CASE REPORT

A 31-year-old woman with infertility and polycystic ovaries diagnosed with non-classic congenital adrenal hyperplasia due to a novel CYP21 mutation

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ABSTRACT. A 31-yr-old woman presenting with a history of hirsutism, amenorrhea, and infertility was previously assumed to have polycystic ovary syndrome. A new gynecological-endocrine evaluation demonstrated elevated testosterone/SHBG ratio, serum 17-hydroxyprogesterone (17-OHP), and urinary pregnantriol. She was diagnosed with non-classic congenital adrenal hyperplasia. In spite of treatment with dexamethasone and fludrocortisone in doses that suppressed adrenal androgens and 17-OHP into normal range or below, she did not ovulate. Clomiphene citrate and then FSH/hCG treatment in several cycles gave no consistent ovulation. Progesterone levels remained elevated throughout the cycles indicating a possible contribution from the adrenals. Oral glucose tolerance was normal, but the homeostasis model assessment index indicated insulin resistance. With metformin 1500 mg daily the index decreased remarkably from 2.77 to 0.96 with a few ovulations but no pregnancy occurred. Three cycles of IVF treatment thereafter were unsuccessful. Three months after the last *in vitro* fertilization (IVF) cycle, still on dexamethasone, fludrocortisone, and metformin, her menstruations became regular and she thereafter became pregnant. During pregnancy metformin was discontinued and dexamethasone replaced with prednisolone. Mild gestational diabetes developed and insulin was given. A healthy boy was born at term by elective Cesarean section. A CYP21-gene analysis had not indicated any of the known mutations but after gene sequencing a novel mutation was found, namely R233G. This case confirms the necessity of adding an analysis of 17-OHP when evaluating women with hirsutism and menstrual disturbances and if an elevated value is found, the advantage of performing a mutation analysis to facilitate counseling and decisions on treatment.
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INTRODUCTION

21-hydroxylase-deficient non-classic congenital adrenal hyperplasia (non-classic CAH) has been suggested to represent the most frequent monogenic autosomal recessive disorder in man (1). It is due to mutations within the cytochrome P450 21-hydroxylase (CYP21) gene. Non-classic CAH affects 0.1% of Caucasian populations, 1-2% of Hispanics and Yugoslavs and 3-4% of Ashkenazi (Eastern European) Jews (1). In a study of 400 hirsute French women, 6% had hormone profiles compatible with non-classic CAH (2). Among 873 women looking into symptoms of androgen excess in Alabama, USA, 1.6% were diagnosed to have non-classic CAH and 0.6% to have classic CAH, both genetically verified (3). The difference in prevalence may reflect ethnic dissimilarities of the two studied populations.

By definition, patients with non-classic 21-hydroxylase deficiency do not have clinical signs and symptoms at birth, and may not be detected in neonatal

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screening programs with 17-hydroxyprogesterone (17-OHP). Instead they often develop androgenic symptoms during the peri-pubertal period or as adults (4), in contrast to classic 21-hydoxylase deficiency which usually demonstrates signs and symptoms at birth or shortly thereafter (5). In non-classic CAH females over 10 yr of age the presenting symptoms reported were hirsutism (59%), oligomenorrhea (54%), acne (33%), infertility (13%), clitoromegaly (10%), alopecia (8%), primary amenorrhea (4%), and premature pubarche (4%) (6).

Biochemical evaluation shows elevation of the steroid precursor 17-OHP in serum either at baseline or after ACTH stimulation. In addition, the urinary excretion of the metabolite pregnantriol may be elevated. The diagnosis may then be confirmed by genetic testing.

Herein we report the clinical course in a female patient evaluated for infertility who was diagnosed as non-classic CAH and was found to be the first and hitherto only case known with a new CYP21-gene mutation, R233G.

CASE REPORT

A 31-yr-old married woman of Swedish origin was referred because of infertility. She was a non-smoker and worked as a registered nurse. She had no family history of endocrine disorders including diabetes. Menarche occurred at 9 yr of age, thereafter she had oligomenorrhea until 15 yr of age when she lost weight, 10 kg to a body mass index (BMI) of 20.2 kg/m^2, due to dieting. She developed amenorrhea and was prescribed combined oral contraceptives, experiencing monthly bleedings until the age of 17 yr when the pill was discontinued. Hirsutism and recurrence of amenorrhea then followed.

A gynecological-endocrine evaluation at 19 yr of age indicated modest hyperandrogenism with slightly elevated serum concentrations of testosterone, DHEAS, and androstenedione and an elevated testosterone/SHBG ratio (0.11; reference <0.05). Serum PRL, LH, FSH, and LH/FSH ratio were normal as well as serum cortisol after low dose dexamethasone suppression. Gynecological examination revealed a moderate clitoromegaly. Pelvic organs were normal at laparoscopy. She was considered to have polycystic ovary syndrome (PCOS) and treatment was once again combined oral contraceptives.

The next evaluation took place 12 yr later when she was 31 yr old, still amenorrhoic after discontinuing the pill for 1.5 yr during which time she had tried to become pregnant. She was moderately hirsute with a small moustache, had no acne or signs of virilization except for a moderate clitoromegaly and reported no history of salt loss crisis. Her height was 168 cm and BMI 24.8 kg/m^2. Blood pressure was 110/70 mmHg. Vaginal ultrasonography showed bilateral ovaries with increased stroma and multiple follicles consistent with PCO. Serum PRL, TSH, T_4 and antibodies against thyroid peroxidase were all within normal range. Serum testosterone was normal (2.7 mmol/l; reference 0.3-3.0) but the testosterone/SHBG ratio was elevated (0.084; reference <0.05), serum 17-OHP was markedly elevated (293 nmol/l; reference <10) as well as the urinary metabolite pregnantriol (47 nmol/24-h; reference <8) and 24-h urinary excretion of cortisol was almost normal (95 nmol/24-h; reference 100-430). A sperm analysis of her husband indicated slight subfertility probably without clinical significance. Her diagnosis was non-classic CAH and she received dexamethasone 0.25 mg daily but did not ovulate in spite of increasing the dose to 0.5 mg and adding fludrocortisone 0.05 mg daily. Clomiphene citrate in several cycles was without effect. Several cycles of FSH/hCG administration resulted in a good estrogen response but no consistent ovulation. Progesterone levels remained elevated (10-20 nmol/l; reference: follicular phase and menopause <3.0, luteal phase >13.0) throughout the cycles with unfavourable endometrial development. This indicates a possible contribution from the adrenals in spite of increased dexamethasone dose to 1 mg per day while keeping the fludrocortisone dose at 0.05 mg daily. Serum 17-OHP and 24-h urinary pregnantriol now showed normal low values (2.6 nmol/l and 0.8 µmol/l respectively). Serum testosterone and DHEAS were suppressed below the detection level of the analyses. The CYP21-gene analysis was made with negative result for the known mutations. She discontinued the corticosteroid treatment for 1 yr and became amenorrhoic again. Dexamethasone 0.5 mg and fludrocortisone 0.05 mg were resumed and a new evaluation was performed including a normal oral glucose tolerance test (P-glucose at 2-h was 4.6 mmol/l). However, the homeostasis model assessment (HOMA) index, calculated from fasting P-glucose and insulin, almost reached the level of insulin resistance (2.73; ≥2.77 indicating insulin resistance) (7). In order to increase insulin sensitivity metformin 1500 mg daily was prescribed. The HOMA-index decreased to 0.96 during medication. She experienced a few ovulations but no pregnancy occurred. Once more several cycles of FSH treatment were tried. Finally 3 cycles of *in vitro* fertilization (IVF)-treatment were performed; 1 with transfer of 2 fresh embryos and 2 more cycles with transfer of 2 frozen embryos. Unfortunately no pregnancy was achieved.

The patient continued with dexamethasone, fludrocortisone, and metformin. Three months after the last IVF cycle, regular monthly menstruations recurred and she became pregnant after another 3 months. A healthy boy was born at term by elective Cesarean section. During pregnancy metformin was discontinued and dexamethasone replaced with prednisolone 5 mg daily. A mild gestational diabetes developed during the last trimester and short acting insulin was given before meals. Glucose levels and glycated hemoglobin were normal during the rest of the pregnancy.

A second CYP21-gene analysis did not indicate any of the known mutations but after gene sequencing a novel mutation was found, namely R233G in hemi- or homozygous form (genotype R233G/deletion or R233G/ R233G) (8).

MATERIALS AND METHODS

Mutation analyses of the CYP21 gene was first performed by allele-specific PCR on high molecular weight DNA from peripheral leucocytes. This method detects the 9 CYP21 mutations that are responsible for 95% of all disease alleles in 21-hydoxylase deficiency (9). As none of them was detected the complete CYP21 gene was sequenced (8).

Radioimmunoassay methods were used for the determination of serum 17-OHP (CIS Bio International, Gif sur Yvette, France) and androstenedione (DiaSorin S.p.A., Saluggia, Italy). Serum testosterone and insulin were measured by fluoroimmunoassay (AutoDelfia, Wallac Inc, Turku, Findland). Serum DHEAS was measured on Nichols Advantage automatic immune analyser (Nichols Institute Diagnostics, San Clemente, California, USA). The urinary steroid metabolite, pregnantriol, was determinated by gas chromatography and gas chromatography-mass-spectrometry (10). The HOMA-index [insulin/($22.5e^{-\ln glu}$)] using a single fasting sample of insulin and glucose was calculated as an estimation of insulin resistance (11). A threshold of 2.77 for insulin resistance has been suggested (7). The other laboratory tests were measured using routine assays.

DISCUSSION

Both in the National Institutes of Health (NIH) 1990 criteria and in the Rotterdam 2003 revised criteria as well as in the Position Statement from the Androgen Excess Society, an exclusion of other aetiologies such as CAH, androgen-secreting tumors and Cushing's disease is a prerequisite for the diagnosis of PCOS (12-14). Our patient was first evaluated several years before these criteria came into use, hence she was diagnosed as having PCOS instead of non-classic CAH and did not obtain a correct diagnosis until evaluation for infertility at an age of 31 yr.

The ultrasonographic abnormalities of the ovaries found in our patient may be similar in women with PCOS and non-classic CAH (15, 16). In a recent Turkish study 3 out of 43 patients (7%) with PCO were diagnosed as having non-classic CAH (17) while others have reported both higher (19%) (18) and lower (1%) (19) prevalence, probably according to different ethnic populations. Thus, clinical presentation of 21-hydoxylase-deficient non-classic CAH may be indistinguishable from other conditions with elevated androgens such as PCOS. Consequently, an analysis of basal and often also ACTH stimulated levels of 17-OHP should be included in the diagnostic work-up of women presenting with signs of androgen excess. However, the hitherto suggested cut-off levels of basal and ACTH-stimulated 17-OHP of >15nmol/l and >30 nmol/l respectively, have been questioned because lower baseline values are found in a few patients with genetically verified non-classic CAH, that can only be detected with ACTH stimulation (20).

Another common feature seen both in women with PCOS and non-classic CAH is a significant reduction in insulin sensitivity, although not clinical diabetes, among young women with non-classic CAH as compared with controls of similar age and weight (21, 22). Several studies have shown that insulin resistance is also an integral feature of PCOS. The associated hyperinsulinemia, suppression of SHBG, and abnormal androgen secretion and therewith abnormal follicular development leads to dysfunctional ovarian and menstrual activity. The pathogenesis has not been settled. Some authors suggest that elevated androgens hamper insulin sensitivity, while others favor the hypothesis that hyperinsulinemia stimulates ovarian androgen production (23, 24). In our patient insulin sensitivity was substantially improved when she received metformin in addition to dexamethasone and fludrocortisone, and she experienced a few spontaneous ovulations. This positive effect of metformin has been confirmed in a recent structured literature review of metformin treatment in clomiphene resistant patients with PCOS (25).

Data regarding reproductive problems associated with non-classic CAH mainly originates from studies where patients had been referred for symptoms of hyperandrogenism and/or infertility. It is obvious that these data are not reliable. It has been shown from family studies that many individuals with non-classic CAH have minimal symptoms and do not seek medical attention (5). In a case study conducted in France (26), 53 females referred for hirsutism (62%), sterility (17%) or irregular menses (21%) were diagnosed with non-classic 21-hydroxylase deficiency. Only 20 desired a pregnancy and 10 of them became pregnant before the diagnosis without any treatment. Nine conceived when treat-

ed with hydrocortisone. Our patient did neither conceive during treatment with dexamethasone and fludrocortisone alone nor when repeated cycles of clomiphene citrate or FSH/hCG were added. During the latter treatment she produced estrogens but a concomitant progesterone production most likely from the adrenals inhibited the quality of cervical mucus and endometrial development. This phenomenon has been earlier described in classic CAH patients (27). No pregnancy occurred during three IVF cycles in spite of good quality embryos, probably also due to an unsuitable endometrium inhibiting implantation. Although mineralcorticoids are not necessary in treating non-classic CAH patients in our clinical practices, we often add a low dose of fludrocortisone to the glucocorticoid medication in an effort to keep the dose of the latter as low as possible, minimizing the risk of glucocorticoid side effects.

The first genetic evaluation performed in our patient used the allele-specific PCR on high molecular weight DNA from peripheral leucocytes that detects the 9 CYP21 mutations responsible for 95% of all disease-causing alleles in CAH (9). There is generally a good correlation between the various genetic abnormalities and the clinical phenotype (28, 29). Three mutations in CYP21 account for the majority of cases of non-classic CAH when present in homozygous form or as part of a compound genotype, including another CYP21 mutation. These are V281L (30), P453S (31, 32), and P30L (33). Three other rare mutations have been reported in association with non-classic CAH, namely P105L (34), R339H (35) and V304M (36). As none of these were detected a complete CYP21 gene sequencing was later performed (8).

Our patient demonstrated a novel mutation in association with non-classic CAH, R233G, which was present in either homozygous (R233G/R233G) or hemizygous form (R233G/deletion), the latter most probable as no consanguinity was known in the family. The knowledge that the diagnosis of non-classic CAH was confirmed by mutation analysis later made the patient pursue her treatment with dexamethasone, fludrocortisone, and metformin.

The advantage of pursuing a mutation analysis in women with not only classic but also non-classic CAH has been discussed recently by Moran et al. (37). In their international multicenter study the outcomes of 203 pregnancies among 101 women with 21-hydoxylase deficient non-classic CAH was reviewed. The risk of a mother with non-classic CAH giving birth to a child with classic CAH is 2.5% and non-classic CAH 14.8%. The knowledge of the mutations is obviously important in counseling and treatment.

In conclusion, although the frequency of non-classic CAH varies between different populations, this case confirms the necessity of adding an analysis of 17-OHP when evaluating women with hirsutism and menstrual disturbances. The cut-off levels both of basal 17-OHP and ACTH stimulated 17-OHP used for the diagnosis of non-classic CAH have however been questioned lately (20). Hence, when the diagnosis is strongly suspected because of high levels of androgens and increased 17-OHP, a mutation analysis is recommended and, if the first is negative, a gene sequencing in order to facilitate decisions on counseling and treatment. The patient finally got pregnant after a period of treatment with a combination of dexamethasone, fludrocortisone and metformin. It is possible that if this type of treatment had been persisted during a longer period without other interventions she could have got pregnant years earlier.

REFERENCES

1. Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequency of nonclassical steroid 21-hydroxylase deficiency. Am J Hum Genet 1985, 37: 650-67.
2. Kuttenn F, Couillin P, Girard F, et al. Late-onset adrenal hyperplasia in hirsutism. N Engl J Med 1985, 313: 224-31.
3. Azziz R, Sanchez LA, Knochenhauer ES, et al. Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab 2004, 89: 453-62.
4. Falhammar H, Thorén M. An 88-year-old woman diagnosed with adrenal tumor and congenital adrenal hyperplasia: connection or coincidence? J Endocrinol Invest 2005, 28: 449-53.
5. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-Hydoxylase Deficiency. Endocr Rev 2000, 21: 245-91.
6. Moran C, Azziz R, Carmina E, et al. 21-Hydroxylase-deficient nonclassical adrenal hyperplasia is a progressive disorder: a multicenter study. Am J Obstet Gynecol 2000, 183: 1468-74.
7. Bonora E, Kiechl S, Willeit J, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 1998, 47: 1643-9.
8. Robins T, Carlsson J, Sunnerhagen M, Wedell A, Persson B. Molecular model of human CYP21 based on mammalian CYP2C5: structural features correlate with clinical severity of mutations causing congenital adrenal hyperplasia. Mol Endocrinol 2006, 20: 2946-64.
9. Wedell A, Luthman H. Steroid 21-hydroxylase deficiency: two additional mutations in salt-wasting disease and rapid screening of disease-causing mutation. Hum Mol Genet 1993, 2: 499-504.
10. Axelson M, Sahlberg BL, Sjövall J. Analysis of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-mass spectrometry. J Chromatogr 1981, 224: 355-70.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostatis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985, 28: 412-9.
12. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome; towards a rational approach. In: Dunaif A, Givena JR, Haseltine F. eds. Polycystic ovary syndrome. Boston: Blackwell Scientific 1992, 377-84.
13. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome. Fertil Steril 2004, 81: 19-25.
14. Azziz R, Carmina E, Dewailly D, et al. Position statement: criteria for defining polycystic ovary syndrome: an androgen excess society guideline. J Clin Endocrinol Metab 2006, 91: 4237-45.
15. Lobo RA, Goebelsmann U. Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydoxylase deficiency mimicking polycystic ovarian disease. Am J Obstet Gynecol 1980, 138: 720-6.
16. New MI. Nonclassical congenital adrenal hyperplasia and the polycystic ovarian syndrome. Ann N Y Acad Sci 1993, 687: 193-205.
17. Kamel N, Tonyukuk V, Emral R, Corapcioglu D, Bastemir M, Güllü S. The prevalence of late onset congenital adrenal hyperplasia in hirsute women from central Anatolia. Endocrin J 2003, 50: 815-23.
18. Benjamin F, Deutsch S, Saperstein H, Seltzer VL. Prevalence of and markers for attenuated form of congenital adrenal hyperplasia and hyperprolactinemia masquerading as polycystic ovarian disease. Fertil Steril 1986, 46: 215-21.
19. Cobin RH, Futterweit W, Fiedler RP, Thornton JC. Adrenocorticotropic hormone testing in idiopathic hirsutism and polycystic ovarian disease: a test of limited usefulness. Fertil Steril 1985, 44: 224-6.
20. Bachega T, Billerbeck A, Marcondes J, Madureira G, Arnold I, Mendonca B. Influence of different genotypes on 17-hydoxyprogesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 2000, 52: 601-7.
21. Speiser PW, Serrat J, New MI, Gerner JM. Insulin insensitivity in adrenal hyperplasia due to nonclassical steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 1992, 75: 1421-4.
22. Saygili F, Oge A, Yilmaz C. Hyperinsulinemia and insulin insensitivity in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: the relationship between serum leptin levels and chronic hyperinsulinemia. Horm Res 2005, 63: 270-4.
23. Dunaif A. Insulin resistance and polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997, 18: 774-800.
24. Livingstone C, Collison M. Sex steroids and insulin resistence. Clin Sci (Lond) 2002, 102: 151-66.
25. Siebert TI, Kruger TF, Steyn DW, Nosarka S. Is the addition of metformin efficacious in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome? A structured literature review. Fertil Steril 2006, 86: 1432-7.
26. Feldman S, Billaud L, Thalabard JC, et al. Fertility in women with late-onset adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 1992, 74: 635-9.
27. Helleday J, Siwers B, Ritzén EM, Carlström K. Subnormal androgens and elevated progesterone levels in women treated for congenital virilizing 21-hydroxylase deficiency. J Clin Endocrinol Metab 1993, 76: 933-6.
28. Wedell A, Thilén A, Ritzén EM, Stengler B, Luthman H. Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. J Clin Endocrinol Metab 1994, 78: 1145-52.
29. Jääskeläinen J, Levo A, Voutilainen R, Partanen J. Population-wide evaluation of disease manifestation in relation to molecular genotype in steroid 21-hydroxylase (CYP21) deficiency: good correlation in a well defined population. J Clin Endocrinol Metab 1997, 82: 3293-7.
30. Speiser PW, New MI, White PC. Molecular genetic analysis of nonclassical 21-hydoxylase deficiency associated with HLA-B14,DR1. N Engl J Med 1988, 319: 19-23.
31. Owerbach D, Sherman L, Ballard AL, Azziz R. Pro-453 to Ser mutation in CYP21 is associated with nonclassic steroid 21-hydroxylase deficiency. Mol Endocrinol 1992, 6: 1211-5.
32. Wedell A, Ritzén ME, Haglund-Stengler B, Luthman H. Steroid 21-hydroxylase deficiency: three mutated alleles and establishment of phenotype-genotype relationships of common mutations. Proc Natl Acad Sci U S A 1992, 89: 7232-6.
33. Tunsie-Luna MT, Speiser PW, Dumic M, New MI, White PC. A mutation (Pro-30 to Leu) in CYP21 represents a potential nonclassic steroid 21-hydroxylase deficiency allele. Mol Endocrinol 1991, 5: 685-92.
34. Nikoshkov A, Lajic S, Holst M, Wedell A, Luthman H. Synergistic effect of partially inactivated mutations in steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 1997, 82: 194-9.
35. Helmberg A, Tusie-Luna MT, Tabarelli M, Kofler R, White PC. R339H and P453S: CYP21 mutations associated with nonclassic steroid 21-hydoxylase deficiency that are not apparent gene conversion. Mol Endocrinol 1992, 6: 1318-22.
36. Lajic S, Clauin S, Robins T, et al. Novel mutations in CYP21 detected in individuals with hyperandrogenism. J Clin Endocrinol Metab 2002, 87: 2824-9.
37. Moran C, Azziz R, Weintrob N, et al. Reproductive outcome of women with 21-hydroxylase-deficient nonclassic adrenal hyperplasia. J Clin Endocrinol Metab 2006, 91: 3451-6.