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What May Be the Markers of the Male Equivalent of Polycystic Ovary Syndrome?

[This paper is dedicated to Vratislav Schreiber at the occasion of his 80th birthday]

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Summary

Polycystic ovary syndrome (PCOS), the most common endocrinopathy in women (with a prevalence of 5-10 %), is characterized by hormonal and metabolic imbalance. Complexity of symptoms of close relatives of women with PCOS and genetic autosomal trait initiated a hypothesis about the existence of a male equivalent of PCOS. Premature alopecia was suggested as one of the signs of a male phenotype of this syndrome. The present study investigated a group of 30 men, in which premature hair loss started before 30 years of age. In all patients, their hormonal profile was determined. It was possible to form two subgroups. The first one showed similar hormonal changes as women with PCOS, the other had either no anomalies in steroid spectrum or just only lower level of sexual hormones binding globulin (SHBG). Both subgroups did not differ in either BMI or age. In all men with premature alopecia insulin tolerance test was also carried out and the occurrence of allele 3 INS VNTR was investigated, which is one of the candidate genes for PCOS. The subgroup with hormonal changes resembling those of women with PCOS showed a significantly higher insulin resistance than the group without these changes. About one third of the premature balding men showed the combination of hormonal shifts and higher insulin resistance. This frequency corresponds to the prevalence of PCOS in women. There was no significant difference between the two subgroups in the occurrence of allele 3 INS VNTR.

Key words

Premature androgenic alopecia • Polycystic ovary syndrome • SHBG • Insulin tolerance • Male phenotype of PCOS

Introduction

Polycystic ovary syndrome (PCOS) belongs to the most common endocrinopathies in women of fertile age. Its prevalence is reported between 5-10 % (Asuncion *et al.* 2000, Dunaif and Thomas 2001). PCOS in women is defined as anovulation and hyperandrogenism and it is characterized not only by imbalance in sexual hormones but in many cases also by higher insulin resistance and often also by obesity. It represents therefore a substantial risk for diabetes mellitus type 2 and cardiovascular diseases development (Livingstone and Collison 2002).

This syndrome is not defined identically in different countries. The most commonly used definition is the one proposed in 1990 as a combination of hyperandrogenism and chronic anovulation after

excluding other causes of hyperandrogenemia (non-classical block steroidal 21 hydroxylase, hyperprolactinemia or androgen-producing neoplasia). According to this definition typical morphological changes on ovaries may but need not be present. These changes mean presence of at least eight subcapsularly located follicular cysts with the average diameter of up to 10 mm. This definition differs the endocrine and metabolic syndrome from pure morphological changes, which are also described in ovaries of woman with other diseases than PCOS. This definition also does not require often occurring criterion of higher ratio of luteinizing hormone (LH) to follicle stimulating hormone (FSH) as reflection of the higher pulse frequency of LH secretion in PCOS. The imbalance of gonadotropin secretion in these women is common but not specific – it may occur also in other hyperandrogenic states. The PCOS is today recognized widely as nosological unit characterized mainly by metabolic anomalies, especially by shifts in spectrum of sexual hormones and in many cases also by decreased insulin sensitivity and in a large number of the cases also by obesity. PCOS carries also various health risks, some of which are related to women's reproductive functions (infertility, risk pregnancy, and expected higher risks of endometrial and ovary carcinoma) and some are general (impaired glucose tolerance or even diabetes mellitus. type 2, a shift in circulating lipids, hypertension, higher risk of cardiovascular diseases).

Different theories about pathophysiology PCOS suppose a defect in hypothalamus-pituitary-ovary axis, in ovarian and adrenal steroidogenesis or in insulin hypersecretion. Unfortunately, none of these theories explains PCOS pathogenesis in its complexity.

Recently, the main interest in PCOS research has been oriented to genetics of the syndrome. Knowledge of genetic causes of PCOS is based on observation of families with high frequency of the syndrome in female relatives. Hitherto, these findings assume oligogenic or polygenic autosomal type of inheritance. A number of candidate genes have been proposed (Xita *et al.* 2002, Vaňková *et al.* 2002).

Should the polycystic ovary have genetic autosomal background it is evident that the genetic predisposition may also occur in close male relatives of patients with manifested PCOS (Yildiz *et al.* 2003). However, there are only a few references in literature that deal with male phenotype of PCOS. Premature hair loss and marked hypertrichosity were suggested as possible male symptoms of this syndrome (Ferriman and Purdie 1979, Carey *et al.* 1993, Govind *et al.* 1999, Stárka *et al.* 2000, Legro 2000). Unfortunately, the signs were not followed systematically in male relatives in families with frequent occurrence of PCOS. Laboratory findings of typical disturbances in steroids and gonadotropins or of insulin resistance in men suspected of having the male phenotype PCOS are rare.

The male equivalent of PCOS is now subject of interest and for its exact identification genetic studies could be of great help (Ellis and Harrap 2001). Further, it is necessary to add that both PCOS and androgenic alopecia carry the risk factor of cardiovascular diseases, glucose metabolism disorders (Lesko *et al.* 1993, Herrera *et al.* 1995, Lotufo *et al.* 2000, Ford *et al.* 1996, Stárka *et al.* 2004) and androgenic alopecia also the risk of prostate carcinoma (Giles *et al.* 2002, Denmark-Wahnefried *et al.* 2000, Hawk *et al.* 2000).

Premature hair loss starting before 30 years of age occurs in approximately 30 % of men (Sinclair and Dawber 2001). According to some studies PCOS prevalence in women is between 5-10 %. From these data it can be concluded that only a part of prematurely balding men correspond to the possible male phenotype of PCOS. The goal of this study was to find characteristic features for this subgroup.

Methods

We examined a group of 37 men, who searched medical help for premature hair loss; some of them had undergone hair transplantation. Only patients whose hair loss started before the age of 30 years were included. Hair loss was characterized by recess of the frontotemporal hairline or balding at the vertex (Sinclair and Dawber 2001). The men did not have any endocrine disease, took neither hormonal therapy, nor medication for improving quality of hair. BMI of these patients was up to 30 kg/m^2, i.e. within the frame of normal weight or slight overweight. In all patients basic hormonal spectrum was examined. The total testosterone (T), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), dehydro-epiandrosterone (DHEA), epitestosterone (epiT), dihydro-testosterone DHT), cortisol, estradiol, sexual hormone binding globulin SHBG, prolactin, thyreotropin (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the index of free testosterone was calculated (FAI = [(testosterone/SHBG) x 100]). Testosterone was determined by standard radioimunoassay (RIA) using antiserum anti-testosterone-3-carboxymethyloxim: BSA and testosterone-3-carboxymethyloxim-tyrosylmethyl-ester-[^{125}I] as a tracer.

Intra-assay and inter-assay coefficients variants were 7.2 % and 10 %, respectively, and sensitivity was 0.21 nmol/l. Androstenedione (A) was determined by standard RIA with antiserum anti-androstenedione-6-carboxy-methyloxim: BSA and [^{3}H] androstenedione as tracer. Intra-assay and inter-assay coefficients variants were 8.1 % a 10.2 % and sensitivity was 0.39 nmol/l. Estradiol (E2), cortisol and 17-hydroxyprogesterone were determined by RIA kit from Immunotech (Marseilles, France). Sexual hormones binding globulin (SHBG) was assayed by IRMA kit (Orion, Espoo Finland). TSH was assayed by commercial kit Abbott Laboratories utilizing automatic system Abbott Axsym (Abbott Park, IL, USA). Commercial kits Immunotech (Marseilles, France) were used for the determination of prolactin, LH, FSH (IRMA kit), DHEA and DHEAS (RIA kit). DHT and epitestosterone were determined by original methodology (Bílek *et al.* 1987, Hampl *et al.* 1990). Male population of a randomly selected sample from the survey of iodine deficit in the Czech Republic was used as a control group for hormonal evaluation. The group was formed by 256 men in the age category matched to the men with alopecia. Glucose was measured using the glucose-oxidase method (glucose analyzer, Beckman, Fullerton, CA).

In 30 patients characterized by hormonal analysis, insulin tolerance test was carried out according to Young *et al.* (1996) in the fasting state between 7.00 and 9.00 h. Regular insulin (Actrapid HM, 0.1 IU/kg) was applied i.v. The venous blood samples to determine glycemia were taken from a cannula applied in the cubital vein at -3rd, 0, 2nd, 4th and then, each minute up to 15th minute. After the end of the test, the patient was released not sooner until a normoglycemia had been achieved. The rate for plasma glucose disappearance was calculated according to the formula $0.693/\tau$, where τ was calculated from the slope of least square analysis of the plasma glucose concentrations from the 4th to 15th minute (when the glucose concentration declined linearly).

Blood samples were taken for DNA isolation and the frequency of occurrence of allele 3 INS VNTR by molecular-genetic technique (Vaňková *et al.* 2002). Since there is a significant difference in the length of allele I and allele III, an indirect assay with the aid of point polymorphism –23 HphI in the insulin gene area was used. If the enzyme splits PCR product, it is allele I, if it does not split PCR product, it is allele III. Reactive conditions: 50 ng genomic DNA in volume of 25 µl PCR contains 3 mM $MgCl_2$ (PE), 200 mM dNTPs (Takara), 0.24 µM primers, 0.03 U AmpliTaq Gold (PE), 10xPCR Buffer (PE) and 11.75 µl ddH_2O. Sequence of primers: forward AGC AGG TCT GTT CCA AGG, reverse CTT GGG TGT GTA GAA GAA GC. PCR reaction conditions: initial denaturation 94 °C 10 min, 37 cycles of denaturation 95 °C 30 s, annealing (first 10 cycles touch down 65-59 °C -30 s) 59 °C 30 s, extension 72 °C 1 min, 72 °C 10 min. 5 µl of PCR product was split with aid of 4 U restrictive endonuclease Hph I (Fermentas) in 20 µl reaction in 37 °C overnight, then the fragments were divided into 2.5 % MetaPhor agarose gel (Sigma) in 0.5x TBE buffer and visualized by ethidium bromide.

Positivity for allele I is implied by the presence of fragments of 122 bp, 185 bp and 53 bp size (existence of restrictive place), whereas positivity for allele III is implied by fragments of 122 bp and 238 bp size (absence of restrictive place).

For the final statistical analysis only those patients (n = 30) who had complete hormonal pattern analysis, insulin tolerance test and allele 3 INS VNTR determination were evaluated.

Statistical analysis

Regarding the low number of subjects and the relatively large number of variables as well as age relationships in some variables, the data were divided into 3 groups in relation to the reference range. The values -1, 0 and +1 were allocated to the patients with the level of the followed substance below, within or above the reference range, i.e. a three-value scale was created for each substance. In the case when alopecia did not have the connection with serum level of the substance, a mean value of such variable should not significantly differ from zero. Significance of the difference was tested using the sign test.

The other method for evaluation, testing the significance of frequency of the patients outside of the reference range, assumed a matched control subject with normal levels of all measured substances for each patient. In some hormones, both the over- and under-limit subjects were present in the patient's group, which excluded the application of Fischer's exact test that is not sensitive to low number of subjects but it requires 2×2 contingency table. The χ^2 test could not be used due to the low number of subjects.

Differences of mean values between subgroups A and B tested for insulin resistance were evaluated using robust Mann-Whitney test. For statistical analysis of the frequency of carriers of allele 3 in subgroups A and B Fisher's exact test was used.

Table 1. The differences in frequency of the subjects below, within and above the normal reference range in hormonal spectrum between men with premature androgenic alopecia with (group B) or without (group A) the presumed hormonal signs of PCOS.

Variable	Normal reference range	GROUP A								GROUP B								Mann-Whitney test
		n				Mean	SD	Difference from 0 (Sign test)	Fisher's exact test*	n				Mean	SD	Difference from 0 (Sign test)	Fisher's exact test*	
		Total	Reference range: Below	Reference range: Within	Reference range: Above					Total	Reference range: Below	Reference range: Within	Reference range: Above					
SHBG	34-66 nmol/l	19	10	9	0	–0.526	0.513	**p<0.004**	**p<0.0004**	11	11	0	0	–1.000		**p<0.0001**	**p<0.0001**	**p<0.04**
Follitropin (FSH)	2-10 U/l	19	0	19	0	0.000		NS	NS	11	7	4	0	–0.636	0.505	**p<0.03**	**p<0.004**	**p<0.004**
Androstenedione	1.7-8.6 nmol/l	19	0	15	4	0.211	0.419	NS	NS	11	0	11	0	0.000		NS	NS	NS
Epitestosterone	0.9-7.8 nmol/l	16	2	14	0	–0.125	0.342	NS	NS	10	1	9	0	–0.100	0.316	NS	NS	NS
Dihydrotestosterone	0.9-3.6 nmol/l	19	1	16	2	0.053	0.405	NS	NS	11	1	10	0	–0.091	0.302	NS	NS	NS
Testosterone	13.5-31.1 nmol/l	19	3	15	1	–0.105	0.459	NS	NS	11	3	8	0	–0.273	0.467	NS	NS	NS
17-OH-progesterone	1.4-5.4 nmol/l	19	0	18	1	0.053	0.229	NS	NS	11	0	11	0	0.000		NS	NS	NS
DHEA	10.8-32.6 nmol/l	17	3	14	0	–0.176	0.393	NS	NS	9	3	3	3	0.000	0.866	NS	NS	NS
DHEAS	7.2-16.1 µmol/l	17	11	4	2	–0.529	0.717	**p<0.03**	**p<0.0001**	10	5	5	0	–0.500	0.527	NS (p<0.08)	**p<0.04**	NS
Luteotropin (LH)	0.5-10 U/l	19	0	19	0	0.000		NS	NS	11	0	11	0	0.000		NS	NS	NS
Free androgen index	27-90	19	1	17	1	0.000	0.333	NS	NS	11	0	6	5	0.455	0.522	NS (p<0.08)	**p<0.04**	NS (p<0.06)

The second method for evaluation, testing the significance of frequency of the patients outside of the reference range, assumed a matched control subject with normal levels of all measured substances for each patient. In some hormones, both the over- and under-limit subjects were present in the patients group, which excluded application of Fisher's exact test based on 2×2 contingency table even as it is not sensitive to low number of subjects. To enable the use of the test even in the aforementioned case, the frequency of the patients with normal values was pooled with that found in the less frequent abnormality and the pooled proportion was compared with that in matched control subject being 100 % and supposing equal number of subject in the controls as in the patients.

Results

Results of hormonal examinations in 37 men with premature alopecia were evaluated as shown in Table 1. The hormonal levels found were compared to those found in the control set which consisted of randomly selected men from survey of iodine deficit in the Czech Republic. From the group of 30 patients who underwent hormonal analysis and also insulin tolerance test, we selected a subgroup (n = 19, group A), which did not show any hormonal changes or just low SHBG. The second subgroup comprised men (n = 11, group B) who had similar changes as described in the literature in women with PCOS. Patients in this subgroup had low SHBG and low FSH or low SHBG and high free testosterone index (FAI) compared to controls. The two subgroups differed neither in age nor in BMI. In these two subgroups we compared tendency to insulin resistance by k_{ITT} as insulin tolerance criterion. Subgroup (B) (premature balding men with decreased SHBG and FSH values and/or higher FAI index) showed significantly higher insulin resistance than group (A), evaluated by Mann-Whitney test on a significance level p=0.03 (Fig. 1).

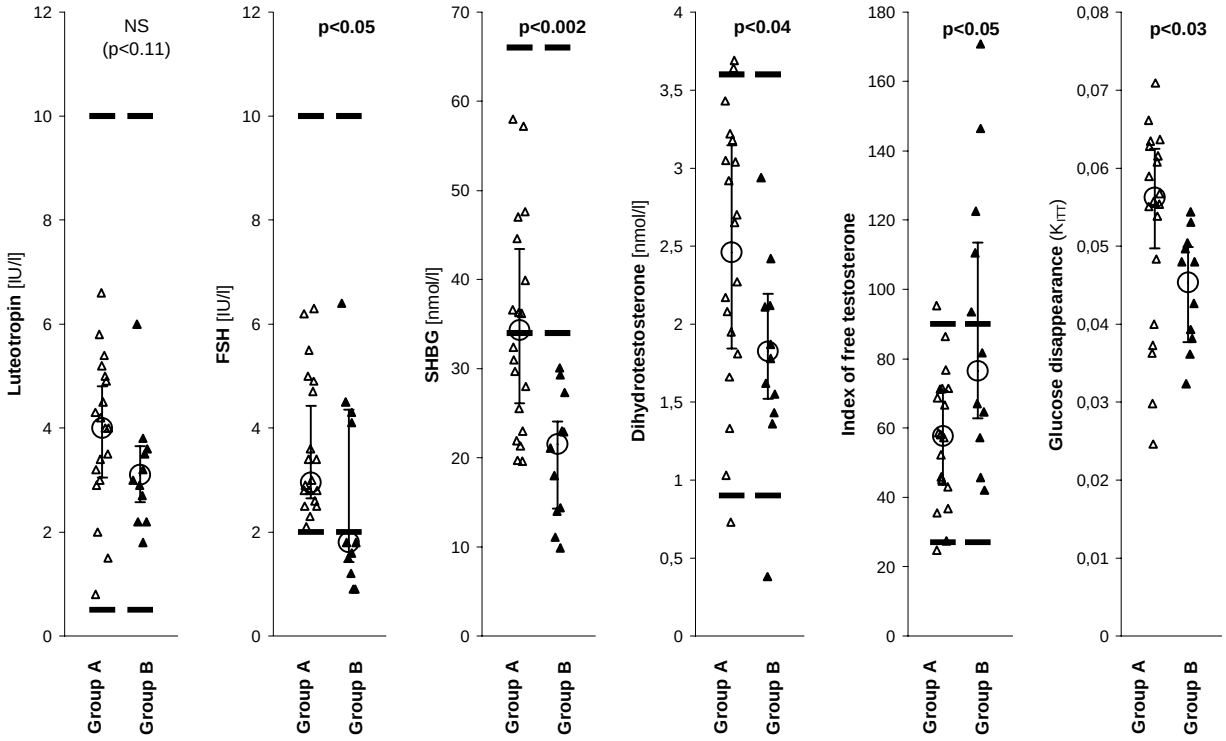

Fig. 1. The differences between subgroup A (balding men without hormonal signs of PCOS) and B (balding men with hormonal pattern resembling that of the women with PCOS). The empty and full triangles represent the subjects in the group A and B, respectively. The circles with error bars denote the medians with quartiles and the bold horizontal bars indicate limits of the normal ranges.

All patients who underwent insulin tolerance test were tested by molecular-biological methods for gene VNTR INS, which is supposed to be in relation with insulin resistance or diabetes mellitus. The frequency of carriers of allele 3 in both subgroups A and B did not differ (Fisher's exact test, data not shown): the frequency of the allele 3 in group A was 9/18, in group B 6/10.

Patients with premature alopecia and with similar hormonal changes as women with PCOS have higher insulin resistance than men with precocious alopecia without these changes. In the observed group of balding men, there was about one third of thus characterized subjects. This frequency corresponds approximately to PCOS prevalence in women.

Discussion

Polycystic ovary syndrome belongs to the most common endocrine diseases in women. It is characterized by hormonal and metabolic imbalance with a genetic background. Great number of communications has described hormonal features of this syndrome combined with markers of insulin resistance. However, a generally acceptable definition of this syndrome has not been found

yet. The formal definition of PCOS from 1990 as anovulation combined with hyperandrogenism is based only on selected signs of the disease and is strictly limited to female patients. The same limitations has the extended definition from 2003 (Rotterdam ESHRE/ASRM 2004). Attention is now driven to genetics of this syndrome. Genetic base of syndrome PCOS led to ideas about the existence of a male equivalent of this syndrome (Legro 2000).

A part – approximately one third of the men under investigation – of the prematurely balding men expressed subnormal levels of SHBG and an imbalance between LH and FSH, like women with PCOS. We also found lower levels of circulating epitestosterone, a steroid with mild antiandrogenic activity (Stárka *et al.* 1991), the content of which is decreased in the hair of balding men (Choi *et al.* 2001). However, we were not able to demonstrate an increase of frequency of higher concentrations of total testosterone in balding men. On the contrary, in some cases subnormal levels of total, but not of free testosterone could be found. The grounds for reduced importance of "hyperandrogenism" in males in relation to PCOS were discussed by Legro (2000).

From our results, it is obvious that in those males with premature hair loss (under the age of 30), whose hormonal changes resemble those of women with PCOS (low SHBG, disproportion between gonadotropins – higher LH or low FSH, and high FAI) are present, significantly higher frequency of decreased insulin sensitivity could be demonstrated. This is also another sign of PCOS in women. These men might therefore represent the male equivalent of PCOS but also the expression of metabolic syndrome X, in which decreased testosterone levels are found in males (Andres 2004). In our study there was about one third of such affected men. If we assume the prevalence of premature hair loss in about 30 % of the male population, it corresponds approximately to the prevalence of PCOS in females.

The men, who are supposed to represent the male phenotype of PCOS, should share similar health risks as women with PCOS syndrome, i.e. higher risk of development of diabetes mellitus type 2 and cardiovascular diseases. In men with alopecia, a higher occurrence of these diseases (Lotufo *et al.* 2000, Herrera *et al.* 1995, Lesko *et al.* 1993, Ford *et al.* 1996) was described. They are also endangered by higher risk of prostate carcinoma (Giles *et al.* 2002, Denmark-Wahnefried *et al.* 2000, Hawk *et al.* 2000).

Thus a final proof of existence of the male equivalent of PCOS might be important not only for closer studies of pathogenesis and genetic background of PCOS but also for general medical praxis. Irrespective whether a part of men with premature balding represents the male phenotype of PCOS respective metabolic syndrome X or not, the occurrence of androgenic alopecia especially before the age of 30 years, may be considered as a mark of potential increased risks of serious diseases in later age.

We did not find any difference in the frequency of allele I and III of VNTR INS gene between the subgroup suspected as male equivalent of PCOS and balding men without the sign of hormonal changes or decreased insulin sensitivity. However, the association of this important genetic marker of insulin action was not unequivocally demonstrated in female PCOS (Vaňková *et al.* 2002).

Conclusions

Based on our results we can conclude that the patients with premature alopecia and hormonal changes resembling partially those typical for women with PCOS have higher insulin resistance than men with precocious alopecia without these hormonal changes. We may expect that premature hair loss before 30 years of age, with lower levels of SHBG, disproportion between FSH and LH and with insulin resistance, might probably represent the male equivalent of PCOS.

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Reprint requests

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