Chapter 5

PARL Leu262Val and fasting insulin levels in UK populations

5.1 Summary

Mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes, and increases with age. Expression levels of the presenilins-associated rhomboid-like protein (PARL), which plays a role in mitochondrial morphology and function, is associated with insulin sensitivity in Israeli sand rats and humans. The *PARL* gene 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. 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. Based on a dominant model, I found no association between the Leu262Val polymorphism and fasting insulin levels (p=0.79) or BMI (p=0.98). Nor did I observe the previously reported interaction between age and genotype on fasting insulin (p=0.14). Despite having greater statistical power, my data do not support the previously reported association between *PARL* Leu262Val and fasting plasma insulin levels, a measure of insulin resistance. My findings indicate that this variant is unlikely to importantly contribute to insulin resistance in UK populations (Fawcett et al. 2006).

5.2 Introduction

5.2.1 Mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes (T2D)

Insulin resistance and the development of T2D have been associated with lower mitochondrial mass and function in key metabolic tissues. Mitochondrial oxidative capacity (Bjorntorp et al. 1967; Kelley et al. 2002; Petersen et al. 2004; Simoneau and Kelley 1997; Simoneau et al. 1999) and basal and insulin-stimulated ATP synthesis (Szendroedi et al. 2007) are reduced in skeletal muscle of patients with type 2 diabetes, their insulin-resistant offspring (Petersen et al. 2005), and in non-alcoholic fatty liver disease and hepatic insulin resistance (Perez-Carreras et al. 2003; Pessayre 2007; Zhang et al. 2007). Age-associated insulin resistance is accompanied by a reduction in mitochondrial oxidative and phosphorylation activity (Petersen et al. 2003). Smaller mitochondrial size and density have also been observed in skeletal muscle and adipose tissue of insulin-resistant, obese, and type 2 diabetic subjects (Kelley et al. 2002; Morino et al. 2005; Ritov et al. 2005; Ukropcova et al. 2007), suggesting impaired mitochondrial biogenesis. Indeed, the expression of genes involved in mitochondrial biogenesis and function, such as peroxisomal proliferator activator receptor γ coactivator 1α (*PGC1α*) and nuclear respiratory factor 1 (*NRF1*), have been shown to be reduced in skeletal muscle and adipose tissue of patients with insulin resistance, obesity and/or diabetes (Bogacka et al. 2005; Mootha et al. 2003; Patti et al. 2003; Semple et al. 2004). Furthermore, knock-down in pancreatic ß-cells of nuclear and mitochondrial genes involved in mitochondrial function results in reduced β-cell mass, impaired insulin secretion and development of overt diabetes (Silva et al. 2000; Soejima et al. 1996). These studies show that disruption of genes involved in mitochondrial function may contribute to insulin resistance and type 2 diabetes.

5.2.2 Expression of PARL correlates with insulin sensitivity

The importance of mitochondrial function in insulin action and secretion led Walder et al. (Walder et al. 2005) to use microarray analysis to screen for genes involved in mitochondrial function that were differentially expressed between skeletal muscle of lean, normal glucose tolerant, and obese type 2 diabetic *Psammomys obesus*. One gene, the presenilins-associated rhomboid-like (Parl) gene, was of particular interest for two reasons. First, expression of Parl was reduced by 50% in skeletal muscle of obese, type 2 diabetic animals compared to lean glucose tolerant animals. Second, the human homolog, *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 (Kissebah et al. 2000) (Figure 5.1). Parl expression was also negatively correlated with blood glucose and plasma insulin levels in Israeli sand rats (Walder et al. 2005). 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 (Walder et al. 2005). This lends support to the idea that *PARL* is required for mitochondrial function and thereby promotes insulin sensitivity.

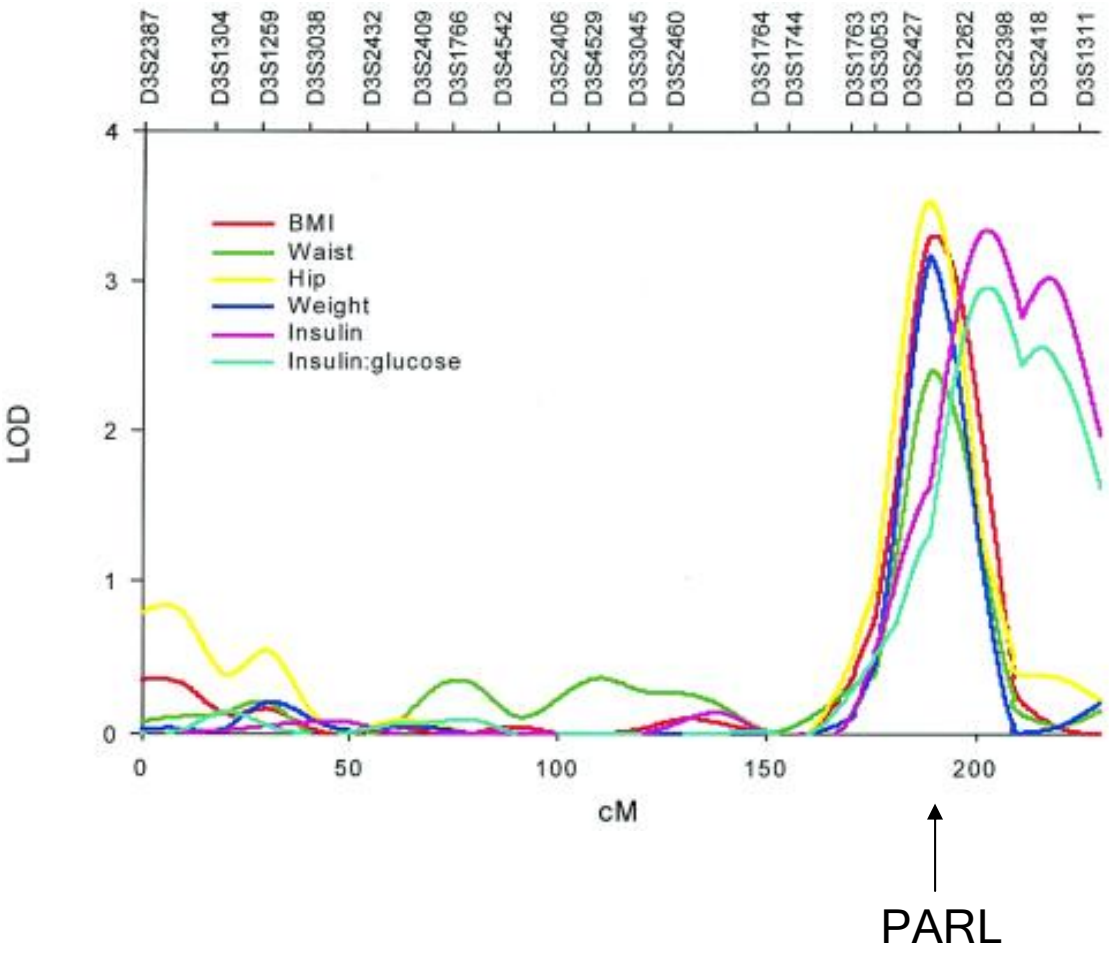

Figure 5.1 Evidence for linkage of the *PARL* locus to obesity and diabetes traits in a US study

5.2.3 A SNP in *PARL* is associated with fasting insulin levels in a US cohort

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 (Figure 5.2), was genotyped in 1031 US white individuals, a sub-group of the original population in which the QTL was discovered (Kissebah et al. 2000; Walder et al. 2005). 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 Leu262Val and BMI (Walder et al. 2005).

Figure 5.2 Schematic of the *PARL* protein
L262V = location of the SNP tested in this study. Exons are shown in alternating light and dark purple, and purple boxes indicate transmembrane domains. The protein is 379 amino acids long.

5.2.7 Aims of this study

The aim of my study was to investigate whether *PARL* Leu262Val genotype is associated with fasting plasma insulin levels in participants from two UK population-based cohorts.

5.3 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 5.1. I 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. I also performed a meta-analysis of Ely and Hertfordshire studies adjusting for cohort to increase power to detect an effect of genotype on insulin levels, but no significant association was found (p = 0.79) (Table 5.2).

Table 5.1 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/m2)	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/m2)	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 5.2 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.

I found no association between genotype and BMI in Ely (p = 0.65), Hertfordshire (p = 0.75) or in the combined dataset (p = 0.94) (Table 5.1). 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 the pooled analysis adjusted for study (p = 0.14) (data not shown).

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 (Walder et al. 2005). Therefore I 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), I found no statistically significant association between *PARL* genotype and fasting insulin (p = 0.23). Secondly, I wanted to test for an interaction between genotype and BMI on fasting insulin (in other words, I wanted to determine whether the way fasting insulin varies by genotype depends on BMI). To test this I fitted an interaction term (genotype*BMI) to the regression model. However, the coefficient of the interaction term was not statistically significant (the change in fasting insulin in response to genotype did not depend on BMI) (p = 0.88).

Finally, I 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 (Table 5.3 and 5.4). Only one nominally significant association was detected with insulin increment in Ely subjects but this did not replicate in Hertfordshire participants and was not significant in a joint analysis (Table 5.4).

Table 5.3 Summary of glucose traits and insulin increment in Ely and Hertfordshire study participants by *PARL* Leu262Val genotype

				P value
	GG	GC	CC	
Ely				
Fasting glucose	5.12 ± 0.04	5.07 ± 0.03	5.12 ± 0.05	0.47
2-hour glucose	6.46 ± 0.13	6.44 ± 0.09	6.48 ± 0.14	0.94
Insulin increment	39.49 ± 1.52	36.44 ± 0.91	35.87 ± 1.25	0.03
Hertfordshire				
Fasting glucose	5.97 ± 0.05	5.89 ± 0.03	5.91 ± 0.04	0.15
2-hour glucose	7.68 ± 0.13	7.39 ± 0.09	7.48 ± 0.12	0.05
Insulin increment	603.09 ± 20.55	629.07 ± 18.0	621.20 ± 25.6	0.66

Data are means ± SE. The P value indicates the results of regression analysis assuming dominance of the C allele. All statistical analyses were performed on log transformed data.

Table 5.4 Analysis of associations between *PARL* Leu262Val genotype and glucose traits and insulin increment in pooled Ely and Hertfordshire cohorts

	n	B ± SE	P value
Fasting glucose	3715	-0.008 ± 0.005	0.12
2-hour glucose	3599	-0.02 ± 0.01	0.13
Insulin increment	2900	-0.05 ± 0.03	0.08

B = Regression coefficient is the mean change (± SE) in the trait of interest when the C allele is present. All analyses were performed on log transformed data and included adjustment for cohort term.

5.4 Discussion

Based on 3666 study participants with full genotypic and phenotypic data, I found no evidence of an association between the *PARL* Leu262Val SNP and fasting insulin levels. Nor did I replicate the genotype- age interaction on fasting insulin levels (Walder et al. 2005), 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 my 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 (Walder et al. 2005). 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 ± 7.2 kg/m^2 in the US cohort compared to 27.3 ± 4.5 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, I repeated my 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.

My study may have lacked the statistical power to detect the association of Leu262Val genotype with plasma insulin levels. However, I estimated that the pooled study would have had a 93% probability of detecting the previously reported effect size (a fasting insulin decrease of 7.84 pmol/l in GC and CC genotypic classes

compared to GG homozygotes), given the frequency of the C allele in my study (0.49) and my sample size of 3666 with a type 1 error rate of 0.05. 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%. I therefore cannot exclude a false negative finding in this stratified analysis.

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.

I detected a nominally significant difference in age between those Ely subjects with a C allele and those without (Table 5.1), 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.

The PARL data from Ely and Hertfordshire cohorts was published in 2006 (Fawcett et al. 2006). Since then, data from genome-wide association studies have become publicly available allowing me to evaluate the role of this polymorphism in the context of type 2 diabetes. Leu262Val is not on the Affymetrix 500k SNP chip but is present on Illumina 317k *P*-value based meta-analysis of WTCCC, DGI, and FUSION studies by the DIAGRAM consortium showed no statistical association of rs3732581 with type 2 diabetes ($P = 0.07$) (Zeggini et al. 2008).

In summary, my results suggest that *PARL* Leu262Val is unlikely to importantly contribute to insulin resistance in UK populations. Furthermore, meta-analyses of data from whole-genome association studies show no association of this SNP with type 2 diabetes risk.

5.5 Materials and Methods

5.5.1 Description of cohorts

The MRC Ely study and Hertfordshire cohort study are population-based cohorts comprised of white men and women of European ancestry. Participants underwent standard anthropometric measurements and a 75-g OGTT. These cohorts are described in more detail in Chapter 2.

5.5.2 Genotyping

The Leu262Val SNP (rs3732581) was genotyped in Ely using an ABI PRISM SNaPshot™ multiplex kit (Applied Biosystems, Foster City, CA). First, a~400 bp product containing the Leu262Val SNP locus was amplified by PCR (Chapter 2.3.2) (Forward primer: AGGAGAAGTATTCATGCCCG, reverse primer: ACTGCACCAAGTGATGGTCC) and purified as described in 2.3.4 except that 3.86 units SAP (USB) and 2.90 units Exo1 (USB) were used. Each SNaPshot extension reaction, containing 0.24 µM of probe (CAGGTGTTATTTCCAATTTTGTCAGTTAC) designed to complement sequence adjacent to the SNP, 2 µl PCR product, and 4 µl SNaPshot, was carried out by repeating the following cycle 40 times: 96°C for 10 seconds, 50°C for 10 seconds, and 60°C for 30 seconds. The extension product was incubated with 1 unit of SAP at 37°C for 1 hour then 80°C for 15 minutes. 0.5 µl of the product was mixed with 1 µl GeneScan 120LIZ™ Size Standard (Applied Biosystems) and 8.5 µl dimethylformamide Hi-Di, denatured at 96°C for 2 minutes, and then electrophoresed on an ABI 3100 genetic analyser (Applied Biosystems). Analysis was carried out using GeneMapper v3.0 software (Applied Biosystems, UK). One-hundred-and-twenty 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 by Matthew Sims at MRC epidemiology unit. Allele calling was

performed on the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, UK). One-hundred-and-fifty-five water blanks and 91 duplicates were included. Genotyping success rate was 96% and there was no discordance between replicate samples.

5.5.3 Statistical analysis

Linear regression analysis in Stata v8 (College Station, TX) was used to assess the association between genotype and fasting plasma insulin or BMI in 1608 Ely and 2058 Hertfordshire cohort samples with full genotypic and phenotypic data. Based on the previous reported association, my primary analysis was for a dominant genetic model and I tested genotype–age interactions using log-likelihood ratio tests. In secondary exploratory analyses, I 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. I also investigated whether the association between genotype and fasting insulin was modified by BMI. To increase power I undertook all analyses on both cohorts together, adjusting for study.