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Mutations in *CDMP1* cause autosomal dominant brachydactyly type C

We are interested in identifying genes that are responsible for the 'fine tuning' of skeletal structure. Brachydactylies—disorders in which individual bones, or groups of bones, in the hands and feet can be altered in size or shape—may occur when this 'fine tuning' has gone awry[1]. Consequently, genetic studies in families with hereditary brachydactyly could identify genes important to this process. Here we report that autosomal dominant brachydactyly type C (Bd-C) is caused by mutation in the morphogen *CDMP1* (ref. 2), also known as *GDF5* (ref. 3), a TGF-β superfamily member. Several mutations we have found are predicted to result in functional haploinsufficiency. The murine autosomal recessive phenotype brachypodism *(bp)* has previously been shown to be caused by homozygosity for functional null alleles in *Cdmp1*(ref. 3); a heterozygous phenotype in *bp* mice was not observed[4,5]. Our results indicate that in humans, dominant phenotypes may result from heterozygous mutations in the TGF-β superfamily. Further, they suggest that normal variation in the formation of analogous skeletal elements (for instance, middle phalanges) result from site-specific and species-specific differences in threshold requirements for growth factors.

The clinical description of the family in which we initially mapped the Bd-C locus has been published[6]. Twelve individuals across five generations were affected; shortening of the second, third and fifth middle phalanges was the principal finding (Fig. 1). We mapped the Bd-C locus (lod 6.2 at $\theta=0$ using *D20S470*) to an interval previously shown to contain *CDMP1*. PCR amplification and sequencing of the two *CDMP1* exons in this family, led to the identification of a heterozygous C→T transition at nucleotide 901 of the mRNA coding sequence, converting the codon specifying arginine at amino acid residue 301 to a stop codon (Arg301Stop). Additional *CDMP1* mutations were then found in five other, unrelated persons with Bd-C (Fig. 2).

In addition to brachypodism, *CDMP1* mutations also cause two autosomal recessive human disorders, the Hunter-Thompson (H-T)[7] and Grebe (GS) acromesomelic dysplasias[8]. However, in contrast to these disorders, which affect both the middle and distal segments of the upper and lower extremities, the phenotype in our six families is dominant, principally involving the distal segment. That functional haploinsufficiency of *CDMP1* is the likely mechanism of mutational effect in several families might appear to contradict earlier work. Three different mutations causing frameshifts and/or premature stops have been identified in *bp*[3] (Fig. 2), and a complex tandem insertion leading to a frameshift has been identified in a single family with H-T (Fig. 2; ref. 7). In both *bp* and H-T, the mutations were predicted to cause loss of functional CDMP-1 activity; consequently, the absence of a heterozygote phenotype suggested that functional haploinsufficiency would be silent. Our results argue against this interpretation, as two of the Bd-C frameshift mutations occur close to the 5′ end of the cDNA and are likely to cause functional haploinsufficiency similar to the *bp* mutations (Fig. 2). Furthermore, literature regarding *bp* and H-T does not clearly exclude the possibility of a heterozygous phenotype in either of the disorders. Although detailed comparisons between *bp* homozygote and heterozygote littermates have been published[4,5], similar comparisons between heterozygote and wild-type mice have not been reported; intriguingly, hindlimb dissection in *bp* heterozygotes did reveal anomalous tendon insertions in three of four hindlimbs studied, raising the possibility of a subtle heterozygous effect[5]. Neither detailed clinical descriptions of the hands, nor upper extremity radiographs of H-T heterozygotes have been published.

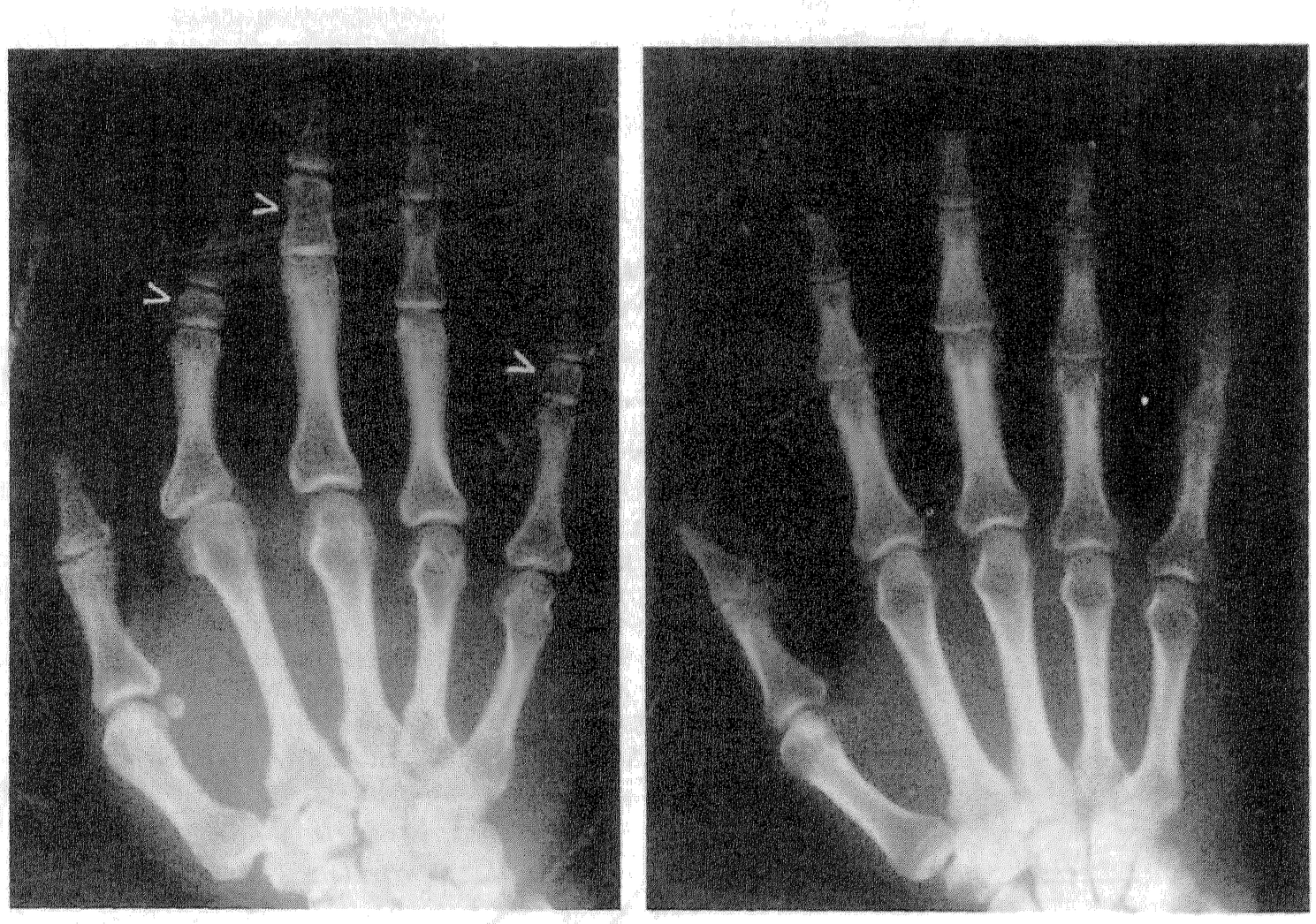

Fig. 1 Right-hand radiographs from an affected individual (left) with brachydactyly type C due to the Arg301Stop mutation and from an unaffected control (right). Note the small second, third and fifth middle phalanges in the affected individual (arrowheads).

Both Bd-C family members with the Arg438Cys mutation also had absent middle phalanges in their second through fifth toes, suggesting that this mutation may have an antimorphic effect—in contrast to the hypomorphic effect postulated for the frameshift and nonsense mutations[9,10]. Whether phenotypic variation in Bd-C is due to different mechanisms of *CDMP1* mutational effect, or stochastic or multifactorial variation, must await detailed clinical characterization of families with known mutations and cell biological studies of the mutational effects. Interfamilial variation may also result from locus heterogeneity, as a second Bd-C locus has been assigned to chromosome 12q (ref. 11). Support for an antimorphic effect for certain *CDMP1* mutations comes from Thomas *et al.*, who show that expression *in vitro* of the *CDMP1* mutation (Cys400Tyr) which causes GS (Fig. 2) has a dominant negative effect upon the secretion of wild-type CDMP-1 and other TGF-β family members[8].

Gradient effects of TGF-β family members have been previously identified as important during pattern formation in

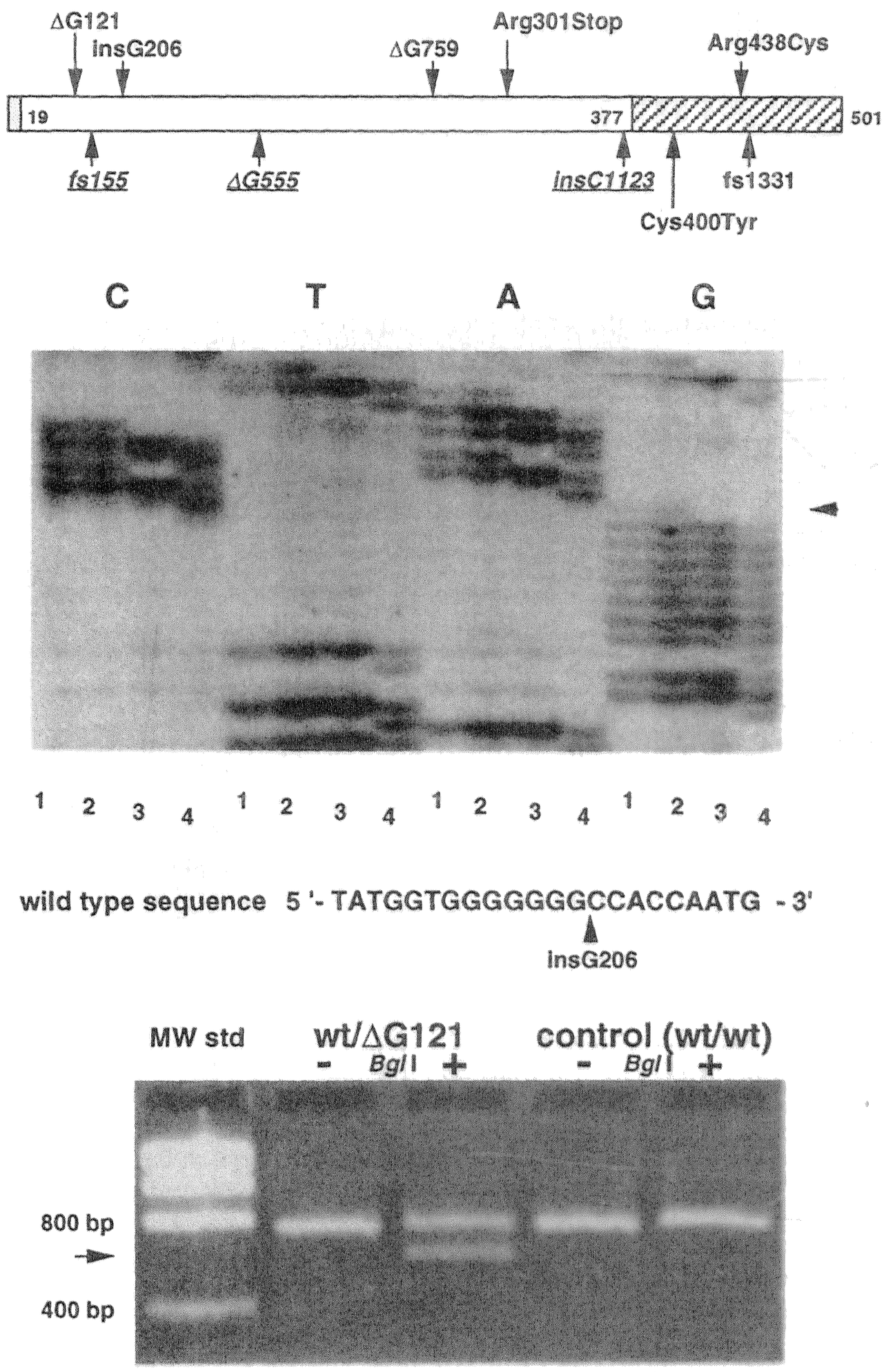

Fig. 2 Schematic of the CDMP-1 protein with sites of the known human and murine mutations. The protein has a 19-amino-acid leader sequence, a 358-amino-acid 'pro domain' and a 124-amino-acid 'active domain'. The active-domain cleavage site begins at amino-acid residue 377. The five human Bd-C mutations are shown above the drawing. The human H-T and GS mutations are shown below. The three mouse *bp* mutations (underlined) are also shown below. Δ, simple deletion; ins, simple insertion; fs, frameshift associated with a complex deletion and/or insertion. Amino-acid residue numbering begins at the methionine translation initiation site; nucleotide numbering also begins with this codon. Below the schematic, a sequencing gel demonstrates the two most 5′ Bd-C mutations. Wild-type sequence is in lane 3. Double sequence in lanes 1, 2 and 4 result from a single nucleotide insertion (lanes 1 and 2) or deletion (lane 4). Two unrelated patients (lanes 1 and 2), both from the United Kingdom, have a single G insertion at nucleotide 206 (arrowhead), causing a frameshift and a premature termination codon 25 amino-acid residues downstream. Double sequence observed in lane 4 results from a single G deletion at nucleotide 121 (not shown), causing a frameshift and premature termination codon 46 residues downstream. This deletion creates a novel *Bgl* I restriction site, which is shown in an ethidium bromide–stained agarose gel containing exon 1 amplimers from the patient heterozygous for the DG138 mutation (wt/DG138) and an unaffected control (wt/wt). The wild-type (741-bp) allele is uncut by *Bgl*I, whereas the mutant allele is cut into fragments of 572 bp (arrow) and 169 bp (not shown). None of the five Bd-C mutations were observed in 100 control chromosomes. All mutations were detected after direct sequencing of PCR-amplified genomic DNA. Exon 1 was PCR amplified using primers FPL-1 (5′–CGCTGCTGCCGCTGTTCTCTTTGGTGT-CATTCAGC–3′; –48 to –14 nt 5′ of the ATG translation start site) and 1.1 (5′–GCCCCTCCATTCAT-GCAG–3′; +63 to +46 nt 3′ of the donor splice site). Exon 2 was amplified using primers 2.1C (5′–GAATGGGGCAGAGGTGAAAG–3′; –124 to –105 nt 5′ of the acceptor splice site) and FPL-2 (5′–AGCTTCCTGACCCCTCTGTGATTCCA–3′; +84 to +60 nt 3′ of the termination codon). Internal primers were used for sequencing.

Drosophila[12]. Our results suggest that subtle morphologic differences between individual skeletal elements of hands and feet, and between humans and mice, may be due in part to differences in threshold effects for CDMP-1. Other autosomal recessive skeletal phenotypes in mice have also been associated with TGF-β superfamily member mutations[13–15]. Heterozygous murine phenotypes have not been reported. On the basis of our observations with *CDMP1*, however, the existence of clinically apparent human heterozygous phenotypes associated with other TGF-β superfamily members cannot be precluded.

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