Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

ESR1 polymorphism is associated with plasma lipid and apolipoprotein levels in Caucasians of the Rochester Family Heart Study

Kathy L. E. Klos,[1,*] Eric Boerwinkle,* Robert E. Ferrell,† Stephen T. Turner,§ and Alanna C. Morrison*

Human Genetics Center,* University of Texas Health Science Center at Houston, Houston, TX; Department of Human Genetics,† Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; and Division of Nephrology and Hypertension,§ Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, MN

Abstract We evaluated six estrogen receptor 1 (ESR1) polymorphisms for association with ten plasma lipid and apolipoprotein traits in 1,847 individuals (941 females and 906 males) in the multi-generation Rochester Family Heart Study using a generalized estimating equation approach. Apolipoprotein A-I (apoA-I), apoA-II, and HDL-cholesterol (HDL-C) were associated with exon 4 rs1801132 (Pro325Pro) genotype ($P = 0.0044$, $P = 0.0048$, and $P = 0.0035$, respectively). Positive correlation between levels of apoA-I, apoA-II, and HDL-C and the number of G alleles was observed in females ($P = 0.0120$, $P = 0.0032$, and $P = 0.0030$), but not males ($P > 0.05$). Because few studies have evaluated the effect of ESR1 gene polymorphisms on lipid traits in children, we also stratified our sample at the age of 15 years. There was evidence of association between intron 1 single-nucleotide polymorphisms rs9322331 and rs9340799 and apoC-II, and triglycerides (TGs) in youths 15 years and younger. In youths, evidence of association between rs9322331 and rs9340799 and apoC-II was stronger in males ($P = 0.0036$ and $P = 0.0124$) than in females ($P > 0.05$), whereas evidence of association with TG was stronger in females ($P = 0.0030$ and $P = 0.0024$) than in males ($P > 0.05$). These findings suggest that ESR1 variation plays an age- and sex-dependent role in determining plasma lipid and apolipoprotein levels.—Klos, K. L. E., E. Boerwinkle, R. E. Ferrell, S. T. Turner, and A. C. Morrison. **ESR1 polymorphism is associated with plasma lipid and apolipoprotein levels in Caucasians of the Rochester Family Heart Study. *J. Lipid Res.* 2008.** 49: **1701–1706.**

Supplementary key words estrogen receptor 1 • HDL • LDL • triglycerides

High levels of serum LDL-cholesterol (LDL-C) and low levels of HDL-C are independent risk factors for coronary artery disease (CAD). Women have a lower lifetime risk of CAD than do men, and the difference in risk may be attributed in part to a generally later onset of high LDL-C levels (>100 mg/dl) and a slower decline in HDL-C level (1, 2). The influence of estrogen has been proposed as one of the underlying factors in the differential risk profiles of women and men, making estrogen receptor α a candidate for predicting plasma lipid and apolipoprotein levels (3, 4).

Estrogen receptor α, encoded by the estrogen receptor 1 (ESR1) gene on chromosome 6, has been demonstrated to influence the expression of proteins involved in the regulation of plasma lipid metabolism, such as apolipoprotein E (apoE), LDL receptor, and scavenger class B type 1 receptor (5–7). DNA variation in the ESR1 gene region has been associated with variation among individuals for components of the plasma lipid profile, including apoA-I, apoB, HDL-C, LDL-C, triglyceride (TG) levels, and HDL and LDL particle size (8–11). Results from studies of association between plasma lipids and ESR1 polymorphisms have been mixed, however, with negative results reported for association with apoA-I, HDL-C, LDL-C, total cholesterol (TC), and TG. (9–11).

In this study, we evaluate six ESR1 single-nucleotide polymorphisms (SNPs) for their association with ten measures of plasma lipids and apolipoproteins. We took advantage of the multi-generation pedigree structure of the Rochester Family Heart Study (RFHS) to evaluate associations within sex and after stratifying at age 15. We find strong evidence that ESR1 polymorphisms influence varia-

Support for this work was provided by National Heart, Lung, and Blood Institute Grant R01- HL-077491.

Manuscript received 30 October 2007 and in revised form 6 December 2007 and in re-revised form 20 February 2008 and in re-re-revised form 25 April 2008.

Published, JLR Papers in Press, April 30, 2008.
DOI 10.1194/jlr.M700490-JLR200

[1] To whom correspondence should be addressed.
e-mail: kathy.klos@uth.tmc.edu

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one table.

Copyright © 2008 by the American Society for Biochemistry and Molecular Biology, Inc.

Downloaded from www.jlr.org by guest, on June 14, 2012

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

TABLE 1. Mean (standard deviation) of plasma lipid and apolipoprotein traits of Rochester Family Heart Study individuals used in these analyses

	Child Generation		Parents		Grandparents	
	Female	Male	Female	Male	Female	Male
N	354	389	304	293	344	275
Age (years)	15.93 (4.44)	15.13 (4.67)[a]	42.31 (4.71)	44.28 (5.10)[a]	69.30 (6.88)	70.56 (6.14)[a]
BMI	21.51 (4.41)	20.80 (4.05)[a]	24.75 (4.76)	27.28 (4.41)[a]	27.33 (5.76)	28.20 (4.11)
ApoA-I (mg/dl)	133.52 (19.27)	129.40 (18.68)[a]	144.90 (19.89)	132.57 (17.50)[a]	149.94 (22.14)	132.67 (18.65)[a]
ApoA-II (mg/dl)	33.29 (5.47)	33.01 (5.51)	34.79 (5.28)	34.87 (5.42)	35.14 (5.94)	32.49 (5.67)[a]
ApoB (mg/dl)	69.49 (15.06)	65.76 (12.68)[a]	74.34 (16.08)	83.63 (17.22)[a]	90.16 (18.91)	87.65 (17.37)
ApoC-II (mg/dl)	1.91 (0.69)	1.99 (0.69)	1.99 (0.69)	2.68 (1.19)[a]	2.91 (0.91)	2.62 (0.99)[a]
ApoC-III (mg/dl)	14.24 (4.46)	13.29 (4.15)[a]	14.33 (4.10)	16.48 (5.49)[a]	19.98 (6.03)	17.68 (6.76)[a]
ApoE (mg/dl)	5.02 (1.32)	4.78 (1.16)[a]	4.99 (1.17)	5.39 (1.54)[a]	6.18 (1.52)	5.56 (1.43)[a]
HDL-C (mg/dl)	46.58 (10.90)	43.12 (10.74)[a]	52.16 (12.63)	40.35 (10.30)[a]	50.60 (15.23)	38.81 (9.65)[a]
LDL-C (mg/dl)	98.68 (28.65)	94.03 (24.08)[a]	115.56 (29.71)	135.91 (30.23)[a]	147.66 (33.63)	138.79 (31.99)[a]
TC (mg/dl)	158.85 (31.29)	151.07 (26.60)[a]	180.78 (30.81)	198.13 (34.90)[a]	221.68 (36.55)	202.43 (36.42)[a]
TG (mg/dl)	86.30 (40.79)	82.41 (46.09)	81.42 (35.40)	127.02 (84.98)[a]	131.28 (57.48)	133.34 (70.58)

BMI, body mass index; apoA-I, apolipoprotein A-I; HDL-C, HDL-cholesterol; TC, total cholesterol; TG, triglyceride.
[a] Males and females within generation significantly different at $P \leq 0.05$.

tion in measures of HDL metabolism in women and evidence of an influence on TG-related traits in youths 15 years or younger.

MATERIALS AND METHODS

Beginning in 1984, RFHS pedigrees were ascertained without regard to health through households with two or more children enrolled in primary and secondary schools of Rochester, MN. Sampling details, baseline characteristics, and clinic protocol of the RFHS have been described by Moll et al. (12) and Turner et al. (13). Consistent with the ethnic profile of the region, the majority of individuals were self-identified Caucasian. The sample used in this study also included one American Indian, two Orientals, and seven individuals who indicated "Other" as their ethnic identity. Individuals with ethnicity other than Caucasian were excluded. Plasma TC and TG levels were measured by standard enzymatic methods (14, 15). Plasma apoA-I, apoA-II, apoC-II, apoC-III, and apoE were measured by radioimmunoassay (16). Plasma apoB was measured by ELISA (17). LDL-C levels were calculated using the equation of Friedwald, Levy, and Frederickson (18). LDL particles were precipitated with polyethylene glycol 6000, and an aliquot of the supernatant was used for determination of HDL-C by an enzymatic method, as described in Kaprio et al. (17). Individuals who had not fasted for at least 8 h prior to the clinic evaluation were excluded. Participants were asked about their current use of exogenous hormones and were classified into four categories: no use of exogenous hormones, use of oral contraception, use of estrogen (not as oral contraception), use of progesterone (not as oral contraception), and use of both estrogen and progesterone (not as oral contraception). The study protocol was approved by the appropriate institutional review boards and all participants gave informed consent.

SNPs rs9322331, rs2234693, rs9340799, rs12664989, rs712221, rs1801132, and rs3798577 were selected for genotyping based on a history of use in investigations of ESR1 genotype-phenotype association. The rs12664989 SNP was not polymorphic in this sample and, therefore, was not evaluated for association. Genotyping was performed using the fluorescence polarization method (19). Primers are available from the authors upon request. Each plate was run with sequenced genotype controls. Genotypes were assigned by two independent readers, and disagreements were resolved by consensus. Genotype and phenotype information was available on 1,847 individuals (1,941 females and 906 males). Relative allele frequency was determined by direct counting, and Hardy-Weinberg equilibrium was evaluated using a χ^2 test in the unrelated grandparent generation (n = 528). Pairwise linkage disequilibrium (LD) was evaluated using the Haploview program.

Because of non-normality, apoC-II, apoC-III, apoE, LDL-C, and TG were log-transformed prior to analyses. Association between quantitative traits and SNP genotypes was evaluated using linear regression models. A generalized estimating equation approach was used to estimate model parameters because of the potential for correlation due to relatedness. Age, sex, and hormone use were incorporated as covariates. Body mass index (BMI) was not used as a covariate because of evidence that ESR1 polymorphisms influence BMI. (8, 20, 21) (see supplementary Table I).

TABLE 2. Relative minor allele frequencies, P values for tests of Hardy-Weinberg equilibrium in the grandparent generation of the Rochester Family Heart Study pedigrees, and sample sizes (N) of strata defined by age and sex

			All		Youths ≤ 15 years		Adults > 15 years	
	MAF	HWE	Females	Males	Females	Males	Females	Males
Rs9322331	0.33	0.044	945	904	183	230	762	674
Rs2234693	0.44	0.008	930	887	181	225	749	662
Rs9340799	0.36	0.360	929	890	182	227	747	663
Rs712221	0.38	0.576	906	882	182	227	724	655
Rs1801132	0.24	0.390	934	898	178	227	756	671
Rs3798577	0.49	0.797	945	890	183	229	762	665

MAF, minor allele frequencies; HWE, Hardy-Weinberg equilibrium.

Downloaded from www.jlr.org by guest, on June 14, 2012

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

TABLE 3. Pairwise LD in the grandparent generation of Rochester Family Heart Study pedigrees for six ESR1 SNPs presented as D′ in the upper right diagonal and as r^2 in the lower left diagonal

	Rs9322331	Rs2234693	Rs9340799	Rs712221	Rs1801132	Rs3798577
Rs9322331	—	0.95	0.99	0.72	0.28	0.11
Rs2234693	0.56	—	0.92	0.56	0.45	0.01
Rs9340799	0.89	0.59	—	0.71	0.25	0.12
Rs712221	0.40	0.25	0.42	—	0.36	0.13
Rs1801132	0.01	0.05	0.01	0.03	—	0.09
Rs3798577	0.01	0.00	0.01	0.01	0.00	—

LD, linkage disequilibrium; ESR1, estrogen receptor 1; SNP, single-nucleotide polymorphism.

Genotypes were recoded for these analyses as the number of rare alleles. Inflation of the type I error rate due to multiple testing is a well-known problem. However, because of the extensive correlation among these phenotypes, a Bonferroni adjustment for 60 independent tests (6 SNPs × 10 traits) may be too conservative. Therefore, we chose to adjust for 6 independent SNP tests, resulting in a significance threshold of $P \leq 0.008$, but to remain skeptical of associations with a *P* value >0.001 without supporting evidence from the literature. All *P* values are reported (see supplementary Table I) to allow readers to judge significance by their own standards.

This study did not collect information on age at menarche or on menopause status. The age of 15 years was selected to stratify individuals into adults and youths in order to be inclusive of a possible later age at menarche reported to be associated with ESR1 genotype (22). Although none of the females 15 or younger were taking oral contraceptives, it is likely that some youths had reached maturity. Because we were unable to otherwise identify these individuals, their presence was not considered for these analyses.

RESULTS

Table 1 presents the characteristics of individuals used in these analyses by sex- and generation-strata. Within generation, females and males differed for all characteristics except apoA-II levels in children and parents, logTG levels in children and grandparents, and logapoC-II level in children. All traits differed among generations ($P < 0.05$).

Relative allele frequencies and sample sizes of strata evaluated in this study are presented in **Table 2**. There was no evidence that allele frequency differed across generations ($P > 0.05$ by χ^2 test). SNP genotype classes did not deviate from expectations under Hardy-Weinberg equilibrium ($P > 0.008$). Pairwise LD among the six SNPs evaluated for association is presented in **Table 3**. The three intron 1 SNPs, rs9322331, rs2234693, and rs9340799, were in strong LD with one another (D' from 0.92 to 0.99) and in moderate LD with rs712221 (D' from 0.56 to 0.72).

Table 4 presents *P* values from selected genotype-phenotype association tests. (*P* values from all tests are presented in supplementary Table I.) ApoA-I, apoA-II, and HDL-C levels were associated with rs1801132 genotype in the complete pedigrees ($P < 0.005$). After sex stratification, evidence of association with these phenotypes was confined to females, but there was no evidence of an rs1801132 × sex interaction effect ($P > 0.05$). **Fig. 1** shows the genotype mean levels of plasma apoA-I, apoA-II, and HDL-C in females. For these HDL-related traits, genotype class mean level increased with the number of G alleles (mean apoA-I levels in mg/dl by rs1801132 genotype in females were CC = 140.89 ± 21.96, CG = 143.46 ± 20.69, and GG = 146.04 ± 20.44; apoA-II levels were CC = 33.96 ± 5.61, CG = 34.89 ± 5.56, and GG = 35.01 ± 5.91; HDL-C levels were CC = 48.61 ± 13.23, CG = 50.79 ± 12.83, and GG = 51.12 ± 12.63).

Within youths aged 15 and younger (n = 438), rs9322331 and rs9340799 were associated with apoC-II and TG and rs9322331 with apoC-III ($P < 0.008$). Further stratification by sex indicated that evidence of association with TG level was stronger in females (rs9322331, $P = 0.0030$ and rs9340799, $P = 0.0024$) than in males (rs9322331, $P = 0.0649$ and rs9340799, $P = 0.2520$), and evidence of association with apoC-II was stronger in males (rs9322331, $P = 0.0036$ and rs9340799, $P = 0.0124$) than in females (rs9322331, $P = 0.0772$ and rs9340799, $P = 0.1264$). However, genotype mean trends were very similar for both sexes (**Fig. 2**). Evidence of apoC-III association with rs932231 was similar in females ($P = 0.0113$) and males ($P = 0.0327$). Figure 2 shows the rs9322331 genotype

TABLE 4. *P* values from tests of association for SNPs rs9322331, rs9340799, and rs1801132 with plasma levels of apoA-I, apoA-II, apoC-II, apoC-III, HDL-C, and TGs

	All	Females	Males	≤15 yrs	>15 yrs
Rs9322331					
ApoA-I	0.7376	0.4663	0.5777	0.5349	0.4886
ApoA-II	0.8320	0.9660	0.6974	0.6384	0.7448
HDL-C	0.9474	0.9514	0.9230	0.2905	0.7756
logApoC-II	0.9100	0.4781	0.7978	**0.0013**	0.2020
logApoC-III	0.9989	0.6760	0.6646	**0.0038**	0.2808
logTG	0.2319	0.1413	0.4777	**0.0012**	0.9693
Rs9340799					
ApoA-I	0.6138	0.9490	0.4533	0.1732	0.7024
ApoA-II	0.8498	0.7675	0.4052	0.6370	0.7451
HDL-C	0.6856	0.7305	0.8849	0.1018	0.6551
logApoC-II	0.5537	0.5900	0.5407	**0.0041**	0.4015
logApoC-III	0.9817	0.9273	0.3549	0.0766	0.3806
logTG	0.2138	0.1688	0.4006	**0.0055**	0.9874
rs1801132					
ApoA-I	**0.0044**	0.0120	0.1044	0.0662	0.0259
ApoA-II	**0.0048**	**0.0032**	0.2163	0.0106	0.0533
HDL-C	**0.0035**	**0.0030**	0.2878	0.1131	0.0128
logApoC-II	0.7280	0.3745	0.1109	0.9773	0.5764
logApoC-III	0.7803	0.3258	0.6408	0.9499	0.9029
logTG	0.3097	0.5401	0.4723	0.0354	0.6393

P values ≤ 0.008 are emphasized with bold-face type.

Downloaded from www.jlr.org by guest, on June 14, 2012

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

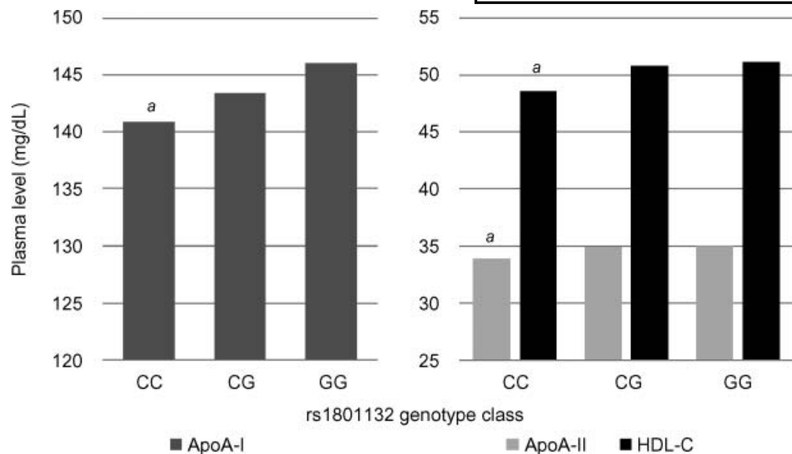

Fig. 1. Mean levels of plasma apolipoprotein A-I (apoA-I), apoA-II, and HDL-cholesterol (HDL-C) by estrogen receptor 1 (ESR1) rs1801132 genotype class in females. [a] Evidence of genotype-phenotype association in females ($P < 0.025$).

mean levels of plasma apoC-II and TG in youths (in females, mean apoC-II levels in mg/dl by rs9322331 genotype were AA = 1.74 ± 0.60, AG = 1.95 ± 0.68, and GG = 2.08 ± 0.71, and in males, they were AA = 1.71 ± 0.45, AG = 1.97 ± 0.71, and GG = 2.09 ± 0.57; TG levels were AA = 68.36 ± 24.31, AG = 77.78 ± 31.27, and GG = 96.24 ± 74.83 in females and AA = 64.09 ± 19.40, AG = 73.82 ± 33.68, and GG = 78.92 ± 36.27 in males). ApoC-III trends were very similar (in females, mean apoC-III levels in mg/dl by rs9322331 genotype were AA = 11.85 ± 2.31, AG = 13.12 ± 3.23, and GG = 14.29 ± 4.36, and in males, they were AA = 11.80 ± 2.51, AG = 12.48 ± 3.52, and GG = 13.27 ± 3.06).

Within youths homozygous for the rs7340799 G-allele, rs9322331 genotype was associated with apoC-II (P = 0.0179), apoC-III (P = 0.0011), and TG ($P < 0.0001$); however, within rs9322331 G-allele homozygotes, rs7340799 genotype was not associated with any trait ($P > 0.05$). Although SNP coverage in this region of ESR1 was not exhaustive, it appears that rs9322331 is better representative of the ESR1 genotype effect on these traits than is rs9340799. Caution should be taken with the small sample sizes (in youths, only 14 rs9322331 G- allele carriers were rs9340799 G-allele homozygotes, for example).

There was no evidence of association confined to adults over the age of 15, at a significance threshold of P = 0.008. Further tests of that strata within sex were not performed.

DISCUSSION

Variation in the ESR1 gene encoding the estrogen receptor α protein has been investigated for the ability to predict plasma lipid and apolipoprotein levels in several studies. To date, evidence has been presented to support a role for ESR1 gene variation in determining levels of apoA-I, apoB, HDL-C, LDL-C, TC, and TG (8–10, 23). ESR1 has also been associated with HDL and LDL particle size distributions and changes in plasma HDL-C levels in response to hormone replacement therapy (11, 24, 25). Negative results have also been reported, however, including lack of association with apoA-I, HDL-C, LDL-C, TC, and TG (9–11). These mixed results may, in part, be because of the difficulty of detecting small to moderate gene effects in samples of small size (we are aware of only five studies in which ESR1 effect on one or more measures of plasma lipid metabolism was evaluated in samples of >500 individuals). In addition, endogenous estrogen level is age- and sex-dependent, which should raise an expectation of differential effects among samples that differ for these characteristics. Most of these relationships await

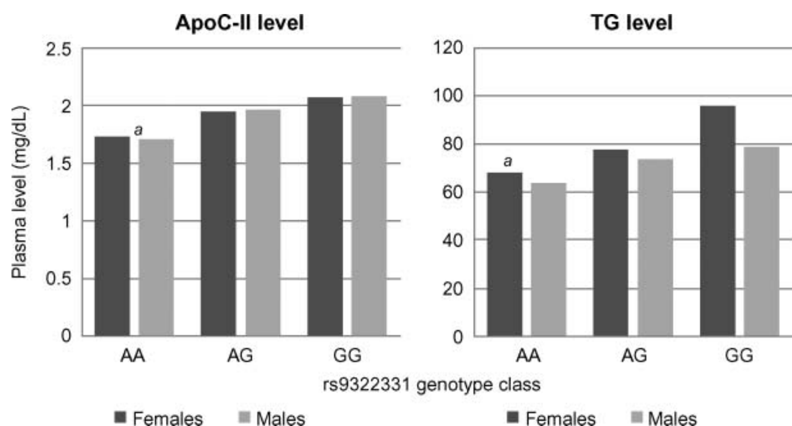

Fig. 2. Mean levels of plasma apoC-II and triglycerides (TG) by ESR1 rs9322331 genotype class in females and males. [a] Evidence of genotype-phenotype association ($P < 0.008$).

Downloaded from www.jlr.org by guest, on June 14, 2012

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

support from additional studies. This study identifies an ESR1 gene influence on inter-individual variation in plasma levels of apoA-I, apoA-II, and HDL-C, and in children on plasma apoC-II, apoC-III, and TG.

The full sample and the sex-stratified samples

The finding that measures of HDL metabolism (apoA-I, apoA-II, and HDL-C) were associated with ESR1 gene variation in females across a wide age range is consistent with previous reports. In women (n = 854, mean age = 52 years) of the Framingham Heart Study, rs1801132 was found to be associated with variation in HDL particle size ($P = 0.015$) and concentration of intermediate HDL particles ($P = 0.005$) (11). A suggestive association between HDL-C level and rs1801132 ($P = 0.062$) is of interest in light of the stronger findings of the current study.

In menstruating Caucasian females aged 42 to 52 of the Study of Women's Health Across the Nation (SWAN), rs3798577 C-allele homozygotes had lower apoA-I levels than T-carriers. An rs3798577 effect on apoA-I was also observed in SWAN Japanese subjects (23). No association was observed for this SNP with any plasma lipid or apolipoprotein trait in the RFHS ($P > 0.05$). Sowers et al. (23) rightly point out that ethnic differences in genotype-phenotype association may be due to differences in plasma lipid profile. It is also possible that rs3798577 is in LD with a polymorphism not present in a sufficient number of individuals to evaluate in the RFHS.

Although previous findings were reported based on samples of adults, the strong association with HDL-related traits in our study was in a sample of combined youths and adults. Many of the studies that report a lack of association between HDL-C level and ESR1 genotype were in postmenopausal women (26–28). This suggests that the effects of ESR1 variation on HDL-related traits in females may begin in childhood and extend to peri-menopause. Evidence from examining longitudinal data in a population-based sample would be very useful in answering this question.

We did not observe the rs12664989, rs712221, and rs9322331 effect(s) on LDL-C reported for African-Americans of the Insulin Resistance Atherosclerosis Family Study ($P = 0.016$, $P = 0.029$, and $P = 0.034$) (8). In the RFHS, rs12664989 was monomorphic, and best evidence of association for rs712221 was with apoA-I and apoA-II levels in females of all ages ($P = 0.0928$ and $P = 0.0894$, respectively). Association of rs9322331 genotype and plasma lipid levels in the RFHS was confined to youths aged 15 and younger, and did not include LDL-C ($P > 0.05$).

The age-stratified samples

ESR1 intron 1 SNPs rs9322331 and rs9340799 were associated with plasma TG-related traits within those RFHS individuals aged 15 and younger. We are aware of only one other study investigating the relationship between ESR1 genotype and lipid traits in children. Kikuchi et al. (9) evaluated seven lipid and apolipoprotein traits in 102 Japanese youths (age 10 to 15) for association with rs9340799 and rs2234693. They found that rs9340799 was associated with apoB ($P = 0.008$) and LDL-C levels ($P = 0.031$), but not with TC, HDL-C, apoA-I, or apoE. Unlike Kikuchi et al. (9), we did not find association between rs9340799 and apoB or LDL-C ($P > 0.05$). The strongest associations with rs9340799 in the RFHS were for apoC-II and TG, traits not evaluated by Kikuchi et al. These results in RFHS youths support evidence of the role of ESR1 gene variation in influencing plasma lipid profiles in children and suggest that this role is important in males as well as females.

We were limited in this study by an inability to classify females by their menopause status. Most studies of postmenopausal women (particularly those not currently taking exogenous hormones) have found no evidence for association between ESR1 genotype and plasma lipid levels (27, 28, 29). Our inability to remove these women from among the premenopausal, peri-menopausal, and postmenopausal women taking exogenous hormones may have reduced our power to identify associations particular to the adult strata.

In summary, we report association with HDL-related traits in non-age-stratified pedigrees with a pattern of sex-specific association, suggesting that the effect was largely confined to females. Associations with apoC-II, apoC-III, and TG were observed in youths, and sex may be a modifier of those effects as well. We feel that these results, like the multivariate results of Gallagher et al. (8) support the hypothesis of multiple variants in ESR1 that, individually or collectively, have a pleiotropic effect on plasma lipids and apolipoproteins. Overall, it is likely that ESR1 variation plays an age- and sex-dependent role in determining plasma lipid and apolipoprotein levels in the general population, but the molecular mechanism of these associations remains to be addressed.

The authors thank the participants of the Rochester Family Heart Study for their time and effort.

REFERENCES

1. Blumenthal, R. S. 2002. Overview of the adult treatment panel (ATP) III guidelines. *Advanced Studies Med.* **2:** 148–157.
2. Gardner, C. D., D. L. Tribble, D. R. Young, D. A. Ahn, and S. P. Fortmann. 2000. Population frequency distributions of HDL, HDL2, and HDL3 cholesterol and apolipoproteins A-I and B in healthy men and women and associations with age, gender, hormonal status, and sex hormone use: The Stanford Five City Project. *Prev. Med.* **31:** 335–345.
3. Deroo, B. J., and K. S. Korach. 2006. Estrogen receptors and human disease. *J. Clin. Invest.* **116:** 561–570.
4. Huxley, V. H. 2007. Sex and the cardiovascular system: the intriguing tale of how women and men regulate cardiovascular function differently. *Adv. Physiol. Educ.* **31:** 17–22.
5. Srivastava, R. A. K., N. Srivastava, M. Averna, R. C. Lin, K. S. Korach, D. B. Lubahn, and G. Schonfeld. 1997. Estrogen up-regulates apolipoprotein E (ApoE) gene expression by increasing ApoE mRNA in the translating pool via the estrogen receptor α-mediated pathway. *J. Biol. Chem.* **272:** 33360–33366.
6. Li, C., M. R. Briggs, T. E. Ahlborn, F. B. Kraemer, and J. Liu. 2001. Requirement of Sp1 and estrogen receptor α interaction in 17β-estradiol-mediated transcriptional activation of the low

Downloaded from www.jlr.org by guest, on June 14, 2012

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2008/05/01/M700490-JLR200.DC1.html

density lipoprotein receptor gene expression. *Endocrinology.* **142:** 1546–1553.

7. Lopez, D., M. D. Sanchez, W. Shea-Eaton, and M. McLean. 2002. Estrogen activates the high-density lipoprotein receptor gene via binding to estrogen response elements and interaction with sterol regulatory element binding protein-1A. *Endocrinology.* **143:** 2155–2168.
8. Gallagher, C. J., C. D. Langefeld, C. J. Gordon, J. K. Campbell, J. C. Mychalecky, M. Bryer-Ash, S. S. Rich, D. W. Bowden, and M. M. Sale. 2007. Association of the estrogen receptor-α gene with the metabolic syndrome and its component traits in African-American families. The Insulin Resistance Atherosclerosis Family Study. *Diabetes.* **56:** 2135–2141.
9. Kikuchi, T., N. Hashimoto, T. Kawasaki, and M. Uchiyama. 2000. Association of serum low-density lipoprotein metabolism with oestrogen receptor gene polymorphism in healthy children. *Acta Paediatr.* **89:** 42–45.
10. Sosa, M., E. Jódar, E. Arbelo, C. Domínguez, P. Saavedra, A. Torres, E. Salido, J. M. Limiñana, M. J. G. de Tejada, and D. Hernández. 2004. Serum lipids and estrogen receptor gene polymorphisms in male-to-female transsexuals: effects of estrogen treatment. *Eur. J. Intern. Med.* **15:** 231–237.
11. Demissie, S., L. A. Cupples, A. M. Shearman, K. M. Gruenthal, I. Peter, C. H. Schmid, R. H. Karas, D. E. Housman, M. E. Mendelsohn, and M. E. Ordovas. 2006. Estrogen receptor-α variants are associated with lipoprotein size distribution and particle levels in women: The Framingham Heart Study. *Atherosclerosis.* **185:** 210–218.
12. Moll, P. P., V. V. Michels, W. H. Weidman, and B. A. Kottke. 1989. Genetic determination of plasma apolipoprotein AI in a population-based sample. *Am. J. Hum. Genet.* **44:** 124–139.
13. Turner, S., W. Weidman, V. Michels, T. Reed, C. Ormson, T. Fuller, and C. F. Sing. 1989. Distribution of sodium-lithium countertransport and blood pressure in Caucasians five to eighty-nine years of age. *Hypertension.* **13:** 378–391.
14. Barr, S. E., B. A. Kottke, and S. J. T. Mao. 1981. Improved method for determination of triglycerides in plasma lipoproteins by an enzymic kit method. *Clin. Chem.* **27:** 1142–1144.
15. National Institutes of Health. 1974. Lipid Research Clinics Program Manual of Laboratory Operations. DHEW NIH Publication No. 75–628.
16. Kottke, B. A., P. P. Moll, V. V. Michels, and W. H. Weidman. 1991. Levels of lipids, lipoproteins, and apolipoproteins in a defined population. *Mayo Clin. Proc.* **66:** 1198–1208.
17. Kaprio, J., R. E. Ferrell, B. A. Kottke, M. I. Kamboh, and C. F. Sing. 1991. Effects of polymorphisms in apolipoproteins E, A-IV, and H on quantitative traits related to risk for cardiovascular disease. *Arterioscler. Thromb.* **11:** 1330–1348.
18. Friedewald, W. T., R. I. Levy, and D. S. Frederickson. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma. *Clin. Chem.* **18:** 499–501.
19. Chen, X., L. Levine, and P-Y. Kwok. 1999. Fluorescence polarization in homogeneous nucleic acid analysis. *Genome Res.* **9:** 492–498.
20. Deng, H. W., J. Li, J. L. Li, R. Dowd, K. M. Davies, M. Johnson, G. Gong, H. Deng, and R. R. Recker. 2000. Association of estrogen receptor-alpha genotypes with body mass index in normal healthy postmenopausal Caucasian women. *J. Clin. Endocrinol. Metab.* **85:** 2748–2751.
21. Okura, T., M. Koda, F. Ando, N. Niino, S. Ohta, and H. Shimokata. 2003. Association of polymorphisms in the estrogen receptor alpha gene with body fat distribution. *Int. J. Obes. Relat. Metab. Disord.* **27:** 1020–1027.
22. Stavrou, I., C. Zois, J. P. Ioannidis, and A. Tsatsoulis. 2002. Association of polymorphisms of the oestrogen receptor alpha gene with the age of menarche. *Hum. Reprod.* **17:** 1101–1105.
23. Sowers, M. R., J. P. Symons, M. L. Jannausch, J. Chu, and S. R. Kardia. 2006. Sex steroid hormone polymorphisms, high-density lipoprotein cholesterol, and apolipoprotein A-1 from the Study of Women's Health Across the Nation (SWAN). *Am. J. Med.* **119:** 561–568.
24. Shearman, A. M., S. Demissie, L. A. Cupples, I. Peter, C. H. Schmid, J. M. Ordovas, M. E. Mendelsohn, and D. E. Housman. 2005. Tobacco smoking, estrogen receptor α gene variation and small low density lipoprotein level. *Hum. Mol. Genet.* **14:** 2405–2413.
25. Herrington, D. M., T. D. Howard, G. A. Hawkins, D. M. Reboussin, J. Xu, S. L. Zheng, B. Brosnihan, D. A. Meyers, and E. R. Bleeker. 2002. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. *N. Engl. J. Med.* **346:** 967–974.
26. Almeida, S., M. Fiegenbaum, F. M. de Andrade, M. C. Osório-Wender, and M. H. Hutz. 2006. *ESR1* and *APOE* gene polymorphisms, serum lipids, and hormone replacement therapy. *Maturitas.* **54:** 119–126.
27. Lawlor, D. A., N. Timpson, S. Ebrahim, I. N. M. Day, and G. D. Smith. 2006. The association of oestrogen receptor α-haplotypes with cardiovascular risk factors in the British Women's Heart and Health Study. *Eur. Heart J.* **27:** 1597–1604.
28. Silvestri, S., B. Thomsen, A. Gozzini, Y. Bagger, C. Christiansen, and M. L. Brandi. 2006. Estrogen receptor α and β polymorphisms: is there an association with bone mineral density, plasma lipids, and response to postmenopausal hormone therapy? *Menopause.* **13:** 451–461.
29. NCEP Expert Panel. 2001. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. **285:** 2486–2497.

Downloaded from www.jlr.org by guest, on June 14, 2012