Published in final edited form as:

J Clin Lipidol. 2016 ; 10(1): 101–108.e3. doi:10.1016/j.jacl.2015.09.003.

Premature Coronary Heart Disease and Autosomal Dominant Hypercholesterolemia: Increased Risk in Women with *LDLR* Mutations

Zahid Ahmad, MD^{1,3}, Xilong Li, MS², Jedrek Wosik, MD³, Preethi Mani, MD³, Elisabeth Joye Petr, MD³, George McLeod, MD³, Shatha Murad, MD³, Li Song, MD³, Beverley Adams-Huet, MS², and Abhimanyu Garg, MD^{1,3}

¹Division of Nutrition and Metabolic Diseases, Center for Human Nutrition, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390

²Department of Clinical Sciences, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390

³Department of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390

Abstract

Background—For patients with autosomal dominant hypercholesterolemia (ADH), it remains unclear whether differences exist in the risk of premature coronary heart disease (CHD) between patients with confirmed mutations in low-density lipoprotein receptor (*LDLR*) vs those without detectable mutations.

Objective—This study sought to assess the risk of premature CHD in ADH patients with mutations in *LDLR* (referred to as Familial Hypercholesterolemia, FH) vs those without detectable mutations (unexplained ADH), stratified by sex.

Methods—Comparative study of premature CHD in a multiethnic cohort of 111 men and 165 women meeting adult Simon-Broome criteria for ADH.

Results—Women with FH (n = 51) had an increased risk of premature CHD compared to unexplained ADH women (n = 111) (hazard ratio [HR] 2.74 [95% CI: 1.40, 5.34], p = 0.003) even after adjustment for lipid levels and traditional CHD risk factors (HR 2.53 [1.10, 5.83], p = 0.005). Men with FH (n = 42), in contrast, had a similar risk of premature CHD when compared to unexplained ADH men (n = 66) (unadjusted: HR 1.48 [0.84, 2.63], p = 0.18; adjusted: HR 1.04

Corresponding Authors: Zahid Ahmad, MD, Address: 5323 Harry Hines Blvd, Mail Code 8537, Dallas, TX 75390, Phone: 214 648 2377, Zahid.ahmad@utsouthwestern.edu, Abhimanyu Garg, MD, Address: 5323 Harry Hines Blvd, Mail Code 8537, Dallas, TX 75390, Phone: 214 648 2895, Abhimanyu.garg@utsouthwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DISCLOSURE SUMMARY: Z.A. has received honorarium for educational talks sponsored by Sanofi and Genzyme; and participated in advisory board meetings sponsored by Genzyme and Regeneron. AG received research grants from Pfizer, Bristol-Myers-Squibb and Astra-Zeneca, Aegerion and is a consultant for Bristol-Myer Squibb, Astra Zeneca, Amgen and Eli Lilly.

[0.46, 2.37] $p = 0.72$). To address whether mutation status provides additional information beyond LDL-C level, we analyzed premature CHD risk for FH vs unexplained ADH at each quartile of LDL-C: the findings were significant for women (Q1: HR 4.90 [1.69-14.19]; Q2: HR 3.44 [1.42-8.32]; Q3: HR 2.79 [1.25-6.23]; Q4: HR 1.99 [0.95-4.17]), but not for men.

Conclusion—Our findings suggest that genetic confirmation of ADH may be important to identify patient's risk of CHD, especially for female *LDLR* mutation carriers.

Keywords

familial hypercholesterolemia; lipids; premature coronary heart disease; LDLR; APOB

INTRODUCTION

Autosomal dominant hypercholesterolemia is characterized by severe elevations in low-density lipoprotein-cholesterol (LDL-C) with a concomitant 10-20 fold-increased risk of premature coronary heart disease (CHD) compared with the general population (1).

Thus far, three genes have been found to cause the disorder: low density lipoprotein receptor (*LDLR*, Online Mendelian Inheritance in Man [OMIM] # 143890, referred to as having familial hypercholesterolemia [FH]), apolipoprotein B-100 (*APOB*, OMIM # 107730, referred to as familial defective APOB), and proprotein convertase subtilisin-like kexin type 9 (*PCSK9*, OMIM # 603776, referred to as FH3) (2). Among patients with detectable mutations, *LDLR* mutations (FH) represent ~90% of cases, and recent large-scale exome sequencing studies have identified *LDLR* mutations as the most common genetic defect among all individuals with premature CHD (3).

In ADH cohorts, mutation detection rates vary - as high as 90% in ethnically homogenous populations (4-8) and as low as 40% in our ongoing study of ADH in a multiethnic US cohort (9). For the 10-60% of ADH patients without detectable causal mutations (unexplained), a few differences have been reported when compared with FH patients: unexplained ADH patients have lower baseline LDL-C levels, higher high density lipoproteincholesterol (HDL-C) levels, and lower prevalence of tendon xanthomas (6,9-14).

It remains unclear whether differences in CHD risk exist between FH and unexplained ADH patients. Prior investigations failed to compare FH patients with unexplained ADH patients, and rather focused on CHD risk only among FH patients (e.g. those with LDL-C ≥ 309 mg/dL vs LDL-C < 309 mg/dL (15) or those with *LDLR* missense vs *LDLR* null mutations (16)).

Our previous analysis suggested a trend towards increased CHD risk in FH patients (9). Here, we expand our comparison of CHD risk in FH vs unexplained ADH patients. Because sex differences exist in lipoprotein levels (e.g. HDL-C) and in the pathophysiology, risk factors, and clinical presentation of CHD (17,18), we stratified by sex and adjusted for additional confounders such as lipid levels and CHD risk factors.

METHODS

Patients

All patients gave written informed consent, and the Institutional Review Board of UT Southwestern Medical Center approved the protocol.

ADH patients were ascertained from specialty lipid clinics in the Dallas, Texas area as previously described (9). All patients were 18 years or older. We used modified Simon-Broome criteria (19): pretreatment LDL-C was greater than the 95th percentile for age and sex (corresponding to 190 mg/dL for most adults), with one of the two following criteria: 1) tendon xanthoma (proband or 1st degree relative), or 2) either 1st degree relative with premature CHD (less than 55 years in men or 65 years in women) or pretreatment LDL-C greater than the 95th percentile for age and sex. When 1st degree relatives were not willing to participate in the study (which occurred for 69% of probands), an autosomal dominant inheritance pattern was inferred based on family history of hypercholesterolemia in more than one generation. Exclusion criteria included any secondary cause of the dyslipidemia (e.g. obstructive liver disease, hypothyroidism or nephrotic syndrome).

In accordance with standardized definitions (20), a patient had CHD if they had a history of acute myocardial infarction or myocardial ischemia, unstable angina, stable angina pectoris, or coronary revascularization (coronary artery bypass graft surgery or percutaneous coronary intervention). CHD events after age 55 years in men or age 65 years in women were excluded from this study. Medical charts were reviewed by physicians, unaware of genetic screening results, to confirm the age and diagnosis of CHD as well as to collect additional data on CHD risk factors such as diabetes or hypertension. Diabetes and hypertension were considered present if diagnosed by a physician using standard diagnostic methods (e.g. diabetes: fasting plasma glucose values ≥ 126 mg/dL, two-hour post oral glucose challenge values ≥ 200 mg/dL, or hemoglobin A1C values $\geq 6.5\%$). Menopausal status and smoking status (current, former, pack-years) were self-reported.

All patients were examined for tendon xanthomas by the study investigators; xanthomas were considered to be present if tendons were diffusely enlarged or had focal nodularity.

Candidate Gene Analysis

All 18 exons and the flanking intronic regions of *LDLR* and exon 26 of *APOB* were amplified and sequenced (Sanger) as previously described (9). Deletions and duplications of one or more exons of *LDLR* were detected with the SALSA Multiplex Ligation-dependent Probe Amplification (MLPA) kit.

Lipids and Lipoproteins

Medical charts were reviewed to obtain untreated baseline lipid levels. All centers measured fasting total cholesterol, triglycerides and HDL-C using enzymatic assays in commercial laboratories. LDL-C was estimated with the Friedewald equation.

Statistical analysis

Comparisons of quantitative variables were made with two-way analysis of variance models to simultaneously test for group and sex effects and for interaction between group and sex. Categorical variable comparisons between groups were made with Cochran-Mantel-Haenszel stratifying by sex; homogeneity of strata was assessed with the Breslow-Day test. Cox proportional hazard regression models (Cox PH) were used for comparing risk of premature CHD and censoring age at 55 years for men and 65 years for women (ages corresponding to the definition of premature CHD (21) and to control for LDL-C, HDL-C, diabetes, hypertension, and smoking. Survival functions were derived with Kaplan-Meier estimators and Cox PH model estimates. Continuous variables are summarized as mean (SD) and median unless otherwise specified. A two-sided p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Because of the small number (n = 6) of ADH patients with *APOB* mutations - who usually display a milder phenotype than FH patients (2) - comparative analyses included only patients with *LDLR* mutations (FH) vs unexplained ADH patients.

RESULTS

Patient Population

Two hundred seventy six unrelated patients with ADH (111 men and 165 women; 83 Non-Hispanic Caucasian, 109 African-American, 54 Hispanics and 30 others) met entry criteria and agreed to participate. Heterozygous *LDLR* mutations were found in 93 patients via Sanger sequencing or MLPA. Similar to our previous findings (9), nearly all the *LDLR* mutations were reported as pathogenic in the University College London *LDLR* FH database (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/) (22). Since *LDLR* mutation type (e.g. null vs missense) can influence ADH phenotype (23), we investigated for differences in mutation types between men and women (Supplementary Table 1). No differences existed in mutation types between sexes: missense (%) men 48 vs women 63; null (%), including nonsense, copy number, splice site, and frameshift mutations) men 52 vs women 37, p = 0.21.

Sequencing of exon 26 of *APOB* revealed 6 patients with a p.R3527Q mutation (3 men, 3 women; mean $\pm$ SD age 51 ± 20 years, baseline total cholesterol 348 ± 55 mg/dL, HDL-C 67 ± 17 mg/dL, LDL-C 252 ± 52 mg/dL, median [IQR] triglycerides 113 [84-186] mg/dL, 0% xanthomas, 0% premature CHD).

No mutations were detected in 177 patients (unexplained ADH).

FH vs “Unexplained ADH” in the Overall Cohort

FH patients, as compared to unexplained ADH patients, were younger (mean $\pm$ SD: 51 ± 12 vs 56 ± 11 years, p = 0.0006), had higher LDL-C levels (297 ± 64 vs 240 ± 40 mg/dL; p < 0.0001), lower levels of HDL-C (49 ± 15 vs 54 ± 16 mg/dL, p = 0.008) and a higher prevalence of tendon xanthomas (42 vs 16 %, p < 0.0001). No differences existed in body mass index, (BMI, 31 ± 6 vs 31 ± 6 kg/m², p = 1.0), ethnicity/race (African American 37 vs 42%; non-Hispanic Caucasian 28 vs 29%; Hispanic 26 vs 16%; others 7 vs 12%; p = 0.21), and prevalence of traditional CHD risk factors (diabetes 27 vs 28 %, p = 1.0; hypertension

63 vs 68%, $p = 0.49$; and smoking – current and former, 41 vs 46%, $p = 0.60$). FH patients, compared to unexplained ADH patients, had a higher hazard ratio for CHD (2.24 [1.45, 3.48], $p = 0.003$; Supplementary Figure 1).

FH vs “Unexplained ADH,” Stratified by Sex

To address confounding, we stratified the cohorts by sex (Table 1). No differences existed in BMI, ethnicity/race, prevalence of traditional CHD risk factors (diabetes, hypertension and smoking), and prevalence of peripheral arterial disease or cerebrovascular disease between FH and unexplained ADH patients after stratification.

FH men were of similar age as unexplained ADH men. At the time of enrollment, treated LDL-C levels remained higher in FH men compared to those in unexplained ADH patients (147 ± 49 vs 123 ± 43 mg/dL, $p < 0.05$, Supplementary Table 2), although both groups had similar % LDL-C reduction (-49 ± 13 vs -49 ± 17) and were treated with similar lipid-lowering drugs (Supplementary Table 3). The prevalence of tendon xanthomas was nearly triple in those with FH (46% vs 16%, $p = 0.002$). Cox PH analysis revealed a similar hazard ratio (HR) of premature CHD between FH and unexplained ADH men (HR [95% CI]: 1.48 [0.84- 2.63], $p = 0.18$, Figure 1A). After adjusting for LDL-C, HDL-C, and the prevalence of traditional CHD risk factors (diabetes, hypertension, smoking), the analysis remained nonsignificant (HR 1.04 [0.46-2.37] $p = 0.72$, Figure 1C, Supplementary Table 4).

FH women were younger than unexplained ADH women (51 ± 13 vs 58 ± 9 years, $p < 0.001$), and fewer FH women were post-menopausal (63% vs 88%, $p < 0.001$). Treated LDL-C levels were similar in FH and unexplained ADH patients (143 ± 37 vs 131 ± 39 mg/dL), although FH women had slightly more % LDL-C reduction (-52 ± 12 vs -46 ± 13 mg/dL, $p < 0.05$) and were on more aggressive treatment (Supplementary Table 3, % statin use 80 vs 22%, $p < 0.01$; % statin + ezetimibe use 28 vs 10%, $p < 0.01$). The prevalence of tendon xanthomas was nearly double in those with FH (38% vs 16%; $p = 0.003$). Cox PH analysis indicated FH women to have higher risk of premature CHD when compared to unexplained ADH women (HR 2.74 [1.40-5.34], $p = 0.003$, Figure 1B). After adjusting for LDL-C, HDL-C, and the prevalence of traditional CHD risk factors (diabetes, hypertension, smoking), the analysis remained significant (HR 2.53 [1.10-5.83], $p = 0.005$, Figure 1D, Supplementary Table 5). Because of the difference in % post-menopausal between the groups, we performed a subgroup analysis of only post-menopausal women ($n = 31$ FH and $n = 92$ Unexplained ADH) adjusted for LDL-C; the results (HR 2.85 [1.01-8.02]) resembled the entire group of women (HR 2.74 [1.40-5.34]).

Risk of CHD in FH Men vs FH Women, Unexplained ADH Men vs Unexplained ADH Women

In unadjusted analysis of both FH and unexplained ADH groups, the HR for premature was lower in women than men (FH women vs men, HR 0.44 [0.22, 0.88], $p = 0.02$, Figure 2B; unexplained ADH women vs men, HR 0.18 [0.09, 0.36], $p < 0.0001$, Figure 2A). After adjusting for LDL-C, HDL-C, and the prevalence of traditional CHD risk factors (diabetes, hypertension, smoking), the comparison was nonsignificant in FH patients (women vs men HR 0.81 [0.35, 1.84], $p = 0.61$, Figure 2D), suggesting that men and women with FH have similar chances of suffering from premature CHD. The major variable that accounted for

this adjusted nonsignificant HR was HDL-C (HR 0.95 [0.91, 0.99], $p = 0.008$). In contrast, the adjusted analysis in unexplained ADH men vs women remained significant (HR 0.16 [0.07, 0.37], $p = 0.002$, Figure 2C).

Interaction between *LDLR* and LDL-C on risk of Premature CHD

To address whether the identification of a *LDLR* mutation provides any additional ability to predict the presence of premature CHD beyond knowing the LDL-C level, we analyzed premature CHD risk for FH compared to unexplained ADH at each quartile of LDL-C (Figure 3). In men, the premature CHD failed to achieve significance in any LDL-C quartile (Q1: HR 1.16 [0.46-2.90]; Q2: HR 1.27 [0.59-2.73]; Q3: HR 1.35 [0.65-2.78]; Q4: HR 1.53 [0.68-3.42]). For women the risk was inversely related to LDL-C levels for all quartiles of LDL-C (Q1: HR 4.90 [1.69-14.19]; Q2: HR 3.44 [1.42-8.32]; Q3: HR 2.79 [1.25-6.23]; Q4: HR 1.99 [0.95-4.17]).

DISCUSSION

The key finding from our analyses, that women with mutations in *LDLR* (FH) have a 2.5 fold higher risk of premature CHD compared to women with unexplained ADH, identifies an additional phenotypic difference between women with and without identifiable ADH mutations. The risk of premature CHD in FH women resembled that of men (after adjustment for CHD risk factors) and remained significant even in the post-menopausal subgroup. FH women in the lowest LDL-C quartile had almost 4 fold higher risk of CHD compared to unexplained ADH women, suggesting that knowledge of mutation status may add information for patients with lower ADH-range LDL-C levels. We expected similar findings in ADH men, yet no difference existed.

Only one prior publication, from a subset of a larger French ADH cohort, reported a higher prevalence of premature CHD in FH patients ($n=65$) compared to unexplained ADH patients ($n = 27$) (68% vs 41%, $p = 0.016$) (10). The study lacked sex stratification, survival analysis, or adjustment for lipid levels and other CHD risk factors. Similarly, most other studies of CHD in ADH patients lacked robust comparative analyses(10,23-25), genetic testing (26-28), stratification by sex (10,24,25), adjustment for traditional risk factors(10,23-25), or sufficient number of unexplained ADH patients (6).

Several factors may explain our discrepant observations in men vs women. First, FH women may receive less aggressive treatment than men due to the teratogenicity of lipid lowering medications or due to assumptions that all premenopausal women are protected against CHD. In general, premenopausal women rarely receive treatment for dyslipidemia (29) and anecdotally, FH women are not taken seriously when presenting with chest pain (30) and have even suffered acute myocardial infarctions during pregnancy (31). Second, our cohort of FH men may be missing severe cases that died at younger ages from sudden cardiac death. Unlike women, FH and unexplained ADH men had similar ages at the time of enrollment into our study, a finding that raises suspicion for survival bias. Third, *LDLR* mutations may mitigate the protection against CHD that healthy women enjoy during premenopausal years; if true, genetic testing may have benefit in young women but not men.

A future prospective population-based or cohort study may help confirm that genetic testing plays a role in predicting CHD outcomes and also if CHD differences exist in men.

Regarding the need of genetic testing in ADH patients, guidelines differ (1,32,33) and most studies have focused only on the psychological benefits and drawbacks. Thus far, genetic testing for ADH has been shown to improve patients' perceived accuracy of diagnosis (34), but few other benefits have been established. Our data suggest a potential utility for genetic testing as a CHD risk factor in ADH women above and beyond a clinical diagnosis.

Some limitations of our study merit comment. First, our lack of findings in men may be due to insufficient power from the smaller size of the male subgroup. Assuming larger sample sizes would achieve statistical significance, the effect size of CHD risk in FH men would still be less than half of FH women (men's adjusted HR 1.04 vs women's adjusted HR 2.53), suggesting a sex difference exists. Second, our study is retrospective and lacks control over variables; to address this in our survival analyses, we adjusted for possible confounding variables. Third, we did not sequence *PCSK9*, *LDLR* introns for cryptic mutations, or more recently implicated ADH genes - signal transducing adaptor family member 1 (*STAP1*) (35) and apolipoprotein E (*APOE*). These are thought to be extremely rare causes of ADH (6,11,13,24,36-40) and in our earlier ADH cohort, we failed to identify any patients with *PCSK9* mutations (9). Thus, it's unlikely that our data would be altered significantly by the presence of such patients. Fourth, our study lacked measurements of nontraditional CHD risk factors such as lipoprotein (a), phospholipase associated A2, and C-reactive protein. However, predictability of these risk factors above and beyond the traditional CHD risk factors remains controversial (41).

For most patients we recruited, family members were unwilling or unavailable to participate, which limited our ability to confirm autosomal dominant inheritance patterns and could result in the inclusion of phenocopies. Despite fulfilling clinical criteria for ADH, unexplained ADH patients may suffer from hypercholesterolemia due to the influence of polygenic (42) and environmental factors (e.g. high saturated fat, *trans* monounsaturated fat, and cholesterol intake; weight gain; and menopausal status in women). Also, we cannot exclude race/ethnicity as a confounder. Overall, though, our experience represents real-world US care: families may be geographically separated; few healthcare models address screening of relatives; and the overall US population is multiethnic.

In conclusion, our findings suggest that genetic confirmation of ADH may be important to identify patient's risk of CHD, especially for female *LDLR* mutation carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank Jaime Almandoz, MD, Peter McCullough, MD, and Scott Grundy, MD, PhD for thoughtful discussions; Jonathan Cohen, PhD; and Helen Hobbs, MD for ongoing support and guidance; Claudia Quittner for collecting and processing samples; Tommy Hyatt, Sarah Masood, and Pei-Yun Tseng for technical assistance. We also thank the FH Foundation for helping to raise awareness of FH in the US.

FUNDING SOURCES: The work was supported by grants from the Southwest Medical Foundation, Center for Human Nutrition at UT Southwestern and from the National Institutes of Health (NIH) K23 HL114884, NIH HL020948 and CTSA Grant UL1TR001105 for REDCap.

Abbreviations List (in order of appearance)

ADH	autosomal dominant hypercholesterolemia
LDLR	low density lipoprotein receptor
OMIM	Online Mendelian Inheritance in Man
FH	familial hypercholesterolemia
APOB	apolipoprotein B-100
PCSK9	proprotein convertase subtilisin-like kexin type 9
CHD	coronary heart disease
LDL-C	LDL cholesterol
HDL-C	high density lipoprotein-cholesterol
MLPA	Multiplex Ligation-dependent Probe Amplification
Cox PH	Cox proportional hazard regression models

REFERENCES

1. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013; 34:3478–90a. [PubMed: 23956253]
2. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest*. 2003; 111:1795–803. [PubMed: 12813012]
3. Do R, Stitzel NO, Won HH, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. *Nature*. 2014
4. Khachadurian AK, Uthman SM. Experiences with the homozygous cases of familial hypercholesterolemia. A report of 52 patients. *Nutrition and metabolism*. 1973; 15:132–40. [PubMed: 4351242]
5. Jenkins T, Nicholls E, Gordon E, Mendelsohn D, Seftel HC, Andrew MJ. Familial hypercholesterolaemia--a common genetic disorder in the Afrikaans population. *S Afr Med J*. 1980; 57:943–7. [PubMed: 7404060]
6. van der Graaf A, Avis HJ, Kusters DM, et al. Molecular basis of autosomal dominant hypercholesterolemia: assessment in a large cohort of hypercholesterolemic children. *Circulation*. 2011; 123:1167–73. [PubMed: 21382890]
7. Moorjani S, Roy M, Gagne C, et al. Homozygous familial hypercholesterolemia among French Canadians in Quebec Province. *Arteriosclerosis*. 1989; 9:211–6. [PubMed: 2923577]
8. Seftel HC, Baker SG, Jenkins T, Mendelsohn D. Prevalence of familial hypercholesterolemia in Johannesburg Jews. *Am J Med Genet*. 1989; 34:545–7. [PubMed: 2624266]
9. Ahmad Z, Adams-Huet B, Chen C, Garg A. Low Prevalence of Mutations in Known Loci for Autosomal Dominant Hypercholesterolemia in a Multi-Ethnic Patient Cohort. *Circ Cardiovasc Genet*. 2012; 5:666–75. [PubMed: 23064986]
10. Marduel M, Carrie A, Sassolas A, et al. Molecular spectrum of autosomal dominant hypercholesterolemia in France. *Hum Mutat*. 2010; 31:E1811–24. [PubMed: 20809525]

11. Civeira F, Ros E, Jarauta E, et al. Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia. *Am J Cardiol.* 2008; 102:1187–93. 1193, e1. [PubMed: 18940289]
12. Khoo KL, van Acker P, Defesche JC, et al. Low-density lipoprotein receptor gene mutations in a Southeast Asian population with familial hypercholesterolemia. *Clin Genet.* 2000; 58:98–105. [PubMed: 11005141]
13. Taylor A, Wang D, Patel K, et al. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. *Clin Genet.* 2010; 77:572–80. [PubMed: 20236128]
14. Varret M, Abifadel M, Rabes JP, Boileau C. Genetic heterogeneity of autosomal dominant hypercholesterolemia. *Clinical genetics.* 2008; 73:1–13. [PubMed: 18028451]
15. Besseling J, Kindt I, Hof M, Kastelein JJ, Hutten BA, Hovingh GK. Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14,000 mutation carriers. *Atherosclerosis.* 2014; 233:219–23. [PubMed: 24529147]
16. Gaudet D, Vohl MC, Couture P, et al. Contribution of receptor negative versus receptor defective mutations in the LDL-receptor gene to angiographically assessed coronary artery disease among young (25–49 years) versus middle-aged (50–64 years) men. *Atherosclerosis.* 1999; 143:153–61. [PubMed: 10208490]
17. Polk DM, Naqvi TZ. Cardiovascular disease in women: sex differences in presentation, risk factors, and evaluation. *Curr Cardiol Rep.* 2005; 7:166–72. [PubMed: 15865855]
18. Bairey Merz CN, Shaw LJ, Reis SE, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part II: gender differences in presentation, diagnosis, and outcome with regard to gender-based pathophysiology of atherosclerosis and macrovascular and microvascular coronary disease. *J Am Coll Cardiol.* 2006; 47:S21–9. [PubMed: 16458167]
19. Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. *BMJ.* 1991; 303:893–6. [PubMed: 1933004]
20. Food and Drug Administration. Standardized definitions for end point events in cardiovascular trials. 2010
21. Grundy SM. United States Cholesterol Guidelines 2001: expanded scope of intensive low-density lipoprotein-lowering therapy. *Am J Cardiol.* 2001; 88:23J–27J. [PubMed: 11423053]
22. Leigh SE, Foster AH, Whittall RA, Hubbart CS, Humphries SE. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. *Ann Hum Genet.* 2008; 72:485–98. [PubMed: 18325082]
23. Bertolini S, Pisciotta L, Rabacchi C, et al. Spectrum of mutations and phenotypic expression in patients with autosomal dominant hypercholesterolemia identified in Italy. *Atherosclerosis.* 2013; 227:342–8. [PubMed: 23375686]
24. Miyake Y, Yamamura T, Sakai N, Miyata T, Kokubo Y, Yamamoto A. Update of Japanese common LDLR gene mutations and their phenotypes: Mild type mutation L547V might predominate in the Japanese population. *Atherosclerosis.* 2009; 203:153–60. [PubMed: 18718593]
25. Al-Khateeb A, Zahri MK, Mohamed MS, et al. Analysis of sequence variations in low-density lipoprotein receptor gene among Malaysian patients with familial hypercholesterolemia. *BMC Med Genet.* 2011; 12:40. [PubMed: 21418584]
26. Hopkins PN, Stephenson S, Wu LL, Riley WA, Xin Y, Hunt SC. Evaluation of coronary risk factors in patients with heterozygous familial hypercholesterolemia. *Am J Cardiol.* 2001; 87:547–53. [PubMed: 11230837]
27. Stone NJ, Levy RI, Fredrickson DS, Verter J. Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia. *Circulation.* 1974; 49:476–88. [PubMed: 4813182]
28. Hill JS, Hayden MR, Frohlich J, Pritchard PH. Genetic and environmental factors affecting the incidence of coronary artery disease in heterozygous familial hypercholesterolemia. *Arterioscler Thromb.* 1991; 11:290–7. [PubMed: 1998646]
29. Schisterman EF, Mumford SL, Chen Z, et al. Lipid concentrations and semen quality: the LIFE study. *Andrology.* 2014; 2:408–15. [PubMed: 24596332]
30. M V. Fighting Genetic High Cholesterol: Katherine Wilemon's Story. *Everday Health.*

31. Bezgin T, Elveran A, Dogan C, et al. Acute ST-elevation myocardial infarction in early puerperium due to severe left main coronary stenosis in a woman with familial hyperlipidaemia. *Cardiovasc J Afr.* 2013; 24:e13–6. [PubMed: 24217125]
32. Youngblom, E.; Knowles, JW. Familial Hypercholesterolemia.. In: Pagon, RA.; Adam, MP.; Ardinger, HH., et al., editors. *GeneReviews(R)*. Seattle (WA): 1993.
33. Robinson JG. Management of familial hypercholesterolemia: a review of the recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Manag Care Pharm.* 2013; 19:139–49. [PubMed: 23461430]
34. Marteau T, Senior V, Humphries SE, et al. Psychological impact of genetic testing for familial hypercholesterolemia within a previously aware population: a randomized controlled trial. *Am J Med Genet A.* 2004; 128A:285–93. [PubMed: 15216550]
35. Fouchier SW, Dallinga-Thie GM, Meijers JC, et al. Mutations in STAP1 are associated with autosomal dominant hypercholesterolemia. *Circ Res.* 2014; 115:552–5. [PubMed: 25035151]
36. Medeiros AM, Alves AC, Francisco V, Bourbon M. Update of the Portuguese Familial Hypercholesterolaemia Study. *Atherosclerosis.* 2010; 212:553–8. [PubMed: 20828696]
37. Chmara M, Wasag B, Zuk M, et al. Molecular characterization of Polish patients with familial hypercholesterolemia: novel and recurrent LDLR mutations. *J Appl Genet.* 2010; 51:95–106. [PubMed: 20145306]
38. Lombardi MP, Redeker EJ, van Gent DH, Smeele KL, Weerdesteijn R, Mannens MM. Molecular genetic testing for familial hypercholesterolemia in the Netherlands: a stepwise screening strategy enhances the mutation detection rate. *Genet Test.* 2006; 10:77–84. [PubMed: 16792510]
39. Dedoussis GV, Skoumas J, Pitsavos C, et al. FH clinical phenotype in Greek patients with LDL-R defective vs. negative mutations. *Eur J Clin Invest.* 2004; 34:402–9. [PubMed: 15200491]
40. Wang J, Huff E, Janecka L, Hegele RA. Low density lipoprotein receptor (LDLR) gene mutations in Canadian subjects with familial hypercholesterolemia, but not of French descent. *Hum Mutat.* 2001; 18:359. [PubMed: 11668627]
41. Garg A. What is the role of alternative biomarkers for coronary heart disease? *Clinical endocrinology.* 2011; 75:289–93. [PubMed: 21521314]
42. Talmud PJ, Shah S, Whittall R, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet.* 2013; 381:1293–301. [PubMed: 23433573]

Highlights

Autosomal dominant hypercholesterolemia (ADH) patients have severely elevated LDL-C.

ADH results in an increased risk of premature coronary heart disease (CHD).

We tested whether mutation status and gender influence CHD risk.

Female LDLR mutation carriers had a higher risk of CHD than non-mutation carriers.

Figure 1.

Age of onset of premature coronary heart disease (CHD) in patients with autosomal dominant hypercholesterolemia (ADH) due to Familial Hypercholesterolemia (FH, due to mutations in low density lipoprotein receptor) vs Unexplained (no disease-causing mutations in *LDLR* or *APOB*).

(A) men, unadjusted and (B) women, unadjusted;

(C) men, adjusted and (D) women adjusted for LDL-C, HDL-C as well as diabetes, hypertension, and smoking status.

Premature CHD is defined as the diagnosis of CHD at ages ≤ 55 years in men or ≤ 65 years in women. Each step down represents the occurrence of the patient's CHD diagnosis.

Symbols represent censored observations. Cox proportional hazard models were used for comparing risk of premature CHD and censoring age at 55 years for men and 65 years for women. Kaplan-Meier estimators for unadjusted survival curves and Cox proportional hazard model estimates for covariate adjusted survival curves are shown. HR, hazard ratios with 95% confidence intervals are shown in parentheses.

Figure 2.

Age of onset of premature coronary heart disease (CHD) in men vs women.

(A) **Unexplained**, (no disease-causing mutations in *LDLR* or *APOB*), unadjusted and (B) **Familial Hypercholesterolemia (FH)**, due to mutations in low density lipoprotein receptor), unadjusted

(C) **Unexplained**, adjusted and (D) **FH** adjusted for LDL-C, HDL-C as well as diabetes, hypertension, and smoking status.

Figure 3.
Hazard Ratio for Premature CHD at each quartile of LDL-C in patients with autosomal dominant hypercholesterolemia (ADH) due to mutations in low density lipoprotein receptor (*LDLR*, referred to as familial hypercholesterolemia [FH]) vs. unexplained (no disease-causing mutations in *LDLR* or *APOB*).

Table 1

Characteristics of ADH patients due to heterozygous *LDLR* mutations (FH) vs. those lacking an identifiable genetic cause (unexplained), stratified by sex

	Men		Women	
	FH	Unexplained	FH	Unexplained
n	42	66	51	111
Age (yrs)	50 (11)	52 (12)	51 (13) ^c	58 (9)
BMI (kg/m²)	31 (5)	30 (5)	31 (7) ^e	31 (7)
Ethnicity				
African-American (%)	38.1	39.4	35.3	44.1
Caucasian (%)	35.7	28.8	21.6	28.8
Hispanic (%)	19.1	19.7	33.3	14.4
Other(%)	7.1	12.1	9.8	12.6
CHD Risk Factors				
Diabetes (%)	26	23	28	31
Hypertension (%)	75	72	54	66
Current Smoker (%)	15	13	10	14
Former Smoker (%)	46	43	24	34
Post-menopausal (%)	NA	NA	63 ^c	88
Age at menopause (yrs)	NA	NA	42 (9)	43 (9)
Phenotypic Features				
Xanthomas (%)	46 ^b	16	38 ^b	16
Peripheral Arterial Disease (%)	15	11	4	6
Cerebrovascular Disease (%)	15	13	12	11
Premature CHD n (%)	22 (52)	25 (39) ^f	16 (31)	19 (17)
Age of onset (yrs)	41 (8)	43 (7) ^e	44 (11)	52 (10)
Baseline Lipids (mg/dL)				
Total Cholesterol	377 (79) ^c	317 (47)	379 (67) ^c	328 (41)
Triglycerides	187 (92) ^d	169 (69)	142 (66) ^a	167 (79)
HDL-C	43 (15) ^{ad}	49 (11) ^f	52 (14)	57 (18)
LDL-C	294 (64) ^c	238 (44)	300 (65) ^c	239 (37)

ADH denotes autosomal dominant hypercholesterolemia; *LDLR*, low density lipoprotein receptor; FH, familial hypercholesterolemia; BMI, body mass index; CHD, coronary heart disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Continuous variables are summarized as mean (SD)

^a p<0.05

^b p<0.01

^c p<0.001, FH versus unexplained ADH for the same sex from two-way ANOVA for continuous variables, Fisher Exact test for categorical variables.

^d
p<0.05

^e
p<0.01

^f
p<0.001, Male versus Female for the same group (male FH vs female FH; male Unexplained vs female Unexplained)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript