Letter to the Editor

Expansion of the germline analysis for the *INHA* gene in Indian women with ovarian failure

Sir,

Previously we had reported mutational analysis of the mature peptide region of inhibin genes (*INHA*, *INHBA* and *INHBB*) in Indian women with ovarian failure (Dixit *et al*., 2004). This article has reported a significant association of the c.769G>A (p.Ala257Thr) missense variant in *INHA* gene (inhibin alpha) in Indian women with ovarian failure. Our article demonstrated the presence of this variant in 11.2% cases of premature ovarian failure (POF) and 9.1% cases of primary amenorrhoea (PA) with their complete absence in controls. This published article described a case–control study based on the 80 cases of POF, 33 cases of PA and 100 controls. The potential importance of this variant encouraged us to further analyse the germline status of the complete coding region of *INHA* gene with an increased population size. The other two inhibin genes *INHBA* and *INHBB* were not found to be associated with ovarian failure and hence were not considered for further analysis. This letter reports an enhanced mutational analysis of the complete coding region of *INHA* gene in 133 cases of POF, 63 cases of PA and 200 controls including the previously reported individuals. Our published article described the sequence analysis of only second half of the second exon, which codes for the mature peptide region. This letter additionally introduces the sequence analysis of the first exon and first half of the second exon covering the signal peptide as well as the proregion. The first exon was amplified using INHAEX1F (5′GTCGCTTGAGGCGAAATCC3′) and INHAEX1R (5′GTCTCCCAGCGCATACTTC3′) primers. The first half of the second exon was amplified using INHAEX2AF (5′CCGGAGGGCGTGGAGCAGAGT3′) and INHAEX2AR (5′GGCGCAGAGCAGAGGGAGACCAA3′) primers. The second half of the second exon (mature peptide region) was amplified as mentioned earlier (Shelling *et al*., 2000). The updated prevalence of c.769G>A variant is 10.5% of cases of POF (14 out of 133, Fisher's exact test, $P = 0.000017$), 10% of cases of PA (six out of 60, Fisher's exact test, $P = 0.0007$), with its presence in one out of 200 controls. All the mutant individuals were heterozygous except one homozygous, which was identified in our published article. The earlier reported SNP c.1–124A>G (rs11893842) was revealed with the genotypic distribution in POF cases (AA = 66, AG = 53 and GG = 14), PA cases (AA = 32, AG = 22 and GG = 6) and controls (AA = 110, AG = 74 and GG = 16) (Harris *et al*., 2006). This SNP represented almost similar genotypic distribution among the patient and control population. The earlier reported variant c.1–16C>T (mentioned as 129C>T by Montgomery *et al*., 2000; Marozzi *et al*., 2002) was present in 16 cases (12%) of POF, seven cases (11.7%) of PA and 26 (13%) controls. The two earlier reports illustrated a significant under-representation of the T allele in cases with ovarian failure (Marozzi *et al*., 2002; Harris *et al*., 2006). The present data do not find a significant difference in the prevalence of T allele among the patients and controls. This allelic difference may be due to the population diversity. A novel silent variant c.327C>T was revealed in four cases (6.67%) of PA, three cases (2.25%) of POF and 10 (5%) controls. One control was homozygous for this variant, and all the remaining individuals were heterozygous. The presence of c.531C>T variant was corroborated to have a complete linkage with the c.1–16C>T variant similarly as reported by Montgomery *et al*. (2000). Interestingly, four novel missense variants were revealed in the proregion. Three missense variants c.275G>A (p.Ser92Asn), c.525C>G (p.His175Gln) and c.545C>A (p.Ala182Asp) were exclusively associated with the patients, and one missense variant c.487G>A (p.Val163Met) was present in a control. The c.275G>A (p.Ser92Asn) missense variant was heterozygous with its presence in a POF patient. Upon aligning the available protein sequences against human serine^{92} demonstrated the similar residue (in rhesus macaque, dog and cow) or arginine residue (in horse and pig) or threonine residue (rat and mouse) but never asparagine. This patient also had the homozygous truncation variant c.631C>T (p.Glu211X) in the *BMP15* gene as reported in our recently published article (Dixit *et al*., in press). This patient had facial paralysis with 85.0 IU/l FSH levels and 37.0 IU/l LH levels. She had only withdrawal of bleeding upon medication. The ovaries were not visualized in the patient. She underwent two IVF cycles but failed both times. The c.525C>G (p.His175Gln) missense variant was heterozygous with its presence in a POF patient. The patient attained menopause at 30 years and was diagnosed with high FSH and LH levels as 78 and 47 IU/l, respectively. The proband's mother was also heterozygous for this variant and attained menopause at 36 years with high gonadotrophin levels. The patient's father and her sister were genotypically normal and had no history of infertility. The alignment of available protein sequences against histidine^{175} revealed that most of the other vertebrate sequences were containing arginine but never glutamine. The c.545C>A (p.Ala182Asp) missense variant was homozygous with its presence in a PA patient. She had FSH and LH levels as 9 and 7 IU/l, respectively. She never attained menarche and presented under-developed secondary sexual characters. The protein alignment depicted the highly conserved nature of wild-type alanine^{182} among the various vertebrates. Thus, the substitution of alanine may possibly lead to functional defects. One missense variant c.487G>A (p.Val163Met) was revealed in a control. This variant was

heterozygous. The sequence alignment results demonstrated an increased presence of methionine over valine among vertebrates. Thus, this substitution may not have any effect on the protein activity. A novel variant c.1098+18C>A was also detected in the 3′UTR region with its presence in a PA case. The Human Genome Variation Society (HGVS) guidelines were followed for the nomenclature of variants. All the experiments were performed following standard protocols as mentioned in our previously published article. In a nutshell, the present letter not only provides the updated information about the prevalence of c.769G>A variant but also provides the presence of various novel variants in the *INHA* proregion and comparative information about the reported polymorphisms in the analysed region. The data in this letter will surely strengthen the significance of our previously published article.

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