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COMMENTARY

Expanding the spectrum of skeletal dysplasia with immunodeficiency: a commentary on identification of biallelic *EXTL3* mutations in a novel type of spondylo-epi-metaphyseal dysplasia

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The association of skeletal dysplasia with immunodeficiency has been recognized as a distinctive feature of Schimke immune-osseous dysplasia (MIM: 242900), cartilage hair hypoplasia (MIM: 250250) and phosphoglucomutase 3 deficiency (MIM: 172100).[1] In this issue, Guo *et al.*[2] report on a novel form of autosomal recessive skeletal dysplasia with immunodeficiency, due to mutations of the exostostin-like 3 (*EXTL3*) gene (MIM: 605744). This report follows shortly similar publications by two other groups.[3,4] Altogether, these studies unequivocally identify EXTL3 deficiency as a novel form of epispondylometaphyseal dysplasia with immunodeficiency and severe neuromotor development delay.

Description of the clinical, imaging and laboratory data among the 14 patients reported in these studies (Table 1) permits to define the phenotypic spectrum of the disease. Severe platyspondyly, brachydactyly and kyphoscoliosis were cardinal features.[2–4] Narrowing of the craniocervical canal resulting in spinal cord compression and requiring neurosurgery early in life was observed in several infants. The vast majority of the patients presented facial dysmorphisms, with coarse features, upslanting palpebral fissures, frontal bossing, prominent nose and broad nasal tip.[2–4] Truncal hypotonia and severe motor development delay were common, and several patients had a history of seizures. Liver and kidney cysts have been also frequently reported.[2–4] The immunodeficiency of the syndrome is largely restricted to T-cell lymphopenia. Two of the patients had undetectable or very low levels of T-cell receptor excision circles at birth,[4] indicative of severe combined immune deficiency. Some patients manifested Omenn syndrome (with oligoclonal T cells, erythroderma, eosinophilia and elevated serum IgE);[3,4] and eosinophilia and hyper-IgE were observed also in other patients without features of Omenn syndrome,[3,4] suggesting that immune dysregulation may be part of the disease phenotype.

Importantly, identification of this novel genetic disorder has also permitted characterization of the molecular and cellular mechanisms underlying the skeletal dysplasia and immune deficiency of the syndrome. EXTL3 is an *N*-acetylglucosaminyltransferase (GlcNac transferase), and plays a critical role in heparan sulfate (HS) and heparan sulfate proteoglycan (HSPG) biosynthesis.[5] HSPGs modulate the activity of a variety of morphogenetic proteins that play a critical role in skeletal, hematopoietic immune system development, including fibroblast growth factors (FGFs), bone morphogenetic protein, sonic hedgehog and various interleukins (ILs).[6] The *EXTL3* mutations identified in the patients affect GlcNac transferase activity,[2] and alter production of HS,[3] that show longer and abnormally sulfated chains.[4] Furthermore, cellular responses to FGF2, IL-7 and IL-2 were also abnormal.[4] Both the biochemical abnormalities of HS composition and the increased signaling to FGF2 were rescued upon transfer of a normal copy of the gene into the patient's cells,[4] indicating that the *EXTL3* mutations observed in the patients are hypomorphic. Consistent with this notion, null mutations of the *Extl3* gene are embryonically lethal in mice,[7] whereas the boxer (*box*) zebrafish carrying a hypomorphic *extl3* mutation is viable, but presents severe defects of pectoral fin development[8] and impaired thymopoiesis,[4] reminiscent of the human skeletal and immunological phenotype.

Gain of function mutations of the *FGFR2* and *FGFR3* genes have been reported in various genetic disorders affecting the skeletal development.[9] The increased signaling in response to FGF2 observed in *EXTL3*-mutated patients may lead to similar effects.

With regard to the immunological abnormalities, Oud *et al.*[3] have shown that EXTL3 is expressed in hematopoietic stem cells and at early stages of T-cell development. Using patient-derived induced pluripotent stem cells (iPSCs), Volpi *et al.*[4] have demonstrated that *EXTL3* mutations compromise development and expansion of early hematolymphoid progenitor cells. Altogether, these data suggest that the T-cell immunodeficiency of the syndrome may reflect impaired colonization of the thymus by common lymphoid progenitor cells and/or their expansion in the thymus. However, a possible contribution of thymic epithelial cell dysfunction cannot be dismissed, since patient-derived iPSCs showed impaired differentiation toward thymic epithelial progenitor cells.[4] Importantly, some patients

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Table 1 Clinical and laboratory features of 14 patients with EXTL3 deficiency[2–4]

Clinical and laboratory features	*Number (%) of patients with manifestation*
Skeletal	
Platysplondyly	12 (85.7)
Brachydactyly	10 (71.4)
Short stature	9 (64.3)
Kyphoscoliosis	9 (64.3)
Craniocervical stenosis	7 (50%)
Pelvis abnormalities (broad ischia, sloping acetabular roof and coxa valga)	8 (57.1%)
Neuromuscular	
Motor neurodevelopment delay	8 (57.1)
Truncal hypotonia	6 (42.8)
Seizures	6 (42.8)
Immunohematologic abnormalities	
T-cell lymphopenia	8 (57.1)
Omenn syndrome	4 (28.6)
Eosinophilia	6 (42.8)
Hyper-IgE	3 (21.4)
Facial dysmorphisms	12 (85.7)
Liver cysts	6 (42.8)

with EXTL3 deficiency showed no signs of immunodeficiency,[3] and progressive and spontaneous improvement of T-cell count and immune function was detected in some other patients.[4] It remains to be determined whether this variability of the immunological phenotype is due to differences in the severity of distinct *EXTL3* gene mutations. Alternatively, it is possible that the partial nature of the hematopoietic (defect may be compensated over time, so that the thymus niche is eventually filled with a sufficient number of progenitor cells to allow relatively robust T-cell output). The longitudinal study of additional patients, and development of novel, knock-in animal models, may help address these questions. Similarly, the precise mechanisms underlying motor developmental delay, and the occurrence of liver and kidney cysts, remain undefined.

From a therapeutic standpoint, hematopoietic stem cells transplantation has been shown to correct the immune deficiency, but has no effects on the skeletal and neuromotor abnormalities. Therefore, caution should be used in proposing such approach, especially since the T-cell immunodeficiency may spontaneously improve over time.

In conclusion, the identification of this novel genetic disease is remarkable in many regards: (a) it illustrates one more time how valuable are unbiased genomic approaches based on whole-exome sequencing, as long as families sharing similar features are identified upon careful annotation of phenotypic data; (b) it confirms the value of *in vitro* iPSC-based and *in vivo* animal models for the characterization of disease pathophysiology; and (c) it identifies a critical role for HSPGs in human skeletal and immune system development. It can be anticipated that additional genetic conditions due to abnormalities of HSPG composition and function will be identified in the near future.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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