Vascular Calcifications in Homozygote Familial Hypercholesterolemia


Background—Patients with homozygous familial hypercholesterolemia (hmzFH) attributable to LDL receptor gene mutations have shown a remarkable increase in survival over the last 20 years. Early onset coronary heart disease (CHD) and calcific aortic valve stenosis are the major complications of this disorder. We now report extensive premature calcification of the aorta in patients with hmzFH.

Methods and Results—We examined 25 hmzFH patients from Canada; mean age was 32 years (range 5 to 54), and mean baseline cholesterol before treatment was 19±5 mmol/L (737±206 mg/dL). Aortic calcification was quantified using computed tomography (CT). An elevated mean calcium score was found in patients by age 20 and correlated with age (r²=0.53, P=0.001). One quarter (24%) of patients underwent aortic valve surgery.

Conclusions—We document premature severe aortic calcifications in all adult hmzFH patients studied. These presented considerable surgical management challenges. Strategies to identify and monitor aortic calcification in hmzFH by noninvasive techniques are required, as are clinical trials to determine whether additional or more intensive therapies will prevent the progression of such calcifications. Whether vascular calcifications in hmzFH subjects are related to sustained increases in LDL-C levels or to other mechanisms, such as abnormal osteoblast activity, remains to be determined. (Arterioscler Thromb Vasc Biol. 2008;28:777-785)

Key Words: aorta ▪ calcification ▪ familial hypercholesterolemia

Familial hypercholesterolemia (FH) is considered to be a prototypical monogenic disorder. Careful study of rare patients with the homozygous FH (hmzFH) led to the identification of the low-density lipoprotein (LDL) receptor and its gene (LDLR), a seminal discovery in cell and molecular biology.1 Since then, 3 other genes have been found to cause a phenotype similar to FH: the apolipoprotein (apo) B gene (APOB), the proprotein convertase subtilisin/kexin type 9 gene (PCSK9), and the autosomal recessive hypercholesterolemia gene (ARH).2-3 The hallmarks of the disease are the cutaneous manifestations of tendinous xanthomata, xanthelasmas, and arcus corneus. The elevated serum concentration of LDL cholesterol (LDL-C) is considered causal for the premature coronary heart disease (CHD) that is found by the third decade of life in patients with heterozygous FH (htzFH) and in childhood in hmzFH.4,5 In adults with hmzFH, calcific aortic stenosis is identified as a complication, often requiring a surgical approach, with aortic valve replacement representing the most frequent surgical procedure.6-10

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The prevalence of htzFH is ≈1:500, based on a single study of families with premature CHD,11 giving a frequency of hmzFH of ≈1:1 000 000. The diagnosis of hmzFH is usually based on family history, presence of cutaneous and tendinous manifestations (xanthelasmas and xanthomas), severity of LDL-C elevation, and, in most cases, molecular diagnosis. In populations with a founder effect, such as the French Canadians, the prevalence of LDLR mutations can be much higher; for instance it reaches ≈1:80 in certain geographical regions in the province of Quebec.12

Earlier treatment options for hmzFH patients were limited, and death was typically attributable to ischemic cardiovascular diseases before the second or third decade of life. The advent of selective extracorporeal removal techniques prolonged survival and improved the quality of life.13-15 Bi-monthly treatments are recommended and time-averaged serum load of LDL-C can be effectively reduced by 60% to 65%, and even more with weekly treatments. Cost considerations and access limit broader use of the techniques currently...
This technology has been available for more than 2 decades; in earlier studies, 5-year benefit was achieved by conventional plasma exchange apheresis in genetically matched siblings.\(^{13,16,17}\) Since the last published data from French-Canadian patients,\(^{5}\) the mean age of patients reported has increased from a mean of 15 years (1 to 26 years) to 31 (5 to 54 years) in the present study. Although this represents an increase in survival, it does not necessarily describe disease-free survival. Patients with hmzFH develop both premature CHD, which is markedly slowed by extracorporeal LDL removal techniques and aortic valve disease, leading to critical aortic stenosis. The recent description of 2 cases (patients R and W in Table 1)\(^{18}\) who underwent extensive reconstructive ascending aorta surgery for severe calcific disease during aortic valve replacement (AVR) and repeat coronary arterial bypass graft (CABG) surgery, respectively, prompted us to review these conditions in cases of hmzFH in Canada. Anecdotal reports, mostly in the surgical literature of severe aortic calcifications in hmzFH, confirm that this is a novel finding in this unique patient population.\(^{19–22}\) As patients with hmzFH enter the fourth and fifth decades of life, cardiovascular complications will remain the most challenging aspect of their care. Because many therapeutic options are surgical in nature (eg, CABG, repeat CABG, and AVR), the presence of aortic calcifications represents an important cause of procedure-related morbidity and mortality. Calcific aortic disease must be ascertained in hmzFH, and techniques to prevent its progression must be studied and implemented.

### Materials and Methods

#### Study Subjects

In the present study, patients with hmzFH were followed in 2 major health institutes in the province of Quebec and in 4 other Canadian institutions. All patients had a molecular diagnosis using established techniques\(^{23–27}\) or fulfilled the criteria of hmzFH.\(^{23}\) All patients’ charts were reviewed using preestablished criteria. Anthropometric, clinical data, time of diagnosis, initiation of treatment, duration of specific treatments were collected, and all pertinent surgical reports were reviewed.

### Table 1. Ethnic Background, Mutation Analysis, and LDL-R Activity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ethnicity</th>
<th>Mutational Analysis*</th>
<th>% LDLR Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Lebanese</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>Anglo-Saxon</td>
<td>Asp211Gly (exon 4) + 2061insC (exon 14)**</td>
<td>&lt;2: ND</td>
</tr>
<tr>
<td>C</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1)</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>D</td>
<td>French-Canadian</td>
<td>Trp666Gly (exon 3)</td>
<td>25–100: 25–100</td>
</tr>
<tr>
<td>E</td>
<td>Anglo-Saxon</td>
<td>Cys88Tyr (exon 2) + IVS7-8 T&gt;C (splicing site)**</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>F</td>
<td>Latin-American</td>
<td>Del &gt;15 kb (exon 1 to exon 6)</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>G</td>
<td>French-Canadian</td>
<td>del &gt;15 kb</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>H</td>
<td>Anglo-Saxon</td>
<td>del &gt;7 kb</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>I</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1)</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>J</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1)</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>K</td>
<td>Anglo-Saxon</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L</td>
<td>Lebanese</td>
<td>Cys660Term (exon 14)</td>
<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>M</td>
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<td>&lt;2: &lt;2</td>
</tr>
<tr>
<td>N</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1) + Cys646Try (exon 14)**</td>
<td>?: &lt;2</td>
</tr>
<tr>
<td>O</td>
<td>Lebanese</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1) + Trp666Gly (exon 3)**</td>
<td>&lt;2: 25–100</td>
</tr>
<tr>
<td>Q</td>
<td>Iraqi</td>
<td>Cys281Trp (exon 5)</td>
<td>ND</td>
</tr>
<tr>
<td>R</td>
<td>Hungarian</td>
<td>Ala431Thr (exon 9)</td>
<td>5–15: 5–15</td>
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<td>S</td>
<td>French-Canadian</td>
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<td>25–100: 25–100</td>
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<tr>
<td>T</td>
<td>Bulgarian</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>U</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1) + Trp666Gly (exon 3)**</td>
<td>&lt;2: 25–100</td>
</tr>
<tr>
<td>V</td>
<td>French-Canadian</td>
<td>del &gt;15 kb (promoter to exon 1) + Trp666Gly (exon 3)**</td>
<td>&lt;2: 25–100</td>
</tr>
<tr>
<td>W</td>
<td>Latin-American</td>
<td>IVS7+1 G &gt;C (splicing site)</td>
<td>ND</td>
</tr>
<tr>
<td>X</td>
<td>Chinese-Canadian</td>
<td>Pro644Lei (exon 14)</td>
<td>20: 20</td>
</tr>
<tr>
<td>Y</td>
<td>French-Canadian</td>
<td>Trp666Gly (exon 3)</td>
<td>25–100: 25–100</td>
</tr>
</tbody>
</table>

*All mutation references and % LDL receptor (LDLR) activity can be found in the University College of London, Division of Cardiovascular Genetics website. www.ucl.ac.uk/fh. ND: Not determined. **Compound heterozygous.
Although hmzFH incidence is high in populations like the French-Canadians (1 in 270 000 or higher), a drop in birth rates in French-Canadian families, decrease in marriages within structured endogamy, genetic counseling, and an increase in immigration rate have all contributed to the genetic diversification observed in the present study. Information was obtained from laboratory data and medical records. Cases were managed and investigated according to established guidelines and the standard of care in effect at the time.

Patients gave signed informed consent for biochemical and molecular testing.

**Lipoprotein Analysis and Management**

Lipid and lipoprotein lipid measurements were performed in the laboratories of each institution on fasting blood samples, using conventional techniques. In all cases, the laboratories standards adhere to the Center for Disease Control or other national standardization programs for cholesterol and triglyceride measurements.

LDL concentration was estimated using the Friedewald equation.

Extracorporeal LDL removal techniques included plasmapheresis, LDL-apheresis, LDL immunoprecipitation, selective LDL filtration, or heparin precipitation technique. These techniques remove apoB-containing lipoprotein particles in a few hours and have been shown to reduce inflammatory marker and improve endothelial function and decrease cardiovascular events. The procedure is performed every 2 weeks. Canada has 2 centers for selective LDL apheresis using the extracorporeal LDL removal technique based on the Heparin-induced Extracorporeal LDL Precipitation (HELP) technique (Braun Medical): 1 in Quebec City and 1 in Edmonton, Alberta. The HELP treatment protocol implemented at Quebec City in 1998 and at Edmonton in 2006 to replace plasmapheresis methods or plasma filtration at a molecular weight cut-off point of 2000 kDa. These 2 systems service the provinces of Quebec and Alberta, whereas LDL-apheresis and plasmapheresis was available in many other sites. In our experience, the HELP technique is well tolerated by patients with very few side-effects; no major complications have been directly associated with the technique, and it is more selective in removing LDL-C particles while sparing HDL-C. Only 3 patients were treated with LDL-apheresis and 2 others with plasmapheresis elsewhere in Canada. Pre- and posttreatment LDL-C were determined after each treatment, and the average of both was considered as the therapeutic level achieved at the particular time point.

**Molecular Testing**

Mutational analysis was carried out using whole blood and a multiplex polymerase chain reaction (PCR) reaction performed to detect population specific mutations in the LDLR gene. Only 6 mutations account for ~85% of LDL receptor mutations in the French-Canadian patients. Exon-by-exon sequence analysis (EBESA) or multiplex ligation-dependent probe amplification (MLPA) mutation search was required to identify mutation in non-French-Canadian individuals (Table 1). To date, no homozygous mutations in the APOB or PSCK9 genes have been detected in these hmzFH subjects.

**Computed Tomography (CT) Evaluation of Aortic Calcifications and Calcium Score**

Scans were carried out on a multi-slice-detector spiral CT (General Electric), without contrast infusion; the scan covered the entire aorta length from the aortic valve to 10 cm below the aortic bifurcation. Scans settings were as follows: slice thickness 2.5 mm, acquired at 2.0-mm interval, 0.7-s tube rotation time, pitch 1.375, 120 kV, and 450 mA. The calcium score was determined using dedicated software (Smart Score, GE Medical Systems). Two trained physicians and an experienced vascular radiologist (Z.A., K.A., and D.V., respectively) were assigned to review and score all CT scans. Measurements were standardized by dividing the aorta into 7 segments defined by anatomic landmarks (valve, ascending, arch, descending, suprarenal, infrarenal, and bifurcation; Figure 3). The final aortic calcium score represents the sum of the 7 segments (Table 2). Because of concerns about the radiation dose (approximately 500 mSv), children below age 12 were not scanned.

**Results**

We studied 25 hmzFH patients (13 men, 12 women; Table 1 and Figure 1) followed since 1968; mean follow up was 18 years (range, 2 to 39). In most patients, homozygous (or compound heterozygous) mutations of the LDLR gene confirmed the diagnosis of hmzFH (Table 1). Among the 12 patients in the present study are of French Canadian ancestry, the mutations identified are in keeping with previous reports. In other patients, mutations either reflected ethnic origins or were novel mutations identified by EBESA and MLPA. The residual LDLR activity of the affected allele was taken from fibroblast studies reported from the Hospital for Sick Children in Toronto and from previous reports (www.ucl.ac.uk/fh). One patient (G) was lost to follow-up.

Mean age was 32±14 years (range 5 to 54) and the initial average total cholesterol value before intensive treatment was 19±5 mmol/L (737±206 mg/dL). Mean apoB level was 2.80 g/L. None of the patients tested was homozygote for the APOE E2 allele. Stigmata of dyslipidemia such as tendinous xanthomas and xanthelasmas were present initially in most patients, but regressed markedly during therapy, as previously reported. Consanguinity and the influence of endogamy were observed in 16 patients; 15 patients had known family history of dyslipidemia and premature CHD.

Table 2 shows the initial lipoprotein profile in all subjects. Posttreatment total cholesterol and LDL-C levels were taken as reflecting optimal therapy either with extracorporeal LDL removal and medications or medications alone. In 4 patients, the response to medical therapy (high-dose statin plus the intestinal cholesterol absorption inhibitor ezetimibe) was considered sufficient by the attending physician not to initiate extracorporeal LDL removal. Only the 5-year-old boy has not yet been considered to receive invasive therapy by his treating physician and family. Extracorporeal LDL removal using the HELP system is used currently in 15/25 patients. In patients treated every 2 weeks by the HELP system, LDL-apheresis and plasmapheresis, on-treatment total cholesterol, and LDL-C levels were determined as (pretreatment value + posttreatment value)/2.

Figure 1 shows total cholesterol levels in each patient during the entire period of follow-up. In some patients, the increase in cholesterol levels during follow-up reflects temporary cessation of treatment, specifically unavailability of the HELP system for patients T, U, V, and W, the desire to become pregnant for patient R, and lack of compliance for patients I, J, and K). Major cardiovascular events, defined as symptomatic angina, acute coronary syndrome, and myocardial infarction, confirmed by coronary angiography (docu-
mented stenosis >50% of the cross sectional coronary stenosis of a major epicardial coronary artery or aortic stenosis documented by echocardiography or hemodynamic measurements) were present in 20 subjects (mean age at diagnosis 25 years; range 12 to 38 years). Nine of the 20 patients with a diagnosis of CHD (45%) had CABG surgery for multiple vessel disease, and 1 patient required a repeat CABG; 11/25 (44%) patients were diagnosed with aortic stenosis or aortic root disease. Of these, 6/11 (55%) required aortic valve replacement.

The timing of cardiovascular events in each patient is shown in Figure 1. Two patients underwent experimental (and ultimately unsuccessful) retroviral-mediated hepatic \textit{LDLR} gene therapy in 1992 and 1993, at ages 29 and 41, respectively,\textsuperscript{29} and 2 others each had a portocaval shunt in 1980 and 1981, at ages 28 and, because of severe aortic stenosis (aortic valve area [AVA] 0.5 cm\textsuperscript{2}, and evidence of left ventricular dysfunction) at age 43, the patient underwent aortic root reconstruction and

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
Patient & Age, Sex & Diagnosis Date & Chol & LDL-C & HDL-C & Trig\textsuperscript{a} & Chol & LDL-C & HDL-C & CAD & Valvular Disease & LDL-removal\textsuperscript{b} & Chol Year Score & Aortic-Calcium-Score \\
& & & mmol/L & mmol/L & mmol/L & mmol/L & mmol/L & mmol/L & mmol/L & & & Starting Date & & \\
\hline
A & 5 M & 02-05-11 & 30.2 & 26.0 & 0.6 & 2.4 & 24.0 & 22.0 & No & No & Medication only & & 156 & NA \\
B & 12 M & 02-07-01 & 18.1 & 16.4 & 1.1 & 1.3 & 9.4 & 8.2 & No & No & 06-03-07 & 205 & NA \\
C & 13 M & 95-12-07 & 22.4 & 21.2 & 0.7 & 1.1 & 5.6 & 4.7 & No & No & 03-09-19 & 222 & 47 \\
D & 14 M & 04-10-28 & 24.0 & 21.5 & 1.5 & 2.2 & 5.1 & 3.7 & CABG & AR & 06-03-19 & 156 & 27 \\
E & 19 M & 97-01-01 & 14.0 & 12.0 & 0.9 & 1.3 & 3.8 & 3.4 & IHD & AS & 97-01-01 & 205 & 1123 \\
F & 20 M & 91-07-01 & 20.0 & 18.0 & 0.8 & 1.2 & 3.7 & 3.2 & IHD & AR & 92-07-01 & 202 & 895 \\
G & 21 F & 88-07-01 & 29.0 & 26.0 & 0.6 & 1.0 & 5.2 & 4.0 & CABG & AR & 88-07-01 & 178 & 1457 \\
H & 24 F & 96-11-07 & 13.9 & 12.6 & 0.6 & 1.6 & 8.4 & 6.7 & No & AS & 96-11-07 & 314 & 804 \\
I & 25 M & 96-11-07 & 13.1 & 12.1 & 0.5 & 1.3 & 7.9 & 7.9 & IHD & AVR & 96-11-07 & 300 & 11703 \\
J & 26 F & 91-07-01 & 25.0 & 23.0 & 2.4 & 10 & 5.3 & 5.0 & PCI & No & 98-07-01 & 517 & 2260 \\
K & 27 M & 91-08-26 & 12.3 & 9.2 & 0.4 & 5.9 & 4.5 & 3.4 & IHD & AS & 01-06-13 & 315 & 1259 \\
L & 36 M & 71-04-05 & 17.8 & 16.2 & 0.8 & 2.0 & 11.2 & 9.5 & No & AVR & Medication only & & 491 & NA \\
\hline
M & 36 F & 79-08-27 & 23.9 & 23.0 & 0.5 & 1.0 & 4.6 & 3.4 & PCI & AVR & 02-03-04 & 637 & 6444 \\
O & 38 F & 03-11-21 & 16.6 & 14.5 & 1.0 & 2.2 & 3.7 & 2.6 & MI,PCI & No & 03-12-12 & 608 & 8154 \\
P & 38 F & 78-12-04 & 17.4 & 14.9 & 1.0 & 3.3 & 3.5 & 2.0 & No & No & 03-05-12 & 505 & 3095 \\
Q & 39 M & 05-06-29 & 18.0 & 15.3 & 1.4 & 2.9 & 6.9 & 5.3 & CABG & No & 06-01-19 & 677 & 2687 \\
R & 43 F & 87-01-14 & 10.6 & 9.4 & 1.0 & 0.4 & 7.5 & 5.8 & MI,CABG & AVR & Medication only & & 404 & 10646 \\
S & 43 F & 83-02-18 & 17.4 & 16.1 & 0.7 & 1.3 & 5.3 & 3.8 & MI,CABG & No & Medication only & & 557 & 11610 \\
T & 45 M & 68-07-01 & 28.0 & 25.0 & 0.7 & 1.2 & 3.8 & 2.9 & CABG & AR & 96-07-01 & 763 & 4072 \\
U & 46 F & 78-05-02 & 18.4 & 17.1 & 0.8 & 1.2 & 3.7 & 2.3 & No & AVR & 01-03-13 & 733 & 7457 \\
V & 47 F & 78-05-01 & 17.7 & 15.5 & 1.7 & 1.9 & 4.3 & 2.2 & IHD & No & 01-02-21 & 770 & 18024 \\
W & 48 F & 03-01-16 & 15.4 & 14.1 & 0.8 & 1.1 & 9.3 & 7.7 & MI,CABG & AS & 04-07-01 & 635 & 15525 \\
X & 50 F & 72-07-01 & 18.0 & 15.9 & 0.8 & 2.7 & 7.4 & 6.0 & CABG & No & 06-01-20 & 756 & 28922 \\
Y & 54 F & 74-02-18 & 13.4 & 12.2 & 0.9 & 0.6 & 10.0 & 8.3 & CABG & No & Medication only & & 672 & 15691 \\
\hline
mean & 32±14 & 19±5 & 17±5 & 0.9±0.4 & 2.3±2 & 7±4 & 6±4 & & & & 462±210 & 7270±7381 \\
\hline
\end{tabular}
\caption{Lipid Profile, Cardiovascular Disease, and Aortic-Calcium-Score}
\end{table}

*LDL-removal: (HELP indicates heparin-induced extracorporeal lipoprotein precipitation, LDL-apheresis, or plasmapheresis). **Score underestimated because of surgical removal of the aortic valve and portions of ascending aorta: Chol, cholesterol; Trig, triglycerides; CABG, coronary artery bypass grafting; IHD, ischemic heart disease; AS, aortic stenosis; AR, aortic regurgitation; MR, mitral regurgitation; AVR, aortic valve replacement; PCI, percutaneous coronary intervention; MI, myocardial infarction. Dates as yy/mm/dd; NA, not available.

We have previously reported 2 cases (patients R and W, Table 2) who underwent complex aortic surgery during AVR and CABG, respectively, because of extensive calcifications.\textsuperscript{18} Figure 2 shows endoluminal calcifications of the ascending aorta resected during a Cabrol procedure for AVR in patient R. Of interest, this patient underwent CABG at age 28 and, because of severe aortic stenosis (aortic valve area [AVA] 0.5 cm\textsuperscript{2}, and evidence of left ventricular dysfunction) at age 43, the patient underwent aortic root reconstruction and
AVR with a metallic valve. The coronary grafts were all patent at the time of the second surgery. Despite the lack of progression of the CHD over a 14-year period with high-dose statin and ezetimibe, aortic valve disease and calcifications progressed. Patient G underwent aortic root reconstruction by a Bentall procedure with a bioprosthesis at age 15 (2001). Similarly, patient J had AVR with a mechanical valve, followed by a Bentall procedure at age 21 (2003), as did patient U who, at age 45 (2006), underwent AVR with a metallic valve by a Bentall procedure. Ross procedures were performed on patient M at age 29 (2000) and in patient N at age 21 with coronary artery surgical angioplasties (1991). Patient R underwent AVR with a metallic valve by a Cabrol procedure at age 43 (2006). Surgical reports from 4 of the 6 AVR confirm heavily calcified valves at the time of surgical intervention. The mean age of patients undergoing surgery for the first time was 32 years (range 12 to 40 years).

Calcification of the aorta, quantified by calcium score, was observed in all but the 2 youngest scanned patients (aged 13
and 14 years). Serial CT scans of the entire aorta were performed in all subjects between October 2006 and June 2007 (ages at the time of CT scanning is shown in Table 2). Figure 3 shows a representative patient (patient V in Table 1 and Figure 1) with extensive calcifications in the ascending, transverse, descending, and abdominal aorta and into the iliac vessels. The calcium score is shown in Table 2 (5 patients indicated with ** in table 2 underwent reconstruction of the ascending aorta with a prosthetic graft; in these patients the overall calcium score is underestimated because that segment could not be included in the analysis).

The mean cholesterol-year score, calculated as previously described, was 462±210 year-chol (mmol/L). The cholesterol-year score is a reflection of lifetime burden of hypercholesterolemia. The mean calcium-score by CT was 7270±7381 (range 27 to 28 922) in the 22 patients scanned. A strong correlation between age and calcium score was found (r=0.73; r²=0.53; P=0.0001). No significant correlation was found between total cholesterol at baseline and the calcium score (r=-0.39, r²=0.15; P=0.07). There was also a significant correlation between the cholesterol-year score and the aortic calcium score (r=0.59; r²=0.35; P=0.0038).

**Discussion**

Here, we report that premature, severe, and extensive aortic calcifications are a characteristic feature of patients with hmzFH, despite appropriate medical therapy including extracorporeal LDL removal techniques resulting in marked sustained reductions in LDL-C levels. The presence of these calcifications poses therapeutic challenges in patients requiring a surgical approach for CHD or aortic valve disease. The need for complex surgical procedures increases mortality and morbidity in these patients in the second to third decade of life. The lack of correlation between total cholesterol and aortic calcium score and the strong correlation with age and calcium score raises the concern that vascular calcifications may progress independent of marked decreases in total and LDL cholesterol levels. Experimentally, this was similarly

![Figure 2](image1.png)

*Figure 2. Calcification within the Tunica Intima of the ascending aorta from (patient R), taken at the time of aortic valve replacement. A, 60× magnification; B, 100× magnification. Hematoxilin and eosin stain.*

![Figure 3](image2.png)

*Figure 3. A, Representative computed tomographic scans of the aorta. B, Computerized reconstruction of calcifications (indicated by arrows).*
found in rhesus monkeys and in maccaca mulatta,\textsuperscript{31} where diet-induced hypercholesterolemia led to significant atherosclerosis and vascular calcifications, followed by regression of atherosclerosis, but not of calcifications despite a decrease in plasma cholesterol on a regression diet. Furthermore, in a clinical trial to prevent the progression of aortic stenosis, statins did not change the rate of coronary calcifications, as determined by helical CT.\textsuperscript{32}

Extensive calcifications of the ascending aorta constitute a significant challenge for the surgical approach. Such a condition is associated with a clear increase in morbidity for the patient. Previous anecdotal reports of extensive aortic root calcifications have been published, mostly in the surgical literature.\textsuperscript{19–22} The incidence of complications such as calcific embolization to the central nervous system or peripheral arterial circulation, patient-valve mismatch in the case of patients with a small aortic root, and early coronary graft failure from proximal anastomotic occlusion is increased. To alleviate such risks, extra-anatomic cannulation for the institution of cardiopulmonary bypass and resection of the ascending aorta under deep hypothermic circulatory arrest is often necessary. These techniques necessitate longer surgical time and are associated with higher risks of all perioperative complications such as prolonged extracorporeal circulation and hypothermia, bleeding, renal dysfunction, capillary leak, and possible neurological deficit because of long cardiopulmonary pump time. Preoperative identification of the condition greatly facilitates planning of the appropriate surgical strategy. The diagnosis of hmzFH should be an indication for preoperative scanning of the thoracic segments of the aorta as other modalities of aortic visualization such as trans-thoracic or transesophageal echocardiography are less precise and will miss some important segments of the aorta.

Pathophysiology

Vascular calcifications in patients with FH have previously been reported, especially in association with aortic valve calcification.\textsuperscript{5,7,9,10,33–35} More recently, anecdotal reports have emphasized the importance of ascending aortic calcifications in hmzFH during cardiac surgery and the need for complex surgery.\textsuperscript{18–22} The pathogenesis of vascular calcifications has been reviewed recently, in light of recent discoveries in the literature.\textsuperscript{43–45} Our long-term clinical data now indicate that despite marked LDL-C lowering, vascular calcifications progress and contribute to significant morbidity associated with surgical interventions. One possibility is that signaling pathways are dysregulated in osteoblast-like LDLR\textsuperscript{−/−} cells,\textsuperscript{36–39} as observed in Ldlr\textsuperscript{−/−}, APOB\textsubscript{100/100} mice.\textsuperscript{45} Another possibility to explain the rapid and apparently irreversible vascular calcifications is the prevention of differentiation of preosteoclasts because of an increase on osteoprotegerin observed in hypercholesterolemic states.\textsuperscript{46} Osteoprotegerin prevents the RANK ligand to bind to its cognate receptor on preosteoclasts, delaying the differentiation into mature osteoclasts and thus the desorption of calcium within the arterial wall. These pathways constitute potential therapeutic targets to prevent such vascular calcifications.

Tissue cholesterol deposits—tendinous or planar xanthomas and xanthelasmas—often regress with lipid-lowering therapy in FH patients. Experienced clinicians have noted that once calcified, tendinous xanthomas are unlikely to regress. Although xanthomas and xanthelasmas regress in hmzFH patients treated with LDL-C lowering,\textsuperscript{16,17} the evolution of vascular calcifications appears to be independent of LDL-C levels. This provides compelling rationale to examine the long-term effects of early and sustained intervention in hmzFH to prevent or delay vascular calcifications.

Recommendations

The treatment of hmzFH has improved markedly since the inception of extracorporeal LDL removal techniques, which now constitutes the standard of care in patients with poor response to drug therapy.\textsuperscript{14} Although statins have limited effectiveness in receptor-negative hmzFH, their use is still associated with a further decrease in LDL-C, most likely by increasing residual LDLR activity and by decreasing hepatic apoB secretion in the case of receptor-negative mutations, such as the >15-kb deletion, common in French-Canadian patients. The optimal frequency of extracorporeal LDL removal treatments is still a matter of controversy, with cost remaining a major restriction to more frequent (ie, weekly) treatments.

Noninvasive imaging techniques should be used serially in hmzFH patients to assess coronary artery disease, along with transthoracic echocardiography to assess the aortic valve and left ventricular function. On the basis of the present report, clinical studies should be initiated to examine the rate of progression of aortic calcifications and their possible prevention. Whenever surgical treatment is contemplated, preoperative CT scanning should be performed routinely to facilitate the planning of the surgical strategy.
Limitations of the Study
This study was not prospective. We cannot determine reliably whether aortic calcifications were related to the LDL-C lifetime burden or to other factors, nor can we determine whether present treatment options can alter the natural history of vascular calcium deposits. Indeed, previous authors have suggested that LDL removal techniques do not decrease aortic calcifications. Nonetheless, we have shown that extensive and severe aortic calcifications occur in most patients with hmnzFH in the second decade of life, despite optimal medical therapy of the hyperlipoproteinemias. The calcifications represent a considerable management challenge when patients require surgery to treat aortic calcification or aortic stenosis, 2 well-described complications of hmnzFH.

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Disclosures
None.

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Vascular Calcifications in Homozygote Familial Hypercholesterolemia

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*Ten: Tendinous Xanthoma; Tub: Tuberos xanthoma; Dys: Dyslipidemia; Endogamy: practice of marrying within a social group; CABG: Coronary Artery Bypass Grafting; IHD: Ischemic Heart Disease; AS: Aortic Stenosis; AR: Aortic Regurgitation; MR: Mitral Regurgitation; AVR: Aortic Valve Replacement; PCI: Percutaneous coronary intervention; MI: Myocardial Infarction.