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

UNIVERSITÀ DEGLI STUDI DI TORINO

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

Gambino R, Fanni G, Togliatto G, Ponzo V, Goitre I, Cassader M, Brizzi MF*, Bo S*

Corresponding authors:
Simona Bo
Maria Felice Brizzi

Abstract (**50 words**)
Patients with type 2 diabetes mellitus, carrying a single Sirtuin 1 (SIRT1) nucleotide polymorphism, the *rs12778366,* displayed lower SIRT1 expression after resveratrol supplementation but no differences on their metabolic or inflammatory variables. This suggests that this polymorphism does not impact on carriers' clinical outcomes.

1. Introduction

Resveratrol is a polyphenolic compound found in several plants, such as *Polygonum cuspidatum* roots, peanuts, berries and red grapes [1]. In preclinical studies, it has been demonstrated 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 [2]. The effects of resveratrol in humans are controversial [3], probably due to the unfavorable pharmacokinetics (such as low bioavailability, influenced by food matrix and gut microbiota) and the not well-defined pharmacodynamics [4–6]. We have recently failed to find either anti-inflammatory or insulin-sensitizer effects of resveratrol in patients with type 2 diabetes mellitus (T2DM) [7].
Genetic background could play a major role in the individual response to resveratrol. *Rs12778366* is a single nucleotide polymorphism (SNP) located in the SIRT1 gene promoter region, affecting its transcription [8]. Few studies evaluated the role of this polymorphism on SIRT1 activity with contrasting results [8–12]; so far whether the wild-type allele (T) enhances or reduces SIRT1 activity is controversial.
The aim of this paper was to evaluate the influence of *rs12778366* SNP on the response to resveratrol supplementation in T2DM patients.

2. Methods

This is an observational study nested on a randomized controlled trial, which has previously been described [7,13]. Briefly, 192 T2DM patients (age ≥40 years, BMI<35 kg/m^2) were recruited from the Diabetic Clinic of the University of Turin. Exclusion criteria were: treatment with insulin, anticoagulants, steroids, anti-inflammatory drugs different from aspirin, any antioxidant substance, alcohol/substance abuse, uncompensated diabetes, diabetes-related chronic complications, acute cardiovascular events/procedures, chronic or life-threatening diseases, pregnancy, peanuts/grapes/wine/mulberries allergy. All procedures respected the Helsinki Declaration principles. The study was approved by the local ethics committee. All participants provided written informed consent.
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. All the capsules were identical in size, shape, color, and taste. Compliance with the study protocol was monitored by monthly phone calls and pill counting.
Patients and researchers were blinded to the bottle content. Patients were stratified by acetylsalicylic acid use and glycated hemoglobin levels (cut-point=7%), according to a computer-generated randomization sequence [7].

The primary outcome was the association between *rs12778366* polymorphic allele and SIRT1 levels. Secondary outcomes were associations among this SNP and the measured metabolic and inflammatory variables.
Anthropometric measurements, percent body fat (determined by dual X-ray densitometry), arterial blood pressure values, and blood samples (for the determination of metabolic variables, C-reactive

protein (CRP), Interleukin-6, pentraxin) were collected both at baseline and at trial end, after an overnight fast [7,13]. The laboratory measurements, centralized and blindly performed, have been previously described [7,13,14]. SIRT1 expression was determined by Western Blot analyses from peripheral blood mononuclear cells; values are reported as relative amount [14].
Genotyping for *rs12778366* SNP utilized the real-time allele discrimination method (TaqMan Allelic Discrimination Assay; Applied Biosystems, Foster city, CA).

2.1 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.

3. Results and Discussion
The frequency of the C (variant) allele was 0.11, which was similar to data observed in other cohorts [9–12,15]. The SNP was in Hardy-Weinberg equilibrium (chi-square test; $p>0.05$).
Since only 4 patients carried the homozygote allele variant, they were combined with the 33 heterozygotes (Table 1).

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

3.2 Resveratrol and SIRT1 activity
At the study end, respectively 4/6/3 patients from the placebo, resveratrol 40mg and resveratrol 500mg arms dropped out. We focused on the resveratrol-receiving arms only, which were combined (n=121).
SIRT1 expression was significantly increased in the *wild-type* allele group (Table 2).
SIRT1 acts in multiple tissues: i) on beta-pancreatic cells, by mitochondrial uncoupling protein-2 repression and insulin secretion increase; ii) on hepatocytes, by upregulation of peroxisome-proliferator-activated receptor γ coactivator-1α, forkhead-box transcription factors and liver X receptor protein activity, thus modulating respectively glucose and cholesterol biosynthesis; iii) on fat cells, by repressing peroxisome proliferator-activator receptor-γ with reduced lipogenesis and increased lipolysis [2,8,16-17]. The expression of SIRT1 in different tissues enables its differential activity in responses to different metabolic and nutritional signals [16].
We had previously found that not all patients receiving resveratrol showed increased SIRT-1 activation [14]. Accordingly, the present results showed that resveratrol enhanced SIRT1 expression in *wild-type* allele carriers only, thus explaining our previous unexpected findings.

3.3 Resveratrol and metabolic/inflammatory outcomes
After resveratrol supplementation, no significant difference on the metabolic/inflammatory variables were evident among variant and *wild-type* alleles' patients (Table 2). Only one study analyzed the role of this polymorphisms after resveratrol supplementation, and no outcome change was detected [15].
Overall, very few and contrasting data are available about this SNP, since the wild-type allele was found to play detrimental [9], neutral [11] or mixed roles [12] on metabolic outcomes. This uncertainty is consistent with a minor role on clinical outcomes. This is supported by the finding that a significant association with clinical outcomes was detected in the variant allele carriers when coexistent conditions are present [8-10].

3.4 Limitations
Several limitations should be recognized. The study power was originally calculated to detect an effect size of 0.5 on CRP value [7]. The sample size may therefore be too small to find subgroup differences. SIRT1 expression levels were not available from all participants. The study has been focused on *rs12778366* polymorphism only.

4. Conclusions

In T2DM patients after resveratrol supplementation, the *rs12778366* polymorphism is associated with lower SIRT1 expression but not with other metabolic or inflammatory variables.

Conflict of interest

The authors declare no conflict of interests.

Authors contribution

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.

References

[1] Park EJ, Pezzuto JM. The pharmacology of resveratrol in animals and humans. Biochim Biophys Acta - Mol Basis Dis 2015;1852:1071–113. doi:10.1016/j.bbadis.2015.01.014.

[2] Yang T, Fu M, Pestell R, Sauve AA. SIRT1 and endocrine signaling. Trends Endocrinol Metab 2006;17:186–91. doi:10.1016/j.tem.2006.04.002.

[3] Visioli F. The resveratrol fiasco. Pharmacol Res 2014;90:87. doi:10.1016/j.phrs.2014.08.003.

[4] Tomé-Carneiro J, Larrosa M, González-Sarrías A, Tomás-Barberán FA, García-Conesa MT, Espín JC. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des 2013;19:6064–93.

[5] Sahebkar A, Serban C, Ursoniu S, Wong ND, Muntner P, Graham IM, et al. Lack of efficacy of resveratrol on C-reactive protein and selected cardiovascular risk factors--Results from a systematic review and meta-analysis of randomized controlled trials. Int J Cardiol 2015;189:47–55. doi:10.1016/j.ijcard.2015.04.008.

[6] Novelle MG, Wahl D, Diéguez C, Bernier M, De Cabo R. Resveratrol supplementation: Where are we now and where should we go? Ageing Res Rev 2015;21:1–15. doi:10.1016/j.arr.2015.01.002.

[7] Bo S, Ponzo V, Ciccone G, Evangelista A, Saba F, Goitre I, et al. Six months of resveratrol supplementation has no measurable effect in type 2 diabetic patients. A randomized, double blind, placebo-controlled trial. Pharmacol Res 2016;111:896–905. doi:10.1016/j.phrs.2016.08.010.

[8] Rai E, Sharma S, Kaul S, Jain K, Matharoo K, Bhanwer AS, et al. The Interactive Effect of SIRT1 Promoter Region Polymorphism on Type 2 Diabetes Susceptibility in the North Indian Population. PLoS One 2012;7:7–12. doi:10.1371/journal.pone.0048621.

[9] Figarska SM, Vonk JM, Boezen HM. SIRT1 Polymorphism, Long-Term Survival and Glucose Tolerance in the General Population. PLoS One 2013;8:1–6. doi:10.1371/journal.pone.0058636.

[10] Albani D, Mazzuco S, Chierchia A, Fusco F, Boeri L, Martines R, et al. The SIRT1 promoter polymorphic site rs12778366 increases IL-6 related human mortality in the prospective study “Treviso Longeva (TRELONG).” Int J Mol Epidemiol Genet 2015;6:20–6.

[11] Han J, Wei M, Wang Q, Li X, Zhu C, Mao Y, et al. Association of Genetic Variants of SIRT1 With Type 2 Diabetes Mellitus. Gene Expr 2015;16:177–85. doi:10.3727/105221615X14399878166195.

[12] Peng Y, Zhang G, Tang H, Dong L, Gao C, Yang X, et al. Influence of SIRT1 polymorphisms for diabetic foot susceptibility and severity. Medicine (Baltimore) 2018;97:e11455. doi:10.1097/MD.0000000000011455.

[13] Bo S, Ponzo V, Evangelista A, Ciccone G, Goitre I, Saba F, et al. Effects of 6 months of resveratrol versus placebo on pentraxin 3 in patients with type 2 diabetes mellitus: a double-blind randomized controlled trial. Acta Diabetol 2017;54:499–507. doi:10.1007/s00592-017-0977-y.

[14] Bo S, Togliatto G, Gambino R, Ponzo V, Lombardo G, Rosato R, et al. Impact of sirtuin-1 expression on H3K56 acetylation and oxidative stress: a double-blind randomized controlled trial with resveratrol supplementation. Acta Diabetol 2018;55:331–40. doi:10.1007/s00592-017-1097-4.
[15] Siedlinski M, Boer JMA, Smit HA, Postma DS, Boezen HM. Dietary factors and lung function in the general population: Wine and resveratrol intake. Eur Respir J 2012;39:385–91. doi:10.1183/09031936.00184110.
[16] Gomez-Cabrera MC, Zaragoza R, Pallardo F V., Viña JR. SIRT1 regulation of insulin-signalling pathways in liver, white adipose tissue and pancreas during fasting or calorie restriction. Trends Endocrinol Metab 2007;18:91–2. doi:10.1016/j.tem.2007.02.008.
[17] Sosnowska B, Mazidi M, Penson P, Gluba-Brzózka A, Rysz J, Banach M. The sirtuin family members SIRT1, SIRT3 and SIRT6: Their role in vascular biology and atherogenesis. Atherosclerosis 2017;265:275–82. doi:10.1016/j.atherosclerosis.2017.08.027.
[18] Chiu P, Tang -Tsun, Ng Y-F, Ho S, Gyda M, Chan S-W. Review Resveratrol and cardiovascular health-Promising therapeutic or hopeless illusion? Pharmacol Res 2014;90:88–115. doi:10.1016/j.phrs.2014.08.001.

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 (BMI) (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
HbA1c (%)	6.9±1.1	7.2±1.4	0.15
Insulin (µU/ml)	16.6±8.0	17.6±11.9	0.53
Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (mmol/l x 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
AST (U/l)	21.5±6.2	21.8±6.7	0.82
ALT (U/l)	17.9±8.6	17.9±8.4	0.99
GGT (U/l)	27.3±14.9	40.0±44.6	0.17*
Adiponectin (ng/ml)	8748.3±5879.4	10613.3±7731.1	0.22*
C-reactive protein (CRP) (mg/l)	3.7±8.4	2.9±3.4	0.48*
IL-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 (TAS) (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-vales 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***
BMI (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
HbA1c (%)	+0.20	-0.05	0.22
Insulin (µU/ml)	+1.8	-0.93	0.37
Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (mmol/l x 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
AST (U/l)	+2.0	+2.0	0.26
ALT (U/l)	0.0	+1.0	0.12
GGT (U/l)	+1.0	+2.0	0.23
Adiponectin (ng/ml)	+8.1	-176.9	0.88
C-reactive protein (CRP) (mg/l)	-0.02	-0.01	0.32
IL-6 (pg/ml)	-0.13	+0.35	0.39
Pentraxin-3 (PTX3) (ng/mL)	+0.05	+0.23	0.16
Total antioxidant status (TAS) (µmol/L)	+12.0	+15.5	0.55
Sirtuin-1 (SIRT1) (ra)**	+0.25	-0.25	0.017

Patients on resveratrol 40mg and resveratrol 500mg were merged. Patients with at least one C variant allele were merged.

Ra=relative amounts

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

**available data on 64 and 19 patients, respectively