



Case Report

Spondylo-epi-metaphyseal dysplasia due to a homozygous missense mutation in the gene encoding Matrilin-3 (T120M)

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ABSTRACT

Introduction: Spondylo-epi-metaphyseal dysplasia (SEMD) represents a group of osteo-chondrodysplasias characterized by vertebral, epiphyseal as well as metaphyseal abnormalities. Several genes have been identified underlying the different forms.

Methodology and results: Two relatives (cousins) in a family were found to have disproportionate short stature with clinical and radiological features suggestive of SEMD. Metabolic bone profile was normal including parathyroid hormone and 25(OH)vitamin D3. Exome sequencing revealed a missense mutation (p. T120M) in the von-Willebrand factor A-domain of the Matrilin 3 (MATN3) gene that segregates with the disease in the family.

Conclusion: We identified a homozygous missense mutation in MATN3, an important structural component of the extracellular matrix of cartilage, as the genetic cause of SEMD in this pedigree. MATN3 mutations have been more commonly associated with multiple epiphyseal dysplasia than SEMD. Recognition of this mutation will aid in enhancing the understanding and expanding the spectrum of this particular skeletal dysplasia.

1. Introduction

Spondylo-epi-metaphyseal dysplasia (SEMD OMIM 608728) is a form of osteo-chondrodysplasia characterized by vertebral, epiphyseal and metaphyseal anomalies. Multiple clinical entities exist within this subgroup as listed in the revised international classification of bone disorders (Warman et al., 2011). Inheritance in such cases follows a Mendelian pattern with autosomal dominant, autosomal recessive or X-linked recessive forms having been described. Mutations causing SEMD can affect structural proteins of the extracellular matrix (aggrecan, matrilin), cation channels (TRPV4, zinc transporter), post-translational processing (sulfation proteins, lysosomal disorders) or chromatin regulation (SMARCA1).

Of these, SEMD due to matrilin-3 gene (MATN3) mutation is extremely rare (Borochowitz et al., 2004). Mutations in MATN3 have been described as monogenic causes of multiple epiphyseal dysplasia (Von willebrand factor domain) and hand osteoarthritis (EGF domain) and (Chapman et al., 2001; Stefánsson et al., 2003). This report highlights SEMD in a pair of cousins attributable to bi-allelic MATN3 mutations, expanding the spectrum of both MATN3 mutations and autosomal recessive SEMD.

2. Patients and methods

In this family, two affected individuals were found to have disproportionate short stature with clinical and radiological features suggestive of SEMD.

2.1. Patient 1

The proband was a 13-year-old girl who presented with growth failure (Fig. 1). She was born to healthy non-consanguineous parents of normal stature. Clinical evaluation revealed disproportionate short stature with a height of 98 cm, height SDS of -8.2 , upper segment 48.5 cm, lower segment 49.5 cm (US:LS = 0.97) and arm span of 95 cm. She had a normal neuromotor development. Her parents noticed bowing of both knees and elbows since infancy which was progressive. Her neurological examination, cardiovascular examination, hearing and visual acuity were otherwise normal. She had no fractures, chronic renal disease, delayed dentition or dental abscesses. There was no clinical evidence of rickets. Pubertal status was normal with menarche attained at the age of 12 years. Her family history was significant with similar complaints in her cousin brother. The pedigree chart of the

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Fig. 1. 1a- Clinical image of both patients (13 year old proband and her 10 year old cousin brother) showing disproportionate short stature and short limbs with genu varum.
 1b- Follow-up clinical photograph of the proband at 18 years of age showing persistent short stature and severe genu varum.
 1c- Homozygous *MATN3* mutation causing severe phenotype in the proband with normal phenotype in both flanking parents who are heterozygous for this mutation.

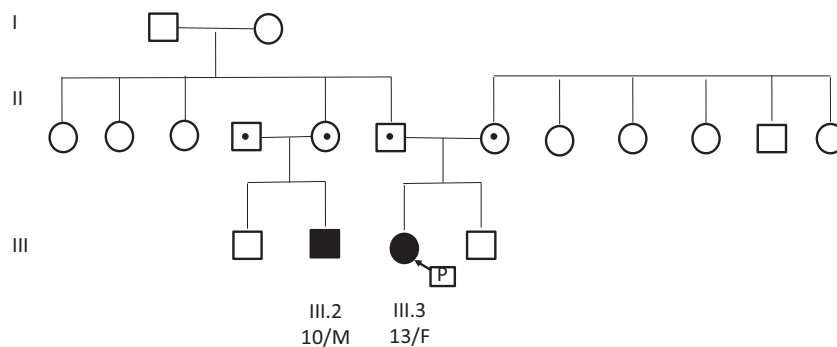


Fig. 2. Pedigree of the family with the two affected cousins affected by an autosomal recessive form of SEMD due to *MATN3* mutation. Heterozygous carriers (parents) are depicted by a dot in the middle.

family is depicted in Fig. 2. Routine biochemistry, thyroid function test, IGF-1 and thyroid ultrasound of the proband were normal. She had hypovitaminosis D (< 4 ng/ml) with normal iPTH of 34.4 pg/ml. Radiographs (Fig. 3) showed brachydactyly with metaphyseal widening, cupping and terminal spurs. Dysplastic epiphyses were also noted both at the wrists and knees. Lateral radiograph of the lumbar spine showed bullet shaped oval vertebrae with flattening of surfaces (platyspondyly) and mild posterior humping. A ^{99m}Tc MDP bone scan showed increased tracer uptake at epiphyseal ends of all long bones except humeral heads. She was advised vitamin D and calcium supplementation in standard doses. Re-evaluation at 1 year showed normalization of biochemical profile with further disproportion of limbs and bowing of legs. Follow-up after 5 years showed a persistent severe short stature and bowing of the limbs (Fig. 5).

2.2. Patient 2

This boy, cousin of the proband, presented at 10 years of age with similar complaints of growth failure. He also had healthy parents of normal stature and there was no history of consanguinity. He was born

at term and had a normal perinatal course. Later at 1 year of age, his parents noticed increasing curvature of the legs followed by progressive curvature of the arms. At presentation, he had disproportionate short stature with height of 94 cm corresponding to -7.4 SDS, upper segment 46 cm, lower segment 48 cm (US:LS = 0.95) and arm span 91 cm. There was again no history of fractures, no stigmata of rickets or dental anomalies. General physical and rest of systemic examination were within normal limits. Investigations revealed a normal bone age as well as normal biochemistry, thyroid function tests and IGF1. Serum 25(OH) vitamin D3 was 12.0 ng/ml and intact parathyroid hormone was 16.50 pg/ml. Radiographs showed similar findings as the proband, however the epi-metaphyseal changes and platyspondyly were less pronounced(Fig. 4). A ^{99m}Tc MDP bone scan showed decreased tracer uptake at epiphyseal ends of long bones except humeral head on both sides. He was also prescribed cholecalciferol and calcium supplementation. Re-evaluation at 1 year showed further disproportion of limbs, with bowed legs. His repeat biochemical profile was normal.

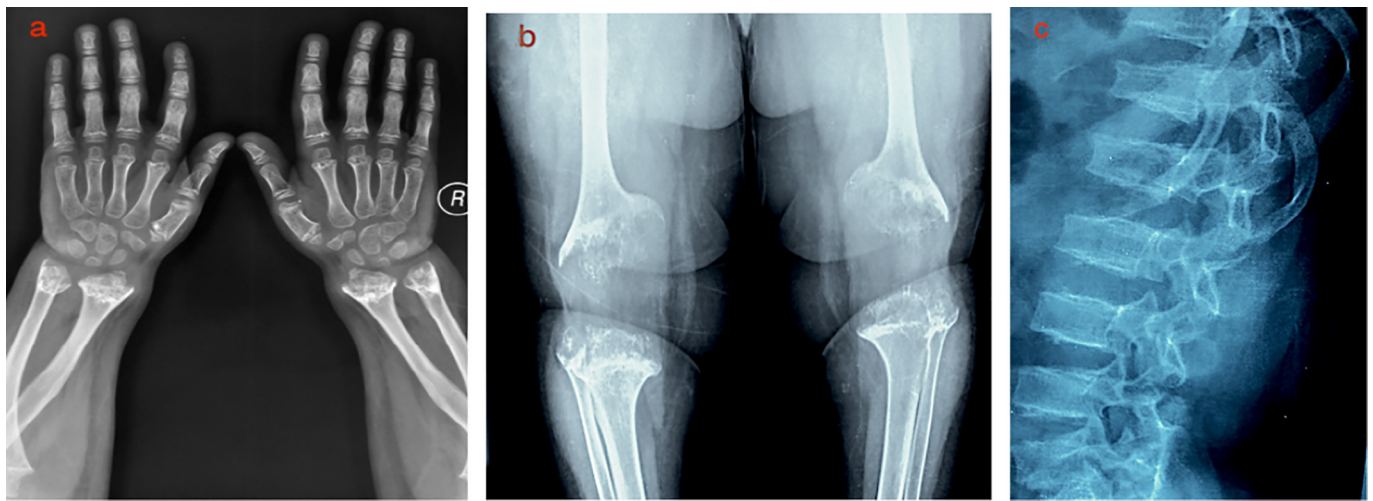


Fig. 3. Radiographs of the proband taken at the age of 13 years. Both hands show brachydactyly with metaphyseal widening and cupping of distal ulna and radius (3a). The epiphyses are small and dysplastic. Both knees are abnormal and reveal severe epimetaphyseal changes (3b). The lumbar vertebrae are flattened (platyspondyly) with mild posterior humping (3c).

2.3. Genetic analysis

Genomic material was isolated from peripheral blood leukocytes of both patients and their parents after written, informed consent. The extracted DNA was subjected to exome sequencing by NGS (Next generation sequencing) in one patient. Segregation of identified variants were checked by Sanger sequencing. Bioinformatic analysis conducted later identified a homozygous missense mutation (c.359C > T) (T120M) in the *MATN3* gene that segregates with the disease in the family. Both sets of parents were detected to be heterozygous carriers for this specific mutation (Fig. 6). In silico analysis supported the pathogenicity of this variant with a CADD-score of 27.7 and Mutation taster and SIFT predicting it to be damaging. Two additional variants were identified in the *MATN3* gene. These variants are synonymous variants involving aminoacids A149 and E205 and were homozygous in the patients and heterozygous in the parents. Exome analysis did not reveal any other mutations or variants known to cause SEMD.

3. Discussion

This report highlights a rare autosomal recessive cause of severe SEMD due to a homozygous missense mutation (T120M) in the von Willebrand factor (vWF) A domain of the *MATN3* gene. Genetic analysis identified this rare mutation in both affected patients (homozygous) and revealed their parents as carriers (heterozygous). There is no formal proof for a relationship between all four parents but we identified two

additional *MATN3* variants for which all parents were heterozygous. This supports the hypothesis that in all cases the mutation is on the same genetic background. It also expands the current repertoire of knowledge regarding the genetics and phenotype of this rare condition.

SEMD is a heterogeneous group of osteo-chondrodysplasias with common characteristics in the form of vertebral and epi-metaphyseal disorders. Metaphyseal involvement is the most variable component. Distinct genetic forms with autosomal dominant and recessive transmission are characterized (Cormier-Daire, 2008). The pattern of bony involvement and extra skeletal features in a given patient ultimately help to narrow down to a specific diagnosis. It is classified as a distinct entity under the International Nosology and classification of genetic dysplasias, but may be regarded as a MED (Multiple epiphyseal dysplasia) like condition with some genetic and clinico-radiologic heterogeneity.

The matrilin family is an important component of extracellular matrix (ECM) proteins consisting of four members that are differentially expressed in skeletal tissues (Deak et al., 1999). The mechanism by which matrilins stabilise the ECM structure is by self-association to form supramolecular filamentous networks. Matrilin 1 and matrilin 3 are similar in terms of expression in the physis (growth plate) but the latter is different owing to its high expression during early development (Belluoccio et al., 1998). Matrilin 3, like other members of its family, consists of a vWFA domain, epidermal growth factor (EGFR) domain and a C-terminal coiled-coil domain (Frank et al., 2002), but unlike other members, is highly specific to cartilage tissue. Missense *MATN3*

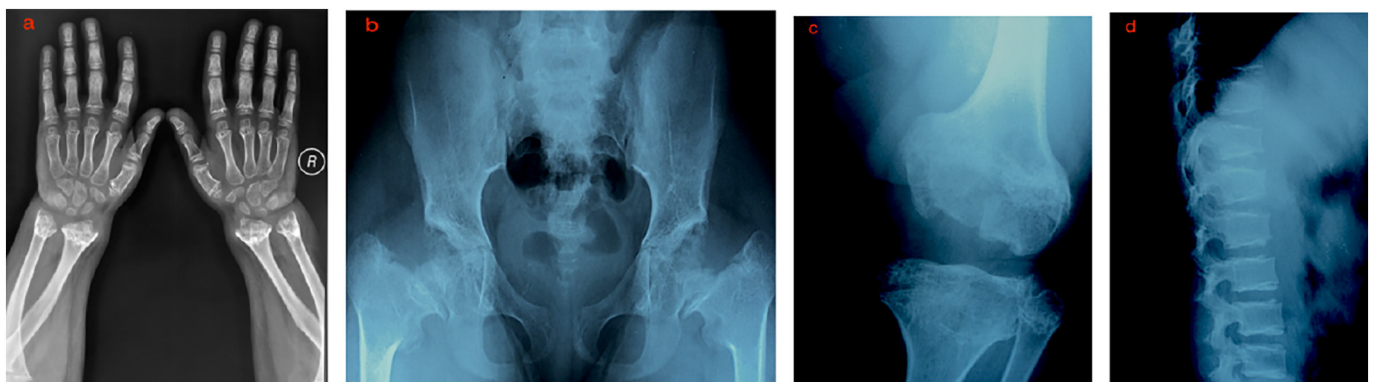


Fig. 4. Radiographs of the male cousin taken at the age of 10 years. Similar findings as in the proband (4a, 4c), however the platyspondyly is less pronounced (4d) as are the epi-metaphyseal changes in the knees. The pelvis is abnormal with coxa vara (4b).

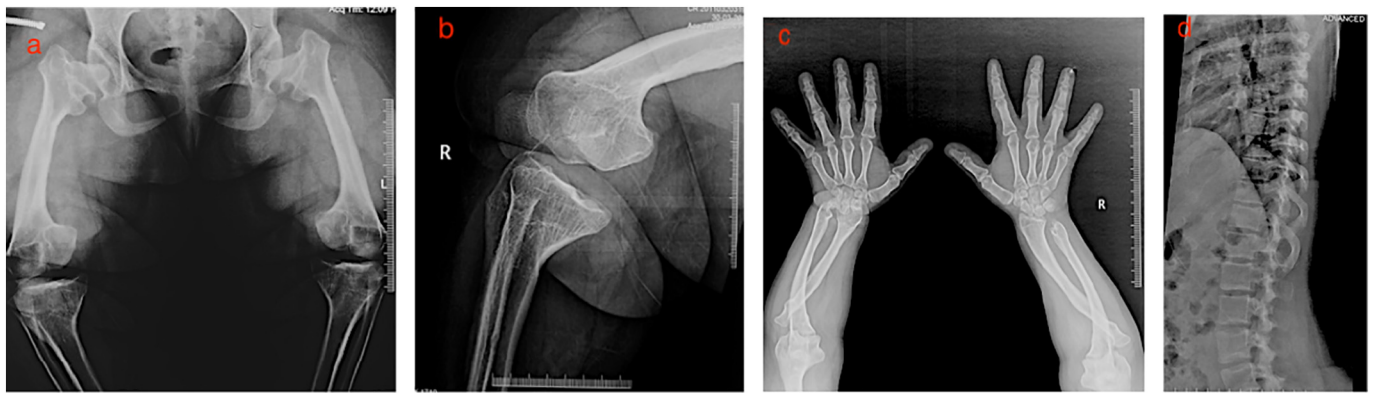


Fig. 5. Representative follow-up radiographs of the proband at 18 years of age showing persistent poor development of epiphyses at the knees and elbows (5a–c). Flattened vertebral bodies are also noted (5d).

mutations disturb protein trafficking to the Golgi apparatus, cause cytosolic accumulation of the matrilin 3 protein and affect the ECM as well as chondrocyte differentiation (Otten et al., 2005), ultimately causing osteo-chondrodysplasia.

Clinical presentation of the matrilin type of SEMD is fairly similar to the other SEMDs. Growth parameters at birth in individuals affected with this condition are usually normal, but rarely, short birth length may be noted. Postnatal growth failure is marked early in life with characteristic bowing and shortening of the limbs. Same was observed in both our patients who had disease onset in infancy. Vision and hearing with *MATN3* SEMD are normal, unlike other SEMDs which can be marked by corneal, lenticular, retinal defects or sensorineural hearing loss. Extra skeletal features described with other SEMDs like diabetes, hypothyroidism, renal anomalies or valvular heart defects are also lacking in this subtype. Our patients also lacked any significant

extra skeletal features. Similarly, psychomotor development and craniofacial features are usually normal. On the other hand, genu varum, lumbar lordosis, pectus excavatum and limited elbow extension are common. The overall phenotype of an affected patient is that of a disproportionate severe short stature with short and bowed limbs.

Radiological survey reveals vertebral platyspondyly, short and stocky limbs with widened metapyseal ends, cupping and lateral spurs in affected patients. Epiphyses are dysplastic and underossified. X-Ray pelvis reveals hypoplastic pubic bones and ischia. A trident configuration of the iliac bone (medial spike of the acetabular roof) has been described in some, which may disappear later (Stefánsson et al., 2003). The epi-metaphyseal changes often worsen with time and corrective surgery of the lower limbs have been successfully reported during adolescence (Stefánsson et al., 2003).

Homozygous missense vWFA mutations of the *MATN3* gene have

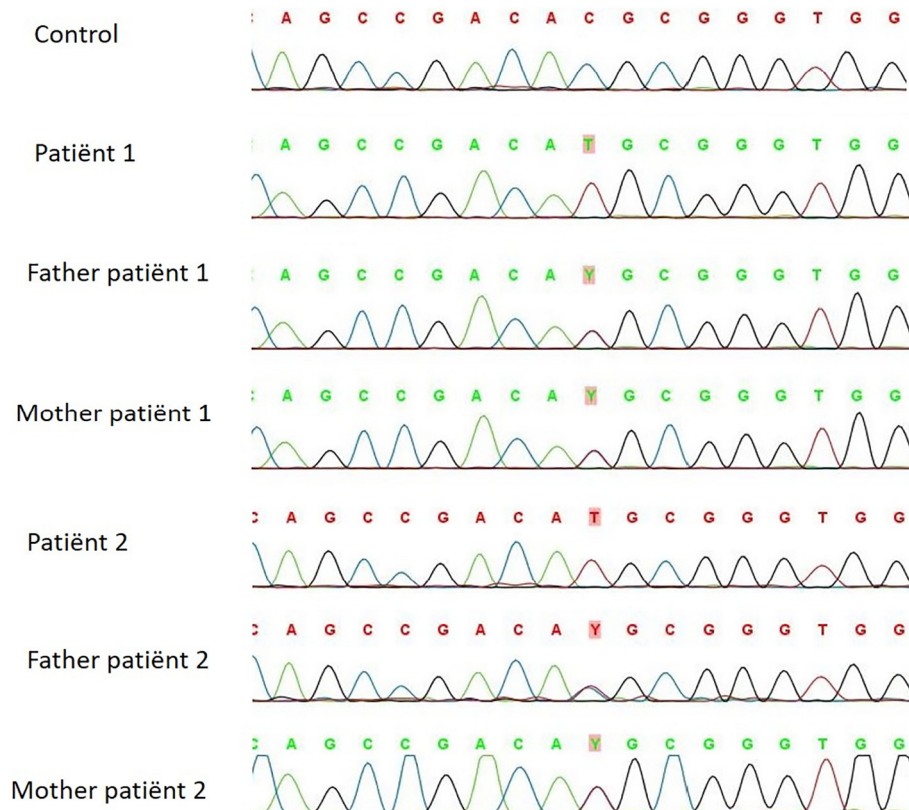


Fig. 6. Electropherogram of the affected patients and their parents demonstrating a homozygous missense mutation (c.359C > T) (p.T120M) in the *MATN3* gene and heterozygous carrier state in both parents.

been reported in MED cases while those in the EGF domain (missense type replacing threonine with methionine) have been associated with hand osteoarthritis. Heterozygous parents or siblings have been reported to be normal both clinically and radiologically. *MATN3* mutations were first identified as possibly causative for skeletal dysplasias by Chapman et al. (Chapman et al., 2001). Its role was further delineated in familial MED by Mostert et al. and Jackson et al. (Mostert et al., 2003; Jackson et al., 2004). Borochowitz et al. reported a homozygous C304S *MATN3* mutation in familial SEMD in an Arabic family with consanguinity (Borochowitz et al., 2004). Mutations in *MATN3* are usually associated with mild skeletal disease (with all variants except the R121W which shows variability) and normal or only mildly compromised stature (Mabuchi et al., 2004; Seo et al., 2014). Our kindred showed two affected cousins with similar missense mutation at position 120 in the *MATN3* gene that caused a substitution of threonine by methionine, leading to SEMD. However, both patients showed severe disease phenotype and severe short stature, which could be attributed to extensive spinal involvement or the effect of the other identified variants which could be modifying the phenotype.

Long term follow-up of the *MATN3* type of SEMD has shown a good prognosis primarily due to absence of extraskelatal involvement. The peculiar feature of this *MATN3* mutation causing SEMD was that it was a severe disease phenotype. The limitations of this study include the low patient number and the fact that we did not perform functional analysis. However, *MATN3* causing SEMD is a rare disease and prior functional analysis of this variant has been demonstrated to cause intracellular retention of the mutant protein in patients with MED (Cotterill et al., 2005).

4. Conclusion

This report depicts an extremely rare genetic cause of spondylo-epi-metaphyseal dysplasia due to a missense mutation in the gene encoding matrilin-3. Severe short stature and bowed legs without significant extraskelatal involvement are potential clues to diagnose this uncommon entity.

Contributorship statement

LD drafted the manuscript and did clinical assessment as well as follow-up of the patients. VD did data recording and retrieval. WVH performed the genetic analysis and drafted the manuscript. AB oversaw patient management. YG helped in patient management. ES helped in genetic analysis. GM supervised genetic analysis and edited the manuscript. SKB oversaw patient management and edited the manuscript. All authors reviewed and accepted the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Belluocchio, D., Schenker, T., Baici, A., Trueb, B., 1998. Characterization of human Matrilin-3 (*MATN3*). *Genomics* 53, 391–394.
- Borochowitz, Z.U., Scheffer, D., Adir, V., Dagoneau, N., Munnich, A., Cormier-Daire, V., 2004. Spondylo-epi-metaphyseal dysplasia (SEMD) matrilin 3 type: homozygote matrilin 3 mutation in a novel form of SEMD. *J. Med. Genet.* 41 (5), 366–372 May 1.
- Chapman, K.L., Mortier, G.R., Chapman, K., Loughlin, J., Grant, M.E., Briggs, M.D., 2001. Mutations in the region encoding the von Willebrand factor A domains of matrilin-3 are associated with multiple epiphyseal dysplasia. *Nat. Genet.* 28, 393–396.
- Cormier-Daire, V., 2008. Spondylo-epi-metaphyseal dysplasia. *Best Pract. Res. Clin. Rheumatol.* 22 (1), 33–44 Mar 1.
- Cotterill, S.L., Jackson, G.C., Leighton, M.P., Wagener, R., Mäkitie, O., Cole, W.G., Briggs, M.D., 2005. Multiple epiphyseal dysplasia mutations in *MATN3* cause misfolding of the A-domain and prevent secretion of mutant matrilin-3. *Hum. Mutat.* 26 (6), 557–565 Dec.
- Deak, F., Wagener, R., Kiss, I., Paulsson, M., 1999. The matrilins: a novel family of four oligomeric extracellular matrix proteins. *Matrix Biol.* 18, 55–64.
- Frank, S., Schulthess, T., Landwehr, R., Lustig, A., Mini, T., Jenö, P., Engel, J., Kammerer, R.A., 2002. Characterization of the matrilin coiled-coil domains reveals seven novel isoforms. *J. Biol. Chem.* 277 (21), 19071–19079 May 24.
- Jackson, G.C., Barker, F.S., Jakkula, E., Czarny-Ratajczak, M., Mäkitie, O., Cole, W.G., Wright, M.J., Smithson, S.F., Suri, M., Rogala, P., Mortier, G.R., 2004. Missense mutations in the β strands of the single A-domain of matrilin-3 result in multiple epiphyseal dysplasia. *J. Med. Genet.* 41 (1), 52–59 Jan 1.
- Mabuchi, A., Haga, N., Maeda, K., Nakashima, E., Manabe, N., Hiraoka, H., Kitoh, H., Kosaki, R., Nishimura, G., Ohashi, H., Ikegawa, S., 2004. Novel and recurrent mutations clustered in the von Willebrand factor A domain of *MATN3* in multiple epiphyseal dysplasia. *Hum. Mutat.* 24 (5), 439–440 Nov.
- Mostert, A.K., Dijkstra, P.F., Jansen, B.R., Van Horn, J.R., De Graaf, B., Heutink, P., Lindhout, D., 2003. Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation: further delineation of the phenotype including 40 years follow-up. *Am. J. Med. Genet. A* 120 (4), 490–497 Aug 1.
- Otten, C., Wagener, R., Paulsson, M., Zaucke, F., 2005. Matrilin-3 mutations that cause chondrodysplasias interfere with protein trafficking while a mutation associated with hand osteoarthritis does not. *J. Med. Genet.* 42 (10), 774–779 Oct 1.
- Seo, S.G., Song, H.R., Kim, H.W., Yoo, W.J., Shim, J.S., Chung, C.Y., Park, M.S., Oh, C.W., Jeong, C., Song, K.S., Kim, O.H., 2014. Comparison of orthopaedic manifestations of multiple epiphyseal dysplasia caused by *MATN3* versus *COMP* mutations: a case control study. *BMC Musculoskelet. Disord.* 15 (1), 84 Dec.
- Stefánsson, S.E., Jónsson, H., Ingvarsson, T., Manolescu, I., Jónsson, H.H., Ólafsdóttir, G., Pálsdóttir, E., Stefánsdóttir, G., Sveinbjörnsdóttir, G., Frigge, M.L., Kong, A., 2003. Genomewide scan for hand osteoarthritis: a novel mutation in matrilin-3. *Am. J. Hum. Genet.* 72 (6), 1448–1459 Jun 1.
- Warman, M.L., Cormier-Daire, V., Hall, C., Krakow, D., Lachman, R., LeMerrer, M., Mortier, G., Mundlos, S., Nishimura, G., Rimoin, D.L., Robertson, S., 2011. Nosology and classification of genetic skeletal disorders: 2010 revision. *Am. J. Med. Genet. A* 155 (5), 943–968 May.