Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype–phenotype relationship, and clinical outcome

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Aims	Homozygous autosomal dominant hypercholesterolaemia (hoADH), an orphan disease caused by mutations in low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), or proprotein convertase subtilisin–kexin type 9 (*PCSK9*), is characterized by elevated plasma low-density lipoprotein-cholesterol (LDL-C) levels and high risk for premature cardiovascular disease (CVD). The exact prevalence of molecularly defined hoADH is unknown. Therefore, we investigated the prevalence and phenotypical characteristics of this disease in an open society, i.e. the Netherlands.
Methods and results	The database of the nationwide ADH molecular diagnostic center was queried to identify all molecularly defined hoADH patients. Carriers of non-pathogenic mutations were excluded. Medical records were analysed for data regarding lipid levels and CVD events. Of 104 682 individuals screened for molecular defects, 49 were classified as hoADH (0.05%); 20 were true homozygotes, 25 were compound heterozygotes for *LDLR* mutations, and 4 were homozygous for *APOB* mutations. No bi-allelic *PCSK9* mutation carriers were identified. Consequently, the prevalence of hoADH was estimated to be ~1 : 300 000. Mean LDL-C levels prior to lipid-lowering treatment were 12.9 ± 5.1 mmol/L (range 4.4–21.5 mmol/L). Surprisingly, only 50% of the patients met the clinical criteria for hoADH (LDL-C >13.0 mmol/L); 29% of patients suffered from a CVD event.
Conclusion	The prevalence of molecularly defined hoADH is much higher and the clinical phenotype is more variable than previously assumed. In light of the fact that novel therapies are, or will be registered for the treatment of hoADH patients, an uniform definition of hoADH either as a phenotypic or molecular entity is warranted in order to identify patients who are considered to be eligible for these novel agents.
Keywords	Homozygous • Autosomal dominant hypercholesterolemia • Familial hypercholesterolaemia • Prevalence • Phenotype

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Introduction

Autosomal dominant hypercholesterolaemia (ADH) is caused by mutations in the genes encoding the low-density lipoprotein receptor (*LDLR*; OMIM #606945), apolipoprotein B (*APOB*; OMIM #107730), or proprotein convertase subtilisin−kexin type 9 (*PCSK9*; OMIM #607786). Homozygous ADH (hoADH) is either caused by homozygosity or compound heterozygosity for mutations in these genes and is characterized by increased levels of low-density lipoprotein-cholesterol (LDL-C) and physical signs of cholesterol deposits in the skin, eyes, and/or tendons that are known as xanthelasmas, arcus lipoides corneae, or tendon xanthomas, respectively. As a consequence of the lifelong exposure to elevated LDL-C levels, hoADH patients typically suffer from cardiovascular disease (CVD) at very young age.[1,2] Fortunately, the clinical outcome has changed dramatically for hoADH patients since the introduction of 3-hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors ('statins') in the 1990s.[3,4] Statins lower LDL-C by decreasing the intracellular production of cholesterol synthesis and by hepatic up-regulation of the *LDLR*, and the latter effect is reduced in hoADH patients. Therefore, additional therapy, e.g. LDL-apheresis, is often warranted in these patients to further lower LDL-C levels.[5]

To date, limited data are available about the exact prevalence of hoADH. The historical and most widely cited study reported a prevalence of one in a million, but the disorder is much more frequent in populations with a founder effect such as South Africa and French Canada.[6−8] It is also important to realize that previous estimates were mostly based on clinical, and not on molecular criteria.[9]

Since the 1990s, a cascade screening programme is exploited in the Netherlands with the aim to identify all ADH patients. Similar programmes have been started in a number of countries including Scotland and Wales.[10,11] The unprecedented number of participants in this nationwide programme and the fact that virtually all general practitioners, paediatric lipidologists, cardiologists, and internists are aware of, and actively collaborating within this programme, enabled us to investigate the prevalence and clinical phenotype of molecular defined hoADH in our country.

Methods

Patients and data collection

The molecular diagnostic laboratory of the Academic Medical Center (AMC) in Amsterdam, the Netherlands, serves as a nationwide DNA diagnostic center for ADH. Patients are referred for molecular diagnostics by their physician. Once a pathogenic mutation is identified, the patient is designated as an index case. To identify all affected family members of the index case, cascade screening is performed by the Foundation for Identification of Persons with Inherited Hypercholesterolaemia, subsidized by the Dutch government. The database of the diagnostic center, comprising all molecular diagnostic results, was queried to identify all subjects with hoADH. Homozygous autosomal dominant hypercholesterolaemia was defined as homozygosity or compound heterozygosity for mutations in *LDLR*, *APOB*, or *PCSK9*. Since the Hardy−Weinberg Equilibrium was used to calculate the prevalence of bi-allelic mutation carriers and since this equilibrium assumes a monogenic disorder, double heterozygous ADH patients (carrying a mutation in two different genes, e.g. carriers of a *LDLR* and an *APOB* mutation) were excluded. Carriers of non-pathogenic mutations, patients who were deceased and patients who were living abroad were also excluded.

Medical records were reviewed, and data on physical characteristics, lipid profiles, lipid-lowering therapy (LLT), and cardiovascular events were collected after receiving informed consent. This study was approved by the Medical Ethics Committee of the Academic Medical Center, Amsterdam, the Netherlands.

Results are described for all hoADH patients combined, unless stated otherwise.

Molecular diagnostic procedures

DNA analysis was performed as previously described.[12] Pathogenicity of mutations was defined according to the criteria for functionality as published by Huijgen *et al.*[13] 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 non-pathogenic' and therefore also excluded from this study. Mutations were described according to the nomenclature as proposed by den Dunnen and Antonarakis.[14] For *LDLR* and *APOB* mutations, the numbering was based on the cDNA with nucleotide +1 being A of the ATG initiation codon.

Prevalence of hoADH and statistical analysis

All continuous variables are expressed as means ± standard deviation. To assess differences in lipoprotein levels, continuous variables were analysed using *t*-tests and Mann-Whitney *U* tests, where appropriate. A *P*-value of <0.05 was considered to be statistically significant. Patients were classified into three groups according to the type of *LDLR* mutation: Group 1: two Class 1 mutations (null alleles or large rearrangements); Group 2: one Class 1 mutation and one defective mutation, and Group 3: two defective mutations.[1] Since all identified *APOB* mutations in this study interfere with binding of the LDL particle to the *LDLR*, they were classified as defective mutations (Group 3).

Prevalence was estimated under the assumption that the Hardy−Weinberg equilibrium applies to the Dutch population. In this equilibrium, homozygosity $= p^2$, heterozygosity $= 2pq$, unaffected $= q^2$, and $p + q = 1$. Theoretically, the presence of a few large families with consanguinity would inflate the prevalence. Therefore, we repeated the analyses after excluding patients from consanguineous marriages. Confidence Interval Analysis version 1.0 (London, UK) was used to calculate confidence intervals for the point estimates. All other statistical analyses were performed in IBM SPSS statistics, Inc., version 19.0 (Chicago, IL, USA).

Results

The database of the diagnostic laboratory comprised data from 104 682 individuals who were screened for ADH mutations, of which 178 were found to be carriers of two bi-allelic mutations in either *LDLR* or *APOB*. Of those, 63 patients were identified through the cascade screening programme and 115 were referred (index cases). We excluded 129 patients; 94 carriers of non-pathogenic mutations, 25 patients with double heterozygous ADH (mutation in both *APOB* and *LDLR*), 9 patients who resided outside the Netherlands, and 1 patient deceased (*Figure 1*). In total, 49 patients (36 referred and 13 identified through the genetic cascade screening programme) from 39 families were included in the current analysis. This cohort comprised of 20 homozygotes and 25 compound heterozygotes for *LDLR* mutations (hoFH and compHeFH, respectively), and 4 homozygotes for *APOB* mutations (hoFDB) (*Table 1*). Four hoFH patients from two different families were offspring of
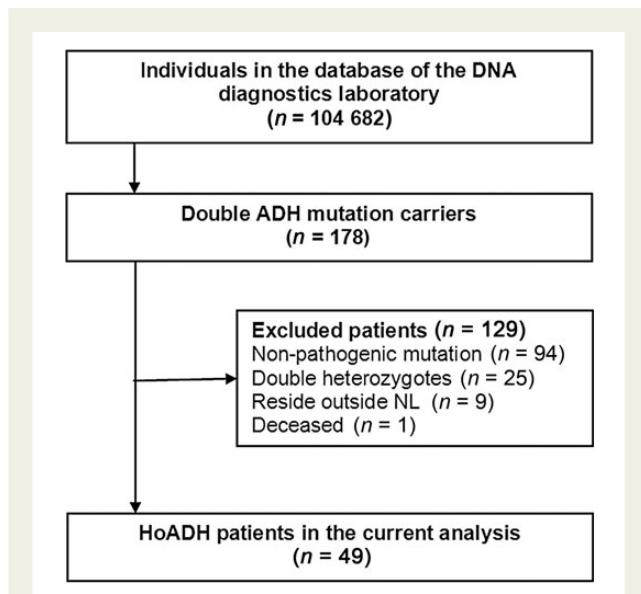
Figure 1 Flow diagram of patient selection. *n*, indicates the number of patients.

consanguineous parents. No compound heterozygous *APOB* or bi-allelic *PCSK9* mutation carriers were identified. At the moment of data collection, the mean age was 37.4 ± 19.2 years (range 3−77 years), and at molecular diagnosis, the mean age was 28.2 ± 19.8 years (range 0−68 years). Lipid-lowering therapy was used by all patients for whom data on medication were present (*n* = 45). Three homozygous and one compound heterozygous patient (all *LDLR* mutants) underwent regular LDL-apheresis.

Prevalence

Based on 16 722 387 inhabitants,[15] the prevalence of hoFH and compHeFH in the Dutch population ranged from 1 in 371 608 (95% CI 1 : 287 356−1 : 526 316) to 1 in 407 863 (95% CI 1 : 312 500−1 : 588 235) persons, after excluding patients from consanguineous parents. Assuming the Hardy−Weinberg equilibrium in the Dutch population, in which $p^2 = 1/407\,863$, $p = 1/639$, and $q = 1 - p$, the prevalence of heterozygous FH (heFH) (2 *pq*) is 1 in 319 persons. The prevalence of hoFDB is 1 in 4 180 597 (95% CI 1 : 2 109 705−1 : 209 205 021), which translates to a prevalence of heterozygous FDB of 1 in 1023 persons.

Based on the calculated prevalences, the number of heterozygous ADH patients in the Netherlands is 68 636 (16 722 387 inhabitants *heFH prevalence + 16 722 387*heFDB prevalence); translating into a heterozygous ADH prevalence of 1 in 244 individuals (1/319 + 1/1023).

Phenotypes

Individual LDL-C levels prior and after LLT are shown in *Figure 2*. Mean LDL-C levels prior to LLT were 12.9 ± 5.1 mmol/L (range 4.4−21.5 mmol/L); LDL-C levels did not differ between hoFH (12.6 ± 5.8 mmol/L, range 4.4−20.8 mmol/L, *n* = 14) and compHeFH (13.4 ± 4.7 mmol/L, range 6.9−21.5 mmol/L, *n* = 17) patients.

In hoFDB patients, LDL-C levels prior to LLT were calculated to be 9.0 mmol/L. Lipid levels prior and after LLT were similar

between index cases and patients identified through the screening programme.

The type of mutation had a significant impact on statin naïve LDL-C levels; significantly, higher LDL-C levels were observed in patients with one or two null alleles compared with patients without null alleles (17.7 ± 2.6 vs. 9.1 ± 2.9 mmol/L; *P* < 0.001).

Almost half (49%) of our patients were found to have LDL-C levels <13.0 mmol/L, and as such they did not meet the clinical criteria for hoADH.[9] About 76% of the patients did not meet another frequently used criterion of an LDL-C level >7.8 mmol/L while receiving LLT.[9]

Thirty per cent of the hoADH patients suffered from a cardiovascular event. The average age of onset was 34.2 ± 17.1 years (range 13−69 years). All premature CVD events occurred in bi-allelic LDLR mutation carriers; 86% of events occurred prematurely (before age 55 in men; before age 60 in women). Three patients suffered from coronary artery disease (CAD) in their second decade of life (ages: 13, 14, and 20 years), and three other patients experienced CAD events in their third decade. One hoFDB patient suffered from peripheral vascular disease, but only at the age of 69 years.

All the 45 patients, for whom data about LLT were present, were using LLT (*Table 1*). Of these patients, 30 (67%) were additionally treated with ezetimibe, 4 (9%) with bile acid sequestrants, and 4 (9%) with nicotinic acid. Fibrates were not used by any of the hoADH patients. Thirteen (29%) patients were treated with statin monotherapy of whom one patient was treated by LDL-apheresis. Twenty-six (58%) patients were treated with two different medications of whom 3 (7%) were also treated with LDL-apheresis. Six (13%) patients were treated with triple therapy. Only 19 (42%) were treated with maximum statin dose combined with ezetimibe. The target LDL-C level (< 2.5 mmol/L) as being recommended by the current ESC/EAS guideline was not reached in any of the patients.[5] The NICE guideline for ADH patients does advise an LDL-C reduction of 50% or more,[16] and this criterion was met in only 43% of the patients.

For a detailed description of individual hoADH patients, see Supplementary material online, *Tables S1* and *S2*.

Discussion

In the current analysis, we established the prevalence of molecularly defined hoADH as ∼1 : 300 000 (1/407 863 hoFH/compHeFH + 1/4 180 597 hoFDB) inhabitants in the Netherlands, which is at least three times more frequent as previously described.[6] The prevalence of hoFDB was found to be 1 in 4 million Dutch inhabitants which, to the best of our knowledge, was not previously addressed in a cohort size like ours.[17] We also observed a significant phenotypical variability in patients diagnosed with molecularly defined hoADH and, in particular, the majority of patients did not fulfil the phenotypic criteria for hoADH.[9]

In 1973, Goldstein *et al.* estimated the prevalence of heterozygous ADH at 1 : 500, which translates into a hoADH prevalence of 1 in a million. This estimate, which was based on the frequency of ADH among relatives of survivors of myocardial infarction,[6] has been widely cited, but limited numbers of studies have specifically addressed the prevalence of heterozygous ADH or homozygous ADH. Higher prevalences, ranging from 1 : 30 000 to 1 : 275 000, have been described in other populations, e.g. French Canadians, South Africans, and Japanese, probably resulting from founder

Table 1 Patient characteristics

	Homozygotes		Compound heterozygotes LDLR
	LDLR	**APOB**	
Number of patients	20	4	25
Age (range)	35.9 (3.3–76.0)	56.5 (33.1–77.5)	35.0 (3.1–65.2)
Female sex	60%	75%	44%
Cardiovascular disease[a] (percentage)	6 (30)	1 (25)	7 (28)
CHD	5 (25)	0 (0)	7 (28)
Stroke/TIA	1 (5)	0 (0)	0 (0)
PVD	1 (5)	1 (25)	0 (0)
Lipid levels not on LLT[b] (SD)			
TC	13.6 ($\pm$5.2)	10.9 ($\pm$1.8)	15.3 ($\pm$4.5)
LDL-C	12.6 ($\pm$5.8)	7.8	13.4 ($\pm$4.7)
Lipid levels on LLT[b] (SD)			
TC	7.3 ($\pm$2.8)	7.2 ($\pm$2.8)	8.2 ($\pm$3.5)
LDL-C	5.7 ($\pm$2.8)	5.0 ($\pm$2.0)	6.6 ($\pm$3.5)
LLT			
Drug therapy[c]	19 (95)	3 (75)	23 (92)
Statins only	8 (40)	1 (25)	4 (16)
Statin + cholesterol absorption inhibitor	10 (50)	2 (50)	13 (52)
Other combination of oral LLT	1 (5)	–	6 (24)
LDL-apheresis	3 (15)	0 (0)	1 (4)

Data shown are numbers (percentage) or means ($\pm$ standard deviation or range).

Lipid levels shown are in mmol/L.

CHD, coronary heart disease; PVD, peripheral vascular disease; LLT, lipid-lowering therapy; TIA, transient ischaemic attack; LDLR, low-density lipoprotein receptor; APOB, apolipoprotein B; SD, standard deviation; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol.

[a]Data about cardiovascular events were available for 20 homozygotes for LDLR mutations, for 3 homozygotes for apoB mutations, and for 24 compound heterozygotes for LDLR mutations.

[b]TC levels not on LLT were available from 20 homozygous LDLR mutation carriers, 22 compound heterozygous LDLR mutation carriers, and 3 homozygous APOB mutation carriers. LDL-C levels not on LLT were available from 14 homozygous LDLR mutation carriers, 17 compound heterozygous LDLR mutation carriers, and 1 homozygous APOB mutation carriers. TC levels on LLT were available from 18 homozygous LDLR mutation carriers, 23 compound heterozygous LDLR mutation carriers, and 2 homozygous APOB mutation carriers. LDL-C levels on LLT were available from 18 homozygous LDLR mutation carriers, 22 compound heterozygous LDLR mutation carriers, and 3 homozygous APOB mutation carriers.

[c]Data about drug therapy used were available from 20 homozygotes for LDLR mutations, 3 homozygotes for APOB mutations, and 23 compound heterozygotes for mutations in the LDLR.

effects or consanguinity.[7,8,18,19] The latter is exemplified by a study in Lebanon, where consanguinity rates were over 60%.[18] Also, patients with two mutations in two different genes (so called 'double heterozygotes') and patients with autosomal recessive hypercholesterolaemia were included in some studies addressing the prevalence of hoADH.[18,19] So, very few accurate data were available to date to be able to determine the hoADH prevalence.

Since 1991, a systematic and nationwide screening programme for causative mutations underlying ADH has been performed in the Netherlands, and since 1994, a large genetic cascade screening programme is implemented to identify affected ADH family members of index cases. Based on the prevalence described by Goldstein and co-workers, ~25 400 ADH patients (corresponding to 76%) of the anticipated number of ~33 400 patients were identified in our country by December 2011.[6,20] Our finding of a hoADH prevalence of ~1 in 300 000 and a calculated prevalence of heterozygous ADH of ~1 in 200 Dutch inhabitants is in line with a previous estimate of the prevalence of heterozygous ADH in the Netherlands[21] and

suggests that we, in fact, have only identified around one-third of the heterozygous ADH cases. The true prevalence of heterozygous ADH might even be higher, which would be in line with data from a recent Danish study that reported a prevalence of 1 : 137.[22] It should be noted, however, that, for this Danish study, clinical ADH criteria were used, which is a combination of lipid levels, clinical symptoms, and family history. The Dutch population is an open society and we used a strict model to estimate the prevalence of hoADH. As a consequence, these data could probably be extrapolated to other societies in Europe and the USA.

Phenotypic diagnostic criteria have been used to diagnose hoADH, and LDL-C levels >13.0 mmol/L are generally accepted as a major criterion for the presence of hoADH.[1,9] It is of note, however, that a minority of patients in our study met this criterion, and the range of LDL-C levels (4.4–21.5 mmol/L) in our study overlaps to a significant extent with LDL-C levels observed in heterozygous ADH patients.[23] Interestingly, we identified a total of 69 heterozygous ADH patients [of 13 080 patients (0.53%), of whom
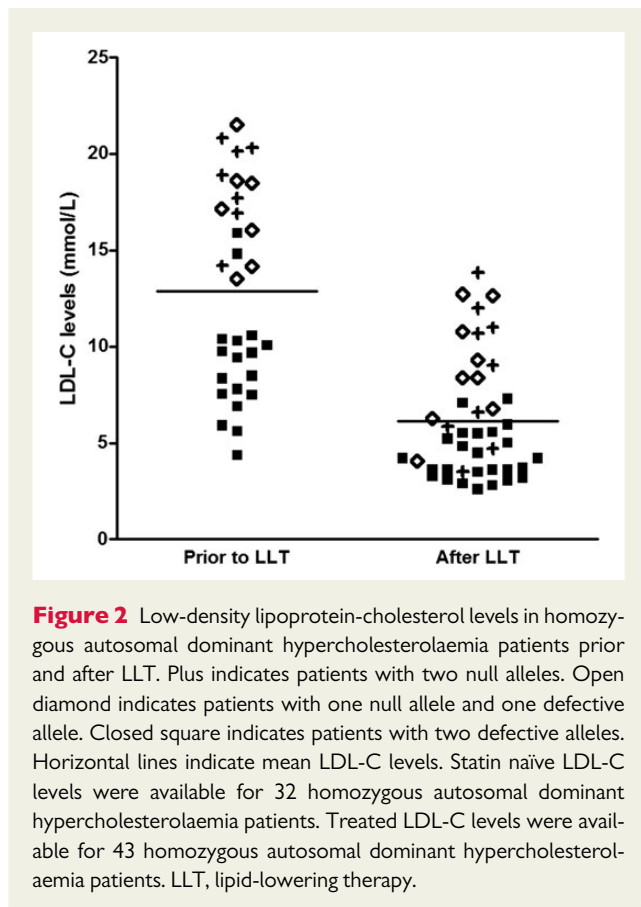
Figure 2 Low-density lipoprotein-cholesterol levels in homozygous autosomal dominant hypercholesterolaemia patients prior and after LLT. Plus indicates patients with two null alleles. Open diamond indicates patients with one null allele and one defective allele. Closed square indicates patients with two defective alleles. Horizontal lines indicate mean LDL-C levels. Statin naïve LDL-C levels were available for 32 homozygous autosomal dominant hypercholesterolaemia patients. Treated LDL-C levels were available for 43 homozygous autosomal dominant hypercholesterolaemia patients. LLT, lipid-lowering therapy.

6 index cases] with untreated LDL-C levels >13 mmol/L in the database of the Foundation for the Identification of Persons with Inherited Hypercholesterolaemia. Based on the clinical criteria, these patients should be considered to suffer from homozygous ADH. This clearly further shows the overlap between heterozygous and homozygous ADH. The potential misperception that a patient with LDL-C levels much lower than expected for hoADH cannot be a carrier of two pathogenic mutations has likely resulted in an underestimation of the prevalence of hoADH. It is therefore no surprise that lipid levels in our study were on average lower than generally assumed in hoADH patients.[9]

Moreover, LDL-C levels observed in our study were also significantly lower than observed in a large retrospective cohort study in hoADH patients performed by Raal and co-workers. In their study, comprising 149 hoFH patients, LDL-C levels were 16.4 ± 3.9 mmol/L and the mean age of hoADH patients in this South African cohort was 26.8 ± 14.6 years, compared with a mean age in our study of 37.4 ± 19.2 years.[4] The majority of patients in the South African study were molecularly diagnosed with hoADH. The large difference with our study is the fact that, in their study, sequencing of *LDLR* and *APOB* was only performed upon a clinical suspicion of hoADH. The latter will result in an inflation of the clinical phenotype associated with the molecular defect. The question, however, remains whether the underestimation of the prevalence of molecularly defined hoADH has any clinical relevance, since it has been shown that cardiovascular risk in ADH patients is driven by LDL-C levels and not by the presence or absence of a mutation *per se*.[24]

Early identification and treatment have been shown to prevent CVD events to occur in patients with ADH.[3] In line, genetic testing and consequent monitoring of lipid levels for the identification and treatment of ADH patients are recommended.[25] In addition, genetic testing of relatives of patients with ADH has shown to be more cost-effective than currently used clinical screening strategies.[26]

It is of note, however, that some guidelines for apheresis recommend consideration of this therapy in homozygous FH patients, independent of the level of plasma LDL-C.[27] Moreover, the approval of US Food and Drug Administration and the European Medicines Agency has been obtained for the lomitapide (Juxtapid®) treatment of hoADH. Mipomersen (Kynamro®) has only been approved for this indication in the USA. The question remains whether only those with a molecular diagnosis should be considered for these novel medications, since this would exclude patients with a clear clinical hoADH phenotype for whom a molecular defect cannot be identified, while we would consider these patients in dire need for a novel therapy.[28]

The origin of the phenotypic variation of hoADH in this study is not completely understood. We used a virtually unbiased approach, focusing on the molecular diagnostics, and the finding of near-normal lipid levels in some of these patients might suggest non-penetrance of molecular defects or counteracting (molecular) mechanisms that lower LDL-C. Worldwide >1700 mutations in the *LDLR* have been described to cause ADH.[29] The severity of the disease partly depends on the residual activity of the *LDLR* and therefore on the severity of the underlying mutation. The impact of ADH causing mutations on LDL-C levels observed in our study is in line with a recently published manuscript about the clinical consequences of mutations in an Italian ADH population.[30] In addition, the phenotypic variability could also be explained by concomitant mutations with effects on LDL-C levels, i.e. in *APOB*, *ANGPTL3*, or *PCSK9* or to lifestyle factors.[31–33]

Some limitations should be taken into account while interpreting the results of our analysis. First, clinical data were obtained from medical records and we were not able to retrieve all clinical data. Secondly, referral bias might be in play since index cases were referred based on clinical abnormalities. However, the subsequent cascade screening was not subjected to bias; all first degree family members were approached irrespective of their clinical status. Therefore, the occurence of 'positive' referral bias was significantly reduced. On the contrary, the results of our study are likely also to be subjected to survival bias. The overall referral bias will, therefore, probably be counterbalanced and thus, is unlikely to have a major influence on the outcome of our study. Third, our data were derived from a relatively small number of patients compared with the entire population of our country. Therefore, it is likely that a number of hoADH patients have not been identified, despite the high awareness of ADH among referring physicians. This, in addition to the fact that we had exclude double heterozygous ADH mutation carriers would imply that the prevalence is even higher than we report in this study.

To the best of our knowledge, this is the first study investigating the phenotypic variation of molecularly defined hoADH. From a clinical and screening perspective, diagnosing molecularly defined hoADH (instead of using phenotypic criteria) is of importance, since both parents and all children of the hoADH patient will be heterozygous carriers.

Therefore, our observations are relevant for the diagnostic strategy in family members of patients with molecularly defined

hoADH. More importantly, it is pivotal to define hoADH either as a clinical or a molecular entity, since this will have a huge impact on the identification of the patients who are deemed eligible for reimbursement for novel agents that lower LDL-C beyond statins (e.g. PCSK9 inhibitors, lomitapide, and antisense APOB therapy).

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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