Western University
Scholarship@Western

Department of Medicine Publications | Medicine Department

9-1-2004

Genetic variation in PPARG encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis

Khalid Z. Al-Shali
Robarts Research Institute

Andrew A. House
Western University

Anthony J.G. Hanley
University of Toronto

Hafiz M.R. Khan
Robarts Research Institute

Stewart B. Harris
Western University

See next page for additional authors

Follow this and additional works at: https://ir.lib.uwo.ca/medpub

Citation of this paper:
Al-Shali, Khalid Z.; House, Andrew A.; Hanley, Anthony J.G.; Khan, Hafiz M.R.; Harris, Stewart B.; Zinman, Bernard; Mamakeesick, Mary; Fenster, Aaron; Spence, J. David; and Hegele, Robert A., "Genetic variation in PPARG encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis" (2004). *Department of Medicine Publications*. 289.
https://ir.lib.uwo.ca/medpub/289

Authors

Khalid Z. Al-Shali, Andrew A. House, Anthony J.G. Hanley, Hafiz M.R. Khan, Stewart B. Harris, Bernard Zinman, Mary Mamakeesick, Aaron Fenster, J. David Spence, and Robert A. Hegele

This article is available at Scholarship@Western: https://ir.lib.uwo.ca/medpub/289

Genetic Variation in *PPARG* Encoding Peroxisome Proliferator-Activated Receptor γ Associated With Carotid Atherosclerosis

Khalid Z. Al-Shali, MBBS; Andrew A. House, MD; Anthony J.G. Hanley, PhD; Hafiz M.R. Khan, PhD; Stewart B. Harris, MD; Bernard Zinman, MD; Mary Mamakeesick, RPN; Aaron Fenster, PhD; J. David Spence, MD; Robert A. Hegele, MD

Background and Purpose—Peroxisome proliferator-activated receptor γ is a crucial molecule in atherogenesis because it is associated with metabolic risk factors such as obesity and diabetes and also plays a key role in subcellular metabolism of arterial wall macrophage foam cells. Genetic variation in *PPARG* has been associated with metabolic and cardiovascular end points.

Methods—We investigated the relationship between 2 common *PPARG* polymorphisms, namely P12A and c.1431C>T, and carotid atherosclerosis in a sample of 161 Canadian aboriginal people. Dependent variables were carotid intima media thickness (IMT), assessed using B-mode ultrasonography, and total carotid plaque volume (TPV), assessed using 3D ultrasound.

Results—Using multivariate analysis, we found that subjects with ≥1 *PPARG* A12 allele had less carotid IMT than others (0.72 ± 0.03 versus 0.80 ± 0.02 mm; $P=0.0045$), with no between-genotype difference in TPV. In contrast, subjects with the *PPARG* c.1431T allele had greater TPV than others (124 ± 18.4 versus 65.1 ± 23.7 mm^3; $P=0.0079$), with no between-genotype difference in IMT.

Conclusions—The findings show an association between *PPARG* genotypes and carotid arterial phenotypes, and further reflect the prevailing view that the *PPARG* A12 allele protects against deleterious phenotypes. Also, whereas IMT and TPV are somewhat correlated with each other, they might also represent distinct traits with discrete determinants representing different stages of atherogenesis. **(*Stroke*. 2004;35:2036-2040.)**

Key Words: atherosclerosis ■ carotid arteries ■ polymorphism ■ ultrasonography

Downloaded from http://ahajournals.org by on March 15, 2023

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors.[1] It is an important regulator of adipogenesis; by forming a heterodimer with retinoid X receptor, it triggers the adipocyte differentiation program by binding to specific transcription elements in various metabolic genes.[2,3] Furthermore, the pharmacological PPARγ agonist thiazolidinedione (TZD) drugs appear to be antiatherogenic at multiple levels, including generalized improvement of metabolism, and beneficial effects on vascular wall components, such as macrophages.[4–6] For instance, TZD activation of PPARγ induces cholesterol efflux from macrophages by inducing ATP-binding cassette protein A1.[7] For these reasons, association analysis has been performed using common single nucleotide polymorphisms (SNPs) in *PPARG*, such as the Pro12Ala (P12A; MIM 601487.0002) SNP in the adipocyte-specific PPARγ2 isoform,[8–11] and the silent exon 6 c.1431C>T (codon 478 CAC [His]→CAT[His]; MIM 601487.0009) SNP.[12–14] A meta-analysis of 8 case–control studies and 2 family-based studies found that the *PPARG* A12 allele was associated with significantly reduced risk of type 2 diabetes.[9] The *PPARG* A12 allele was also associated with significantly reduced risk of myocardial infarction.[11] In contrast, the *PPARG* c.1431T allele has been less consistently associated with traits such as obesity[12] and coronary heart disease (CHD),[13,14] although associations of vascular metabolic traits with this allele have tended to be unfavorable.

Because of these associations involving *PPARG* alleles A12 and c.1431T, we investigated their association with atherosclerosis in 161 Canadian aboriginal subjects, an isolated founder population.

Methods

Study Sample

The Sandy Lake community is located at the 55th parallel of latitude in the subarctic boreal forest of central Canada. Residents are self-defined as Oji-Cree on the basis of their language, which is derived from Ojibway and Cree. Baseline demographic, clinical, and biochemical attributes from an ongoing study of diabetes risk and

Received April 30, 2004; final revision received June 3, 2004; accepted June 28, 2004.

From the Robarts Research Institute (K.Z.A.-S., H.M.R.K., A.F., J.D.S., R.A.H.), London, Ontario, Canada; the Department of Medicine (A.A.H.), University of Western Ontario, London, Ontario, Canada; the Department of Medicine and Samuel Lunenfeld Research Institute (A.J.G.H., B.Z.), Mount Sinai Hospital and University of Toronto, Ontario, Canada; the Thames Valley Family Practice Research Unit (S.B.H.), University of Western Ontario, London, Ontario, Canada; and the Sandy Lake Health and Diabetes Project (M.M.), Ontario, Canada.

Correspondence to Dr Robert A. Hegele, Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, 406-100 Perth Dr, London, Ontario, Canada N6A 5K8. E-mail hegele@robarts.ca

© 2004 American Heart Association, Inc.

Stroke **is available at http://www.strokeaha.org** **DOI: 10.1161/01.STR.0000138784.68159.a5**

complications have been described.[15–17] Briefly, all community members aged ≥10 years were studied with medical history and physical examination. The participation rate was 72%. Fasting blood samples were sent to Toronto for analysis. In 2001, 278 adult community members free of coronary heart disease had ultrasound (US) assessment of the carotid arteries. Of these, 161 had baseline demographic data and sufficient DNA for *PPARG* genotyping, and this subset was demographically representative of the overall sample (data not shown). All subjects provided informed consent, and the study received approval from the Sandy Lake Band Council and the institutional review boards of the University of Toronto and the University of Western Ontario.

DNA Analysis

We used published methods to genotype the *PPARG* P12A and c.1431C>T SNPs.[8–14] Briefly, for the P12A SNP genotype, we amplified exon B using primers 5′-ACT CTG GGA GAT TCT CCT ATT GGC and 5′-CTG GAA GAC AAA CTA CAA GAG, with the underlined mismatched base introducing an *Hae*III recognition site, allowing for restriction digestion and size polymorphism to distinguish between alleles. Samples were amplified for 28 cycles, each of which consisted of denaturing at 94°C for 20 s, annealing at 56°C for 20 s, and extension at 72°C for 20 s. After *Hae*III (New England Biolabs), digestion of the resulting 155-bp fragment, the P12 allele yielded 2 fragments with sizes 132 and 23 bp, although the A12 allele yielded only a single 155-bp fragment.

For the *PPARG* c.1431C>T SNP genotype, we amplified *PPARG* exon 6 using primers 5′-CTG AAT GTG AAG CCC ATT GAA and 5′-GTG GCT CAG GAC TCT CTG CTA G. Samples were amplified for 30 cycles, each of which consisted of denaturing at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. After *Pml* I (New England Biolabs) digestion of the resulting 251-bp fragment, the c.1431C allele yielded 2 fragments with sizes 145 and 106 bp, although the c.1431T allele yielded only a single 251-bp fragment.

General US Logistics

US images were obtained using an HDI-5000 US machine and an L12–5 transducer (both from Advanced Technology Laboratories) that had been flown to the Sandy Lake community and housed within the diabetes research center. A single certified operator used the same instrument during a 4-week period to obtain carotid US images suitable for determination of intima media thickness (IMT) and total carotid plaque volume (TPV) from each participant.

IMT Measurement

A single observer, blinded to subjects' vascular risk, measured combined thickness of intima and media of the far wall of both common carotid arteries. The anterolateral longitudinal far walls of the common carotid arteries were recorded with the head 45° in the contralateral direction and the probe between 30° and 45° to the horizontal. The sonographer used the minimum gain necessary to clearly visualize lumen-intima and media-adventitia echoes, which were of best quality when the image plane was parallel to the carotid artery axis. These views were played back using an image processing board and a specialized recorder with digital memory permitting digitization of a full video frame in still mode. Still images were analyzed using computerized edge-detection software (Prowin).[18] Using a step-wise algorithm, conditional sets of "edges" (consisting of lumen-intima and media-adventitia echoes) were located within the image and then tested for "edge strength." Weak edge points were deleted, thus minimizing identification of spurious edge points resulting from image noise. Once all acceptable edge points were identified, boundary gaps were filled by linear interpolation. The distance between lumen-intima and media-adventitia boundaries was then measured to calculate IMT. Mean IMT was computed from 80 to 120 measurements over a 10-mm span ending 5 mm proximal to the transition between the common carotid and bulb regions. Intraoperator and interoperator coefficients of variation of 3.0% and 3.1%, respectively, and intraoperator and interoperator intraclass correlations were both 0.97.

TPV Measurement

Two-dimensional US images of the carotid arteries were obtained from each of the 278 Oji-Cree subjects enrolled in the study. The resulting 2D images that were parallel to each other within a known regular spatial interval and constant transducer angle for each subject were reconstructed immediately into a 3D volume to verify scan quality.[19,20] Three-dimensional US images were acquired with a mechanical linear scanning system and analyzed with L3Di visualization software (Life Imaging Systems Inc). Each 3D image was displayed using multiplanar texture mapping, allowing plaques to be viewed in various orientations. Plaque volumes were measured using manual planimetry: each 3D image was "sliced" transversely at an interslice distance of 1 mm, moving from 1 plaque edge to the other. Plaques were identified on the basis of visible changes in morphology in which local thickening of the intimal layer exceeded 1.0 mm. Plaque boundaries were traced using a mouse-driven cross-haired cursor. Slice areas were summed and multiplied by interslice distance to calculate plaque volume. TPV was the sum of all plaque volumes between the clavicle and angle of the jaw for both carotids. Intraobserver and interobserver reliability were 0.94 (n=40) and 0.93 (n=40), respectively.

Statistical Analysis

SAS version 6.12 (SAS Institute) was used to evaluate the association between IMT and plaque volume and the *PPARG* P12A and c.1431C>T SNPs. Distributions of IMT and TPV measurements were significantly non-normally distributed in this data set. Therefore, for parametric statistical analyses, IMT and TPV variables were transformed and subjected to analysis of normality. After transformation, 1/IMT and the square root of TPV were normally distributed. The transformed IMT and TPV were used for parametric statistical analyses, but the untransformed values are presented in the tables. ANOVA, which was performed using the general linear models procedure, was used to determine the sources of variation, with *F* tests computed from the type III sums of squares. This form of sums of squares is applicable to unbalanced study designs and reports the effect of an independent variable after adjusting for all other variables included in the model.[16–18] The dependent variables were transformed mean IMT and TPV from both carotid artery systems in each subject. Independent variables were *PPARG* P12A genotype, *PPARG* c.1431C>T genotype, age, sex, body mass index (BMI), diabetes status, total cholesterol, current smoking, and hypertension. The general linear model procedure for least-squares means was used to determine the level of significance in pairwise plaque volume comparisons as well as pairwise IMT and TPV comparisons between genotype classes. Least-squares means, also known as population marginal means, are the values for class means after adjustment for all covariates in the model. Deviation of genotype frequencies from those predicted by the Hardy–Weinberg law was tested by χ^2 analysis. Linkage disequilibrium between *PPARG* genotypes was estimated using a modification of the method of Hill and Robertson as described.[21]

Results

Baseline Demographic Features

Clinical attributes of the 161 subjects overall and according to sex are shown in Table 1. None of the discrete or quantitative traits were significantly different between males and females, although there was a trend toward a higher proportion of females affected with diabetes or impaired glucose tolerance ($P=0.063$). The simple Pearson correlation coefficient between untransformed carotid artery quantitative traits was 0.474 ($P<0.0001$). This increased somewhat to 0.644 ($P<0.0001$) when transformed values (ie, 1/IMT and square root of TPV) were used. However, the correlations between

Downloaded from http://ahajournals.org by on March 15, 2023

TABLE 1. Baseline Demographics of 161 Oji-Cree Study Subjects

	All Subjects	Males	Females
Discrete traits			
No.	161	66	95
Female	59.1%		
Diabetes or IGT	51.6%	39.4%	60.0%
Treated hypertension	31.1%	37.9%	26.3%
Current smoking	14.5%	12.1%	16.1%
Quantitative traits (mean±SD)			
Age (y)	38.2±15.3	37.6±15.6	38.7±15.1
BMI (weight/height; kg/m^2)	29.1±5.0	27.9±4.5	29.9±5.1
Total cholesterol (mmol/L)	4.76±0.91	4.83±0.96	4.71±0.88
Carotid IMT (mm)	0.78±0.18	0.80±0.19	0.76±0.17
Carotid TPV (mm^3)	101±195	124±235	84.5±160

IGT indicates impaired glucose tolerance.

Diabetes refers to physician-diagnosed diabetes and current treatment with insulin or an oral hypoglycemic agent (verified by nursing station chart review), or to physician-diagnosed diabetes or a fasting blood sugar of >11.1 mmol/L. IGT was diagnosed according to 2-hour postload plasma glucose between 7.8 and 11.1 mmol/L. Hypertension refers to documented hypertension on treatment or systolic blood pressure >140 (average of 2 measurements) or diastolic blood pressure >90 mm Hg. Each measurement was performed twice, and the average of the 2 was used in the analysis.

None of the between-gender comparisons was statistically significant.

US measurements, although highly significant, were only moderate in degree ($r<0.7$).

Allele and Genotype Frequencies

Genotype and allele frequencies for *PPARG* P12A and c.1431C>T SNPs are shown in Table 2. There was no significant deviation of the frequencies of both *PPARG* genotypes from those predicted by the Hardy–Weinberg law. The correlation coefficient *r* of nonrandom allelic association on the basis of Hill and Robertson's linkage disequilibrium constant was 0.34 ($\chi^2=133$; $P<0.0001$). Thus, there was significant but modest and incomplete linkage disequilibrium between these 2 SNP markers. We constructed haplotypes using phase information derived from inheritance but found no significant associations with the haplotypes (data not shown). Therefore, the 2 SNPs were treated as separate variables in subsequent analyses.

Determinants of Carotid IMT and TPV

ANOVA in Table 3 showed that transformed IMT was significantly associated only with age and with *PPARG* P12A genotype in this sample, each with a nominal $P<0.05$. Sex and hypertension tended to be associated with IMT. ANOVA in Table 3 also showed that transformed TPV was significantly associated with age, BMI, the presence of diabetes, and *PPARG* c.1431C>T genotype in this sample, each with a nominal $P<0.05$.

TABLE 2. *PPARG* Genotype and Allele Frequencies

Genotype	Frequency	Allele Frequencies
P12/P12	0.826 (133/161)	P12: 0.913
A12/P12	0.174 (28/161)	A12: 0.087
c.1431C/C	0.465 (75/161)	c.1431C: 0.711
c.1431T/C	0.491 (79/161)	c.1431T: 0.289
c.1431T/T	0.043 (7/161)	

TABLE 3. Determinants of Carotid Ultrasound Traits in Oji-Cree (ANOVA)

Source of Variation	Degrees of Freedom	*F*	*P*>*F*
Dependent variable: inverse of mean carotid intima media thickness			
Age	1	76.0	<0.0001
Sex	1	2.58	NS (0.11)
BMI	1	0.33	NS (0.57)
Diabetes	1	0.87	NS (0.35)
Total cholesterol	1	1.29	NS (0.26)
Current smoking	1	0.88	NS (0.35)
Treated hypertension	1	3.29	NS (0.07)
PPARG P12A genotype	1	4.57	0.034
PPARG c.1431C>T genotype	1	0.14	NS
Dependent variable: square root of total carotid plaque volume			
Age	1	95.9	<0.0001
Sex	1	0.83	NS (0.36)
BMI	1	3.81	0.053
Diabetes	1	11.1	0.0011
Total cholesterol	1	0.14	NS (0.71)
Current smoking	1	1.43	NS (0.23)
Treated hypertension	1	0.00	NS (0.95)
PPARG P12A genotype	1	0.08	NS (0.78)
PPARG c.1431C>T genotype	1	7.40	0.0073

P>*F*, indicates *P* value of a greater between-group *F* value from ANOVA using type III sums of squares.

The significant associations detected using ANOVA were evaluated by comparison of least-squares means for genotype classes (Table 4). Subjects heterozygous for the A12 allele had significantly less carotid IMT than homozygotes for the P12 allele (0.72±0.03 versus 0.80±0.02 mm; $P=0.0045$),

TABLE 4. Carotid Ultrasound Traits Shown According to *PPARG* Genotype in Oji-Cree

	PPARG Genotype		
	P12/P12	A12/P12	*P*
No.	133	28	
IMT (mm)	0.80±0.02	0.72±0.03	0.0045
TPV (mm^3)	104±13.7	86.7±30.4	NS (0.82)
	c.1431C/C	c.1431C/T and T/T	
No.	75	86	
IMT (mm)	0.75±0.02	0.76±0.02	NS (0.71)
TPV (mm^3)	65.1±23.7	124±18.4	0.0079

Untransformed least-squares group means±SEs are shown. *P* value of nonrandom difference between least-square group means in pairwise comparisons using the general linear models procedure. *P* values were calculated for normalized dependent variables.

Downloaded from http://ahajournals.org by on March 15, 2023

but there was no association between this genotype and carotid TPV. In contrast, subjects heterozygous or homozygous for c.1431T allele (ie, dominant model for c.1431T) had significantly more carotid TPV than homozygotes for the c.1431C allele (124 ± 18.4 versus 65.1 ± 23.7 mm^3; $P=0.0079$), but there was no association between this genotype and carotid IMT.

Discussion

In Canadian Oji-Cree, we report: (1) cardiovascular risk factors and noninvasive measurements of carotid arterial structural changes, namely IMT and TPV; (2) moderate correlation between IMT and TPV determinations; (3) allele frequencies of *PPARG* P12A and c.1431C>T genotypes, and modest pairwise linkage disequilibrium between them; (4) an association of IMT with age and *PPARG* P12A genotype, with A12 heterozygotes having lower mean IMT; and (5) an association of TPV with age, BMI, diabetes, and *PPARG* c.1431T, with c.1431T carriers having significantly higher TPV than homozygotes for c.1431C. Thus, *PPARG* variation is associated with carotid artery structural changes measured noninvasively. The association of reduced IMT with the A12 allele may be related to improved profile of intermediate metabolites attributed to this allele,[8] although the association of increased TPV with the silent, nonfunctional c.1431T allele is probably the result of linkage disequilibrium with another DNA change.

Although the genetic association findings will require replication in other study samples, our findings are consistent with some previous observations. For instance, we showed that the A12 allele was associated with a lower mean IMT. A recent study in 154 Japanese subjects with type 2 diabetes found a similar relationship between carotid IMT and the A12 allele.[22] Furthermore, the A12 allele was associated with 25% reduction in myocardial infarction risk in a prospective study.[11] The A12 allele also seems to be beneficial with respect to metabolic traits; a meta-analysis showed A12 to be associated with decreased diabetes risk.[9] Another study showed that A12 was associated with improved insulin sensitivity.[10] In our relatively small study sample, *PPARG* A12 was not associated with either type 2 diabetes or obesity measures (data not shown). Together, association studies support the idea that *PPARG* A12, which has in vitro functional consequences,[23] is associated with less severe vascular or metabolic phenotypes.

We also found that the *PPARG* c.1431T allele was associated with increased TPV after adjustments for age, BMI, sex, diabetes status, total cholesterol, current smoking, hypertension, and *PPARG* codon 12 genotype. However, associations between this SNP and vascular phenotypes have been somewhat inconsistent. The c.1431T allele has been associated previously with reduced[13] and unchanged[14] risk of CHD. Furthermore, the c.1431T allele has been associated previously with increased[24,25] and decreased[26] obesity indices. These inconsistencies may be attributable to the fact that this SNP is silent at the amino acid level and is unlikely to have any direct mechanistic link with specific phenotypes. Instead, this silent SNP might be in linkage disequilibrium with an unmeasured functional variation either within or flanking the *PPARG* gene or in another gene at this locus. Because there was only modest linkage disequilibrium between the *PPARG* P12A and c.1431C>T SNPs in this sample, and because there was no independent association of P12A with TPV, it is unlikely that the P12A SNP explains the association of c.1431T with TPV in this sample.

Although both US traits were significantly associated with age, IMT was significantly associated only with *PPARG* P12A genotype, although TPV was significantly associated with BMI, diabetes, and *PPARG* c.1431C>T genotype. Furthermore, the simple correlation between IMT and TPV, although statistically significant, was moderate. Thus, different US-derived measures of carotid artery morphology, although somewhat correlated, probably represent distinct intermediate traits with unique determinants and relationships with risk factors. The use of any particular US trait as a surrogate marker for "atherosclerosis" might lead to different conclusions regarding the role of specific risk factors in a particular patient sample. Recently, we showed that total carotid plaque area and percent carotid stenosis measured by US were moderately well correlated and had different associations with specific risk factors.[27] In the same way, IMT and TPV in this study likely reflected different attributes of atherosclerosis.

The results of this small study indicate that although genetic variation in *PPARG* is associated with atherosclerosis, specific relationships with IMT and TPV differ somewhat. In addition to small sample size, the limitations include the relatively young age of the sample, the generalizability of the study, and the fact that the internal carotid arteries and bifurcations were not assessed. *PPARG* is emerging as a focal determinant of metabolic and vascular pathways that determine atherosclerosis risk. If replicated, associations with *PPARG* could be used in risk prediction algorithms. Also, IMT and TPV may be different stages along a continuum that might reflect different attributes of the atherosclerotic process, and therefore, their use as surrogates for atherosclerosis might lead to different conclusions in a particular sample. Future work in individual study samples, with careful and extensive collection of intermediate phenotypes and genetic markers, may help clarify whether these traits actually reflect different aspects of atherosclerosis.

Acknowledgments

This work was supported by grants from the Heart and Stroke Foundation of Ontario, the Canadian Institutes for Health Research (CIHR; FRN 44087), the Canadian Genetic Diseases Network, and the Blackburn Group. The authors acknowledge the expert assistance of Maria DiCicco, Janine Boere, Edith Fiddler, and Ken Goodwin. R.A.H. and A.F. hold Canada Research Chairs (tier I). R.A.H. is a Career Investigator of the Heart and Stroke Foundation of Ontario. A.J.G.H. was supported through a CIHR Postdoctoral fellowship. S.B.H. is a Career Scientist of the Ontario Ministry of Health.

References

1. Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol*. 1997;8:159–166.
2. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell*. 1994;79:1147–1156.

Downloaded from http://ahajournals.org by on March 15, 2023

3. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med*. 2004;10:355–361.
4. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2000;106:523–531.
5. Collins AR, Meehan WP, Kintscher U, Jackson S, Wakino S, Noh G, Palinski W, Hsueh WA, Law RE. Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and nondiabetic low density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001;21: 365–371.
6. Chen Z, Ishibashi S, Perrey S, Osuga Ji, Gotoda T, Kitamine T, Tamura Y, Okazaki H, Yahagi N, Iizuka Y, Shionoiri F, Ohashi K, Harada K, Shimano H, Nagai R, Yamada N. Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression and HDL. *Arterioscler Thromb Vasc Biol*. 2001;21:372–377.
7. Argmann CA, Sawyez CG, McNeil CJ, Hegele RA, Huff MW. Activation of peroxisome proliferator-activated receptor gamma and retinoid X receptor results in net depletion of cellular cholesteryl esters in macrophages exposed to oxidized lipoproteins. *Arterioscler Thromb Vasc Biol*. 2003;23:475–482.
8. Stumvoll M, Haring H. The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. *Diabetes*. 2002;51:2341–2347.
9. 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*. 2000;26:76–80.
10. Hara M, Alcoser SY, Qaadir A, Beiswenger KK, Cox NJ, Ehrmann DA. Insulin resistance is attenuated in women with polycystic ovary syndrome with the Pro(12)Ala polymorphism in the PPARgamma gene. *J Clin Endocrinol Metab*. 2002;87:772–775.
11. Ridker PM, Cook NR, Cheng S, Erlich HA, Lindpaintner K, Plutzky J, Zee RY. Alanine for proline substitution in the peroxisome proliferator-activated receptor gamma-2 (PPARG2) gene and the risk of incident myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2003;23:859–863.
12. Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Lebel P, Dallongeville J, Deeb S, Auwerx J, Amouyel P. A genetic polymorphism of the peroxisome proliferator-activated receptor gamma gene influences plasma leptin levels in obese humans. *Hum Mol Genet*. 1998;7:435–440.
13. Wang XL, Oosterhof J, Duarte N. Peroxisome proliferator-activated receptor gamma C161→T polymorphism and coronary artery disease. *Cardiovasc Res*. 1999;44:588–594.
14. Bluher M, Klemm T, Gerike T, Krankenberg H, Schuler G, Paschke R. Lack of association between peroxisome proliferator-activated receptor-gamma-2 gene variants and the occurrence of coronary heart disease in patients with diabetes mellitus. *Eur J Endocrinol*. 2002;146:545–551.
15. Harris SB, Gittelsohn J, Hanley A, Barnie A, Wolever TM, Gao J, Logan A, Zinman B. The prevalence of NIDDM and associated risk factors in native Canadians. *Diabetes Care*. 1997;20:185–187.
16. Triggs-Raine BL, Kirkpatrick RD, Kelly SL, Norquay LD, Cattini PA, Yamagata K, Hanley AJ, Zinman B, Harris SB, Barrett PH, Hegele RA. HNF-1alpha G319S, a transactivation-deficient mutant, is associated with altered dynamics of diabetes onset in an Oji-Cree community. *Proc Natl Acad Sci U S A*. 2002;99:4614–4619.
17. Mok A, Cao H, Zinman B, Hanley AJ, Harris SB, Kennedy BP, Hegele RA. A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. *J Clin Endocrinol Metab*. 2002;87:724–727.
18. Selzer RH, Hodis HN, Kwong-Fu H, Mack WJ, Lee PL, Liu CR, Liu CH. Evaluation of computerized edge tracking for quantifying intima-media thickness of the common carotid artery from B-mode ultrasound images. *Atherosclerosis*. 1994;111:1–11.
19. Landry A, Spence JD, Fenster A. Measurement of carotid plaque volume by 3-dimensional ultrasound. *Stroke*. 2004;35:864–869.
20. Landry A, Fenster A. Theoretical and experimental quantification of carotid plaque volume measurements made by three-dimensional ultrasound using test phantoms. *Med Phys*. 2002;29:2319–2327.
21. Hegele RA, Plaetke R, Lalouel JM. Linkage disequilibrium between DNA markers at the low-density lipoprotein receptor gene. *Genet Epidemiol*. 1990;7:69–81.
22. Iwata E, Yamamoto I, Motomura T, Tsubakimori S, Nohnen S, Ohmoto M, Igarashi T, Azuma J. The association of Pro12Ala polymorphism in PPARgamma2 with lower carotid artery IMT in Japanese. *Diabetes Res Clin Pract*. 2003;62:55–59.
23. 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*. 1998;20:284–287.
24. 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*. 1999; 84:3708–3712.
25. Doney A, Fischer B, Frew D, Cumming A, Flavell DM, World M, Montgomery HE, Boyle D, Morris A, Palmer CN. Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight. *BMC Genet*. 2002;3:21.
26. Knoblauch H, Busjahn A, Muller-Myhsok B, Faulhaber HD, Schuster H, Uhlmann R, Luft FC. Peroxisome proliferator-activated receptor gamma gene locus is related to body mass index and lipid values in healthy nonobese subjects. *Arterioscler Thromb Vasc Biol*. 1999;19:2940–2944.
27. Spence JD, Hegele RA. Noninvasive phenotypes of atherosclerosis: similar windows but different views. *Stroke*. 2004;35:649–653.

Downloaded from http://ahajournals.org by on March 15, 2023