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Association of the Arg72Pro Polymorphism in p53 with Progression of Diabetic Nephropathy in T2DM Subjects

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Abstract

Objective: In addition to its anticancer function, p53 (regulated by murine double minute 2 oncoprotein, MDM2) has recently been shown to control intracellular metabolic processes. It participates in the regulation of glucose, fatty and amino acid and purine metabolism, influences mitochondrial integrity and oxidative phosphorylation, insulin sensitivity, antioxidant response and autophagy. With respect to the possible impact of genetic variability in p53 and MDM2 on metabolic compensation the aim of the study was to analyse the effect of common germ line Single Nucleotide Polymorphisms (SNPs) - Arg72Pro in the *TP53* and SNP309 in the *MDM2* - on the progression of Diabetic Nephropathy (DN), cardiovascular morbidity and mortality and all-cause mortality in Type 2 Diabetes Mellitus (T2DM) subjects.

Methods: The cross-sectional study comprised a total of 309 (a sum of 155 and 154) unrelated Caucasian diabetic patients with diabetes duration at least 10 years and variable renal function at baseline (309, mean age was 67.2 ± 10.8 years). The stage of diabetic nephropathy was defined according to the urinary albumin excretion and glomerular filtration rate. Patients were followed-up for median 37 (20-59) months. The following end-points were considered: (a) progression of DN, (b) major cardiovascular event (non-fatal or fatal myocardial infarction or stroke, limb amputation, revascularization), (c) all-cause mortality. Genotypes were determined by PCR-based methodology. Time-to-event analysis using Kaplan-Meier curves and log-rank test was used.

Results: We found significant difference between CG+GG vs. CC genotypes of the p53 Arg72Pro SNP for DN progression (P=0.046, log-rank test). Carriers of genotypes containing G allele (previously associated with susceptibility to T2DM) had faster progression of DN than CC genotype carriers. We did not find any significant difference between genotypes of MDM2 SNP for any of the end-points studied.

Conclusions: Presented findings in general support the role of p53 in the pathogenesis of metabolic diseases, namely progression of hyperglycemia-related morbidity. Nevertheless, further studies are warranted to elucidate the eventual causal involvement of p53 pathway in the development of diabetic complications.

Keywords: p53; Murine double minute 2 oncoprotein; Diabetic nephropathy; Cardiovascular disease; Mortality; Single nucleotide polymorphism; Association

Abbreviations: CKD: Chronic Kidney Disease; DN: Diabetic Nephropathy; MDM2: Murine Double Minute 2 Oncoprotein; PPP: Pentose Phosphate Pathway; T2DM: Type 2 Diabetes Mellitus

Introduction

The physiological role of p53 protein in the prevention of cancer development through blocking cell cycle progression, regulating cellular senescence or apoptosis is well established [1]. In recent years though, the emerging role of p53 in metabolic regulation has been a topic of great interest. Not only p53 is activated by stress signals, it also seems to complexly control energy metabolism under normal conditions [2]. By regulating gene expression and other indirect means p53 participates in the regulation of glucose, fatty acid, amino acid (glutaminolysis) and purine metabolism, influences mitochondrial integrity and oxidative phosphorylation, insulin sensitivity, antioxidant response, autophagy and mammalian Target of Rapamycin (mTOR) signalling to name a few [3]. Importantly, p53 induces expression of TP53-Induced Glycolysis And Apoptosis Regulator (TIGAR) which stimulates Pentose Phosphate Pathway (PPP) with subsequent production of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) necessary for reduction of glutathione thus supporting an efficient antioxidant defence [4]. Activation of PPP is especially relevant in diabetes where it may have protective effect counteracting the negative consequences of hyperglycaemia. PPP can process glucose intermediates accumulating due to hyperglycaemia that activate metabolic pathways largely responsible for the development and progression of microvascular diabetic complications [5].

A host of tumour-associated mutations have been described in the *TP53* gene (chromosome 17p13.1) resulting in very different activities from wild-type p53 such as either loss- or gain-of-function [6]. Apart from somatic mutations, inherited germ line common polymorphisms in the *TP53* gene may also contribute to the physiological and pathophysiological functions of p53. One of them - a guanine to cytosine exchange in exon 4 (rs1042522) of the *TP53* causing substitution of arginine to proline in the codon 72 – has been widely studied in the context of various tumours since the two variants exhibited markedly different pro-apoptotic potential [7]. Due to the previously reported positive association of p53 polymorphism with susceptibility to Type 2 Diabetes Mellitus (T2DM) in a large scale candidate gene association study [8] and results of experimental studies indicating that p53 expression in adipose tissue is involved in the development of insulin resistance [9] the focus broadened to non-oncological fields as well. Recently, two large studies reported association of allele Arg72 with T2DM [10] and with the degree of insulin resistance in T2DM [11]

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and there are also reports of its association with coronary artery disease [12,13].

The activity of p53 is negatively controlled by Murine Double Minute 2 Oncoprotein (MDM2) which promotes p53 degradation and is considered as a major negative regulator of p53 [14]. The levels of MDM2 strongly affect p53 response and even small increase in MDM2 level can attenuate p53 function with possible tumour development [15]. First intron of the *MDM2* gene contains second promoter with binding site for p53 [16]. This intron contains a thymine to guanine exchange at position 309 which creates SP1 transcription factor binding site and increase basal levels of MDM2 [15].

Considering increasingly appreciated role of p53 in the regulation of metabolism and its close interaction with MDM2 we were interested whether common functional Single Nucleotide Polymorphisms (SNPs) in the *TP53* and *MDM2* genes may affect development and progression of diabetes-related morbidity and mortality. Specifically, the aim of the study was to analyse the effect of SNPs Arg72Pro in the *TP53* and SNP309 in the *MDM2* on the progression of Diabetic Nephropathy (DN), cardiovascular morbidity and mortality and all-cause mortality in T2DM subjects.

Material and Methods

Subjects

The study comprised a total of 309 unrelated Caucasian T2DM patients with diabetes duration at least 10 years and variable degree of impairment of renal function (155 men and 154 women, mean age was 67.2 ± 10.8 years) from South Moravia region of Czech Republic. The baseline stage of DN was defined according to the Urinary Albumin Excretion (UAE). Our study sample consisted of: normoalbuminuric subjects (UAE <30mg/24h, n=27), subjects with persistent microalbuminuria (UAE 30-300mg/24h, n=104), with persistent proteinuria (UAE >300mg/24h, n=130) and patients with End-Stage Renal Disease (ESRD, glomerular filtration rate <15ml/min/1.73m^2 and/or permanent renal replacement therapy, n=48) followed up in specialized nephrology units of Brno University hospitals. Clinical characteristics of the study participants are shown in Table 1. Patients were followed-up for median 37 (IQR 20 – 59) months. The following end-points were considered: (a) progression of DN (i.e. transition from any given baseline DN stage to a more advanced stage of albuminuria or ESRD, subjects with ESRD at baseline were omitted from this analysis since no further progression was possible), (b) major cardiovascular event (i.e. non-fatal or fatal myocardial infarction or stroke, limb amputation, revascularization), (c) all-cause mortality. All patients gave their signed consent prior to their inclusion in the study. The study was performed according to the recommendations of the Declaration of Helsinki and approved by the Ethical Committee of Faculty of Medicine, Masaryk University Brno.

DNA isolation and genotyping

DNA was extracted from peripheral blood samples by the phenol-chloroform method and stored at -20°C until further analysis. Genotyping of *TP53* SNP was performed using TaqMan® SNP Genotyping Assay (C_2403545_10) in ABI Prism® 7000 Sequence Detection System with genotype discrimination performed using SDS 2.3 software (all from Applied Bio systems, Foster City, CA). *MDM2* SNP was detected as previously described [17].

Statistical analysis

Comparisons between DN groups were performed by Kruskal-Wallis ANOVA. For both SNPs, Hardy-Weinberg Equilibrium (HWE) was tested by chi-square test. Time-to-event analysis using Kaplan-Meier curves and log-rank test was used. For all standard analyses Statistica for Windows (Statsoft inc., Tulsa, OK, USA) was used. P value<0.05 was considered statistically significant.

Results and Discussion

At the end of follow-up period, cumulative incidence of DN progression was 25.7% (of these 1.6% progressed from normoalbuminuria to microalbuminuria, 1.6% from normo- to proteinuria, 31.2% from microalbuminuria to proteinuria, 15.6% from microalbuminuria to ESRD and 50% from proteinuria to ESRD). Cumulative incidence of major cardiovascular event was 22.6% and of all-cause mortality 24.7%, respectively. Ascertained genotype frequencies were: CC 57%, CG 39%, GG 4% for SNP Arg72Pro in *TP53* and TT 40%, TG 51%, GG 9% for SNP309 in *MDM2*.

To assess the eventual impact of both SNPs on studied end-points we used Kaplan-Meier analysis. Results of the analyses are shown in Table 2. Given the low number of GG genotypes for the *TP53* Arg72Pro SNP, we applied a dominant model and found significant difference between CG+GG vs. CC genotypes for DN progression (P=0.046, log-rank test). DN progression was faster in carriers genotypes containing G allele (associated previously with higher risk of T2DM [10]), i.e. genotypes GG and GC compared to CC homozygotes, Kaplan-Meier curves are shown in Figure 1. We did not find any significant difference between genotypes of *MDM2* SNP for any of the end-points studied.

Protein p53 has been traditionally viewed as a tumour suppressor activated by genotoxic stress with subsequent cell cycle arrest and repair of the damage or induction of apoptosis if the extent of changes was too

Parameter (unit)	normoalbuminuria (n = 27)	microalbuminuria (n = 104)	proteinuria (n = 130)	ESRD (n = 48)	P
Age (years)	66 [61 - 72]	68 [57 - 77]	66 [58 - 75]	69 [64 - 75]	0.179
Duration of diabetes (years)	14 [10 - 22]	8 [5 - 14]	14.5 [8 - 20]	17 [12 - 22]	<0.001
FPG (mmol/l)	7.7 [6.2 - 10.2]	7.9 [7.3 - 10.5]	9.3 [7.5 - 11.3]	7.8 [6.3 - 11]	0.173
HbA_{1C} (%)	6.4 [5.2 - 7.5]	6.45 [5.35 - 8.1]	8.1 [6 - 9.6]	6.2 [5.2 - 7.8]	0.062
Total cholesterol (mmol/l)	4.9 [4.3 - 6.3]	4.9 [4.3 - 5.7]	5.2 [4.3 - 6.2]	4.8 [3.9 - 5.5]	0.323
Triglycerides(mmol/l)	1.88 [1.32 - 2.97]	2 [1.32 - 2.71]	2.15 [1.52 - 3.52]	2.11 [1.62 - 3.11]	0.027
Creatinin (μmol/l)	90 [83 - 110]	114 [92 - 147]	139 [104 - 175]	420 [250 - 550]	<0.001
Proteinuria (g/24h)	0.11 [0.08 - 0.13]	0.14 [0.08 - 0.22]	1.33 [0.4 - 3.06]	-	<0.001
GFR (ml/min per 1.73m^2)	1.62 [1.32 - 2.3]	1.1 [0.7 - 1.6]	0.88 [0.71 - 1.38]	-	<0.001
UAE (mg/24h)	10 [8 - 18]	50 [21 - 160]	181 [97 - 545]	-	<0.001

Data are expressed as median [interquartile range]. Comparisons were made by Kruskal-Wallis ANOVA.
Abbreviations: ESRD, end-stage renal disease; FPG, fasting plasma glucose; GFR, glomerular filtration rate; UAE, urinary albumin excretion.

Table 1: Clinical characteristics of the study participants.

Gene (rs number)	Genotype	P		
		DN progression	CVE	ACM
TP53 (rs1042522)	CC vs.CG vs. GG	0.05	NS	NS
	CC vs.CG + GG	0.046	NS	NS
	CC + CG vs. GG	NS	NS	NS
MDM2 (rs2279744)	TT vs. TG vs. GG	NS	NS	NS
	TT vs. TG +. GG	NS	NS	NS
	TT + TG vs. GG	NS	NS	NS

Comparisons were made by log-rank test.
Abbreviations: DN: Diabetic Nephropathy; CVE: Cardiovascular Event; ACM: All-Cause Mortality.

Table 2: Time-to-event analysis of individual SNPs.

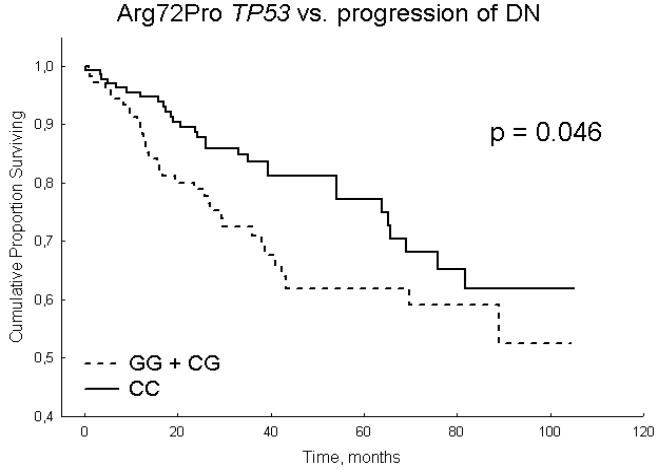

Figure 1: Kaplan-Meier curves for renal end-point for pooled genotypes CC vs. GG+GC DN, diabetic nephropathy.

large. However, in the last several years substantial evidence showed that p53 also has an important role in the regulation of metabolic pathways. This enabled a major shift of the Warburg effect paradigm and brought p53 into the focus in many other disciplines apart from oncology, e.g. diabetes and metabolic diseases. Hyperglycaemia has been shown to induce mobilization of p53 to the mitochondrial membrane with subsequent changes in mitochondrial membrane potential [18]. Hyperglycaemia also activates p53 in human endothelial cells [19]. Interestingly, oscillating glucose is more effective in p53 activation than constant high glucose [19] supporting the view of high glucose variability as an additional cellular stressor. Altogether, however, data elucidating the role of p53 in metabolic diseases like diabetes mellitus and its consequences are scarce so far. To our knowledge this is the first study exploring possible relationship between p53 and MDM2 SNPs and the progression of adverse effects of diabetes, namely diabetic nephropathy, cardiovascular morbidity and mortality.

Conclusions

In the current study we ascertained significant association of common polymorphism (specifically genotypes containing allele previously associated with higher risk of T2DM) with progression of diabetic nephropathy in subjects with T2DM. Elucidation of mutual contribution of *TP53* genetic effect, long-term metabolic compensation and glycemic variability for progression of diabetic nephropathy definitely warrants further studies.

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Conflict of Interest

None declared

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