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Diversity of *Bmp15* and *Gdf9* Genes in White Goat of Guizhou Province and Evolution of the Encoded Proteins

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Abstract: Members of the transforming growth factor-beta superfamily, growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), have crucial roles in fecundity of sheep. Our previous investigation confirmed that the fecundity mutations of sheep presented in highly prolific White goat individuals of Guizhou province. To illuminate other polymorphisms in *Bmp15* and *Gdf9* genes and the relationship of these mutations with function, we cloned and characterized the coding region of *Bmp15* and *Gdf9*. Molecular models of BMP15 and GDF9 mature peptide of White goat were constructed based on the homology of human BMP7 experimental tertiary structure. Two exons encoded prepropeptide of 394 amino acids in BMP15 and 453 residues in GDF9, respectively. Apart from the $FecX^B$ mutation (S99I) in BMP15 and V79I mutation in GDF9 confirmed in White goat previously, other seven and three polymorphism sites were detected from BMP15 and GDF9 mature peptides, respectively. S32G, N66H, S99I/P99I and G107R in BMP15 could be important for the binding of dimer to receptors. Changes of P78Q and V79I in GDF9 might affect the binding of dimer to receptor type 1. Comparing the length of BMP15 and GDF9 prepropeptide in vertebrates, an increase in length of BMP15 presented along with the protein evolution from fish to mammal and the divergence of the N-terminus residues in matured BMP15 peptide might contribute to the sensitive control on the fertility of animal species with low ovulation rate. These findings gave a valuable explanation for the correlation of mutations in *Bmp15* and *Gdf9* genes with the control on fecundity of White goat and supported the notion that they were the pivotal factors in female fertility of White goat in Guizhou province.

Key words: *Bmp15*; *Gdf9*; Gene; Evolution; White goat

贵州白山羊 *Bmp15* 和 *Gdf9* 基因多态性及其编码蛋白的进化分析

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摘要：BMP15和GDF9是转化生长因子β（TGFβ）超家族的成员，对绵羊的繁殖性状有直接的调节作用，从中发现的多个高产突变位点直接提高了排卵数和产羔数。在之前的研究中，作者从贵州白山羊中找到了一个高产突变位点。为了进一步揭示*Bmp15*和*Gdf9*基因突变与繁殖性状之间的关系，对贵州白山羊*Bmp15*和*Gdf9*基因编码区进行了克隆，以人BMP7的晶体结构为模板构建了贵州白山羊BMP15和GDF9成熟肽的三维模型。贵州白山羊*Bmp15*和*Gdf9*基因分别编码394和453个氨基酸的蛋白前体。对BMP15和GDF9成熟肽序列进行分析发现，除了之前确认的BMP15中的$FecX^B$ 突变（S99I）和GDF9中的V79I突变之外，还从贵州白山羊的BMP15和GDF9成熟肽分别发现7个和3个位点突变。其中，BMP15成熟肽的S32G、N66H、S99I/P99I和G107R突变可能影响二聚体与受体的结合；GDF9成熟肽的P78Q和V79I影响二聚体与I型受体的亲和力，将值得进一步深入研究。对*Bmp15*和*Gdf9*基因编码的蛋白前体序列进行聚类分析，结果显示在鱼类到哺乳类的进化过程中，BMP15出现长度逐渐增加的现象，以BMP15成熟肽N端长度增加为主。这种演变可能使BMP15对低排卵哺乳动物繁殖力的控制更为灵敏。该文的研究结果为贵州白山羊*Bmp15*和*Gdf9*基因变异与繁殖力的关系提出了合理的解释，并支持这两个因子是贵州白山羊高产性状重要调节因子的观点。

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Gene insertions and deletions have been widely acknowledged to play an essential role in the evolution of genes in vertebrates (Gemmell & Slate, 2006). Most mutations in protein are associated with disease, only a small number of examples show the heterozygote advantage. The good example is *Bmp15* and *Gdf9* genes, which are identified to be related with female fecundity in several animals. *Bmp15* and *Gdf9* genes encode for bone morphogenic protein 15 (BMP15, also known as GDF9B) and growth differentiation factor 9 (GDF9), respectively. Both of them are members of the transforming growth factor β (TGFβ) superfamily, in which over 35 proteins have been found with pivotal role in cellular growth and differentiation (Juengel et al, 2004). These two oocyte-derived molecules, BMP15 and GDF9, are closely related in their primary structures and share a nearly identical spatiotemporal expression pattern in the ovary of animals (Juengel et al, 2004). However, these two molecules are encoded by distinct genes and *Bmp15* gene maps to chromosome X but *Gdf9* to autosome 5 in sheep (Davis, 2005). Active peptides of BMP15 and GDF9 contain 125 and 135 amino acids respectively (Davis, 2005; Galloway et al, 2000). Currently, it is hypothesized that BMP15 and GDF9 may form non-covalent heterodimers or homodimers to regulate the fertility of small ruminants (Hanrahan et al, 2004). The dimers combine and activate two types of membrane-bound serine/threonine kinases on the membrane of target cells in ovary. BMP15 is reported to bind to ALK-6 and BMPR-II (Moore et al, 2003), while GDF9 integrates to ALK-5 (Mazerbourg et al, 2004) and BMPR-II (Vitt et al, 2002). Mutations in *Bmp15* and *Gdf9* genes have been shown to increase ovulation rate and ultimately litter size in sheep (Hanrahan et al, 2004). Five separate mutations in the mature *Bmp15* coding region have been described (Davis, 2005) with heterozygous advantage in sheep. One copy of the Inverdale ($FecX^I$), Hanna ($FecX^H$), Belclare ($FecX^B$), Galway ($FecX^G$) or Lacaune ($FecX^L$) allele increases about 0.6 lambs per ewe compared to the wildtype (Hanrahan et al, 2004; Bodin et al, 2007). Conversely, homozygous carriers of *Bmp15* mutation have small undeveloped ovaries and are infertile. Similar to *Bmp15*, invalid *Gdf9* gene also blocks follicular growth in homozygotes, resulting in sterility in sheep, and one mutated $FecG^H$ allele lends to increase ovulation rates in heterozygous ewes (Hanrahan et al, 2004). Furthermore, ewes heterozygous for mutations in both *Gdf9* and *Bmp15* are fertile and the effects of these mutations on ovulation rate are additive. It appears that both of *Bmp15* and *Gdf9* are important in the control of ovulation rate in sheep. However, the relative importance of these growth factors differs in mice. *Gdf9* is essential for normal follicular development whereas *Bmp15* is not because *Bmp15* knock-out mice are fertile only with the ovulation and fertilization of oocytes impaired. It suggests that the regulation of *Bmp15* and *Gdf9* genes on fertility is species-specific in mammals. In nonmammalian vertebrates, both of *Bmp15* and *Gdf9* have been detected in ovaries of hen (Elis et al, 2007; Johnson et al, 2005) and fish (Clelland et al, 2006; Liu & Ge, 2007) but the correlation with their fertility remains to be elucidated.

Goat normally has a low ovulation rate, which is similar to sheep. The mRNAs and proteins of *Gdf9*, *Bmp15* and BMP receptors have been detected in goat ovarian follicles at all stages (Silva et al, 2004). Polymorphisms of *Bmp15* and *Gdf9* of White goat in Guizhou province had been confirmed in our previous investigations (Du et al, 2008; Lin et al, 2007). In addition, it is characterized by litter size with extreme variation among individuals from one to five lambs, which is consistent with the phenotype of segregating major genes. Therefore, White goat is a desirable model to study the diversity of these genes for control of ovarian folliculogenesis. Thus, to illuminate polymorphisms in *Bmp15* and *Gdf9* genes and the relationship of these mutations with function, the coding regions of *Bmp15* and *Gdf9* genes of White goat were cloned and a comparison with other animals was also undertaken in the present study.

1 Material and Methods

1.1 Animal and samples

Five prolific White goats chosen for this experiment were healthy and recorded to give triplets birth. They kindly provided by Qinglong goat breeding farm in Guizhou province of China. Genomic DNA was prepared respectively from the blood sample of goats as the protocol of E.Z.N.A. TM SE Blood DNA Kit (Omega) described. Electrophoresis on 0.7% agarose gel showed this material to be of high molecular weight.

1.2 Amplification and cloning of *Bmp15* and *Gdf9*

genes

The sense and antisense primers were based on the conserved sequences of *Bmp15* and *Gdf9* genes in goat and sheep deposited in GenBank (Tab. 1). Genes were amplified by polymerase chain reaction (PCR) with primers (50 pmol each), 100 ng of genomic DNA, 2.5 units of Ex *Taq* DNA polymerase (Takara), 0.2 mmol/L of each dNTP, and 10 μL of 10×reaction buffer adjusted to a final reaction volume of 100 μL with $3dH_2O$. An initial long (5 min) denaturation step at 94˚C was followed by 30 s at 50—56˚C and 2 min at 72˚C, and then 30 cycles of 1 min at 94˚C, 30 s at 52—60˚C, and 1 min at 72˚C. The reaction was stopped after a final extension time of 10 min at 72˚C. The size and purity of the PCR products were estimated by subjecting the samples to 1% agarose gel electrophoresis.

PCR products were cloned into the pMD18-T simple vector according to the procedures of the PCR cloning kit (Takara) and transformed into the competent *Escherichia coli* cells TG1 {*supE* Δ(*hsdM-mcrB*)*5 thi* Δ (*lac-proAB*) F'[*tra* Δ*36proA+B+lacIqZDM15*]}. The positive clones were detected by colony PCR. The plasmid DNA carrying the PCR products was prepared by the procedure of E.Z.N.A. Plasmid Miniprep kit (Omega) and subjected to sequencing by Applied Biosystems 3730 automatic DNA sequencer. The sequencing was carried out by Shanghai Invitrogen DNA Sequencing Service. To correct for potential errors arising from PCR amplification, sequencing was carried out on at least two clones, derived from independent PCRs.

1.3 Sequence analysis and tertiary model

Tab. 1 Primers for amplification of *Bmp15* and *Gdf9* genes in White goat of Guizhou

Primer name		Primers sequence (5'→3')	PCR products (bp)
Bmp15 exon1	sense	GATGCAAAGAGGACAATTTAGAAGACC	574
	antisense	CCCACCAGAACAATATAGTATGATAACTC	
Bmp15 exon2	sense	TGCAGGCTCCTGGCACATACAGAC	862
	antisense	TCACCTGCATGTGCAGGACTGGG	
Gdf9 exon1	sense	GGAAGAAGACTGGTATGGGGAAATG	461
	antisense	CTGCTCCTACACACCTGCCGC	
Gdf9 exon2	sense	TCTTCTCAGGAACCTTTCCATCAGT	995
	antisense	GTTTTACTTGACAGGAGTCTGTTAACGAC	

Searches for homologous DNA and protein sequences were conducted with the BLAST software against the nonredundant GenBank database (http://www.ncbi.nlm.nih.gov/blast/blastn/). Phylogenetic analysis was performed with MEGA4.0 program using Neighbor-Joining method. The confidence level for each branch was estimated by interior branch testing with 1000 random replications using Clustal W method. Predictions of the structure were performed online with the Phyre program (Bennett-Lovsey et al, 2008) and deepview/Swiss-pdb Viewer v4.0 (Arnold et al, 2006).

1.4 Nucleotide sequence

Two sequences of *Bmp15* and *Gdf9* gene from White goat in Guizhou province has been submitted to the GenBank database libraries and given the accession numbers of FJ429281 and FJ429282.

2 Results

2.1 Identification of *Bmp15* and *Gdf9* genes in White goat

After comparing of DNA sequences encoding *Bmp15* and *Gdf9* genes, specific oligonucleotides were designed as primers to amplify fragments contained exons from genomic DNA of White goat. PCR products were cloned and sequenced (Fig. 1). Assembly sequences were obtained from repeated results of five prolific goats. Based on the blast by NCBI database, it contained complete exon1 (328 bp) and exon2 (857 bp) in *Bmp15*-like gene, with partial 5'-flank region (215 bp) and intron1 (38 bp). Complete exon1 (397 bp) and exon2 (965 bp) of *Gdf9*-like gene in White goat were screened as well, together with 5'-flank (50 bp), part of intron 1 (23 bp) and 3'-flank region (21 bp). They were 99%—100% identical to *Bmp15* of Jining, Yunling and Boer goat breeds, and 99% to *Gdf9* of Jining goat breed. The joined exon 1 and exon 2 of *Bmp15*- and *Gdf9*-like sequences encoded for 394aa of *Bmp15*- and 453 aa of *Gdf9*-like prepropeptide respectively by conceptual translation, which were very similar to that of other goat breeds. Highest peptide sequence identity was 98.7%—100% to that of Boer, Jining and Yunling goat breeds. It confirmed that the amplified fragments from White goat were *Bmp15* and *Gdf9* gene. The deduced precursor protein of BMP15 and GDF9 contained the proteolytic

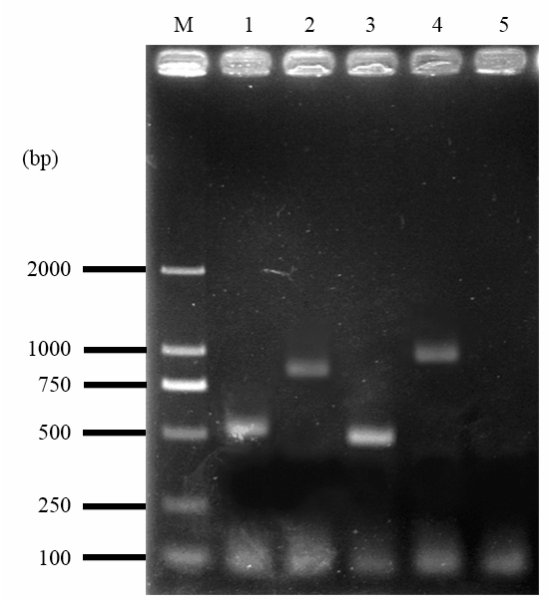

Fig. 1 PCR products of exons fragments of *Bmp15* and *Gdf9* in White goat

Lane M: DL2000 DNA marker; Lane 1: exon1 fragments of *Bmp15* (574 bp); Lane 2: exon 2 fragments of *Bmp15* (862 bp); Lane 3: exon 1 fragments of *Gdf9* (461 bp); Lane 4: exon 2 fragments of *Gdf9* (995 bp). Lane 5: negative control.

processing site of RXXR in proregions to yield mature peptides, with 125 residues in BMP15 and 135 residues in GDF9. Each peptide contained a conserved 6-Cys domain which made up three pairs of disulfide bonds forming the cysteine-knots. In mature BMP15 peptide, seven amino acids changes were detected from the five female goats with D17G, P50L, L65H, H70Y, V85A, P99I and G107R. It was interested that three amino acids, phenylalanine, serine or isoleucine, appeared at residue 99 of the matured BMP15 (denoted as P99I/S99I). Fewer substitutions of amino acids were found out from the mature GDF9 peptide sequences, which were V18A, P78Q, V79I and T81A. Furthermore, GDF9 peptide sequence in mammals was much more conserved than that of BMP15. For example, the similarity of mature peptide between White goat and mouse was 87.4% in GDF9 while just 68.1% in BMP15. Taking the deposited sequences of BMP15 and GDF9 in White goat as represents, a multiple alignment containing amino acids sequences of different goat breeds was generated and a phylogenetic tree was constructed with other mammals and opossum, platypus, chicken and fish (Figs. 2, 3). The

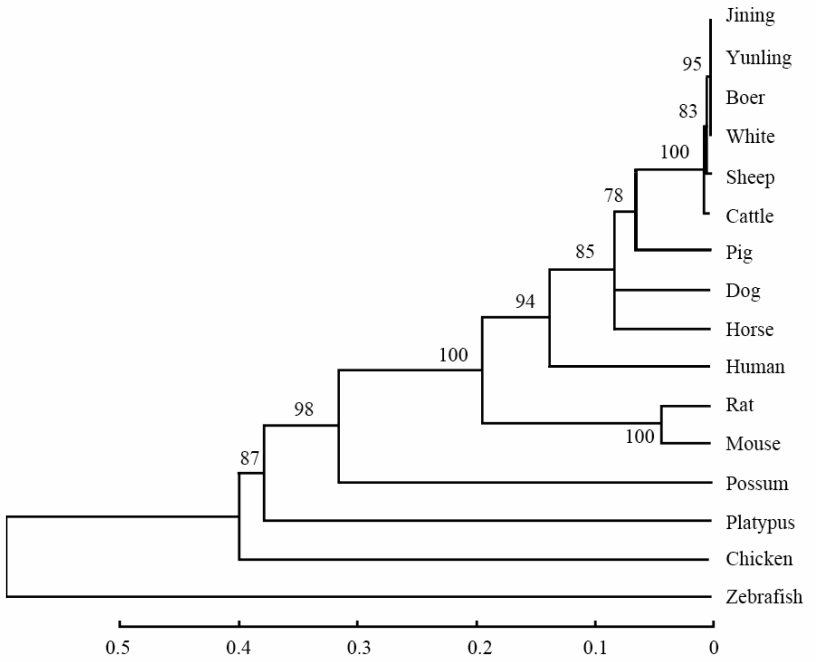

Fig. 2 The phylogenetic tree of BMP15 prepropeptide by MEGA4.0 program using Neighbor-Joining method

The bootstrap consensus inferred from 1 000 replicates. The percentage of replicate trees in which the associated taxa clustered together was shown next to the branches. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and Pairwise deletion. BMP15 prepropeptide (394aa) of White goat was much closer to the other three goat breeds (Yuling, Jining and Boer goats). The cited sequences in GenBank were as following, White goat: FJ429281; Jining goat: ABH08952; Yunling goat: ACF24868; Boer goat: ACF24873; sheep: AAF81688; cattle: AAS99651; pig: AAL58885; horse: XP_001496273; dog: XP_549005; human: AAC99768; mouse: AAC99766; rat: CAB41039; opossum: AAO12760; platypus: XP_001510645; chicken: AAU29611; zebrafish: AAI15086.

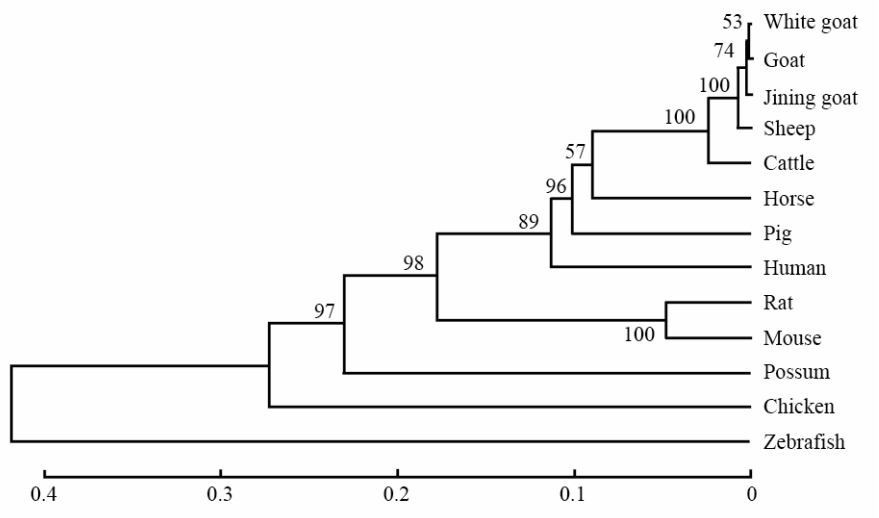

Fig. 3 Phylogenetic tree of GDF9 prepropeptide by program MEGA4.0 using Neighbor-Joining method

All of correction and option was the same with that of BMP15. GDF9 prepropeptide of White goat was clustered together with other two goat breeds (Jining goat and goat described by Wu). The cited sequences in GenBank were as following, White goat: FJ429282; goat described by Wu: AAU09020; Jining goat: ABR10699; sheep: AAC28089; cattle: AAG38106; horse: XP_001504477; pig: AAT48523; human: AAH96230; mouse: AAH52667; rat: AAD16406; opossum: XP_001372019; chicken: NP_996871; zebrafish: AAI08014.

genetic relationships among sequences were assessed by MEGA4.0 program (Tamura et al, 2007) using Neighbor-Joining method**.** BMP15 and GDF9 prepropeptide of White goat, Jining goat, Yunling goat and Boer goat constituted a branch in the phylogenetic tree.

2.2 Analysis of the divergence of BMP15 and GDF9 prepropeptide

Higher identity of BMP15 prepropeptide sequence was presented between White goat and other mammals with percentage of 54.2%—99% while lower one was found between White goat and chicken (45.1%), platypus (40.7%) and fish (28.2%) (Fig. 2). In GDF9, the distances of White goat from other mammals were much closer than that from avian (58.3%) and fish (41.1%) (Fig. 3).

Compared among vertebrates taking the sequence of White goat deposited in the GenBank as template, the deletion mutations in BMP15 prepropeptide increased from mammals to fish (Fig. 4a, Fig. 5) and resulted in shorter BMP15 prepropeptide from mammals with 391—394 residues to chicken with 350 residues, in which deleted amino acids were 3 in human, 13 in opossum, and 45 in chicken. The BMP15 prepropeptide of zebrafish was reported as 394 residues which resulted from deletion of 19 amino acids and compensated by insertion of 18 residues. On the other hand, the length of GDF9 prepropeptide kept in 441—453 except for the length of zebrafish decreased to 418 residues, but both of deletion and insertion occurred more frequently from mammals, opossum, chicken to fish (Fig. 4b).

2.3 The tertiary model of BMP15 and GDF9 in White goat

The identity of mature peptide between BMP15 and

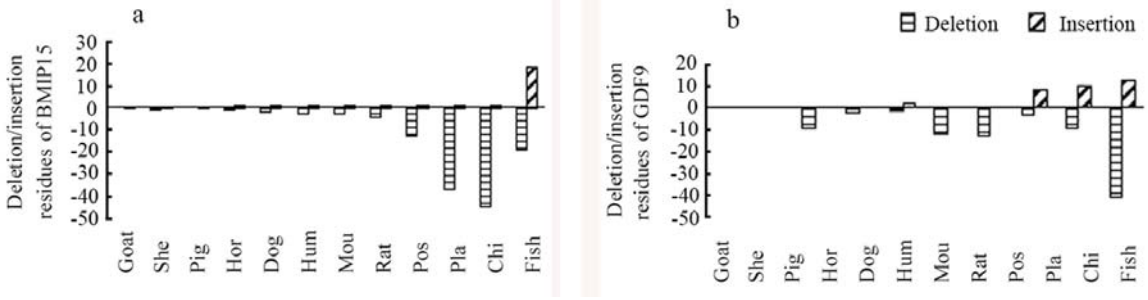

Fig. 4 Profiles of deletion and insertion mutations in BMP15 (a) and GDF9 (b) prepropeptides

The deletion numbers of BMP15 or GDF9 prepropeptide were showed downward column by negative values. Insertion mutation numbers produced in BMP15 or GDF9 prepropeptides standed upward by positive values.

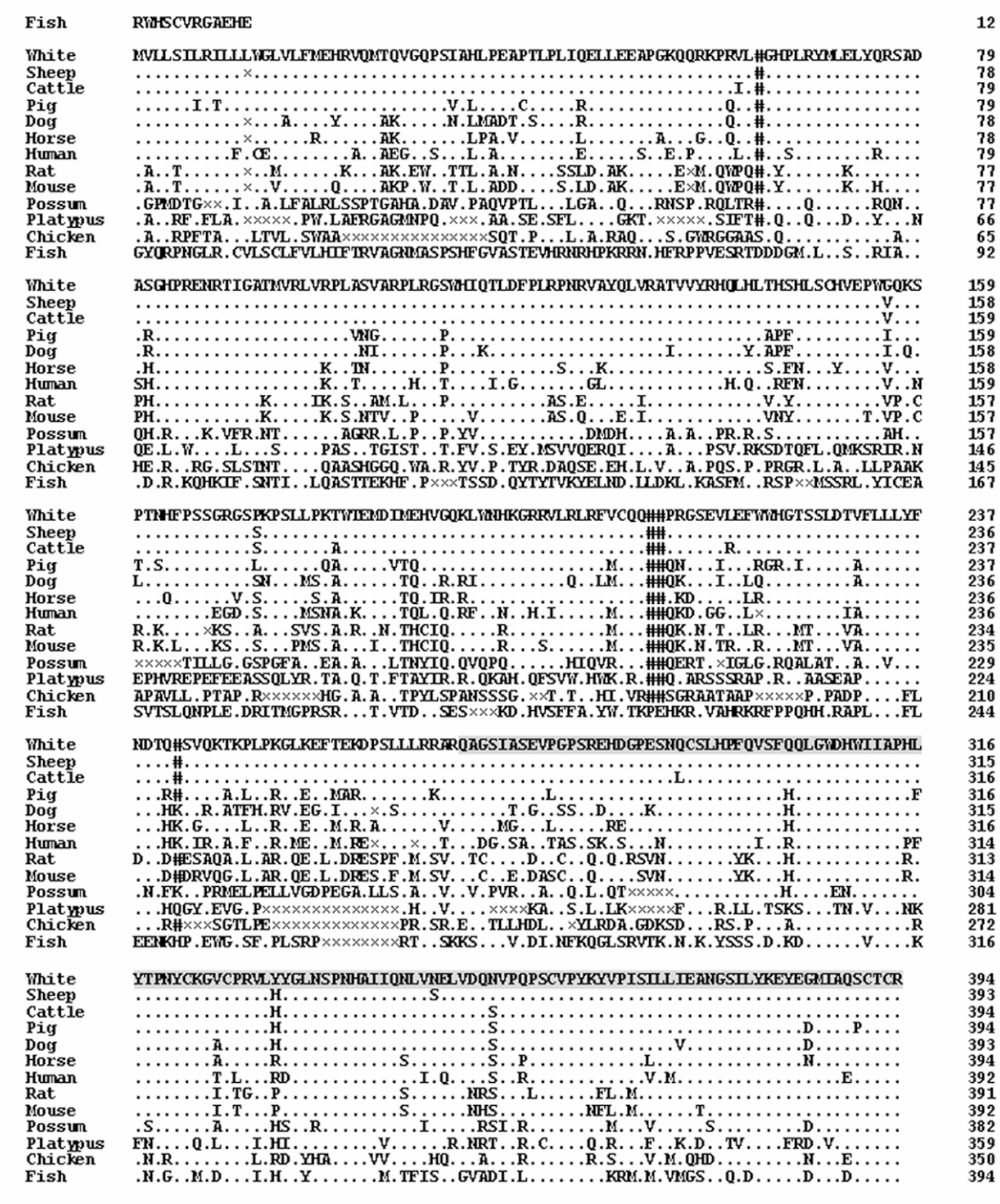

Fig. 5 Compare of BMP15 preproprotein in vertebrates with that of White goat as template

The sign # denoted insertion, × as deletion and the dot (.) showed the same residue as template at the first line. The matured peptide of BMP15 in White goat (125aa) was shaded in grey. All of the sequence of amino acid of BMP15 was the same as Fig. 2.

GDF9 was 58.7%. In the comparison of human BMP7 (hBMP7) with TGFβ2, the three-dimensional structure is very similar even though their sequence identity is only 36% (Griffith et al, 1996). Thus, in the light of crystal structure available for hBMP7 (pdb ID: c1lx5A), the tertiary structures of mature BMP15 started from 22 to 125 and GDF9 from 32 to 135 were built using Phyre program on line (Fig. 6, 7). In large part, the analogy to a curled left hand for TGFβ2 (Daopin et al, 1992) was true in BMP15 and GDF9 monomers as well (Fig. 7a, 7b). The BMP15 or GDF9 cysteine-knots constituted the core of the monomer and consisted of three disulfide bonds: C24–C90, C53—C122 and C57—C124 in BMP15 and C34—C100, C63—C132 and C67—C134 in GDF9 (Fig.

6). Four strands of antiparallel β-sheet, which emanated from the knots, formed two fingerlike projections (Fig. 7). Helix α2 (Fig. 6) located on the opposite end of the knots and lay vertically to the axis of two fingers thereby forming the heel of the left hand. The smaller finger 1 contained β1, α1, β2 and β3, and the larger finger 2 comprised of β4, β5, β6, and β7 (Fig. 6, 7). N-terminus of BMP15 and GDF9 corresponded to the thumb of the hand (not shown). The palm of the hand included the cysteine-knots and 31 residues contained in helix α2 (Fig. 6, 7). The dimer of BMP15 and/or GDF9 was formed by a palm-to-palm orientation of the two monomers with an uncovalent binding (Fig. 7c).

3 Discussion

Bmp15 and *Gdf9* genes are known to be specifically expressed in oocytes and to be essential for female fertility in sheep. Partial defects in *Bmp15* and *Gdf9* are associated with the increased ovulation rate in sheep (Galloway et al, 2000). We have investigated the naturally occurring mutation in *Bmp15* and *Gdf9* genes of White goat in Guizhou province and confirmed that the mutation of $FecX^B$ was present in White goat population (Lin et al, 2007). In addition, a different mutation from sheep was found in exon 2 of *Gdf9* gene which was only detected from prolific White goat (Du et al, 2008). It was confirmed that heterozygous carriers contained one copy of mutation at 791 bp (G/A) in *Gdf9* exon 2 which resulted in the substitution of valine to isoleucine at residue 79 of GDF9 mature peptide (V79I).

To detect other diversities of *Bmp15* and *Gdf9* genes, complete coding regions of exons in *Bmp15* and *Gdf9* were cloned from five White goats of Guizhou province. Sequencing results showed that, like other TGFβ superfamily members, *Bmp15* and *Gdf9* of White goat were translated as prepropeptides composed of a signal peptide, a large proregion and a smaller mature peptide of 125 residues in BMP15 or 135 residues in

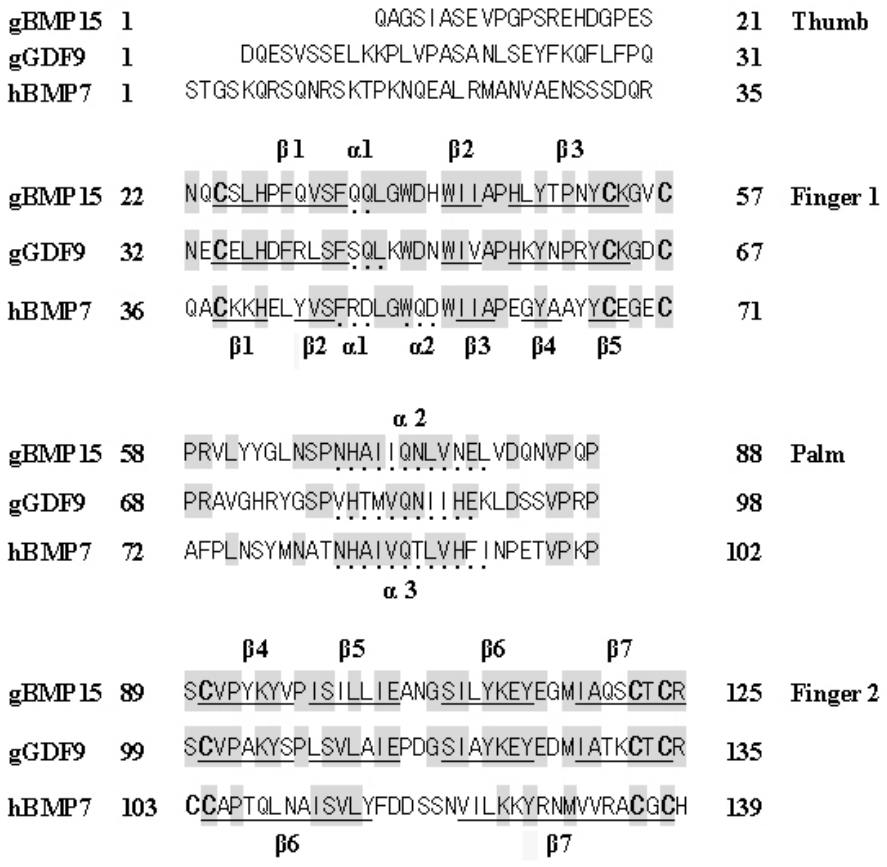

Fig. 6 Structure-based sequence alignment of 6-Cys domains in White goat BMP15 (gBMP15), White goat GDF9 (gGDF9) and human BMP7 (hBMP7, pdb ID: c1lx5A)

Size of **C** (cysteine) in sequences was larger than the others in black font, while residues conserved in the mature peptides were shaded in grey. Sequences in β-sheets (β1-β7) were underlined and helixes (α1-α3) were dotted. The second structures in both gBMP15 and gGDF9 (on the sequence of gBMP15) were similar while divergent from hBMP7 (under the sequence of hBMP7). Position of thumb, fingers and palm were denoted at the right side of the sequences.

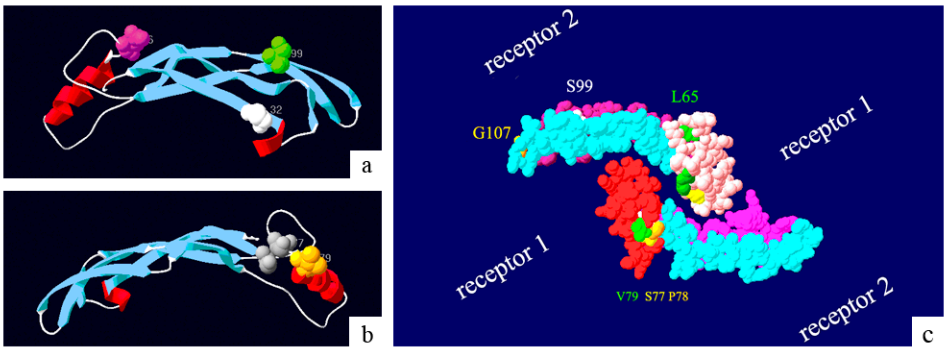

Fig. 7 Homology-based model of BMP15 and GDF9 mature peptides in White goat

Residue 22−125 of BMP15 (FJ429281) and 32−134 of GDF9 (FJ429282) were modeled on the basis of homology to the experimentally determined tertiary structure of human BMP7 (Griffith et al, 1996) with two fingers in blue and helix α2 in red located in the palm. Heterodimer model of BMP15 and GDF9 in White goat was constructed on the basis of homology to TGFβ1 dimer (Greenwald et al, 2003). ***a***: monomer model of BMP15 with substitutions at S32 (white balls) at finger 1, N66 (purple balls) at palm and S99 (green balls) at finger 2 were highlighted. ***b***: monomer model of GDF9 with substitutions at S77 (grey balls) and V79 (orange balls) were distinguished. ***c***: dimer of gBMP15 (up) and gGDF9 (down). Diversity residues of BMP15 were highlighted as: P50 (could not be seen from the outside of the dimer as it located inside of finger 1), L65 (green balls, inside of the palm), H70 (dark green balls, surface of the palm), V85 (yellow balls, near to H70), S99 (white balls), G107 (orange balls). Polymorphism sites of GDF9 were showed as: S77 (yellow balls), P78 (orange balls), V79 (green balls) and T81 (white balls, inside of the palm). The position of receptors was denoted according to McNatty et al (2004).

GDF9. Deduced mature peptides of BMP15 and GDF9 contained cysteine-knots which were conserved in TGFβ superfamily. Apart from the detected polymorphisms in matured BMP15 (S99I) and GDF9 (V79I) (Du et al,2008; Lin et al, 2007), seven and three substitutions were found in mature peptide sequences of BMP15 and GDF9 respectively in White goat population. And another two changes of BMP15 (S32G and N66H) were found from that of Jining and Yunlin goat breeds. The effect of these polymorphisms in BMP15 and GDF9 on their function would be an interesting issue to address in the future and some clues from their structure analysis might be helpful.

The structure of BMP15 or GDF9 peptide has not yet been determined by experiment assay. Based on human BMP7 structure (Griffith et al, 1996), three-dimension structures of BMP15 and GDF9 monomers of White goat were modeled by computer on line (Fig. 7). Each of them could be compared to a left hand with a palm and two fingers. To date, it still remains to be elucidated that these molecules exist as monomers or dimers *in vivo*. Evidences from TGFβ members show that for the proteolytic cleavage of prepropeptides to occur, the prepropeptide must first dimerize (Hogan, 1996). BMP15 and GDF9 lack a cysteine which is conserved in TGFβ superfamily required for the intersubunit disulphide bridge (McPherron et al, 1993). However, *in vitro* studies using cell expression systems indicate that both BMP15 and GDF9 can form non-covalent homodimers and BMP15/GDF9 heterodimers (Liao et al, 2003). Therefore, a heterodimeric BMP15 and GDF9 of White goat was modeled in the light of TGFβ1 dimer (Hinck et al, 1996). GDF9 and BMP15 molecules linked non-covalently by palm contacted each other, and four receptors binding around the dimer (Fig. 7c).

In these seven polymorphism sites of BMP15 in White goat individuals, D17G was on the thumb of the molecule which could not be proposed as the thumb structure needed further experimental data. P50L located inner part of finger1 and L65H was inside of the palm. Both of them might not affect the stability of dimer and/or the affinity of the dimer binding to its receptors. H70Y was at the side part of the palm and it might have little role on the function of BMP15 for it was far away from the connection region of the dimer and its receptors (Fig. 7c). V85A was at the connection region of the dimer which might be unimportant because both of valine and alanine are hydrophobic. G107R was at the tip of finger 2 which was the connection region of dimer with receptor type 2 like BMPR-II. S99I/P99I was between the seam of finger 1 and finger 2 and protruded from the back of the dimer which was important for the dimer binding to its receptor 2 (Fig. 7c). This coincides with previous reports that S99I in sheep ($FecX^B$) affects receptor BMPR-II binding by structural determination of ligand-receptor complexes with BMP2, BMP7, and

activin A, and by site-directed mutagenesis (Greenwald et al, 2003; Thompson et al, 2003). For the difference of BMP15 between White goat in Guizhou province and Jining, Yunlin goat breeds, S32G and N66H were found at the surface of finger1 and palm of BMP15, respectively (Fig. 7a). The ovulation of sheep is potently inhibited when immunized by directed against the peptide fragments contained S32 and N66 residues of BMP15 (McNatty et al, 2007). Thus, like S99, S32 and N66 might participate in the binding of BMP15 with receptors but not the formation of dimer.

For GDF9 of White goat, like D17G in BMP15, V18A was in the thumb of the handed-like molecule, which was also observed in goat *Gdf9* described by Wu et al. (GenBank no. AAU09020). T81A was hidden in the palm of GDF9 (Fig. 7c) which might be unimportant. However, two sites, P78Q and V79I, were adjacent to S77 which was $FecG^H$ mutation (S77F) found from sheep (Fig. 7b). All of these three residues occupied the side part of the GDF9 palm. Previous finding has showed that $FecG^H$ mutation changes an uncharged polar serine residue to a nonpolar phenylalanine in a region of the binding to ALK5 (type I receptor of TGFβ superfamily) (Kirsch et al, 2000). This change at S77 also affects the H80 in helix α2 of the mature GDF9 peptide. In BMP7, this conserved histidine H80 is important in dimmer binding which exhibits hydrogen bonding to three residues of the homodimer of BMP7 (Griffith et al, 1996) and heterodimer of TGFβ3 (Mittl et al, 1996). On the other hand, P78Q changed from nonpolar proline to polar glutamine, and the diversity at residue 79 from valine to isoleucine increased a mehyl group at the side chain of amino acid. Further, site I79 was detected only from the prolific population of White goat (Du et al, 2008). It was reasonable that polymorphism sites, P78Q and V79I, might have an influence to the GDF9 binding to receptor1 (Fig. 7c) such as ALK5.

Deduced amino acids sequences of BMP15 and GDF9 in White goat were further compared with that of other animals. The length polymorphism presented in both of BMP15 and GDF9 prepropeptides. Compared with GDF9, higher frequency of deletion mutations was found in the prepropeptides of BMP15 in opossum, platypus, chicken and fish (Fig. 4). Noticeably, one, five and nine residues deletion was observed in chicken, possum and platypus of BMP15 mature peptides respectively (Fig. 5). It was interested that all of these deletions was located in the thumb of the handed-like model of BMP15 (Fig. 5, 6). Fragments of residue 1－21 at the N-terminal of BMP15 mature region are potent at inhibiting ovulation in sheep by immune neutralization (McNatty et al, 2007). It showed that the high divergence of BMP15 in nonmammalian vertebrates from mammals might be related to the affinity of ligand and receptor or the selection of receptors to bind. Currently, function researches on *Bmp15* and *Gdf9* are limited in mammals and the information is not always consistent with each other. In mice, GDF9 but not BMP15 is essential for normal follicular development (David, 2005). However, in sheep, both GDF9 and BMP15 are essential for normal follicular growth (Juengel et al, 2004). In humans, ovarian insufficiency is associated with *Bmp15* gene mutation (Di et al, 2004), and Hreinsson et al. (2002) obtained an increased number of secondary follicles by adding GDF9 to the ovarian tissue culture medium. Nevertheless, the natural mutation related to the increase of ovulation size was only reported in sheep (Hanrahan et al, 2004) and goat (Du et al, 2008; Lin et al, 2007). Taken together, these findings showed that evolution of *Bmp15* and *Gdf9* gene existed from fish to mammal and displayed difference in the regulation of fertility in vertebrates. The regulation of *Bmp15* and *Gdf9* genes on ovulation rate appeared to be more sensitive on species with low ovulation rate phenotype (such as sheep, goat, human) than those with higher ovulation rate vertebrates (e.g., rat, mouse, dog and pig) especially species of laying eggs, such as chicken and fish. Insertions and deletions within BMP15 and GDF9 peptides might be associated with divergent function on ovulation rate in vertebrates. These findings in the present study gave a valuable explanation for the correlation of mutation in *Bmp15* and *Gdf9* genes with the control on fecundity of goat and supported the notion that these factors had an important role in female fertility of White goat in Guizhou province.

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