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Mutational analysis of human bone morphogenetic protein 15 in Chinese women with polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the common defects that cause ovary dysfunction and link to the aberrant process of folliculogenesis. Bone morphogenetic protein 15 (*BMP15*) is expressed in human oocytes and functions importantly to regulate early follicle growth and fertility. Previous studies have discovered several mutations in the screening of *BMP15* in premature ovarian failure but none in PCOS. In this current study, we focused on the mutational analysis of the coding region of *BMP15* among 216 Chinese PCOS patients. Five novel missense mutations in *BMP15* were discovered, namely, c.34C>G, c.109G>C, c.169C>G, c.288G>C, and c.598C>T. These results are the first to indicate that *BMP15* gene mutations may be potentially associated with PCOS patients.

1. Introduction

Polycystic ovary syndrome (PCOS) affects 5% to 10% of women in the reproductive age worldwide [1], which is a major cause of anovulation, infertility, and menstrual irregularities. The evidence suggests that the mechanism of PCOS involves ovary dysfunction that links to the aberrant process of folliculogenesis. Polycystic ovary syndrome follicles are generally smaller than normal ones because of the excessive development of prophase follicles and the lack of selection of dominant follicles in the PCOS ovary, but not through the recruitment of primordial follicles [2].

Several genes have been thought to be associated with stages of follicle development that been documented in PCOS by mutational analysis, such as *FSHR* [3,4], *IR* [5], *CYP19* [6], follistatin [7], *GnRH* [8], *FEM1A* [9], and *WNT-4* [10]. However, these studies mostly failed to provide sufficient evidence on the potential candidate gene associated to PCOS.

It is known that transforming growth factor–β proteins are involved in various developmental processes including repro-

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Table 1 – Variants of *BMP15* in 216 Chinese women with PCOS

Mutation	Exon	Sequence variation	Amino acid variation	No. of cases (n = 216)	No. of controls (n = 200)
Novel	1	c.C34G	p.Leu12Val	1/216	0/200
Novel	1	c.G109C	p.Ala37Pro	1/216	0/200
Novel	1	c.C169G	p.Pro57Ala	1/216	0/200
Novel	1	c.G288C	p.Arg96Ser	1/216	0/200
Novel	2	c.C598T	p.His200Tyr	1/216	2/200
Common	1	c.A308G	p.Asn103Ser	1/216	0/200
Common	2	c.788insTCT	p.262insLeu	21/216	30/200

ductive functions [11]. As a member of the transforming growth factor–β superfamily secreted by the mammalian oocyte that contributes in early follicular development, bone morphogenetic protein 15 (*BMP15*, also called *GDF9B*) is essential for ovarian follicular development, ovulation, and fertility [12,13].

Human *BMP15* is located on Xp11.1 that regulates follicle growth and degeneration through autocrine or paracrine process and affects the selection of dominant follicle and the formation of atretic follicles [14].

BMP15 has been evaluated in animal models. *BMP15* in sheep has been suggested as a breeding marker for its prolificacy by influencing the rates of ovulation [12,13]. In mice, deletion of *BMP15* results in reduced female fertility with the primary defects in ovulation and fertilization [15].

BMP15 may be the candidate gene for PCOS. Oocyte cells regulate the activities of oocyte follicles and begin the process of secretion before the individual oocytes generate. So far, *BMP15* regulates follicle growth and decline through autocrine or paracrine way, affecting the choice of dominant follicle as well as the formation of follicular atresia. *BMP15* acts not only to stimulate primary follicles to the development of small preantral follicles, but also to increase the number of preantral follicles, with the ability to promote granulosa cell meiosis [16]. Animal experiments show that *BMP15* gene mutations attenuate follicular development, thus affecting. Polycystic ovary syndrome is a syndrome of ovarian dysfunction. Its cardinal features are hyperandrogenism and polycystic ovary morphology.

Previous studies detected several variations of *BMP15* in humans, mostly found among patients with premature ovarian failure (POF) (supplementary table 1). To our knowledge, this current study is the first to provide a mutation analysis on screening a cohort of 216 Chinese patients with PCOS for variations in the entire coding sequences of *BMP15* to discover whether they could likewise function as potential disease-associated genes in this syndrome.

2. Materials and methods

A total of 216 PCOS patients and 200 unrelated healthy controls were recruited from the First Affiliated Hospital, Anhui Medical University, China. Controls were individuals of proven fertility, with normal menstrual cycles and ovarian morphology, and without a history of subfertility treatment. The study protocol was approved by the Ethics Committee of the National Research Institute for Family Planning, and informed consent was obtained from all participants.

Women with PCOS were diagnosed following the criteria of the Rotterdam Revised 2003 (2 of 3) diagnosis: oligomenorrhea or amenorrhea for at least 6 months; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume [10 mL]). Congenital adrenal hyperplasia, Cushing syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction were excluded. Total testosterone, progesterone, follicle-stimulating hormone, luteinizing hormone, and estradiol of each woman were determined through laboratory analysis. Body mass index was calculated as weight/(height)^2 (kilograms per square meter) to assess obesity (clinical characteristics of the study population are shown in supplementary table 2).

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Kit according to the manufacturer's protocol. The coding region of *BMP15* was amplified in all patients and control individuals by polymerase chain reaction (PCR) using the primer set listed in supplementary table 3.

Each PCR product was tested and sequenced on an automated sequencer (ABI 3730XL; Applied Biosystems) to perform mutation analysis. Presence of all sequence variants was confirmed by performing 2 independent PCRs and subsequent DNA sequencing.

3. Results

In the coding region of *BMP15*, 7 variants were revealed (Table 1). Five novel missense mutations (c.34C>G, c.109G>C, c.169C>G, c.288G>C, and c.598C>T) were discovered along with the 2 earlier reported variants: c.308A>G and c.788insTCT; the latter resulted in a leucine insertion at position 262 (shown in supplementary table 1). One missense variant (c.598C>T) was discovered in both PCOS patients (1/216) and controls (2/200). The other 4 mutations were presented exclusively among PCOS subjects and discretely in different patients.

4. Discussion

BMP15 is synthesized as a preproprotein; after cleavage, the proproteins of growth differentiation factor 9 and *BMP15* form homodimers or heterodimers [17]. They are coexpressed in human oocytes to play key roles in the molecular dialogue between the oocyte and the surrounding somatic cells, promoting granulosa cell mitosis and cumulus expansion by

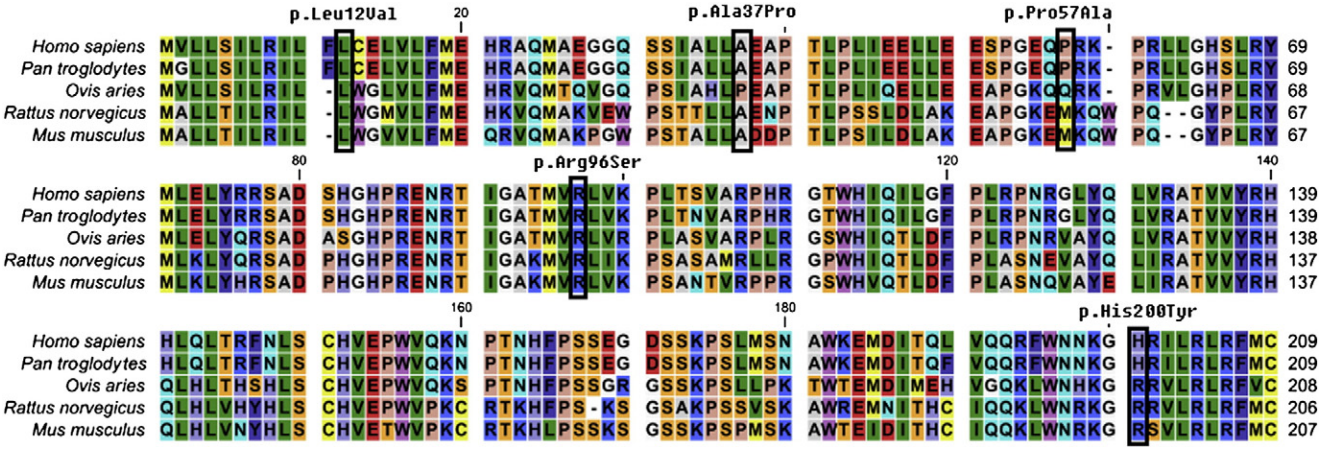

Fig. 1 – The conservatism of *BMP15* among species.

a paracrine effect [18,19]. The highly specific localization of *BMP15* within the ovarian follicles suggests that this potential growth factor might be involved in the regulation of folliculogenesis and fertility in humans.

Previous studies based on the mutational analysis of *BMP15* mostly focused on patients with POF, with several variants published (shown in supplementary table 1); however, there was only one report that included a screening of 38 Japanese PCOS patients but found no missense mutation [20]. Furthermore, one study on association analysis of *BMP15* among Spanish women also failed to provide any association between *BMP15* gene and PCOS [21]. Probably, ethnic/genetic differences between Chinese and Spanish subjects led to the discordant experimental results.

Because of the high prevalence of PCOS and because this syndrome constitutes the most common cause of anovulatory infertility and hirsutism, several attempts have been made to determine the presence of causal mutations. Yet, few had managed to provide adequate evidence on the presence of a potential candidate gene [3-10]. Urbanek et al [22] used linkage analysis to study 37 candidate genes, but found only evidence for linkage with follistatin.

To our knowledge, the current study is the first to provide evidence that *BMP15* mutations may be associated with PCOS.

Seven variants in *BMP15* with 5 novel missense ones were identified among 216 PCOS patients in this study. Compared with the fact that great quantities of mutations were discovered in patients with POF in previous studies, it could be inferred that *BMP15* may function through the common pathway in the pathogenesis of both PCOS and POF.

Variant p.Leu12Val and p.Pro57Ala both led to the substitution of the amino acids with smaller R groups. Missense mutation c.109G>C caused a substitution of alanine to proline that might function as the terminator to the folding of the protein. Another mutation, c.288G>C, resulted in a replacement of a basic amino acid arginine with a neutral and hydrophilic amino acid serine at position 96.

All variants we discovered in the present study were compared among *Homo sapiens*, *Pan troglodytes*, *Mus musculus*, *Rattus norvegicus*, and *Ovis aries*. Details were shown in Fig. 1. The detailed clinical characteristics of subjects carrying the novel missense mutations mentioned above were listed in supplementary table 4.

When studying the mutants that naturally happen in sheep, previous researchers found out that the mutations impair the correct folding of *BMP15*, leading to mutant proteins and forming unstable proregion heterodimers that are subjected to targeted degradation before completion of processing, especially to damage the folding of the protein with the mutational heterodimers.

With missense mutations in *BMP15*, resultant proteins formed from the missense messenger RNA will not be able to form homodimers or heterodimers, reducing normal *BMP15* dimer levels; therefore, the pattern of expression of *BMP15* may be altered in the PCOS and PCO oocytes during follicle development [23]. Mutations in *BMP15* may play an important role in the cessation of folliculogenesis that leads to the accumulation of large numbers of small antral follicles, thereby causing development of few graafian follicles and generating the PCO phenotype [24].

The decline of *BMP15* proteins that regulate the differentiation of granulosa cells is thought to affect early follicular development in ovary, whereas PCOS is characterized by the abnormal development of prophase follicles.

In conclusion, the current study was the first to discover variants in *BMP15* in association with PCOS. It should be noted that most prior studies focusing on human *BMP15* have been performed among patients with ovarian failure. Further research on *BMP15* is required to confirm or refute our data and supply more rigorous evidence for the role of *BMP15* in PCOS. Further research among other populations is also required to establish whether, in populations other than Chinese, *BMP15* can also serve as a genetic marker for PCOS as suggested in this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2010.10.006.

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