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From genotype to therapy

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Publication date
2017
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Citation for published version (APA):
Sjouke, B. (2017). *Extreme phenotypes in hypercholesterolemia: From genotype to therapy*. [Thesis, fully internal, Universiteit van Amsterdam].

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Chapter 3

Homozygous Autosomal Dominant Hypercholesterolemia in the Netherlands: Prevalence, Genotype-Phenotype Relationship and Clinical Outcome

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European Heart Journal 2015 Mar 1; 36(9): 560-5.

ABSTRACT

Background Homozygous autosomal dominant hypercholesterolemia (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 analyzed 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.

INTRODUCTION

Autosomal dominant hypercholesterolemia (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 program is exploited in the Netherlands with the aim to identify all ADH patients. Similar programs have been started in a number of countries including Scotland and Wales.[10,11] The unprecedented number of participants in this nationwide program and the fact that virtually all general practitioners, paediatric lipidologists, cardiologists, and internists are aware of, and actively collaborating within this program, 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 Hypercholesterolemia, 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 hypercholesterolemia 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 an *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 cosegregation 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 analyzed 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 program 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 program) from 39 families were included in the current analysis. This cohort comprised of 20 homozygotes and 25 compound heterzygotes 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 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.

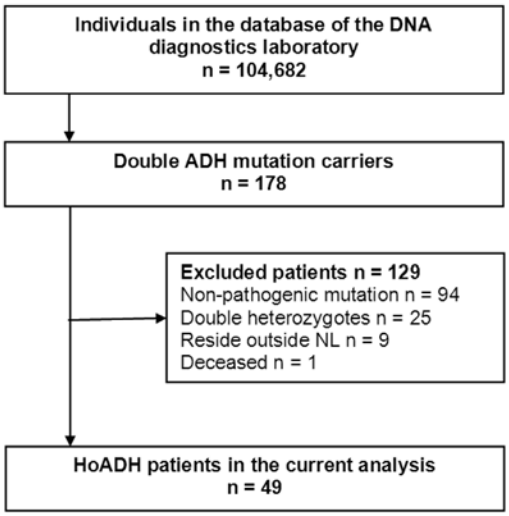

Figure 1. Flow Diagram of Patient Selection.
N indicates number of patients

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* (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† (SD)			
– TC	13.6 (± 5.2)	10.9 (± 1.8)	15.3 (± 4.5)
– LDL-C	12.6 (± 5.8)	7.8	13.4 (± 4.7)
Lipid levels on LLT† (SD)			
– TC	7.3 (± 2.8)	7.2 (± 2.8)	8.2 (± 3.5)
– LDL-C	5.7 (± 2.8)	5.0 (± 2.0)	6.6 (± 3.5)
LLT			
– Drug therapy‡	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 lipid-lowering therapy	1 (5)	–	6 (24)
– LDL-apheresis	3 (15)	0 (0)	1 (4)

Data shown are numbers (percentage) or means (± standard deviation or range). Abbreviations: CHD = coronary heart disease; PVD = peripheral vascular disease; LLT = lipid-lowering therapy; TIA = transient ischemic attack. Lipid levels shown are in mmol/L. * Data about cardiovascular events was available for 20 homozygotes for *LDLR* mutations, for 3 homozygotes for *APOB* mutations and for 24 compound heterozygotes for *LDLR* mutations. † 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 carrier. 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. ‡ Data about drug therapy used was available from 20 homozygotes for *LDLR* mutations, 3 homozygotes for *APOB* mutations and 23 compound heterozygotes for mutations in the *LDLR*.

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.

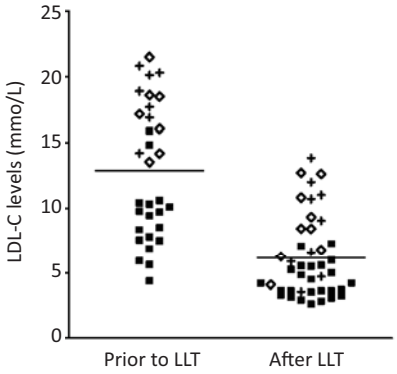

Figure 2. LDL-C Levels in HoADH Patients Prior and After Lipid-Lowering Therapy.
+ Indicates patients with two null alleles ◊ Indicates patients with one null allele and one defective allele ■ Indicates patients with two defective alleles. Horizontal lines indicate mean LDL-C levels. Statin naïve LDL-C levels were available for 32 hoADH patients. Treated LDL-C levels were available for 43 hoADH patients. LLT = lipid-lowering therapy.

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 program.

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*, *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 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 hypercholesterolemia 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 program for causative mutations underlying ADH has been performed in the Netherlands, and since 1994, a large genetic cascade screening program is implemented to identify affected ADH family members of index cases. Based on the

prevalence described by Goldstein and coworkers, ~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 6 index cases] with untreated LDL-C levels > 13 mmol/L in the database of the Foundation for the Identification of Persons with Inherited Hypercholesterolemia. 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 coworkers. 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 occurrence 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).

REFERENCES

1. Goldstein JL, Hobbs HH, Brown MS. Familial Hypercholesterolemia. In: Scriver C, Beaudet A, Sly W, Valle D, eds. The metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001:2863-913.
2. Kolansky DM, Cuchel M, Clark BJ, et al. Longitudinal evaluation and assessment of cardiovascular disease in patients with homozygous familial hypercholesterolemia. American Journal of Cardiology 2008;102:1438-43.
3. Versmissen J, Oosterveer DM, Yazdanpanah M, et al. Efficacy of statins in familial hypercholesterolemia: a long term cohort study. British Medical Journal 2008;337:a2423.
4. Raal FJ, Pilcher GJ, Panz VR, et al. Reduction in mortality in subjects with homozygous familial hypercholesterolemia associated with advances in lipid-lowering therapy. Circulation 2011;124:2202-7.
5. Catapano AL, Reiner Z, G. DB, et al. ESC/EAS Guidelines for the management of dyslipidaemias The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis 2011;217:3-46.
6. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. Journal of Clinical Investigation 1973;52:1544-68.
7. Seftel HC, Baker SG, Sandler MP, et al. A host of hypercholesterolemic homozygotes in South Africa. British Medical Journal 1980;281:633-6.
8. Moorjani S, Roy M, Gagne C, et al. Homozygous familial hypercholesterolemia among French Canadians in Quebec Province. Arteriosclerosis 1989;9:211-6.
9. Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: current perspectives on diagnosis and treatment. Atherosclerosis 2012; 223:262-8.
10. Finnie RM. Cascade screening for familial hypercholesterolemia in Scotland. British Journal of Diabetes and Vascular Disease 2010;10:123-5.
11. Datta BN, McDowell IF, Rees A. Integrating provision of specialist lipid services with cascade testing for familial hypercholesterolemia. Current Opinion in Lipidology 2010;21:366-71.
12. 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.
13. Huijgen R, Kindt I, Fouchier SW, et al. Functionality of sequence variants in the genes coding for the low-density lipoprotein receptor and apolipoprotein B in individuals with inherited hypercholesterolemia. Human Mutation 2010;31:752-60.
14. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Human mutation 2000;15:7-12.
15. Statistics Netherlands (in Dutch: Centraal Bureau voor de Statistiek). 2012. at http://www.cbs.nl/nl-nl/menu/cijfers/default.htm.
16. Wierzbicki AS, Humphries SE, Minhas R. Familial hypercholesterolemia: summary of NICE guidance. British Medical Journal 2008; 337:a1095.
17. Myant NB. Familial defective apolipoprotein B-100: a review, including some comparisons with familial hypercholesterolemia. Atherosclerosis 1993;104:1-18.
18. Fahed AC, Safa RM, Haddad FF, et al. Homozygous familial hypercholesterolemia in Lebanon: a genotype/phenotype correlation. Molecular Genetics and Metabolism 2011;102:181-8.
19. Mabuchi H, Nohara A, Noguchi T, et al. Molecular genetic epidemiology of homozygous familial hypercholesterolemia in the Hokuriku district of Japan. Atherosclerosis 2011;214:404-7.
20. StOEH. StOEH Annual Report 2011, 2012.
21. Lansberg PJ, Tuzgol S, van de Ree MA, Defesche JC, Kastelein JJ. [Higher prevalence of familial hypercholesterolemia than expected in adult patients of four family practices in Netherlands]. Nederlands Tijdschrift voor Geneeskunde 2000; 144:1437-40.
22. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. Journal of Clinical Endocrinology and Metabolism 2012;97:3956-64.
23. Huijgen R, Hutten BA, Kindt I, Vissers MN, Kastelein JJ. Discriminative ability of LDL-cholesterol to identify patients with familial

hypercholesterolemia: a cross-sectional study in 26,406 individuals tested for genetic FH. Circulatiom Cardiovascular Genetics 2012;5:354-9.
24. Huijgen R, Vissers MN, Kindt I, et al. Assessment of carotid atherosclerosis in normocholesterolemic individuals with proven mutations in the low-density lipoprotein receptor or apolipoprotein B genes. Circulation Cardiovascular Genetics 2011;4:413-7.
25. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolemia is under-diagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: Consensus Statement of the European Atherosclerosis Society. European Heart Journal 2013;34:3478-90a.
26. Nherera L, Marks D, Minhas R, Thorogood M, Humphries SE. Probabilistic cost-effectiveness analysis of cascade screening for familial hypercholesterolemia using alternative diagnostic and identification strategies. Heart 2011;97:1175-81.
27. Szczepiorkowski ZM, Winters JL, Bandarenko N, et al. Guidelines on the use of therapeutic apheresis in clinical practice--evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. Journal of Clinical Apherersis 2010;25:83-177.
28. Ahmad Z, Adams-Huet B, Chen C, Garg A. Low prevalence of mutations in known Loci for autosomal dominant hypercholesterolemia in a multiethnic patient cohort. Circulation Cardiovascular Genetics 2012;5:666-75.
29. 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. Annals of Human Genetics 2008;72:485-98.
30. 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.
31. Huijgen R, Sjouke B, Vis K, et al. Genetic variation in APOB, PCSK9, and ANGPTL3 in carriers of pathogenic autosomal dominant hypercholesterolemic mutations with unexpected low LDL-Cl Levels. Human Mutation 2012;33:448-55.
32. Tonstad S, Leren TP, Sivertsen M, Ose L. Determinants of lipid levels among children with heterozygous familial hypercholesterolemia in Norway. Arteriosclerosis Thrombosis and Vascular Biology 1995;15:1009-14.
33. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nature Genetics 2005;37:161-5.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Characteristics of Patients with Homozygous Familial Hypercholesterolemia and Homozygous Familial Defective Apolipoprotein B.

Common nomenclature	Official nomenclature cDNA/protein	Gene	Age	Sex	Current treatment	First event (age)	TC untreated	LDL-C untreated	TC treated	LDL-C treated
1658delACT	c.1658_1660delACT/ p.Tyr553del	*LDLR*	24	M	Atorvastatin 80	None	17.8	16.9	11.1	9.0
313+1/2	c.313+1G>A	*LDLR*	6	F	Rosuvastatin 20, ezetimibe 10	None	21.9	20.8	15.0	13.8
D333G	c.1061A>G/p.Asp354Gly	*LDLR*	40	F	Atorvastatin 160, ezetimibe 10, LDL-apheresis	CAD (14)	20.2	NA	6.7–2.1*	4.9–1.0*
D333G	c.1061A>G/p.Asp354Gly	*LDLR*	32	F	Atorvastatin 80, ezetimibe 10, LDL-apheresis	CAD (13)	22.4	20.3	10.3–2.6*	8.4–1.3*
G186G	c.621C>G/p.Gly186Gly splice defect	*LDLR*	31	M	Atorvastatin 80, ezetimibe 10	None	12.0	10.4	6.9	5.5
G186G	c.621C>G/p.Gly186Gly splice defect	*LDLR*	29	F	Rosuvastatin 40, ezetimibe 10	ACS (20)	16.5	14.8	6.6	5.0
G-20R GGG>AGG signal peptide	c.4G>A/p.Gly2Arg	*LDLR*	67	M	Simvastatin 40	None	8.6	5.9	5.7	3.2
G571E	c.1775G>A/p.Gly592Glu	*LDLR*	63	F	Simvastatin 20	NA	8.0	NA	5.0	3.6
insertion exon 11-12	c.1586+?_1845+?_dup	*LDLR*	3	M	Rosuvastatin 10	None	15.6	14.2	12.1	10.7
insertion exon 11-12	c.1586+?_1845+?_dup	*LDLR*	11	F	Rosuvastatin 10, LDL-A	None	21.6	20.1	7.4–2.9*	6.2–2.0*
L590F	c.1833G>C/p.Leu611Phe	*LDLR*	50	F	NA	NA	6.7	4.4	NA	NA
L590F	c.1833G>C/p.Leu611Phe	*LDLR*	72	M	Atorvastatin 20	MI (32)	9.0	NA	6.2	4.8

Common nomenclature	Official nomenclature cDNA/protein	Gene	Age	Sex	Current treatment	First event (age)	TC untreated	LDL-C untreated	TC treated	LDL-C treated
L590F	c.1833G>C/p.Leu611Phe	*LDLR*	75	M	Rosuvastatin 40, ezetimibe 10	CAD (63), PVD (74)	13.0	NA	5.1	3.6
N543H/2393del9	c.1690A>C;2393_2401del/ p.N564H;L799_F801del	*LDLR*	38	F	Atorvastatin 80	None	> 12.0	NA	5.4	3.5
N543H/2393del9	c.1690A>C;2393_2401del/ p.N564H;L799_F801del	*LDLR*	24	F	Rosuvastatin 10	None	6.9	5.6	5.8	4.2
N543H/2393del9	c.1690A>C;2393_2401del/ p.N564H;L799_F801del	*LDLR*	48	M	Atorvastatin 80, colesevelam 3750	TIA (36)	12.0	NA	NA	NA
N543H/2393del9	c.1690A>C;2393_2401del/ p.N564H;L799_F801del	*LDLR*	25	F	Atorvastatin 60, ezetimibe 10	None	9.3	7.6	5.3	3.6
S285L	c.917C>T/p.Ser306Leu	*LDLR*	28	M	Atovastatin 60, ezetimibe 10	None	11.8	10.6	8.5	7.1
V502M	c.1567G>A/p.Val523Met	*LDLR*	26	F	Atorvastatin 40, ezetimibe 10	None	18.2	15.9	7.0	5.5
V502M	c.1567G>A/p.Val523Met	*LDLR*	14	F	Atorvastatin 10, ezetimibe 10	None	9.7	8.4	7.2	6.0
R3500Q	c.10580G>A/p.Arg3527Gln	*APOB*	62	F	NA	None	13.0	NA	NA	NA
R3500Q	c.10580G>A/p.Arg3527Gln	*APOB*	52	F	Atorvastatin 80, ezetimibe 10	None	9.6	7.8	5.2	3.6
R3500Q	c.10580G>A/p.Arg3527Gln	*APOB*	77	M	Rosuvastatin 20, ezetimibe 10	Abdominal aneurysm (69)	10.1	NA	NA	4.2
R3500W	c.10579C>T/p.Arg3500Trp	*APOB*	32	F	NA	NA	NA	NA	9.2	7.3

Abbreviations: CAD = coronary artery disease; F = female; M = male; MI = myocardial infarction; NA = not available; PVD = peripheral vascular disease; TIA = transient ischemic attack; ACS = acute coronary syndrome. Lipid levels shown are in mmol/L. Drug doses are in mg/day unless otherwise stated. * Presented cholesterol ranges are pre- and post-LDL-apheresis levels. Lipid levels are shown in mmol/L.

Supplementary Table 2. Characteristics of Patients with Compound Heterozygous Familial Hypercholesterolemia.

Common nomenclature		Official nomenclature cDNA/protein		Age	Sex	Treatment	First event (age)	TC untreated	LDL-C untreated	TC treated	LDL-C treated
Mutation 1	Mutation 2	Mutation 1	Mutation 2								
2.5kb deletion exon 7-8	4.4 kb duplication exon 9-12 in intron 12	c.940+?_-?_1186+?_del	c.1186+?_1845+?dup	21	M	Atorvastatin 80, ezetimibe 10, nicotinic acid/ laropiprant 2000/40	None	20.1	18.9	13.2	11.0
2.5kb deletion exon 7-8	4.4 kb duplication exon 9-12 in intron 12	c.940+?_1186+?_del	c.1186+?_1845+?dup	18	F	Atorvastatin 80, colesevelam 625, nicotinic acid/laropiprant 1000/20	None	18.9	17.7	14.5	12.0
2.5kb deletion exon 7-8	G322S	c.940+?_1186+?del	c.1027G>A/ p.Gly343Ser	36	F	Atorvastatin 80, Ezetimibe 10, Nicotinic acid 2000	None	15.3	13.5	8.0	4.1
13kb deletion promoter and exon 1	S285L	c.1-?_67+?del	c.917C>T/p.Ser306Leu	36	F	Atorvastatin 80, ezetimibe 10, LDL-apheresis	None	19.8	NA	10/14–4.0*	4.0–3.0*
13kb deletion promoter and exon 1	S285L	c.1-?_67+?del	c.917C>T/p.Ser306Leu	34	M	Rosuvastatin 10†, ezetimibe 10	None	17.8	NA	13.9	12.7
16kb deletion exon 12-18	R574Q	c.1705+?_2580+?del	c.1784G>A/p. Arg595Gln	24	M	Rosuvastatin 40, ezetimibe 10, colesevelam 3750	None	18.3	16.0	7.8	6.8
190+4A>T	1480del121bp	c.190+4A>T	c.1480_1586+14del/ p.Val494LeufsX6	55	M	Atorvastatin 80, ezetimibe 10	CAD(34)	18.0	NA	8.5	6.6

Common nomenclature		Official nomenclature cDNA/protein		Age	Sex	Treatment	First event (age)	TC untreated	LDL-C untreated	TC treated	LDL-C treated
Mutation 1	Mutation 2	Mutation 1	Mutation 2								
314-1	S285L	c.314-1G>A	c.917C/T/p.Ser306Leu	2	F	Rosuvastatin 5	No	16.6	14.2	13.8	12.6
2204ins13bp	N543H/ 2393del9	c.2191_2203dup/ p.Ala735GlyfsX51	c.[1690A>C;2393_ cx2401del]/ p.[N564H;L799_ F801del]	33	M	Rosuvastatin 40, ezetimibe 10	CAD (26)	23.0	21.5	10.8	8.4
2417insG	D283N	c.2416dup/ p.Val806GlyfsX11	c.910G>A/p.Aps304Asn	9	M	Rosuvastatin 20, ezetimibe 10	None	20.1	18.5	10.3	9.3
2417insG	G352D	c.2416dup/ p.Val806GlyfsX11	c.1118G>A/ p.Gly373Asp	27	F	Atorvastatin 80, ezetimibe 10, nicotinic acid/ laropiprant 1000/20	CAD (25)	19.9	18.8	12.6	10.8
2417insG	G352D	c.2416dup/ p.Val806GlyfsX11	c.1118G>A/ p.Gly373Asp	16	M	Atorvastatin 80, ezetimibe 10	None	18.9	17.2	9.9	8.4
C201X	E336G	c.666C>A/ p.Cys222*	c.1070A>G/ p.Glu357Gly	49	M	NA	AP (33)	NA	NA	NA	NA
D245E	N543H/ 2393del9	c.796G>A/ p.Asp266Glu	c.[1690A>C;2393_ 2401del]/ p.[N564H;L799_ F801del]	31	F	Rosuvastatin 20, ezetimibe 10	None	11.3	10.1	6.5	4.5
D245E	N543H/ 2393del9	c.796G>A/ p.Asp266Glu	c.[1690A>C;2393_ 2401del]/ p.[N564H;L799_ F801del]	29	F	Rosuvastatin 40	None	11.3	10.0	7.3	5.2
D321N	G352D	c.1024G>A/ p.Asp342Asn	c.1118G>A/ p.Gly373Asp	58	M	Atorvastatin 40	MI (54)	8.7	6.9	4.5	2.8

Common nomenclature		Official nomenclature cDNA/protein		Age	Sex	Treatment	First event (age)	TC untreated	LDL-C untreated	TC treated	LDL-C treated
Mutation 1	Mutation 2	Mutation 1	Mutation 2								
D461N	N407K	c.1444G>A/ p.Asp482Asn	c.1284C>G/ p.Asn428Lys	57	M	Atorvastatin 40 mg/day, ezetimibe 10	None	9.0	NA	4.7	3.1
D461N	N407K	c.1444G>A/ p.Asp482Asn	c.1284C>G/ p.Asn428Lys	61	M	Atorvastatin 80, ezetimibe 10	MI (37)	9.5	7.5	4.3	2.9
G186G	S285L	c.621C>G/p.=? Splice defect	c.917C>T/ p.Ser306Leu	36	F	Rosuvastatin 40, ezetimibe 10	None	12.0	9.7	5.5	3.9
G186G	G314V	c.621C>G/p.=? Splice defect	c.1004G>T/ p.Gly335Val	37	M	Rosuvastatin 40, ezetimibe 10, colesevelam	AP (23), CAD (29)	NA	NA	6.8	5.6
G361V	T41M	c.1145G>T/p.Gly382Val	c.185C>T/ p.Thr62Met	46	F	Atorvastatin 20, ezetimibe 10	None	10.0	8.5	3.8	2.6
I564N	R236W/D568N	c.1754_1755delinsAT/ p.Ile585Asn	c.[769C>T;1765G>A]/ p.[Arg257Trp; p.Asp589Asn]	17	M	Rosuvastatin 10	None	11.2	9.4	4.8	3.1
Q12X	V815I	c.97C>T/p.Gln33*	c.2506G>A/ p.Val836Ile	34	F	NA	NA	NA	NA	NA	NA
Q12X	V815I	c.97C>T/p.Gln33*	c.2506G>A/ p.Val836Ile	29	M	Rosuvastatin 40	None	15.0	NA	5.0	NA
S285L	V415A	c.917C>T/p.Ser306Leu	c.1307T>C/ p.Val436Ala	65	F	Rosuvastatin 40, ezetimibe 10	None	11.3	10.3	4.9	3.3

Abbreviations: CAD = coronary artery disease; F = female; M = male; AP = angina pectoris; MI = myocardial infarction; NA = not available; Lipid levels shown are in mmol/L. Drug doses are in mg/day unless stated otherwise. * Presented ranges are pre- and post-LDL apheresis levels. †In uptitration phase to rosuvastatin 40 mg/day