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Symposium: Diet, nutrition and exercise in reproduction

Genetic and environmental origins of obesity relevant to reproduction

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Abstract

Obesity has a negative impact on reproductive health, particularly in women with polycystic ovarian syndrome (PCOS). Obesity itself is the product of both genetic and environmental influences, although the current 'epidemic' of obesity is largely related to changes in diet and lifestyle. Single gene defects leading to obesity and disordered reproductive function are rare but can are informative about metabolic pathways involved in appetite regulation. There is good evidence that PCOS has an important genetic background, which probably involves the interaction of several genes. The phenotype of PCOS and its impact on reproductive function is profoundly affected by obesity, which, in turn has both genetic and environmental influences. Understanding the genetic basis of PCOS is important but improvements in diet and lifestyle are the best means of improving reproductive function.

Keywords: *androgens, anovulation, candidate genes, complex trait, insulin resistance, polycystic ovarian syndrome (PCOS)*

Obesity and reproductive function

It has been recognized for thousands of years that obesity has deleterious effects on reproductive function in women. In an essay by Hippocrates on the Scythians which appears under the heading 'The influence of climate, water supply and situation on health' their reproductive function is described in the following terms: 'The girls get amazingly flabby and podgy.....People of such constitution cannot be prolific..... fatness and flabbiness are to blame. The womb is unable to receive the semen and they menstruate infrequently and little. As good proof of the sort of physical characteristics that are favourable to conception, consider the case of serving wenches. No sooner do they have intercourse with a man than they become pregnant, on account of their sturdy physique and their leanness of flesh' (Chadwick and Mann, 1983). Reports in the more recent medical literature have confirmed the link between nutrition and reproduction (Hartz *et al.*, 1979; Kopelman, 1988; Franks *et al.*, 1996), but whilst the mechanism by which under-nutrition impacts on ovarian function is well understood (it clearly involves disturbance of the hypothalamic control of gonadotrophin secretion), the mechanism of ovarian dysfunction associated with obesity remains uncertain (Franks *et al.*, 1996).

Genetic influences on obesity

The current 'epidemic' of obesity in western societies is clearly related to changing patterns of nutrition but there is little doubt that genetic factors play an important part in the susceptibility of an individual to obesity. Data from studies of monozygotic and dizygotic twins and family studies provide a clear indication that genes play a role in the development of obesity (Loos and Bouchard, 2003), even though nutritional factors

clearly play a huge part in the worryingly steep increase in obesity rates over the last decade.

Monogenic causes of obesity provide important information about the aetiology of obesity, the biochemical pathways involved in appetite regulation, and the mechanism of reproductive dysfunction (Farooqi and O'Rahilly, 2000). A good example of such a single gene defect is leptin deficiency. Children with a mutation in the leptin gene are morbidly obese and have delayed puberty (Montague *et al.*, 1997; Farooqi and O'Rahilly, 2000; Farooqi *et al.*, 2001). The main reason for weight gain is a failure of satiety and unrestricted eating behaviour. Leptin deficiency probably contributes to the aetiology of delayed puberty, since leptin has been implicated in positive regulation of gonadotrophin-releasing hormone (GnRH) (Shalitin and Phillip, 2003). Another example is obesity and delayed puberty associated with a mutation in the gene coding for prohormone convertase 1 (PC1) (Jackson *et al.*, 1997; Farooqi and O'Rahilly, 2000). Deficiency of PC1 affects processing of pro-opiomelanocortin (POMC) and although the cause of obesity is not conclusively known, there is good evidence that deficiency, or impaired action of one of the ligands derived from POMC, melanocortin, leads to hyperphagia.

Nevertheless, such single gene defects are very rare and the genetic contribution to obesity, whilst highly important, is usually complex (Loos and Bouchard, 2003). Several candidate genes have been implicated in the aetiology of obesity including those encoding peroxisome proliferator-activated receptor gamma (PPARγ), β-adrenergic receptors and uncoupling proteins. In genome-wide scans, further susceptibility loci have been identified on chromosomes 2p, 3q, 5p, 6p, 7q, 10p, 11q, 17p and 20q. Loos and Bouchard have suggested classifying the genetic impact on obesity in terms of four levels: genetic obesity, strong genetic predisposition, slight genetic predisposition and genetically resistant (Loos and Bouchard, 2003).

The most important example of a complex trait disorder associated with obesity and disordered reproductive function is polycystic ovarian syndrome (PCOS). PCOS provides a key paradigm for understanding the interaction of genetic and environmental factors controlling nutrition and reproduction (Franks *et al.*, 1996; Abbott *et al.*, 2002). Whilst PCOS is not invariably linked to obesity [the proportion of subjects with PCOS who are overweight or obese varies from series to series according to the selection criteria used for diagnosis (Franks, 1995)], there is evidence that PCOS is associated with a disorder of energy balance that predisposes to obesity (Robinson *et al.*, 1992; Franks *et al.*, 1996). The next section describes a model for the pathogenesis of PCOS that proposes that it has its origins during fetal or early postnatal development and in which the clinical manifestations, and especially ovulatory status, are affected by the interaction of genetic and environmental factors.

PCOS: genes, environment, obesity and reproduction

PCOS is the commonest cause of anovulatory infertility and its influence on reproductive function is profoundly affected by nutritional status (Franks, 1995). Specifically, overweight and obese women with PCOS are more likely to have menstrual disturbance and less likely to respond to induction of ovulation than their lean counterparts (Kiddy *et al.*, 1990; White *et al.*, 1996). Calorie restriction in obese women with PCOS not only improves the metabolic profile, but also greatly increases the chance of spontaneous ovulation and restoration of potential for fertility (Kiddy *et al.*, 1992; Clark *et al.*, 1995, 1998; Moran *et al.*, 2006 [see page 569 in this issue]). The mechanism of anovulation remains uncertain but the relative hyperinsulinaemia that is characteristic of obese women with PCOS appears to play an important part (Franks *et al.*, 2001; Bellver *et al.*, 2006 [see page 562 in this issue]).

Developmental origin of PCOS

It has been postulated that PCOS is a genetically determined ovarian disorder that is characterized by excessive androgen production (Abbott *et al.*, 2002). The heterogeneity of clinical and endocrine features may be explained by the interaction of this abnormality with the effects of other genes and with the environment (**Figure 1**). The proposed central role for androgens in the aetiology of the syndrome is supported by evidence from an informative primate model for PCOS, the prenatally androgenized Rhesus monkey (Abbott *et al.*, 2004). In adult life, these animals show many of the features typical of PCOS; the animals demonstrate hypersecretion of LH, ovarian hyperandrogenism, insulin resistance and anovulation in relation to increased body weight (Abbott *et al.*, 2002, 2004). Recent data from a similar model in another, non-primate species, the prenatally androgenized ewe, support these findings (Birch *et al.*, 2001). These animals also show abnormalities of gonadotrophin secretion, of preantral follicular development and abnormal glucose/insulin homeostasis. With these data in mind, it is reasonable to propose that many, if not most of the manifestations of PCOS can be attributed to 'downstream' effects of exposure to excess androgen during development. The key questions with regard to the relevance to PCOS, however, are what is the source of the excess androgen and when during development does the exposure occur?

It is unlikely that in human PCOS maternal androgen excess has any significant bearing on the androgen status of the fetus. The high concentration of sex hormone-binding globulin (SHBG) in the maternal circulation, together with the high activity of placental aromatase, acts as an effective barrier to androgenization of a female fetus. In both Rhesus and sheep models, the pharmacological doses of androgen employed effectively flooded this protective barrier. It is much more likely that there is an intrinsic source of excess androgen, i.e. the ovary or adrenal (or perhaps both). There is certainly evidence for a primary (intrinsic) abnormality of theca cell steroidogenesis in PCOS (Gilling-Smith *et al.*, 1994; Wood *et al.*, 2003), but even this phenomenon may be secondary to an abnormality of ovarian follicular development. Specifically, the polycystic ovary is characterized by increased density of preantral follicles and an increase in the proportion of early growing follicles (Webber *et al.*, 2003; Maciel *et al.*, 2004). Significantly, such abnormalities are found in follicles that have not yet acquired a theca cell layer, suggesting that abnormal theca function may be a consequence rather than

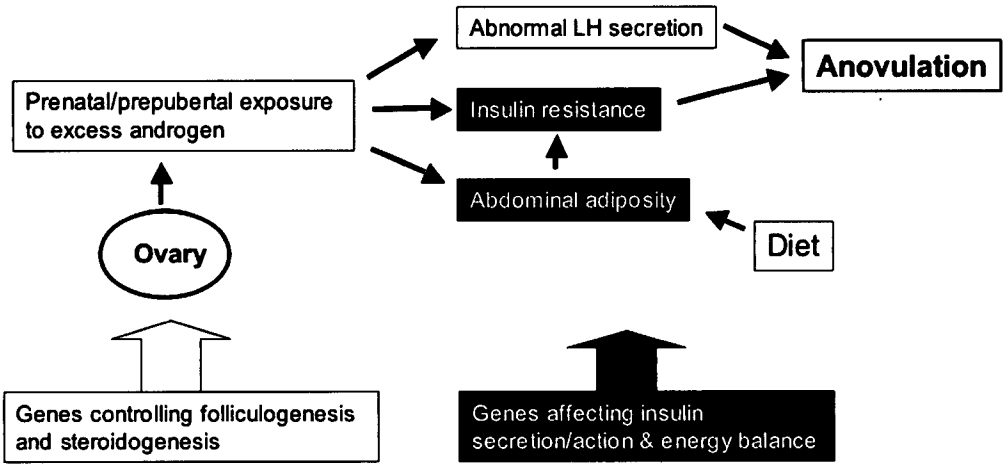

Figure 1. Interaction of genes and environment in the aetiology of polycystic ovarian syndrome and pathogenesis of anovulation. It is suggested that the polycystic ovary is genetically predisposed to hyper-secrete androgens and that exposure to excess androgens (either pre- or post-natally) contributes to abnormal LH secretion, abdominal adiposity and insulin resistance (Abbott *et al.*, 2002). Other genetic factors will influence insulin secretion and action, adipocyte function and energy balance but dietary factors have an important overriding effect. Both abnormal LH secretion and hyperinsulinaemia contribute to the onset and maintenance of anovulation.

the cause of disordered folliculogenesis (Webber *et al.*, 2003). Nevertheless, the polycystic ovary is effectively predisposed to secrete excess androgens and it is likely that genetic factors have a major part to play in this phenomenon. It is possible that the prenatal ovary in a fetus predisposed to PCOS is already steroidogenically active but this seems somewhat implausible given that the human fetal ovary appears to be relatively quiescent in terms of steroid synthesis. It is more plausible that the excess production of androgens occurs during the physiological, but temporary, activation of the hypothalamic–pituitary–gonadal axis in infancy, and is reinforced at the onset of puberty. Puberty is also a time when body fat increases, abdominal fat distribution is selectively increased and an increase in insulin resistance occurs as part of normal pubertal changes (Franks, 2002). Androgens appear to play a significant part in body fat distribution and in girls predisposed to PCOS excess androgens will tend to exaggerate this phenotype (Abbott *et al.*, 2002). Symptoms of PCOS typically present first during adolescence and as obesity becomes more prevalent in the population, so the number of young women with symptoms of PCOS is increasing. This is particularly worrying because of the attendant insulin resistance and increased risk of type 2 diabetes (Dunaif, 1997). Another aspect of the putative developmental origin of PCOS (that may involve either genetic or environmental factors during gestation) is fetal growth and birth weight. Low birth weight has been reported to be associated with development of symptoms of PCOS during adolescence (Ibanez *et al.*, 1998), although this was not borne out in a study of a large cohort of women born in Finland in 1966 (Laitinen *et al.*, 2003).

Genes and PCOS

There is plentiful evidence that PCOS has a genetic basis (Franks *et al.*, 1997; Legro *et al.*, 1998; Franks and McCarthy, 2004). It is significantly more common within families than in the general female population; intrinsic differences between PCOS subjects and control subjects have been reported in ovarian folliculogenesis, steroidogenesis and insulin secretion or action, and there is familial transmission of polycystic ovaries, hyperandrogenaemia and insulin resistance. Importantly, a recently reported study based on the Dutch Twin Registry showed a much higher concordance of clinical features of PCOS between monozygotic than between dizygotic twins (Vink *et al.*, 2005). The mode of inheritance of PCOS remains unclear. It is, of course, a disorder that presents during the reproductive years and thus affected status is difficult to ascertain in premenarchal girls and in postmenopausal women. Furthermore, the male phenotype is not evident and although some studies have suggested that premature balding is more common within families of women with PCOS than in the general population, this is not accepted as a clear male counterpart of PCOS (Franks *et al.*, 1997). In studies of relatively large pedigrees in affected families, it was originally proposed that PCOS was an autosomal dominant condition (Carey *et al.*, 1993), suggesting a single gene defect, but it now seems more plausible that PCOS represents an oligogenic (or truly polygenic) disorder (Franks *et al.*, 1997; Diamanti-Kandarakis and Piperi, 2005; Escobar-Morreale *et al.*, 2005).

Ovarian morphology, hyperandrogenaemia and insulin resistance all appear to be inherited traits in families of women with PCOS and it is reasonable to propose that genes regulating the relevant biochemical pathways have a significant part to play. In a model of the developmental origin of PCOS, it is suggested that the major candidate pathway is that affecting folliculogenesis and/or androgen production (Abbott *et al.*, 2002). The subsequent hyperandrogenism gives rise to the reproductive and metabolic abnormalities, as in the androgenized Rhesus monkey or sheep. But even if this is indeed the major pathway of pathogenesis, it is very likely that other genetic (and environmental) factors play a

part in determining the phenotype. Of particular relevance in the context of this review are genes that affect obesity and hence metabolic manifestations of PCOS, i.e. those involved in energy balance, adipocyte function, insulin secretion and action (Franks and McCarthy, 2004).

Candidate genes in PCOS

Both association and linkage studies have been used to determine whether there is any relationship between variants in the genes of interest and disease risk within populations or families. Association studies have involved both the case–control approach and family-based association methods such as the transmission disequilibrium test (TDT) (Spielman and Ewens, 1996, 1999) in which transmissions from parents to their affected offspring are the focus of analysis. TDT methods have the advantage of avoiding spurious positive associations that can be obtained in case–control studies when the two populations are not matched for ethnic background (so-called 'population stratification'). TDT also offers the prospect of assessing 'parent-of-origin' effects wherein there is preferential transmission of disease alleles from either the mother or the father to affected offspring. This indeed may be the case in relation to the insulin gene in PCOS, as described below.

Whilst TDT relies on one particular configuration of PCOS families, other family structures form the usual substrate for linkage analyses. Such analyses depend on the fact that polymorphic markers within, or closely linked to a disease-susceptibility locus should show a tendency to segregate with the disease in families. A number of computer-assisted methods are available for linkage analysis. Traditionally, parametric (LOD-score based [logarithm of the odds (to the base 10) for estimating linkage distance]) analytical methods have been used when there is clear evidence to support a particular mode of inheritance: although, as has been seen, some of the available family data do support an autosomal dominant mode of inheritance to PCOS, there remains the concern that incorrect specification of the model could lead to reduced power to detect linkage. For this reason, for most linkage studies, a non-parametric method of analysis has been used (the GENEHUNTER programme) (Kruglyak *et al.*, 1996) which requires no assumptions to be made about the mode of inheritance.

Problems associated with the candidate gene approach in PCOS

In the search for candidate genes in any complex trait, it is clear that studies need to be of sufficient power to account for the likelihood that the variants to be detected will have modest relative risks. For example, in type 2 diabetes, the two best-substantiated susceptibility variants, PPARγ Pro12Ala (Altshuler *et al.*, 2000) and KCNJ11 E23K (Gloyn *et al.*, 2003), have odds ratios of 1.15–1.20. The consequences of inadequate study design (particularly small subject cohorts) have been a proliferation of reports of positive associations, difficult or impossible to replicate in other data sets (Altshuler *et al.*, 2000; Hirschhorn *et al.*, 2002): most of these are likely to be false-positives. Given the effort required to define the phenotype of cases and controls for PCOS, studies of this disease (including some of our own previous studies!) have proven particularly prone to these failings. The largest published linkage study in PCOS to date included only 39 affected sib pairs (Urbanek *et al.*, 1999). These sample sizes are substantially less than those available for most other complex traits. It is clear that new standards for association studies in complex traits are required in an effort to improve the reliability of published association studies (Editorial, 1999; Cardon and Bell, 2001). These place an emphasis on adequately sized cohorts, replication, appropriate statistical methodologies and, where possible, functional validation.

Genes involved in the secretion and action of insulin

Insulin gene variable number tandem repeat (INS-VNTR)

There is evidence that the insulin gene (INS) variable number tandem repeat (VNTR) is a major susceptibility locus for PCOS (Waterworth *et al.*, 1997). The INS-VNTR lies in the 5′ regulatory region of the gene; it has been shown to be involved in regulation of insulin gene expression and has been implicated in the aetiology of type 2 diabetes and of obesity. In an initial study, it was found that class III alleles in the VNTR were associated with anovulatory PCOS in two independent populations and using two different methods of analysis (case-control studies and by the use of affected family based controls; AFBAC). With the aid of the GENEHUNTER linkage analysis programme, it was also established that there was excess allele sharing at the INS-VNTR locus. The geometric mean of fasting serum insulin concentrations was significantly higher in families in which linkage was demonstrated than in those families without evidence of linkage (Waterworth *et al.*, 1997). These findings suggested a functional role for the VNTR variant in the expression of hyperinsulinaemia/insulin resistance in PCOS. In contrast, however, Urbanek and colleagues (1999) found no evidence for excess allele sharing at this locus in their population. In a recent follow-up study, the role of INS-VNTR was re-evaluated using both a large case–control series and using TDT for parent–offspring trios. On this occasion, no evidence was found for association of the INS-VNTR with PCOS (Powell *et al.*, 2005). This is a salutary illustration of the key importance of employing sufficiently large series of subjects to either exclude or confirm the role of candidate genes in a complex trait disorder.

Genes involved in insulin action

The insulin receptor gene and chromosome 19p

Screening of the insulin receptor gene has been undertaken in two well-characterized populations of hyperinsulinaemic women with PCOS. Conway and colleagues (Conway *et al.*, 1994) examined the tyrosine kinase domain of the insulin receptor gene in 22 patients but found no abnormalities. Talbot and co-workers (Talbot *et al.*, 1996) performed molecular scanning of the entire coding region of the gene in 24 hyperinsulinaemic subjects with PCOS and again no significant mutations were detected. Mutations of the insulin receptor gene are therefore unlikely to be a major cause of insulin resistance

in PCOS. Urbanek and colleagues (Urbanek *et al.*, 1999) did find evidence of association of the insulin receptor gene locus with PCOS in their TDT analysis, but this effect proved to be non-significant after correction for multiple testing.

Two small case–control studies showed an association between a locus close to (but not in linkage disequilibrium with) the insulin receptor on the short arm of chromosome 19 (Tucci *et al.*, 2001; Villuendas *et al.*, 2003). Recently, in a much larger and appropriately powered study, Urbanek and colleagues have confirmed the association between this locus on chromosome 19p13.2 (specifically at D19S884) (Urbanek *et al.*, 2005). As yet, the functional significance of this susceptibility locus remains uncertain and the relationship of genotype to metabolic phenotype will be of interest.

Genes encoding insulin receptor substrates and other proteins involved in insulin action

There have been a few studies implicating variants of the genes encoding insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) (El Mkadem *et al.*, 2001; Ehrmann *et al.*, 2002b). In the France-based study (El Mkadem *et al.*, 2001), a relationship was found between polymorphisms in both IRS-1 and IRS-2 and metabolic indices in women with PCOS but the number of cases and controls was small. In the American study (Ehrmann *et al.*, 2002b), there was a respectable number of cases (about 200) but in this series, no association was found between IRS-1 variants and PCOS, and although there was a link between glucose homeostasis and variants of IRS-2, the biochemical changes were minor.

Other genes implicated in the metabolic abnormalities of PCOS have been investigated, prompted, in the main, by findings in populations with type 2 diabetes. These findings have been reviewed extensively elsewhere (Diamanti-Kandarakis and Piperi, 2005; Escobar-Morreale *et al.*, 2005). These include calpain 10 (Ehrmann *et al.*, 2002a; Haddad *et al.*, 2002), PPARγ (Hara *et al.*, 2002) and resistin (Urbanek *et al.*, 2003). The results have been largely negative although there is some evidence of an association between variations in PPARγ and insulin sensitivity in women. Again, large case–control studies are lacking, but the Pro12Ala polymorphism in PPARγ2 has emerged as a potential candidate locus.

In conclusion, there is good evidence that PCOS has an important genetic background, which is probably oligogenic in origin. The phenotype of PCOS and its impact on reproductive function is profoundly affected by obesity, which, in turn has both genetic and environmental influences. With the possible exception of the Chr19p polymorphism (whose phenotypic expression still remains to be clearly elucidated), the key candidate loci involved in the aetiology of the syndrome are still unknown. Identification of such susceptibility loci will help both in the understanding of the pathogenesis of PCOS and in conceiving more specific therapeutic approaches.

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