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BRIEF COMMUNICATION

The Peroxisome Proliferator-Activated Receptor γ2 Pro12Ala Mutation Is Associated with Early Onset Extreme Obesity and Reduced Fasting Glucose

We screened the peroxisome proliferator activated receptor γ2 (PPARγ2) for sequence variants in 165 unrelated obese (BMI ≥ 30 kg/m^2) Caucasian women, and 49 normal weight Caucasian female controls (BMI < 27 kg/m^2). The allele frequency of the Pro12Ala mutation was higher in obese(18.18%) than in normal weight women (8.16%) ($\chi^2_{(1)} = 5.68$, $P = 0.017$). Among obese women, the Pro12Ala mutation lowered age of obesity onset (Pro/Pro, 13.2 ± 9.4 years; Pro/Ala+Ala/Ala 8.6 ± 7.1 years, $P = 0.005$), was associated with lower fasting glucose and was protective against type II diabetes.

Peroxisome proliferator activated receptor-γ (PPARγ) is a nuclear receptor that can form a heterodimer with the retinoid X receptor (RXR) and act as a transcription factor with many target genes (1). PPARγ has its highest expression level in adipocytes, and has been shown to play an important role in adipocyte differentiation (2–4).

Two PPARγ isoforms (PPARγ 1 and PPARγ 2) are identical except that PPARγ2 has 28 extra amino acids on the N-terminus (5). Since Yen *et al.* (6) found the PPARγ2 Pro12Ala mutation, several studies have examined possible associations between this mutation and obesity- and diabetes-related phenotypes, including body weight, plasma leptin levels, and insulin resistance (7–12). Of those, three studies reported associations with body mass index (BMI), but the results are conflicting. Deeb *et al.* (7) found that individuals homozygous for the mutation had lower BMI, while Beamer *et al.* (8) found the association to be in the opposite direction. Ek *et al.* (9) found that the homozygous (Pro12Ala) mutation carriers had higher BMI and higher weight gain than wild type carriers. Koch *et al.* (10) reported that the codon12 mutation was associated with increased insulin sensitivity. However, other studies reported negative results (11,12).

To evaluate the function of the PPARγ2 Pro12Ala mutation, we examined this sequence variant and its relation to clinical characteristics in obese (n = 165) and normal weight (n = 49) Caucasian women.

RESEARCH DESIGN AND METHODS

Subjects. One hundred sixty-five unrelated obese (BMI ≥ 30 kg/m^2) Caucasian women were selected from an ongoing study of the genetics of human obesity (13), including 147 extremely obese individuals with BMI ≥ 40 kg/m^2 and 18 moderatly obese individuals (30 kg/m^2 ≤ BMI < 40 kg/m^2). Briefly, all subjects had a family history of obesity, with at least one obese sibling (BMI ≥ 30 kg/m^2) and at least one parent and one sibling who were of normal weight. Forty-nine unrelated normal weight (BMI < 27 kg/m^2) Caucasian women individuals were selected from a study of individuals with and without psychiatric illness (14). All subjects gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania. Table 1 describes characteristics of our obese and normal weight subjects.

Phenotypes. Fasting blood glucose was measured by Quest Diagnostics (Horsham, PA). BMI was calculated by dividing weight (kg) by the square of height (meters). Only BMI was available for controls. Age of obesity onset and diabetes status were based on self-report.

Screening for sequence variants. DNA was extracted by the high-salt method (15), and 20 ng of

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TABLE 1
Characteristics of Obese and Normal Weight Groups[a]

	Obese	Normal weight
Number of subjects	165	49
Age (mean ± SD, years)	38.5 ± 7.5	32.2 ± 13.3
BMI (mean ± SD, kg/m^2)	49.6 ± 10.1	23.0 ± 2.4
Fasting glucose (mean ± SD, mg/dL)	101.4 ± 58.6	—
Ala/Ala	5	0
Pro/Ala	50	8
Pro/Pro	110	41

[a] Obese is defined as BMI ≥ 30 kg/m^2 and normal weight BMI < 27 kg/m^2.

DNA was used for PCR amplification. PCR primer sequences from Deeb *et al.* (7) were used. Primers were end-labeled with γ^{33}P-ATP, and PCR was performed according to conditions published elsewhere (16). PCR products were loaded on 6% polyacrylamide gel (Acr/Bis = 37.5:1), and electrophoresis was run at 4W for 6–7 h. Selected samples with shifted bands were reamplified and purified using QIAquick PCR Purification Kit (QIAGEN, Valencia,CA) and sequenced with an ABI 377 automatic DNA sequencer (Perkin–Elmer, Norwalk, CT). (Sequencing was completed by the Genetics Core Facility at the University of Pennsylvania.)

Statistical analysis. We compared the allele frequencies in obese and control individuals using a two-sided χ^2 test. Logistic regression was used to estimate the odds ratio for obesity risks based on having one or two copies of the mutation. An F test was used to determine the difference in age of obesity onset in carriers. A t test was used to test the difference in mean between the obese and normal weight groups for BMI and fasting glucose. For all analyses SPSS 6.1 was used.

RESULT AND DISCUSSION

Genotype frequencies are given at the bottom of Table 1. The obese cases are in Hardy–Weinberg equilibrium. Allele frequencies for the Pro12Ala mutation were 18.18% (60/330) in the 165 obese individuals, compared with 8.16% (8/98) in the 49 normal weight controls ($\chi^2_{(1)} = 5.68$, $P = 0.017$). After adjusting for age, obese individuals were 1.7 times (95% CI 1.1–2.6) more likely to be have one or more copies of the mutation.

Previously, two groups reported conflicting relations of PPARγ2 Pro12Ala mutations with BMI. Deeb *et al.* (7) reported that the Pro12Ala mutation in exon B of PPARγ2 gene was associated with *lower* BMI and improved insulin sensitivity in Finnish and Japanese Americans. On the other hand, for a sample with extreme obesity (mean BMI = 36.5), Beamer *et al.* (8) observed a *higher* mean BMI in individuals with the Pro12Ala mutation. However, for the lean-to-moderately-obese individuals (mean BMI = 26.5), they did not observe a significant difference in mean BMI with respect to the mutation status. Recently, several other studies showed contradictory effects of the Pro12Ala mutation on BMI and type II diabetes status (17–20). Our results are in agreement with the findings by Beamer *et al.* (8), and it is plausible that these differences in findings can be attributed to the selection of study populations.

We observed that the Pro12Ala mutation was associated with earlier age of obesity onset. Mean age at onset declined from 13.2 ± 9.4 years for obese individuals with homozygous wild type sequence (Pro/Pro, $n = 93$) to 8.6 ± 7.1 years for those with the Pro12Ala mutation (Pro/Ala + Ala/Ala, $n = 43$), ($F = 8.14$, $P = 0.005$). These results suggest the PPARγ2 Pro12Ala mutation may lead to obesity at a younger age, perhaps by facilitating adipocyte differentiation.

We further observed that the Pro12Ala mutation was protective against type II diabetes but not type I diabetes. Within the obese group, the Pro12Ala mutation was associated with lower fasting blood glucose level. Obese women having at least one copy of the mutant sequence (Pro/Ala or Ala/Ala) had lower mean fasting glucose (83.6 ± 25.0 mg/dL, $n = 45$) compared with obese women without the mutation (110.2 ± 67.7 mg/dL, $n = 92$, $P = 0.02$). Diabetes status was reported by 157 and 155 obese individuals, respectively, for type I and type II diabetes. Two of 51 obese women with the Pro12Ala mutation had type II diabetes, but 15 of 104 wild type individuals had type II diabetes ($\chi^2 = 3.86$, $P = 0.049$). However, this protective effect was not observed with type I diabetes (2/48 vs 3/104, in mutation and in wild type individuals, respectively. $\chi^2 = 0.17$, $P = 0.680$). This pattern suggests that the mutation may increase insulin sensitivity and thereby inhibit the development of type II diabetes.

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REFERENCES

1. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* **358:**771–774, 1992.
2. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid- activated transcription factor [published erratum appears in Cell 1995 Mar 24;80(6):following 957]. *Cell* **79:**1147–1156, 1994.
3. Hu E, Tontonoz P, Spiegelman BM. Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. *Proc Natl Acad Sci U S A* **92:** 9856–9860, 1995.
4. Wu Z, Xie Y, Bucher NL, Farmer SR. Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. *Genes Dev* **9:**2350–2363, 1995.
5. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* **272:**18779–18789, 1997.
6. 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.
7. 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.
8. 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-gamma2 gene with obesity in two Caucasian populations. *Diabetes* **47:**1806–1808, 1998.
9. Ek J, Urhammer SA, Sorensen TI, Andersen T, Auwerx J, Pedersen O. Homozygosity of the Pro12Ala variant of the peroxisome proliferation- activated receptor-gamma2 (PPAR-gamma2): Divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* **42:**892–895, 1999.
10. Koch M, Rett K, Maerker E, Volk A, Haist K, Deninger M, Renn W, Haring HU. The PPARγ2 amino acid polymorphism Pro12Ala is prevalent in offspring of Type II diadetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* **42:**758–762, 1999.
11. Mori Y, Kim-Motoyama H, Katakura T, Yasuda K, Kadowaki H, Beamer BA, Shuldiner AR, Akanuma Y, Yazaki Y, Kadowaki T. Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity,fat distribution,and insulin sensitivity in Japanese men. *Biochem Biophys Res Commun* **251:**195–198, 1998.
12. 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.
13. Price RA, Reed DR, Lee JH. Obesity related phenotypes in families selected for extreme obesity and leanness. *Int J Obes Relat Metab Disord* **22:**406–413, 1998.
14. Li WD, Reed DR, Lee JH, Xu W, Kilker R, Sodam B, Price RA. Sequence variants in the promotor and 5′ untraslated region of the leptin gene are associated with obesity in women. *Ann Hum Genet* **63:**227–234, 1999.
15. Lahiri DK, Nurnberger JI, Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* **19:**5444, 1991.
16. Lee JH, Reed DR, Li WD, Xu W, Joo EJ, Kilker RL, Nanthakumar E, North M, Sakul H, Bell C, Price RA. Genome scan for human obesity and linkage to markers in 20q13. *Am J Hum Genet* **64:**196–209, 1999.
17. 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.
18. Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. *Biochem Biophys Res Commun* **268:**178–182, 2000.
19. 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.
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.

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