and P.S.R. assembled population projections; and all authors analyzed results and wrote the letter.

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To the editor:

First characterization of platelet secretion defect in patients with familial hemophagocytic lymphohistiocytosis type 3 (FHL-3)

Familial hemophagocytic lymphohistiocytosis (FHL), a rare autosomal recessive disorder of lymphocyte cytotoxicity, is caused by mutations in genes encoding perforin (FHL-2) or proteins important for intracellular trafficking/exocytosis of perforin-containing lytic granules: Munc13-4 (FHL-3), syntaxin 11 (FHL-4), and Munc18-2 (FHL-5).[1] FHL-1 is due to an unidentified gene defect located on chromosome 9. Munc13-4 (a Rab27a effector) coordinates exocytosis in hematopoietic cells.[2,3] Munc13-4 deficiency (FHL-3) results in defective cytolytic granule exocytosis.[4] Interestingly, platelets from Munc13-4–deficient mice showed a severe secretion defect of α- and dense granules.[5] In patients with FHL-3, bleeding symptoms have been rarely reported because patients may have not been challenged (ie, surgery).[6] An 8-month-old Chinese FHL-3 patient died because of gastrointestinal hemorrhage.[7] A platelet secretion defect in patients with FHL-5 has been described previously.[8,9]

Therefore, we analyzed platelet function in 2 unrelated male patients with genetically confirmed FHL-3. Both patients presented with clinical symptoms indicative of hemophagocytic lymphohistiocytosis (HLH) and fulfilled the diagnostic criteria.[10] Functional analyses showed absent degranulation of cytotoxic T cells and reduced degranulation of natural killer cells. Patient 1 (diagnosed at the age of 4 months) showed compound heterozygous mutations in the *UNC13D* gene accounting for FHL-3: c.551G>A (p.W184X) in exon 6 and c.118-308C>T in intron 1. After treatment according to the HLH 2004 protocol, haploidentical hematopoietic stem cell transplantation was performed at the age of 16 months.[10] Patient 2 (diagnosed at the age of 5 months) had severe central nervous system involvement and showed compound heterozygous splice donor site mutations of *UNC13D*: c.753+1G>T of exon 9 and c.1389+1G>A of exon 15. The patient received treatment according to the HLH 2004 protocol and underwent hematopoietic stem cell transplantation from a matched unrelated donor at the age of 9 months. He died of HLH relapse due to graft failure 4 months later. No major bleeding episodes were observed in either patient. The platelet studies were performed shortly before transplantation when patients were stable and did not receive medication, which induced secretion defects. Platelet count was normal at the time of platelet studies.

Flow cytometric analyses of platelets from both patients revealed severely diminished to absent platelet α- (CD62P) and dense granule (CD63) secretion in response to thrombin, collagen, and thrombin receptor activating peptide (TRAP) in platelet-rich plasma (Figure 1A-D; TRAP not shown). Collagen-induced adenosine triphosphate secretion (lumiaggregometry) in whole blood was absent (Figure 1E), whereas agonist-induced fibrinogen binding (data not shown) and aggregation (Figure 1F) were normal for a child of that age. In addition, flow cytometric analyses of platelets from patient 2 showed absent mepacrine uptake and release (a marker of uptake and release of dense body contents), as well as absent expression of the lysosomal marker CD107a (LAMP-1) in response to collagen and TRAP (data not shown).

These data demonstrate a selective impairment of platelet granule secretion in patients with FHL-3, and thus support an important role for Munc13-4 in human platelet degranulation. Although bleeding symptoms in FHL-3 patients can be mild, our findings clearly demonstrate that Munc13-4 deficiency is more than a genetic disorder of cytotoxicity.

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Figure 1. Platelet function analyses. Flow cytometric quantification of platelet granule secretion in platelet-rich plasma was stimulated using increasing concentrations of thrombin (0, 0.05, 0.1, 0.2, 0.5, and 1.0 U/ml) (A-B) or collagen (0, 0.25, 0.5, 1, and 2 μg/ml) (C-D). After fixation cells were washed and incubated with fluorescein isothiocyanate-conjugated anti-CD62 (A), phycoerythrin-conjugated anti-CD62 (C), or fluorescein isothiocyanate-conjugated anti-CD63 (B,D). Surface fluorescence was analyzed with a flow cytometer (FACSCalibur; Becton Dickinson). Data are expressed as linear arbitrary units. Analyses were performed with patients' platelets and platelets from a healthy control. Platelet adenosine triphosphate release in response to collagen was monitored by lumiaggregometry in whole blood (E). Relative luminescence in percent was plotted as a function of time. Analyses were performed with platelets from patient 2 and platelets from a healthy control. Collagen-induced platelet aggregation of platelets from patient 2 and platelets from a healthy control was analyzed using a PAP4-aggregometer (F).
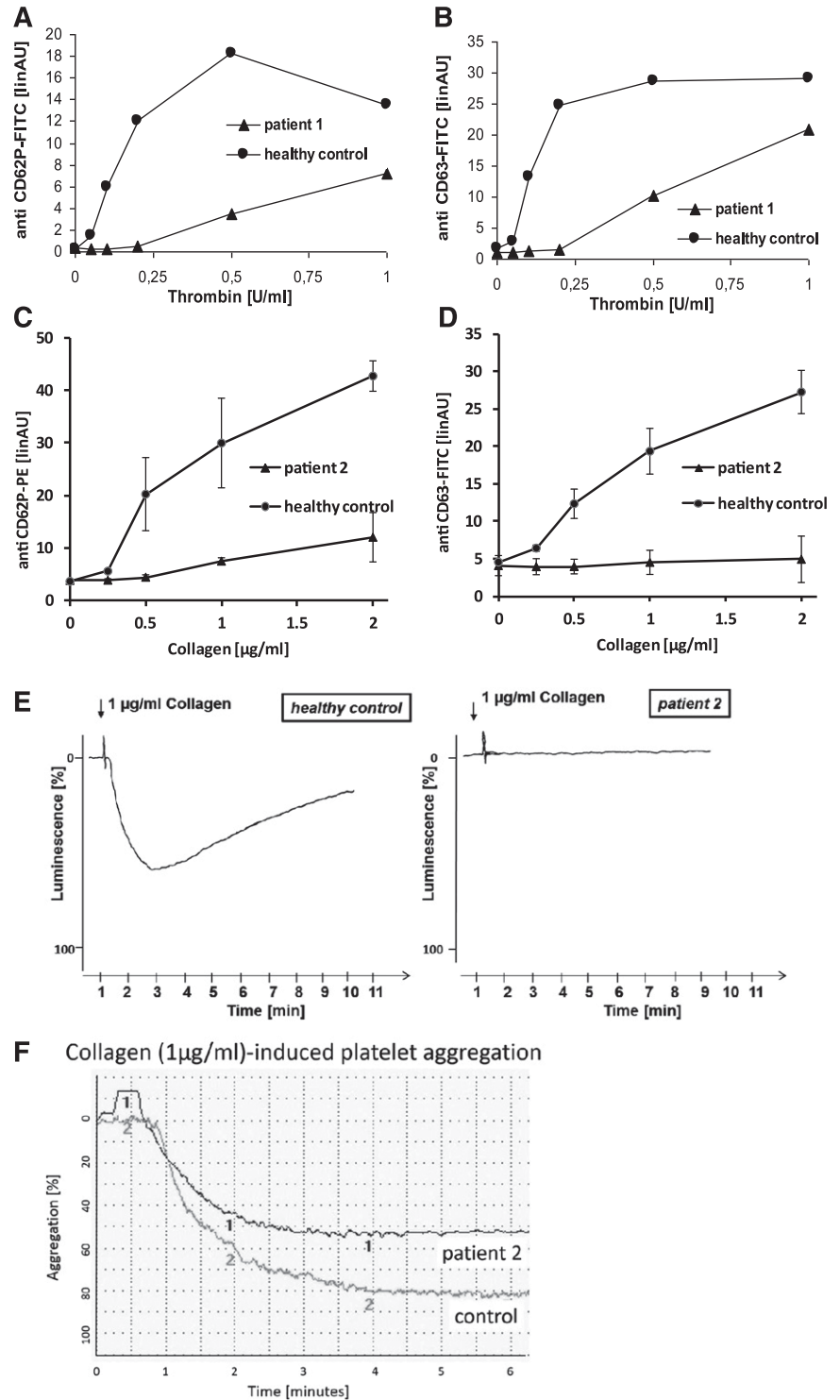
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To the editor:

Complications in children and adolescents with Chuvash polycythemia

In the Chuvash form of congenital polycythemia,[1] a homozygous germ-line VHL^{R200W} mutation leads to impaired degradation of the α subunits of the hypoxia inducible transcription factors (HIF-1α and HIF-2α) and augmented hypoxic responses during normoxia including inappropriately elevated erythropoietin levels.[2] Mutated VHL^{R200W} reportedly binds with increased avidity to suppressor of cytokine signaling 1, which hinders Janus kinase (JAK) 2 degradation, leading to erythropoietin-hypersensitive growth of erythroid progenitors.[3] JAK2 inhibitors have been shown to correct the Chuvash polycythemia phenotype in a mouse model[3] and anecdotally in people. In addition to elevated red cell mass, Chuvash polycythemia is marked by headache, vertigo or dizziness, leg varices, increased systolic pulmonary artery pressure, aberrant iron metabolism, hemorrhage, thrombosis not correlating with hematocrit elevation, and early mortality.[1,4,5] There is no demonstrated effective therapy for reducing symptoms, complications, or mortality.

The complications of Chuvash polycythemia have not been addressed specifically in children and adolescents, but available evidence raises concern for untoward consequences. In the first description of this disorder in the 1970s, 103 patients were diagnosed at a median age range of 10-19 years and followed for a median of 6 years.[1,6] Eleven patients died, 2 of them <20 years of age. In a follow-up of this cohort in 2001, mortality (often attributable to cerebral infarction or hemorrhage) was >25% by age 40 years.[4]

We enrolled 30 subjects with Chuvash polycythemia (VHL^{R200W} homozygotes) and 16 Chuvash controls (VHL wild type) <21 years of age into a registry in 2005-2006 to prospectively evaluate their clinical course. The health status of these subjects was reevaluated in June 2014. Approval was obtained from the institutional review boards of Cheboksary Children's Hospital and Chuvash Republic Clinical Hospital 1 for the study, and the subjects or parents gave written informed consent according to the Declaration of Helsinki.

Baseline characteristics of this cohort are summarized in Table 1. The VHL^{R200W} homozygotes (age range of 6-20 years) tended to be older than the VHL wild-type controls (age range of 3-20 years) but the sex distributions were similar. Eight VHL^{R200W} homozygotes had had intermittent phlebotomies, 13 were being treated with aspirin, and 13 were being treated with cinnarizine, an antihistamine and calcium channel blocker used to promote cerebral blood flow. Prominent symptoms at study entry in the VHL^{R200W} homozygotes included headache in 22, lower extremity pain in 15, and dizziness in 13. Frequent physical findings included plethora and a tendency to lower systolic blood pressure. The median hemoglobin concentration was 19.1 g/dL in the VHL^{R200W} homozygotes vs 14.0 g/dL in the controls, and the erythropoietin concentrations were higher in the VHL^{R200W} homozygotes despite the higher hemoglobin concentrations. Headache was reported in 13 (100%) of VHL^{R200W} homozygotes treated with cinnarizine vs 9 (53%) of those not treated ($P = .004$), and dizziness was a symptom in 6 (46%) of homozygotes treated with cinnarizine vs. 5 (29%) of those not treated ($P = .3$). Similarly, headache was a symptom in 12 (92%) of VHL^{R200W} homozygotes treated with aspirin vs 10 (59%) in those not treated ($P = .09$), and dizziness was a symptom in 5 (38%) of homozygotes treated with aspirin vs 6 (35%) of those not treated ($P = .6$).

We were able to obtain follow-up information for 29 of the VHL^{R200W} homozygotes and all 16 controls in 2014 at a median of 8 years (range 1-9 years) after entry into the registry (Table 1). Nine subjects (31%), 3 of 15 females and 6 of 14 males, and no controls have had complications. In 7 of these subjects, the complications are plausibly related to Chuvash polycythemia. One male died at the age of 17 years from thromboembolism. Two subjects had cardiac complications at age 19 and 24 years (i.e., chest pain with exertion and paroxysmal atrial tachycardia, respectively). Two subjects were found to have benign cysts: 1 investigated for severe headache at age 21 years had a retro-cerebellar arachnoid cyst, and the other had