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RESEARCH ARTICLE

The Pro12Ala polymorphism of PPARγ2 modulates beta cell function and failure to oral glucose-lowering drugs in patients with type 2 diabetes

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Abstract

Background: We evaluate whether the Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ2 (PPARγ2) has a role in the progression of diabetes by modulating the occurrence of treatment failure to glucose-lowering drugs.

Methods: We studied 215 patients with type 2 diabetes participating in the Thiazolidinediones Or Sulphonylureas and Cardiovascular Accidents Intervention Trial study. All participants were insufficiently controlled (glycated haemoglobin [HbA_{1c}] 7.0%-9.0%) with metformin 2 g/day and were randomly allocated to add-on pioglitazone or a sulfonylurea. Treatment failure was defined as HbA_{1c} ≥8% on two consecutive visits, 3 months apart.

Results: Carriers or non-carriers of the polymorphism had similar age, body mass index, and diabetes duration. Ala carriers had lower fasting plasma insulin, better insulin sensitivity (Homeostasis Model Assessment [HOMA]2-%S), and worse beta cell secretion (HOMA2-%B) than non-carriers. During 24 months of follow-up, 32.5% among the Ala carriers and 8.6% among non-carriers ($P < 0.001$) developed treatment failure with a cumulative incidence of 18.6 vs 4.6/100 person-years. Those patients who developed treatment failure were older, had a younger age at diabetes diagnosis (48 ± 10 vs 52 ± 7 years; $P = 0.032$), higher HbA_{1c} (8.1 ± 0.5 vs 7.7 ± 0.5%; $P < 0.001$), and lower HOMA2-%B (30 ± 12 vs 46 ± 29; $P = 0.015$) at study entry, as compared to those who did not develop treatment failure. At multivariate analysis, the Pro12Ala polymorphism was significantly associated with treatment failure (hazard ratio [HR] 4.45; 95% confidence interval [CI] 1.79-11.1; $P < 0.001$); HbA_{1c} at study entry was the other independent predictor of failure in this study population.

Conclusion: The Pro12Ala polymorphism is associated with a greater insulin sensitivity, reduced beta cell function and a substantially increased risk of treatment failure.

KEYWORDS

beta cell function, insulin sensitivity, pioglitazone, PPARγ2, treatment failure, type 2 diabetes

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1 | INTRODUCTION

The attainment and maintenance of optimal glucose control reduces the risk of long-term complications of diabetes and it is, therefore, a major goal in the management of diabetes.[1] However, the progressive nature of the disease makes it difficult to maintain target levels of glycated haemoglobin (HbA_{1c}). Despite lifestyle and pharmacologic interventions, glucose levels increase overtime and generally the escalation of drug doses and the use of combination therapies, or insulin, is necessary, with progressive increase in the complexity of treatment and worsening of the quality of life.[2,3] The determinants of treatment failure remain insufficiently understood, in particular, there is limited information about the durability of glycaemic response when oral glucose-lowering agents are used as add-on treatments to metformin in patients with type 2 diabetes (T2D) no longer controlled with metformin monotherapy.[4] Insulin resistance and impaired insulin secretion are key factors in the development and worsening of hyperglycaemia, but other factors are also at play.[5-7] Furthermore, T2D is a heterogeneous condition characterized by different degrees of insulin resistance/deficiency.[8] Not surprisingly, the treatment response differs among patients and the need for add-on treatment(s) vary considerably in speed from person to person. Individual factors predicting these different treatment courses are scarcely investigated: among others, genetic factors may play a relevant role. We focus on the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ2 (PPARγ2) gene. This is one the best-replicated genetic risk factor for T2D.[9] PPARγ2 encodes for a transcription factors that belong to the family of nuclear receptors; it regulates several genes involved in carbohydrates and lipids metabolism, promotes adipocytes differentiation and fatty acids uptake and modulates insulin sensitivity. Multiple studies have reproducibly shown that the common Pro12Ala polymorphism is associated with greater insulin sensitivity and protection from development of T2D in the general population.[9] Apparently at variance with this evidence, prospective studies in people with disglycaemia have shown that the Ala12 variant is associated with a greater risk of transition from impaired glucose tolerance to diabetes.[10-12] Whether this polymorphism has a role also in the progression of the disease in people with overt diabetes by modulating the persistence of the therapeutic response to glucose-lowering drugs and the need for treatment escalation is not known.

The aims of the study are to evaluate whether the Pro12Ala polymorphism of PPARγ2 is associated with the incidence of secondary failure to second-line glucose-lowering drugs used as add-on to metformin in patients with T2D, and to evaluate some of the possible mechanisms underlining this association.

2 | MATERIALS AND METHODS

2.1 | Participants and study design

The study was conducted at one of the 57 centres participating in the "Thiazolidinediones Or Sulphonylureas and Cardiovascular Accidents Intervention Trial (TOSCA.IT)," a randomized controlled trial designed to evaluate the cardiovascular effects of second-line glucose-lowering drugs. The study protocol and the main results have been published.[13,14] Briefly, patients with T2D in the age range 50 to 75 years, insufficiently controlled (HbA_{1c} values 7.0%-9.0%) with metformin at the dosage of 2 g/day were randomly allocated to add-on pioglitazone or a sulphonylurea. The metformin dose remained unchanged throughout the study, whereas the add-on drugs were titrated according to a standard protocol on the basis of home glucose monitoring and HbA_{1c} values. Drug compliance was assessed at each visit. HbA_{1c} was measured every 6 months, for HbA_{1c} values ≥8% lifestyle recommendations were reinforced and an extra visit was scheduled after 3 months. Treatment failure was one of the pre-specified secondary end points of the study and was defined as HbA_{1c} ≥ 8% on two consecutive visits performed 3 months apart while the patients were taking the maximum tolerated dosage of the prescribed drug(s).

For the purposes of the present work, the study population consists of 215 patients enrolled in the centre of Naples and with 24 months of follow-up. The study protocol was approved by Federico II University Ethics Committee, all participants provided written informed consent, including consent for genetic analyses, before entering the study.

2.2 | Measurements

History of diabetes and use of drugs were assessed by questionnaire. At baseline and annual follow-up visits, anthropometric measures were taken according to a standard protocol. Fasting blood samples were obtained. Plasma lipids and HbA_{1c} were measured at a central laboratory. The single nucleotide polymorphism (SNP) rs1801282 was genotyped by TaqMan SNP Genotyping Assay as previously described[15] on 7900HT real-time polymerase chain reaction (PCR) System (Applied Biosystems, Branchberg, NJ, USA). To monitor quality control, three DNA samples were genotyped by Sanger sequencing (3730 DNA analyser; Applied Biosystems, Branchberg, NJ, USA) and included in each 384-well reaction plate; genotype concordance was 100%. Primer sequences are available upon demand. For a subgroup of 150 participants, plasma insulin was measured at the CNR laboratory in Pisa (Roche Diagnostics, Germany) on frozen plasma collected prior to randomization to the study drugs and kept at −70°C. The updated version of the Homeostasis Model Assessment (HOMA)[16,17] was used to calculate HOMA2-%S and HOMA2-%B which reflect insulin sensitivity and the beta cell secretory capacity.

2.3 | Statistical analysis

Data were presented as mean (± SD) for quantitative variables and as number and percentages for categorical variables. Continuous variables were compared between groups by Student's *t*-test or Mann-Whitney *U* test (HOMA2-%S; HOMA2-%B). Categorical variables were compared by χ^2 test. Plasma insulin concentration was log

transformed before comparisons. The original values are given in the tables and were used for HOMA calculations Hardy-Weinberg equilibrium was evaluated using the goodness-of-fit chi-square test. Kaplan Meier curves were used to plot the cumulative incidence of treatment failure over time, the two groups with or without the Pro12Ala polymorphism were compared using the log-rank. Univariate Cox proportional hazard regression analysis was performed to estimate the crude Hazard Ratios for treatment failure. Multivariate Cox regression analysis was performed to further examine factors associated with treatment failure. A mediation analysis was performed, using the approach implemented in the "mediation" R package, to test whether the relationship between the Pro12Ala polymorphism and the occurrence of treatment failure was also mediated by HOMA2-%B. All statistical analyses were performed by using R (R Core Team, 2018). The level of significance was set at $\alpha = 0.05$.

3 | RESULTS

The study population consists of 215 males and females with T2D in the age range 50 to 75 years. The frequencies of the genotypes were as follows: 175 (81.4%) Pro12Pro, 38 (17.7%) Pro12Ala, 2 (0.9%) Ala12Ala and were in accord with the Hardy-Weinberg equilibrium ($P > 0.05$). For analytical purposes, those people with the Pro12Ala or Ala12Ala genotype were pooled in one group defined as Ala carriers and were compared with the non-Ala carriers. The clinical characteristics of the participants' carriers or non-carriers of the Ala allele are given in Table 1. Attained age, age at diabetes diagnosis, body mass index (BMI), proportion of people with abdominal obesity, and HbA_{1c} were comparable between the two groups (Table 1). Ala carriers had lower fasting plasma insulin, worse beta cell secretory capacity (HOMA2-%B), and greater insulin sensitivity (HOMA2-%S). Assigned glucose-lowering treatments (pioglitazone or sulphonylureas) were equally distributed between the two groups.

During 24 months of follow-up, a total of 28 participants (12%) developed secondary failure. The cumulative incidence of secondary failure was 32.5% among Ala carriers and 8.6% among non-Ala carriers ($P < 0.001$) with a rate of failure of 18.6 and 4.6/100 person-years, respectively. The Kaplan Mayer estimated incidence curves of failure to treatment are given in Figure 1 for the total population (panel A) and by assigned treatment (panel B, pioglitazone; panel C, sulphonylureas). The incidence of failure overtime is significantly higher among Ala carriers in the total group and in each of the treatment arms. The Ala carriers have a more than fourfold increased risk of treatment failure as compared to non-carriers (hazard ratio [HR] 4.16; 95% confidence interval [CI] 1.98-8.76; $P < 0.0001$) in the total group, estimated HRs in the group treated with pioglitazone or sulphonylureas were 5.39 (95% CI 1.95-14.87) and 3.34 (95% CI 1.06-10.54), respectively. The incidence curves start diverging quite early, with a clear difference between Ala carriers or non-carriers already evident within the first year of follow-up.

The patient characteristics associated with treatment failure are given in Table 2. Those patients who developed treatment failure were significantly younger, were diagnosed with diabetes at a younger age, had significantly higher HbA_{1c} and significantly lower HOMA2-%B at study entry, than those who did not develop treatment failure. No significant differences were observed with regard to diabetes duration, prevalence of obesity or abdominal obesity and estimated insulin sensitivity (HOMA2-%S) (Table 2). A multivariate analysis including age at diagnosis, HbA_{1c}, Pro12Ala

TABLE 1 Baseline patient's characteristics and incidence of treatment failure by polymorphism

	PPARγ2 polymorphism		
	Pro12Ala + Ala12Ala (n = 40)	Pro12Pro (n = 175)	*P*
Age (years)	61 ± 6	61 ± 6	0.383
Males (%)	57	55	0.450
BMI (kg/m^2)	29.7 ± 4.0	30.3 ± 4.4	0.387
With abdominal obesity[a] (%)	65	73	0.394
Age at diabetes diagnosis (years)	52 ± 9	52 ± 8	0.615
Diabetes duration (years)	9 ± 6	10 ± 6	0.820
Assigned to pioglitazone/sulphonylureas (n)	20/20	82/93	0.413
HbA_{1c} (%)	7.8 ± 0.5	7.7 ± 0.6	0.811
Treatment failure (%)	13 (32.5)	15 (8.6)	**0.001**
Plasma insulin (mU/l)[b]	11 ± 7	14 ± 8	**0.023**
HOMA2-%B[b]	34 ± 25	46 ± 28	**0.046**
HOMA2-%S[b]	88 ± 53	66 ± 38	**0.013**

Note: Data are given as mean and SD, or percentages.

BMI, body mass index; HbA_{1c}, glycated haemoglobin; HOMA2-%B, Homeostasis Model Assessment beta cell function; HOMA2-%S, Homeostasis Model Assessment insulin sensitivity; PPARγ2, peroxisome proliferator-activated receptor gamma2.

[a]Waist circumference ≥ 82 cm for women or ≥ 102 cm for men.

[b]Available for a subsample of 150 participants.

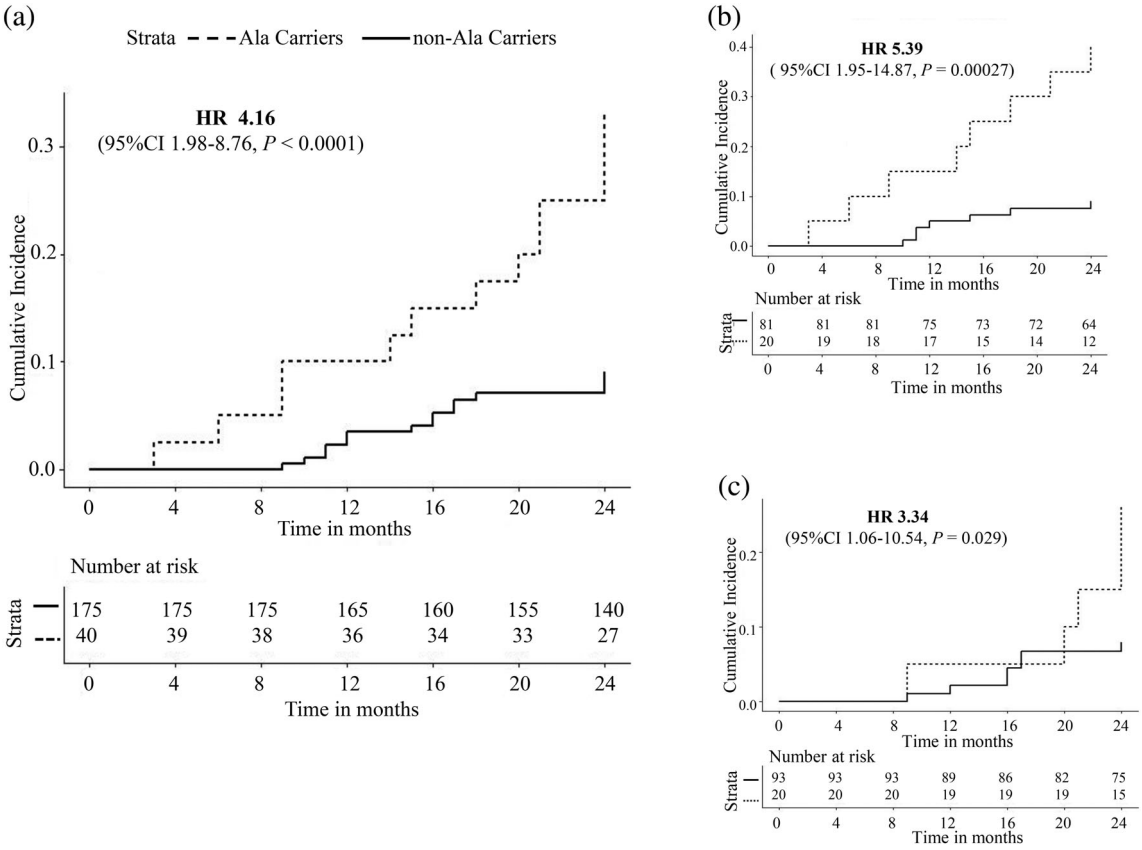

FIGURE 1 Cumulative incidence (Kaplan Meier) of treatment failure in carriers or non-carriers of the Ala allele in the total study population (panel A) and by assigned treatment (panel B: Pioglitazone; panel C: Sulphonylureas)

TABLE 2 Baseline patient's characteristics by occurrence of treatment failure

	Treatment failure		
	YES (n = 28)	NO (n = 187)	*P*
Attained age (years)	59 ± 7	62 ± 6	**0.016**
Males (%)	46	57	0.218
BMI (kg/m^2)	30.7 ± 4.0	30.1 ± 4.4	0.494
Abdominal obesity (%)	79%	70%	0.245
Age at diabetes diagnosis (years)	48 ± 10	52 ± 7	**0.032**
HbA_{1c} (%)	8.1 ± 0.5	7.7 ± 0.5	**0.001**
Assigned to pioglitazone/sulphonylureas (n) (%)	16/12 57/43	86/101 46/54	0.312
Pro12 Ala (n) (%)	13 46	27 14	**0.0001**
Plasma insulin (mU/l)[a]	11 ± 6	14 ± 9	0.253
HOMA2-%B[a]	30 ± 12	46 ± 29	**0.015**
HOMA2-%S[a]	79 ± 54	69 ± 39	0.327

Data are given as mean and SD, or percentages.
BMI, body mass index; HbA_{1c}, glycated haemoglobin. HOMA2-%B, Homeostasis Model Assessment beta cell function; HOMA2-%S, Homeostasis Model Assessment insulin sensitivity.
[a]Available for a subsample of 150 participants.

polymorphism and HOMA2-%B confirmed the Pro12Ala polymorphism and HbA_{1c} at study entry as significant and independent predictors of treatment failure (Table 3). A Cox regression analysis was performed also to test for the interaction between treatment and the PPARγ2 polymorphism, but the interaction term was not statistically significant (*P* = 0.523). The mediation analysis showed that

TABLE 3 Multivariate Cox proportional hazard model for the association between selected variables and treatment failure

	HR (95% CI)	*P*
Age at diabetes diagnosis (× 1 year)	0.96 (0.92-1.01)	0.089
HbA_{1c} (%)	6.07 (2.18-16.9)	**<0.001**
PPARγ2 polymorphism (Ala carriers vs non-Ala carriers)	4.45 (1.79-11.1)	**0.001**
HOMA2-%B	0.99 (0.97-1.01)	0.365

HbA_{1c}, glycated haemoglobin; HOMA2-%B, Homeostasis Model Assessment beta cell function; HR, hazard ratio; PPARγ, peroxisome proliferator-activated receptor gamma.

the relationship between PPARγ2 polymorphism and occurrence of failure was also significantly mediated by HOMA2-%B ($P = 0.034$).

4 | DISCUSSION

We have shown for the first time that in people with T2D, the Pro12Ala polymorphism of PPARγ2 is associated with greater insulin sensitivity, lower beta cell function, and a higher incidence of failure to treatment with second-line glucose-lowering drugs (pioglitazone or sulphonylureas) as add-on to metformin. The Pro12Ala polymorphism was associated with a more than fourfold higher risk of treatment failure. The PPARγ2 has been extensively studied in regard to diabetes and the proline for alanine substitution in codon 12 (Pro12Ala) has been reproducibly associated with a decreased risk for T2D in the general population.[9] Apparently at variance with these data, prospective studies in people with impaired glucose tolerance—Finnish diabetes study,[10] DPP,[11] STOP-NIDDM[12]—report a greater risk of progression to diabetes in carriers of the Ala allele. The mechanisms through which this genetic variant modifies the diabetes risk have not been fully elucidated. Available data provide substantial evidence that the Pro12Ala polymorphisms is associated with a better insulin sensitivity[18] which may partly explain the lower risk of diabetes associated with the Ala allele in the general population. This is, however, apparently in contrast with the observation that in people with impaired glucose tolerance the polymorphism increases the risk of further deterioration of glucose homeostasis and development of diabetes.[10-12] The present study expands current knowledge by documenting, for the first time, that in Caucasians with T2D the Pro12Ala polymorphism is associated with the progression of the disease and a higher incidence of treatment failure. Moreover, the study identifies the impairment of insulin secretion as a possible mechanism linking the Ala12 polymorphism with the occurrence of treatment failure, much in line with the notion of beta cell function being a determinant of deterioration of glucose control overtime. In the UKPDS study, beta cell function was already decreased by 50% by the time of diabetes diagnosis and continued to decline over the six-year observation period, notwithstanding on-going glucose-lowering therapy.[19] Furthermore, the San Antonio Metabolism Study showed that people with impaired glucose tolerance have already lost 80% to 85% of their beta cell function.[20] Our results are also in keeping with the study by Mori et al who showed, in a Japanese population, that people with diabetes carriers of the Ala12 variant have a reduced capacity of insulin secretion as compared to non-carriers.[21] To our knowledge, the finding by Mori et al has never been replicated in a different ethnic group. Thus, based on available data, including findings of the present study, it is plausible to hypothesize that the Ala allele protects from diabetes in the general population, likely due to its association with a greater insulin sensitivity. However, the polymorphism is also associated with a greater impairment of beta cell secretory capacity which may have a greater impact on glucose homeostasis in individuals with impaired glucose tolerance or T2D in whom insulin demand is increased due to the superimposition of glucotoxicity and lipotoxicity. Interestingly, in carriers of the Pro12Pro polymorphism the experimental elevation of free fatty acids by means of a lipid infusion induced, as expected, an increase in the insulin secretion, evaluated with the hyperglycaemic clamp; conversely a marked fall in the insulin secretion was observed in carriers of the Ala allele.[22]

PPARγ2 is the target of thiazolidinediones, a class of glucose-lowering drugs. In the ACT NOW study, treatment with pioglitazone improved insulin sensitivity and beta cell function[23] in comparison to placebo, thus suggesting a potentially greater benefit in people with the more severe impairment of beta cell function. Whether the PPARγ2 Pro12Ala polymorphism modulates the response to thiazolidinediones remains, however, an open question.[24,25] We have not observed any drug/genotype interaction effect, but, due to lack of power, we were unable to exhaustively explore the hypothesis of a differential response to drugs. Other study limitations include the fact that the study was conducted in Caucasians; the ethnic factor may be important in the relationship between the Pro12Ala polymorphism and diabetes[26] and therefore the results are not necessarily applicable to other ethnic groups.

In summary, the study documents for the first time the metabolic phenotype associated with the Pro12Ala polymorphism of PPARγ2 in Caucasians with T2D and shows that the Ala allele is a strong predictor of failure to treatment with second-line glucose-lowering drugs (pioglitazone or sulphonylureas) independent of age, BMI, abdominal obesity age at diagnosis, and HbA_{1c}. A more severe impairment of beta cell function may partly mediate the more frequent occurrence of secondary failure in carriers of the 12Ala polymorphism.

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CONFLICT OF INTEREST

The Authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

AUTHORS' CONTRIBUTIONS

OV, MM, GR and AAR designed the study and contributed to the analysis and interpretation of data. GDP and MM wrote the first draft

of the report. OV, GR, AG and AAR provided relevant intellectual contribution to the development of the report. SC, MV, GDP and MR collected data. MC and PP carried out the genetic analyses. PD performed the statistical analysis. All authors provided substantial contribution to the interpretation of data, critically revised the report, and gave final approval of the version to be submitted for publication. OV is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request and can be obtaining by contacting the corresponding author by E-mail at ovaccaro@unina.it.

ETHICS APPROVAL

The study protocol was approved by the Federico II University Ethics Committee and signed informed consent was obtained from all participants, including consent for genetic analyses. This study is registered with ClinicalTrials.gov, number NCT00700856.

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REFERENCES

1. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008;359:1577-1589. https://doi.org/10.1056/NEJMoa0806470.
2. Turner RC, Cull CA, Frighi V, Holman RR, Group UKPDS. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies. (UKPDS 49). *JAMA*. 1999;281:2005-2012. https://doi.org/10.1001/jama.281.21.2005.
3. Kahn SE, Lachin JM, Zinman B, et al. Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. *Diabetes*. 2011;60:1552-1560. https://doi.org/10.2337/db10-1392.
4. Montvida O, Shaw JE, blonde L, Paul SK. Long term sustainability of glycaemic achievements with second-line anti-diabetic therapies in patients with type 2 diabetes: a realworld study. *Diabetes Obes Metab*. 2018;20:1722-1731. https://doi.org/10.1111/dom.13288.
5. Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycemia, insulin sensitivity and insulin secretion preceding the diagnosis of type 2 diabetes: the Whitehall II study. *Lancet*. 2009;373:2215-2221.
6. Langenberg C, Lotta LA. Genomic insights into the causes of type 2 diabetes. *Lancet*. 2018;391:2463-2474. https://doi.org/10.1016/S0140-6736(09)60619-X.
7. Dendup T, Feng X, Clingan S, Astell-Burt T. Environmental risk factors for developing type 2 diabetes mellitus: a systematic review. *Int J Environ Res Public Health*. 2018;15:78. https://doi.org/10.3390/ijerph15010078.
8. Dennis JM, Henley WE, Jones AG, Hattersley AT. Disease progression and treatment response in data-driven subgroups of type 2 diabetes compared with models based on simple clinical features: an analysis using clinical trial data. *Lancet Diabetes Endocrinol*. 2019;7:442-451.
9. Witka BZ, Oktaviani DJ, Marcellino M, Barliana MI, Abdulah R. Type 2 diabetes-associated genetic polymorphisms as potential disease predictors. *Diabetes Metab Syndr Obes*. 2019;12:2689-2706.
10. Andrulionyte L, Zacharova J, Chiasson JL, Laakso M. Common polymorphisms of the PPAR-gamma2 (Pro12Ala) and PGC-1alpha (Gly482Ser) genes are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial. *Diabetologia*. 2004;47:2176-2184. https://doi.org/10.1007/s00125-004-1577-2.
11. Florez JC, Jablonski KA, Sun MW, et al. Effects of the type 2 diabetes associated PPARG P12A polymorphism on progression to diabetes and response to troglitazone. *J Clin Endocrinol Metab*. 2007;92:1502-1509. https://doi.org/10.1210/jc.2006-2275.
12. Lindi VI, Uusitupa MIJ, Lindstrom J, et al. Association of the Pro12Ala polymorphism in the PPAR-γ2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish diabetes prevention study. *Diabetes*. 2002;51:2581-2586. https://doi.org/10.2337/diabetes.51.8.2581.
13. Vaccaro O, Masulli M, Nicolucci A, et al. Effects on the incidence of cardiovascular events of the addition of pioglitazone versus sulfonylureas in patients with type 2 diabetes inadequately controlled with metformin (TOSCA. IT): a randomised, multicentre trial. *Lancet Diabetes Endocrinol*. 2017;5:887-897. https://doi.org/10.1016/S2213-8587(17)30333-9.
14. Vaccaro O, Masulli M, Bonora E, et al. Addition of either pioglitazone or a sulfonylurea in type 2 diabetic patients inadequately controlled with metformin alone: impact on cardiovascular events. A randomized controlled trial. *Nutr Metab Cardiovasc Dis*. 2012;22:997-1006. https://doi.org/10.1016/j.numecd.2012.09.003.
15. Capasso M, Ayala F, Russo R, Avvisati RA, Asci R, Iolascon A. Predicted functional single-nucleotide polymorphism of bone morphogenetic protein-4 gene affects mRNA expression and shows a significant association with cutaneous melanoma in southern Italian population. *J Cancer Res Clin Oncol*. 2009;135:1799-1807. https://doi.org/10.1007/s00432-009-0628-y.
16. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*. 1998;21:2191-2192. https://doi.org/10.2337/diacare.21.12.2191.
17. University of Oxford. HOMA calculator. https://www.dtu.ox.ac.uk/homacalculator/. Accessed March 19, 2020.
18. Stumvoll M, Haring H. The peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism. *Diabetes*. 2002;51:2341-2347. https://doi.org/10.2337/diabetes.51.8.2341.
19. Wright A, Burden AC, Paisey RB, Cull CA, Holman RR, U.K. Prospective Diabetes Study Group. Sulfonylurea inadequacy: efficacy of addition of insulin over 6 years in patients with type 2 diabetes in the UK Prospective Diabetes study (UKPDS 57). *Diabetes Care*. 2002;25:330-336. https://doi.org/10.2337/diacare.25.2.330.
20. Gastaldelli A, Ferrannini E, Miyazaki Y, DeFronzo RA. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia*. 2004;47:31-39. https://doi.org/10.1007/s00125-003-1263-9.
21. Mori H, Ikegami H, Kawaguchi Y, et al. The Pro12 → Ala substitution in PPAR-γ is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes*. 2001;50:891-894. https://doi.org/10.2337/diabetes.50.4.891.
22. Stefan N, Fritsche A, Häring H, Stumvoll M. Effect of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of peroxisome-proliferator–activated receptor-2 gene. *Diabetes*. 2001;50:1143-1148. https://doi.org/10.2337/diabetes.50.5.1143.
23. DeFronzo RA, Tripathy D, Schwenke DC, et al. Prevention of diabetes with pioglitazone in ACT NOW physiologic correlates for the ACT NOW study. *Diabetes*. 2013;62:3920-26:3920-3926. https://doi.org/10.2337/db13-0265.

24. Kawaguchi-Suzuki M, Frye R. Current clinical evidence on pioglitazone pharmacogenomics. *Front Pharmacol*. 2013;4:1-12. https://doi.org/10.3389/fphar.2013.00147.
25. Mannino GC, Andreozzi F, Sesti G. Pharmacogenetics of type 2 diabetes mellitus, the route toward tailored medicine. *Diabetes Metab Res Rev*. 2019;35:e3109. https://doi.org/10.1002/dmrr.3109.
26. Ludovico O, Pellegrini F, Di Paola R, et al. Heterogeneous effect of peroxisome proliferator-activated receptor gamma2 Ala12 variant on type 2 diabetes risk. *Obesity*. 2007;15:1076-1081. https://doi.org/10.1038/oby.2007.617.

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