Association between MIR499 gene polymorphism and diabetic neuropathy in type 2 diabetes

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Background

are non-coding RNAs that regulate gene expression through degradation of target
f translation. They are increasingly implicated in the development of diabetes and
(*Karolina 2012, Shantikumar 2012*).
miRNAs and diabetes mainly focused on expression studies. Genetic variants (such as
rmorphisms, SNPs) in miRNA genes may disrupt specific miRNA-mRNA target site
sult in an aberrant regulation of target gene expression (Figure 1). Recently, we found
nd MIR27a associated with an increased and decreased risk of type 2 diabetes
vely (*Ciccacci 2013*). Moreover, we firstly described MIR128a, MIR146a and MIR27a
diabetic neuropathy susceptibility (*Ciccacci 2014*).
miRNA, mainly expressed in the muscle, heart, and brain, and implicated in a variety of
uch as cardiovascular disease, cancer, rheumatoid arthritis, and metabolic
e viewed the preferential expression of mir-499 in heart and areas of central autonomic
iguous) (*Wang 2012*), its involvement in both cardiovascular disease and metabolic
d the association of MIR499 A/G rs3746444 SNP with ischemic stroke (*Zhu 2015*) and
ion prognosis (*Li 2015*), as a reason to analyse genetic variability of MIR499 with regard

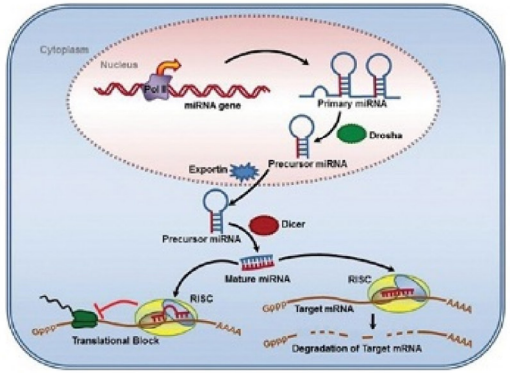
ed a genotype–phenotype correlation analysis to investigate whether rs3746444 SNP is associated with susceptibility to diabetic DPN) and/or cardiovascular autonomic neuropathy (CAN) in type 2

Patients

2 diabetes, age 18-80 years.

athies, other causes of pain than DPN; advanced peripheral arterial disease, limb

esponsible of autonomic dysfunction;
nalignancies, recent cardiovascular events, heart failure, renal failure, liver disease;
rders and any condition preventing understanding of the questionnaires.
patients, 150 were included according to the selection criteria.



A biogenesis and
silencing (*Joshi*

Methodology

al and metabolic parameters. Micro- and macrovascular complications: history of
vascular events; microalbuminuria and eGFR; ophthalmoscopic examination; peripheral

hy
s and signs: Questionnaire of Michigan Neuropathy Screening Instrument (MNSI-Q)
europathy Score (MDNS) (*Feldman 1994*).
esting: Vibration perception threshold (VPT) (Biothesiometer); Cold (CWT) and warm
holds (WTT) (TSA-II Neurosensory Analyzer);
pable): at least 2 abnormalities among symptoms and signs, VPT, or thermal thresholds

uropathy
mic tests: Heart rate response to deep breathing (EI), to standing (LS), and to Valsalva
tatic hypotension test (OH); CAN score based on test abnormalities (range 0-8).
confirmed CAN: ≥1 abnormal test and ≥2 abnormal tests (*Spallone 2011*).
was extracted using a Qiagen blood DNA mini kit. The miR499 gene was amplified by
qMan genotyping assay.

Results

Genetic analysis of miR499 rs3746444 SNP confirmed the presence of 2 alleles (A and G) and 3 genotypes. AA, AG, and GG genotypes were present in 56%, 38%, and 6% of patients, respectively.

Table 1 and 2: After ANOVA adjustment for sex, age, BMI, duration, and HbA1c, GG genotype was associated with higher insulin dose, CAN score, MDNS, and VPT in comparison with AA+AG genotypes.

Figure 2-3. After correction for sex, age, BMI, duration, and HbA1c, GG genotype was associated with the presence of early CAN, confirmed CAN, DPN and abnormal Thermal Thresholds (WTT and/or CTT).

Table 3. In a logistic regression analysis, including in three different models sex, age, BMI, duration, HbA1c, insulin dose, physical activity, LDL cholesterol, systolic BP, microalbuminuria, retinopathy, and GG genotype as independent variables, CAN was predicted by duration and GG genotype.

Table 4. In a similar logistic regression analysis, abnormality in TT was predicted by duration, retinopathy, HbA1c and GG genotype, while the association with DPN remained significant only in the first model with sex, age, BMI, duration, and HbA1c.

Table 5. In a multiple regression analysis, GG genotype was the major determinant of CAN score.

Table 1. Anthropometric. clinical, metabolic and neurological parameters of patients according to MIR499 SNP genotypes

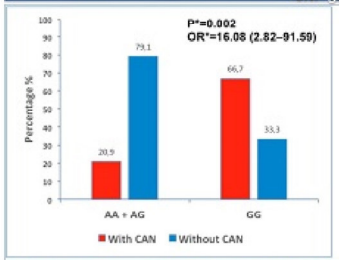
Patients	MIR499 genotype			P
	AA	AG	GG	GG Vs. AA+AG
Males/females	52/32	32/25	5/4	0.81
Age (years)	63.4 ± 7.7	64.6 ± 9.21	62.89 ± 3.66	0.72
Disease duration (years)	11.70 ± 8.80	14.51 ± 10.3	10.11 ± 8.82	0.41
BMI (kg/m2)	30.5 ± 4.62	31.04 ± 5.45	32.49 ± 8.07	0.31
Insulin treated (%)	25	38.6	55.6	0.12
Insulin dose (units/Kg)	**0.12 ± 0.26**	**0.23 ± 0.63**	**0.42 ± 0.44**	**0.04**
HbA1c (%)	7.34 ± 1.55	7.15 ± 1.38	7.48 ± 1.56	0.67
Total cholesterol (mg/dl)	173.95 ± 35.62	168.82 ± 41.43	163.56 ± 60.09	0.54
HDL cholesterol (mg/dl)	47.75 ± 15.46	45.04 ± 10.69	42.67 ± 13.03	0.4
Triglycerides (mg/dl)	149.4 ± 107.9	177.95 ± 365.06	121.68 ± 29.47	0.63
eGFR (ml/min/1.7 m²)	92.04 ± 29.11	90.88 ± 34.15	94.56 ± 60.10	0.79
With microalbuminuria (%)	33.3	30.6	33.3	0.95
Casual systolic BP (mm Hg)	137.98 ± 20.17	136.84 ± 15.23	138.1 ± 20.07	0.94
Casual diastolic BP (mm Hg)	78.67 ± 9.88	75.96 ± 10.33	74.44 ± 11.02	0.4
With hypertension (%)	84.3	87.7	77.8	0.52
With peripheral vascular disease (%)	8.4	12.5	33.3	0.07
With retinopathy (%)	23.1	32	57.1	0.08
With cardiovascular disease (%)	21.4	21.1	11.1	0.47
With coronary artery disease (%)	18.3	15.8	11.1	0.72
Current smokers (%)	23.8	9.1	22.2	0.74
Regular physical activity (%)	67.9	49.1	44.4	0.35
Alcohol consumption (%)	37.7	29.2	25	0.6

Table 2. Neurological parameters of patients according to MIR499 SNP genotypes

Patients	MIR499 genotype			P*
	AA	AG	GG	GG Vs AA+AG
MNSI-Q	1.78 ± 2.16	2.78 ± 2.88	3.25 ± 2.43	0.243
MDNS	4.66 ± 4.13	5.62 ± 4.82	8.86 ± 5.79	0.009*
Monofilament (correct answers)	8.86 ± 2.14	8.45 ± 1.93	7.06 ± 3.16	0.025*
VPT hallux (Volt)	18.21 ± 9.99	20.68 ± 11.22	28.86 ± 12.76	0.001*
CTT dorsal foot (C°)	29.04 ± 2.75	28.72 ± 5.08	26.99 ± 3.78	0.146
WTT dorsal foot (C°)	36.85 ± 3.92	37.29 ± 4.26	39.86 ± 2.57	0.067
With DPN (%)	39	52	77.8	0.082
Expiration/Inspiration Ratio	1.24 ± 0.14	1.24 ± 0.10	1.17 ± 0.13	0.097
Lying to Standing (30:15)	1.15 ± 0.09	1.17 ± 0.12	1.10 ± 0.15	0.087
Valsalva ratio	1.45 ± 0.29	1.44 ± 0.23	1.28 ± 0.16	0.126
Orthostatic Hypotension (mmHg)	9.76 ± 7.74	8.16 ± 7.74	14.72 ± 14.81	0.053
CAN score	0.99 ± 1.46	0.81 ± 1.47	3.0 ± 2.74	<0.0001*
With CAN (%)	21.2	20.4	66.7	0.006
With confirmed CAN (%)	7.5	7.5	44.4	0.006

* P was adjusted for sex, age, duration BMI, and HbA1C

Figure 2. Percentage of patients with and without CAN (early or cofirmed SNP genotypes



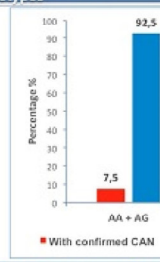
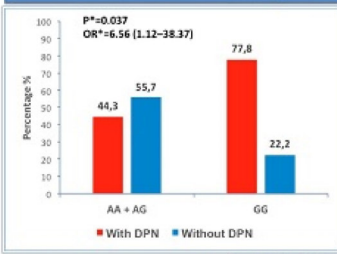
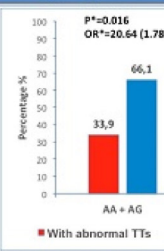


Figure 3. Percentage of patients with and without DPN or abnormal The according to MIR499 SNP genotypes.





* p and OR were adjusted for BMI, HbA1C, dise

Table 3. Logistic regression analysis with CAN, DPN, and abnormality in Thermal Thresholds (TTs) as dependent variables.

Early CAN

Variables	B	P	OR (95 % CI)	R^2
Duration	0.135	0.000	1.145 (1.07-1.22)	0.28
MIR499 (GG Vs AA+AG)	2.952	0.002	19.14 (3.02-121.2)	

Second model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG), systolic BP, LDL, physical activity.

Variables	B	P	OR (95 % CI)	R^2
Duration	0.070	0.055	1.072 (0.99-1.15)	0.27
MIR499 (GG Vs AA+AG)	3.115	0.006	22.524 (2.5-205.7)	

Third model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG), insulin treatment, microalbuminuria, retinopathy.

Confirmed CAN

Variables	B	P	OR (95 % CI)	R^2
Duration	0.122	0.009	1.13 (1.03-1.24)	0.21
HbA1c	0.709	0.022	2.03 (1.11-3.72)	
MIR499 (GG Vs AA+AG)	3.69	0.001	39.9 (4.14-384.2)	

Second model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG), systolic BP, LDL, physical activity.

DPN

Variables	B	P	OR (95 % CI)	R^2
Age	0.05	0.047	1.05 (1.00-1.11)	0.21
Duration	0.07	0.003	1.07 (1.02-1.13)	
MIR499 (GG Vs AA+AG)	1.88	0.037	6.56 (1.12-38.4)	
HbA1c	0.29	0.043	1.34 (1.01-1.78)	

First model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG).

Abnormal TTs

Variables	B	P	OR (95 % CI)	R^2
Duration	0.091	0.006	1.09 (1.03-1.17)	0.31
MIR499 (GG Vs AA+AG)	3.00	0.031	20.03 (1.31-306.9)	

Second model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG), systolic BP, LDL, physical activity.

Variables	B	P	OR (95 % CI)	R^2
Retinopathy (yes/not)	1.23	0.049	3.44 (1.01-11.72)	0.33
MIR499 (GG Vs AA+AG)	3.03	0.037	20.64 (1.20-355)	
HbA1c	0.50	0.019	1.65 (1.08-2.50)	

Third model: independent variables are age, disease duration, BMI, HbA1c, gender, MIR499 (GG Vs AA+AG), insulin treatment, microalbuminuria, retinopathy.

Table 4. Multiple linear regressio score as dependent variable (r^2=0.

Independent variables	
Sex (male)	
Age (years)	
BMI (Kg/m2)	
Physical activity (with)	
Duration (years)	
HbA1c (%)	
Insulin dose (units/Kg)	
Systolic BP (mmHg)	
LDL cholesterol (mg/dl)	
eGFR (ml/min/1.73m²)	
Retinopathy (with)	
MIR499 GG genotype	

Conclusions

There is an emerging evidence of a link between mir499 and both cardiovascular function and its autonomic
Moreover, MIR499 rs3746444 SNP has been associated with cardiovascular disease and diabetes.
Thus, we analysed in a small cohort of type 2 Italian patients the genetic variability of MIR499 with
neuropathy.
We found MIR499 rs3746444 polymorphism associated with susceptibility to diabetic neuropathy, in partic
severity.
This novel finding, albeit requiring replication in larger cohorts, might suggest a role of mir499 autonomic dysfunction in diabetes.

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