MicroRNA-126 and micro-/macrovascular complications of type 1 diabetes in the EURODIAB Prospective Complications Study

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Abstract:	AIMS. Increasing evidence suggest a potential role of circulating miRNAs as clinical biomarkers and loss of miRNA-126 has been proposed as a predictor of type 2 diabetes onset. However, a systematic analysis of circulating miRNAs in type 1 diabetic patients with micro/macrovascular complications has not yet been performed. METHODS. A cross-sectional nested case-control study from the EURODIAB Prospective Complications Study of 455 type 1 diabetic patients was performed. Case subjects (n=312) were defined as those with one or more complications of diabetes; control subjects (n=143) were those with no evidence of any complication. A differential miRNA expression profiling was performed in pooled serum samples from cases and controls. Furthermore, miR-126 levels were quantified by qPCR in all individual samples and associations with diabetic complications investigated. RESULTS. 25 miRNAs differed in pooled samples from cases and controls. miR-126 levels were significantly lower in case than in control subjects, even after adjustment for age and sex. In logistic regression analyses, miR-126 was negatively associated with all complications (OR=0.85, 95% CI 0.75-0.96) as well as with each micro/macro-vascular complication examined separately. This was likely dependent of diabetes as associations were no longer significant after adjustment for both hyperglycemia and diabetes duration. However, a significant 25% risk reduction, independent of age, sex, A1C and diabetes duration, was still observed for proliferative retinopathy (OR=0.75, 95% CI 0.59-0.95). CONCLUSIONS: In this large cohort of type 1 diabetic subjects, we found that miR-126

	levels are associated with vascular complications of diabetes, particularly with proliferative retinopathy.
Response to Reviewers:	Reviewer #1: The Authors conducted a systematic analysis of circulating miRNAs in type 1 diabetic patients with and without micro/macrovascular complications. Differential miRNA profiling showed that expression of 25 miRNAs differed in pooled serum samples from cases and control subjects. In particular, miR-126 levels were significantly lower in case than in control subjects, even after adjustment for age and sex. In logistic regression analyses, miR-126 was negatively associated with all complications as well as with each micro/macrovascular complication examined separately. However, associations were no longer significant after adjustment for both hyperglycemia and diabetes duration, except for proliferative retinopathy, thus suggesting that loss of miR-126 might be a signature of type 1 diabetes, as previously shown for type 2 diabetes. The paper is interesting, though it has some limitations: 1. The cross sectional design, which did not allow to assess temporal relationship, though the large EURODIAB database should contain the information on the time of onset of each complications and this could be better analysed using individual samples. We agree with the Reviewer that a prospective study would have given more information on the temporal relationship between changes in microRNA levels and development of complications. However, we could not perform a prospective study on the cohort of type 1 diabetic patients from the EURODIAB study because serum samples collected at the baseline examination were not stored for subsequent analyses; therefore, miRNA measurements could only be performed in serum samples collected at the follow-up visit. Moreover, time of onset of complications was not recorded in the EURODIAB PCS as patients were re-assessed 7 years after the baseline visit rather than annually. To acknowledge this limitation, pointed by the Reviewer, a sentence has been included in the discussion (page 10 lines 2-4). 2. As loss of miR-126 seems to be related to the presence of diabetes rather than of its complications, the appropriate control group should be composed of age- and sex-maged non-diabetic individuals. Results of our logistic regression analyses showed that the association between the risk of most complications and miR-126 loss was dependent of long exposure to hyperglycemia (diabetes duration, HbA1c). This finding is in line with the results of the Brunick study in type 2 diabetic patients and with several other reports in vivo and in vitro suggesting that hyperglycemia lowers circulating miR-126 levels. Therefore, we agree with the Reviewer that loss of miR-126 is likely related to hyperglycemia, independently of its underlying cause (i.e. type 1, type 2, IGT). However, the study question was not if miR-126 is link to hyperglycemia in type 1 diabetes, but if miR-126 is associated with diabetic complications beyond the effect of hyperglycemia and may serve as additional biomarker, which was not the case except for retinopathy. Therefore, we did not include a control group of non-diabetic patients as this would have not help clarifying the role of miR-126 in diabetic complications. In addition, the nested case-control study was performed almost twenty years ago and non-diabetic control subjects recruited now could not be compared to patients, who were recruited long time ago.

MicroRNA-126 and Micro/Macrovascular Complications of Type 1 Diabetes in the EURODIAB Prospective Complications Study.

Running title: miRNAs and type 1 diabetes complications

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ABSTRACT

AIMS. Increasing evidence suggest a potential role of circulating miRNAs as clinical biomarkers and loss of miRNA-126 has been proposed as a predictor of type 2 diabetes onset. However, a systematic analysis of circulating miRNAs in type 1 diabetic patients with micro/macrovascular complications has not yet been performed.

METHODS. A cross-sectional nested case-control study from the EURODIAB Prospective Complications Study of 455 type 1 diabetic patients was performed. Case subjects (n=312) were defined as those with one or more complications of diabetes; control subjects (n=143) were those with no evidence of any complication. A differential miRNA expression profiling was performed in pooled serum samples from cases and controls. Furthermore, miR-126 levels were quantified by qPCR in all individual samples and associations with diabetic complications investigated.

RESULTS. 25 miRNAs differed in pooled samples from cases and controls. miR-126 levels were significantly lower in case than in control subjects, even after adjustment for age and sex. In logistic regression analyses, miR-126 was negatively associated with all complications (OR=0.85, 95% CI 0.75-0.96) as well as with each micro/macro-vascular complication examined separately. This was likely dependent of diabetes as associations were no longer significant after adjustment for both hyperglycemia and diabetes duration. However, a significant 25% risk reduction, independent of age, sex, A1C and diabetes duration, was still observed for proliferative retinopathy (OR=0.75, 95% CI 0.59-0.95).

CONCLUSIONS: In this large cohort of type 1 diabetic subjects, we found that miR-126 levels are associated with vascular complications of diabetes, particularly with proliferative retinopathy.

Keywords: type 1 diabetes, microRNAs, retinopathy, complications.

INTRODUCTION

Micro- and macrovascular complications are a major cause of morbidity and mortality in type 1 diabetic patients and there is an increasing quest to find novel biomarkers to identify and treat individuals at high risk.

MicroRNAs (miRNAs) are small, non-protein-encoding RNAs that post-transcriptionally regulate gene expression via suppression of target mRNAs [1]. MiRNAs are critically involved in many biological processes and accumulating evidence points to an important role of miRNAs in the pathogenesis of both diabetes and diabetes-related complications [2-4]. MiRNAs are also present in the circulation in a remarkable stable form as packaged in microvesicles that protect them from endogenous RNase activity. Circulating miRNAs can display unique expression profiles in pathological conditions, suggesting that distinctive miRNA signatures may be exploited as innovative diagnostic/prognostic tools [5-8].

Despite the growing interest in miRNAs, there is relatively little knowledge on circulating miRNAs in both diabetes and diabetic chronic complications. A distinct circulating miRNA profile has been shown in children with newly diagnosed type 1 diabetes [9] and reduced miR-126 levels were a significant predictor of type 2 diabetes in a systematic analysis of circulating miRNAs from the population-based Bruneck study [10]. Moreover, both miR-27b and miR-320a have been proposed as biomarkers of diabetic retinopathy in type 1 diabetes patients [11], while levels of specific TGF-β1-regulated miRNAs (let-7c-5p, miR-29a-3p, let-7b-5p, miR-21-5p) appear to predict progression to end-stage renal disease (ESRD) in proteinuric type 1 diabetes patients with normal renal function [12]. However, a comprehensive analysis of the relationship between serum miRNAs and micro/macrovascular diabetic complications is still lacking.

In this study, we performed a differential miRNA profiling in pooled serum samples of type 1 diabetes patients from the nested case-control study of the EURODIAB Prospective Complications Study (PCS). Results for miR-126 were validated in individual samples from all subjects and associations with micro/macro-vascular complications of diabetes explored.

MATERIALS AND METHODS

Patient Sample

The EURODIAB PCS (1997-1999) is a follow-up of the EURODIAB IDDM Complications Study (1989-1991), which was designed to explore risk factors for diabetes complications in 3,250 randomly selected people with type 1 diabetes, aged 15-60 years, attending 31 diabetes centers in 16 European countries [13,14].

A cross-sectional nested case-control study was designed at the 1997-1999 follow-up examination [15-19]. The response rate at follow-up examination was 57.8% (20). Case subjects were selected to have the greatest complication burden possible to provide sufficient numbers for subgroup analyses. Thus, case subjects were all those with cardiovascular diseases or retinopathy, or albuminuria at follow-up. Control subjects were selected to be completely free of complications. This design allowed us to compare individuals with single or multiple complications with individuals free of complications, according to the study question, as efficiently as possible. Applying these criteria, this yielded 312 cases and 143 control subjects with full data on complications and samples available for analyses.

Patient evaluation for the presence of cardiovascular risk factors [hypertension, body mass index (BMI), waist-to-hip ratio (WHR), smoking, cholesterol, triglycerides, A1c] is described elsewhere (15,19). Retinopathy was graded according to the EURODIAB protocol [20]. Albumin excretion rate (AER), assessed on two 24-h urine collections by immunoturbidimetric method, was categorized as normoalbuminuria (<20 μg/min), microalbuminuria (20-200 μg/min), and macroalbuminuria (≥200 μg/min). Estimated glomerular filtration rate (eGFR) was determined using the four-component abbreviated equation from the Modification of Diet in Renal Disease study [21]. Subjects with an eGFR <60 ml/min/1.73m^2 were defined as having chronic kidney disease (CKD). Distal symmetrical polyneuropathy (DSP) was diagnosed based on (i) presence of one or more neuropathic symptoms, (ii) absence of two or more ankle or knee reflexes, and (iii) abnormal vibration perception threshold, measured by centrally calibrated biothesiometers (Biomedical, Newbury, OH) on the right big toe and on the right medial malleolus. Cardiovascular disease (CVD) was defined as physician-diagnosed myocardial infarction, angina, coronary artery bypass graft, or stroke and/or ischemic changes on centrally Minnesota-coded electrocardiogram. Soluble vascular cell adhesion molecule (sVCAM-1) levels were measured by a commercially available ELISA (R&D Systems, Oxon, UK) [15].

Differential miRNA expression profiling in pooled serum samples

Pooled serum samples from cases and controls were used to assess expression of 377 individual miRNAs. Total RNA was extracted using the Trizol reagent (Invitrogen, Milan, Italy) and RNA quality assessed by capillary

electrophoresis on an Agilent-2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). MiRNAs were reverse transcribed using the Megaplex Primer Pool A, Human Pool A v2.1 (Applied Biosystems). RT reaction products were pre-amplified using the Megaplex PreAmp Primers (Primers A v2.1) and the TaqMan® PreAmp Master Mix. MiRNA expression profiling was performed by Human TaqMan miRNA Array A on an 7900HT Fast Real-Time PCR System. Raw Ct values were calculated using the SDS software and standardized to both U6 snRNA and the spike-in C.elegans-miR-39. MiRNAs were excluded if both samples had Ct values ≥ 35/undetermined. Relative expression was calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$). MiRNAs were considered differentially expressed if they exhibited greater than twofold expression differences.

miR-126 Expression in individual samples

Expression of miR-126 was quantitated in individual serum samples from all 455 patients. Diluted pre-amplification products were combined with Taqman miRNA Assay and Taqman Universal PCR Master Mix No AmpErase UNG, then a qPCR was performed using a specific Taqman microRNA Assay (002228) on an Applied Biosystems 7900HT thermocycler. All samples were run in triplicates and standardized to both U6 snRNA and the spike-in C.elegans-miR-39 using the SDS2.2 software.

Statistical analyses.

Variables distributed normally are presented as means (standard deviation, SD), while variables with skewed distribution were analyzed after log transformation (miR-126, triglycerides, AER, creatinine, sVCAM-1,) and presented as geometric means and interquartile range. Logistic regression analyses were used to estimate the odd ratios (ORs) of miR-126 for any complication (AER ≥20 μg/min, retinopathy, neuropathy, CVD), independently of confounders and known risk factors. The likelihood ratio test was used to compare nested models examining the role of age, sex, diabetes duration, A1C, and sVCAM-1. In light of the hypothesis of a different role of miR-126 in the pathogenesis of different micro/macrovascular complications, logistic regression models were also fitted separately for each complication/subgroup.

RESULTS

Characteristics of case and control subjects

The study population (n = 455) had a mean age of 39.5 years, average diabetes duration of 21.5 years, and an equal proportion of men and women. Case subjects with vascular complications had a more adverse risk factor profile than control subjects (Table 1). Of the 312 case subjects, nephropathy was present in 179 (41.3% micro-

and 58.7% macroalbuminuria, retinopathy in 249 (background 47.8% and proliferative 52.2%), DSP in 176 (56.4%). Most people, however, had more than one complication; indeed, 161 (51.6%) individuals had both AER ≥20 μg/min and retinopathy, 113 (36.2%) had both AER ≥20 μg/min and DSP, and 108 (34.6%) had AER ≥20 μg/min, DSP, and retinopathy. CVD was present in 126 subjects (40.4%), all of whom also had at least one microvascular complication who had CVD only.

Differential miRNA expression profiling

Differential miRNA profiling showed that expression of 25 miRNAs differed in pooled serum samples from cases and control subjects. As detailed in the supplementary online Table, 10 miRNAs (miR-139-5p, miR-133a, miR-106a, miR-16, miR-222, miR-17, miR-140-3p, miR-574-3p, miR-486-3p, miR-885-5p) were up-regulated and 15 down-regulated (miR-155, miR-92a, miR-126, miR-483-5p, miR-29a, miR-320, miR-145, miR-146a, miR-191, miR-342, miR-223, miR-24, miR-150, miR-486-5p, miR-484) in cases as compared to controls.

miR-126 expression

Out of the 25 differentially expressed miRNAs, miR-126 was chosen for further analysis by qRT-PCR in all recruited subjects because miR-126 has been involved in pathophysiological processes of relevance to diabetic complications, such as angiogenesis, vascular repair, and inflammation [3].

miR-126 was measurable in all 455 samples with a right-skewed distribution of values. Consistent with the profiling results, qRT-PCR analysis showed that miR-126 levels were significantly lower in cases than in control subjects (Table 1) and results were unchanged after adjustment for age and sex (p=0.045). In cases with micro-macroalbuminuria (0.78 vs 1.28, p=0.03) or retinopathy (0.77 vs 1.29, p=0.02) or DSP (p=0.016) age- and sex-adjusted miR-126 levels were also lower than in controls.

Logistic regression analyses

Logistic regression analyses were performed to assess whether lower levels of miR-126 conferred an increased OR of having any complication, independently of main risk factors and confounders. In the unadjusted model (Model 1), miR-126 levels were negatively associated with all complications (OR=0.85, 95% CI 0.75-0.96) as well as with CVD, nephropathy, DSP and retinopathy examined separately. After inclusion of age and sex into the model (Model 2), the association was only marginally significant for all complications, but retained statistical significance for nephropathy and retinopathy. This was likely dependent of hyperglycemia and diabetes duration as associations were no longer significant after further adjustment for these variables (Model

3). Of interest, a 21% and 25% risk reduction independent of age, sex, A1C and diabetes duration was still observed for macroalbuminuria (OR=0.79, 95% CI 0.63-1.00) and proliferative retinopathy (OR=0.75, 95% CI 0.59-0.95), respectively (Model 3). After inclusion into the model of sVCAM-1, an established miR-126 target [22], OR for proliferative retinopathy only remained marginally significant (OR=0.79, 95% CI 0.61-1.02) (Model 4). A minority of case subjects (n: 29) were under treatment with aspirin and their exclusion did not modified results [macroalbuminuria: OR 0.79 (0.62-1.00) (Model 3) and OR 0.87 (0.67-1.12) (Model 4); proliferative retinopathy: OR 0.75 (0.58-0.95) (Model 3) and OR: 0.79 (0.61-1.02) (Model 4)].

DISCUSSION

Our study is the most extensive study to date on circulating miRNAs in serum samples from type 1 diabetic patients and our results expand upon a growing body of literature that highlights the role of miRNAs in diabetic complications.

Using an unbiased approach, we assessed the differential expression of 377 miRNAs in pooled serum samples from type 1 diabetic patients with and without vascular complications. Among them, 25 miRNAs were differentially expressed in subjects with vascular complications; however, these results may underestimate real differences as pooling of samples may mask opposing changing in individual samples.

In our profiling, levels of both miR-29a and miR-155 were lower in cases compared to controls. Of interest, a previous study has shown that miR-29a is diminished in proteinuric compared to normoalbuminuric type 1 diabetic patients and a predictor of rapid progression towards ESRF [12]. Moreover, a lower content of miR-155, which is known to suppress angiotensin II receptor activity [23,24], was previously found in urinary exosomes from type 1 diabetic patients with microalbuminuria as compared to normoalbuminuric patients [25].

At variance with a recent study on biomarkers of both incidence and progression diabetic retinopathy in type 1 diabetic patients, our profiling did not reveal differences in miR-27 expression and levels of pro-angiogenetic miR-320 were even lower in cases as compared to controls [11]. However, profiling data on pooled serum from patients with multiple complications of diabetes cannot be compared to data obtained from individual measurements performed on a well-selected cohort of patients with retinopathy, particularly in respect to miRNAs affecting angiogenesis that is known to undergo opposing deregulation in different vascular beds of diabetic complications.

Several other differentially expressed miRNAs were previously linked to vascular diseases in either type 2 diabetic patients or non-diabetic subjects. We found that circulating levels of endothelial cell-enriched (miR-126 and miR-92a), inflammation-associated (miR-155), and smooth muscle-enriched (miR-145) miRNAs were lower in type 1 diabetic patients with complications. Consistently, previous studies have shown that these miRNAs were significantly reduced in patients with stable of coronary artery diseases (CAD) compared to healthy controls [26]. In our study, miR-16 was enriched in pooled samples from case subjects and a rise in serum miR-16 levels has been previously reported in type 2 diabetic patients with chronic complications [27]. Furthermore, in a prospective study, baseline miR-16 levels correlated with amputation and restenosis at follow-up in type 2 diabetic patients who underwent re-vascularization [28]. Finally, miR-133a levels, which are enhanced in non-diabetic patients with CHD and positively correlated with the severity of the coronary artery stenosis [29], were also greater in pooled serum from case subjects.

Surprisingly, miR-885-5p, the miRNA displaying the most pronounced upregulation in cases, has not been previously studied in the context of either diabetes or vascular diseases; however, mir-885-5p has potent anti-proliferative, pro-apoptotic, and pro-senescence effects; therefore, its enrichment might be of relevance and deserves further investigation [30].

Among differential expressed miRNAs, we have chosen miR-126 for further assessment by measurement in all individual serum samples from the EURODIAB nested case-control study. Consistent with the profiling results, mean miR-126 levels were significantly lower in case than in control subjects and negatively associated with all complications in logistic regression analysis.

miR-126 is highly enriched in endothelial cells and plays a pivotal role in maintaining endothelial homeostasis and vascular integrity [31]. Furthermore, miR-126 controls endothelial inflammation, at least in part by lowering VCAM-1 expression [22,32]. As endothelial cell activation and inflammation are common features of micro-macrovascular diseases in diabetes, this may provide a plausible link between miR-126 loss and diabetes complications. In line with this hypothesis, microvesicles released from endothelial cells cultured under standard conditions promote vascular repair via transfer of miR-126; by contrast, microvesicles from endothelial cells exposed in vitro to high glucose to mimic hyperglycemia show reduced miR-126 content and impaired regenerative potential [33]. Whether miR-126 is a marker of diabetic complications or a mediator of vascular injury in diabetes remains, however, to be established.

A previous pioneer work has identified a plasma miRNA signature for type 2 diabetes that includes loss of endothelial miR-126 [10] and results have been later confirmed by an independent laboratory [34]. Our results expand these findings and suggest that a measurable reduction of circulating miR-126 may be a marker not only of pre-diabetes, but also of diabetes-induced endothelial dysfunction. In logistic regression analyses, the inverse associations between miR-126 and vascular complications remained only marginally significant after adjustment for A1C and diabetes duration, suggesting a major role of hyperglycemia in mediating the relationship between miR-126 and both micro and macrovascular complications of diabetes. Although this finding may be important on the pathogenic standpoint, it reduces miR-126 relevance as potential new clinical biomarker for early diagnosis of complications.

In logistic regression analysis, the OR of proliferative retinopathy was 25% lower for each unit increment of miR-126 levels. This was statistically significant and independent of age, sex, A1C, and diabetes duration. Although results from subgroup analyses have to be taken with caution, this finding is of potential relevance as miR-126 controls expression/signalling of angiogenetic factors, such as VEGF and SDF-1 [35-38], and downregulation of miR-126 has been proven in the retina from diabetic rats [37] and causally linked to angiogenesis in animal models of cancer [35,36]. The association between miR-126 and proliferative retinopathy was partially dependent of sVCAM-1. In this regard it is noteworthy that overexpression of SDF-1, a miR-126 target, has been shown to contribute to proliferative retinopathy development, at least in part, through VCAM-1 upregulation on retinal endothelial cells, resulting in enhanced recruitment of endothelial cell precursors from hematopoietic stem cells [39].

Anti-platelet treatment may represent a potential confounding factor in the assessment of circulating levels of platelet-derived miRNAs and platelets are another important source of circulating miR-126 besides endothelial cells [40]. Therefore, we cannot exclude the possibility that anti-platelet therapy administered to type 1 diabetic patients with vascular complications may explain their lower circulating miR-126 levels. However, in the EURODIAB PCS only a minority of cases were under anti-platelet treatment and exclusion of these subjects from the analyses did not modify results, making less likely this possibility.

There are certain limitations to our study. Firstly, this is a cross-sectional study and this restricts our ability to assess temporal relationships between miR-126 and vascular complications and to identify causal biological mechanisms underlying this association. However, no data on miR-126 in large groups of type 1 diabetes

patients exist; therefore this study may serve as a reasonable starting point to explore the role of this miRNA in type 1 diabetes. Although the Eurodiab PCS is a prospective study, serum samples collected at the baseline examination were not stored for subsequent analyses; therefore, miRNA measurements could only be performed in serum samples collected at the follow-up visit. Secondly, the number of controls was lower than the overall number of cases, thus reducing the power of analyses; comparisons between controls and cases with single complications allowed a more favorable case/control ratio, but multiple comparisons within the same case-control study base might have caused significant results due to chance. Thirdly, although serum samples were adequately stored, the possibility of protein degradation cannot be excluded, though miRNAs are stable in biological fluids. Unlike previous studies, a key strength here is the ability to account for confounding by other risk factors and complications, and the large sample size provides sufficient power for these analyses. In addition, our patients were from a representative sample of people with type 1 diabetes across Europe, and our results, therefore, are likely to be generalisable. This is the first study profiling miRNAs in a large group of type 1 diabetic patients and our results provide evidence that miR-126 levels are associated with vascular complications of diabetes and in particular with proliferative retinopathy. Further studies are required to determine causal relationships and elucidate underlying mechanisms.

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CONFLICT OF INTEREST: None

ETHICAL APPROVAL: All procedures followed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT: Informed consent was obtained from all individual participants included in the study.

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Table 1. Characteristics of the 455 subjects with type 1 diabetes subjects recruited in the cross-sectional nested case-control study of the EURODIAB PCS.

	Case subjects	Control subjects	P
N	312	143	
Age (years)	41.5 ± 10.1	35.4 ± 7.3	<0.0001
Diabetes duration (years)	24.5 ± 9.4	15.1 ± 6.7	<0.0001
Males (%)	52.5%	48.2%	0.45
BMI (Kg/m^2)	25.0 ± 3.5	23.7 ± 2.6	<0.0001
WHR	0.89 ± 0.13	0.87± 0.16	0.35
A1C (%)	8.9 ± 1.6	7.7 ± 1.2	<0.0001
Systolic blood pressure (mmHg)	126.7± 21.6	114.3 ± 12.9	<0.0001
Diastolic blood pressure (mmHg)	75.8 ± 11.3	73.2 ± 10.3	0.04
Hypertension (%)	53.5%	10.5%	<0.0001
Total cholesterol (mmol/l)	5.45 ± 1.21	4.88 ± 1.08	<0.0001
LDL-cholesterol (mmol/l)	3.60 ± 1.11	3.04 ± 0.95	<0.0001
HDL-cholesterol (mmol/l)	1.59 ± 0.44	1.66 ± 0.42	0.10
Triglycerides (mmol/l)	1.18 (0.81-1.58)	0.82 (0.65-1.05)	<0.0001
miR-126	0.85 (0.35-2.48)	1.30 (0.64-3.11)	<0.011

Data are expressed as mean ± SD, percentage or geometric mean (interquartile range) for log-transformed data.

Table 2. Odds ratios for diabetes complications by miR-126 levels in the nested case-control study within the EURODIAB PCS study

	MODEL 1	MODEL 2	MODEL 3	MODEL 4
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
All complications	0.85 (0.75-0.96)	0.89 (0.77-1.03)	0.91 (0.77-1.07)	0.93 (0.79-1.10)
CVD (*n*=126)	0.82 (0.70-0.96)	0.87 (0.72-1.06)	0.89 (0.72-1.10)	0.91 (0.73-1.13)
Nephropathy (*n*=179)	0.83 (0.72-0.95)	0.84 (0.72-0.99)	0.88 (0.73-1.06)	0.95 (0.78-1.17)
Microalbuminuria (*n*=74)	0.88 (0.74-1.05)	0.89 (0.73-1.08)	0.96 (0.77-1.20)	0.99 (0.78-1.25)
Macroalbuminuria (*n*=105)	0.77 (0.65-0.91)	0.78 (0.63-0.97)	0.79 (0.63-1.00)	0.86 (0.67-1.10)
DSP (*n*=176)	0.84 (0.73-0.97)	0.89 (0.75-1.06)	0.89 (0.74-1-08)	0.92 (0.75-1.11)
Retinopathy (*n*=249)	0.82 (0.72-0.94)	0.84 (0.72-0.99)	0.86 (0.73-1.04)	0.88 (0.73-1.07)
Background Retinopathy (*n*=119)	0.85 (0.73-1.00)	0.89 (0.73-1.07)	0.91 (0.74-1.11)	0.91 (0.74-1.12)
Proliferative Retinopathy (*n*=130)	0.78 (0.67-0.91)	0.75 (0.61-0.92)	0.75 (0.59-0.95)	0.79 (0.61-1.02)

Model 1: unadjusted
Model 2: adjusted for age, sex
Model 3: adjusted for age, sex, diabetes duration, A1C,
Model 4: adjusted for age, sex, diabetes duration, A1C, sVCAM-1