Particular forms of polycystic ovary syndrome with severe hyperandrogenism, acanthosis nigricans, and marked insulin resistance, defining the type A insulin resistance syndrome, are due to insulin receptor gene mutations. However, the majority of affected individuals do not have such mutation, arguing for the genetic heterogeneity of this syndrome. The familial partial lipodystrophy of the Dunnigan type, one of the diseases due to mutations in the lamin A/C (LMNA) gene, is characterized by a lipodystrophic phenotype and shares some clinical and metabolic features with the type A syndrome. We describe here the case of a nonobese 24-year-old woman affected with type A syndrome without clinical lipodystrophy. We linked this phenotype to a novel heterozygous missense mutation in the LMNA, predicting a G602S amino acid substitution in lamin A. This mutation cosegregated with impaired glucose tolerance, insulin resistance, and acanthosis nigricans in the absence of clinical lipodystrophy in the family. The skin fibroblasts from the proband exhibited nuclear alterations similar to those described in other laminoopathies, and showed several defects in the insulin transduction pathway. This study further extends the vast range of diseases linked to LMNA mutations and identifies another genetic cause for the type A insulin resistance syndrome. Diabetes 54:1873–1878, 2005

Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women of reproductive age and the main cause of anovulatory infertility (1). It is associated with hyperandrogenemia, metabolic alterations including insulin resistance, and an increased risk of type 2 diabetes and cardiovascular disease. The primary cause of PCOS remains elusive, although there is evidence that genetic factors have an important role (2). Particular forms of PCOS with acanthosis nigricans, severe insulin resistance, and hyperandrogenism, defining the type A insulin resistance syndrome, are due to insulin receptor gene mutations (3). However, many affected individuals do not have such a mutation, which lends support to the genetic heterogeneity of this syndrome (4).

Lamins are the main components of the nuclear lamina, a filamentous network located between inner nuclear membrane and chromatin that plays a fundamental role in nuclear organization in all differentiated cells (5). Mutations in lamins A and C, which are alternatively spliced products of the lamin A/C (LMNA) gene, are responsible for several genetic diseases, including familial partial lipodystrophy of the Dunnigan type (FPLD) (5). Women with FPLD often have irregular menses and hyperandrogenemia, as well as severe insulin resistance, dyslipidemia, and atherosclerotic vascular disease (6–8). These common clinical and metabolic features of FPLD and type A insulin resistance syndrome led us to search for mutations of the LMNA gene in a patient with a typical type A phenotype.

We describe the case of a nonobese 24-year-old woman with severe hyperandrogenemia who had clinical, hormonal, and morphological ovarian features of PCOS, acanthosis nigricans, and profound insulin resistance but was free of clinical lipodystrophy. Type A insulin resistance syndrome was therefore diagnosed. We linked this phenotype to a novel heterozygous missense mutation in the LMNA gene, predicting a G602S amino acid substitution in lamin A. Her skin fibroblasts exhibited nuclear alterations similar to those described in other laminoopathies and showed several defects in the insulin transduction pathway.

These findings suggest that a mutation in lamin A can affect insulin signaling and may be responsible for in vivo...
profound insulin resistance, leading to severe hyperandro-
genism and oligomenorrhea but without significantly af-
fecting adipose tissue distribution.

CASE REPORT
The propositus (subject II-3, Fig. 1A and B), a 24-year-old
African-Creole woman originating from Reunion Island,
was referred to our department with oligomenorrhea and
hirsutism. Spontaneous thelarche and menarche occurred
at ages 11 and 12 years, respectively. She has since had
irregular spotting every 4 months on average, but not
spontaneous regular menses. At the age of 14 she devel-
oped acne, seborrhea, and increasing hair growth on the
face and nipples. Between the ages of 16 and 23, she
received combined oral contraceptive treatment that in-
duced cyclical withdrawal bleeding, but oligomenorrhea
recurred after interruption of this treatment. Her personal
medical history was otherwise unremarkable; in particu-
lar, she did not have any period of overweight. Her birth
weight was normal, and she did not have premature pubarche.

Her height was 170 cm, weight 67 kg (BMI 23.2 kg/m²),
and blood pressure 110/60 mmHg. Physical examination
revealed an android habitus with moderate excess hair on
the upper lip, chin, submental and periareolar areas, and
midline of the lower abdominal wall, together with a male
balding pattern (Fig. 1B). Applying the Ferriman-Gallwey
standard, the patient achieved a score of 13 (normal = 5).
The breasts were of adult size. Evidence of acanthosis
nigricans was observed in the axillae (Fig. 1C), neck, and
back. The waist-to-hip circumference ratio was normal
(0.84). No lipoatrophy of the limbs or faciocervical fat
accumulation was present. Examination of the genitalia
disclosed a mildly enlarged clitoris. Neuromuscular, car-
diac, and cutaneous examinations showed no abnormali-
ties.

Hormone tests (Table 1) showed a normal serum dehy-
droepiandrosterone sulfate level but elevated levels of
plasma androstenedione and total and bioavailable testos-
terone. The plasma sex hormone–binding globulin level
(inmunoradiometric assay methodology) was low. The
plasma estradiol concentration was normal. The ratio of
luteinizing hormone to follicle stimulating hormone was
elevated, in both the basal and gonadotropin-releasing
hormone–stimulated state. Pulsatile luteinizing hormone
secretion was evaluated at 10-min intervals for 6 h (9). An
increased amplitude and frequency of luteinizing hormone
pulses was observed. The free urinary cortisol and the
serum prolactin levels were normal. Basal and corticotropin-
stimulated (0.25 mg i.v.) 17-hydroxyprogesterone levels
were normal, ruling out late-onset 21-hydroxylase defi-
ciency. The IGF-1 level was normal for age. Pituitary-adrenal

![FIG. 1. Clinical characteristics of the propositus. A: Pedigree subjects bearing the LMNA G602S mutation are represented as filled symbols. The propositus is indicated by an arrow. B: Anterior view. C: Axillary acanthosis nigricans. D: Enlarged polycystic left ovary, transvaginal sonography. E: Cross-sectional computed tomography at the level of the L4 vertebra and the mid-thigh in a control woman, the proband, and a woman with typical FPLD due to the LMNA R482W mutation and similar BMI (22.8, 23.2, and 23.1 kg/m², respectively).]

### TABLE 1
Hormone tests from the proband

<table>
<thead>
<tr>
<th>Test</th>
<th>Proband</th>
<th>Normal range (in premenopausal women)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydroepiandrosterone sulfate (µmol/l)</td>
<td>7.4</td>
<td>1.7–15.7</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>11.2</td>
<td>3.2–6.0</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>3.7</td>
<td>1.0–2.4</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>1.0</td>
<td>0.07–0.51</td>
</tr>
<tr>
<td>Sex hormone–binding globulin (nmol/l)</td>
<td>12</td>
<td>25–70</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>125</td>
<td>92–390b</td>
</tr>
<tr>
<td>Luteinizing hormone (IU/l) (basal/GnRH stimulated)</td>
<td>10.5/59.5</td>
<td>2.8–7.0/8.0–25*</td>
</tr>
<tr>
<td>FSH (IU/l) (basal/GnRH stimulated)</td>
<td>2.1/5.8</td>
<td>2.5–6.0/2.0–9*</td>
</tr>
<tr>
<td>Pulsatile luteinizing hormone secretion during 6 h (means ± SE in normal women)</td>
<td>5.7</td>
<td>3.8 ± 0.3*</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>5.6</td>
<td>2.7 ± 0.32*</td>
</tr>
<tr>
<td>Amplitude of pulses (IU/l)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In early follicular phase. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone.
and pituitary-thyroid function was normal. Computed tomography of the adrenal glands showed no abnormalities.

Pelvic and transvaginal sonography showed a normal uterus but polycystic enlarged ovaries (right: 7.6 cm², 10.9 ml; left: 12.4 cm², 13.5 ml; normal volume <5.5 cm², <10 ml) with stromal hypertrophy (Fig. 1D). More than 20 subcapsular follicles measuring between 3 and 6 mm in diameter were seen on the largest plane of each ovary.

A standard 75-g oral glucose tolerance test was performed after a 12-h overnight fast to determine plasma glucose and insulin (measured with immunoradiometric assay) levels. At baseline, glycemia was 5.1 mmol/l and insulinemia 180 pmol/l (normal range: 12–54 pmol/l); at 120 min, glycemia was 9.8 mmol/l and insulinemia 4,928 pmol/l. The patient was therefore markedly insulin resistant and had altered glucose tolerance. Tests for circulating insulin receptor autoantibodies were negative. The HbA1c was normal (5.2%). The serum total cholesterol level was normal (2.2 mmol/l), but the HDL cholesterol was low (0.92 mmol/l) and the triglyceride level slightly elevated (2.2 mmol/l).

Body fat content, evaluated by means of whole-body dual-energy X-ray absorptiometry (DEXA), was 28.4% of body weight, in keeping with the value predicted from BMI, sex, and age (10). The serum leptin level was 13.0 ng/ml (normal for BMI), whereas serum adiponectin was very low (1.7 μg/ml, normal: 7.1 ± 0.5 [means ± SE]) (11). Cross-sectional computed tomography at the level of the L4 vertebra (Fig. 1E) showed a normal amount of subcutaneous fat and a normal visceral-to-total fat ratio (0.13 in the proband, 0.26 in a control woman, as compared with 0.93 in a woman with FPLD due to a R482W LMNA mutation; normal values: 0.25 ± 0.14 [means ± SD]) (12). The mid-thigh computed tomography scan showed the presence of subcutaneous adipose tissue, the thickness of which was slightly thinner than in a control woman free of hyperandrogenism, but which strikingly contrasted with the complete lipatrophy observed in a typical FPLD woman (Fig. 1E). Similar values for truncal and peripheral skinfolds were observed in the proband and in normal women, unlike those of typical FPLD R482W LMNA–mutated women or atypical lipodystrophic women bearing the R582H LMNA mutation (13,14) (Fig. 2A). In addition, the proband’s segmental body distribution of fat, assessed by DEXA, was close to that reported for normal women (15) but very different from that observed in three typical FPLD R482W LMNA–mutated women with similar BMI (22.1, 23.1, and 25.3 kg/m² from left to right), measured by DEXA, as previously described (6,16). While sequencing of the 10th and 90th percentile values for control women, used by Garg et al. (13), except for biceps skinfold thickness published by Araujo-Vilar et al. (14). Whiskers represent the range values observed in seven typical FPLD women bearing the R482W LMNA mutation (13), whereas skinfold thickness from two atypical FPLD women with the R582H LMNA mutation are shown as white circles (13). B: The segmental body distribution of fat was assessed by DEXA using the same equipment (Lunar model DPX; GE Medical Systems) in the proband and in three women of similar BMI (22.1, 23.1, and 25.3 kg/m² from left to right), diagnosed in our department with typical FPLD due to the LMNA R482W mutation. *Normal values (means ± SD, n = 6) were reported by Mazess et al. (15).

Kidney and liver function test results were normal. Levels of serum total hemolytic complement and its C3 and C4 fractions were normal. Echocardiography and 24-h electrocardiogram monitoring showed no abnormalities.

The proband’s father (subject I-1, Fig. 1A) was diagnosed with type 2 diabetes in his forties and also had acanthosis nigricans. He was hemiplegic following a stroke at age 54. Systematic clinical and biological investigations of the other family members led to the diagnosis of insulin-resistant diabetes (fasting glucose: 10.2 mmol/l; fasting insulin: 222 pmol/l) and hypertriglyceridemia (plasma triglycerides: 8.7 mmol/l) in a 26-year-old brother (subject II-2), who also had acanthosis nigricans. Neither the father nor this brother was obese, bald, or lipodystrophic (see online appendix [available at http://diabetes.diabetesjournals.org]). The other siblings (subjects II-1 and II-4) had normal fasting glucose, insulin, cholesterol, and triglyceride levels. The proband’s mother (subject I-2) and sister (subject II-4) had no history of hirsutism, menstrual disorders, or infertility. There was no evidence of consanguinity. All the subjects provided written informed consent for these studies.

We amplified and directly sequenced all exons and surrounding intronic sequences of the insulin receptor (INSR) and LMNA genes from the proband’s genomic DNA, as previously described (6,16). While sequencing of the INSR gene revealed no alterations in the proband, a heterozygous GGC-to-AGC transition at codon 602 (exon 11) of the LMNA gene was detected, predicting a glycine-to-serine amino acid substitution in the COOH-terminal tail of the lamin A isoform (Fig. 3A and B). This DNA
variation, which has not been previously reported, was absent in 394 chromosomes from unrelated control subjects, including 194 from Creole subjects with African ancestry from Reunion Island. The same LMNA mutation was found in the father (subject I-1, Fig. 1A) and the younger brother (subject II-2) but not in other relatives. Therefore, the LMNA G602S substitution cosegregates with impaired glucose tolerance, insulin resistance, and acanthosis nigricans in the family.

Primary fibroblast cultures were established by skin biopsy from the proband and from two nonobese, nonobestic women aged 20 and 33 years. Immunofluorescence microscopy and protein expression studies were performed as described elsewhere (17). Some of the proband’s fibroblasts showed nuclear herniations and altered staining patterns of lamin A/C and B, which constitute the lamina meshwork located on the inner surface of the nuclear envelope (5) (Fig. 4A). Emerin, a known lamin A/C partner protein, consistently colocalized with lamin A/C in mutated fibroblasts, even in regions in which the lamina network was disorganized (not shown). These abnormalities of the lamina structure are similar to those described in other laminopathies (17–21). The cellular amount and apparent molecular masses of lamins and emerin, evaluated by Western blotting, were normal (data not shown). Fibroblast responsiveness to insulin was assessed by examining early and late events in insulin signaling pathways, namely tyrosine and serine phosphorylation of the insulin receptor β subunit (IRβ) and insulin receptor substrate-1 (IRS-1), and activation of protein kinase B (Akt/PKB) and mitogen-activated protein kinase (extracellular-regulated kinase [ERK] 1/2), respectively (see online appendix for details). The amount of IRβ, its substrate IRS-1, and the molecular effectors Akt/PKB and ERK1/2 was normal in the proband’s fibroblasts (Fig. 4B). In contrast, insulin signal transduction pathway activation was impaired in the patient’s fibroblasts: IRβ and IRS-1 were both tyrosine and serine phosphorylated in the basal state, no further stimulation occurred following insulin treatment, and insulin activation of Akt/PKB and ERK1/2 was impaired (Fig. 4B).

DISCUSSION
The patient described here was referred to our department with oligomenorrhea and hirsutism. Her clinical history, hormonal test results, and ovarian morphology established the diagnosis of PCOS (1,2,22). However, clinical examination revealed a masculine body habitus, temporal balding, and clitoromegaly (related to a severe hyperandrogenemia) and acanthosis nigricans (linked to a striking insulin resistance).

These features, characteristic of type A syndrome, in a
nonobese women led us to search for and exclude mutations or circulating autoantibodies affecting the insulin receptor (3). Conversely, we discovered an LMNA gene mutation predicting a heterozygous G602S substitution in lamin A protein. LMNA mutations have previously been shown to be responsible for FPLD, which is mainly characterized by abnormal fat distribution with lipodystrophy of the limbs and a relative fat excess in the face, neck, and abdomen, features considered important factors for the development of insulin resistance (23). As our patient was markedly insulin resistant but had no clinical evidence of lipodystrophy, we sought intrinsic defects in insulin signaling pathways by testing her cultured fibroblasts. The IRβ and its substrate IRS-1 were tyrosine phosphorylated in the basal state, which can lead to desensitization of the insulin signal (24,25). Furthermore, the basal-serine phosphorylation of IRβ and IRS-1 was enhanced, and this also inhibits the cellular response to insulin (26). Insulin resistance associated with PCOS has previously been linked to increased serine phosphorylation of IRβ, but the responsible serine kinase is unknown (2).

Interestingly, the type A insulin resistance phenotype and the lamin A mutation cosegregated in the propositus’ family with a dominant pattern. Further work is needed to unravel the precise pathophysiological sequence, but it may involve dysregulation of the serine/threonine kinase PKCα, which both inhibits insulin signaling (26) and binds lamin A (27). The low level of adiponectin could also contribute to the state of insulin resistance. Whatever the mechanism of insulin resistance, the resulting hyperinsulinemia may have led to the ovarian disorders observed in this patient (2).

This case further extends the vast range of clinical disorders linked to LMNA mutations, which notably comprise FPLD, skeletal and/or cardiac muscle dystrophies, axonal neuropathy, and several premature aging syndromes, including Hutchinson-Gilford progeria (5). Our findings show that an LMNA mutation could be responsible for type A insulin resistance in the absence of clinical lipodystrophy. Although this patient’s phenotype was unusual, the fibroblast nuclear shape defects and lamin localization were typical of those observed in other laminopathies. This new LMNA mutation is located in a region highly conserved among species, very close to the mutation responsible for Hutchinson-Gilford progeria (20,21).

The combination of PCOS with severe hyperandrogenism, acanthosis nigricans, and insulin resistance in a nonobese woman calls for molecular studies not only of the insulin receptor but also of the LMNA gene. Whether LMNA gene mutations represent a frequent cause for nonobese, insulin-resistant PCOS remains an open issue.

REFERENCES


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