Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy

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Patients with Dunnigan-type familial partial lipodystrophy (FPLD) are born with normal fat distribution, but after puberty experience regional and progressive adipocyte degeneration, often associated with profound insulin resistance and diabetes. Recently, the FPLD gene was mapped to chromosome 1q21–22, which harbours the LMNA gene encoding nuclear lamins A and C. Mutations in LMNA were shown to underlie autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD-AD), which is characterized by regional and progressive skeletal muscle wasting and cardiac effects. We hypothesized that the analogy between the regional muscle wasting in EDMD-AD and the regional adipocyte degeneration in FPLD, in addition to its chromosomal localization, made LMNA a good candidate gene for FPLD. DNA sequencing of LMNA in five Canadian FPLD probands indicated that each had a novel missense mutation, R482Q, which co-segregated with the FPLD phenotype and was absent from 2000 normal alleles \( (P = 1.1 \times 10^{-13}) \). This is the first report of a mutation underlying a degenerative disorder of adipose tissue and suggests that LMNA mutations could underlie other diseases characterized by tissue type- and anatomical site-specific cellular degeneration.

INTRODUCTION

Dunnigan-type familial partial lipodystrophy (FPLD; OMIM 151660) is a rare autosomal dominant disease, which is part of a heterogeneous group of disorders characterized by complete or partial absence of adipose tissue (1,2). Patients with FPLD are born with normal fat distribution, but then lose subcutaneous fat from their extremities, trunk and gluteal region after the onset of puberty (1–3). Also, excess fat may become deposited within the face, neck, back and labia majora (1–3). Furthermore, patients with FPLD have normal stores of intermuscular, intra-abdominal, intrathoracic and bone marrow fat (1–3). Acanthosis nigricans, hirsutism, menstrual abnormalities and polycystic ovarian disease can also occur (1,2). Profound insulin resistance with diabetes can develop later in life, and FPLD subjects can also have dyslipidaemia and coronary heart disease, which is secondary to the metabolic disturbances (1,2).

Recently, three groups have independently mapped the FPLD gene to chromosome 1q21–22 (4–6). Included among >100 known genes within this region is the lamin A/C gene (LMNA), which undergoes alternative splicing to produce two nuclear laminar proteins: lamin A and lamin C (7). Nuclear lamins are part of the intermediate filament multigene family, and lamins A and C are present in most differentiated mammalian cells (7). Lamins participate in filament construction, chromatin organization, spatial arrangement of nuclear pores, nuclear growth and anchorage of nuclear membranes (7). Four mutations in LMNA were found in families with autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD-AD), which is characterized by regional and progressive skeletal muscle wasting and cardiac effects (8). We hypothesized that mutations in LMNA might lead to the analogous regional and progressive degeneration of adipocytes that is characteristic of patients with FPLD.

RESULTS

We evaluated five probands from five Canadian kindreds with FPLD (Fig. 1). DNA sequencing revealed that all probands with FPLD were heterozygous for a G/A change at codon 482 in exon 8, which predicted the replacement of arginine (CGG) by glutamine (CAG) (Fig. 2). The control subject in the screening experiment was homozygous for the wild-type sequence (Fig. 2). There were no other coding sequence or flanking region abnormalities in LMNA detected in any subject. Sequencing of the coding and flanking sequences of 11 other genes on chromosome 1q21 revealed no DNA variants that both altered the coding sequence and were unique to FPLD probands (data not shown). Genotyping confirmed that all FPLD probands were LMNA Q482/R482 heterozygotes (Fig. 1). In sharp contrast, all 1000 normal control subjects were LMNA R482/R482 homozygotes \( (\chi^2 = 1005, P = 1.1 \times 10^{-13}) \). Furthermore, the mutation completely co-segregated with either a definite or probable diagnosis of FPLD (Fig. 1). One male subject, OFPLD-1-13, had an uncertain phenotype based on clinical criteria, but was clearly not a carrier of the LMNA R482 mutation by genotyping.

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Complete clinical data were available from 22 LMNA Q482/ R482 heterozygotes and 23 R482/R482 homozygotes from the FPLD families, and are shown in Table 1. The genotype classes did not differ with respect to age, gender and body mass index (BMI). Compared with LMNA R482/R482 homozygotes, there were significantly more Q482/R482 heterozygotes who had definite FPLD and frank diabetes (Table 1). Also, compared with LMNA R482/R482 homozygotes, Q482/R482 heterozygotes had significantly higher serum insulin and C-peptide (data not shown). The LMNA Q482/R482 heterozygotes with diabetes were significantly older than heterozygotes without diabetes (51.4 ± 11.3 versus 34.4 ± 15.8 years, *P* = 0.02), but were not different with respect to BMI (data not shown).

**DISCUSSION**

Our results indicate that the LMNA R482Q mutation is the molecular basis for the FPLD phenotype in these Canadian kindreds. The LMNA R482Q mutation had an extremely strong statistical association with FPLD, since it was absent from 2000 normal alleles and was present only in FPLD families. Moreover, within FPLD families, LMNA R482Q was found only within subjects with definite or probable FPLD, and not within those family members who were definitely unaffected. Carriers of LMNA R482Q had significantly more diabetes, with elevated serum concentrations of insulin and C-peptide, which is consistent with the conspicuous insulin resistance that is pathognomonic for FPLD. Finally, phenotype in LMNA Q482/R482 homozygotes was variably penetrant, and the development of diabetes in LMNA Q482/R482 heterozygotes was age dependent.

The results indicate that different mutations in LMNA can underlie the disparate clinical entities of EDMD-AD and FPLD, analogous to the relationship between different mutations in the RET proto-oncogene and the disparate clinical entities of multiple endocrine neoplasia type 2, related sporadic tumours and Hirschsprung disease (9). The LMNA Q6X, R453W, R527P and
L530P mutations underlie muscle wasting in EDMD-AD (7). The LMNA R482Q mutation underlies site-specific degeneration of adipocytes in FPLD. The position of the mutant residue within LMNA appears to be a crucial determinant of both the affected cell type and the anatomical distribution of the affected cells. This suggests a high degree of functional specificity for particular lamin A/C residues and raises the possibility that LMNA mutations could underlie other diseases characterized by degeneration of specific cell types in particular anatomical distributions.

Lamins A and C are members of the intermediate filament multigene family. Both are absent from early embryos and undifferentiated cells, but are present in most terminally differentiated cells. Lamins A and C polymerize to form part of the nuclear lamina, a structural meshwork of 10 nm filaments on the nucleoplasmic side of the inner nuclear membrane (7). Lamins A and C form dimers through their rod domains and interact with the nucleoplasmic side of the inner nuclear membrane (7). Lamins the nuclear lamina, a structural meshwork of 10 nm filaments on differentiated cells. Lamins A and C polymerize to form part of undifferentiated cells, but are present in most terminally multigene family. Both are absent from early embryos and distributions.

The presence of the LMNA Q482 mutation in five probands suggests the possibility of a founder effect for this mutation in Canadian FPLD kindreds. Haplotype analysis strongly suggests that there is a common chromosomal haplotype for all subjects with LMNA (data not shown). In addition, there is likely to be a common ancestor for OFPLD-1, OFPLD-2 and NBFPPLD-1, who would be nine generations removed from the most recent generations in these kindreds. If this common ancestry can be confirmed, then it may be possible to extend these kindreds in order to perform a large multigenerational family study and to evaluate such attributes as epistasis or gene–environment interactions that could affect penetrance of the FPLD phenotype in LMNA Q482 carriers.

**MATERIALS AND METHODS**

**Patients**

After informed consent was obtained, we performed clinical evaluations and drew blood samples from five probands and members of their families, shown in Figure 1. We studied: 29 members of a three-generation Ontario family (OFPLD-1), of whom 13 were definitely or probably affected; six members of a two-generation Ontario family (OFPLD-2), of whom three were definitely or probably affected; seven members of a three-generation New Brunswick family (NBFPPLD-1), of whom four were definitely or probably affected; a definitely affected mother–daughter pair from New Brunswick (NBFPPLD-2); and a single definitely affected New Brunswick proband (NBFPPLD-3). All families were of Northern European descent and had lived in Canada for several generations. Each family member was assessed for characteristic physical attributes of FPLD and provided a fasting serum sample for biochemical determinations.

The phenotype was classified as ‘definitely affected’, ‘probably affected’ or ‘definitely unaffected’ based on clinical and biochemical criteria. The absence of subcutaneous fat tissue from upper and lower extremities and an extremely muscular appearance commencing in adolescence was the essential criterion for a definitive diagnosis of FPLD. Other important phenotypic criteria included the presence of excess adipose tissue in the face and neck, giving a pseudo-Cushingoid appearance. Additional supportive criteria included the presence of acanthosis nigricans, hirsutism, menstrual abnormalities and laboratory data confirming the presence of diabetes, hypertension, elevated insulin, elevated C-peptide and/or abnormal lipoproteins. Control DNA was obtained from 1000 unaffected normal subjects representing six ethnic groups (276 Caucasians, 243 South Asians, 169 Africans, 160 Chinese, 76 Oji-Cree and 76 Inuit). Statistical comparisons were made using SAS software (11).

**DNA analysis**

DNA was extracted from all family members; it was sequenced in subjects with a certain diagnosis of FPLD, and in an unrelated, unaffected normal control subject. Primers for DNA amplification and sequencing were derived using published sequence information for all 12 exons, all intron–exon boundaries and the 5′- and 3′-untranslated regions of LMNA (12). After the LMNA R482Q mutation was identified, a rapid genotyping assay was developed, which involved amplification of a 1069 bp fragment that
contained exon 8, using primers 5'-GCAAGATACACCCAGAGCC-3' and 5'-ACACCTGGTCTCCGTTC-3'. This was followed by digestion of the amplification products with MspI and electrophoresis in 2% agarose. Digestion of the amplification product from the wild-type allele, R482, produced two variant fragments of size 480 and 69 bp, in addition to invariant fragments with sizes 381, 80 and 59 bp. Digestion of the product from the mutant allele, Q482, produced a single fragment of size 549 bp, in addition to the invariant fragments.

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