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SHORT COMMUNICATION

Rs12778366 single nucleotide polymorphism of Sirtuin 1 (SIRT1) and response to resveratrol supplementation in patients with type 2 diabetes mellitus

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Introduction

Resveratrol is a polyphenolic compound found in several plants, such as *Polygonum cuspidatum* roots, peanuts, berries and red grapes [S1, Online-Resource-1]. In preclinical studies, resveratrol has been shown to act as an activator of Sirtuin-1 (SIRT1), a NAD^+ histone deacetylase, member of the sirtuins family, which plays a crucial role, among others, in glucose metabolism, nutrient sensing and inflammation shutdown [S2]. The potential role of SIRT1 variants has been investigated in various dysmetabolic and inflammatory diseases [S3]; moreover, the relationship between several single nucleotide polymorphisms (SNPs) and SIRT1 expression has recently been explored [S4–S7].

The effects of resveratrol in humans are controversial [S8], probably due to the unfavorable pharmacokinetics (such as low bioavailability, influenced by food matrix and gut microbiota) and the lack of well-defined pharmacodynamics [1, S9–S12]. We recently failed to demonstrate resveratrol-associated anti-inflammatory or insulin sensitizer effects in patients with type 2 diabetes mellitus (T2DM) [1].

Genetic background could play a major role in the individual response to resveratrol. *Rs12778366,* a SNP located in the promoter region of SIRT1 affecting its transcription [2], is one of the few SNPs studied after resveratrol supplementation with a potential impact in glucose metabolism [3, 4]. However, contrasting results have been reported on its role on metabolic and inflammatory outcomes [S13–S14]; moreover, whether the variant allele (C) enhances or reduces SIRT1 activity is still debated.

We aimed to evaluate the impact of *rs12778366* SNP on the response to resveratrol supplementation in T2DM patients.

Methods

This is an observational study nested on a previously described randomized controlled trial [1, 5, S15]. Briefly, 192 T2DM patients (age ≥ 40 years, BMI < 35 kg/m^2) were recruited from the Diabetic Clinic of the University of Turin. Patients were randomized to one capsule/day of resveratrol 500 mg/day ($n = 65$), one capsule/day of resveratrol 40 mg/day ($n = 65$), or one capsule/day of placebo (totally inert micro-cellulose) ($n = 62$) for 6 months [1]. Detailed enrollment criteria, intervention, measurements and laboratory methods are reported in Online-Resource-1.

The primary outcome was the association between *rs12778366* SNP and SIRT1 levels. Secondary outcomes were the associations among this SNP and the analyzed metabolic and inflammatory variables.

All procedures respected the Helsinki Declaration principles. The study was approved by the local ethics committee. All participants provided written informed consent.

Anthropometric measurements, percent body fat (determined by dual X-ray densitometry), arterial blood pressure values, and blood samples (for the determination of metabolic and inflammatory variables) were collected both at baseline and at trial end, after an overnight fast [1]. The laboratory measurements were centralized and blindly

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performed [1, 5, S15]. SIRT1 expression, determined by Western Blot from peripheral blood mononuclear cells, is reported as relative amount [5]. Genotyping for *rs12778366* SNP utilized the real-time allele discrimination method (Online-Resource-1).

Statistical analysis

Difference by genotypes were evaluated by the Student's *t* test or, in case of non-normally-distributed variables, by Mann–Whitney *U* test; the Chi-square test was used for categorical variables.

Results and discussion

The frequency of the C (variant) allele was 0.11, which was similar to the results reported in other cohorts [3, 4, S13–S14, S16]. The SNP was in Hardy–Weinberg equilibrium (Chi-square test; $p > 0.05$).

Since only four patients carried the homozygote allele variant, they were combined with the 33 heterozygotes (Table 1).

Baseline data

No significant difference in metabolic/inflammatory variables and SIRT1 levels was detected between patients expressing the C-allele and the *wild-type* alleles (TT) (Table 1).

Resveratrol and SIRT1 activity

At the study end, four patients from the placebo, six from resveratrol 40 mg and three from resveratrol 500 mg arms dropped out. The arms receiving resveratrol were combined and evaluated ($n = 121$).

SIRT1 expression was significantly reduced in the variant C-allele carriers (Table 2), while increased in the T allele homozygotes and similarly in the two resveratrol arms (Table 1S, Online-Resource-2). Differences between C-allele carriers and T allele homozygotes were significant in the resveratrol-40 mg arm ($p = 0.01$). The low number of individuals in the resveratrol-500 mg arm did not allow the evaluation of dose–response relationships (Table 1S).

SIRT1 acts in multiple tissues: (1) on beta-pancreatic cells, by increasing mitochondrial uncoupling protein-2 repression and insulin secretion; (2) on hepatocytes, by upregulation of peroxisome proliferator-activated receptor γ coactivator-1α, forkhead-box transcription factors and liver X receptor protein activity, thus modulating glucose and cholesterol biosynthesis, respectively; (3) on fat cells, by repressing peroxisome proliferator activator receptor-γ with reduced lipogenesis and increased lipolysis [2, S2, S17–S19]. SIRT1 expression in different tissues explains its differential activity in response to different metabolic and nutritional signals [S17]. Indeed, SIRT1 is just one of the actors of the inflammatory process, acting in this complex interplay as a functional NF-κB antagonist [S20].

Table 1 Characteristics of the participants by *rs12778366* polymorphism

	TT	TC/CC	*p*
Number	155	37	
Age (years)	64.8 ± 8.5	66.3 ± 7.5	0.31
Males (%)	64.5	70.3	0.51
Actual smokers (%)	21.3	13.5	0.29
Diabetes duration (years)	9.8 ± 8.1	9.4 ± 6.3	0.87*
Body mass index (kg/m^2)	28.9 ± 3.9	28.6 ± 3.9	0.73
Waist circumference (cm)	102.0 ± 10.7	102.2 ± 10.0	0.94
Fat mass (%)	32.4 ± 7.6	31.9 ± 7.5	0.72
Systolic blood pressure (mmHg)	132.9 ± 9.7	133.4 ± 9.4	0.80
Diastolic blood pressure (mmHg)	81.0 ± 7.8	82.2 ± 8.3	0.42
Fasting glucose (mg/dl)	141.5 ± 40.0	147.4 ± 40.8	0.42
Glycated hemoglobin (%)	6.9 ± 1.1	7.2 ± 1.4	0.15
Insulin (μU/ml)	16.6 ± 8.0	17.6 ± 11.9	0.53
Homeostasis model assessment for insulin resistance (mmol/l × μU/ml)	5.7 ± 3.3	6.8 ± 5.9	0.97*
C-peptide (nmol/l)	0.90 ± 0.45	0.82 ± 0.54	0.37
Total cholesterol (mg/dl)	176.0 ± 37.0	184.5 ± 43.0	0.23
HDL cholesterol (mg/dl)	45.2 ± 13.4	49.7 ± 16.8	0.08
LDL cholesterol (mg/dl)	105.1 ± 33.1	106.1 ± 36.1	0.88
Triglycerides (mg/dl)	132.5 ± 84.0	138.3 ± 123.8	0.61*
Free fatty acids (mmol/l)	0.66 ± 0.21	0.67 ± 0.17	0.65
Uric acid (mg/dl)	5.4 ± 1.4	5.2 ± 1.2	0.44
Aspartate aminotransferase (U/l)	21.5 ± 6.2	21.8 ± 6.7	0.82
Alanine aminotransferase (U/l)	17.9 ± 8.6	17.9 ± 8.4	0.99
γ-glutamyltransferase (U/l)	27.3 ± 14.9	40.0 ± 44.6	0.17*
Adiponectin (ng/ml)	8748.3 ± 5879.4	10,613.3 ± 7731.1	0.22*
C-reactive protein (mg/l)	3.7 ± 8.4	2.9 ± 3.4	0.48*
Interleukin-6 (pg/ml)	3.5 ± 2.9	2.6 ± 1.3	0.17*
Pentraxin-3 (ng/mL)	0.79 ± 0.42	0.81 ± 0.72	0.31*
Total antioxidant status (μmol/L)	295.3 ± 40.7	283.7 ± 39.4	0.12
Sirtuin-1 (SIRT1) (ra)**	0.98 ± 0.64	1.31 ± 0.95	0.16*
Placebo (%)	34.2	24.3	
Resveratrol 40 mg (%)	31.0	46.0	
Resveratrol 500 mg (%)	34.8	29.7	0.21

Ra = relative amounts

p values obtained by Student's *t* test or Chi-square test as appropriate

**p* values obtained by Mann–Whitney *U* test

**Available data on 105 and 23 patients, respectively

Table 2 Median changes in metabolic, inflammatory variables and SIRT1 levels after resveratrol supplementation by genotype

	TT	TC/CC	*p*
Number	95	26	
Age	64.5 ± 8.1	66.3 ± 7.5	0.29
Males (%)	61.1	73.1	0.26
Actual smokers (%)	20.0	11.5	0.32
Diabetes duration (years)	9.9 ± 8.4	9.8 ± 6.8	0.68*
	Median change	Median change	*p**
Body mass index (kg/m^2)	0.0	−0.23	0.47
Waist circumference (cm)	+0.5	0.0	0.47
Fat mass (%)	+0.7	+0.6	0.65
Systolic blood pressure (mmHg)	0.0	0.0	0.73
Diastolic blood pressure (mmHg)	0.0	−5.0	0.10
Fasting glucose (mg/dl)	0.0	+4.0	0.34
Glycated hemoglobin (%)	+0.20	−0.05	0.22
Insulin (μU/ml)	+1.8	−0.93	0.37
Homeostasis model assessment for insulin resistance (mmol/l × μU/ml)	+0.6	−0.6	0.48
C-peptide (nmol/l)	−0.02	−0.015	0.75
Total cholesterol (mg/dl)	0.0	+8.0	0.72
HDL cholesterol (mg/dl)	+2.0	−1.0	0.32
LDL cholesterol (mg/dl)	+3.0	−0.5	0.93
Triglycerides (mg/dl)	+14.0	+16.5	0.42
Free fatty acids (mmol/l)	+0.01	+0.01	0.60
Uric acid (mg/dl)	+0.2	−0.1	0.24
Aspartate aminotransferase (U/l)	+2.0	+2.0	0.26
Alanine aminotransferase (U/l)	0.0	+1.0	0.12
γ-glutamyltransferase (U/l)	+1.0	+2.0	0.23
Adiponectin (ng/ml)	+8.1	−176.9	0.88
C-reactive protein (mg/l)	−0.02	−0.01	0.32
Interleukin-6 (pg/ml)	−0.13	+0.35	0.39
Pentraxin-3 (ng/mL)	+0.05	+0.23	0.16
Total antioxidant status (μmol/L)	+12.0	+15.5	0.55
Sirtuin-1 (SIRT1) (ra)**	+0.25	−0.25	0.017

Patients on resveratrol 40 mg and resveratrol 500 mg were merged

Ra = relative amounts

**p* values obtained by Mann–Whitney *U* test

**Available data on 64 and 19 patients, respectively

We have previously shown that not all patients receiving resveratrol displayed increased SIRT1 activation [4]. Accordingly, the present results show that resveratrol enhanced SIRT1 expression in *wild-type* allele carriers only, thus clarifying our previous unexpected findings.

Resveratrol and metabolic/inflammatory outcomes

After resveratrol supplementation, no significant differences in the metabolic/inflammatory variables were found between variant and *wild-type* alleles' patients (Table 2). These data are consistent with the study aimed to investigate resveratrol supplementation in this SNP carriers, which did not find differences on the assessed outcomes (lung function) [3]. Limited and contrasting data are so far available on this SNP, since the wild-type allele was found to play detrimental [4], neutral [S14], or mixed roles [S16] on metabolic outcomes. This uncertainty is consistent with a minor role on clinical outcomes and is supported by the finding that a significant association with clinical outcomes was detected in the variant allele carriers when coexistent conditions are present [2, 4, S13].

Limitations

Several limitations should be recognized. The study power was originally calculated to detect an effect size = 0.5 on CRP value [1]. The sample size may be therefore too small to find subgroup differences. Moreover, the physiological high inter-individual variability of the inflammatory and metabolic variables would require a large sample size to detect differences, avoiding type-2 error. SIRT1 expression levels were not available from all participants. The study was only focused on *rs12778366* polymorphism.

Conclusions

Resveratrol supplementation in T2DM patients carrying the *rs12778366* polymorphism was associated with a low SIRT1 expression but no differences in their metabolic or inflammatory variables.

Authors contributions RG participated in the conception of the study, data analysis, interpretation of the findings of the study, manuscript writing and revision. GF participated in the data analysis, interpretation of the findings, manuscript writing and revision. GT participated in the data analysis, interpretation of the findings, and manuscript revision. VP participated in the data collection, interpretation of the findings and manuscript revision. IG participated in the data collection, interpretation of the findings and manuscript revision. MC participated in the design of the study, interpretation of the findings and manuscript revision. MFB participated in the conception of the study, data analysis, interpretation of the findings and manuscript revision. SB participated in the conception and design of the study, data collection and revision, manuscript writing and revision. All authors have approved the final article.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

Ethical approval All procedures respected the Helsinki Declaration principles. The study was approved by the local ethics committee.

Informed consent All participants provided written informed consent.

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