Aim: The aim of this study was to compare coronary calcium scores and aortic calcium scores between patients with severe hypercholesterolemia having a DNA-based diagnosis of FH (FH group) versus patients with severe hypercholesterolemia without the FH gene mutation (NFH group).

Method: A total of 89 FH and 50 NFH patients underwent CT with coronary and thoracic aorta calcium scoring. Their CCS and TCS in ascending aorta (TCSasc) and descending aorta (TCSdesc) were determined and compared between the two patient groups.

Results: TCSasc was significantly higher in the FH group when compared to the NFH group (30.6 ± 59 vs 4.7 ± 13.4, p < 0.001). After adjusting for age, sex, smoking, blood pressure, history of diabetes mellitus and LDL cholesterol levels, FH gene mutation was an independent risk factor of having non-zero TCSasc 3.6 (95% CI, 1.4 – 9.5, p < 0.01), high TCSasc 9.6 (95% CI, 2.4 – 38.2, p < 0.01) and high CCS of 4.1 (95% CI, 1.2 – 13.2, p < 0.05).

Conclusion: We found that when computed tomography calcium scores were used as an assessment, patients with familial hypercholesterolemia displayed an increased burden of ascending aorta atherosclerosis when compared to patients with nonfamilial severe hypercholesterolemia. This phenomenon appears to be more dependent on the presence of FH genotype than hypercholesterolemia itself.

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Key words: Atherosclerosis, Familial hypercholesterolemia, Multidetector computed tomography

Introduction

Familial hypercholesterolemia (FH) is a genetic disease affecting 1 in 500 individuals and is associated with premature coronary artery disease (CAD) because of high plasma cholesterol levels. The coronary calcium score (CCS), which can be determined by computed tomography (CT), is a well-known indicator of coronary artery plaque burden. CCS provides a better estimate of the burden of disease than luminal stenosis, as determined by coronary angiography because less-obstructive plaques cause more acute coronary syndromes than obstructive plaques due to their greater number. Negative results of CCS has a strong negative predictive value for both coronary obstruction and clinical events in the general population. CCS is recommended to estimate the risk of coronary events in asymptomatic individuals, particularly those with intermediate risks, but high prognostic and diagnostic values were also shown in high-risk patients. To date, only a few studies are available that report about CCS in patients with FH.
Similarly, the thoracic aorta calcium score (TCS) is an established marker of atherosclerosis. As with CCS, the prevalence of TCS increases with age, is associated with coronary risk factors, and correlates closely with CCS. According to Hoeg et al., in FH homozygous patients, calcification in the proximal ascending aorta precedes calcification in the coronary arteries. Whether this localization of atherosclerosis is specific for FH patients because of FH gene mutation or is rather just related to having high low-density lipoprotein cholesterol (LDL-C) levels remain unclear.

It is known that even in the presence of a genetic mutation causing FH, the expression of atherosclerosis is markedly affected by other factors, generally environmental ones. It has also been shown that FH heterozygotes with the same low-density lipoprotein receptor (LDLR) mutation can have widely different plasma levels of LDL-C. Even after adjustment for traditional risk factors, including LDL-C levels, an association between FH gene mutations and the prevalence of CAD was found. It is possible that the genes responsible for hyperlipidemia can act upon atherogenesis not only by an evident increase in the serum lipid profile but also by other gene-gene and gene-environmental interactions. Although the nature of the FH molecular defect has a significant impact on the severity of hypercholesterolemia to date, there is no convincing evidence that molecular diagnosis has any important therapeutic implications. The issue remains whether the presence of the FH genotype is associated with the intensity and location of atherosclerosis in patients with severe hypercholesterolemia.

The aim of this study was a comparison of CCS and TCS between patients with severe hypercholesterolemia and a DNA-based diagnosis of FH (FH group) versus patients with severe hypercholesterolemia without the FH gene mutation (NFH group).

**Methods**

In our study, we included patients who had a suspicion of FH and who were admitted to our outpatient preventive clinic between 2010 and 2013. On all FH patients having at least 3 points according to the Dutch Lipid Clinic Network, we performed a mutation analysis of LDLR and apolipoprotein B-100 (APOB) as previously described. We evaluated a total of 156 subjects having a DNA-based diagnosis of FH. All cases with previous clinically apparent CAD were excluded. CAD was defined by at least one of the following factors: a documented, past history of myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, angina, wall motion abnormalities on an echocardiogram, or positive results of exercise testing which was performed on all patients with results of CCS above 100. The other exclusion criteria were as follows: age <30 years, secondary hypercholesterolemia due to thyroid or liver diseases, renal insufficiency (estimated creatinine clearance <50 ml/min), and pregnancy. Finally, 89 patients (37 men and 52 women) with a genetic confirmation of FH were included, with a mean age of 50.2±11 years. CT scans were also performed in an NFH group consisting of 50 persons with a cholesterol level above 5 mmol/L, who were selected from among patients without an FH mutation, and who were diagnosed during the same period. NFH cases were of the same sex and age as the FH group. The exclusion criteria were the same as for FH patients. After signing a written informed consent, ECG-gated CT was performed on all included patients.

CT was performed using a 64 row CT scanner (GE). Contiguous 2.5-mm slices with ECG gating, without intravenous contrast enhancement, were then made during inspiration scanning from the aortic arch to the diaphragm. Each scan was assessed by an experienced radiologist who was blinded to all clinical data. The CCS and TCS scores were calculated for each participant using the volumetric method by means of SmartScore 4.0 software (GE). Calcium scores were obtained for the following regions: left main, left anterior descending, left circumflex, and right coronary arteries; ascending aorta; and the thoracic part of the descending aorta to the level of the diaphragm. Diabetes was defined as a fasting blood glucose level greater than 7 mmol/L or the use of hypoglycemic medications. Smoking history was defined as positive for former or present smokers who had smoked more than 1 pack-year.

Pretreatment cholesterol levels were obtained from the patients’ records. The current lipids levels were obtained from each patient by standard methods from patients who were fasting for at least 12 h.

The Local Ethics Committee approved the study protocol, and a written informed consent was obtained before entering the study from all FH and NFH patients.

**Statistical Analysis**

Continuous variables are shown as means and standard deviations, except for calcium score results that are also shown as medians and quartiles values. Categorical variables are expressed by numbers and percentages. Data normality was tested using the Kolmogorov–Smirnov test. The data sets were compared...
of the patients, the p.G592E mutation located within exon 12 was observed, whereas major rearrangements and frameshift or nonsense mutations were found in 8 (8.9%) of the individuals. In 23 (25.3%) of the patients, other missense mutations (p.G20R, p.C34G, p.C89R, p.D168G, p.S177L, p.F282L, p.G373C, p.N564S, and p.P608T) were found. In our group, 16 (18%) intronic variants were detected, some of which might be of substantial importance for LDL receptor activity. In addition, 16 (18%) of the patients presented a heterozygous substitution at codon 3527 of APOB (p.R3527Q). In one individual, the p.H3570Y APOB substitution was found.

The results of CCS and TCS calculated using volumetric methods are presented in Fig. 1 and Table 2. TCS in the ascending aorta was significantly higher in the FH group. There were no significant differences between CCS and TCS in the descending aorta. We found no statistical difference in body mass index (BMI), history of diabetes and hypertension, high-density lipoprotein cholesterol (HDL-C) levels, or triglycerides (TG) levels between the FH and NFH groups (Table 1), although the FH group had higher maximum total cholesterol (TC) and LDL-C levels. The difference of cholesterol-year scores was of bor-

### Table 1. Clinical characteristics of FH and NFH groups

|                | FH N=89 | NFH N=50 | p     |
|----------------|---------|----------|-------|
| Age (years)    | 50.2 ± 11.9 | 51.5 ± 9.9 | NS    |
| Gender         | 37 M, 52 F | 23M, 27 F | NS    |
| BMI (kg/m²)    | 26.4 ± 4.3 | 26.9 ± 4.1 | NS    |
| TC max (mmol/L)| 9.4 ± 2.2 | 8.1 ± 1.5 | P<0.001|
| LDL-C max （mmol/L）| 7.1 ± 1.7 | 5.9 ± 1.1 | P<0.001|
| HDL-C max （mmol/L）| 1.5 ± 0.4 | 1.5 ± 0.3 | NS    |
| TG max （mmol/L）| 1.5 ± 0.9 | 1.6 ± 0.8 | NS    |
| Chol-year score (mmol-year/L) | 452.8 ± 136.5 | 402.7 ± 152.2 | 0.05 |
| TC (mmol/L)    | 7.3 ± 2.2 | 7.4 ± 1.5 | NS    |
| LDL-C (mmol/L)| 5.3 ± 2.1 | 5.1 ± 1.3 | NS    |
| HDL-C (mmol/L)| 1.4 ± 0.3 | 1.5 ± 0.3 | NS    |
| TG (mmol/L)    | 1.4 ± 0.7 | 1.5 ± 0.7 | NS    |
| SBP (mmHg)     | 132.6 ± 16 | 134.3 ± 14.5 | NS    |
| DBP (mmHg)     | 83.4 ± 11.1 | 82.7 ± 8.7 | NS    |
| Diabetes † (n) | 3 (3%) | 5 (10%) | NS    |
| Smoking ‡ (n)  | 29 (33%) | 21 (42%) | NS    |
| Statin treatment on 1st visit (n) | 49 (55%) | 20 (40%) | NS    |

Abbreviations: FH - familial hypercholesterolemia NFH - nonfamilial hypercholesterolemia, NS – not significant, BMI - body mass index, TC – total cholesterol, LDL-C low-density lipoprotein cholesterol, HDL-C - high-density lipoprotein cholesterol, TG – triglycerides, max – maximum levels without pharmacotherapy, SBP – systolic blood pressure, DBP – diastolic blood pressure, † data not available in 6 patients, ‡ treatment with insulin or oral anti-diabetic medicine, ‡ smoking - ever, >1 pack-year

using the two-sided t-test or Mann–Whitney U-test for non-parametric variables depending on the variables distribution. Categorical variables were compared using Fisher’s exact test. To determine the association of non-zero and high calcium score results with the presence of an FH mutation, a multivariate logistic regression model was fitted to obtain odds ratios (ORs) after adjustment for confounders. The analyses were performed using Statistica 10 software. P value <0.05 was considered statistically significant.

### Results

The clinical characteristics of the 89 FH and 50 NFH patients are presented in Table 1. The average age of both groups did not differ significantly and was 50.2 years for the FH group and 51.5 for the NFH group. In the group of 89 FH patients studied, isolated LDLR and APOB mutations were found in 69 (77.5%) and 17 (19.1%) of the individuals, respectively. We found the coexistence of LDLR and APOB alterations in three individuals (3.4%). The most frequent LDLR alteration was the c.662A>G point mutation in exon 4, resulting in p.D221G substitution, which was detected in 14 (15.4%) of the patients. In 8 (8.9%) of the patients, the p.G592E mutation located within exon 12 was observed, whereas major rearrangements and frameshift or nonsense mutations were found in 8 (8.9%) of the individuals. In 23 (25.3%) of the patients, other missense mutations (p.G20R, p.C34G, p.C89R, p.D168G, p.S177L, p.F282L, p.G373C, p.N564S, and p.P608T) were found. In our group, 16 (18%) intronic variants were detected, some of which might be of substantial importance for LDL receptor activity. In addition, 16 (18%) of the patients presented a heterozygous substitution at codon 3527 of APOB (p.R3527Q). In one individual, the p.H3570Y APOB substitution was found.

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Discussion

We found that patients with documented heterozygous FH have a higher calcium score in the ascending aorta than patients with severe hypercholesterolemia without FH gene mutation. We did not observe statistically significant differences of calcium scores between either group in the descending aorta and coronary arteries. The location of atherosclerosis depends on many predisposing factors, and the cause of this dependence still remains the subject of research. It is possible that atherosclerosis exhibits selective responses in different vascular beds. Our data confirmed that atherosclerosis of the ascending aorta seems to be more specific for FH patients even when compared to hypercholesterolemic patients without FH gene mutation.

Previous reports focused on differences between patients with FH and a control group which consisted of healthy subjects without hypercholesterolemia. We compared patients with a genetic diagnosis of FH to patients having severe hypercholesterolemia, but without FH gene mutation. Nevertheless, we still observed significant differences in maximum LDL-C levels, but

derline statistical significance. Untreated LDL-C and HDL-C as well TG levels were not available for six patients whose pretreatment and treated total cholesterol levels did not differ significantly from those with complete data.

We sought to determine whether the FH mutation was connected with higher results of calcium scores. For this analysis, we selected CCS and TCS results with non-zero or high results if the value was in the top 20% of the calcium score distribution. After adjusting for age, sex, smoking, blood pressure, history of diabetes mellitus, and LDL-C levels, compared with patients with no identified mutation, those with an FH mutation had an OR 3.7 of having a high CCS score (p < 0.05) and 1.3 of having non-zero CCS (ns, non-significant). FH mutation was also a risk factor of having high TCSasc (OR 8.8, p < 0.01) or non-zero TCSasc (OR 3.6, p < 0.01). We did not observe such dependencies in the thoracic part of the descending aorta (OR 1.1 and 0.8 respectively, ns; Table 3). Of the traditional risk factors, age and arterial hypertension were significant for higher CCS, whereas age and LDL-C pretreatment levels were significant for higher TCS (data not presented).

**Fig. 1.** Calcium score distribution across CCS, TCSasc and TCSdesc ranges in FH and NFH groups.

Data in particular ranges are expressed as % of each calcium score subgroup. Note significant difference in fractions of zero calcium score for TCSasc (*p* < 0.01, two proportion Z test)

**Abbreviations:** FH - familial hypercholesterolemia, NFH – nonfamilial hypercholesterolemia, CCS – coronary calcium score, TCSasc – TCS in ascending aorta, TCSdesc – TCS in descending aorta
levels of cholesterol from an early age than NFH patients. Another explanation of observed differences is that the association between calcium scores and cholesterol levels is probably not only a linear one. Jensen et al. found that age-adjusted calcium scores still increased slightly with age, suggesting that coronary calcification is a self-perpetuating process. His diagnostic model for the prediction of CAD in FH patients based on cholesterol-year scores was the weakest model in his study, more than his model based on a combination of traditional risk factors, including age, sex, smoking, hypertension, untreated cholesterol, and BMI. Moreover, our FH heterozygotes may share other genetic or environmental factors that may have affected the obtained results. It is possible that FH patients carry additional alleles that increase the pathogenic effects of hypercholesterolemia or NFH mutations in genes responsible for severe hypercholesterolemia are not so atherogenic. Phenotypic variation has been observed in families or populations sharing the same LDLR or APOB mutation. Pereira et al. examined FH heterozygotes in three Cuban families of Spanish descent, in which one-third of the family members carried the LDLR V408M mutation common in the Afrikaner population. Although all the subjects had elevated LDL-C, cardiovascular complications were rarely observed in the Cuban subjects compared with Afrikaners.

There are only a few reports about CCSs in asymptomatic FH patients in the medical literature. In our study of 89 asymptomatic statin-treated patients, far less than for healthy subjects. We initially concluded that differences in calcium scores were connected with higher cholesterol levels in the FH group. A multivariate analysis was performed to test this hypothesis. After adjusting for age, sex, smoking, blood pressure, history of diabetes mellitus, and LDL-C levels, FH mutation remained an independent risk factor of patients having a high result of CCS and TCS and non-zero results of TCS. Therefore, it is possible that the FH genotype affects atherogenesis independently of serum cholesterol concentration, particularly in coronary arteries and the ascending aorta. Humphries et al. also found an association between LDLR and proprotein convertase subtilisin/kexin type 9 (PCSK9) gene mutations and the prevalence of CAD in a group of 409 FH patients. This association was present even after adjusting for traditional CAD risk factors, including pretreatment levels of total cholesterol. It was found that polymorphism of the apo E genotype in a group of 720 young males after adjustment for cholesterol levels and smoking status is still responsible for 3%–11% of the variance for total atherosclerotic lesions in the thoracic and abdominal aorta and concluded that genotypic effects on lesions may not be due entirely to differences in serum lipid levels. The explanation for this phenomenon can be that a single measurement of pretreatment LDL-C and even cholesterol-year scores may not represent the true difference in cholesterol burden. It is a known fact that the cholesterol level increases with age. The dynamics of these changes can vary between FH and NFH groups. In particular, FH patients may be exposed to higher

### Table 2. Volumetric calcium score of coronary arteries and thoracic aorta in FH and NFH groups

|                     | FH          | NFH         | p value |
|---------------------|-------------|-------------|---------|
| **CCS (mm³)**       | median      | 4           | 3.5     | NS      |
|                     | mean ± stdev| 72 ± 186.8  | 25.1 ± 72 |         |
| Q1                  | 0           | 0           |         |         |
| Q3                  | 61          | 1           |         |         |
| **TCSasc (mm³)**    | median      | 0           | 0       | <0.001  |
|                     | mean ± stdev| 31.3 ± 59   | 5.2 ± 12 |         |
| Q1                  | 0           | 0           |         |         |
| Q3                  | 32          | 0           |         |         |
| **TCSdesc (mm³)**   | median      | 0           | 0       | NS      |
|                     | mean ± stdev| 23.9 ± 67.4 | 9.7 ± 21.5 |         |
| Q1                  | 0           | 0           |         |         |
| Q3                  | 1           | 8           |         |         |

Abbreviations: FH - familial hypercholesterolemia, NFH – nonfamilial hypercholesterolemia, CCS – coronary calcium score, TCSasc – TCS in ascending aorta, TCSdesc – TCS in descending aorta, NS – not significant, stdev – standard deviation, Q1 – first quartile, Q3 – third quartile
with FH, we found that 45% had a zero CCS and 33% had a zero TCS. The results of our CCS are consistent with those obtained by Miname et al. The prevalence of calcified coronary plaque in his study was 48% in asymptomatic FH patients. The FH group they analyzed had a mean age of 45 ± 15 years; therefore, the patients were a little younger than ours, and the group comprised of 64% women, which was close to our group (58%). The diagnosis of FH in the Miname et al. study was clinical and based on US MEDPED criteria. Our FH cases had LDL-C above 5 mmol/L, and this diagnosis was confirmed using genetic testing, which gives a definite diagnosis. The prevalence of zero CCS results in our group was noticeably higher than the 20% observed by Neefjes et al. in their group of FH patients of a similar age 52 ± 8 years. However, in their group, there were more men in comparison to our patient groups (64% vs. 42%). Moreover, the maximum total cholesterol levels in their group were higher than in ours (9.7 vs. 9.0 mmol/l).

### Limitations of the Study

Some NFH patients with a very high LDL-C level are likely to have FH mutations that were not detected which can influence observed differences. This applies in particular to the PCSK9 mutation that has not been examined, but which occurs in only 1.7%–3% of FH patients. Studies performed in our center on another group of 413 unrelated Polish patients with hypercholesterolemia and having at least 3 points on the Dutch Lipid Clinic Network score and testing negative for LDLR and APOB alterations showed that mutation of the PCSK9 gene was present in only 1.2% (n = 5) of individuals (unpublished data). Therefore, there is only a minimal chance that PCSK9 mutation influenced observed differences. Many of our subjects were on statin treatment, which could have modified their natural history of coronary plaque evolution. Using the CCS parameter as a marker of atherosclerosis can identify only calcified plaques, but previous reports have shown that the average prevalence of purely non-calcified atherosclerotic plaque (NCAP) in the general population was only around 6%. According to Neefjes et al., NCAP was present in 4% of FH patients. Furthermore, patients with diagnosed CAD were excluded from our study. Most of the CAD patients previously had coronary stent implantations that could significantly affect their CCS analysis. All our patients were asymptomatic, but despite the absence of symptoms, 25% of the patients with FH exhibited obstructive CAD shown by CT angiography, which occurred at a relatively young age. Because the exclusion of CAD is dependent upon clinical documentation history only, which may not be precise, we performed exercise testing in patients with high CCS who revealed no ischemia.

### Conclusions

FH patients have an increased burden of ascending aorta atherosclerosis as assessed by CT calcium scores comparison with patients with nonfamilial severe hypercholesterolemia, even after adjustment for cholesterol levels. FH gene mutation is also an independent risk factor of having a high result of CCS. These findings demonstrate that the selective location and intensity of atherosclerosis in the ascending aorta and coronary arteries in FH patients appears to be more

### Table 3. Odds ratio for high and non-zero CCS and TCS in patients with FH mutation, adjusted for risk factors

|                | High result OR (95%CI) | Non-zero result OR (95%CI) |
|----------------|------------------------|----------------------------|
| **TCSasc**     | 8.8 (2.1-36.8, p < 0.01) | 3.6 (1.4-9.5, p < 0.01) |
| N = 29/110     | N = 54/85               |
| **TCSdesc**    | 0.8 (0.3-2.3, NS)       | 1.1 (0.4-3.2, NS)         |
| N = 25/114     | N = 33/106              |
| **CCS**        | 3.7 (1.1-12.4, p < 0.05) | 1.3 (0.5-2.9, NS)         |
| N = 27/112     | N = 62/77               |

Abbreviations: FH - familial hypercholesterolemia, CCS – coronary calcium score, TCSasc – TCS in ascending aorta, TCSdesc – TCS in descending aorta, NS – not significant. Adjusted for age, sex, smoking (never vs ever >1 pack-year), systolic blood pressure at recruitment, history of diabetes mellitus and pretreatment LDL-C level (or group average if data not recorded, n = 6).
dependent on the presence of the FH genotype than hypercholesterolemia itself.

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**Conflicts of Interest**

The authors declare that there is no conflict of interest.

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