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Identification of Novel Nucleotide Changes in INHBB Gene by Mutation Screening in Females with Ovarian Dysgenesis : A Case Report

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Abstract

Background: Inhibin and activin regulate the follicle stimulating hormone level by their antagonistic actions and thus have been considered as strong candidate genes in the etiology of ovarian dysgenesis. In the present study, two cases of primary amenorrhea with poorly developed secondary sexual characteristics were reported. The purpose of the study was to identify mutations in candidate gene.

CasePresentation: In this paper, clinical, genetic, biochemical, and molecular findings in female patients with primary amenorrhea were reported. Whole blood culture and G-banding for karyotyping, sequencing, and in silico analysis were performed following the standard protocol. Both cases were cytogenetically characterized as normal females with 46,XX, chromosome constitution. Hormonal assay revealed high level of follicle stimulating hormone and luteinizing hormone. DNA sequence analysis of inhibin identified two novel heterozygous missense mutations of c.975T>A and c.1156G>A which were translated into p.I310N and p.D386N, respectively. These identified positions were highly conserved across species during

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Table 1. Primer list used for sequence analysis of INHA, INHBA, and INHBB gene

Primers	Sequences (5'→3')	Product size (bp)	Annealing temperature (°C)
INHA_EX1F	TGCGTCAGAGATAGGAGGTCT	531	60
INHA_EX1R	CCATGCTGTGCCTTGCTTTT		
INHA_EX2.1F	CTCCTGCTGAAGAGGAGGG	546	58
INHA_EX2.1R	CGCAGAGCAGAGGGAGAC		
INHA_EX2.2F	CCTGGTGGCCCACACTC	539	62
INHA_EX2.2R	AACTCTGCCTTTCCTCCCAG		
INHBB_EX1F	CAGTGACCCGCGACCTC	797	62
INHBB_EX1R	GAGCTAGCAAGGAGAGGGAG		
INHBB_EX2.1F	AGCAGAGAGTGTGTTTCCCC	513	60
INHBB_EX2.1R	ACTCCAGGCCTCGCTTG		
INHBB_EX2.2F	GTGGACCCAGGCGAAGAG	495	60
INHBB_EX2.2R	GCTGGAAGAAGCCCACC		
INHBA_EX1F	AGTTCCTCCTGGGACTGTCA	700	60
INHBA_EX1R	ACAGCCACAAACCTACAGCA		
INHBA_EX2.1F	AACTCTTGCTCCCTTTCCCC	322	58
INHBA_EX2.1R	ACATCGGCTGGAATGACTGG		
INHBA_EX2.2F	TGACCTTGCCATCACACTCC	663	58
INHBA_EX2.2R	ACAGAAGACTCCTGCTTGCC		

3130 Genetic Analyzer (Applied Biosystems, USA) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to WKH PDQXIDFWXUHU¶V instructions were screened in 150 control individuals from the same population by Sanger sequencing.

In silico analysis Obtained nucleotide substitutions were further analyzed using MutationTaster online tool (www.mutationtaster.org) and other prediction tools like predictSNP, SIFT, Panther, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, and MAPP. Hydrophobicity profile and helix forming properties of wild-type and mutant INHBB protein were also examined by ProtScale program available at the ExPASy web server.

Homology modeling of inhibin βB subunit The inKLE-subunit was homology modeled by using coordinates from Protein Data Bank. Three-protein structures named 3rjr_A, 5hly_A, and 5hlz_A were utilized for modeling using Modeller 9.15 and selection of the best model was done by using four different web servers and tools, i.e. RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) for Ramachandran plot analysis, ERRAT program (http://services.mbi.ucla.edu/ERRAT/) for verifying protein structures determined by crystallography, PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) for structural analyses, and Modeller 9.15 to find out the best model among all. The figures were produced using standalone tool, DeepView-SwissPdbViewer (http://spdbv.vital-it.ch/).

Results

Cytogenetic analysis revealed 46,XX karyotype signifying the normal chromosome constitution in both cases (Figures 2A, 3A). Hormonal assay from serum showed FSH and LH levels of 90.3 and 18.5 mIU/ml, respectively in case 1. In case 2, the measured level of FSH and LH was 55.95 and 16.09 mIU/ml, respectively. Higher level of both FSH and LH, biomarker of gonadal dysgenesis, was observed in both cases. Resequencing of INHA, INHBA and INHBB genes identified two novel non-synonymous substitutions of c.975T>A in case 1 and c.1156G>A in case 2. These variations were translated into p.I310N and p.D386N, respectively (Figures 2B, 3B). Both isoleucine at position 310 and aspartic acid at 386 position were also highly conserved across species during evolution except a few of them (Figures 2C, 3C). P.I310N variation was predicted to be pathogenic by several in silico prediction tools (Mutation Taster, SIFT, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, MAPP and predictSNP) (Figure 2D) while p.D386N mutation was predicted as neutral (Figure 3D).

The study of intramolecular hydrogen bonding pattern of p.I310N mutation revealed the formation of three new hydrogen bonds in mutant

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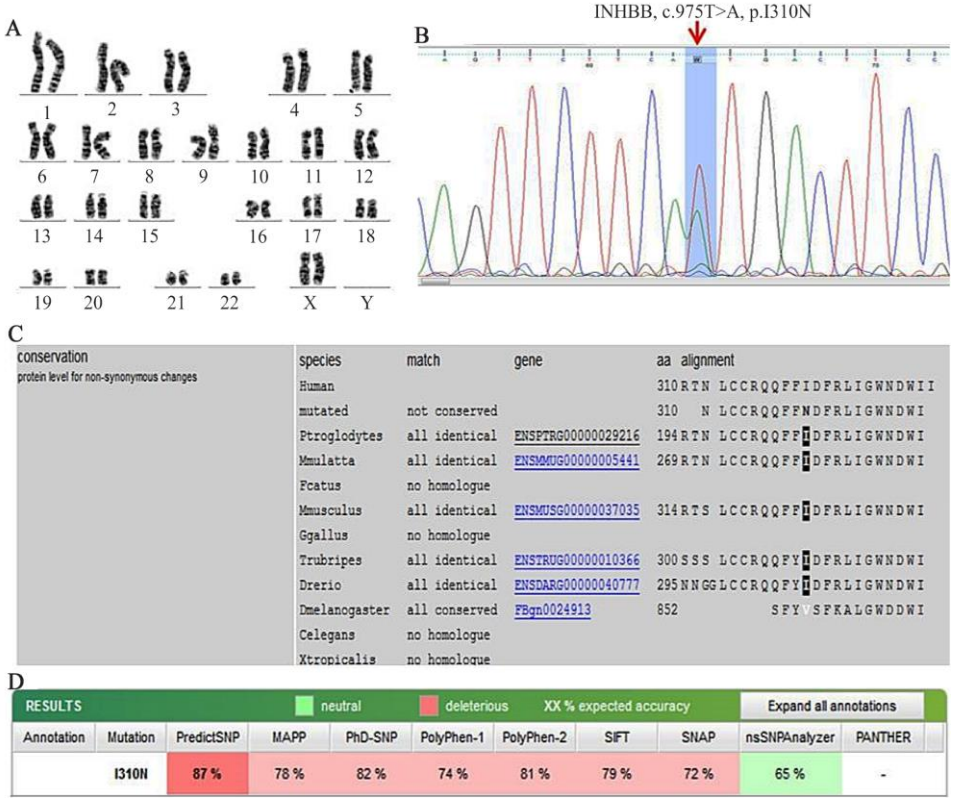

Figure 2. A) Representative karyotype (46,XX) of case 1, B) Electropherogram of the c.975T>A (p.I310N), C) The multiple sequence alignment of the p.I310N mutant protein reveals the conservation of isoleucine at this position throughout evolution, D) In silico prediction of both variants using prediction tools of SIFT, SNAP, PolyPhen-1, PolyPhen-2, PhD-SNP, MAPP, and PredictSNP

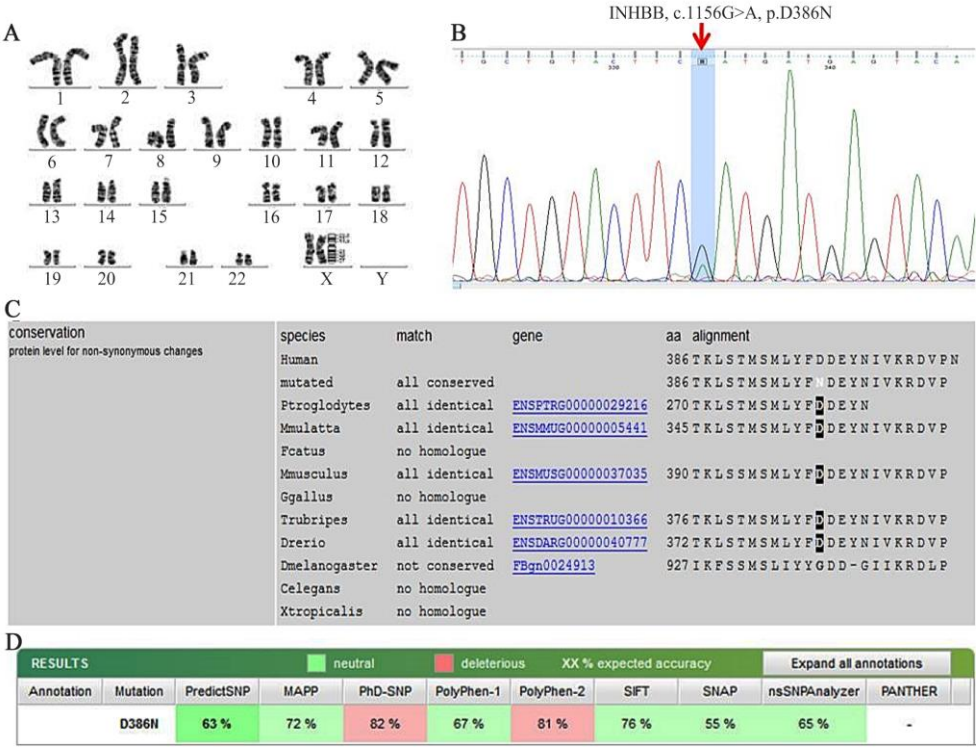

Figure 3. A) Representative karyotype (46,XX) of the second case, B) Electropherogram of the c.1156G>A (p.D386N), C) Triple sequence alignment of the p.D386N mutant protein reveals the conservation of isoleucine at this position throughout evolution, D) In silico prediction of both variants using prediction tools of SIFT, SNAP, PolyPhen-1, PolyPhen-2, PhD-SNP, MAPP, and PredictSNP

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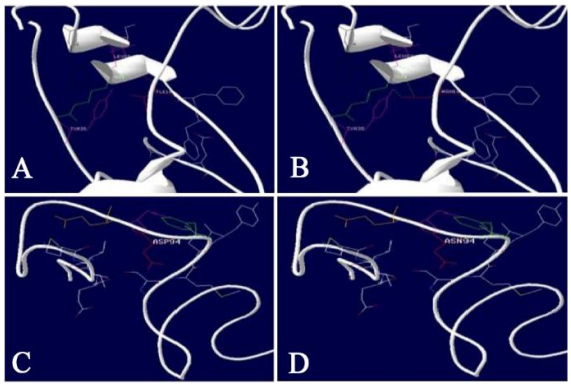

Figure 4. The tertiary structure of the INHBB protein predicted by Modeller and visualized on the standalone tool DeepView - SwissPdbViewer, A) the wild-type INHBB protein with I310, B) the mutant N310 INHBB protein formation of three new hydrogen bonds occurs with TYR327, ARG313 and LEU314, C) the wild-type INHBB protein with D386, D) the mutant N386 INHBB protein shows no change in hydrogen bonding pattern

tein with TYR327, ARG313, and LEU314 (Figures 4A, 4B). The analysis of alteration in hydrophobicity of the protein due to this mutation revealed minor changes in protein microenvironment (Figures 5A, 5B). On the other hand, intramolecular hydrogen bonding pattern and hydrophobicity analysis of p.D386N mutation showed the neutral effect (Figures 4C, 4D, 5C, 5D).

Discussion

The present study reports two mutations (p.I310N and p.Q386K) identified in females with primary amenorrhea. The first identified mutation results in alteration in intramolecular hydrogen bonding pattern as well as protein microenvironment due to hydrophobicity change while the second mutation does not show any pathogenic effect. The alteration in protein microenvironment might interfere with normal function of inhibin B protein that leads to disturbance in inhibin and activin balance. Activin and inhibin balance plays an important role in regulation of ovarian cycle through FSH level. As the alpha subunit involves in multiple complexes with antagonistic functions, the consequences of mutations related to loss-of-function or gain-of-function of protein might be difficult to predict. However, there is no report till date that mutation

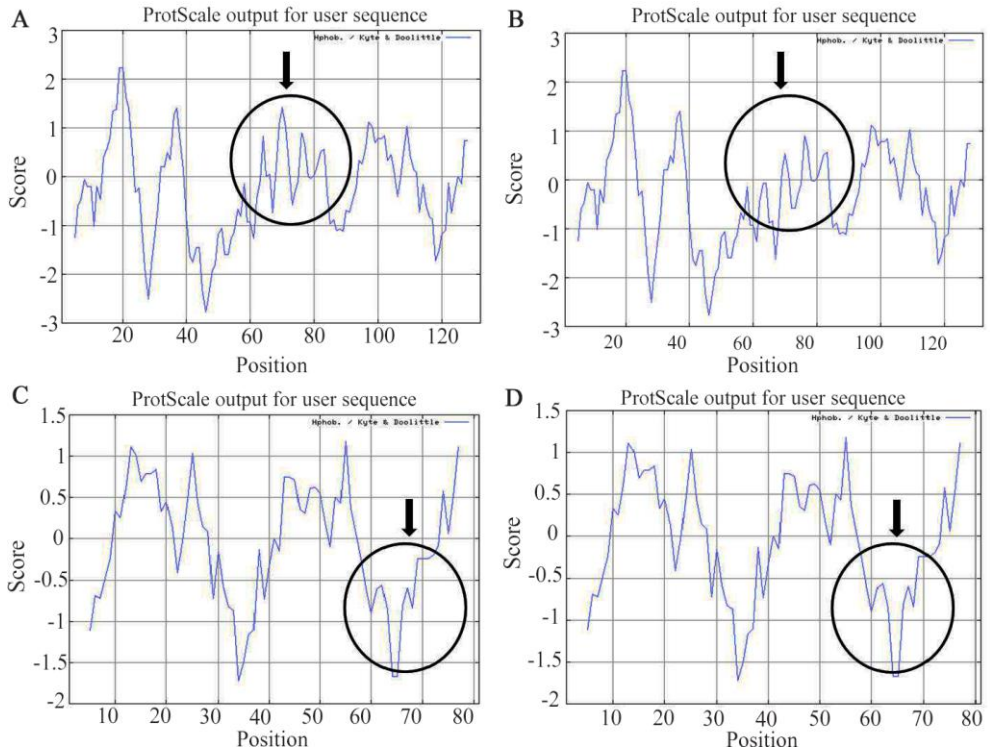

Figure 5. Hydrophobicity profile of wild-type and mutant protein predicted by the ProtScale program at the ExPASy server, A) the black circle represents the hydrophobicity around I310 in wild-type protein, B) the black circle represents the hydrophobicity around N310 in the mutant protein showing a mild shift in hydrophobicity compared to the wild-type, C) black circle represents the hydrophobicity around D386 in the wild-type protein, B) the black circle represents the hydrophobicity around N386 in the mutant protein showing no shift in hydrophobicity compared to the wild-type

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in in KLEL subunit is involved in ovarian dysgenesis in females. An independent study reported that p.M370T substitution is involved in spermatogenic impairment in males (12). Furthermore, very low level of inhibin B in prepubertal girls is a biomarker for premature ovarian failure (13, 14). INHA has been more studied in ovarian failure and several mutations have been reported by different studies (10, 11, 15-17).

A nucleotide substitution (INHA 769G>A) in LQKLELQ . VXEXQLW LV ZHOO VWXGLHG LQ GLIIHUHQW SRSX lations with reference to premature ovarian failure (8, 11, 15-17). Dixit et al. also reported three different mutations (p.S92N, p.H175Q, p.A182D) in INHA gene in patients with premature ovarian failure (10). A p.H175Q mutation in INHA was observed by Dixit et al. in a patient who experienced menopause at the age of 30 years with high level of FSH and LH (78 IU/l and 47 IU/l, respectively). Interestingly, another patient of this study with p.A182D mutation in INHA never menstruated and had underdeveloped secondary sexual characteristics.

Conclusion

Our study has demonstrated for the first time that mutation in INHBB subunit might lead to ovarian dysgenesis. Mutations in inhibin gene might affect the FSH level either through inhibition by inhibin or stimulation by activin through disturbing the ratio between inhibin and activin. Inhibin is suggested as a marker for ovarian reserve in premature ovarian failure patients and early screening of inhibin gene is necessary for identification of ovarian reserve to manage ovarian failure.

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Conflict of Interest

The authors have no conflict of interest.

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