

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.

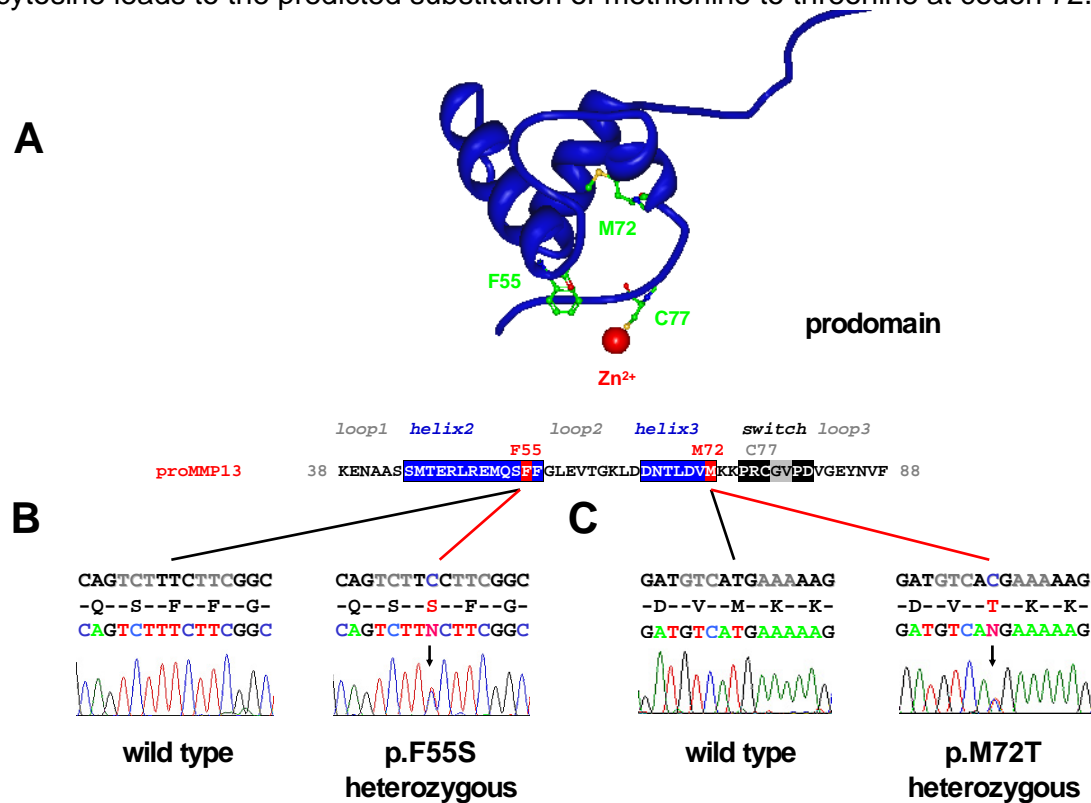


Figure S2. Evolutionary Conservation of MMP13 Prodomain Residues

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.

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xenopus tropicalis      RMRNVKSIETKLEMQSFFGLEVTGKLNEDTLDIMKQPRCGVDPVGEYNFF
ornithorhynchus anatinus  KKR-ASTAASKLREMQAFFGLEVTGKLNEDTLDV MKQPRCGVDPVGEYNFF
dasypus novemcinctus    KKAAASSMGDRLRQMQSFFGLKVTGKLDDDTLDV MKRPRCGVDPVGEYNVF
procavia capensis      KKNAARSVADRLREMQTFFGLEVTGKLDDNTLEIMKKPRCGVDPVGEYNVF
echinops telfairi      KKITGLSLADRLREMQSFFGLEVTGQLDDNTLDIMKKPRCGVDPVGEYNVF
loxodonta africana     KKNAVSSVVDRLREMQSFFGLEVTGKLDDNTLNI MKKPRCGVDPVGEYNVF
erinaceus europaeus    RKSAANSMSGDRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
canis familiaris       KKSAAAGSVADRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
myotis lucifugus       KKTAASSMVDRLREMQSFFGLEVTGKLDDKTLDIMKKPRCGVDPVGEYNVF
pteropus vampyrus      KKTAASSMADRLREMQSFFGLEVTGKLDDN-LDIMKKPRCGVDPVGEYNVF
tursiops truncatus     KKTAASSVVDRLREMQSFFGLEVTGRLDDNTLDIMKKPRCGVDPVGEYNVF
vicugna pacos          KKTAAGSVVDRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
bos taurus              KKTAASSVIDRLREMQSFFGLEVTGRLDDNTLDIMKKPRCGVDPVGEYNVF
equus caballus         KKTAANSVVDRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
tupaia belangeri       KKTAASSMVDRLREMQSFFGLEVTGKLDDADTLDMKKPRCGVDPVGEYNVF
ochotona princeps      RENAAGSMAKRLREMQSFLGWVETGKLDDNTLAIMK-PRCGVDPVGEYNVF
oryctolagus cuniculus  KKNAAGSMVDRLREMQSFFGLEVTGKLDDNTLAIMKQPRCGVDPVGEYNVF
cavia porcellus        KKSAGSSMVDRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
spermophilus tridecemlineatus  KKNAASSMVDRLREMQSFFGLEVTGKLDDSTLDIMKKPRCGVDPVGEYNVF
dipodomys ordii        KKSAAAGSMVERLREMQSFFGLEVTGQLDDNTLDV MKKPRCGVDPVGEYNVF
rattus norvegicus      KKSTVTSTVDRLREMQSFFGLDVTGKLDDPTLDIMRKPRCGVDPVGEYNVF
mus musculus           KKSTVTSTVDRLREMQSFFGLEVTGKLDDPTLDIMRKPRCGVDPVGEYNVF
otolemur garnettii     KKTSASSMIDRLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
microcebus murinus     KKTSASSMVDRLREMQSFFGLEVTGKLDDNTLDIMKKPRCGVDPVGEYNVF
tarsius syrichta       KKNAASSMVDRLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
macaca mulatta         KENAASSMTRDLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
pongo pygmaeus         KENAASSMTRDLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
pan troglodytes        KENAASSMTERLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
homo sapiens proMMP13  38 KENAASSMTERLREMQSFFGLEVTGKLDDNTLDV MKKPRCGVDPVGEYNVF
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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.

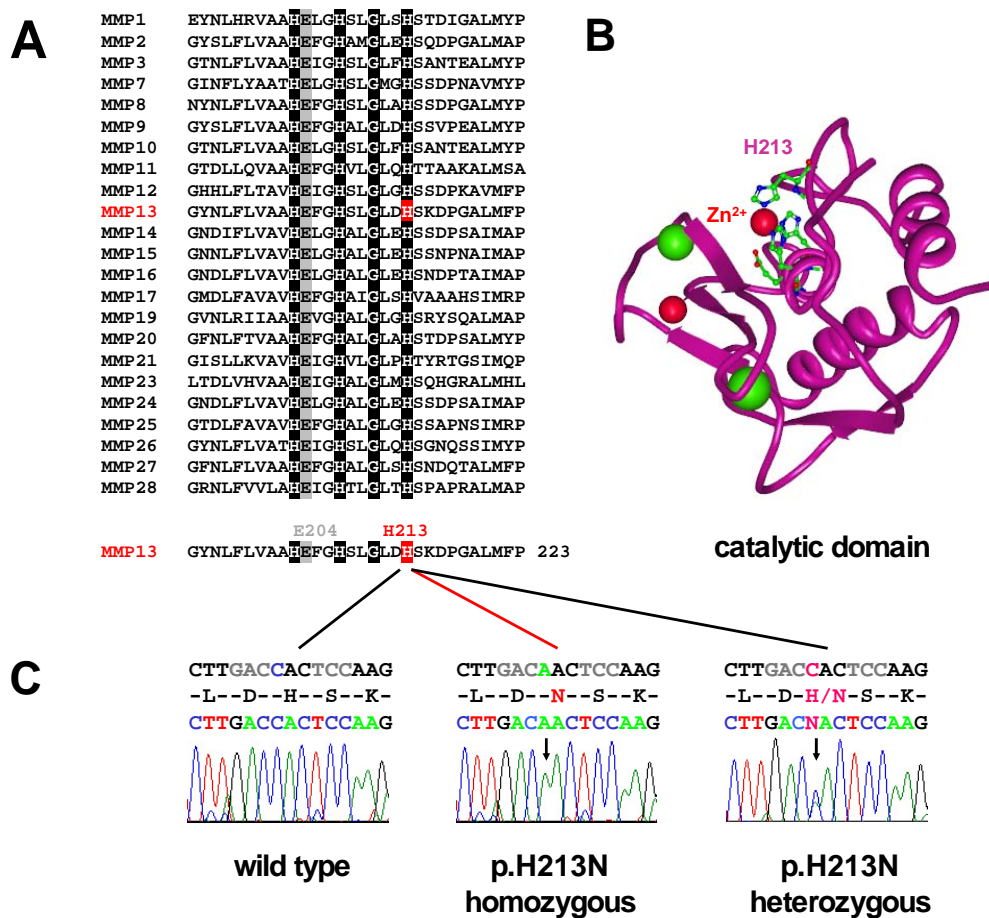


Figure S4. MMP9 Sequence Changes in Recessive MAD

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.

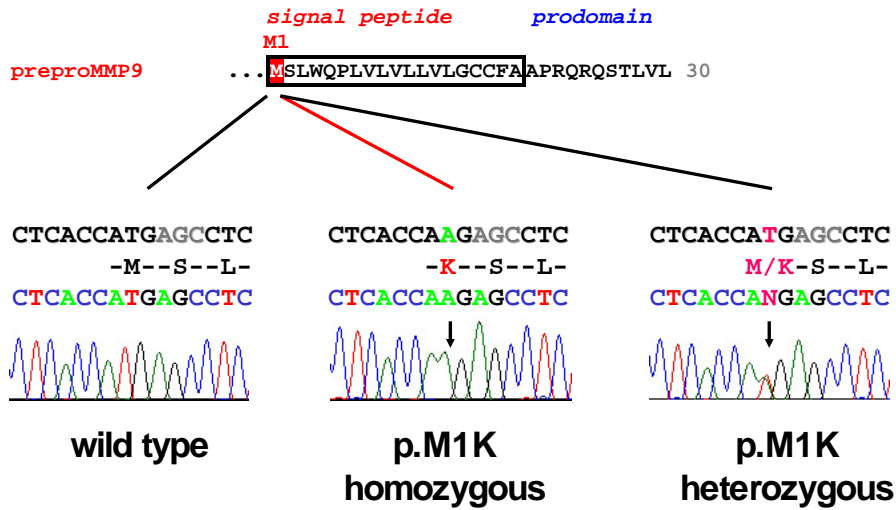


Figure S5. Schematic Drawing Illustrating the Proposed Role of MMPs in MAD Pathophysiology

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.

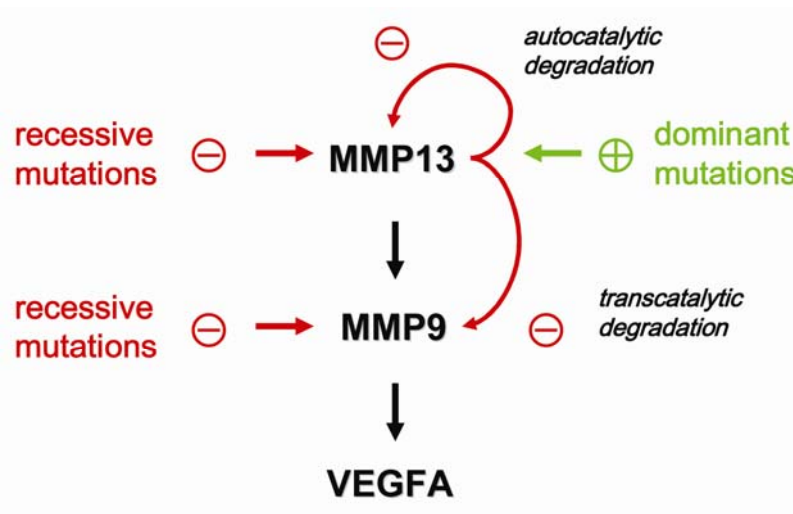


Table S1. Primers Used for Amplification and Sequence Analysis of MMP9
(A) Amplicons of exons and intervening sequences; the PCR product of exon 13 includes the complete 3' UTR. **(B)** Amplicons of 5' promoter regions and sequencing primers.

A

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

B

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