Original Investigation

Association Between Familial Hypercholesterolemia and Prevalence of Type 2 Diabetes Mellitus

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IMPORTANCE Familial hypercholesterolemia is characterized by impaired uptake of cholesterol in peripheral tissues, including the liver and the pancreas. In contrast, statins increase the cellular cholesterol uptake and are associated with increased risk for type 2 diabetes mellitus. We hypothesize that transmembrane cholesterol transport is linked to the development of type 2 diabetes.

OBJECTIVE To assess the association between type 2 diabetes prevalence and familial hypercholesterolemia.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study in all individuals (n = 63 320) who underwent DNA testing for familial hypercholesterolemia in the national Dutch screening program between 1994 and 2014.

EXPOSURES Deleteriousness and nondeleteriousness of familial hypercholesterolemia mutations were based on literature or laboratory function testing. Low-density lipoprotein (LDL) receptor mutations were considered more severe than apolipoprotein B gene (*APOB*) mutations, and receptor-negative LDL receptor mutations were considered more severe than receptor-deficient mutations.

MAIN OUTCOMES AND MEASURES Prevalence of type 2 diabetes.

RESULTS The prevalence of type 2 diabetes was 1.75% in familial hypercholesterolemia patients (n = 440/25 137) vs 2.93% in unaffected relatives (n = 1119/38 183) (*P* < .001; odds ratio [OR], 0.62 [95% CI, 0.55-0.69]). The adjusted prevalence of type 2 diabetes in familial hypercholesterolemia, determined using multivariable regression models, was 1.44% (difference, 1.49% [95% CI, 1.24%-1.71%]) (OR, 0.49 [95% CI, 0.41-0.58]; *P* < .001). The adjusted prevalence of type 2 diabetes by *APOB* vs LDL receptor gene was 1.91% vs 1.33% (OR, 0.65 [95% CI, 0.48-0.87] vs OR, 0.45 [95% CI, 0.38-0.54]), and the prevalence for receptor-deficient vs receptor-negative mutation carriers was 1.44% vs 1.12% (OR, 0.49 [95% CI, 0.40-0.60] vs OR, 0.38 [95% CI, 0.29-0.49]), respectively (*P* for trend <.001 in both comparisons).

CONCLUSIONS AND RELEVANCE In a cross-sectional analysis in the Netherlands, the prevalence of type 2 diabetes among patients with familial hypercholesterolemia was significantly lower than among unaffected relatives, with variability by mutation type. If this finding is confirmed in longitudinal analysis, it would raise the possibility of a causal relationship between LDL receptor-mediated transmembrane cholesterol transport and type 2 diabetes.

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Familial hypercholesterolemia is a dominantly inherited disease with impaired hepatic cholesterol uptake, characterized by high plasma levels of low-density lipoprotein (LDL) cholesterol.[1] Patients with familial hypercholesterolemia are at increased risk for premature cardiovascular disease (CVD) and are therefore under strict clinical follow-up. It has been noticed that familial hypercholesterolemia patients might be less prone to develop type 2 diabetes mellitus, although this was never substantiated.

In contrast, the risk for type 2 diabetes is increased in statin users,[2] and genetic variation at the 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR* [NCBI Entrez Gene 3156]) locus is associated with type 2 diabetes as well.[3] Statins inhibit *HMGCR*, increasing the expression of LDL receptors (*LDLR*) in many tissues and promoting transmembrane cholesterol transport.[4] This is exactly opposite to the genetically impaired cellular cholesterol uptake in familial hypercholesterolemia. So, perturbation in cellular cholesterol transport might be implicated in the pathogenesis of type 2 diabetes.

We hypothesized that the prevalence of type 2 diabetes is decreased in patients with familial hypercholesterolemia because their pancreatic beta cells have decreased cholesterol uptake and therefore improved function and survival. We tested this hypothesis using the registry of the national Dutch familial hypercholesterolemia screening program. It is also possible to explore a dose-response relationship, since familial hypercholesterolemia is caused by a large number of mutations with different consequences for the clinical phenotype of a carrier.[5] In general, carriers of mutations in the apolipoprotein B (*APOB* [NCBI Entrez Gene 338]) gene express a less severe phenotype (ie, lower levels of LDL cholesterol and lower risk for CVD compared with carriers of the LDL receptor (*LDLR* [NCBI Entrez Gene 3949]) mutation.[6,7] Furthermore, *LDLR* mutations can be divided into receptor-negative and receptor-defective mutations, and the latter commonly result in a milder phenotype.[8,9] Demonstrating such a relationship between mutation severity and clinical outcome would support the argument for causality.

The aim of our study was to compare the prevalence of type 2 diabetes between patients with familial hypercholesterolemia and their unaffected relatives.

Methods

Study Population and Collection of Data

The source population was extracted from the familial hypercholesterolemia screening program registry in the Netherlands—a nationwide and government-subsidized cascade screening program initiated in January 1994 to identify all domestic patients with familial hypercholesterolemia. The screening cascade starts with an individual with high suspicion for being a carrier of a familial hypercholesterolemia mutation (index patient). If a mutation is demonstrated, DNA analysis is performed in first-degree relatives as well. Once a familial hypercholesterolemia mutation is demonstrated in one of the relatives, the patient's first-degree relatives are subsequently approached for further analysis. Data are collected during a single visit. A certified genetic field worker (a member of the screening program, educated about familial hypercholesterolemia and trained to use the questionnaire and perform venous blood sampling) interviewed participants at home using a standardized questionnaire and gathered demographic and clinical data as well as information about medication use. Additionally, a blood sample was drawn for DNA analysis and to measure lipids and lipoproteins. The latter was done occasionally before 2004 and became standard practice as of 2004.

Participants were eligible for our study if they were screened for familial hypercholesterolemia mutations from January 1994 until January 2014. Index patients, both with and without a mutation causing familial hypercholesterolemia, were included. Patients with homozygous familial hypercholesterolemia were excluded. Only carriers of deleterious mutations were classified as patients with familial hypercholesterolemia. Mutations were considered nondeleterious based on evidence in the literature or when functionality tests by the laboratory of Experimental Vascular Medicine in the Academic Medical Center showed nonfunctionality. A list of nondeleterious mutations is provided in eTable 1 in the Supplement. All participants provided written informed consent. This study was approved by the medical ethics committee of the Academic Medical Center in Amsterdam.

Lipid Profile and Mutation Analysis

The lipid profile was measured with the LDX-analyzer (Cholestech Corporation).[10] LDL cholesterol levels were calculated with the Friedewald formula unless triglycerides were greater than 400 mg/dL (4.5 mmol/L).[11] In patients using lipid-lowering therapy at the time of screening, the untreated LDL cholesterol level was calculated according to a validated equation.[12]

DNA of the tested individuals was isolated from 10 mL of freshly collected blood containing ethylenediamine tetraacetic acid as anticoagulant. The method of mutation analysis has been described previously.[13,14]

Primary Outcome

The primary outcome was presence of type 2 diabetes. Type 2 diabetes was defined by self-reported diagnosis; the presence of diabetes was recorded by the genetic field worker using a standardized questionnaire. Patients who were likely to have type 1 diabetes, which was defined as diabetes diagnosed before the age of 30 years and/or diabetes requiring insulin therapy diagnosed before the age of 40 years,[15,16] were classified as having non-type 2 diabetes.

Statistical Analysis

Differences in descriptive characteristics between familial hypercholesterolemia patients and unaffected relatives were evaluated by means of logistic and linear regression (for dichotomous and continuous variables, respectively).

We explored the difference in type 2 diabetes prevalence between patients with familial hypercholesterolemia and unaffected relatives using univariable logistic regression analy-

Table 1. Demographic and Clinical Characteristics of All Participants

	Patients With Familial Hypercholesterolemia (n = 25 137)	Unaffected Relatives (n = 38 183)	*P* Value
Male sex, No. (%)	11 920 (47.4)	18 340 (48.0)	.13
Age, mean (SD), y	38 (20.6)	43 (20.0)	<.001
Body mass index, mean (SD)[a]	23.5 (5.4)	24.4 (5.3)	<.001
Current smoker, No. (%)	4917 (19.8)	11 222 (29.4)	<.001
Statin use, No. (%)	7271 (28.9)	3431 (9.0)	<.001
Statin intensity categories, No. (%)[b]			
Low[b]	513 (2.0)	440 (1.2)	<.001[c]
Moderate	4754 (18.9)	2657 (7.0)	
High	2006 (8)	336 (0.9)	
History of cardiovascular disease, No. (%)[d]	1864 (7.4)	1916 (5.0)	<.001
Cholesterol, mean (SD), mg/dL			
Low-density lipoprotein[d,e]	204 (76)	121 (43)	<.001
High-density lipoprotein	46 (14)	49 (14)	.02
Triglycerides, median (IQR), mg/dL	97 (65-143)	107 (72-161)	<.001

Abbreviation: IQR, interquartile range.

SI conversions: To convert high- and low-density lipoprotein cholesterol to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.0113.

[a] Body mass index is calculated as weight in kilograms divided by height in meters squared.

[b] Low-intensity statin is defined as cerivastatin 0.1 mg or 0.4 mg; fluvastatin 20 or 40 mg; lovastatin 20 mg; pitavastatin 1 mg; pravastatin 10 or 20 mg; or simvastatin 10 mg. Moderate-intensity statin is defined as atorvastatin 10 or 20 mg; fluvastatin 80 mg; lovastatin 40 mg; pitavastatin 2 or 4 mg; rosuvastatin 5 or 10 mg; or simvastatin 20 or 40 mg. High-intensity statin is defined as atorvastatin 40 or 80 mg; rosuvastatin 20 or 40 mg; or simvastatin 80 mg (in line with current American Heart Association guidelines).[18]

[c] Value is calculated as *P* for trend.

[d] Cardiovascular disease is defined as myocardial infarction, coronary artery bypass graft, percutaneous transluminal angioplasty, or ischemic cerebrovascular accident.

[e] Indicates untreated low-density lipoprotein cholesterol.

sis. Second, we adjusted for potential confounders by means of a multivariable logistic regression model. Third, we evaluated a possible dose-response relationship between the severity of familial hypercholesterolemia mutations and prevalence of type 2 diabetes. In general, carriers of mutations in the gene for *APOB* express a less severe phenotype compared with carriers of the *LDLR* mutation carriers.[6,7] Furthermore, *LDLR* mutations are traditionally divided into 5 classes[17] that are either receptor-negative (class 1) or receptor-defective (class 2-5) mutations. Receptor-defective *LDLR* mutations commonly result in a milder phenotype compared with receptor-negative *LDLR* mutations.[8,9] We therefore determined type 2 diabetes prevalence in carriers of *APOB* mutations and carriers of *LDLR* mutations, compared with unaffected relatives, and tested for a linear trend. To do so, we defined a continuous variable based on mutation type; unaffected relatives were allocated as 0, carriers of the *APOB* mutation as 1, and carriers of the *LDLR* mutation as 2. Using multivariable logistic regression, we then tested if and in which direction this continuous variable was associated with type 2 diabetes. Receptor-defective and receptor-negative *LDLR* mutations were evaluated by the same method. In both analyses, we adjusted for potential confounders.

A potential confounder was defined as a demographic or clinical characteristic that differed significantly between patients with familial hypercholesterolemia and unaffected relatives. We did not use the LDL cholesterol level as potential confounder since differences between both groups regarding this variable are almost exclusively determined by familial hypercholesterolemia mutation status; adjusting for the LDL cholesterol level would therefore obscure the association between familial hypercholesterolemia and type 2 diabetes. We explored the statistical effect of potential confounders on various subgroups by performing stratified analyses for statin use (both users vs nonusers, as well as low and moderate vs high-intensity dose statin users [according to current American Heart Association guidelines[18]; **Table 1**]), history of CVD, and smoking status. All multivariable logistic regression models were performed using complete cases only, assuming missing data were missing completely at random. We performed a sensitivity analysis using multiple imputation to evaluate this assumption (eAppendix 1, eTable 5 in the Supplement).

Based on the multivariable logistic regression models, we estimated the adjusted type 2 diabetes prevalence in patients with familial hypercholesterolemia and with different types of mutations by applying the multivariable parameter estimates to the mean values of unaffected relatives. We calculated prevalence ratios by the method described by Santos[19] and multiplied these with the observed prevalence in unaffected relatives to estimate the adjusted prevalence. The 95% CI of the adjusted prevalence was determined in 200 bootstrap samples.

All regression analyses were performed using the generalized estimating equation method to account for family relations. The exchangeable correlation structure was used for these models. *P* values were 2-sided and significance level was set at less than .05. Continuous variables with a skewed distribution were log-transformed before the analyses. All analyses were calculated using the R statistical package, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Description of the Study Population

From January 1994 until January 2014, 63 385 individuals underwent DNA analysis for familial hypercholesterolemia. Of these, we excluded 65 (0.1%) patients with homozygous familial hypercholesterolemia. The remaining participants comprised our study population, of whom 25 137 (39.7%) were patients with familial hypercholesterolemia and 38 183 (60.3%) were unaffected relatives.

Of the patients with familial hypercholesterolemia, 3475 (13.8%) were *APOB* mutation carriers, 21 606 (86.0%) had the *LDLR* mutation, and 56 (0.2%) had a proprotein convertase subtilisin/kexin type 9 ([*PCSK9*] NCBI Entrez Gene 255738]) mutation. Among patients with an *LDLR* mutation, 12 567 (58.2%) were receptor-deficient and 9039 (41.8%) were receptor-negative mutations.

Demographic and clinical characteristics are shown in Table 1. Patients with familial hypercholesterolemia were younger, had lower body mass index (BMI [calculated as weight in kilograms divided by height in meters squared]), high-density lipoprotein (HDL) cholesterol, and triglycerides levels, but higher levels of LDL cholesterol, showed greater statin use, and smoked less. Sex distribution was similar.

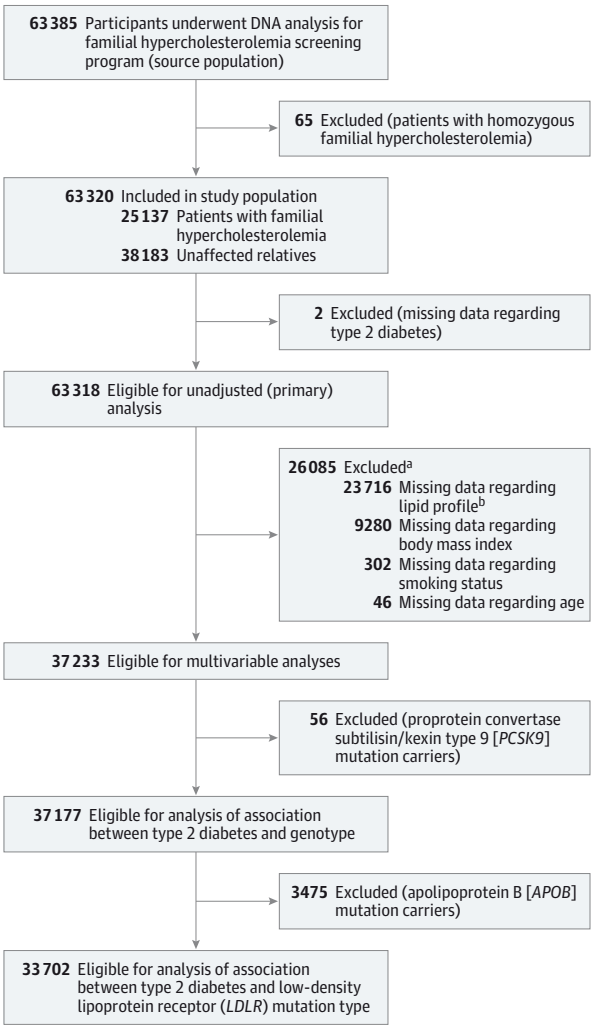
Data regarding type 2 diabetes were missing in 2 (<0.1%) persons. As a result, 63 318 individuals were eligible for our primary analysis. Data regarding age, BMI, and smoking status were missing in 46 (0.1%), 9280 (14.7%), and 302 (0.5%) cases, respectively. Because measurement of the lipid profile has only been customary since 2004, values were missing in 23 716 (37.5%) of the cases. In total, 37 233 (58.8%) complete cases were eligible for the multivariable logistic regression analyses. The **Figure** describes the flow of the study population and individuals who were available for the analyses. In eTable 2 (in the Supplement), we have summarized the annual number of patients with familial hypercholesterolemia who were traced in the screening program. A specification of statins that were used is provided in eTable 3 (in the Supplement).

Association Between Familial Hypercholesterolemia and Type 2 Diabetes

We identified 1559 patients with type 2 diabetes. The observed prevalence of type 2 diabetes was significantly lower in patients with familial hypercholesterolemia vs their unaffected relatives (1.75% [95% CI, 1.59%-1.91%], [n = 440 of 25 137] vs 2.93% [95% CI, 2.76%-3.10%], [n = 1119 of 38 183]; $P < .001$). After adjustment for age, BMI, HDL cholesterol, triglycerides, statin use, smoking status, and history of CVD, the prevalence of type 2 diabetes in patients with familial hypercholesterolemia was 1.44% (difference, 1.49% [95% CI, 1.24%-1.71%]) (**Table 2**). This inverse association between familial hypercholesterolemia and type 2 diabetes was consistent in all evaluated subgroups (eTable 4 in the Supplement).

We further examined the association of type 2 diabetes prevalence and mutation severity. The prevalence of type 2 diabetes was lower in carriers of *APOB* mutations compared with unaffected relatives (adjusted odds ratio [OR], 0.65 [95% CI, 0.48-0.88]; $P < .001$; [adjusted prevalence, 1.91%]; difference, 1.02 [95% CI, 0.41-1.49]), and this difference was more pronounced in patients with *LDLR* mutations (adjusted OR, 0.45 [95% CI, 0.38-0.54]; $P < .001$; [adjusted prevalence, 1.33%]; difference, 1.60 [95% CI, 1.36-1.81]). The overall *P* for trend was less than .001. Also, the severity of the *LDLR* mutation was inversely associated with type 2 diabetes prevalence. The adjusted ORs for type 2 diabetes in patients with receptor-defective and receptor-negative mutations were 0.49 (95% CI, 0.40-0.60; [adjusted prevalence, 1.44%]; difference, 1.49 [95% CI, 1.18-1.75]; $P < .001$), compared with their unaffected relatives and 0.38 (95% CI, 0.29-0.50; [adjusted prevalence, 1.12%]; difference, 1.81 [95% CI, 1.50-2.05]; $P < .001$), with an overall *P* for trend of less than .001. All associations are summarized in Table 2. Type 2 diabetes prevalence in different familial hypercholesterolemia groups, both observed as well as ad-

Figure. Patient Screening and Selection for Associations Between Presence of Type 2 Diabetes and Familial Hypercholesterolemia



[a] Data may not sum because participants could be excluded for more than 1 reason.

[b] Measurement of lipid profile became customary after 2004.

Table 2. Associations Between the Presence of Type 2 Diabetes and Familial Hypercholesterolemia

	Prevalence of Type 2 Diabetes				
	Familial Hypercholesterolemia		Unaffected Relatives		
	No. /Total	% (95% CI)	No. /Total	% (95% CI)	OR (95% CI)
Overall comparison					
Unadjusted	440/25 137	1.75 (1.59-1.91)	1119/38 183	2.93 (2.76-3.10)	0.62 (0.55-0.69)[a]
Adjusted[b]	177/12 300[c]	1.44 (1.22-1.69)	812/24 898[c]	3.26 (3.04-3.48)	0.49 (0.42-0.58)[a]
Affected gene					0.67 (0.61-0.73)[d]
No mutation			812/24 898[c]	3.26 (3.04-3.48)	1 [Reference]
APOB					
Unadjusted	84/2125[c]	2.42 (1.91-2.93)			
Adjusted[b]	41/2125[c]	1.91 (1.44-2.52)			0.65 (0.48-0.87)[a]
LDLR					
Unadjusted	353/10 126[c]	1.63 (1.46-1.80)			
Adjusted[b]	135/10 126[c]	1.33 (1.12-1.57)			0.45 (0.38-0.54)[a]
Type of *LDLR* mutation					0.58 (0.51-0.66)[d]
None			812/24 898[c]	3.26 (3.04-3.48)	1 [Reference]
Receptor-defective					
Unadjusted	226/6320[c]	1.80 (1.57-2.03)			
Adjusted[b]	91/6320[c]	1.44 (1.18-1.75)			0.49 (0.40-0.60)[a]
Receptor-negative					
Unadjusted	127/3806[c]	1.41 (1.16-1.65)			
Adjusted[b]	43/3806[c]	1.12 (0.88-1.43)			0.38 (0.29-0.49)[a]

Abbreviations: *APOB*, apolipoprotein B; OR, odds ratio; *LDLR*, low-density lipoprotein receptor.

[a] Analyzed as a categorical variable.

[b] Adjusted for age, body mass index, high-density lipoprotein cholesterol, triglycerides (log transformed), use of statins, smoking, cardiovascular disease, and family relations. Adjusted prevalence in patients with familial hypercholesterolemia and each subgroup are calculated by multiplying the prevalence ratio with the observed prevalence in non-familial hypercholesterolemia patients (ie, 2.93%).

[c] Sample size for the multivariable regression analyses.

[d] Linear trend.

justed for age, BMI, HDL cholesterol, triglycerides, use of statins, smoking status, and history of CVD, are summarized in Table 2.

Discussion

Summary of Results

In this observational study in 25 000 patients with familial hypercholesterolemia and 38 000 unaffected relatives, the prevalence of type 2 diabetes was significantly lower in patients with familial hypercholesterolemia than in their unaffected relatives. Furthermore, we observed an inverse dose-response relationship between the severity of the familial hypercholesterolemia causing mutation and prevalence of type 2 diabetes.

To our knowledge, no study has specifically examined the relationship between type 2 diabetes and familial hypercholesterolemia. One previous smaller study, in which coronary artery disease was compared between patients with familial hypercholesterolemia (n = 102) and age- and sex-matched controls (n = 102), reported a significantly lower prevalence of type 2 diabetes in the patients with familial hypercholesterolemia.[20] Furthermore, in a subset of the current study cohort, we recently reported that type 2 diabetes was significantly less prevalent in patients with severe familial hypercholesterolemia vs those with nonsevere familial hypercholesterolemia.[12]

Pancreatic Beta Cells and Cellular Cholesterol Uptake

Unlike familial hypercholesterolemia, the association between statin use and prevalence of type 2 diabetes has been examined extensively.[2,21] Statins increase the risk for type 2 diabetes by 9% and although various biological mechanisms are proposed to explain this association,[22,23] the precise mechanism is unclear. However, the fact that *HMGCR* variants are associated with changes in LDL cholesterol levels and type 2 diabetes risk[3] suggests a role for cellular cholesterol metabolism. Moreover, HMGCR inhibition by statins primarily increases LDLR expression,[4] which might be a proxy for the relationship between HMGCR activity and type 2 diabetes development—underscoring a relationship between intracellular cholesterol and type 2 diabetes risk.

Our findings support the hypothesis that the common pathway in familial hypercholesterolemia and statin therapy—cellular cholesterol uptake—plays a role in the development of type 2 diabetes, perhaps because increased intracellular cholesterol levels are detrimental for pancreatic beta cell function. It was shown that addition of LDL cholesterol to cultured medium of isolated rat islet beta cells resulted in cell death,[24,25] and this was LDLR dependent.[25] Furthermore, a study by Rütti et al[26] found decreased glucose-stimulated insulin secretion in rodent pancreatic islets that were incubated with LDL cholesterol, and this was abolished in LDLR knockout animals. They also examined the effect of LDL cho-

lesterol on human beta cells; incubation of islets derived from pancreas donors with LDL cholesterol was detrimental in this situation as well.[26] Further support for this mechanism comes from studies examining $ABCA_1$, a key transmembrane protein that promotes cellular cholesterol efflux.[27] A study by Brunham et al[28] showed that pancreas-specific *ABCA1* knockout mice exhibited increased plasma glucose levels and impaired insulin secretion. The relationship between intracellular cholesterol concentration and pancreatic beta cell function was strengthened by the finding that miRNA22 inhibition, causing increased *ABCA1* expression, improved beta cell function.[29] Furthermore, a study by Vergeer et al[30] showed that altered intracellular cholesterol homeostasis by *ABCA1* defects results in impaired insulin secretion in humans.

The small absolute difference in prevalence of type 2 diabetes between patients with familial hypercholesterolemia and unaffected relatives will not have a major influence on individual risk for type 2 diabetes. However, the substantial relative difference of 50%, together with previous findings, might suggest an effect of intracellular cholesterol metabolism on pancreatic beta cell function. Nevertheless, a plethora of pathways contribute to development of type 2 diabetes,[31] and therefore, the mechanism we discuss here can only be 1 part of the pathogenesis of this highly complex disease. For example, the study by Swerdlow et al[3] suggests that body weight might be a mediator on the pathway between both HMGCR single-nucleotide polymorphisms, as well as statin use, with type 2 diabetes. We explored whether BMI is a mediator of the relationship between familial hypercholesterolemia and type 2 diabetes and found that this is indeed a possibility (eTable 6 in the Supplement). However, because we lack information on the time-relationship between BMI and type 2 diabetes, we cannot distinguish whether BMI is a confounder or a mediator.

Clinical Relevance

One important aspect in the pathogenesis of type 2 diabetes is pancreatic inability to sufficiently secrete insulin, either as a result of dysfunction or loss of beta cells.[31] To improve pancreatic function and clinical outcome in type 2 diabetes, a wide range of therapies have been developed. These strategies stimulate the pancreas to secrete more insulin, but do not focus on function or survival of beta cells per se.[32] These novel therapies decrease the risk for microvascular complications of type 2 diabetes, but seem to have little effect on the risk for macrovascular complications.[33,34] As a result, new strategies are needed. The findings in our study raise the possibility of a role for cellular cholesterol metabolism in the pathogenesis of type 2 diabetes. If these findings are confirmed in longitudinal studies, they might provide support for development of new approaches to the prevention and treatment of type 2 diabetes by improving function and survival of pancreatic beta cells.

Methodological Considerations

Some methodological aspects of our study merit discussion. First, we used a cross-sectional study design, suitable for studying prevalence but not for establishing a causal relationship between exposure and outcome of interest. In particular, it is not clear whether the exposure occurs prior to the outcome. In our study however, familial hypercholesterolemia (the exposure) is caused by a genetic defect that influences the full spectrum of the phenotype from birth onwards. Type 2 diabetes (the outcome) is a condition that arises later in life and will therefore always be preceded by the carrier status (gene mutation) of an individual.

Second, due to the observational nature of this study, the association between familial hypercholesterolemia and type 2 diabetes could be obscured by confounding. We adjusted for known confounders, but some potential confounders may not be assessed accurately or assessed at all. Nevertheless, we consider the influence of residual and unmeasured confounding to be minimal in this data set, since the genetic defect exhibits Mendelian inheritance. Consequently, assignment of the genetic defect, as well as inheritance to subsequent generations, occurs in a random fashion. These random assignments of genotypes minimize the risk for confounding. The fact that a familial hypercholesterolemia mutation always precedes the phenotypic expression of type 2 diabetes, in combination with our finding of a dose-response relationship between the severity of familial hypercholesterolemia mutations and type 2 diabetes, might suggest a causal relationship between decreased transmembrane cholesterol transport and type 2 diabetes. However, confounding in our cross-sectional design might have led to an association between familial hypercholesterolemia and type 2 diabetes via a noncausal path. Furthermore, one could argue that patients with familial hypercholesterolemia adapt a healthier lifestyle after receiving the diagnosis of familial hypercholesterolemia, which might influence their susceptibility for developing type 2 diabetes. For instance, a Mediterranean diet is implicated in lowering the risk for type 2 diabetes.[35] However, the reason for the majority of participants (93.0%) in the screening program to be screened was the notion that a first-degree relative was diagnosed with familial hypercholesterolemia, ie, participants were unaware of their familial hypercholesterolemia mutation status at the moment of data collection. We therefore reason that the influence of awareness of the familial hypercholesterolemia mutation on lifestyle and subsequent risk for type 2 diabetes is insignificant.

Third, we included consecutive participants of the familial hypercholesterolemia screening program. Because patients with familial hypercholesterolemia are at increased risk for premature CVD, some patients will have died before they could have participated in the screening program. The prevalence of type 2 diabetes might have been higher in these patients since CVD patients have a higher prevalence of type 2 diabetes. As a result, the observed prevalence in patients with familial hypercholesterolemia could be underestimated. Conversely, an overestimation of the observed prevalence can be argued as well; type 2 diabetes prevalence increases with age and patients with familial hypercholesterolemia who died prematurely might have been less likely to have developed type 2 diabetes.[36] Taken together, we do not consider survival bias to have significantly influenced our results.

Fourth, the diagnosis of type 2 diabetes was self-reported and might therefore not be accurate. However, a study by Okura et al[37] found substantial agreement between questionnaire responses and medical records for type 2 diabetes (total agreement, 97.2% [κ, 0.76; 95% CI, 0.70-0.82]) in a well-characterized, population-based cohort with a long record archival period. Additionally, the type of diabetes was not specified by our questionnaire. We considered patients with age younger than 30 at time of diabetes diagnosis, or younger than 40 in combination with insulin use, as having type 1 diabetes and classified them as non–type 2 diabetes patients. Since type 2 diabetes rarely occurs before the age of 30 and type 1 diabetes is seldom first diagnosed in patients older than 30,[15] we think we adequately addressed this issue. Moreover, the fact that insulin is initiated in patients with type 2 diabetes on average 10 years after the diagnosis supports the age limit of 40 years in combination with the use of insulin.[16] Our sensitivity analyses, in which all patients with diabetes were classified as type 2 diabetes patients, showed consistent results (eAppendix 1, eTable 5 in the Supplement).

Major strengths of our study include the size and consecutive nature of the study cohort, the recruitment of all participants into the screening program, and the minimal confounding due to the random assignment of the genotype underlying the phenotype, based on the inheritance pattern. Individuals were referred to the program for clinical suspicion of familial hypercholesterolemia or for their relationship to a mutation carrier. The latter is by far the most frequent reason for inclusion (n = 58 914 [93.0%]) and as a result, risk of selection bias is probably minimal. We therefore think that our study population is representative for both familial hypercholesterolemia as well as for the general population in the Netherlands.

Conclusions

In a cross-sectional analysis in the Netherlands, the prevalence of type 2 diabetes among patients with familial hypercholesterolemia was significantly lower than among unaffected relatives, with variability by mutation type. If this finding is confirmed in longitudinal analysis, it would raise the possibility of a causal relationship between LDLR-mediated transmembrane cholesterol transport and type 2 diabetes.

ARTICLE INFORMATION

Author Contributions: Dr Kastelein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Drs Hutten and Hovingh contributed equally to this work.
Study concept and design: Besseling, Hutten, Hovingh.
Acquisition, analysis, or interpretation of data: Besseling, Kastelein, Defesche, Hutten, Hovingh.
Drafting of the manuscript: Besseling, Defesche, Hutten, Hovingh.
Critical revision of the manuscript for important intellectual content: Besseling, Kastelein, Defesche, Hutten, Hovingh.
Statistical analysis: Besseling, Hutten.
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Administrative, technical, or material support: Besseling, Defesche, Hovingh.
Study supervision: Hutten, Hovingh.

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