MAGNETIC RESONANCE IMAGING OF Atherosclerotic MANIFESTATIONS IN FAMILIAL HYPERCHOLESTEROLEMIA

Sami Soljanlahti

Helsinki Medical Imaging Center
and
Department of Radiology
University of Helsinki
Helsinki, Finland

Academic Dissertation

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki in Auditorium XII, Helsinki University, on 31 October 2008, at 12 noon.

Helsinki 2008
Supervisors
Docent Taina Autti
Department of Radiology
University of Helsinki
Helsinki, Finland

and

Docent Kirsi Lauherma
Helsinki Medical Imaging Center
Hospital for Children and Adolescents
Helsinki University Central Hospital
Helsinki, Finland

Reviewers
Professor Petri Kovanen
Wihuri Research Institute
Helsinki, Finland

and

Docent Pekka Niemi
University of Turku
Turku, Finland

Opponent
Professor Ritva Vanninen
Department of Radiology
University of Kuopio
Kuopio, Finland

ISBN 978-952-10-5043-5 (PDF)

Yliopistopaino
2008
CONTENTS

LIST OF ORIGINAL PUBLICATIONS .............................................. 6

ABBREVIATIONS .................................................................... 7

ABSTRACT ........................................................................... 8

INTRODUCTION ................................................................. 9

REVIEW OF THE LITERATURE ............................................. 10
  Pathogenesis Of Atherosclerosis ....................................... 10
  Familial Hypercholesterolemia .......................................... 11
  Type 2 Diabetes Mellitus .................................................... 12
  Imaging Atherosclerosis .................................................... 12
  MRI Measurement of arterial wall thickness ....................... 13
  MRI Measurement of arterial elasticity .............................. 13
  Intracranial MRI ............................................................... 13

AIMS OF THE STUDY ......................................................... 15

SUBJECTS AND METHODS ................................................ 16
  Subjects ........................................................................... 16
  Magnetic resonance imaging .......................................... 18
    Methods ....................................................................... 18
    Data analysis .................................................................. 18
  Ultrasound ....................................................................... 21
  Laboratory analyses ......................................................... 21
  Statistical methods .......................................................... 21

RESULTS ............................................................................ 22
  Intracranial MRI .............................................................. 22
  Aortic MRI ....................................................................... 23
  Ultrasound ........................................................................ 24
  Laboratory analyses ........................................................ 24

DISCUSSION ...................................................................... 27
  Intracranial MRI .............................................................. 27
  Aortic MRI ....................................................................... 28
  Limitations ...................................................................... 29

CONCLUSIONS .................................................................... 29

ACKNOWLEDGEMENTS ....................................................... 30

REFERENCES ...................................................................... 32
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals and on unpublished data presented in the results.


The original publications are presented with permission from the publishers.
ABBREVIATIONS

AA  Ascending aorta
AMI  Acute myocardial infarction
BMI  Body-mass index
BSA  Body surface area
CCA  Common carotid artery
CHD  Coronary heart disease
CT  Computed tomography
CVD  Cardiovascular disease
DA  Descending aorta
DM  Diabetes Mellitus
DSA  Digital subtraction angiography
ECG  Electrocardiogram
FH  Familial hypercholesterolemia
FH-NK  Familial hypercholesterolemia – North Karelia (mutation)
FLAIR  Fluid-attenuated inversion recovery
FOV  Field of view
GRE  Gradient echo
HDL  High-density lipoprotein
hsCRP  High-sensitivity C-reactive protein
IMT  Intima-media thickness
IVMRI  Intravascular magnetic resonance imaging
IVUS  Intravascular ultrasound
LDL  Low-density lipoprotein
Lp(a)  Lipoprotein (a)
MRA  Magnetic resonance angiography
MRI  Magnetic resonance imaging
PD  Proton-density
PWV  Pulse wave velocity
SD  Standard deviation
TSE  Turbo spin echo
SE  Spin echo
TE  Echo time
TI  Inversion time
TOF  Time of flight
TR  Repetition time
US  Ultrasound
WMHI  White matter hyperintensity
ABSTRACT

Cardiovascular diseases (CVD) are, in developed countries, the leading cause of mortality. The majority of premature deaths and disability caused by CVD are due to atherosclerosis, a degenerating inflammatory disease affecting arterial walls. Early identification of lesions and initiation of treatment is crucial because the first manifestations quite often are major disabling cardiovascular events. Methods of finding individuals at high risk for these events are under development. Because magnetic resonance imaging (MRI) is an excellent non-invasive tool to study the structure and function of vascular system, we sought to discover whether existing MRI methods are able to show any difference in aortic and intracranial atherosclerotic lesions between patients at high risk for atherosclerosis and healthy controls.

Our younger group (age 6-48) comprised 39 symptomless familial hypercholesterolemia (FH) patients and 25 healthy controls. Our older group (age 48-64) comprised 19 FH patients and 18 type 2 diabetes mellitus (DM) patients with coronary heart disease (CHD) and 29 healthy controls. Intracranial and aortic MRI was compared with carotid and femoral ultrasound (US) and with risk factors assessed from a venous blood sample.

In neither age-group did MRI reveal any difference in the number of ischemic brain lesions or white matter hyperintensities (WMHs) - possible signs of intracranial atherosclerosis - between patients and controls. Furthermore, MRI showed no difference in the structure or function of the aorta between FH patients and controls in either group. DM patients had lower compliance of the aorta than did controls, while no difference appeared between DM and FH patients. However, ultrasound showed greater plaque burden and increased thickness of carotid arterial walls in FH and DM patients in both age-groups, suggesting a more advanced atherosclerosis.

The mortality of FH patients has decreased substantially after the late 1980’s when statin treatment became available. With statins, the progression of atherosclerotic lesions slows. Statins may provide effects beyond cholesterol lowering, ones which may stabilize atherosclerotic plaques, thus preventing strokes and other atherothrombotic events. We think that this, in concert with improvements in treatment of other risk factors, is one reason for the lack of differences between FH patients and controls in MRI measurements of the aorta and brain despite the more advanced disease of the carotid arteries assessed with US. Furthermore, whereas atherosclerotic lesions between different vascular territories correlate, differences might still exist in the extent and location of these lesions among different diseases. Small (<5 mm in diameter) WMHs are more likely a phenomenon related to aging, but the larger ones may be the ones related to CVD and may be intermediate surrogates of stroke. Most MRI studies are done with rather few patients, and the image quality in aortic imaging, although constantly improving, is not yet optimal and thus is a source of bias.
INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of mortality in developed countries, and if current demographic and lifestyle changes continue, they will soon take the lead position globally (Yusuf et al. 2001). Atherosclerosis is an inflammatory disease of the arterial wall mainly responsible for the CVD mortality in westernized countries, where it accounts for 50% of all deaths, mainly through ischemic complications: coronary heart disease (CHD) and stroke (Lusis 2000). In Finland, CHD mortality has decreased from 1973 to 2001 over 70% (Jousilahti 2003), which is largely due to lower cholesterol and blood pressure levels and to favorable changes in smoking habits (Vartiainen et al. 1994). Other improvements, as well, in primary and secondary prevention have accounted for decreases in CHD mortality (Salomaa et al. 2003). Nevertheless, CVD holds the second place after alcohol-related causes as a cause of mortality in those 15 to 64 years old, and holds first place in those over 65 in Finland.

Despite evolving treatment strategies and information on the new risk factors, patients with such diseases as familial hypercholesterolemia (FH) (Anonymous 1999) and diabetes mellitus (DM) (Roglic et al. 2005) still have higher cardiovascular mortality than the general population. Both hypercholesterolemia and diabetes are conventional risk factors for atherosclerosis as well as age, male gender, hypertension, smoking, obesity, and family history of premature CVD (Frucht et al. 2004). Lesion formation begins in early childhood, whereas the clinically symptomatic phase starts normally in middle age. Early identification of lesions and initiation of treatment is emphasized because quite often the first manifestation of the disease is a catastrophic cardiovascular event such as stroke, acute myocardial infarction, or even sudden death (Naghavi et al. 2003).

The insufficiency of risk factor assessment in finding individuals at risk for major cardiovascular events has led to development of methods for direct imaging of atherosclerotic lesions. Its capability to find high-risk lesions in all vascular territories without ionizing radiation is the advantage of magnetic resonance imaging (MRI) in screening and follow-up of atherosclerosis (Yuan and Kerwin 2004).

This study was conducted to determine whether available non-invasive MRI and ultrasound (US) methods for assessment of atherosclerotic lesions can show differences between FH patients and controls and DM patients.
REVIEW OF THE LITERATURE

PATHOGENESIS OF ATHEROSCLEROSIS

The walls of both muscular and elastic arteries consist of three distinct layers. The innermost, the intima, consists of endothelium, connective tissue, and smooth muscle cells. The intima is surrounded by mainly smooth muscle containing media enveloped by the adventitia which is composed mainly of connective tissue. Atherosclerosis principally affects the arterial intima, and atherosclerotic lesions are traditionally classified according to the American Heart Association (AHA) histological classification (Stary et al.1994, 1995) (Figure 1). The initial lesions normally form near branch points and curvatures of the arteries because those geometries are associated with flow disturbances and resulting gradients in wall shear stress (Gimbrone 1999). Nowadays, the initial step in development of atherosclerosis is thought to be endothelial dysfunction caused by several factors: elevated low-density lipoprotein (LDL) cholesterol, hypertension, DM, infectious microorganisms, or free radicals caused by cigarette smoking (Ross 1999). The loss of endothelial homeostasis leads, in the intima, to an influx and retention of LDL cholesterol, accumulation of macrophages and T lymphocytes, and proliferation of smooth muscle cells. If the offending agents are not removed or neutralized by the inflammatory response, inflammation persists, leading to formation of atherosclerotic plaque.

The first lesions (fatty streaks) consist of lipid-laden macrophages called foam cells and can be seen as early as during fetal development (Napoli et al.1997). Normally these appear in the aorta of everyone during the first decade of life, in the coronaries during the second decade, and in the cerebral arteries during the third or fourth decades (Lusis 2000). These fatty streak lesions are not clinically significant but serve as precursors for more advanced lesions if the inflammation persists. The lesions between the initial fatty streak lesion and the

Figure 1. Histological classification of atherosclerotic lesions
end-stage critically stenotic plaque contain many different potentially harmful lesion types. The AHA classification provides no equivalent for all of these lesions that are prone to thrombus formation, ones nowadays called “vulnerable plaques” (Naghavi et al. 2003). The final stages in this AHA classification (lesion types V and VI) are associated with a thick fibrous cap. Nowadays, however, the thin fibrous cap lesion (Virmani et al. 2000) is considered the most common vulnerable lesion associated with plaque rupture. It is the most common plaque complication, causing 70% of fatal acute myocardial infarcts (AMI) and sudden coronary deaths (Naghavi et al. 2003). However, the atherosclerotic process may continue without atherothrombotic events; in time, the hydrolytic enzymes, cytokines, chemokines, and growth factors secreted by the constituents of intima and inflammatory cells result in formation of fibrotic tissue and formation of a thick fibrous cap making the plaque more stable. Due to dilation of the artery and outward remodeling of the vessel wall (Glagov et al. 1987), the lesions do not normally compromise blood flow until the very end of the disease process. Then the growing plaque starts to occlude the arterial lumen, causing ischemia in the region the artery supplies. However, due to the slow progression of the lesion, ischemic symptoms may be mild or even absent because of the development of a protective collateral circulation (Fuster et al. 1992, 1994).

In addition to plaque formation, atherosclerosis is related to arterial stiffness (Van Popele et al. 2001). In atherosclerosis, endothelial dysfunction (Davignon and Ganz 2004) has been proposed as a possible mechanism for arterial stiffness. The mechanism is the reduced synthesis of nitrous oxide (NO), a major determinant of arterial tone along with collagen, smooth muscle, and elastin. Atherosclerosis may, in addition, possess other mechanisms for arterial stiffening, like inflammation-induced fibrosis and calcification at later stages of the disease. Patients with hypercholesterolemia (Stefanadis et al. 2000) and CHD (Stefanadis et al. 1995) show increased aortic stiffness in intravascular ultrasound (IVUS). Increased arterial stiffness measured with US occurs along with many established cardiovascular risk factors (Lehman et al. 1998).

The term “arteriosclerosis” is often confusingly used as a synonym for “atherosclerosis”. These two terms, however, represent distinct but overlapping conditions (Safar 2007). Unlike focal intimal lesions of (early) atherosclerosis, atherosclerotic changes (degradation of elastin, proliferation of collagen, and deposition of calcium) take place in arterial media, are diffuse and lead to arterial stiffening. The main risk factors for arteriosclerosis are DM and aging (Johnson et al. 2006).

**FAMILIAL HYPERCHOLESTEROLEMIA**

Familial hypercholesterolemia is an inherited metabolic disorder caused by mutations in the LDL receptor gene located on the short arm of chromosome 19. More than 1200 mutations (Human Gene Mutation Database) exist, classified into five classes based on the different phenotypes of the mutant proteins. Mutated LDL receptor protein impairs the removal of LDL cholesterol from plasma in the liver and other organs. This results in hypercholesterolemia, the severity of which depends on environmental factors and mutation type. Receptor-negative mutations cause a more severe phenotype than do receptor-defective ones. FH heterozygotes have one normal and one mutant allele, and this enables LDL removal at approximately half the normal rate, causing a two-to-three fold elevation in blood cholesterol level compared to that of the general population. Homozygotes have two mutated alleles, resulting in total or near-total inability to remove LDL cholesterol from plasma; this causes severe hypercholesterolemia leading to death usually by myocardial infarction before the age of 20 (Goldstein et al. 2001).

Heterozygous FH is one of the most common inherited metabolic diseases, with a worldwide prevalence of 1/500. These patients show a higher risk for cardiovascular mortality (Anonymous 1999), whereas their all-cause
mortality is not necessarily higher than that of the general population (Anonymous 1999, Sijbrands et al. 2001). This emphasizes the role of environmental factors. Cholesterol-lowering treatment, especially introduction of HMG-CoA reductase inhibitors, i.e., statins in the late 1980’s, has lowered cardiovascular mortality rates (Anonymous 1999) and stroke risk (Hutter et al. 2004) which in these heterozygotes was high before initiation of statin treatment (Bansal et al. 1986, Kaste and Koivisto1988). Nowadays, statins are recommended and considered safe for FH patients over age 8 (Vuorio et al. 2004, Wiegman et al. 2004, Avis et al. 2007). In addition to dietary treatment, patients from the age of 2 can use cholesterol absorption-inhibiting stanol esters and from age 6 onwards add resins to further prevent cholesterol absorption (Vuorio et al. 2004).

In Finland, 90% of the FH patients have one of the seven founder mutations (FH-Helsinki, FH-North Karelia, FH-Turku, FH-Pori, FH-Pogosta, FH-11, or FH-12). In North Karelia, 84% of the FH is caused by the FH-North Karelia (FH-NK) mutation (Kontula 2005) which leads to failure of LDL receptor protein production and classical clinical FH with two- to three-fold elevated LDL levels and resulting skin and tendon xanthomas and accelerated atherosclerosis. The age of onset of CHD in FH-NK has been 42 years for men and 48 for women (Vuorio et al. 1997).

**TYPE 2 DIABETES MELLITUS**

Type 2 diabetes mellitus is a multifactorial metabolic disease characterized by insulin resistance and by a high level of blood glucose. According to estimates, its global prevalence will grow from 2.8% in the year 2000 to 4.4% in 2030 (Wild et al. 2004). Type 2 DM is a risk factor for atherosclerosis, and chronically elevated glucose levels are postulated to lead - independently of other risk factors - to development of atherosclerosis (Selvin et al. 2005). Type 2 DM causes mortality higher than that of the general population (Almdal et al. 2004, Roglic et al. 2005), approximately half of which is attributed to cardiovascular diseases (Morgan et al. 2000). Risk for stroke (Tuomilehto et al. 1996, Folsom et al. 1999, Almdal et al. 2004) and AMI (Almdal et al. 2004) is elevated.

**IMAGING ATHEROSCLEROSIS**

We can choose from multiple commercially available modalities for imaging atherosclerotic lesions: ultrasound (US), magnetic resonance imaging (MRI), computed tomography (CT) and digital subtraction angiography (DSA). Angiographic techniques, including DSA, the current gold standard for coronary, carotid, and peripheral lesion diagnostics, are able to show only luminal changes; they thereby are unable to show wall structure and indicate early lesions not yet altering the lumen. CT is able to show the structure with good resolution, and wall area measurements are in close and highly significant agreement with MRI (and with histology). However, perhaps due to better spatial resolution, MRI is more sensitive and specific in identifying atherosclerotic plaques (Viles-Gonzales et al. 2004). Furthermore, CT can serve to quantify calcium in the coronary arteries for assessment of atherosclerotic burden (Becker et al. 2001), although its radiation exposure makes it unsuitable for screening and follow-up. For those purposes, intravascular modalities (IVMRI, IVUS), able to provide good resolution, are also unsuitable because of their invasiveness. Wall-area measurements between IVUS and MRI correlate well with each other (as well as with histology), but spatial resolution of IVUS is better than that of MRI (Chiesa et al. 2004). Transcutaneous ultrasound is widely used but limited to vascular regions near the body surface. However, carotid intima-media thickness (IMT) assessed with US is considered a robust and reproducible (Montauban van Swijndregt et al.1999) indicator of overall atherosclerosis (de Groot et al. 2004, Klabak-Ziembicka et al. 2007). It is therefore widely used in statin treatment studies as an indicator for atherosclerosis progression or regression (Smilde et al. 2001, de Sauvage Nolting et al. 2003). MRI is able to show both morphological and functional changes in the vessels. It offers no radiation exposure and can visualize every
vascular territory. However, MRI is still quite expensive and has limited availability compared to US.

**MRI measurement of arterial wall thickness**

Most wall-area and thickness measurements are performed with different spin echo (SE) sequences. Different contrast weightings are often used to obtain complementary information on the tissues of the arterial wall because plaque structure is also of interest. Which weighting gives the best result in wall measurements is unclear. Zhang et al. studied T1-, T2- and proton-density (PD) -weighted images in carotid artery wall area measurements. They stated that the image (T1-, T2- or PD-weighted) with the highest quality should be the one to serve for area measurements (Zhang et al. 2001).

Periadventitial fat must be separated from the adventitia in measurement of aortic wall area or thickness. Some have used fat saturation to suppress the signal from periadventitial fat (Chan et al. 2001), while others have not (Li et al. 2004). In the aorta, the wall area and thickness measurements are reproducible (Chan et al. 2001, Li et al. 2004). The current trend toward an increased imager field strength is making MRI a more accurate tool in assessing blood-vessel properties. Wall-area measurements in the carotid arteries show better signal-to-noise and contrast-to-noise ratios and improved image quality at 3T than at 1.5T measurements (Yarnykh et al. 2006).

**MRI measurement of arterial elasticity**

Aortic elasticity is studied mainly with two MRI approaches (Metafratzi et al. 2002). The simpler approach is to assess change in aortic diameter or area in relation to distending pulse pressure with SE (Mohiaddin et al. 1989) or gradient echo (GRE) (Mohiaddin et al. 1993) sequences and to calculate different elasticity indexes from these (Oliver and Webb 2003). SE sequences have better contrast, allowing sharp definition of the arterial wall and lumen. However, slow-flowing blood may produce a high signal which makes delineation of the luminal border difficult. A GRE sequence provides a better temporal resolution in exchange for the poorer contrast between intima and lumen. Brachial blood pressure is normally used in the elasticity calculations. Young people have higher peripheral than central blood pressure, which leads to underestimation of the elasticity, but this gradient normally disappears with aging. New methods capable of assessing blood pressure non-invasively from MRI data are being proposed and prove to be comparable to methods using brachial blood pressure measurements (Vulliemoz et al. 2002, Laffon et al. 2005).

The second way to assess elasticity is to measure pulse wave velocity (Mohiaddin et al. 1993). The rationale behind this is the fact that fluids travel faster in a rigid tube. In MRI, flow can be measured with velocity-encoded GRE images (Nayler et al. 1986, Lotz et al. 2002). The pulse wave velocity is assessed by dividing the distance between two measuring sites by the time required for the pulse wave to travel between these sites.

**Intracranial MRI**

The blood supply to cerebral white matter originates mainly from the pial network of the surface of the brain via penetrating arteries ending near the walls of the lateral ventricles. The area immediately adjacent to these receives its blood supply through ventriculofugal vessels originating from subependymal arteries. That anastomoses between the penetrating and ventriculofugal arteries are scarce or absent means that white matter, which is highly vulnerable to ischemia (Pantoni et al. 1996), is exposed to blood-supply disturbances (Pantoni and Garcia 1997).

Magnetic resonance angiography (MRA) is able to show the luminal changes (narrowing, obstruction, wall irregularities) in intracranial carotid arteries and major cerebral arteries. Its resolution is insufficient to study submillimeter arteries, however.

MRI is very sensitive to morphological changes in brain tissue, and quite often small hyperintense lesions are visible in T2-weighted MR images even in asymptomatic subjects. Most of these white matter changes are thought to be of ischemic origin (Pantoni and
Garcia1997, Pantoni 2002). These lesions consist of small infarcts and small non-specific foci which have many synonyms in the literature: leuko-araiosis, high-signal intensity lesions, signal hyperintensities, white matter findings, white matter changes, incidental high signal-foci, unidentified bright objects (UBOs) and white matter hyperintensities (WMHIs). WMHIs appear hyperintense in T2-and PD weighted images and hypointense in T1-weighted images. The highly T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence is nowadays the best means of finding WMHIs because it shows fluid as hypointense, thereby preventing any mixing of WMHIs with perivascular spaces or cysts also seen as hyperintensities in conventional T2-weighted images (Barkhof and Scheltens 2002).

Among other risk factors for WMHIs are high carotid IMT (Manolio et al. 1999), hypercholesterolemia (Breteler et al. 1994), and diabetes (Erkinjuntti et al. 1994, Ylikoski et al. 1995). WMHIs show increased risk for stroke in the (elderly) general population (Vermeer et al. 2003, Kuller et al. 2004) and increased risk for stroke and myocardial infarction in patients with established atherosclerotic disease (Gerdes et al. 2006). White matter lesions in MRI have quite low specificity for several neuropathological conditions. Possible differential diagnoses are therefore numerous, including inflammatory, infectious, toxic, traumatic, and unknown etiologies (Barkhof and Scheltens 2002), in addition to ischemia. Fortunately, the fact that many white matter-affecting diseases have characteristic clinical manifestations, unlike incidental WMHIs of ischemic origin, does facilitate the differential diagnosis. Furthermore, ischemic WMHIs related to cardiovascular risk factors account for over 95% of WMHIs found incidentally (Barkhof et al. 1998). Clinically, WMHIs have been linked to motor and gait disturbances, cognitive impairment, urinary dysfunction, and mood disorders (Pantoni and Garcia 1995). Current evidence suggests that treatment of cardiovascular disease risk factors may prevent development or progression of WMHIs (Schwartz et al. 2005).
AIMS OF THE STUDY

Despite the improved risk factor treatment and lifestyle changes, atherosclerosis accounts for the majority of strokes and myocardial infarcts which are the major sources of morbidity and disability in most of the westernized world. MRI is an excellent tool for assessing the atherosclerotic burden in all vascular beds; however, we know little about the condition of large arteries and silent, possibly atherosclerosis-related brain lesions in familial hypercholesterolemia and diabetes mellitus patients.

The overall aim of this study was to use magnetic resonance imaging for assessment of atherosclerotic changes in patient groups at high risk for atherosclerosis and compare them to ultrasound measures and risk factors.

The specific aims were:

(I) To study subclinical brain lesions possibly of atherosclerotic origin in familial hypercholesterolemia patients.

(II) To investigate aortic and carotid findings of subclinical atherosclerosis in familial hypercholesterolemia.

(III) To assess the extent of brain lesions possibly of atherosclerotic origin in familial hypercholesterolemia patients with coronary heart disease.

(IV) To compare atherosclerotic findings in the aorta and carotid arteries among familial hypercholesterolemia and diabetes mellitus patients with coronary heart disease.
SUBJECTS AND METHODS

SUBJECTS

(I & III) During a one-year-study period (year 2000) we invited all FH-NK patients under age 50 who had children and who were registered at the outpatient lipid clinic of North Karelia Central Hospital (Joensuu, Finland) to take part in the study. We found 60 FH-NK patients, and 41 of them, aged 6 to 48, agreed to participate. Two patients were excluded because one had a foreign metal body in his eye and the other was claustrophobic.

Of the remaining patients (in the study), one 45-year-old had had an acute myocardial infarction, and one 46-year-old patient had undergone coronary bypass surgery. The others had no history of diabetes, manifestations of atherosclerosis, or neurological disorders. Statin medication was used by 28 patients; of the 11 patients not receiving statins, 9 were 15 years old or younger, and of the other 2, one could not tolerate statin treatment, and one did not use statins regularly.

The control group comprised 13 family members of the patients without the FH-NK mutation and 12 other Finnish controls chosen among acquaintances of the staff of the Helsinki Medical Imaging Center (Helsinki, Finland). The controls were aged 12 to 50 with no history of diabetes or manifestations of atherosclerosis or neurological disorders. In total, 64 subjects, of whom 39 were FH-NK patients and 25 controls, took part. See Table 1 for characteristics.

(II & IV) In 2003 we searched the patient register of the North Karelia Central Hospital (Joensuu, Finland) for FH-NK patients between 48 and 64 with CHD verified from medical records and by physical examination. We found 30 FH-NK patients, of whom eight were excluded: three also had diabetes, one had a body-mass index (BMI) of 46, two had an unreliable diagnosis of CHD, one was unable to travel, and one could not be reached. Of the remaining 22, 19 agreed to participate.

By the same register and same CHD criteria, we found 22 age- and gender-matched type 2 DM patients; 20 of these agreed to participate, but two were excluded because they used no statin medication. All FH-NK patients and 17 of the 18 type 2 DM patients had a history of acute myocardial infarction, coronary bypass or balloon angioplasty. Two type 2 DM patients and two FH-NK patients had each had a transient ischemic attack (TIA), and one FH-NK patient had had an ischemic stroke. Other patients had no history of neurological diseases.

The control group comprised 30 subjects (aged 49-63) recruited from among the acquaintances of the staff of the Helsinki Medical Imaging Center (Helsinki, Finland). None of these controls had CHD, DM, or a history of any vascular or neurological disease. One control was excluded from the final analyses when MRI revealed Moya Moya disease. In total, 66 subjects, of whom 19 were FH-NK patients, 18 type 2 DM patients and 29 controls, took part. See Table 1 for characteristics.

Studies were approved by the ethics committee of our institution, with the informed consent of all subjects.
<table>
<thead>
<tr>
<th></th>
<th>FH-NK (age 6-48)</th>
<th>Controls (age 12-50)</th>
<th>P-value</th>
<th>FH-NK (age 48-64)</th>
<th>Controls (age 49-63)</th>
<th>DM (age 51-64)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males / females, number</td>
<td>n=39</td>
<td>n=25</td>
<td></td>
<td>n=19</td>
<td>n=29</td>
<td>n=18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16/23</td>
<td>13/12</td>
<td></td>
<td>11 / 8</td>
<td>13 / 16</td>
<td>11 / 7</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>30.0 ± 13.3</td>
<td>30.6 ± 11.3</td>
<td>0.847</td>
<td>55.9 ± 4.5</td>
<td>56.0 ± 3.8</td>
<td>57.9 ± 3.7</td>
<td>0.210</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3 ± 4.1</td>
<td>23.0 ± 2.4</td>
<td>0.443</td>
<td>26.7 ± 2.8</td>
<td>25.9 ± 3.5</td>
<td>32.6 ± 4.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>121 ± 15</td>
<td>118 ± 11</td>
<td>0.402</td>
<td>137 ± 23</td>
<td>130 ± 14</td>
<td>143 ± 21</td>
<td>0.081</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>71 ± 11</td>
<td>69 ± 10</td>
<td>0.580</td>
<td>78 ± 10</td>
<td>79 ± 9</td>
<td>83 ± 13</td>
<td>0.179</td>
</tr>
<tr>
<td>Statins, users</td>
<td>28</td>
<td>0</td>
<td></td>
<td>19</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Statin usage duration, year</td>
<td>9.9 ± 3.3</td>
<td>0</td>
<td></td>
<td>15.1 ± 1.3</td>
<td>0</td>
<td>5.2 ± 2.9</td>
<td>0.000*</td>
</tr>
<tr>
<td>CHD, number of patients</td>
<td>2</td>
<td>0</td>
<td></td>
<td>19</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Duration of CHD, year</td>
<td>0</td>
<td>13.3 ± 7.6</td>
<td></td>
<td>0</td>
<td>5 ± 3.1</td>
<td>0</td>
<td>0.000*</td>
</tr>
<tr>
<td>Smokers</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

FH-NK, familial hypercholesterolemia-North Karelia; DM, Diabetes mellitus; BMI, Body mass index; CHD, Coronary heart disease.

*FH-NK and DM compared. Values are means ± SD.
MAGNETIC RESONANCE IMAGING

Methods

In the younger group (age 6-50) (I & II) MRI was performed with a 1.5T Siemens Vision imager (Erlangen, Germany).

The head (I) was studied with a head coil and an axial FLAIR sequence (TR/TE/TI/FOV/Matrix/Slice thickness = 9999 ms/105 ms/2500 ms/173 x 256 mm/190 x 256/5 mm) and a coronal T2-weighted turbo spin echo (TSE) sequence (TR/TE/FOV/Matrix/Slice thickness = 3000 ms/85 ms/173 x 230 mm/190 x 256/5 mm) were obtained. The intracranial arteries were studied with routine MRA using a 3D time of flight (TOF) sequence (TR/TE/FOV/Matrix = 35 ms/7.2 ms/150 x 200 mm/200 x 512).

The aorta (II) was studied with a phased array coil; 23 axial images of the aorta starting from the top of the aortic arch were obtained with a T1-weighted, fat-saturated, TSE sequence (TR/TE/Flip Angle/FOV/Matrix/Slice thickness = 2 RR/20 ms/90°/200 x 200 mm/128 x 256/6 mm) in free breathing. Then a breath-hold oblique sagittal TSE sequence (TR/TE/Flip Angle/FOV/Matrix/Slice Thickness = 700 ms/30 ms/180°/400 x 300 mm/130 x 256/7 mm) was taken to assess the imaging plane at the level of the right pulmonary artery for the velocity-encoded flow-measurement sequence (TR/TE/Flip Angle/FOV/Matrix/Slice Thickness/VENC = 26 ms/5 ms/30°/300 x 300/256 x 256/6 mm/150 cm/s). An electrocardiogram (ECG) gating was used with all sequences.

In the older group (age 48-64) (III & IV) MRI was performed with a 1.5T Siemens Sonata imager (Erlangen, Germany).

The head (III) was studied with a head coil and an axial FLAIR sequence (TR/TE/TI/FOV/Matrix/Slice thickness = 9000 ms/119 ms/2500 ms/201 x 230 mm/448 x 512/5 mm) and a coronal T2-weighted TSE sequence (TR/TE/FOV/Matrix/Slice thickness = 4200 ms/97 ms/173 x 230 mm/192 x 512/5 mm) were obtained. The intracranial arteries were studied with routine MRA using a 3D TOF sequence (TR/TE/FOV/Matrix = 34 ms/4.5 ms/165 x 220 mm/187 x 384).

The aorta (IV) was studied with a phased array coil. A breath-hold oblique sagittal TSE sequence (TR/TE/Flip Angle/FOV/Matrix/Slice Thickness = 47 ms/1.6 ms/65°/298 x 340 mm/148 x 256/6 mm) was taken to assess the imaging planes for cine sequence (TR/TE/Flip Angle/FOV/Matrix/Slice Thickness = 28 ms/3.2 ms/30°/223 x 340 mm/156 x 256/5 mm) at the level of the right pulmonary artery and at the level of the renal artery. ECG gating was used with all sequences.

Data analysis

(I & III) All parenchymal and vascular abnormalities of the brain were recorded and rated independently by two experienced radiologists blinded to the clinical and laboratory data. The size and number of earlier infarcts, including lacunes, and of WMHs were recorded. Only those hyperintensities seen by both radiologists in the highly T2-weighted FLAIR images (Figure 2) were taken into account. Because even healthy young people may have small (≤5 mm) incidental white matter hyperintensities (Autili et al.1994), the hyperintensities were placed in two diameter categories: ≤5 mm and >5 mm. In the older group (age 48-64) (III) WMHs were also categorized as deep or peripheral according to their location. Lumen diameter and wall contour of the arterial walls were assessed from MRA. In the younger group (age 6-50) (I), MR data for two patients could not be analyzed due to technical problems, and MRA data of two FH patients of the older group (age 48-64) (III) were unavailable.

(II & IV) In the younger group (age 6-50), the aortic images (II) were analyzed by two reviewers blinded to the clinical and laboratory data. Lumen and wall contours of the aorta starting from the first image below the left subclavia and ending at the last one above the celiac trunk were manually traced with MRtrico software (author: Chris Rorden, University of South Carolina, Columbia, SC, USA) from T1-weighted images (Figure 3A) if they had sufficient contrast to allow the visual definition of >50% of the circumference of the aortic wall. The subject was excluded from analysis if ≥50% of the slices failed to meet these criteria. Then the area of lumen and wall on each slice was assessed and their volumes calculated by multiplying the area by the sum of slice thickness
(6 mm) and slice gap (6 mm). To obtain the volume of the entire aortic wall and lumen, those volumes were added together. The values were adjusted by body surface area (BSA) = [(height (cm) x weight (kg))/3600]² of the patient. In total, the T1-TSE data of 53 subjects (30 patients and 23 controls) underwent analysis. The distance between the two measurement sites of the cine sequence was assessed from the oblique sagittal image (Figure 3B). Compliance of the ascending and descending aorta was calculated from the anatomical cine images (Figure 3C) (Mohiaddin et al. 1989), and the velocity-encoded cine images (Figure 3D) were analyzed with NIH Image software (National Institutes of Health, Bethesda, MD, USA) to obtain pulse wave velocity (PWV) (Mohiaddin et al. 1993).

In the older group (age 48-64) (IV), the aortic images were analyzed with Siemens Leonardo workstation’s Argus software (Erlangen, Germany). Compliance of the ascending, descending thoracic, and descending abdominal aorta was calculated from cine images (Mohiaddin et al. 1989). Due to poor image quality, one DM patient and one control were excluded from the analysis of compliance of the ascending aorta and descending thoracic aorta.
Figure 3. Aortic MRI

A) T1-weighted axial turbo spin echo image. The volume of lumen and vessel wall of the descending aorta (DA) between the first slice below left artery subclavia and to the last one above the celiac trunk was assessed from consecutive images.

B) Oblique sagittal turbo spin echo image from the level of the right pulmonary artery (RPA) to assess the imaging plane (white line) for the flow-encoded sequence. Distance between imaging planes at ascending and descending aorta was assessed as mean of distances along the inner and outer rim of the aorta.

C, D) Axial flow-encoded sequence from the level of the right pulmonary artery. C) From the anatomical images, the compliance in the ascending (AA) and descending (DA) aorta was measured. D) From the velocity-encoded images, pulse wave velocity was assessed between ascending (AA) and descending (DA) aorta. Different shades of black and white represent different velocities.
ULTRASOUND

All US examinations were performed by the same radiologist using a Siemens Acuson 128 XP/10 imager (Erlangen, Germany) equipped with a 8 to 12 MHz linear transducer. Each subject was examined in the supine position.

In the younger group (I & III), the intima-media thickness (IMT) of the far wall of the left common carotid artery (CCA) was measured 1 cm below the bifurcation with high-resolution B-mode (Pignoli et al. 1986), and the mean of three consecutive measurements was calculated. CCA strain (%) was calculated by the equation: maximal diameter of CCA minus minimal diameter of CCA, and this was divided by the minimal diameter of CCA multiplied by 100 (Pearson et al. 1996). The mean was calculated for five consecutive measurements.

In the older group (II & IV), IMT was measured for the far walls of both common carotid arteries 1 cm below the bifurcation and of the left femoral artery at the level of the inguinal ligament with high-resolution B-mode (Pignoli et al. 1986), and the mean of three consecutive measurements was calculated. The carotid bifurcations and the left femoral artery were examined for plaques. Doppler flow measurements were performed to assess the severity of arterial stenoses caused by the plaques. Stenosis of 70% or more of the diameter was considered significant (Grant et al. 2003). Ultrasound data for two controls were unavailable.

In the older group (III & IV), insulin, fasting glucose, and glycosylated hemoglobin were also determined.

HsCRP values >10 mg/ml were considered to reflect acute infection rather than underlying inflammation (Ridker 2003), and a 30-year-old FH patient and some 4 DM patients were thus excluded from hsCRP analysis.

In the older group (III & IV), one control was excluded from the laboratory assessments because her anti-inflammatory therapy was initiated between the MR examination and laboratory tests.

STATISTICAL METHODS

Continuous variables with normal distribution were compared with t-test or analysis of variance, as appropriate. When assumptions of normality were unfulfilled, non-parametric tests (Mann-Whitney U and Kruskall-Wallis) were used (I-IV). Categorical variables were compared with Fisher’s exact test (II). For lumen and wall volume measures, an intraclass correlation coefficient was assessed to evaluate inter-rater reliability (III).

The analyses were performed with the NCSS 2000 program (NCSS, Kaysville, UT, USA) and SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered statistically significant.

LABORATORY ANALYSES

After overnight fasting, each subject provided a venous blood sample. Serum total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, homocysteine, high-sensitivity C-reactive protein (hsCRP), and lipoprotein (a) -Lp (a)- were determined by standard methods. The low-density lipoprotein (LDL) cholesterol value was calculated by the Friedewald formula. The cholesterol-years score was calculated to take into account each participant’s lifelong cholesterol burden (Hoeg et al.1994). A DNA test (Koivisto et al. 1993) was performed on all FH-NK patients to detect the LDL receptor mutation. Blood pressure was measured twice with an automatic meter from the left arm of the patient lying supine, with the mean of two measurements used in calculations.
RESULTS

INTRACRANIAL MRI

In the younger group of subjects (age 6-50) (I), except for one aneurysm of the a. cerebri media in one FH-NK patient, no lumen diameter narrowing or irregularity of the vessel walls appeared on MRA; whereas in the older group (age 48-64) (III) narrowing of the lumen or wall irregularity appeared in three FH-NK patients. Four DM patients showed narrowing of the lumen or wall irregularity (previously unpublished data). One FH-NK patient of the older group (II), who had suffered a clinical stroke 15 years before initiation of statin treatment, showed post-infarct changes in the left middle cerebral artery area of his brain, and one DM patient showed changes of possible hemorrhagic etiology in his right putamen. Other subjects in both age-groups showed no infarcts in MRI.

The younger group of subjects (I), had 28 WMHIs in total, and all were <5 mm in diameter. These were distributed among six FH-NK patients (16%) and six controls (24%). No significant difference existed in their occurrence between patients and controls (P = 0.44). In the older group (III), too, no difference existed in the number of WMHIs between patients and controls (Table 2). At least one WMHI ≤5 mm in diameter was visible in 11 (58%) FH-NK, 15 (83%) DM patients, and in 22 (76%) controls. WMHIs >5 mm accounted for 8% (51 examples) of all WMHIs and at least one was found in 5 (26%) FH-NK, 6 (33%) DM patients, and in 6 (21%) controls. The total number of WMHIs ranged from 0 to 36 in FH, 0 to 64 in DM patients, and 0 to 85 in controls; no WMHIs appeared in 7 (37%) FH-NK, 3 (17%) DM patients, and in 6 (21%) controls. Figure 4 shows all WMHIs of both age-groups as a function of age.

Table 2. White matter hyperintensities in 48 to 64-year-old controls and FH-NK and DM patients.

<table>
<thead>
<tr>
<th></th>
<th>FH-NK (n=19)</th>
<th>CONTROLS (n=29)</th>
<th>DM (n=18)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMHIs Total, number</td>
<td>4 (0-8)</td>
<td>3 (1-13)</td>
<td>3.5 (1-16)</td>
<td>0.586</td>
</tr>
<tr>
<td>≤5 mm, number</td>
<td>2 (0-7)</td>
<td>3 (0.5-13)</td>
<td>3 (1-12)</td>
<td>0.476</td>
</tr>
<tr>
<td>&gt;5 mm, number</td>
<td>0 (0-1)</td>
<td>0 (0-0)</td>
<td>0 (0-2.25)</td>
<td>0.485</td>
</tr>
<tr>
<td>Peripheral, number</td>
<td>2 (0-6)</td>
<td>3 (0.5-12)</td>
<td>3.5 (1-12)</td>
<td>0.530</td>
</tr>
<tr>
<td>Deep, number</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0 (0-0)</td>
<td>0.831</td>
</tr>
</tbody>
</table>

FH-NK, familial hypercholesterolemia-North Karelia; DM, Diabetes mellitus; WMHI, white matter hyperintensity. Values are medians, with interquartile ranges in brackets. Previously unpublished data in bold.
AORTIC MRI

In the younger group (age 6-50) (II), no significant difference appeared for any of the aortic measures (BSA-adjusted volume of the aortic lumen and wall, PWV, or the compliance of AA and DA) between FH-NK patients and controls (Table 3 & Figure 5). The intraclass correlation coefficient was 0.979 for lumen and 0.523 for wall volume. In the older group (age 48-64) (IV), no difference in the compliance of the aorta appeared between FH-NK patients and controls, whereas DM patients had less compliant descending thoracic and abdominal aortas than did controls (Figure 5).

Table 3. Morphologic and functional aortic measures of FH-NK patients and controls aged 6-50.

<table>
<thead>
<tr>
<th></th>
<th>FH-NK</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=39</td>
<td>n=25</td>
<td></td>
</tr>
<tr>
<td>Volume of aortic wall/ BSA, mm(^3/m(^2)</td>
<td>10 563 ± 1826</td>
<td>10 200 ± 1358</td>
<td>0.427</td>
</tr>
<tr>
<td>Volume of aortic lumen/ BSA, mm(^3/m(^2)</td>
<td>29 252 ± 6865</td>
<td>29 063 ± 5641</td>
<td>0.915</td>
</tr>
<tr>
<td>Pulse wave velocity, m/s</td>
<td>3.86 ± 0.85</td>
<td>3.87± 0.82</td>
<td>0.964</td>
</tr>
</tbody>
</table>

FH-NK, familial hypercholesterolemia-North Karelia; BSA, body surface area. Values are means ± SD.
**RESULTS**

![Aortic compliance bar chart](chart.png)

**Figure 5. Aortic compliance in younger (aged 6-50) and older (48-64) subjects**
FH-NK, familial hypercholesterolemia-North Karelia; DM, Diabetes mellitus; AA, ascending aorta; TDA, descending thoracic aorta; ADA, descending abdominal aorta. Bars indicate mean, whiskers ±SD.

**ULTRASOUND**

In the younger group, age 6 to 48 (I & II), the mean IMT of the far wall of the left CCA was significantly greater in the FH-NK patients than in the controls (Figure 6), and no carotid plaques appeared. The strain of the CCA showed no significant (P=0.502) difference between patients (15.18 ± 6.92 %) and controls (12.95 ± 3.33 %).

In the older group, age 48 to 64 (III & IV), IMTs were significantly greater in the FH-NK and DM patients than in the controls in all vessels studied (Figure 6). No significant differences in IMTs existed between FH-NK and DM patients. One or both carotid bifurcations contained plaque in 11 FH-NK patients and 9 DM patients; femoral artery plaque appeared in 7 FH-NK, 3 DM patients, and in one control. Five FH patients showed stenosis, occlusion or both in their carotid arteries but none of the DM patients or controls showed significant stenosis or occlusion.

**LABORATORY ANALYSES**

In both age groups (I-IV), LDL cholesterol and cholesterol-years score was higher in FH-NK patients than in controls or DM patients (Table 4).

In the younger group, age 6 to 50 (I & II), Lp(a) level of FH-NK patients was higher than that of the controls, but no difference existed in their homocysteine and hsCRP levels (previously unpublished data).

In the older group, age 48 to 64 (III & IV), DM patients had higher triglycerides, fasting glucose, insulin, and percentage of glycylated hemoglobin than did FH-NK patients and controls (Table 4).
Figure 6. IMT in younger (aged 6-50) and older (48-64) subjects
FH-NK, familial hypercholesterolemia-North Karelia; DM, Diabetes mellitus; IMT, intima-media thickness; LCCA, left common carotid artery; RCCA, right common carotid artery; LFA, left femoral artery. Bars indicate mean, whiskers ±SD.
<table>
<thead>
<tr>
<th></th>
<th>FH-NK</th>
<th>Controls</th>
<th>P-value</th>
<th>FH-NK</th>
<th>Controls</th>
<th>DM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(age 6-48)</td>
<td>(age 12-50)</td>
<td>(age 6-50)</td>
<td>(age 48-64)</td>
<td>(age 49-63)</td>
<td>(age 51-64)</td>
<td>(age 48-64)</td>
</tr>
<tr>
<td></td>
<td>n=39</td>
<td>n=25</td>
<td></td>
<td>n=19</td>
<td>n=29</td>
<td>n=18</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.24 ± 1.70</td>
<td>3.89 ± 1.03</td>
<td>0.001</td>
<td>6.0 ± 1.0</td>
<td>5.6 ± 0.8</td>
<td>4.7 ± 0.9</td>
<td>0.000 (FH=CTRL; FH&gt;DM; CTRL&gt;DM)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.68 ± 1.41</td>
<td>2.27 ± 0.82</td>
<td>0.000</td>
<td>4.1 ± 0.9</td>
<td>3.3 ± 0.7</td>
<td>2.5 ± 0.7</td>
<td>0.000 (FH&gt;CTRL; FH&gt;DM; CTRL&gt;DM)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.13 ± 0.41</td>
<td>1.19 ± 0.30</td>
<td>0.282</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>1.2 ± 0.2</td>
<td>0.000 (FH&lt;CTRL; FH=DM; CTRL&gt;DM)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.94 ± 0.48</td>
<td>0.93 ± 0.32</td>
<td>0.736</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.6</td>
<td>2.1 ± 0.9</td>
<td>0.001 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
</tr>
<tr>
<td>Cholesterol-years, mmol-y/L</td>
<td>243 ± 122</td>
<td>137 ± 74</td>
<td>0.001</td>
<td>516 ± 96</td>
<td>313 ± 58</td>
<td>368 ± 89</td>
<td>0.000 (FH&gt;CTRL; FH&gt;DM; CTRL=DM)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2 ± 0.6</td>
<td>5.1 ± 0.4</td>
<td>8.7 ± 3</td>
<td>0.000 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.5 ± 3.7</td>
<td>6 ± 4.5</td>
<td>31.1 ± 31.4</td>
<td>0.000 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylated hemoglobin, %</td>
<td>5.7 ± 0.2</td>
<td>5.9 ± 0.3</td>
<td>8.2 ± 1.6</td>
<td>0.000 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoprotein (a), g/L</td>
<td>0.23 ± 0.19</td>
<td>0.12 ± 0.07</td>
<td>0.000</td>
<td>0.20 ± 0.21</td>
<td>0.21 ± 0.20</td>
<td>0.22 ± 0.21</td>
<td>0.359</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>10.1 ± 4.1</td>
<td>12.7 ± 7.8</td>
<td>0.152</td>
<td>12.2 ± 2.5</td>
<td>13.1 ± 4.8</td>
<td>15 ± 3</td>
<td>0.047 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>0.79 ± 0.93</td>
<td>0.71 ± 0.95</td>
<td>0.660</td>
<td>2.22 ± 2.34</td>
<td>1.35 ± 1.92</td>
<td>3.07 ± 2.45</td>
<td>0.001 (FH=CTRL; FH&lt;DM; CTRL&lt;DM)</td>
</tr>
</tbody>
</table>

FH-NK, familial hypercholesterolemia-North Karelia; DM, Diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HsCRP, high-sensitivity C-reactive protein. Values are means ± SD. Previously unpublished data in bold
DISCUSSION

INTRACRANIAL MRI

None of our younger FH patients showed stenoses in their intracranial arteries assessed with MRA (I). Asymptomatic stenoses occurred in 16% of our older FH patients with CHD (III) and in 22% of the DM patients with CHD. These are in line with the results of Uehara et al. (1996), who found asymptomatic stenoses in 11 (16%) of their 40- to 78-year-old patients with CHD.

Neither age-group had any signs of silent or symptomatic infarcts (apart from the signs of the old symptomatic infarct in one FH patient and the changes of possible hemorrhagic etiology in one DM patient of the older group) on brain MR images. In contrast, Giele et al. (2004) found silent infarcts in 51 (17%) of their patients, mean age 58, with manifest cardiovascular disease of any etiology. The severity of the cardiovascular disease of our older FH and DM patients and of their patients was quite similar. In a Framingham cohort study, 12.3% of 1302 subjects free of clinically evident neurological disease showed silent infarcts in MRI (DeCarli et al. 2005). They were, however, a bit older (60.6 ± 9.4) than our FH and DM patients, but were healthier as to cardiovascular diseases.

The role of diabetes as a risk factor for silent brain lesions of vascular origin is controversial. Two studies from the 1990’s showed DM to be an independent risk factor for silent brain lesions (Kobayashi et al. 1997) or showed them to be associated (Ylikoski et al. 1995). A newer study showed no difference in the occurrence of silent lesions between patients with DM, impaired glucose tolerance, or normal glucose tolerance (Saitoh et al. 2002). No mention of statin treatment was made in any of these studies, and among them, MR technique, nomenclature, and classification of the lesions differ. Our findings are in line with the newer findings, although we suspect that our patients may have had more severe atherosclerotic disease, because they all had verified CHD. DM patients’ risk for stroke is elevated (Tuomilehto et al. 1996, Folsom et al. 1999, Almdal et al. 2004), whereas the risk for statin-treated FH patients is not (Huxley et al. 2003, Hutter et al. 2004). A few studies done before the statin era show elevated stroke risk in FH patients (Bansal et al. 1986, Kaste and Koivisto 1988). Those studies were lacking a control group, and 77% of patients in that 1988 study had cardiovascular or cerebrovascular disease. One Japanese study (Mabuchi et al. 1986) showed no stroke risk elevation when their FH patients were compared to controls. However, statins reduce the number of strokes (Law 2003), which in addition to lowering cholesterol levels, is thought to result from their pleiotropic effects on blood vessels (Vaughan et al. 2001, Miida et al. 2007). We may only speculate on the effects of statins on silent lesions, since placebo-controlled studies are no longer ethically justifiable. We must also keep in mind the possibility of the healthy survivor effect, meaning that only the fittest have survived, and those with more serious lesions are deceased.

White matter changes seen as hyperintensities on T2-weighted MRI are thought to be of ischemic origin (Pantoni and Garcia 1997, Pantoni 2002). Among our younger FH patients (I), only six showed WMHIs, none of which was >5 mm in diameter. Furthermore, no difference existed between patients and controls. In our opinion, this was surprising, since patients’ cholesterol burden expressed as cholesterol-years score and amount of overall atherosclerosis expressed as intima-media thickness were both higher than those of the controls (Table 4, Figure 6). Both carotid atherosclerosis (IMT) (Manolio et al. 1999) and hypercholesterolemia (Breteler et al. 1994) are thought to be risk factors for WMHIs. However, our findings are in line with those of a recent study in which no brain abnormalities, including WMHIs, appeared in 3T MRI of five statin-treated homozygous FH patients and their controls, all aged 12 to 36 (Schmitz et al. 2007).

In the older group (III) no difference existed in total number of WMHIs or number of WMHIs when compared according to location (deep or peripheral) or size (≤5 mm or >5 mm) between FH, DM, and control groups (Table 2). These results are in line with the silent infarct findings’ frequency, which was also lower in our patients than in the studies of deCarli et al. (2005) and Giele et al. (2004). We think that this is a result of improved risk-factor treatment, statins in particular. Probably the number of the WMHIs of our controls from both studies did not differ from those of Salonen et al. (1997), who found WMHIs <5 mm in diameter in 45% of their
healthy subjects aged 30 to 50 and in 100% of their neurologically healthy subjects aged 55 to 86. Comparison between these studies is, however, difficult because they used T2-weighted SE images which normally overestimate WMHIs by showing also the perivascular spaces as bright, unlike with the newer FLAIR sequence we used. The Salonen group stated, however, that about half their lesions were undetectable on PD-weighted images, which better match the FLAIR sequence that is nowadays normally used in the diagnostics of WMHIs (Barkhof and Scheltens 2002). Further, in a study done with 67 healthy 4- to 50-year-old subjects, WMHIs <5 mm in diameter appeared in T2-weighted images in 27% (Autti et al. 1994). We therefore speculate that at least the WMHIs <5 mm in diameter may not be the result of atherosclerosis, high cholesterol burden, or diabetes, proposed risk factors for WMHIs; they may be merely a phenomenon related to aging of the brain. The increase in WMHIs along with aging was also evident in our controls (Figure 4), which is in line with the fact that age is considered the most common risk factor for WMHIs (Erkinjuntti et al. 1994, Longstreth et al. 1996, Jeerkathil et al. 2004, Basile et al. 2006). The punctate small WMHIs have also been considered non-progressive in a 6-year follow-up study and thus have been considered benign (Schmidt et al. 2003). This parallels the finding that not all of the small hyperintensities have pathological correlates (Fazekas et. al 1993) and that WMHIs have many other etiologies, possibly complementary to the ischemic one, including apoptosis, blood-brain barrier alterations, chronic edema, and genetic factors (Pantoni 2002). In the follow-up study, the larger (confluent) lesions progressed (Schmidt et al. 2003), and these may be the ones that progress to ischemic stroke, the intermediate surrogates of which maybe WMHIs (Inzitari 2003).

AORTIC MRI

In neither age-group (II & IV) did any difference in elastic properties emerge between FH patients and controls, despite patients’ higher cholesterol-years score indicating higher cholesterol burden, and their higher intima-media thickness indicating more severe overall atherosclerosis. This agrees with the findings of Toikka et al. (1999) showing no difference in aortic elasticity between 10 heterozygous FH patients and their controls, all 34 years old on average. Some MRI studies, however, have revealed CHD patients’ aortas to be stiffer (Mohiaddin et al. 1989; Forbat et al. 1998), whereas other MRI-derived (Kupari et al. 1994) and US-derived (Lehman et al. 1992) evidence exists that in the early phases of atherosclerotic disease, accumulation of lipids in the absence of calcification and large collagen deposits may actually make the wall more compliant. One MRI study showed improvement in the aortic elasticity of hypercholesterolemic patients with and without CHD after 12 months of Fluvastatin therapy (Forbat et al. 1998). In 24 CHD patients, mean age 66, cholesterol reduction with statins improved aortic elasticity as measured with MRI after 3 months, and this improvement was sustained in a 12-month follow-up (Lee et al. 2008).

Diabetes affects the arteries by two different mechanisms. First, it promotes atherosclerotic changes, and second, it leads to arteriosclerosis. These two overlapping and sometimes erroneously interchangeably discussed mechanisms make feasible the lower compliance of our diabetes patients’ descending and abdominal aortas (IV). In one recent MRI study, aortic elasticity was lower for 14 type 2 DM patients, mean age 55 and without diabetic complications, than it was in their 16 controls (van der Meer et al. 2007). Furthermore, our diabetes patients were obese, and obesity has been shown to reduce aortic elasticity assessed with MRI in otherwise healthy adults aged 20 to 40 (Dianas et al. 2003).

In the younger group (II) we also calculated the aortic wall and lumen volume derived from wall- and lumen-area measurements; no difference existed between FH patients and controls. In contrast, subclinical aortic atherosclerosis expressed as greater aortic wall area has appeared in 62 (38%) of women and 64 (41%) of men free of clinically evident CHD (Jaffer et al. 2002), patients not tested for CHD and much older than our FH patients and controls (aged 36 to 78 compared to 6 to 48 for our FH patients). One explanation for the lack of lesions despite the higher carotid IMT of our patients may be the effect of modern risk factor treatment, statins in particular. The aortic and carotid wall...
areas regressed 11% in 21 asymptomatic hypercholesterolemic patients during 12 months of simvastatin therapy (Corti et al. 2002). In another MRI study, on 19 asymptomatic hypercholesterolemic patients, 18% regression of wall area of the thoracic aorta in 12 months of atorvastatin therapy appeared at a 20-mg daily dosage (Yonemura et al. 2005); this study also showed that a 5-mg daily dose was unable to reduce wall area. US has shown regression of atherosclerotic lesions with higher doses of statins to be greater than regression at lower doses (Smilde et al. 2001). Furthermore, while the degrees of atherosclerosis among vascular beds show an association (Sternby 1969), the possibility that different diseases may produce different degrees of lesions in different vascular areas must be kept in mind.

LIMITATIONS

The strength of our studies (I-IV) was that the same DNA-verified NK mutation in our FH patients resulted in similar phenotypes. Because cardiovascular medication appears to modify atherosclerotic lesions in favor of less progression, we therefore consider having the records of the cardiovascular medication of our older patients a strength; many other imaging studies had no medical records available.

First, our rather small sample size is a source of bias, but we consider that the number of subjects was sufficient for the assumptions made. Second, the gender distribution of the controls in the older group could have biased the results. Third, because males normally develop a more severe atherosclerosis, we therefore assume that no bias occurred toward underestimation of its severity in FH and DM (III & IV). Fourth, due to poor image quality of 11 subjects’ aortic wall volume (derived from the aortic wall area), their measurements were excluded; this may be a source of bias. However, aortic wall area measurements with MRI are reproducible (Chan et al. 2001, Li et al. 2004), and also our inter-rater correlation coefficients (0.979 for lumen and 0.523 for wall volume) were quite good, although the delineation of the borders of the aortic wall was more difficult than was delineation of the border of the lumen.

CONCLUSIONS

We found no difference between FH-NK patients and controls in possibly atherosclerotic manifestations assessed with MRI, neither in the aorta nor in the brain, whereas the patients showed more advanced atherosclerosis in their carotid and femoral US and a higher cholesterol burden expressed by cholesterol-years score. Improved risk factor treatment, statins in particular, is one explanation for the lack of lesions, together with the assumption that although atherosclerotic lesions between different vascular territories correlate, differences may still appear in the extent and location of these lesions in different diseases. Furthermore, some evidence exists that statins may even cause regression of calcifications (Callister et al. 1998, Williams et al. 1998), but no information exists as to their effects on severely stenosed arteries. Small (<5 mm in diameter) WMHs seem to be a finding related to normal aging, while the larger ones may be the ones related to CVDs and may be intermediate surrogates of stroke and may therefore be the ones requiring attention in further studies. The main limitation of the study was the relatively small number of patients. Moreover, image quality in aortic imaging (although constantly improving) is not yet optimal and may fall short in the task of demonstrating small differences.
ACKNOWLEDGEMENTS

This study was carried out at the Department of Radiology of the University of Helsinki, Finland and at Helsinki Medical Imaging Center, Helsinki, Finland. I wish to acknowledge Professor Emeritus Carl-Gustaf Standertskjöld-Nordenstam, Professor Leena Kivisaari, Docent Jaakko Kinnunen, Docent Juhani Ahovuo, Docent Kaarina Partanen, Docent Pekka Tervahartiala and CMO Jyrki Putkonen for providing me with the facilities for performing the MRI and US studies.

This study (and I) was supervised by Docent Taina Autti and Docent Kirsu Lauerman. I am indebted to Kirsi for giving me the opportunity to work quite independently yet not leaving me totally alone in the dark. I felt that Taina always believed in me and encouraged me giving me the last spark to complete this work. Her capability in finding crucial articles is to be acknowledged.

I wish to express my sincere thanks to:
My excellent reviewers Professor Petri Kovanen and Docent Pekka Niemi who gave me valuable advice and whose critical comments enhanced this work greatly.

My co-authors: Laura Hyttinen for her efforts in searching for the FH and DM patients, Pekka Keto for guiding me in the aortic imaging, Raili Raininko for her excellent comments on brain MRI and her efforts in rating the brain images even during her holidays, Hannu Turtola for providing the appropriate sample of FH patients, Alpo Vuorio for his never-ending ideas and enthusiasm and expertise in FH.

My colleagues at Sputnik: Miia Holmström for enjoyable company, Reetta Kivisaari for showing that projects finally do end sometime, Sari Kivistö for good laughs even in the most stressful situations, Minna Mannerkoski for enjoyable coffee and chats and for sharing the moments of despair during the work.

My friends for being my guinea pigs in the preliminary MRI tests and for their company and optimism towards my work. Special thanks goes for Tero for saving me from computer-related distress and Jartsa for his artistic touch in Figures and layout of this work.

To our senior radiographers: Pentti Pölönen, Timo Päivärinta and Aki Syrjälä who taught me how to use the MRI imagers.

To my excellent teacher in written and spoken English and author-editor of this thesis, Carol Norris, without whom this work would have looked and sounded more Finglish than English.

All the FH and DM patients and controls without whose kind cooperation this thesis could not have been completed.

My deepest thanks go to my family and especially to my parents Arja and Matti for providing me support and never failing to believe in me even if I sometimes did.

My most sincere thanks goes to Maija for enduring me in the last turbulent times of “polishing” this work.

This work was supported by grants from the Pehr Oscar Klingendahl foundation, the Finnish Cultural Foundation, the Finnish Medical Society Duodecim, the Radiological Society of Finland, the Schering Research Foundation and the Helsinki University Central Hospital research funds.
REFERENCES


REFERENCES


REFERENCES


REFERENCES


REFERENCES


