Authorship

Contribution: W.Z. collected and analyzed the data and wrote the manuscript; Y.Z. designed and supervised the study, analyzed the data, and revised the manuscript; B.H., L.J., J.Y., J.D., S.W., and Y.L. collected the data and critically discussed the manuscript; C.Z. and Z.G. performed IHC and analyzed data; B.D. headed the CAR-T generation team; and A.H.C. provided CAR-T technique and support.

Conflict-of-interest disclosure: A.H.C. is a founding member of Shanghai YaKe Biotechnology Ltd., a biotechnology company focusing on research and development of tumor cellular immunotherapy. The remaining authors declare no competing financial interests.

Correspondence: Yonghong Zhang, Department of Pediatric Lymphoma, Beijing Boren Hospital; Department of Hematology Oncology, National Center for Children's Health, Beijing Children's Hospital, Capital Medical University, Beijing 100070, China; e-mail: zhangyongh@borenhospital.com.

Footnotes

For original data, please contact the corresponding author.

The online version of this article contains a data supplement.

REFERENCES

1. Molyneux EM, Rochford R, Griffin B, et al. Burkitt's lymphoma. *Lancet*. 2012;379(9822):1234-1244.
2. Pagano L, Caira M, Valentini CG, Fianchi L. Clinical aspects and therapy of sporadic burkitt lymphoma. *Mediterr J Hematol Infect Dis*. 2009;1(2): e2009030.
3. Egan G, Goldman S, Alexander S. Mature B-NHL in children, adolescents and young adults: current therapeutic approach and emerging treatment strategies. *Br J Haematol*. 2019;185(6):1071-1085.
4. Minard-Colin V, Auperin A, Pillon M, et al. Results of the randomized Intergroup trial Inter-B-NHL Ritux 2010 for children and adolescents with high-risk B-cell non-Hodgkin lymphoma (B-NHL) and mature acute leukemia (B-AL): Evaluation of rituximab (R) efficacy in addition to standard LMB chemotherapy (CT) regimen [abstract]. *J Clin Oncol*. 2016;34(15). Abstract 10507.
5. Cairo M, Auperin A, Perkins SL, et al. Overall survival of children and adolescents with mature B cell non-Hodgkin lymphoma who had refractory or relapsed disease during or after treatment with FAB/LMB 96: a report from the FAB/LMB 96 study group. *Br J Haematol*. 2018;182(6):859-869.
6. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725-733.
7. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371(16):1507-1517.
8. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017; 377(26):2531-2544.
9. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med*. 2017;377(26):2545-2554.
10. Avigdor A, Shouval R, Jacoby E, et al. CAR T cells induce a complete response in refractory Burkitt lymphoma. *Bone Marrow Transplant*. 2018; 53(12):1583-1585.
11. Rivers J, Annesley C, Summers C, et al. Early response data for pediatric patients with non-Hodgkin lymphoma treated with CD19 chimeric antigen receptor (CAR) T-cells [abstract]. *Blood*. 2018;132(suppl 1). Abstract 2957.
12. Cheson BD, Pfistner B, Juweid ME, et al; International Harmonization Project on Lymphoma. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25(5):579-586.
13. Pan J, Yang JF, Deng BP, et al. High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. *Leukemia*. 2017;31(12):2587-2593.
14. Pan J, Niu Q, Deng B, et al. CD22 CAR T-cell therapy in refractory or relapsed B acute lymphoblastic leukemia. *Leukemia*. 2019;33(12): 2854-2866.
15. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome [published correction appears in *Blood*. 2015;126(8):1048]. *Blood*. 2014;124(2):188-195.
16. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. 2018;15(1):47-62.
17. Yang ZZ, Grote DM, Ziesmer SC, et al. Soluble IL-2Rα facilitates IL-2-mediated immune responses and predicts reduced survival in follicular B-cell non-Hodgkin lymphoma. *Blood*. 2011;118(10):2809-2820.
18. Shah NN, Maatman T, Hari P, Johnson B. Multi targeted CAR-T cell therapies for B-cell malignancies. *Front Oncol*. 2019;9:146.

DOI 10.1182/blood.2019002008

TO THE EDITOR:

A homozygous deletion in the *SLC19A1* gene as a cause of folate-dependent recurrent megaloblastic anemia

Michael Svaton,[1,*] Karolina Skvarova Kramarzova,[1,*] Veronika Kanderova,[1] Andrea Mancikova,[2] Petr Smisek,[3] Pavel Jesina,[4] Jakub Krijt,[4] Blanka Stiburkova,[4,5] Robert Dobrovolny,[4] Jitka Sokolova,[4] Violeta Bakardjieva-Mihaylova,[1] Elena Vodickova,[6] Marketa Rackova,[1] Jan Stuchly,[1] Tomas Kalina,[1] Jan Stary,[3] Jan Trka,[1] Eva Fronkova,[1,†] and Viktor Kozich[4,†]

[1]Childhood Leukemia Investigation Prague, Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; [2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic; [3]Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; [4]Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic; [5]Institute of Rheumatology, Prague, Czech Republic; and [6]Department of Clinical Hematology, University Hospital Motol, Prague, Czech Republic

Megaloblastic anemia resulting from ineffective hematopoiesis in the bone marrow (BM) is one of the main hematologic signs of folate or vitamin B_{12} deficiency. Functional deficiencies of these 2 vitamins originate from nutritional, gastrointestinal, or genetic factors, and their clinical symptoms result from the impaired synthesis of nucleotides in hematopoietic cells and S-adenosylmethionine in the nervous system.[1] Folates are pteroyl(poly)glutamate derivatives with various 1-carbon moieties at the pterine ring, with

Table 1. Laboratory markers during the second episode of anemia

Markers	Biological material	Reference range	Before treatment with folic acid*	With folic acid treatment†
Blood count				
Hemoglobin, g/L		135-175	**61-99**	131
Red blood cells $\times 10^{12}$/L		4-5.8	**2.02-3.21**	4.34
Hematocrit, %		40-50	**17.1-30.4**	41
Mean cell volume, fL		82-98	82.1-97.3	94.5
Reticulocytes, %		0.5-2.5	0.62-**3.71**	0.52
Routine biochemistry				
Bilirubin, μmol/L	Serum	2-17	**42.2-60.6**	17.4
Lactate dehydrogenase, μkat/L	Serum	1.67-3.17	**13.38-115.66**	2.6
Ferritin, μg/L	Serum	17-304	**413.2-954.8**	**1036.3**
Iron, μmol/L	Serum	7.2-29	**49.4-50.5**	**6.7**
B vitamins and related metabolites				
Folate, nmol/L	Serum	4.53-21.5	7.3-14.3	***>45***
Folate, nmol/L	Erythrocytes	1185-2841	**1155.2**	2175.4
Vitamin B_{12}, ng/L	Serum	197-771	**138-*2000***	***866***
Holotranscobalamin II, pmol/L	Serum	19-119	44	**151**
Total homocysteine, μmol/L	Plasma	5.2-11.3	**31.5 to ≥50**	9.49
Cystathionine, nmol/L	Plasma, serum	80-1000	187-217	99
Methionine, μmol/L	Plasma, serum	12-40	26-27	17
Sarcosine, μmol/L	Plasma, serum	0.7-3	**5.2**	1.85
Methylmalonic acid, nmol/L	Plasma	<270	136.8	204.5
Methylmalonic acid, mmol/mol creatinine	Urine	<15	<15	<15
AICAr, mmol/mol creatinine	Urine	0.04-1.01	**1.87**	0.18

Levels out of reference range are shown in bold; supraphysiological concentrations resulting from administration of respective vitamins are shown in italics.

*Ranges with the minimal and maximal observed levels during the period in which therapy with cyanocobalamin only was administered (days 1155-1414; single values are from day 1261).

†The levels at follow-up of 1 month of combined cyanocobalamin and folic acid therapy (day 1444, 3 days after the last cyanocobalamin injection).

the major circulating form being 5-methyltetrahydrofolate. Folates are delivered to tissues by at least 5 transporters with different kinetic properties and variable expression.[2,3] Two inherited disorders in folate transport, hereditary folate malabsorption and cerebral folate deficiency resulting from mutations in the *SLC46A1*[4] and *FOLR1*[5] genes, have been reported in humans.

Reduced folate carrier (RFC; FOLT1) is encoded by the *SLC19A1* gene and facilitates the cellular uptake of anionic folates,[6] of folate analogs such as methotrexate (MTX), and 2′3′-cyclic-GMP-AMP, a second messenger that activates the antiviral stimulator of interferon genes pathways.[7] Loss of its function leads to MTX resistance in cancer cells.[8,9] A murine model of RFC deficiency showed embryonic lethality before E9.5. These mice could be partially rescued by supplementing the pregnant dams with folic acid; however, all liveborn mice died within 12 days because of the absence of hematopoiesis in BM, spleen, and liver.[10] To our knowledge, no inherited disease caused by loss-of-function mutations in the *SLC19A1* gene has been described in humans.

In our study, we presented the first case of recurrent severe megaloblastic anemia in a patient with a homozygous *SLC19A1* mutation. The patient's history was unremarkable with normal growth and development until age 15 years, when he presented with the first episode of severe anemia (hemoglobin [Hb], 5 g/dL), mild hemolysis, hyperhomocysteinemia of 34.7 μmol/L, low total vitamin B_{12} levels of 138 ng/L, and normal serum folates of 10.7 nmol/L. He denied having any bleeding, limb numbness, any other neurologic symptoms, or symptoms of glossitis. He reported poor eating habits with decreased intake of food for 6 months preceding his presentation, avoidance of vegetables, and avoidance of morning meals. He responded well to treatment with cyanocobalamin and folate, but he returned at age 17 years with a second episode of severe anemia (Hb, 7.8 g/dL) and signs of hemolysis with elevated bilirubin (46 μmol/L), hyperhomocysteinemia, and low total vitamin B_{12} levels (for laboratory and clinical details, see Table 1 and supplemental Data, available on the *Blood* Web site). BM aspiration showed 48% megaloblastic erythroid precursors with dysplastic signs, and the peripheral blood smear revealed sporadic macro-ovalocytes, frequent schistocytes, and hypersegmented neutrophils (supplemental Figure 1). Flow cytometry of the BM showed abundant erythropoietic precursors. Despite parenteral cyanocobalamin therapy, anemia and hyperhomocysteinemia persisted. The laboratory abnormalities and clinical signs normalized within a month but only after adding folic acid (10 mg/day) to the patient's therapy. In contrast, carefully monitored withdrawal of folic acid resulted in an immediate increase in homocysteine to levels of 17 to 31 μmol/L, despite persisting normal folate levels in serum (Figure 1A).

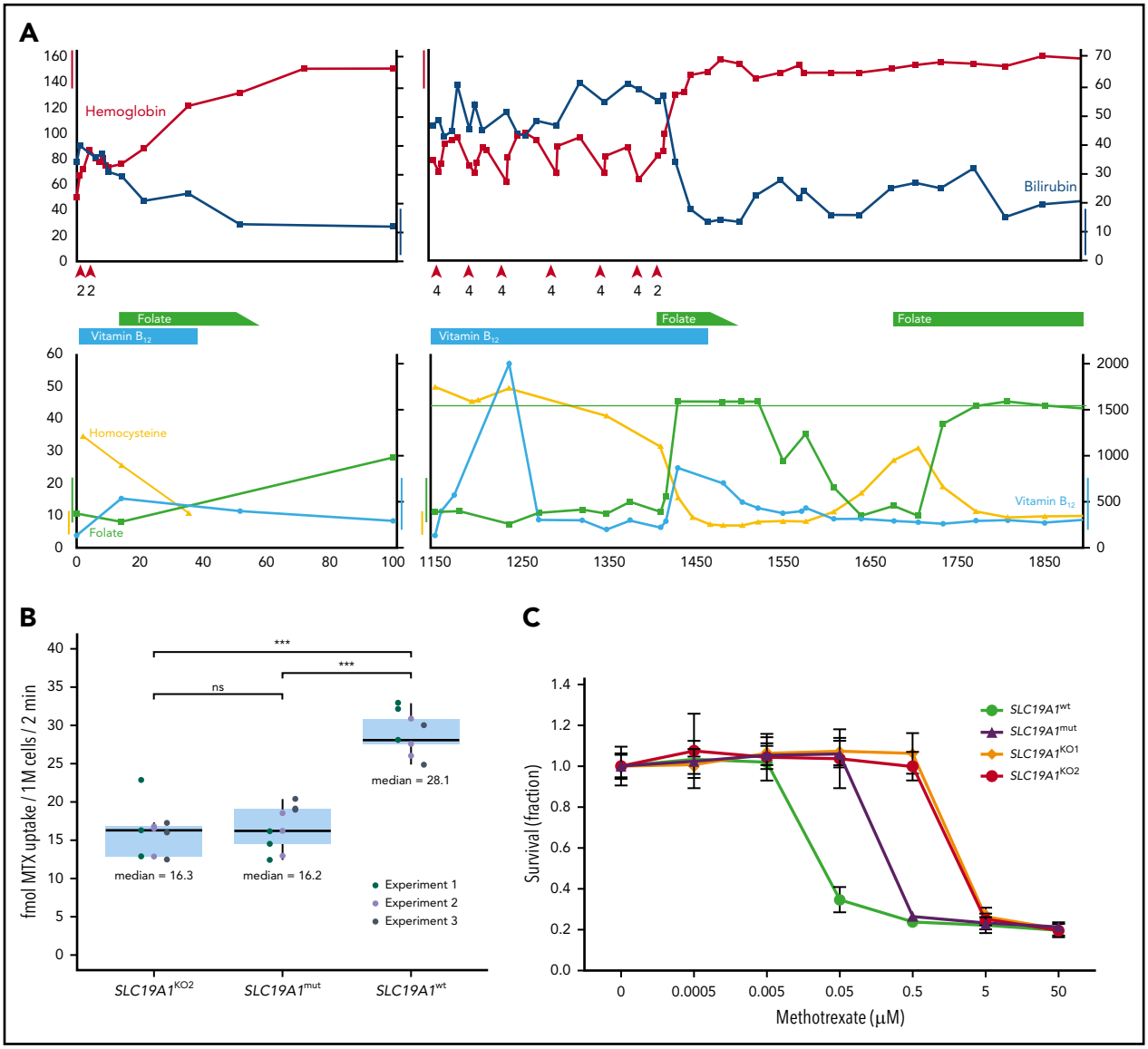

Figure 1. Course of patient's disease and functional evaluation of the *SLC19A1* mutation. (A) The graphs show the results of laboratory tests obtained after the patient's first and second disease attacks and the course of treatment. The x-axis represents days from his first hospital admission (interrupted for a period between day 100 and 1150, when he was monitored in the outpatient department, and laboratory results were normal). The hemoglobin levels (g/L; red) are plotted on the left y-axis, and total bilirubin (μmol/L; blue) is plotted on the right y-axis. The serum folate levels (nmol/L; green) are plotted in the lower part of the graph, with homocysteine (μmol/L; orange) on the left y-axis and vitamin B_{12} levels (ng/L; blue) on the right y-axis. Reference ranges are marked by bars in the corresponding colors on both axes. Treatment is indicated between both graphs, with erythrocyte transfusions marked by red arrows and the number of units given. (B) MTX uptake assay into wild-type and monoclonal gene-edited K562 model cell lines. The graph shows the amount of [3H]MTX per 1 million cells after 2-minute incubation with 0.5 μmol/L [3H]MTX. MTX uptake into the *SLC19A1*^{KO} and *SLC19A1*^{mut} cells was significantly decreased in comparison with the *SLC19A1*^{wt}. The data points were obtained from 3 independent experiments (each with 3 technical replicates) and tested by two-way analysis of variance with Bonferroni corrections; horizontal lines in the boxplots indicate medians and the 25th and 75th percentiles. (C) Sensitivity of wild-type and monoclonal gene-edited K562 model cell lines to MTX. Cell proliferation assays of the K562 cell lines with CRISPR/Cas9 introduced homozygous (*SLC19A1*^{mut}) mutation found in the patient compared with a wild-type control and to a complete *SLC19A1* KO (2 representative clones carrying different frameshift mutations). The assay was performed in 3 separate experiments, each of which was performed in 6 replicates. The results are shown as the mean and standard deviation of all values, which were normalized to the appropriate controls in each experiment. ns, not significant. ***$P < .001$.

Whole exome sequencing revealed a homozygous 3bp deletion, c.634_636delTTC (rs757838708), in the *SLC19A1* gene inherited from heterozygous parents. This variant leads to a deletion of phenylalanine residue 212 (p.Phe212del) located in a highly conserved Lys204-Arg214 peptide sequence that is crucial for the function of RFC[11] (supplemental Figure 2).

To test the pathogenicity of the p.Phe212del mutation, we generated a model system using genome-editing techniques to produce a monoclonal homozygous *SLC19A1*^{mut} K562 cell line and 2 clones with full *SLC19A1* gene knockouts (KOs) as a result of frameshift mutations (supplemental Figure 4C-D). First, we tested the capacity of the mutated RFC protein to transport radioactively labeled MTX into cells. The ability of the *SLC19A1*^{mut} clone to transport MTX into K562 cells was significantly decreased by ~42% compared with the *SLC19A1*^{wt} clone ($P < .001$), and it was similar to that of the *SLC19A1*^{KO} clone (Figure 1B). The residual MTX transport into *SLC19A1*^{mut} and *SLC19A1*^{KO} cells

represents the capacity of all other endogenously expressed folate transporters[12] with the exception of RFC. Confocal microscopy using a monoclonal anti-RFC antibody showed the presence of antigen in the *SLC19A1*^{mut} clone, which indicated sufficient (although slightly decreased) expression of the p.Phe212del mutant (supplemental Figure 5). Next, we examined the resistance of these cell lines to the cytotoxic effects of MTX. As expected, the homozygous *SLC19A1*^{mut} clone showed an increased resistance to MTX compared with the *SLC19A1*^{wt} cells (50% inhibitory concentration [IC_{50}], 0.287 vs 0.036 μM). However, the IC_{50} of the *SLC19A1*^{mut} clone was still about an order of magnitude lower compared with that of the 2 clones with a complete *SLC19A1* KO (IC_{50}, 3.078 and 1.681 μM; Figure 1C). In summary, these experiments clearly and congruently showed that homozygous deletion of the Phe212 residue impairs transport of MTX and increases the resistance of genetically modified cells to this cytotoxic anti-folate drug, which uses RFC to enter the cells.

This patient presented a diagnostic conundrum that was resolved owing to next-generation sequencing analysis. The biochemical findings at the beginning of both episodes of anemia were dominated by low concentrations of serum vitamin B_{12}, probably caused by poor nutrition and/or increased demand for cobalamin in the remethylation pathway. The absence of serum folate deficiency obscured the role of folates in the patient's anemia; however, folates were decreased in erythrocytes in the single analysis performed when the patient was not treated with folic acid. The classical metabolic consequences of folate deficiency such as decreased serum methionine and increased cystathionine were not present, but there was markedly increased total homocysteine and sarcosine in blood and the purine de novo synthesis intermediate 5-amino-4-imidazolecarboxamide riboside (AICAr) in urine,[13-15] as well as their normalization only after introducing folic acid treatment, which strongly indicates a functional folate deficiency in tissues.[13,16]

The episodic clinical course in the patient was milder than that observed in the KO mouse model.[10] Because of the existence of multiple folate transporters and their complex biology,[2] it is conceivable that under conditions of normal dietary folate intake, the other folate transporters may compensate for the impaired RFC transport activity.[17] Indeed, an increased expression of the *SLC46A1* and *FOLR2* genes (7- to 14-fold messenger RNA increase in the patient compared with healthy controls) was observed in the patient's bone marrow as well as in the gene-edited mutant and KO K562 cells (supplemental Figure 6). These data indicate a grossly increased total capacity of alternative (albeit less kinetically favorable) folate transporters that may compensate for the impaired function of the p.Phe212del RFC under conditions of sufficient folate intake and moderate cellular needs (see supplemental Results). However, inadequate folate intake combined with increased demand for folates reported in adolescent males[18,19] may have contributed to episodes of anemia in our patient.

Our study describes the first human patient with recurrent megaloblastic folate–dependent anemia resulting from a homozygous p.Phe212del mutation in the *SLC19A1* gene. Several lines of evidence support the causal role of this mutation in the pathogenesis of anemia in this patient. First, the role of RFC in hematopoietic cells was clearly demonstrated in a KO mouse model.[10] Second, the low frequency of this mutation in available population databases (see supplemental Results) and its location in the conserved cytosolic loop of RFC supports the hypothesis of its pathogenicity. Third, detailed functional testing in CRISPR/Cas9-edited K562 cells clearly showed the decreased ability of the mutant protein to transport 5-methyltetrahydrofolate analog MTX; because of the intrinsic limitations of the cellular transport studies, the degree of functional impairment could not be exactly quantitated and some residual activity of p.Phe212del RFC cannot be ruled out. Fourth, the clinical observation of severe anemia with megaloblastic changes in the patient's BM suggestive of impaired maturation and ineffective hematopoiesis as well as the signs of demyelination on electromyography are indeed compatible with clinically significant tissue folate deficiency. Finally, the reversal of anemia and rapid drop of total homocysteine and AICAr concentration only after the addition of folic acid to cyanocobalamin therapy during the second episode is typical for folate deficiency.[20]

In summary the above data support the key role of defective folate transport to hematopoietic cells in the development of anemia in this patient. We propose that there may be additional individuals with germline mutations in *SLC19A1*; however, their phenotypes could be masked either by mandatory folate fortification or by the commonly used combined treatment with vitamin B_{12} and folic acid in those with unresolved megaloblastic anemia without properly elucidated primary cause.

Acknowledgments

This work was supported in part by grant no. 17-04941Y from the Czech Science Foundation (K.S.K. and M.S.), grants no. PRIMUS/19/MED/004 (K.S.K. and M.R.) and no. GAUK 502119 (M.R.) from Charles University, and grants no. NV18-07-00430 (E.F. and J. Stary) and no. NV19-01-00307 (V. Kozich) from the Czech Health Research Council. Institutional support was provided by programs 00064203 (University Hospital Motol, Prague, Czech Republic), RVO-VFN 64165 (General University Hospital in Prague), RVO 00023728 (Institute of Rheumatology), Progress Q26 (Charles University), ERDF OPPK CZ.2.16/3.1.00/28007, and CZ.2.16/3.1.00/24505. The research infrastructure was supported by program LO1604 (Ministry of Education, Youth and Sports, Czech Republic).

Authorship

Contribution: M.S., K.S.K., and B.S. performed and planned the research, analyzed and interpreted the data, and wrote the manuscript; V. Kanderova, V.B.-M., E.V., A.M., J.K., M.R., R.D., J. Sokolova, J. Stuchly, and T.K. performed the research and analyzed the data; P.S. and P.J. collected and interpreted the data and clinically evaluated the patient; E.F., V. Kozich, J.T. and J. Stary designed the study and wrote the paper; and all authors revised the paper and approved the final version.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: M.S., 0000-0003-2966-3687; K.S.K., 0000-0003-4860-7453; A.M., 0000-0003-4487-1943; J.K., 0000-0002-1738-654X; B.S., 0000-0003-2465-1476; R.D., 0000-0003-0081-156X; J. Sokolova, 0000-0002-0453-3336; T.K., 0000-0003-4475-2872; E.F., 0000-0002-6900-8145; V. Kozich, 0000-0001-5820-5277.

Correspondence: Viktor Kozich, Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital, Ke Karlovu 455/2, 128 08 Prague 2, Czech Republic; e-mail: viktor.kozich@vfn.cz; and Eva Fronkova, Childhood Leukemia Investigation Prague, Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University and University

Hospital Motol, V Uvalu 84, 150 06 Prague 5, Czech Republic; e-mail: eva.fronkova@lfmotol.cuni.cz.

Footnotes

*M.S. and K.S.K. contributed equally to this article as joint first authors.

†E.F. and V. Kozich contributed equally to this article as joint senior authors.

For original data, please contact michael.svaton@lfmotol.cuni.cz.

The online version of this article contains a data supplement.

REFERENCES

1. Newman AC, Maddocks ODK. One-carbon metabolism in cancer. *Br J Cancer*. 2017;116(12):1499-1504.
2. Alpers DH. Absorption and blood/cellular transport of folate and cobalamin: Pharmacokinetic and physiological considerations. *Biochimie*. 2016;126:52-56.
3. Whetstine JR, Flatley RM, Matherly LH. The human reduced folate carrier gene is ubiquitously and differentially expressed in normal human tissues: identification of seven non-coding exons and characterization of a novel promoter. *Biochem J*. 2002;367(pt 3):629-640.
4. Qiu A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell*. 2006;127(5):917-928.
5. Steinfeld R, Grapp M, Kraetzner R, et al. Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism. *Am J Hum Genet*. 2009;85(3):354-363.
6. Matherly LH, Hou Z, Deng Y. Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. *Cancer Metastasis Rev*. 2007;26(1):111-128.
7. Ritchie C, Cordova AF, Hess GT, Bassik MC, Li L. SLC19A1 Is an Importer of the Immunotransmitter cGAMP. *Mol Cell*. 2019;75(2):372-381.e5.
8. Zhao R, Sharina IG, Goldman ID. Pattern of mutations that results in loss of reduced folate carrier function under antifolate selective pressure augmented by chemical mutagenesis. *Mol Pharmacol*. 1999;56(1):68-76.
9. Zhao R, Goldman ID. Resistance to antifolates. *Oncogene*. 2003;22(47):7431-7457.
10. Zhao R, Russell RG, Wang Y, et al. Rescue of embryonic lethality in reduced folate carrier-deficient mice by maternal folic acid supplementation reveals early neonatal failure of hematopoietic organs. *J Biol Chem*. 2001;276(13):10224-10228.
11. Liu XY, Witt TL, Matherly LH. Restoration of high-level transport activity by human reduced folate carrier/ThTr1 thiamine transporter chimaeras: role of the transmembrane domain 6/7 linker region in reduced folate carrier function. *Biochem J*. 2003;369(pt 1):31-37.
12. Biswal BK, Verma RS. Differential usage of the transport systems for folic acid and methotrexate in normal human T-lymphocytes and leukemic cells. *J Biochem*. 2009;146(5):693-703.
13. Herbert V, Streiff RR, Sullivan LW, McGeer PL. Deranged purine metabolism manifested by aninoimidazolecarboxamide excretion in megaloblastic anaemias, haemolytic anaemia, and liver disease. *Lancet*. 1964;2(7349):45-46.
14. Deodhar SD, Pittman G. A study of the metabolism of 4-amino-5-imidazolecarboxamide (AIC) in folic acid deficiency in rats. *Cleve Clin Q*. 1966;33(4):191-194.
15. Baggott JE, Morgan SL, Sams WM, Linden J. Urinary adenosine and aminoimidazolecarboxamide excretion in methotrexate-treated patients with psoriasis. *Arch Dermatol*. 1999;135(7):813-817.
16. Allen RH, Stabler SP, Lindenbaum J. Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. *Metabolism*. 1993;42(11):1448-1460.
17. Qiu A, Min SH, Jansen M, et al. Rodent intestinal folate transporters (SLC46A1): secondary structure, functional properties, and response to dietary folate restriction. *Am J Physiol Cell Physiol*. 2007;293(5):C1669-C1678.
18. Monsen AL, Refsum H, Markestad T, Ueland PM. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. *Clin Chem*. 2003;49(12):2067-2075.
19. Pfeiffer CM, Johnson CL, Jain RB, et al. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988-2004. *Am J Clin Nutr*. 2007;86(3):718-727.
20. Antony AC. Evidence for potential underestimation of clinical folate deficiency in resource-limited countries using blood tests. *Nutr Rev*. 2017;75(8):600-615.

DOI 10.1182/blood.2019003178