LETTER TO JMG

A recurrent R718W mutation in *COMP* results in multiple epiphyseal dysplasia with mild myopathy: clinical and pathogenetic overlap with collagen IX mutations

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Multiple epiphyseal dysplasia (MED) is clinically and genetically a heterogeneous disorder that affects growth centres and results in delayed and irregular mineralisation of the ossification centres.[1 2] Recessively inherited MED (rMED; MIM 226900) accounts for a significant proportion of MED cases and is associated with mutations in the sulphate transporter gene, DTDST/SLC26A2.[3 4] More often, MED is inherited as a dominant trait. Thus far, five different genes have been implicated in dominantly inherited MED: the gene for cartilage oligomeric matrix protein, *COMP* (MIM 600310); the genes for the α1, α2, and α3 chains of collagen IX, *COL9A1* (MIM 120165), *COL9A2* (MIM 120260), and *COL9A3* (MIM 120270); and the gene for matrilin-3, *MATN3* (MIM 602109). Patients with the severe forms of MED have short stature and major disability because of joint pain and stiffness. In the milder forms, height can be normal and joint complaints minimal.

Mutations in *COMP* typically lead to the severe forms of dominant MED (MIM 132400) and can also cause a related but more severe disorder—pseudoachondroplasia (PSACH, MIM 177170). COMP is a pentameric extracellular glycoprotein that belongs to the thrombospondin protein family.[5–7] It consists of a coiled coil N-terminal domain responsible for pentamerisation, four epidermal growth factor (EGF)-like repeats, eight thrombospondin type 3 (T3) repeats, and a large C-terminal globular domain. Mutations in *COMP* that cause MED are located in the T3 repeats.[1 2] Mutations in these repeats alter the conformation of the protein and affect its ability to bind calcium.[8–10] No mutations have been reported in the N-terminal domain or the EGF-like domains in MED. Only four mutations causing MED have been found in the C-terminal domain—two (T585R and T585M) in patients with unclassified MED,[1 11] and the other two (R718W and N742fsX743) in patients with ''severe MED'' and ''ribbing type MED'', respectively.[12]

Altogether eight mutations have been identified in the collagen IX genes in MED patients.[11 13–18] All reported mutations are clustered in the splice donor or acceptor site of exon 3 of *COL9A2* or *COL9A3* or in the splice acceptor site of exon 8 of *COL9A1*. The consequence of these mutations is skipping of exon 3 within the COL3 domain, leading to an in-frame 12 amino acid deletion from either the α2(IX) or α3(IX) chain, respectively; or in the case of the α1(IX) chain, skipping of exon 8 and/or exon 10, leading to an in-frame 25, 21, or 49 amino acid deletion within the COL3 domain. Patients with collagen IX mutation are typically of normal >to near normal height. Dysplastic changes are mainly seen in the knees, and the hips are relatively spared.[1 19] In contrast, the presence of dysplastic capital femoral epiphyses and severely irregular acetabuli is suggestive of COMP mutations.[1 19]

Key points

- A heterozygous R718W mutation in the *COMP* gene was ascertained in a three generation family in which two children presented with muscular weakness, a moderate rise in creatine kinase, and knee joint epiphyseal dysplasia. The same mutation was identified in a second family with dominantly inherited multiple epiphyseal dysplasia (MED) with similar radiographic changes.
- Mild myopathy is not exclusively associated with collagen IX-MED but can occur in COMP-MED as well.
- In both families, radiographic features had suggested a collagen IX mutation but screening of *COL9A1*, *COL9A2*, and *COL9A3* yielded negative results. Radiographic criteria for distinction between collagen IX-MED and COMP-MED may not be reliable and a pragmatic mutation screening approach may be safer.
- Analysis of radiographic changes at the knee joint in seven individuals with the same *COMP* mutation showed the time window in which epiphyseal changes are recognisable and their dynamic nature.
- The clinical and radiographic overlap between collagen IX-MED and COMP-MED points to a common supramolecular complex pathogenesis.

We undertook a clinical and molecular study of two families with an MED phenotype very similar to those individuals previously reported with mutations in the collagen IX genes. Surprisingly, affected members in both families had a mutation in *COMP*, R718W, suggesting that this mutation and mutations in collagen IX may share the same molecular pathogenesis.

METHODS

Subjects

We studied two MED families, family 1 and family 2 (fig 1). Family 1 was a three generation family in which two children presented with muscular weakness, moderately raised creatine kinase, and joint pain. Family 2 was a two generation family in which a 12 year old girl presented with joint, and especially knee, pain. All subjects were examined clinically and radiographically. Affected and unaffected family members were informed about the nature of the

Abbreviations: CSGE, conformation sensitive gel electrophoresis; MED, multiple epiphyseal dysplasia; PSACH, pseudoachondroplasia

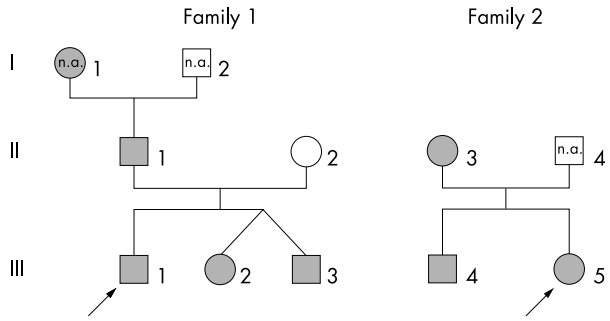

Figure 1 Pedigree of the two families studied. Shaded individuals were carriers of the R718W mutation. Individual I-1 was not available for molecular analysis but her history of bilateral hip replacement indicates that she probably carries the mutation. NA, not available for analysis.

molecular investigations aimed at determining the cause of the muscle and joint disease in some family members, and gave consent to venepuncture and molecular analysis. Genomic DNA was extracted using standard methods.

Mutation analysis

Collagen 9 gene analysis

Sequences corresponding to exons 8 to 10 of *COL9A1*,[20] exons 2 to 4 of *COL9A2*,[20] and *COL9A3*[21] and the corresponding intronic flanking sequences were amplified by polymerase chain reaction (PCR) to obtain products of 252 to 396 base pairs (bp) to be used for mutation screening by conformation sensitive gel electrophoresis (CSGE) (table 1). The amplifications were carried out in a volume of 20 µl that contained 20 to 40 ng genomic DNA, 5 to 10 pmol of the PCR primers, 1.5 mM of $MgCl_2$, 0.2 mM of deoxynucleotide triphosphates (dNTPs), and one unit of AmpliTaq Gold DNA polymerase (Applied Biosystems). The thermocycling and the CSGE analysis conditions were the same as described earlier[22 23] with the exception that the CSGE gels were stained with SYBR Gold nucleic acid gel stain (Eugene, USA) instead of ethidium bromide. All samples were also analysed by direct sequencing of PCR products with fluorescence labelled dideoxy nucleotides and on-line fluorescence detection using ABI PRISM^{TM}377 or 3100 sequencer apparatus and the BigDye terminator cycle sequencing kit (Applied Biosystems). In family 2, all 32 exons and the boundaries of *COL9A2* and *COL9A3*, and all 38 exons and the boundaries of *COL9A1* were screened by CSGE from the affected individuals.[11]

Sequences corresponding to all 19 exons and exon boundaries of *COMP*[24] were amplified by PCR to obtain products of 259 to 401 bp (table 1). These PCR products were analysed by CSGE followed by direct sequencing as indicated above.

RESULTS

Clinical findings

Family 1

The eldest boy in this sibship (fig 1, subject III-1) was noted by his paediatrician to have mild muscular weakness around the age of three years. At five years, he was referred for neuropaediatric consultation and was found to have a moderately raised plasma creatine kinase (between 440 and 1647 U/l in six different blood samples; normal for age, <195 U/l). Clinical examination confirmed mild muscular weakness without other major findings. Subsequently he complained of joint pain and was referred to a rheumatologist for investigation of possible myositis or other rheumatological disorder. Skeletal radiographs were taken and epiphyseal changes at the knees were noted, prompting a review of the findings of the skeletal dysplasia group. A diagnosis of epiphyseal dysplasia with mild myopathy was made and molecular investigation of collagen IX genes was recommended. His clinical course was mild, with occasional joint complaints but no significant limitation or handicap in his daily life.

The younger siblings of III-1 were fraternal twins—a girl and a boy (III-2 and III-3 in fig 1). The boy had motor features similar to those of his elder brother and he was included in the neuropaediatric consultation. He was also found to have raised creatine kinase (411 and 315 U/l on two occasions; normal, <195 U/l) and, following identification of epiphyseal dysplasia in his elder brother, he was found to have similar radiographic changes (figs 2 and 3).

The other twin, a girl (III-2 in fig 1), was clinically healthy. Neither her paediatrician nor her parents considered her to have muscular or other clinical features like those of her brothers. When mutation analysis revealed that she was also carrying the *COMP* mutation, *x* rays of her hand and knee were done. These showed the unequivocal presence of epiphyseal dysplasia (fig 3). Her plasma creatine kinase on that occasion was 208 U/l (normal for her age, <171 U/l).

The height of all three children was in the upper range of normal. Family history revealed that the parents originated from the Balkans. The father (subject II-1) had precocious osteoarthritis of both hip joints. He had been a construction worker but had to be placed on disability leave in his early thirties because of pain and disability. He required bilateral hip joint replacement at age 33 years. His mother (grandmother of the affected siblings; subject I-1 in fig 1) had a similar history of osteoarthritis and had had bilateral hip replacement surgery in her forties. Radiographic data were not available; blood could be obtained from the father but not from the grandmother, who lived abroad. Plasma creatine kinase in the father was 222 U/l (normal for age, 180 U/l).

Family 2

A 12 year old girl (individual III-5 in fig 1) was referred for recurrent joint pain. Her mother (II-3) also gave a history of frequent joint pain, and her older brother (III-4) mentioned occasional knee pain. Radiographs were obtained. Knee *x* rays showed changes suggestive of MED in the girl (fig 3, panel D), and magnetic resonance imaging (MRI) was done (fig 4). Knee radiographs of the mother and brother showed only mild changes (fig 3, panels E and F). The family members consented to donate blood for molecular analysis but considered their knee affliction to be of little significance and they where lost to follow up; their plasma creatine kinase could not be obtained. The radiographic pattern at the knee, with the clefts on the lateral sides of the distal femoral and proximal tibial epiphyses (''*Gletscherspalte*'', glacier crevice; fig 3, panel D) was considered similar to that described in published reports in association with collagen IX mutations. However, no mutations were identified and investigations were stopped. Only a few years later, upon identification of family 1 (above), the DNA samples were resubmitted to *COMP* mutation analysis which revealed the presence of the same mutation (see below) in the index case, in her mother, and also in her brother, who had mild radiographic changes.

Radiological findings

The radiographic features associated with the R718W mutation are shown in figs 2 to 4. The hands showed some delay in epiphyseal maturation with slightly irregular contours of the carpal elements and a small radial distal epiphysis. The proximal femoral epiphyses in case III-3 (at age seven years) have low-normal size and regular contours, while in case III-5 (at age 14 years), they are small and have an irregular contour. The knees show the most significant

Table 1 Polymerase chain reaction primers and conditions for analysis of the COL IX candidate exons and all *COMP* exons

Gene	Amplified region	PCR primer sequence (5′-3′)	Primer location (5′)	Product size (bp)	Ann temp
COL9A1	exon 8	F: CTG GAA GGT AAC TTT TAC CCC AC	IVS7–108	252	60˚C
		R: GGA GCC CTG GGA AAA GAA TAG G	IVS8+69		
	exons 9+10	F: CCC CAT CTG TGA AGT GAG CTC	IVS8–97	397	60˚C
		R: CAC AAA ACA CAC ACT TAC TCG TAC	IVS10+71		
COL9A2	exon 2	F: CAC AGT GCC TGC TAT ACA GAA G	IVS1–149	298	60˚C
		R: CCA CAG CGC TCA CAA AGT TCT C	IVS2+70		
	exons 3+4	F: GGC CTG TGT GTG CCC ACT TGG	IVS2–68	330	60˚C
		R: CAG TGA AGA TGC CAG AGC CAG G	IVS4+79		
COL9A3	exon 2	F: CTC ACC GAG GAG AGC GGC GGT CGT C	IVS1–119	275	64˚C
		R: CTC TCC ATC AGG CCG GGG AGC	IVS2+87		
	exon 3	F: GCT GAT TTG GAG GCC AGC GCT GC	IVS2–94	277	64˚C
		R: CAG GCC TGC TCC AAG CCC TCT G	IVS3+147		
	exon 4	F: TGA GCC GGG TCT GCC AGA CAG	IVS3–154	326	60˚C
		R: TGA GCA GGC CCA GCT GGA CAA C	IVS4+97		
COMP	exon 1	F: CCG GCC GTG CCT TGG GGA TAA ATA	5′UTR -78	283	65˚C
		R: GCC CGG CAC GTC CCC TAC GAA CAG	IVS1+120		
	exon 2	F: TGT TTG GGG CTC ACG GAC TGT TCG	IVS1–176	379	65˚C
		R: GAC CCC CTT CCC TCC CCA TCT CCT	IVS2+117		
	exon 3	F: GGG GTC GGC GGG TAG GGA GTC CTT	IVS2–125	320	62˚C
		R: ATT CCC GTT CGT CCG TCC ACC TCT	IVS3+143		
	exon 4	F: GGG GCG TGG CCA TGA TAG GCT CTG	IVS3–78	351	65˚C
		R: GGC GAA CAC TCC CAG TGG AGG	IVS4+100		
	exon 5	F: GGC CCT TGA ACT TTC CAC ATC C	IVS4–95	391	62˚C
		R: CCC CTC CAT CCT GCC CCA AAC C	IVS5+158		
	exon 6	F: AGG ATG CGG AGG AAG TGT GGA	IVS5–82	259	65˚C
		R: CAC TGG AAG GAG CCC TGC GC	e7+14		
	exon 7	F: AAC TCC GTG TGC ATC AAC ACC CG	e6+52	315	63˚C
		R: GCC GCC ACC CAA CCC CGC CTC AAG	IVS7+44		
	exon 8	F: CAT GCA GAC TGC GTC CTA GAG C	e7+115	336	62˚C
		R: GCA CAG TCA CGC AGT TGT CCT GG	e9+19		
	exon 9	F: GCC AGT GCC GTA AGG TGG GT	e8+92	274	64˚C
		R: CCT CGG CCT CCC AAA GTG CT	IVS9+62		
	exon 10	F: TTT GGG AAT CTG AGG AGT GTG AC	IVS9–71	335	60˚C
		R: TAC CCC ATC CGG CTT CCA AAA CCA	IVS10+104		
	exon 11	F: ATG AAG TTG GGA CTC TGT TCC AGG	IVS10–108	322	58˚C
		R: GCA GTG TAA AAT GCT CTA AGC TGG	IVS11+96		
	exon 12	F: GGT GGG GGT TTC CTT GGG GTC AG	IVS11–111	365	62˚C
		R: GTC GTC GTC GTC GCA GGC ATC AC	e13+109		
	exon 13	F: GCG TCC GGG TAG CCT TTG A	IVS12–70	380	62˚C
		R: CGC CCA CGC CGT CCC CTG AGA G	e14+15		
	exon 14	F: GGG GCT CCT GGC GCG GGG TCT A	IVS13–72	309	62˚C
		R: AGG CCT CAC TGT GGG GGT TCT A	IVS14+58		
	exon 15	F: GAC GCG CAG ATT GAC CCC AAC TGG	e14+141	290	64˚C
		R: CCC GGG CCT GCC CCT TCT CTG	IVS15+76		
	exon 16	F: CAG GGG GCA GGT TTG GGT TCT	IVS15–86	371	60˚C
		R: GGG GGC TCT AAG TGG CTG TAA AG	IVS16+88		
	exon 17	F: CCG CCC ACC ACC TGC TGC TC	IVS16–101	401	60˚C
		R: CTG CCC TGT ATC TTT CCT ATC GTA	IVS17+127		
	exon 18	F: GGG CTG GCC ACT GAA GCT CTG AGA	IVS17–74	399	64˚C
		R: GCC GCG GTG AGG GTG GCT GTC AT	e19+93		
	exon 19	F: TCA TCT GGG CCA ACC TGC GTT ACC	e18+107	381	64˚C
		R: CCT GGC CCG ACC CTC CCT CAG A	3′UTR+68		

Ann temp, annealing temperature; bp, base pairs; PCR, polymerase chain reaction.

changes. A lateral view of the knee of subject III-1 at age 11 ½ years shows a moderately small patella with an accessory cranial ossification centre and markedly irregular femoral epiphyses with frayed contours and the possible presence of a loose body. Figure 3 delineates the sequence of radiographic changes at the knee. At ages six and seven years there are moderately small epiphyses with slightly irregular contours. Between the ages of 11 and 13 years (probably coinciding with the pubertal growth spurt and osseous maturation), an additional ossification centre appears at the medial condyle and extends to form a ring at the periphery of the epiphysis; it is at this stage that the aspect is characteristic (resembling a ''crevice'' in a glacier) and can be seen both in the distal femoral and the proximal tibial epiphyses. At later ages, the ''crevice'' sign is no longer visible but the epiphyses are flatter than normal.

Mutation analysis

Because the phenotype of the affected individuals studied here was similar to that caused by collagen IX gene mutations, these genes were analysed for mutations. All reported mutations of collagen IX genes are clustered in the splice sites of exon 3 of *COL9A2* or *COL9A3*, or the splice acceptor site of exon 8 of *COL9A1*.[1 2] Therefore the candidate exons were analysed by CSGE and sequencing from the index case of family I (fig 1), his affected father, and his unaffected mother. Analysis of the candidate exons of *COL9A1*, *COL9A2*, and *COL9A3* genes identified five sequence variations. Two of them were in *COL9A1* (IVS8^{+13}C>T, IVS10^{+45}G>A), two in *COL9A2* (IVS2–^{42}T>C, IVS4^{+36}C>A), and one in *COL9A3* (e2^{+15}C>A; P29P). They were not disease causing because no co-segregation with the variation and phenotype was observed or they were also present in control samples (data

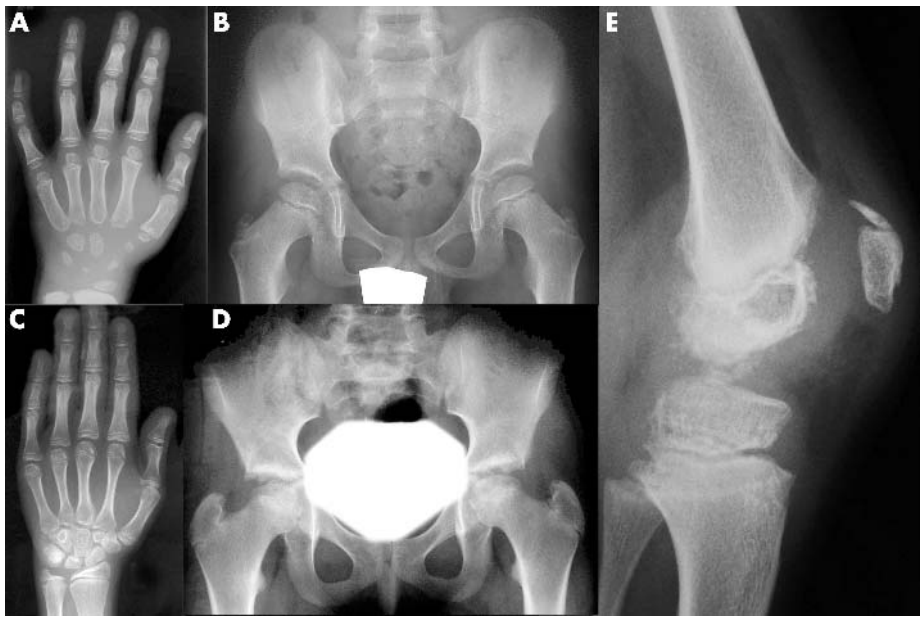

Figure 2 Radiographic features associated with the R718W mutation. (A) and (B), subject III-3 at age seven years; (C) and (D), subject III-5 at age 14 years; (E), subject III-1 at age 11½ years. *Hands*: (A) Skeletal age is mildly delayed (five to six years) and the shape of the carpal bones is edgy and not rounded. (C) Phalangeal maturation is adequate for this postpubertal girl; the carpal bones are smaller than normal. *Pelvis*: (B) Both the femoral capital epiphyses and the greater trochanters are slightly smaller than usual but regular in shape. (D) The femoral heads are markedly smaller than normal and their shape is mildly irregular. The acetabular roof shows the beginning of degenerative changes. Pelvic shape is unremarkable in both (B) and (D). *Knee*: (E) The lateral knee film shows marked irregularities of the epiphyseal contours, wedging of the metaphyses, and a small patella with an accessory cranial ossification centre. This lateral view corresponds to the second AP view in fig 3. A remarkably similar radiograph has been published in association with collagen IX associated multiple epiphyseal dysplasia (Lohiniva *et al*,[14]; see fig 3 in that report).

not shown). The analysis did not reveal any putative disease causing variations.

Because no mutations were found in the candidate exons of collagen IX genes in this family in spite of an unequivocable MED phenotype, mutation screening was then extended to *COMP*. *COMP* mutations are among the most common causes of the dominant form of MED. All 19 exons and the boundaries of *COMP* were analysed by CSGE from three individuals in family 1 (fig 1, II:1, II:2, and III:1). In addition, all exons and the boundaries were sequenced from the proband. A unique CSGE pattern in exon 18 of *COMP* was present in the affected boy (III:1) and his affected father (II:1). Sequencing of the product identified a c.2152 C>T transition converting the codon CGG for R718 (arginine) to TGG for W (tryptophan) (fig 5). A previously reported polymorphism, IVS18^{+53} T>C,[11] was also present in the same PCR product in the affected family members and seen on CSGE analysis as well (fig 5). CSGE analysis of the other exons did not reveal any other heteroduplexes, and no other sequence variations were found in sequencing. After identifying the R718W mutation in two affected family members (II:1, III:1), the analysis of the twins showed that they had also inherited the R718W mutation and the IVS18^{+53}T>C polymorphism from their father. The unaffected mother did not have the R718W mutation or the polymorphism. The fact that the polymorphism was seen in one family only, and that the mutation itself occurs at a CpG dinucleotide, suggests independent occurrence.

Initially, all exons and the boundaries of *COL9A1*, *COL9A2*, and *COL9A3* were analysed from the three affected indivi-

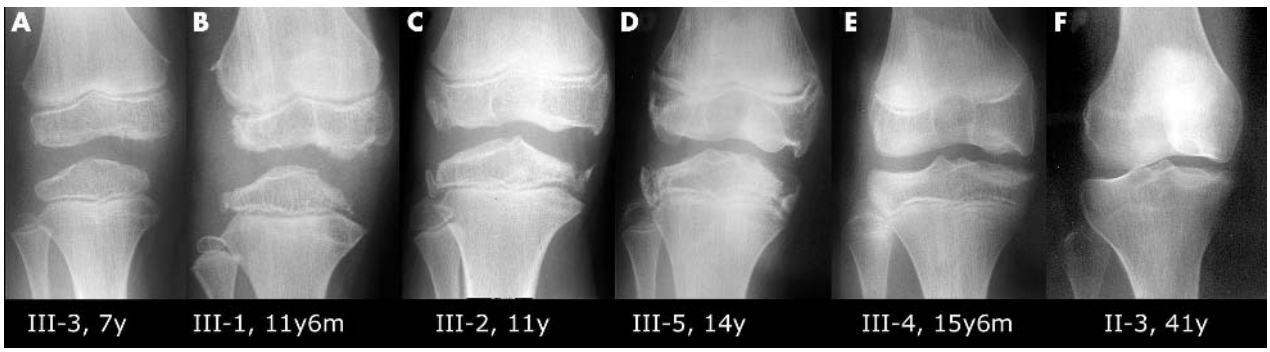

Figure 3 Anteroposterior knee radiographs of six individuals with the R718W mutation at different ages (as indicated in the figure). (A) The epiphyses are small but regular, there is mild metaphyseal wedging. (B) Wedging is more pronounced and the contour of the epiphyses is irregular. (C) Progress of ossification has resulted in accessory medial and lateral nuclei at the proximal tibia, with what looks like a cleft between the regular and the accessory nuclei (''crevice''). At the distal femoral epiphysis, ossification is more advanced, leaving lateral and medial epiphyseal ''spurs''. (D) The proximal tibial epiphysis shows here a distinct ossific ''ring'' surrounding the normal central nucleus. This image corresponds to the magnetic resonance images in fig 4. (E) Epiphyseal ossification is complete. Apart from small dimensions and edginess of the distal femoral epiphysis, the previous changes are no longer discernible. (F) In the adult, the epiphyses are small but otherwise undiagnostic. m, months; y, years.

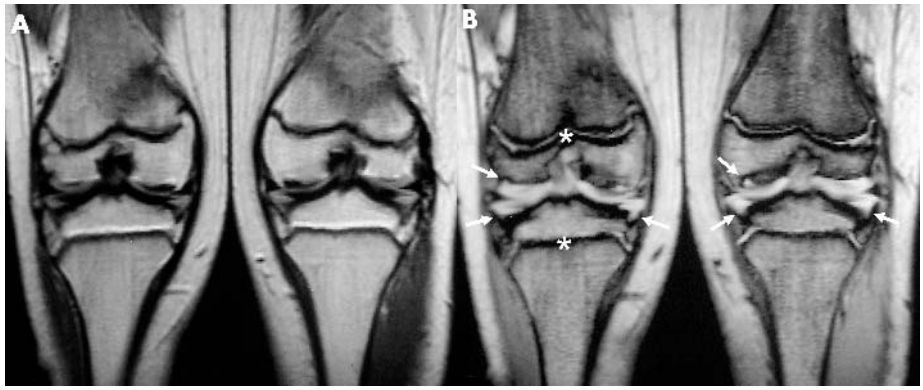

Figure 4 Magnetic resonance imaging of the knees in individual III-5 at age 14 years (corresponding to the fourth radiograph in fig 3). Coronal sections of both knees are shown. (A) Proton density spin echo sequence (TR (time of repetition) 1800, TE (time of echo) 30) shows symmetrical flattening and irregular contour of femoral and tibial epiphyses. The signal intensity of the bone marrow is homogeneous, corresponding to normal fatty tissue. (B) Gradient echo sequence (TR 540, TE 16, flip angle 75) shows hyaline cartilage as a hyperintense (bright) structure, while menisci, ligaments, and bone marrow appear darker grey; dense bone appears black. Note the abundance of cartilage around the flattened and misshapen epiphyseal ossification centres. Cartilage is also seen within the ''crevices'' of both the femur and tibia (arrows). Note also the growth plates (marked by asterisks) of the distal femur and proximal tibia which have abnormal shapes but a qualitatively normal signal.

duals from family 2. The analysis failed to identify any disease causing mutations. When the R718W mutation was identified in family 1 because the phenotype was similar to family 2, the three affected members of family 2 were analysed for the presence of R718W mutation in *COMP* by CSGE and sequencing. The same R718W mutation was identified in all three individuals. Subsequent CSGE analysis of the other 18 exons and the boundaries of *COMP* did not indicate any other sequence variations in these family members. Sequencing of the remaining 18 *COMP* exons from the proband's sample did not reveal any other sequence variations.

DISCUSSION

We identified seven individuals in two families who were heterozygous for an amino acid substitution in the C-terminal globular domain of *COMP*. In spite of its relatively large size, mutations in the C-terminal domain producing a skeletal phenotype seem to be relatively rare and may tend to cluster around sensitive microdomains. According to the review of genotype–phenotype correlations by Briggs and Chapman,[1] mutation of threonine-585 has been observed in two unrelated individuals with MED, and two closely situated mutations, glutamate-583 and histidine-587 have been observed in patients with PSACH. Recently, three novel mutations were reported in the C-terminal domain of *COMP*: a nucleotide insertion leading to premature termination at codon 742 (giving MED); a substitution of glutamate 719 with aspartate (resulting in severe PSACH); and substitution of arginine 718 with tryptophan, which is the same mutation we found in our study subjects.[12 25] Apparently, arginine 718 and glutamate 719 define a second functionally important microdomain in the C-terminal domain of COMP.

Mabuchi *et al* observed R718W segregating in a family, but little clinical detail or radiographic data were given, except for height at –3 SD and a diagnosis of ''Fairbank'' type MED.[12] While saturating the mutation map of the molecule contributes to the genotype–phenotype database and deline-

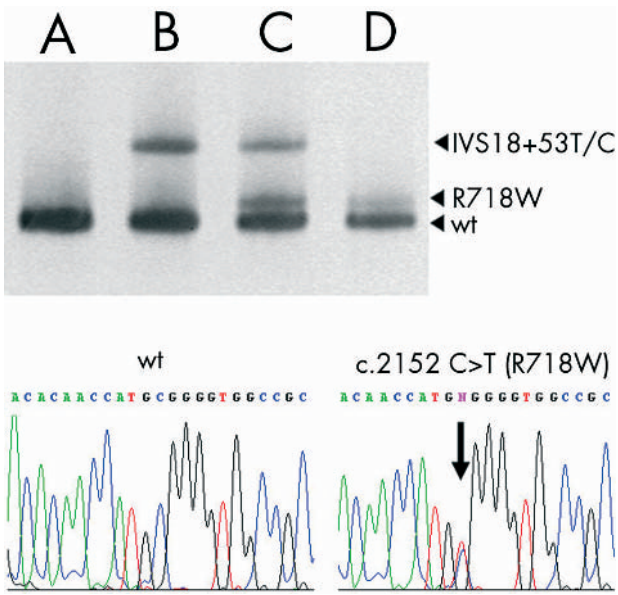

Figure 5 Top panel: CSGE analysis of COMP exon 18: lane A, no changes; lane B, IVS18^{+53} T/C; lane C, R718W and IVS18^{+53} T/C; lane D, R718W. Bottom panel: Genomic sequence showing the wild type and the c.2152 C>T mutation at the heterozygous state.

ates functional regions of the molecule, the clinical and radiographic findings in the two families reported here provide novel information, such as the association of this mutation with myopathy, the surprising overlap between radiographic findings in this family with those reported for collagen IX associated MED, and the dynamic changes at the distal femoral epiphyses that delineate a diagnostic time window.

Muscle weakness was the presenting clinical sign in the two index cases in family 1, and clear elevation of plasma creatine kinase in both confirmed the presence of myopathy. Creatine kinase levels were also slightly raised in their younger sister and their father. Myopathy has previously been reported in association with MED and *COL9A3* mutation[13]; interestingly, in that family also two boys had clinically relevant myopathy, while female mutation carriers were affected subclinically. In that family, elevation of plasma creatine kinase was less marked than in the two boys studied here. In addition, the proband in another family with a *COL9A3* mutation, an 11 year old boy, was initially evaluated by a neuropaediatrician because of stiffness in the knees, clumsiness, and muscle weakness in the legs.[14] It is not unusual for children who are later diagnosed as having PSACH to present before the onset of short stature with neuromuscular symptoms, most often gait disturbances. In view of the molecular interaction between COMP and collagen IX, it may be that a common supramolecular complex in muscle (possibly at the junction between myocytes and adjacent matrix structures) is affected. Little is known so far about a possible function of collagen IX and COMP in muscle and this aspect remains to be investigated.

A review of the radiographic features of a series of patients with dominant MED suggested a distinct pattern, allowing differentiation between forms caused by mutations in the collagen IX genes and forms caused by mutations in COMP.[19] The knee ''crevice'' sign can be recognised in other report of MED—for example, in Hatori *et al* [26] (case 6 in fig 5 in that paper), and, significantly, in collagen IX associated MED.[14 16] The accessory cranial ossification centre of the patella has also been observed in collagen IX-MED[14] (compare fig 3 in that report with the findings presented here). This was what prompted us to investigate collagen IX before COMP. Apparently neither sign is specific for collagen IX associated MED as it can also be seen in COMP associated MED. Thus although these observations do not refute the distinction proposed by Unger *et al* that patient groups may tend to show differences,[19] there is considerable overlap. It is true that two affected individuals (I-1 and II-1) developed severe hip arthritis in adulthood, which is more typical of COMP-MED. At present there is not enough follow up information on adult patients with collagen IX-MED to determine whether severe hip involvement in adult life is a clear distinguishing criterion between the two forms of MED.

The lack of specificity of the radiographic signs during childhood and adolescence, and the presence of myopathy, points to a common molecular pathogenesis. Conceivably, the function of a supramolecular complex is disrupted in dominant MED, with similar consequences irrespective of whether the primary defect is in one component (COMP) or the other (collagen IX). From a diagnostic perspective, we suggest that it may be more practical to investigate the *COMP* gene first and the collagen IX genes only after *COMP* mutation screening has proven negative.

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