LETTER TO JMG

Spondylo-epi-metaphyseal dysplasia (SEMD) matrilin 3 type: homozygote matrilin 3 mutation in a novel form of SEMD

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Osteochondrodysplasia is a heterogeneous group of conditions caused by impaired development of osseous skeleton. Within this group, spondylo-epi-metaphyseal dysplasia (SEMD) represents a subgroup which includes a number of conditions associated with vertebral, epiphyseal, and metaphyseal anomalies. The International Classification recognises at least 15 distinct entities within this group as defined by a combination of clinical, radiological, and molecular data.[1] Mutations in the genes encoding structural proteins of the cartilage extracellular matrix (that is, cartilage oligomeric matrix protein, type II collagen, perlecan) or involved in posttranslational processing and transport (lysosomal storage disorders, sulfation protein (*PAPSS2*), regulator of chromatin (*SMARCAL1*), transcription initiation factor kinase (*EIFKA3*)) have been previously shown to be responsible for different forms of SEMD.[1–6] However, a large number of SEMD conditions are still defined on purely descriptive features. Here, we describe five patients from a large consanguineous family of Arabic-Muslim origin with short-limb dwarfism with distinct skeletal dysplasia which, to our knowledge, has not been described previously. The manifestations and a constellation of radiographic bony abnormalities are unique in this entity. As there are abnormalities in the vertebral bodies, long bones, and specifically the lower limbs involving epiphyses and metaphyses, we suggest that this entity be called spondylo-epi-metaphyseal dysplasia, bowed-legs type. This hitherto unreported form of autosomal recessive SEMD is caused by homozygous mutation in matrilin 3 (MATN3), a component of the extracellular matrix of cartilage. This finding not only provides a molecular explanation for a novel form of SEMD but also expands the phenotype range of MATN3 defects which have been previously identified in autosomal dominant epiphyseal dysplasia and idiopathic hand osteoarthritis.[7 8]

METHODS

In this family, five affected individuals were found to have disproportionate dwarfism with similar clinical and radiological features. Patient 1 (figs 1 and 2) was born to healthy parents of Arabic-Muslim origin who are first cousins and of normal stature (father 175 cm (75th centile) and mother 168 cm (50th centile)), after a term pregnancy and normal delivery (birth weight 3100 g, birth length 45 cm (-2 SD), head circumference 33 cm). At 1 year, she had short-limbed short stature (height 68 cm (-3 SD), weight 8 kg (3rd centile), head circumference 43 cm (-3 SD), arm span 71 cm, palm length 5.2 cm (50th centile), middle finger length 3.2 cm (3rd centile)). Neurological examination, psychomotor development, hearing, and visual acuity were otherwise normal. Routine chemistry, thyroid function tests, and renal ultrasounds were normal as well. Re-evaluation at 6 years showed further disproportion of limbs, with bowed

Key points

- We investigated a large consanguineous Arabic-Muslim family in which five affected individuals were diagnosed with a novel form of autosomal recessive spondylo-epi-metaphyseal dysplasia (SEMD). All five affected individuals presented with disproportionate early-onset dwarfism, bowing of the lower limbs, lumbar lordosis, and normal hands. Skeletal findings included short, wide, and stocky long bones with severe epiphyseal and metaphyseal changes, hypoplastic iliac bones, and flat-ovoid vertebral bodies.

- Genome wide homozygosity mapping mapped the disease locus gene to chromosome 2p25–24 with a maximum LOD score of 3.46 at locus D2S305. Two recombination events defined the minimal critical region interval between loci D2S320 and D2S215 (2.24 Mb). Using a candidate gene approach, we identified, in all affected individuals, a homozygous substitution (973T>A) in the gene encoding for Matrilin 3 (MATN3), a cartilage specific protein. The mutations changed a cysteine into a serine in the EGF-domain of MATN3, in a region highly conserved across species.

- We conclude that homozygous MATN3 mutations are responsible for this novel form of SEMD.

legs, waddling gait, wide joints (especially the knees), with limited elbow extension, pectus excavatum, and lumbar lordosis. At age 10 years, she underwent bilateral osteotomy of both proximal tibiae due to severe bowing. Examination at age 12 years showed the same changes (height at -4 SD), severe waddling gait, and very pronounced bowing of lower limbs.

Patient 2, the brother of the propositus (figs 1 and 2), had short limbs at birth (length 45 cm (-2 SD), head circumference 34 cm). At 4 months, he had bowed lower legs, and mild lumbar lordosis (length 55 cm, -3 SD). On follow-up, he was noted to have short limbs, severe short stature, small chest, lumbar lordosis, and waddling gait. No hyperlaxity of joints was noted.

Patient 3 is the second brother of the propositus (fig 1). At 33 weeks of gestation, ultrasound had detected short limbs.

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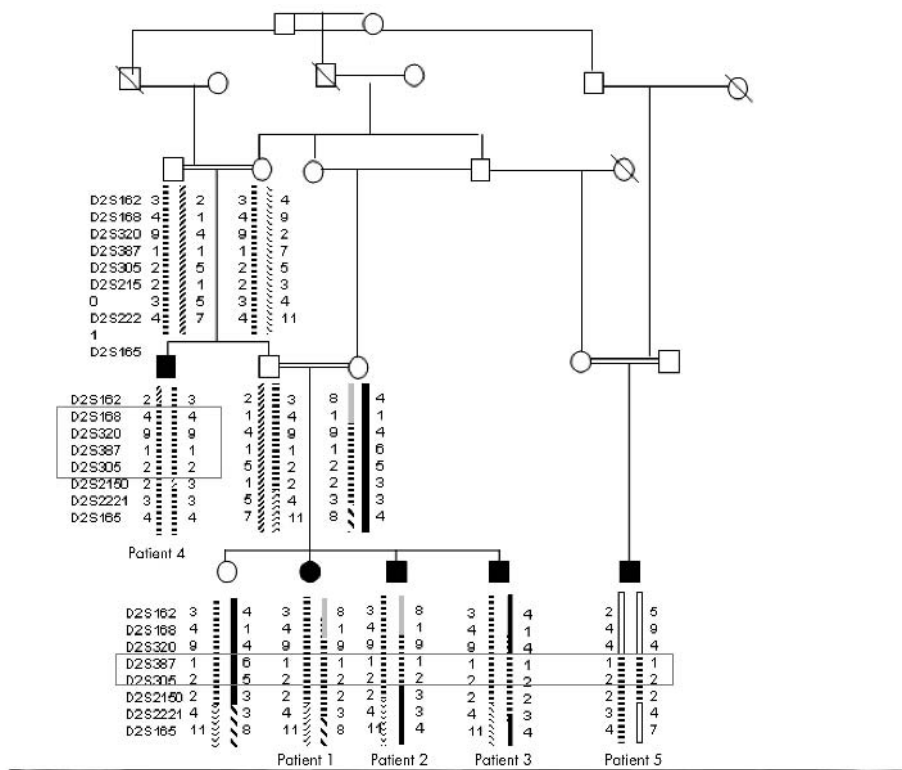
Figure 1 Pedigree of the family and genotype analysis of eight markers within the linked region on chromosome 2.
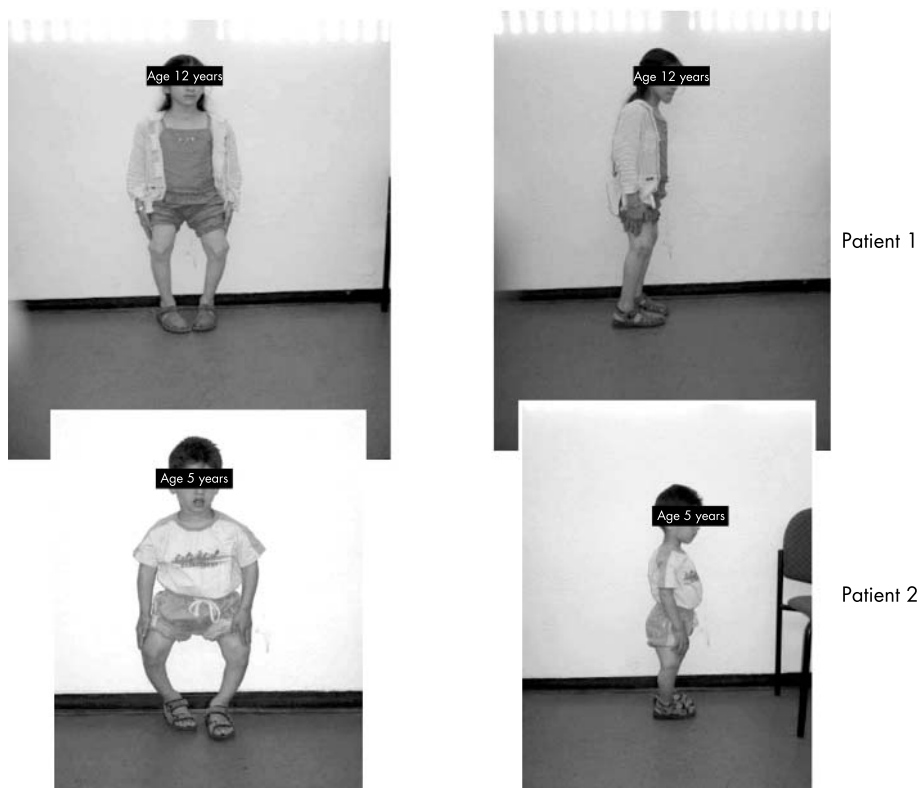

Figure 2 Patients 1 and 2: total AP and lateral view of the body. Note the short trunk and severe bowing of legs.

He was delivered normally at 39 weeks and examination at birth showed short limbs (length 44 cm (-2 SD), head circumference 33 cm). Recent examination at 4 months showed short limbs and bowed thighs. No chest narrowing or joint hyperlaxity was noted.

Patient 4, the brother of the father of the propositus (fig 1), was born to parents who were first cousins. He was first examined at 23 years of age (head circumference 52.5 cm (-2.5 SD), height 136 cm (-5 SD), arm span 147 cm, palm length 11 cm (90th centile), middle finger length 7.6 cm (50th centile)). He had a waddling gait since he started walking (at 1 year), short limbs with severe short stature, bowing of lower limbs, and severe lumbar lordosis. At 13 years of age, he underwent bilateral osteotomy of both proximal tibiae for severe bowing. On follow-up at age 32, he is married with four children, all with regular height.

Patient 5 is the half-cousin of the propositus (fig 1). He was born to parents who were first cousins after a 41 week pregnancy (birth weight 3000 g). Waddling gait was noted when he began walking at approximately 1 year of age. At age 11.5 years, he had short-limbed short stature (height of 117 cm (-4 SD), head circumference 51 cm (10th centile), arm span 122 cm, palm length 5.2 cm (50th centile), middle finger length 3.2 cm (3rd centile)). He had a flat face, normal shaped chest, short limbs, hyperlordosis of the lumbar area, wide joints, with limited extension of elbows, and bowed lower limbs. On follow-up at age 23 years (height 130 cm), the same clinical features were noted. He conducted a normal life and drove a car.

x Ray radiographs of patient 3 at birth were similar to those at 3 months (fig 3A) and showed ribs of normal length, with posteriorly marked cupping. In the lateral projection, most of the vertebral bodies had an oval shape. In the AP projection, vertebral bodies were flat. The long tubular limb bones (except those of the hands and feet) were abnormally short with a stocky appearance and relatively wide metaphyses with lateral spurs. The epiphyses of the knees were irregularly shaped and small, and those of the proximal femurs were markedly underossified for age. The tubular bones of the hands were normal. The iliac wings were dysplastic and square with narrowing of the greater sciatic notch. The acetabular roof was flat, projecting medially from the roof with a bony spike, giving rise to a trident configuration of the iliac bone. The pubic bones were hypoplastic with small and underossified ischia. Epiphyseal and metaphyseal changes as well as spinal changes increased in severity with time (fig 3B,D).

It should be emphasised that in the family reported here, none of the obligate heterozygote parents or the carrier healthy sib sister of the propositus (fig 1) ever complained of knee or hip pain or hand osteoarthritis or underwent surgery for early-onset osteoarthritis. A complete skeletal radiographic evaluation of the propositus' parents as adults, and of the healthy sib sister as a teenager, did not reveal any skeletal changes or any epiphyseal dysplasia.

Approval was obtained from the National Helsinki Committee for Genetic Research in Humans, and informed consent was given by family members or parents of the affected minors. Homozygosity mapping of the disease gene was performed using a panel of 400 markers at an average distance of 10 cM. Genomic DNA was extracted from leukocytes according to standard procedures. The MLINK program of the linkage software package was used to calculate two-point LOD scores between the disease phenotype and each of the markers, assuming a recessive disease with an allele frequency of 0.001.

A series of 18 primers was used to amplify the eight coding exons of the MATN3 gene (including intron–exon boundaries) as previously described.[7]

Amplified products were purified and sequenced using the fluorescent dideoxyterminator method on an ABI 3100 automatic sequencer.

RESULTS

Using a homozygosity mapping strategy, we found linkage of the disease gene on chromosome 2p25–24 (Z_{max} = 3.46 at $\theta = 0$ at locus D2S305, table 1).
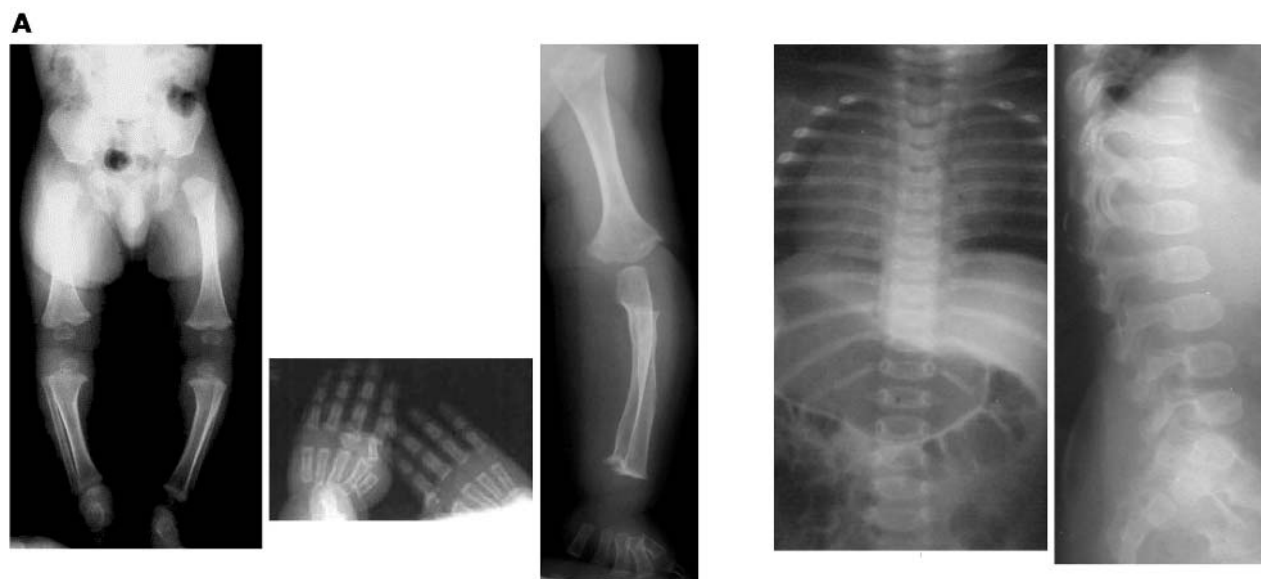

Figure 3A Skeletal findings in affected individuals. At 3 months (patient 3). The long tubular limb bones are abnormally short with a stocky appearance and relatively wide metaphyses with lateral spurs. The epiphyses of the knees are irregularly shaped and small, and those of the proximal femurs are markedly underossified for age. The tubular bones of the hands are normal. Ribs are of normal length, with posteriorly marked cupping, and vertebral bodies are oval on lateral projection and flattened on AP projection. The iliac wings are dysplastic and square with narrowing of the greater sciatic notch. The acetabular roof is flat, projecting medially from the roof with a bony spike, giving rise to a trident configuration of the iliac bone. The pubic bones are hypoplastic with small and underossified ischia.

A recombination event between loci D2S320 and D2S387 in patients 3 and 5 defined the proximal boundary of the genetic interval and another recombination event between loci D2S305 and D2S2150 in patients 2 and 4 defined the distal boundary of the candidate region (2.4 Mb, fig 1).

A search of the human genome resources of the National Center for Biotechnology Information (NCBI) disclosed several putative disease genes within the candidate region including lysosomal-associated protein transmembrane 4 (LAPTM4A), matrilin 3 (MATN3), and Syndecan 1 (SDC1). Of these genes, MATN3 was considered the strongest candidate gene owing to its restrictive expression in cartilage.[9][10] Moreover, dominant mutations in the region encoding the von Willebrand domain (vWFA) of MATN3 have been recently associated with multiple epiphyseal dysplasia.[7]

Figure 3B Skeletal findings in affected individuals. At 1 year (patient 2). Epiphyses are irregularly shaped and small in all long bones, especially at the proximal femurs. Metaphyses are wide with same changes as at 3 months. Vertebral bodies continue to have the flattened-oval shape seen at the earlier age as well as posteriorly marked cupping.
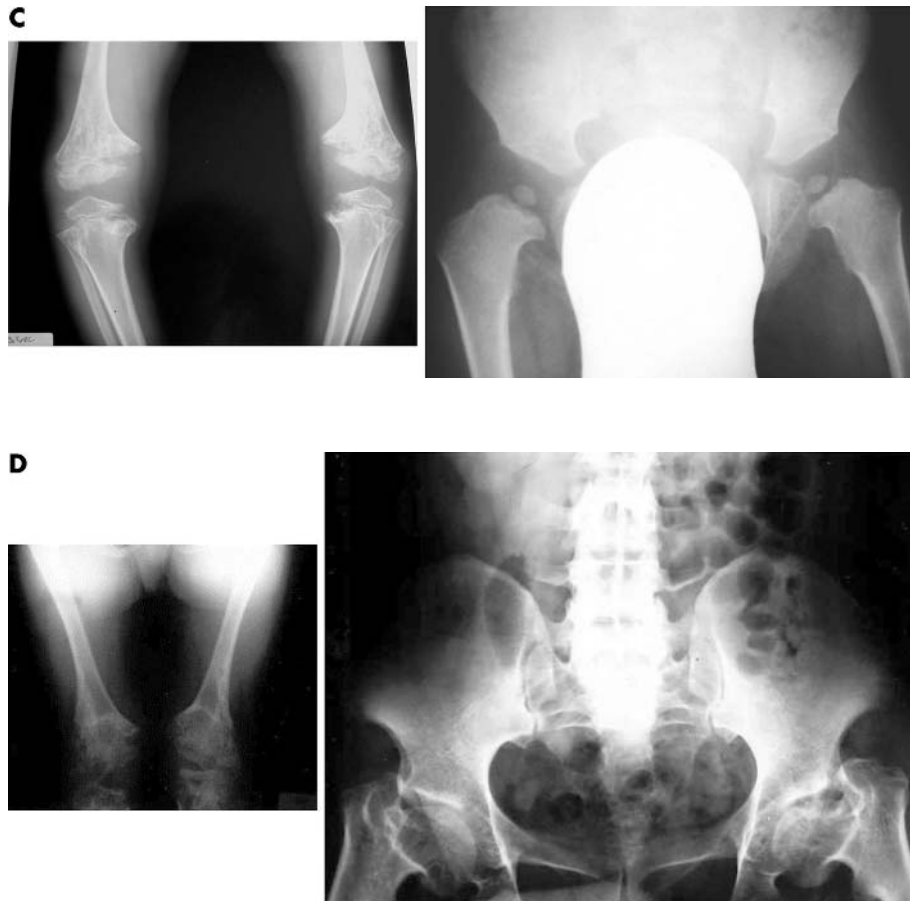
Figure 3C, D Skeletal findings in affected individuals. (C) At 12 years (patient 1). Note the severe epi-metaphyseal changes of long bones and the severe small proximal epiphyses of femur. Iliac bones are hypoplastic with steep acetabular roofs, and with no trident configuration as seen at an earlier age. (D) At 25 years (patient 5). Note the severe epi-metaphyseal changes seen in all long bones.

Sequence analysis of MATN3 (GenBank accession number NM_002381) in the affected individuals revealed a homozygous substitution (973 T>A), which changed a cysteine into a serine in the first EGF domain of MATN3 (C304S).

The mutation cosegregated with the disease (fig 4) and was not found by direct sequencing in 100 chromosomes from the same ethnic origin.

DISCUSSION

We report here homozygous mutations (C304S) in the region encoding for the first EGF like-domain of matrilin-3 in an apparently new autosomal recessive form of SEMD.

Matrilin-3 belongs to a family of four oligomeric extracellular proteins and has a restricted expression pattern to cartilage.[9–12] Although the tissue specific expression patterns of matrilin-1 and -3 are similar, their levels of expression are different during embryonic development and matrilin-3 appears to be more highly expressed than matrilin-1 during early development of the epiphyseal growth plate.[11] The functions of matrilins have not been completely defined but they may play a role in stabilising the extracellular matrix structure. They can self-associate into supramolecular structures resulting in the formation of filamentous networks which can be associated with collagen fibrils. Matrilin-1 has been shown to bind to collagen type 2 in cartilage and also to interact with agregan.[13] All matrilins share a similar structure including a Von Willebrand factor A domain, a varying number of epidermal growth factor domains, and a C-terminal coiled-coil domain.[9 10 14] While matrilin-1 only contains one EGF domain, matrilin-3 lacks one A domain and contains four EGF-like domains. The matrilins form homo- and hetero-multimers mediated though the C-terminal coiled-coil domain and matrilin-3 has been shown to form a heteromultimer only with matrilin-1.[10 14] The vWFA domain is a collagen binding domain and engineered mutations within the metal ion-dependent

Table 1 Two-point LOD scores for linkage of an SEMD locus to chromosome 2p24

Loci	Distance	Recombination fraction					Z_{max}	θ
		0.00	0.05	0.10	0.20	0.30		
D2S320	1.22	$-\infty$	−0.942	−0.59	−0.39	−0.29	−0.29	0.30
D2S387	0.07	3.15	2.87	2.58	1.95	1.27	3.15	0.00
D2S305	1.1	3.46	2.18	1.89	1.31	0.75	3.46	0.00
D2S2150		$-\infty$	−0.824	−0.183	−0.079	0.19	0.19	0.30

Distance is given in megabases according to the Human Genome working draft, http://genome.usc.edu.
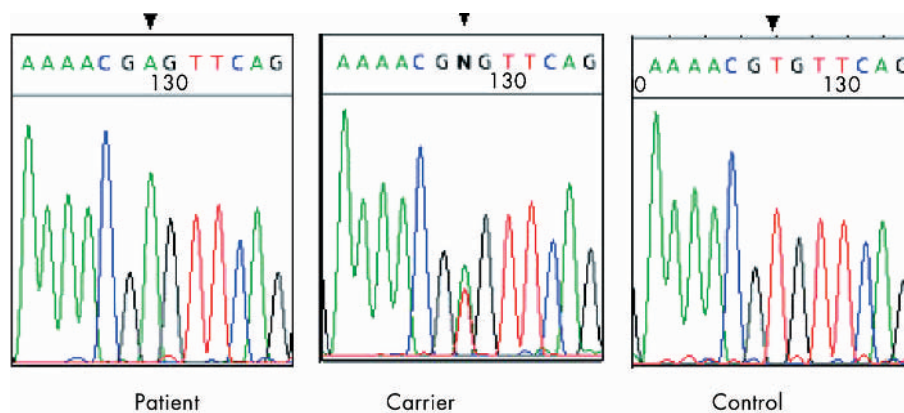
Figure 4 Sequence analysis of MATN3 in the family. The homozygous mutant (973T>A), heterozygous and normal sequences are shown.

adhesion site motif of the vWFA domain of matrilin-1 reduces its ability to associate with the ECM network.[13] The mutations that we have identified in the SEMD family reported here changed a cysteine residue located in the first EGF domain. Each EGF-like domain contains six cysteines and all of them (including the C304) are highly conserved across species. Although the role of these cysteine residues in matrilin-3 has not yet been demonstrated, they are presumably involved in intermolecular disulfide bonds as previously shown for cysteine residues in EGF-like domains in other human diseases such as Marfan syndrome or CADASIL.[15][16]

This finding also expands the phenotypic spectrum of matrilin-3 mutations. Indeed, dominant mutations in the region encoding the von Willebrand domain (vWFA) of matrilin-3 have been identified in families with multiple epiphyseal dysplasia (MED).[7][17] These mutations were missense mutations (V194D, R121W, A128P) located within the vWFA domain of the protein. More recently, a dominant mutation in the region encoding the first EGF domain has been found to cosegregate with hand osteoarthritis (OA) in several families.[8] The mutation was a missense mutation changing a threonine to a methionine. In the family reported here, none of the heterozygote parents or the healthy sibs of the affected individuals ever complained of knee or hip pain or hand osteoarthritis or underwent surgery for early-onset osteoarthritis nor did radiographic evaluation reveal any degree of epiphyseal dysplasia. It seems therefore that a heterozygote state does not have any clinical or radiographic effects in this disorder. Although mutations in MATN3 causing MED or this novel form of SEMD are all missense, their consequences are certainly distinct as they are clearly not located in the same domains (that is, the von Willebrand/EGF domain). By contrast, the mutation identified in hand OA is also located in the first EGF domain, but presumably it does not have the same effect on the function of protein based on the amino-acid change (that is, threonine change in hand OA/cysteine change in SEMD).

Similar situations, where heterozygotes for a recessive mutations do not exhibit the dominant phenotype, have been observed.[18–21] Indeed, heterozygous ROR2 mutations cause brachydactyly type B, whereas homozygous mutations cause Robinow syndrome and heterozygous parents of those with Robinow syndrome do not have brachydactyly.[18][19] Similarly, heterozygous IHH mutations cause brachydactyly type A1 (BDA1), whereas homozygous mutations cause acrocapital femoral dysplasia (ACFD) with no manifestations in the heterozygote parents.[20][21] The mutations in IHH causing either BDA1 or ACFD are both missense mutations, but their accurate locations within the amino terminal domain differ.

One possible explanation is that dominant mutations may exert a dominant negative effect whereas homozygous mutations would cause a loss of function.

We conclude that MATN3 is another component of the intracellular matrix involved in the SEMD group. A better understanding of the interactions of MATN3 with the other extracellular matrix proteins, particularly COMP, type II and IX collagens, will certainly shed light on the pathogenesis of these chondrodysplasias.

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Conflicts of interest: none declared.

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ECHO ....

Female preclinical presenilin-1 mutation carriers unaware of their genetic status have higher levels of depression than their non-mutation carrying kin

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Please visit the *Journal of Medical Genetics* web-site [www.jmedgenet.com] for a link to the full text of this article.

Objectives: To study depressive symptoms in preclinical presenilin-1 (PS1) related Alzheimer's disease.

Methods: Participants were 33 Mexican women at risk for inheriting PS1 mutations who were not demented. They were interviewed, underwent cognitive testing, and completed the Beck depression inventory (BDI). PS1 mutation status was determined. Mean BDI scores were compared between PS1 mutation carriers and non-carriers. The percentage of subjects who reported seeing a psychiatric professional, and the percentage complaining of memory loss were compared between groups. Regression analysis was used to determine whether mutation status predicted BDI scores after adjusting for age, education, mini-mental state examination, and subjective memory function.

Results: PS1 mutation carriers (n = 17) scored significantly higher than non-carriers (n = 16) on the BDI (mean score, 14.4 *v* 6.5, p = 0.017); 24% of mutation carriers and 12.5% of non-carriers admitted having sought help from a psychiatric professional (NS). Mutation status remained a significant predictor of BDI scores after adjusting for potential covariates. Though not demented, mutation carriers tended to score lower than non-carriers on several neuropsychological tests.

Conclusions: Depressive symptoms can occur early in the course of PS1 related Alzheimer's disease, at least in women. This supports the hypothesis that depression may occur as a direct result of the neuropathology underlying Alzheimer's disease.

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