Follow-up study of two sisters with type A syndrome of severe insulin resistance gives a new insight into PCOS pathogenesis in relation to puberty and pregnancy outcome: a case report

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We report two sisters with profound insulin resistance associated with a novel heterozygous missense mutation in exon 19 (His1130Arg) of the insulin receptor gene. The eldest was seen after puberty at age 15 and she presented a severe form of polycystic ovary syndrome (PCOS) with biological hyperandrogenism (HA) mimicking a virilizing tumour. However, she has been able to ovulate under clomiphene citrate (CC) and to achieve two uneventful pregnancies. The patient had no glucose tolerance abnormality during pregnancies. The outcome of pregnancy was good except for a low birthweight. The youngest sister was seen earlier in life (at age 11) before puberty. First, she developed polycystic ovaries (PCO), seen under ultrasound scan, and later also developed full PCOS. This second finding gave us the opportunity to observe that PCO developed before and at the beginning of puberty despite low LH levels. We postulate that the development of PCO was the consequence of an LH-independent intra-ovarian HA likely induced by the severe hyperinsulinism in the context of genetic abnormalities.

Key words: insulin resistance/PCOS/pregnancy/puberty

Introduction

Polycystic ovary syndrome (PCOS) is a common but complex endocrine disorder. It is a major cause of anovulation and consequent subfertility. It is also associated with several metabolic disturbances, characterized by hyperinsulinism (HI) and insulin resistance, both contributing to increased risk of type 2 diabetes later in life. Some authors proposed that a genetically determined androgen hypersecretion by the ovary during, or likely long before, puberty would be the primary event leading to clinical and biochemical features of PCOS at puberty (Abbott et al., 2002). By ‘programming’ the hypothalamic–pituitary axis, the hyperandrogenism (HA) would then favour excess LH secretion. It could also predispose to insulin resistance by masculinizing the abdominal fat (Abbott et al., 2002).

In some situations however, insulin resistance is the primary abnormality, such as the type A syndrome of severe insulin resistance and acanthosis nigricans due to mutation in the insulin receptor (IR) gene (Kahn et al., 1976; Kahn, 1982; Grigorescu et al., 1984). Severe-to-moderate insulin resistance is also observed in another clinical form, referred to as HAIRAN syndrome (hyperandrogenism, insulin resistance and acanthosis nigricans), where IR mutations are not detected and obesity is often associated (Barbieri and Hornstein, 1988). The definitive mode of inheritance of type A syndrome has not been fully defined since both recessive and dominant patterns of inheritance have been reported. In the current understanding, missense mutations of the gene coding for IR are the most commonly reported genetic abnormalities (Taylor et al., 1990; Accili et al., 1992; Barbieri, 1994; Rique et al., 2000a). Both type A and HAIRAN syndromes characterized by severely insulin-resistant situation with compensatory HI (Barbieri and Hornstein, 1988) may constitute a subphenotype of PCOS covering almost 5% of all women with HA. Clinical signs of type A syndrome appeared early in life prior to puberty. The development of HA occurs generally post-pubertally when increased levels of gonadotropins act synergistically with elevated levels of endogenous insulin, thus initiating excessive ovarian androgen production (Friedman et al., 1987; Omar et al., 2004). Little is known, however, about the time of apparition of polycystic ovaries (PCO). The consequences of severe insulin resistance and HI on the reproductive function in women raise therapeutic concerns. For instance, it has been shown that metformin...
(Seale et al., 2000) or troglitazone (Elkind-Hirsch and McWilliams, 1999) administration may be useful for treatment of infertility in these patients. In the literature, there are few data about the pregnancy outcome in women with type A or HAIRAN, and most of them are retrospective data.

We report here on a case of two sisters who had profound insulin resistance associated with heterozygous novel missense mutation in the exon 19 (His1130Arg) of the IR gene, clearly indicating type A syndrome. The eldest sister was seen after puberty and gave us the opportunity to analyse the impact of severe insulin resistance on ovulation and on pregnancy outcome. The second observation gave us the unique opportunity to evaluate respective roles of HI and LH on the genesis of PCO and polycystic ovary syndrome (PCOS) during puberty.

To our knowledge, this has been seldom reported in the literature, and it has never been established that insulin might stimulate the ovary in the absence of gonadotropins.

**Case report**

**Case 1**

A 15-year-old girl (index case or proband) was referred to our department for the evaluation of hirsutism and primary amenorrhoea. She had a familial history of maternal type 2 diabetes. Her childhood was uneventful (birthweight 2600 g), with no precocious pubarche. Physical examination revealed severe insulin resistance on ovulation and on pregnancy outcome. The second observation gave us the unique opportunity to evaluate respective roles of HI and LH on the genesis of PCO and polycystic ovary syndrome (PCOS) during puberty.

To our knowledge, this has been seldom reported in the literature, and it has never been established that insulin might stimulate the ovary in the absence of gonadotropins.

Laboratory evaluation revealed a serum total testosterone (TT) level of 6.27 nmol/l (normal range, 0.34–2.32), delta-4-androstenedione (A) level of 14.25 nmol/l (normal range, 1.75–7.7), dehydroepiandrosterone sulphate (DHEAS) level of 3.1 μmol/l (normal range, 2.9–12) and sex hormone-binding protein (SHBP) level of 24.8 nmol/l (normal range, 30–60). Serum basal 17-α-hydroxyprogesterone (17-OHP) was slightly elevated (7.69 nmol/l), but the increase was normal (12.9 nmol/l) 60 min after Synacthen injection (0.250 mg, intravenous). LH and FSH basal levels were 5.6 IU/l (normal range, 2–6) and 5.1 IU/l (normal range, 1–8), respectively. Serum estradiol (E2) level was 176 pmol/l (normal range, 92–367). LH and FSH basal levels were 5.6 IU/l (normal range, 2–6) and 5.1 IU/l (normal range, 1–8), respectively. Serum estradiol (E2) level was 176 pmol/l (normal range, 92–367). Normal free urinary cortisol excluded a Cushing syndrome. Basal serum prolactin was normal.

Routine serum biochemical profile was otherwise unremarkable, including liver and kidney function tests. An oral glucose tolerance test (OGTT, 75 g) was then performed, and blood samples were collected at fast and after 2 h for glucose and insulin determination. Glucose tolerance was normal, with normal fasting and stimulated glycaemia (3.80 and 6.10 mmol/l, respectively). Marked fasting and stimulated insulin levels were observed (72.0 and 436 μIU/ml, respectively) (fasting normal range 0.9–9.7 μIU/ml). Serum high-density lipoprotein (HDL) cholesterol was only slightly decreased (0.010 mmol/l, normal range 0.10–0.020).
Laboratory evaluation revealed a serum TT level of 1.32 nmol/l, an A level of 3.91 nmol/l, a DHEAS level of 0.08 μmol/l, and an SHBP level of 0.66 nmol/l. LH and FSH levels were <0.5 and 2.5 IU/l, respectively. Serum E2 level was 51 pmol/l.

At OGTT, glucose tolerance was normal, but basal and stimulated insulin levels were excessive (27.4 and 1090 mU/l, respectively). Total cholesterol, HDL cholesterol, low-density lipoprotein cholesterol and triglycerides were normal.

Ultrasonography through abdominal route revealed the presence of enlarged ovaries (right ovarian area: 6.8 cm² and left ovarian area: 8.1 cm²) suggesting PCO. No precise follicle count could be done because of the lack of spatial resolution, as occurs frequently by the abdominal route. No treatment was proposed, and the patient was simply followed-up.

One year later, physical examination revealed signs of HA, mainly acne and hirsutism (F-G score: 18). TT level was 9.67 nmol/l and A level was 19.3 nmol/l. Serum basal 17-OHP and DHEAS were normal (2.87 nmol/l and 1.4 μmol/l, respectively). LH and FSH levels were 4.5 and 2.0 IU/l, respectively. In order to stop the progression of HA, the patient was then placed under a continuous treatment with cyproterone acetate (CA) 25 mg/day. She was also given percutaneous estradiol (1 mg/day) in order to complete the pubertal development, since CA induces gonadotropin suppression. She did not receive metformin, given the partial and insufficient efficacy of this drug in her sister. Six months later, physical examination revealed a reduction of the hirsutism scored at 10 with the F-G score. TT level was 1.18 nmol/l and A level was 2.55 nmol/l. Serum basal 17-OHP and DHEAS levels were 1.33 nmol/l and <0.8 μmol/l, respectively. LH and FSH levels were 0.5 and 1.4 IU/l, respectively. However, the ultrasonic appearance of the ovaries was unchanged. She completed her puberty at the age of 14 years (Tanner stage 5), with a final height of 161 cm.

**Genetic studies**

All 22 exons and flanking introns of the IR gene (Chr19 p13.2) were screened for potential mutation. We found that the two sisters and the mother were heterozygous for a novel missense in exon 19 (His1130Arg).

**Materials and methods**

**Hormonal immunoassays**

Serum glucose was determined by a glucose oxidase method. Serum insulin levels were measured by an RIA (Bi–insulin RIA, Diagnostics Pasteur, Marnes de Coquette, France) using an anti-pig insulin polyclonal antibody and human [125I] insulin as a tracer. Results are expressed as μIU/ml in terms of WHO 66/304 Reference Preparation. This RIA displayed 40% cross-reactivity with human proinsulin. Intra- and inter-assay coefficients of variation were less than 6 and 9%, respectively.

Serum gonadotropin levels were evaluated by two immunoassays provided by Cis Bio International (Gif-sur-Yvette, France). These immunometric assays used monoclonal antibodies raised against LH and FSH. Results are expressed as mIU/ml in terms of the first International Reference Preparation 68/40 (LH) and the Second International Reference Preparation 78/549 (FSH). Intra- and inter-assay coefficients of variation were less than 5% for the two immunoassays.

Estradiol concentrations were measured in unextracted serum by RIA using kits provided by Biomérieux (Lyon, France). The detection limit was 12 pg/ml. The intra- and inter-assay coefficients of variation were 9.8 and 8.7%, respectively.

TT concentration was measured by radioimmunooassay using the Coat-A-Count total testosterone (Diagnostic Product Corporation, Garenne, Colombes, USA). SHBP was measured using immunometric assay on the Immulite 2000 analyser (Diagnostic Product Corporation). 17-OHP was measured by RIA (Schering-Cis Bio International) and A by RIA (Immunotech-Beckman, Marseille, France).

**Screening for mutation in IR**

Genomic DNA was isolated from peripheral blood cells using BACC 3 DNA isolation kit (Amersham, Orsay, France).

The 22 exons and flanking introns of the IR gene were amplified using PCR and specific primers adapted from Seino et al. (1990). PCR conditions were denaturation at 96°C for 6 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 30 s, extension at 72°C for 50 s, and post-extension at 72°C for 10 min. The amplification of exon 1 was carried out in the presence of 5% v/v dimethylsulphoxide. The PCR products were purified using Microcon PCR (Amicon, Millipore, Bedford, MA, USA) and then sequenced directly from both ends using the same primers as used for PCR and ampliTaq FS ABI PRISM Dye Terminator cycle sequencing kit (ABI, Foster City, CA, USA) on an ABI 373 A DNA sequencer as previously described in detail (Rouard et al., 1997).

**Discussion**

As the two sisters were heterozygous carriers for His1130Arg mutation in the IR, we defined the clinical phenotype as classical type A insulin-resistance syndrome. Although functional analysis was not performed in this study, it is very likely that the mutation would affect the kinase activity of the β-subunit of the IR. The mutation His1130Arg is located just upstream of the catalytic base from residues 1132–1137 (Asp-Leu-Ala-Arg) of the kinase domain and is directly involved in the binding of the ATP moiety together to residues 1131 (Arg), 1132 (Asp) and 1133 (Leu). The closest mutation was detected at position 1131, with severe alteration of the kinase activity. Further studies, mainly through pointed mutagenesis, are necessary to demonstrate the deleterious effect on kinase. It should be emphasized that in our current experience, current functional testing of IR function on circulating cells (insulin binding and kinase activity) is of poor diagnosis value, since both binding and activation of the kinase are altered in patients with severe insulin resistance, due to both intrinsic defect and severe down-regulation of the IR on the cell surface. Therefore, we could reasonably conclude that the genetic alteration in this family may account most likely for the phenotype of severe insulin resistance observed in the two sisters. It is now currently accepted that other modifier genes may also be involved in the full spectrum of manifestations of this syndrome, including IRS-1 and IRS-2 polymorphisms (Ait El Mkadem et al., 2001).

In face of a such clear-cut situation of type A syndrome, the goal of our investigation was to observe the effects of severe HI and/or insulin resistance on puberty, fertility and pregnancy outcome. Indeed, case 2 (younger sister) could be evaluated before starting puberty. Unexpectedly, asymptomatic PCO
were found at ultrasound, despite low serum pre-pubertal LH level. It is only when LH level increased above the detection threshold of the assay that the patient became hyperandrogenic. We speculate therefore that the development of PCO without PCOS may occur with minimal pre-pubertal levels of LH. Either by itself or through amplification of LH, HI may have stimulated the ovarian steroidogenesis (Moghetti et al., 1996), thus creating an intra-ovarian HA, which is considered as the key factor for development of PCO (Jonard and Dewailly, 2004). At this stage, this HA would not be perceived in peripheral blood, as suggested by the absence of clinical HA and the normality of serum androgen levels. Later on, normal adult levels of serum LH would have allowed sufficient production of androgens to induce systemic clinical and biological symptoms of ovarian HA. However, this remains speculative since no such observation of PCO without PCOS occurring early during puberty has been reported so far in the situation of severe HI due to a genetic insulin resistance. PCO have been described during the follow-up of premenarchal girls developing later on a full-blown PCOS, but they had already a slight HA and their LH level tended to be exaggerated (Venturoli et al., 1995). Although HI is here more severe, this observation is in reminiscence to the one of girls whom HI, resulting from reduced fetal growth leads to precocious pubarche and then to functional ovarian HA in late puberty (Ibanez et al., 1996). In addition, the follow-up of case 2 offered the unique opportunity to observe that an excessive LH serum level was not a prerequisite for the constitution of PCO, and we speculate that this occurred because of the intra-ovarian HA. Alternatively, one could suggest that insulin itself induced PCO independently of circulating gonadotropins and androgens, through an effect on insulin/IGF receptors. However, experimental data reporting such an effect in the absence of LH are scarce in the literature.

The eldest sister was first referred while she had already a full-blown and severely hyperandrogenic PCOS. Interestingly, she was not resistant to CC, despite her severe HI. Since she was concomitantly treated by metformin, this could be explained by the favourable effect of this drug on the anovulation of PCOS (Vandermolen et al., 2001). However, metformin acts on insulin sensitivity downstream of the receptor signalling events (Wiernsperger and Bailey, 1999), and it is unclear therefore whether the normal sensitivity to CC was the result of this treatment in this particular case. Indeed, no favourable effect of metformin was seen on OGGT results in case 1 in contrast to data from others obtained in a similar situation (Rique et al., 2000b). We also expected the patient to be resistant to CC because she was amenorrheic and had a severe HA, both features being considered as strong predictors of CC resistance (Imani et al., 2002). On the other hand, these negative factors were counterbalanced by the patient’s young age and absence of being overweight.

The moderate impact of severe HI and/or insulin resistance on pregnancy outcome in case 1 also deserves discussion. PCOS women with common insulin resistance at the pregestational stage have been shown to be prone to impaired gestational glucose tolerance or gestational diabetes mellitus (GDM) during pregnancy (Lanzone et al., 1996; Weerakiet et al., 2004). In the literature, there are no data about the outcome of pregnancy in women with PCOS and severe insulin resistance. Our patient developed no mild gestational hyperglycaemia nor GDM, despite metformin being stopped, and she received no specific nutritional advice. Our patient did not develop PIH nor pre-eclampsia, although the frequency of PIH is significantly elevated in PCOS women (11.5%) compared to control (0.3%) (Fridstrom et al., 1999), as well as one of pre-eclampsia in the common form of insulin-resistant PCOS (13.5%) compared to controls (7.0%) (Bjercke et al., 2002). The pathophysiological explanation of such findings needs further studies. Since our patient was not obese, it might be questioned whether metabolic complications occurring during pregnancy in insulin-resistant women with PCOS have more to do with overweight than with HI by itself. It remains, however, that she had uterine Doppler abnormalities with elevated resistance index and a Notch, suggesting a vasculorenal syndrome, and that she gave birth at term to children who were small for gestational age (<10th percentile).

In summary, these two observations give a new insight into the difficult issue of the relationship between insulin and PCOS. Our data suggest that PCO but not PCOS may occur independently of LH during puberty, perhaps through the sole action of insulin.

References


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