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Functional genetic polymorphisms and female reproductive disorders: Part I: polycystic ovary syndrome and ovarian response

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BACKGROUND: The identification of polymorphisms associated with a disease can help to elucidate its pathogenesis, and this knowledge can be used to improve prognosis for women with a particular disorder, such as polycystic ovary syndrome (PCOS). Since an altered response to ovarian stimulation is also a characteristic of the disease, further knowledge about its aetiology could help in defining the parameters that determine the response of an individual to ovarian stimulation. METHODS: PubMed and EMBASE databases were systematically searched for gene association studies published until the end of August 2007, using search criteria relevant to PCOS and ovarian response to stimulation. Data from additional papers identified through hand searches were also included; 139 publications were reviewed. RESULTS: Several genes involved in ovarian function and metabolism are associated with increased susceptibility to PCOS, but none is strong enough to correlate alone with susceptibility to the disease, or response to therapy. A single-nucleotide polymorphism in exon 10 of the FSH receptor (FSHR) gene, *FSHR* p.N680S, was consistently identified as having a significant association with ovarian response to FSH. CONCLUSIONS: No consistent association between gene polymorphism and PCOS could be identified. The *FSHR* gene may play a significant role in the success of ovarian stimulation, and can be used as a marker to predict differences in FSHR function and ovarian response to FSH. Genotyping the *FSHR* p.N680S polymorphism may provide a means of identifying a population of poor responders before *in vitro* fertilization procedures are initiated.

Keywords: female reproduction; genetic polymorphisms; polycystic ovary syndrome

Introduction

Genetic studies contribute towards our understanding of disease pathogenesis and hold the promise of improving our ability to individualize treatment for patients. In this review, we first explain the rationale and methodology of studies that identify gene–disease associations. We then review the extensive body of research aimed at identifying contributing genetic factors in polycystic ovary syndrome (PCOS) and assess genetic studies that have investigated patients' ovarian response to gonadotrophin treatment using assisted reproductive techniques. This provides the first systematically selected summary of published studies about all genes that have so far been investigated in these areas. We then discuss the limitations of such studies and provide recommendations for using this information in the future, with the aim of guiding the design and interpretation of future studies.

Genetic association studies

Genetic association studies are used to investigate genes or genetic markers that might be associated with a particular disease phenotype or trait. These studies rely on the identification and characterization of natural variants or polymorphisms in the DNA sequence among individuals. If an association is present, a particular variant

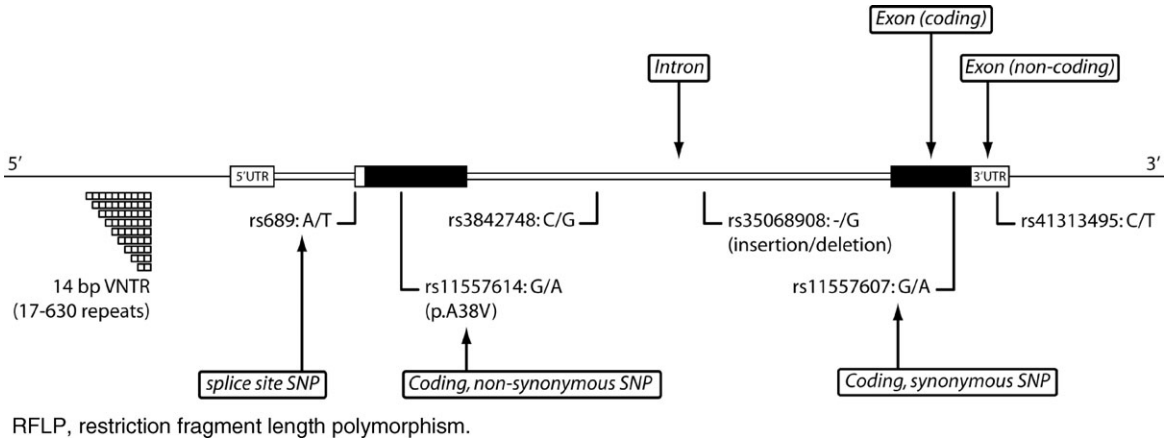


Figure 1: Graphic representation of types of genetic variants, showing insertion/deletion (ins/del) polymorphisms, both coding and non-coding SNPs, and repeat polymorphisms such as tandem repeats or VNTR.
Variants are shown occurring within a gene (in this example the *INS* gene), but can also occur outside of genes. Other types of genetic variations that affect larger regions, such as copy number variations, are not shown. SNP, single-nucleotide polymorphism; VNTR, variable number of tandem repeats.

will be seen more often than expected by chance in an individual carrying the trait. Variants fall into several categories, depending on where they occur relative to a gene. First, variants can occur within or outside a gene. Those that occur within a gene may occur within an exon, an intron or regulatory regions. Regulatory regions control gene transcription and include the promoter region upstream of the gene and the 3′-untranslated region downstream of the protein-coding region (Fig. 1). Variants within a gene can be functional or silent. Functional variation within a gene can be the direct cause of a phenotype abnormality or may increase susceptibility to a disease. Functional variants in coding regions can change the protein sequence; in non-coding regions they may have effects on RNA transcription and processing (Kim *et al*., 2005; Wang *et al*., 2005). Silent variants occur in protein-coding DNA but do not change the sequence of a gene product. In the majority of cases, variation that occurs outside a gene is used as a tag to identify nearby functional variation within a gene (Fig. 2). Such analyses are based on a chromosomal property called linkage disequilibrium. Linkage disequilibrium refers to the observation that in the general population, two DNA variants that are located close to each other tend to be observed together more frequently than two variants that are located further apart. Variants may be single-base changes, known as single-nucleotide polymorphisms (SNPs), or they may occur as insertions or deletions of one base or more.

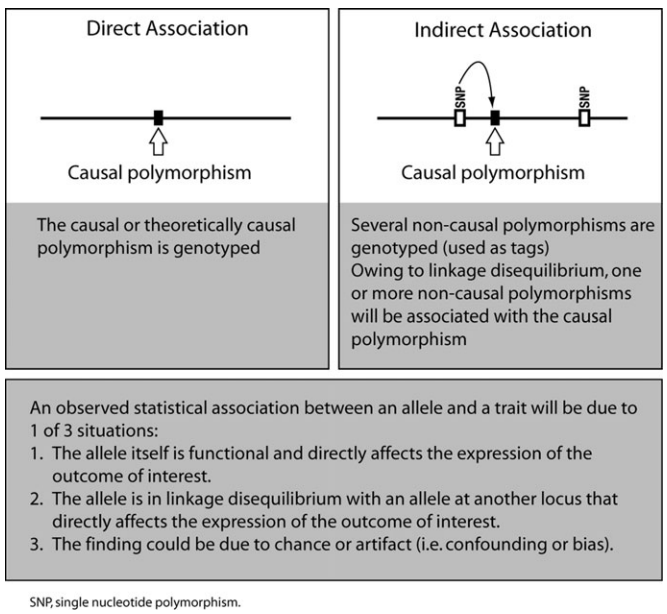


Figure 2: Principles of genetic association, and possible explanations for an observed association.

Until recently, the high cost of testing for genetic variation has meant that most analyses have concentrated on the study of a limited number of functional genetic variants, primarily SNPs, in specific genes. Candidate genes for genotyping are selected according to their function: they encode proteins that are thought to have a role in the disease or response to treatment. Variants within the candidate genes are most often selected because they occur within exons and would result in a change in amino acid sequence in the protein. Alternatively, they are located in non-coding regions, but change a transcription factor-binding site or influence splicing efficiency, affecting the expression of a protein. The main advantage of the candidate SNP approach is that such studies are affordable, as only a limited number of variants are studied and a relatively small sample size can be used. In the context of genome-wide analyses, the main advantages of using SNPs are their abundance in the genome, and the possibility of conducting genotyping in a high-throughput manner. To date, a wealth of results has been obtained from studies addressing the problem of potential associations between genetic polymorphisms and PCOS or ovarian response to gonadotrophins.

Materials and Methods

We systematically searched the PubMed and EMBASE databases for gene association studies published until the end of August 2007, using the terms ‘PCOS’, ‘polycystic and (ovary or ovaries)’, ‘ovarian and response’, ‘OHSS’ or ‘ovarian and hyperstimulation’, combined with ‘polymorphism or polymorphisms’ or ‘mutation or mutations’. The search was not limited by language of publication. Two authors (B.C.J.M.F. and M.S.) then selected relevant studies using the

following criteria: more than one patient, inclusion of a control group and with at least the abstract written in English. Also included were additional papers identified through hand searches carried out by the same authors.

All results of selected studies are comprehensively summarized in functional group-specific tables by gene of interest, with a brief description in the text. As the frequency of genetic differences varies between ethnic or geographic populations, each study is based on a specific patient population to minimize heterogeneity. Therefore, the tables also include critical details that give information about the context (ethnic background) and likely strength (based on sample size) of the study, to provide a source of reference.

Results

Polycystic ovary syndrome

PCOS affects about 1 in 10 women of reproductive age, and is the most common endocrine condition in this group. The syndrome is associated with multiple endocrinological and metabolic abnormalities, with hyperandrogenaemia and anovulation as the central hallmarks (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004a, b). About 80% of women with PCOS have abnormal menstrual cycles (Conway *et al*., 1989), many are anovulatory (Laven *et al*., 2002) and, in those who manage to conceive successfully after treatment, there is an increased risk of complications during pregnancy as well as neonatal complications (Boomsma *et al*., 2006).

Twin and familial studies suggest that there is a genetic component to PCOS, with a polygenic pattern of inheritance (Jahanfar and Eden, 1996; Diamanti-Kandarakis and Piperi, 2005; Fratantonio *et al*., 2005). There is also a strong environmental component (e.g. diet and exercise) (Abbott *et al*., 2002). A series of familial studies conducted in the USA have demonstrated a strong linkage between susceptibility to PCOS and the dinucleotide marker D19S884 in the chromosomal region 19p13.2 (Urbanek *et al*., 2000a, 2005, 2003; Stewart *et al*., 2006). The identity of the gene or genetic element responsible for this effect is not yet known. As complex traits such as PCOS result from the interaction of several genetic variations with environmental factors, individual genetic variations have a modest effect that is more difficult to detect than for monogenic traits.

The search for PCOS susceptibility genes has focused mainly on genes involved in sex hormone regulation, insulin sensitivity, cardiovascular risk or steroid biosynthesis and detoxification (Ehrmann, 2005; Escobar-Morreale *et al*., 2005; Fratantonio *et al*., 2005). The results from these studies are summarized in Tables I–IV and are described below.

Sex hormones and hormone regulators

Because PCOS is characterized by endocrinological abnormalities, polymorphisms in genes encoding sex hormones or regulators of their activity have been investigated (Table I).

Hormone receptors. Two variants in the gene encoding the β2 adrenergic receptor (*ADRB2)* were studied in Japanese women: the p.Q27E variant was linked to PCOS susceptibility, but the p.R16G variant was not (Kurabayashi *et al*., 2006). Within the β3 adrenergic receptor (*ADRB3*) gene, the p.W64R polymorphism does not appear to confer an increased risk of PCOS (Perez-Bravo *et al*., 2005; Kurabayashi *et al*., 2006), although it may affect triglyceride regulation in women with this condition (Perez-Bravo *et al*., 2005). Conflicting findings have been reported from studies investigating the association between the length of the CAG repeat microsatellite in the androgen receptor (*AR*) gene and PCOS susceptibility (Hickey *et al*., 2002; Jaaskelainen *et al*., 2005); however, short alleles of this microsatellite appear to be associated with increased androgenic activity (Mifsud *et al*., 2000), and this may lead to PCOS. One study has shown that an AccI restriction fragment length polymorphism (RFLP) in the gene that encodes the beta subunit of the gonadotrophin follicle-stimulating hormone (*FSHB*) may be associated with PCOS susceptibility (Tong *et al*., 2000). A larger number of studies have investigated polymorphisms in the gene that encodes the receptor for FSH rather than the gene for the hormone itself. The *FSHR* gene contains two important SNPs in exon 10, which are in linkage disequilibrium and change two amino acids at positions 307 and 680. However, although the p.N680S genotype influences ovarian response (see section on ovarian response to gonadotrophins), this common polymorphism does not seem to play a major role in PCOS. In Japanese women with PCOS, the S allele was more prevalent than in normal subjects (Sudo *et al*., 2002), but no differential allelic distribution was found in Caucasian (Conway *et al*., 1999) or Chinese women (Tong *et al*., 2001).

Sex hormone-binding globulin. Women with PCOS have subnormal levels of SHBG, and a microsatellite repeat in the promoter of the *SHBG* gene has been linked to PCOS susceptibility in Greek (Xita *et al*., 2003) but not in Slovenian (Ferk *et al*., 2007) women, although in both populations, serum SHBG levels were strongly influenced by the genotype. The p.D327N polymorphism in this gene was not found to influence PCOS susceptibility in studies in Slovenia or the Czech Republic (Bendlova *et al*., 2007; Ferk *et al*., 2007).

Regulators of hormone activity. Studies of the following genes that also encode hormones or proteins regulating hormone activity have failed to show an association with PCOS susceptibility: Dopamine D3 receptor (*DRD3*) (Kahsar-Miller *et al*., 1999), follistatin (*FST*) (Urbanek *et al*., 2000b; Calvo *et al*., 2001; Jones *et al*., 2007), guanine nucleotide-binding protein G (s) subunit alpha (*GNAS*) (Hahn *et al*., 2006), leptin receptor (*LEPR*) (Oksanen *et al*., 2000) and luteinizing hormone (*LHB*) (Rajkhowa *et al*., 1995; Elter *et al*., 1999). However, there was an association between phenotype and two of the SNPs investigated in the *FST* gene (Jones *et al*., 2007). Furthermore, although not associated with susceptibility, the genotype distribution of the signal transduction protein gene *GNAS* was found to be associated with body mass index (BMI) and insulin resistance in women with PCOS (Hahn *et al*., 2006; Jones *et al*., 2007).

Steroid metabolism and biosynthesis

Several studies have investigated whether polymorphisms in enzymes involved in the biosynthesis and metabolism of sex steroids confer PCOS susceptibility (Table II).

Sex steroid synthesis. Type 5 17β-hydroxysteroid dehydrogenase is an important enzyme for testosterone biosynthesis, and a polymorphism in the gene encoding this enzyme (*AKR1C3*) has been

Table I. Polymorphisms of genes encoding sex hormones and hormone regulators.

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)	
ADRB2 (5q31-q32, β2 adrenic receptor: calcium signalling, may play a role in insulin resistance)	p.R16G	rs1042713		Japanese women (59, 97) (Kurabayashi *et al.*, 2006)	
	p.Q27E	rs1042714	Japanese women (59, 97) (Kurabayashi *et al.*, 2006)		
ADRB3 (8p12-p11.2, β3 adrenic receptor: hormone receptor)	p.W64R	rs4994		Chilean women (106, 82) (Perez-Bravo *et al.*, 2005)	Chilean women—triglyceride levels (106, 82) (Perez-Bravo *et al.*, 2005)
				Japanese women (59, 97) (Kurabayashi *et al.*, 2006)	
AR (Xq11.2-q12, androgen receptor: hormone receptor)	CAG repeat	rs5902610, rs25885096	Australian women (122, 83) (Hickey *et al.*, 2002)	Finnish women (106, 112) (Jaaskelainen *et al.*, 2005)	Asian women—T levels (91, 12) (Mifsud *et al.*, 2000)
DRD3 (3q13.3, dopamine D3 receptor: regulates sex hormone secretion)	MscI RFLP (p.S9G)	rs6280		Caucasian women (152, 96) (Kahsar-Miller *et al.*, 1999)	
FSHB (11p13, follicle-stimulating hormone β: sex hormone)	AccI RFLP (1736T/C)	rs6169	Chinese women (135, 105) (Tong *et al.*, 2000)		
FSHR (2p21-p16, follicle-stimulating hormone receptor: hormone receptor)	p.A307T	rs6165	Japanese women (18, 258) (Sudo *et al.*, 2002)	Chinese women (124, 236) (Tong *et al.*, 2001) Caucasian women (93, 51) (Conway *et al.*, 1999)	
	p.N680S	rs6166	Japanese women (18, 258) (Sudo *et al.*, 2002)	Chinese women (124, 236) (Tong *et al.*, 2001) Caucasian women (93, 51) (Conway *et al.*, 1999)	
FST (5q11.2, follistatin: inhibits follicle-stimulating hormone release)	951G/A	rs1746136		Spanish women (34, 15) (Calvo *et al.*, 2001)	
	343xT/A	rs722910		Families, mixed descent (249[a]) (Urbanek *et al.*, 2000b)	
	D5S474, D5S822, D5S628			Families, mixed descent (249[a]) (Urbanek *et al.*, 2000b)	

		rs3797297		Australian women (173, 107) (Jones *et al.*, 2007)	Australian women—FAI and SHBG levels (173, 107) (Jones *et al.*, 2007)
		rs11745088		Australian women (173, 107) (Jones *et al.*, 2007)	Australian women—DHES-S level (173, 107) (Jones *et al.*, 2007)
		rs1423560, rs3203788, rs1062809, rs1127760, rs1127761		Australian women (173, 107) (Jones *et al.*, 2007)	
GNAS (20q13.32, guanine nucleotide-binding protein G(s) subunit alpha isoform: GPCR signal transduction)	393T/C	rs7121		German caucasian women (278, 209) (Hahn *et al.*, 2006)	German caucasian women—BMI/weight, insulin resistance (278, 209) (Hahn *et al.*, 2006)
LEPR (1p31, leptin receptor: regulates hormone activity)	p.K109R	rs1137100		Finnish women (38, 122) (Oksanen *et al.*, 2000)	
	p.R223Q	rs1137101		Finnish women (38, 122) (Oksanen *et al.*, 2000)	
	p.N656K	rs8179183		Finnish women (38, 122) (Oksanen *et al.*, 2000)	
	ins/del CTTTA in 3′-UTR			Finnish women (38, 122) (Oksanen *et al.*, 2000)	
LHB (19q13.32, luteinizing hormone: sex hormone)	p.W8R (p.W28R)	rs1800447		Turkish women (30, 30) (Elter *et al.*, 1999), UK women (153, 212) (Rajkhowa *et al.*, 1995)	
	p.I15T (p.I35T)	rs3434826		Turkish women (30, 30) (Elter *et al.*, 1999), UK women (153, 212) (Rajkhowa *et al.*, 1995)	
SHBG (17p13-p12, sex hormone-binding globulin: mediates hormonal activity)	TAAAA repeat	rs35785886	Greek women (185, 324) (Xita *et al.*, 2003)	Slovenian women (123, 110) (Ferk *et al.*, 2007)	Greek women—FAI and SHBG levels (185, 324) (Xita *et al.*, 2003) Sloven women—SHBG levels (123, 110) (Ferk *et al.*, 2007)
	p.D327 N	rs6259		Czech Republic women (248, 109) (Bendlova *et al.*, 2007) Slovenian women (123, 110) (Ferk *et al.*, 2007)	

The list (ordered alphabetically) shows genes that have been investigated for their role in polycystic ovary syndrome. Studies showing positive or no associations of these genes with disease susceptibility and positive associations with phenotype (clinical characteristics of the condition, e.g. endocrinological abnormalities) are presented. Genes are listed alphabetically. [a]Number of PCOS families. BMI, body mass index; PCOS, polycystic ovary syndrome; RFLP, restriction fragment length polymorphism.

Table II. Polymorphisms of genes encoding enzymes involved in steroid metabolism and biosynthesis.

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype	Treatment response
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)		
AKR1C3 (17q11-q21, 5 17β-hydroxysteroid dehydrogenase: testosterone biosynthesis, oestrogen metabolism)	−71G/A	rs3763676	North American women (121, 128) (Qin *et al.*, 2006)			
CYP1A1 (15q22-q24, cytochrome P450 1A1 enzyme: steroid biosynthesis, oestrogen metabolism, Phase I detoxification)	MspI RFLP (6235T/C) (3801T/C)	rs4646903	South Indian women (180, 72) (Babu *et al.*, 2004)			
CYP11A1 (15q23-q24, cytochrome P450 11A enzyme: steroid biosynthesis)	TTTTA VNTR (D15S520)		Greek women (80, 90) (Diamanti-Kandarakis *et al.*, 2000) UK (371, 350) and Finnish women (1589[a]) (Gaasenbeek *et al.*, 2004) Chinese women (201, 147) (Hahn *et al.*, 2006; Wang *et al.*, 2006a) UK women (97, 51aPCO, 59) (Gharani *et al.*, 1997)		Chinese women— BMI (201, 147) (Hahn *et al.*, 2006; Wang *et al.*, 2006a), UK women—T levels (97, 51aPCO, 59) (Gharani *et al.*, 1997)	
	AAAT repeat (intron 1)			UK (371, 350) and Finnish women (1589[a]) (Gaasenbeek *et al.*, 2004)		
CYP11B2 (8q21-q22, aldosterone synthetase: steroid biosynthesis, regulates ovarian renin–angiotensin system)	−344C/T	rs1799998	Chinese women (92, 60) (Zhao *et al.*, 2003)			
CYP17A1 (10q24.3, 17α-hydroxylase: oestrogen biosynthesis)	MspA1 RFLP (−34T/C)	rs743572		Polish women (55, 56) (Marszalek *et al.*, 2001) Greek women (50, 50) (Diamanti-Kandarakis *et al.*, 1999) UK women (69, 124) (Techatraisak *et al.*, 1997)		
CYP19A1 (15q21.1, aromatase: steroid biosynthesis)	TTTA VNTR (D15S103)			UK women (97, 59) (Gharani *et al.*, 1997)		
	SNP44	rs12907866		Spanish women (186, 71) (Petry *et al.*, 2005)		
	SNP50	rs2414096			Spanish women—'PCOS score' (186, 71) (Petry *et al.*, 2005)	
	SNP60	rs17601241		Spanish women (186, 71) (Petry *et al.*, 2005)		
	SNP64	rs4646		Spanish women (186, 71) (Petry *et al.*, 2005)		
EPHX1 (1q42.1, microsomal epoxide hydrolase: detoxification, component of the anti-oestrogen binding site complex)	p.Y113H	rs1051740	Finnish women (rs1051740, rs2234922 haplotype) (112, 115) (Korhonen *et al.*, 2003b)			
	p.H139R	rs2234922	Finnish women (rs1051740, rs2234922 haplotype) (112, 115) (Korhonen *et al.*, 2003b)			

GATA6 (18q11.2, GATA binding protein 6: transcription factor)		rs7235350, rs9957475, rs9944560, rs3764962, rs1941083		Australian women (173, 107) (Jones *et al.*, 2006)	
GSTM1 (1p13.3, glutathione S-transferase M1: phase 2 detoxification)	Null deletion			South Indian women (180, 72) (Babu *et al.*, 2004)	
GSTT1 (22q11.23, glutathione S-transferase T1: phase 2 detoxification)	Null deletion			South Indian women (180, 72) (Babu *et al.*, 2004)	
H6PD (1p36, hexose-6 phosphate dehydrogenase: steroid biosynthesis)	p.R453Q	rs6688832	Spanish women (116, 76) (San Millan *et al.*, 2005)	UK women (191, 261) (Draper *et al.*, 2006)	Spanish women—basal F and 17OHP levels (116, 76) (San Millan *et al.*, 2005)
HSD11B1 (1q32-q41, 11β-hydroxysteroid dehydrogenase: steroid biosynthesis)	83557ins/delA			Spanish women (116, 76) (San Millan *et al.*, 2005) UK women (202,263) (Draper *et al.*, 2006)	
	83597T/G	rs12086634		UK women (202, 263) (Draper *et al.*, 2006)	
HSD17B6 (12q13, 17β-hydroxysteroid dehydrogenase 6: steroid biosynthesis)		rs898611	Australian women (173, 107) (Jones *et al.*, 2006)		Australian women—fasting insulin and glucose levels, BMI (173, 107) (Jones *et al.*, 2006)
		rs2277339, rs10459246		Australian women (173, 107) (Jones *et al.*, 2006)	
		rs7967600		Australian women (173, 107) (Jones *et al.*, 2006)	Australian women—fasting insulin and glucose levels, BMI (173, 107) (Jones *et al.*, 2006)
		rs1870673		Australian women (173, 107) (Jones *et al.*, 2006)	Australian women—BMI, T levels (173, 107) (Jones *et al.*, 2006)
		rs1227117		Australian women (173, 107) (Jones *et al.*, 2006)	Australian women—BMI (173, 107) (Jones *et al.*, 2006)
SRD5A1 (5p15, 5 α-reductase isoform: steroid biosynthesis)		Haplotype: rs3797179, rs39848	North American women (287, 187) (Goodarzi *et al.*, 2006)		North American women—hirsutism, mFG score (287, 187) (Goodarzi *et al.*, 2006)
		Haplotype: rs472402, rs2677933, rs248805, rs3822430, rs10060745			North American women—hirsutism, mFG score (287, 187) (Goodarzi *et al.*, 2006)
SRD5A2 (2p23, 5 α-reductase isoform: steroid biosynthesis)		Haplotype: rs11889731, rs7571644, rs12470143, rs12467911, rs2300697	North American women (287, 187) (Goodarzi *et al.*, 2006)		

Continued

Table II. *Continued*

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype	Treatment response
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)		
		rs11675297, rs2754530		North American women (287, 187) (Goodarzi *et al.*, 2006)		
	p.V89L	rs523349	North American women (287, 187) (Goodarzi *et al.*, 2006)			

The list (ordered alphabetically) shows genes that have been investigated for their role in polycystic ovary syndrome. Studies showing positive or no associations of these genes with disease susceptibility and positive associations with phenotype (clinical characteristics of the condition, e.g. endocrine abnormalities) and/or treatment response are presented. [a]Not a case–control study.

linked to PCOS susceptibility in North American women (Qin *et al*., 2006). A polymorphism in the *CYP1A1* gene, which encodes the enzyme cytochrome P450 1A1, has shown an association with PCOS susceptibility in Indian women (Babu *et al*., 2004). Cytochrome P450 11A (*CYP11A1*) is a rate-limiting enzyme involved in the synthesis of androgens. Studies have indicated that a pentanucleotide repeat in the gene is associated with PCOS susceptibility (Gharani *et al*., 1997; Diamanti-Kandarakis *et al*., 2000; Gaasenbeek *et al*., 2004; Wang *et al*., 2006a; Jones *et al*., 2007). However, the large study of Gaasenbeek *et al*. found only a weak association between the pentanucleotide repeat and PCOS, and no association with another promoter microsatellite (Gaasenbeek *et al*., 2004). In Chinese women, a polymorphism in the promoter region of the aldosterone synthetase gene (*CYP11B2*), which affects the balance of the ovarian renin–angiotensin system, has been linked to PCOS susceptibility (Zhao *et al*., 2003). A polymorphism in the gene for 17α-hydroxylase (*CYP17A1)*, which is active in estrogen biosynthesis, was not associated with PCOS susceptibility (Techatraisak *et al*., 1997; Diamanti-Kandarakis *et al*., 1999; Marszalek *et al*., 2001). The *CYP19A1* gene encodes a key component of aromatase, which catalyses the production of estrogens from androgens. Although polymorphisms in this gene were not found to directly affect susceptibility to PCOS in women from the UK (Gharani *et al*., 1997) or Spain (Petry *et al*., 2005), the SNP50 polymorphism in this gene did influence PCOS severity in the Spanish study (Petry *et al*., 2005).

Sex steroid metabolism. The low activity haplotype (H113–R139) of the gene encoding the detoxification enzyme microsomal epoxide hydrolase (*EPHX1*) was significantly associated with PCOS susceptibility in Finnish women (Korhonen *et al*., 2003b). A variant of the hexose-6-phosphate dehydrogenase gene (*H6PD*), which has been implicated in a rare cortisone reductase deficiency that is characterized by a PCOS-like phenotype, has been linked to PCOS susceptibility in Spanish (San Millan *et al*., 2005), but not in UK women (Draper *et al*., 2006). Polymorphisms in the gene encoding 17β-hydroxysteroid dehydrogenase 6 (*HSD17B6*) have been found to be associated with either PCOS or with the clinical phenotype (Jones *et al*., 2006). A North American genetic association study of the two isoforms of the steroid biosynthesis enzyme 5α-reductase found that haplotypes in both *SRD5A1* and *SRD5A2* were associated with PCOS susceptibility, but that only variants in the *SRD5A1* gene were associated with severity of hirsutism (Goodarzi *et al*., 2006).

Polymorphisms in genes encoding 11β-hydroxysteroid dehydrogenase (HSD11B1) (San Millan *et al*., 2005; Draper *et al*., 2006), the glutathione S-transferases M1 or T1 (*GSTM1*, *GSTT1*) (Babu *et al*., 2004) and the transcription factor GATA-binding protein 6 (GATA6) (Jones *et al*., 2006) have also been investigated but failed to show an association with PCOS.

Genes involved in type 2 diabetes and cardiovascular disease

The increased risk of type 2 diabetes and cardiovascular disease in women with PCOS has led to numerous association studies in genes related to these diseases (Table III).

Adiponectin. Patients with obesity, type 2 diabetes, insulin resistance or PCOS have abnormally low levels of adiponectin, and therefore several studies have investigated the effect of two

Table III. Polymorphisms of genes encoding proteins involved in type 2 diabetes and cardiovascular disease.

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype	Treatment response
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)		
Genes encoding proteins involved in insulin pathways						
ADIPOQ (3q27, adiponectin: mediates insulin resistance)	45G/T	rs2241766	German women (57, 567) (Haap *et al.*, 2005)	Greek women (132, 100; 100, 140) (Panidis *et al.*, 2004; Xita *et al.*, 2005) Caucasian women (72, 42) (San Millan *et al.*, 2004)	Greek women—insulin level (100, 140) (Panidis *et al.*, 2004; Xita *et al.*, 2005)	
			Finnish women (143, 245) (Heinonen *et al.*, 2005)			
	276G/T	rs1501299		Greek women (132, 100; 100, 140) (Panidis *et al.*, 2004; Xita *et al.*, 2005)	Greek women—insulin exposue, adiponectine levels, BMI (100, 140) (Panidis *et al.*, 2004; Xita *et al.*, 2005)	
				Caucasian women (72, 42) (San Millan *et al.*, 2004) Finnish women (143, 245) (Heinonen *et al.*, 2005)		
APOE (19q13.2, apolipoprotein E: mediates insulin resistance)	3937C/T and 4075C/T (e2, e3 and e4 alleles)	rs429358+rs7412		Finnish women (58, 91) (Heinonen *et al.*, 2001)		
CAPN10 (2q37.3, calpain-10: mediates insulin resistance)	4841T/C (UCSNP44)	rs2975760	Spanish women (148, 93) (Gonzalez *et al.*, 2003)	German women (57, 567) (Haap *et al.*, 2005)	Spanish women—hirsutism score (81, 37) (Escobar-Morreale *et al.*, 2002)	
				Spanish women (81, 37) (Escobar-Morreale *et al.*, 2002)		
	4852G/A (UCSNP43)	rs3792267		German women (57, 567) (Haap *et al.*, 2005)	Spanish women—hirsutism score (81, 37) (Escobar-Morreale *et al.*, 2002)	
				Spanish women (148, 93; 81, 37) (Gonzalez *et al.*, 2003) North American women (181, 422) (Ehrmann *et al.*, 2002a)		
	7920ins/del32 bp (UCSNP19)			Spanish women (148, 93) (Gonzalez *et al.*, 2003) North American women (181, 422) (Ehrmann *et al.*, 2002a)		
	UCSNP45	rs41266971	Spanish women (81, 37) (Escobar-Morreale *et al.*, 2002)	German women (57, 567) (Haap *et al.*, 2005)		

Continued

Table III. *Continued*

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype	Treatment response
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)		
	UCSNP63	rs5030952		Spanish women (148, 93) (Gonzalez *et al.*, 2003)	Spanish women (148, 93)—cholesterol level (Gonzalez *et al.*, 2003)	
				North American women (181, 422) (Ehrmann *et al.*, 2002a)		
ENPP1 (6q22-q23, plasma cell differentiation antigen glycoprotein (PC-1): mediates insulin resistance)	p.K121Q	rs1044498	Finnish women (143, 115) (Heinonen *et al.*, 2004)	Caucasian women (72, 42) (San Millan *et al.*, 2004)		
				Japanese women (123, 180) (Baba et al., 2007)		
FOXC2 (16q22-16q24, forkhead box C2: transcription factor, mediates insulin resistance)	−512C/T	rs34221221		German women (57, 567) (Haap *et al.*, 2005)		
GYS1 (19q13.3, glycogen synthetase 1 (muscle): mediates insulin resistance)	XbaI RFLP (C/T)	rs8103451		UK women (90, 62) (Rajkhowa *et al.*, 1996)		
IGF1 (12q22-q23, insulin-like growth factor-1: insulin signalling)	CA repeat (promoter)			Caucasian women (72, 42) (San Millan *et al.*, 2004)		
IGF2 (11p15.5, insulin-like growth factor-2: insulin signalling)	ApaI RFLP (17200G/A)	rs680	Caucasian women (72, 42) (San Millan *et al.*, 2004)			
IGF1R (15q26.3, insulin-like growth factor-1 receptor: insulin signalling)	AGG repeat at position 967			Caucasian women (72, 42) (San Millan *et al.*, 2004)		
IGF2R (6q26, insulin-like growth factor-2 receptor: insulin signalling)	ins/delACAA in 3′ UTR			Caucasian women (72, 42) (San Millan *et al.*, 2004)		
INS (11p15.5, insulin: hormone)	VNTR		UK women, linkage studies (54, 78 and 2 family-based linkage studies; 74, 150) (Franks *et al.*, 1999; Michelmore *et al.*, 2001)	UK (255 families+185, 1062) and Finnish women (530 [72 confirmed by US], 1069) (Powell *et al.*, 2005) Spanish women (40, 38) (Calvo *et al.*, 2002) Czech women (38, 22) (Vankova *et al.*, 2002)		
INSR (19p13.3-p13.2, insulin receptor: hormone regulation)	p.H1058H T/C (3364T/C)	rs1799817	Chinese women (120, 40) (Chen *et al.*, 2004) Non-obese North American women (99, 136) (Siegel *et al.*, 2002) Korean women (134, 100) (Lee *et al.*, 2007)	Korean women (174, 93) (Lee *et al.*, 2006)	Chinese women—weight/BMI (120, 40) (Chen *et al.*, 2004)	
	p.C1008C T/C (3128T/C)	No dbSNP ID	Chinese women (109, 107) (Jin *et al.*, 2006)		Chinese women—insulin sensitivity (109, 107) (Jin *et al.*, 2006)	
	109482A/G, 109665C/T,	No dbSNP ID, rs6510959,		Korean women (134, 100) (Lee *et al.*, 2007)		

	125498A/G, 127527G/A, 143485G/C, 161822G/A, 168828T/A	rs2303672, rs2059806, rs2252673, rs2860175, No dbSNP ID				
	176477C/T	No dbSNP ID	Korean women (134, 100) (Lee *et al.*, 2007)			
IRS1 (2q36, insulin receptor substrate-1: insulin signalling)	p.G972R	rs1801278	Chilean women (146, 97) (Sir-Petermann *et al.*, 2004)	German women (57, 567) (Haap *et al.*, 2005)	Chilean women—insulin resistance (146, 97) (Sir-Petermann *et al.*, 2004)	Metformin response in Turkish women (60[a]) (Ertunc *et al.*, 2005)
			Japanese women (123, 180) (Baba *et al.*, 2007)	North American women (227[a]) (Ehrmann *et al.*, 2002b)	North American women, in carriers of CYP21 heterozygous mutation (114, 95) (Witchel *et al.*, 2005)	
			Turkish women (60, 60) (Dilek *et al.*, 2005)	Spanish caucasian women (103, 48) (Villuendas *et al.*, 2005)	Spanish caucasian women—BMI, insulin resistance (103, 48) (Villuendas *et al.*, 2005)	
				Caucasian women (53, 102) (El Mkadem *et al.*, 2001)	Caucasian women—insulin resistance (53, 102) (El Mkadem *et al.*, 2001)	
IRS2 (13q34, insulin receptor substrate-2: insulin signalling)	p.G1057D	rs1805097		German women (57, 567) (Haap *et al.*, 2005)	Caucasian American women—blood glucose level (227[a]) (Ehrmann *et al.*, 2002b)	
				Spanish caucasian women (103, 48) (Villuendas *et al.*, 2005)	Caucasian women—blood glucose level (53, 102) (El Mkadem *et al.*, 2001)	
				Caucasian women (53, 102) (El Mkadem *et al.*, 2001)		
PON1 (7q21.3, paraoxonase 1: antioxidant enzyme, linked to insulin resistance and cardiovascular disease)	−108C/T	rs705379	Caucasian women (72, 42) (San Millan *et al.*, 2004)			
	p.L55M	rs854560		Caucasian women (72, 42) (San Millan *et al.*, 2004)	Caucasian women—BMI and insulin resistance (72, 42) (San Millan *et al.*, 2004)	
	p.Q192R	rs662		Caucasian women (72, 42) (San Millan *et al.*, 2004)		
PPARG (3p25, peroxisome proliferator-activated receptor-γ: transcription factor, mediates insulin resistance, regulates *CCL5* expression)	p.P12A	rs1801282	Finnish women (135, 115) (Korhonen *et al.*, 2003a)	German women (57, 567) (Haap *et al.*, 2005)	Turkish women—glucose metabolism (60, 60) (Tok *et al.*, 2005)	
				Caucasian women (72, 42) (San Millan *et al.*, 2004)	German women—insulin sensitivity; hirsutism score (102, 104) (Hahn *et al.*, 2005)	

Continued

Table III. *Continued*

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype	Treatment response
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)		
				Chinese women (201, 147) (Wang *et al.*, 2006b)	North American women—insulin sensitivity (218[a]) (Hara *et al.*, 2002)	
				North American women (285, 187) (Antoine et al., 2007)		
				Italian women (100, 100) (Orio *et al.*, 2003a)		
	p.H447H, 1431C/T	rs3856806		North American women (285, 187) (Antoine et al., 2007)	Italian women—BMI (100, 100) (Orio *et al.*, 2003a)	
PPARGC1A (4p15.2, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha: transcriptional coactivator for steroid receptors and nuclear receptors)	p.S482G	rs8192678		Chinese women (201, 147) (Wang *et al.*, 2006b)		
PPP1R3A (7q31.1, protein phosphatase-1: mediates insulin resistance)	5 bp ins/del in the 3′UTR (ARE-1/ARE-2)	rs5886683+rs5886684			North American women—insulin sensitivity (186[a]) (Alcoser *et al.*, 2004)	
PTPN1 (20q13.1-q13.2, protein tyrosine phosphatase 1B: insulin signalling)	981C/T	rs11575938		Caucasian women (72, 42) (San Millan *et al.*, 2004)		
	1484ins/delG			Caucasian women (72, 42) (San Millan *et al.*, 2004)		
RETN (19p13.2, resistin: mediates insulin resistance)	−179C/G (−180C/G)	rs1862513		Greek women (320, 180) (Xita *et al.*, 2004)	Greek women—BMI (320, 180) (Xita *et al.*, 2004)	
SORBS1 (10q23.3-q24.1, sorbin: may play a role in insulin resistance)	p.T228A	rs2281939		Caucasian women (72, 42) (San Millan *et al.*, 2004)	Caucasian women—BMI (72, 42) (San Millan *et al.*, 2004)	
Mediators of vascular function or genes linked to cardiovascular risk						
AGT (1q42-q43, angiotensin I: mediates vascular homeostasis)	p.M235T	rs699	Italian women (95, 64) (Zulian *et al.*, 2005)		Italian women—insulin sensitivity (95, 64) (Zulian *et al.*, 2005)	
MTHFR (1p36.3, methylenetetrahydrofolate reductase: olate metabolism, linked to cardiovascular disease)	677C/T (p.A222V)	rs1801133		Italian women (70, 70) (Orio *et al.*, 2003b)		
PLAT (8p12, tissue plasminogen activator: fibrinolysis system, linked to cardiovascular disease)	Alu repeat ins/del 311 bp	rs4646972		Turkish women (91, 100) (Karadeniz *et al.*, 2007)	Turkish women—cholesterol and LDL levels (91, 100) (Karadeniz *et al.*, 2007)	

SERPINE1 (7q21.3-q22, plasminogen-activator inhibitor-1 (PAI-1): pibrinolysis system, linked to cardiovascular disease)	−675ins/delG (4G/5G)	Several IDs: rs1799768, rs34857375, rs1799762, rs1799889	Greek women (98, 64) (Diamanti-Kandarakis *et al*., 2004)	Caucasian women (72, 42; 106, 102) (San Millan *et al*., 2004; Walch *et al*., 2005a)	North American women—rate of miscarriages (149, 234) (Glueck *et al*., 1999) Greek women—SERPINE1 (PAI-1) levels (98, 64) (Diamanti-Kandarakis *et al*., 2004)
				Turkish women (91, 100) (Karadeniz *et al*., 2007)	

The list shows genes that have been investigated for their role in polycystic ovary syndrome. Genes are grouped according to their function (and then alphabetically). Studies showing positive or no associations of these genes with disease susceptibility and positive associations with phenotype (clinical characteristics of the condition, e.g. endocrine abnormalities) are presented. [a]Replicated in a family study (52 probands). NB: when only the number of cases is given, the study was not case control. BMI, body mass index; *CCL5*, formally know as *RANTES*, Regulation upon Activation Normal T cell Expressed and Secreted; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; RFLP, restriction fragment-length polymorphism; VNTR, variable number of tandem repeats.

SNPs in the adiponectin gene (*ADIPOQ*) on PCOS susceptibility. Whereas there was an association with the 45G/T polymorphism in German and Finnish women (Haap *et al*., 2005; Heinonen *et al*., 2005), there was no association for this polymorphism or the 276G/T polymorphism in Greek or Caucasian women (Panidis *et al*., 2004; San Millan *et al*., 2004; Heinonen *et al*., 2005; Xita *et al*., 2005) (NB: 276G/T was not investigated by Haap *et al*.). Both polymorphisms may, however, affect insulin levels (Xita *et al*., 2005).

Calpain-10. The gene for the cysteine protease calpain-10 (*CAPN10*) is associated with type 2 diabetes, and potential associations between five different polymorphisms and PCOS have been investigated. In the *CAPN10* gene, two different Spanish studies found an association between either the SNP UCSNP44 (Gonzalez *et al*., 2003) or UCSNP45 (Escobar-Morreale *et al*., 2002) and PCOS susceptibility. These associations were not observed in a German study (Haap *et al*., 2005). No associations were found between the UCSNP43, UCSNP19, UCSNP63 polymorphisms and PCOS (Ehrmann *et al*., 2002a; Gonzalez *et al*., 2003), although there were some links with the symptoms of PCOS (Escobar-Morreale *et al*., 2002; Gonzalez *et al*., 2003).

Plasma cell differentiation antigen. Genes encoding components of the insulin signalling system have been investigated in relation to PCOS across a number of studies. Plasma cell differentiation antigen glycoprotein (*PC-1*, also known as *ENPP1*, ectonucleotide pyrophosphatase/phosphodiesterase 1) is a mediator of insulin receptor signalling and has been implicated in insulin resistance. However, studies investigating an association between PCOS susceptibility and the p.K121Q polymorphism have reported both positive (Heinonen *et al*., 2004) and negative (San Millan *et al*., 2004; Baba *et al*., 2007) results. Although early case–control studies in the UK indicated a link between PCOS susceptibility and the insulin gene variable number of tandem repeats microsatellite length (Franks *et al*., 1999; Michelmore *et al*., 2001), a large subsequent study in more than 3000 women from the UK and Finland found no such association (Powell *et al*., 2005). In addition, studies conducted in the Czech Republic (Vankova *et al*., 2002) and Spain (Calvo *et al*., 2002) have found no association between this variant and PCOS.

Insulin receptor gene. Three polymorphisms in the insulin receptor gene (*INSR*) have shown a positive association with PCOS: 3364T/C (p.H1058H) in Chinese (Chen *et al*., 2004) and American women (Siegel *et al*., 2002), but not Korean women (Lee *et al*., 2006); 3128T/C (p.C1008C) in Chinese women (Jin *et al*., 2006); and 176477C/T in Korean women (Lee *et al*., 2007). The 2007 study by Lee *et al*. investigated a total of nine polymorphisms but only found an association with 176477C/T. The 3364T/C and 3128T/C polymorphisms were also associated with the phenotype of PCOS (Chen *et al*., 2004; Jin *et al*., 2006).

Insulin receptor substrates. The insulin receptor substrates IRS-1 and IRS-2 are proteins that participate in the insulin signal transduction pathway. Extensive investigations on a possible association between the p.G972R polymorphism in the *IRS-1* gene and PCOS have been carried out, with both postive (Sir-Petermann *et al*., 2004; Dilek *et al*., 2005; Baba *et al*., 2007) and negative (El Mkadem *et al*., 2001; Ehrmann *et al*., 2002b; Haap *et al*., 2005; Villuendas *et al*., 2005) results. The polymorphism was

Table IV. Polymorphisms of genes encoding proteins involved in tissue remodelling or inflammatory processes.

Gene (locus, protein name and its function)	Variant		Association with susceptibility		Phenotype
	Name	dbSNP ID	Positive (number of cases, number of controls)	Negative (number of cases, number of controls)	
Genes involved in tissue remodeling					
MMP1 (11q22.3, matrix metalloproteinase-1: endometrial lesion formation)	1607ins/delG	rs11292517	Caucasian women (62, 94) (Walch *et al.*, 2005b)		
Inflammatory mediators					
HLA-A (6p21.3, major histocompatibility complex class I, A: antigen processing and presentation)	A11		Japanese women (56, 237) (Kaibe *et al.*, 2006)		
HLA-B (6p21.3, major histocompatibility complex class I, B: antigen processing and presentation)	B39		Protective effect in Japanese women (56, 237) (Kaibe *et al.*, 2006)		
HLA-C (6p21.3, major histocompatibility complex class I, C: antigen processing and presentation)	All alleles			Japanese women (56, 237) (Kaibe *et al.*, 2006)	
HLA-DRB1 (6p21.3, major histocompatibility complex class I, DR beta 1: antigen processing and presentation)	*0403		Japanese women (68, 292) (Kaibe *et al.*, 2006)		
HFE (6p21.3, haemochromatosis protein (MHC class I family): antigen processing and presentation, iron binding)	p.H63D	rs1799945		Spanish women (78, 43) (Botella-Carretero *et al.*, 2006)	
	p.C282Y	rs1800562		Spanish women (78, 43) (Botella-Carretero *et al.*, 2006)	
IL1A (2q14, interleukin-1 alpha: cytokine)	−889C/T	rs1800587	Austrian caucasian women (105, 102) (Kolbus *et al.*, 2007)		Austrian caucasian women—FSH serum level, LH/FSH ratio (105, 102) (Kolbus *et al.*, 2007)
IL1B (2q14, interleukin-1 beta: cytokine)	−511C/T, exon 5	rs16944		Austrian caucasian women (105, 102) (Kolbus *et al.*, 2007)	
	3953G/A (exon5 *TaqI* RFLP, E1/E2)	rs1143634		Austrian caucasian women (105, 102) (Kolbus *et al.*, 2007)	
IL6 (7p21, interleukin-6: cytokine)	−597G/A	rs1800797	Spanish caucasian women (85, 25) (Villuendas *et al.*, 2002)		
	−572G/C	rs1800796		Spanish caucasian women (85, 25) (Villuendas *et al.*, 2002)	
	−174G/C	rs1800795	Spanish caucasian women (85, 25) (Villuendas *et al.*, 2002)	Austrian caucasian women (62, 94) (Walch *et al.*, 2004)	Austrian caucasian women—BMI, T levels, OGTT results (62, 94) (Walch *et al.*, 2004) German women—androstendione levels (50, 0) (Mohlig *et al.*, 2004)
IL6R (1q22, interleukin-6 repector alpha: cytokine receptor)	CA repeat			Spanish women (88, 45) (Escobar-Morreale *et al.*, 2003)	
IL6ST (5q11.2, gp130, interleukin-6 repector beta: cytokine receptor)	p.G148R	rs3729960	Spanish women (88, 45) (Escobar-Morreale *et al.*, 2003)		
TNF (6p21.3, tumour necrosis factor alpha: cytokine)	−850C/T (−857C/T)	rs1799724		Cauasian Finnish women (87, 115) (Korhonen *et al.*, 2002)	Australian women—OGTT results (122, 28) (Milner *et al.*, 1999)

	−308G/A	rs1800629		Chinese women (118, 54) (Mao *et al*., 2000) Australian women (122, 28) (Milner *et al*., 1999)
TNFRSF1B (1p36.3-p36.2, tumour necrosis factor receptor 2: cytokine receptor)	p.M196R	rs1061622	Spanish women (87, 36) and Italian women (64, 29) (76, 36) (Peral *et al*., 2002)	Spanish women (87, 36) and Italian women (64, 29)—hyperandrogenism (Peral *et al*., 2002)
	CA repeat		Spanish women (87, 36) and Italian women (64, 29) (76, 36) (Peral *et al*., 2002)	
	1663G/A	rs1061624	Spanish women (87, 36) and Italian women (64, 29) (76, 36) (Peral *et al*., 2002)	
	1668T/G	rs5030792	Spanish women (87, 36) and Italian women (64, 29) (76, 36) (Peral *et al*., 2002)	
	1690T/C	rs3397	Spanish women (87, 36) and Italian women (64, 29) (76, 36) (Peral *et al*., 2002)	

The list shows genes that have been investigated for their role in polycystic ovary syndrome. Genes are grouped according to their function (and then alphabetically), and studies showing positive or no associations of these genes with disease susceptibility and positive associations with phenotype (clinical characteristics of the condition, e.g. endocrine abnormalities) are presented.

associated with symptoms of PCOS across four different studies (El Mkadem *et al*., 2001; Sir-Petermann *et al*., 2004; Villuendas *et al*., 2005; Witchel *et al*., 2005). Metformin, an insulin-sensitizing drug that is often used in women with PCOS to induce ovulation, is believed to act via phosphorylation of IRS proteins. This could explain why a study in Turkish women with PCOS indicated that the IRS-1 genotype may influence a patient's response to metformin therapy (Ertunc *et al*., 2005). In contrast, investigations into the p.G1057D polymorphism in the *IRS-2* gene have failed to show an association with susceptibility to PCOS (El Mkadem *et al*., 2001; Haap *et al*., 2005; Villuendas *et al*., 2005), but have linked this polymorphism with blood glucose levels in women with PCOS (El Mkadem *et al*., 2001; Ehrmann *et al*., 2002b).

Insulin-like growth factors. An ApaI RFLP in the insulin-like growth factor-2 (*IGF-2*) gene has been linked to PCOS susceptibility in Caucasian women (San Millan *et al*., 2004), whereas polymorphisms in genes encoding IGF-1, IGF-1 receptor and IGF-2 receptor were not associated (San Millan *et al*., 2004). Of three variants investigated in the paraoxonase gene (*PON1*) (San Millan *et al*., 2004), the −108C/T variant was linked to PCOS susceptibility in Caucasian women. The p.L55M polymorphisms was not associated with PCOS, but was linked with a higher BMI and greater insulin resistance in women who were homozygotic for the 55M polymorphism compared with carriers of the common 55L allele. This polymorphism is believed to contribute to impaired insulin function by increasing levels of oxidative stress in women with PCOS. The remaining polymorphism investigated (p.Q192R) was associated with neither susceptibility nor phenotype.

Peroxisome proliferator-activated receptor-γ. Peroxisome proliferator-activated receptor-γ (*PPARG*) regulates the expression of several anti-atherosclerotic proteins. One study of the polymorphism p.P12A showed an association with PCOS susceptibility in Finnish women (Korhonen *et al*., 2003a), but five other studies found no such association (Orio *et al*., 2003a; San Millan *et al*., 2004; Haap *et al*., 2005; Wang *et al*., 2006b; Antoine *et al*., 2007). Three studies found effects on glucose metabolism or insulin sensitivity in carriers of this polymorphism (Hara *et al*., 2002; Hahn *et al*., 2005; Tok *et al*., 2005) and indications that genotype also determines levels of hirsutism (Hahn *et al*., 2005). The 1431C/T (p.H447H) polymorphism in this gene has been linked to obesity in women with PCOS (Orio *et al*., 2003a) but not to susceptibility to PCOS (Antoine *et al*., 2007). So far, there has only been a single study of the *PPARG* coactivator 1 alpha (*PPARGC1A*) gene and no association was shown between the polymorphism studied (p.S482G) and PCOS (Wang *et al*., 2006b).

Glucose metabolism and insulin resistance. An insertion/deletion polymorphism in the AT-rich element within the 3′-untranslated region of the muscle-specific glycogen-targeting subunit of the protein phosphatase-1 gene (*PPP1R3A*), which has been associated with insulin resistance and diabetes, has been shown to influence insulin resistance and androgen levels in women with PCOS (Alcoser *et al*., 2004). Also related to the phenotype of PCOS is the −179C/G polymorphism in the promoter region of the resistin gene (*RETN*) (Xita *et al*., 2004) and the p.T228A polymorphism in the sorbin and SH3 domain containing 1 (*SORBS1*) gene (San Millan *et al*., 2004). Both were linked to higher BMI in carriers,

but neither was linked with susceptibility (San Millan *et al*., 2004; Xita *et al*., 2004).

A number of variants of other genes involved in insulin resistance, type 2 diabetes and/or obesity have been investigated but failed to show an association with PCOS: apolipoprotein E (*APOE*) (Heinonen *et al*., 2001), forkhead box C2 (*FOXC2*) (Haap *et al*., 2005), glycogen synthetase 1 (*GYS1*) (Rajkhowa *et al*., 1996) and protein tyrosine phosphatase 1B (*PTPN1*) (San Millan *et al*., 2004).

Genes linked to cardiovascular risk. Polymorphisms in a limited number of genes linked to cardiovascular risk have been studied to assess a possible role in PCOS (Table III). In one study, the p.M235T polymorphism in the gene encoding angiotensin I (*AGT*), a potent vasoconstrictor, was linked to PCOS susceptibility and a genotype-dependent difference was seen in insulin sensitivity in women with PCOS (Zulian *et al*., 2005). Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the homocysteine pathway, and the p.A222V polymorphism in the *MTHFR* gene increases circulating homocysteine levels. As elevated levels of plasma homocysteine are associated with an increased risk of cardiovascular disease, a study investigated whether this polymorphism increases PCOS susceptibility in Italian women, but failed to show an effect (Orio *et al*., 2003b). As part of the fibrinolytic system, the gene encoding tissue plasminogen activator (*PLAT*) has been investigated in relation to PCOS. Although no association between the Alu repeat insertion/deletion and susceptibility was found, PCOS patients that were homozygous for the insertion (i/i genotype) had lower levels of low-density and total cholesterol than other genotypes (Karadeniz *et al*., 2007). Plasminogen activator inhibitor-1 (SERPINE1) is also involved in blood coagulation and has been implicated in cardiovascular disease. The 4G/5G polymorphism in the *SERPINE1* gene has been linked with PCOS susceptibility in one study (Diamanti-Kandarakis *et al*., 2004), but no association was found in three other studies (San Millan *et al*., 2004; Walch *et al*., 2005a; Karadeniz *et al*., 2007). Women with PCOS who are carriers of the polymorphism were, however, found to have an increased risk of miscarriage (Glueck *et al*., 1999) and higher plasminogen activator inhibitor-1 levels (Diamanti-Kandarakis *et al*., 2004).

Genes involved in tissue remodelling and inflammatory processes

Tissue remodelling. Matrix metalloproteinases (MMPs) are thought to play a critical role in the remodelling of the extracellular matrix during follicular development (Goldman and Shalev, 2004). The 1607G insertion/deletion polymorphism in the *MMP1* gene has been associated with an increased risk of PCOS in Caucasian women (Walch *et al*., 2005b) (Table IV). The association of genes of the histocompatibility family with PCOS has been studied in Japanese women. Whereas women with PCOS were more likely to be carriers of the *HLA-A11* or *HLA-DRB1*0403* alleles, they were less likely to be carriers of the *HLA-B39* allele when compared with healthy individuals (indicating a protective effect). No association was found for the alleles tested within *HLA-C* (Kaibe *et al*., 2006). No association was found between two polymorphisms in the gene encoding haemochromatosis protein, in the MHC Class I family (*HFE*), and PCOS (Botella-Carretero *et al*., 2006).

Inflammatory cytokines. Circulating levels of several inflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), correlate with insulin resistance and obesity (Milner *et al*., 1999; Mohlig *et al*., 2004). It has also been proposed that another inflammatory cytokine, IL-1, influences the processes of ovulation, fertilization and implantation. A study in Austrian women showed that the −889C/T polymorphism in the *IL1A* gene is associated with PCOS, which was not the case for the −511C/T and 3953G/A polymorphisms in the *IL1B* gene (Kolbus *et al*., 2007). Within the interleukin-6 (*IL6*) gene, the 597G/A polymorphism has been shown to be associated with PCOS susceptibility (Villuendas *et al*., 2002); the 572G/C polymorphism not associated (Villuendas *et al*., 2002) and the −174G/C was both associated with susceptibility (Villuendas *et al*., 2002) and not associated with (Walch *et al*., 2004), but related to, the PCOS phenotype (Mohlig *et al*., 2004; Walch *et al*., 2004). An investigation into polymorphisms in the genes of the IL-6 receptor complex found no association between the presence of a microsatellite CA repeat in the *IL6R* gene, but an association between PCOS and the p.G148R polymorphism in the IL-6 signal transducer (*IL6ST*) gene (Escobar-Morreale *et al*., 2003).

Polymorphisms in the promoter region of the TNF-α gene (*TNF*) do not appear to affect PCOS susceptibility (Milner *et al*., 1999; Mao *et al*., 2000; Korhonen *et al*., 2002), but the −308G/A polymorphism is linked with glucose tolerance in women with PCOS (Milner *et al*., 1999). The actions of TNF-α are mainly mediated by type 1 or type 2 TNF receptors. Of the five polymorphisms in the TNF receptor 2 gene (*TNFRSF1B*) investigated in relation to PCOS in Spanish and Italian women, only p.M196R was found to be associated with PCOS susceptibility and hyperandrogenism, but the other four polymorphisms investigated were not (Peral *et al*., 2002).

Ovarian response to gonadotrophins

Ovarian stimulation is usually performed by administering exogenous FSH following pituitary down-regulation. Ovarian response to FSH, however, varies widely among women undergoing ovarian stimulation (Georgiou *et al*., 1997) with wide-reaching clinical consequences. If ovarian response to stimulation is insufficient, the cycle may need to be cancelled. Similarly, if ovarian response is excessive, cycle cancellation may be necessary to avoid the risk of ovarian hyperstimulation syndrome (OHSS), a potentially life-threatening complication. Application of pharmacogenetics to the problem of predicting ovarian response utilizes information about the genetic make-up of an individual patient. This information may then be used in conjunction with existing clinical factors to predict treatment response (Fauser *et al*., 2008).

FSH receptor

FSH induces the proliferation of granulosa cells and the synthesis of the androgen-converting enzyme aromatase, and also plays a crucial role in secondary follicle recruitment and selection of the dominant follicle (Fauser and Van Heusden, 1997). The action of FSH is mediated by FSHR, a member of the G-protein-coupled receptor family, which is expressed solely by granulosa cells. The *FSHR* gene harbours more than 900 SNPs, arranged in two major linkage disequilibrium blocks (Fig. 3). Two non-synonymous SNPs in strong linkage disequilibrium (p.A307T and p.N680S)

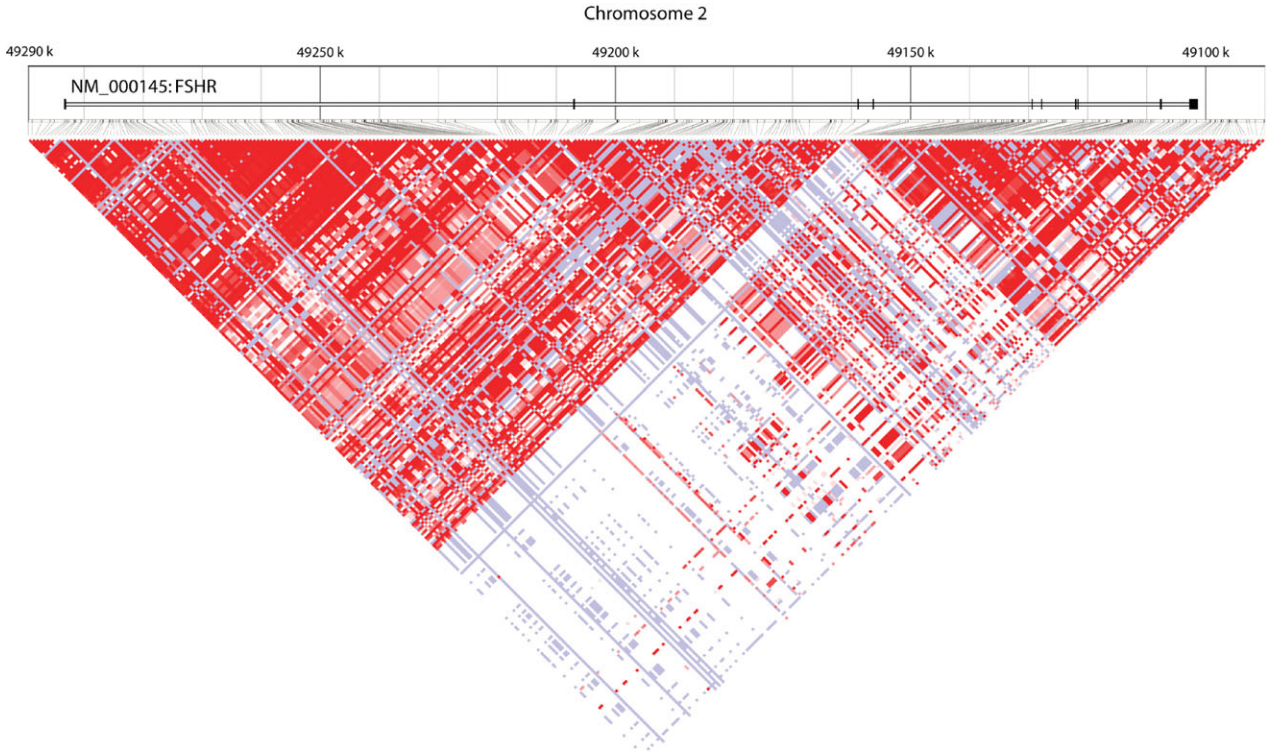


Figure 3: LD plot of the genomic region on chromosome 2 harbouring the FSHR gene, obtained from the HapMap website (www.hapmap.org). More than 900 SNPs are currently listed in the National Centre for Biotechnology Information SNP database. These SNPs are grouped into two major LD blocks (red triangles). FSHR, follicle-stimulating hormone receptor; LD, linkage disequilibrium.

have been identified in exon 10 of the *FSHR* gene (Simoni *et al*., 1999). As the interaction between FSH and its receptor plays a key role in ovarian stimulation, a number of groups have investigated the effect of polymorphisms in the *FSHR* gene on ovarian response (Table V).

Studies in women with normal ovarian function demonstrate convincingly that SNPs in exon 10 modulate FSHR function and the ovarian response to FSH. This effect was first observed in a partly retrospective, non-randomized study of German women undergoing controlled ovarian hyperstimulation for assisted reproduction. The amount of FSH needed for controlled ovarian hyperstimulation to achieve similar peak estradiol levels was significantly lower in women with the N/N genotype at position 680 of the *FSHR* gene compared with women carrying the S/S or N/S genotypes, indicating a lower ovarian sensitivity to FSH *in vivo* for the S680 allele (Perez Mayorga *et al*., 2000). Similar results were later obtained by other investigators who studied populations from different ethnic backgrounds (Sudo *et al*., 2002; de Castro *et al*., 2003, 2004; Behre *et al*., 2005; Falconer *et al*., 2005; Jun *et al*., 2006; Loutradis *et al*., 2006). In accordance with a genetic control of *FSHR* p.N680S genotype status on *in vitro* fertilization (IVF) outcome, de Castro *et al*. (2003) reported that women with the S/S genotype have significantly higher rates of cycle cancellation and poor response compared with carriers of the N/S or N/N genotypes. These consistent findings in various populations indicate that the effects of the *FSHR* p.N680S polymorphism are independent of ethnic background and may also be present in other, previously uninvestigated, populations. Recently, it was shown that in women undergoing IVF treatment, the clinical pregnancy rate in women with the N/N genotype is significantly higher compared with those with the S/S genotype (Jun *et al*., 2006). However, another study using a similar study design showed opposite results, with higher pregnancy rates in women with the S/S genotype (Klinkert *et al*., 2006) (Table VI). These contrasting data should be interpreted with caution, and larger, well-designed and properly powered studies should be conducted before drawing conclusions about the effects of the *FSHR* genotype on pregnancy rates.

In a study involving menstrual cycle monitoring in women with normal, mono-ovulatory cycles, Greb *et al*. (2005) were able to show that during the luteo-follicular transition, serum levels of estradiol, progesterone and inhibin A were significantly lower and FSH started to rise earlier in women with the S/S genotype compared with women who carried the N/N genotype for the *FSHR* p.N680S polymorphism (Fig. 4). In addition, FSH levels were steadily and significantly higher during the follicular phase in the S/S genotype group, whereas no differences were observed between groups with regard to estradiol, inhibin B and growth velocity of the dominant follicle, showing that higher levels of endogenous FSH are necessary to achieve ovulation in carriers of the S/S genotype. Menstrual cycles were significantly longer in women with the S/S genotype, with a difference of about 2 days between these women and those with the N/N genotype. Thus, this study demonstrated that the S/S genotype results in a higher ovarian threshold for FSH, decreased negative feedback to the pituitary and a longer menstrual cycle (Greb *et al*., 2005). Other studies have observed that the genotype for the codon 680 polymorphism was associated with differences in cycle length (Perez Mayorga *et al*., 2000), basal FSH levels (Sudo *et al*., 2002; Laven *et al*., 2003; Falconer *et al*., 2005; Jun *et al*., 2006; Loutradis *et al*.,

Table V. Polymorphisms in genes encoding sex hormones and hormone regulators, enzymes involved in metabolism and biosynthesis, or paracrine factors.

Gene	Locus	Protein name	Protein function	Variant		Association with ovarian response		Phenotype (cases, *controls*)
				Name	dbSNP ID	Positive (cases, *controls*)	Negative (cases, *controls*)	
Sex hormones and hormone regulators								
AMH	19p13.3	Anti-Müllerian hormone	Hormone	p.I49S	rs10407022			Caucasian women—E_2 levels (53, 45) (Kevenaar *et al.*, 2007)
AMHR2	12q13	Anti-Müllerian hormone type II receptor	Hormone receptor	−482A/G	rs2002555			Caucasian women—E_2 levels levels (53, 45) (Kevenaar *et al.*, 2007)
ESR1	6q25.1	Oestrogen receptor α	Hormone receptor	PvuII RFLP (−397T/C) (g.938T/C)	rs2234693		Spanish women (170) (Mao *et al.*, 2000; de Castro *et al.*, 2004) Chinese women (200, 200) (Sundarrajan *et al.*, 1999)	Caucasian women—follicule/oocyte ratio, pregnancy rate (100, 100) (Georgiou *et al.*, 1997) Chinese women—serum oestratdiol levels, follicule/oocyte ratio, pregnancy rate (200, 200) (Sundarrajan *et al.*, 1999)
ESR2	14q23.2	Oestrogen receptor β	Hormone receptor	AluI RFLP (1730A/G) (39A/G)	rs4986938		Spanish women (170) (Mao *et al.*, 2000; de Castro *et al.*, 2004)	
FSHR	2p21-p16	Follicle-stimulating hormone receptor	Hormone receptor	p.N680S (in complete LD with p.A307T, rs6165)	rs6166	German women (93) (Perez Mayorga *et al.*, 2000; Behre *et al.*, 2005)	Dutch women (105) (Klinkert *et al.*, 2006)	German women—peak oestradiol levels (93) (Behre *et al.*, 2005)
						Japanese women (58) (Sudo *et al.*, 2002)		German women—circulating FSH, levels, number of follicules, lutheal phase and menstrual cycle length (23) (Perez Mayorga *et al.*, 2000; Greb *et al.*, 2005)
						Spanish women (102) (de Castro *et al.*, 2003; de Castro *et al.*, 2004)		Japanese women (58)—basal FSH (Sudo *et al.*, 2002)
						Korean women (263) (Jun *et al.*, 2006)		Dutch women (148) (Laven *et al.*, 2003) Swedish women (68) (Falconer *et al.*, 2005)
						Greek women (125 (Loutradis *et al.*, 2006)		Korean women—basal FSH, pregnancy rate (263) (Jun *et al.*, 2006)
								Greek women (125)—FSH levels, follicule and oocyte number (Loutradis *et al.*, 2006) Dutch women—pregnancy rate (105) (Klinkert *et al.*, 2006)
Enzymes involved in metabolism and biosynthesis								
CYP19A1	15q21.1	Aromatase	Steroid biosynthesis	1672C/T	rs10046		Spanish women (170) (de Castro *et al.*, 2004)	
Paracrine factors								

BMP15	Xp11.2	Bone morphogenic protein 15	Oocyte and follicular development	−673C/T	No dbSNP ID	Spanish women (307) (Moron *et al.*, 2006)	
					−9C/G	rs3810682	Spanish women (307) (Moron *et al.*, 2006)
				905G/A	rs3897937	Spanish women (307) (Moron *et al.*, 2006)	
				308A/G (p.N103S)	rs41308602		Spanish women (307) (Moron *et al.*, 2006)
MTHFR	1p36.3	Methylenetetrahydrofolate reductase	Folate metabolism, linked to cardiovascular disease	677C/T (p.A222V)	rs1801133	German women (105) (Thaler *et al.*, 2006)	North American women (223) (Rosen *et al.*, 2007) German women—E_2 levels, ocyte number (105) (Thaler *et al.*, 2006)
				1298A/C (p.E429A)	rs1801131	North American women (223) (Rosen *et al.*, 2007)	

The list shows genes that have been investigated for their role in ovarian response. Genes are grouped according to their function (and then alphabetically), and studies showing positive or no associations of these genes with ovarian response and/or phenotype (e.g. basal FSH, follicular oestradiol levels) are presented.

2006), pregnancy rates (Jun *et al*., 2006; Klinkert *et al*., 2006) and follicular/oocyte number (Loutradis *et al*., 2006) (Table V).

Behre *et al*. (2005) conducted a prospective interventional study using a randomized controlled trial design and showed that the codon 680 polymorphism of the *FSHR* gene caused a differential estradiol response to FSH. In this study, the same FSH dose for ovarian hyperstimulation resulted in significantly lower serum levels of estradiol in women with the S/S genotype than in women with the N/N genotype. This lower response could be overcome by increasing the FSH dose (Behre *et al*., 2005) (Fig. 5). Despite differences in estradiol levels, no significant differences were detected in the number of follicles or retrieved oocytes, fertilization rate, cumulative embryo score or pregnancy rate. This finding suggests that, using standard protocols, FSH might be overdosed in individual women, which may put them at risk of OHSS. Indeed, a retrospective association study demonstrated that the *FSHR* S680 allele was represented to a higher degree in women developing OHSS, and that the N680 allele was associated significantly with the severity of OHSS (Daelemans *et al*., 2004). Since OHSS is relatively rare, none of the studies performed so far in individual centres have sufficient power to demonstrate or refute convincingly any association with the *FSHR* gene; this is an issue that should be analysed retrospectively in a multicentre study.

Taken together, these data indicate that the *FSHR* gene may play a significant role in the success of ovarian stimulation. Women with the *FSHR* S680 allele comprise 60–75% of women undergoing IVF (allelic distribution is similar to that of the general population), and are characterized by higher basal FSH serum concentrations, the need for a higher amount of exogenous FSH and a higher risk of hypo- or hyper-response. Thus, genotyping the *FSHR* p.N680S polymorphism may provide a means of identifying a population of poor responders before IVF procedures are initiated. Since the basis of the poor responder status of these women is a reduced response of the *FSHR* to FSH stimulation, a stimulation protocol designed to overcome the partial resistance to FSH response should be sufficient to improve significantly the success of IVF in these women. The clinical effectiveness of such an approach should be confirmed in randomized controlled trials.

It is important to note that studies in women with ovarian dysfunction did not find any association between *FSHR* polymorphisms and ovarian response to FSH; e.g. in premature ovarian failure (Conway *et al*., 1999; Sundblad *et al*., 2004). No significant difference in the FSH dose needed for monofollicular development was detected between women with different *FSHR* genotypes in a non-randomized trial and normogonadotrophic anovulatory women treated with low-dose FSH for ovulation induction (Laven *et al*., 2003). Therefore, it seems that the differential estradiol response caused by different *FSHR* alleles is evident so far only in women with normal ovarian function. Another study demonstrated that the S680 allele is significantly more frequent in women with a normal menstrual cycle and elevated FSH levels than in women with normal FSH levels, corroborating the idea of a higher FSH threshold (de Koning *et al*., 2006). Finally, an intriguing association between the A307–S680 haplotype and ovarian cancer susceptibility was recently reported (Yang *et al*., 2006).

Table VI. Comparison of the study designs and outcomes of two conflicting studies investigating the relation between the FSHR polymorphism at amino acid position 680 (Asn/Ser) and pregnancy rate.

	Jun *et al.* (2006)	Klinkert *et al.* (2006)
Number of patients	263	105
Status regarding ICSI patients	Not excluded	Excluded
Frequency of unexplained infertility (%)	35.6	25.0
Genotype associated with significantly higher FSH levels	Ser/Ser	No difference
Genotype associated with significantly higher clinical pregnancy rate	Asn/Asn	Ser/Ser

ICSI, intracytoplasmic sperm injection.

Other genes

In addition to studies investigating the influence of *FSHR* genotype on ovarian responsiveness, other genetic factors have been analysed and reported to be involved in modulating ovarian sensitivity to FSH. Important candidates are polymorphisms in the *ER1* (Georgiou *et al*., 1997; Sundarrajan *et al*., 1999; de Castro *et al*., 2004), and *ER2* genes (de Castro *et al*., 2004) as well as in the anti-Müllerian hormone (AMH) and AMH type II receptor genes (Kevenaar *et al*., 2007) and in *MTHFR* (Thaler *et al*., 2006; Rosen *et al*., 2007). As ovarian responsiveness to FSH may be a polygenic trait, future studies should investigate the combined role of all these factors in large numbers of well-characterized patients undergoing ovarian hyperstimulation, using stringent genetic epidemiological criteria.

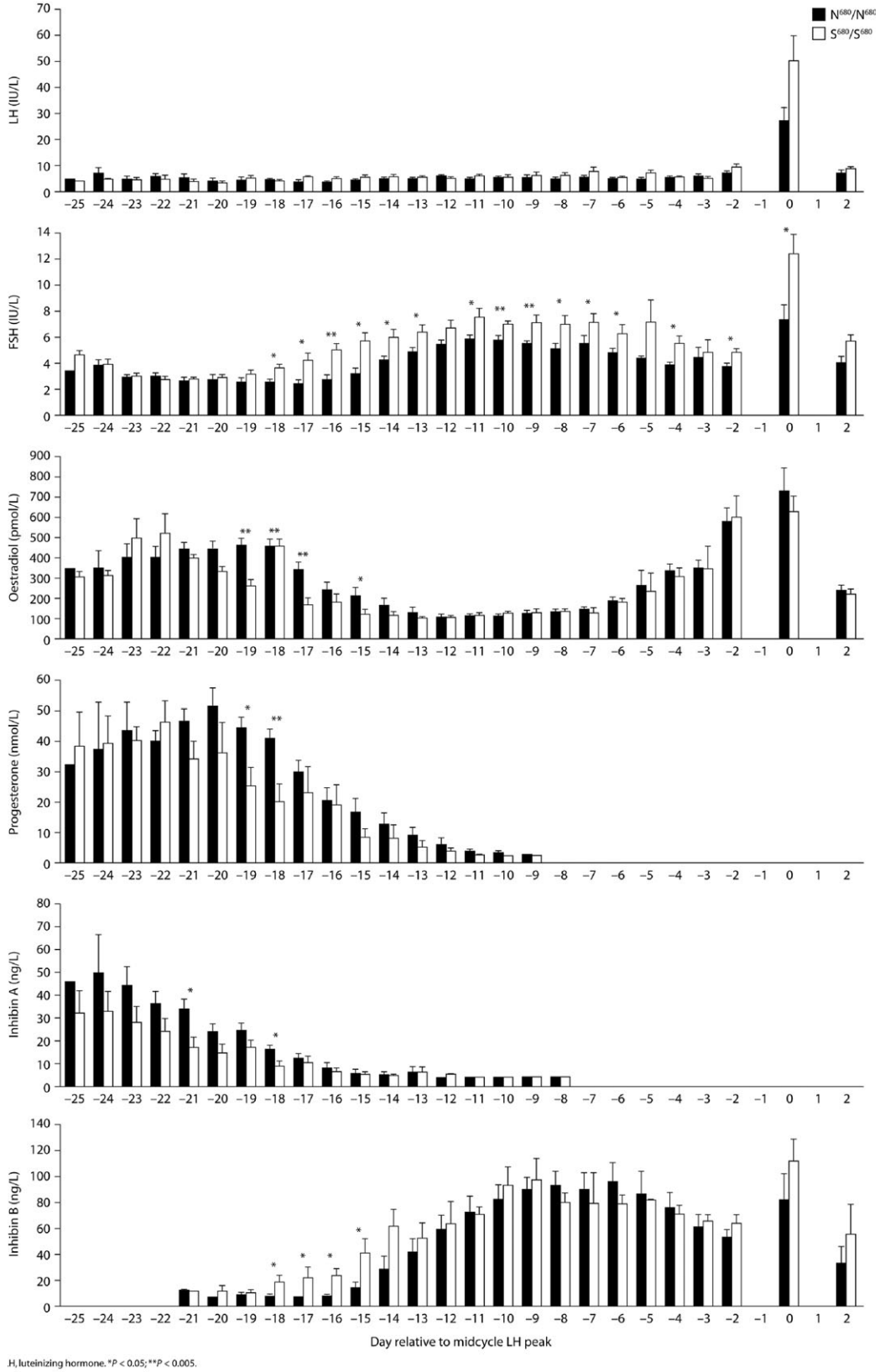
Finally, several studies have explored the effects of polymorphisms in genes for metabolic enzymes on ovarian response (Table V). De Castro *et al*. (2004) studied the 1672C/T polymorphism in the *CYP19A1* gene, which encodes an enzyme involved in estrogen synthesis, and found that it does not influence ovarian response to exogenous FSH. Bone morphogenetic protein 15 (BMP-15), a member of the transforming growth factor-β superfamily, is a paracrine factor expressed exclusively in the ovaries and is involved in oocyte and follicular development. A recent study investigated the association between OHSS and polymorphisms in the *BMP15* gene (Moron *et al*., 2006). Although individual polymorphisms did not show a strong association with the risk of OHSS, the TGGG haplotype was a risk factor and the CCAA haplotype was found to confer protection against OHSS (Moron *et al*., 2006).

Discussion

This systematic review emphasizes the inconsistency of results obtained by studies that have investigated associations between polymorphisms and PCOS or ovarian response. This may be due to methodological reasons, such as population stratification (genetic heterogeneity, population history), inappropriate disease/trait definition, subject selection issues or chance findings. One of the major problems with candidate gene studies concerns sample size, which is frequently insufficient. The sample sizes of many such studies have been calculated based on knowledge acquired while studying monogenic traits, which have a very strong effect. Unlike monogenetic traits, complex traits result from the interaction of several genetic variations and environmental factors, thus individual genetic variations have a much more modest effect (Lander and Schork, 1994). This may not have been taken into consideration, resulting in studies that are too small to reveal a genetic association in the context of a complex trait. Furthermore, it has been clearly demonstrated that studies with small populations, those that investigate a genetic variation with a small effect, or those that have a flexible design that is prone to bias are less likely to be replicated (Ioannidis, 2005). Similar conclusions were drawn by Gorroochurn *et al*. (2007), who showed that for commonly observed *P*-value thresholds ($P = 0.02–0.01$, when $\alpha = 0.05$), replication probabilities are surprisingly low (around 60–70% chance of replication).

Inconsistent results may also be due to characteristics inherent to polymorphisms, such as incomplete penetrance, genetic heterogeneity and gene–gene or gene–environment interactions. Other shortcomings of the use of candidate genes/markers to study association include the fact that only a very tiny part of the genome is being studied, and this is done independently of any interactions that may be involved. In addition, candidate gene selection relies on prior knowledge, making it impossible to reveal an association with genes that have unknown function or that have not been known to be implicated in the disease/trait being studied.

To overcome some of these limitations, the use of genome-wide scans is an experimental tool that is becoming an increasingly realistic option. Over the past 5–10 years, refinement of technology involving polymerase chain reaction, development of microarray technology and the remarkable progress in the characterization of the human genome sequence have enabled the study of thousands of DNA variations in a single experiment. Commercial genotyping tools currently allow the study of almost a million SNPs per sample in a single assay, representing roughly 10% of the estimated total number of SNPs in the human genome. Although not all known SNPs are represented on one genotyping microarray, linkage disequilibrium allows nearly 90% of the human genome to be studied with current technology (Schork *et al*., 2000; Locke *et al*., 2006; Roeder *et al*., 2006). Therefore, by performing an assay for a particular SNP, it is also possible to indirectly test for the presence of other variants that are in linkage disequilibrium. Although less advanced, the same technology is also being used to study quantitative (or copy number) gene variation on a genome-wide basis; this type of variation has recently been found to impact a much larger part of the genome than originally thought (Iafrate *et al*., 2004). Before this technique offers clinically useful data, however, key methodological points must be addressed. These include increasing the sample size compared with single gene/marker studies and resolving statistical problems inherent to multiple testing. Although it is obvious that a number of the studies reviewed had a small sample size, it is quite difficult to give a generic sample size that will fit all studies, as this depends on the frequency and number of the markers being tested and the importance of the genetic effect of the associated marker; genome-



H, luteinizing hormone. *P < 0.05; **P < 0.005.

Figure 4: Menstrual cycle-dependent serum levels of LH (A), FSH (B), estradiol (C), progesterone (D), inhibin A (E), and inhibin B (F) referenced to the day of the LH surge (0) in women with the Asn680/Asn680 (n=12) and the Ser680/Ser680 (n=9) genotype from the luteo-follicular transition phase (LH -25) until ovulation (LH +2). Means are displayed as bars, and error bars show SEM; *P<0.05; **P<0.005.
Serum hormone concentrations during the menstrual cycle in women carrying either N^{680}/N^{680} or S^{680}/S^{680} allele variants of the N680S polymorphism in the FSH receptor gene [Reproduced with permission from Greb *et al*. (2005), © 2005 The Endocrine Society].

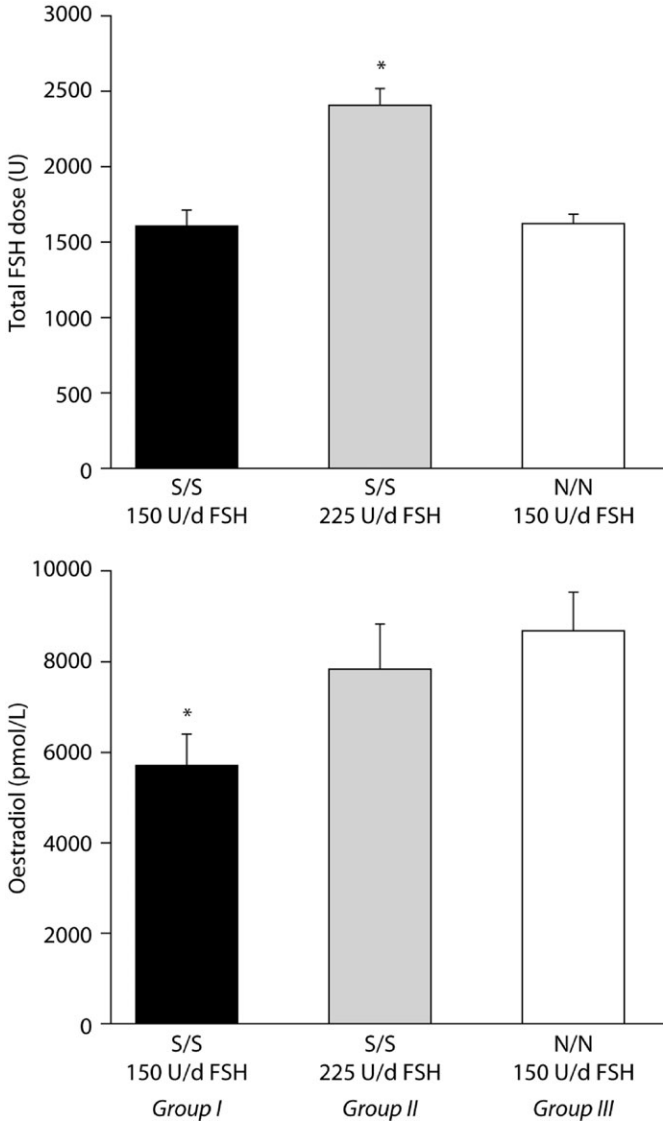


Figure 5: Serum levels of oestradiol before ovulation induction were significantly lower in women with the Ser/Ser allele variant (group I, n=24) compared to the Asn/Asn allele variant (group III, n=44) of the FSH receptor (lower panel: *significant difference between group I and III). This difference in ovarian response could be overcome by increasing the daily FSH dose from 150U/day to 225U/day (upper panel:*significant higher total FSH dose) in women with the Ser/Ser allele variant (group II, n=25); lower panel: no significant difference between group II and III.
Total FSH dose required (upper) and serum estradiol concentration (lower) in normo-ovulatory women undergoing controlled ovarian hyperstimulation, grouped according to N680S genotype for the FSH receptor gene [Reproduced with permission from Behre *et al*. (2005), ©2005 Lippincott Williams & Wilkins].

wide studies are often carried out on more than 1000 individuals. If the association studies have been properly designed, replication of the results in an independent population is one of the best methods to confirm an association.

Regardless of whether a candidate gene or a genome-wide approach is used, we suggest that in order to improve the quality of results, genetic association studies should be carried out in two phases: an exploratory phase, followed by a validation phase. Whenever possible, exploratory studies should be carried out using a genome-wide approach to maximize the chances of identifying an association. Ideally, these exploratory studies should be replicated, and only markers that are positive in at least two exploratory studies should be considered for validation. As explained previously, the identification of an association between a certain allele or genotype and a given disease/trait does not necessarily mean that there is an aetiological link. Due to the occurrence of linkage disequilibrium, in a number of cases, the associated genetic variation will only be a marker, and not the direct cause, of the trait; for this reason, care must be taken when drawing conclusions from the results. The recommended second phase consists of validating the small set of markers that were associated with the disease/trait in the exploratory studies. Depending on the objective, validation of the markers can take several forms. In some cases, it may be enough to replicate the association with this marker in a different study population (e.g. if a genetic marker is predictive of response to treatment or of disease state). On the other hand, if the aim is to study the impact of genetic variants on molecular mechanisms, then it will be necessary to first either sequence or screen the DNA region around the associated variant with a higher density of markers in order to pinpoint the particular variation that is the causative variation (resulting in, e.g. changes in amino acid sequence or changes to the promoter region affecting gene expression). Once identified, this causative variation will only be truly validated by results of *in vitro* and *in vivo* assays, such as site-directed mutagenesis or reporter gene assays.

On the basis of the data we have summarized, there is currently only one polymorphism for which a sufficient number of studies have consistently identified a significant association. Studies in women from various ethnic backgrounds with normal ovarian function demonstrate convincingly that SNPs in exon 10 of the FSH receptor gene can be used as markers to predict differences in FSHR function and ovarian response to FSH (Perez Mayorga *et al*., 2000; Sudo *et al*., 2002; de Castro *et al*., 2003, 2004; Falconer *et al*., 2005; Jun *et al*., 2006; Loutradis *et al*., 2006), although ovarian response is a polygenic trait and the interaction with other gene polymorphisms remains to be investigated. On the contrary, no consistent association between gene polymorphism and PCOS could be identified.

Conclusions

By pursuing the most promising polymorphisms identified in this review, we hope that the key factors involved in the pathogenesis of PCOS will be identified in the not-too-distant future, and that this knowledge can be used to improve prognosis for women with this disorder. These women are also part of a wider group who would potentially benefit from further analyses to confirm the link between variants in the *FSHR* gene and ovarian response to gonadotrophins. Strong evidence for an association could ultimately lead to stimulation protocols that are carefully personalized to each woman's individual needs, according to the particular combination of polymorphisms inherited.

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