Low Prevalence of Mutations in Known Loci for Autosomal Dominant Hypercholesterolemia in a Multiethnic Patient Cohort

Zahid Ahmad, MD; Beverley Adams-Huet, MS; Chiyuan Chen, PhD; Abhimanyu Garg, MD

Background—Autosomal dominant hypercholesterolemia (ADH), characterized by elevated plasma levels of low-density lipoprotein (LDL)-cholesterol, is caused by variants in at least 3 different genes: LDL receptor (LDLR), apolipoprotein B-100, and proprotein convertase subtilisin-like kexin type 9. There is paucity of data about the molecular basis of ADH among ethnic groups other than those of European or Japanese descent. Here, we examined the molecular basis of ADH in a multiethnic patient cohort from lipid clinics in a large, urban US city.

Methods and Results—A total of 38 men and 53 women, aged 22 to 76 years, met modified Simon-Broome criteria for ADH and were screened for mutations in the exons and consensus splice sites of LDLR, and in selected exons of apolipoprotein B-100 and proprotein convertase subtilisin-like kexin type 9. Deletions and duplications of LDLR exons were detected with multiplex ligation-dependent probe amplification. Heterozygous variants in LDLR were identified in 30 patients and in apolipoprotein B-100 in 1 patient. The remaining 60 patients (65%) had unexplained ADH. A higher proportion of blacks (77%) than either non-Hispanic whites (57%) or Hispanics (53%) had unexplained ADH. Compared with patients with LDLR variants, those with unexplained ADH had lower levels of LDL-cholesterol (292±47 mg/dL versus 239±42 mg/dL, respectively; P<0.0001) and higher levels of high-density lipoprotein cholesterol (45±12 mg/dL versus 54±13 mg/dL, respectively; P=0.003).

Conclusions—Our findings suggest that additional loci may contribute to ADH, especially in understudied populations such as blacks. (Circ Cardiovasc Genet. 2012;5:666-675.)

Key Words: familial hypercholesterolemia ■ genetics ■ lipids ■ lipoproteins

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Elevated plasma levels of low-density lipoprotein-cholesterol (LDL-C) are an important contributor to the pathogenesis of coronary heart disease (CHD), one of the major causes of morbidity and mortality in the United States. Approximately 50% of the interindividual variation in plasma levels of LDL-C is attributable to genetic variation. The major portion of this genetic variation is polygenic, reflecting the cumulative effects of multiple sequence variants in any given individual.

Editorial see p 599
Clinical Perspective on p 675

Received December 21, 2011; accepted September 8, 2012.
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The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.112.963587/-/DC1.

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Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org

DOI: 10.1161/CIRCGENETICS.112.963587

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25% of ADH patients in the general population do not have mutations in the known loci.

Most of these screening studies have been performed in Europe or in Japan and are from relatively homogenous populations with regard to ethnicity and race. As a result, there is a paucity of data in subjects of African origin and other minorities. Therefore, we examined the molecular basis of ADH in a multiethnic cohort from a large, urban US city.

Methods

Patients

All patients and their family members 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, TX, area according to modified Simon-Broome criteria. Specifically, pretreatment LDL-C was greater than the 95th percentile for age and sex, and 1 of the 2 following criteria: (1) tendon xanthoma (proband or first-degree relative) or (2) either first-degree relative with premature CHD (<55 years of age in men or 65 years of age in women) or pretreatment LDL-C greater than the 95th percentile for age and sex. When first-degree relatives were not available to participate in the study, an autosomal dominant inheritance pattern was inferred based on family history of hypercholesterolemia in >1 generation.

Ethnicity/race was self-reported according to the guidelines of the National Institutes of Health. In addition, patients were allowed to report their maternal and paternal ancestral origins.

Detailed family history regarding hypercholesterolemia, CHD, cerebrovascular accidents, and xanthomas was obtained. Patients were examined for the presence of arcus senilis and tendon xanthomas. A grading scheme was used for arcus senilis as previously described. Xanthomas were considered to be present if tendons were examined for the presence of arcus senilis and tendon xanthomas. A grading scheme was used for arcus senilis as previously described. Xanthomas were considered to be present if tendons appeared diffusely enlarged or had focal nodularity. The Achilles tendon width was calculated from the mean of measurements using Lange calipers (Beta Technology Incorporated, Cambridge, MD) in the superior, middle, and inferior aspects bilaterally.

Exclusion criteria for the study included pretreatment serum triglyceride (TG) concentrations of >500 mg/dL or any secondary cause of the dyslipidemia (ie, uncontrolled diabetes mellitus, obstructive liver disease, hypothyroidism, or nephrotic syndrome).

DNA Isolation

Genomic DNA was isolated from whole blood using the Easy-DNA kit (Invitrogen, Carlsbad, CA).

Candidate Gene Analysis

All 18 exons and the flanking intronic regions of LDLR, exon 26 of APOB, and exons 2, 4, and 7 of PCSK9 were amplified and sequenced using Applied Biosystems’ 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA). Deletions and duplications of ≥1 exons of LDLR were detected with the SALSA Multiplex Ligation-Dependent Probe Amplification kit (MRG Holland, the Netherlands) as previously described, and data were analyzed with GeneMarker software from SoftGenetics version 1.95 (State College, PA). The genes were sequenced sequentially: LDLR, APOB, and then the search for a deletion/duplication of LDLR. Finally, PCSK9 was sequenced.

ApoE Genotyping

Specific 5′-nucleotidase assays for the ApoE sequence variations were developed using the TaqMan system (Applied Biosystems). The assays were performed on an HT7900 Real-Time PCR system with probes and reagents purchased from Applied Biosystems. For the ApoE_112 variant, 2 primers (forward 5′-CGGAACTGGAGAACAACACTGA-3′ and reverse 5′-GGTGCTCTGGCAGCAT-3′) and 2 fluoro-
genic minor groove binder probes (fam-TGGAGGAGCTGCCG and vic-ATGGAGGAGCTGCCG) were used to amplify either the wild-type or mutant allele. Similarly, for the ApoE_158 variant, 2 primers (forward 5′-TCCACCTGGGCAAGCT-3′ and reverse 5′-CCCCGGGCTGTTGACTC-3′) and 2 fluoro-
genic minor groove binder probes (fam-CTGCAGAAAGTGCC and vic-CTGCAGAAAGTGCC) were used. For both variants, amplification consisted of 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Lipids and Lipoproteins

Medical charts were reviewed to obtain untreated lipid levels. All centers measured fasting total cholesterol, TG, and high-density lipoprotein-cholesterol (HDL-C) using enzymatic assays in commercial laboratories. LDL-C was estimated with the Friedewald equation when serum TG were <500 mg/dL.

Historical untreated serum lipid values were not available in 6 patients; however, their clinical picture was consistent with ADH, and therefore they were included in candidate gene analysis but excluded from statistical analyses of untreated lipid levels.

Plasma PCSK9 Measurement

Plasma concentrations of PCSK9 were determined as previously described.

In Vitro PCSK9 (D129N) Experiments

Construction of PCSK9(D129N) Expression Vector

All DNA manipulations were performed using standard molecular biology techniques. A D129N change was introduced into the porcine cytomagelovirus PCSK9-FLAG vector by site-directed mutagenesis using the primer 5′-GGCTTCTGGTGAAG GATGAGTGCCGCCTGGTACACT-3′ and vic-ATGGAGGAGCTGCCGTTG-3′ as described; the resulting plasmid was designated porcine cytomagelovirus-PCSK9(D129N)-FLAG.

Tissue Culture Medium

Medium A contained Dulbecco’s Modified Eagle Medium (Cellgro) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, and 1 g/L glucose. Medium B contained Medium A supplemented with 10% (v/v) fetal calf serum. Medium C contained Medium A supplemented with 2.5% (v/v) newborn calf lipoprotein-deficient serum, 10 μmol/L sodium compactin, and 50 μmol/L sodium mevalonate.

Purification of Epitope-Tagged PCSK9 Proteins

For stable lines expressing PCSK9(D129N), human embryonic kidney 293S cells were cultured in Medium B in 60-mm dishes and transfected with 0.2 μg porcine cytomagelovirus PCSK9 using FuGENE 6 transfection reagent (Roche Applied Science, Indianapolis, IN). Colonies surviving neomycin selection were assessed for PCSK9 secretion by immunoblot analysis of the medium using an anti-FLAG M2 antibody. FLAG-tagged human PCSK9(wild type), PCSK9(D129N), and PCSK9(D374Y) proteins were purified from the stably transfected human embryonic kidney 293S cells as described.

PCSK9 Protein Activity in HepG2 Cells

HepG2 cells (ATCC, HB-8065) were plated at 4×10⁵ cells/60-mm dish in Medium B on day 0. On day 3, Medium B was replaced with Medium C. After 18 hours, cells were treated with purified FLAG-tagged PCSK9 proteins for 4 hours. After incubation with exogenous PCSK9, cell surface proteins were biotinylated and isolated from whole cell extracts as described. Mouse anti-human LDLR immunoglobulin G (IgG) HL-1 and mouse anti-human transferrin receptor IgG (Invitrogen, Carlsbad, CA) were used for immunoblot analysis.
An IRDye800-conjugated donkey antimouse antibody (LI-COR Biosciences, Lincoln, NE) was used as the secondary antibody. Proteins were visualized and quantified using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences) as described.\textsuperscript{23} Transferrin receptor was used as the loading control for the calculation of relative LDLR protein levels.

**Statistical Analysis**

Comparisons of quantitative variables were made with 2-way ANOVA models to test simultaneously 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 models were used for comparing risk of premature CHD and censoring age at 55 years of age for men and 65 years of age for women (ages corresponding to the definition of premature CHD). Serum TG values were log transformed prior to analysis. Continuous variables are summarized as mean (SD) and median unless otherwise specified. A 2-sided \( P \) value <0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

**Results**

Ninety-one unrelated patients with ADH (38 men and 53 women) met entry criteria and agreed to participate. Ages ranged from 22 to 76 years, and body mass index from 16 kg/m\(^2\) to 58 kg/m\(^2\). Total cholesterol ranged from 254 mg/dL to 557 mg/dL, LDL-C from 175 mg/dL to 397 mg/dL, TG from 52 mg/dL to 498 mg/dL, and HDL-C from 26 mg/dL to 95 mg/dL.

**Candidate Gene Analysis**

Heterozygous LDLR mutations were found in 27 patients via Sanger sequencing. Multiplex ligation-dependent probe amplification detected 3 patients with small heterozygous LDLR deletions (Figure 1). Overall, 28 different heterozygous mutations were represented among these 30 patients (Table 1). Of the 6 patients with missing untreated serum lipids values, 4 had LDLR mutations. Four new LDLR variants were detected; these variants were not present in the University College London LDLR FH database (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/).\textsuperscript{28} A 44-year-old non-Hispanic black man with an untreated LDL-C of 234 mg/dL, premature arcussenilis, tendon xanthomas, and history of ischemic stroke harbored a heterozygous p.F11S (c.95T>C) variant. Another heterozygous variant p.Q324P (c.1034A>C) was found in a 54-year-old black woman with premature arcussenilis, tendon xanthomas, and a family history of premature CHD. Although her untreated serum lipid values were available, her LDL-C was 171 mg/dL while taking maximum doses of colesevelam and atorvastatin. A heterozygous variant, p.L435P (c.1367T>C) was found in a 63-year-old black man with an untreated LDL-C of 279 mg/dL and arcussenilis. Finally, a heterozygous p.D339H (c.1078G>C) mutation was found in a 72-year-old Hispanic woman with an untreated LDL-C of 250 mg/dL and history of ischemic stroke. None of these LDLR variants were found in single nucleotide polymorphism databases.

Sequencing of APOB revealed 1 patient, who was of German descent, with heterozygous p.R3527Q (c.10580G>A), also known as p.R3500Q according to the previous numbering system) mutation. Another patient, of black descent, was heterozygous for another missense mutation, p.W3594R (c.10780T>C), which has not been reported previously. However, her brother, who was also hypercholesterolemic, did not inherit the mutation (Figure 2A).

Sequencing of PCSK9 revealed 1 proband with the missense mutation p.D129N (c.385 G>A). This PCSK9 variant had been previously identified in a single individual with hypercholesterolemia. Functional studies in cells transfected with PCSK9(D129N) suggested that it degraded LDLRs more efficiently than wild-type PCSK9, although the effect was modest.\textsuperscript{29} Here, we found that this variant did not cosegregate with the ADH phenotype in the family (Figure 2B). To study the activity further, this PCSK9 variant, recombinant PCSK9(D129N) protein was purified and the activity of this protein was compared with wild-type PCSK9 and PCSK9(D374Y), a confirmed gain-of-function mutation.\textsuperscript{46} As shown in Figure 2C, the ability of PCSK9(D129N) to degrade LDLRs in HepG2 cells was equivalent to than of wild-type PCSK9, and both were significantly less potent than PCSK9(D374Y).

A total of 66% patients lacked any mutation in the 3 candidate genes screened, which included 27 men and 33 women. Ethnic and racial breakdown of the patients is shown in Figure 3. Of the 35 black patients, only 8 (23%) had mutations in the known ADH genes. Unexplained ADH was found in 57% of non-Hispanic whites, 50% of Native Americans, and 53% of Hispanic patients in our study. Only 2 of 9 patients of Asian origin were found to have disease-causing mutations, and 1 Ethiopian man did not have any disease-causing mutation.

**ApoE Genotyping**

No patients had the E2/E2 genotype. Using reference populations,\textsuperscript{31} we tested whether the E4 allele is more prevalent in blacks and whites, respectively, and found no significant difference from the reference populations.

**Comparison of FH and Unexplained ADH**

We compared the clinical characteristics and laboratory data between those with LDLR mutations (FH) and those in whom no mutations were found (unexplained ADH) (Table 2). Compared with patients with LDLR variants, those with unexplained ADH had lower levels of LDL-C (292±47 mg/dL vs 239±42 mg/dL, respectively; \( P<0.0001 \)) and higher levels of HDL-C (45±12 mg/dL vs 54±13 mg/dL, respectively, \( P=0.003 \)) (Table 2 and Figure 4A and 4B). Serum TG were higher in men with FH compared with the unexplained ADH group, but the opposite was observed in women (\( P \) for interaction, 0.005). Frequency of tendon xanthomas was nearly double in those with FH. There were no significant differences in the Achilles tendon width in the 2 groups, but Achilles tendon width had good predictive ability for the presence of xanthomas (area under the curve =0.85). Also, the 2 groups were not different with regard to the frequency of arcussenilis, grade of arcussenilis, premature CHD in first-degree relatives, and plasma PCSK9 levels. There was no difference in the prevalence of premature CHD in male probands; however, women with unexplained ADH had a lower prevalence of premature CHD than women with LDLR mutations and...
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men with unexplained ADH (Figure 4C and 4D). Both groups showed a similar response to LDL lowering medications, and the use of lipid-lowering medications was similar in both the groups (online-only Data Supplement Tables I and II).

Discussion

The most striking finding of our study was our inability to identify a disease-causing mutation in LDLR, APOB, or PCSK9 in 66% of our patients with ADH. In examining the molecular basis of ADH in a multiethnic patient cohort from lipid clinics in a large US city, we found that a higher proportion of blacks (77%) than either non-Hispanic whites (57%) or Hispanics (53%) had unexplained ADH. Our findings suggest that new ADH loci exist, especially among understudied populations such as blacks. Nearly all patients with a gene defect had FH, and differences in clinical features were observed between FH and unexplained ADH.

Black adults are more likely than non-Hispanic whites to be diagnosed with CHD, and they are more likely to die from heart disease. This disparity has been linked to hypertension, but only limited data are available about the frequency of LDLR disease-causing variants in ADH patients of African origin. Previously, Thiart et al reported data on 16 unrelated Africans from South Africa and found 10 with mutations in LDLR, but 4 shared the same 6-bp deletion in exon 2. These 4 Africans were not from the same tribe, and the authors excluded the likelihood of a recurrent mutational event, suggesting that there is a common ancestry. Four individuals of African/Afro-Caribbean descent were included in a study from the United Kingdom, and only 1 harbored an LDLR mutation. Thus, our finding of a low frequency of LDLR mutations (23%) in blacks suggests that other mechanisms may be a common cause of the ADH phenotype in patients of African origin.

Our mutation detection rate among Hispanics corresponds well to previous small studies that showed 40% to 61% of Mexican ADH patients have mutations in LDLR or APOB. In addition, our study included 8 patients of Asian origin, only one of whom was found to have a mutation. Previous small studies of Asian cohorts have also found low mutation detection rates. In Malaysian ADH cohorts, only 26% to 42% of patients have been found to have LDLR mutations. Studies of patients from other Asian

Figure 1. Capillary electrophoresis of multiplex ligation-dependent probe amplification low-density lipoprotein receptor gene products in the 3 patients found to have heterozygous deletions. Gray lines show control data whereas black lines show data from the patients. Heterozygous deletions are indicated by peaks that are half as high as control peaks. A, A 25-year-old Hispanic woman with untreated low-density lipoprotein-cholesterol (LDL-C) of 339 mg/dL and premature arcus senilis was found to have heterozygous deletion of exons 4 to 6. B, A 47-year-old white man with xanthomas (untreated lipids not available) was found to have heterozygous deletion of promoter and exon 1. C, A 49-year-old black man with untreated LDL-C 271 mg/dL, tendon xanthomas, and premature coronary heart disease was found to have heterozygous deletion of exon 7.
origins (Taiwanese, Philippines, Asian/Indian origin living in United Kingdom) have found mutation detection rates between 20% and 60%. Non-Hispanic whites in our study had a mutation detection rate lower than expected, perhaps attributable to admixture in US citizens who self-report to be white.

Mutations in \textit{LDLR} account for the majority of ADH cases, and >1000 unique disease-causing variants have been reported (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/). Worldwide, the prevalence of heterozygous FH attributable to \textit{LDLR} variants is estimated to be 1 in 500. Consistent with previous observations, \textit{LDLR} mutations accounted for nearly all patients with ADH for whom a molecular basis was ascertained in our study. Even though >1000 disease-causing mutations in \textit{LDLR} have been reported, we found 4 new missense variants: p.F11S, p.Q324P, p.D339H, and p.L435P. Of note, p.F11C, p.Q324X, and p.L435H have been previously reported to cause FH. Also, in our cohort, there were only 2 recurrences of mutations.

The most common familial defective \textit{APOB} mutation in \textit{APOB} occurs in exon 26 (p.R3527Q), with the majority of affected patients being of central European descent and an estimated frequency of 1/1000. Although some other variants in \textit{APOB} have been reported in patients with high LDL-C levels, there has not been a robust documentation of segregation with the ADH phenotype within pedigrees and proof of decreased LDLR binding affinity in functional assays. Similarly, we found a p.W3594R \textit{APOB} variant that did not cosegregate with hypercholesterolemia in the family. The molecular mechanism of ADH in this pedigree (Figure 1A) remains unknown.

Because exons 2, 4, and 7 harbor the well-defined FH3-causing variants (p.S127R, p.F216L, p.D374Y), we chose to sequence only these. We identified a family with heterozygous \textit{PCSK9} p.D129N variant. This mutation was previously

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NA indicates not available.

*Numbering system for exon variations does not include 21 amino acids in the signal sequence (original amino acid number).

†Mutation at DNA level. Nucleotide numbering is according to the genomic reference sequence (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/refseq/LDLR_codingDNA.html) and includes the promoter region.

‡Not present in University College London low-density lipoprotein receptor familial hypercholesterolemia database (www.ucl.ac.uk/ldlr/LOVDv.1.1.0).
reported in a single individual with extremely elevated choles-
terol.41 However, the D129N variant did not cosegregate with
hypercholesterolemia in our family, and our functional studies
using recombinant PCSK9(D129N) and wild-type PCSK9
protein showed that there was no significant difference in
their ability to degrade LDLRs. Therefore, the molecular
mechanism of ADH in this pedigree (Figure 1B) also remains
unknown.

Our study suggests that the unexplained ADH group may
be distinct compared with the FH group. Clinically, those with
unexplained ADH had lower LDL-C levels, higher HDL-C
levels, and reduced frequency of tendon xanthomas (Table 2).
Also, men with FH had the highest TG level despite body mass
indexes that were similar to the other groups.

Although differences in LDL-C and xanthoma prevalence
are consistent with multiple prior reports, HDL-C differences
have not been widely reported in previous systematic screening
studies.7,12–14,21 In a French ADH study,12 which only 19%
of ADH patients were unexplained, similar differences
in HDL-C were noted. HDL particle size and the ratio of
HDL-C to ApoA1 is significantly lower in FH patients than in
normal subjects.42,43 Previous studies have shown an increased
fractional catabolic rate of ApoA144 and higher cholesterol
ester transfer protein levels.43 Alternatively, low HDL-C may
be secondary to the decreased uptake of TG-rich lipoproteins
by deficient LDLR.

Remarkably, men in the unexplained ADH group had
similar onset of premature CHD as the FH group, indicating
that men with severe hypercholesterolemia should be treated
aggressively with regard to preventing CHD regardless of any
knowledge of LDLR defects. The CHD rate in the unexplained
ADH females, however, was about 2-fold lower than that seen
in the unexplained ADH males and FH females. It is not clear

Figure 2. Lack of cosegregation with the hypercholesterolemia phenotype in families found to have apolipoprotein B-100 (APOB) and
proprotein convertase subtilisin-like kexin type 9 (PCSK9) variants. Arrows indicate probands. Filled circles and squares designate women and
men (respectively) with hypercholesterolemia. A, Black pedigree with APOB p.W3594R heterozygous variant. No disease-causing variants were
found in low-density lipoprotein receptor (LDLR) or PCSK9. B, Hispanic pedigree with PCSK9 p.D129N heterozygous variant. No disease-causing
variants were found in LDLR or APOB. C, Degradation of LDLR by exogenous PCSK9 proteins. HepG2 cells were cultured in sterol-supplemented medium (see Experimental Procedures) and incubated with the indicated amount of wild-type PCSK9 (WT),
PCSK9(D129N), and PCSK9(D374Y) protein for 4 hours. After biotinylation, isolated cell surface protein lysates were subjected to SDS-PAGE
and immunoblot analysis. LDLR was detected with an anti-LDLR monoclonal antibody (HL-1). Transferrin receptor was detected as a control for
equal protein loading. Secondary detection used an infrared dye (IRDye800)-labeled antibody. Blots were visualized and quantified using the LI-
COR Odyssey infrared imaging system. LDLR levels were normalized to transferrin receptor expression. For these data, results shown are from
representative experiments repeated at least 3 times with similar results. CHD indicates coronary heart disease. Arcus indicates arcus senilis.

Figure 3. Self-reported ethnicity/race based on 91 patients with autosomal dominant hypercholesterolemia (ADH). Maternal and
paternal ancestor’s origins were used if provided by the patient. Familial hypercholesterolemia (FH) denotes patients found to have
low-density lipoprotein receptor (LDLR) mutations. Familial defective apolipoprotein B (FDB) denotes patients found to have apo-
lipoprotein B-100 (APOB) mutations. Unexplained ADH denotes patients with ADH but no mutations in the known loci. n indicates
number of subjects.
why this group has lower CHD. Similar to the general population, CHD in women with ADH tends to lag behind men with ADH by 10 years.45

Limitation

Limitations of this study are that we did not sequence the LDLR promoter or introns for cryptic mutations, and we limited the sequencing of APOB and PCSK9 to exons with known disease-causing variants. Although the prevalence of LDLR cryptic mutations is not clear, a recent study of LDLR promoter variants in a large cohort of European (Spanish) patients with ADH found their prevalence to be <1%.46 Future studies with next-generation sequencing techniques, such as whole-genome sequencing, may help elucidate the contribution of cryptic LDLR mutations in ADH patients. Unlike LDLR variants, only a few variants in APOB and PCSK9 cause ADH, accounting for <14% of patients.7–15,47 The probability of novel mutations in nonsequenced exons of either of these genes is extremely unlikely and may not significantly affect the frequency of unexplained ADH.

Our results have the lowest mutation detection rate when compared with previous studies, likely because of the diverse genetic background of our patients, particularly the inclusion of many black patients. Other possibilities could be inclusion of phenocopies such as familial combined hyperlipidemia, type 3 hyperlipoproteinemia, secondary hypercholesterolemia, and nongenetic factors. However, all patients were carefully screened for secondary hypercholesterolemias, and none of our patients had the ApoE2/E2 genotype, which can result in type 3 hyperlipoproteinemia. The likelihood of familial combined hyperlipidemia is minimal because serum TG levels were not significantly different between those with and without LDLR mutations. Nongenetic factors that may be causing elevations in LDL-C include age, postmenopausal status, diet, and obesity. For example, diets high in saturated fats, trans fats, and cholesterol may cluster in families and incorrectly suggest inheritance. Unexplained ADH may also be related to increased function of PCSK9 attributable to nongenetic influences,48 but has not been clinically reported.

Another possibility for the low mutation detection rate is that a polygenic inheritance is responsible for the ADH phenotype. This may be particularly true among those individuals in our cohort who had LDL-C greater than the 95th percentile but <230 mg/

<table>
<thead>
<tr>
<th>Table 2. Comparison of Clinical Features and Lipid and Lipoprotein Values in Patients With Familial Hypercholesterolemia Versus Unexplained ADH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FH (LDLR) Patients</strong></td>
</tr>
<tr>
<td>No. of patients*</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Ethnicity, %</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>White non-Hispanic</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
</tr>
<tr>
<td>TG, mg/dL</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
</tr>
<tr>
<td>PCSK9, ng/mL</td>
</tr>
<tr>
<td>Achilles tendon width, mm</td>
</tr>
<tr>
<td>Arcus senilis, %</td>
</tr>
<tr>
<td>Tendon xanthomas, %</td>
</tr>
<tr>
<td>Lipid treatment, %</td>
</tr>
<tr>
<td>Premature CHD, %</td>
</tr>
<tr>
<td>Age of onset of CHD, y</td>
</tr>
<tr>
<td>Premature CHD in relatives, %</td>
</tr>
</tbody>
</table>

FH indicates familial hypercholesterolemia; LDLR, low-density lipoprotein receptor; ADH, autosomal dominant hypercholesterolemia; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; PCSK9, proprotein convertase subtilisin/kexin 9; and CHD, coronary heart disease. Continuous variables are summarized as mean (SD). P values are from 2-way ANOVA for continuous variables, Breslow-Day test for categorical variables, and Cox proportional hazard models for comparing premature CHD onset. Untreated lipid and lipoprotein values were available in 9 FH (LDLR) males, 17 FH (LDLR) females, 25 unexplained ADH males, and 33 unexplained ADH females.

*One patient with the apolipoprotein B-100 heterozygous mutation was not included.

+P<0.05, +P<0.01, +P<0.001, FH(LDLR) vs unexplained ADH for the same sex.

+P<0.05, +P<0.01, +P<0.001, men vs women for the same group.

+Includes statins, ezetimibe, bile acid sequestrants, niacin, fish oil, and sitostanol.

+Premature CHD is defined as the diagnosis of CHD at age <55 years in men or 65 years in women.
dl, because the mutation detection rate was 0 of 26 in this subgroup (Figure 3). Because there is at least a 5% chance that any first-degree relative will have LDL-C above the 95th percentile, collecting data on a large number of relatives to show nearly 50% prevalence of high LDL-C levels among the relatives of the unexplained ADH group may provide hard evidence for an autosomal dominant inheritance pattern. However, we were unable to ascertain the precise prevalence of high LDL-C levels among the relatives of the unexplained ADH group because of lack of data. Regardless, the majority of the unexplained ADH group had severe hypercholesterolemia with LDL-C >230 mg/dL and included many patients with tendon xanthomas and evidence of autosomal dominant inheritance.

**Conclusion**

We found a low mutation detection rate of the known genes that cause ADH among a multiethnic cohort of patients who met widely accepted criteria for a clinical diagnosis of ADH. Our findings suggest the existence of new ADH loci, especially in understudied populations such as blacks. The discovery of new ADH genes could lead to further insight into lipid metabolism and new therapeutic targets.

**Acknowledgments**

We thank Scott Grundy, MD, PhD, for ongoing support and guidance; Jean Wilson, MD, for critical review of the article; Jay D. Horton, MD, Jonathan Cohen, PhD, and Helen Hobbs, MD, for the genetic analysis and providing advice; Claudia Quittner and Barbara Gilbert for collecting and processing samples; Drs Frederick Dunn, Amit Khera, and David Balis for referring patients; and Tommy Hyatt, Sarah Masood, Rashmin Asher, Tuyet Dang, and Norma Anderson for technical assistance.

**Sources of Funding**

The work was partly supported in part by grants from the Department of Internal Medicine, UT Southwestern and from the National Institutes of Health (HL020948 Helen Hobbs, Jonathan Cohen, and Jay Horton).

**Disclosures**

Dr Garg is a consultant for Pfizer, on the speaker bureau for Merck, and has grant support from Amylin, Inc.
References


Patients with autosomal dominant hypercholesterolemia (ADH) present with very high low-density lipoprotein-cholesterol levels accompanied by tendon xanthomas and premature coronary heart disease. Worldwide, several countries have undertaken systematic screening efforts to identify patients with mutations in the genes known to cause ADH, and disease-causing mutations have been found in up to 95% of ADH patients. However, most of these screening studies have been performed in Europe or in Japan and are from relatively homogenous populations with regard to ethnicity and race. As a result, there is a paucity of data in subjects of African origin and other minorities. Therefore, we examined the molecular basis of ADH in a multiethnic patient cohort from a large, urban US city (Dallas, TX). We identified 91 unrelated ADH patients and screened them for mutations in the known genes: low-density lipoprotein-cholesterol receptor, apolipoprotein B-100, and proprotein convertase subtilisin-like kexin type 9. Sixty ADH patients did not have mutations in any of these genes (unexplained ADH). A higher proportion of blacks (77%) than either non-Hispanic whites (57%) or Hispanics (53%) had unexplained ADH. Compared with patients with low-density lipoprotein receptor variants, those with unexplained ADH had lower levels of low-density lipoprotein-cholesterol (292±47 mg/dL versus 239±42 mg/dL, respectively; \( P<0.0001 \)) and higher levels of high-density lipoprotein-cholesterol (45±12 mg/dL versus 54±13 mg/dL, respectively, \( P=0.003 \)). Our findings suggest that additional loci may contribute to ADH, especially in understudied populations such as blacks.

Low Prevalence of Mutations in Known Loci for Autosomal Dominant Hypercholesterolemia in a Multiethnic Patient Cohort
Zahid Ahmad, Beverley Adams-Huet, Chiyuan Chen and Abhimanyu Garg

_Circ Cardiovasc Genet._ 2012;5:666-675; originally published online October 11, 2012; doi: 10.1161/CIRCGENETICS.112.963587

_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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http://circgenetics.ahajournals.org/content/5/6/666

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SUPPLEMENTAL MATERIAL

Low prevalence of mutations in known loci for autosomal dominant hypercholesterolemia in a multi-ethnic patient cohort

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Authors: Beverley Adams-Huet, MS; Chiyaun Chen, Abhimanyu Garg, MD

1Division of Nutrition and Metabolic Diseases, Center for Human Nutrition; 2Department of Clinical Sciences; 3Departments of Molecular Genetics; 4Department of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390
Table S1. Lipid and lipoprotein profile on lipid lowering therapy

<table>
<thead>
<tr>
<th></th>
<th>FH (LDLR) Patients</th>
<th>Unexplained ADH Patients</th>
<th>Group x Gender Interaction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>19</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>204 (26)</td>
<td>215 (36)</td>
<td>182 (45)</td>
<td>207 (40)</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>145 (26) $^a$</td>
<td>135 (34)</td>
<td>116 (36)</td>
<td>123 (31)</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>137 (57)</td>
<td>140 (68)</td>
<td>127 (55)</td>
<td>162 (51)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>39.4 (6.9) $^d$</td>
<td>51.8 (10.8)</td>
<td>43.7 (8.6) $^e$</td>
<td>55.4 (18.0)</td>
</tr>
<tr>
<td>% LDL-C change from untreated value</td>
<td>-48.5 (13.3)</td>
<td>-53.1 (13.0)</td>
<td>-52.2 (14.9)</td>
<td>-48.3 (10.8)</td>
</tr>
<tr>
<td>Absolute LDL-C change from untreated value</td>
<td>-143 (65)</td>
<td>-159 (55)</td>
<td>-130 (51)</td>
<td>-116 (36)</td>
</tr>
</tbody>
</table>

Continuous variables are summarized as mean (SD). P-values are from two-way ANOVA for continuous variables and the Breslow-Day test for categorical variables.

$^a$ p<0.05, FH (LDLR) versus unexplained ADH for the same gender.
$^d$ p<0.05, $^e$ p<0.01, Male versus Female for the same group

Includes statins, ezetemibe, bile acid sequestrants, niacin, fish oil, and/or sitostanol.
Table S2. Percentage of patients on lipid lowering medication

<table>
<thead>
<tr>
<th>Lipid lowering medication</th>
<th>FH (LDLR) Patients</th>
<th>Unexplained ADH Patients</th>
<th>Median daily dose (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Rosuvastatin (%)</td>
<td>94.7</td>
<td>76.0</td>
<td>35.7</td>
</tr>
<tr>
<td>Simvastatin (%)</td>
<td>68.4</td>
<td>16.0</td>
<td>35.7</td>
</tr>
<tr>
<td>Atorvastatin (%)</td>
<td>5.3</td>
<td>4.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Pravastatin (%)</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Ezetimibe (%)</td>
<td>52.6</td>
<td>44.0</td>
<td>35.7</td>
</tr>
<tr>
<td>Niacin (%)</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Bile acid sequestrants* (%)</td>
<td>10.5</td>
<td>8.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Fish oil (%)</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

* Colestipol, cholestyramine, and colesveleam were used