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SH2B3 alterations in a novel genetic condition, juvenile myelomonocytic leukemia, and myeloproliferative neoplasia

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Haematopoiesis is a highly dynamic process evolving across human lifespan. The foetal and perinatal period is one of particular physiological changes and evolution. During these developmental stages, constitutional genetic conditions can cause in imbalances in potent regulators of maintenance and differentiation of stem and progenitor cells giving rise to specific haematological phenotypes in neonates and young infants. A prominent example is Down syndrome with its trisomy 21-mediated perturbation of foetal haematopoiesis. Approximately 10% of newborns with Down syndrome present with transient abnormal myelopoiesis (TAM) characterized by increased peripheral blood blast cells and pathognomonic somatic mutations in the transcription factor *GATA1*.(1) While 10% to 20% of cases transform to full-blown leukaemia within the first four years of age, the majority of cases resolve without treatment. Another common developmental disorder, which can present with a transient myeloproliferative disease (MPD) in the first months of life, is Noonan syndrome caused by germline pathogenic variants of the RAS/mitogen-activated protein kinase (MAPK) pathway. Most of these patients with Noonan syndrome carry germline mutations in *PTPN11* (2). While the mutational landscape of TAM in Down syndrome is at least in part elucidated, the mechanism of Noonan syndrome-associated MPD remains largely obscure.

Recently, Perez-Garcia et al. (3) and Blombery et al. (4) reported on another transient MPD presenting shortly after birth in patients with biallelic germline mutations in the *SH2B3* gene. *SH2B3* encodes the lymphocyte adapter LNK (also named SH2B3), a member of the SH2B adapter family of proteins, which also comprises APS (SH2B2) and SH2B (SH2B1) (Figure). SH2B proteins share a common architecture of a N-terminal dimerization domain, central pleckstrin homology domain (PH) and C-terminal Src homology 2 (SH2) domain. LNK is widely expressed in human tissues, with the highest expression in haematopoietic cells (reviewed in (5)). It functions as a negative regulator of multiple cytokine and growth factor receptor signalling pathways including the JAK-STAT pathway. LNK directly binds JAKs via its SH2 domain and plays a particular important role in the negative regulation of TPO and EPO signalling. Lnk-deficient mice exhibit a MPD-like phenotype with splenomegaly, an expanded haematopoietic stem cell pool with enhanced stem cell renewal, increased cytokine sensitivity, and abnormal accumulation of megakaryocytes, erythrocytes and B-lymphocytes in bone marrow and/or spleen. Somatic inactivating *SH2B3* mutations have been reported in

a number of haematopoietic malignancies, most commonly in myeloproliferative neoplasms (MPN) and in acute lymphoblastic leukaemia. LNK also acts as a potent inhibitor of JAK2V617F constitutive activity in MPN(6), which may explain the increasing frequency of loss-of function (LOF) *SH2B3* alterations in patients with MPN from 5 to 10% up to 13% in MPN leukemic transformation. Limited information is available regarding the clinical significance of germline variants.

In this edition of Haematologica, two cooperative study groups from France and North America present data on germline *SH2B3* alterations in patients referred to the respective reference diagnostic laboratories for juvenile myelomonocytic leukaemia (JMML).(7, 8) JMML is a unique myelodysplastic/myeloproliferative neoplasia of early infancy characterized by constitutive activation of the RAS signal transduction pathway. Approximately 95% of patients with JMML harbour either germline events in *NF1* or *CBL* that progress to neoplasia with acquired biallelic inactivation of the respective genes in haematopoietic cells, or heterozygous somatic gain-of-function mutations of *PTPN11*, *NRAS*, and *KRAS* in the absence of germline disease.(9) Arfeuille et al. performed sequencing studies in two pair of siblings suspected of neonatal JMML but lacking RAS pathway mutations, and unravelled biallelic LOF *SH2B3* germline variants. Subsequent targeted sequencing of *SH2B3* in a large cohort of consecutive French patients identified eight patients from six families carrying biallelic deleterious *SH2B3* alterations. Three of the six families were consanguineous and family studies were consistent with an autosomal recessive inheritance. Wintering et al. report on two additional cases with biallelic germline conditions from North America.(8) With the three kindred previously published (3, 4), there is now sufficient evidence for recognizing a novel genetic condition characterized by biallelic *SH2B3* germline alterations. This disorder presents as MPD in the first few months of life with clinical and haematological features resembling JMML, normal karyotype, absence of somatic mutations, and a DNA methylation pattern resembling that of foetal bone marrow. Most patients have spontaneous normalization of blood counts, while splenomegaly may persist. Following this initial phase of MPD with thrombocytopenia and a reduced number of megakaryocytes, some patients experienced rapid development of megakaryocytic hyperplasia with persistent thrombocytosis.(4, 7) The molecular mechanism involved in this puzzling evolution is unknown; unravelling its nature may contribute to the understanding of

age-specific signalling networks. Interestingly, children with biallelic *SH2B3* germline alterations also appear to be at a significant risk for autoimmune disease like autoimmune hypothyroidism or diabetes mellitus later in life.(3, 4, 7)

Like other genetic disorders with MPD features occurring in the neonatal age, the novel biallelic *SH2B3* germline condition presents with transient leukoerythroblastosis, thrombocytopenia and extramedullary haematopoiesis resembling the haematological and clinical phenotype observed in JMML. Thus, it is not surprising that biallelic *SH2B3* germline variants accounted for almost half of the French cases suspected of JMML that remained unresolved on the genetic level.(7) Interestingly, two patients of the North American cohort presented at the age of two and four months with a monoallelic germline *SH2B3* germline variant and a variant allele frequency (VAF) in haematopoietic cells of 63% and 100%, respectively (8). Like in biallelic cases, acquired somatic driver mutations were absent. While allogeneic haematopoietic stem cell transplantation was performed in both patients, it is s tempting to speculate that monoallelic germline disease with neonatal acquisition of biallelic inactivation in haematopoietic cells may possibly run a clinical course similar to that observed in the biallelic germline disease.

The adapter protein LNK lacks catalytic activity. The mechanism by which LNK negatively regulates signalling is not fully understood. LNK can promote degradation of signalling molecules like JAK2 by recruiting the CBL E3 ubiquitin ligase thereby inactivating its target protein. CBL is one of the canonical RAS pathway genes involved in JMML. CBL deficiency enhances JAK2 signalling and upregulates RAB27B, a GTPase critical for plasma membrane localization and palmitoylation of NRAS.(10) With such interplay between the RAS/MAPK and JAK/STAT signalling pathways, presence of secondary *SH2B3* mutations in patients with JMML are unsurprising. Somatic *SH2B3* alterations are seen in high-risk JMML cases and are generally accompanied by other subclonal mutations; in a genetic mouse model, they have been shown to exacerbate disease severity.(11) Of note, in both the French and North American cohort, presentation and clinical course in older children with monoallelic pathogenic *SH2B3* germline variants was not different from that noted in JMML patients with somatic *SH2B3* mutations.(7, 8) This observation is consistent with findings in MPN in adults suggesting that *SH2B3* mutations, either germline or acquired, can cooperate with

acquired driver mutations in *JAK2*, *CALR*, or *MPL* to determine the MPN disease phenotype.(12) Interestingly, both monoallelic germline disease in older children and somatic *SH2B3* mutations were associated with somatic *PTPN11* driver mutations.(7, 8) In two patients of the French cohort, acquired chromosome 12q uniparental disomy developed resulting in copy-neutral loss of heterozygosity of both the *PTPN11* and *SH2B3* gene.(7)

SH2B3 variants described were missense, nonsense or frameshift and were distributed throughout the gene clustering in the PH and SH2 domain that are essential for LNK function. Modelling a frameshift variant of a patient with biallelic germline condition in zebrafish with CRISPR-Cas9 gene editing, Blomberg et al. had demonstrated that treatment of the *sh2b3* crispant fish with the JAK inhibitor ruxolitinib could prevent the myeloproliferative phenotype.(4) In this edition of Haematologica, Wintering et al. expanded the drug discovery screening methodology by using iPSC-derived JMML-like haematopoietic progenitor cells (HPCs). With this approach, they show that HPCs with alterations in *SH2B3* were more sensitive to JAK1/2 inhibition compared to HPCs not harbouring mutations in *SH2B3*.(8) Therapy of two children with ruxolinitib led to resolution of splenomegaly in both patients. In one patient with two secondary *SH2B3* mutations, the VAF of both *SH2B3* variants decreased, while the size of the *PTPN11*-mutated clone remained unchanged and a new *NRAS* mutation became detectable. In the other patient with monoallelic *SH2B3* germline disease and copy neutral loss of heterozygosity in haematopoietic cells, the VAF of the *SH2B3* mutation remained at 100%.(8)

Similar to what has been reported for *JAK2*^{V617F}-positive MPN(13), clonal hierarchy in cases of JMML with *SH2B3* alteration is complex. *SH2B3* mutations can be acquired early or late during the course of clonal evolution and are not mutually exclusive to mutations in the known canonical RAS pathway driver mutations. Multiple *SH2B3* mutations in *trans* can arise independently suggesting that both *SH2B3* alleles are vulnerable to functionally relevant mutations. Arfeuille et al. report on difficulties in determining, which lesion was the initiating driver.(7) Single cell sequencing performed by Wintering et al. revealed a heterozygous somatic *SH2B3* mutation branching into a *PTPN11*-mutated population and a homozygous SH2B3 population.(8) Little is known about clonal haematopoiesis in children, but it is

conceivable that the *PTPN11*-driver mutation occurred in the background of pre-existing somatic mosaicism.

Further insight into the regulatory function of LNK in intracellular signalling will help to decipher its role in the pathogenesis of haematological malignancies. There is currently little evidence that *SH2B3* alterations act as classical driver mutations in MPN or JMML, although their role in the phylogenetic origin of these myeloproliferative disorders remains puzzling. Biallelic *SH2B3* germline disease needs to be added to the list of heterogeneous rare genetic conditions presenting as transient MPD in newborns or young infants. Whether the possible subsequent development of thrombocytosis involves an impending risk for neoplasia later in life is one of the questions suitable for a larger cohort study on *SH2B3* germline disease.

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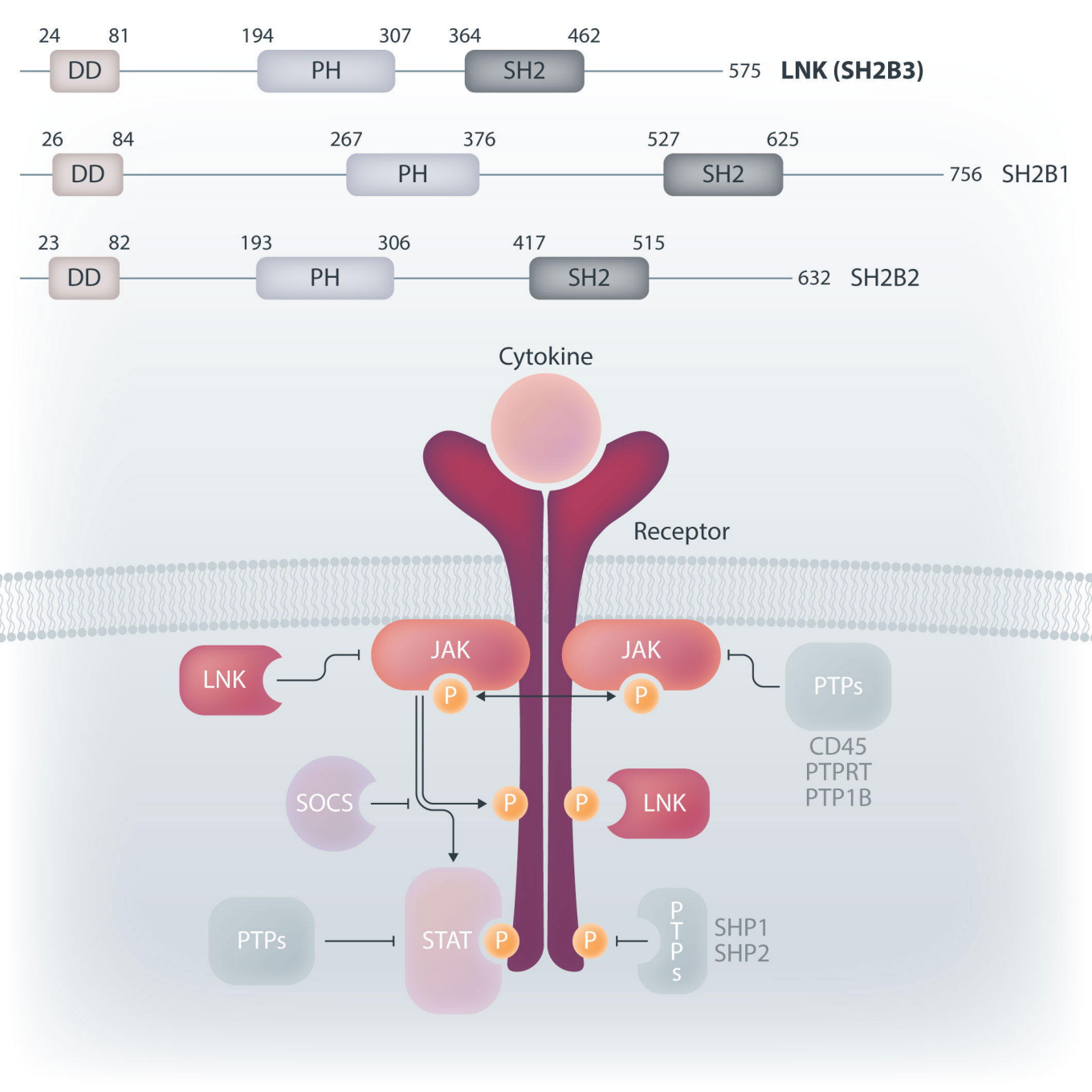
Figure: LNK and the adapter family of proteins (from (5) used with permission)

Top: The SH2 domain-containing adaptor family shares a domain architecture with N-terminal dimerization domain (DD), central pleckstrin homology domain (PH) and C-terminal Src homology 2 (SH2) domain. Bottom: LNK directly binds JAK and receptors via its SH2 domain thereby inhibiting downstream signaling (shown semi-transparently).


24
81
194
307
364
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DD
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LNK (SH2B3)
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84
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625
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SH2
756
SH2B1
23
82
193
306
417
515
DD
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SH2
632
SH2B2
Cytokine
Receptor
LNK
JAK
JAK
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PTPs
CD45
PTPRT
PTP1B
SOCS
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LNK
PTPs
STAT
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P
PTPs
SHP1
SHP2