

## HIGHLIGHT

## Common variants in the regulatory region of the insulin gene are associated with fasting plasma insulin levels in juvenile obesity

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Variable number of tandem repeat polymorphisms (VNTRs) are widely dispersed throughout the genome. They consist of repeats of stereotypic DNA sequences and are generally found in non-coding regions of DNA. For many VNTRs the number of repeat units display large inter-individual variation, which has made them useful tools in forensic medicine and gene mapping studies. In the majority of cases, the function of these VNTR polymorphisms remains a mystery.

The insulin VNTR (INS-VNTR) is located 596 base pairs upstream (–596) of the translation initiation codon of the insulin gene on chromosome 11. It was first described by Bell *et al.* in 1981 (1). The INS-VNTR consists of varying numbers of a 14–15 base pair repeat and is broadly divided into three allelic groups: the short class I alleles consisting of 26–63 repeats, the intermediate class II alleles (approximately 80 repeats) and the long class III alleles consisting of 141–209 repeats (2). The class II alleles have been found predominantly in Black populations and are rarely found in Caucasians and little data are available for this particular class of alleles. In addition to length polymorphisms there are sequence differences within the various repeat units. The polymorphisms of the INS-VNTR are not randomly distributed and have been grouped into lineages based upon sequence and length (3, 4). Moreover, the INS-VNTR polymorphism is in strong linkage disequilibrium with several neighbouring single nucleotide polymorphisms (5). One of these is a T/A polymorphism, which can be identified by the restriction enzyme HphI, and is located 23 nucleotides upstream of the insulin gene translation initiation codon. In Caucasian populations the –23 HphI T or A alleles can be used as surrogate genetic marker for class I and III VNTR alleles respectively. Several reports on associations between particular INS-VNTR alleles and diseases and phenotypic traits have been published, some of which are listed in Table 1.

The molecular mechanisms behind the associations with particular INS-VNTR alleles are not clear. Furthermore, due to strong linkage disequilibrium between the various polymorphisms in this region there is, with the exception of type 1 diabetes (6, 7), not formal proof to conclude that it is the INS-VNTR and not one of the neighbouring polymorphisms that are

primarily involved. There is, however, evidence from both *in vitro* (8, 9) and *in vivo* studies (7, 10) that the INS-VNTR has an effect on mRNA transcription of the insulin gene where the various alleles are associated with different levels of transcription.

Recently, Le Stunff *et al.* (11) demonstrated an association between the INS-VNTR and fasting plasma insulin levels in obese children. Two cohorts of 458 and 157 children of Mediterranean and Central European origin respectively with a mean age of 12 years and body mass index (BMI) above the 85th percentile before the age of 6 years (mean BMI approximately 30 kg/m<sup>2</sup>) were recruited to the study. They used the insulin gene –23 HphI T and A alleles as markers for VNTR class I and III alleles respectively. Fasting insulin levels were 13% and 29% higher among children homozygous for the VNTR class I allele marker compared with the children homozygous for the class III marker or class I/III heterozygotes. No significant differences in fasting insulin levels were observed between children homozygous for the VNTR class III marker and class I/III heterozygotes. Allele frequencies were the same as in a large cohort of lean controls, which indicated that the INS-VNTR marker was not primarily associated with juvenile obesity, at least not when classifying alleles broadly as VNTR class I and III respectively.

The association between fasting insulin levels and VNTR classes was even stronger when the study was limited to the 105 and 44 morbidly obese children from the two cohorts (BMI  $\geq$  99.6th percentile). Fasting insulin levels among children with mean BMI 37 kg/m<sup>2</sup> were approximately 70% higher in homozygotes for the VNTR class I marker compared with the class I/III heterozygotes and class III homozygotes. For the whole cohort of children, Le Stunff *et al.* (11) demonstrated a stronger influence on the increase in fasting insulin levels secondary to overweight among VNTR class I homozygotes compared with the other genotypes. It was more marked among obese boys than girls, but the relative effect of genotype on the interaction between BMI and insulin levels was not significantly different among the sexes. BMI accounted for approximately 50% of the variance of fasting plasma insulin levels in the group homozygous for the VNTR class I marker, compared with less than 10% among the heterozygotes

**Table 1** Diseases and traits reported to be associated with the INS-VNTR polymorphism.

Disease/Phenotypic trait	Associated INS-VNTR allele	References
Type 1 diabetes	Class I alleles confer susceptibility Class III alleles confer dominant protection	6, 7
Type 2 diabetes	Class III alleles confer susceptibility	12, 13
Polycystic ovary syndrome	Class III alleles confer susceptibility	16
Birth weight	Class III alleles associated with higher birth weight than class I alleles	17
Obesity	Increased class I allele size confer susceptibility	14
Thymic expression of insulin	Class III alleles lead to higher insulin expression in the majority of cases	10, 15

and the class III homozygotes. The authors speculate that class I homozygotes may adjust their insulin secretion to an increase in fat accumulation better than the class I/III heterozygotes and class III homozygotes, and that class III alleles have a dominant negative effect on obesity-induced increase in insulin secretion. Previous studies have shown that the INS-VNTR class III allele is associated with a higher risk of type 2 diabetes (12, 13). The study by Le Stunff *et al.* (11) indicated that the hyperinsulinaemia in class I homozygous obese children may enhance their capacity to maintain normal blood glucose levels and protect against type 2 diabetes.

Increased insulin secretion is associated with increased fat deposition. Le Stunff *et al.* (11) showed that VNTR class I homozygotes with their relatively higher insulin levels also gained weight more rapidly than class I/III heterozygotes and class III homozygotes during adolescence. A putative role of INS-VNTR classes as markers of juvenile obesity is nevertheless dubious because there were no differences in allele frequencies between the obese children and a group of lean controls. However, in the study by Le Stunff *et al.* (11) they did not type the INS-VNTR at high resolution and hence could not split the INS-VNTR class I and III alleles respectively into lineages or more precisely defined alleles. In a previous study of adults, no differences in BMI between the INS-VNTR classes were detected, whereas the length of the class I allele correlated with increasing BMI (14).

Genomic variation in the regulatory regions of the insulin gene has been associated with increased susceptibility to both type 1 and type 2 diabetes (7, 12, 13). A protective effect of class III VNTR against type 1 diabetes was associated with increased insulin expression in the thymus (10, 15). However, as Le Stunff *et al.* (11) have shown, in juvenile obesity class I VNTR homozygotes seemed to have  $\beta$ -cells with an increased capacity to produce insulin and potentially protect against type 2 diabetes. The mechanisms behind these differences have not been resolved. A possible explanation is that transcription factors that regulate insulin gene expression in the thymus and  $\beta$ -cells may differ in their ability to interact with short or long INS-VNTRs and partly determine an individual's predisposition to either type 1 or type 2 diabetes.

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