Association of Pro12Ala Polymorphism in Peroxisome Proliferator–Activated Receptor γ With Pre-Diabetic Phenotypes

Meta-analysis of 57 studies on nondiabetic individuals

ANKE TÖNJES, MD[1]
MARKUS SCHOLZ, PHD[2]
MARKUS LOEFFLER, MD[2]
MICHAEL STUMVOLL, MD[1]

OBJECTIVE — The provariant of the Pro12Ala polymorphism in peroxisome proliferator–activated receptor (PPAR)γ has been identified as a risk allele for type 2 diabetes. The purpose of the present study was to reveal a significant association with pre-diabetic phenotypes in nondiabetic individuals based on a systematic meta-analysis of all available published evidence.

RESEARCH DESIGN AND METHODS — We performed a classical meta-analysis of data from ~32,000 nondiabetic subjects in 57 studies to assess the effect of the Pro12Ala polymorphism on pre-diabetic traits.

RESULTS — In the global comparison, there were no differences in BMI, glucose, insulin, or homeostasis model assessment of insulin resistance between the Pro/Pro and X/Ala genotype. However, in the Caucasian subgroup, the X/Ala genotype was associated with significantly increased BMI. In the obese subgroup (BMI >30 kg/m^2), fasting glucose ($P = 0.041$) and insulin resistance (by homeostasis model analysis) ($P = 0.020$) were significantly greater in the Pro/Pro group. In subjects with the homozygous Ala/Ala genotype, fasting insulin was significantly lower compared with the Pro/Pro genotype ($P = 0.040$, $N_{Ala/Ala} = 154$).

CONCLUSIONS — Across all studies, the Pro12Ala polymorphism had no significant effect on diabetes-related traits. Only in selected subgroups, such as Caucasians and obese subjects, did we see an association of the Ala allele with greater BMI and greater insulin sensitivity. This demonstrates the importance for appropriate stratification of analyses by environmental or other genetic factors. Meta-analysis of Ala/Ala homozygotes more clearly demonstrated the association with greater insulin sensitivity of carriers of the Ala allele.

Diabetes Care 29:2489–2497, 2006

The Pro12Ala polymorphism in peroxisome proliferator–activated receptor (PPAR)γ, caused by a missense mutation in exon B of the adipocyte-specific γ2 isoform, was identified in 1997 and is thought to confer reduced transcriptional activity (1). Several more genetic variants in PPARγ are known but are much less frequent (2). The prevalence of the Ala allele varies from ~4% in Asian (3) to ~28% in Caucasian (4) populations. Since PPARγ2 is exclusively expressed in adipose tissue, the prevalent Pro12Ala polymorphism was originally studied for an association with obesity. Soon, evidence emerged suggesting that the Ala variant is associated with increased insulin sensitivity in nondiabetic Caucasians (5). Consequently, association with type 2 diabetes was examined, and an early meta-analysis (6) strongly suggested that the Pro variant is a risk allele. This analysis included 16 genetic association studies and used a family-based design to control for population stratification. A more recent meta-analysis (7) confirmed these findings, and the Pro12Ala polymorphism is now considered the best replicated genetic risk factor of common type 2 diabetes.

The key components in the pathogenesis of type 2 diabetes are insulin resistance and β-cell dysfunction (8,9). To further clarify the mechanisms underlying the association of Pro12Ala with type 2 diabetes, however, it is necessary to examine nondiabetic populations, because hyperglycemia itself can induce insulin resistance and secretory dysfunction (10,11). Therefore, we performed a meta-analysis of 57 suitable studies containing data related to the insulin resistance of cohorts with normal or impaired glucose tolerance. The hypothesis is that the Pro/Pro genotype is associated with a more "diabetic" phenotype in this population (e.g., greater insulin resistance).

RESEARCH DESIGN AND METHODS

— Appropriate studies were collected by a PubMed search (before July 2005) using the phrase "(pro12ala or p12a) and (stud* or diabetes or insulin sensitivity or insulin secretion)." Additionally, reference lists from articles were searched for further suitable studies. Two articles could not be obtained, one from a Chinese journal (12) and the other one from a Polish journal (13). The retrieval contained 151 articles.

We used the following exclusion criteria: review articles without new data; multifold data or interim data of studies, except when interim analyses contained data not presented in the final analysis; studies only including patients with type 2 diabetes; patients with polycystic ovarian syndrome; hyperlipidemia; studies including children; missing case numbers or measurements of deviation; and missing genotype-specific data of the quanti-

From the [1]IIIrd Medical Department, University of Leipzig, Leipzig, Germany; and the [2]Institute of Medical Statistics and Epidemiology University of Leipzig, Leipzig, Germany.
Address correspondence and reprint requests to Michael Stumvoll, MD, University of Leipzig, IIIrd Medical Department, Philipp-Rosenthal-Str. 27, 04103 Leipzig, Germany. E-mail: michael.stumvoll@medizin.uni-leipzig.de.
Received for publication 7 March 2006 and accepted in revised form 4 August 2006.
A.T. and M.S. contributed equally to this work.
Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
DOI: 10.2337/dc06-0513

Table 1—*Studies included in meta-analysis*

Author (ref.)	Race	Lean/obese	*n*	Age (years)	Sex distribution (% female)	X/Ala frequency (%)	Comments
Altshuler (6)	Caucasian	Mixed	349	31	51	25	NGT, estimated minimal case numbers
Andrulionyte (30)	Caucasian	Obese	770	54.8	50	23	IGT
Baratta (31)	Caucasian	Mixed	338	37.9	56	12	—
Beamer (32) a	Caucasian	Mixed	517	64.4	39	21	BLSA
Beamer (32) b	Caucasian	Obese	169	43.3	66	17	JHU-WMC
Buzzetti (33)	Caucasian	Obese	1,215	42.7	70	17	—
Clement (34)	Caucasian	Lean	295	53	66	17	SU.VI.MAX, only control subjects taken
Deeb (5) a	Caucasian	Mixed	973	70	63	29	Elderly group
Deeb (5) b	Caucasian	Mixed	333	44.8	50	23	Middle-aged group
Douglas (35)	Caucasian	Mixed	191	61.4	68	36	Spousal control subjects, only significant results presented
Ek (26) a	Caucasian	Lean	364	25.2	49	26	Danish group
Ek (26) b	Caucasian	Mixed	616	70	—	26	Swedish group
Eriksson (15)	Caucasian	Mixed	476	69.6	64	32	—
Eurlings (36)	Caucasian	Mixed	124	51.1	60	25	Familial combined Hyperlipidemic spouses
Evans (37)	Caucasian	Obese	429	45.7	—	25	—
Fornage (38) a	Caucasian	Lean	1,954	25.6	59	24	CARDIA study
Fornage (38) b	African	Mixed	1,844	24.4	54	4	CARDIA study
Franks (39)	Caucasian	Mixed	506	53.1	55	21	—
Frederiksen (4)	Caucasian	Mixed	2,245	53.3	51	26	—
Ghoussaini (40) a	Caucasian	Lean	865	51.0	62	22	NGT
Ghoussaini (40) b	Caucasian	Obese	507	47.2	68	22	Obese group
Hara (20)	Asian	Lean	541	69.1	53	8	Unselected population
Hegele (41)	Indian	Mixed	171	30.4	100	13	Canadian Oji-Cree
Kahara (42)	Asian	Lean	123	45.2	0	5	—
Kawasaki (21)	Asian	Lean	116	53.4	61	5	—
Kolehmainen (43)	Caucasian	Obese	22	43.8	67	27	—
Li (44)	mixed	Mixed	686	20–38	—	20	Bogalusa Study
Lindi (23)	Caucasian	Obese	ca.490*	55.2	67	31	IGT
Lindi (45)	Caucasian	Mixed	150	49.2	49	24	KANWU study
Luan (46)	Caucasian	Mixed	592	53.9	56	21	Isle of Ely Study
Mancini (47)	Caucasian	Mixed	312	45.2	—	18	NGT
Meirhaeghe (48)	Caucasian	Mixed	1,133	35–64	50	21	MONICA study
Mori (3)	Asian	Lean	1,212	71.3	57	8	—
Mousavinasab (49)	Caucasian	Mixed	252	17–28	0	31	Soldiers study
Muller (28)	Indian	Obese	241	27.5	44	14	Pimas
Nicklas (50)	Caucasian	Obese	ca. 50*	60.2	100	21	Postmenopausal women, before weight loss
Oh (51)	Asian	Mixed	229	48.0	36	8	—
Ostergard (27)	Caucasian	Mixed	54	33.6	44	41	Only control subjects taken
Pisabarro (52)	Caucasian	Obese	45	39.0	71	13	—

Continued on facing page

ties of interest (BMI, fasting plasma glucose, and insulin; 2-h glucose and insulin during oral glucose tolerance test [OGTT]; and homeostasis model assessment of insulin resistance [HOMA-IR]). The 57 studies suitable for meta-analysis are shown in Table 1.

Data analysis

We assumed normal distribution for fasting glucose and 2-h glucose during OGTT and a log normal distribution for all other quantities, compatible with the original data from Tschritter et al. (14). Hence, means and SDs of BMI, fasting insulin, 2-h insulin during OGTT, and HOMA-IR were transformed to means and SDs of corresponding logarithmic quantities using standard formulas.

Logarithmic transformation provided the mean of log insulin when geometric means were presented. In the study of

Table 1—*Continued*

Author (ref.)	Race	Lean/obese	*n*	Age (years)	Sex distribution (% female)	X/Ala frequency (%)	Comments
Poirier (53)	Caucasian	Lean	675	18–28	0	25	Family history of myocardial infarction
Poulsen (54)	Caucasian	Mixed	553	66.5	—	22	Twin study
Robitaille (55)	Caucasian	Mixed	ca.600*	43.1	57	19	Quebec Family Study
Rosmond (24)	Caucasian	Mixed	268	58	0	31	—
Sanchez (56) a	Caucasian	Mixed	ca.460*	48.7	55	17	All subjects group
Sanchez (56) b†	Caucasian	Obese	ca.140	52.0	65	18	Obese group
Stumvoll (29)	Caucasian	Mixed	318	33	60	24	—
Tai (19)	Asian	Lean	3,618	37.1	55	9	NGT + IGT group
Takata (57)	Asian	Lean	247	24.2	41	7	—
Thamer (58)	Caucasian	Mixed	648	36	63	23	—
Tschritter (14)	Caucasian	Lean	229	32.0	51	23	Clamp data
Vaccaro (59) a	Caucasian	Lean	280	45.6	42	15	Lean group
Vaccaro (59) b	Caucasian	Obese	141	45.6	44	23	Obese and obese with early onset
Valve (2)	Caucasian	Obese	141	43.0	100	24	—
Wang (22)	Asian	Mixed	153	—	—	7	NGT + IGT
Weiss (60)	Caucasian	Mixed	73	58.4	56	16	Sedentaries, baseline
Yamamoto (61)	Asian	Lean	595	48.1	20	5	—
Yamamoto (62)	Asian	Lean	81	43.8	0	5	Patients with hypertension
Yang (63)	Asian	Mixed	1,708	50.8	56	7	—

*Different number of cases for different quantities. †Part a completely includes part b; therefore, they were only separately included in the analysis. Studies included into analysis in alphabetical order. BLSA, Baltimore Longitudinal Study of Aging; CARDIA, Coronary Artery Risk Development in Young Adults; IGT, impaired glucose tolerance; JHU-WMC, Johns Hopkins University Weight Management Center; KANWU, Kuopio, Arthus, Naples, Wallongog, Uppsala; MONICA, Monitoring of Trends and Determinants in Cardiovascular Diseases; NGT, normal glucose tolerance; SU.VI.MAX, Supplementation en Vitamines et Mineraux Antioxydants Study.

Erikson et al. (15), means and SDs for log glucose were calculated under the assumption of log normally distributed glucose. We calculated the corresponding means and SDs for glucose but treated the later quantities as parameter estimates for a normally distributed quantity according to our distribution assumption.

If only quartile range or CI as a measure of deviation were available, SD was estimated under the above distribution assumptions. More precisely, for normally distributed quantities, we used the differences of quartile range or CI boundaries to calculate SD. For log normally distributed quantities, the differences of the logarithmic boundaries of quartile range or CI were taken to calculate SD.

Altshuler et al. (6) did not provide genotype-specific case numbers after a written request. In this case, we used the available allele frequencies to estimate a lower boundary of the case numbers. Hence, the influence of this study in our analysis is underestimated.

Subjects with Pro/Ala and Ala/Ala were referred to as X/Ala for the pooled analysis. Where possible, a separate analysis of the Ala/Ala genotype in comparison with Pro/Pro was performed. In some

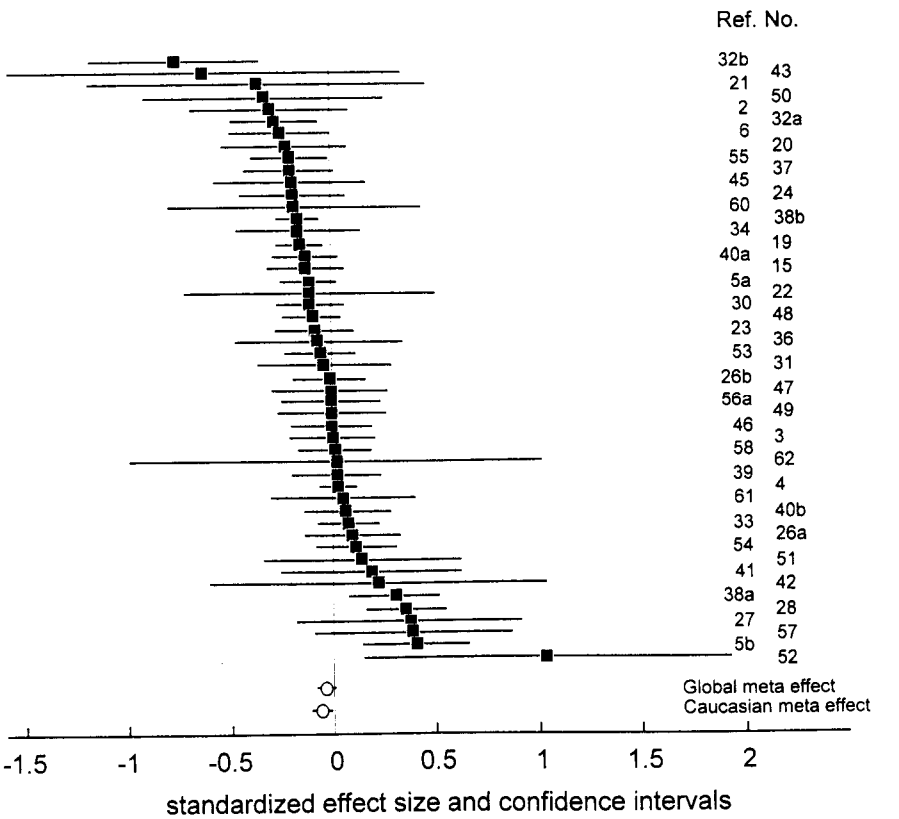

Figure 1—*BMI on eligible studies (Pro/Pro versus X/Ala). Estimated standardized effect sizes and CIs are given for the single studies for global comparison and Caucasian subgroup. Positive standardized effect size indicates that the corresponding quantity is greater in Pro/Pro than in X/Ala.*

Table 2—*Standardized effect sizes for comparison Pro/Pro versus X/Ala*

	Pro/Pro-X/Ala (standardized effect size)				
				Lean (mean BMI <25 kg/m^2)	Obese (mean BMI >30 kg/m^2)
	Global	Caucasians	Asians		
Log (BMI)	−0.037 (−0.087 to 0.012); P = 0.139; r, 50; $N_{Pro/Pro}$ = 24,074; $N_{X/Ala}$ = 5,350	−0.062 (−0.111 to −0.012); P = 0.015; r, 37; $N_{Pro/Pro}$ = 15,129; $N_{X/Ala}$ = 4,570*	−0.087 (−0.174 to −0.001); P = 0.048; f, 10; $N_{Pro/Pro}$ = 6,354; $N_{X/Ala}$ = 561†	0.015 (−0.040 to 0.070); P = 0.602; f, 14; $N_{Pro/Pro}$ = 9,416; $N_{X/Ala}$ = 1,550	0.081 (0.003–0.158); P = 0.041; f, 12; $N_{Pro/Pro}$ = 3,099; $N_{X/Ala}$ = 824†
Fasting glucose	0.022 (−0.019 to 0.062); P = 0.296; r, 49; $N_{Pro/Pro}$ = 23,615; $N_{X/Ala}$ = 4,930	0.011 (−0.041 to 0.063); P = 0.673; r, 34; $N_{Pro/Pro}$ = 13,015; $N_{X/Ala}$ = 3,965	0.069 (−0.009 to 0.147); P = 0.085; f, 11; $N_{Pro/Pro}$ = 7,937; $N_{X/Ala}$ = 686	—	−0.010 (−0.124 to 0.104); P = 0.864; f, 4; $N_{Pro/Pro}$ = 1,251; $N_{X/Ala}$ = 395
2-h glucose	0.018 (−0.079 to 0.115); P = 0.713; r, 12) $N_{Pro/Pro}$ =5,121; $N_{X/Ala}$ =1,163	−0.019 (−0.131 to 0.094); P = 0.745; r, 8; $N_{Pro/Pro}$ = 2,976; $N_{X/Ala}$ = 959	—	−0.055 (−0.114 to 0.003); P = 0.062; f, 12; $N_{Pro/Pro}$ = 8,056; $N_{X/Ala}$ = 1,403	0.163 (−0.009 to 0.334); P = 0.064; r, 12; $N_{Pro/Pro}$ = 3,075; $N_{X/Ala}$ = 813
Log(fasting insulin)	0.031 (−0.024 to 0.085); P = 0.268; r, 51; $N_{Pro/Pro}$ = 23,467; $N_{X/Ala}$ = 5,145	0.017 (−0.037 to 0.071); P = 0.536; r, 37; $N_{Pro/Pro}$ = 13,981; $N_{X/Ala}$ = 4,278	−0.076 (−0.160 to 0.008); P = 0.078; f, 10; $N_{Pro/Pro}$ = 6,823; $N_{X/Ala}$ = 588	—	0.038 (−0.256 to 0.333); P = 0.798; r, 4; $N_{Pro/Pro}$ = 1,251; $N_{X/Ala}$ = 395
Log(2-h insulin)	0.087 (−0.031 to 0.205); P = 0.150; r, 9; $N_{Pro/Pro}$ = 4,520; $N_{X/Ala}$ = 1,046	0.078 (−0.038 to 0.194); P = 0.186; r, 7; $N_{Pro/Pro}$ = 2,734; $N_{X/Ala}$ = 883	—	−0.065 (−0.144 to 0.015); P = 0.111; f, 8; $N_{Pro/Pro}$ = 3,746; $N_{X/Ala}$ = 776	0.102 (0.016 to 0.188); P = 0.020; f, 5; $N_{Pro/Pro}$ = 2,453; $N_{X/Ala}$ = 674†
Log(HOMA-IR)	0.079 (−0.077 to 0.234); P = 0.322; r, 23; $N_{Pro/Pro}$ = 13,667; $N_{X/Ala}$ = 2,919	0.158 (−0.059 to 0.376); P = 0.154; r, 13; $N_{Pro/Pro}$ = 8,038; $N_{X/Ala}$ = 2,454	−0.030 (−0.159 to 0.100); P = 0.652; f, 8; $N_{Pro/Pro}$ = 3,317; $N_{X/Ala}$ = 247	—	—

Data are standardized effect sizes (95% asymptotic CI); P values; choice of a random-effects model (r) in case of significant inhomogeneity or a fixed-effects model (f) in case of no significant inhomogeneity; and the number (N) of studies included into the corresponding analysis as well as number of individuals per genotype subgroup are provided. *Result remains significant after jackknifing; †significant result.

studies, sex and age subgroups were pooled by calculating the common mean and SD. Analysis was globally performed for all eligible studies and within the subgroups of Caucasian, Asian, lean, and obese individuals. To avoid overlap of subgroups, instead of via a cutoff point, we determined obesity by classifying mean BMI <25 kg/m^2 as lean, mean BMI >30 kg/m^2 as obese, and discharged groups in between.

Statistics

For statistical analysis, we applied the general approach for meta-analyses proposed by Whitehead (16,17). Hence, test statistic is the χ_1^2-distributed U:

$$U = \left(\sum_i \hat{\theta}_i w_i \right)^2 / \sum_i w_i$$

where $\hat{\theta}_i$ is the effect size and w_i is the weight of the i-th study.

We calculated dimensionless standardized effect sizes $\hat{\theta}_i$, which are the differences of the means of the compared groups divided by their common SD. Consequently, the weights depend only on case numbers. This method is advised when different unit systems are used, consistency among laboratories cannot be guaranteed, or if absolute study results cannot be directly compared (e.g., because of large differences between study populations and/or different adjustment methods). Results are shown in Figs. 1–3, providing standardized effect sizes and corresponding CIs.

We tested for the null hypothesis of no difference between the genotypes. The effect of the meta-analysis and its CI is also shown in comparison with the effects of the single studies. Negative values for the meta-effect size indicate that the corresponding quantity is smaller in Pro/Pro genotypes compared with X/Ala genotypes.

Inhomogeneity is analyzed by Q statistics. We calculated fixed-effects models and, in the case of significant inhomogeneity, random-effects models. Studies inconsistent with the other results were identified by radial plots (18) (data not shown). Regression of these plots were used to detect a possible publication bias (65).

Finally, robustness of significant results was investigated by a jackknife procedure. If significance was lost by leaving out single studies, we interpreted the result as having insufficient evidence for an effect. A substantial effect is indicated in cases of improvement of homogeneity

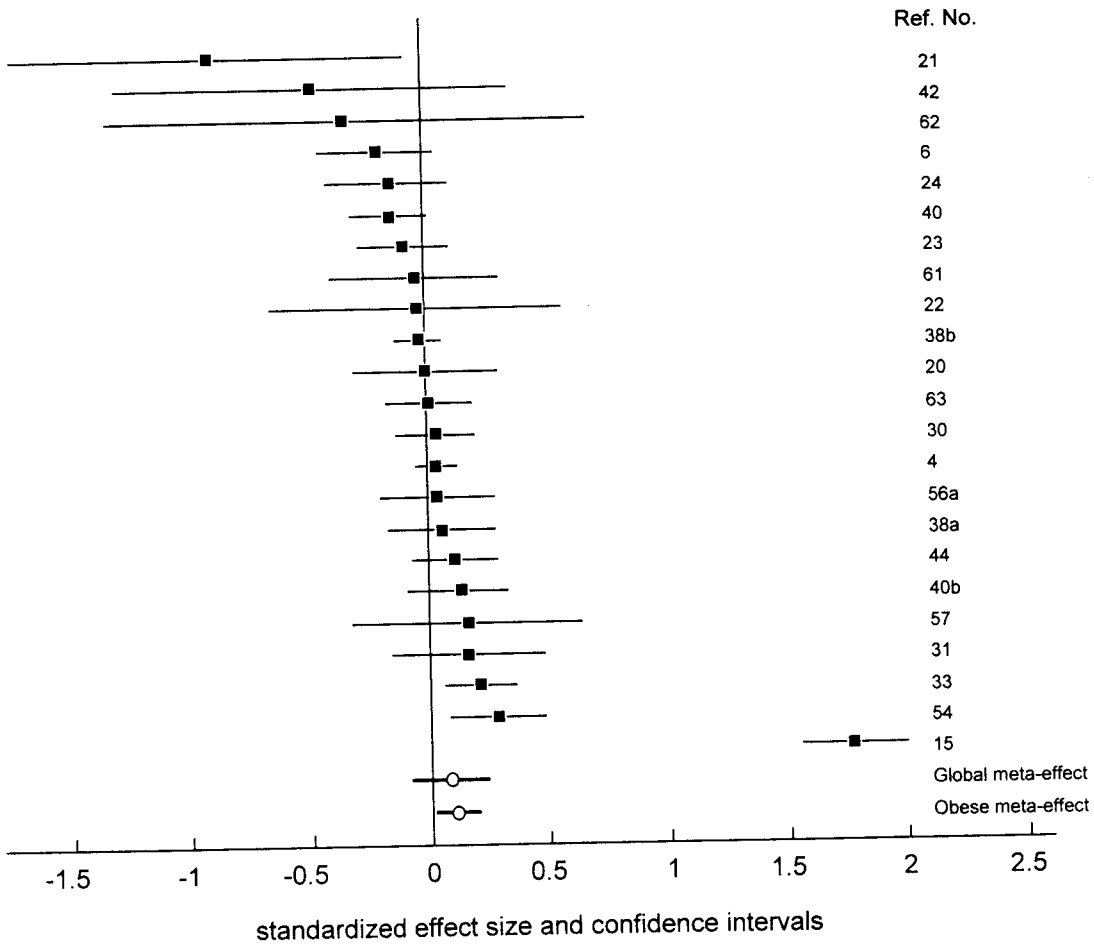

Figure 2—*HOMA-IR on eligible studies (Pro/Pro versus X/Ala). Estimated standardized effect sizes and CIs are given for the single studies, for global comparison and obese subgroup. Positive standardized effect size indicates that the corresponding quantity is greater in Pro/Pro than in X/Ala.*

and significance by leaving out a single study. All calculations except the power calculations were performed using MATLAB 7.0.4.365 (The MathWorks, Natick, MA). The powers were calculated with nQuery Advisor 6.0 (Statistical Solutions, Saugus, MA).

RESULTS — The Eggers test did not indicate any obvious publication bias.

X/Ala versus Pro/Pro (Table 2)

BMI was not different in the global analysis. In the Caucasian subgroup, however, BMI was greater in X/Ala compared with Pro/Pro ($P = 0.015$), which held up to jackknifing (Fig. 1). The BMI in Asians was also greater in the X/Ala group, but after leaving out one of the studies of Tai et al. (19), Hara et al. (20), Kawasaki et al. (21), or Wang et al. (22), the effect became nonsignificant. We found no effect of the Pro12Ala polymorphism with respect to fasting insulin, neither globally nor in any of the subgroups.

Fasting glucose was not different in the global comparison. In the obese subgroup, however, fasting glucose was higher in the Pro/Pro group ($P = 0.041$), and leaving out the study of Lindi et al. (23) substantially increased significance (standardized effect size 0.127, $P = 0.0033$) and homogeneity ($P = 0.76$). There was no significant effect on 2-h insulin and glucose during OGTT, neither globally nor in any subgroup.

HOMA-IR was significantly higher in Pro/Pro compared with X/Ala in the obese subjects, though not globally, indicating greater insulin sensitivity in obese carriers of the Ala allele. This effect became stronger (standardized effect size 0.148, $P = 0.0025$) with higher homogeneity of the remaining studies after leaving out the study of Lindi et al. (23) (Fig. 2).

Analysis of Ala/Ala

In 12 studies, the authors presented full genotype information, which allowed the analysis of the homozygous Ala/Ala genotype. Because of insufficient data quantity, the parameters HOMA-IR, 2-h glucose, and insulin were not analyzed in this part. Furthermore, only global testing of all pooled subgroups was performed. A total of up to 160 subjects with the Ala/Ala genotype were available for meta-analysis. Neither BMI (standardized effect size −0.125, $P = 0.353$) nor fasting glucose (standardized effect size −0.088, $P = 0.282$) were significantly different between the genotypes after global testing.

Fasting insulin was significantly lower in Ala/Ala compared with the Pro/Pro genotype (standardized effect size 0.168, $P = 0.040$). However, this effect vanished after jackknifing. The effect also vanished after calculating a random-effects model, which might have been more appropriate in view of the borderline study homogeneity ($P = 0.052$ for test of inhomogeneity). On the other hand, excluding the study of Rosmond et al. (24), which reported extremely high fasting insulin values in the Ala/Ala group discrepant from all other studies, makes the effect highly significant (standardized effect size 0.227, $P = 0.0067$) and the

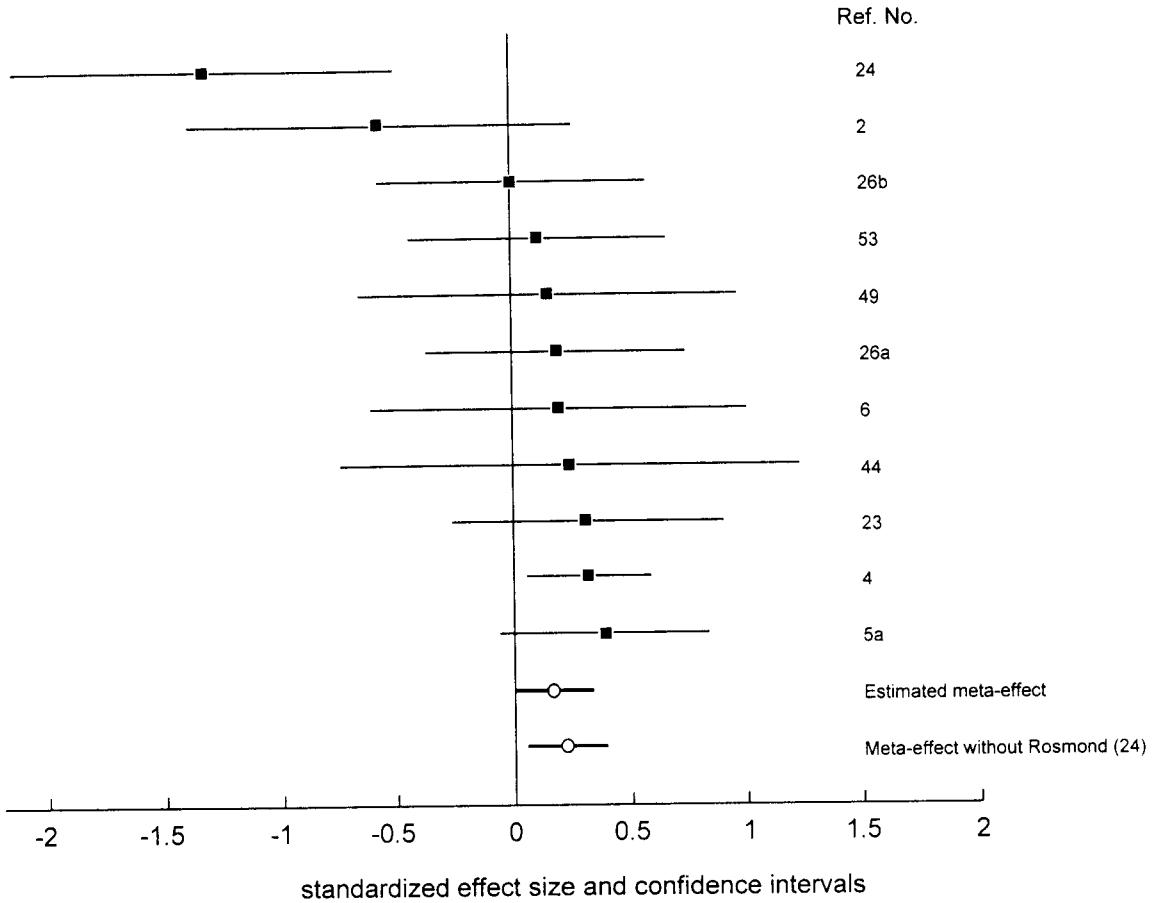

Figure 3—*Fasting insulin on eligible studies (Pro/Pro versus Ala/Ala). Estimated standardized effect sizes and CIs are given; the meta-analysis effect and its CI is shown for global comparison and separately after excluding the study of Rosmond et al. (24). Positive standardized effect size indicates that the corresponding quantity is greater in Pro/Pro than in X/Ala.*

remaining studies highly homogeneous ($P = 0.81$ for inhomogeneity test; Fig. 3).

CONCLUSIONS — The BMI of individuals with the Pro/Pro genotype did not significantly differ from individuals with the X/Ala genotype in the global analysis. Interestingly, in the Caucasian subgroup, the X/Ala genotype was associated with significantly increased BMI. The absence of a statistical effect in the Asian subgroup might be explained by gene-gene and gene-environmental interactions (25). Also, the lower frequency of the Ala allele in Asians and the lower sample size in comparison with the Caucasian subgroup may play a role. Masud et al. (64) found a greater BMI in Ala carriers in obese subjects, but this study included patients with diabetes.

Surrogate markers of insulin resistance, such as fasting insulin and HOMA-IR, were not significantly different between the Pro/Pro and X/Ala genotypes globally. In the obese subgroup (BMI >30 kg/m^2), however, fasting glucose was significantly higher in the Pro/Pro group. Analogously, HOMA-IR was also significantly higher in the obese subgroup with the Pro/Pro genotype. These effects became even stronger after leaving out the study of Lindi et al. (23), which only included obese subjects with impaired glucose tolerance. Two-hour postchallenge glucose and insulin concentrations were not significantly different between genotypes, neither in the global nor in the subgroup analysis, but the case numbers are much lower.

Subsequently, we investigated the influence of Ala/Ala homozygosity when full genotype information was given. Fasting insulin was higher in the Pro/Pro compared with the Ala/Ala group. Jackknifing revealed that the study of Rosmond et al. (24) was responsible for significant inhomogeneity among the studies. That study reported extremely high fasting insulin values in the Ala/Ala group, in discordance with all other studies. After excluding the study of Rosmond et al. (24), which contained only six individuals with Ala/Ala, the effect became highly significant and the remaining studies highly homogeneous. It is important to note that these six individuals had a significantly higher BMI, which would easily explain the differences in fasting insulin and insulin sensitivity. Therefore, we interpret the results of the Ala/Ala meta-analysis as an indication of greater insulin sensitivity of this genotype.

To ultimately assess the role of greater insulin resistance in the Pro/Pro group in comparison with the Ala/Ala group, a meta-analysis of euglycemic-hyperinsulinemic clamp data would be desirable. This could not be performed because the four available studies (26–29) were too heterogenous and could not be subjected to jackknifing. Two-hour glucose and insulin were not analyzed in Ala/Ala individuals due to insufficient data.

Another shortcoming of our meta-analysis is the classical meta-analysis approach with respect to the quantities considered. Because several studies used different adjustment methods, a meta-

regression of the data might have been more appropriate. However, this would require access to original data of the individual studies, which was not possible. Because of different adjustment methods or different study populations, we always calculated standardized effect measures, which are more robust than absolute effect measures.

To evaluate the sensitivity of our meta-analysis, we estimated the statistical power and the absolute effects under the assumption of variance homogeneity among the studies. With a power of 80% for log(BMI), a global effect size of 0.8% difference (related to the group with the greater value) between the Pro/Pro and X/Ala groups could be discovered. On the other hand, with calculation of the common variance, the estimated effect was only a 0.7% difference. For fasting glucose, the discoverable effect was 0.045 mmol/l but the estimated effect was only 0.023 mmol/l. For logarithm of fasting insulin, the discoverable effect was a 2.7% difference but the estimated effect was only 2%. Consequently, even very small effects could be detected with our analysis but were not found globally. Furthermore, the estimates of the global effects were very precise (narrow CI) despite some inhomogeneity of the studies.

In conclusion, across all studies the Pro12Ala polymorphism had no significant effect on diabetes-related traits within a nondiabetic population. Only in selected subgroups, such as Caucasians and obese subjects, did we see an association of the Ala allele with greater BMI and greater insulin sensitivity. This demonstrates the importance for appropriate stratification of analyses by as many factors as possible. Meta-analysis of Ala/Ala homozygotes more clearly demonstrated the association with greater insulin sensitivity of the Ala allele.

References

1. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR: Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241: 270–274, 1997
2. Valve R, Sivenius K, Miettinen R, Pihlajamaki J, Rissanen A, Deeb SS, Auwerx J, Uusitupa M, Laakso M: Two polymorphisms in the peroxisome proliferator-activated receptor-gamma gene are associated with severe overweight among obese women. *J Clin Endocrinol Metab* 84:3708–3712, 1999
3. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Hara K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M: The $Pro^{12}\rightarrow$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* 50:891–894, 2001
4. Frederiksen L, Brodbaek K, Fenger M, Jorgensen T, Borch-Johnsen K, Madsbad S, Urhammer SA: Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome. *J Clin Endocrinol Metab* 87:3989–3992, 2002
5. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J: A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284–287, 1998
6. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
7. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177–182, 2003
8. Chiasson JL, Rabasa-Lhoret R: Prevention of type 2 diabetes: insulin resistance and β-cell function. *Diabetes* 53 (Suppl. 3): S34–S38, 2004
9. Kahn SE: The relative contributions of insulin resistance and β-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia* 46:3–19, 2003
10. Hartling SG, Roder ME, Dinesen B, Binder C: Proinsulin, C-peptide, and insulin in normal subjects during an 8-h hyperglycemic clamp. *Eur J Endocrinol* 134:197–200, 1996
11. Kahn SE, Bergman RN, Schwartz MW, Taborsky GJ Jr, Porte D Jr: Short-term hyperglycemia and hyperinsulinemia improve insulin action but do not alter glucose action in normal humans. *Am J Physiol* 262:E518–E523, 1992
12. Fu M, Chen H, Li X, Li J, Wu B, Cheng L, Cai M, Fu Z: [Association of Pro12Ala variant in peroxisome proliferator-activated receptor-gamma2 gene with type 2 diabetes mellitus]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 19:234–238, 2002 [Article in Chinese]
13. Kobalava ZD, Nosikov VV, Tolkacheva VV, Chernysheva GV, Volkova EG, Shustov SB, Ignat'ev IV, Moiseev VS: [Clinical genetic determinants of carbohydrate metabolism disturbances in patients with hypertension and excessive weight.]. *Kardiologiia* 45:37–43, 2005 [Article in Russian]
14. Tschritter O, Fritsche A, Stefan N, Haap M, Thamer C, Bachmann O, Dahl D, Maerker E, Teigeler A, Machicao F, Haring H, Stumvoll M: Increased insulin clearance in peroxisome proliferator-activated receptor gamma2 Pro12Ala. *Metabolism* 52: 778–783, 2003
15. Eriksson J, Lindi V, Uusitupa M, Forsen T, Laakso M, Osmond C, Barker D: The effects of the Pro12Ala polymorphism of the PPARgamma-2 gene on lipid metabolism interact with body size at birth. *Clin Genet* 64:366–370, 2003
16. Whitehead A, Whitehead J: A general parametric approach to the meta-analysis of randomized clinical trials. *Stat Med* 10: 1665–1677, 1991
17. Hedges LV: *Statistical Methods for Meta-Analysis*. Orlando, FL, Academic Press, 1985
18. Galbraith RF: A note on graphical presentation of estimated odds ratios from several clinical trials. *Stat Med* 7:889–894, 1988
19. Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, Tan CE, Ordovas JM: Differential effects of the C1431T and Pro12Ala PPARgamma gene variants on plasma lipids and diabetes risk in an Asian population. *J Lipid Res* 45:674–685, 2004
20. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T: The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 271: 212–216, 2000
21. Kawasaki I, Tahara H, Emoto M, Shoji T, Shioji A, Okuno Y, Inaba M, Nishizawa Y: Impact of Prol2Ala variant in the peroxisome proliferator-activated receptor (PPAR) gamma2 on obesity and insulin resistance in Japanese type 2 diabetic and healthy subjects. *Osaka City Med J* 48:23–28, 2002
22. Wang C, Zhai F, Chi Y, Wang G: [Association of Pro12Ala mutation in peroxisome proliferator-activated receptor gamma 2 with obesity and diabetes in Chinese population]. *Wei Sheng Yan Jiu* 33:317–320, 2004 [Article in Chinese]
23. Lindi VI, Uusitupa MI, Lindstrom J, Louheranta A, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Tuomilehto J: Association of the Pro12Ala polymor-

phism in the PPAR-γ2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* 51:2581–2586, 2002
24. Rosmond R, Chagnon M, Bouchard C: The Pro12Ala PPARgamma2 gene missense mutation is associated with obesity and insulin resistance in Swedish middle-aged men. *Diabetes Metab Res Rev* 19: 159–163, 2003
25. Ochoa MC, Marti A, Azcona C, Chueca M, Oyarzabal M, Pelach R, Patino A, Moreno-Aliaga MJ, Martinez-Gonzalez MA, Martinez JA: Gene-gene interaction between PPAR gamma 2 and ADR beta 3 increases obesity risk in children and adolescents. *Int J Obes Relat Metab Disord* 28 (Suppl. 3):S37–S41, 2004
26. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O: Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. *Diabetologia* 44:1170–1176, 2001
27. Ostergard T, Ek J, Hamid Y, Saltin B, Pedersen OB, Hansen T, Schmitz O: Influence of the PPAR-gamma2 Pro12Ala and ACE I/D polymorphisms on insulin sensitivity and training effects in healthy offspring of type 2 diabetic subjects. *Horm Metab Res* 37:99–105, 2005
28. Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ: A functional variant in the peroxisome proliferator–activated receptor γ2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. *Diabetes* 52:1864–1871, 2003
29. Stumvoll M, Stefan N, Fritsche A, Madaus A, Tschritter O, Koch M, Machicao F, Haring H: Interaction effect between common polymorphisms in PPAR-gamma2 (Pro12Ala) and insulin receptor substrate 1 (Gly972Arg) on insulin sensitivity. *J Mol Med* 80:33–38, 2002
30. 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* 47:2176–2184, 2004
31. Baratta R, Di Paola R, Spampinato D, Fini G, Marucci A, Coco A, Vigneri R, Frittitta L, Trischitta V: Evidence for genetic epistasis in human insulin resistance: the combined effect of PC-1 (K121Q) and PPARgamma2 (P12A) polymorphisms. *J Mol Med* 81:718–723, 2003
32. Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ, Andres R, Roth J, Shuldiner AR: Association of the Pro12Ala variant in the peroxisome proliferator–activated receptor-γ2 gene with obesity in two Caucasian populations. *Diabetes* 47:1806–1808, 1998
33. Buzzetti R, Petrone A, Ribaudo MC, Alemanno I, Zavarella S, Mein CA, Maiani F, Tiberti C, Baroni MG, Vecci E, Arca M, Leonetti F, Di Mario U: The common PPAR-gamma2 Pro12Ala variant is associated with greater insulin sensitivity. *Eur J Hum Genet* 12:1050–1054, 2004
34. Clement K, Hercberg S, Passinge B, Galan P, Varroud-Vial M, Shuldiner AR, Beamer BA, Charpentier G, Guy-Grand B, Froguel P, Vaisse C: The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. *Int J Obes Relat Metab Disord* 24:391–393, 2000
35. Douglas JA, Erdos MR, Watanabe RM, Braun A, Johnston CL, Oeth P, Mohlke KL, Valle TT, Ehnholm C, Buchanan TA, Bergman RN, Collins FS, Boehnke M, Tuomilehto J: The peroxisome proliferator–activated receptor-γ2 Pro12A1a variant: association with type 2 diabetes and trait differences. *Diabetes* 50:886–890, 2001
36. Eurlings PM, van der Kallen CJ, Vermeulen VM, de Bruin TW: Variants in the PPARgamma gene affect fatty acid and glycerol metabolism in familial combined hyperlipidemia. *Mol Genet Metab* 80: 296–301, 2003
37. Evans D, de Heer J, Hagemann C, Wendt D, Wolf A, Beisiegel U, Mann WA: Association between the P12A and c1431t polymorphisms in the peroxisome proliferator activated receptor gamma (PPAR gamma) gene and type 2 diabetes. *Exp Clin Endocrinol Diabetes* 109:151–154, 2001
38. Fornage M, Jacobs DR, Steffes MW, Gross MD, Bray MS, Schreiner PJ: Inverse effects of the PPAR(gamma)2 Pro12Ala polymorphism on measures of adiposity over 15 years in African Americans and whites: the CARDIA study. *Metabolism* 54:910–917, 2005
39. Franks PW, Luan J, Browne PO, Harding AH, O'Rahilly S, Chatterjee VK, Wareham NJ: Does peroxisome proliferator-activated receptor gamma genotype (Pro12ala) modify the association of physical activity and dietary fat with fasting insulin level? *Metabolism* 53:11–16, 2004
40. Ghoussaini M, Meyre D, Lobbens S, Charpentier G, Clement K, Charles MA, Tauber M, Weill J, Froguel P: Implication of the Pro12Ala polymorphism of the PPAR-gamma 2 gene in type 2 diabetes and obesity in the French population. *BMC Med Genet* 6:11, 2005
41. Hegele RA, Cao H, Harris SB, Zinman B, Hanley AJ, Anderson CM: Peroxisome proliferator-activated receptor-gamma2 P12A and type 2 diabetes in Canadian Oji-Cree. *J Clin Endocrinol Metab* 85: 2014–2019, 2000
42. Kahara T, Takamura T, Hayakawa T, Nagai Y, Yamaguchi H, Katsuki T, Katsuki K, Katsuki M, Kobayashi K: PPARgamma gene polymorphism is associated with exercise-mediated changes of insulin resistance in healthy men. *Metabolism* 52:209–212, 2003
43. Kolehmainen M, Uusitupa MI, Alhava E, Laakso M, Vidal H: Effect of the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor (PPAR) gamma2 gene on the expression of PPARgamma target genes in adipose tissue of massively obese subjects. *J Clin Endocrinol Metab* 88: 1717–1722, 2003
44. Li S, Chen W, Srinivasan SR, Boerwinkle E, Berenson GS: The peroxisome proliferator–activated receptor-γ2 gene polymorphism (Pro12Ala) beneficially influences insulin resistance and its tracking from childhood to adulthood: the Bogalusa Heart Study. *Diabetes* 52:1265–1269, 2003
45. Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K, Storlien L, Riccardi G, A Rivellese A, the KANWU Study Group: Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. *Mol Genet Metab* 79:52–60, 2003
46. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ: Evidence for gene-nutrient interaction at the PPAR γ locus. *Diabetes* 50:686–689, 2001
47. Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, Riccardi G, Colantuoni V: Pro12Ala substitution in the peroxisome proliferator–activated receptor-γ2 is not associated with type 2 diabetes. *Diabetes* 48:1466–1468, 1999
48. Meirhaeghe A, Tanck MW, Fajas L, Janot C, Helbecque N, Cottel D, Auwerx J, Amouyel P, Dallongeville J: Study of a new PPARgamma2 promoter polymorphism and haplotype analysis in a French population. *Mol Genet Metab* 85:140–148, 2005
49. Mousavinasab F, Tahtinen T, Jokelainen J, Koskela P, Vanhala M, Oikarinen J, Keinanen-Kiukaanniemi S, Laakso M: Common polymorphisms in the PPAR-gamma2 and IRS-1 genes and their interaction influence serum adiponectin concentration in young Finnish men. *Mol Genet Metab* 84:344–348, 2005
50. Nicklas BJ, van Rossum EF, Berman DM, Ryan AS, Dennis KE, Shuldiner AR: Genetic variation in the peroxisome proliferator–activated receptor-γ2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50:2172–2176, 2001
51. Oh EY, Min KM, Chung JH, Min YK, Lee MS, Kim KW, Lee MK: Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Ko-

rean diabetic and obese subjects. *J Clin Endocrinol Metab* 85:1801–1804, 2000
52. Pisabarro RE, Sanguinetti C, Stoll M, Prendez D: High incidence of type 2 diabetes in peroxisome proliferator–activated receptor γ2 Pro12Ala carriers exposed to a high chronic intake of trans fatty acids and saturated fatty acids. *Diabetes Care* 27:2251–2252, 2004
53. Poirier O, Nicaud V, Cambien F, Tiret L: The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor gamma2 gene is not associated with postprandial responses to glucose or fat tolerance tests in young healthy subjects: the European Atherosclerosis Research Study II. *J Mol Med* 78:346–351, 2000
54. Poulsen P, Andersen G, Fenger M, Hansen T, Echwald SM, Volund A, Beck-Nielsen H, Pedersen O, Vaag A: Impact of two common polymorphisms in the PPAR γ gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both? *Diabetes* 52:194–198, 2003
55. Robitaille J, Despres JP, Perusse L, Vohl MC: The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. *Clin Genet* 63: 109–116, 2003
56. Gonzalez Sanchez JL, Serrano Rios M, Fernandez Perez C, Laakso M, Martinez Larrad MT: Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. *Eur J Endocrinol* 147:495–501, 2002
57. Takata N, Awata T, Inukai K, Watanabe M, Ohkubo T, Kurihara S, Inaba M, Katayama S: Pro12Ala substitution in peroxisome proliferator-activated receptor gamma 2 is associated with low adiponectin concentrations in young Japanese men. *Metabolism* 53:1548–1551, 2004
58. Thamer C, Machicao F, Fritsche A, Stumvoll M, Haring H: No influence of the PPARgamma2 Pro12Ala genotype on serum adiponectin concentrations in healthy Europeans (Letter). *Metabolism* 52:798, 2003
59. Vaccaro O, Mancini FP, Ruffa G, Sabatino L, Colantuoni V, Riccardi G: Pro12Ala mutation in the peroxisome proliferator-activated receptor gamma2 (PPAR-gamma2) and severe obesity: a case-control study. *Int J Obes Relat Metab Disord* 24:1195–1199, 2000
60. Weiss EP, Kulaputana O, Ghiu IA, Brandauer J, Wohn CR, Phares DA, Shuldiner AR, Hagberg JM: Endurance training-induced changes in the insulin response to oral glucose are associated with the peroxisome proliferator-activated receptor-gamma2 Pro12Ala genotype in men but not in women. *Metabolism* 54:97–102, 2005
61. Yamamoto Y, Hirose H, Miyashita K, Nishikai K, Saito I, Taniyama M, Tomita M, Saruta T: PPAR(gamma)2 gene Pro12Ala polymorphism may influence serum level of an adipocyte-derived protein, adiponectin, in the Japanese population. *Metabolism* 51:1407–1409, 2002
62. Yamamoto J, Kageyama S, Nemoto M, Sasaki T, Sakurai T, Ishibashi K, Mimura A, Yokota K, Tajima N: PPARgamma2 pro12Ala polymorphism and insulin resistance in Japanese hypertensive patients. *Hypertens Res* 25:25–29, 2002
63. Yang WS, Hsiung CA, Ho LT, Chen YT, He CT, Curb JD, Grove J, Quertermous T, Chen YD, Kuo SS, Chuang LM: Genetic epistasis of adiponectin and PPAR-gamma2 genotypes in modulation of insulin sensitivity: a family-based association study. *Diabetologia* 46:977–983, 2003
64. Masud S, Ye S, the SAS group: Effect of the peroxisome proloferator activated receptor-γ gene Pro12Ala variant on body mass index: a meta-analysis. *J Med Genet* 40: 773–780, 2003
65. Egger M, Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315:629–634, 1997