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Postnatal Expression of Bone Morphogenetic Proteins and Their Receptors in the Mouse Testis

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Summary

TGF-β superfamily members including bone morphogenetic proteins (BMPs) and their receptors (BMPR-1A, -1B and -2) have been shown to be important for reproductive function in both males and females, while information on the role of BMPs in males is limited. Functional studies on select BMPs and BMP receptors have demonstrated vital roles for these proteins in somatic and germ cell proliferation, steroidogenesis and overall fertility. In order to gain insight into the importance of these genes during postnatal reproductive development in males, our study was undertaken to specify the distribution of BMP and BMPR mRNA in male reproductive and steroidogenic tissues and quantify these genes in the testis using the mouse as our model. We screened testis at two, four, six and eight weeks of age for the expression of ten BMPs and three BMP receptors using RT-qPCR. All three BMP receptor mRNAs – *Bmpr1a*, *Bmpr1b* and *Bmpr2*, and ten BMP mRNAs – *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* were expressed in mouse testis at all stages screened. Testicular expression of genes varied within age groups and at specific developmental stages. Our study establishes an extensive BMP system in mouse reproductive and steroidogenic tissues.

Key words

Male reproduction • Growth factors • Bone morphogenetic proteins • Testis

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Introduction

Bone morphogenetic proteins (BMPs) belong to the decapentaplegic-Vg-related (DVR) family, which forms the largest subgroup of growth factors in the transforming growth factor-β (TGF-β) superfamily (McDonald and Hendrickson 1993). BMP signaling occurs *via* heterodimerization of type I (BMPR-1A, BMPR-1B) and type II (BMPR-2) serine/threonine receptors (Koenig *et al.* 1994, Ebisawa *et al.* 1999), which activate SMAD1, SMAD5 and/or SMAD8 signal transducers (Hoodless *et al.* 1996, Liu *et al.* 1996, Nishimura *et al.* 1998, Aoki *et al.* 2001, Kersten *et al.* 2005). Reproductive functions of BMPs and their putative receptors in males include the modulation of testosterone synthesis (Teixeira *et al*. 1999), germ cell maturation (Zhao *et al.* 1998), sperm quality (Hu *et al.* 2004), integrity of reproductive tissues (Hu *et al*. 2004, Zhao *et al.* 1998) and epithelial secretory function (Settle *et al*. 2001).

In mice, knockouts for *Bmpr1a* (*Alk-3*) and *Bmpr2* are embryonically fatal (Mishina *et al.* 1995, Beppu *et al.* 2000), while *Bmpr1b* (*Alk-6*) deficiency leads to infertility in males and females (Yi *et al.* 2001). In male mice *Bmpr1b* deficiency resulted in compromised fertility attributed to defective development of the seminal vesicles (Yi *et al.* 2001). Seminal vesicles have been shown to express *Bmpr1a* and *Bmpr1b* mRNA in immature mice (Settle *et al.* 2001), however, whether these mutants had altered testicular function has not been reported. On the other hand, partial dysfunction of

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Bmpr1b as seen in the Booroola merino strain of sheep results in significantly fewer primordial follicles (Ruoss *et al.* 2009) yet higher ovulation rate in females and no apparent effects in males (Piper and Bindon 1982, Wilson *et al*. 2001).

Expression of mutant *Bmp4* (Hu *et al.* 2004), *Bmp7* (Zhao *et al.* 2001), *Bmp8a* (Zhao *et al.* 1998) and *Bmp8b* (Zhao *et al.* 1996) resulted in either compromised fertility or infertility in male mice, while *Bmp15* null male mice were reported to have normal fertility (Yan *et al.* 2001). *Bmp2*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmpr1a* have been shown to be expressed in embryonic mouse testis (Dewulf *et al.* 1995, Ross *et al.* 2007). Mouse mRNA expression of *Bmp2* and *Bmp4* was detected in immature testis (Itman and Loveland 2008), *Bmp7*, *Bmp8a* and *Bmp8b* in immature and adult testis (Zhao *et al.* 1998, 2001, Itman and Loveland 2008), *Bmp5* and *Bmp6* in adult testis (Lyons *et al.* 1989, Marker *et al.* 1997), while in adult mouse testis *Bmp15* mRNA and *Bmp3b (Gdf10)* were reported to be absent (Dube *et al.* 1998, Katoh and Katoh 2006).

Findings about the expression of BMP receptor mRNAs in the mouse testis or cells derived thereof have been inconclusive, perhaps due to assay sensitivity. Pellegrini *et al.* (2003), Dewulf *et al.* (1995) and ten Dijke *et al.* (1994) reported not finding *Bmpr1b* mRNA in mouse testis using Northern blotting and *in situ* hybridization, while Gouedard *et al.* (2000) reported *Bmpr1b* expression in mouse testis and testicular cell lines MA-10 cells and SMAT-1 cells derived from Leydig cell tumors and immature Sertoli cells respectively using polymerase chain reaction (PCR). In Sertoli cells of immature mice Puglisi *et al.* (2004) detected *Bmpr1a* using the Ribonuclease Protection Assay and *Bmpr2* expression by Northern blot, while on the contrary Pellegrini *et al.* (2003) did not readily detect the same mRNAs in Sertoli cells. In spermatogonia of immature mice Pellegrini *et al.* (2003) identified BMPR-IA protein and *Bmpr1a* and *Bmpr2* transcripts using Northern blot, and while Puglisi *et al.* (2004) also found *Bmpr1a* expressed in spermatogonia, they did not detect *Bmpr2*.

Pellegrini *et al.* (2003) demonstrated that BMP-4 increased proliferation of spermatogonia while its transcripts were expressed by Sertoli cells but not germ cells, indicating BMP-4 had a paracrine function in germ cell signaling. *Bmp4* expression decreased progressively from postnatal day 4 to 17 (Pellegrini *et al.* 2003). In mice before 3 weeks of age *Bmp8a* and *Bmp8b* mRNAs have been detected in spermatogonia and spermatocytes and at 3 weeks *Bmp8a* and *Bmp8b* mRNAs were localized in stage 6-8 round spermatids, demonstrating a development shift (Zhao and Hogan 1996, Zhao *et al.* 1996). *Bmp8b* homozygous mutants had greater germ cell degeneration than *Bmp8a* mutants, additionally homozygous $Bmp8b^{tm1b1h}$ mutant mice exhibited progressive depletion of germ cells due to increased germ cell apoptosis and were rendered infertile (Zhao *et al.* 1996, 1998), indicating a function in germ cell survival and maintenance.

As significant roles for BMPs and their receptors are emerging in male reproductive function, the aim of this study was to investigate the distribution of these genes in adult reproductive and steroidogenic tissues using RT-PCR and quantify the relative gene expression of BMP receptors – *Bmpr1a*, *Bmpr1b* and *Bmpr2*, and BMPs – *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* in the mouse testis at two, four, six and eight weeks of age using reverse transcription quantitative PCR (RT-qPCR) analysis of mRNA. By examining the expression profiles of BMP/BMP receptor mRNAs at four specific time points we provide greater clarity to what functions individual genes may serve at specific developmental stages, as well as providing comparisons between different genes to enhance understanding of relative abundance and how that may affect normal physiology of the testis.

Methods

Animals

The University of New England Animal Ethics Committee authorized the use of animals needed to conduct this research, which was in accordance with the National Health and Medical Research Council: Australian code of practice for the care and use of animals for scientific purposes 7th Edition 2004. Male Swiss Quackenbush mice (Physiology Animal House, University of New England, NSW, Australia) were housed in sanitary conditions in a light controlled room (12:12) at a constant temperature of 21 °C and had access to a constant supply of standard rodent chow and water.

At the age of 2, 4, 6 and 8 weeks mice were sacrificed by asphyxiation with CO_2 and approximately 100 mg testis placed in RNALater (Ambion, Austin, TX) and incubated at 4 °C overnight and then stored at −80 °C until RNA extraction. Epididymis, vas deferens, seminal vesicles, coagulating gland, prostate, adrenal gland and

visceral adipose tissue of mature mice were also collected for RNA extraction and processed as for testis. Seminal vesicle and testis weights of mice sacrificed weekly from 2 to 8 weeks of age were measured to have a biological indicator of reproductive development. At 2 weeks mice were considered immature, at 4 weeks early pubertal, at 6 weeks late pubertal and at 8 weeks mature. N=5.

RNA extraction, RT PCR and qPCR

RNA was extracted using TRI Reagent (Sigma-Aldrich Co, St. Louis, MO) according to manufactures instructions. RNA integrity was checked on a 1 % RNA agarose gel (CSBC 2011) and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE).

Reverse transcription was performed by annealing 2 µg of total RNA with 20 ng Oligo(dT)$_{15}$Primer (Fisher Biotec, Subiaco, WA) at 70 °C for 5 min and then placed on ice. This was followed by extension carried out at 40 °C for 60 min using 10 mM dNTP Mix (Fisher Biotec), 1 x RT Buffer, 400 U M-MLV Reverse Transcriptase (Promega, Alexandria, NSW) and 1 U rRNasin(R)RNase Inhibitor (Promega).

Gene specific primers (Table 1) (GeneWorks Pty Ltd, Hindmarsh, SA) were designed using NCBI Primer-BLAST (Ye *et al.* 2009). A routine PCR using 80 ng cDNA was carried out to confirm the specificity of primers and their expected product lengths were checked using 2 % agarose gel electrophoresis to confirm the presence and amplification of the gene of interest. The PCR products were gel eluted and sequenced at Ramachiotti Centre for Genomics, UNSW, Sydney for confirmation.

Quantitative PCR (qPCR) reactions were set up in duplicate using 16 ng cDNA, in Fast EvaGreen qPCR Mix (Biotium, Hayward, CA) using a CAS-1200 automated PCR Setup robot (Corbett Robotics, Eight Mile Plains, QLD) and qPCR performed using a Rotor-Gene R6 6000 Real-time Analyzer (Corbett Life Science, Concord, NSW).

Data analysis

Data analysis of qPCR Ct values was performed by the $2^{-\Delta\Delta Ct}$ method using *β-actin* as the reference gene. Statistical analysis was performed using a general linear model procedure in SAS statistical software (SAS Institute Inc., Cary, NC, USA). The data were evaluated using one-way ANOVA followed by the Student-Newman Keuls *post hoc* test. Values were considered to be significantly different at $P<0.05$ and presented as mean ± standard error (SE).

Table 1. The forward and reverse primer sequences used for qPCR.

Gene	NCBI reference #	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon length (bp)
β-actin	NM_007393.3	CGTCGACAACGGCTCCGGCATG	TGGGCCTCGTCACCCACATAG	150
Bmpr1a	NM_009758.4	AGGTCAAAGCTGTTCGGAGA	CTGTACACGGCCCTTTGAAT	178
Bmpr1b	NM_007560.3	TCAATGTCGTGACACTCCCATTCCT	TGCTGTACCGAGGTCGGGCT	245
Bmpr2	NM_007561.3	CACCCCCTGACACAACACCACTC	GACCCCGTCCAATCAGCTCCAG	243
Bmp2	NM_007553.2	ACCCCCAGCAAGGACGTCGT	AAGAAGCGCCGGGCCGTTTT	197
Bmp3	NM_173404.3	AGCAGTGGGTCGAACCTCGGA	ACCCCCACCGCTCGCACTAT	199
Bmp3b	NM_145741.2	GGCAACACCGTCCGAAGCTTCC	AGGAGGCGGCAGGATGCGTT	199
Bmp4	NM_007554.2	GACTACTGGACACCAGACTAGTCC	CTTCCCGGTCTCAGGTATCA	180
Bmp5	NM_007555.3	ATCAGGACCCCTCCAGGATGCC	TGATCCAGTCCTGCCATCCCAGATC	120
Bmp6	NM_007556.2	GCAGAGTCGCAACCGGTCCA	GGTGCAATGATCCAGTCCTGCC	153
Bmp7	NM_007557.2	CAAGCAGCGCAGCCAGAATCG	CAATGATCCAGTCCTGCCAGCCAA	161
Bmp8a	NM_007558.2	TTGGCTGGCTGGACTGGGTCA	GCTGTCATAGTACAGCACAGAGGTG	209
Bmp8b	NM_007559.4	GGCTGGCTGGACTCTGTCATTGC	AGCTCAGTAGGCACACAGCACAC	176
Bmp15	NM_009757.4	GCCGTCGGCCAACACAGTAAG	AGAAGGTAAGTGCTTGGTCCGGCA	202

Results

Testis and seminal vesicle weight

As a measure of reproductive development we measured testis and seminal vesicle (SV) weight in 2- to 8-week-old mice as shown in Figure 1 panel A and B. Testis and SV weight increased significantly ($P<0.05$) on a weekly basis from 2 weeks to 7 weeks at which time point weight had plateaued and was no longer significantly different at 8 weeks.

Comparative analysis of BMP receptor gene expression

The relative changes in the BMP receptor gene expression at the 4 time points examined are shown in Figure 2. *Bmpr2* and *Bmpr1a* were the most abundant genes at all ages tested, while *Bmpr1b* was expressed in considerably lower amounts. Overall the pattern of change with age was similar in all three receptors with a marked decline in expression at 4 weeks followed by a slight rise at 8 weeks. At 2 weeks *Bmpr1a* expression was relatively high at 60.3±12 %, while at 4 weeks *Bmpr1a* expression had been reduced 23 fold ($P=0.0003$) and continued to decline although not significantly to six weeks. By 8 weeks *Bmpr1a* significantly increased by 2 fold ($P=0.016$) over the expression at 6 weeks (Fig. 2). At 2 weeks of age *Bmpr1b* was expressed at significantly lower amounts (1.4±0.4 %) than either *Bmpr1a* (60.3±12 %) or *Bmpr2* (295±75 %). The drop in expression level at 4 and 6 weeks from 2 weeks of age was also much higher (182 and 255 fold) than the decline seen with *Bmpr1a* but similar to that observed with *Bmpr2*. The expression of *Bmpr2* was the highest of the 3 receptors at 2 weeks (296±75 %), and followed a similar expression pattern with age as did *Bmpr1b*, with a large drop at 4 weeks and remaining low at 6 weeks followed by a slight but significant rise ($P<0.0001$) at 8 weeks.

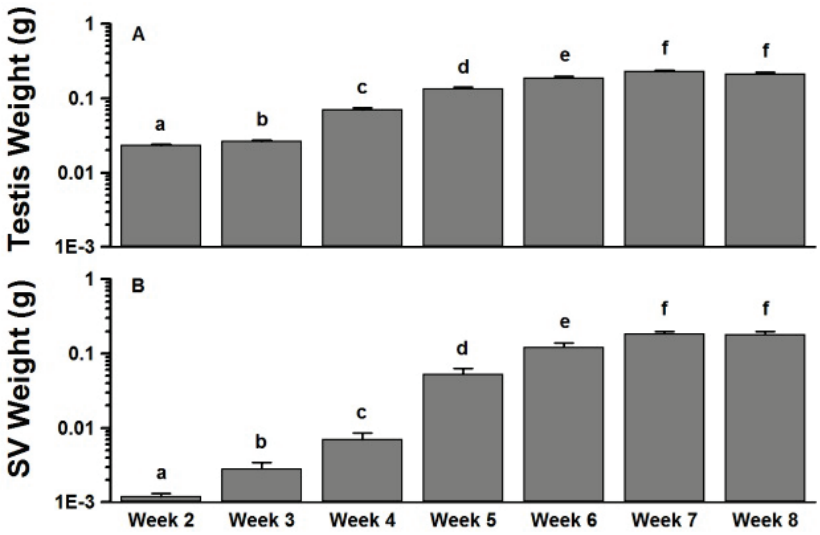

Fig. 1. (Panel A) Testis and **(Panel B)** seminal vesicle weights (g) for mice aged 2, 3, 4, 5, 6, 7 and 8 weeks. Results expressed as Mean ± SE (n=5). Different superscripts denote different levels of significance ($P<0.05$).

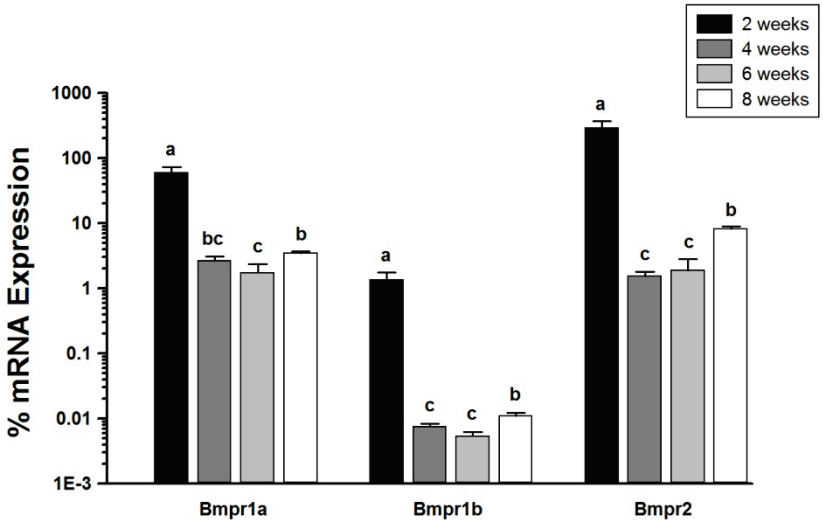

Fig. 2. *Bmpr1a*, *Bmpr1b* and *Bmpr2* expression in testis for mice aged 2, 4, 6 and 8 weeks. Results were calculated as a percentage of the housekeeping gene *β-actin* and presented as Mean ± SE (n=5 in duplicate). Different superscripts denote different levels of significance ($P<0.05$) of individual genes throughout postnatal development.

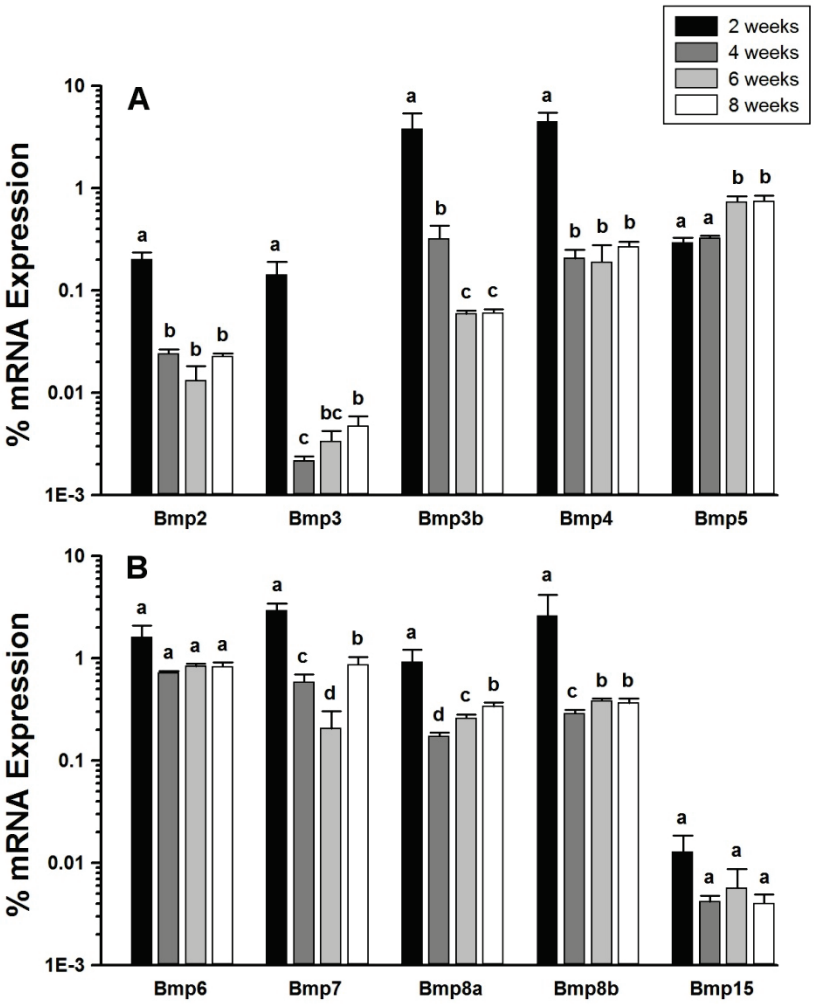

Fig. 3. (Panel A) *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4* and *Bmp5,* **(Panel B)** *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* mRNA levels in testis for mice aged 2, 4, 6 and 8 weeks. Results were calculated as a percentage of the housekeeping gene *β-actin* and presented as Mean ± SE (n=5 in duplicate). Different superscripts denote different levels of significance (P<0.05) of individual genes throughout postnatal development.

Comparative analysis of BMP gene expression in mouse testis during postnatal development

The relative changes in BMP gene expression at the 4 time points examined are shown in Figure 3 panel A and B. With the exception of *Bmp5* all genes reduced their expression from 2 to 4 weeks. *Bmp15* was the least expressed gene at 2 weeks. Genes that significantly increased their expression from 4 to 8 weeks were *Bmp3*, *Bmp5*, *Bmp7*, *Bmp8a* and *Bmp8b*. Genes that did not change significantly from 4 to 8 weeks were *Bmp2*, *Bmp4*, *Bmp6* and *Bmp15*, while *Bmp3b* was the only gene to significantly decrease its expression by 8 weeks. At 4 weeks *Bmp3b* was reduced by 12 fold (P≤0.04), *Bmp4* by 22 fold (P≤0.0007), *Bmp7* by 5 fold (P=0.0008), *Bmp8a* by 5 fold (P≤0.02) and Bmp8b by 9 fold (P=0.0463), while *Bmp2* was reduced 8 fold to less than 0.1 % (P≤0.0001), and *Bmp3* was reduced 65 fold to less than 0.01 % were P=0.009. *Bmp3* expression declined the most radically of all the BMP mRNAs becoming the least expressed gene. By 6 weeks *Bmp5*, *Bmp8a* and *Bmp8b* significantly increased their expression were P≤0.0001, P=0.003 and P≤0.004 respectively, and *Bmp3b* and *Bmp7* expression reduced significantly were P≤0.03 and P=0.02 respectively from 4 weeks. Expression of *Bmp2*, *Bmp3*, *Bmp4*, *Bmp6* and *Bmp15* did not alter and *Bmp3* remained the least expressed gene. At 8 weeks expression of *Bmp7* and *Bmp8a* increased by 4 fold (P≤0.005) and 1 fold (P=0.04) respectively. Expression of *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp8b* and *Bmp15* did not change, however *Bmp15* was the least expressed gene at 8 weeks, being expressed at only 0.004 % of the housekeeping gene.

Bmp receptor and bmp screening in male reproductive and steroidogenic tissues

Having detected mRNA expression of *Bmpr1a*, *Bmpr1b*, *Bmpr2*, *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* in mouse testis, we examined the expression of these genes in the epididymis, vas deferens, seminal vesicles, coagulating gland, prostate, adrenal gland and visceral adipose tissue of adult mice (Fig. 4). All genes were detected in all

tissues screened with the exception of *Bmp3* and *Bmp5* in vas deferens of which expression was too low to detect.

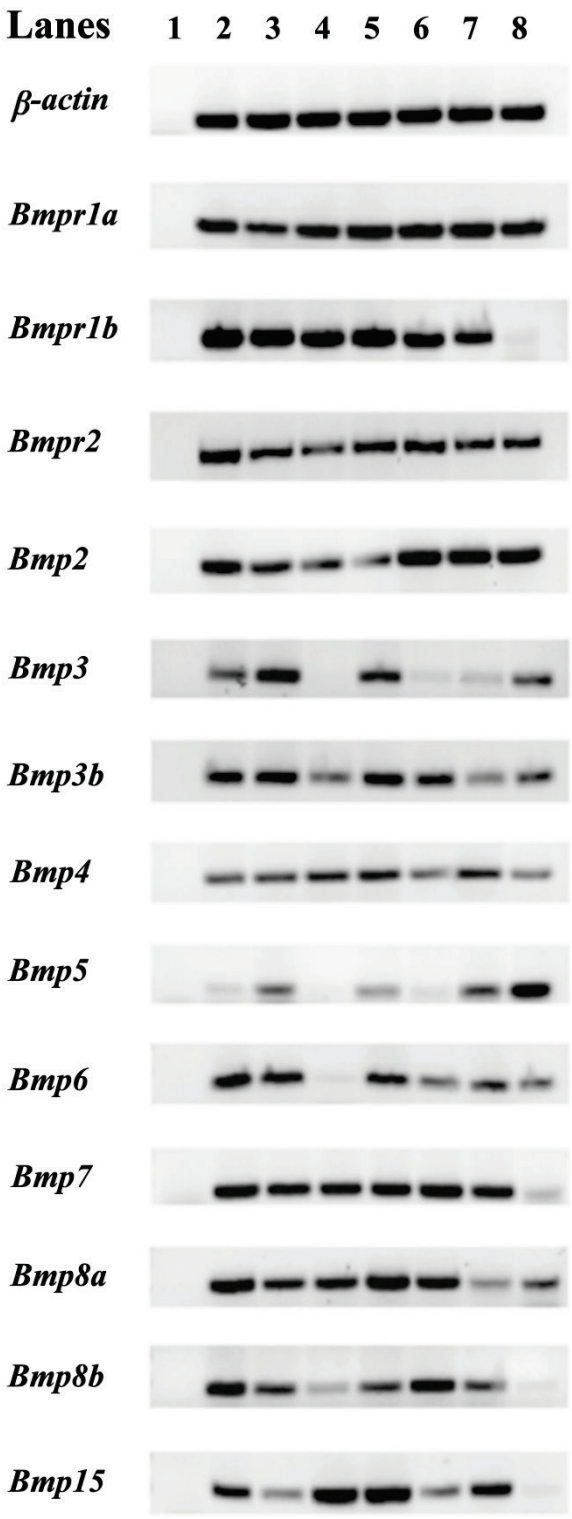

Fig. 4. BMP receptor (*Bmpr1a*, *Bmpr1b*, *Bmpr2*) and BMP (*Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b*, *Bmp15*) mRNA expression in adult male reproductive and steroidogenic tissues. Lane: 1 = Negative control, 2 = Epididymis, 3 = Vas deferens, 4 = Seminal vesicle, 5 = Prostate, 6 = Coagulating gland, 7 = Adrenal gland, 8 = Adipose tissue

Discussion

We have demonstrated that there is widespread expression of BMPs and BMP receptors in male reproductive and steroidogenic tissues, which included finding testicular expression of *Bmpr1b*, *Bmpr2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6* and *Bmp15* at all developmental stages. It is likely that *Bmpr1b* mRNA had been reported absent in mouse testis by numerous researchers (ten Dijke *et al.* 1994, Dewulf *et al.* 1995, Pellegrini *et al.* 2003) with one exception (Gouedard *et al.* 2000), because its expression is low both relative to the housekeeping gene and to *Bmpr1a* and *Bmpr2* as determined in this study using highly sensitive RT-qPCR. This is supported in part by Belville *et al.* (2005) who found that *Bmpr1b* was expressed at a significantly lower level than *Bmpr1a* in SMAT-1 cells. We found *Bmpr1a* and *Bmpr2* mRNA in relatively high levels at all developmental stages, however BMP receptor expression was significantly higher in immature animals than in adult animals. Similarly, Puglisi *et al*. (2004) reported that *Bmpr1a* and *Bmpr2* expression in the testis decreased significantly with age, however they were unable to detect *Bmpr2* mRNA by 30 days of age (~4 weeks) or older. Given that *Bmpr2* was one of the most abundant genes tested in our study the reason for this discrepancy is unclear.

Bmp2, *Bmp4* and *Bmp7* mRNAs were present at all ages screened but had their highest expression in immature testis. Itman and Loveland (2008) reported *Bmp2*, *Bmp4* and *Bmp7* mRNA in 5-day-old mouse testis, and demonstrated that BMP-2 and BMP-4 treatment stimulated signaling of SMAD 1, 5 and 8 in Sertoli cell and spermatogonial cultures. Furthermore, BMP-2 had a role in the proliferation of spermatogonia in concert with FSH but not alone, and BMP-7 had a role in the proliferation of Sertoli cells in the presence of FSH (Puglisi *et al.* 2004), while BMP-4 increased proliferation of spermatogonia independent of gonadotrophins (Pellegrini *et al.* 2003). BMP-4 has been shown to be important for sperm quality with heterozygous mutation of *Bmp4* resulting in diminished sperm counts and motility (Hu *et al.* 2004). This demonstrates a great diversity of actions by these closely related BMPs and demonstrates how they can have vastly different functions under different conditions including the presence or absence of gonadotrophin stimulation.

Gene expression of *Bmp7* was highest in immature testis, lowest during early puberty and then

significantly increased during late puberty and more so in adult testis. This suggests upon translation BMP-7 may have a role in the initiation of germ cell proliferation and maintenance of late stage spermatogenesis. Our findings are in agreement with Zhao *et al.* (2001) who found abundant expression of *Bmp7* in spermatogonia of immature mice, while in adult mice *Bmp7* mRNA was found mainly in spermatids and suggested to have a supporting role for maintenance of spermatogenesis. Similar to Zhao *et al.* (1998) we found *Bmp8a* was significantly less expressed than *Bmp8b* in pubertal testis but not in adult testis. This supports the suggestions that BMP-8a is important in late stage spermatogenesis with mRNA being identified in round spermatids (Zhao *et al.* 1998), while BMP-8b was shown to be necessary for both initiation and maintenance of spermatogenesis being expressed in spermatogonia and spermatids of pubertal mice and at high levels in round spermatids of adult mice (Zhao and Hogan 1996).

In humans *BMP3B* (*GDF10*) mRNA has been detected in the testis (Hino *et al.* 1996) and by using *in-silico* expression analysis Katoh and Katoh (2006) identified *BMP3B* in human testis but reported the gene absent in mouse testis. We found low-level expression of *Bmp3* and significantly higher expression of *Bmp3b* in all age groups. *Bmp3b* expression was highest in immature mice, reduced significantly during early puberty and late puberty and remained unchanged in adult testis. Based on its expression patterns we hypothesis *Bmp3b* may have a role in germ cell proliferation while factors released from spermatids and/or spermatozoa present at 6 weeks of age (Seok *et al.* 2004) may down regulate its expression.

Bmp5 mRNA has been detected in adult mouse spermatogonia (Marker *et al.* 1997) and of interest *Bmp5* was the only gene we tested that had a lower expression at 2 weeks than all other age groups and increased significantly between 4 and 6 weeks which suggests a possible role in late stage spermatogenesis. *Bmp6* has been reported to be expressed in mature mouse testis (Lyons *et al.* 1989), and in our study *Bmp6* expression stayed relatively high compared with other BMP genes indicating a likely role in testicular functioning. We also detected *Bmp15* mRNA in mouse testis, which was previously not detected using Northern blotting (Dube *et al.* 1998). Compared to other BMP genes, *Bmp15* was expressed at a low level throughout development.

In humans *BMPR2* and *BMP15* mRNAs have been detected in the testis (Rosenzweig *et al.* 1995, Aaltonen *et al.* 1999), and *BMPR1B* expression was shown to be elevated in testicular cancer (Fustino *et al.* 2011). As the expression of BMPs is widespread in the testis, over-expression of receptors is likely to result in heightened sensitivity to low expressed ligands resulting in altered cell responses. Altered expression of BMPs is also characteristic of prostate cancers (Harris *et al.* 1994, Barnes *et al.* 1995, Buijs *et al.* 2007). Given that altered BMPR-1B signaling by putative BMPs is implicated in reproductive cancers including testicular cancer (Miyazaki *et al.* 2004, Bokobza *et al.* 2009, Fustino *et al.* 2011, Neumann *et al.* 2011), which is one of the most common cancers affecting young Caucasian men in developed countries (Rosen *et al.* 2011), a mouse model profiling the postnatal expression of BMP and BMP receptor genes in the testis may be useful for understanding normal physiology and provide a comparison for altered physiology in cancerous tissues.

This study establishes an extensive BMP system in mouse testis throughout postnatal development at the mRNA level. Further examination of BMP and BMP receptor mRNAs in Leydig cells, Sertoli cells and germ cells will need to be carried out to elucidate what their individual roles are likely to be. Additionally, studies of protein expression will be needed to confirm the translation and abundance of the BMP and BMP receptor proteins not already detected. Given the predominance of BMP and BMP receptor genes reported in our study, and based on available research findings, it is likely that many of these genes have vital roles in germ and somatic cell proliferation, cellular homeostasis and steroid production, aspects we are currently investigating.

Conflict of Interest

There is no conflict of interest.

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References

AALTONEN J, LAITINEN MP, VUOJOLAINEN K, JAATINEN R, HORELLI-KUITUNEN N, SEPPA L, LOUHIO H, TUURI T, SJOBERG J, BUTZOW R, HOVATTA O, DALE L, RITVOS O: Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. *J Clin Endocrinol Metab* **84**: 2744-2750, 1999.

AOKI H, FUJII M, IMAMURA T, YAGI K, TAKEHARA K, KATO M, MIYAZONO K: Synergistic effects of different bone morphogenetic protein type I receptors on alkaline phosphatase induction. *J Cell Sci* **114**: 1483-1489, 2001.

BARNES J, ANTHONY CT, WALL N, STEINER MS: Bone morphogenetic protein-6 expression in normal and malignant prostate. *World J Urol* **13**: 337-343, 1995.

BELVILLE C, JAMIN SP, PICARD JY, JOSSO N, DI CLEMENTE N: Role of type I receptors for anti-Mullerian hormone in the SMAT-1 Sertoli cell line. *Oncogene* **24**: 4984-4992, 2005.

BEPPU H, KAWABATA M, HAMAMOTO T, CHYTIL A, MINOWA O, NODA T, MIYAZONO K: BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev Biol* **221**: 249-258, 2000.

BOKOBZA SM, YE L, KYNASTON E, MANSEL RE, JIANG WG: Reduced expression of BMPR-IB correlates with poor prognosis and increased proliferation of breast cancer cells. *Cancer Genomics Proteomics* **6**: 101-108, 2009.

BUIJS JT, RENTSCH CA, VAN DER HORST G, VAN OVERVELD PGM, WETTERWALD A, SCHWANINGER R, HENRIQUEZ NV, TEN DIJKE P, BOROVECKI F, MARKWALDER R, THALMANN GN, PAPAPOULOS SE, PELGER RCM, VUKICEVIC S, CECCHINI MG, LOWIK CWGM, VAN DER PLUIJM G: BMP7, a putative regulator of epithelial homeostasis in the human prostate, is a potent inhibitor of prostate cancer bone metastasis in vivo. *Am J Pathol* **171**: 1047-1057, 2007.

CSBC: Protocols/Gene Expression/RNA extraction and quality control:/RNA quality control/Protocol – ethidium bromide Medium to large-scale RNA extractions. Cambridge Systems Biology Centre CSBC – FlyChip. Accessed 2011 at http://www.flychip.org.uk/ Updated 6/9/2011.

DEWULF N, VERSCHUEREN K, LONNOY O, MOREN A, GRIMSBY S, VANDE SPIEGLE K, MIYAZONO K, HUYLEBROECK D, TEN DIJKE P: Distinct spatial and temporal expression patterns of two type I receptors for bone morphogenetic proteins during mouse embryogenesis. *Endocrinology* **136**: 2652-2663, 1995.

DUBE JL, WANG P, ELVIN J, LYONS KM, CELESTE AJ, MATZUK MM: The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol Endocrinol* **12**: 1809-1817, 1998.

EBISAWA T, TADA K, KITAJIMA I, TOJO K, SAMPATH TK, KAWABATA M, MIYAZONO K, IMAMURA T: Characterization of bone morphogenetic protein-6 signaling pathways in osteoblast differentiation. *J Cell Sci* **112**: 3519-3527, 1999.

FUSTINO N, RAKHEJA D, ATEEK CS, NEUMANN JC, AMATRUDA JF: Bone morphogenetic protein signaling activity distinguishes histological subsets of pediatric germ cell tumors. *Int J Androl* **34**: 218-233, 2011.

GOUEDARD L, CHEN YG, THEVENET L, RACINE C, BORIE S, LAMARRE I, JOSSO N, MASSAGUE J, DI CLEMENTE N: Engagement of bone morphogenetic protein type IB receptor and Smad1 signaling by anti-Mullerian hormone and its type II receptor. *J Biol Chem* **275**: 27973-27978, 2000.

HARRIS SE, HARRIS MA, MAHY P, WOZNEY J, FENG JQ, MUNDY GR: Expression of bone morphogenetic protein messenger RNAs by normal rat and human prostate and prostate cancer cells. *Prostate* **24**: 204-211, 1994.

HINO J, TAKAO M, TAKESHITA N, KONNO Y, NISHIZAWA T, HATSUO H, KANGAWA K: cDNA cloning and genomic structure of human bone morphogenetic protein-3B BMP-3b. *Biochem Biophys Res Commun* **223**: 304-310, 1996.

HOODLESS PA, HAERRY T, ABDOLLAH S, STAPLETON M, O'CONNOR MB, ATTISANO L, WRANA JL: MADR1, a MAD-related protein that functions in BMP2 signaling pathways. *Cell* **85**: 489-500, 1996.

HU J, CHEN YX, WANG D, QI X, LI TG, HAO J, MISHINA Y, GARBERS DL, ZHAO GQ: Developmental expression and function of Bmp4 in spermatogenesis and in maintaining epididymal integrity. *Dev Biol* **276**: 158-171, 2004.

ITMAN C, LOVELAND KL: SMAD expression in the testis: an insight into BMP regulation of spermatogenesis. *Dev Dyn* **237**: 97-111, 2008.

KATOH Y, KATOH M: Comparative integromics on BMP/GDF family. *Int J Mol Med* **17**: 951-955, 2006.

KERSTEN C, SIVERTSEN EA, HYSTAD ME, FORFANG L, SMELAND EB, MYKLEBUST JH: BMP-6 inhibits growth of mature human B cells; induction of Smad phosphorylation and upregulation of Id1. *BMC Immunol* **6**: 9, 2005.

KOENIG BB, COOK JS, WOLSING DH, TING J, TIESMAN JP, CORREA PE, OLSON CA, PECQUET AL, VENTURA F, GRANT RA, CHEN GX, WRANA JL, MASSAGUE J, ROSENBAUM JS: Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. *Mol Cell Biol* **14**: 5961-5974, 1994.

LIU FL, HATA A, BAKER JC, DOODY J, CARCAMO J, HARLAND RM, MASSAGUE J: A human Mad protein acting as a BMP-regulated transcriptional activator. *Nature* **381**: 620-623, 1996.

LYONS K, GRAYCAR JL, LEE A, HASHMI S, LINDQUIST PB, CHEN EY, HOGAN BLM, DERYNCK R: Vgr-1, a mammalian gene related to Xenopus Vg-1, is a member of the transforming growth factor β gene superfamily. *Proc Natl Acad Sci U S A* **86**: 4554-4558, 1989.

MARKER PC, SEUNG K, BLAND AE, RUSSELL LB, KINGSLEY DM: Spectrum of Bmp5 mutations from germline mutagenesis experiments in mice. *Genetics* **145**: 435-443, 1997.

MCDONALD NQ, HENDRICKSON WA: A structural superfamily of growth factors containing a cysteine knot motif. *Cell* **73**: 421-424, 1993.

MISHINA Y, SUZUKI A, UENO N, BEHRINGER RR: Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Gene Dev* **9**: 3027-3037, 1995.

MIYAZAKI H, WATABE TTK, MIYAZONO K: BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. *Oncogene* **23**: 9326-9335, 2004.

NEUMANN JC, CHANDLER GL, DAMOULIS VA, FUSTINO NJ, LILLARD K, LOOIJENGA L, MARGRAF L, RAKHEJA D, AMATRUDA JF: Mutation in the type IB bone morphogenetic protein receptor alk6b impairs germ-cell differentiation and causes germ-cell tumors in zebrafish. *Proc Natl Acad Sci U S A* **108**: 13153-13158, 2011.

NISHIMURA R, KATO Y, CHEN D, HARRIS SE, MUNDY GR, YONEDA T: Smad5 and DPC4 are key molecules in mediating BMP-2-induced osteoblastic differentiation of the pluripotent mesenchymal precursor cell line C2C12. *J Biol Chem* **273**: 1872-1879, 1998.

PELLEGRINI M, GRIMALDI P, ROSSI P, GEREMIA R, DOLCI S: Developmental expression of BMP4/ALK3/SMAD5 signaling pathway in the mouse testis: a potential role of BMP4 in spermatogonia differentiation. *J Cell Sci* **116**: 3363-3372, 2003.

PIPER L, BINDON BM: Genetic segregation for fecundity in Booroola Merino sheep. In: *Proceedings of the World Congress on Sheep and Beef Cattle Breeding.* BARTON RA, SMITH WC (eds), Dunmore Press, Palmerston North, 1982, pp 395-400.

PUGLISI R, MONTANARI M, CHIARELLA P, STEFANINI M, BOITANI C: Regulatory role of BMP2 and BMP7 in spermatogonia and Sertoli cell proliferation in the immature mouse. *Eur J Endocrinol* **151**: 511-520, 2004.

ROSEN A, JAYRAM G, DRAZER M, EGGENER SE: Global trends in testicular cancer incidence and mortality. *Eur Urol* **60**: 374-379, 2011.

ROSENZWEIG BL, IMAMURA T, OKADOME T, COX GN, YAMASHITA H, TEN DIJKE P, HELDIN CH, MIYAZONO K: Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc Natl Acad Sci U S A* **92**: 7632-7636, 1995.

ROSS A, MUNGER S, CAPEL B: Bmp7 regulates germ cell proliferation in mouse fetal gonads. *Sex Dev* **1**: 127-137, 2007.

RUOSS C, TADROS A, O'SHEA T, MCFARLANE JR, ALMAHBOBI G: Ovarian follicle development in Booroola sheep exhibiting impaired bone morphogenetic protein signaling pathway. *Reproduction* **138**: 689-696, 2009.

SEOK OS, AHN JM, MAYO KE, CHO BN: Developmental changes in inhibin-α gene expression in the mouse testis. *Mol Cells* **17**: 67-72, 2004.

SETTLE S, MARKER P, GURLEY K, SINHA A, THACKER A, WANG Y, HIGGINS K, CUNHA G, KINGSLEY DM: The BMP family member Gdf7 is required for seminal vesicle growth, branching morphogenesis, and cytodifferentiation. *Dev Biol* **234**: 138-150, 2001.

TEIXEIRA J, FYNN-THOMPSON E, PAYNE AH, DONAHOE PK: Mullerian-inhibiting substance regulates androgen synthesis at the transcriptional level. *Endocrinology* **140**: 4732-4738, 1999.

TEN DIJKE P, YAMASHITA H, ICHIJO H, FRANZEN P, LAIHO M, MIYAZONO K, HELDIN CH: Characterization of type I receptors for transforming growth factor-beta and activin. *Science* **264**: 101-104, 1994.

WILSON T, WU XY, JUENGEL JL, ROSS IK, LUMSDEN JM, LORD EA, DODDS KG, WALLING GA, MCEWAN JC, O'CONNELL AR, MCNATTY KP, MONTGOMERY GW: Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol Reprod* **64**: 1225-1235, 2001.

YAN C, WANG P, DEMAYO J, DEMAYO FJ, ELVIN JA, CARINO C, PRASAD SV, SKINNER SS, DUNBAR BS, DUBE JL, CELESTE AJ, MATZUK MM: Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* **15**: 854-866, 2001.

YE J, COULOURIS G, ZARETSKAYA I, CUTCUTACHE I, ROZEN S, MADDEN TL: Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* **13**: 134, 2012.

YI SY, LAPOIT PS, YOON BS, CHEN JYC, LU JKH, LYONS KM: The type I BMP receptor BmprIB is essential for female reproductive function. *Proc Natl Acad Sci U S A* **98**: 7994-7999, 2001.

ZHAO GQ, HOGAN BLM: Evidence that mouse Bmp8a Op2 and Bmp8b are duplicated genes that play a role in spermatogenesis and placental development. *Mech Dev* **57**: 159-168, 1996.

ZHAO GQ, DENG K, LABOSKY PA, LIAW L, HOGAN BLM: The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. *Gene Dev* **10**: 1657-1669, 1996.

ZHAO GQ, LIAW L, HOGAN LM: Bone morphogenetic protein 8A plays a role in the maintenance of spermatogenesis and the integrity of the epididymis. *Development* **125**: 1103-1112, 1998.

ZHAO GQ, CHEN YX, LIU XM, XU Z, QI X: Mutation in Bmp7 exacerbates the phenotype of Bmp8a mutants in spermatogenesis and epididymis. *Dev Biol* **240**: 212-222, 2001.