EDITORIAL

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

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Hematopoiesis is a highly dynamic process evolving across the human lifespan. The fetal and perinatal periods are ones of particular physiological change and evolution. During these developmental stages, constitutional genetic conditions can cause imbalances in potent regulators of maintenance and differentiation of stem and progenitor cells giving rise to specific hematologic phenotypes in neonates and young infants. A prominent example is Down syndrome with its trisomy 21-mediated perturbation of fetal hematopoiesis. Approximately 10% of newborns with Down syndrome present with transient abnormal myelopoiesis characterized by increased peripheral blood blast cells and pathognomonic somatic mutations in the transcription factor *GATA1*.[1] While 10% to 20% of cases transform into full-blown leukemia within the first 4 years of life, 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 transient abnormal myelopoiesis in Down syndrome is at least in part elucidated, the mechanism of Noonan syndrome-associated MPD remains largely obscure.

Perez-Garcia *et al.*[3] and Blombery *et al.*[4] have 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 1). SH2B proteins share a common architecture of an N-terminal dimerization domain, central pleckstrin homology (PH) domain and a C-terminal Src homology 2 (SH2) domain. LNK is widely expressed in human tissues, with the highest expression in hematopoietic cells.[5] It functions as a negative regulator of multiple cytokine and growth factor receptor signaling pathways including the JAK-STAT pathway. LNK directly binds JAK via its SH2 domain and plays a particularly important role in the negative regulation of thrombopoietin and erythropoietin signaling. Lnk-deficient mice exhibit a MPD-like phenotype with splenomegaly, an expanded hematopoietic 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 hematopoietic malignancies, most commonly in myeloproliferative neoplasms (MPN) and in acute lymphoblastic leukemia. LNK also acts as a potent inhibitor of *JAK2*^{V617F} constitutive activity in MPN,[6] which may explain the increasing frequency of loss-of function *SH2B3* alterations in patients with MPN from 5-10% 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 leukemia (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 harbor either germline events in *NF1* or *CBL* which progress to neoplasia with acquired biallelic inactivation of the respective genes in hematopoietic cells, or heterozygous somatic gain-of-function mutations of *PTPN11*, *NRAS*, and *KRAS* in the absence of germline disease.[9] Arfeuille *et*

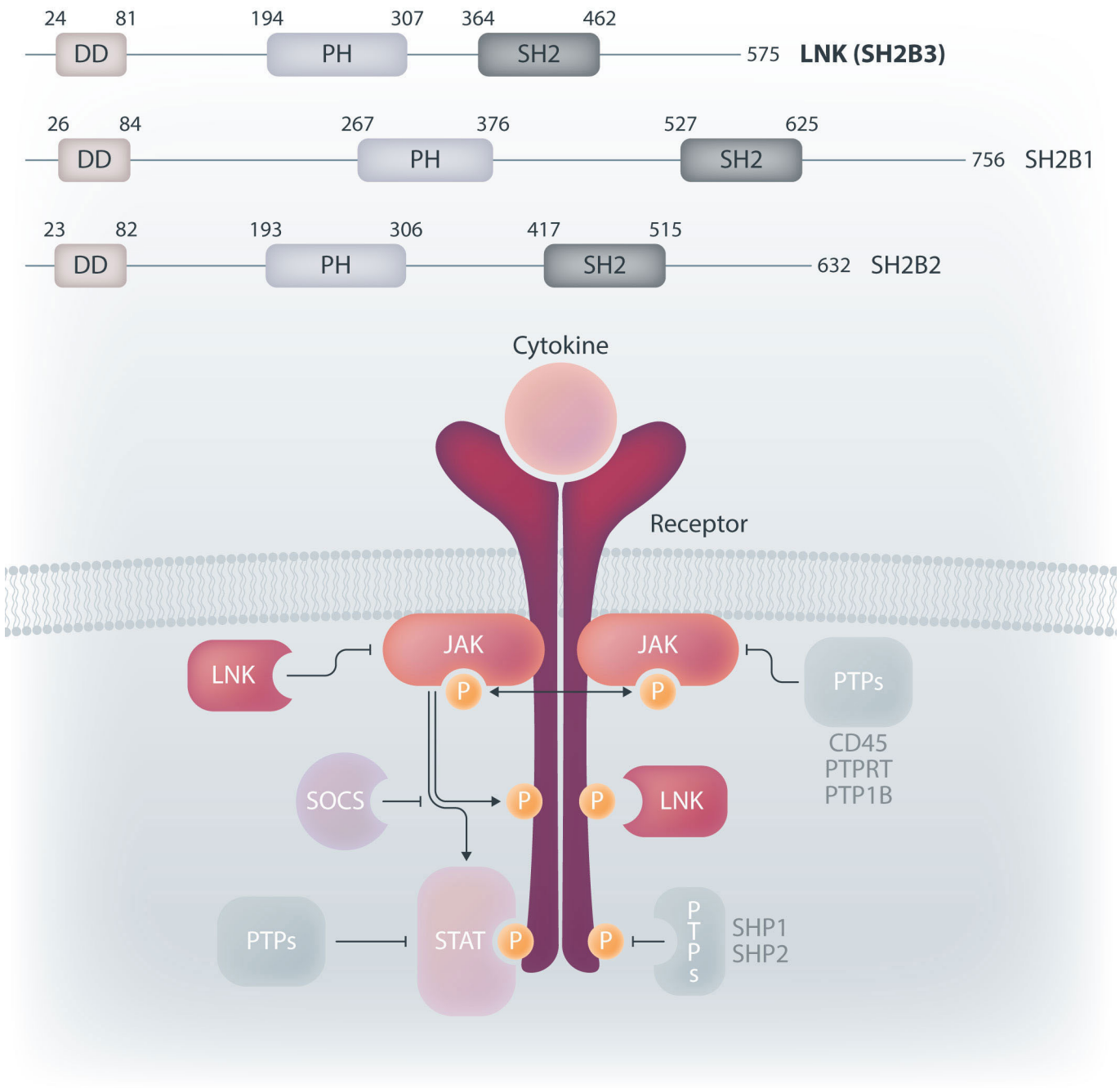

Figure 1. LNK and the adapter family of proteins. Top. The SH2 domain-containing adapter family shares a domain architecture with an N-terminal dimerization domain, central pleckstrin homology domain and a C-terminal Src homology 2 (SH2) domain. Bottom. LNK directly binds JAK and receptors via its SH2 domain thereby inhibiting downstream signaling (shown semi-transparently). Figure adapted from Morris *et al.*[5] with permission. DD: dimerization domain; PH: pleckstrin homology; SH2: Src homology 2; LNK: lymphocyte adapter protein; JAK: Janus kinases; SOCS: suppressor of cytokine signaling; STAT: signal transducers and activators of transcription; PTP: protein tyrosine phosphatases.

al. performed sequencing studies in two pairs of siblings suspected of having neonatal JMML but lacking RAS pathway mutations, and identified biallelic loss-of-function *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 to recognize a novel genetic condition characterized by biallelic *SH2B3* germline alterations. This disorder presents as a MPD in the first few months of life with clinical and hematologic features resembling JMML, normal karyotype, absence of somatic mutations, and a DNA methylation pattern similar to that of fetal 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 experience rapid development of megakaryocytic hyperplasia with persistent thrombocytosis.[4,7] The molecular mechanism involved in this puzzling evolution is unknown; unraveling its nature may contribute to the understanding of age-specific signaling networks. Interestingly, children with biallelic *SH2B3* germline alterations also appear to be at a significant risk of autoimmune diseases, such as autoimmune hypothyroidism and diabetes mellitus, later in life.[3,4,7]

Like other genetic disorders with MPD features occurring in the neonatal period, the novel biallelic *SH2B3* germline

condition presents with transient leukoerythroblastosis, thrombocytopenia and extramedullary hematopoiesis, resembling the hematologic and clinical phenotypes observed in JMML. Thus, it is not surprising that biallelic *SH2B3* germline variants accounted for almost half of the French cases suspected of being JMML that remained unresolved on the genetic level.[7] Interestingly, two patients in the North American cohort presented at the age of 2 and 4 months with a monoallelic germline *SH2B3* germline variant and a variant allele frequency in hematopoietic cells of 63% and 100%, respectively.[8] As in biallelic cases, acquired somatic driver mutations were absent. While allogeneic hematopoietic stem cell transplantation was performed in both patients, it is tempting to speculate that monoallelic germline disease with neonatal acquisition of biallelic inactivation in hematopoietic 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 signaling is not fully understood. LNK can promote degradation of signaling molecules such as 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 signaling 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 signaling pathways, the presence of secondary *SH2B3* mutations in patients with JMML is 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 cohorts, the presentation and clinical course in older children with monoallelic pathogenic *SH2B3* germline variants were not different from those noted in JMML patients with somatic *SH2B3* mutations.[7,8] This observation is consistent with findings in MPN in adults suggesting that *SH2B3* mutations, whether 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* genes.[7]

The *SH2B3* variants described were missense, nonsense or frameshift and were distributed throughout the gene clustering in the PH and SH2 domains that are essential for LNK function. Modeling a frameshift variant of a gene from a patient with a biallelic germline condition in zebrafish with CRISPR-Cas9 gene editing, Blomberg *et al.* 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 induced pluripotent stem cell-derived JMML-like hematopoietic progenitor cells (HPC). With this approach, they showed that HPC with alterations in *SH2B3* were more sensitive to JAK1/2 inhibition compared to HPC not harboring 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 variant allele frequency 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 hematopoietic cells, the variant allele frequency 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 hematopoiesis in children, but it is conceivable that the *PTPN11*-driver mutation occurred on the background of pre-existing somatic mosaicism.

Further insight into the regulatory function of LNK in intracellular signaling will help to decipher its role in the pathogenesis of hematologic 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 individuals with *SH2B3* germline disease.

Disclosures

No conflicts of interest to disclose.

Contributions

CMN and ME designed the outline of the editorial. CMN wrote the manuscript.

References

1. Roberts I. Leukemogenesis in infants and young children with trisomy 21. Hematology Am Soc Hematol Educ Program. 2022;2022(1):1-8.
2. Niemeyer CM. RAS diseases in children. Haematologica. 2014;99(11):1653-1662.
3. Perez-Garcia A, Ambesi-Impiombato A, Hadler M, et al. Genetic loss of SH2B3 in acute lymphoblastic leukemia. Blood. 2013;122(14):2425-2432.
4. Blombery P, Pazhakh V, Albuquerque AS, et al. Biallelic deleterious germline SH2B3 variants cause a novel syndrome of myeloproliferation and multi-organ autoimmunity. EJHaem. 2023;4(2):463-469.
5. Morris R, Butler L, Perkins A, Kershaw NJ, Babon JJ. The role of LNK (SH2B3) in the regulation of JAK-STAT signalling in haematopoiesis. Pharmaceuticals (Basel). 2021;15(1):24.
6. Gery S, Cao Q, Gueller S, Xing H, Tefferi A, Koeffler HP. Lnk inhibits myeloproliferative disorder-associated JAK2 mutant, JAK2V617F. J Leukoc Biol. 2009;85(6):957-965.
7. Arfeuille C, Vial Y, Cadenet M, et al. Germline bi-allelic SH2B3/LNK alteration predisposes to a neonatal juvenile myelomonocytic leukemia-like disorder. Haematologica. 2024;109(8):2542-2554.
8. Wintering A, Hecht A, Meyer J, et al. LNK/SH2B3 as a novel driver in juvenile myelomonocytic leukemia. Haematologica. 2024;109(8):2533-2541.
9. Niemeyer CM, Flotho C. Juvenile myelomonocytic leukemia: who's the driver at the wheel? Blood. 2019;133(10):1060-1070.
10. Ren JG, Xing B, Lv K, et al. RAB27B controls palmitoylation-dependent NRAS trafficking and signaling in myeloid leukemia. J Clin Invest. 2023;133(12):e165510.
11. Morales CE, Stieglitz E, Kogan SC, Loh ML, Braun BS. Nf1 and Sh2b3 mutations cooperate in vivo in a mouse model of juvenile myelomonocytic leukemia. Blood Adv. 2021;5(18):3587-3591.
12. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. Br J Haematol. 2017;178(5):689-698.
13. Lasho TL, Tefferi A, Finke C, Pardanani A. Clonal hierarchy and allelic mutation segregation in a myelofibrosis patient with two distinct LNK mutations. Leukemia. 2011;25(6):1056-1058.