HIGHLIGHT

Identification of follistatin as a possible trait-causing gene in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder, which affects up to 5–10% of women of reproductive age (1). It is characterized by hyperandrogenism and chronic anovulation, which results in reduced fertility (2). The syndrome is also associated with a 7-fold increased risk for type 2 diabetes mellitus (3).

Familial clustering of cases suggests that genetic factors play an important role in its etiology (4). However, the mode of inheritance of PCOS has not been firmly established. Although studies of families with several cases of PCOS have produced results suggesting an autosomal dominant trait (5–7), others did not (8). There are obvious problems which make genetic studies of PCOS hard to perform (9, 10): (a) heterogeneity and lack of universally acceptable clinical and biochemical criteria for diagnosis of the disorder; (b) difficulty in spanning more than one generation in the segregation studies because PCOS primarily affects premenopausal women; (c) lack of a common acceptable male phenotype; and (d) high prevalence (about 20%) of polycystic ovaries in the normal population (11, 12). In addition, several pathways are implicated in the etiology of PCOS and, therefore, several candidate genes may be responsible for this 'complex' genetic trait, making the identification of each contributing gene very difficult.

In a recent paper, Urbanek *et al.* (13) have shown a paradigmatic approach to the genetic study of such a complex disorder, based on the use of appropriately selected standard techniques for linkage analysis, and a good understanding of the physiology and relevant metabolic pathways of gonadal function, these include the regulatory pathways of steroid hormone synthesis (14, 15), gonadotropin action (2), insulin signaling (16–18), and those regulating body weight (19). Thirty-seven genes were chosen from these pathways (Table 1) and examined as candidate genes in 150 families with PCOS (148 of European origin and two of Caribbean origin). The authors performed linkage and association studies, using 'affected-sib pair' (ASP) analysis (20) and the transmission/disequilibrium test (TDT) (21, 22), respectively. ASP analysis is a well-known allele-sharing method, the results of which are particularly powerful for specifying a complete genetic model (i.e. identifying each gene that contributes to a disease), as in the case

of PCOS. Indeed, allele-sharing methods are non-parametric (that is, assume no model for the inheritance of the trait), ignore unaffected people, and, because they look for alleles or chromosomal segments shared only by affected individuals, are able to test whether affected relatives inherit an identical-by-descent (IBD) region more often than expected under random Mendelian segregation. Thus, using ASP analysis within nuclear families, the presence of a trait-causing gene is revealed by more than the expected 50% IBD allele sharing. To further support the results obtained by ASP analysis, Urbanek *et al.* also performed an association study using TDT (13). This method tests whether a particular allele occurs at higher frequency among affected than unaffected individuals, thus involving population correlation rather than cosegregation within a family, as in the case of ASP analysis. TDT represents one of the most advantageous association studies as it is not influenced by population structure or heterogeneity, unlike case-control studies.

Several polymorphic markers were tested using genetic analysis in the study of Urbanek *et al.* (13). Some of these markers were typed within the candidate genes, but for those genes without intragenic polymorphisms, 45 closely linked (within 1–4 centimorgan) short tandem repeats polymorphisms (STRPs) were used. The closer the polymorphism is to the disease gene, the less likely is recombination between the two at meiosis, and therefore the two are more likely to be inherited together.

The clinical study of Urbanek and co-workers was very well designed and the patients were appropriately selected. Previous genetic studies of PCOS have failed to show consistent results, probably because of a difficult phenotype definition (14, 15, 23). Urbanek and co-workers, however, were able to overcome the problem by selecting a subgroup of patients (index cases and relatives) likely to be more etiologically homogeneous on the basis of the presence or absence of hyperandrogenemia (HA), i.e. elevated levels of total testosterone and testosterone not bound to sex hormone-binding proteins (more than 2 s.d. above the control mean; in their assay the thresholds were 2 nmol/l and 52 pmol/l for total and unbound testosterone respectively). This condition was particularly useful for patient selection

Table 1 Panel of PCOS candidate genes

Pathway	Candidate gene	Chromosomal location
Steroid hormone	Androgen receptor	Xq11.2
	CYP11A-cytochrome P450 side-chain cleavage enzyme	15q23–24
	CYP17-cytochrome P450 17 α -hydroxylase/17,20-desmolase	10q24.3
	CYP19-cytochrome P450 aromatase	15q21
	17 β -hydroxysteroid dehydrogenase type I	17q11–21
	17 β -hydroxysteroid dehydrogenase type II	16q24.2
	17 β -hydroxysteroid dehydrogenase type III	9q22
	3 β -hydroxysteroid dehydrogenase, type I and II	1p31.1
	Steroidogenic acute regulatory protein	8p11.2
Gonadotropin action	Activin receptor 1	12q13.12
	Activin receptor 2A	2q22.2
	Activin receptor 2B	3p22.2
	Follistatin	5p14
	Inhibin A	2q33.34
	Inhibin β -A	7p13–15
	Inhibin β -B	2cen–2q13
	Inhibin C	12q13
	Sex hormone binding globulin	17p13.2
	Luteinizing hormone/choriogonadotropin receptor	2p21
Obesity and energy regulation	Follicle-stimulating hormone receptor	2p21
	Mothers against decapentaplegic homolog 4	18q21
	Melanocortin 4 receptor	8q21.32
	Leptin	7q31.3–32.1
	Leptin receptor	1p31
Insulin action	Pro-opiomelanocortin	2p23
	Uncoupling protein 2+3	11q13
	Insulin-like growth factor I	12q22–23
	Insulin-like growth factor I receptor	15q25–26
	Insulin-like growth factor binding protein 1+3	7p13–7p12
	Insulin gene VNTR	11p15.5
	Insulin receptor	19p13.3
	Leydig insulin-like protein 3	19p13.1
	Insulin receptor substrate 1	2q36–37
	Peroxisome proliferator-activated receptor- γ	3p25–24.2

as it represents the major reproductive endocrine phenotype of PCOS, as demonstrated by previous studies on PCOS families performed by the same group (5).

Among the 37 analysed genes, the authors found promising evidence for linkage with two genes: follistatin and cholesterol side-chain cleavage enzyme (CYP11A). However, only the linkage with follistatin remained significant after correction for multiple testing (multiplying the *P* value by 33, the number of regions tested; these regions correspond to 33 distinct chromosomal locations where the 37 genes map). In any case, the evidence for linkage with CYP11A, albeit not significant after correction, confirms the findings previously published by Gharani *et al.* (15) and is worthy of further study because multiplying by the full 33 tests probably provides too stringent a correction. The linkage with follistatin was demonstrated by the high IBD levels (72%) observed for D5S623, the marker closest to follistatin (<0.5 centimorgan), in the affected sib-pairs. However, the TDT did not show evidence of disease

association with follistatin. How can this discrepancy be explained? The power to detect association by using a marker depends on several factors: strength of the linkage disequilibrium between marker and disease, the recombination fraction between marker and disease, the increase in risk attributable to the particular susceptibility locus under consideration, and the penetrance of the different disease locus genotypes. It follows that genetic markers may reveal linkage without showing allelic association with the disease, especially if, as in the case of follistatin, the marker is not tightly linked (<0.5 centimorgan).

An important point to discuss is how mutations of follistatin may be responsible for PCOS phenotype. Follistatin is a high-affinity binding protein capable of binding activin and neutralizing its biological activity *in vitro* and *in vivo* (24, 25). Activin, a member of the transforming growth factor- β superfamily, and follistatin are expressed in several tissues, such as ovary, pituitary, adrenal cortex and pancreas. This similar

pattern of tissue distribution suggests that follistatin is likely to be an auto-/paracrine regulator of the hormonal effects of activin within the tissues. In particular, activin promotes ovarian follicular development, enhances LH binding sites and progesterone production, playing a role in preventing premature luteinization of the ovarian follicles, inhibits thecal cell androgen production, and increases pituitary follicle-stimulating hormone (FSH) secretion and insulin secretion by pancreatic β -cells. An increase in circulating level or biological activity of follistatin may, therefore, cause follicular development to be arrested, the process of follicular luteinization or atresia to be favored, ovarian androgen production to be increased, FSH circulating levels to be reduced, and insulin release to be impaired. These effects are all characteristic features of PCOS. Moreover, data from transgenic mice overexpressing follistatin seem to confirm these effects (26). Indeed, mice overexpressing follistatin show suppressed serum FSH levels and arrested ovarian folliculogenesis, as observed in patients with PCOS.

In conclusion, the paper by Urbanek *et al.* (13), by the combination of a well-designed genetic and clinical study, with appropriate clinical classification of the patients, shows how to screen a large number of candidate genes, without testing each gene for mutation, and provides a paradigmatic approach for genetic dissection of complex traits.

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