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The *PARL* Leu262Val is not associated with fasting insulin levels in UK populations

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Abstract

Aims/hypothesis: *PARL* (encoding presenilins-associated rhomboid-like protein) maps to chromosome 3q27 within a quantitative trait locus (QTL) that influences components of the metabolic syndrome. Recently, an amino acid substitution (Leu262Val, rs3732581) in *PARL* was associated with fasting plasma insulin levels in a US white population (N = 1031). This variant was also found to modify the positive association between age and fasting insulin. The aim of this study was to test the replication of these findings in two UK population-based cohorts.

Methods: Participants from the MRC Ely and Hertfordshire cohort studies were genotyped for this variant using a SNaPshot primer extension assay and Taqman assay respectively. Full phenotypic and genotypic data were available for 3666 study participants.

Results: Based on a dominant model, we found no association between the Leu262Val polymorphism and fasting insulin levels ($p=0.79$) or BMI ($p=0.98$). Nor did we observe the previously reported interaction between age and genotype on fasting insulin ($p=0.14$).

Conclusions/interpretation: Despite having greater statistical power, our data do not support the previously reported association between *PARL* Leu262Val and fasting plasma insulin levels, a

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measure of insulin resistance. Our findings indicate that this variant is unlikely to importantly contribute to insulin resistance in UK populations.

Keywords

association study; fasting insulin; insulin resistance; polymorphism; *PARL*

Introduction

The presenilins-associated rhomboid-like protein (encoded by *PARL*) is a mitochondrial intramembrane protease conserved in eukaryotes and prokaryotes [1,2]. In yeast, deletion of *Rbd1/Pcp1* (the yeast homolog of *PARL*) leads to respiratory defects, impaired growth and small fragmented mitochondria. These phenotypes can be rescued by mammalian *PARL* suggesting a conserved role in mitochondrial membrane remodelling [3,4]. In *Psammomys obesus*, expression of the *PARL* homolog is reduced by 50% in skeletal muscle of obese, type 2 diabetic animals compared to lean glucose tolerant animals. *PARL* expression is also negatively correlated with blood glucose and plasma insulin levels [5]. Consistent with these observations, in a Mexican American population, *PARL* expression was positively correlated with insulin sensitivity and citrate synthase activity, a marker of mitochondrial oxidative capacity [5]. Furthermore, *PARL* maps to chromosome 3q27 within a region that has been linked to quantitative traits that are components of the metabolic syndrome, such as body mass index (BMI), waist and hip circumference, body weight, fasting plasma insulin and insulin/glucose ratio [6].

Based on its chromosomal position, and animal and human data suggesting that *PARL* expression is associated with insulin sensitivity, variation at this gene was previously investigated for association with fasting plasma insulin and BMI in a US population. A common SNP in exon 7 resulting in a leucine to valine substitution at amino acid residue 262, was genotyped in 1031 US white individuals, a sub-group of the original population in which the QTL was discovered [5,6]. In a dominant model, *PARL* genotype was associated with fasting plasma insulin levels and modified the positive association between age and fasting insulin. There was no statistically significant association between this variant and BMI [5].

The aim of our study was to investigate whether *PARL* Leu262Val genotype had a similar association with fasting plasma insulin levels in participants from two UK population-based cohorts.

Subjects, materials and methods

Definition of cohorts

The Medical Research Council (MRC) Ely Study is a population-based cohort study of the aetiology and pathogenesis of type 2 diabetes and related metabolic disorders in the UK [7]. This analysis included 1721 white Euroid men and women aged 35-79 years without diagnosed diabetes. Participants underwent standard anthropometric measurements and a 75-g oral glucose tolerance test. Plasma glucose was measured using the hexokinase method.

Plasma specific insulin was determined by two-site immunometric assays with either ^{125}I or alkaline phosphatase labels. Cross-reactivity was <0.2% with intact proinsulin at 400 pmol/l and <1% with 32-33 split proinsulin at 400pmol/l. Inter-assay CVs were 6.6% at 28.6 pmol/l (n=99), 4.8% at 153.1 pmol/l (n=102) and 6.0% at 436.7 pmol/l (n=99) respectively.

Informed consent was obtained from all participants and ethical approval for the study was granted by the Cambridge Local Research Ethics Committee.

The Hertfordshire Cohort Study was recruited from the cohort of people born in Hertfordshire between 1931 and 1939. The cohort definition has been described previously [8]. Intact insulin was measured by in-house immunofluorimetric two-site assays (DELFI system) based on published methods [9]. Inter-assay imprecision ranged from 6-10% (CV%) for low, medium and high quality control samples for intact insulin and from 7-15% for insulin precursors. Glucose was assayed on an Advia 1650 autoanalyser (Bayer Diagnostics, UK). In this study we attempted to genotype 2341 samples from North and East Hertfordshire.

Genotyping

DNA was available for 1706 Ely cohort samples and 2341 Hertfordshire cohort samples. The Leu262Val SNP (rs3732581) was genotyped in Ely using an ABI PRISM SNaPshot™ multiplex kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Analysis was carried out using GeneMapper v3.0 software (Applied Biosystems, UK). 120 water blanks were included and 86 DNA samples (2.2%) were in duplicate on the plates. Genotyping success rate was 95% and there was no discordance between replicate samples. Hertfordshire cohort samples were genotyped using a custom TaqMan® SNP assay (Applied Biosystems, UK) on 10ng of DOP DNA. Allele calling was performed on the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, UK). 155 water blanks and 91 duplicates were included. Genotyping success rate was 96% and there was no discordance between replicate samples.

Statistical Analysis

All analysis was carried out in Stata v8 (College Station, TX). Deviation of Leu262Val genotype from Hardy-Weinberg equilibrium was assessed using a χ^2 test. Only participants with genotype, fasting insulin and BMI data were included in the analysis: 1608 Ely samples, and 2058 Hertfordshire samples (total = 3666). Linear regression analysis was used to assess the association between genotype and fasting plasma insulin or BMI in Ely and Hertfordshire cohorts separately. Based on the previous reported association, our primary analysis was for a dominant genetic model and we tested genotype-age interactions using log-likelihood ratio tests. In secondary exploratory analyses, we assessed the association between Leu262Val genotype and three further quantitative traits: fasting plasma glucose, 2-hour glucose, and 30min insulin increment in an OGTT test (the difference between 30 minute and fasting insulin concentrations divided by the 30 minute glucose concentration in an OGTT test), using regression analysis. We also investigated whether the association between genotype and fasting insulin was modified by BMI. To increase power we undertook all analyses on both cohorts together including an indicator term for study.

Results

The *PARL* Leu262Val variant was in Hardy-Weinberg equilibrium in the combined Ely and Hertfordshire dataset ($P = 0.94$). Demographic, anthropometric and biochemical characteristics according to Leu262Val genotype for the separate cohorts are shown in Table 1. We did not detect any statistically significant association between genotype and fasting plasma insulin, either in the Ely ($p = 0.49$) or Hertfordshire ($p = 0.23$) populations, or in the meta-analysis adjusting for cohort ($p = 0.79$) (Table 2).

We found no association between genotype and BMI in Ely ($p = 0.65$), Hertfordshire ($p = 0.75$) or in the combined dataset ($p = 0.94$). Furthermore, no age-dependent effects of

genotype on fasting insulin levels were detected in either of the separate cohorts ($p = 0.40$ for Ely, and $p = 0.21$ for Hertfordshire) or in our pooled analysis adjusted for study ($p = 0.14$).

The statistically significant association between genotype and fasting insulin reported by the original study was based on a US cohort with higher mean BMI than our UK cohorts [5]. In an exploratory analysis we investigated the impact of BMI on the association between genotype and fasting insulin in the pooled dataset. In an analysis restricted to individuals in the upper tertile for BMI (mean BMI = 32.3, $N = 1276$), we found no statistically significant association between PARL genotype and fasting insulin ($p = 0.23$). Secondly, we fitted a BMI-genotype interaction term, but this was not significantly associated with fasting plasma insulin ($p = 0.88$).

Finally, we assessed the association between this genetic variant and other quantitative metabolic traits (fasting glucose levels (pmol/l), two-hour glucose levels (pmol/l), and 30 min insulin increment (pmol/mmol) with PARL genotype. No statistically significant associations were found (data not shown).

Discussion

Based on 3666 study participants with full genotypic and phenotypic data, we found no evidence of an association between the PARL Leu262Val SNP and fasting insulin levels. Nor did we replicate the genotype- age interaction on fasting insulin levels [5], suggesting that this genetic variant does not importantly contribute to variation in fasting insulin levels in UK populations.

There are several reasons that may explain the lack of agreement between our data and that which was previously published. The original study showed that plasma insulin levels were significantly higher in GG compared to GC/CC genotypic groups and that plasma insulin increased more rapidly with age in GG homozygotes [6]. The study population consisted of extended families recruited from Take Off Pounds Sensibly (TOPS) membership and the mean BMI in this population was greater than the two UK cohorts included in this analysis ($33.4 \pm 7.2 \text{ kg/m}^2$ in the US cohort compared to $27.3 \pm 4.5 \text{ kg/m}^2$ in the UK cohorts). To examine the possibility that PARL Leu262Val is more strongly associated with fasting plasma insulin in more overweight people, we repeated our analyses in the restricted population of Ely and Hertfordshire participants in the upper tertile of the distribution of BMI. No statistically significant impact of genotype on fasting insulin was detected, nor was there evidence for an interaction between BMI and genotype on fasting insulin levels. These data indicate that failure to replicate the previous result is unlikely to be due to population differences in BMI.

The difference in results could also be explained by other genetic and/or environmental differences between the US and UK study populations. For example, the degree of linkage disequilibrium between the PARL SNP and the putative unmeasured true functional variants may vary between the cohorts. Alternatively, our study may have lacked the statistical power to detect the association of Leu262Val genotype with plasma insulin levels. However, we estimated that our pooled study would have had a 92% probability of detecting the previously reported effect size. The power of the previously reported study was less due to the smaller sample size. In our stratified analyses our power to detect an association of the previously reported effect size in the upper tertile of BMIs ($N = 1276$) was 64%. We therefore cannot exclude a false negative finding in this stratified analysis.

We detected a nominally significant difference in age between those Ely subjects with a C allele and those without (Table 1), which is probably a chance finding resulting from

multiple testing as it is not apparent in the combined Ely and Hertfordshire dataset. Although there was a difference in average fasting plasma insulin between the Ely and Hertfordshire cohorts, most probably related to between-laboratory variation, this difference was not associated with genotype and would not have affected our results as we included an adjustment for study cohort in our pooled analysis.

In summary, our results suggest that PARL Leu262Val is unlikely to importantly contribute to insulin resistance in UK populations. However, given the relationship between PARL expression levels and states of insulin resistance this gene remains an interesting candidate for type 2 diabetes and obesity. A wider range of SNPs could be typed in additional larger case-control and/or quantitative trait cohorts to elucidate the role of this gene in metabolic traits.

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Abbreviations

BMI	Body mass index
MRC	Medical research council
PARL	Presenilins-associated rhomboid-like
QTL	Quantitative trait locus
SNP	Single nucleotide polymorphism
TOPS	Take Off Pounds Sensibly

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Table 1

Demographic, anthropometric, and biochemical characteristics of study participants by PARL Leu262Val genotype in Ely and Hertfordshire cohorts

				P value
	GG	GC	CC	
Ely				
N	412	832	364	
Women (%)	217 (52.7)	444 (53.4)	205 (56.3)	0.58
Age (Years)	62.2 ± 0.46	60.9 ± 0.31	60.8 ± 0.46	0.01
Fasting insulin (pmol/l)	60.1 ± 2.01	59.3 ± 1.30	55.4 ± 1.82	0.49 [*]
BMI (kg/m ²)	27.3 ± 0.24	27.3 ± 0.16	27.1 ± 0.27	0.65
Hertfordshire				
N	547	990	521	
Women (%)	257 (47.0)	476 (48.1)	245 (47.0)	0.77
Age (Years)	65.7 ± 0.12	65.6 ± 0.09	65.7 ± 0.12	0.91
Fasting insulin (pmol/l)	88.5 ± 2.58	92.1 ± 2.09	97.5 ± 3.64	0.23 [*]
BMI (kg/m ²)	27.2 ± 0.18	27.4 ± 0.14	27.0 ± 0.19	0.75

Data are means ± SE, unless otherwise indicated. The P value indicates the results of regression analysis assuming dominance of the C allele.

^{*} analysis performed on log transformed data

Table 2

Analysis of associations between PARL Leu262Val genotype and fasting insulin levels or BMI in pooled Ely and Hertfordshire cohorts

	n	B ± SE	P value
Fasting insulin	3666	-1.76 ± 2.26	0.79 *
BMI	3666	-0.01 ± 0.17	0.95

B = Regression coefficient is the mean change (± SE) in the trait of interest when the C allele is present.

* analysis performed on log transformed data and included adjustment for cohort term