Assocation of Kir6.2 and INS VNTR variants with glucose homeostasis in young obese

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Le Fur, Sophie, Delphine Fradin, Pascal Boileau, and Pierre Bougnères. Assocation of Kir6.2 and INS VNTR variants with glucose homeostasis in young obese. Physiol Genomics 22: 398–401, 2005. Although insulin secretion is commonly increased and glucose tolerance decreased in young obese patients, there is a wide individual variability of these parameters. We investigated whether common variants at the Kir6.2 (KCNJ11) and insulin variable number of tandem repeat (INS VNTR) loci are associated with insulin or glucose levels in 388 obese children. The E23K and INS VNTR alleles showed no significant association when each locus was examined individually but a clear effect when the two loci were combined for analysis. In obese children with Kir6.2 KK and class III VNTR alleles, fasting glucose was slightly but consistently greater (4.76 ± 0.05 mM) than in those with Kir6.2 EE and class I/I VNTR alleles (4.63 ± 0.06 mM) or other genotypes (4.64 ± 0.03 mM, P = 1.10 ^ -3). Obese children with KK and class III VNTR genotypes also had an early response to oral glucose diminished by ~36% [insulogenic index (IGI) = 50 ± 4] compared with Kir6.2 EE and class I/I (IGI = 78 ± 7, P = 0.026) or other genotypes (IGI = 69 ± 3, P = 0.001). In young European obese, the polymorphisms of Kir6.2 and INS VNTR are thus associated with a trend for lower insulin and higher glucose levels, which may reveal a possible epistatic genetic effect that may influence a prediabetic trait in young obese children.

INSULIN SECRETION DEPENDS ON MULTIFACTORIAL FACTORS. In addition to genetic predisposition, age, adiposity, physical activity, and diet composition are known to influence the production of insulin by β-cells. Among genetic effectors of insulin secretion, Kir6.2 and insulin variable number of tandem repeat (INS VNTR) exert a regulatory role in β-cells at different levels, and therefore their variants could combine their effects to set the individual insulin response to glucose.

The Kir6.2 gene (KCNJ11) encodes subunits of a potassium channel that regulates the transmembrane potential of pancreatic β-cells and the exocytosis of insulin. The E23K single nucleotide polymorphism (SNP) is one of the three common missense variants of the Kir6.2 gene in Caucasians. There is a debate regarding the role of this variant in the predisposition to type 2 diabetes (T2D). The first study failed to show an association of E23K with T2D in white English patients (23), but subsequent studies found this association in other cohorts: white Europeans from Denmark (20), France (12), and United Kingdom (11) and white Americans living in Utah (8). In French whites (12) and in the UK Prospective Diabetes Study (10), KK homozygosity was increased among T2D patients. In United Kingdom white subjects, Gloyn et al. (11) reported that the E23K allele was associated with T2D, although to a limited extent [odds ratio (OR) 1.18 [95% interval of confidence (IC) 1.04–1.34]], but did not show any familial association with T2D. In a multiethnic meta-analysis of studies in Europeans, Hispanics, East Asians, Africans, and Americans, Barroso et al. (2) found that E23K achieved nominal statistical significance for association with T2D only under a recessive model and without correction for the multiple hypotheses examined (OR 1.49, P = 0.03). E23K was not associated with detectable alterations in glucose-stimulated insulin secretion in two independent populations from the Netherlands (25). Nielsen et al. (20) also failed to find a significant association of E23K with T2D in Danish patients but observed an association of the K allele with decreased insulin secretion in nondiabetic Danes (P = 0.02).

In Caucasians, the VNTR polymorphism located within the insulin gene promoter (INS VNTR) is associated with changes in the transcriptional activity of the insulin gene (14), and with the variation of fasting insulin level or response to glucose in European obese children (17). There is a debate also with respect to the role of the INS VNTR in the predisposition to T2D. According to Rotwein et al. (22), the class III VNTR alleles appeared as genetic markers for T2D in a heterogeneous United States population made of white, Pima, and black patients. The class III/III genotype was also found associated with T2D in the United Kingdom (4, 21). In a meta-analysis of six case-control studies, the III/III genotype was associated with a 40% increase of the relative risk for T2D (21), a result confirmed by Meigs et al. (19) with an OR of 1.89 (95% IC 1.01–3.52) in the Framingham Heart Study. However, in a subsequent large case-control study of Dutch people, the class III VNTR allele was not associated with T2D (13).

To examine the genotypic effects of the polymorphisms at the two loci on insulin secretion, we performed the present study in obese juveniles, because their rapid accumulation of fat creates a situation of mounting insulin resistance leading to a compensatory increase of insulin secretion (4). Thus insulin secretion varies over a remarkably large phenotypic range. We think that this situation is favorable to the detection of effects of gene variants involved in the variability of insulin secretion.

MATERIALS AND METHODS

Patients. The phenotype-genotype association study was performed in 388 obese children from families originating from Western Europe. The geographic origin of the patients was carefully assessed through family history, analysis of patronymic names, and birthplaces. Inclusion criteria were a body mass index (BMI) exceeding the 85th centile before the age of 6 yr, a monotonic weight curve since birth, and no weight diminution during the course of obesity (17).

Genotyping. The obese children were genotyped for the E23K polymorphism of Kir6.2, as reported (20), and for the −23Hph1 polymorphism located within the insulin gene promoter as described (4). In Caucasians, Hph1 “+” alleles (A) are in near complete linkage disequilibrium (LD) with class I alleles of the neighboring VNTR.
with only 0.20% recombinants, and “−” alleles (T) with class III alleles. Therefore, we tested the VNTR by using -23 Hph1 as a surrogate. Both E23K and insulin VNTR polymorphisms fulfilled Hardy-Weinberg expectations.

**Procedures.** After 12 h of overnight fasting following 3 days of standardized diet (caloric and carbohydrate content) in hospital, plasma insulin was measured in unstressed condition. Oral glucose tolerance test (OGTT) consisted of the ingestion of 1.75 g/kg glucose (75 g maximum) in 200 ml of lemon-flavored water at 10°C and venous blood sampling at 0, 30, 60, 90, and 120 min after the end of the ingestion. Plasma insulin was measured in each sample in duplicate with standard radioassay (17).

Impaired glucose tolerance (IGT) is defined as a fasting plasma glucose level of <6.9 mM and, during OGTT, a 2-h plasma glucose level of 7.7–11 mM.

**Statistical analysis.** The insulin and glucose values during OGTT were used to calculate the insulinogenic index (IGI), which reflects the early phase of insulin secretion, as the ratio of the 30-min insulin increment (in pmol/l) to the 30-min glucose concentration (in mmol/l) (27).

The composite insulin sensitivity index (ISIcomp, called ISI in this article) was calculated according to the following formula: 10,000 / square root of [(fasting insulin × fasting glycemia) × (mean insulin concentration during OGTT) × (mean glycemia during OGTT)] (18).

Multiple linear regression analysis examined the contribution of age, gender, and BMI to these indexes. The Mann-Whitney test and the Kruskal-Wallis analysis of variance by rank were applied to compare, respectively, two and several independent samples when a classical t-test could not be used. Data were evaluated using Statview 5.0 (SAS Institute). Results were corrected for multiple tests by Bonferroni correction. Values are reported as means ± SE.

**RESULTS**

The distribution of Kir6.2 and INS VNTR genotypes was comparable in the 388 obese Caucasian juveniles and in 178 nonobese controls (data not shown). This suggests that these variants do not predispose to early onset obesity in the studied European population.

The clinical and metabolic parameters stratified by either Kir6.2 or VNTR genotypes are shown in Table 1. Neither the E23K variant of Kir6.2 nor the VNTR polymorphism was associated with the studied glucose-insulin parameters in the locus-by-locus analysis, although mean values indicated a trend to lower insulin values in Kir6.2 23K carriers and in VNTR class III carriers independently, confirming previous observations in a larger sample (17).

Table 2 shows that, when the polymorphisms at these two loci were combined in a joint analysis, a marked difference in insulin secretion in studied children, subgrouped in 2-loci genotypic groups based on E23K Kir6.2 variant and class I or III VNTR. *P = 6.10⁻⁴ vs. Kir6.2 E/E and class I/III VNTR, and †P = 1.10⁻³ vs. Others. †P = 0.026 vs. Kir6.2 E/E and class I/III VNTR, and †P = 1.10⁻³ vs. Others.

Fasting plasma glucose was slightly greater in subjects carrying both Kir6.2 KK and class III VNTR alleles (4.76 ± 0.05 mM) than in children carrying Kir6.2 EE and class I/I VNTR alleles (4.63 ± 0.06 mM, P = 6.10⁻⁴) or other genotypes (4.64 ± 0.03 mM, P = 1.10⁻³) after adjustment on the BMI. The magnitude of the difference was small, but the consistency of glycemic values within genotypic groups makes it very significant.

Fasting plasma insulin was comparable in the three groups (Table 2) and lower than observed in United States children of comparable BMI (25).

The IGI, reflecting the early response of β-cells to oral glucose, was lower in children carrying both Kir6.2 KK and class III VNTR alleles (50 ± 4) than in those with Kir6.2 EE and class I/I VNTR alleles (79 ± 7, P = 0.026) or other genotypes (69 ± 3, P = 1.10⁻³) after adjustment to the BMI.

ISI was comparable in the three groups, with a trend to higher values in Kir6.2 KK and VNTR class III carriers (90 ± 14) vs. patients with Kir6.2 EE and class I/I VNTR alleles (84 ± 12) and subjects with other genotypes (70 ± 2) (data not shown).

Table 2. Clinical and biological characteristics of the insulin secretion in studied children

<table>
<thead>
<tr>
<th></th>
<th>KK/III or III/III</th>
<th>E/E/I/I</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>73</td>
<td>293</td>
</tr>
<tr>
<td>Age, yr</td>
<td>11.9 ± 0.6</td>
<td>11.5 ± 0.3</td>
<td>11.9 ± 0.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.2 ± 1.3</td>
<td>28.5 ± 0.5</td>
<td>29.7 ± 0.3</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>84 ± 12</td>
<td>93 ± 6</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>4.76 ± 0.05*</td>
<td>4.63 ± 0.06</td>
<td>4.64 ± 0.03</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>50.4 ± †</td>
<td>78 ± 7</td>
<td>69 ± 3</td>
</tr>
</tbody>
</table>

Data are presented as unadjusted means ± SE. Clinical and biological characteristics of insulin secretion in studied children, subgrouped in 2-loci genotypic groups based on E23K Kir6.2 variant and class I or III VNTR.

Table 1. Clinical and biological characteristics of the studied children stratified by genotype at each locus

<table>
<thead>
<tr>
<th></th>
<th>Kir6.2</th>
<th>VNTR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K/K</td>
<td>E/K</td>
</tr>
<tr>
<td>n</td>
<td>53</td>
<td>180</td>
</tr>
<tr>
<td>Age, yr</td>
<td>11.9 ± 0.4</td>
<td>11.8 ± 0.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.2 ± 0.7</td>
<td>29.7 ± 0.4</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>100 ± 8</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>4.64 ± 0.05</td>
<td>4.68 ± 0.04</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>61 ± 6</td>
<td>69 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as unadjusted means ± SE. BMI, body mass index; VNTR, variable no. of tandem repeat.
DISCUSSION

Investigation of gene variants that could influence insulin secretion may be important to the understanding of the genetic susceptibility of obese adolescents to later diabetes mellitus. T2D is common among adults with long-lasting obesity (16). Although not observed in the present cohort, IGT, an intermediate stage in the natural history of T2D, is highly prevalent (>20%) among children and adolescents with severe obesity living in the United States (25). Instead of studying the genetics of T2D or IGT as primary phenotypes, we studied genotype-phenotype association at the level of insulin secretion, considered as a prediabetic trait (5).

The INS VNTR polymorphism influences the transcription of the insulin gene (14). In fetal and adult pancreas, the insulin transcripts in cis with class III VNTR alleles are expressed at ~20% lower levels than class I alleles (14). Class III alleles are associated with altered pulsatility (1) and decrease of insulin secretion. Class III alleles may thus contribute to T2D pathogenesis by inducing a low level of insulin biosynthesis, as observed in several studies. Here, class III alleles were associated with a trend for a lower insulin response that was not significant, unlike previous results in a larger cohort. We suspect that the smaller size of the present sample explains the lack of significance.

The E23K variant is located in the NH2-terminal domain of Kir6.2 involved in ATP binding and regulation of K-channel activity. The 23K allele is associated with decreased sensitivity to ATP facilitating channel opening (24). Transgenic mice with overactive Kir6.2 subunits develop insulin deficiency (15). These observations are consistent with an association of 23K allele with increased T2D risk. Failure of several studies to replicate this association can be due to disease heterogeneity, differences in the genetic background, population stratification, and number of individuals tested in the different cohorts (9). These difficulties are increased if the variant has a small functional effect at the whole body level. The current study detected only a slight nonsignificant association between the E23K polymorphism of the Kir6.2-sulfonylurea receptor channel and insulin response to oral glucose.

Our results support that polymorphisms at the INS VNTR and Kir6.2 loci join their effects for influencing insulin output and plasma glucose. The KK class III subgroup is associated with higher fasting glucose and lower insulin response in a consistent manner, with no outlying value for these parameters. On the basis of these observations, it is possible that the discordances regarding the effects of the 23K allele on insulin secretion or T2D predisposition are partially explained by the varying prevalence of VNTR class III alleles in the studied cohorts. Indeed, the prevalence of class III alleles varies in the European population from 0.17 in Finnish (3) to 0.42 in Spanish cohorts (7). Two-loci association studies are difficult to perform, largely because the small size of the genotypic groups reduces the statistical power necessary for bona fide comparison.

GRANTS

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