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Short Report

A common variation of the *PTEN* gene is associated with peripheral insulin resistance

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Abstract

Aim. – Phosphatase and tensin homologue (PTEN) reduces insulin sensitivity by inhibiting the phosphatidylinositol 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homologue (Akt) pathway. This study investigated how a common single nucleotide polymorphism near *PTEN*, previously associated with fasting levels of plasma insulin and glucose, influences in vivo glucose metabolism and insulin signalling. The primary outcome measure was the gene variant's association with peripheral glucose disposal rate and, secondarily, whether this association was explained by altered activities of PTEN targets PI3K and Akt.

Methods. – A total of 183 normoglycaemic Danes, including 158 twins and 25 singletons, were genotyped for *PTEN* rs11202614, which is in complete linkage disequilibrium with rs2142136 and rs10788575, which have also been reported in association with glycaemic traits and type 2 diabetes (T2D). Hepatic and peripheral insulin sensitivity was measured using tracer and euglycaemic–hyperinsulinaemic clamp techniques; insulin secretion was assessed by intravenous glucose tolerance test; and muscle biopsies were taken during insulin infusion from 150 twins for measurement of PI3K and Akt activities.

Results. – The minor G allele of *PTEN* rs11202614 was associated with elevated fasting plasma insulin levels and a decreased peripheral glucose disposal rate, but not with the hepatic insulin resistance index or insulin secretion measured as the first-phase insulin response and disposition index. The single nucleotide polymorphism was not associated with either PI3K or Akt activities.

Conclusion. – A common *PTEN* variation is associated with peripheral insulin resistance and subsequent risk of developing T2D. However, the association with insulin resistance is not explained by decreased proximal insulin signalling in skeletal muscle.

Keywords: Genetics; Insulin resistance; Insulin signalling; PTEN; Skeletal muscle

Abbreviations: Akt, v-akt murine thymoma viral oncogene homologue; FFM, fat-free mass; GWAS, genome-wide association studies; HGP, hepatic glucose production rate; IRS-1-PI3K, insulin receptor substrate-1-associated PI3K; LD, linkage disequilibrium; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue; Rd clamp, insulin-stimulated glucose disposal rate; SNP, single nucleotide polymorphism.

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1. Introduction

The product of the tumour suppressor gene phosphatase and tensin homologue (*PTEN*) negatively regulates insulin sensitivity by inhibiting the pathway of phosphatidylinositol 3-kinase (PI3K) and v-akt murine thymoma viral oncogene homologue (Akt). Consequently, decreased *PTEN* activity in peripheral tissues promotes glucose uptake and storage as well as cellular growth. A previous study found that patients suffering from the rare neoplasia-associated Cowden syndrome, caused by loss-of-function mutations in *PTEN*, had increased adiposity due to excessive glucose uptake and lipid storage in adipose tissue. Muscle and adipose tissue biopsies obtained from these patients suggested enhanced PI3K/Akt signalling compared with healthy controls [1].

Common non-coding variations of the *PTEN* locus can affect *PTEN* expression, with each variant most likely contributing a small effect. Thus far, human epidemiological studies investigating associations between common *PTEN* variations and glucose metabolism have only involved surrogate measures of insulin resistance, with no molecular characterization of insulin signalling. An early candidate-gene study of Danish patients with insulin-resistant type 2 diabetes (T2D) identified four *PTEN* single nucleotide polymorphisms (SNPs), but none of them were associated with the disease [2]. A similar study reported that the less frequent *PTEN*-9C>G polymorphism (rs11202592) was associated with T2D in a Japanese population [3]. In addition, two common SNPs—rs2142136 and rs11202614—in the proximity of *PTEN* have been associated with fasting plasma insulin and glucose levels in genome-wide association studies (GWAS) of normoglycaemic participants [1,4]. Recently, pooled meta-analyses of GWAS found a suggestive association between T2D and rs10788575 at the same *PTEN* locus [5].

The present study aimed to obtain a detailed glucose metabolic characterization of the common *PTEN* variations associated with quantitative glycaemic traits in GWAS. To achieve this, a population of 195 Danes, whose glucose metabolism had been examined using euglycaemic–hyperinsulinaemic clamp and tracer techniques, and whose muscle biopsies provided data on key insulin-signalling molecular activities, was investigated. Our primary hypothesis and outcome measure was the association of the fasting insulin- and fasting glucose-raising minor G allele of *PTEN* rs11202614 with decreased peripheral insulin sensitivity and, secondarily, whether this association could be attributed to decreased PI3K and Akt activities in skeletal muscle.

2. Materials and methods

2.1. Participants

The Danish study population, comprising 98 monozygotic and dizygotic twin pairs and 32 singleton spouses of the twins, was recruited in the years 1997–1999 as previously described in detail elsewhere [6–8]. Participants were recruited to represent two age groups (28.0 ± 2.3 years and 61.5 ± 3.1 years), but were pooled in the present analyses after adjusting for age group. None of the participants had been previously diagnosed with diabetes; however, an oral glucose tolerance test (OGTT) revealed that 24 of them had impaired glucose tolerance and four had T2D according to World Health Organization (WHO) criteria. These subjects were excluded from the present study, as were five others with no DNA samples, leaving 195 (102 males and 93 females) participants. Body mass index (BMI) was 25.0 ± 3.7 kg/m^2 for this normoglycaemic sample subset.

The study was approved by the relevant regional scientific ethical committees, and all participants gave their informed written consent.

2.2. Clinical examination

Participants were subjected to a 2-h euglycaemic–hyperinsulinaemic clamp test (40 mU/m^2/min), using a 3-^{3}H tritiated glucose infusion to determine rates of peripheral glucose disposal (Rd) and hepatic glucose production (HGP). Rd and HGP were calculated at 10-min intervals during steady-state periods using Steele's non-steady-state equations [6]. The hepatic insulin resistance index was calculated as basal HGP × fasting insulin. The clamp test was preceded by an intravenous glucose tolerance test (IVGTT). The first-phase insulin response in relation to glucose concentration during the initial 10 min of the IVGTT (Phi1) was calculated as $AUC_{insulin(0–10\ min)}/AUC_{glucose(0–10\ min)}$ (AUC = area under the curve), and the disposition index was calculated as Phi1 × Rd clamp as a proxy for glucose-stimulated insulin secretion in relation to the level of peripheral insulin sensitivity. Body fat percentage and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry, and metabolic rates were expressed per kg of FFM.

2.3. Muscle biopsy

Biopsies were taken from vastus lateralis muscle in 150 twins at baseline and during the steady-state period of the euglycaemic–hyperinsulinaemic clamp [7]. Only the insulin-stimulated biopsies were used in this study. Activities of insulin receptor substrate-1-associated PI3K (IRS-1-PI3K), Akt1 and Akt2 were measured as described previously [9,10].

2.4. Genotyping

The *PTEN* SNPs previously reported in GWAS for T2D and related traits—rs10788575, rs2142136 and rs11202614—are in complete linkage disequilibrium (LD, $R^2 = 1$) according to the 1000 Genomes CEU reference. Genotyping for rs11202614 was done using the KASP assay (LGC Genomics, Hoddesdon, Herts, UK); the success rate was 94%. The minor allele frequency was 16.3%, which is close to the expected value (18.9%), according to the 1000 Genomes CEU reference. Genotype distribution obeyed the Hardy–Weinberg equilibrium ($P = 1.0$). As a control for the genotyping, participants were also genotyped for rs2142136, which showed 100% agreement with rs11202614 as expected for complete LD. Furthermore, monozygotic twins