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File ID	123191
Filename	Chapter 2: Results from a family- and DNA-based screening programme for familial hypercholesterolaemia
Version	Final published version (publisher's pdf)

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type	Dissertation
Title	Health services research at work for national health policy
Author	A.H.A. ten Asbroek
Faculty	Faculty of Medicine
Year	2006
Pages	225
ISBN	9071433757 ; 9789071433757

FULL BIBLIOGRAPHIC DETAILS:
http://dare.uva.nl/record/199437

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Chapter

2

Results from a family and DNA based active identification programme for familial hypercholesterolaemia

A.H.A. ten Asbroek, P.J. Marang-van de Mheen,
J.C. Defesche, J.J.P. Kastelein, L.J. Gunning-Schepers

J Epidemiol Community Health 2001 55 500-502

Introduction

Heterozygous familial hypercholesterolaemia (FH) is a common inborn error of lipoprotein metabolism, which strongly predisposes for coronary artery disease (CAD) and premature cardiac death [1]. In 1994, a family based active identification programme for FH was implemented in the Netherlands [2]. It is based on DNA diagnosis of the LDL-receptor gene mutation which enables us to search selectively for patients in a high-risk population. The programme initially targets first and second-degree relatives of FH probands (diagnosed at Lipid Research Clinics throughout the country) and extends further into the family only when new patients are identified. The programme aims to identify mutation carriers and to refer them to Lipid Research Clinics for extensive individual risk assessment and, if necessary, treatment. Since no carefully collected data are available for cholesterol levels among the general population of LDL-receptor gene mutation carriers, the large majority of whom are asymptomatic, we studied the prevalence of hypercholesterolaemia among screenees with a proved LDL-receptor gene mutation.

Methods and Results

Between 1994 and 1998 2814 adults were screened. The estimated response rate was constant over the years at 90% [3;4]. For reasons of comparison with available population data for total serum cholesterol levels [5], we selected those who were between 20 and 60 years of age (1856 screenees). Depending on the available funds in the screening programme, which were lacking in certain periods, single cholesterol measurements were taken at the time of screening. Therefore, we analysed the data of all 1005 persons who had DNA test results as well as cholesterol measurements. These were a non-selective sample of the 1856 screenees between 20 and 60 years of age. Cholesterol was measured using commercially available kits (Boehringer Mannheim, Mannheim, Germany). Genomic DNA was isolated from the leukocyte fraction of 10 ml of freshly collected blood, followed by polymerase chain reaction (PCR) and restriction enzyme analysis.

From the perspective of the screening programme, the screenees that are already treated can not be considered as new cases and they do not benefit from the screening programme in the same manner as newly identified cases. Therefore, we present the prevalence of hypercholesterolaemia among all screenees as well as the prevalence of hypercholesterolaemia among those not yet treated with HMG-CoA reductase inhibitors.

Hypercholesterolaemia was defined as either an untreated total cholesterol (TC) level above the 95th percentile for age and sex in the Dutch population (table1) [5], or as receiving HMG Co-A reductase inhibitors. We also show the total cholesterol distribution for the untreated screenees using conventional cut-off points (<6.5, 6.5-7.9, >=8 mmol/l). All LDL-receptor

Table 1. MORGEN Project Data 1996-1997: mean total serum cholesterol (TC) and standard deviation (SD) as well as 95th percentile (P95) in the general Dutch population by sex and age group.

MEN				
Age group (years)	20-29	30-39	40-49	50-59
N	589	890	1210	1030
Mean TC (SD)	4.4 (0.9)	5.0 (1.0)	5.5 (1.0)	5.5 (1.0)
P95	5.8	6.7	7.2	7.1
WOMEN				
Age group (years)	20-29	30-39	40-49	50-59
N	843	1140	1450	1139
Mean TC (SD)	4.6 (0.8)	4.8 (0.8)	5.1 (0.9)	5.7 (1.0)
P95	6.1	6.3	6.7	7.4

Table 2. Prevalence of hypercholesterolaemia[1] (HC) by sex, age group and DNA test result in all screenees and prevalence of hypercholesterolaemia, mean total serum cholesterol(TC), standard deviation (SD) and total serum cholesterol distribution, using conventional cut-off values (6.5 and 8.0 mmol/l) as well as the 95th percentile (P95)[2] in untreated screenees.

MEN		20-29 years			30-39 years		
		all	fh+	fh-	all	fh+	fh-
All[3]	N (%)	105 (100)	35 (33.3)	70 (66.7)	141 (100)	63 (44.7)	78 (55.3)
	% HC	39.0	82.9	17.1	46.1	76.2	21.8
Untreated	N (%)	101(100)	31(30.7)	70 (69.3)	116 (100)	41(35.3)	75 (64.7)
	Mean TC (SD)	5.5 (1.4)	6.9 (1.3)	4.9 (0.9)	6.3 (1.5)	7.5 (1.4)	5.7 (1.1)
	TC< 6.5 (%)	81 (80.2)	13 (41.9)	68 (97.1)	68 (58.6)	10 (24.4)	58 (77.3)
	6.5 <= TC< 8.0 (%)	11 (10.9)	9 (29.0)	2 (2.9)	34 (29.3)	19 (36.3)	15 (20.0)
	TC >= 8.0 (%)	9 (8.9)	9 (29.0)	0	14 (12.1)	12 (29.3)	2 (2.7)
	% > P95	36.6	80.6	17.1	34.5	63.4	18.7
WOMEN		**20-29 years**			**30-39 years**		
		all	**fh+**	**fh-**	**all**	**fh+**	**fh-**
All	N	103 (100)	40 (38.8)	63 (61.2)	168 (100)	60 (35.7)	108 (64.3)
	% HC	44.7	90.0	15.9	39.3	83.3	14.8
Untreated	N	89 (100)	26 (29.2)	63 (70.8)	146 (100)	40 (27.4)	106 (72.6)
	Mean TC (SD)	5.7 (1.7)	7.5 (1.5)	5.0 (1.1)	5.7 (1.7)	7.3 (1.4)	5.1 (1.1)
	TC< 6.5 (%)	65 (73.0)	8 (30.8)	57 (90.5)	106 (72.6)	12 (30.0)	94 (88.7)
	6.5 <= TC< 8.0 (%)	12 (13.5)	8 (30.8)	4 (6.3)	29 (19.9)	18 (45.0)	11 (10.4)
	TC >= 8.0 (%)	12 (13.5)	10 (38.5)	2 (3.2)	11 (7.5)	10 (25.0)	1 (0.9)
	% > P95	36.0	84.6	15.9	30.1	75.0	13.2

1 Hypercholesterolaemia was defined as either an untreated total cholesterol (TC) level above the 95th percentile for age and sex in the Dutch population (table1), or as receiving HMGco-A ruductase inhibitors.

gene mutation carriers were heterozygotes. None of the screenees had been tested for a LDL-receptor gene mutation before. Table 2 shows the results for the screened population.
It is evident that each age category contains LDL-receptor gene mutation carriers who do not have hypercholesterolaemia: 19.8% in all men, 32.3% in untreated men and 16.7% in women, 28.7% in untreated women. Furthermore, it is shown that the prevalence of mutation carriers among all screenees tends to be lower in the older age groups. This is probably the result of selective mortality. However, the prevalence of mutation carriers among untreated screenees is also lower in the older age groups. This is not purely the result of selective mortality but it is mainly due to the fact that an increasing proportion of those screened in the older age groups is already treated with cholesterol lowering drugs, and more in mutation carriers than in those without a mutation since they have generally higher cholesterol levels. This might also explain why the prevalence of hypercholesterolaemia in untreated female mutation carriers is lower in the older age groups.

Discussion

These data have not been shown before in such a large and well-defined cohort, and have important consequences for case finding strategies for inherited disorders of lipoprotein

40-49 years			50-59 years			All ages (20-59 years)		
all	fh+	fh-	all	fh+	fh-	all	fh+	fh-
134 (100)	38 (28.4)	96 (71.6)	98 (100)	26 (26.5)	72 (73.5)	478 (100)	162 (33.9)	316 (66.1)
35.8	76.3	19.8	32.7	92.3	11.1	38.9	80.2	17.7
109 (100)	17 (15.6)	92 (84.4)	79 (100)	10 (12.7)	69 (87.3)	405 (100)	99 (24.4)	306 (75.6)
6.2 (1.2)	7.4 (1.0)	6.0 (1.1)	6.3 (1.0)	7.7 (0.7)	6.1 (0.9)	6.1 (1.3)	7.3 (1.3)	5.7 (1.1)
71 (65.1)	4 (23.5)	67 (72.8)	52 (65.8)	0	52 (75.4)	272 (67.2)	27 (27.3)	245 (80.1)
30 (27.5)	8 (47.1)	22 (23.9)	19 (24.1)	6 (60.0)	13 (18.8)	94 (23.2)	42 (42.4)	52 (17.0)
8 (7.3)	5 (29.4)	3 (3.3)	8 (10.1)	4 (40.0)	4 (5.8)	39 (9.6)	30 (30.3)	9 (2.9)
21.1	47.1	16.3	16.5	80.0	7.2	27.9	67.7	15.0
40-49 years			50-59 years			All ages (20-59 years)		
all	fh+	fh-	all	fh+	fh-	all	fh+	fh-
146 (100)	43 (29.5)	103(70.5)	110 (100)	31(28.2)	79 (71.8)	527(100)	174 (33.0)	353 (67.0)
34.2	83.7	13.6	31.8	74.2	15.2	37.4	83.3	14.7
124 (100)	22 (17.7)	102(82.3)	89 (100)	13 (14.6)	76 (85.4)	448 (100)	101(22.5)	347 (77.5)
5.9 (1.2)	7.3 (1.5)	5.6 (0.9)	6.5 (1.1)	7.8 (1.4)	6.3 (0.9)	5.9 (1.4)	7.4 (1.4)	5.5 (1.1)
93 (75.0)	7 (31.8)	86 (84.3)	45 (50.6)	1 (7.7)	44 (57.9)	309 (69.0)	28 (27.7)	281 (81.0)
26 (21.0)	10 (45.5)	16 (15.7)	37 (41.6)	8 (61.5)	29 (38.2)	104 (23.2)	44 (43.6)	60 (17.3)
5 (4.0)	5 (22.7)	0	7 (7.9)	4 (30.8)	3 (3.9)	35 (7.3)	29 (28.7)	6 (1.7)
22.6	68.2	12.7	15.7	38.5	11.8	26.3	71.3	13.3

2 95th percentile for total cholesterol in the Dutch general population as observed in the MORGEN-study (Source: RIVM Bilthoven The Netherlands) (see table 1).
3 All= treated and untreated screenees

metabolism with a known molecular basis. Our study shows that if in a high risk population of yet untreated, mainly asymptomatic mutation carriers, a single TC level would be used for the diagnosis of FH rather than the current gold standard, i.e. the presence of a LDL-receptor gene mutation, the diagnosis would be missed in more than a quarter of the FH patients.

As is shown by others, FH is not fully penetrant from birth onwards [6]. It has not been documented before, however, to which extent the genetic disorder causes hypercholesterolaemia in a population of mainly asymptomatic adult relatives of genetically diagnosed patients as is shown by the present study.

The high prevalence of "lower" TC levels in our cohort may well reflect a combination of factors like patients' adherence to low calorie diets for weight loss, intercurrent illness of infectious nature, a better general health of participants in a screening programme, or a protective genetic constitution. In addition, because cholesterol levels vary with LDL-receptor mutation [7], we cannot exclude that our findings of normal cholesterol levels in LDL-receptor gene mutation carriers are the result of screening for mutations that may result in a milder than expected phenotype as reported elsewhere, albeit very unlikely. Nevertheless, even though a single measurement of cholesterol is not very reliable when assessing the individual cardiovascular disease risk, for the purpose of assessing the cholesterol values on a population level a single measurement can be used [8].

The prevalence of hypercholesterolaemia in those without a LDL-receptor gene mutation is higher than in the general population, which indicates that in this high-risk population probably also other factors than the LDL-receptor gene mutation contribute to the prevalence of hypercholesterolaemia.

The importance of our findings depends largely on whether patients with a LDL-receptor gene mutation but without hypercholesterolaemia, experience an increased risk of coronary heart disease and whether they need the same rigorous treatment as other FH patients. This is currently unknown. Since damage to the vascular wall in FH patients is likely to be the result of the number of cholesterol-years [9], a single TC level below the 95th percentile could be falsely reassuring. Comprehensive appraisal of cardiovascular disease risk and cholesterol screening at regular intervals is advised. In a follow up study, we plan to further explore explanations for our findings and to assess whether the risk of coronary heart disease is increased in this group of mutation carriers in whom FH does not seem to be fully penetrant.

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