The Peroxisome Proliferator–Activated Receptor-γ2 Gene Polymorphism (Pro12Ala) Beneficially Influences Insulin Resistance and Its Tracking From Childhood to Adulthood

The Bogalusa Heart Study

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The peroxisome proliferator–activated receptor (PPAR)-γ2 gene polymorphism Pro12Ala has been associated with increased insulin sensitivity in some but not all studies. Little is known about its effect on the tracking of insulin resistance status over time. These aspects were examined in a community-based sample of 686 white young adults, aged 20–38 years, and 426 white children, aged 4–17 years, and a subsample of a cohort (n = 362) who participated both as children and adults, with an average follow-up period of 13.4 years. Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR) using fasting insulin and glucose. The frequency of the variant Ala12 allele was 0.104 in whites vs. 0.017 in blacks. After adjusting for sex, age, and BMI, adult subjects with the genotype Pro/Pro, Pro/Ala, and Ala/Ala, respectively, showed significant decreasing trends in fasting insulin (11.7, 10.3, and 8.8 μU/ml; P = 0.002) and HOMA-IR (2.4, 2.1, and 1.7; P = 0.006). Similar but nonsignificant trends were noted in childhood. A significant genotype-BMI interaction effect on insulin (P = 0.020), glucose (P = 0.007), and HOMA-IR (P = 0.001) was found in adulthood, with carriers versus noncarriers showing attenuated association with BMI. The genotype-BMI interaction effect on these variables tended to be similar in childhood. With respect to tracking over time, of individuals in the top age- and sex-specific quartile of HOMA-IR in childhood, 48.7% (38/78) of noncarriers vs. 16.7% (2/12) of the carriers (P = 0.035) remained in the same quartile in adulthood. A similar trend was observed for insulin (2/13 vs. 35/77, P = 0.037). In conclusion, the Pro12Ala polymorphism of the PPAR-γ2 gene beneficially influences insulin resistance and its tracking from childhood to adulthood. Further, the Ala12 allele attenuates the adverse association between adiposity and insulin resistance measures. *Diabetes* 52:1265–1269, 2003

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CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment of insulin resistance; PPAR, peroxisome proliferator–activated receptor.

Insulin resistance is an important risk factor for type 2 diabetes and cardiovascular disease (CVD) (1,2). The peroxisome proliferator–activated receptor (PPAR)-γ, which is a member of the nuclear hormone receptor superfamily of transcription factors and functions as a heterodimer with a retinoid X receptor, plays a pivotal role in the regulation of energy storage, adipocyte differentiation, insulin sensitivity, and lipoprotein metabolism (3,4). The PPAR-γ gene located in chromosome 3q25 produces two isoforms: PPAR-γ1 and PPAR-γ2 (5–7). Human PPAR-γ2, which is expressed almost exclusively in adipose tissue, has 28 additional amino acids at its NH_2-terminus, which makes its ligand-independent activation domain much more potent than that of PPAR-γ1 (6,8). Within this functional domain, a missense mutation that results in a substitution of proline by alanine in codon 12 (Pro12Ala) has been found (9) and associated with improved insulin sensitivity and decreased risk of type 2 diabetes in many (10–17) but not all studies (18–21). Further, studies have also shown that the beneficial effect of this variant on insulin resistance depends on obesity status (10,22). Most of these studies have been performed in middle-aged and elderly white and Japanese subjects.

Insulin resistance/hyperinsulinemia in youth is a primary antecedent abnormality for the risk of developing type 2 diabetes (23–25). Studies have shown that levels of CVD risk factors, including insulin, persist (track) from childhood to adulthood (26–29). Of note, genetic factors are considered to play a role in this regard (30–32). Whether Pro12Ala polymorphism in the PPAR-γ2 gene influences tracking of insulin resistance status from childhood to adulthood is not known. Therefore, information on measures of insulin resistance and their tracking may be helpful in diabetes risk assessment in youth. As part of the Bogalusa Heart Study, a long-term community-based study of CVD risk beginning in childhood (33), the present study examines *1*) the effect of Pro12Ala genotypes on measures of insulin resistance and their tracking from childhood to adulthood and *2*) the body fatness–genotype interaction effect on insulin resistance measures.

TABLE 1
Levels of fasting insulin, glucose, and HOMA-IR in childhood and adulthood by the Pro12Ala genotype of PPAR-γ2 in whites: the Bogalusa Heart Study

	Pro/Pro	Pro/Ala	Ala/Ala	P*
Childhood				
n	351	72	3	—
Insulin (μU/ml)	10.4 ± 7.2	9.0 ± 3.7	8.4 ± 1.4	0.277
Glucose (mg/dl)	81.2 ± 6.8	82.0 ± 5.3	82.7 ± 7.6	0.492
HOMA-IR	2.1 ± 1.5	1.8 ± 0.8	1.7 ± 0.2	0.356
Adulthood				
n	547	135	4	—
Insulin (μU/ml)	11.7 ± 7.9	10.3 ± 6.2	8.8 ± 2.9	0.002
Glucose (mg/dl)	79.3 ± 10.4	79.5 ± 8.4	80.0 ± 4.2	0.894
HOMA-IR	2.4 ± 1.9	2.1 ± 1.4	1.7 ± 0.6	0.006

Data are means ± SD unless otherwise indicated. *Adjusted for age, sex, and BMI.

RESEARCH DESIGN AND METHODS

Study population. Two cross-sectional surveys were conducted from 1988 to 1996 on young adults (n = 2,571), aged 20–38 years, residing in the biracial (65% white, 35% black) community of Bogalusa, Louisiana. Of those, 1,093 unrelated individuals (771 whites, 322 blacks) who had PPAR-γ2 Pro12Ala genotype data were available to estimate the allele frequency. Among nondiabetic whites with genotype data, 686 had fasting insulin and glucose values in adulthood; 426 had these values in childhood (age range 4–17 years), as determined from earlier surveys (1981–1988). These data were used to examine cross-sectional genotype-phenotype associations. Only individuals (n = 362) who had fasting insulin and glucose values both in childhood and in adulthood were used for tracking analysis. The average follow-up period for tracking was 13.4 years. There was no significant difference with respect to age, BMI, and the study variables in both childhood and adulthood among individuals who had genotype data and those who did not.

The participation rates ranged from >60% for the adult cohort to >80% for school-aged children. All subjects in this study gave informed consent approved by the Institutional Review Board of the Tulane University Health Sciences Center.

Examinations. All examinations followed the same protocols (34). Subjects were instructed to fast for 12–14 h before the screening, and compliance was determined by an interview on the screening day. Height and weight were measured twice to ±0.1 cm and ±0.1 kg, respectively, and the average values were used to calculate BMI (weight in kilograms divided by the square of height in meters) as a measure of body fatness.

Insulin and glucose analyses. A commercial radioimmunoassay kit was used for measuring plasma immunoreactive insulin (Padebas Pharmacia, Piscataway, NJ). This insulin assay has 41% cross-reactivity with proinsulin, which is disproportionately low in nondiabetic subjects, and <0.2% cross-reactivity with C-peptide. According to the manufacturer, the detection limit of insulin level was <2.0 μU/ml. Plasma glucose was measured by an enzymatic method using the Beckman Instant Glucose Analyzer (Beckman Instruments, Palo Alto, CA). On the basis of blind duplicate determination, intraclass correlation coefficients of reliability ranged from 0.94 to 0.98 for insulin and 0.86 to 0.98 for glucose.

Insulin resistance status was assessed according to the homeostasis model assessment of insulin resistance (HOMA-IR) formula described previously (35): fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.

Genotyping. Genotyping of the PPAR-γ2 Pro12Ala polymorphism was performed using the TaqMan assay (Applied Biosystems). An 89-bp product was amplified using 0.9 μmol/l each of the forward primer 5′-AAACCCCTATTC CATGCTGTTATG-3′ and the reverse primer 5′-GCAGACAGTGTATCAGT GAAGGAATC-3′, 50 ng DNA, 5.0 mmol/l $MgCl_2$, and 1× TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a 22-μl reaction volume. After an initial step of 2 min at 50°C and 10 min at 95°C to activate the AmpliTaq Gold, the products were amplified using 40 cycles of 15 s at 92°C and 1 min at 60°C. A total of 0.1 μmol/l of each of the sequence-specific probes 5′-6FAM-CTCCTATTGACCCAGAAA-MGB-3′ and 5′-VIC-TCCTATTGACGCAGAAA-MGB-3′ was used in the allele discrimination assay, and allele detection and genotype calling were performed using the ABI 7700 and the Sequence Detection System software (Applied Biosystems). Based on the analysis of 67 blind duplicate pairs, there was 98.5% concordance in Pro12Ala genotyping.

Statistical analyses. Statistical analyses were performed using SAS version 8.0. Gene counting was used to estimate allele frequencies within each race. Estimates of Hardy-Weinberg equilibrium were tested using the goodness-of-fit χ^2 test. Insulin and HOMA-IR levels were log-transformed in the analysis to improve normality. The general linear model was used to examine the effects of the Pro12Ala polymorphism on levels of insulin resistance measures (fasting insulin and glucose and HOMA-IR). The genotype-BMI interaction effect on insulin resistance measures was examined by comparing associations between BMI and insulin resistance measures in carriers versus noncarriers of the Ala12 allele. The significance of the interaction was tested using the general linear homogeneity of slopes model. The persistence (tracking) of high or low levels of insulin, glucose, and HOMA-IR from childhood to adulthood was evaluated by sex- and age-specific extreme quartiles. The differences in the proportions of tracking in the extreme quartiles between carriers and noncarriers were examined by the χ^2 test and logistic regression model. Fisher's exact test was applied where appropriate.

RESULTS

Among 771 whites, 618 (80.2%) displayed the Pro/Pro genotype, 146 (18.9%) the Pro/Ala genotype, and 7 (0.9%) the Ala/Ala genotype, with a frequency of 0.104 for the variant Ala12 allele. Among 322 blacks, there were 11 heterozygous and 0 homozygous subjects for the variant allele. The genotype distributions were in the Hardy-Weinberg equilibrium both in whites and blacks. Because of the very low frequency of the Ala12 allele in blacks (0.017), further analyses were restricted to whites only.

As shown in Table 1, after adjusting for age, sex, and BMI, significant decreasing trends in fasting insulin (P = 0.002) and HOMA-IR (P = 0.006) were noted with increasing gene dosage of the Ala12 allele in adulthood. However, these genotype-related trends were similar but not statistically significant in childhood.

A significant genotype-BMI interaction effect on the positive association of BMI with insulin (P = 0.020), glucose (P = 0.007), and HOMA-IR (P = 0.001) was found in adulthood, with carriers of the Ala12 allele showing an attenuated relationship with BMI. A similar but marginally significant interaction effect was observed in childhood for insulin (P = 0.091), HOMA-IR (P = 0.064), and glucose (P = 0.194) (data not shown). As an example, Fig. 1 showed this interaction effect with respect to HOMA-IR.

Tracking by the Pro12Ala genotype was examined in terms of persistence in ranking at the bottom or top age- and sex-specific quartiles of insulin, glucose, and HOMA-IR. As shown in Table 2, of the 12 carriers in the top quartile of HOMA-IR in childhood, only 16.7% (2/12) remained in the top quartile as adults, compared with 48.7% (38/78) of noncarriers (P = 0.035). A similar result was observed for insulin (2/13 vs. 35/77, P = 0.037). Glucose did not show such a trend. The influence of the genotype

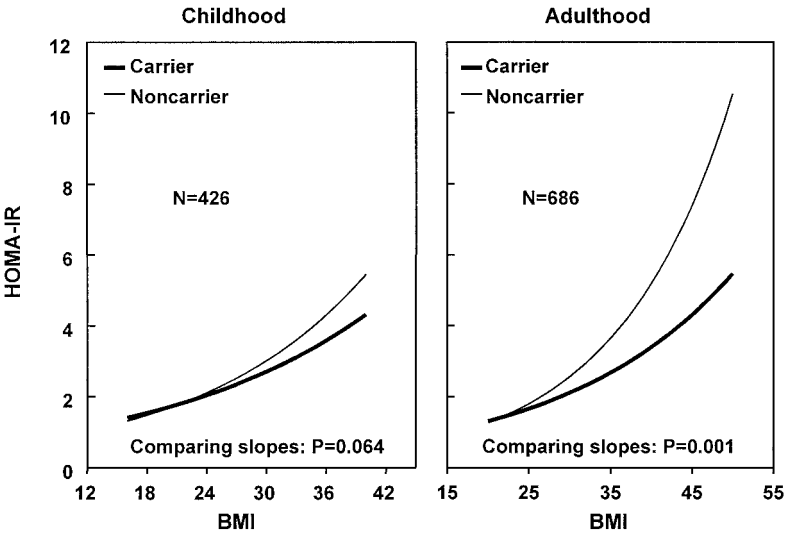

FIG. 1. Changes in HOMA-IR with BMI in white children and young adults by the Pro12Ala genotype of PPAR-γ2: the Bogalusa Heart Study.

on tracking of insulin or HOMA-IR in the top quartile was independent of baseline age, sex, and baseline BMI in a logistic regression model (data not shown). However, no such difference was observed for the three variables in the bottom quartile (data not shown).

DISCUSSION

The present study demonstrates the association of the Ala12 allele with lower levels of insulin and HOMA-IR in whites. In addition, the results show that the Ala12 allele attenuates both the adverse relationship between obesity and insulin resistance measures and the persistence (tracking) of high levels of insulin and HOMA-IR from childhood to adulthood. It is noteworthy that these observations are derived from an unselected community-based sample, representative of the population.

Several but not all studies have found that the Ala12 variant of PPAR-γ2 is associated with higher insulin sensitivity measures (10–15). In the present study, white young adult carriers of the variant had lower levels of fasting insulin and HOMA-IR. A significant decreasing trend in these two variables with increasing gene dosage of the Ala12 allele occurred in this group, thereby supporting the notion that the polymorphism may be a modulator of insulin resistance in the general population (13,17). Given that insulin resistance is a well-established risk factor for CVD and type 2 diabetes, to some extent, carriers of the variant Ala12 allele may have a reduced risk for developing these diseases.

The modulating effect of Pro12Ala polymorphism on insulin sensitivity is considered primarily through its influence on body fatness because adjusting for adiposity eliminated the association (13). However, as in previous studies (11,14), the present study found that the association between Pro12Ala polymorphism and measures of insulin resistance persisted after adjusting for BMI. In addition, BMI showed no significant difference between carriers and noncarriers of the Ala12 allele (data not shown). Moreover, the annual change of BMI or weight also showed no significant difference between the two groups in this study (data not shown), although weight gain is known to play a role in this regard (36).

The observed trends in genotype-phenotype associations in adulthood were similar, but not significant, in childhood. The reason for this is not clear, and no previous data on children are available for comparison. To speculate, a relatively lower range of BMI and HOMA-IR in childhood versus adulthood (Fig. 1) may partly account for the lack of association in childhood because the phenotypic effect of the polymorphism was stronger in obese subjects in this study. Further, since the study cohort in childhood spanned prepubertal and pubertal periods, hormonal effects could vary during these periods; small sample size is a limitation to explore this aspect in

TABLE 2
Tracking of HOMA-IR in the bottom and top quartiles from childhood to adulthood in carriers versus noncarriers of the Ala12 allele: the Bogalusa Heart Study

		Ranking in adulthood			
	n	Bottom quartile	2nd quartile	3rd quartile	Top quartile
Ranking in childhood					
Bottom quartile					
Carriers	14	4 (28.6)	6 (42.9)	3 (21.4)	1 (7.1)
Noncarriers	73	22 (30.1)	18 (24.7)	25 (34.2)	8 (11.0)
Top quartile					
Carriers	12	3 (25.0)	3 (25.0)	4 (33.3)	2 (16.7)*
Noncarriers	78	10 (12.8)	17 (21.8)	13 (16.7)	38 (48.7)*

Data in parentheses are %. *Carriers vs. noncarriers: $P = 0.035$.

the current study. Further studies are needed in this direction.

In the current study, the Pro12Ala polymorphism modulated the well-known adverse associations of body fatness with insulin resistance measures in that increases in fasting insulin, glucose, and HOMA-IR with increasing BMI were blunted significantly in young adults. Similar trends were also observed in childhood, although they were not statistically significant (Fig. 1). Whether differences in sample size and/or body fatness of children versus adults may account for this lack of significant interaction in the former is not clear. Earlier studies have found improved insulin sensitivity in obese carriers of the Ala12 variant (10,22). The mechanisms underlying the current findings are not clear. Because obesity is one of the most important risk factors for CVD and type 2 diabetes, obese subjects who are presumably at a higher risk may be protected by the relatively stronger phenotypic effect of the Ala12 allele on insulin resistance measures.

With respect to persistence of levels over time, the current study showed that carriers of the variant Ala12 allele tended to maintain lower rather than higher ranking of insulin resistance from childhood to adulthood. This result is consistent with the observed phenotypic effect of the variant Ala12 allele. Taken together, the current findings underscore the beneficial effects of the Ala12 allele on levels of insulin resistance measures, cross-sectionally and longitudinally. However, as a caveat, it should be noted that this study lacks an independent replication of the findings to overcome the weaknesses of genetic association studies (37).

In conclusion, the Ala12 allele beneficially influences insulin resistance status and its tracking from childhood to young adulthood in whites. Further, the Ala12 allele attenuates the adverse association between adiposity and insulin resistance measures in the same group. The Ala12 allele may be potentially useful as an informative genetic marker for susceptibility to type 2 diabetes and CVD.

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