

Exclusion of the Dymeclin, and PAPSS2 genes in a novel form of spondyloepimetaphyseal dysplasia and mental retardation.

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Spondyloepimetaphyseal dysplasias (SEMD) represent a heterogeneous group of conditions composed of at least fifteen well defined entities. The classification is based on clinical, radiological and molecular findings. Among them, several conditions also include a mental retardation (MR) syndrome, namely Wolcott Rallison syndrome, Dyggve-Melchior-Clausen syndrome (DMC) and lysosomal storage disorders. Here, we report on a novel form of SEMD with MR in two Pakistani sisters born to first cousin parents. SEMD, MR, microcephaly, ataxia, facial dysmorphism, and hirsutism of back and legs were noted in the two children. Skeletal findings included flat vertebral bodies with irregular vertebral plates, irregular and flared metaphyses with vertical striations, small and irregular epiphyses, small carpal bones and narrow iliac wings without lacy pelvis iliac crest. Similarities with DMC prompted us to test and eventually exclude the DMC gene, dymeclin, by direct sequencing. Similarly, we excluded the PAPSS2 gene (3-prime-alpha phosphoadenosine 5-prime-phosphosulphate synthase 2) responsible for SEMD Pakistani type. The combination of features observed in the two sisters does not fit with any previously reported SEMD and represents therefore a novel form of autosomal recessive SEMD with MR.

Running Title: Novel syndrome with SEMD and mental retardation.

Keywords: Spondyloepimetaphyseal dysplasia, mental retardation, autosomal recessive.

## INTRODUCTION

Spondyloepimetaphyseal dysplasias (SEMD) represent a heterogeneous group of conditions composed of at least fifteen well defined entities. These various forms of SEMD have been characterized based on clinical, radiological and molecular features but, only few of them are associated with MR <sup>1</sup> namely i) Wolcott-Rallison syndrome <sup>2-3</sup> (WR, MIM 226980) due to mutations in the EIF2AK3 gene encoding a translation initiation factor 2-alpha kinase 3 and regulating the synthesis of unfolded proteins in the endoplasmic reticulum <sup>4</sup>, ii) Dyggve-Melchior-Clausen syndrome <sup>5</sup> (DMC, MIM 223800) due to mutations in the dymeclin gene encoding a protein of hitherto unknown function and iii) lysosomal storage disorders <sup>6</sup>. Here, we report on what we believe to be a novel autosomal recessive syndrome of SEMD and MR in two Pakistani sisters.

## PATIENTS REPORT

**Patient 1**, a girl, was born to first-cousin Pakistani parents after term pregnancy and normal delivery (birthweight 3 Kg, height and OFC were unknown). Hypotonia, nystagmus with poor vision and feeding difficulties were noted in the neonatal period. She was able to control her head at 11 months and to sit unaided at 2 years but her gait was unstable. At 8 ½ years, she had no speech, ataxia and was unable to walk unaided (weight = 18.8 Kg; -2 SD ; height = 104 cm, -4.5 SD and OFC = 46.5 cm , -5 SD). No abnormal body proportion was observed. Facial dysmorphism included coarse facies, low anterior and posterior hairline, brachycephaly, simple and flat ears, small and well shaped eyebrows, synophrys, bilateral epicanthi, broad nose, full lips with eversion of the lower lip, short neck, and hirsutism of

back and legs (Figure 1A). At the last examination (11 years), she could walk unaided but with ataxia and her coarse facial appearance had worsened.

**Patient 2**, her younger sister, was born after an uneventful pregnancy (birthweight = 2500 g, length and OFC were unknown). She could sit at 2 years of age and walk unaided at 3 ½ years. She was first seen at 32 months of age for hypotonia with trunkal and gait ataxia (weight = 9.8 Kg, -2.5 SD; length = 83.5 cm, -2 SD; and OFC = 43 cm, -5 SD). She also had repetitive behaviour and no speech. Facial features included brachycephaly, low anterior and posterior hairline, bilateral epicanthus, simple and flat ears with everted lobules, broad nose and full lips with eversion of the lower lip. She also had abnormal pigmentary skin changes on the legs along the Blaschkolines. No abnormal body proportion was observed. At 5 years of age, her coarse facial appearance and failure to thrive had worsened (weight = 11 Kg, -3.5 SD; length = 90 cm, -4 SD; and OFC = 45 cm, -5 SD - Figure 1B).

#### LABORATORY INVESTIGATIONS

Heart and kidney ultrasounds, brain MRI, fundus examination, evoked visual potential, electromyogram, standard and high resolution blood karyotype and FISH analyses of the telomeres were normal on the two sibs. Plasma and urinary aminoacids and organic acids, plasma T3, T4, TSH, protein glycosylation tests (western blot analysis) and blood and fibroblast lysosomal enzymes (  $\beta$ -Glucosidase,  $\beta$ -Galactosidase, Hexosaminidase A and B,  $\alpha$ -L-Iduronidase,  $\alpha$ -Glucosaminidase,  $\alpha$ -Galactose 6 sulfatase, Arylsulfatase A and B,  $\alpha$ - and  $\beta$ -Mannosidase,  $\alpha$ -Fucosidase,  $\alpha$ -Neuraminidase and  $\alpha$ -Nacetylgalactosaminidase) were unremarkable. Molecular analysis of the MECP2 gene was also normal. Electroretinogram of patient 1 detected an abnormal macular signal but normal peripheral retina.

## METHODS

### **Molecular studies :**

C DNA was extracted from peripheral blood lymphocytes according to standard techniques <sup>7</sup>. Because of non informative haplotype analysis of the DMC locus, direct sequencing of the DMC gene, dymeclin, was performed using primers previously described <sup>8</sup>. Intragenic polymorphic microsatellite marker was used to analysis the PAPSS2 (3-prime-alpha phosphoadenosine 5-prime-phosphosulphate synthase 2) gene, which accounts for the SEMD Pakistani type (*PAPSS2 5' GGC AGT AAG TTT GCA GTA GA; PAPSS2 3' GTC TAC AAC CCA AGT CT*).

### **Electron microscopy and ultrastructural analyses:**

A 4mm punch skin biopsy including dermis was performed in both sisters. The sample was fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium-tetroxyde, dehydrated in a graded ethanol series and embedded in epon 812. Semi-thin sections (1µm) were stained with toluidine blue. Ultra-thin sections were selected, contrasted with uranyl acetate and lead citrate and examined with a Philips CM 10 transmission electron microscope.

## RESULTS

D 3-4 Skeletal X-rays of patient 1 showed radial incurvation and spondyloepimetaphyseal dysplasia including flat vertebral bodies with irregular vertebral plates and posterior scalloping, irregular and flared femoral superior and inferior, radial and ulnar as well as fibulae and tibial superior metaphyses with vertical striations, small and irregular femoral

superior and inferior as well as tibial superior epiphyses, small carpal bones, flat acetabular roofs, short femoral neck, disproportionate long fibulae, and narrow iliac wings without lacy pelvis iliac crest (Figure 2A).

In patient 2, skeletal X-rays showed similar, but less severe, bone involvement including radial incurvation and spondyloepimetaphyseal dysplasia with flat vertebral bodies and posterior scalloping, irregular vertebral plates, irregular and flared femoral superior and inferior, radial and ulnar as well as fibulae and tibial superior metaphyses with vertical striations, small and irregular femoral superior and inferior as well as tibial superior epiphyses with small carpal bones, flat acetabular roofs, short femoral neck, disproportionate long fibulae, and narrow iliac wings without lacy pelvis iliac crest (Figure 2B).

C            Molecular studies allow us to exclude the Dymeclin gene by direct sequencing and the PAPSS2 gene by linkage analysis (Figure 3).

Electron microscopy analysis of the skin biopsy of affected children revealed an abnormally large number of small vacuoles near the cytoplasmic membrane in fibroblasts, macrophages (Figure 4 A - C) and cells of the duct canal portion of the sweat glands (Figure 4 D - E). Moreover, large vacuoles containing either myelinic or granular osmiophilic bodies were observed in most cutaneous cell types studied, particularly in fibroblasts, macrophages, keratinocytes, melanocytes, secretory and duct canal portions of the sweat glands. Other features included dilated endoplasmic reticulum in fibroblasts while Golgi apparatus and mitochondria were normal.

## DISCUSSION

We report here on SEMD, severe MR, microcephaly, facial dysmorphism and ataxia in two Pakistani sisters born to first-cousin parents. Severity of the clinical and radiological manifestations worsened in the course of the disease in the two sibs. The association of SEMD and MR prompted us to consider the diagnosis of DMC and Smith-McCort syndrome<sup>8-10</sup> but molecular genetic studies and distinct radiological features (ie lacy pelvis iliac crest and double vertebral humps) ruled out this diagnosis. Similarly, the absence of insulin-dependent diabetes mellitus and distinctive radiological features ruled out the diagnosis of WR syndrome as well.

The radiological features were reminiscent of the SEMD Pakistani type (especially vertebral anomalies and epiphyseal dysplasia)<sup>11</sup> and the combination of these finding with the ethnic origin of the family and the likely autosomal recessive mode of inheritance prompted us to study and eventually exclude the PAPSS2 locus<sup>12</sup>.

Metaphyseal striations is a rare radiological feature observed in several skeletal conditions but only a few of them are associated with mental retardations. Among them, SPONASTRIME dysplasia (MIM: 271510) is characterised by Spondylar (i.e. platyspondyly and irregular vertebral end-plates), nasal anomalies, striations of metaphyses and epiphyseal dysplasia. Mental retardation has been described in two families<sup>13-14</sup> but the absence of vertebral body abnormalities has suggested that these patients have a different condition<sup>15</sup>. Moreover, the combination of dysmorphic and radiological features, observed in SPONASTRIME dysplasia or related condition are clearly distinct from this new SEMD form reported here.

Despite negativity of extensive blood and urinary metabolic screening, the progressivity of organ involvement and electron microscopy studies of the skin (abnormally

large number of small vacuoles in fibroblasts, macrophages, keratinocytes, melanocytes, secretory and duct canal portion of sweat glands) observed in our patients, may argue in favour of a novel hitherto unknown metabolic disorder.

We suggest therefore that the combination of features observed in this sibship corresponds to a novel form of autosomal recessive SEMD with MR, especially as severity of symptoms worsened in the course of the disease.

#### ACKNOWLEDGEMENTS

We thank Jean-Paul Monnet and Nicole Lièvre for their technical assistance for electron microscopy. Financial support from the association “Vaincre les maladies lysosomales” is gratefully acknowledged.

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## LEGENDS OF FIGURES

### Figure 1:

A: Front and lateral view of patient 1 at 11 years of age. Note facial dysmorphism including coarse facies, low anterior and posterior hairline, brachycephaly, simple and flat ears, small and wellshaped eyebrows, synophrys, bilateral epicanthi, broad nose, full lips with eversion of the lower lip, short neck, and hirsutism on back and legs.

B: Front and lateral view of patient 2 at 7 years of age. Facial dysmorphism is similar but less coarse than that of her older sister.

### Figure 2:

A: Skeletal anomalies observed in patient 1 at 10 years of age. Note radial incurvation, flat vertebral bodies with irregular vertebral plates and posterior scalloping body, irregular and flared metaphyses with vertical striations, small and irregular epiphysis, small carpal bones and narrow iliac wings without lacy pelvis iliac crest. Note also flat acetabular roofs, short femoral neck and disproportionately long fibulae.

B: Bone x-rays of patient 2 at 6 years of age, showed less severe but quite similar bone involvement than observed in her older sister.

Figure 3 :

Electrophoregram and segregation of the PAPSS2 intragenic polymorphic microsatellite marker in our family.

Figure 4 :

A-B and C: Electron microscopy analysis of the skin biopsy of patient 1:

Cytoplasm of a macrophage (A) and a fibroblast (B) display abnormally numerous phagocytosis vacuoles (  $\longrightarrow$  ) along the cytoplasmic membrane and large vacuoles which contain either myelinic bodies (  $\blackrightarrow$  ) or granular osmiophilic bodies (  $\bullet\blackrightarrow$  ).

Details showing the fusion of the phagocytosis vacuoles with cytoplasmic membrane (  $\longrightarrow$  ) (C1 – C2), dilated GER (C2) and normal Golgi apparatus (C1) in 2 other fibroblasts

Ma = nucleus of Macrophage, Ne = nucleus of Schwann cell in a small Nerve, Cap = nucleus of an endothelial cell in a Capillary, Fi = nucleus of a Fibroblast, GER = granular endoplasmic reticulum, Go = Golgi apparatus

D and E: Electron microscopy analysis of the skin biopsy of patient 2:

Cells of the two layers of the duct canal of sweat glands contain numerous small vacuoles (  $\longrightarrow$  ) usually along the cytoplasmic membrane and larger vacuoles which contain granular osmiophilic bodies (  $\bullet\blackrightarrow$  ).

D = low magnification

E = higher magnification of the insert

F GER = dilated granulous endoplasmic reticulum

☆ = lumen of the duct canal of a sweat gland