Clinical Investigation and Reports

Premature Atherosclerosis Associated With Monogenic Insulin Resistance

Robert A. Hegele, MD, FRCPC

Background—The common insulin resistance syndrome, with obesity, dyslipidemia, hyperglycemia, and hypertension, is associated with increased risk of atherosclerosis. Early atherosclerosis in rare monogenic forms of insulin resistance, however, has not been extensively documented. Cardiovascular end points were thus evaluated in subjects with Dunnigan-type familial partial lipodystrophy (FPLD) due to mutations at LMNA codon 482.

Methods and Results—FPLD subjects ≥35 years old were stratified by genotype for either the LMNA R482Q or R482W mutation. Twenty-three subjects were heterozygous mutation carriers, and 17 were R482/R482 homozygous family control subjects. All LMNA mutation carriers had FPLD with insulin resistance. In addition, LMNA mutation carriers had significantly more type 2 diabetes, hypertension, and dyslipidemia than normal family control subjects. Eight LMNA mutation carriers had coronary heart disease (CHD), compared with 1 normal control subject (OR 5.9, 95% CI 1.2 to 30.2). Six LMNA mutation carriers had CHD end points before age 55 years, and 4 of these, all women, had been hospitalized for CABG surgery between the ages of 35 and 54 years.

Conclusions—Rare LMNA mutations that underlie FPLD with insulin resistance and hyperinsulinemia are also associated with early CHD, notably in women. This suggests that abnormalities of the nuclear envelope can result in a phenotype that recapitulates most of the important attributes of the common insulin resistance syndrome, including accelerated cardiovascular disease. FPLD thus appears to be an appropriate human monogenic model for the common insulin resistance syndrome. (Circulation. 2001;103:2225-2229.)

Key Words: diabetes ■ genetics ■ obesity ■ risk factors

Impairment of the ability of insulin to stimulate glucose uptake and inadequate compensation for altered insulin sensitivity underlie type 2 diabetes mellitus (DM) and atherosclerosis. Moreover, the compensatory hyperinsulinemia necessary to maintain glucose tolerance in insulin-resistant individuals is frequently associated with a cluster of metabolic abnormalities, which is sometimes referred to as the “insulin resistance syndrome” or “metabolic syndrome X.” The insulin resistance syndrome is seen in individuals with central obesity and is characterized by glucose intolerance, dyslipidemia, and high blood pressure. These manifestations increase the risk of coronary heart disease (CHD). Defining the mechanisms that underlie insulin resistance might help to develop new strategies to treat or prevent insulin resistance and its complications.

An approach to understanding a complex phenotype, such as insulin resistance, is to study an extreme monogenic form. For instance, understanding of the role of LDL cholesterol in CHD was advanced by studying patients with familial hypercholesterolemia. This led to the discovery of receptor-mediated endocytosis via the LDL receptor in cholesterol metabolism, providing a rationale for the development of the statin drugs, which were subsequently shown to reduce LDL cholesterol and CHD in the general population. By analogy, investigation of a monogenic form of insulin resistance might lead to improved understanding and treatment of the common form.

Dunnigan-type familial partial lipodystrophy (FPLD) is a rare autosomal dominant form of insulin resistance. FPLD patients are born with normal fat distribution but lose fat from their extremities and gluteal region after the onset of puberty. This results in prominent, well-defined musculature and phlebectasia in these areas, with central accumulation of fat, because facial, truncal, and visceral adipose depots are not dystrophic. The presence of insulin resistance in FPLD, with hyperinsulinemia and often type 2 DM, dyslipidemia, and hypertension, recapitulates some of the features of the common insulin resistance syndrome. There is evidence of vascular disease, including CHD, stroke, and peripheral vascular disease in subjects with FPLD, but not specifically early CHD. Demonstrating a link between FPLD and early CHD has become especially important since the discovery that mutant LMNA, which encodes nuclear lamins A/C, underlies FPLD. Furthermore, the LMNA codon 482 missense mutation in FPLD was strongly associated with hyperinsulinemia, dyslipidemia (high triglycerides and low HDL cho-
lesterol), hypertension, and type 2 DM.10 Documenting early CHD in patients with this monogenic form of insulin resistance would provide further rationale to understand how nuclear envelope defects can produce such complications. This study reports premature CHD in Canadian subjects with FPLD due to mutations in LMNA.

Methods

Study Subjects

Subjects from 3 Canadian FPLD kindreds were examined.8–11 The FPLD mutation was LMNA R482Q in the first kindred, from which 49 subjects (22 mutation carriers) had been evaluated.8,10 The FPLD mutation was R482Q in the second kindred, from which 4 subjects, all mutation carriers, were evaluated.9,11 The FPLD mutation was LMNA R482W in the third kindred, from which 6 subjects, all mutation carriers, had been evaluated.11 For the present study of the relationship between LMNA mutations and CHD risk, only subjects ≥35 years old were included. In the largest kindred,8,10 mutation carriers were matched with normal family control subjects without LMNA mutations. This left a total sample size of 40 subjects: 23 were FPLD subjects with mutant LMNA, of whom 18 had the R482Q mutation, and 17 were matched family control subjects with a normal LMNA gene. Height, weight, and body mass index (kg/m²) were determined. A diagnosis of type 2 DM (by pre-1997 criteria) and/or the use of oral hypoglycemic agents and/or insulin was recorded. The use of antihypertensive medications was recorded. Dyslipidemia was defined as untreated plasma triglyceride above the 95th and/or HDL cholesterol below the 5th percentile for age and sex. A history of ever having smoked cigarettes was recorded. A history of CHD, defined as a medical diagnosis and/or treatment for angina and/or myocardial infarction, and/or coronary angioplasty, and/or CABG surgery, was recorded. The study was approved by the University of Western Ontario Institutional Review Board.

Biochemical and Genetic Determinations

Assays of fasting plasma concentrations of lipids, lipoproteins (including direct measurement of LDL cholesterol after ultracentrifugation) and apolipoproteins (apo), glucose, HbA1C, insulin, and C-peptide were performed by established procedures.10 DNA was extracted as described.10 Screening for LMNA mutations, which had been characterized by sequencing of genomic DNA from probands, was performed by amplification with the oligonucleotide primers 5′-GCAAGATAACCCAAAGGCC-3′ and 5′-ACACCTGGGTTCCTTGGTC-3′. The 1069-bp amplification product was digested with MspI, and the digestion products were electrophoresed in 2% agarose gels. Digestion of the amplification product from the wild-type LMNA allele, R482, produced 2 variant fragments of size 480 and 69 bp, in addition to invariant fragments (381, 81, and 59 bp). Digestion of the product from either mutant LMNA allele, Q482 or W482, produced a single fragment of size 549 bp, in addition to the invariant fragments.8,10

Statistical Analyses

Clinical and biochemical traits for mutation carriers were compared with matched family control subjects. Differences between genotypes in qualitative traits were compared by Fisher’s exact test using SAS version 6.12 (SAS Institute). Because of the small numbers of subjects and the nonnormal distribution of the biochemical variables, nonparametric analysis was carried out with the Kruskal-Wallis χ² approximation test of significance of the Wilcoxon rank sums. Differences between genotypes in log-transformed quantitative traits were also compared by Student’s t tests from the general linear models procedure in SAS, with Bonferroni adjustment. A value of P<0.05 was taken as the nominal level of significance for all pairwise comparisons.

Results

Clinical and Biochemical Attributes of Study Sample

Qualitative traits and unadjusted means±SEM for quantitative traits are shown in Table 1. The significant between-genotype differences in quantitative traits from nonparametric analysis were each also significant at a value of P<0.05 with Bonferroni analysis, so only the unadjusted probability values from the nonparametric analysis are shown in Table 1. The 2 genotype classes were well matched by sex and age. Body mass index was not different, confirming that fat distribution and not total fat mass was altered in FPLD.10 Subjects with mutant LMNA had significantly more type 2 DM, treated hypertension, and dyslipidemia defined by age- and sex-specific population cut points (Table 1). Fasting plasma insulin, glucose, and C-peptide were significantly higher in subjects with mutant LMNA. Fasting triglycerides were significantly higher, and fasting HDL cholesterol and apoA-I were significantly lower, in subjects with mutant LMNA. Fasting LDL cholesterol was significantly lower in subjects with mutant LMNA, but apoB was not significantly different.

CHD End Points in Study Subjects

All CHD end points were significantly more frequent in FPLD subjects with mutant LMNA (P=0.033, Table 1). Eight subjects with mutant LMNA had CHD end points: all had hypertension and dyslipidemia, and all but 1 had type 2 DM...
The main finding of this study is that a monogenic form of insulin resistance—namely, FPLD due to mutant LMNA—was associated with an increased risk of early CHD, with significant odds ratios compared with family control subjects without LMNA mutations and without insulin resistance. The association with CHD seen in the FPLD subjects with hyperinsulinemia is consistent with the epidemiological association of hyperinsulinemia with CHD. The findings also suggest that FPLD is an appropriate monogenic model for common insulin resistance, because FPLD subjects have both the characteristic metabolic disturbances and early CHD.

The magnitude of the increased risk of early CHD in subjects with mutant LMNA is further highlighted by examination of a hard CHD end point, such as hospitalization for CABG. Among female LMNA mutation carriers, 1 in 3.75 (4 in 14) had been hospitalized between the ages of 35 and 54 years for CABG. In contrast, hospitalization data from the general Canadian population in 1996 indicated that 1 woman in 7350 (603 in 4429258) had been hospitalized between the ages of 35 and 54 years for CABG. Among female LMNA mutation carriers with insulin resistance had a rate of hospitalization of the ages of 35 and 54 years for CABG that was orders of magnitude higher than that in the general Canadian population. This increase is at least of the same order of magnitude as the increased risk of premature atherosclerosis due to LDLR gene mutations in familial hypercholesterolemia. Taken together with the earlier observations by Garg,7 these findings clearly indicate the increased risk of atherosclerosis associated with FPLD.

The most likely basis of the increased risk of early CHD is the associated metabolic abnormalities in FPLD subjects. The FPLD subjects with mutant LMNA and early CHD each had hyperinsulinemia, together with diabetes, hypertension, and dyslipidemia, all of which increase CHD risk. Although these results could simply reflect the increased CHD seen in typical diabetes, the monogenic nature of FPLD and the cluster of marked metabolic perturbations are characteristic differentiating factors for this disease. In addition, the LMNA mutations might have influenced atherosclerosis progression independently of the intermediate proatherogenic phenotypes of insulin resistance assessed here. For example, there might have been unmeasured proatherogenic consequences of mutant LMNA, such as increased serum plasminogen activator inhibitor-1, which is also seen in subjects with common

### Table 2. Clinical Attributes of LMNA Codon 482 Mutation Carriers With CHD

<table>
<thead>
<tr>
<th>Present Age, y</th>
<th>Sex</th>
<th>LMNA Genotype</th>
<th>Clinical End Points</th>
<th>Early CHD</th>
<th>Type 2 DM</th>
<th>Hypertension</th>
<th>Dyslipidemia</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>F</td>
<td>0482/R482</td>
<td>Stable angina at 39 y</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myocardial infarction at 44 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4-Vessel CABG at 44 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>F</td>
<td>0482/R482</td>
<td>Stable angina at 58 y</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intermittent claudication at 43 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stable angina at 46 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>F</td>
<td>0482/R482</td>
<td>Stable angina at 66 y</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myocardial infarction at 46 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-Vessel CABG at 53 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>M</td>
<td>W482/R482</td>
<td>Myocardial infarction at 46 y</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>44</td>
<td>F</td>
<td>W482/R482</td>
<td>Unstable angina at 38 y</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-Vessel CABG at 38 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>F</td>
<td>W482/R482</td>
<td>Unstable angina at 34 y</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myocardial infarction at 34 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-Vessel CABG at 34 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Table 2). The 1 subject without mutant LMNA and CHD (subject FPLD-1 VIII:4 in Reference 13) had stable angina with onset at age 59 years. This subject was not a smoker but had hypertension and dyslipidemia. The odds ratios of CHD in carriers of mutant LMNA compared with normal family control subjects was 5.9 (95% CI 1.2 to 30.2). When early onset of CHD end points under age 55 years was considered, 6 FPLD subjects with mutant LMNA were affected, compared with no control subjects without the LMNA mutation (P = 0.026, Table 1).

The most likely basis of the increased risk of early CHD is the associated metabolic abnormalities in FPLD subjects. The FPLD subjects with mutant LMNA and early CHD each had hyperinsulinemia, together with diabetes, hypertension, and dyslipidemia, all of which increase CHD risk. Although these results could simply reflect the increased CHD seen in typical diabetes, the monogenic nature of FPLD and the cluster of marked metabolic perturbations are characteristic differentiating factors for this disease. In addition, the LMNA mutations might have influenced atherosclerosis progression independently of the intermediate proatherogenic phenotypes of insulin resistance assessed here. For example, there might have been unmeasured proatherogenic consequences of mutant LMNA, such as increased serum plasminogen activator inhibitor-1, which is also seen in subjects with common
insulin resistance. There might also have been cellular consequences of the LMNA mutations within the arterial wall, such as altered differentiation of smooth muscle cells. The most parsimonious explanation of these observations, however, would be the clustering of diabetes, hypertension, and dyslipidemia in the insulin-resistant FPLD subjects with mutant LMNA.

The evidence for accelerated atherosclerosis in other forms of lipodystrophy is minimal, probably because of the small numbers of affected subjects. For example, Berardinelli-Seip congenital lipodystrophy (OMIM 269700), an autosomal recessive disease whose gene is on chromosome 9q34, is even rarer than FPLD. Necropsy studies have provided some evidence for premature CHD and early death in a small number of patients with Berardinelli-Seip disease. Studies of patients with other human lipodystrophies have presented virtually no information on their possible association with atherosclerosis. The presence of early atherosclerosis in other genetic forms of insulin resistance, such as that due to mutations in the insulin receptor gene, has also not been documented. Finally, the relevance to insulin resistance and CHD of single nucleotide polymorphisms in genes whose products are involved in insulin metabolism, such as insulin receptor substrate-1, is under evaluation.

The evidence for atherosclerosis in murine models of lipodystrophy is also minimal. For example, one of the best-studied murine models of lipodystrophy, the fld mouse, has elevated plasma insulin and triglycerides, fatty liver, and neuropathy, and a 2-fold increase in aortic arch atherosclerotic lesions. Vascular pathology has not been systematically evaluated in these models, however. Thus, it would be important to document atherosclerosis in these newer murine models of lipodystrophy with insulin resistance. If accelerated atherosclerosis can be demonstrated, these mice may become even more useful models for human insulin resistance.

The 2 LMNA codon 482 mutations studied here, and a third, namely LMNA R482L, indicate that mutation in this residue specifically affects adipose tissue. Other LMNA missense mutations affect skeletal and cardiac myocytes in the autosomal dominant forms of Emery-Dreifuss muscular dystrophy (EMD2) and dilated cardiomyopathy (CMD1A). These conditions are not associated with adipose abnormalities, insulin resistance, diabetes, or atherosclerosis. Furthermore, no FPLD subject had any evidence of skeletal myopathy, cardiomyopathy, or cardiac conduction abnormalities. Also, serum creatine kinase levels were all within 1.5 times the upper limit of normal for each FPLD subject with mutant LMNA (data not shown). Thus, LMNA mutations can have independent effects on the heart, including conduction system anomalies, cardiomyopathy, and CHD. The LMNA mutations in EMD2 and CMD1A probably have direct effects on cardiac myocytes, whereas the CHD in FPLD is related to the insulin resistance. A common single nucleotide polymorphism in LMNA has recently been associated with small differences in obesity-related phenotypes.

The cardinal plasma lipoprotein abnormality in LMNA mutation carriers with FPLD was elevated triglycerides and depressed HDL cholesterol and apoA-I. The lipid disturbances preceded the glucose abnormalities in LMNA mutation carriers. Interestingly, the directly measured plasma LDL cholesterol and apoB were not increased in FPLD subjects with mutant LMNA, compared with normal subjects. Thus, high triglyceride and low HDL cholesterol, but not elevated LDL cholesterol, are seen in FPLD subjects with CHD. This observation appears to be especially relevant in light of the recent demonstration that treatment of the high-triglyceride/low–HDL cholesterol profile, with no change in LDL cholesterol, was associated with reduction in CHD.

Thus, mutations in LMNA associated with FPLD and insulin resistance are also associated with early CHD. The relevance of the mechanism(s) underlying the metabolic derangements of FPLD to common obesity and insulin resistance remains to be established. It is not altogether clear that the LMNA gene product is strictly a nuclear envelope protein, because some work suggests that lamins A and C can occur as components of interchromatin granule clusters. This suggests additional mechanisms by which the lamins may influence adipose tissue function and insulin metabolism. In any event, FPLD appears to be an appropriate human model for the study of common insulin resistance, whose main metabolic disturbances and association with atherosclerosis it recapitulates. Monogenic disorders of adipocyte biology and insulin resistance, such as FPLD, might help to elucidate new metabolic pathways and mechanisms for the common forms of obesity, diabetes, and atherosclerosis. The findings also suggest that disordered structure/function of the nuclear envelope can contribute to insulin resistance with atherosclerosis.

Acknowledgments
This work was supported by grants from MRC Canada (MT13430), the Canadian Diabetes Association (in honor of Reta Maude Gilbert), and the Canadian Genetic Diseases Network. Dr Hegele is a Career Investigator of the Heart and Stroke Foundation of Ontario and holds a Canada Research Chair in Human Genetics. Drs T.J. McDonald, I. Hramiak, and M.C. McSween were kind to have referred their patients; Henian Cao provided technical help; and Carol Anderson helped with manuscript preparation.

References
Premature Atherosclerosis Associated With Monogenic Insulin Resistance

Robert A. Hegele

Circulation. 2001;103:2225-2229
doi: 10.1161/01.CIR.103.18.2225

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/103/18/2225

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/