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Report

Mandibuloacral Dysplasia Is Caused by a Mutation in *LMNA*-Encoding Lamin A/C

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Mandibuloacral dysplasia (MAD) is a rare autosomal recessive disorder, characterized by postnatal growth retardation, craniofacial anomalies, skeletal malformations, and mottled cutaneous pigmentation. The *LMNA* gene encoding two nuclear envelope proteins (lamins A and C [lamin A/C]) maps to chromosome 1q21 and has been associated with five distinct pathologies, including Dunnigan-type familial partial lipodystrophy, a condition that is characterized by subcutaneous fat loss and is invariably associated with insulin resistance and diabetes. Since patients with MAD frequently have partial lipodystrophy and insulin resistance, we hypothesized that the disease may be caused by mutations in the *LMNA* gene. We analyzed five consanguineous Italian families and demonstrated linkage of MAD to chromosome 1q21, by use of homozygosity mapping. We then sequenced the *LMNA* gene and identified a homozygous missense mutation (R527H) that was shared by all affected patients. Patient skin fibroblasts showed nuclei that presented abnormal lamin A/C distribution and a dysmorphic envelope, thus demonstrating the pathogenic effect of the R527H *LMNA* mutation.

Mandibuloacral dysplasia (MAD [MIM 248370]) is a rare autosomal recessive disorder characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of the cranial suture, joint contractures, and types A and B patterns of lipodystrophy (Cavallazzi et al. 1960; Young et al. 1971; Freidenberg et al. 1992; Simha and Garg 2002). We identified five consanguineous pedigrees that included nine individuals affected by MAD. All families originated from a sparsely populated area in central Italy, suggesting the existence of a founder effect (Tenconi et al. 1986; Tudisco et al. 2000). Patients were born at full term after an uneventful pregnancy. Development was considered unremarkable during the first 4–5 years of life. At this time, growth retardation, clavicular dysplasia, delayed closure of the cranial suture, and fingertip rounding were noted. Reexamination of patients revealed subtotal alopecia (only in male patients), micrognathia, premature loss of teeth, and dental overcrowding. Hepatomegaly, acanthosis nigricans in the axillae and groin, together with mottled areas of hyperpigmentation over the lower trunk, upper thighs, and distal lower limbs were found. Patients also presented acro-osteolysis, stiff joints, and hypoplastic clavicles (fig. 1). In all patients, dual-energy x-ray absorbtiometry (DEXA) revealed loss of subcutaneous fat in the extremities and identified fat accumulation in the trunk, face, submental region, and occiput (buffalo hump). All patients displayed normal fasting plasma-glucose concentrations; however, oral glucose-tolerance tests revealed marked basal and post-load hyperinsulinemia, indicating insulin resistance. These metabolic characteristics and similar partial lipodystrophy, are the main features of the Dunnigan-type familial partial lipodystrophy (FPLD), which has been shown to be caused by mutations in the lamin A/C gene (*LMNA* [MIM 150330])

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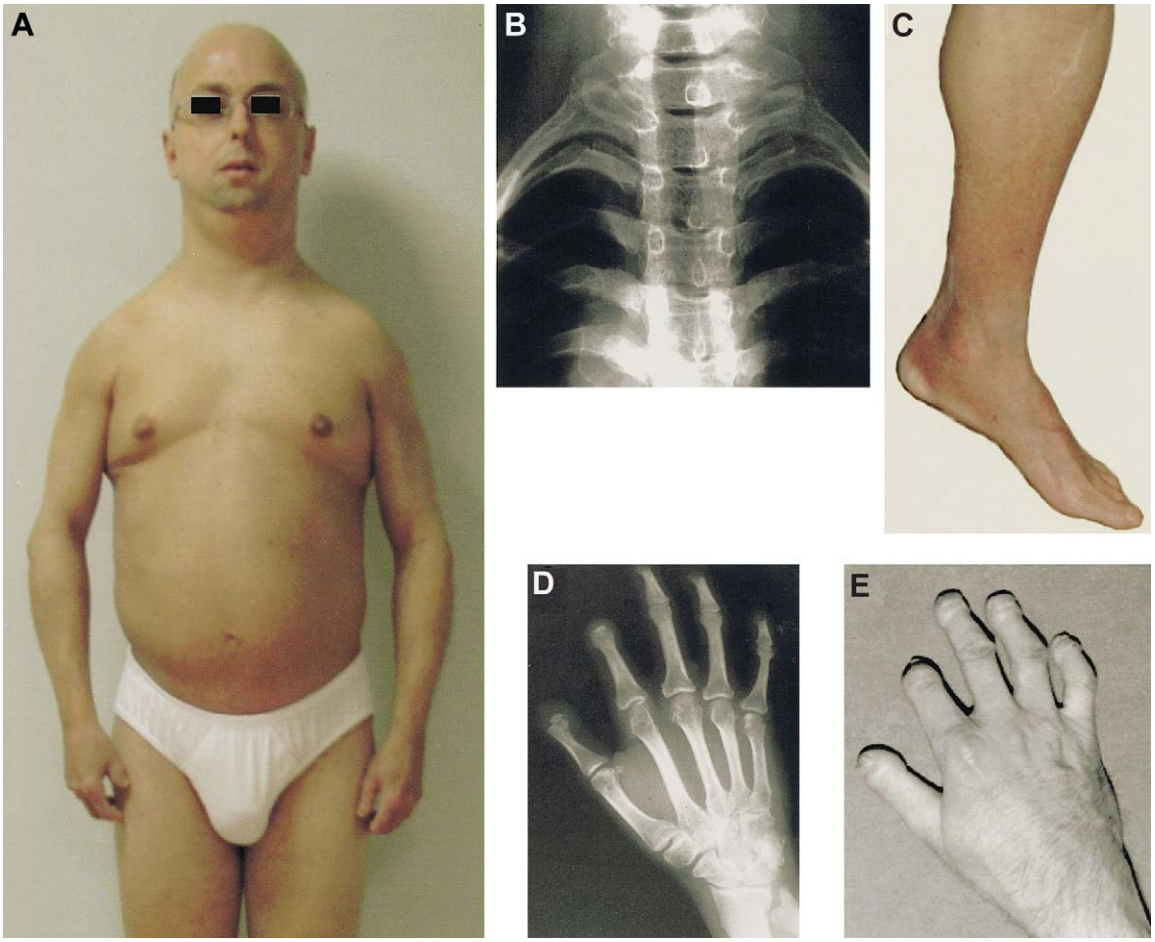

Figure 1 Clinical spectrum of MAD. *A,* Alopecia, micrognathia, and hanging shoulders (patient VII−1, family 1). *B,* X-ray films showing hypoplastic clavicles (patient 4). *C,* Lipodystrophy, hyperpigmentation, and acroosteolysis in lower limbs (patient VII-1). *D*, X-ray films showing marked osteolysis of the distal phalanges (patient VII−1). *E,* short distal phalanges, "joint stick deformities" of the fingers with areas of skin atrophy over the hand (patient VII−1).

(Shackleton et al. 2000). In addition, recessive mutations of this gene cause more-severe phenotypes (Raffaele di Barletta et al. 2000; De Sandre-Giovannoli et al. 2002). For these reasons, we thought it worthwhile to screen for the *LMNA* gene in our patients with MAD. We therefore focused our attention on 1q21 chromosome region where *LMNA* gene is located. This gene encodes lamins A and C (lamin A/C) by alternative splicing. These proteins are major components of the nuclear lamina, a fibrous network underlying the inner surface of the nuclear envelope (Lin and Worman 1993). *LMNA* mutations cause five distinct diseases: autosomal dominant and recessive Emery-Dreifuss muscular dystrophy (EDMD) (Bonne et al. 1999; Raffaele di Barletta et al. 2000), limb-girdle muscular dystrophy type 1B (Muchir et al. 2000), dilated cardiomyopathy type 1A (Fatkin et al. 1999; Bécane et al. 2000), autosomal recessive Charcot-Marie-Tooth disease type 2 (De Sandre-Giovannoli et al. 2002) and Dunnigan-type FPLD (Cao and Hegele 2000; Shackleton et al. 2000; Speckman et al. 2000). The last condition is characterized by loss of subcutaneous fat from the extremities and excess fat in the face, neck, and trunk (Dunnigan et al. 1974). This pattern of lipodystrophy is very similar to the one observed in the patients we examined and in affected individuals reported elsewhere (Simha and Garg 2002). Moreover, patients with MAD, as well as those with FPLD, display insulin resistance and associated metabolic derangements, including diabetes and dyslipidemia (Simha and Garg 2002).

To test linkage of MAD to the *LMNA* locus, we analyzed D1S1664, D1S305, D1S2715, D1S303, D1S2721, and D1S2624 microsatellite markers. Alleles were amplified using fluorescently labeled primers and were separated on an ABI 310 genetic analyzer (Applied Biosystems). Genotypes were determined using the GENOTYPER 2.0 software (Applied Biosystems), and haplo-

types were derived by minimizing recombination events. Multipoint analysis was implemented using the MAPMAKER/HOMOZ software (Kruglyak et al. 1995).

Genotyping results are reported in figure 2*A*, which shows that markers D1S2715 and D1S2721 are homozygous and identical by descent in all affected individuals. Accordingly, multipoint analysis identified a maximum LOD score of 9.05 in the interval defined by D1S2715 and D1S2721. Control DNA from healthy unrelated individuals of Italian origin was used to determine allele frequencies of six markers: two microsatellite markers (D1S2715 and D1S2721) and four SNPs localized in *LMNA* (three silent exonic SNPs: A287 [861T/C], D446 [1338T/C], and H566 [1698C/T] [Vigouroux et al. 2000]; and one intronic SNP [IVS6 +16G/A]) [unpublished data, P. Richard and G.B.]) for which families with MAD shared a common haplotype (fig. 2*A*). This disease-bearing haplotype was not detected in healthy control individuals ($n = 96$). Taken together, these results strongly suggest that all Italian families with MAD originate from a common founder.

We subsequently sequenced *LMNA* exons in patients with MAD and in their consanguineous relatives. In all nine patients, we identified the same homozygous transition, 1580G→A, which changes arginine 527 to histidine (R527H) (fig. 2*B*). Because the mutation removes

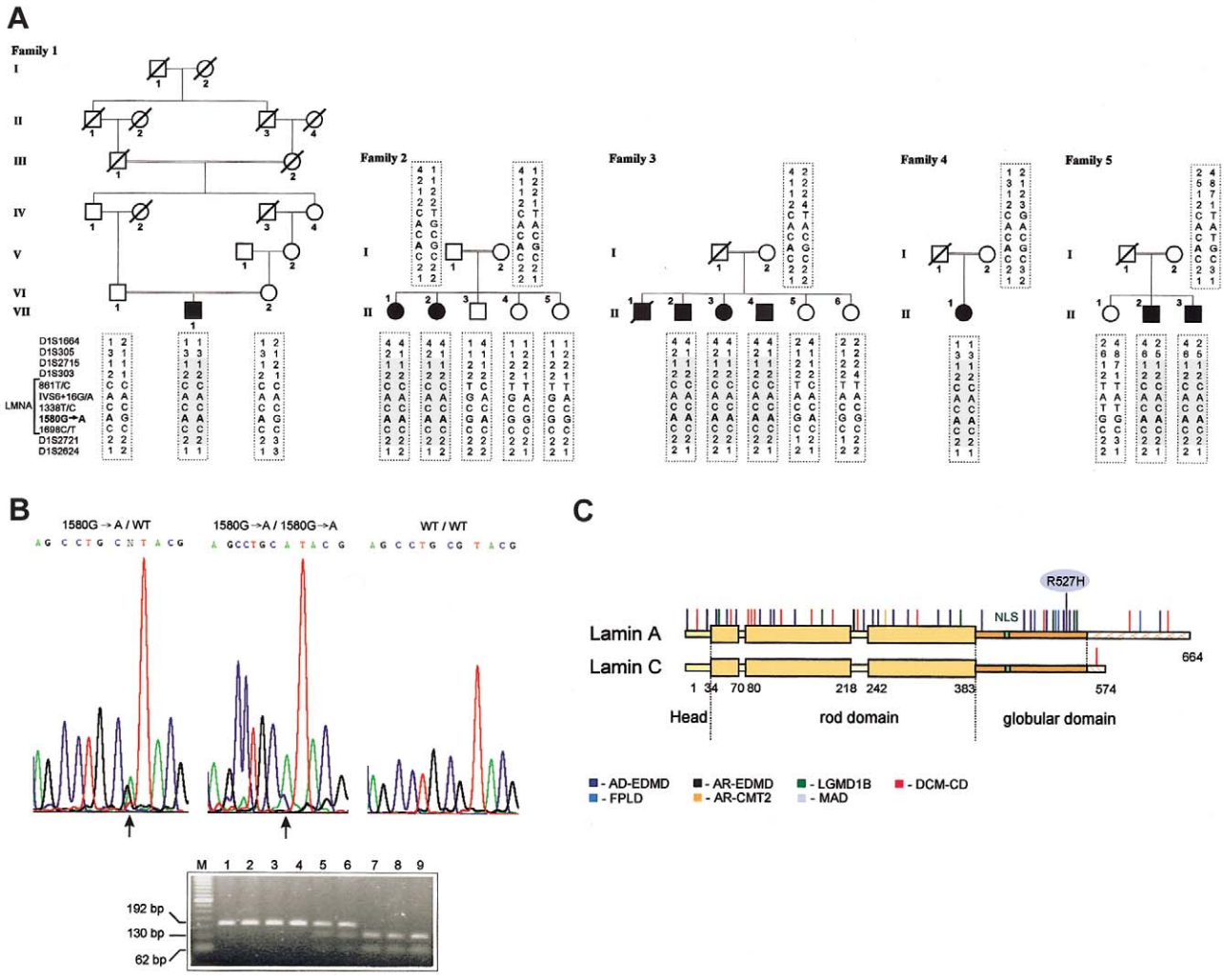

Figure 2 A mutation in *LMNA* that underlies MAD. *A*, Haplotype analysis in four families with MAD, using nine polymorphic markers on 1q21.2-q21.3. The shared disease-associated haplotype is boxed. *B*, Sequence analysis showing a homozygous G→A substitution at cDNA position 1580. Upon digestion with *Rsa*I, amplicons of exon 9, which normally produce two fragments (130 bp and 62 bp) (*lanes 7, 8,* and *9*, control subjects), yield only one in affected individuals (*lanes 1, 2, 3,* and *4*, F1-VII-1, F2-II-2, F3-II-2, and F4-II-1, respectively) and three in heterozygous carriers (*lanes 5* and *6*, F2-I-1 and F2-I-2, respectively). *C*, Schematic representation of mutations located within lamin A/C proteins and their phenotypic effects. AD = autosomal dominant; AR = autosomal recessive; CD = conduction disease; DCM = dilated cardiomyopathy.

an *Rsa*I site, we were able to screen for the presence of mutation in all family members (fig. 2*B*) and all samples from unrelated control individuals from the same ethnic background, for confirmation. The mutation was present in a heterozygous state in healthy parents and was absent in 300 unaffected individuals.

To confirm the pathogenic effect of R527H mutation involved in MAD, we analyzed cultured skin fibroblasts from patient VII-1 of family 1 and his unaffected mother (VI-2), who were found to carry the R527H lamin A/C mutation in homozygous and heterozygous states, respectively. Cells were fixed and permeabilized (Yang et al. 1997), DNA was stained with 4′, 6-diamino-2-phenylindole, and lamin A/C was labeled using a lamin A/C–specific antibody (mAb 4A7 [kindly provided by G. Morris]). In control fibroblasts, nuclei are round or ovoid, and lamin A/C is localized at the nuclear envelope (fig. 3*A* and 3*B*). In heterozygous R527H fibroblasts of the mother, most nuclei have normal morphology, but ~10% of the nuclei show a honeycomb immunofluorescence pattern for lamin A/C distribution (fig. 3*C* and 3*D*). It is noteworthy that parents do not display any of the bone, cutaneous, or fat abnormalities of the affected son, and their appearance is completely normal; furthermore, although metabolic investigations were not performed, results of a thorough physical examination were unremarkable. In homozygous R527H fibroblasts of the patient, 10% of the nuclei show lobulation of their nuclear envelope (fig. 3*E* and 3*F*), with a honeycomb labeling of lamin A/C. Elsewhere, similar abnormalities of skin-fibroblast nuclei have been reported in patients with FPLD who harbor R482Q or R482W mutations (Vigouroux et al. 2001) and in other patients with autosomal dominant EDMD (authors' unpublished data). Earlier observations (unpublished data, A.M. and G.B.) demonstrated that the R527H *LMNA* mutation identified in patients with MAD perturbs the correct assembly of nuclear lamina, but this mechanical instability is not specific to this disease. These results expand our knowledge of laminopathies and provide evidence of a wide range of effects of this protein on human development and differentiation. The R527 amino acid is located in the C-terminal domain common to lamin A and lamin C, which has an immunoglobulin-like three-dimensional structure (Dhe-Paganon et al. 2002; Krimm et al. 2002). R527 is localized at the external surface of the domain, and thus R527H substitution would disrupt the surface structure of the protein, altering binding fundamental sites. Interestingly, Lloyd et al. (2002) have identified a binding site of lamin A for the adipocyte-differentiation factor sterol-response element–binding protein 1 (SREBP1) between residues 227 and 487. This argues for the possibility that fat loss observed in FPLD and MAD may be caused by reduced binding of the adipocyte-differentiation factor SREBP1 to lamin A. In addition, it is interesting to note that we described heterozygous R527P mutation in patients with autosomal dominant EDMD, a condition that specifically affects striated muscle tissue (Bonne et al. 1999), and that, in some of these patients, partial lipodystrophy was observed (van der Kooi et al., in press). In contrast, patients with MAD who bear the R527H mutation displayed neither symptoms nor signs of muscle involvement: pa-

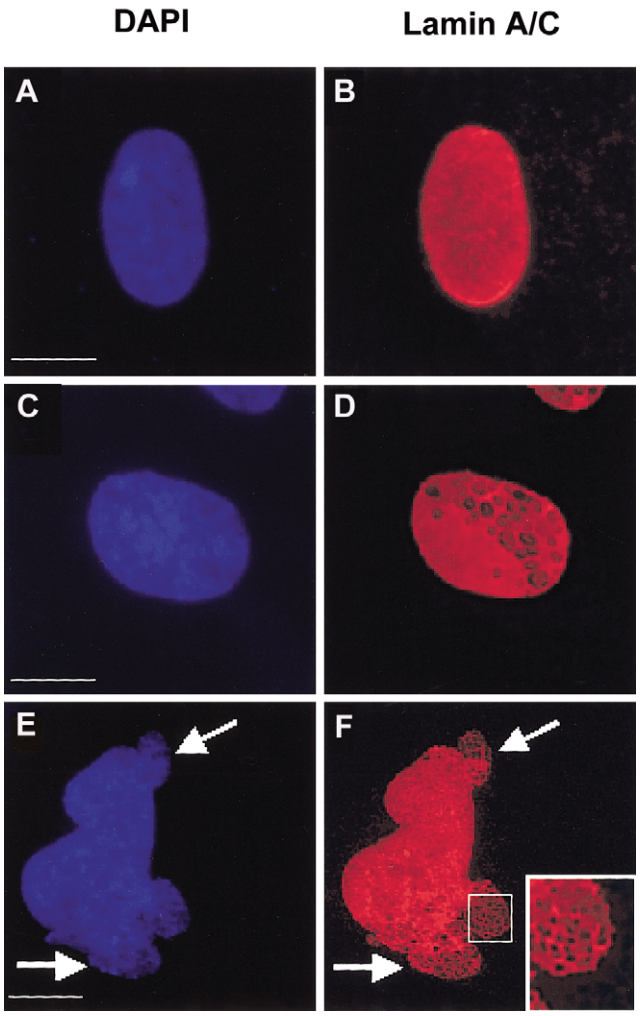

Figure 3 Immunofluorescence analysis of wild-type, heterozygous and homozygous R527H cultured skin fibroblasts. *A* and *B*, Control fibroblasts, with most nuclei having a normal ovoid shape. *C* and *D*, Heterozygous R527H fibroblasts, with most nuclei having a normal ovoid shape. Lamin A/C labeling shows a honeycomb pattern of distribution. *E* and *F*, Homozygous R527H fibroblasts, showing abnormal nucleus morphology, with some lobules of the nuclear envelope (*arrows*). Lamin A/C was located all around the nuclear envelope, in which lamin A/C labeling shows a honeycomb pattern of distribution. Immunofluorescence microscopy was performed by use of an Axiophot microscope (Carl Zeiss). The pictures were processed with Adobe Photoshop, version 5.5. Bar = 5 μm. Labeling procedures are described in the text.

tients showed no detectable muscle wasting or weakness, and their creatine kinase levels were in the normal range. In addition, patients did not report any cardiac symptoms, and results of electrocardiograms were normal. The molecular structures of both arginine and histidine are basic, and therefore such change could explain the absence of phenotypic effects in individuals carrying heterozygous R527H. Conversely, proline is hydrophobic and is known to introduce structural change, and thus a R527P mutation is expected to produce an abnormal phenotype. Such phenotypic variability was previously reported for H222P and H222Y mutations, which were associated with autosomal dominant and autosomal recessive EDMD, respectively (Bonne et al. 2000; Raffaele di Barletta et al. 2000). Similar instances of allele specificity that produce different developmental syndromes have been reported for disorders related to fibroblast growth receptor factor and in the *p53* homologue *p63* (van Bokhoven and McKeon 2002). In the case of lamin A/C, allelic disorders may be caused by the differential disruption of domains involved in tissue-specific interactions. Indeed, several transcription factors involved in tissue differentiation are associated with the nuclear matrix, and it has been demonstrated that mutations that interfere with this process can severely affect transcription-factor function (Stenoien et al. 2000). On this basis, we speculate that the primary defect that characterizes lamina disruption may have downstream effects on chromatin structure or gene expression. The effects of the latter may be responsible for the tissue-specific pathology in human laminopathies.

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Electronic-Database Information

Accession numbers and URLs for data herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MAD [MIM 248370] and *LMNA* [MIM 150330])

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