Genetic studies on gonadotropin regulating gene(s) polymorphisms and its association with polycystic ovary syndrome

Introduction

Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrine disorder of women. It was initially described as an association of polycystic ovaries (PCO) with amenorrhea, hirsutism, and obesity in women presented with infertility. The PCOS stems from several factors intrigued with abnormal synthesis and secretion of steroid, insulin, gonadotropin hormones involved in maintaining reproductive physiology and metabolic homeostasis. Apart from major phenotypes such as hyperandrogensim and ovarian dysfunction, PCOS is being associated with broad spectrum of metabolic abnormalities such as insulin resistance and hyperinsulinemia, abdominal obesity, and dyslipidemia, endometrial hyperplasia and cardiovascular diseases. Albeit, due to the broad spectrum of symptoms with overlapping biochemical parameters with other disorders, the diagnosis of PCOS, go undetected until puberty in the pediatric population.

Globally, 2-15% of women in their reproductive ages are found to have PCOS. Variation in frequency of PCOS mainly depends on diagnostic criteria, ethnicity, lifestyle, geographic location and diet. Though the direct cause of PCOS remains unknown, both environmental and genetic factors are implicated.

Abnormal secretion and regulation of gonadotropins, namely the luteinizing hormone (LH) and follicle stimulating hormone (FSH), and associated excess secretion and action of ovarian steroid hormones leading to sub-fertility in PCOS women. Further they have an abnormal gonadotropin secretion elevated LH/FSH ratio, increased
frequency and amplitude of LH pulsations due to rapid hypothalamic GnRH secretion, a defect at the level of the hypothalamus. Comprehensive evidence suggests that several mutations or polymorphisms in genes involved in steroid, gonadotropin and insulin pathways closely interact to set the stage for possible development of PCOS. Expression of clinical and biochemical phenotypes vary among individuals and influenced by interactions of gene-gene and gene-environmental factors and largely depends on ethnicity and life style. Therefore, diagnosis of PCOS is critical to evaluate the basic pathogenesis mechanism affecting in infertile women and management of those affected individuals.

Aim

To evaluate candidate genes single nucleotide polymorphisms (SNP) involved in the gonadotropin action, regulatory pathway and its risk for PCOS.

Objectives:

I. To obtain systematic phenotypic data with respect to PCOS among patients and a sample of normal unaffected control population.

II. To study the SNP in the gonadotrophin releasing hormone (GnRH1), β-subunit of leutinizing hormone, (LHβ), follicle stimulating hormone receptor (FSHR), sex hormone binding globulin (SHBG) and androgen receptor (AR) genes.

III. To investigate genotype –association with PCOS.

Organization of the thesis

The thesis comprises eight chapters. The first chapter addresses diagnostic criteria, prevalence in different ethnic groups, and genetic factors related to gonadotropin,
steroid and insulin metabolism implicated in the pathogenesis of PCOS. An etiology of PCOS has been established that it results from the combined abnormalities on organs of reproductive physiology (hyperandrogenism, anovulation, polycystic ovaries) and metabolism (Insulin resistance, obesity and dyslipidemia). Of which abnormal patterns of hormone secretion in hypothalamic-pituitary-gonadal (HPG) axis and associated signaling pathways is listed as the major cause. Hyperandrogenesim, an androgen excess condition, have been shown to be associated with broad spectrum of disorders (amenorrhea, anovulation, PCOS, infertility, insulin resistance, type 2 diabetes mellitus, obesity, metabolic syndrome, cardiovascular and dyslipidemia). However, it is a dominant phenotype observed in 50-80% of women with PCOS. Thus, PCOS is a complex infertility disorder of unclear etiology associated with interplay of several hormonal and metabolic deregulations. Altered expression of genes regulating steroid, gonadotrophin synthesis, energy homeostasis and chronic inflammation suggest a strong influence of genetic factors in the pathogenesis. However, inconsistent results from genetic studies attribute mainly due to variability in expression of phenotypes, lack of unique diagnostic criteria and ethnicity.

The Chapter 2 describes the materials and methods adapted in the present study. The present genetic case-control study was approved by the Institutional ethics committee, Sri Ramachandra University (Ref. No: IECNI/08/Aug/05/32). The study subjects were recruited from SMART clinic Department of Obstetrics and Gynaecology, Sri Ramachandra University. The Rotterdam criteria-2003 was adopted to recruit the PCOS subjects (n=97). Healthy women without any history of infertility and clinical
signs of hyperandrogenism were included as controls (n=101). History of the subjects, demographic and phenotypic information and blood samples were collected after obtaining written informed consent. Around 2 - 3mL of blood samples were collected into EDTA vacutainer and stored at -20°C until further processing. The DNA isolation from whole blood was carried out by phenol chloroform method. Gradient PCR method was adopted to optimize the PCR conditions. SNP of GnRH1 (rs6185) was done by allele-specific PCR and LHβ (rs1800447, rs34349826, rs5030774), FSHR (rs132905, rs6165, rs6166), SHBG (rs9913778, rs6258, rs6259), AR (rs5919393, rs5919411, rs12014709) was examined with restriction fragment length polymorphism (RFLP) and DNA sequencing.

The Chapter 3, provides anthropometric and phenotypic evaluation between PCOS and controls of the study subjects. The average age is 26.20±4.12 in PCOS and 26.11±4.10 in controls. The average (Mean±SD) for weight (kg), height (m) and body mass index (kg/m²) among the PCOS is 56.92±9.58, 1.54±0.06 and 24.14±4.09 respectively. Similarly, it is 56.15±8.77, 1.54±0.06 and 23.84±4.06 for controls. None of the parameters show any significant difference between PCOS and control groups (P>0.05).

Phenotypic data such as menarche, days between menstrual cycles, androgenetic alopecia and acne has been evaluated between PCOS and control groups. Age of menarche attained by PCOS group were 20.6% in 10-12 years, 60.8% between 13-15 years and 18.6% in >16 years; at the same age group, it was 17.8%, 78.2% and 4% in the control subjects. While, control group shows a regular menstrual cycle occurring in normal intervals (25 to 34 days at an average of 28 days counting from first day of
menstruation to next one), among the cases 36.1% experienced 25-37 days and 63.9% are >37 days. Androgenetic alopecia was observed in 38.1% of PCOS and 61.9% did not show any signs, where as in controls 8.9% have the signs and 91.1% did not show any signs. In PCOS, 73.2% found to have acne and in 26.8% of subjects did not show any signs of acne, whereas in controls 39.6% have acne and in 60.4% did not.

The chapter 4 presents the results of gonadotropin releasing hormone (GnRH1) gene SNP and its association in the pathogenesis of PCOS. It is well established fact that the GnRH is the major regulator on differential secretion of LH and FSH from pituitary and any changes in those synthesis or secretion affect the normal physiology of reproduction through its downstream molecules. As it was reported that deregulated endocrine pathway as a cause of PCOS, rs6185(G/C) SNP was examined. The observed genotypes are C/C, C/G and G/G genotypes were 73.2%, 21.6% and 5.2% in PCOS and 75.2%, C/G (21.8%) and 3% in controls respectively. Allele and genotypes distribution did not show any significant difference between PCOS and controls (P>0.05).

In Chapter 5, the SNP's rs1800447 (T/C; Trp8Arg), rs34349826 (T/C; Ile15Thr) and rs5030774 (G/A; Gly102Ser) in luteinizing hormone beta (LHβ) gene was investigated. PCR-RFLP results showed the presence of only homozygous T/T (100%) genotypes for rs1800447 and rs34349826 and G/G (100%) genotype for rs5030774 in both PCOS and control subjects. The other genotypes C/T and C/C for rs1800447 and rs34349826 and G/A and A/A for rs5030774 are completely absent and found to be monomorphic suggest the variant LH is very rare among the Indian population.
Chapter 6 discusses the results of FSHR gene polymorphisms rs1394205(G/A), rs6165(G/A) and rs6166(G/A) obtained in the study population. The genotypes frequencies of rs1394205 A/A, A/G and G/G genotypes are found to be 16.5%, 37.1% and 46.4% in PCOS and 13.9%, 44.5% and 41.6% in controls respectively. For SNP rs6165, A/A, A/G and G/G genotypes are 25.8%, 41.2% and 33.0% in PCOS and 22.8, 36.6 and 40.6% in controls respectively. Similarly, for SNP rs6166, A/A, A/G and G/G genotypes was found to be 25.8%, 66.0% and 8.2% in PCOS and 30.7%, 51.2% and 17.8% in controls. The rs6165 in controls and rs6166 in PCOS are found to be deviated from HWE (P<0.05). The obtained results demonstrates that the allele and genotype frequency for rs1394205(G/A) and rs6165(G/A) did not show significant difference between PCOS and controls (P >0.05). In contrast, rs6166 heterozygote (A/G) frequencies are found to be more than the homozygotes in PCOS subjects. Polymorphism rs6166 found to be associated with PCOS in recessive model (Odds ratio (CI): 0.41 (0.17-1.00); P-value: 0.04).

Optimum concentration of free steroids to act on target organs including the ovary is regulated by the levels of sex steroid binding protein (SHBG). Therefore, it was of interest to investigate the distribution of SHBG gene polymorphisms and its association on the pathogenesis of PCOS (Chapter-7). PCR-RFLP analysis of SNP rs9913778 shows the presence of C/T and C/C genotypes 20.6% and 79.4% in PCOS and 15.8 and 84.2% in controls respectively; however T/T genotypes are completely absent in both study groups. Another SNP rs6258 (C/T) shows the presence of only C/C genotypes (100%) and neither C/T nor T/T genotypes in both PCOS and controls. PCR product of rs6259 subjected to restriction digestion with HinfI, showed
the presence of only G/A and G/G genotypes 2.1% and 97.9% in PCOS and 4% and 96% in controls respectively. Furthermore, all SNPs followed Hardy-Weinberg equilibrium (P>0.05). No significant difference was observed for SNPs allele frequencies and genotypes distribution between PCOS and controls. Both SNPs did not show any association with PCOS (P>0.05). Similarly, haplotype association analysis for rs9913778/rs6259 showed a combination of three haplotypes CA, TG and CG, also did not found significant risk haplotype for PCOS (P>0.05). Despite the study did not found any association of *SHBG* gene tagging SNPs with PCOS status; it was of interest to observe that genotypes of both SNPs rs9913778 and rs6259 are uniformly distributed between PCOS and control subjects.

Even optimum concentrations of steroid hormones available at the target site to mediate its action, functional receptors are essential. An attempt was made to study the frequencies of *AR* SNPs (rs5919393, rs5919411, and rs12014709) (Chapter-8). In the present study, SNP rs5919393 and rs5919411 are monomorphic and rs12014709 (G/T) is completely absent in PCOS and less prevalent (2%) in control subjects.

Anthropometric data did not show any significance difference between PCOS and control groups and phenotypic data showed the presence of phenotypes associated with PCOS. Among the 13 SNP’s examined on *GnRH1* (rs6185), *LHβ* (rs1800447, rs34349826, rs5030774), *FSHR* (rs132905, rs6165, rs6166), *SHBG* (rs9913778, rs6258, rs6259), *AR* (rs5919393, rs5919411, rs12014709); *FSHR* gene SNP rs6166 shows an association with PCOS.