Chapter IV

Gonadotropin releasing hormone (GnRH1) gene

SNP and its association with PCOS
4.0. Introduction

Gonadotropin releasing hormone (GnRH), is a decapeptide released from hypothalamus in a pulsatile fashion into hypophyseal portal vascular system and acts on anterior pituitary gonadotrophs. GnRH mediates its biological functions by binding through its receptor expressed on gonadotrophs and modulates differential secretion of LH and FSH, which exert their actions on the ovaries; regulate theca cell androgen synthesis and ovarian folliculogenesis. While, speed of GnRH pulses favor LH secretion and FSH synthesis [1], GnRH pluses are regulated by negative feedback action by progesterone under the influence of estrogens [2]. The GnRH1 is located on chromosome 8, consists of 3 coding exons which encode a 92 aminoacid preprohormone that is processed to produce the GnRH decapptide and the GnRH-associated peptide.

Altered synthesis, secretion and biological actions of GnRH have negative impact on the normal development of reproductive phenotypes. GnRH deficiency, pathological condition lead to low or inappropriate secretion of LH and FSH, thereby affecting the maturation of reproductive phenotypes (absence or incomplete puberty) and non-reproductive phenotypes such as craniofacial, skeletal, neurologic, renal, and olfactory abnormalities [3]. High levels of plasma LH concentrations and hyper-responsiveness of the pituitary to GnRH have been found in the majority of women with PCOS [4]. Several mutations have been reported in the GnRH1, and resulted with GnRH deficiency associated disorders [5, 6, 7]. Till date no major genetic variants have been reported in GnRH1, however one functional SNP located in exon-1 sequence of GnRH1 results in amino acid change Trp16ser (rs6185) was reported in clinical end-
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points in relation to altered estrogen exposure [8, 9]. As the GnRH is a major regulator of the pituitary and ovarian functions, abnormalities in the GnRH regulation may alter the steroidogenesis and ovulation in PCOS.

4.1. Methodology

The study population consists of PCOS women (n=97) and healthy women (n=101) with normal reproductive physiology were recruited in the study by adopting the criteria as explained in the chapter 2.1 and 2.2. About 3mL of intravenous blood sample was collected from the study subjects and DNA was extracted by phenol chloroform method as mentioned in the methodology section 2.5.1. Genotyping of GnRH1 SNP rs6185 (C/G) was done by AS-PCR using allele specific primers (Table 2.1) as explained in methodology section 2.5.7 and 2.5.8. Genotypes were confirmed by PCR amplification followed by agarose gel electrophoresis (section 2.5.3). Further PCR-agarose gel electrophoresis results were confirmed using direct sequencing of PCR products as explained in section 2.5.9. Suitable statistical tools were applied to draw inference as explained in the methodology section 2.5.10. Genotype frequencies and allele proportions were calculated and tested for Hardy-Weinberg equilibrium (HWE). Association of SNP with PCOS done by performing logistic regression analysis using allelic, dominant and recessive models and the risk estimated by calculating odds ratio (CI 95%); p-value<0.05 considered as significant.

4.2. Results

Genotyping of GnRH1 SNP rs6185 by AS-PCR showed the presence of homozygous wild (C/C), heterozygous (C/G) and homozygous variant (G/G) genotypes in both PCOS and control subjects (Figure 2.2.A) and these results were further confirmed by
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direct sequencing of PCR products (Figure 2.7. A, B and C). AS-PCR GnRH1 gene SNP rs6185(C/G) observed and expected genotypes and minor and major allele proportions were given in Table 4.1. SNP rs6185 genotypes, G/G, C/G and C/C were found to be 5.2, 21.6 and 73.2% in PCOS and 3.0, 21.8, 75.2% in control subjects respectively (Figure 4.1). SNP rs6185 follows HWE in both PCOS and controls (p-value>0.05) (Table 4.1). Association analysis performed by allelic model did not show any significant association with PCOS (OR (95%CI): 1.18 (0.68 – 2.05); p-value>0.05). Similarly, dominant model (OR (95%CI): 1.11 (0.59 – 2.11); p-value>0.05) and recessive model (OR (95%CI): 1.78 (0.41 – 7.61); P-value>0.05) did not show any significant association with PCOS in the present study (Table 4.2.)

Table 4.1. GnRH1 SNP rs6185 genotype frequency and allele distribution in PCOS and control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotypes (n)</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>HWE P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td>C/G</td>
<td>C/C</td>
<td>G</td>
</tr>
<tr>
<td>PCOS (n=97)</td>
<td>Observed</td>
<td>5 (5.2)</td>
<td>21 (21.6)</td>
<td>71 (73.2)</td>
</tr>
<tr>
<td>Expected</td>
<td>2.5</td>
<td>26.0</td>
<td>68.5</td>
<td></td>
</tr>
<tr>
<td>Controls (n=101)</td>
<td>Observed</td>
<td>3 (3)</td>
<td>22 (21.8)</td>
<td>76 (75.2)</td>
</tr>
<tr>
<td>Expected</td>
<td>1.9</td>
<td>24.1</td>
<td>74.9</td>
<td></td>
</tr>
</tbody>
</table>

Values within the parentheses represent the percentage (%).
NS: Not significant (P-Value>0.05)
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Figure 4.1. Distribution of GnRH1 SNP rs6185 genotypes in PCOS and control subjects

Table 4.2. Association analysis of GnRH1 SNP with PCOS

<table>
<thead>
<tr>
<th>SNP</th>
<th>Model Test</th>
<th>OR (CI95%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6185</td>
<td>Allelic</td>
<td>1.18 (0.68 – 2.05)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>1.11 (0.59 – 2.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>1.78 (0.41 – 7.61)</td>
<td></td>
</tr>
</tbody>
</table>

NS: Not significant (P-Value>0.05)
4.3. Discussion

GnRH is the principal hormone regulating the pituitary gonadotropins, thereby affecting the ovarian physiology. Lack of negative feedback regulation on GnRH pulse frequencies, can lead to excess secretion of LH; which in turn aggravates androgen biosynthesis at ovarian theca cells and results in hyperandrogenism, a key etiological factor in the pathogenesis of anovulation and infertility in PCOS women [10]. In the present study, AS-PCR analysis of GnRH1 SNP rs6185 C/G genotypes are uniformly distributed between PCOS and controls subjects (Table 4.1). Genetic association analysis by allelic, dominant and recessive models did not show any significant association with PCOS (Table 4.2) (p>0.05).

Role of GnRH1 SNPrs6185 variants in the signal peptide region of GnRH is not yet disclosed. This SNP had been reported in Mediterranean women with PCOS; nonetheless, they did not found any association with PCOS and the rs6185 genotypes are not correlated with biochemical levels of LH and FSH. However, homozygous variant (G/G) and heterozygous (C/G) genotypes are found to be associated with lower testosterone levels and fasting insulin [11]. Iwasaki et al. [8] have described an association between this SNP rs6185 (C/C) and higher bone mineral density, considered to be an indirect marker for estrogen activity in postmenopausal women. Piersma et al. [9] reported an association between rs6185 (G/G) with increased lymph node involvement and shorter disease free survival in Caucasian breast cancer patients [9]. GnRH1 SNP rs6185 is found to be associated with periodontitis in Japanese population [12]. SNP rs6185 (C/G) is reported in few other studies related to age at
menarche and age at menopause [13], papillary thyroid carcinoma [14], however did not find any significant association.

In conclusion, *GnRH1* SNP rs6185 genotypes are uniformly distributed in both PCOS and control subjects and did not found any significant association with PCOS in the present study despite the fact that this SNP is associated with many other disorders. In depth analysis on the genotype of this SNP and changes in the function of *GnRH1* may provide additional knowledge in the area.

References


