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A Functional Polymorphism in the Glucocorticoid Receptor Gene and Its Relation to Cardiovascular Disease Risk in Familial Hypercholesterolemia

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Context: Individuals with the functional ER22/23EK variant in the glucocorticoid receptor gene are relatively resistant to the downstream consequences of glucocorticoids. Evidence suggests that carriers have a more favorable cardiovascular risk profile, but the relationship between this ER22/23EK variant and cardiovascular disease has not been hitherto assessed.

Objective: We, therefore, determined whether carriership of the ER22/23EK improves cardiovascular disease risk in patients with severe hypercholesterolemia.

Design, Setting, and Participants: In a multicenter cohort study, 2024 patients with heterozygous familial hypercholesterolemia, aged 18 yr and older, were genotyped for the ER22/23EK polymorphism. Patients were identified at lipid clinics throughout The Netherlands between 1989 and 2002.

Main Outcome Measures: The primary outcome measure was cardiovascular disease.

Results: Seventy-six (7.8%) of 977 men and 72 (6.9%) of 1047 women were carriers of the ER22/23EK variant. A total of 395 men and 247 women had a cardiovascular event. In contrast to expected results, we observed no significant association of the ER22/23EK variant with cardiovascular disease risk (men: relative risk, 0.75; 95% confidence interval, 0.50–1.14; $P = 0.2$; women: relative risk, 1.37; 95% confidence interval, 0.82–2.28; $P = 0.2$). However, we found a significant interaction between gender and the polymorphism on cardiovascular disease ($P = 0.02$).

Conclusions: In this large cohort of individuals with very high risk of cardiovascular disease, the association between the functional ER22/23EK polymorphism and cardiovascular risk was not significant overall, although it varied significantly by gender. (***J Clin Endocrinol Metab* 91: 4131–4136, 2006**)

FAMILIAL HYPERCHOLESTEROLEMIA (FH) is an autosomal dominant disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene (1). The prevalence of heterozygotes in Caucasian populations averages about 1:500, positioning FH among the most common monogenetic disorders in man (2). Patients with FH have severely increased LDL cholesterol levels and a pronounced susceptibility to premature cardiovascular disease (CVD) (1, 2). Despite the monogenetic nature of this disorder, however, large variation in CVD risk is observed (3, 4). Age, gender, smoking, body mass index (BMI), and the presence of hypertension and diabetes mellitus contribute to this variation of CVD risk (5, 6). In addition, polymorphisms in candidate genes have also been shown to modulate the expression of the clinical phenotype (7–15), supporting the concept that the hereditary component of CVD is determined by multiple genes, each explaining only a limited proportion of the risk (16). The present challenge is to identify those markers that improve risk prediction in FH to tailor preventive strategies to the individual risk level.

Variants in the glucocorticoid receptor gene have been associated with a plethora of cardiovascular risk factors (17). One of these variants consists of two linked, single-nucleotide changes in codons 22 and 23 of exon 2. The mutation in codon 22 at nucleotide position 198 does not result in an amino acid change [both coding for a glutamic acid (E)], whereas the other sequence change in codon 23 at nucleotide position 200 causes a change from arginine (R) to lysine (K). Therefore, this variant has been named ER22/23EK. Upon dexamethasone suppression testing, carriers of the ER22/23EK variant express higher serum cortisol concentrations as well as a smaller decrease in cortisol levels, suggesting a relative resistance to glucocorticoids (18). Recently, the pathophysiological basis of this resistance was elucidated (19, 20). Alternative translation initiation occurs that results in two isoforms of the glucocorticoid receptor: a long (GR-A) and a short (GR-B) isoform (21). The GR-B protein has stronger gene transcription-activating effects (20). The ER22/23EK polymorphism affects translation, resulting in a shift toward the less active GR-A variant (19, 20). In fact, association studies have shown that carriers have 1) lower plasma total and LDL cholesterol levels, 2) increased insulin sensitivity, 3) beneficial body composition, and 4) lower plasma C-reactive protein levels and a better survival (18, 22, 23).

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Abbreviations: BMI, Body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RR, relative risk.

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These findings suggest that carriers of this functional ER22/23EK variant in the glucocorticoid receptor gene may have a reduced risk of CVD. Nevertheless, this relationship has not been assessed. In the present study, we therefore investigated the effect of this polymorphism on CVD in patients heterozygous for FH.

Patients and Methods

Study design and study population

Between 1989 and 2002, lipid clinics throughout The Netherlands submitted blood samples of 9300 patients who were clinically suspected for FH to a central laboratory for LDL receptor mutation analysis (6). Out of this database, we randomly selected 4000 patients and diagnosed FH according to previously published criteria (6). We excluded subjects with secondary causes of hypercholesterolemia and those with hypercholesterolemia caused by other genetic defects, such as familial defective apolipoprotein B. A total of 2400 unrelated patients aged 18 yr and older fulfilled the diagnostic criteria for heterozygous FH. Over 99% of these patients were Caucasian. The institutional review board of each participating hospital approved the study protocol, and informed consent was obtained from all patients.

Data collection

Patients' medical records were used to acquire information about age, gender, smoking, BMI, and the presence of hypertension (patients with a documented diagnosis using antihypertensive medication or a systolic blood pressure $>$ 140 mm Hg or a diastolic blood pressure $>$ 90 mm Hg at three consecutive office visits) and diabetes mellitus (patients using antidiabetic medication or fasting plasma glucose $>$ 6.9 mmol/liter). Additional information was sought from general practitioners and hospitals that patients had visited formerly and with questionnaires to ensure data completeness.

Lipid levels were determined in fasting patients not using lipid-lowering medication for at least 6 wk. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by standard methods. LDL cholesterol was calculated with the Friedewald formula. Forty patients had triglyceride levels more than 4.5 mmol/liter, and in these individuals LDL cholesterol concentrations were directly measured by standard methods.

CVD and coronary heart disease (CHD) definitions

CVD was defined as coronary, cerebral, or peripheral artery disease using internationally accepted criteria as published before (6). CHD was defined by the presence of 1) myocardial infarction, 2) percutaneous coronary intervention or other invasive procedures, 3) coronary artery bypass grafting, or 4) angina pectoris.

Molecular analysis

DNA was available from 2024 heterozygous FH patients for the present analyses. Genomic DNA was extracted from peripheral blood leukocytes according to a standard protocol (24). The ER22/23EK polymorphism in the glucocorticoid receptor gene was detected by allelic discrimination using TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA), primers (forward, 5′-AGAAGAAAACCCCAGCAGTGT-3′, and reverse, 5′-CAGTAGCTCCTCCTCTTAGGGTTTTA-3′), probes (Applied Biosystems), and a TaqMan ABI Prism 7900 Sequence Detection System (Applied Biosystems). The probes used were 5′-FAM-CACATCTCCCTTTTCCTGA-3′ and 5′-VIC-CACATCTCCCCTCTCCTGA-3′ (Applied Biosystems). Reaction components and amplification parameters were based on the manufacturer's instructions using an annealing temperature of 60 C.

Statistical analysis

All data were analyzed using SPSS for Windows software package version 11.5.0 (SPSS Inc., Chicago, IL). Because the hormone dynamics and the risk of CVD differ considerably between men and women, we stratified our analyses by gender. As expected, the number of ER22/23EK homozygotes was limited; the analyses of heterozygous and homozygous carriers were therefore combined. Contingency tables were used with χ^2 tests to compare observed genotype frequencies with those expected under Hardy-Weinberg equilibrium. Differences between ER22/23EK carriers and noncarriers and men and women were tested with χ^2 statistics for dichotomous variables or independent-sample t test for continuous variables. Statistical testing of triglyceride levels was performed after logarithmic transformation. We used multiple logistic regression analysis to adjust statistical tests for age.

The association between the ER22/23EK polymorphism and the occurrence of CVD and CHD was evaluated using a Cox proportional hazard regression analysis. The proportional hazards assumption was tested by drawing log minus log plots of the survival function and was met for all Cox proportional hazard models. Follow-up started at birth and ended at the first occurrence of established fatal or nonfatal CVD. Patients without CVD were censored at the date of the last lipid clinic visit or at the date of death attributable to causes other than CVD. In the primary model, we adjusted for year of birth and smoking. Additional variables, which had significant effects on CVD risk in univariate Cox regression analyses, were investigated in the secondary model. Young patients with the polymorphism may not have had the chance to express their high CVD risk. Hence, inclusion of young carriers of the polymorphism may cause underestimation of CVD risk. Therefore, we tested the effect of age by adjusting for age tertiles in the Cox regression analysis. The interaction between the ER22/23EK variant and gender in the total population was statistically tested in the Cox regression analysis with adjustment for variables that were significantly different between genders. Throughout, a two-tailed P value of < 0.05 was interpreted as statistically significant.

Results

Patient characteristics

In 977 men heterozygous for FH, 76 (7.8%) individuals were heterozygous for the ER22/23EK polymorphism, but no homozygous carriers were detected. In 1047 women heterozygous for FH, 70 (6.7%) were heterozygous for the ER22/23EK variant whereas two (0.2%) were homozygous. In women with CVD, the ER22/23EK variant deviated from Hardy-Weinberg equilibrium ($\chi^2 = 9.182$; $P = 0.002$) because of the small number of homozygotes; omitting one of them resulted in equilibrium. The genotype distributions in the total cohort and in all other subgroups (Table 1) did not differ from Hardy-Weinberg equilibrium ($\chi^2 < 1.610$; $P > 0.2$).

The clinical and biochemical characteristics of carriers and noncarriers of the ER22/23EK polymorphism are presented in Table 2. In men, no differences existed between patients with and without ER22/23EK. Female carriers of ER22/23EK were 4.6 ± 1.7 (SD) yr younger at the first lipid clinic visit compared with women without the polymorphism ($P = 0.006$). Other variables were not significantly different between female carriers and noncarriers.

Males visited the lipid clinic 2.3 ± 0.6 (SEM) yr earlier, were more often smokers (difference, 12.0 ± 2.1%), and had a higher BMI (difference, 0.7 ± 0.2 kg/m^2), lower plasma LDL cholesterol (difference, 0.24 ± 0.09 mmol/liter) and HDL cholesterol (difference, 0.23 ± 0.02 mmol/liter) levels, and higher plasma triglyceride concentrations (difference, 0.34 ± 0.05 mmol/liter) compared with women, all as expected.

Risk factors for CVD

During 44,044 person-years, 395 (40.4%) men had onset of CVD, and a total of 247 (23.6%) women had their first cardiovascular event during 51,331 person-years. The mean age

TABLE 1. Genotype and allele frequencies for the ER22/23EK polymorphism in the glucocorticoid receptor gene

	Men [n (%)]		Women [n (%)]	
	CVD	No CVD	CVD	No CVD
Genotypes				
Wild type	368 (93.2)	533 (91.6)	231 (93.5)	744 (93.0)
Heterozygous ER22/23EK	27 (6.8)	49 (8.4)	14 (5.7)	56 (7.0)
Homozygous ER22/23EK	0 (0.0)	0 (0.0)	2 (0.8)	0 (0.0)
Total	395 (100.0)	582 (100.0)	247 (100.0)	800 (100.0)
Alleles				
Wild type allele	763 (96.6)	1115 (95.8)	476 (96.4)	1544 (96.5)
ER22/23EK allele	27 (3.4)	49 (4.2)	18 (3.6)	56 (3.5)
Total	790 (100.0)	1164 (100.0)	494 (100.0)	1600 (100.0)

of onset of CVD (± SD) was 45.7 ± 9.3 yr in men and 52.8 ± 11.6 yr in women.

Separate analyses of a number of variables, which may act as confounder or intermediate trait in the relationship between the ER22/23EK polymorphism and CVD risk, are presented in Table 3. In men and women, year of birth, smoking, and lower plasma HDL cholesterol concentrations were significantly associated with increased CVD risk. The presence of diabetes mellitus also contributed to an increased risk of CVD in women but not in men. Hypertension, BMI, plasma LDL cholesterol levels, and triglyceride concentrations were not significantly associated with CVD among men or women.

CVD and the ER22/23EK polymorphism

In the total population, we found no significant association between the ER22/23EK variant and CVD with adjustment for year of birth, gender, and smoking [relative risk (RR) 0.92; 95% confidence interval (CI), 0.67–1.27; $P = 0.6$). Unadjusted survival rates of men and women with and without the ER22/23EK polymorphism are shown in Table 4. Figure 1 illustrates the association between the ER22/23EK polymorphism and the cumulative incidence of CVD during lifetime follow-up in patients with FH after adjustment for year of birth and smoking and stratified by gender. The ER22/23EK polymorphism was not significantly associated with CVD in either gender; male carriers had a 0.75 (95% CI, 0.50–1.14; $P = 0.2$) times decreased risk of CVD, whereas female carriers had a 1.37 (95% CI, 0.82–2.28; $P = 0.2$) times increased CVD risk. As shown in Table 5, additional adjustment of plasma HDL cholesterol concentrations in men and plasma HDL cholesterol concentrations and diabetes mellitus in women did not change the results. To test for effects of the ER22/23EK variant on CVD risk with age, we repeated the multiple Cox regression analyses of the primary model with additional adjustment for age tertiles. After this additional adjustment, the CVD risk estimates remained virtually identical in men (RR, 0.75; 95% CI, 0.49–1.13; $P = 0.2$) and in women (RR, 1.41; 95% CI, 0.84–2.35; $P = 0.2$).

Although we found no significant relationship between the ER22/23EK variant and CVD overall, the effect of the polymorphism on CVD risk seemed opposite in men and women. Therefore, we tested the interaction between the ER22/23EK variant and gender in the total population using Cox regression analysis with adjustment for variables that were significantly different between genders (age, smoking, BMI, and plasma LDL cholesterol, HDL cholesterol, and triglyceride levels). Indeed, the effect of the ER22/23EK variant on CVD risk was significantly different for men and women ($P = 0.02$).

CHD and the ER22/23EK polymorphism

CHD was the most frequent cardiovascular event; in 350 men (88.6%) and 200 women (81.0%), CHD was the first event. Cerebral artery disease occurred as the first event in only 20 men (5.1%) and 20 women (8.1%); in 25 men (6.3%)

TABLE 2. Characteristics of male and female FH patients with and without the ER22/23EK polymorphism in the glucocorticoid receptor gene

	Men			Women		
	Noncarriers (n = 901)	ER22/23EK carriers (n = 76)	*P* value[a]	Noncarriers (n = 975)	ER22/23EK carriers (n = 72)	*P* value[a]
Age at first lipid clinic visit (yr)	43.6 ± 0.4	43.3 ± 1.2	0.8	46.2 ± 0.4	41.5 ± 1.6	0.006
Age at last lipid clinic visit (yr)	48.7 ± 0.4	47.7 ± 1.2	0.4	51.0 ± 0.5	47.7 ± 1.7	0.07
Smoking, ever (%)	79.3 ± 1.4	76.1 ± 5.1	0.6	67.7 ± 1.6	57.6 ± 6.1	0.08
Hypertension (%)	8.6 ± 0.9	6.8 ± 2.9	0.6	10.1 ± 1.0	8.5 ± 3.3	0.9
Diabetes mellitus (%)	5.5 ± 0.8	3.9 ± 2.2	0.7	6.3 ± 0.8	6.9 ± 3.0	0.6
BMI (kg/m^2)	25.5 ± 0.1	25.7 ± 0.4	0.5	24.8 ± 0.1	24.3 ± 0.5	0.4
Total cholesterol (mmol/liter)	9.36 ± 0.07	9.41 ± 0.24	0.8	9.67 ± 0.06	9.76 ± 0.28	0.4
LDL cholesterol (mmol/liter)	7.24 ± 0.07	7.22 ± 0.26	0.9	7.46 ± 0.07	7.70 ± 0.27	0.2
HDL cholesterol (mmol/liter)	1.09 ± 0.01	1.14 ± 0.04	0.3	1.32 ± 0.01	1.30 ± 0.05	0.6
Triglycerides (mmol/liter)	1.96 ± 0.03	2.15 ± 0.11	0.4[b]	1.64 ± 0.03	1.64 ± 0.13	0.9[b]

Values are given as means ± SEM. SI conversion factors: to convert total cholesterol, LDL cholesterol, and HDL cholesterol to mg/dl, multiply by 38.67; for triglycerides, multiply by 89.15.

[a] Additional adjustment for age.

[b] Statistical testing after logarithmic transformation.

TABLE 3. Risk factors of CVD in 977 men and 1047 women with familial hypercholesterolemia

Variables	Men			Women		
	RR	95% CI	*P* value	RR	95% CI	*P* value
Year of birth	1.05	1.04–1.07	<0.001	1.08	1.06–1.10	<0.001
Smoking	1.56	1.14–2.15	0.006	1.53	1.15–2.05	0.004
Diabetes mellitus	1.06	0.76–1.48	0.7	1.68	1.19–2.37	0.004
Hypertension	1.32	1.00–1.75	0.05	1.10	0.79–1.52	0.6
BMI	1.00	0.97–1.04	0.8	1.04	1.00–1.08	0.07
LDL cholesterol	0.99	0.92–1.06	0.7	1.00	0.93–1.08	0.9
HDL cholesterol	0.53	0.35–0.80	0.002	0.47	0.29–0.75	0.001
Triglycerides	1.15	0.99–1.35	0.08[a]	1.02	0.83–1.26	0.8[a]

[a] Statistical testing after logarithmic transformation.

and 27 women (10.9%), the first event was peripheral artery disease.

In the total population, again we observed no significant relationship between the polymorphism and CHD after adjustment for year of birth, gender, or smoking (RR, 0.93; 95% CI, 0.67–1.31; $P = 0.7$). With adjustment for year of birth and smoking, the ER22/23EK polymorphism was not significantly associated with CHD in men (RR, 0.72; 95% CI, 0.46–1.12; $P = 0.1$) or women (RR, 1.60; 95% CI, 0.94–2.73; $P = 0.09$). Adjustment for additional variables resulted in similar risk estimates (Table 5). In line with the results for CVD, the effect of the ER22/23EK variant on CHD risk was significantly different between men and women ($P = 0.006$).

Discussion

We hypothesized that the functional ER22/23EK variant in the glucocorticoid receptor gene might decrease CVD risk. In this large cohort at severely increased risk, however, the association between the ER22/23EK variant and the occurrence of CVD or CHD was not significant overall. Nonetheless, in a *post hoc* analysis, we found opposite effects of the polymorphism on CVD and CHD in men and women.

Exogenous as well as endogenous glucocorticoid excess in humans contributes to the development of hypertension, dyslipidemia, impaired glucose tolerance, and central adiposity, and may alter thrombosis and fibrinolysis (25, 26). Glucocorticoids exert their function primarily via binding to the cytoplasmic glucocorticoid receptor, which then transfers into the nucleus where it enhances or represses transcription of specific target genes (27). The ER22/23EK polymorphism in the glucocorticoid receptor gene results in a relative glucocorticoid resistance and has been associated with a beneficial cardiovascular risk profile defined by lower plasma total and LDL cholesterol levels, increased insulin sensitivity, and beneficial body composition (18, 21). In addition, carriers had lower plasma C-reactive protein levels as well as better overall survival (22). In our FH cohort, 7.3% of the individuals carried at least one copy of the variant, in concordance with genotype frequencies described in previous studies (5.2–8.9%). In contrast to expected results, we found no significant association between the ER22/23EK polymorphism and CVD or CHD risk. The severe hypercholesterolemia in our patients might offer a clue as to why we observe these results. The potential beneficial effect of this polymorphism may be partly a result of lower LDL cholesterol levels (18). We did not observe, however, such differences; the dominant dyslipidemia of FH may outweigh potential beneficial effects on cholesterol metabolism. In the general population, variants in the glucocorticoid receptor gene may be better risk predictors for CVD. Alternatively, we made a type II error in the separate analyses of men and women; differences were not observed with statistical significance because of lack of power because of small numbers. The results of our interaction analysis between the polymorphism and gender in the total population support the latter alternative. Based on literature, we had *a priori* hypothesized that the ER22/23EK variant decreased CVD risk, and the study was powered to detect a significant association between the polymorphism and cardiovascular risk in the total population.

Although we found no significant relationship between the ER22/23EK polymorphism and CVD or CHD overall, the influence of the genetic variant appears to be different between sexes. This variant has been preferentially analyzed in male individuals, and most association studies did not analyze men and women separately. In a cohort of young adults followed from the age of 13–36 yr, the body composition in carriers of the ER22/23EK variant was indeed different between sexes; only male carriers were taller and leaner and had more muscle strength (21). The reason for these differential effects of the ER22/23EK variant between men and women is yet unknown. In rodents, the hypothalamic-pituitary-adrenal axis responds to variations in circulating sex

TABLE 4. CVD-free survival of patients with familial hypercholesterolemia according to the ER22/23EK polymorphism in the glucocorticoid receptor gene

	Men		Women	
	Noncarriers	ER22/23EK carriers	Noncarriers	ER22/23EK carriers
CVD-free survival[a]				
40 yr	62.9 ± 1.9	63.0 ± 6.5	87.1 ± 1.3	85.7 ± 5.2
50 yr	35.2 ± 2.4	43.9 ± 8.5	70.1 ± 2.0	63.3 ± 8.7
60 yr	20.5 ± 2.9	31.4 ± 12.2	49.2 ± 2.8	41.0 ± 11.8
Median survival time	54.7	56.8	69.6	66.0

[a] Cumulative CVD-free survival probability ± SEM.

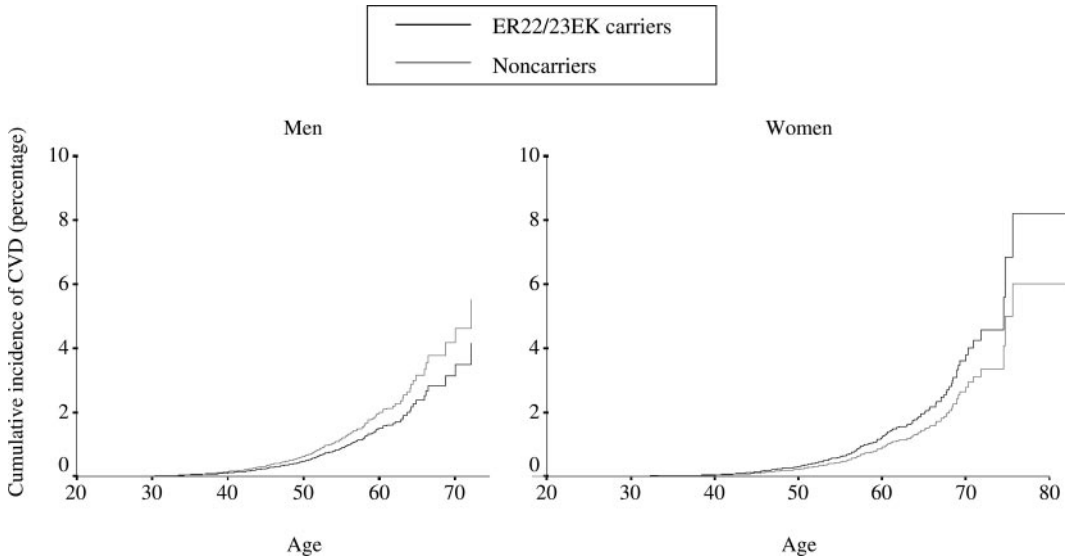

FIG. 1. CVD hazard curve according to the ER22/23EK polymorphism in 977 men and 1047 women with heterozygous FH.

steroid concentrations (28). This regulation differs between sexes; estrogen primarily exerts stimulatory effects on stress-induced ACTH and glucocorticoid release, whereas testosterone inhibits stress-related hypothalamic-pituitary-adrenal axis activity (28–30). As speculated previously, in a relative glucocorticoid resistance condition, as is the case for ER22/23EK carriers, the high circulating estrogen levels in women may annul the potential beneficial effects of the ER22/23EK variant (21). Otherwise, high variability in the expression of genes located at the X chromosome between men and women has been reported and may also account for these gender differences (31). An example of such a gene is Mediator subunit MED14. Garabedian and co-workers (32, 33) found that MED14 interacts with the glucocorticoid receptor and increases its transcriptional activation in a gene-specific manner. Interestingly, MED14 is X-linked and fails to undergo X-chromosome inactivation. Therefore, the researchers suggested that MED14 levels could be higher in females than in males, which may represent a mechanism underlying gender-specific differences in the expression of glucocorticoid receptor target genes (33). These observations support that separate analyses of men and women are indicated for studies on the ER22/23EK variant.

The monogenetic background but large variation in CVD risk determines the strength of the present study. Patients with heterozygous FH have 8.5 times increased cardiovascular risk (4). The atherosclerotic burden of the disorder, however, exhibits wide variation, and many untreated FH patients experience little or no excess mortality (3). As in the general population, CVD in FH patients is the result of a dynamic interplay among multiple genes in addition to gene-environment interactions (3–5). The disorder is therefore considered to be an exemplary model to analyze secondary (or modifier) genes involved in CVD.

However, there are also some limitations to our association study. It depended on medical records, questionnaires, and information retrospectively obtained from physicians as the primary source of data. Certain information of interest, such as postmenopausal status, was not available. Furthermore, the influence of the ER22/23EK polymorphism on potential intermediate traits (*e.g.* dexamethasone suppression test, insulin and C-reactive protein levels, body composition, and

TABLE 5. Relative risks for CVD and CHD according to ER22/23EK genotype

Genotype	RR (95% CI)			
	Univariate	Model 1	Model 2	Model 3
Men CVD				
Wild type	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
ER22/23EK	0.86 (0.58–1.27)	0.75 (0.50–1.14)	0.73 (0.46–1.17)	0.75 (0.49–1.13)
Women CVD				
Wild type	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
ER22/23EK	1.27 (0.76–2.10)	1.37 (0.82–2.28)	1.35 (0.74–2.44)	1.41 (0.84–2.35)
Men CHD				
Wild type	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
ER22/23EK	0.83 (0.55–1.25)	0.72 (0.46–1.12)	0.72 (0.44–1.19)	0.71 (0.46–1.11)
Women CHD				
Wild type	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
ER22/23EK	1.45 (0.86–2.45)	1.60 (0.94–2.73)	1.62 (0.86–3.02)	1.64 (0.96–2.80)

Model 1, adjusted for year of birth and smoking; model 2, adjusted for year of birth, smoking, and HDL cholesterol levels in men, and adjusted for year of birth, smoking, HDL cholesterol levels, and diabetes in women; model 3, adjusted for year of birth, smoking, and age tertiles.

hormone levels) could not be determined. Our study included patients that were referred to lipid clinics, and this could lead to selection bias in two different ways. First, patients with the most detrimental genetic profiles might have died before referral, although we did not observe such premature deaths in a previously reported mortality analysis (3). Nonetheless, we cannot exclude that polymorphisms causing early death could have been missed, leading to underestimation of the risk. Second, patients that presented themselves with premature symptoms of atherosclerosis are more easily referred to lipid clinics; this could lead to selection on CVD in our cohort study. However, we used data of a nationwide screening program, and to prevent selection biases, we selected only patients from the 48 larger outpatient lipid clinics that are characterized by clinically more diverse patient populations. Finally, our findings in heterozygous FH cannot be extrapolated to other populations. The observed opposite effects of the ER22/23EK variant on CVD and CHD in our FH cohort should be validated or excluded on wider population-based studies.

In conclusion, in this large, retrospective cohort study among patients with heterozygous FH, the association between the functional ER22/23EK polymorphism and CVD risk or CHD risk was not significant overall, although it varied significantly by gender.

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