Monogenic polycystic ovary syndrome due to a mutation in the lamin A/C gene is sensitive to thiazolidinediones but not to metformin

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Abstract

Context: Despite the very high prevalence of the polycystic ovary syndrome (PCOS), the underlying pathogenetic mechanism has remained obscure.

Objective: To determine the cause of two sisters’ PCOS associated with severe insulin resistance.

Design: Clinical case report.

Methods: Two sisters who presented with hyperandrogenism and menstrual disorders in the context of PCOS, and were subsequently found to be severely insulin resistant. Physical examination revealed muscular hypertrophy with a paucity of fat in the extremities, trunk and gluteal regions, in spite of excess fat deposits in the face, neck and dorsocervical region. Known genes involved in familial partial lipodystrophy were screened. At the same time, metformin (1700 mg/day) was commenced. After 2–3 years of uninterrupted therapy, lack of clinical improvement led to the introduction of pioglitazone (30 mg/day).

Results: Both sisters were found to be heterozygous for the R482Q mutation in the lamin A/C gene (LMNA) gene, establishing the definitive diagnosis as Dunnigan-type familial partial lipodystrophy complicated by severe insulin resistance and secondary PCOS. Treatment with pioglitazone resulted in progressive amelioration of insulin resistance, hyperinsulinaemia and hyperandrogenaemia. Menses also improved, with restoration of a eumenorrhoeic pattern, and the framework of ultrasound PCO was in complete remission.

Conclusions: Assessment of insulin sensitivity and adipose tissue topography should be a key part of the initial evaluation of patients with PCOS. Identifying such forms of PCOS with monogenic insulin resistance as the primary pathogenic abnormality may have practical implications for therapy, since they respond to thiazolidinediones, but not to metformin.

European Journal of Endocrinology 159 347–353

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 4–7% of women of reproductive age, making it one of the most common human endocrinological disorders. The classical phenotype is characterized by hirsutism, oligomenorrhoea and anovulatory infertility and the most robust biochemical marker is hyperandrogenaemia, with 70% of all cases presenting with elevated circulating testosterone and/or androstenedione levels. Nevertheless, other endocrine abnormalities, particularly insulin resistance and hyperinsulinaemia, are present in the majority of patients affected by the disorder.

Despite its very high prevalence, and despite some evidence for a genetic predisposition, the underlying pathogenic mechanism of PCOS has remained obscure. One suggested hypothesis is that hyperandrogenaemia is primary, with insulin resistance a secondary consequence of increasing abdominal fat deposits and modified skeletal muscle structure. However, primary disorders of insulin action also recapitulate the entire phenotype of PCOS, often in severe form, and insulin has been implicated as a direct modulator of ovarian and adrenal androgen production, sex hormone-binding globulin (SHBG) synthesis and hypothalamic–pituitary function.

We report here the identification and response to treatment of two sisters who presented with hyperandrogenism and menstrual disturbances, and were subsequently found to be severely insulin resistant due to familial partial lipodystrophy in association with the R482Q mutation in the lamin A/C gene. This is an important reminder that the assessment of insulin sensitivity and adipose tissue topography is a key part of the initial evaluation of patients with PCOS. Identifying such monogenically-determined PCOS with severe insulin resistance may have practical implications for therapy.
resistance as the primary pathogenic abnormality may not only have practical repercussions for the management of individual patients, but may also yield clues to the genetic aetiopathogenesis of more prevalent forms of the condition.

Materials and methods

Anthropometry, functional tests and laboratory evaluations

Height, weight, waist and hip circumference were obtained in both cases according to standardized procedures (8). Each subject also underwent an estimate of fat mass and fat-free mass by bioimpedance (Akern-BIA, Pontassieve, Florence, Italy).

In case 1, presenting with oligomenorrhea, functional tests and laboratory evaluations were performed starting from day 2 or 3 of the menstrual cycle in order to complete it on day 8 or 9. On the contrary, they were performed randomly in case 2 presenting with amenorrhea. Blood samples were drawn and all tests were performed in both cases from 2000 to 0900 h after a 12-h overnight fasting.

On the day after baseline blood samples for hormonal and metabolic parameters had been obtained, an oral glucose tolerance test (OGTT) (75 g Curvosio, Sclavo, Cinisello Balsamo, Italy) was performed, and samples for hormone assay were immediately chilled on ice, centrifuged and serum and plasma aliquots collected and frozen at −20 °C until assayed.

On the second day, a 1–24 adrenocorticotrophin (ACTH) stimulation test (Synacthen, 250 μg e.v.) was performed, and samples for 17OH-progesterone determinations were obtained at baseline and 60 min after stimulation. These samples were immediately chilled on ice, centrifuged and plasma aliquots were collected and frozen at −20 °C until assayed. The same day, at 1300 h, 1 mg dexamethasone was orally administered. The day after blood samples were taken again in the morning (from 0800 to 0830 h) to measure cortisol concentrations. Written informed consent was obtained from each of the two sisters.

Assays

Plasma glucose levels were determined by the glucose oxidase technique immediately after the blood drawing. Hormones, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Apo A1, apo B, lipids and C-reactive protein were measured as previously described (9). Leptin and adiponectin were determined using customized autoDELFIA assays that have previously been described (10). The free androgen index (FAI) was calculated as the ratio between total testosterone and SHBG, according to Vermeulen et al. (11). To investigate insulin sensitivity in the basal condition, the homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated (12); in addition, from the results of the OGTT, the composite insulin sensitivity index (IS_comp) was determined (13). Insulin response to the OGTT was expressed as area under the curve, which was calculated by the trapezoidal method.

The intra-assay coefficients of variation in our laboratories were 3.0% for insulin, 4.8% for luteinizing hormone (LH), 1.9% for follicle-stimulating hormone (FSH), 7.0% for testosterone, 6.0% for androstenedione, 13% for 17OH-progesterone, 10% for cortisol, 6.5% for SHBG, 3.9% for leptin and 5.2% for adiponectin.

Genotyping

All exons of LMNA gene were amplified using Promega GoTaq Green (Promega) according to the manufacturer’s instructions. Thirty-five cycles (60 s at 95 °C, 60 s at the annealing temperature, and 60 s at 72 °C) were performed using a PTC-225 Peltier thermal cycler (MJ Research, Watertown, MA, USA). PCR products were verified electrophoretically and sequenced using ABI BigDye Terminator (version 3.1) reagents with electrophoresis on an ABI Prism 3100-Avant genetic analyzer (PE Applied Biosystems, Foster City, CA, USA). Subsequent sequence analysis was performed using Sequencher 4.8 software (Gene Codes, Ann Arbor, MI, USA). Primers and annealing temperatures are available on request.

Results

Case 1

A 21-year-old girl was referred for evaluation of hirsutism and polymenorrhea. Her childhood was uneventful, with adrenarche at 11 years and menarche at 12 years of age. At 16 years of age hirsutism and oligomenorrhea prompted endocrine evaluation elsewhere, and hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal function was found to be normal. The karyotype was 46,XX. Magnetic resonance imaging (MRI) (T1-weighted) revealed a small area of low signal density in the right side of an otherwise unremarkable pituitary, compatible with a microadrenonoma. Treatment with a compound preparation of cyproterone acetate + ethinyl oestradiol for one year from age 19 to 20 produced no discernible benefit.

Physical examination revealed hirsutism (Ferriman-Gallwey score of 17 (14)) and moderate acanthosis nigricans on the neck, and in the axillae and inguinal areas (15). Puberty was complete (Tanner stages of B3, P5). Body mass index (BMI) was 26 kg/m², with a waist circumference of 81 cm and waist-to-hip ratio (WHR) of 0.87. There was markedly abnormal adipose tissue topography, with a paucity of fat in the extremities, trunk and gluteal regions, with prominent musculature in the limbs. Mammary adipose tissue was preserved, and there was excess fat accumulation in the face, neck and
The results of initial hormonal evaluation revealed elevated serum total testosterone and androstenedione levels, low SHBG, and a subsequent elevated FAI (Table 1). FSH basal levels were normal (3.0 mU/ml; reference value < 8.5 mU/ml), while LH basal levels were slightly elevated (8.7 mU/ml; reference value < 8.0 mU/ml). Serum prolactin and thyroid hormones were normal. Morning (0800 h) cortisol was suppressed by more than 90% by 1 mg dexamethasone at midnight. The response of 17OH-progesterone to 1–24 ACTH was normal (basal value = 71 ng/dl; reference value < 150 ng/dl; 60 min value = 135 ng/dl; reference value < 450 ng/dl).

Because of the clinical evidence of insulin resistance, fasting blood and OGTT were undertaken (Table 1). Glucose tolerance was normal (16), whereas fasting and glucose-stimulated insulin levels were high.

HOMA-IR and ISI composite values were in keeping with significant insulin resistance (Table 1). Lipid profile showed high serum triglycerides, normal total cholesterol and decreased serum high density lipoprotein (HDL)-cholesterol concentrations (Table 1). Apo A1 and Apo B were in the normal range. C-reactive protein was in the upper limit of the normal range (Table 1).

Transabdominal pelvic ultrasonography showed polycystic ovaries (17), while repeated pituitary MRI confirmed the presence of a microadenoma (8 mm). Once again no abnormality was detected on biochemical evaluation of hypothalamic–pituitary function.

A diagnosis of PCOS, partial lipodystrophy, insulin resistance and a non-secretory pituitary microadenoma was made, and metformin at a dose of 1700 mg/day was commenced. After 2 years of uninterrupted therapy, lack of clinical improvement led to introduction of pioglitazone at a dose of 30 mg/day, with progressive amelioration of total testosterone and FAI, insulin resistance, hyperinsulinaemia and lipid profile, a marked decrease of C-reactive protein levels and normalization of ultrasound ovarian morphology (Table 1). Menses also improved.

Figure 1 (A) Shows an anterior view of case 1 at the age of 21 and (B) shows case 2 at the age of 16.
with restoration of a eumenorrhoeic pattern, but acanthosis nigricans and hirsutism remained unimproved. For this reason, in the fifth year flutamide (a nonsteroidal antiandrogen) was added to the regimen at a dose of 250 mg/day, with progressive improvement of hirsutism. Over all the years, no significant modifications of BMI and body fat distribution were observed (Table 1). However, a slight increase of fat mass and, conversely, a slight decrease of fat free mass were detected (Table 1). Each pharmacological treatment was well tolerated and no side effects were observed.

**Case 2**

The 15 year-old sister of case 1 was referred for secondary amenorrhoea. During childhood she suffered from recurrent renal colic due to nephrolithiasis. She had adrenarche at 12 years of age. Menarche was at 13 years, followed by amenorrhoea and a rapid increase of body weight (12 kg in 10 months). Her karyotype was 46,XX.

Physical examination revealed acanthosis nigricans on neck, axillary and inguinal areas, but no hirsutism (Ferriman-Gallwey score = 6). Puberty was incomplete (Tanner stages B4, P4). BMI was 25 kg/m², with a waist circumference 75 cm and WHR 0.82. Muscular hypertrophy was apparent, with loss of fat from the extremities, trunk and gluteal regions, in spite of excess fat deposition in the face, neck, axillae and back (Fig. 1B).

Hormonal analysis revealed elevated total testosterone and androstenedione levels, decreased SHBG concentrations, and a slight increase of FAI (Table 1). FSH and LH basal levels were normal (2.4 and 6.6 mU/ml respectively), as well as serum prolactin and thyroid hormones. The normal responses of cortisol to dexamethasone (1 mg overnight) and of 17OH-progesterone to 1–24ACTH excluded adrenal diseases. Biochemical analysis showed elevated aminotransferase activities, and a slight increase of FAI (Table 1). FSH and LH basal levels were normal (2.4 and 6.6 mU/ml respectively), as well as serum prolactin and thyroid hormones.

Physical examination revealed acanthosis nigricans on neck, axillary and inguinal areas, but no hirsutism (Ferriman-Gallwey score = 6). Puberty was incomplete (Tanner stages B4, P4). BMI was 25 kg/m², with a waist circumference 75 cm and WHR 0.82. Muscular hypertrophy was apparent, with loss of fat from the extremities, trunk and gluteal regions, in spite of excess fat deposition in the face, neck, axillae and back (Fig. 1B).

Hormonal analysis revealed elevated total testosterone and androstenedione levels, decreased SHBG concentrations, and a slight increase of FAI (Table 1). FSH and LH basal levels were normal (2.4 and 6.6 mU/ml respectively), as well as serum prolactin and thyroid hormones. The normal responses of cortisol to dexamethasone (1 mg overnight) and of 17OH-progesterone to 1–24ACTH excluded adrenal diseases. Biochemical analysis showed elevated aminotransferase activities (ALT = 87 U/l, AST = 34 U/l; reference values < 31 and < 32 U/l respectively), normal serum triglycerides and total cholesterol levels, and marked decrease of serum HDL-cholesterol concentrations (Table 1). Plasma concentrations of Apo A1 and Apo B were in the normal range. C-reactive protein was in the upper limit of the normal range (Table 1).

Glucose tolerance, evaluated by OGTT, was normal, while fasting and glucose-stimulated insulin levels were high (Table 1). HOMA-IR and ISI composite values confirmed a severe insulin resistance (Table 1).
microadenoma in the sister, we performed pituitary MRI which revealed a pituitary microadenoma (5 mm) in the left part of the gland. Pituitary function was normal, as documented by fasting hormone values.

As in the case of her sister a diagnosis was made of PCOS, partial lipodystrophy, insulin resistance and a non-secretory pituitary microadenoma, and metformin was commenced at a dose of 1700 mg/day. After 3 years of uninterrupted therapy pioglitazone at a dose of 30 mg/day was added due to persisting amenorrhoea and to lack of improvement in hyperinsulinaemia, with rapid appearance of menstrual cycles and the normalization of metabolic parameters and of androgen levels (Table 1). Moreover, the normalization of ultrasound ovarian morphology was observed (Table 1). Over all years no modifications of BMI and body fat distribution phenotype were observed (Table 1). However, an increase of fat mass and, conversely, a decrease of fat free mass were detected (Table 1). Each pharmacological treatment was well tolerated and no side effect was observed.

**Genetic screening and diagnosis of Dunnigan-type familial partial lipodystrophy**

The concordant phenotype between the two sisters of partial lipodystrophy with muscular hypertrophy and marked insulin resistance led us to screen known genes involved in familial partial lipodystrophy. Both sisters were found to be heterozygous for the previously described R482Q mutation in the LMNA gene (18), establishing the definitive diagnosis as Dunnigan-type familial partial lipodystrophy complicated by severe insulin resistance and secondary PCOS.

Taking into account the new diagnosis, we completed biochemical evaluations with measurements of leptin and adiponectin. Leptin was normal in case 1 and mildly suppressed in case 2 (9.3 μg/l in case 1, 6.7 μg/l in case 2; sex and BMI-adjusted reference range = 8.6–38.9 μg/l), whereas total adiponectin was low in case 2 (1.9 μg/ml; sex and BMI-adjusted reference range = 3.5–15.5 μg/ml), but preserved in case 1 (6.5 μg/ml). Finally, the two patients underwent liver ultrasonography that showed moderate hepatic steatosis in case 2, but not in case 1, and a complete cardiovascular screening that did not reveal any macro-vascular complication.

**Familial genotyping for the LMNA R482Q mutation**

Genotyping of the mother showed her not to carry the mutation, while the father was deceased. Thus, it is likely to have been inherited from the paternal side of the family, and consistent with this the father died at young age from cerebrovascular disease, while paternal grandmother, who also died from a stroke had similar physical features to the two sisters presented.

**Discussion**

In the two sisters described, insulin resistance and PCOS occurred in the context of familial partial lipodystrophy due to a heterozygous R482Q missense mutation in LMNA gene. LMNA gene encodes lamins A and C, type V intermediate that make up a fibrous layer just beneath the inner nuclear membrane, providing a framework for nuclear envelope organization and an anchoring site for interphase chromatin (19–21). Increasing evidence suggests that nuclear lamins are also involved in cellular functions such as DNA synthesis, transcription and apoptosis (22). Although there is no immediately obvious link between perturbed organization of the nuclear lamina and loss of adipose tissue, recent work suggests that some mutations in LMNA, particularly those localized to the globular C-terminal domain of the lamin A/C protein, produce mechanical abnormalities of the nucleus and aberrant interaction with key adipogenic transcription factors (23). Furthermore, some mutations affecting codon 482 of LMNA result in accumulation of unprocessed pre-lamin A at intranuclear sites. This reduces the amount of DNA-bound sterol regulatory element binding protein 1 (a key regulator of lipogenesis) with consequent downregulation of peroxisome proliferator-activated receptor (PPAR-γ) expression (24). Expression of PPAR-γ plays a key role not only in adipocyte differentiation, but also in the entraining of adipose tissue metabolism to nutritional state, by upregulating the genes that mediate fatty acid uptake and trapping (25). This defect, in association with insufficient adipocyte tissue capacity to buffer dietary fatty acids, leads to the deposition of triglycerides and acyl-CoA in insulin-sensitive tissues, with consequent lipotoxicity and insulin resistance.

The aetiology of the highly prevalent form of PCOS remains unknown. The hypothesis that has received most attention is that hyperandrogenaemia is the primary event and that insulin resistance, when associated, may contribute to the expression of PCOS. However, the very aggressive PCOS seen in patients with either hereditary or acquired defects in insulin receptor function (26) clearly establishes the principle that insulin resistance may at least in some cases be the primary pathogenic abnormality of PCOS. Insulin resistance and compensatory hyperinsulinaemia have been directly implicated in regulating ovarian, and probably adrenal, androgen production and in increasing androgen availability in the target tissues through synergism with LH and ACTH on steroidogenic enzyme activity. This has been suggested to be exacerbated by suppression of SHBG synthesis and secretion by hyperinsulinaemia, with attendant increase in bioavailable androgens (3). Similarly, hyperinsulinaemia decreases hepatic insulin-like growth factor (IGF) binding protein-1 (IGFBP1), thereby increasing IGF-1 availability in target tissues, which has been suggested
to drive development of ovarian cysts and ovarian enlargement (27). However, in insulin receptoropathies, unlike in other forms of insulin resistance, both SHBG and IGFBP1 are either preserved or frankly elevated (28), suggesting that these last two mechanisms are not dominant in the pathogenesis of PCOS in this context. The cases described here, similarly to that described previously by Young et al. (29), illustrate clearly that monogenic insulin resistance caused by a primary defect in adipose tissue development and function, like insulin resistance caused by primary insulin signalling defects, may produce PCOS. Indeed, hyperandrogenism and oligomenorrhoea are the commonest reasons for presentation to a medical practitioner of patients with monogenic insulin resistance, and careful clinical examination for acanthosis nigricans or abnormal adipose tissue topography are key components of the initial evaluation of any patient with PCOS.

Identifying such forms of PCOS secondary to monogenic insulin resistance may have practical implications for therapy, as shown for the first time by our case. For instance, it has been shown that thiazolidinediones (TZD), a class of insulin-sensitizing drugs that selectively acts on PPAR-γ (30, 31) are successful in the treatment of insulin resistance in LMNA-linked lipodystrophy. This is supported by our case, in which insulin resistance and hyperinsulinaemia, as well as hyperandrogenism and menstrual abnormalities (expression of PCOS) improved only when pioglitazone was added to metformin. In addition, a complete remission of the pelvic ultrasound framework of PCO was observed.

Insulin sensitization by TZDs through PPAR-γ activation could be interpreted as a consequence of the ability of PPAR-γ to stimulate adipogenic differentiation of preadipocytes into mature adipocytes, in part by reducing pre-lamin A accumulation (32), and to expand depot-selective adipose tissue, including s.c. adipose tissue, with concomitant reduction in visceral depots (30). In addition TZDs act on adipose tissue enhancing its ability to trap dietary fatty acids, sequestering them in adipocytes and removing them from other insulin-sensitive tissues, including skeletal muscle (33).

However, although the main mechanism of the significant effect of pioglitazone on hyperandrogenism in our cases is probably the amelioration of insulin resistance and hyperinsulinaemia, direct effect of TZDs at the ovarian level have also been suggested. In fact, several in vitro studies in animal and human ovarian cells have shown that TZDs directly inhibit activity of ovarian steroidogenic enzymes (34–36).

The finding of non-secretory pituitary microadenomas in both sisters is interesting, and not previously documented in laminopathies. In one recent study performed in mice, nuclear lamin A/C expression was visualized inside the inner nuclear membrane of the mouse anterior pituitary cells (37). However, data linking lamin A/C gene mutations and anterior pituitary adenomas are lacking.

In conclusion, these cases emphasize the importance of identifying monogenically-determined PCOS, here due to a missense mutation (R482Q) in the lamin A/C gene with attendant lipodystrophy and severe insulin resistance. This may have practical repercussions for the management of individual patients. Our data suggest significant therapeutic efficacy of TZDs in the treatment of hyperandrogenism and ovulatory dysfunction in PCOS associated with partial lipodystrophy and severe insulin resistance.

Acknowledgements

This work was supported by a grant from the Sixth EC Program (LSHM-CT-2003-503041).

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