

Association of the PGC-1 α rs8192678 variant with microalbuminuria in subjects with type 2 diabetes mellitus

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Abstract. PPAR- γ co-activator-1 α (PGC-1 α) is a tissue-specific transcriptional co-activator involved in the regulation of antioxidant enzymes. The A-allele of the rs8192678 PGC-1 α (G>A) gene variant has previously been associated with nephropathy in Korean and Indian-Asian type 2 diabetes mellitus (T2DM) samples. Our aim was to examine the association between this variant and urine albumin excretion in European subjects with T2DM.

Genotyping was performed on 583 European subjects with T2DM and examined in relation to urinary albumin, plasma oxidized-LDL and small dense-LDL percentage. We observed a significant association between genotype (GG/GA/AA) and urinary albumin (normoalbuminuria v micro/macroalbuminuria: 48.6/39.7/11.7% v 38.2/51.2/10.5%, $p = 0.02$; for GG v GA/AA, $p = 0.01$). The odds ratio for micro/macroalbuminuria in GA and AA subjects relative to GG were 1.70 [1.15–2.50], $p = 0.008$ and 1.20 [0.66–2.16], $p = 0.56$ respectively (for GA/AA v GG: 1.58 [95%CI: 1.09–2.27], $p = 0.02$). There was a significant association between the A allele and a higher percentage of small dense-LDL particles (GG v GA v AA: 70.8 [58.01–81.06] % v 72.8 [56.18–81.19] % v 78.9 [67.16–85.33] %, $p = 0.03$).

In European subjects with T2DM the GA relative to the GG genotype is associated with a 70% increase in the risk of micro/microalbuminuria. Furthermore, homozygosity for the A-allele is also associated with a preponderance of small dense-LDL particles.

Keywords: PGC-1 α , type 2 diabetes mellitus, microalbuminuria, oxidative stress

1. Introduction

The endothelium plays a major role in vascular homeostasis regulating vascular tone, platelet activity, leukocyte adhesion, inflammation and thrombosis [1, 2]. Endothelial dysfunction is associated with cardiovascular disease (CVD), diabetes mellitus, and chronic renal impairment [3]. In subjects with diabetes, endothelial dysfunction is associated with microalbumin-

uria and proteinuria [4], and therefore microalbuminuria may be regarded as a marker of vascular dysfunction and CVD risk in subjects [5]. Microalbuminuria is also associated with increased oxidative stress and elevated inflammatory cytokines such as interleukin-1, interleukin-6 and TNF- α [6]. CVD risk in diabetes increases with progression from normoalbuminuria to microalbuminuria and macroalbuminuria [5]. Compared to subjects with normoalbuminuria survival for subjects with microalbuminuria is reduced by 20% and for those with macroalbuminuria by 30% [7].

Peroxisome proliferator-activated receptor- γ co-activator-1 α (PGC-1 α) is a tissue-specific transcriptional co-activator [8] involved in the activation of sev-

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eral nuclear hormone receptors essential for the regulation of adaptive thermogenesis, cellular respiration, adipogenesis and oxidative metabolism [9]. Of particular importance, PGC-1 α is involved in the regulation of genes involved in glucose and lipid metabolism [10] as well as antioxidant enzymes such as glutathione peroxidase-1 and superoxide dismutase-2 [11]. The PGC-1 α gene is located on chromosome 4p15.1 [12] and spans 13 exons [13]. The PGC-1 α protein stimulates mitochondrial biogenesis and respiration by coactivating nuclear respiratory factor-1, which in turn binds to the transcription factor peroxisome proliferator-activated receptor- γ and induces the expression of mitochondrial uncoupling protein-2 [8,14] which protects against cellular oxidative damage [15–17].

Genetic variation at position 1444 in exon 8 of the PGC-1 α gene (*rs8192678*) results in a G>A base substitution [18], causing a glycine to serine change in the amino acid sequence (Gly482Ser) and a structural protein change [19]. The minor A-allele (coding a serine residue) results in lower protein levels and reduced activity of the PGC-1 α protein in muscle tissue from subjects with insulin-resistance and diabetes [20,21] and is also associated with lower levels of fatty acid oxidation [22]. Carriers of the A-allele also exhibit increased levels of DNA damage [11]. The AA genotype (Ser/Ser) has been associated with hypertension in male subjects with type 2 diabetes mellitus (T2DM) [18] and furthermore is also associated with an increased risk of T2DM [23]. Two previous studies have investigated this gene variant with respect to diabetic nephropathy. In an Asian Indian sample, the A-allele had an autosomal dominant association with diabetic nephropathy and homozygosity for the A-allele was associated with an eight-fold increase in risk [24]. With a Korean sample, the A-allele was associated with a 1.5-fold increase in nephropathy risk [25].

Our aim was to examine the association between the *rs8192678* (Gly482Ser) PGC-1 α gene variant with urinary albumin as a marker of endothelial dysfunction, in a cross-sectional sample of European subjects with T2DM.

2. Methods

2.1. Subjects

Ethical approval was obtained from the UCL and UCL Hospitals Research Ethics Committee and all subjects gave written informed consent before recruit-

ment. Subjects were recruited as part of the University College London Diabetes and Cardiovascular Study (UDACS), described elsewhere [17,26]. Briefly, this comprises of consecutive subjects recruited from the diabetes clinic at University College London Hospitals (UCLH) NHS Trust between the years 2001–2002. All subjects had diabetes according to WHO criteria [27]. Microalbuminuria was defined as an albumin:creatinine ratio (ACR) of greater than 2.5 mg/mmol in men and 3.5 mg/mmol in women [28–30]. Macroalbuminuria was defined as an ACR > 30 mg/mmol. At the outset subjects with micro/macroalbuminuria were grouped together for analysis. All urine samples were first-voided. None of the subjects were knowingly taking any form of vitamin supplementation. Plasma samples were collected during the twelve months of recruitment and stored immediately at -80°C . Samples were collected during routine diabetes clinic visit. None of the patients were fasting at the time of collection.

2.2. Genotyping for the *rs8192678* gene variant

Genomic DNA was extracted from 5ml EDTA blood samples. Genotyping was conducted using polymerase chain reaction amplification followed by restriction digest (HpaII). Primer sequences were: forward TGCTACCTGAGAGAGACTTTG and reverse CTTTCACTCTCGCTGTCATC [20]. Genotype was confirmed by two independent technicians and any discrepancy resolved by repeat genotyping.

2.3. Measurement of plasma total anti-oxidant status

Plasma total anti-oxidant status (TAOS) was measured by Sampson's modification of Laight's photometric microassay [31], using 2.5 μl citrated plasma samples in 96-well ELISA plates. The TAOS of plasma was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS+) radical. The inter- and intra-assay coefficients of variation were 10.1% and 4.3% respectively. Previously, we have shown that baseline plasma TAOS is associated with prospective risk and has a good correlation with plasma F₂-isoprostanes [32].

2.4. Measurement of plasma Ox-LDL

Plasma oxidized LDL (Ox-LDL) was measured using a commercially available Enzyme-Linked Im-

munosorbent Assay (ELISA) kit supplied by Merckodia (Uppsala, Sweden). In this assay a monoclonal antibody is directed against antigenic determinants in the Ox-LDL molecule (mAB-4E6).

2.5. Measurement of low density lipoprotein particle diameter

Low density lipoprotein (LDL) at d 1.019–1.063 g/ml was isolated from EDTA plasma. Electrophoresis was performed as previously described [33]. Polyacrylamide gels were used to determine LDL peak particle diameter (LDL-PPD) and LDL mean particle diameter (LDL-MPD) and the % SD-LDL was determined from the LDL particle size [34].

2.6. Measurement of plasma C-reactive protein

C-reactive protein (CRP) levels were measured with a highly sensitive in-house ELISA assay with rabbit anti-CRP (Dako, Copenhagen, Denmark) as a catching and tagging antibody, as described previously [35]. The inter-assay and intra-assay CV were 8% and 10% respectively and limit of detection 0.15 mg/L.

2.7. Statistical analysis

Statistical analysis was performed using SPSS (version 10.1, SPSS Inc., Chicago). Data are reported for those individuals with available genotype. Results are presented as mean and standard deviation. Deviations from Hardy-Weinberg equilibrium were considered using chi-squared tests. Allele frequencies are shown with the 95% confidence interval. The association between genotype and urinary albumin excretion was examined by chi-squared testing and by calculating the odds ratios (ORs) with 95% confidence intervals derived by logistic regression analysis. For continuous data that was normally distributed after log transformation, the geometric mean and approximate standard deviation is shown. This included systolic blood pressure, diastolic blood pressure, body mass index (BMI), HbA_{1c}, random glucose, LDL-cholesterol and triglyceride. For duration of diabetes, creatinine, HDL-cholesterol, TAOS, Ox-LDL and % small dense LDL particles, the data could not be transformed to a normal distribution and so the data is shown as median and interquartile range. Analysis of variance (ANOVA) was used to assess the association between genotype and baseline characteristics for data that was normally distributed after log transformation. For duration of

diabetes, creatinine, HDL-cholesterol, TAOS, Ox-LDL and % small dense LDL particles, data was analysed by the Kruskal-Wallis test and Mann-Whitney tests. Chi-squared tests were used to compare differences in categorical variables by genotype. In all cases a p-value of less than 0.05 was considered statistically significant. Two sided statistical testing was performed.

3. Results

3.1. Characteristics of study subjects

As shown in Table 1, micro/macroalbuminuria was associated with the male gender, increasing age, systolic blood pressure, serum creatinine, HbA_{1c} and lower HDL-cholesterol. There were also a higher proportion of ACE inhibitor and aspirin use in those with micro/macroalbuminuria.

3.2. Genotype data

Genotype data was available on 980/1002 (97.8%) subjects. We chose to focus on European patients with T2DM since all previous published studies looking at this variant have focussed on T2DM. There were a total of 583 European subjects with T2DM with available genotype and data on urine protein excretion was available on 575 subjects (98.6%). The genotype distribution for the *rs8192678* (1444G>A) gene variant was in Hardy-Weinberg equilibrium (GG/GA/AA: 254/264/65, $\chi^2 = 0.08$, $p = 0.77$), with a minor A-allele frequency of 0.34 [95%CI: 0.31–0.37]. The mean age of the GG subjects was approximately two years older than the A-allele carriers (GG/GA/AA: 68.0/65.4/66.4 years, $p = 0.03$). Furthermore, there were a significantly lower proportion of males with the GG genotype (GG/GA/AA: 53.5/65.9/56.9% males, $p = 0.02$).

3.3. Genotype association with urinary protein excretion

There was a significant difference in genotype distribution in the urinary albumin excretion groups (GG/GA/AA within normoalbuminuria v micro/macroalbuminuria: 48.6/39.7/11.7% v 38.2/51.2/10.6%, $p = 0.02$; for GG v GA/AA, $p = 0.02$) (Table 1). Relative to the GG genotype the OR for micro/macroalbuminuria in GA and AA subjects were 1.64 [1.12–2.33], $p = 0.005$ and 1.14 [0.66–1.98], $p = 0.64$ respectively. The

Table 1
Baseline differences by urine protein excretion

Variable	Normoalbuminuria (N = 290)	Micro/macroalbuminuria (N = 285)	P
Age (years)	65.6 (10.7)	67.8 (11.2)	0.01
Males % (n)	52.9	66.4	0.001
Duration of diabetes (years)#	8[4–15]	10 [5–17]	1.00
Systolic blood pressure (mmHg)*	138 (19)	143 (22)	0.006
Diastolic blood pressure (mmHg)*	79 (11)	80 (12)	0.21
Body mass index (kg/m ²)*	29.4 (5.7)	39.3 (5.3)	0.79
Creatinine(μ mol/L)#	87 [77–100]	96 [82–120]	< 0.001
HbA _{1c} (%)*	7.5 (1.5)	7.8 (1.8)	0.006
Random glucose mmol/L)*	9.3 (4.0)	10.6 (4.6)	< 0.001
LDL cholesterol (mmol/L)*	2.6 (0.4)	2.6 (0.4)	0.89
HDL cholesterol (mmol/L)#	1.3 [1.1–1.6]	1.2 [1.0–1.5]	0.002
Triglyceride (mmol/L)*	1.9 (1.1)	1.9 (1.1)	0.80
TAOS (%)#	44.8 [36.9–52.1]	43.8 [33.7–52.0]	0.27
Ox-LDL#	45.8 [34.8–54.3]	48.6 [38.1–62.2]	0.01
Mean LDL size (nm)	26.62 (0.88)	26.50 (0.96)	0.29
Peak particle diameter LDL (nm)	26.82 (0.85)	26.73 (0.80)	0.21
% Small dense-LDL#	72.8 [60.0–81.5]	72.2 [55.2–81.3]	0.36
Current smoker % (n)	17.0% (49)	13.9% (39)	0.31
ACE inhibitor use % (n)	38.4% (111)	61.1% (174)	< 0.001
Aspirin use % (n)	44.3% (128)	57.6% (164)	0.001
Insulin use % (n)	26.0% (75)	26.8% (76)	0.85
Statin use % (n)	30.8% (89)	33.1% (94)	0.56
Genotype (GG/GA/AA) % (n)	48.6/39.7/11.7 (141/115/34)	38.2/51.2/10.6 (109/146/30)	0.02

Urine albumin data was available on 575 of the 583 with genotype data (98.6%). # Median and interquartile range shown for HDL, TAOS and Ox-LDL. *Log transformed data. Mean and standard deviation shown or geometric mean and approximate standard deviation for log transformed data. Analysis performed by ANOVA after appropriate transformation of non-normally distributed data and by Kruskal-Wallis for HDL, TAOS and Ox-LDL. χ^2 -test was used to compare groups.

OR for micro/macroalbuminuria in the GA/AA subjects relative to GG subjects was 1.53 [95%CI: 1.10–2.13], $p = 0.01$. After adjusting for the variables associated with urine albumin excretion described in section 3.1 (male gender, age, systolic blood pressure, serum creatinine, HbA_{1c}, lower HDL-cholesterol, ACE inhibitor and aspirin use) the association remained significant with the OR for micro/macroalbuminuria relative to GG in GA and AA subjects were 1.70 [1.15–2.50], $p = 0.008$ and 1.20 [0.66–2.16], $p = 0.56$ respectively, with the OR for micro/macroalbuminuria in the GA/AA subjects relative to GG subjects being 1.58 [95%CI: 1.09–2.27], $p = 0.02$.

3.4. Association with markers of oxidative damage and small dense-LDL

Plasma Ox-LDL was higher in those with micro/macroalbuminuria compared to those with normoalbuminuria (48.6 [38.1–62.2]U/L v 45.8 [34.8–54.3]U/L respectively, $p = 0.01$). No association was observed between urinary albumin excretion and plasma TAOS (43.8 [33.7–52.0]% v 44.8 [36.9–52.1]%,

$p = 0.27$) or LDL particle size (MPD: 26.73 (0.80)nm v 26.82 (0.85)nm, $p = 0.21$).

No association between genotype (GG/GA/AA) and plasma TAOS (45.4 [36.9–51.8]% v 43.0 [33.9–52.8]% v 44.9 [35.0–52.8]%, $p = 0.43$), Ox-LDL (46.1 [35.2–58.7] v 49.0 [39.1–57.9] v 43.4 [32.4–56.0], $p = 0.13$) or LDL -MPD (26.77 (0.71)nm v 26.72 (0.91)nm v 27.02 (0.84)nm, $p = 0.07$) was observed. There was a significant association between the AA genotype the percentage of small dense-LDL particles (GG v GA v AA: 70.8 [58.01–81.06]% v 72.8 [56.18–81.19]% v 78.9 [67.16–85.33]%, $p = 0.03$).

4. Discussion

This study examined the rs8192678 (1444G>A) variant of the PGC-1 α gene in relation to micro/macroalbuminuria in a European sample of subjects with T2DM. We chose from the outset to focus on European subjects because of differences in genotype distribution by ethnic backgrounds (Table 2). Previously a higher prevalence of the GG genotype has been

Table 2
Genotype distribution grouped by ethnic origin

Ethnic Group	GG	GA	AA	A-allele frequency
African	79.2% (61)	18.2% (14)	2.6% (2)	0.12 [0.07–0.17]
European	44.5% (345)	44.4% (344)	11.1% (86)	0.33 [0.31–0.36]
Indian-Asian	54.5% (60)	38.2% (42)	7.3% (8)	0.26 [0.21–0.32]
Asian	22.2% (4)	61.1% (11)	16.7% (3)	0.66 [0.54–0.78]

Genotype data was available on 980/1002 (97.8%). Genotype distribution (% and n within each ethnic group) is shown. Analysis between groups $\chi^2 = 41.2$, $p < 0.000001$.

observed in Africans and of the AA genotype in Asians (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=8192678). As expected we observed that subjects with micro/macroalbuminuria had increased plasma levels of Ox-LDL, a finding that was independent of other risk factors. We also observed that the rs8192678 (1444G>A) A-allele was associated with a 50% increase in the risk of micro/macroalbuminuria. The greatest risk was associated with the GA genotype (70% compared to the GG genotype). Previous studies in Asian Indian [24] and Korean [25] samples have observed an autosomal dominant risk of nephropathy associated with the A-allele. We did not observe a greater risk associated with the AA genotype. One possible explanation may be that if risk was markedly increased in AA subjects they may have experienced fatal CVD events. The subjects in our study sample were at least 10 years older and had a higher BMI than the Korean and Asian Indian studies. This is speculative but as described we observed that the AA genotype was associated with a higher proportion of small dense-LDL particles but we observed no association between genotype and plasma markers of oxidative stress. Small dense-LDL particles are more susceptible to oxidation [36], which may result in the generation of Ox-LDL, endothelial dysfunction, micro/macroalbuminuria [37] and increased CVD risk [38–41]. We also observed that there was greater ACE inhibitor and aspirin use in those with micro/macroalbuminuria compared to those without. These agents have anti-inflammatory and antioxidant properties [2] and this may account for the lack of association between genotype and the plasma markers of oxidative stress. There was no difference in statin use between those with or without micro/macroalbuminuria.

There are limitations to our study. We have chosen from the outset to examine one gene variant in PGC-1 α in relation to micro/macroalbuminuria and intermediate biochemical markers of oxidative stress. We have focussed on plasma TAOS and Ox-LDL as intermediate biochemical phenotypes. There are limitations to these measurements as discussed elsewhere [32,42].

Further work should be performed to look at other variants within the PGC-1 α regulatory region to allow haplotype based analysis. This would also allow linkage disequilibrium across the region to be examined more fully. At first sight, the lack of any significant association between genotype and plasma markers of oxidative stress might appear to conflict with the associations observed for micro/macroalbuminuria and the proportion of small dense-LDL. One possible explanation for this might be the high proportion of aspirin and ACE inhibitor use described above. Furthermore, diabetes with increased obesity, oxidative burden, inflammation and hyperglycaemia might all overwhelm the PGC-1 α genotype ‘strength of signal’ and hence the genotype-intermediate phenotype association may not be observed. Case-control cross-sectional studies are prone to intrinsic bias, for example, due to possible altered rates of disease progression, subsequent progression of secondary phenotypes or genotype associations with death or treatment changes. Therefore, this SNP should be examined in a non-diabetic sample. Such influences are well-recognised confounders [43,44].

This report adds to the current literature relating to PGC-1 α gene variants. Further prospective analysis of the gene variant is required in diabetic and non-diabetic cohorts with measured plasma markers of oxidative stress, however access to such cohorts can be difficult [45].

Acknowledgments

Diabetes UK supported JWS (BDA: RD01/0001357) and the creation of UDACS. SEH is supported by the British Heart Foundation (RG2005 014).

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