Journal of
Bioengineering & Biomedical Science

Research Article Open Access

Characterization of Inhibin Alpha Gene in Patients of Polycystic Ovary Syndrome

Farkhanda Haroon*, Ayesha Maqbool, Rabia Rafiq, Fatima Azhar and Muhammad Nazer Ul Islam Haroon

Virtual University, Pakistan

Abstract

Polycystic ovary syndrome (PCOS) is multi-symptomatic gynecological disorder with high prevalence (5-10%) among human females. Primarily it affects female reproductive system resulting in infrequent menstrual cycles. Visceral adiposity, insulin resistance, hirsutism and infertility are other consequences of PCOS. Genetic as well as environmental factors contribute for the progression of PCOS. Multiple studies revealed association of PCOS with mutations in different genes specifically expressing androgens and androgen receptors. PCOS is an autosomal and X-linked disease. Inhibin A downregulates the follicle stimulating hormone (FSH) to promote ovulation and normal menstrual cycle. Inhibin A is a hetero dimer of *INHA* and *inhibin INHB subunits. INHA* along with Anti-mullerian hormone can be used as a diagnostic marker for PCOS. This study has been conducted using data of fifty participants. They were classified into two groups, control and experimental group. Out of total 50 participants, 30 were PCOS patients and 20 were healthy control subjects. Blood samples of PCOS patients were collected from Pakistan Institute of Medical Sciences. Extracted DNA from blood was used for amplification of exon 2 of *INHA* subunit of Inhibin A and B gene. Restriction digestion of Amplified gene segment was carried out with restriction endonuclease. Restriction fragment length polymorphism (RFLP) results showed 30% (p 0.0178) of PCOS patients having heterozygous mutation (G769A/ rs12720062). Results revealed positive risk of developing PCOS when having A allele at position 769bp in heterozygous state. So, we concluded that there is an association between heterozygosity at rs12720062 and risk of development of PCOS.

Keywords: Hirsutism; Anti-mullerian hormone; Heterozygous mutation

Abbreviations: PCOS: Polycystic Ovary Syndrome; FSH: Follicle Stimulating Hormone; RFLP: Restriction Fragment Length Polymorphism; LH: Luteinizing Hormone; PCR: Polymerase Chain Reaction; POF: Premature Ovarian failure; AMH: Anti-Mullerian hormone

Introduction

Polycystic ovary syndrome (PCOS) also called as Stein Leventhal syndrome is one of the most common gynecological disorders. It is one of the main reasons of infertility or poor fertility among females. It is a multi-symptomatic disorder that is a result of elevated androgens in females. Hyperandrogenism, insulin resistance and irregular menstrual cycles are consequences of PCOS. During menstrual cycle, follicles are ruptured to release egg for fertilization in a process called as ovulation. If follicles fail to rupture, eggs absorb fluid and turn into follicular cysts. On the other hand, after normal ovulation, follicles are dissolved. If follicles are not dissolved and their opening is closed than follicles will turn into corpus luteum cysts. Other types of ovarian cysts include dermoid, cystadenomas and endometriomas cysts. Sometimes ovaries contain large number (12 or more than 12) cysts. In this case, this disorder is called as polycystic ovary syndrome.

Symptoms of cystic ovaries include abdominal bloating, painful bowl movement, pelvic pain, painful intercourse, breast tenderness, nausea and vomiting. If this disorder becomes severe, symptoms like faintness, rapid breathing and sharp pelvic pain are added. 60 to 70 % of PCOS patients suffer from clinical hyperandrogenism [1] which is actually steroidogenic defect of theca cells. In this case follicle stimulating hormone (FSH) and Luteinizing Hormone (LH) level is increased.

Another consequence of PCOS is insulin resistance. Variable number of tandem repeat polymorphism in insulin gene promoter regions is involved in its expression [2]. There is a strong association between class III allele of insulin gene VNTR in 5' region of insulin gene in case of PCOS [3]. This allele is preferentially transmitted from heterozygous fathers. Later studies revealed no association between insulin gene, PCOS and hyperandrogenemia. One of the reasons of these opposing results is ethnic and geographical differences.

Hyperandrogenism is also a major problem of PCOS patients. Some cases of hyperandrogenism have been reported in patients with aromatase deficiency [4]. CYP17A1 enzyme over production and other intracellular signaling defects are responsible for excessive androgen synthesis. GATA6 mRNA was found at high level as it regulates the expression of STAR in procrine ovary. This transcription factor enhances the steroid synthesis in theca cells [5]. Earlier studies have revealed that PCOS is an X linked disorder in which it affects multiple members of successive generations [6]. 47 % female offspring of affected females are affected while all the daughters of affected males (Elevated FSH/LH) are affected [7].

First relatives of patients of hirsutism and enlarged ovaries are at higher risk as compared to first degree relatives of normal females [8]. Later on, studies revealed PCOS as an autosomal as well as X linked inherited disease [9].

A study has shown that healthy sisters of affected females showed evidence of insulin resistance despite of absence of clinical

***Corresponding author:** Farkhanda Haroon, Virtual University, Pakistan, Tel: 00923045447368; E-mail: haroonfarkhanda@gmail.com

Received: September 24, 2019; **Accepted:** October 03, 2019; **Published:** October 11, 2019

Citation: Haroon F, Maqbool A, Rafiq R, Azhar F (2019) Characterization of Inhibin Alpha Gene in Patients of Polycystic Ovary Syndrome. J Bioengineer & Biomedical Sci 9: 259.

hyperandrogenism. It shows that in PCOS affected families, insulin resistance is a dominant trait [10].

Inhibin is a peptide hormone that is synthesized and secreted by ovaries and testis. It has two isoforms named inhibin A and inhibin B. Inhibin is involved in suppression of FSH level through negative feedback during luteal to follicular phase transition of menstrual cycle [11]. Welt and his colleagues investigated levels of inhibin A and B through ELISA and find elevated levels of inhibin A and B in PCOS patients as compared to the regular subjects [12]. Other researchers have shown slightly elevated inhibin A and unaffected inhibin B levels in women suffering from PCOS [13]. In case of PCOS, increased level of circulating Inhibin B was found in persistent small follicles [14]. Kumar et al. worked on transgenic models and concluded that INHA mutant mice possessed higher concentration of FSH [15].

Abnormalities and mutations in inhibin genes are associated with male and female reproductive cancers and reproductive disorders such as premature ovarian failure [16]. Women with variants of INHA suffer from severe symptoms of premature ovarian failure [17]. One of the variants of inhibin A allele named p. A257T is found abundant in Indian female patients of premature ovarian failure [18].

Method

Sampling

We studied 50 adolescent females including 30 PCOS patients recruited from gynae outpatient department of Pakistan institute of medical sciences (PIMS) and 20 control subjects. Diagnostic criteria was presence of 10-12 cysts in ovary seen through ultrasound. Demographical data of patients as well as control subjects was recorded.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from 1ml peripheral blood through inorganic extraction method. 999bp long Exon 2 of Inhibin alpha was amplified by PCR. Reaction mixture for every sample contained total volume of 25µl comprising of 19µl PCR grade water, 2.5µl genomic DNA, 1.3µl forward primer INHA2F (TTTTAAGTCCCTTCATGATGAAA), 1.3µl reverse primer INHA2R (ATACTTGAAAGAGTAACCTCCAT), 2µl PCR buffer, 0.5µl dNTPs and 0.7µl Taq polymerase. Denaturation at 95°C was followed by 25 cycles of annealing at 54°C-59°C for 30 seconds and extension at 72°C for 1minute. 25th cycle included 10 minutes of extension at 72°C (Figure 1).

Restriction fragment length polymorphism (RFLP)

PCR product was digested using restriction enzyme named BsrFI to study polymorphism at SNP named rs12720062. To have an insight of restriction fragments of PCR product, we used online tool named NEB Cutter.

For digestion, we used protocol given by suppliers (Thermo scientific). RFLP reaction mixture contained 18µl of PCR H2O, 10µl PCR product and 2µl of buffer and 1µl of restriction endonuclease BsrFI. PCR product of all samples digested at 37°C for 3 hours.

Results

We analyzed total 50 subjects including 30 PCOS patients and 20 control subjects. PCR product digestion through RFLP showed heterozygous genotype (AG) in 9 experimental subjects and 01 control subject. On the other hand homozygous mutated genotype (AA) was observed in only one experimental subject. Table 1 shows Frequency of different genotypes found in control & experimental subjects (Figure 2).

RFLP analysis for 769G<A mutation (rs12720062) results show linkage of heterozygosity with PCOS. Lane 1 showing marker (10000bp-250bp). Lane 2-4 representing control group samples with wild genotype GG. Lane 5-8 showing wild genotype GG as well as heterozygous genotype AG.

Statistical analysis

Data resulted from RFLP was subjected to statistical analysis to confirm statistical significance. Data was analyzed in terms of p value Chi-square.

As shown in Table 2 Chi square calculations of RFLP results (expected values are displayed in square brackets and individual χ2values are displayed in parentheses)

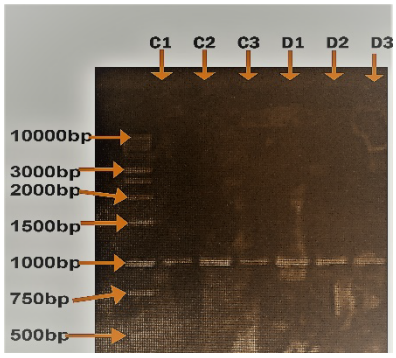

Figure 1: 1.5% agarose gel showing PCR product of length 999bp. Lane 1 showing 10kb marker Lane 2-4. representing PCR product of control group samples. Lane 5-7 showing PCR product of PCOS patients.

	GG	AG	AA
Control group	19	1	0
Diseased group	20	9	1

Table 1: Frequency of different genotypes found in control & experimental subjects.

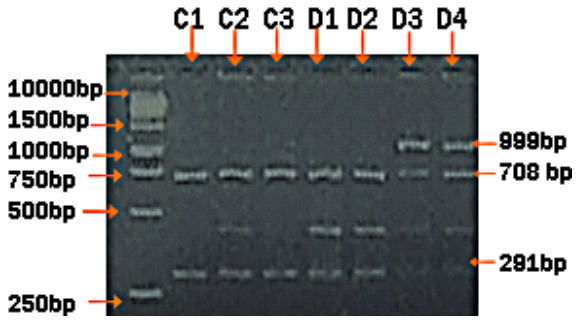

Figure 2: RFLP analysis for 769G<A mutation (rs12720062) results show linkage of heterozygosity with PCOS.

Category	GG (wild genotype)	AG+GG	Marginal row total
Control	19 (15.6) [0.74]	01 (4.4) [2.63]	20
Diseased	20 (24.3) [0.49]	10 (6.6) [1.75]	30
Marginal column total	39	11	50

Table 2: Chi square calculations of RFLP results (expected values are displayed in square brackets and individual χ^2values are displayed in parentheses).

The chi-square statistic ($\chi 2/df$) is 5.6138. The p-value ($\chi 2>5.6138$) is 0.017819. This result is significant at $p < .05$.

Discussion

INHA gene encodes Inhibin α subunit of peptide hormones Inhibin A and Inhibin B. This peptide binds with other INH βA to make dimeric Peptide hormone Inhibin A. In case INH α makes a dimer with INH βB, a peptide hormone called as Inhibin B forms. Inhibins are crucial peptide hormones for proper regulation of menstrual cycle. Inhibin A down regulates the FSH through negative feedback mechanism in late follicular phase (follicular-luteal phase transition) [19]. Inhibin B regulates the development of follicles during follicular phase of menstrual cycle. In response to FSH, Inhibin B is released by antral ovarian follicles in response to FSH during early follicular phase, and indicates the follicular growth [20].

According to a study mutation in INHA at position 769bp converts alanine into threonine and hence changes Inhibin α peptide structure. G769A (p.A257Th) is involved in pathogenesis of Premature Ovarian failure (POF) in Italian, Indian and Iranian ethnicities [17-20]. While other studies showed no significance of such type of linkage of mutation G769A with POF in other ethnicities [21,22].

Pigny et.al. [23] measured inhibin alpha levels in 72 PCOS & 61 control subjects. The findings proved high level of circulating Inhibin alpha in serum. A recent study considering comparison of plethora of androgens levels conducted in 2016 showed high level of FSH and INHA in amenorrhoeic and oligo-amenorrhoeic patients of PCOS. It suggested that INHA and Anti-mullerian hormone (AMH) together can be used as a biomarker for diagnosis of PCOS. As researchers found elevated level of LH, FSH and LH: FSH in PCOS patients [24].

According to Inhibin level in serum can be used as a diagnostic marker of ovarian cancer. Inhibin A level along with that of CA125 can be used as perfect biomarker of different types of ovarian carcinoma with very high specificity [25]. Another study revealed that Inhibin A level in serum of patients with differentiated carcinoma is higher as compared to that of non-differentiated carcinoma. High Inhibin B level can be used to predict probability of survival. These studies reveal the potential role of Inhibin A and B in carcinoma proliferation [26].

Li W et.al [27] conducted a study on 48 big white sows with ovarian cysts and 60 healthy normal sows. This genetic study was performed using techniques like SSCP-PCR and RFLP. They found association between ovarian follicular cysts and mutations in inhibin α in white big sows. They concluded that sows with two significant mutations c.G42A and c.G3222A are at higher risk of developing ovarian cysts while sows with wild alternatives of same locus 42GG & 3222GG are at lower risk of cyst development. As Inhibin alpha is one of the most conserved genes among mammals, these mutations and inhibin alpha gene should be taken under consideration. This type of studies in human females can give insight of pathogenesis of cyst formation in PCOS patients.

Münzker J first ever investigated INHA polymorphism rs12720062 in PCOS patients and found heterozygosity (AG genotype) in 10 out of 233 patients (4.29%). Inhibin corresponds to the level of LH and AMH. Author explains the low level of LH and AMH as a consequence of affected bioreactivity of INHA in patients with heterozygous genotype. Author reported G769A mutation in PCOS patients and found this heterozygous polymorphism in 10 out of 223 diseased subjects. Author suggested that further studies should consider control group along with experimental to confirm statistical significance of this mutation as compared to control subjects and confirm linkage with PCOS [28].

Conclusion

We conducted this study to find association of Inhibin alpha subunit gene with pathogenesis of PCOS. We selected controversial SNP rs12720062 (G769A). Polymorphism in this gene has already been reported as missense mutation in POF patients of different ethnicities. This study included 30 PCOS subjects and 20 healthy control subjects.

We found this mutation in heterozygous state (AG) in 30% (p-value 0.026254) of PCOS patients. We found one patient with mutated homozygous genotype (AA). Her mother was also suffering from PCOS. She is infertile and never conceived. As this genotype has already been proved as a biomarker of POF in different ethnicities so having a patient PCOS patient with this genotype is unique and provokes a lot of questions. On the other hand, we also came across a healthy control subject having heterozygous genotype (AG). Consequences of this mutation in heterozygous condition might include less bio-reactive inhibin and in turn impaired function on down regulation of FSH. Our study shows potential association of missense mutation in heterozygous condition at SNP rs12720062 of INHA with pathogenesis of PCOS.

References

1. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, et al. (2006) Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. J Clin Endocrinol & Metab 91: 4237-4245.
2. Undlien DE, Bennett ST, Todd JA, Akselsen HE, Ikäheimo I, et al. (1995) Insulin gene region-encoded susceptibility to IDDM maps upstream of the insulin gene. Diabetes 44: 620-625.
3. Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, et al. (1997) Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. Lancet 349: 986-990.
4. Harada N, Ogawa H, Shozu M, Yamada K (1992) Genetic studies to characterize the origin of the mutation in placental aromatase deficiency. Am J Hum Genet 51: 666-672.
5. Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, et al. (2003) The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 278: 26380-26390.
6. Givens JR, Wiser WL, Coleman SA, Wilroy RS, Andersen RN, et al. (1971) Familial ovarian hyperthecosis: a study of two families. Am J Obstet Gynec 110: 959-972.
7. Wilroy RS Jr, Givens JR, Wiser WL, Coleman SA, Andersen RN, et al. (1975) Hyperthecosis: an inheritable form of polycystic ovarian disease. Birth Defects Orig Artic Ser 11: 81-85.
8. Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R (2001) Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. Fertil Steril 75: 53-58.
9. Hague WM, Adams J, Reeders ST, Peto TE, Jacobs HS (1988) Familial polycystic ovaries: a genetic disease? Clin Endocrinol 29: 593-605.
10. Diamanti-Kandarakis E, Dunaif A (2012). Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 33: 981-1030.
11. Stouffer RL, Dahl KD, Hess DL, Woodruff TK, Mather JP, et al. (1994) Systemic and intraluteal infusion of inhibin A or activin A in rhesus monkeys during the luteal phase of the menstrual cycle. Biol Reprod 50: 888-895.
12. Welt CK, Taylor AE, Fox J, Messerlian GM, Adams JM, et al. (2005) Follicular arrest in polycystic ovary syndrome is associated with deficient inhibin A and B biosynthesis. J Clin Endocrinol Metab 90: 5582-5587.
13. Tsigkou A, Luisi S, De Leo V, Patton L, Gambineri A, et al. (2008) High serum concentration of total inhibin in polycystic ovary syndrome. Fertil Steril 90: 1859-1863.
14. Lockwood GM, Muttukrishna S, Groome NP, Matthews DR, Ledger WL (1998) Mid-follicular phase pulses of inhibin B are absent in polycystic ovarian syndrome and are initiated by successful laparoscopic ovarian diathermy:

a possible mechanism regulating emergence of the dominant follicle. J Clin Endocrinol Metab 83: 1730-1735.

15. Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, et al. (1999) Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. Mol Endocrinol 13: 851-865.
16. Shelling AN (2012) Mutations in inhibin and activin genes associated with human disease. Mol Cell Endocrinoly 359: 113-120.
17. Shelling AN, Burton K.A, Chand AL, van Ee CC, France JT, et al. (2000) Inhibin: a candidate gene for premature ovarian failure. Hum Reprod 15: 2644-2649.
18. Prakash GJ1, Ravi Kanth VV, Shelling AN, Rozati R, Sujatha M (2010) Mutational analysis of inhibin alpha gene revealed three novel variations in Indian women with premature ovarian failure. Fertil Steril 94: 90-98.
19. Welt CK, Smith ZA, Pauler DK, Hall JE (2001) Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. J Clin Endocrinol Metab 86: 2531-2537.
20. Hillier SG, Wickings EJ, Illingworth PI, Yong EL, Reichert Jr LE, et al. (1991) Control of immunoactive inhibin production by human granulosa cells. J Clin Endocrinol Metab 35: 71-78.
21. Madania A, Alchamat GA, Alhalabi M, Ghoury I, Orabi M, et al. (2018) Inhibin a gene–16C? T and 769 G? A polymorphisms in Syrian women with idiopathic premature ovarian failure. Middle East Fertil Soc J 23: 48-51.
22. Jeong HJ1, Cho SW, Kim HA, Lee SH, Cho JH, et al. (2004). G769A Variation of Inhibin Î±-gene in Korean Women with Premature Ovarian Failure. Yonsei Med J 45: 479-482.
23. Pigny P, Desailloud R, Cortet-Rudelli C, Duhamel A, Deroubaix-Allard D, et al. (1997) Serum α-inhibin levels in polycystic ovary syndrome: relationship to the serum androstenedione level. J Clin Endocrinol Metab 82: 1939-1943.
24. Yetim A, Yetim Ç, Baş F, Erol OB, Çığ G, et al. (2016) Anti-Müllerian hormone and inhibin-A, but not inhibin-B or insulin-like peptide-3, may be used as surrogates in the diagnosis of polycystic ovary syndrome in adolescents: preliminary results. J Clin Res Pediatr Endocrinol 8: 288-297.
25. Robertson DM, Pruysers E, Jobling T. (2007) Inhibin as a diagnostic marker for ovarian cancer. Cancer let 249: 14-17.
26. Walentowicz P, Krintus M, Sadlecki P, Grabiec M, Mankowska-Cyl A, et al. (2014) Serum inhibin A and inhibin B levels in epithelial ovarian cancer patients. PLoS One 9: e90575.
27. Li W, Sun L, Chen S, Chen L, Liu Z, et al. (2015) Association of inhibin-α gene polymorphisms with follicular cysts in large white sows. Theriogenology 84: 839-845.
28. Münzker J (2014) Androgen Patterns in Polycystic Ovary Syndrome (PCOS) (Unpublished Doctoral dissertation) Medical University of Graz, Graz, Austria.