SHORT COMMUNICATION

Chromosomal Localization, Embryonic Expression, and Imprinting Tests for Bmp7 on Distal Mouse Chromosome 2

PAUL C. MARKER,* JENNIFER A. KING,* NEAL G. COPELAND,† NANCY A. JENKINS,† and DAVID M. KINGSLY*†

*Department of Developmental Biology, Beckman Center, B300, Stanford University School of Medicine, Stanford, California 94305-5427, and †Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick, Cancer Research and Development Center, Frederick, Maryland 21702-1201

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Murine Bmp7 has been assigned to distal Chromosome 2 by interspecific backcross mapping. The map location suggests close linkage to classical mouse mutations and places Bmp7 within a chromosome region thought to contain one or more unidentified imprinted genes. A direct test suggests that Bmp7 is not imprinted. An examination of embryonic RNA expression patterns shows that Bmp7 is expressed in a variety of skeletal and nonskeletal tissues. Both embryonic expression patterns and the human chromosomal sublocalization inferred from its mouse location make Bmp7 a candidate for the gene affected in some patients with Holt–Oram syndrome.

In seminal work, Urist discovered that demineralized bone extracts could induce bone formation when implanted ectopically in vivo (25). This initial observation led to the eventual purification and cloning of the first four bone morphogenetic proteins (BMPs; 28). Three of these initially isolated BMPs represented the first members of a new family within the TGFβ superfamily of secreted growth factors. Subsequent work has identified seven additional members of this family (4, 16, 18, 24). The degree of sequence similarity among the TGFβ-related BMPs has led to the division of these BMPs into four subfamilies: the BMP2/BMP4 subfamily, the BMP5/Vgr1(BMP6)/OP1(BMP7)/OP2 subfamily, the BMP3 subfamily, and the GDF5/GDF6/GDF7 subfamily.

While the ectopic bone formation assay demonstrates that at least some BMPs are sufficient to trigger bone formation in vivo, the normal function of BMPs appears to extend beyond a simple role as triggers of bone development. This is suggested by the expression of BMPs in both skeletal and nonskeletal tissues (9, 10, 13, 14, 26, 27) and confirmed by null mutations in Bmp5 that cause a syndrome of both skeletal and nonskeletal abnormalities (10, 11). Because Bmp5 mutations suggest that it is an important regulator of skeletal pattern and plays a role in the development or maintenance of nonskeletal tissues, we became interested in determining whether other members of the BMP5/Vgr1(BMP6)/OP1(BMP7)/OP2 subfamily might have a similar role.

As a first step in this process, we sought to define the chromosomal localization of Bmp7 to uncover any potential correspondence between known mutations and this gene. Although this gene has been referred to as both Op1 and BMP7 in the literature, we will use the term Bmp7 to conform to the nomenclature already used in human mapping studies (7).

A Bmp7 probe was used to follow the segregation of the gene in N2 progeny from an interspecific backcross previously examined for the segregation of over 1300 loci (5, 6). The map position of Bmp7 was determined by comparing its segregation pattern to the patterns for previously mapped loci. Bmp7 was positioned to minimize multiple crossovers across the length of its chromosome (Fig. 1). Bmp7 maps to distal Chromosome 2, 6.8 cM distal of Ada and Sup1, 0.8 cM proximal of Pck1.

Four known mutations have been mapped to the distal region of Chromosome 2: flecking (fc), lethal spotting (ls), ragged (Ra), and wasted (wst) (12). Bmp7 is not the product of the ls locus, as it has recently been shown to encode endothelin-3 (2). Bmp7 is also not a candidate for the genes affected by Ra or wst: interspecific crosses that segregated these mutations placed both distal to Gnas (1), a gene we found to be distal of Bmp7. Based on map position, Bmp7 is a candidate for the gene affected by fc. The fc phenotype is a head spot and occasional belly spot. No information is available for the embryological abnormalities that underlie the fc phenotype. The possible relationship between this mutation and Bmp7 has not been further examined.

In addition to these mutations, distal Chromosome 2 exhibits an imprinting effect when reciprocal chromosome translocations are used to produce progeny with either two maternal or two paternal copies of the chro-
of its map position, Bmp7 is a candidate for the imprinted gene inferred from translocation studies; consequently, we examined Bmp7 for imprinted expression (Fig. 2). Our assay shows that both the maternal and the paternal Bmp7 alleles are expressed in kidney. This result suggests that Bmp7 is not imprinted; however, it does not exclude the possibility that Bmp7 may be imprinted in a tissue-specific or developmental stage-specific manner.

The human BMP7 gene has previously been localized to Chromosome 20 by somatic cell hybrid mapping (7). The authors noted that the localization of Bmp7 to Chromosome 20 made it a potential candidate for the gene affected in some patients with Holt–Oram syndrome, a genetically heterogeneous human genetic disorder that causes heart defects and skeletal abnormalities of the arms and shoulder girdle (for review see 8). Because of the relatively low resolution of somatic cell hybrid mapping, it was not previously possible to ascertain whether Bmp7 mapped to a subregion of Chromosome 20 known to have breakpoints associated with Holt–Oram syndrome. Our positioning of Bmp7 between Ada (localized to 20q12–q13.11) and Pck1 (localized to 20q13.2–q13.31) suggests that human Bmp7 is likely to be on Chromosome 20q13.1–q13.3. Interestingly, one breakpoint of a pericentric inversion associated with a sporadic case of Holt–Oram syndrome is at 20q13.2 (29). Thus, the likely human chromosomal sublocalization of Bmp7 suggests that it maps to a Holt–Oram-associated breakpoint.

![FIG. 1. Position of Bmp7 on mouse Chromosome 2. A probe from the 5' end of the Bmp7 cDNA was obtained from mouse genomic DNA by PCR using AmpliTaq (Cetus) and primers corresponding to bases 12–28 and 422–404 of the Bmp7 cDNA (17). The probe recognized 4.0- and 2.0-kb TaqI restriction fragments in C57BL/6J and a 1.2-kb restriction fragment in Mus spretus DNA. Segregation of this restriction fragment length polymorphism was followed on a 159-animal subset of the 205 N2 progeny generated in the (C57BL/6J × M. spretus)F1 × C57BL/6J backcross conducted at NCI-Frederick. Columns represent chromosome types inherited from the (C57BL/6J × M. spretus)F1 parent and observed in backcross progeny. White boxes represent a M. spretus allele, while black boxes represent a C57BL/6J allele. The observed number of each chromosome type appears beneath the column. Although Bmp7 was typed on 159 N2 backcross progeny, only progeny typed on all depicted loci are shown. The positions of other loci in the M. spretus backcross have been reported previously: Ada, Svp1, Pck1 (22), and Gnas (5). A partial Chromosome 2 linkage map with loci segregated in the interspecific cross is shown (bottom right). The genetic distances between adjacent loci are to the left of the map, while the human map positions of these loci are listed to the far right. Human chromosomal locations were obtained from GDB, a database of human linkage information maintained by the William H. Welch Medical Library (Johns Hopkins University, Baltimore, MD). A partial consensus map of distal Chromosome 2 is also shown (bottom left, from Ref. 23). The black bar (far left) indicates a region defined by reciprocal translocations that contains one or more imprinted genes (19).]

![FIG. 2. Lack of imprinting for Bmp7. Imprinting was assayed with 3-week-old mice from a (mixed inbred × Mus musculus castaneus)F1 × TKDU mating. Progeny that inherited the M. m. castaneus allele of Bmp7 from either male or female F1 parents were selected. RNA was isolated from kidney, the major site of adult Bmp7 expression (17), using RNAzol (Teltest). Reverse transcription was performed on 1 mg of RNA with Superscript (Gibco) and a primer corresponding to bases 1813–1794 of the Bmp7 cDNA (17). A portion of the CDNA was then amplified by PCR using AmpliTaq and primers corresponding to bases 831–849 and 1463–1444 (17). These primers were chosen to span intron–exon boundaries to ensure that trace DNA in RNA samples would not interfere with the assay. Amplification products were gel purified and sequenced directly using Sequenase (USB) and a primer corresponding to bases 1463–1444 (17). ddATP lanes from a sequencing ladder are shown. The first two lanes are parental strains TKDU and castaneus. The third and fourth lanes are from animals heterozygous for the TKDU and castaneus Bmp7 alleles. The first heterozygote (het 1) has a maternally contributed castaneus allele, while the second heterozygote (het 2) has a paternally contributed castaneus allele. Note that both alleles are expressed in the heterozygotes, showing that Bmp7 is not imprinted. Lanes 5–7 are controls with 100:1, 1:100, and 1:1 ratios of TKDU:cas­taneus input RNA. These controls indicate that there is no substantial allele bias during amplification; consequently, our assay can detect imprinted gene expression.]

mosome region (3, 19, 21). Progeny receiving a maternal duplication/paternal deficiency have a long, flat body with an arched back, are virtually inactive, and die shortly after birth. In contrast, progeny receiving a paternal duplication/maternal deficiency exhibit the opposite phenotype. These data indicate that one or more genes on distal Chromosome 2 are imprinted; however, none of the genes previously mapped to this region is known to have imprinted expression.
FIG. 3. Embryonic RNA expression of Bmp7. The Bmp7 mapping probe (see Fig. 1) was cloned into pCR II (Invitrogen) in both orientations. Plasmids were linearized using HindIII (NEB), and riboprobes were synthesized using T7 polymerase as previously described (15). In situ hybridization to sections from Day 11.5, 13.5, and 15.5 embryos was performed as previously described (10). In all cases, adjacent sections were hybridized with a sense probe to control for background staining. Compare G to H for a typical result. (A, B) Bright- and dark-field pictures of a transverse section through a Day 13.5 embryo. Bmp7 is expressed (white grains in B) in the epidermis (ep), the epithelial layer of the esophagus (es), the subepithelial mesenchyme of the lung (lu), the heart (he), the chondrifying ribs (ri), and the developing bones of the forelimb (fl). (C–E) Day 15.5 hindlimb hybridized with Bmp7 probe (D) or Bmp5 probe (C, E). Skeletal expression of Bmp7 correlates with hypertrophying chondrocytes in the tarsals (tar), metatarsals (met), and phalange (ph). Bmp7 is still expressed in
To test further the possible relationship between BMP7 and the Holt–Oram syndrome, we examined the distribution of Bmp7 transcripts at various anatomical sites disrupted by Holt–Oram mutations. Bmp7 expression was observed in all of the structures that are altered in Holt–Oram patients, including the heart, proximal and distal forelimb, clavicle, and scapula (Fig. 3 and data not shown). In addition, expression was seen in a variety of skeletal and nonskeletal structures unaffected in Holt–Oram patients (Fig. 3 and data not shown). Expression at sites not affected by the Holt–Oram syndrome does not preclude the possibility that some Holt–Oram mutations affect Bmp7. This is clearly illustrated by the closely related gene Bmp5 that is expressed in a distinct subset of skeletal and nonskeletal tissues (Fig. 3 and Ref. 10). Null mutations in the mouse Bmp5 gene cause obvious abnormalities at some but not all sites of Bmp5 expression (10). The chromosome location of Bmp7 in mice and humans (7, Fig. 1), its RNA and protein expression pattern (17, 26, Fig. 3), and the ability of Bmp7 protein to induce the formation of cartilage and bone (20) all suggest that the human BMP7 locus should be examined further as a candidate for the gene affected in a subset of Holt–Oram patients.

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the epidermis but is now restricted to the basal layer. In contrast, the closely related gene Bmp5 (E) is expressed in the perichondrium and periosteum of the tarsals, metatarsals, and phalange. (F–H) Frontal section through the cervical and upper-thoracic region of a Day 13.5 embryo: bright field (F); dark field, antisense probe (G); dark field, sense control probe (H). Bmp7 is expressed in the scapulae (sc), intervertebral discs (id), vertebrae, neural tube (nt), and ribs. Other sites of expression include the clavicle, CNS, eye, kidney, Meckel’s cartilage, nasal pits, pancreas, pelvis, stomach, submandibular gland, and tooth buds (data not shown). Another group has previously examined the distribution of human BMP7/OP1 protein using antibodies on human embryos (26). They reported protein expression in the limb bones, around intervertebral disks, in the kidneys and adrenal glands, in the lungs, in the pancreas, and in the skin.