

Alcohol intake modulates the genetic association between HDL cholesterol and the PPAR γ 2 Pro12Ala polymorphism

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Abstract The peroxisome proliferator-activated receptor γ (PPAR γ) Pro12Ala polymorphism affects plasma lipids, but to what extent alcohol intake interferes with this association remains unknown. We randomly recruited 251 nuclear families (433 parents and 493 offspring) in the framework of the European Project on Genes in Hypertension study and genotyped 926 participants in whom all serum lipid variables and information on alcohol consumption were available for PPAR γ 2 Pro12Ala. Genotype-phenotype relations were assessed using generalized estimating equations (GEE) and a quantitative transmission disequilibrium test (QTDT). The Ala12 allele was more frequent in Novosibirsk (0.17) than in Cracow (0.12) and Mirano (0.11) ($P < 0.01$). Using GEE ($P = 0.03$) or QTDT ($P = 0.007$), Italian offspring carrying the Ala12 allele had higher serum HDL cholesterol than noncarriers. HDL cholesterol levels were on average 0.086 mmol/l ($P = 0.001$) higher in drinkers than in nondrinkers. Compared with Pro12 homozygotes, Ala12 allele carriers consuming alcohol had higher serum total and HDL cholesterol, with the opposite trend occurring in nondrinkers. This genotype-alcohol interaction was independent of the type of alcoholic beverage and more pronounced in moderate than in heavy drinkers. **■** We conclude that alcohol intake modulates the relation between the PPAR γ 2 Pro12Ala and HDL cholesterol level and that, therefore, the Pro12Ala polymorphism, pending confirmation of our findings, might affect cardiovascular prognosis.—Brand-Herrmann, S-M., T. Kuznetsova, A. Wiechert, K. Stolarz, V. Tikhonoff, K. Schmidt-Petersen, R. Telgmann, E. Casiglia, J-G. Wang, L. Thijs, J. A. Staessen, and E. Brand on behalf of the European Project

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High density lipoprotein cholesterol exerts various potentially antiatherogenic effects (1, 2). Prospective epidemiological studies identified increased HDL level as an independent negative risk factor for coronary heart disease (3). As a complex trait, serum HDL levels are under endocrine, environmental, and genetic control (4).

Besides various other metabolic actions, the transcription factor peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) plays a role in the regulation of cholesterol homeostasis by controlling the expression of a network of genes that mediate cholesterol efflux from cells and its transport into the plasma (5). By means of both linkage and association analyses, the PPAR γ 2 locus has been demonstrated to affect HDL levels (6). Some investigators already reported an association of the PPAR γ 2 Pro12Ala polymorphism (Ala12 allele) with higher HDL levels (7, 8), whereas others observed an association with lower HDL levels (9). However, alcohol consumption also increases HDL levels (10), and to the best of our knowledge, no pre-

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vious study investigated whether alcohol intake interferes with the reported associations between the PPAR γ 2 polymorphism and serum lipids, which may explain association inconsistencies at least in part. Therefore, we investigated to what extent serum lipid concentrations, in particular HDL cholesterol, are associated with the PPAR γ 2 Pro12Ala polymorphism, alcohol consumption, and their interaction. Caucasian subjects, randomly recruited in three different countries in the framework of the European Project on Genes in Hypertension (EPOGH), were analyzed for this purpose.

MATERIALS AND METHODS

Study population

The EPOGH project was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects (11). The ethics committee of each institution approved the protocol. Participants gave informed written consent.

EPOGH participants recruited at three centers were genotyped for the PPAR γ 2 Pro12Ala polymorphism. Researchers randomly recruited nuclear families of Caucasian origin including offspring with a minimum age of 18 years from the populations of Niepolomice near Cracow (Poland; $n = 325$), Novosibirsk (Russian Federation; $n = 301$), and Mirano near Venice (Italy; $n = 345$). The overall response rate was 61.3%. Of these 971 subjects, we excluded 45 from the analysis. Fourteen participants did not have measurements of serum lipid profile. For 28 subjects, the genomic DNA could not be amplified or the genotype could not be determined with certainty, and we detected three cases of inconsistency with respect to Mendelian segregation. Thus, the number of subjects statistically analyzed totaled 926.

Phenotypes

Weight and height were measured without shoes with the subjects wearing light indoor clothing. The waist-to-hip ratio, determined by means of a tape measure, was the ratio of the smallest circumference at the waist level to the largest circumference at the hip level. The subscapular and triceps skinfolds were measured at the inferior angle of the scapula and at the midportion of the triceps muscle, respectively, by means of a Harpenden Skinfold Caliper (Bedfordshire, UK) providing a constant pressure of 0.01 kg/mm^2 (0.098 N/mm^2) $\pm 10\%$ at all openings of the 90 mm² anvils.

We administered a validated questionnaire (12) to collect information on each subject's medical history, use of medications, as well as smoking and drinking habits. From the type and number of alcoholic beverages used each day, we calculated alcohol consumption in grams per day. To exclude occasional drinkers, we defined current alcohol intake as a consumption of $\geq 5 \text{ g}$ of ethanol per day.

Blood samples were obtained after an overnight fast. Total and HDL cholesterol, triglycerides, and glucose were measured in serum by automated enzymatic methods. LDL cholesterol was calculated by the Friedewald formula.

Determination of genotypes

Genomic DNA was extracted from peripheral blood using standard techniques (13). The region encompassing the PPAR γ 2 Pro12Ala polymorphic site was amplified by polymerase chain reaction as previously described (14). Polymerase chain reaction products were digested using the restriction enzyme *Bst*UI and visualized on ultraviolet light-transilluminated ethidium bromide-stained agarose gels.

Statistical methods

Database management and statistical analyses were performed with SAS version 8.1 (SAS Institute, Cary, NC). Population means and proportions were compared by Tukey's multiple means test and the Chi-square statistic with Bonferroni's adjustment for multiple comparisons, respectively. If the Shapiro-Wilk statistic showed significant departure from normality (serum triglycerides), we analyzed logarithmically transformed variables. We searched for possible covariates of the serum lipid concentrations using stepwise multiple regression with the *F*-value for independent variables to enter and stay in the model set at 0.10.

We performed both population-based and family-based association analyses. In the population-based approach, we tested the association of continuous traits with the genotype of interest by use of generalized estimating equations (GEE). This approach allows adjustment for covariates as well as for the nonindependence of observations within families (15, 16). In GEE, we also tested for heterogeneity across populations using the appropriate interaction terms with the PPAR γ 2 Pro12Ala genotypes.

In the family-based analyses, we performed a quantitative transmission disequilibrium test (QTDT). We evaluated the within- and between-family components by phenotypic variability, using the orthogonal model as implemented by Abecasis, Cardon, and Cookson (17) in the QTDT software (version 2.3; <http://www.sph.umich.edu/csg/abecasis/QTDT>). With similar adjustment to that in the population-based analysis, we investigated the association between phenotypes and allelic transmission using the orthogonal model included in the QTDT package. We adjusted our primary analyses for multiple testing using Bonferroni's method for correlated measurements (<http://home.clara.net/sisa/bonhlp.htm>).

RESULTS

Characteristics of the participants

Table 1 provides the characteristics of participants by country. Overall, the study population included 926 subjects belonging to 251 families. Mean \pm SD age of the 433 founders and 493 offspring was 52.3 ± 5.14 years and 25.7 ± 4.78 years, respectively. The number of siblings per nuclear family was 1 in 36 families, 2 in 188 families, and 3 in 27 families.

The proportion of female participants, body mass index, and prevalence of smoking were similar across centers (Table 1). Italian and Russian participants more frequently reported a daily alcohol consumption of $\geq 5 \text{ g}$ (42.0% and 46.9%, respectively, versus 18.9% in Poland; $P < 0.05$). For subjects consuming $\geq 5 \text{ g}$ of alcohol per day, Fig. 1 shows the alcohol intake per individual and by center. Table 2 provides information on alcohol consumption by center and type of alcoholic beverage. There were significant between-center differences with respect to waist-to-hip ratio and skinfolds (Table 1). Total and LDL cholesterol levels were highest and triglyceride levels were lowest in Mirano, whereas HDL cholesterol was intermediate in Mirano compared with the other centers.

Genotype and allele frequencies

PPAR γ 2 Pro12Ala genotype frequencies did not deviate from Hardy-Weinberg equilibrium in any center ($0.30 < P < 0.45$). The highest Ala12 allele frequency was observed in Novosibirsk (16.5%); the corresponding frequen-

TABLE 1. General characteristics of parents and offspring at the three centers

Variable	Cracow (n = 318)	Novosibirsk (n = 294)	Mirano (n = 314)
Anthropometric characteristics			
Age, years	35.4 ± 13.9	37.9 ± 14.0 ^a	41.2 ± 14.2 ^{a,b}
Female, n (%)	174 (54.7)	164 (55.8)	167 (53.2)
Body mass index, kg/m ²	25.4 ± 5.0	25.3 ± 5.1	25.3 ± 4.4
Waist-to-hip ratio	0.84 ± 0.09	0.81 ± 0.08 ^a	0.85 ± 0.09 ^b
Skinfolds, cm	1.82 ± 0.90	1.54 ± 0.69 ^a	1.62 ± 0.70 ^a
Questionnaire data			
Tobacco use, n (%)	85 (26.7)	88 (29.9)	73 (23.3)
Alcohol intake ≥ 5 g/day, n (%)	60 (18.9)	138 (46.9) ^a	132 (42.0) ^a
Duration of alcohol intake, years	10.5 (3.1–36.3)	12.6 (4.4–34.7)	23.4 (7.8–43.6) ^{a,b}
Treated with β-blockers and/or diuretics, n (%)	47 (14.8)	23 (7.8) ^a	33 (10.5)
Metabolic measurements (fasting)			
Total cholesterol, mmol/l	4.99 ± 1.14	4.95 ± 1.21	5.22 ± 1.14 ^b
HDL cholesterol, mmol/l	1.58 ± 0.39	0.99 ± 0.32 ^a	1.23 ± 0.33 ^{a,b}
LDL cholesterol, mmol/l	2.82 ± 1.02	3.45 ± 1.07 ^a	3.50 ± 0.97 ^a
Triglycerides, mmol/l	1.08 (0.50–2.50)	1.33 (0.74–2.57) ^a	0.96 (0.50–1.90) ^{a,b}
Glucose, mmol/l	4.55 ± 1.03	4.81 ± 0.71 ^a	5.07 ± 1.17 ^{a,b}

Values are arithmetic means ± SD, geometric means (10–90% percentile interval), or number of subjects (%). Skinfold values are the sum of subscapular and triceps skinfolds. *P* values for the between-center differences were adjusted for multiple comparisons by Tukey's test (means) or Bonferroni's method (proportions).

^a *P* < 0.05 versus Cracow.

^b *P* < 0.05 versus Novosibirsk.

cies were 11.8% and 10.7% in Cracow and Mirano, respectively (*P* < 0.01).

Serum lipids and alcohol intake

Stepwise regression analyses confirmed that the serum lipid concentrations differed across centers and that one or more of the serum lipid fractions was in the expected direction, significantly and independently associated with gender, age, body mass index, and use of diuretics and β-blockers. Therefore, we adjusted all analyses for the aforementioned covariates. With these adjustments applied, within and across centers, serum HDL cholesterol concentration increased with current alcohol intake coded as a dichotomous variable or with the amount of alcohol consumed per day. For all centers combined, partial regression coefficients ± SEM were 0.086 ± 0.027 mmol/l (*P* = 0.001) and 0.015 ± 0.008 mmol/l (*P* = 0.0004) per 10 g of daily alcohol intake, respectively. The partial regression coefficients with these two indices of alcohol intake did not show significance with respect to total and LDL cholesterol and serum triglycerides (*P* > 0.30).

Serum lipids and PPARγ2 Pro12Ala polymorphism

With adjustments applied as described above and additionally for current alcohol intake, across all centers and for parents and offspring, there was no association between any of the serum lipid fractions and the PPARγ2 Pro12Ala polymorphism (data not shown).

However, for HDL cholesterol level in offspring, we observed significant heterogeneity across centers with respect to an association with the Pro12Ala polymorphism in GEE analyses (*P* = 0.03). In Mirano, the HDL cholesterol concentration in PPARγ2 Ala12 carriers was 0.132 ± 0.053 mmol/l higher compared with Pro12 homozygotes (1.31 vs. 1.18 mmol/l; *P* = 0.03). No such association was observed for Cracow (1.65 vs. 1.61 mmol/l) or Novosi-

birsk (1.06 vs. 1.03 mmol/l) offspring (*P* ≥ 0.46). In the family-based analyses, transmission of the PPARγ2 Ala allele to Italian offspring was associated with a significant increase of 0.20 mmol/l in serum HDL cholesterol concentration, whereas no such effect was seen in the Slavic centers (Table 3).

Interaction between PPARγ2 Pro12Ala polymorphism and alcohol intake

In GEE analyses, we found a significant interaction between the PPARγ2 Pro12Ala polymorphism and current alcohol intake (Fig. 2) or the amount of alcohol consumed per day in relation to total and HDL cholesterol levels irrespective of whether or not we adjusted for the duration of alcohol intake. We then further explored the possible impact of the duration of alcohol consumption on the association between lipid levels and the PPARγ2 Pro12Ala polymorphism. As a result, we observed no significant effect of the duration of alcohol intake in our models, which also included age, gender, center, body mass index, quantity of alcohol currently consumed, and antihypertensive drug treatment. However, we observed a very strong linear correlation between age and the duration of alcohol consumption. Thus, age by itself is a strong proxy for the duration of alcohol intake. This explains why duration of alcohol intake cannot achieve statistical significance in models already including age as a covariable. Compared with Pro12 homozygotes, Ala12 allele carriers had higher serum total and HDL cholesterol levels if they consumed alcoholic beverages, whereas the opposite trend was observed in those not regularly consuming alcohol. No significant genotype-alcohol interaction was observed for serum LDL cholesterol and triglyceride levels (Fig. 2).

To investigate a possible alcohol "dose effect," we plotted the association between HDL and total cholesterol level and the PPARγ2 Pro12Ala polymorphism in non-

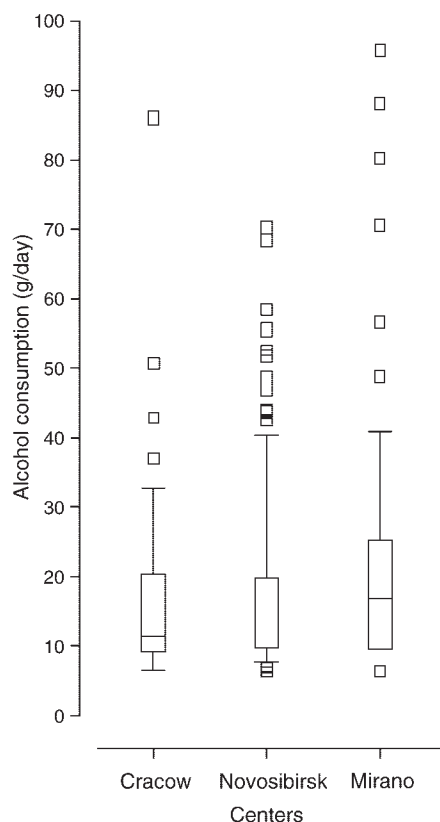


Fig. 1. Box plots of alcohol intake by center in subjects who consumed more than 5 g/d. Statistics include median, interquartile range, 5th to 95th percentile intervals, and outliers. Error bars represent standard deviation.

drinkers and in moderate (5–13 g/day) and heavy (>13 g/day) drinkers, whose alcohol intake was below or above the population median (13 g/day) (**Fig. 3**). Indeed, we observed a significant association between HDL cholesterol and the PPAR γ 2 polymorphism in moderate but not in heavy drinkers ($P = 0.02$ vs. $P = 0.59$).

Further analysis suggested that the genotype-alcohol interaction in relation to total and HDL cholesterol was attributable to alcohol intake per se rather than to a particular type of alcoholic beverage. Among the PPAR γ 2 Ala12 carriers of all centers combined, the fully adjusted HDL cholesterol level was 1.20 mmol/l in nondrinkers com-

TABLE 2. Daily intake by center and type of alcoholic beverage

Type of Alcoholic Beverage	Cracow (n = 60)		Novosibirsk (n = 138)		Mirano (n = 132)	
	Daily Intake		Daily Intake		Daily Intake	
	g/day	% ^a	g/day	% ^a	g/day	% ^a
Beer	10	43	5	1	10	11
Wine	8	2	7	1	16	75
Liquor	16	18	10	18	23	2
Beer + wine	6	7	13	4	18	3
Beer + liquor	26	22	16	49	—	—
Wine + liquor	11	3	9	9	32	8
Beer + wine + liquor	36	5	12	18	36	1

^a Percentage of the population.

TABLE 3. Results of QTDT analysis by center in offspring for HDL cholesterol

Center	QTDT Analysis ^a		
	β	Chi-Square	P^b
Cracow (Pro/Pro, n = 142; Ala carriers, n = 38)	0.026	0.10	0.94
Novosibirsk (Pro/Pro, n = 110; Ala carriers, n = 52)	0.041	0.30	0.82
Mirano (Pro/Pro, n = 123; Ala carriers, n = 28)	0.20	11.5	0.007

QTDT, quantitative transmission disequilibrium test. Analyses were adjusted for sex, age, body mass index, alcohol intake, and antihypertensive treatment (β -blockers and diuretics).

^aThe orthogonal model accounted for between- and within-family variability components. The parameter estimate (β) for the within-family variability indicates the size and direction of the peroxisome proliferator-activated receptor γ Ala12 allele.

^b P values were adjusted for multiple testing using Bonferroni's method.

pared with 1.47 mmol/l ($P = 0.01$), 1.33 mmol/l ($P = 0.01$), 1.37 mmol/l ($P = 0.05$), or 1.38 mmol/l ($P = 0.001$) in those consuming exclusively beer, wine, liquor, or a mixed pattern of consumption, respectively. For total cholesterol, the corresponding levels associated with non-drinking or drinking various types of alcoholic beverages

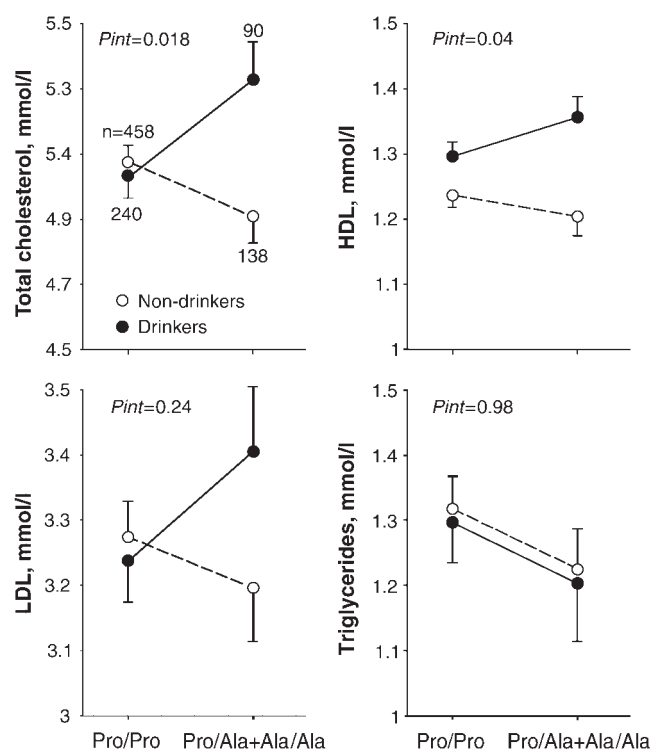


Fig. 2. Interaction of the peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) Pro12Ala polymorphism with alcohol on total, HDL, and LDL cholesterol and triglyceride levels in all subjects. Associations were plotted for two groups, based on alcohol consumption in excess of 5 g/d. The probability of the interaction (P_{int}) between genotype and alcohol intake was derived by generalized estimating equations and accounts for nonindependence within families and covariates. P values were adjusted for multiple testing using Bonferroni's method. Error bars represent standard deviation.

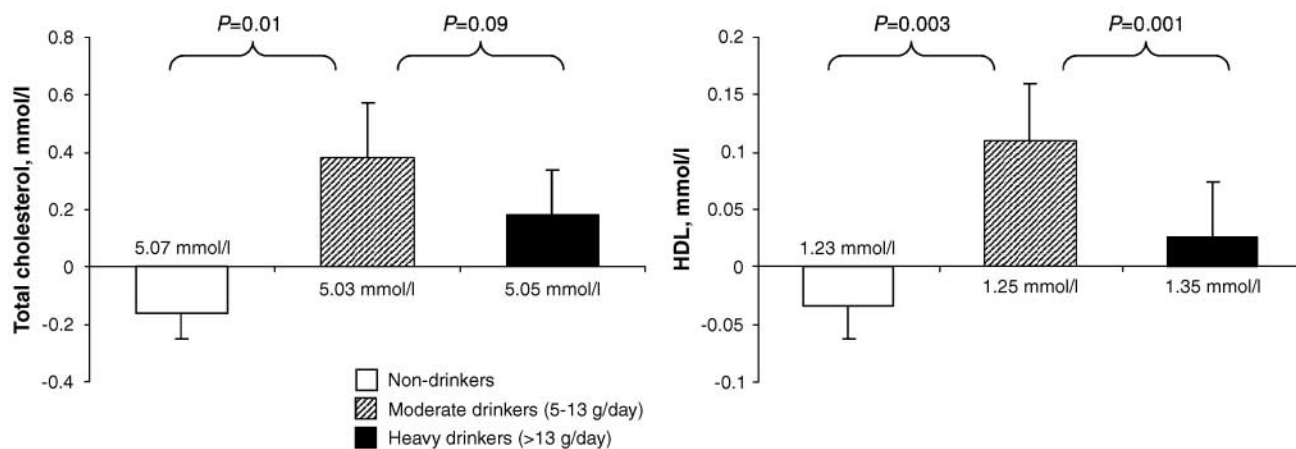


Fig. 3. Differences (SEM) in total (left) and HDL (right) cholesterol associated with the PPAR γ 2 Ala12 allele compared with Pro12 homozygotes. The reference levels in Pro12 homozygotes are given along the horizontal axis. Error bars represent standard deviation.

were 4.91 mmol/l versus 5.46 mmol/l ($P = 0.12$), 5.55 mmol/l ($P = 0.0004$), 5.45 mmol/l ($P = 0.07$), and 5.16 mmol/l ($P = 0.22$), respectively.

DISCUSSION

With respect to the PPAR γ 2 Pro12Ala polymorphism and plasma HDL level, we report significant genotype-alcohol as well as genotype-center interactions. For the entire study population, and regardless of the underlying genotype, drinkers had higher serum HDL levels than non-drinkers. The key finding of the present study is that alcohol intake modulates the genetic association between the PPAR γ 2 Pro12Ala polymorphism and HDL cholesterol level. Across centers, compared with Pro12 homozygotes, Ala12 allele carriers had higher plasma HDL levels if they consumed alcohol on a regular basis, with the opposite effect occurring in nondrinkers. Furthermore, and after adjusting for covariables including alcohol intake, Italian offspring carrying the PPAR γ 2 Ala12 allele had higher serum HDL levels than noncarriers, which was evident both in the family-based GEE and QTDT. These findings highlight that genotype-phenotype relations can only be studied within their ecogenetic context in analyses accounting for lifestyle, environmental factors, and genetic background as reflected by ethnicity. This might explain why a direct association between the PPAR γ 2 Pro12Ala polymorphism and HDL cholesterol was only observed in Italian offspring.

With regard to the (patho)physiological link between PPAR γ and HDL cholesterol, macrophages from PPAR γ conditional knockout mice are phenotypically characterized by reduced expression of lipoprotein lipase, CD36, liver X receptor α , and ABCG1, which leads to a decrease in the basal cholesterol efflux from cholesterol-loaded macrophages to HDL (5). In primary human monocyte-derived macrophages, other investigators (18) reported that PPAR γ activators induce ABCA1 expression, possibly through their inductive effects on the expression of liver X receptor α (19); ABCA1 is implicated in the first steps

of the reverse cholesterol transport pathway and in the control of plasma HDL levels.

The genotype-alcohol interaction was independent of the type of alcoholic beverage, and in keeping with numerous other publications (20, 21), we observed that HDL cholesterol levels increased with alcohol intake analyzed either as a dichotomous or a quantitative variable. The PPAR γ 2 Pro12Ala polymorphism itself affects serum lipid profiles. Indeed, Knoblauch and colleagues (6) recently observed linkage between plasma HDL cholesterol level and the PPAR γ 2 gene locus. Inconsistencies among the reports on associations between HDL cholesterol and PPAR γ 2 Pro12Ala (7–9) can be explained by differences between the populations under study (different diseases, different age classes) or by the lack of accounting for interactions in the genotype-phenotype analysis. In this respect, Memisoglu et al. (22) observed such an interaction with dietary fat intake in that higher total fat intake was directly associated with higher plasma HDL levels in Ala12 carriers. The environmental exposure in the latter study was “high fat intake” as the quantitative variable, compared with the consumption of >5 g of alcohol per day (drinker) as qualitative or the amount of alcohol consumption per day as quantitative (moderate or heavy drinkers) variables in our study. To compare these results, in the study by Memisoglu et al. (22), Ala12 allele carriers had higher HDL cholesterol levels when they “consumed” a high-fat diet (drinker in our study), whereas low fat intake (nondrinker in our study) was associated with low HDL levels in Ala12 allele carriers. In the low fat intake quintile (nondrinker in our study), Pro homozygotes had higher HDL levels compared with Ala allele carriers, which is directly comparable with our results.

The mechanisms underlying the genotype-alcohol interaction remain to be elucidated. Galli and colleagues (23) demonstrated that chronic ethanol feeding inhibited DNA binding and the transcriptional activity of the PPAR/retinoid X receptor (RXR) heterodimer in mice, but these experiments were restricted to PPAR α . In cultured human mesenchymal stem cells differentiating to adipocytes, Weze-

man and Gong (24) observed an upregulation of PPAR γ 2 gene expression after adding alcohol to the culture medium, whereas no such effect was observed for other marker genes representing different differentiation stages (lipoprotein lipase, adipsin, leptin, and adipocyte P2). The fact that in the present study the association between PPAR γ 2 Ala12 and increased plasma HDL level was more pronounced in alcohol consumers could possibly be explained by the proposed PPAR γ 2 gene upregulation attributable to alcohol action (24). In this respect, our present observation supports a gain in function of the Ala12 allele, mimicking some of the effects exerted by synthetic PPAR γ ligands, the thiazolidinediones (e.g., an increase of plasma HDL levels). In the only published functional study to date, Deeb et al. (7) reported that the PPAR γ 2 Pro12 isoform exerts modestly higher basic transcriptional activity compared with PPAR γ 2 Ala12 in transient transfection assays, possibly because of altered binding characteristics with its heterodimerization partner RXR, as shown by in vitro transcribed/translated proteins. However, both isoforms were able to respond to stimulation by BRL49,653 with comparable fold inductions and hence were able to transmit the biological stimulus even under cell culture conditions. It should be noted that in a physiological context, PPAR γ 2 isoforms also encounter different splice variants of RXR with altered physiological capacities (25), so it is conceivable that in a more complex (patho)physiological context, the transcriptional performance of these isoforms differs from that observed by Deeb and colleagues (7).

An analogous genotype-alcohol interaction on HDL cholesterol level has been demonstrated for the cholesteryl ester transfer protein gene polymorphism TaqIB, in that the effect of the TaqIB genotype on (higher) HDL cholesterol levels was only observed in alcohol drinkers, the effect being stronger with greater alcohol intake (26). In the present study, we also report on an alcohol dose effect. However, the association between HDL cholesterol level and the PPAR γ 2 polymorphism was more pronounced in moderate than in heavy drinkers. To explain this observation, we have to consider that for Pro12 homozygotes, HDL level was already significantly higher in heavy drinkers compared with nondrinkers and moderate drinkers (1.35 vs. 1.24 mmol/l). Thus, we hypothesize that in heavy drinkers, high alcohol consumption might mask the increase of HDL level associated with the Ala12 allele.

The present study has to be interpreted within the context of its limitations. Why the direct relation between the PPAR γ 2 Pro12Ala polymorphism and HDL cholesterol was confined to Italian offspring remains to be studied. Direct genetic effects may be more easily detected at a young age. In older people, plasma lipid concentrations are more likely to be influenced by nutritional and environmental factors, which could operate in a long-term manner. On the other hand, the observation of a relation between HDL cholesterol and alcohol intake represents an internal validation of our study measures.

In conclusion, the PPAR γ 2 Ala12 allele increases serum HDL concentration in alcohol consumers. To what extent the Ala12 allele could contribute to cardiovascular protec-

tion should be investigated in prospective studies with moderate alcohol intake, and the mechanisms underlying the PPAR γ 2 Pro12Ala-alcohol interaction need further clarification using appropriate experimental studies. ■

APPENDIX

EPOGH centers and participants are as follows: Belgium (Hechtel-Eksel): E. Balkestein, R. Bollen, H. Celis, E. Den Hond, A. Hermans, L. De Pauw, P. Drent, D. Emelianov, R. Fagard, J. Gasowski, L. Gijssbers, T. Nawrot, L. Thijs, Y. Toremans, J. A. Staessen, S. Van Hulle, J. G. Wang, and R. Wolfs; Bulgaria (Sofia): C. Nachev, A. Postadjian, E. Prokopova, E. Shipkovenska, and K. Vitljanova; Czech Republic (Pilsen): J. Filipovsky, V. Svobodova, and M. Ticha; Czech Republic (Prague): O. Beran, L. Golán, T. Grus, J. Peleška, and Z. Marecková; Italy (Padova): E. Casiglia, A. Pizzioli, and V. Tikhonoff; Poland (Cracow): K. Kawecka-Jaszcz, T. Grodzicki, K. Stolarz, B. Wizner, A. Olszanecka, A. Adamkiewicz-Piejkó, W. Lubaszewski, and J. Życzkowska; Romania (Bucharest): S. Babeanu, D. Jianu, C. Sandu, D. State, and M. Udrea; Russian Federation (Novosibirsk): Y. Nikitin, S. Malyutina, T. Kuznetsova, E. Pello, M. Ryabikov, and M. Voevoda.

Coordination and committees are as follows: project coordinator, J. A. Staessen; scientific coordinator, K. Kawecka-Jaszcz. Steering Committee: S. Babeanu, E. Casiglia, J. Filipovsky, K. Kawecka-Jaszcz, C. Nachev, Y. Nikitin, J. Peleška, and J. A. Staessen. Data Management Committee: T. Kuznetsova, J. A. Staessen, K. Stolarz, L. Thijs, V. Tikhonoff, and J. G. Wang. Publication Committee: E. Casiglia, K. Kawecka-Jaszcz, and Y. Nikitin. Advisory Committee on Molecular Biology: G. Bianchi (Milan), E. Brand (Berlin, Münster), S. M. Brand-Herrmann (Münster), and H. A. Struijker-Boudier (Maastricht). EPOGH-EurNetGen liaisons: A. Dominiczak (Glasgow) and J. A. Staessen (Leuven).

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