Genetic and Phenotypic Heterogeneity in Patients with Mandibuloacral Dysplasia-Associated Lipodystrophy

VINAYA SIMHA, ANIL K. AGARWAL, ELIF ARIOGLU ORAL, JEAN-PIERRE FRYNS, AND ABHIMANYU GARG

Division of Nutrition and Metabolic Diseases (V.S., A.K.A., A.G.), Center for Human Nutrition, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390; Division of Endocrinology and Metabolism (E.A.O.), Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109; and Center for Human Genetics (J.-P.F.), University Hospital of Leuven, 3000–Leuven, Belgium

Mandibuloacral dysplasia (MAD) is a phenotypically heterogeneous, rare autosomal recessive disorder characterized by mandibular and clavicular hypoplasia, acoelosteolysis, delayed closure of cranial sutures, joint contractures, and mottled cutaneous pigmentation. Patients with MAD develop two patterns of lipodystrophy: type A pattern, with loss of subcutaneous fat from the extremities and normal or slight excess in the neck and truncal regions; and type B pattern, with a more generalized loss of subcutaneous fat involving the face, trunk, and extremities. Recently, affected patients from five consanguineous Italian pedigrees with partial lipodystrophy (type A) were reported to have a homozygous R527H mutation in LMNA (lamin A/C) gene. We carried out mutational analysis of LMNA in affected patients from six pedigrees. Affected patients from two pedigrees with type A lipodystrophy had the homozygous R527H mutation in LMNA. The other four affected subjects who had type B lipodystrophy did not have any mutation in the exons and splice site junctions of LMNA; RNA extracted from lymphoblasts of two of these patients also revealed normal sequence. In these four subjects, sequencing of other known genes implicated in lipodystrophies, i.e. AGPAT2, Seipin, and PPARG also revealed no substantial alterations. We conclude that MAD is a genetically and phenotypically heterogeneous disorder. Besides LMNA gene, other as yet unmapped loci could be linked to MAD. (J Clin Endocrinol Metab 88: 2821–2824, 2003)

Mandibuloacral Dysplasia (MAD; Mendelian Inheritance in Man no. 248370) is a rare autosomal recessive disorder characterized by postnatal growth retardation, craniofacial anomalies such as mandibular hypoplasia and birdlike facies, skeletal anomalies such as progressive osteolysis of the terminal phalanges and clavicles, and skin changes such as mottled hyperpigmentation and atrophy (1). Lipodystrophy and metabolic complications associated with insulin resistance have also been reported in those affected with MAD (2, 3). We recently characterized two patterns of lipodystrophy in patients with MAD (4). Some patients have partial lipodystrophy (type A pattern) characterized by marked loss of subcutaneous fat from the extremities with normal or slight excess in the neck and truncal regions, whereas others have a more generalized loss of subcutaneous fat (type B pattern) involving the face, trunk, and extremities (4). Type A pattern of lipodystrophy in patients with MAD is similar to that seen in patients with familial partial lipodystrophy (FPL), Dunnigan variety, an autosomal dominant disorder caused by missense mutations in the lamin A/C (LMNA) gene located on chromosome 1q21–22 (5–11). Recently, a homozygous missense mutation (R527H) in LMNA gene was reported in nine patients with MAD belonging to five consanguinean Italian pedigrees (12). Reported clinical data suggest that all of these patients had Type A pattern of lipodystrophy. It is unclear whether phenotypic heterogeneity in patients with MAD is associated with genetic heterogeneity. We, therefore, screened our affected patients with MAD from six pedigrees with both types of lipodystrophies for mutations in the LMNA gene and investigated genotype-phenotype relationships.

Patients and Methods

The protocol was approved by the appropriate Institutional Review Boards, and all subjects gave informed consent. The clinical features of our patients with MAD have been previously reported (3, 4, 13–16) and are summarized in Table 1. All affected patients were of European ancestry. Pedigrees MAD100, 200, 300, and 400 were recruited from the United States, whereas MAD500 and 600 were from Belgium. MAD100 was of Mexican origin, whereas MAD300 was of Italian origin (Fig. 1).

Blood samples

Blood was collected after a 12-h overnight fast in pedigrees MAD100, 300, and 400 for DNA extraction and for analysis of serum lipoproteins, insulin, leptin, glucose, and a chemistry profile. Lymphoblastoid cell lines or DNA samples from pedigrees MAD200, 500, and 600 were sent to University of Texas Southwestern Medical Center for analysis.

Biochemical analyses

Plasma glucose was measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin and leptin levels were determined by RIA using commercial kits (Linco Research, Inc., St. Charles, MO). Fasting serum samples were analyzed for cholesterol and triglycerides by an enzymatic method using kits (Roche Molecular Biochemicals, Indianapolis, IN). Serum high-density lipoprotein cholesterol was measured enzymatically after lipoproteins containing apolipoprotein B had been precipitated with phosphotungstic acid. Serum chemistry was measured as a part of the systematic multichannel analysis by a commercial laboratory (SmithKline Beecham Clinical Laboratories, Dallas, TX).

Abbreviations: AGPAT2, 1-Acylglycerol-3-phosphate O-acyltransferase 2; FPL, familial partial lipodystrophy; LMNA, lamin A/C; MAD, mandibuloacral dysplasia; PPARG, peroxisome proliferator-activated receptor-γ; SNP, single nucleotide polymorphism.
The reverse transcriptase reaction was performed for 50 min at 50 °C, followed by inactivation at 85 °C for 5 min. Residual RNA was removed by treating the reaction with 2 U of RNase H for 20 min at 37 °C. The cDNA was used for PCR using cDNA-specific primer pairs to amplify LMNA exons 1 to 10. PCR conditions were the same as described earlier (18).

Mutational analysis of additional genes implicated in lipodystrophies

Affected individuals who did not show any mutation in the LMNA gene were further analyzed for mutations in peroxisome proliferator-activated receptor-γ (PPARG), 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2) and Seipin genes as described previously (18, 19).

Results

LMNA mutations were detected in the affected subjects from only two of the six pedigrees, MAD100 and MAD300. In both of these pedigrees, affected subjects had homozygous 1580G>A nucleotide transition causing substitution of arginine at 527 position with histidine (R527H). The mutation segregated in an autosomal recessive manner in these pedigrees. The parents were heterozygous for the mutation, and no heterozygote had the affected phenotype. None of the affected subjects from other pedigrees had any substantial alterations in LMNA gene on direct sequencing. Lymphoblastoid preparations were available for two of these four patients (MAD200.3 and MAD400.5), and the transcripts from these patients revealed normal sequence. Furthermore, sequencing of the exons and splice sites of LMNA revealed no substantial alterations in these patients.

We determined haplotypes associated with the 1580G>A mutation using intragenic SNPs extending 2.5 kb around the site of mutation. The affected subject from our Italian pedigree (MAD300.4) was heterozygous for the SNPs, IVS6 A and IVS8 G, indicating that the mutation may have arisen on two different chromosomes (Table 2). On the other hand, the affected subjects from the MAD100 pedigree of Mexican origin were homozygous for all the intragenic SNPs but showed a different haplotype than that reported earlier in the Italian pedigrees (12).

Table 3 shows the results of metabolic variables related to
insulin resistance in five subjects who were heterozygous for the R527H mutation. One of the subjects (MAD100.1) had type 2 diabetes mellitus, and four of the five had mild fasting hyperinsulinemia. Fasting serum triglycerides were borderline high or high in three of the four adult subjects according to the Adult Treatment Panel III guidelines (20), whereas in the 14-yr-old subject (MAD300.3), serum triglycerides were approximately at the 75th percentile of normal age, sex, and race-matched controls (21). Normal serum triglyceride levels occurred on more than one chromosome and may not conform to the founder effect.

It appears that the disease-causing mutation may have occurred in five subjects who were heterozygous for the R527H mutation. One of the subjects (MAD100.1) had type 2 diabetes mellitus, and four of the five had mild fasting hyperinsulinemia. Fasting serum triglycerides were borderline high or high in three of the four adult subjects according to the Adult Treatment Panel III guidelines (20), whereas in the 14-yr-old subject (MAD300.3), serum triglycerides were approximately at the 75th percentile of normal age, sex, and race-matched controls (21). Normal serum triglyceride levels were seen in MAD300.2. Serum high-density lipoprotein cholesterol was low in one of the heterozygotes (MAD100.1; Ref. 20).

### Discussion

Mandibuloacral dysplasia is a rare, autosomal recessive disorder with protein manifestations involving mainly the skeleton, skin, and adipose tissue. Lipodystrophy and the associated metabolic abnormalities due to insulin resistance are an important feature of this disorder. We had previously described two distinct patterns of lipodystrophy, based on our observations, as well as review of previously reported cases (4). The present study suggests that this phenotypic heterogeneity is also accompanied by genetic heterogeneity. Mutational analysis of LMNA gene in affected patients from six MAD pedigrees revealed a homozygous R527H mutation in three patients belonging to the pedigrees MAD100 and MAD300. Besides the typical features of MAD, all three patients had marked loss of sc adipose tissue from the extremities, whereas truncal fat was normal or increased (Type A lipodystrophy). A similar pattern of lipodystrophy involving loss of sc fat from the extremities and fat accumulation in the trunk, face, submental region, and occiput was described by Novelli et al. (12) in their patients. We did not, however, find this mutation or any other substantial alterations in LMNA gene in four other patients who also had the typical features of MAD, but had generalized loss of sc fat from the extremities, face, and trunk (Type B lipodystrophy). No alternative splicing defects were observed in two of these patients (MAD200.3 and 400.5) whose lymphoblastoid cell lines were available. These patients also showed no mutations in other known lipodystrophy genes. It therefore appears that patients with MAD and type A pattern of lipodystrophy may have the LMNA mutation, whereas patients with MAD and Type B pattern of lipodystrophy may have mutations in other gene(s).

Recently, mutations in AGPAT2 and Seipin genes were reported in patients with autosomal recessive, congenital generalized lipodystrophy linked to 9q34 and 11q13, respectively (18, 22). We also reported a heterozygous missense mutation in PPARG gene in a patient with FPL (19). Subsequently, Hegele et al. (23) confirmed our finding and reported an additional PPARG missense mutation in FPL. Therefore, we sequenced these three genes implicated in patients with lipodystrophies in the four remaining patients who did not have LMNA mutation but found no mutations.

Haplotype analysis in affected patients from MAD100 and 300 revealed different haplotypes from those reported earlier (12). Because the pedigree MAD300 is also of Italian origin, it appears that the disease-causing mutation may have occurred on more than one chromosome and may not conform to the founder effect.

The unaffected members in the MAD pedigrees who were heterozygous for the R527H mutation revealed no phenotypic abnormalities suggestive of MAD or lipodystrophy. However, mild metabolic abnormalities related to insulin resistance such as hyperinsulinemia and hypertriglyceridemia were noted. It has previously been reported that parents with heterozygous R527H LMNA mutation did not display any of the bone, cutaneous, or fat abnormalities seen in their homozygous offspring (12). However, metabolic abnormal-

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**TABLE 2.** Intragenic SNPs surrounding the 1580G>A mutation in the affected patients from our series

<table>
<thead>
<tr>
<th>Patients</th>
<th>Parental origin</th>
<th>LMNA variants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>861 T/C</td>
</tr>
<tr>
<td>MAD 100.3</td>
<td>Paternal</td>
<td>T</td>
</tr>
<tr>
<td>MAD 300.4</td>
<td>Maternal</td>
<td>T</td>
</tr>
<tr>
<td>Novelli et al. (12)</td>
<td>Both</td>
<td>C</td>
</tr>
</tbody>
</table>

**TABLE 3.** Demographic and metabolic features of subjects heterozygous for LMNA R527H mutation

<table>
<thead>
<tr>
<th>Variable</th>
<th>100.1</th>
<th>100.2</th>
<th>300.1</th>
<th>300.2</th>
<th>300.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43</td>
<td>40</td>
<td>48</td>
<td>45</td>
<td>14</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>ND</td>
<td>ND</td>
<td>29.9</td>
<td>25.8</td>
<td>30.4</td>
</tr>
<tr>
<td>Plasma glucose (mmol/liter)</td>
<td>8.2</td>
<td>4.4</td>
<td>6.3</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>Plasma insulin (pmol/liter)</td>
<td>95</td>
<td>92</td>
<td>116</td>
<td>110</td>
<td>85</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/liter)</td>
<td>2.89</td>
<td>4.30</td>
<td>1.88</td>
<td>0.54</td>
<td>1.22</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/liter)</td>
<td>5.27</td>
<td>6.73</td>
<td>6.55</td>
<td>6.08</td>
<td>5.38</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mmol/liter)</td>
<td>0.83</td>
<td>1.72</td>
<td>1.12</td>
<td>2.37</td>
<td>1.43</td>
</tr>
</tbody>
</table>

M, Male; F, female; ND, not determined; BMI, body mass index; HDL, high density lipoprotein.
ities related to insulin resistance were not assessed in these subjects. Interestingly, immunofluorescence analysis of cultured skin fibroblasts showed an abnormal lamin A/C distribution in about 10% of their nuclei. Two other homozygous LMNA mutations (H222Y and R298C) are known to cause autosomal recessive forms of Emery-Dreifuss Muscular Dystrophy (24) and Charcot-Marie-Tooth disorder type 2 (25), respectively. Heterozygous relatives of affected subjects with both of these syndromes were also reportedly normal on clinical examination, although information on their glucose tolerance, serum insulin, and lipoprotein levels have not been reported. It would be interesting to systematically examine additional subjects with heterozygous R527H LMNA mutation for evidence of subtle metabolic abnormalities.

Recently, on the basis of two novel heterozygous missense mutations, R28W and R62G, in exon 1 of LMNA gene in two families with FPL, Dunnigan variety, cardiomyopathy and myopathy, we proposed the occurrence of a multisystem dystrophy syndrome due to LMNA mutations (11). We had also speculated about whether multisystem dystrophy syndrome involves other mesenchymally derived tissue such as bone and cartilage. Recent demonstration of LMNA mutations in patients with MAD (12) and Charcot-Marie-Tooth disorder type 2 (25) suggests that besides adipose tissue, cardiac and skeletal muscle dystrophies, skeletal, cutaneous, and axonal dystrophies are also part of the multisystem dystrophy syndrome.

In summary, patients with MAD and partial lipodystrophy (type A) were found to have homozygous R527H mutation in LMNA gene, whereas patients with MAD and generalized lipodystrophy (type B) did not have any alterations in LMNA gene. Thus, MAD is a genetically heterogenous disorder, and besides LMNA gene, another locus may exist for patients with type B lipodystrophy pattern.

Acknowledgments

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Address all correspondence and requests for reprints to: Abhimanyu Garg, Division of Nutrition and Metabolic Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9052. E-mail: Abhimanyu.garg@utsouthwestern.edu.

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