* VNTR locus, alleles are known as class I (26–63 repeats), class II (about 80 repeats) and class III (141–209 repeats), with frequencies of approximately 0·71 and 0·29 for class I and III, respectively (class II alleles are rare in Caucasians; Stead & Jeffreys, 2002). The −23 A/T *Hph*I polymorphism was used as a surrogate marker for *INS*-VNTR genotyping, as both loci display a linkage desequilibrium higher than 99·7% in Caucasian and Mongoloid populations (Stead & Jeffreys, 2002). The PCR–RFLP (restriction fragment length polymorphism) protocol for −23 A/T *Hph*I genotyping has been described previously (Dunger *et al*., 1998).

Statistical analysis

Genotype distributions were examined using chi-square tests. All variables are expressed as median (interquartile range). Analysis of variance (ANOVA) was used for initial comparisons between groups; the effect of potential confounders [i.e. sex, birthweight, current length/weight/BMI (body mass index) and length/weight gain during the first year] was assessed by analysis of covariance (ANCOVA). All calculations were performed on SPSS 10·0 for Windows (SPSS, Chicago, IL, USA).

Results

INS VNTR genotype frequencies (I/I 55 [55·6%]; I/III 35 [35·3%]; III/III 9 [9·1%]) were in Hardy–Weinberg equilibrium and were similar to those previously reported in Caucasian populations (Bennett & Todd, 1996; Stead & Jeffreys, 2002). Genotype distribution was not different between sexes (χ^2 1·112; $P = 0{\cdot}892$) or birthweight categories (SGA or AGA) (χ^2 2·548; $P = 0{\cdot}863$). Anthropomethric data at birth and at age 1 year sorted by genotype are shown in Table 1; no genotype-based differences were observed.

Fasting and postload plasma glucose levels did not differ among genotypes. Length gain during the first year (in SDS) was correlated with basal and postload insulin levels, as previously reported ($r = 0{\cdot}245$; $P = 0{\cdot}016$). There were no correlations between insulin levels and weight, length or BMI at age 1 year.

Fasting insulin levels were higher in class III *vs.* class I homozygotes, and the same was observed for HOMA values (Table 2, Fig. 1). Class III/III infants also had higher postload insulin secretion than I/I infants, as estimated either by FPIR, AUC insulin, or peak insulin levels during the sIVGTT (Table 2, Fig. 1). All the genotype differences persisted when analysing data from SGA separately, as well as after adjustment for sex, birthweight, length/weight gain and current length/weight or BMI. (data not shown). Finally, dynamic insulin levels in class I/III heterozygotes showed intermediate values, but they did not reach statistical significance when compared to either class I or III homozygotes (Table 2 and Fig. 1).

Discussion

We have shown a significant association between *INS* VNTR genotype and both fasting and stimulated insulin secretion *in vivo*. Class III/III infants secreted more insulin than I/I infants during a sIVGTT, with heterozygous individuals exhibiting intermediate values. This was independent of the previously reported association between insulin levels and longitudinal growth during the first year of life (Colle *et al*., 1976). In contrast to early reports, our studies were carried out in healthy infants, at an early age and none were obese (Cocozza *et al*., 1988; Ahmed *et al*., 1999; Le Stunff *et al*., 2000).

Previous association studies of the *INS* VNTR on fasting insulin levels and stimulated insulin secretion *in vivo* have been

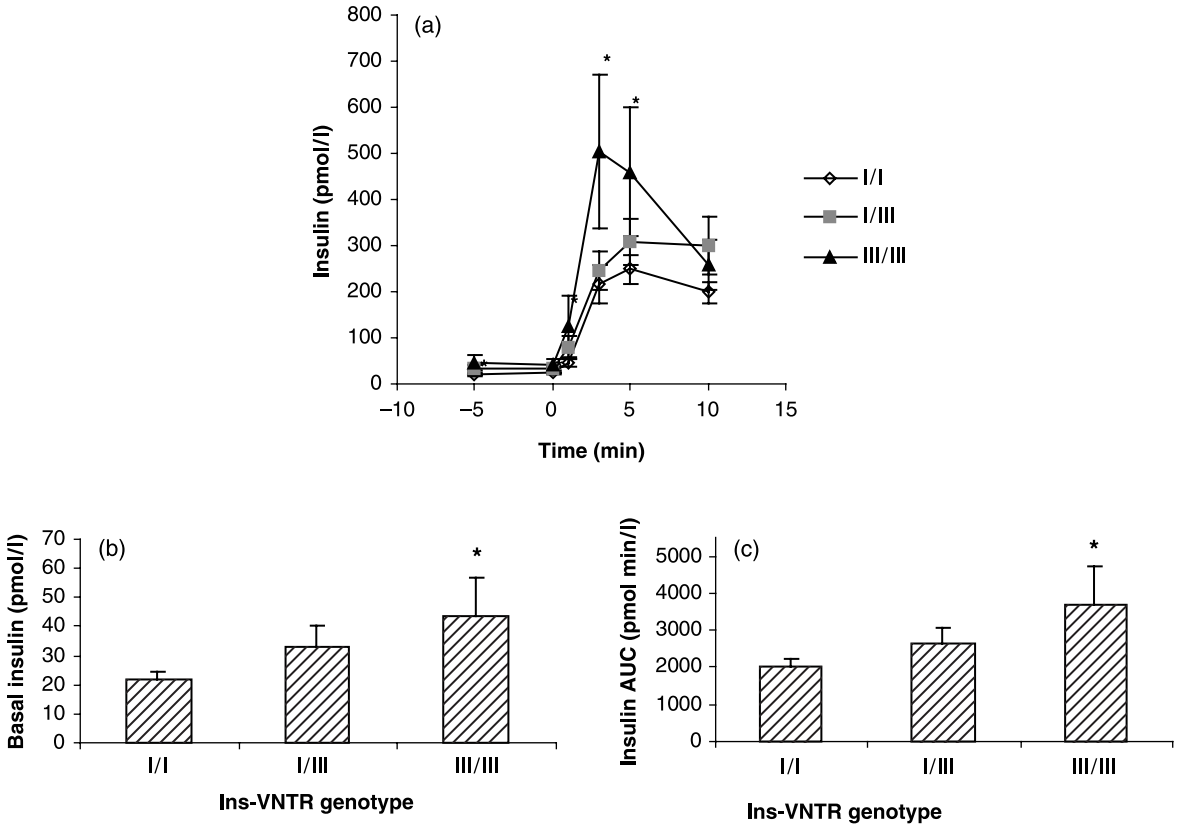

Fig. 1 (a) Serum insulin levels during a short intravenous glucose tolerance test (sIVGTT) in 1-year-old infants, in relation to *INS*-VNTR genotypes. (b) Fasting insulin levels. (c) Insulin area under the curve during the sIVGTT. Data are mean ± SEM. *$P < 0{\cdot}05$ I/I *vs.* III/III.

inconsistent. The I/I genotype has been associated with higher baseline insulin levels in severely obese pubertal children (Le Stunff *et al*., 2000) and with higher insulin and C-peptide secretion in normal adults (Cocozza *et al*., 1988). Conversely, class III alleles have been associated with increased basal and stimulated insulin levels in obese young nondiabetic women, and also with a number of conditions characterized by insulin resistance (Bennett & Todd, 1996). By assessing insulin secretion at a very early age, we minimized potential confounding or interactive effects of factors such as age, pubertal stage and obesity.

In addition, we have studied FPIR after an overnight fast, which may reflect insulin synthesis contributing to the intracellular pool available for immediate secretion (Ahmed *et al*., 1999). Class III alleles could promote insulin secretion by β-cells, either directly through enhanced proinsulin gene expression (Kennedy *et al*., 1995), or indirectly by modulating *IGF2* expression (Paquette *et al*., 1998). However, higher fasting insulin levels and HOMA values suggest lower insulin sensitivity in III/III infants, which in turn could lead to secondary insulin hypersecretion.

While we are unable to clearly dissect whether our findings reflect *INS* VNTR effects primarily on insulin sensitivity or insulin secretion, the presence of both lower insulin sensitivity and hyperinsulinaemia from an early age in III/III subjects may lead to subsequent central fat deposition. However, potential subsequent interactions with appetite, nutrition and other determinants of insulin secretion could impact the development of childhood obesity and adulthood disease risk (Ong *et al*., 2000). Our previous observations of the effects of early length and weight gains on insulin secretion and sensitivity are in agreement with such a multifactorial model (Soto *et al*., 2003). In any case, further exploration of the early development of insulin resistance from infancy through childhood will be important in understanding the early development of adulthood disease risks.

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