human mutations that disrupt transcription factors including KLF1,4 and GATA13 highlight the importance of these factors in HbF expression and human erythropoiesis more generally. Hence, human genetic studies of globin gene regulation are critical to better understand the in vivo requirement for many putative regulators of this process.

Recent studies suggest that the transcription factor SOX6 has a critical role in silencing of the γ-globin (HbF) and related mouse embryonic globin genes.6-8 This activity appears to occur in collaboration with BCL11A. We studied a 10-year-old male patient with a disruption of the SOX6 gene from a balanced translocation (karyotype of 46,XY, t(9;11)(q33.1;p15.1)) found in the course of a genetic workup for craniosynostosis (Figure 1A-B).9 The craniosynostosis appeared to be attributable to this heterozygous disruption that likely results in haploinsufficiency, which is supported by the finding of a SOX6 missense mutation in another patient with craniosynostosis and evidence for a role of this gene in joint and cartilage development.9 The patient had release surgery of the lambdoid and posterior sagittal sutures as an infant and has since been clinically asymptomatic. Approval for this study was obtained from the Ethics Review Board of the Medical Faculty at the University of Cologne. Informed consent was provided according to the Declaration of Helsinki. We were able to obtain a blood sample from this patient for hematologic analysis.

A complete blood count was within normal limits and showed no evidence of anemia or other abnormalities (white blood cells: 5900 cells/mL; red blood cells: 4.53 × 10⁶ cells/mL; Hb: 12.8 g/dL; Hct: 38.5%; and platelets: 212 000/μL). Loss of SOX6 activity derepresses human fetal or mouse embryonic globin gene expression in several experimental systems.6-8 However, capillary electrophoresis of the patient’s blood showed no detectable HbF expression present (0% HbF, 97.8% HbA, 2.2% HbA2) (Figure 1C). We additionally analyzed the percentage of red blood cells containing a significant fraction of HbF, using the F-cell assay (Figure 1D). This revealed that the patient had F cells within the normal range (0.7% for this patient, with a normal range of 0.4%-4%).

These results suggest that heterozygous SOX6 disruption is insufficient to impair normal erythropoiesis or silencing of the HbF genes in vivo. In contrast, lowering the level of SOX6 by ~80% increases HbF expression in cultured human erythroblasts.7 Thus, repression of γ-globin expression may require reduction of SOX6 expression below a critical threshold that is not attained by haploinsufficiency. However, of note, the effect of SOX6 on erythropoiesis appears to be sensitive to the level of expression present.10 It is also possible that dosage compensation of SOX6 gene expression occurs from the intact allele in erythroid cells, in contrast to bone/cartilage cells where heterozygous gene disruption gives rise to the craniosynostosis phenotype. We could not differentiate between these possibilities, because we were unable to get consent to obtain appropriate tissue or cells for expression studies. Regardless, analysis of this patient shows that in contrast to other transcription factors, such as KLF1, heterozygous disruption of SOX6 does not alter erythropoiesis or γ-globin gene expression in vivo. Thus, distinct gene dosage requirements exist for 2 different erythroid transcription factors that function in a common pathway to repress γ-globin expression. Our findings have important implications for developing therapeutic strategies to target the KLF1–BCL11A–SOX6 HbF silencing pathway2,7 and emphasize the utility of using rare human mutations to better understand aspects of globin gene regulation and erythropoiesis.

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To the editor:

Investigation of the ABC transporter MRP1 in selected patients with presumed defects in vitamin B12 absorption

Recently, Beedholm-Ebsen and colleagues reported that the ABCC1 gene is involved in the efflux of cobalamin (Cbl; vitamin B12) from the enterocytes and other cells to the plasma.3 ABCC1 encodes the multidrug resistance protein 1 (MRP1), a member of the ATP-binding cassette (ABC) transporters. MRP1 was shown to export unbound Cbl across the membrane.2

References

Cbl is usually bound to various carrier proteins: to haptocorrin or gastric intrinsic factor (IF) after release from the food, or to haptocorrin and transcobalamin 2 in the serum. For intestinal absorption, the Cbl-IF complex is recognized by cubam, a multi-ligand endocytic receptor on the enterocyte in the intestine. After entering the enterocyte, lysosomal enzymes degrade the IF, and then transcobalamin 2 transports Cbl to the tissues via the blood. 

Hereditary vitamin B12 uptake deficiency is attributed to defects in CUBN, AMN, or GIF. We have examined more than 150 patients or sibships with recessive hereditary Cbl malabsorption (and unpublished results). Approximately 80% were mutated in either AMN or CUBN (causing Imerslund-Gräsbeck syndrome) or in GIF (causing intrinsic factor deficiency). However, some 15%-20% of cases remain unexplained, and they represent an ideal cohort to screen for potential mutations in the ABCC1 gene. These presumed Cbl malabsorption cases were excluded for defects in CUBN, AMN, and GIF because of incompatible genetics and/or direct sequencing, and we chose 18 unrelated patients and the mothers of 2 additional patients from North America, Europe, the Middle East, and North Africa to study ABCC1. Using exon-by-exon genomic DNA sequencing, we screened the entire gene, including flanking splice junctions, for any sequence changes.

We detected a total of 27 changes in ABCC1 compared with the published sequence (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online letter). Four intronic insertions/deletions (14.8%) and 2 missense (7.4%), 6 silent (22.2%), and 15 intronic single nucleotide polymorphisms (SNPs) were identified (55.6%). Twenty-six of the 27 changes have a SNP identifier number (supplemental Table 1). One change in the 5′-UTR has not been described before: c.1-79_66del14bp, which was found to be heterozygous in one of the patients. All identified changes were ruled out as potentially causal for Cbl deficiency because they did not fulfill Mendelian rules for recessive inheritance (even when considering compound heterozygosity for 2 different ABCC1 changes in one patient), were intronic, not affecting the open reading frame, or found in the normal population (SNPbase). Two rare missense SNPs were each found once in heterozygosity. G671V appears to be a conservative change, whereas R723Q is potentially functional, but both were seen in the population at large (SNPbase).

MRP1 is the first known eukaryotic membrane efflux transporter capable of transporting unbound Cbl out of cells. Based on the observation that Mrp1−/− mice present no Cbl-related phenotype, one might conclude that MRP1 plays no role in inherited Cbl deficiency. However, whereas humans with various loss-of-function mutations in AMN develop Imerslund-Gräsbeck syndrome (causing intrinsic factor deficiency), mice present no Cbl-related phenotype, indicating that the same gene may have different functions during embryogenesis in humans and rodents. Analogously, we surmised that mutational screening of ABCC1 in suspected Cbl deficiency was warranted if all known causal genes were excluded beforehand. Based on screening this highly selected patient cohort, we conclude that mutations in the ABCC1 gene, if they exist, are unlikely to cause overt Cbl deficiency in humans. On the other hand, defects in ABCC1 that result in a milder or different phenotype would probably not prompt a clinical follow-up suspecting Cbl malabsorption. Accordingly, alternative Cbl efflux pathways yet to be identified may have to explain the remaining cases of inherited Cbl deficiency.

References

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The online version of this letter contains a data supplement.
Investigation of the ABC transporter MRP1 in selected patients with presumed defects in vitamin B12 absorption

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