BRIEF REPORT — POLYMORPHISM REPORT

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A single-nucleotide polymorphism in the human bone morphogenetic protein-4 (*BMP 4*) gene

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Abstract A polymorphic site has been found in the human bone morphogenetic protein-4 (*BMP4*) gene. To our knowledge, it is the first defined polymorphism in this gene, being present with similar frequencies in Caucasian, Hispanic, and African populations.

Key words *BMP4* · Chromosome 14 · Polymorphism · Osteoporosis · Arthritis

Introduction

Bone morphogenetic protein (*BMP4*) is a member of the *BMP* family, which belongs to the transforming growth factor- β (*TGF- β*) superfamily (Wozney et al. 1988). *BMP4* can induce de novo cartilage and bone formation, and appears to be essential for skeletal development during mammalian embryogenesis (Reddi 1998). *BMP4* is one of the best evolutionary conserved growth factors and is necessary to induce mesoderm formation (Winnier et al. 1995). The structure of the gene and the boundaries of the intronic sequences are known (van den Wijngaard et al. 1996). We report here the first known polymorphic site in this gene and its frequency in three ethnic groups.

Primers for the polymerase chain reaction (PCR)

The primers used for PCR were: *BMP 4*-F: 5'-CCTAACTGTGCCTAG-3' and *BMP 4*-R: 5'-CATAACCTCATAAATGTTTATACGG-3'.

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Polymorphism and allele frequency

HphI polymorphism. *HphI* digestion produced a 197-bp fragment in the A147 allele that lacks the *HphI* site, while the digestion detected 110-bp and 87-bp fragments in the V147 allele having the recognition site (Fig. 1).

Allele frequencies. The estimated A147/V147 allele frequencies in 136 chromosomes from unrelated individuals from different ethnic groups are summarized in Table 1. The polymorphism information content (PIC) was 0.365.

Chromosome localization. The human *BMP4* gene has been assigned to chromosome 14q22–23 (Tabas et al. 1993; van den Wijngaard et al. 1995).

Mendelian inheritance. Mendelian inheritance was confirmed in four three-generation families.

Other comments

To detect the *HphI* restriction fragment length polymorphism, PCR was carried out in a total volume of 25 μ l, containing 200 ng of genomic DNA, 10 mM Tris HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 0.01% of gelatin, 200 μ M dNTPs per, 25 pmol of each primer, and 0.25 units of *Taq* polymerase. Cycle conditions were 94°C for 12 min, then 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, with a final extension step of 7 min in a gene Amp PCR9600 System (Perkin Elmer Cetus, Norwalk, CT, USA). The PCR products (197 bp) were digested with *HphI* and subjected to electrophoresis on a 2% MS-8 (Hispanagar Burgos, Spain), agarose gel. The polymorphic *HphI* site was localized to nucleotide position 538 (T $\rightarrow$ C), resulting in an amino acid change of Val $\rightarrow$ Ala (V147A) in the polypeptide. The polymorphism in the *BMP4* gene may be of interest in the study of human bone and joint diseases (e.g., osteoporosis and arthritis). This single-nucleotide polymorphism (SNP) should also be useful in establishing an SNP genetic map (Wang et al. 1998).

Fig. 1 HphI restriction fragment polymorphism (RFLP). Lanes 1 and 6 are homozygous for V/V. Lanes 2 and 3 are heterozygous for A/V. Lanes 4 and 5 are homozygous for A/A. Lane M, AmpliSize Molecular Ruler (50–2000bp, Bio Rad, Richmond, CA, USA)

Table 1 Polymorphism at amino acid position 147 of the human bone morphogenetic protein (*BMP4*) gene, and gene frequencies in different ethnic groups

Ethnic group	<i>n</i>	Genotypes			Gene frequencies	
		V/V	A/V	A/A	147V	147A
Caucasian	30	10	16	4	0.6	0.4
Hispanic	19	9	8	2	0.68	0.32
African	19	8	9	2	0.65	0.35

V = Valine, A = Alanine

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References

- Reddi AH (1998) Role of morphogenetic proteins in skeletal tissue engineering and regeneration. *Nat Biotechnol* 16: 247–252
- Tabas JA, Hahn GV, Cohen RB, Seaunez HN, Modi WS, Wozney JM, Zasloff M, Kaplan FS (1993) Chromosomal assignment of the human gene for bone morphogenetic protein 4. *Clin Orthop Rel Res* 293: 310–316
- Van den Wijngaard A, Olde Weghuis D, Boersma CJC, van Zoelen EJJ, Geurts van Kessel A, Olijve W (1995) Fine mapping of the human bone morphogenetic protein-4 gene (*BMP4*) to chromosome 14q22–q23 by in situ hybridization. *Genomics* 27: 559–560
- Van den Wijngaard A, van Kraay M, van Zoelen EJJ, Olijve W, Boersma CJC (1996) Genomic organization of the human bone morphogenetic protein-4 gene: Molecular basis for multiple transcripts. *Biochem Biophys Res Commun* 219: 789–794
- Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES (1998) Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 280: 1077–1082
- Winnier G, Blessing M, Labosky PA, Hogan BL (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Gene Dev* 9: 2105–2116
- Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA (1988) Novel regulators of bone formation: Molecular clones and activities. *Science* 242: 1528–1534