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SHORT COMMUNICATION

Neuropeptide Y polymorphism significantly magnifies diabetes and cardiovascular disease risk in obesity: the Hoorn Study

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The leucine7 to proline7 (Leu7Pro) polymorphism in preproneuropeptide Y (preproNPY) has been associated with accelerated atherosclerosis and type II diabetes, both of which are obesity-related diseases. The current study evaluated the impact of obesity on the disease risk linked to the Leu7Pro polymorphism of preproNPY in 393 elderly subjects. In 6 years follow-up, the polymorphism alone did not change the risk for abnormal glucose regulation, while obesity was associated with a significant 3-fold risk (odds ratio (OR) 2.95; 95% confidence interval (CI) 1.81–4.81, $P<0.001$) and the Leu7Pro polymorphism–obesity interaction, with a remarkable 12-fold risk (OR 12.33; 95% CI 1.18–128.35, $P<0.05$). The Leu7Pro polymorphism modified significantly the 10-year incidence of cardiovascular events, causing a 7.6-fold increase in the hazard ratio (HR 7.58; 95% CI 2.87–20.03, $P<0.001$) in the obese but not in the nonobese subjects. The results indicate that obesity may be a pivotal factor in multiplying the disease risk associated with the Leu7Pro polymorphism in preproNPY.
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Introduction and methods

Several single nucleotide polymorphisms (SNPs) have been found in the *NPY* gene (GenBank accession no. NT007819). The SNP *NPY* 1228 T>C (rs16139) leading to an amino-acid chance leucine7 to proline7 (Leu7Pro) in the signal peptide of NPY is relatively common in European populations; about 7–12% of subjects have the minor Pro7 allele in a heterozygous (Leu7Pro7) or, very rarely, homozygous (Pro7Pro7) genotype (Karvonen *et al*., 1998; Ding, 2003). The Pro7 allele has been associated with increased blood pressure, accelerated atherosclerosis and type II diabetes (Karvonen *et al*., 2001; Nordman *et al*., 2005; Ukkola and Kesaniemi, 2007). Since obesity is a major risk factor for diabetes and cardiovascular diseases, among other comorbidities (Poirier *et al*., 2006), and the effect of interaction obesity on genotype has not been well appreciated in all SNP association studies, we wanted to evaluate the impact of the interaction of obesity with the Leu7Pro polymorphism of preproNPY on blood pressure elevation, cardiovascular events and abnormal glucose regulation in elderly people.

The study subjects are part of the Hoorn Study, a population-based cohort study of glucose metabolism and cardiovascular complications. The cohort has been described in detail elsewhere (Mooy *et al*., 1995). The ethical committee of the VU University Medical Centre approved the study and written informed consent was received from all study subjects. A total of 111 obese (body mass index (BMI) $\geqslant 28\,kg\,m^{-2}$, mean age 68.1 ± 7.7 years) and 282 nonobese (BMI $< 28\,kg\,m^{-2}$, mean age 67.1 ± 7.3 years) subjects of the total cohort were genotyped for the Leu7Pro polymorphism of preproNPY as described earlier (Karvonen *et al*., 1998).

The study design of the present follow-up study is shown in Figure 1. The frequency of the Leu7Pro7 genotype was 7.6% (30 out of 393 subjects) and none of the subjects had the Pro7Pro7 genotype. There was no statistically significant

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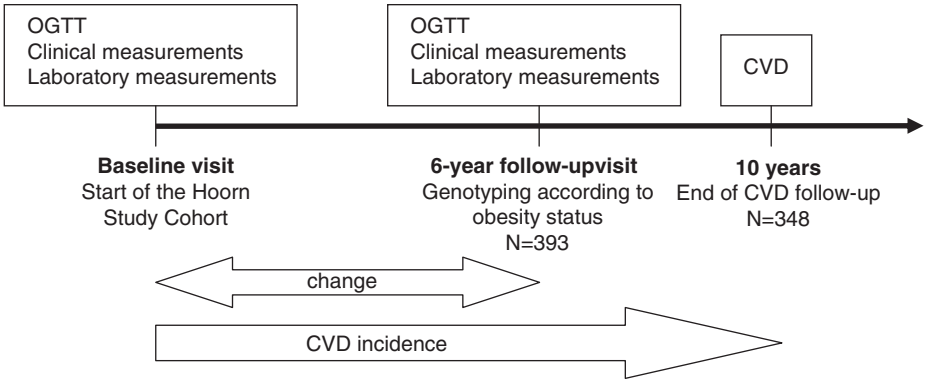

Figure 1 Schematic presentation of the study design. Oral glucose tolerance tests (OGTT) and clinical and laboratory measurements were performed during 6-year intervals. The follow-up for cardiovascular events (CVD) continued for 10 years.

difference in frequency of the Leu7Pro7 genotype between obese and lean subjects (9.0 and 7.1%, respectively; $P=0.519$, χ^2-test). Multiple linear regression analyses were performed to investigate simultaneously the associations of the Leu7Pro7 genotype, obesity and genotype–obesity interaction (explanatory variables) with BMI, body weight, waist to hip ratio, waist and hip circumferences, blood pressure, glucose metabolism, lipid concentrations and with a change in these parameters in 6 years (outcome variables). For investigating simultaneously the effects of the Leu7Pro7 genotype, obesity and genotype–obesity interaction on the risk for abnormal glucose regulation (impaired fasting glucose, impaired glucose tolerance and diabetes mellitus) in 6 years follow-up period, a logistic regression model was used. The Cox's proportional hazard model was used to compare cardiovascular survival in 10 years (fatal or nonfatal events: angina pectoris, myocardial infarction, congestive heart failure, stroke, transient ischaemic attack, peripheral vascular disease and sudden death). All the statistical analyses were performed using SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, IL, USA).

Results and discussion

After a follow-up of 6 years, all (100%) obese subjects with the Leu7Pro7 genotype (10 out of 10 subjects) had abnormal glucose regulation compared to 63.4% (64 out of 101 subjects) of obese subjects with the Leu7Leu7 genotype ($P=0.019$, $p_{corr}=0.046$). In nonobese subjects, 4 out of 20 subjects (20.0%) with the Leu7Pro7 genotype and 107 out of 262 subjects (40.8%) with the Leu7Leu7 genotype had developed abnormal glucose regulation ($P=0.066$, $p_{corr}=0.109$). The analysis indicated that obesity caused a 3-fold risk (odds ratio (OR) 2.95, confidence interval (CI) 1.81–4.81, $P<0.001$) and the Leu7Pro7genotype–obesity interaction caused a 12-fold risk for incident abnormal glucose regulation (OR 12.33, CI 1.18–128.35, $P<0.05$) when adjusted for age, gender and follow-up time in days. No effects of genotype or genotype–obesity interaction on lipid concentrations were detected.

There was a significant positive Leu7Pro7 genotype–obesity interaction effect on the change in systolic ($P=0.002$) and pulse pressure ($P=0.005$) during the 6 years follow-up (adjusted for age, gender, glucose tolerance status at baseline, the duration of follow-up in days and the use of antihypertensive agents). In obese subjects with the Leu7-Pro7 genotype, the mean increase was 27 ± 20 (s.d.) mm Hg in systolic blood pressure and 22 ± 16 mm Hg in pulse pressure; in obese subjects with the Leu7Leu7 genotype, the increase was 10 ± 17 mm Hg in systolic blood pressure and 10 ± 15 mm Hg in pulse pressure. A significant positive Leu7Pro7 genotype–obesity interaction effect was also found with current systolic blood pressure ($P=0.034$) after 6 years (adjusted for age, gender, glucose tolerance status and the use of antihypertensive agents). On the contrary, the Leu7Pro7 genotype alone had a negative association with current systolic ($P=0.004$) and diastolic blood pressure ($P=0.009$) after 6 years. There was a significant positive genotype–obesity interaction effect on 6-year change in BMI ($P=0.030$) and weight ($P=0.033$) when adjusted for age, gender and follow-up time in days. At this time point, the Leu7Pro7 genotype–obesity interaction was positively associated with current body weight ($P=0.046$) and waist circumference ($P=0.048$, adjusted for age and gender).

Obese subjects with the Leu7Pro7 genotype had over seven times higher incidence of cardiovascular events compared to obese subjects without the Pro7 allele (Table 1). During a 10 years follow-up, 83.3% (5 out of 6 subjects) of obese subjects with the Leu7Pro7 genotype had a cardiovascular event, whereas only 39.3% (35 out of 89) of of obese subjects with the Leu7Leu7 genotype has a cardiovascular event ($P=0.039$, $p_{corr}=0.092$). In the nonobese subjects, there was no difference in the incidence of cardiovascular events between the genotypes during the 10 years follow-up (20.0% in subjects with Leu7Pro7 genotype (3 out of 15 subjects) and 26.5% in subjects with Leu7Leu7 genotype (63 out of 238 subjects, $P=0.580$, $p_{corr}=0.802$)).

Table 1 The hazard ratios (HR) for cardiovascular events (CVD) according to genotype and obesity status combinations, adjusted for age, gender, baseline body mass index (BMI), fasting glucose at baseline and hypertension

preproNPY genotype and obesity status	*CVD HR (95% CI)*
Leu7Leu7 genotype (obese)	1.0 (Reference category)
Leu7Pro7 genotype (obese)	7.58 (2.87–20.03)[a]
Leu7Leu7 genotype (nonobese)	1.40 (0.74–2.65)
Leu7Pro7 genotype (nonobese)	0.87 (0.24–3.15)

[a]$P<0.001$.

In the current study, we found in a relatively small cohort that there is a significant effect of interaction of the Leu7Pro7 genotype of preproNPY and obesity on the increase in blood pressure as well as on the risk for abnormal glucose regulation. We also found that this genotype markedly increased the incidence of cardiovascular events in obese but not in nonobese subjects. The results suggest that obesity may be a pivotal factor directing or multiplying the effect of Leu7Pro7 genotype on disease risk and should be evaluated in obesity-related diseases. The mechanisms may involve increased abdominal obesity among the obese, as found in this and earlier studies (Ding, 2003), and/or dysregulation of autonomic and endocrine functions since subjects with the Leu7Pro7 genotype are prone to harmful autonomic cardiovascular changes in response to sympathetic activation (Kallio *et al*., 2003; Jaakkola *et al*., 2005). Furthermore, another polymorphism in the *NPY* gene, which may be in linkage disequilibrium with the Leu7Pro polymorphism, for example, the *NPY*−399 T>C (rs16147) that regulates transcription (Buckland *et al*., 2004), may have a role in the observed clinical findings.

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