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Short Communication

The Gly482Ser polymorphism in the peroxisome proliferator-activated receptor-γ coactivator-1 gene is associated with hypertension in type 2 diabetic men

N. Cheurfa[1] · **A. F. Reis**[1, 2] · **D. Dubois-Laforgue**[3] · **C. Bellanné-Chantelot**[4] · **J. Timsit**[3] · **G. Velho**[1]

[1] INSERM Research Unit 561, Hôpital Saint Vincent de Paul, Paris, France
[2] Laboratory of Molecular Endocrinology, Federal University of São Paulo, São Paulo, Brazil
[3] Department of Immunology and Diabetology, Cochin Hospital, Paris, France
[4] Molecular Biology Unit, Saint Antoine Hospital, Paris, France

Abstract

Aims/hypothesis. Peroxisome proliferator-activated receptor-γ coactivator-1 (PPARGC1) acts as a cofactor for several nuclear hormone receptors in many tissues and organs implicated in blood pressure regulation. Here, we assessed the association between the *Gly482Ser* variant of PPARGC1 and the arterial hypertension frequently found in subjects with type 2 diabetes.
Methods. We studied a group of 479 men and 253 women with type 2 diabetes. Arterial hypertension was present in 70% of the men and in 73% of the women. Genotypes were examined by PCR restriction fragment length polymorphism. A logistic regression analysis was performed to assess the covariables associated with arterial hypertension.
Results. There was an association between *Ser* allele homozygosis and arterial hypertension in type 2 diabetic men (odds ratio of 2.52 vs *Gly* allele homozygosis; 95% CI: 1.32–5.00; p=0.0064), but not in women. The prevalence of arterial hypertension in type 2 diabetic men was 77% vs 73% vs 67% for *Ser-Ser*, *Gly-Ser* and *Gly-Gly* carriers respectively (p=0.021). Age, BMI, the use of insulin, and triglyceride and creatinine levels were also independently associated with arterial hypertension in this cohort.
Conclusions/interpretation. We have observed a sex-specific association between the *PPARGC-1* gene *Gly482Ser* polymorphism and arterial hypertension in type 2 diabetic men. Further studies are needed to investigate the genetic, biochemical and pathophysiological basis of this allelic association.

Keywords Arterial hypertension · Association studies · Polymorphisms · PPARGC1 · Type 2 diabetes

Introduction

Peroxisome proliferator-activated receptor-γ coactivator-1 (PPARGC1) is a coactivator of several nuclear receptors including PPAR-α and γ, thyroid hormone receptor, mineralocorticoid receptor and oestrogen receptors [1]. PPARGC1 is expressed in vascular endothelial and smooth muscle cells, and in the brain, heart and kidney [1, 2], and could play a role in the regulation of blood pressure by interacting with mineralocorticoid and oestrogen receptors. Genetic linkage between chromosome 4p15.1–2, near the *PPARCG1* locus, and systolic blood pressure was reported [3]. Recently, a frequent polymorphism in exon 8 of the *PPARGC1* gene (*GGT>AGT*; *Gly482Ser*) was shown to be associated with arterial hypertension in men, but not in women, in a cohort of middle-aged Austrian subjects, essentially composed of non-diabetic individuals [2]. In the present investigation, we studied the possible association between this single nucleotide polymorphism and the arterial hypertension frequently found in subjects with type 2 diabetes.

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G. Velho (✉)
INSERM Research Unit 561, Hôpital Saint Vincent de Paul,
82 Avenue Denfert Rochereau, 75014 Paris, France
E-mail: gvelho@infobiogen.fr
Tel.: +33-1-44-85-62-34, Fax: +33-1-44-85-62-36

Abbreviations: PCR-RFLP, PCR restriction fragment length polymorphism · PPARGC1, peroxisome proliferator-activated receptor-γ coactivator-1

Table 1. Logistic regression analysis of parameters associated with arterial hypertension in type 2 diabetic subjects

	Odds ratio	95% CI	*p* value
Age			
1st quartile (30–53 years)	1	-	0.0001
2nd quartile (54–62 years)	3.59	2.46–5.27	
3rd quartile (63–70 years)	5.80	4.01–8.46	
4th quartile (71–89 years)	7.09	4.86–12.02	
BMI			
1st quartile (15.7–25.0 kg/m^2)	1	-	0.0005
2nd quartile (25.1–28.1 kg/m^2)	1.48	1.02–2.18	
3rd quartile (28.2–32.1 kg/m^2)	2.53	1.72–3.79	
4th quartile (32.2–55.5 kg/m^2)	4.35	2.95–6.24	
Triglycerides (range: 0.35–7.27 mmol/l)	20.37[a]	6.54–67.79	0.0001
Plasma creatinine (range: 35–780 µmol/l)	69[a]	17–312	0.0001
Insulin therapy	1.81	1.34–2.46	0.0001
Known duration of diabetes (range: 1–50 years)	4.26[a]	1.55–12.03	0.006
Sex (female)	1.28	0.91–1.82	0.15
Gly/Gly alleles[b]	1	-	-
Gly/Ser alleles in men	1.04	0.66–1.64	0.86
Ser/Ser alleles in men	2.52	1.32–5.00	0.0064
Gly/Ser alleles in women	1.38	0.73–2.62	0.33
Ser/Ser alleles in women	0.78	0.34 –1.81	0.55

All quantitative data in the model were log-transformed. However, back-transformed values are given in the table, as they have more clinical relevance. [a] Odds ratio across the range of distribution of the parameter; [b] alleles at codon 482. r^2=0.24, p<0.0001

Subjects and methods

We studied a group of unrelated French Caucasian subjects (479 men and 253 women) with type 2 diabetes, consecutively recruited in the diabetes department of the Necker Hospital, Paris, France. All subjects were negative for islet cell and glutamic acid decarboxylase autoantibodies. Arterial hypertension was defined as: (i) systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg; and (ii) blood pressure below these values in the presence of antihypertensive medication and history of hypertension. To assess the frequency of the polymorphism in the general population, we also studied a group of 102 men (aged 59±19 years) and 125 women (aged 58±17 years) with no known history of diabetes, who were recruited among spouses of the diabetic patients. No data on blood pressure status were available for the non-diabetic subjects.

The *Gly482Ser* polymorphism was examined by PCR restriction fragment length polymorphism (PCR-RFLP) as described previously [4]. Quality control of the PCR-RFLP genotype pattern was assessed by sequencing the PCR product of ten samples, randomly chosen, of each genotype. The study was approved by the ethics committee of the Necker Hospital (CCPPRB Paris Necker) and all participants gave written informed consent. Results are expressed as means ± SD. Differences between groups were assessed by Student's *t* test or by ANOVA. Contingency table chi square test and Fisher's exact test were used to compare allele frequencies and other qualitative data. Odds ratios were computed by logistic regression analysis, and the statistical power of the analysis was determined. For all these analyses, data were log-transformed when adequate and a *p* value of less than 0.05 was considered significant. Statistics were performed using JMP (SAS Institute, Carey, N.C., USA) and "Power and Precision" (Biostat, Englewood, N.J., USA) softwares.

Results

Arterial hypertension was present in 70% of the men and in 73% of the women from our cohort of type 2 diabetic patients. Hypertensive individuals of both sexes, as compared with non-hypertensive individuals, were older (64±10 vs 55±12 years, p<0.0001, data from pooled sexes), had a higher BMI (29.7±5.4 vs 27.0±5.0 kg/m^2, p<0.0001), were older at diagnosis of diabetes (52±11 vs 47±12 years, p<0.0001), had a longer duration of diabetes (12±10 vs 8±7 years, p<0.0001), had higher plasma levels of creatinine (117±97 vs 87±25 µmol/l, p<0.0001) and triglyceride (1.92±1.23 vs 1.61±0.99 mmol/l, p=0.0002), and were more often treated with insulin (35% vs 24%, p=0.002). Systolic blood pressure was 143±16 vs 122±9 mm Hg (p<0.0001) and diastolic blood pressure was 79±10 vs 72±7 mm Hg (p<0.0001) in hypertensive and non-hypertensive subjects respectively. Fasting plasma glucose, HbA_1c and total cholesterol levels were similar in the two groups (data not shown).

The frequency of the *Ser* allele was 0.366 vs 0.315 (p=0.14) in hypertensive and non-hypertensive type 2 diabetic men respectively, and 0.356 vs 0.354 (p=0.96) in hypertensive and non-hypertensive type 2 diabetic women respectively. These allelic frequencies were not different from those observed in the patients' non-diabetic spouses: 0.326 for the men (ANOVA p=0.25) and 0.357 for the women (ANOVA p=0.73). To assess a possible association between the

Gly482Ser variant and arterial hypertension in type 2 diabetic patients, a regressive model that takes into account the phenotypical differences observed in subjects with or without arterial hypertension needs to be used. Thus, a logistic regression analysis was performed to assess the covariables associated with arterial hypertension in this cohort. The presence of arterial hypertension was entered in the model as the dependent variable, and sex, age, BMI, age at diagnosis of diabetes, duration of diabetes, plasma triglyceride and creatinine levels, use of insulin, and genotype at codon 482 were entered as independent covariables (Table 1). As the genetic effect of this variant on arterial hypertension seems to be sex dependent [2], codon 482 genotype was entered in the model nested within the sex variable. This results in the computation of statistical effects for men and women separately, adjusted for multiple comparisons due to the stratification by sex.

Age, BMI, the use of insulin, triglyceride and creatinine levels, and duration of diabetes were independently associated with arterial hypertension in this cohort (Table 1). Homozygosis for the *Ser* allele of codon 482 was also independently associated with arterial hypertension in type 2 diabetic men (odds ratio of 2.52; 95% CI: 1.32–5.00, p=0.0064 as compared with homozygosis for the *Gly* allele), but not in type 2 diabetic women. To assess the robustness of the association between the *Ser* allele and hypertension in men, we computed the power of the logistic regression analysis. For the odds ratios observed for the codon 482 covariable, the power was 0.78 (type 1 error α set at 0.05, two-tail testing). These results are consistent with the differences in the prevalence of arterial hypertension according to genotype observed in the group of men: 77% vs 73% vs 67% in *Ser* allele homozygous, *Ser-Gly* allele heterozygous, and *Gly* allele homozygous subjects respectively (p=0.021 for age-adjusted comparisons, with Bonferroni's correction due to the stratification by sex).

Discussion

In the present work, we report an association between the *Ser* allele of the *Gly482Ser* variant in exon 8 of the *PPARGC1* gene and arterial hypertension in type 2 diabetic men but not in type 2 diabetic women. This allelic association was independent of other variables commonly found to be associated with arterial hypertension, such as age, obesity, dyslipidaemia and renal function. Allelic frequencies were similar in diabetic and non-diabetic subjects, which is consistent with observations in other French Caucasian cohorts [5].

A sex-related effect of this polymorphism on arterial hypertension was recently observed in a cohort of non-diabetic Austrian subjects [2]. However, in that study, the *Ser* allele was associated with a reduction in the prevalence of hypertension in men, with higher allele frequencies in non-hypertensive (0.394) than in hypertensive (0.314) subjects. Different risk alleles in different populations have been observed for other susceptibility genes for type 2 diabetes and might be related to variable degrees of linkage disequilibrium of the polymorphism with the putative functional mutation. Alternatively, our results may merely represent a type 1 error, but we believe this to be a less likely explanation. Indeed, the probability of type 1 error (α) was less than 0.7% (p=0.0064) when we concluded that the odds ratio for *Ser* allele homozygosis as a risk for arterial hypertension in men was included in the 95% CI (1.32–5.00). With respect to type 2 error (β), the power of our study to detect the odds ratio observed for the genotype at codon 482 in men was 0.78. In other words, if homozygosis for the *Ser* allele increased the odds of arterial hypertension by 2.52, there was a 78% chance that we detected this effect in the set of our testing. Moreover, other studies have shown an association between the *Ser* allele, or the *Ser* allele-containing haplotypes, and various components of the metabolic syndrome. Associations with type 2 diabetes were observed in some [4, 6] but not all [5] studies, but associations were also observed with hyperinsulinaemia and insulin resistance [6] and with several obesity-related phenotypes [7]. Of note, the frequency of the *Ser* allele in our cohort of type 2 diabetic patients, with sex and blood pressure status combined (0.354), was in the range of that observed in other cohorts of French type 2 diabetic patients (0.352–0.363) [5], although it was lower than the frequency reported in Danish subjects with type 2 diabetes (0.370 and 0.381) [4]. The allelic frequency in the patients' non-diabetic spouses (0.340 for pooled sexes) was also in the range of that observed in other cohorts of non-diabetic Caucasians (0.304–0.369) [4, 5, 7]. No allelic frequency data in hypertensive and non-hypertensive non-diabetic French Caucasians is currently available for comparison. Of note, with arterial hypertension being twice as frequent in type 2 diabetic patients as in non-diabetic individuals [8], it is possible to speculate that the previously observed associations between the *Gly482Ser* variant and type 2 diabetes [4, 6] may reflect associations with hypertension.

There is no established explanation for the sex-specific association between the *Gly482Ser* variant and arterial hypertension in men, and for that matter, for the sex-specific associations between the same polymorphism and obesity-related traits in non-diabetic women [7]. PPARGC1 acts as a cofactor for several transcription factors in many tissues and organs implicated in blood pressure regulation such as the vascular bed, the heart and the kidneys [1, 2]. PPARGC1 interacts with oestrogen receptors α and β in vascular endothelial cells and enhances their transcriptional activity [2, 9]. Interestingly, mice deficient in oestrogen receptor-β develop arterial hypertension [10].

In conclusion, we have observed a sex-specific association between the *PPARGC1* gene *Gly482Ser* polymorphism and arterial hypertension in type 2 diabetic men. Type 2 diabetes and arterial hypertension are multifactorial polygenic disorders that frequently coexist and could therefore share susceptibility genes. Further studies are needed to investigate the genetic, biochemical and pathophysiological basis of this allelic association.

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