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Juvenile-onset Generalized Lipodystrophy due to a Novel Heterozygous Missense *LMNA* Mutation Affecting Lamin C

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Abstract

The *LMNA* gene contains 12 exons and encodes lamins A and C by alternative splicing within exon 10. While mutations in lamin A specific residues cause several diseases including lipodystrophy, progeria, muscular dystrophy, neuropathy and cardiomyopathy, only three families with mutations in lamin C-specific residues are reported with cardiomyopathy, neuropathy and muscular dystrophy so far. Therefore, we report two brothers with juvenile-onset generalized lipodystrophy due to a lamin C-specific mutation. The proband, a 23-year-old Caucasian male was reported to have generalized lipodystrophy at 3 weeks of age, developed diabetes, hypertriglyceridemia, hypertension and liver problems and died with complications of cirrhosis and kidney failure. His younger brother, a 37-year-old Caucasian male developed generalized lipodystrophy around 2 years of age and was diagnosed with diabetes, hypertriglyceridemia, fatty liver and hypertension at 36 years of age. Their father also died of end stage renal disease at age 52 years. Exome sequencing of the proband revealed an extremely rare missense heterozygous variant c.1711_1712CG>TC; p.(Arg571Ser) in *LMNA* which was confirmed by Sanger sequencing in both the patients. Interestingly, the mutation had no effect on mRNA splicing or relative expression of lamin A or C mRNA and protein in the lymphoblasts. Our observations suggest that mutant lamin C disrupts its interaction with other cellular proteins resulting in generalized lipodystrophy due to defective development and maintenance of adipose tissue.

Keywords

Generalized lipodystrophy; *LMNA*; Lamin C; Diabetes mellitus; Hepatic steatosis; Hypertension

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Introduction

Mutations in lamin A/C (*LMNA*) gene are associated with a wide variety of human disorders including muscular dystrophies, cardiomyopathy; neuropathy; lipodystrophy syndromes and a spectrum of progeroid disorders [Worman & Bonne, 2007]. *LMNA* contains 12 exons and encodes lamins A and C by alternative splicing within exon 10. Exons 11 and 12 contain sequences found in transcripts of lamin A only, and 3' region of exon 10 contains 18 unique nucleotides that encode 6 carboxy-terminal lamin C-specific residues [Lin & Worman, 1993]. The specific roles of lamins A and C in the formation of nuclear lamina, chromatin organization, transcription, DNA replication, DNA damage response and genome stability remain unclear [Gruenbaum et al., 2005]. In humans, mutations in lamin A specific residues cause lipodystrophy, progeria, muscular dystrophy, neuropathy and cardiomyopathy [Worman & Bonne, 2007]. However, only three families have been reported with lamin C-specific mutations to have dilated cardiomyopathy, neuropathy and muscular dystrophy [Fatkin et al., 1999; Ng & Kaye, 2013; Benedetti et al., 2005]. Thus, we report a novel heterozygous lamin C-specific mutation in a family presenting with juvenile-onset generalized lipodystrophy (JGL).

Clinical Reports

JGL100.4

This Caucasian male developed generalized loss of fat with muscular appearing arms and legs and prominent superficial veins around 3 weeks of age and was initially reported as having congenital generalized lipodystrophy (CGL 4200)[Agarwal et al., 2003]. Subsequently, he developed diabetes mellitus, hypertriglyceridemia, hypertension and liver problems. He also had inguinal hernia that needed repair at 6 years of age. At age 22 years, his fasting blood glucose value was 366 mg/dL; hemoglobin A1c, 9.3%; high density lipoprotein (HDL)-cholesterol, 15 mg/dL; and triglycerides, 270 mg/dL. He developed cirrhosis and kidney failure resulting in death at 23 years of age.

JGL100.5

This 37-year-old younger brother of the proband, developed generalized loss of subcutaneous fat at age 2 years (Fig. 1 and supplementary Fig. 1). At age 36 years, he was diagnosed with diabetes, hypertriglyceridemia, fatty liver and hypertension, and was started on fish oil, metformin 1000 mg daily, losartan 50 mg daily and carvedilol 25 mg daily. He underwent right hemi-thyroidectomy for thyroid nodules at age 36 years with biopsy consistent with autoimmune thyroiditis. He had an umbilical hernia repair at age 13 years, left eye corneal transplant at age 32 years for keratoconus and deviated nasal septum repair at age 36 years. He also had intermittent joint pains. He had an automobile accident at age 31 which required metal implants in left humerus, tibia and fibula due to compound fractures. His two daughters, age 6 and 2 years, are healthy. At age 36 years, his weight was 105.5 kg, height 1.9 m, body mass index 29 kg/m², heart rate 92 beats/minute and blood pressure 160/78 mm of Hg. He had generalized loss of subcutaneous fat including the face and soles with prominent superficial veins, muscular extremities and prominent calluses on both the soles (Fig. 1). He had prominent facial bones, hepatomegaly 3 cm below right

coastal margin, splenomegaly 5 cm below left costal margin, healing scar over thyroid and hyperpigmentation around venous prominence in left leg (Fig. 1). He did not have acanthosis nigricans. His skin fold thickness measurements were mostly below the 10th percentile (Fig. 1F). His total body fat by dual energy X-ray absorptiometry (Discovery W, Hologic) was 10.5 % (1stile age matched). Body fat in the right upper extremity was 9.4%, in the right lower extremity was 8.6%, and in the trunk was 10.6%. His total bone mineral density (excluding left arm and left leg) was 1.35 g/cm² (mean $\pm$ SD for non-Hispanic white males being 1.21 $\pm$ 0.106).

His fasting blood glucose was 122 mg/dL; total cholesterol, 147 mg/dL; HDL-cholesterol, 25 mg/dL; triglycerides, 314 mg/dL; aspartate aminotransferase, 21 IU/L; alanine aminotransferase, 38 IU/L; and hemoglobin A1c 6.9%. Abdominal ultrasound revealed significantly enlarged fatty liver measuring 22.3 cm and marked splenomegaly measuring 20 cm. His electrocardiogram was normal but he had a high coronary calcium score of 535 (>90thile for age) on coronary computed tomography. Stress study was notable for hypertensive response (230/90 mm of Hg).

Their father had loss of subcutaneous fat from the face in the photographs, but the extremities were not visualized. He also had premature coronary artery disease, hypertension and kidney disease. He was on dialysis for about 10 years and died at the age of 52 years. Their 56-year-old mother was healthy (Fig. 2A).

Methods

The protocol was approved by Institutional Review Board of UT Southwestern. A written informed consent was obtained from both the patients and their mother and healthy control subjects. Genomic DNA was isolated from whole blood, and whole exome and Sanger sequencing were performed (Supplementary Methods). Total RNA was extracted from lymphoblasts for mutational analysis using allele specific primers and expression studies using real-time PCR; and protein was extracted for immunoblotting (Supplementary methods).

Results

Initial Sanger sequencing of CGL genes, *AGPAT2* and *BSCL2*, revealed no pathogenic variants. We followed this with whole exome sequencing and confirmed the lack of pathogenic variants in *AGPAT2* and *BSCL2*, as well as in *AKT2*, *CAV1*, *CIDEA*, *LIPE*, *PCYT1A*, *PIK3R1*, *PLIN1*, *POLD1*, *PPARG*, *PSMB8*, *PTRF*, or *ZMPSTE24*. Exome sequencing revealed an extremely rare missense heterozygous variant NC_000001.10:g.156107547_156107548delCGinsTC (rs794728612); NM_005572.3:c.1711_1712CG>TC; p.(Arg571Ser) in *LMNA* (Fig. 2B and C). Exome sequencing also confirmed that both the nucleotide substitutions were on the same allele (Fig. 2D). Sanger sequencing confirmed the mutation in both the proband and his brother and absence of mutation in the unaffected mother (Fig. 2E). Closer scrutiny of the ExAC database (<http://exac.broadinstitute.org/>) for the heterozygous *LMNA* 1:156107547 C/T and 1:156107548 G/C variants, revealed that one European (non-Finnish) individual had both the substitutions (minor allele frequency of

0.0001552). There is no mutation at these two sites at 1000 Genomes database (<http://www.internationalgenome.org/>), NHLBI GO Exome Sequencing Project (ESP) data base (<http://evs.gs.washington.edu/EVS/>), UK10K database (<https://www.uk10k.org/studies/cohorts.html>), or Scripps Translational Science Institute Variant Database (<https://genomics.scripps.edu/browser/>). The RNA product amplified from the lymphoblasts of the proband was similar to that of controls and sequencing revealed no alternate splicing (Supplementary Figure 2). Furthermore, no difference among the proband and controls was observed in the expression pattern of lamin A and C transcripts (Fig. 2F) and protein extracted from lymphoblasts (Fig. 2G).

Discussion

We report the first patients with JGL with a lamin C-specific mutation. Previously, [Fatkin et al., 1999] reported a heterozygous c.1711C>A; p.(Arg571Ser) mutation in a family presenting with relatively mild conduction system disease and dilated cardiomyopathy. The same variant was reported by Ng and Kaye [Ng & Kaye, 2013] in a 32-year-old female with atrial fibrillation. Her father had undergone cardiac transplantation for cardiomyopathy. A heterozygous c.1711C>T; p.(Arg571Cys) mutation, was reported in a 14-year-old male with overt neuropathy and myopathy, and his 54-year-old mother with subclinical peripheral nerve involvement by Benedetti *et al.* [Benedetti et al., 2005]. These investigators did not report any lipodystrophy in affected patients.

While both c.1711C>A and c.1711_12CG>TC *LMNA* variants are predicted to cause p.Arg571Ser, why there is marked contrast in the phenotypic manifestations associated with the two variants is unclear. We were unable to show any effects of c.1711_12CG>TC *LMNA* variant on RNA splicing or relative lamin A or C mRNA and protein expression in the lymphoblasts, however, whether this mutation affects the expression of lamins A or C in adipose tissue differently, will require further investigation. Previous studies reveal equal expression of lamin A and C proteins in stromal vascular fraction or mature adipocytes from visceral and subcutaneous adipose tissue, indicating important role of lamin C in adipose tissue development and maintenance [Lelliott et al., 2002; Peinado et al., 2010].

In summary, our cases add to the phenotypic manifestations associated with alterations in lamin C-specific residues. In humans, lamin C may have a wider role and may specifically contribute to function of various tissues including adipose, renal, cardiac, skeletal muscle and nerves.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Clinical features of patient JGL100.5 at 36 years of age.

Anterior (A) and lateral (B) views showing muscular extremities and generalized loss of subcutaneous fat. (C) There is loss of subcutaneous fat and prominent calluses on both the soles (C) and palms (D) and prominent superficial veins in the lower leg (E). F. Skin-fold thickness at various anatomical sites. The shaded bars represent the median, 10th and 90th percentile values of skin-fold thickness for normal adult males [Jackson & Pollock, 2004]. Majority of the measurements were below the 10th percentile

Figure 2.

JGL Pedigree, location of the mutation in the *LMNA* gene and Lamin C protein, exome data, Sanger sequencing and mRNA and protein expression in lymphoblasts.

(A) JGL 100 pedigree showing both the affected patients. Circles denote females and squares represent males. The half-filled symbols represent the affected heterozygotes including the proband's father who was presumed affected but did not undergo genotyping. Unfilled symbols indicate the unaffected subjects. The mother of the proband had the wild type alleles and did not carry the disease-causing variant. A diagonal line across a symbol indicates a deceased subject. The numbers above the symbols indicate the age of the subjects and besides the symbols indicate the pedigree number. (B) Illustration of *LMNA* gene depicting the location of mutation c.1711_1712CG>TC in our patients. The *LMNA* gene contains 12 exons and encodes lamins A and C by alternative splicing within exon 10. Exons 1 through 10 contain sequences encoding 566 amino acids that are shared by both the isoforms. Exons 11 and 12 contain sequences that are found in the transcript of lamin A, and 3' region of exon 10 contains 18 nucleotides that encode 6 lamin C-specific residues. Boxes indicate exons and the lines in between them indicate the introns. Black and hatched regions indicate translated regions of exons, while white regions indicate 5' and 3' untranslated regions. Hatched regions indicate lamin A or lamin C specific exons. (C) Structure of lamins

A and C proteins. Both have identical structure throughout the NH₂-terminal head, α -helical rod domain and proximal carboxy terminal region (blue color), but differ in their distal c-terminal amino acids (lamin C specific region is shown in red and lamin A specific region is shown in purple). Mutation p.(Arg 571Ser) in our patients lies in lamin C region. (D) Whole Exome sequencing of patient 1 (JGL100.4) showing missense heterozygous variant NC_000001.10:g.156107547_156107548delCGinsTC (rs794728612); c.1711_1712CG>TC). Note that both the nucleotide substitutions are on the same allele (Fig. 2D). (E) Chromatogram from Sanger sequencing showing the heterozygous 2-nucleotide substitution in the proband. The unaffected mother did not harbor the mutation. Arrows indicate the site of mutation. (F) The amplified PCR product from control lymphoblasts (N5900) and affected lymphoblasts (JGL 100.4) as analyzed on a 1% agarose gel stained with ethidium bromide. The amplified PCR products are of expected size and upon sequencing carry wild type lamin A and C sequences. Shown also are the amplification of the housekeeping gene, 18S. (G) Immunoblot analysis for lamin A and lamin C proteins of total cellular lysate obtained from lymphoblasts of controls (N300, N5900) and affected subject (JGL100.4). Cellular lysates were loaded in increasing protein concentration and probed with anti-lamin A/C antibody raised against N-terminus of the protein. The same protein blot was stripped and re-probed with GAPDH antibody. In lymphoblasts, the expression of lamin A is less than that of lamin C. There was no difference in the relative expression of lamins A and C in the controls and the affected subject.