Chapter 11

Discussion
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The occurrence of premature ovarian failure cases has been reported as 2% (Coulam et al. 1986) in Caucasian population. Though the disorder does not cause physical pain but gives strong mental and social stigma to the patient women. More than six decades have extensively been devoted by several researchers around the globe to determine the genetic predispositions for the etiology of disorder. The first strong genetic association of p. Ala189Val variant in the FSHR gene with ovarian failure was reported by Aittomaki et al. (1995) in Finnish population but remained uncommon in other world populations. Various researchers performed genetic studies to establish the association of premutation repeats in the FMR2 gene with ovarian failure suggesting the possible involvement of this gene as a risk factor for the disorder. Shelling et al. (2000) reported a strong genetic association of p.Ala257Thr variant in the mature peptide region of inhibin alpha (INHA) gene with ovarian failure in New Zealand, Auckland and Slovenia populations. The similar association studies were further reported in Italian and Indian populations (Marozzi et al. 2002, Dixit et al. 2004). The studies of the INHA gene association provoked us to screen candidate genes which are networked around inhibin function in FSH regulation. We hypothesized a pathway where several candidate genes were linked with inhibin function in FSH regulation (Figures 11 and 12). During the present study, candidate genes of this pathway were screened among ovarian failure cases and compared with controls. These candidate genes included INHA (inhibin alpha), INHBA (inhibin beta A), INHBB (inhibin beta B), GDF9 (Growth Differentiation Factor 9), BMP15 (Bone Morphogenetic Protein 15),
TGFBR3 (betaglycan, inhibin receptor) and ACVR1B (ALK4 receptor/ activin type I receptor) (Figures 11 and 12). We provide the first ever case control studies of these genes involving cases of ovarian failure of Indian population. We established the first ever association studies of GDF9, TGFBR3 and ACVR1B genes with ovarian failure in the world literature.

Figure 14. The pathway representing candidate genes of ovarian failure involved in FSH regulation.
Figure 15. Detailed presentation of inhibin and activin receptor system on gonadotroph cells.

Our study of the \textit{INHA} gene established the presence of c.769G>A (p.Ala257Thr) variant in 10.5% of POF cases, 10% primary amenorrhoea cases with almost absence (1/200) in controls. Our results revealed the presence of this variant with the early onset (below the age of 25 years) of the disorder. This variant possibly diminishes the binding affinity of inhibin alpha subunit with its receptor (betaglycan) and subsequent failure in the competition with activin activity. Three rare missense variants in the proregion viz. c.275G>A (p.Ser92Asn), c.525C>G (p.His175Gln) and c.545C>A (p.Ala182Asp) were exclusively present in ovarian failure cases. Therefore, a total of 12% POF, 11% of PA cases harbor missense variations in \textit{INHA} gene (Figure 13 and Figure 14). The study of the \textit{INHBA} gene revealed a rare missense variants c.896A>C (p.Q299P) in one PA case. The study of the \textit{INHBB} gene did not reveal any missense variant except one silent variant c.942C>T in one POF case.
Our study of *GDF9* gene revealed the presence of two rare missense variants viz. c.199A>C (p.Lys67Glu), c.646G>A (p.Val216Mat) exclusively present with ovarian failure. The c.199A>C variant was present in four POF cases whereas c.646G>A variant was present in two POF cases. Two frequent SNPs, c.398-39C>G, c.447C>T were used for haplotyping and concluded significantly higher frequency of C-T haplotype (p-value = 0.03). Overall, the missense variants were associated with 5% of POF cases (figure 13). The study of *BMP15* gene revealed eleven rare missense variants viz. c.181C>T, c.182G>A, c.226C>T, c.227G>A, [c.538G>T(+)c.539C>T], c.538G>A, c.588T>A, c.617G>A, c.631C>T, c.661T>C, c.727A>G which were present only in ovarian failure cases in a mutually exclusive manner. Cumulatively, these variants were associated with 11% of POF cases and 8% of PA cases (Figure 13 and Figure 14). Haplotype analysis was performed for three frequent variants c.-9C>G, c.308A>G, c.852C>T which concluded significant association of G-G-C haplotype with ovarian failure (P value=0.0075). The variants in the proregions of *GDF9* and *BMP15* genes probably form unstable preprodimers hence are susceptible for proteolytic degradation. Another possibility is the improper folding pattern due to the presence of these variants.

The study of the *TGFBRI3* gene did not reveal any apparently associated missense variant but represented highly polymorphic nature. Haplotype analysis using five frequent and genotypically diverse variants revealed significant association of CCAAT haplotype with ovarian failure. The genetic study of activin receptor gene (*ACVR1B*) revealed significant association of three variants viz. c.389T>C, c.392T>G and c.392T>G. The c.389T>C and c.392T>G were always linked with each other. The c.389T>C and c.392T>G
variants were revealed in 7/133 cases of POF (Fisher’s p value = 0.0015) and 4/63 cases of PA (Fisher’s p value = 0.003) whereas c.581G>A variant was present in 4/133 cases of POF (Fisher’s p value = 0.025). These all variants are probably of gain of function nature. Overall these missense variants were present in 8% of POF cases and 6% of PA cases (Figures 13 and 14).

Figure 16. Graphical distribution of missense variants of all studied genes among POF cases
Figure 17. Graphical distribution of missense variants of all studied genes among PA cases