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**Supplemental Data**

# Mutations in *MMP9* and *MMP13* Determine the Mode of Inheritance and the Clinical Spectrum of Metaphyseal Anadysplasia

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**Figure S1. *MMP13* Sequence Changes in Dominant MAD**
(**A**) The predicted amino acid substitutions in dominant MAD affect conserved residues which determine helix formation and thus prodomain conformation. (**B**) A heterozygous single base substitution (arrow) changes wild type c.249 thymidine of exon 2 to cytosine, and leads to the predicted substitution of phenylalanine to serine at codon 55; (**C**) heterozygous substitution of wild type c.300 thymidine of exon 2 to cytosine leads to the predicted substitution of methionine to threonine at codon 72.

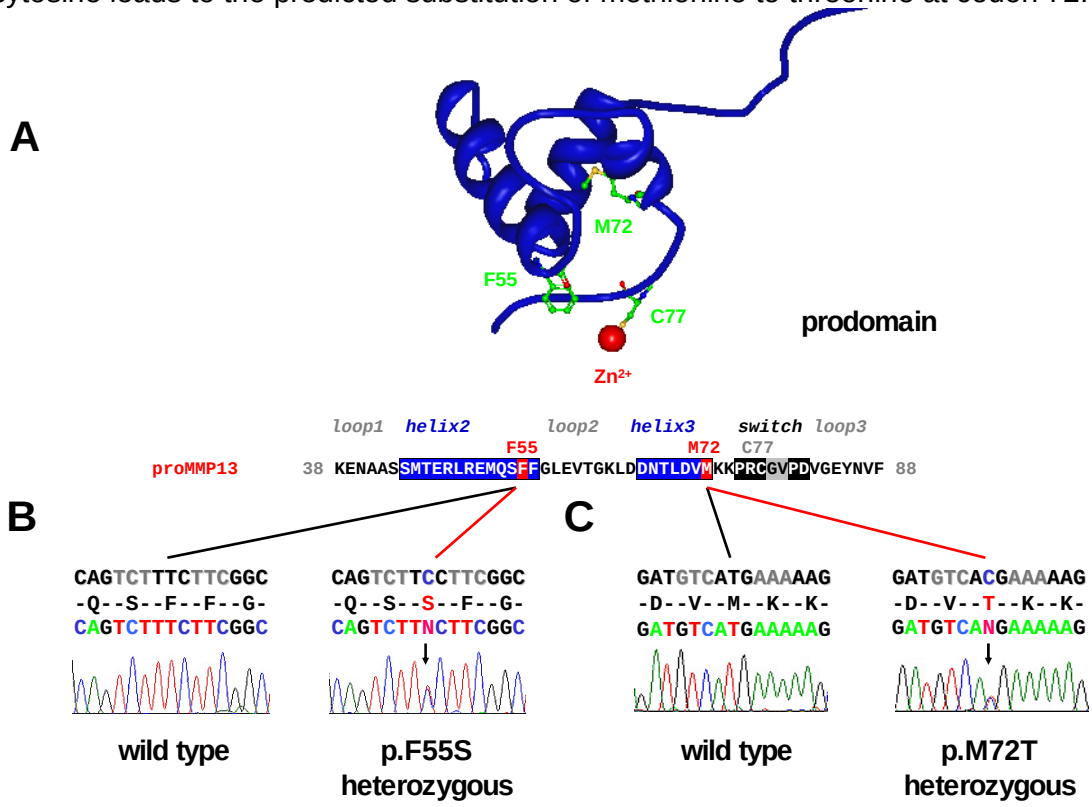

**Figure S2. Evolutionary Conservation of MMP13 Prodomain Residues**
The highly conserved terminal portion of the amino acid sequence of the prodomain of MMP13 is shown from diverse vertebrate species. Both p.F55 and p.M72, indicated in red, are conserved in all species; the consensus 'cysteine switch' sequence PRCxxPD, common to MMPs and ADAMs (a disintegrin and metalloproteinases), is highlighted in black. All sequences were obtained from Ensembl; the comparison is based on a clustalW-generated alignment.

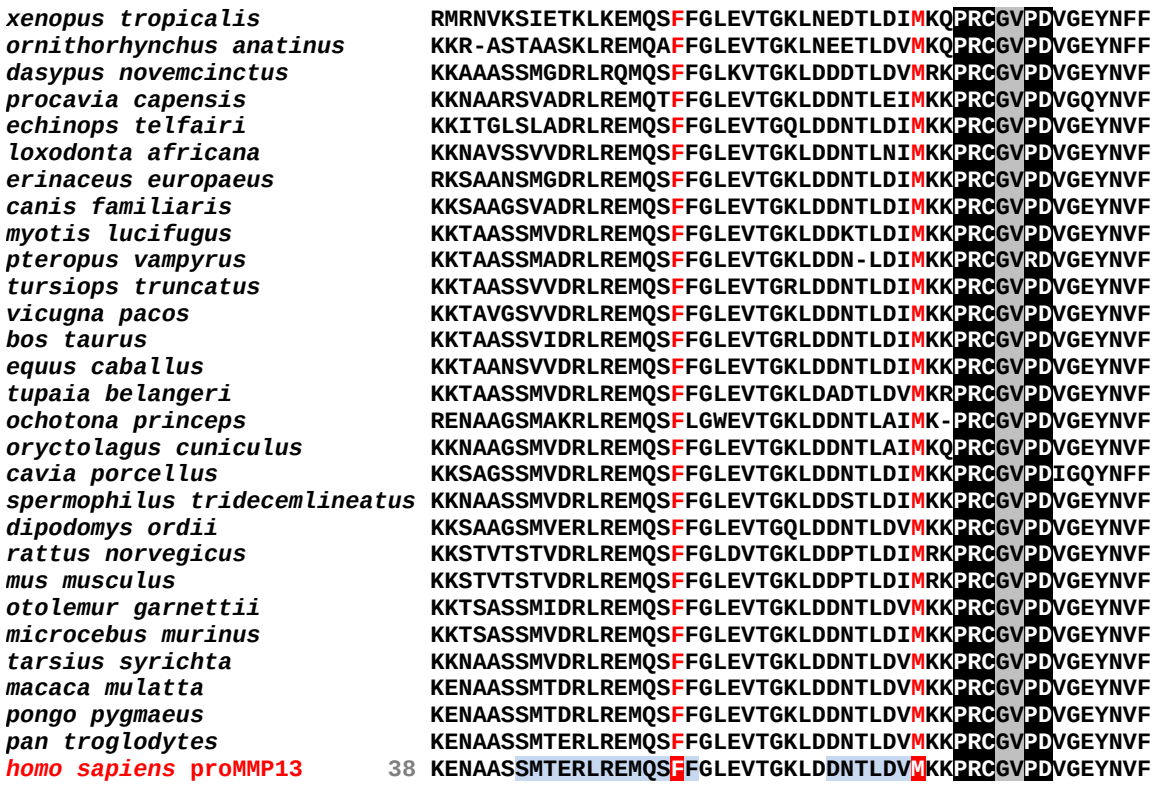

**Figure S3. *MMP13* Sequence Changes in Recessive MAD**
(**A**) The catalytic domain of MMP13 contains within the HExxHxxGxxH motif (highlighted in black) three zinc-binding histidines conserved in all 23 human MMPs. All sequences were obtained from Ensembl; the comparison is based on a clustalW-generated alignment. (**B**) Ribbon diagram of catalytic domain structure of MMP13, the atomic structure and position of the amino acids H203, E204, H207, and H213 ligating the catalytic zinc ion are shown; zinc and calcium ions bound by the catalytic domain are represented as red and green spheres, respectively. (**C**) A single base substitution (arrow) changes wild type *MMP13* c.722 cytosine of exon 5 to adenosine, and leads to the predicted substitution of histidine to asparagine at codon 213.

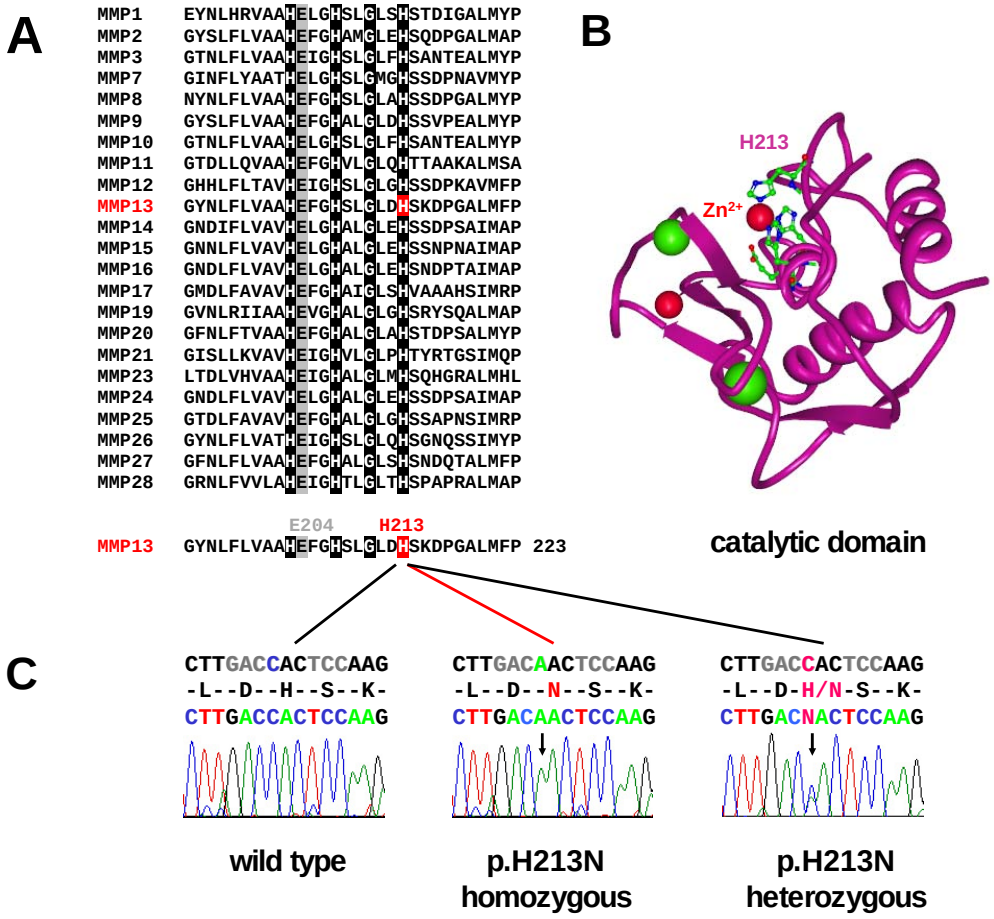

**Figure S4. MMP9 Sequence Changes in Recessive MAD**
Substitution of wild type *MMP9* c.21 thymidine of exon 1 to adenosine leads to the predicted substitution of methionine to lysine at the initiation codon of preproMMP9.

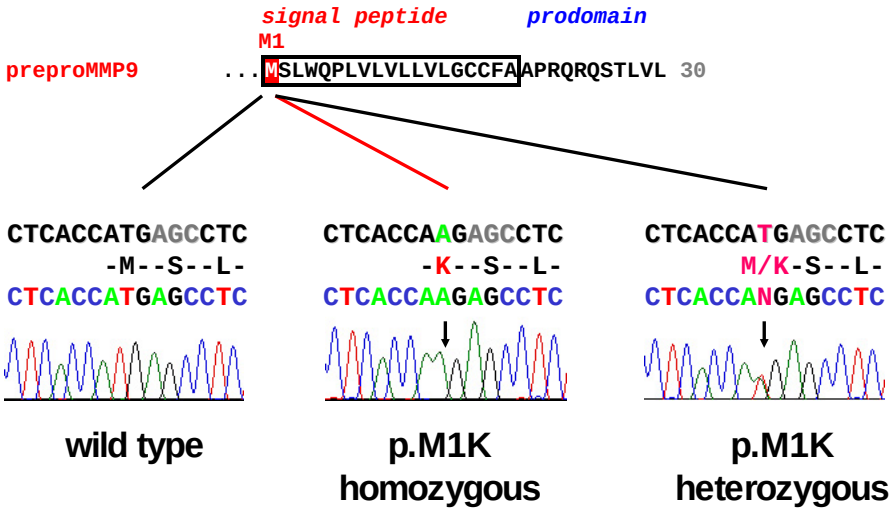

**Figure S5. Schematic Drawing Illustrating the Proposed Role of MMPs in MAD Pathophysiology**

Activation of proMMP9 by MMP13 is necessary for the release of VEGFA from the ECM in the growth plate. In recessive MAD, this cascade is disturbed by isolated loss of either MMP13 or MMP9 function, while dominant prodomain mutations cause ectopic activation of proMMP13; prematurely activated MMP13 in the endoplasmic reticulum degrades both itself (autocatalytic degradation) and the structurally normal MMP9 (transcatalytic degradation). The phenotype of a combined deficiency is only slightly more severe than the individual enzyme deficiencies, supporting the assumption of a common pathway; isolated or combined lesions converge on impaired angiogenesis due to a reduced release of VEGFA, and possibly other bioactive matrix components, in endochondral ossification as the common downstream pathogenic mechanism in MAD.

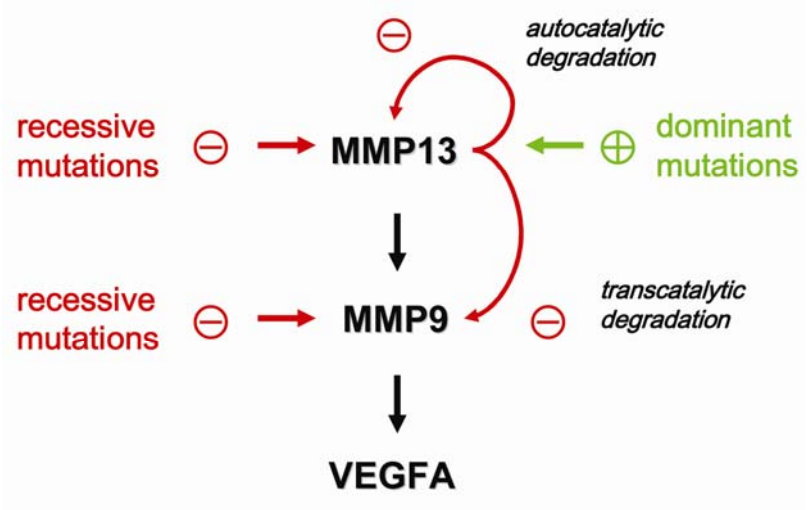

**Table S1. Primers Used for Amplification and Sequence Analysis of MMP9**
(**A**) Amplicons of exons and intervening sequences; the PCR product of exon 13 includes the complete 3' UTR. (**B**) Amplicons of 5' promoter regions and sequencing primers.

## A

| ***MMP9*** **Exon** | **Primer** | **amplicon size** |
|---|---|---|
| 1 | MMP9Ex1F:CTGAGTCAGCACTTGCCTGTC<br>MMP9Ex1R: ACACTCAACACCCTCACGGA | 293 bp |
| 2 | MMP9Ex2F: GCTGATACCGTCTCTCCGAA<br>MMP9Ex2R: AGGACTTGGATTGCAAGAGC | 555 bp |
| 3 | MMP9Ex3F: GCTCTTGCAATCCAAGTCCT<br>MMP9Ex3R: AGGGCTCCGAAATAAGTGC | 583 bp |
| 4 and 5 | MMP9Ex4F: CACTTATTTCGGAGCCCTTG<br>MMP9Ex5R: CATGGATGGTCTGCAGTTCA | 659 bp |
| 6 | MMP9Ex6F2: ACTAGTGCTGTGTGGCCTGC<br>MMP9Ex6R2: TCGTCCTGAGGACCAATGAG | 472 bp |
| 7 and 8 | MMP9Ex7F: CCTAGGCCACCAAGATTGTT<br>MMP9Ex8R: TGCTTCCAGACAGACGTTGA | 680 bp |
| 9 and 10 | MMP9Ex9F: CAGGAATAGGAAGAGTCTCACC<br>MMP9Ex10R: TTGAGCCTCCTTGACTGATG | 639 bp |
| 11 and 12 | MMP9Ex11F: GCGTTCTAGGAGTACGTGCT<br>MMP9Ex12R: GTCCTGGTCTTGGTTGCAT | 603 bp |
| 13 | MMP9Ex13F: CACTCCTCTTATGCCTGCCT<br>MMP9Ex13R: ATCTCCTGACCTCGTGATCC | 546 bp |

**B**

| ***MMP9*** **Promoter** | **Primer** | **amplicon size** |
|---|---|---|
| Mini_1 | MMP9PRF1: ATCCAGGACTTCGTGACTGC<br>MMP9PRR1: CCTGGACACCTCTGTTCTTCA | 2,188 bp |
| Mini_2 | MMP9PRF2: CAGGAATGAGCCACCATACC<br>MMP9PRR1: CCTGGACACCTCTGTTCTTCA | 1,237 bp |
| | MMP9PRseqF1: GGAGGCTTGGCATAAGTGTG<br>MMP9PRseqF2: CGAGGTCCTGAAGGAAGAGA<br>MMP9PRseqF3: TTGGCTGACCACTGGAGGCT | |
| | MMP9PRseqR1: AAGCTGCAGTGTAACCTGGA<br>MMP9PRseqR2: TCTTCCGCAGGCTGAATCTT<br>MMP9PRseqR3: TGACAGGCAAGTGCTGACTC | |