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The genetic basis of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. Familial clustering of cases suggests that genetic factors play an important part in its aetiology. A number of studies of families with several cases of PCOS have produced results suggesting an autosomal dominant trait. Detailed analysis of a large number of affected families has, however, cast some doubt about the mode of inheritance. An autosomal dominant trait remains possible but a more complex aetiology seems more likely. The results of our recent studies support the concept of an oligogenic disorder in which genes affecting metabolic pathways in glucose homeostasis and steroid biosynthesis are both involved. We review evidence for an important role for the insulin gene minisatellite in the aetiology of anovulatory PCOS and for the gene coding for P450 cholesterol side chain cleavage (*CYP11a*) in the mechanism of excessive androgen secretion in women with polycystic ovaries. We propose that the heterogeneity of clinical and biochemical features in PCOS can be explained by the interaction of a small number of key genes with environmental, particularly nutritional, factors.

Key words: anovulation/*CYP11a*/folliculogenesis/insulin/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder which is considered to be the commonest cause of anovulatory infertility and hirsutism (Adams *et al.*, 1986; Hull, 1987). The most widely accepted definition of polycystic ovary syndrome is the association of anovulation (manifesting itself as irregular menses, oligomenorrhoea or amenorrhoea) with clinical or biochemical evidence of androgen excess (Zawadzki and Dunaif, 1992) but the identification of polycystic ovaries ultrasonographically has called into question the validity of this definition (Conway *et al.*, 1989; Franks 1989, 1995). It is now clear that the majority of hirsute women with regular menses have polycystic ovaries (Franks 1989; O'Driscoll *et al.*, 1994). Furthermore, the estimated prevalence of polycystic ovaries, as diagnosed by ultrasonography, in a normal (volunteer) population has been found to be over 20% (Polson *et al.*, 1988; Clayton *et al.*, 1992; Farquhar *et al.*, 1994) and even within this 'normal' group, many of these women will have symptoms which are considered to be typical of the syndrome.

Given the heterogeneous nature of its clinical and biochemical features, it has been suggested that PCOS represents a range of disorders rather than a single entity (Simpson, 1992). Although it seems likely that there is more than one cause of the syndrome, there are, nevertheless, certain biochemical features which are common to all groups of subjects with ultrasonographic evidence of polycystic ovaries irrespective of the clinical presentation. Serum levels of luteinizing hormone (LH) in hirsute women with polycystic ovaries and regular cycles, whilst lower than those in anovulatory subjects, are still significantly higher than normal (Adams *et al.*, 1986; Conway *et al.*, 1989; Franks, 1989). The most consistent endocrine feature in women with polycystic ovaries, however, appears to be hyperandrogenaemia, whether the mode of presentation is as the 'classic' syndrome or as an incidental finding on ultrasound examination (Franks, 1991). There has always been a vigorous debate about the source and aetiology of hyperandrogenaemia in PCOS. The weight of evidence suggests that the ovary is the major source of excess androgen (reviewed by Franks, 1995). Recent data from both clinical investigations and studies of isolated human theca cells implicate a primary ovarian abnormality rather than hypersecretion of androgens as a result of abnormal gonadotrophins (Gilling-Smith *et al.*, 1994, 1997; Ibañez *et al.*, 1996).

In addition to the well-described abnormalities of the pituitary ovarian axis, polycystic ovary syndrome is characterized by significant metabolic abnormalities. These include fasting and glucose-stimulated hyperinsulinaemia, peripheral insulin resistance (affecting predominantly muscle and adipose tissue), abnormalities of energy expenditure (reduced postprandial thermogenesis) and dyslipidaemia (reviewed by Dunaif, 1993; Franks, 1995; Holte, 1996). Furthermore, it has emerged that PCOS represents a major risk factor for non-insulin dependent diabetes mellitus (NIDDM). The prevalence of impaired glucose tolerance or frank diabetes in obese young women with PCOS lies (depending on the population studied) between 11% and 38% (Dunaif, 1993; Dunaif and Finegood, 1996; Holte, 1996). A long-term follow-up study of postmenopausal women with a previous history of PCOS found a 13% prevalence of NIDDM compared with $<2\%$ in the reference population – a seven-fold increase in risk (Dahlgren *et al.*, 1992a). Analysis of cardiovascular risk factors (such as hyperinsulinaemia and abnormal plasma lipids) suggests that

these patients are also at greater risk of developing cardiovascular disease in the future (Dahlgren *et al.*, 1992b). These findings emphasize that the significance of PCOS for women's health extends far beyond the implications for reproductive function, although these are important enough in themselves.

Polycystic ovary syndrome shows strong familial aggregation suggesting a major genetic component to its aetiology. In this paper, published and ongoing studies of the possible genetic basis of polycystic ovary syndrome, from this group, will be reviewed. This review will be set in the context of the clinical and biochemical background outlined above and in the light of previously published clinical and molecular genetic studies. We acknowledge that there is unlikely to be a single cause of the syndrome, but our hypothesis is that much of the clinical and biochemical variability within PCOS can be explained by the interaction of environmental (notably nutritional) factors with a small number of major causative genes which include those involved in androgen production and the secretion and/or action of insulin.

There are obvious problems which make genetic studies of polycystic ovary syndrome difficult to perform (Simpson, 1992; Legro, 1995). The heterogeneity and the lack of universally acceptable clinical or biochemical diagnostic criteria have been discussed. Another major handicap is that this is a disorder which primarily affects women of reproductive age and it is therefore very difficult for segregation studies to span more than one generation. In addition, as discussed below, there is no commonly accepted male phenotype. Lastly, the high prevalence of polycystic ovaries in the population means that large pedigrees, in particular, may include subjects with polycystic ovaries arising from a different genotype from that of the proband. Nevertheless, given modern methods of genetic modelling and molecular genotyoping these problems are not insurmountable, as we hope we will be able to illustrate in this review.

Family studies of polycystic ovary syndrome

A small number of clinical studies have been performed over the last 20 years which have drawn attention to the phenomenon of familial clustering of cases of polycystic ovary syndrome (Cooper *et al.*, 1968; Ferriman and Purdie, 1979; Givens *et al.*, 1988; Hague *et al.*, 1988; Lunde *et al.*, 1989; Carey *et al.*, 1993). Detailed analysis of these studies has been carried out in two excellent recent reviews (Simpson, 1992; Legro, 1995). Given that there is no unequivocal method of diagnosis, it is not surprising that the criteria used to identify probands and affected family members vary considerably between studies. A further confounding factor is that identification of affected family members was made by direct clinical observation in some studies, by questionnaire alone in some and by a combination of the two in others.

In one of the six largest studies (Hague *et al.*, 1988) no attempt was made to identify a male phenotype. In three others, premature balding was suggested as the likely manifestation of affected status in men but this was based, in two of the three, on evidence from questionnaires (Ferriman and Purdie, 1979; Lunde *et al.*, 1989) and, in the other, on a combination of data from direct observation, telephone interview and questionnaires (Carey *et al.*, 1993).

Similarly, there has been no general agreement about the mode of inheritance in PCOS. In four of six studies, segregation analysis gave results that were consistent with autosomal dominant inheritance (Cooper *et al.*, 1968; Ferriman and Purdie, 1979; Lunde *et al.*, 1989; Carey *et al.*, 1993) whilst one study suggested an X-linked mode (Givens *et al.*, 1988). In the other, the prevalence of polycystic ovaries among siblings was too high to be explained by a simple dominant model (Hague *et al.*, 1988). In the face of such inconsistencies, it is probably wise to make no assumptions about the mode of inheritance when performing linkage studies, as suggested below. One further difficulty relates to possible ethnic variations in the prevalence and presentation of the syndrome. None of the six studies has satisfactorily addressed this issue.

The St Mary's family studies

Our preliminary study at this centre, cited briefly in the previous section, focused on segregation analysis in 10 well characterized, multiply-affected families with polycystic ovaries (Carey *et al.*, 1993). It differed from those previously published in that it relied principally on direct interview and observation of relatives rather than on indirect evidence from questionnaires. Results from 50 women of reproductive age and 22 men were analysed. Assignment of affected status was made on the basis of ultrasound evidence of polycystic ovaries in the women and premature onset of fronto-parietal balding in men. In that study, to reduce the chance of false positive results, premature balding was defined as onset before the age of 30 although, conventionally, 40 years has been taken as the lower limit of normal (Ferriman and Purdie, 1979; Lunde *et al.*, 1989).

Although the diagnosis of polycystic ovaries was made ultrasonographically, 92% of affected female family members had at least one clinical (hirsutism, acne, menstrual disturbance) or biochemical feature (raised serum testosterone, LH) of polycystic ovary syndrome. The segregation ratio, expressed as the percentage of affected subjects in each generation (excluding the proband to avoid ascertainment bias), was calculated including data from the men and was found to be 51%, i.e. consistent with an autosomal dominant mode of inheritance. These initial results raised the clear prospect of a single gene effect and set us on a search for an appropriate candidate gene.

Subsequently, we have expanded some of the existing pedigrees and added new ones so that the number of families now includes 23 informative pedigrees. We have reviewed the data and have found that the picture is somewhat more complex than it appeared initially. This is well illustrated by the pedigree shown in Figure 1. In this family, the proband (14) was hirsute, anovulatory and had an elevated serum testosterone level. Both sisters (11, 16) had polycystic ovaries, were non-hirsute but had acne and raised serum testosterone; her brother (13) was prematurely bald. Her mother (5) was postmenopausal but was presumed to have been affected on the basis of a history of hirsutism, irregular menses and because her serum testosterone

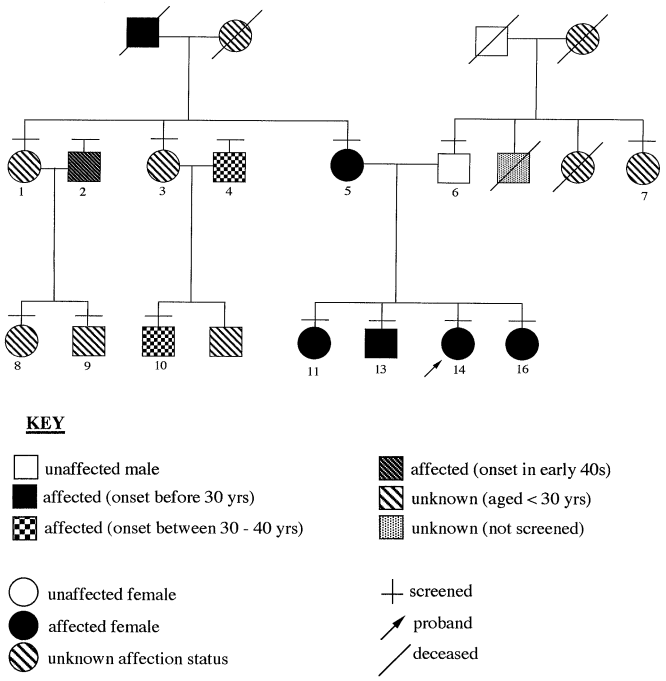

Figure 1. A large pedigree with familial polycystic ovary syndrome. Numbers below and horizontal bars above symbols indicate individuals who were fully screened by interview, examination, ovarian ultrasound (females) and biochemical testing. Affected status in other family members was assigned by history and/or photographic evidence.

was 4.7 nmol/l (normal range for premenopausal women 0.5–2.7 nmol/l). The two maternal aunts (1, 3) were also postmenopausal; neither had a history of hirsutism or menstrual disturbance but one had a serum testosterone of 5 nmol/l. One was married to a man (2) who became bald in his 40s and the other to a man (4) who developed significant hair loss between the ages of 30 and 40. Of their offspring (8, 9, 10), subject 8 was pregnant at the time of the study and the son of subjects 1 and 2 (9) was under 30. The son of parents 3 and 4 (10), like his father (4) became bald between 30 and 40 years of age. The proband's father (6) had no significant hair loss and of the two paternal aunts, one (7) was postmenopausal, but with no history suggestive of PCOS, and one was deceased.

This pedigree exemplifies the following points: (i) symptomatic heterogeneity between the proband and her sisters, all three of whom, nevertheless, had polycystic ovaries and hyperandrogenaemia; (ii) the problems associated with assigning definite affected status to more than one generation because of questions about the reliability of data from postmenopausal women; and (iii) difficulties in assigning affected status to men – especially those who were under 30 or who had noticed onset of balding around the age of 40 years.

Although the results are not incompatible with an autosomal dominant model it would be unwise to consider this mode of inheritance to the exclusion of all others. In this context, the suggestion by Simpson (1992) that PCOS should be treated as a quantitative trait disorder has considerable merit. This does not necessarily imply a truly polygenic aetiology because it would be possible to explain the variable phenotype on the basis of a small number of causative genes (a so-called oligogenic basis for disease). A candidate gene approach therefore remains valid but, rather than perform linkage studies using a single-gene autosomal dominant model, we have recently used a linkage analysis programme which makes no assumption about the mode of inheritance. In the following section the results of association and linkage studies applied to examination of the role of possible candidate genes will be discussed. Given the biochemical phenotype characteristic of women with polycystic ovaries we focused on genes coding for steroidogenic enzymes in the androgen biosynthetic pathway and those involved in the secretion and action of insulin.

Genes coding for steroidogenic enzymes

The 17-hydroxylase/17,20-lyase gene (CYP17)

On the basis of clinical studies which pointed to abnormal regulation of 17-hydroxylase/17,20-lyase (a known rate-limiting step in androgen biosynthesis) (Barnes *et al.*, 1989; Rosenfield *et al.*, 1990), our initial investigations focused on the possible role of *CYP17* (the gene encoding P450c17α). A 459bp fragment in the 5′ untranslated region of *CYP17* was amplified by polymerase chain reaction (PCR). A single base change (a T to C substitution at -34 base pairs from the starting point of translation) was found (Carey *et al.*, 1994). Conveniently, this variant allele includes a restriction site for the enzyme *Msp*-1, thus allowing a simple method of screening DNA by restriction fragment length polymorphism (RFLP) analysis.

Linkage studies were performed in PCOS families using polymorphic markers close to the gene and, on the basis of these, it was possible to exclude *CYP17* as a major causative gene. Nevertheless, using RFLP screening of the -34 allele, preliminary case-control data suggested an association between the variant allele of *CYP17* and PCOS (Carey *et al.*, 1994). These findings were, however, based on a relatively small population of subjects (71 patients and 33 controls) and subsequently we and others have been unable to confirm these results (Gharani *et al.*, 1996; Pugeat *et al.*, 1996; Techatraisak *et al.*, 1997; Franks, 1997). Critically, in none of these studies was any relationship found between the *CYP17* variant and serum androgen levels.

Cholesterol side chain cleavage gene, CYP11a

Our studies of ovarian theca cells in culture have demonstrated that PCO theca cells produce an excess of both androgens and progesterone (Gilling-Smith *et al.*, 1994; Franks *et al.*, 1996a). This prompted us to examine *CYP11a* [encoding P450 side chain cleavage (P450scc)] as a possible candidate gene for abnormal steroidogenesis (Gharani *et al.*, 1997). We therefore examined the segregation of *CYP11a* in 20 families and performed association studies in consecutively recruited, premenopausal, European women with polycystic ovaries on ultrasound and matched control women (with normal ovaries) from a similar ethnic background. We included 97 women with symptomatic PCOS, 51 subjects with polycystic ovaries and no symptoms, and 59 with normal ovaries.

Using an informative, microsatellite marker in the promoter

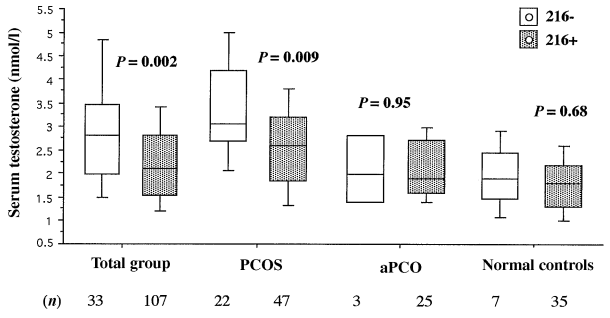

Figure 2. Relationship between serum testosterone and alleles of *CYP11a* in a case-control data-set. Serum testosterone concentrations in women with polycystic or normal ovaries are grouped according to genotype at the promoter region of *CYP11a*. 216+ individuals are those with at least one 216 alelle, 216- are subjects with no 216 allele. 'PCOS' are polycystic ovary syndrome subjects with anovulation and/or hirsutism; aPCO are those with polycystic ovaries but no symptoms. Values shown are 10th, 25th, 50th, 75th and 90th centiles for serum testosterone. Groups were compared by the Mann–Whitney test (from Gharani *et al.*, 1997, with permission).

region of *CYP11a*, genotype analysis was performed after PCR amplification. In the case-control study, subjects were allocated to one of two groups according to the presence or absence of the most common polymorphism, a pentanucleotide repeat $(tttta)_n$, -528bp from the AGT start of translation site. Individuals were designated as 216+ (at least one copy) or 216- (no 216 allele). Our results showed that variation at the *CYP11a* gene was associated with both PCOS and serum testosterone concentrations (Gharani *et al.*, 1997) (Figure 2). On further analysis, it was clear that differences in serum testosterone between 216+ and 216− subjects were maintained in the major subgroup of women with symptomatic PCO (i.e. with polycystic ovary syndrome). In a further analysis, the distribution in genotype was found to vary significantly if subjects were classified according to testosterone levels or by the presence of hirsutism.

Using a number of polymorphic markers in the region of *CYP11a*, we carried out non-parametric linkage analysis using the GENEHUNTER (multipoint linkage) programme (Kruglyak *et al.*, 1996). We found evidence for excess allele sharing (i.e. linkage) at the *CYP11a* locus, generating a maximum non-parametric linkage (NPL) score of 3.03 (P = 0.003). The data from both association and linkage studies suggest that *CYP11a* is a major genetic susceptibility locus for PCOS.

The aromatase gene

In the same population, the possible role of the gene encoding P450 aromatase (*CYP19*) was examined. There have been reports of hyperandrogenism occurring in rare patients with aromatase deficiency (Harada *et al.*, 1992; Ito *et al.*, 1993). In immunohistochemical studies of polycystic ovaries, Takayama *et al.* (1996) were unable to detect aromatase in antral follicles of various sizes. On the other hand, Mason *et al.* (1994) demonstrated enhanced oestradiol production by granulosa cells of antral follicles from polycystic ovaries, suggesting that, functionally, there was no evidence of an intrinsic deficiency of aromatase. Nevertheless, all these studies pointed to abnormal regulation of aromatase in women with hyperandrogenism. We therefore performed both a case control study and linkage analysis. The results revealed no association of alleles of *CYP19* with PCO and no evidence for excess allele sharing (Gharani *et al.*, 1997).

Genes involved in secretion and action of insulin

Numerous metabolic studies have revealed abnormalities of both insulin secretion and action in women with PCOS (reviewed by Dunaif, 1993; Holte, 1996). These studies have shown that there is an interaction between body weight and PCOS, so that individuals with PCOS are more insulin resistant than control subjects, even allowing for the effects of obesity. The results of such studies raise the possibility that genes implicated in the secretion and action of insulin may have a role in the aetiology of PCOS.

The insulin receptor gene

The demonstration of impaired sensitivity to insulin action *in vivo* and *in vitro* naturally led to the hypothesis that genetic abnormalities of the insulin receptor and/or post-receptor signalling were involved in the pathogenesis of familial PCOS. There have been sporadic reports of a PCOS-like phenotype occurring in patients with severe insulin resistance associated with defects of the insulin receptor gene (Moller and Flier, 1988) but Conway *et al.* (1994) were unable to detect any abnormalities of the tyrosine kinase domain of the insulin receptor gene in a population of 22 hyperinsulinaemic women with PCOS. These results are supported by those in a recently published paper by Talbot *et al.* (1996). In this study, molecular scanning of the entire coding region of the insulin receptor gene was carried out on DNA samples from 24 well-characterized women with PCOS. Common polymorphisms were detected, especially in the intron 5′ to exon 3, but no missense or nonsense mutations (i.e. those that would be expected to result in marked impairment of receptor function) were found. The authors concluded that mutations of the insulin receptor gene were rare in women with PCOS.

As far as post-receptor signalling is concerned, it remains to be determined whether there is a genetic basis for the putative abnormality of serine–threonine phosphorylation which characterizes a significant proportion of women with typical PCOS (Dunaif *et al.*, 1995). This observation is particularly intriguing, given that Miller's group have shown that serine phosphorylation is an important process in post-translational regulation of 17,20-lyase activity in steroidogenic tissue (Zhang *et al.*, 1995). These findings have led Miller and colleagues to put forward the hypothesis that a common, perhaps genetically-determined, biochemical abnormality could result in both insulin resistance and hyperandrogenism in patients with PCOS (Zhang *et al.*, 1995).

The insulin gene

Abnormalities of insulin secretion have been reported in recent studies of women with PCOS, with and without a family history of NIDDM (O'Meara *et al.*, 1993; Ehrmann *et al.*,

1995; Holte *et al.*, 1994, 1995; Dunaif and Finegood, 1996). Recent data from the Uppsala group have demonstrated that whereas insulin resistance was largely reversible by weight reduction (in obese PCOS subjects), an abnormality of first phase insulin secretion persisted, despite improved insulin sensitivity, thereby suggesting a fundamental disorder in pancreatic β-cell function (Holte *et al.*, 1995). We have therefore investigated the role of the insulin gene in the aetiology of PCOS. We evaluated the VNTR (variable number tandem repeats) minisatellite which lies 5′ to the insulin gene on chromosome 11p15.5, since variation at this element has been directly implicated in the regulation of insulin secretion, in susceptibility to NIDDM (Bennett *et al.*, 1995) and in hyperinsulinaemia related to central obesity (Weaver *et al.*, 1992). At this locus, there is a bimodal distribution of repeats, class I alleles being short (average 40 repeats) and class III alleles much longer (average 157).

We examined linkage of PCOS to the 11p15.5 locus in 17 families with several cases of PCOS and male pattern balding. We also looked for an association between the insulin gene VNTR (particularly class I and class III alleles) and polycystic ovaries in two additional populations of women (all European) presenting with symptoms of PCOS at two different endocrine centres (Waterworth *et al.*, 1997). We calculated the odds ratios for insulin VNTR genotypes either by using a conventional case-control approach (subjects from the St Mary's Hospital population) or by the use of affected family-based controls (AFBAC) (the Middlesex Hospital).

AFBAC and a related technique, the transmission disequilibrium test (TDT), are applicable if DNA is available from the proband and both parents (Spielman and Ewens, 1996). These methods compare alleles transmitted from parents to affected offspring with those not so transmitted. The latter generate 'control' genotypes or alleles which are matched for ethnicity to those in the sample from the affected case.

We found that class III alleles were associated with PCOS in each of the three populations (Figure 3). An important additional finding was that insulin VNTR class III alleles were most strongly associated with anovulatory PCOS. This is in keeping with the observation that hyperinsulinaemia is a more prominent feature in women with polycystic ovaries who have anovulatory menses (or amenorrhoea) than in equally hyperandrogenaemic subjects with regular menses (Dunaif *et al.*, 1987; Robinson *et al.*, 1993).

Another intriguing finding emerged from TDT analysis of the Middlesex Hospital population and of the 17 families with PCOS. Class III alleles were transmitted significantly more often from fathers than from mothers (Bennett *et al.*, 1997). This 'parent-of-origin effect' suggests genetic imprinting, as has previously been described for 11p15.5 in relation to type 1 (insulin dependent) diabetes (Bennett and Todd, 1996).

In the families, non-parametric linkage analysis was performed with the aid of five polymorphic markers in the region of 11p15.5, using the GENEHUNTER programme. We found evidence for excess allele sharing at the insulin gene VNTR locus, giving a maximum NPL score of 3.250 ($P = 0.002$). Using parametric analysis, we estimated that approximately 60% of families showed linkage to this locus. When we

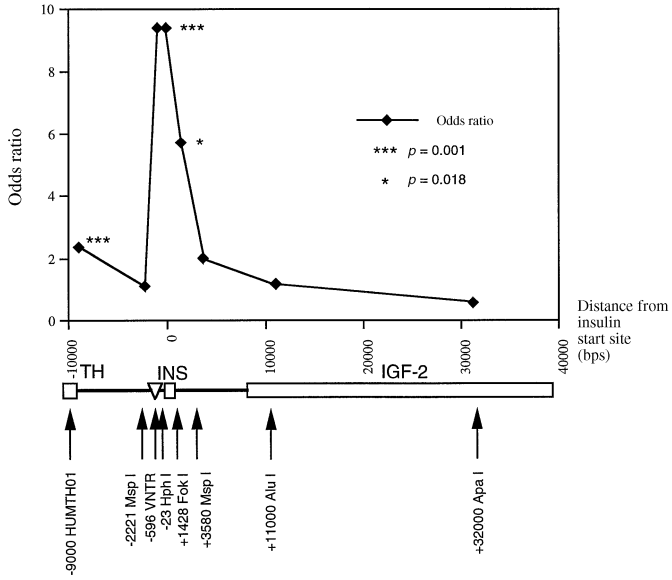

Figure 3. Localization of polycystic ovary syndrome (PCOS) susceptibility to the VNTR of the insulin gene on chromosome 11. Odds ratio (OR) values calculated for probands with anovulatory PCOS compared to non-affected controls. At the insulin gene VNTR locus, class III homozygotes were compared, similarly for alleles at neighbouring polymorphisms in linkage disequilibrium with class III VNTR alleles. 95% CI and *P* values for VNTR were: 2.01–44.2, $P = 0.001$ (from Waterworth *et al.*, 1997, with permission).

assigned data from families according to linkage score, we found that the geometric mean of fasting specific insulin levels was higher in those families with a positive LOD score than those with a negative score (Waterworth *et al.*, 1997).

In summary, in three different populations, we have uncovered strong evidence for both linkage and association between alleles at the VNTR 5′ to the insulin gene and PCOS. We conclude, from these data, that the VNTR of the insulin gene is a major susceptibility locus for PCOS, particularly anovulatory PCOS, and may contribute to the mechanism of hyperinsulinaemia and to the high risk of NIDDM in women with PCOS.

Future studies

To date, the approach we have taken of exploring candidate genes in both association and linkage studies has paid some dividends. Two loci, one related to control of androgen biosynthesis and one related to insulin secretion, have been identified as being of potential aetiological significance. It is important, however, for these findings to be supported in studies of other populations of women with PCOS (paying attention to any effect of ethnic origin) and to consider other methods for future studies. The potential pitfalls of case-control studies in relatively small populations have been illustrated by our experience with *CYP17*. Although we used a similar approach for the studies of *CYP11a*, these findings are likely to prove more robust for the following reasons: (i) the number of subjects examined in the case-control study was greater than in the initial *CYP17* study; (ii) in contrast to *CYP17*, we found a physiological correlation between alleles

of *CYP11a* and serum testosterone, supporting the concept that the variant allele has an effect on androgen production; (iii) non-parametric linkage analysis was undertaken, allowing for the fact that the mode of inheritance of PCOS remains uncertain.

As far as the insulin gene VNTR is concerned, the number of subjects studied in each population was not large but the consistency of results, using different methods in three separate groups of subjects, suggests that this is likely to be a sustainable finding. In studies of the insulin gene VNTR, the results of linkage analyses were similar even if data from the men in these families were omitted. This indicates that the results were not reliant on the, still controversial, assignment of premature balding as the male phenotype.

Nevertheless, consolidation of these findings and the search for other susceptibility genes demands an approach in which most of the disadvantages outlined above can be avoided. We believe that the strategy of assessing candidate genes remains viable. A more extensive 'anonymous' genome-wide scan to identify other susceptibility loci is also valid but requires many more subjects. Of course, the two approaches are not mutually exclusive. Linkage studies using families with several cases of PCOS/male balding are difficult, given the uncertainty, for example, about assignment of post-menopausal women and about the male phenotype. This has prompted us, and others, to consider using affected sibling pairs in which the minimum family unit would be two sisters with documented clinical, biochemical and ultrasonographic evidence of PCOS. Large numbers and resources are needed, especially for a genome-wide scan, but this approach conveniently side-steps the problem of the male phenotype and that of identifying, reliably, unaffected controls. Another strategy in association studies, which we have already found useful in the context of the insulin gene, is the use of AFBAC and TDT, as described above.

Summary

Using a candidate gene approach, we have found evidence for the involvement of two key genes in the aetiology of PCOS. From the results of both linkage and association studies, we suggest that the steroid synthesis gene *CYP11a* and the insulin VNTR regulatory polymorphism are important factors in the genetic basis of PCOS and may go some way to explaining the heterogeneity of the syndrome. Thus, differences in expression of *CYP11a* could account for variation in androgen production in women who have polycystic ovaries. We postulate that those subjects carrying class III alleles at the insulin gene VNTR locus are more likely to be hyperinsulinaemic and to suffer from menstrual disturbances.

These findings remain to be confirmed in larger studies and in other populations but, whatever the outcome of such studies, it is unlikely that these are the only genes to be involved in the aetiology of PCOS. Our earlier hypothesis, based on the initial family studies, that PCO/male balding could be explained by a single gene effect is no longer tenable. Our recent results lend weight to the idea that PCOS is an oligogenic disorder although it is quite possible that, within a given family, there is indeed one major gene which is dominantly inherited. Thus

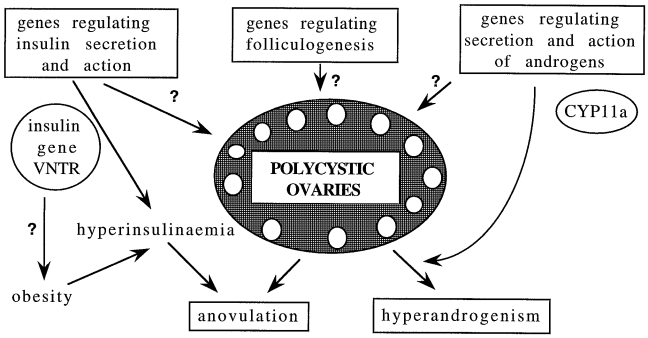

Figure 4. Suggested aetiological factors in polycystic ovary syndrome (PCOS).

PCOS appears to represent a quantitative trait in which a relatively small number of key genes contribute, in conjunction with environmental (particularly nutritional) factors, to the observed clinical and biochemical heterogeneity.

We propose that the underlying problem is the development of the polycystic ovarian morphology with an implicit disorder of folliculogenesis. This predisposes the subject to the development of polycystic ovary syndrome. The gene(s) determining the development of this distinct ovarian morphology remain unknown. *CYP11a* and insulin gene VNTR may act independently or in concert to determine abnormalities of ovarian function and (in the case of insulin) metabolism (Figure 4). It is possible that hyperinsulinaemia contributes to the morphological as well as the biochemical features of the polycystic ovary. Insulin has been shown to be even more effective than insulin-like growth factor-I in stimulating proliferation of ovarian stromal cells (Watson *et al.*, 1997).

Environmental factors can alter the clinical and biochemical presentation in those with a genetic predisposition to PCOS. This is illustrated by the effect of obesity (or, conversely, calorie restriction) on serum insulin levels, insulin sensitivity and menstrual function (Dunaif *et al.*, 1987; Holte *et al.*, 1995; Franks *et al.*, 1996b).

Identification of susceptibility genes in the aetiology of PCOS is not simply an intellectual exercise, as illustrated by the data regarding the insulin gene VNTR. If these findings are substantiated in further phenotype/genotype studies, they offer the prospect of a clinically important genetic marker, not only for PCOS but also for the future risk of NIDDM.

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