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Double-heterozygous autosomal dominant hypercholesterolemia: Clinical characterization of an underreported disease

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INTRODUCTION: Autosomal dominant hypercholesterolemia (ADH), characterized by high-plasma low-density lipoprotein cholesterol (LDL-C) levels and premature cardiovascular disease (CVD) risk, is caused by mutations in *LDLR*, *APOB*, and/or *PCSK9*.

OBJECTIVE: To describe the clinical characteristics of "double-heterozygous carriers," with 2 mutations in 2 different ADH causing genes, that is, *LDLR* and *APOB* or *LDLR* and *PCSK9*.

METHODS: Double heterozygotes were identified in the database of the national referral laboratory for DNA diagnostics of inherited dyslipidemias. We collected the medical data (comprising lipids and CVD events) from double heterozygotes and compared these with data from their heterozygous and unaffected relatives and homozygote/compound heterozygous *LDLR* mutation carriers, identified in a previously described cohort (n = 45).

RESULTS: A total of 28 double heterozygotes (23 *LDLR/APOB* and 5 *LDLR/PCSK9* mutation carriers) were identified. Off treatment, LDL-C levels were significantly higher in double heterozygotes (mean ± SD, 8.4 ± 2.8 mmol/L) compared with 28 heterozygous (5.6 ± 2.2) and 18 unaffected relatives (2.5 ± 1.1; $P \leq .01$ for all comparisons) and significantly lower compared with homozygous/compound heterozygous *LDLR* mutation carriers (13.0 ± 5.1; $P < .001$).

CONCLUSIONS: Double-heterozygous carriers of mutations in ADH genes express an intermediate phenotype compared with heterozygous and homozygous/compound heterozygous carriers and might well be misconceived to suffer from a severe form of heterozygous ADH. The molecular identification of double heterozygosity is of relevance from both a screening and an educational perspective.

Introduction

Autosomal dominant hypercholesterolemia (ADH) or familial hypercholesterolemia (FH) is an autosomal codominant disorder caused by loss-of-function mutations in

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the genes encoding *LDLR* (606945) and *APOB* (107730) or gain-of-function (GOF) mutations in *PCSK9* (607786).[1,2] ADH patients are characterized by elevated low-density lipoprotein cholesterol (LDL-C) levels and, as a consequence, increased risk for premature cardiovascular disease (CVD). All three monogenic forms of ADH exhibit a gene dosage effect with the severest phenotypes observed in homozygous and compound heterozygous carriers, who may, if left untreated, already develop overt coronary heart disease in their second decade of life.[3] LDL-C levels >13.0 mmol/L have been generally accepted for the clinical diagnosis of homozygous/compound heterozygous ADH.[4] However, it has recently been shown that LDL-C levels vary greatly among ADH patients,[5] which is partly related to the gene (ie, LDL-C levels in gain-of-function *PCSK9* mutation carriers are generally higher compared to loss-of-function *LDLR* and *APOB* mutation carriers),[6] the type of gene defect (eg, deficient vs defective mutations) and other not completely understood phenomena. Although the clinical consequences of heterozygous and homozygous mutations in one of ADH causing genes have been described in great detail, little is known about the phenotypes of "double-heterozygous carriers," that is, a combination of a mutation in *LDLR* and *APOB* or *LDLR* and *PCSK9*. Recently, the European Atherosclerosis Society consensus panel stated that LDL-C levels of double heterozygotes are generally less severely increased compared to homozygous carriers of mutations in *APOB*, *PCSK9*, or *LDLR*,[3] but data to bolster this statement are largely lacking in literature, since to date, only ~17 double-heterozygous ADH mutation carriers have been described in case reports.[7–15]

In the Netherlands, a large genetic screening program is carried out to identify affected family members of patients with ADH. We queried the database of the national referral laboratory for DNA diagnostics of inherited dyslipidemias to identify patients with double ADH mutation carriers and compared lipid profiles and the frequency of CVD events among these patients with characteristics of heterozygous and homozygous carriers of ADH mutations.

Methods

Patients, data collection, and molecular diagnostic procedures

The patient identification process has been described previously.[5] Briefly, the database of the national referral laboratory for DNA diagnostics of inherited dyslipidemias at the Academic Medical Center in Amsterdam, the Netherlands, comprising all molecular diagnostic results on ADH patients in our country, was queried to identify all subjects with biallelic ADH mutation carriers. Carriers of nonpathogenic mutations as well as patients who were deceased were excluded.

Pathogenicity of mutations was defined according to the criteria for functionality as previously published.[16] In case not all criteria for functionality were met, and <50 mutation carriers were available to perform co-segregation analysis, the mutation was defined as "possibly nonpathogenic" and therefore also excluded from this study.

In the present study, we included all double-heterozygous ADH mutation carriers that were excluded from a recent previous study (n = 25 plus 1 who was classified as excluded because of residing outside the Netherlands).[5] In addition, during family expansion, we identified 2 other double-heterozygous carriers who are relatives of a previously identified index case.

Medical records were reviewed, and data about physical characteristics, lipid profiles, lipid-lowering therapy (LLT), and CVD events were collected. Written informed consent was received where appropriate. Family members were invited to participate by the index cases and on consent the same parameters were derived from their medical records. Molecular diagnostic procedures (including DNA diagnostics as well as criteria for functionality of mutations) were performed as previously described.[5]

This study was approved by the Medical Ethics Committee of the Academic Medical Center, Amsterdam, the Netherlands. Results are described for all double-heterozygous ADH patients combined, unless stated otherwise.

Statistical analysis

Medical records were analyzed for data on lipid levels and CVD events. Phenotypic characteristics of double-heterozygous ADH mutation carriers, regarding total cholesterol (TC) and LDL-C levels, were compared with heterozygous and unaffected relatives. Moreover, a comparison with 45 homozygous/compound heterozygous *LDLR* mutation carriers who were previously identified[5] was performed. To assess differences in lipoprotein levels, continuous variables were analyzed using *t* tests and Mann–Whitney *U* tests, where appropriate. A *P* value of <.05 was considered to be statistically significant. All statistical analyses were performed in IBM SPSS statistics, Inc., version 23.0 (Chicago, IL).

Results

A total of 28 double-heterozygous ADH mutation carriers were identified.[5] Twenty-eight heterozygous (17 *LDLR*, 10 *APOB*, and 1 *PCSK9* mutation carriers) and 18 unaffected family members were included in the analysis. The total study population comprised individuals from 17 different families (mean number of individuals per family: 4.4 ± 2.4).

Twenty-three patients were double heterozygous for mutations in *LDLR* and *APOB*, and five patients were double heterozygous for mutations in the genes encoding *LDLR* and *PCSK9*. No double heterozygotes for mutations in *APOB* and *PCSK9* were identified.