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Circulating MicroRNA-320a and MicroRNA-486 Predict Thiazolidinedione Response: Moving Towards Precision Health for Diabetes Prevention

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Abstract

Introduction—The aims of this study were to compare microRNA (miR) expression between individuals with and without insulin resistance and to determine whether miRs predict response to thiazolidinedione treatment.

Materials and Methods—In a sample of 93 healthy adults, insulin resistance was defined as steady state plasma glucose (SSPG) ≥180mg/dL and insulin sensitive as <120mg/dL. Response to thiazolidinedione therapy was defined as ≥10% decrease in SSPG. We selected a panel of microRNAs based on prior evidence for a role in insulin or glucose metabolism. Fold change and Wilcoxon rank sum tests were calculated for the 25 miRs measured.

Results—At baseline, 81% (n=75) of participants were insulin resistant. Five miRs were differentially expressed between the insulin resistant and sensitive groups: miR-193b (1.45 fold change (FC)), miR-22-3p (1.15 FC), miR-320a (1.36 FC), miR-375 (0.59 FC), and miR-486 (1.21 FC) (all $p<0.05$). In the subset who were insulin resistant at baseline and received thiazolidinediones (n=47), 77% (n=36) showed improved insulin sensitivity. Six miRs were

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Contributions: EF conceived of the study design and performed molecular data collection and data analysis and wrote the manuscript. BEA contributed to the study design, molecular data collection, analysis and interpretation of the findings, and reviewed/edited the manuscript. FA performed clinical data collection and reviewed/edited the manuscript. CL performed clinical data collection and reviewed/edited the manuscript. KMG assisted with molecular and clinical data collection and reviewed/edited the manuscript. YF contributed to and reviewed/edited the manuscript. GMR performed clinical data collection, contributed to the study design analysis and interpretation of the findings, and reviewed/edited the manuscript.

differentially expressed between responders compared to non-responders: miR-20b-5p (1.20 FC), miR-21-5p, (0.92 FC), miR-214-3p (1.13 FC), miR-22-3p (1.14 FC), miR-320a (0.98 FC), and miR-486-5p (1.25 FC) (all $p<0.05$).

Discussion—This study is the first to report miRs associated with response to a pharmacologic intervention for insulin resistance. MiR-320a and miR-486-5p identified responders to thiazolidinedione therapy among the insulin resistant group.

Keywords

microRNA; insulin resistance; thiazolidinediones; biomarkers; precision health

Introduction

Nearly 40% of insulin resistant individuals will develop type 2 diabetes [1] the majority of individuals with type 2 diabetes are insulin resistant, [2]. Insulin resistant individuals are also at increased risk for a number of other serious diseases, including cardiovascular disease, certain types of cancer, polycystic ovarian syndrome, and non-alcoholic fatty liver. [3-5] In the absence of a simple way to identify individuals who are insulin resistant, [6] clinical benefit may be realized if circulating microRNAs (miRs) can identify insulin resistant persons before the development of frank disease.

Recent studies showed differential expression of circulating miRs in individuals with type 2 diabetes compared to healthy controls. [7-10] MiR-126 is associated with 10-year risk for developing type 2 diabetes. [10] Levels of miR-126 were also observed to decrease in a graded fashion from normal glucose tolerance to impaired fasting glucose to type 2 diabetes. [7] Furthermore, circulating miRs are able to differentiate between individuals with type 2 diabetes who are normal versus overweight. [8] These findings support the possibility that circulating miRs may reflect underlying heterogeneity in the etiology of type 2 diabetes and function as predictors of incident type 2 diabetes.

A second potential clinical application of miRs is the identification of individuals who will respond to insulin sensitizing interventions. Thiazolidinediones (TZDs) are unique pharmacologic agents that improve insulin sensitivity in insulin resistant individuals without type 2 diabetes. [11-15] With optimal treatment, 1.0-1.5% improvements in glycosylated hemoglobin and concurrent improvements in serum lipids and lipoproteins are achieved. [15] Although TZDs are effective, there are potential adverse consequences and inter-individual differences in response to TZDs. [16] As a result, this class of pharmacologic agent has extremely limited use in clinical practice. Identification of individuals who are (1) responders likely to see improvement in insulin sensitivity, and (2) high risk for adverse events could provide sufficient information to allow reintroduction of these effective drugs into clinical practice.

In response to these issues, this study was designed to address two aims. The first was to compare miR expression between non-diabetic individuals with and without insulin resistance determined by a direct measure of insulin-mediated glucose disposal by the insulin suppression test. The second was to determine whether miRs were associated with

improved insulin sensitivity following TZD treatment. The findings of this study provide new information about the identification of circulating miRs as potential biomarkers for insulin resistance and identify miRs that predict which individuals will exhibit improvements in insulin sensitivity following TZD therapy. This study serves as an exemplar for the development of individualized treatment approaches to optimally decrease risk for type 2 diabetes.

Materials and Methods

Study design, setting, and sample

Participant samples and data for the current study were collected during prior studies evaluating the effect of TZDs in generally healthy individuals without type 2 diabetes. The study designs were reported in detail previously. [13, 14, 17] Participants were a multi-ethnic group of male and female adults recruited by convenience from the community around the Stanford University Medical Center. All three studies had a single intervention arm design to test either the effects of rosiglitazone on insulin secretion and clearance [14] and vascular and inflammatory markers [13] or pioglitazone on cardiovascular risk factors [17]. Participants in all studies received either 4 mg rosiglitazone for 4 weeks followed by 8 mg rosiglitazone for 8 weeks (n=55) [13, 14] or 15 mg pioglitazone for two weeks then 30 mg pioglitazone for 2 weeks followed by 45 mg for 8 weeks (n=38) [17]. From these studies, we included only individuals who were in the lowest or highest tertile of insulin resistance at baseline (n=93). The subset who were insulin resistant at baseline and received TZDs (n=47) were evaluated for TZD response. All studies were approved by the Stanford University Institutional Review Board.

Clinical data collection

Measurement of demographic and clinical variables, insulin sensitivity, and collection of blood plasma was done during the baseline visit by trained personnel in the Stanford University Medical Center Clinical Research Center. Total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides were measured by the vertical auto profile II method. [18] Insulin-mediated glucose disposal was quantified by a modification [19] of the insulin suppression test [20]. After an overnight fast, subjects were infused for 180 minutes with octreotide (0.27 μg/m^2/min), insulin (32 mU/m^2/min), and glucose (267mg/m^2/min). Plasma glucose and insulin were measured every 10 minutes during the 150- to 180-minute period, and then averaged to determine steady state plasma glucose and steady-state insulin concentrations. Insulin resistance was defined as the lowest tertile of insulin sensitivity (steady state plasma glucose (SSPG) ≥ 180mg/dL) and insulin sensitivity as the highest tertile of insulin sensitivity (SSPG < 120mg/dL).

Molecular data collection at baseline

Blood used for banking of plasma was collected at baseline through an intravenous catheter during the insulin suppression test protocol. Blood was collected into vacutainers containing the preservative EDTA, centrifuged at 4°C to separate plasma from cellular blood components, and stored at -80°C. Samples were collected between January, 2001 and August, 2008. RNA was isolated from plasma using Trizol and eluted in nuclease free water.

We selected a panel of 25 miRs to measure based on previous data from human studies of blood-based expression of miRs in type 2 diabetes and related conditions. [10, 21-26] Twenty-one miRs (i.e., hsa-miR-122-5p, hsa-miR-126b-3p, hsa-miR-133a-3p, hsa-miR-144-3p, hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-150-5p, hsa-miR-182-5p, hsa-miR-191-5p, hsa-miR-193b-3p, hsa-miR-197-3p, hsa-miR-20b-5p, hsa-miR-21-5p, hsa-miR-223-5p, hsa-miR-27a-3p, hsa-miR-29b-3p, hsa-miR-320a, hsa-miR-34a-5p, hsa-miR-370-3p, hsa-miR-375, hsa-miR-486-5p) were selected based on biological plausibility. The remaining four (i.e., hsa-miR-133b, hsa-miR-214-3p, hsa-miR-22-3p, hsa-miR-296-5p) were selected as potential normalizers based on studies showing low variability of these species in studies of human plasma [10] and whole blood [27].

Initial measurements were done using the Firefly Circulating miRNA Assay (Firefly BioWorks, Cambridge, MA). [28] MiRs were hybridized to complementary oligonucleotides covalently attached to encoded hydrogel microparticles. The bound target was ligated to oligonucleotide adapter sequeneces that serve as universal PCR priming sites. The miR-adapter hybrid molecules were then dehybridized from the particles at 95°C and reverse transcription polymerase chain reaction (RT-PCR) was performed using a fluorescent forward primer. Once amplified, the fluorescent target was rehybridized to the original capture particles and scanned on an EMD Millipore Guava 8HT Flow Cytometer (Merck KGaA Darmstadt, Germany). Triplicate measurements were obtained for each sample and miR target. Of the 25 miRs measured, five (i.e., hsa-miR-133a-3p, hsa-miR-133b, hsa-miR-144-3p, hsa-miR-182-5p, hsa-miR-296-5p) failed to retain a minimum of two valid replicate measures for >90% of samples and were excluded from the analysis. For the remaining 20 miRs, no replicate measure exceeded two standard deviations from the mean of the replicates.

Quantitive polymerase chain reaction (qPCR) was used to validate the findings from the gel-particle hybridization flow cytometry experiments for two miRs. We selected miR-146b-5p, which showed low variability, and miR-486-5p, which showed statistically significant variability between groups. Five replicate measures were obtained for each sample and miR target. Replicates with a Ct value greater than 37 or more than two standard deviations from the mean value for each sample were excluded. For all samples, a minimum of three (of five) technical replicates were required for retention in subsequent analyses.

Statistical analysis

Descriptive statistics were used to describe demographic and clinical characteristics. Student's t-tests and Wilcoxon rank-sum tests were used to test for statistically significant differences between groups. Normalized expression of each sample for each miR target was calculated using the geometric mean expression of the three miRs (i.e., miR-146b-5p, miR-197-3p, miR-29b-3p) that exhibited both reliable detection and low variability (i.e., in the second quartile for both mean expression and difference in expression between the insulin resistant and insulin sensitive groups). Fold change differential expression for the gel-particle hybridization flow cytometry experiments was calculated as the ratio of normalized expression in the insulin resistant compared to insulin sensitive group and the ratio of normalized expression in individuals who failed to achieve a therapeutic response to

TZD therapy compared to individuals who did exhibit a response. Fold change for the qPCR validation experiments was calculated as $2^{-\Delta\Delta Ct}$ using the geometric mean of miR-146b-5p, miR-197-3p, and miR-29b-3p as the normalizer value. [29] Wilcoxon rank sum tests were used to compare normalized expression of miRs between groups. Logistic regression models were used to estimate unadjusted and covariate-adjusted odds ratios. Variables included as covariates included characteristics associated with insulin sensitivity (i.e., age, sex, BMI, HDL-c, triglycerides, blood pressure). MiRs were scaled by quartile in logistic regression models. Blocks of covariates were sequentially added to logistic regression models in order to estimate the independent relationship between individual miRs and insulin sensitivity or TZD response. Statistical analysis was performed used Stata SE Version 12 (College Station, TX) and Microsoft Excel (Redmond, WA).

Results

The sample contained slightly more women (65%) compared to men. Approximately 50% of the sample was Caucasian, 13% were Asian, 8% African American, and 5% Latino. Demographic and clinical characteristics of the insulin resistant and insulin sensitive individuals are shown in Table 1. The insulin resistant group (n=75) was younger, had lower HDL-c, and higher triglycerides, fasting glucose, and BMI. In the subset who were insulin resistant at enrollment and received TZDs (n=47), 77% (n=36) showed at least 10% improvement in steady state plasma glucose following TZD therapy. Rosiglitazone was administered to 57% (n=27) and pioglitazone was administered to 43% (n=20). Of the responders, 58% (n=21) received rosiglitazone and 42% (n=15) received pioglitazone (p=0.8) Baseline demographic and clinical characteristics did not differ between responders and non-responders (Table 2).

Five miRs were differentially expressed in the insulin resistant compared to the insulin sensitive individuals. Four miRs (i.e., miR-193b-3p, miR-22-3p, miR-320a, and miR-486-5p) were increased in insulin resistant compared to insulin sensitive individuals (all $p<0.05$) (Table 3). By contrast, miR-375 had an inverse relationship with insulin resistance ($p<0.05$). When individual miRs were scaled by quartile for logistic regression models, the odds of being insulin resistant compared to insulin sensitive were higher for miR-193b-3p, miR-22-3p, miR-320a, and miR-486-5p (all $p<0.05$) (Table 4). The odds of being insulin resistant compared to insulin sensitive were lower for miR-375 ($p<0.05$). After adjusting for age, sex, BMI, and race, miR-320a and miR-486-5p continued to show a positive association with insulin resistance. In subsequent models adding first HDL-c and triglycerides and then blood pressure, miR-486-5p continued to show a strong positive association with insulin resistance.

Six miRs were differentially expressed in responders compared to non-responders to TZD treatment. Four miRs showed a positive association with response: miR-20b-5p, miR-214-3p, miR-22-3p, and miR-486-5p (all $p<0.05$). The remaining two miRs (i.e., miR-21-5p, miR-320a) were inversely associated with response to TZD treatment (all $p<0.05$) (Table 3). In unadjusted logistic regression models, four miRs were associated with increased odds for response to TZD therapy: miR-20b-5p, miR-214-3p, miR-320a, and miR-486-5p (all $p<0.05$) (Table 4). In multivariate adjusted logistic regression models

adding age, sex, BMI, and race, then HDL-c and triglycerides, then blood pressure, all four miRs remained significantly associated with response to TZD treatment.

The Firefly Bioworks Circulating microRNA Assay has been used in previous studies. [30-32] We selected miR targets, with low variability (i.e., miR-146b-5p) and high (i.e., miR-486-5p variability between samples, to validate measurement of expression by qPCR (Supplementary Table 1). We found similar magnitudes in fold-change for comparisons between insulin sensitive and insulin resistant individuals for miR-486-5p. For the comparison between TZD responders and non-responders, we found similar fold changes for both miR-146b-5p and miR-486-5p. The fold-change for the comparison for miR-146b-5p between insulin resistant and insulin sensitive individuals was not significant by either quantitation method. Our findings are similar or better than the moderate concordance rates reported by prior studies of comparisons between miR quantitation methods. [33, 34] The direction of differential expression observed in our study using the Firefly Bioworks Circulating microRNA assay was consistent with previous observations for a prior study reporting findings for miR-320a using qPCR-based quantitation. [23]

Discussion

In a cross-sectional evaluation of insulin resistant compared to insulin sensitive individuals, we identified five circulating miRs that were associated with insulin resistance. Prior studies described circulating miRs associated with prevalent [22] and incident [10] type 2 diabetes, including two of the miRs we identified as differentially expressed in the insulin resistant compared to insulin sensitive groups (i.e., miR-320a, miR-486-5p). There are several predicted messenger RNA (mRNA) targets of miR-320a and miR-486-5p that are associated with insulin metabolism and related conditions (i.e., type 2 diabetes, obesity, dyslipidemia, blood pressure) [35] (Table 5). In insulin resistant individuals who were treated with TZDs, six miRs measured at baseline were associated with improvement in insulin sensitivity. To our knowledge, this is the first study to evaluate miRs predicting individual response to TZDs.

Identification of readily measured miR biomarkers for insulin resistance has the potential to improve screening and detection of risk for several diseases. [1, 2, 5] In order to evaluate the possibility that miRs can provide novel information about insulin sensitivity beyond what can currently be detected from the presence of related clinical risk factors (i.e., HDL-c, triglycerides, blood pressure), we fit a series of multivariate models. We estimated the relationship between individual miRs and insulin sensitivity after adjusting for HDL-c and triglycerides in order to determine if the relationship between a given miR and insulin sensitivity was influenced substantially by lipoprotein metabolism. We subsequently added blood pressure to the model to determine whether blood pressure contributes to the relationship between a given miR and insulin resistance. We found that miR-486-5p remained statistically significant in all multivariate models, and may contribute novel information about detection of latent insulin resistance. Given the barriers to detecting insulin resistance in clinical settings, the possibility that a circulating miR can indicate underlying insulin sensitivity after controlling for known risk factors is of tremendous potential clinical utility.

Although TZDs are effective for management of type 2 diabetes, there are potential adverse consequences limiting their use and inter-individual differences in response. [16] Four miRs were found to increase the odds of response to TZD treatment. In adjusted models adding clinical covariates (i.e., age, sex, BMI, HDL-c, triglycerides, blood pressure), all four miRs remained statistically significantly associated with failure to respond to TZDs. None of the traditional demographic or clinical characteristics differed between non-responders and responders prior to TZD treatment (Table 2). This suggests that these four miRs predict likelihood of responding to TZD treatment and are not related to common demographic and clinical characteristics. This is promising evidence that circulating miRs may be useful clinical biomarkers to realize individualized treatment approaches to achieve optimal response to pharmacologic agents used to decrease risk for type 2 diabetes.

Two miRs were associated with both insulin resistance and TZD response in multivariate adjusted logistic regression models. Higher levels of miR-486-5p are associated with insulin resistance and response to TZD treatment. We identified higher circulating levels of miR-486-5p associated with prevalent insulin resistance, whereas a prior study found decreased levels in plasma is associated with the presence of type 2 diabetes [10]. The prior study looked at individuals who developed type 2 diabetes after 10-years, whereas our participants had confirmed insulin resistance without type 2 diabetes. A possible explanation for the difference in the observed direction of the relationship the time course of disease progression (i.e., insulin resistance prior to the onset of type 2 diabetes) in relation to when miR-486-5p was measured. Other explanations include the method of miR measurement and differences in how miR levels were normalized. MiR-486-5p is highly prevalent in skeletal muscle [36] and may also regulate smooth muscle [37]. TZDs increase insulin sensitivity in muscle, adipose tissue, and the liver. [15] Although not completely understood, the primary mechanisms of action of TZDs is the stimulation of peroxisome proliferator-activated receptors (PPARs). [15, 38] PPAR-γ is increased in skeletal muscle of individuals who are obese or who have type 2 diabetes, which may relate to the effects of TZDs on insulin sensitivity in skeletal muscle. [39] We observed that responders to TZDs had higher levels of circulating miR-486-5p compared to non-responders. Poor PPAR-γ response to TZDs may be related to excretion of intracellular miR-486-5p and corresponding increases in circulating levels.

The second miR that exhibited an association with insulin resistance and response to TZD therapy is miR-320a. We found increased expression of miR-320a in plasma in insulin resistant compared to insulin sensitive individuals. Similarly, the concentration of this miR is reported to be increased in exosomes from individuals with the metabolic syndrome and type 2 diabetes compared to healthy controls. [23] Increased expression of miR-320a is also associated with response to TZD treatment. PPAR-γ is a predicted target of miR-320a, and up-regulation of PPAR-γ evidenced by increased circulating miR-320a may imply an underlying insufficiency of PPAR-γ and associated response to TZDs. While the transcriptional origins of miR-320a and miR-486-5p are not known, miRs are hypothesized to be co-expressed in related pathways. The observed overlap in mRNA targets between miR-320a and miR-486-5p suggests possibly synergistic effects in modulating insulin sensitivity and TZD response.

MiR-193b-3p and miR-375 identified insulin resistant individuals in multivariate adjusted models. Consistent with a prior study of pre-diabetic compared to normoglycemic participants, [40] we found increased expression of circulating miR-193b-3p in insulin resistant compared to insulin sensitive individuals. A prior study observed that miR-193b-3p is decreased in adipose tissue from obese compared to non-obese individuals and appears to play a role in inflammation of adipose tissue through inhibition of chemokine (C-C motif) ligand 2 (CCL2). [24] MiR-375 is highly expressed in pancreatic islet cells. A study of autopsied pancreatic islet cells found up-regulation of miR-375 in individuals with type 2 diabetes compared to healthy controls and associated histologic changes in pancreatic tissue. [41] We found decreased circulating expression of miR-375 in insulin resistant as opposed to insulin sensitive individuals.

In multivariate adjusted models, two miRs were associated with TZD response but not insulin resistance. MiR-20b-5p was also identified in the study of plasma miRs associated with development of type 2 diabetes after 10-years described above. [10] We found increased expression of miR-20b is associated with response to TZD treatment whereas decreased expression is associated with onset of type 2 diabetes after 10-years [10]. MiR-214-3p was selected for inclusion in this study as a potential normalizer miR as it was previously observed to have low variability in a study of atherogenic dyslipidemia. [27] We observed a positive relationship between miR-214-3p and TZD response in both unadjusted and multivariate logistic regression models. It may be that miR-20b-5p and miR-214-3p are related to biological pathways that are discrete from impaired insulin-induced lipolysis and other known consequences of insulin resistance. Further studies are needed to investigate the biologic pathway(s) regulated by these miRs.

One possible explanation for the discrepant direction of expression between our study and previous findings is the source of the miRs measured. Prior studies evaluated miRs from metabolically active tissues (i.e., adipose, pancreatic), whereas we measured circulating miRs from plasma. Levels of circulating miRs often do not mirror tissue-based expression for the same disease condition. [42, 43] Our findings for miR-193b-3p and miR-375 compared with prior studies support an inverse relationship between circulating and intracellular microRNAs. Circulating miRs are derived from several sources including leukocytes, exosomes and microparticles, HDL-c, protein complexes, and to a lesser extent free in serum/plasma. [44-46] Depending on the source (e.g., HDL-c, protein complex, exosome, and microparticle-derived miRs), the function of circulating miRs is thought to be signaling between tissues, [47, 48] changes in intracellular miR needs in response to physiological demands (e.g., exosome and microparticle-derived miRs), [43, 49] or the result of apoptosis (e.g., free miRs in serum/plasma) [49]. These hypotheses support the previously observed inverse relationships between metabolically active tissue miR levels and circulating miR levels. [42, 43]

Our study has limitations. First, we selected a low threshold for improvement in insulin sensitivity (i.e., ≥10% decrease in steady state plasma glucose) and the non-responders tended to have lower steady state plasma glucose at baseline (222mg/dL vs. 243mg/dL p=0.07), though both groups were well above the accepted threshold for insulin resistance. This was a secondary analysis of several prior studies of two TZD agents with a limited

sample size. However, pharmacologic agent was not significantly associated with odds for response. Our findings in this heterogenous sample support the possibility that miRs may be useful biomarkers in complex clinical scenarios. While TZDs now have extremely limited clinical use, the model for determining whether miRs predict response to pharmacologic agents used to treat risk for type 2 diabetes may be extrapolated to other agents. Similarly, the possibility exists for using miRs to predict individual risk for adverse events in response to effective drugs with limited use. There is increasing recognition of the limitations of qPCR for quantitation of miRs, [50] including poor detection of low abundance miRs in plasma/serum, optimal data normalization, and analytic complexity. Our study used an emerging method for quantitation of miRs that has not yet been widely implemented in clinical research. The gel-particle hybridization flow-cytometry method described in this study showed improved limits of detection and accuracy across replicate measures compared to qPCR.

Conclusions

We screened several miRs with *a priori* evidence for a role in type 2 diabetes and related conditions in order to determine whether individual miRs are associated with insulin resistance and response to TZD treatment. We identified five miRs associated with insulin resistance and six miRs associated with response to TZD treatment. This is promising evidence that miRs may be useful clinical biomarkers for improved risk detection and reduction. Further studies are needed to validate these findings in independent larger samples and to model the relationship of multiple miRs and current clinical risk factors in order to determine the independent contribution of miR(s) and develop overall predictive models. This will provide information to determine if detection of insulin resistance and prediction of response to TZDs through miR quantitation can optimize use of pharmacologic agents to decrease the incidence of complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

miR	microRNA
TZD	thiazolidinedione
HDL-c	high density lipoprotein cholesterol
RT-PCR	reverse transcription polymerase chain reaction

Table 1

Baseline Demographic and Clinical Characteristics of Insulin Resistant Compared to Insulin Sensitive Individuals

Characteristics Mean ± SD, Median (IQR), or n (%)	Insulin Sensitive* (n=18)	Insulin Resistant† (n=75)	p-value
Sex (male)	5 (28)	19 (25)	0.83
Race			0.39
Caucasian	17 (24)	53 (76)	
African American	1(17)	5 (83)	
Asian	0	13 (100)	
Other	0	4 (100)	
Age (years)	49 ± 6	40 ± 14	<0.05
Systolic Blood Pressure (mm Hg)	122 ± 16	129 ± 18	0.15
Diastolic Blood Pressure (mm Hg)	72 ± 11	76 ± 10	0.12
Total Cholesterol (mg/dL)	177 ± 21	191 ± 34	0.12
LDL-c (mg/dL)	107 ± 23	118 ± 32	0.17
HDL-c (md/dL)	56 (19)	39 (12)	<0.01
Triglycerides (mg/dL)	73 (41)	137 (111)	<0.01
Fasting Plasma Glucose (mg/dL)	91 ± 11	100 ± 11	<0.01
Steady State Plasma Glucose (mg/dL)	72 ± 19	245 ± 38	<0.01
BMI (kg/m^2)	26.5 ± 4.3	34.3 ± 7.1	<0.01

*steady state plasma glucose≤120mg/dL

†steady state plasma glucose≥180mg/dL

Abbreviations: BMI, body mass index; HDL-c, high density lipoprotein cholesterol; IQR, interquartile range; mg/dL, milligrams per deciliter; mm Hg, millimeters of mercury; kg/m^2, kilograms per meter^2; LDL-c, low density lipoprotein cholesterol; SD, standard deviation

Table 2

Baseline Demographic and Clinical Characteristics of Thiazolidinedione Responders Compared to Non-responders

Characteristics Mean ± SD, Median (IQR), or n (%)	Responder* (n=36)	Non-Responder† (n =11)	p-value
Sex (male)	36 (13)	45(5)	0.58
Race			0.50
Caucasian	24 (77)	7 (23)	
African American	2 (67)	1 (33)	
Asian	7 (70)	3 (30)	
Other	3 (100)	0	
Age (years)	45 ± 14	46 ± 13	0.89
Systolic Blood Pressure (mm Hg)	131 ± 18	130 ± 23	0.91
Diastolic Blood Pressure (mm Hg)	77 ± 9	76 ± 13	0.74
Total Cholesterol (mg/dL)	187 ± 37	202 ± 40	0.27
LDL-c (mg/dL)	119 ± 35	116 ± 24	0.74
HDL-c (md/dL)	38 (10)	39 (12)	0.93
Triglycerides (mg/dL)	177 (190)	130 (120)	0.58
Fasting Plasma Glucose (mg/dL)	103 ± 11	97 ± 12	0.13
Steady State Plasma Glucose (mg/dL)	243 ± 33	222 ± 31	0.07
BMI (kg/m^2)	31.8 ± 5.7	30.8 ± 2.3	0.55

*Responders exhibited ≥10% improvement in steady state plasma glucose following thiazolidinedione treatment

†Non-responders exhibited <10% response in steady state plasma glucose following thiazolidinedione treatment

Abbreviations: BMI, body mass index; HDL-c, high density lipoprotein cholesterol; IQR, interquartile range; mg/dL, milligrams per deciliter; mm Hg, millimeters of mercury; kg/m^2, kilograms per $meter^2$; LDL-c, low density lipoprotein cholesterol; SD, standard deviation

Table 3

Fold Change MicroRNA Expression

	Insulin Resistant (n=75) vs. Insulin Sensitive (n=18)	p-value*	p-value**	TZD Responder (n=36) vs. Non-Responder (n=11)†	p-value*	p-value**
miR-122-5p	1.35	0.11	0.11	0.95	0.28	0.29
miR-126-3p	1.06	0.73	0.75	1.03	0.38	0.39
miR-146a-5p	1.25	0.13	0.13	0.88	0.09	0.09
miR-150-5p	1.13	0.31	0.32	1.12	0.20	0.20
miR-191-5p	1.04	0.58	0.59	1.00	0.34	0.35
miR-193b-3p	**1.45**	**0.04**	**0.04**	1.13	0.14	0.14
miR-20b-5p	1.25	0.21	0.21	**1.20**	**0.01**	**0.01**
miR-21-5p	1.15	0.26	0.26	**0.92**	**0.02**	**0.02**
miR-214-3p	1.03	0.61	0.62	**1.13**	**0.003**	**0.003**
miR-22-3p	**1.15**	**0.02**	**0.02**	**1.14**	**0.02**	**0.02**
miR-223-3p	0.98	0.53	0.55	1.02	0.26	0.27
miR-27a-3p	1.09	0.35	0.35	0.97	0.23	0.24
miR-320a	**1.36**	**0.001**	**0.001**	**0.98**	**0.007**	**0.005**
miR-34a-5p	0.82	0.08	0.08	1.23	0.28	0.29
miR-370-3p	0.87	0.07	0.08	1.28	0.98	0.98
miR-375	**0.59**	**0.02**	**0.02**	1.32	0.80	0.82
miR-486-5p	**1.21**	**0.02**	**0.01**	**1.25**	**0.005**	**0.004**

*Wilcoxon rank sum test

**Adjusted p-value using permutation tests

†Response is defined as ≥10% improvement in steady state plasma glucose following thiazolidinedione treatment

Abbreviations: TZD, thiazolidinedione

Table 4

MicroRNAs predicting insulin resistance and response to TZD treatment

	Unadjusted		Model 1*		Model 2†		Model 3‡	
	OR§	95% CI	OR§	95% CI	OR§	95% CI	OR§	95% CI
Insulin resistance at baseline (n=93)								
miR-193b-3p	**1.7**	**1.0, 2.7**	1.4	0.8, 2.4	1.1	0.5, 2.3	0.9	0.4, 2.1
miR-22-3p	**1.9**	**1.1, 3.2**	1.7	0.9, 3.3	1.4	0.6, 3.5	1.3	0.5, 3.1
miR-320a	**2.4**	**1.3, 4.2**	**2.3**	**1.1, 4.5**	1.8	0.8, 4.2	1.7	0.7, 3.9
miR-375	0.7	0.4, 1.1	0.8	0.4, 1.3	0.6	0.3, 1.3	0.5	0.2, 1.2
miR-486-5p	**1.9**	**1.1, 3.2**	**2.8**	**1.4, 5.6**	**4.0**	**1.3, 11.8**	**4.2**	**1.3, 13.6**
Response to TZD treatment after 12 weeks (n=47)//								
miR-20b-5p	**2.2**	**1.1, 4.4**	**2.6**	**1.2, 5.5**	**2.6**	**1.1, 6.1**	**2.7**	**1.1, 6.6**
miR-21-5p	1.8	0.9, 3.7	2.0	0.9, 4.4	2.0	0.9, 4.6	2.0	0.8, 4.6
miR-214-3p	**3.3**	**1.4, 7.9**	**3.9**	**1.5, 10.3**	**4.8**	**1.5, 15.0**	**5.8**	**1.5, 22.4**
miR-22-3p	1.9	0.9, 3.9	2.2	0.9, 4.9	2.3	0.9, 5.5	2.3	0.9, 5.9
miR-320a	**2.7**	**1.2, 6.0**	**3.0**	**1.2, 7.4**	**3.4**	**1.2, 9.4**	**3.3**	**1.2, 9.5**
miR-486-5p	**3.0**	**1.4, 6.3**	**3.7**	**1.5, 8.7**	**3.9**	**1.5, 10.0**	**3.8**	**1.4, 10.2**

€ microRNAs are scaled by quartile

* Model 1 is adjusted for age, sex, body mass index, and race

† Model 2 is adjusted for variables in Model 1 and HDL-cholesterol and triglycerides

‡ Model 3 is adjusted for variables in Model 2 and systolic and diastolic blood pressure

// Response is defined as ≥10% improvement in steady state plasma glucose following thiazolidinedione treatment

Abbreviations: OR, odds ratio; TZD, thiazolidinedione

Table 5

Predicted mRNA targets of microRNAs associated with insulin resistance and TZD response

MicroRNA	Predicted target*	Implicated Disease(s)*
miR-320a	NR3C1	Adiposity, abdominal obesity, blood pressure, body mass, cholesterol/lipids, insulin, type 2 diabetes
	PPARGC1A	Aerobic fitness, blood pressure, body mass, glucose tolerance, insulin, metabolic syndrome, obesity, type 2 diabetes
	KLF12	Type 2 diabetes
	IGF1R	Obesity, type 2 diabetes
miR-486-5p	NR3C1	Adiposity, abdominal obesity, blood pressure, body mass, cholesterol/lipids, insulin, type 2 diabetes
	FOX01	Body mass
	FOX03	Body mass
	IGF1	Body mass
	VEGFA	Type 2 diabetes
	IGF1	Blood pressure, obesity, type 2 diabetes
	PTEN	Type 2 diabetes
	NRF1	Type 2 diabetes
	ITGA1	Type 2 diabetes
	PIK3R1	Blood pressure, type 2 diabetes
	KLK2	Blood pressure
	CYP11B1	Blood pressure
	CCR5	Blood pressure

*Abstracted from The miR-Ontology Database (miRò) [35]

Abbreviations: mRNA, messenger ribonucleic acid