determined as platelet diameters on peripheral blood smears in genetically confirmed Bernard-Soulier syndrome and MYH9 disorders were 5.3 (± 1.1) μm (n = 14) and 4.9 (± 1.1) μm (n = 81), respectively. These data are comparable with that of the patient in our study. Because routine automated blood cell counting systems differentiate blood cells by their size and therefore do not recognize giant platelets as platelets, mean platelet volume (MPV) does not reflect actual platelet size. As is quite often the case, an automated counter did not generate MPV in the patient or the mother. We believe that the determination of platelet diameter on peripheral smear is a more reliable way to detect macrothrombocytopenia.

We admit that it is premature to make definite conclusions about how W318 β1-tubulin affects the normal platelet production and results in macrothrombocytopenia. This is mainly because the causative relationship between the mutation and giant platelets was not convincingly investigated in our work. To clarify this, we are planning to establish knockin cell models that equally express wild-type and mutant β1-tubulins identified in human and canine macrothrombocytopenia.

To the editor:

Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation

The different genetic forms of hereditary hemochromatosis share as their pathogenic mechanisms the deficiency or dysregulation of the hormone hepcidin or defects involving the hepcidin receptor, ferroportin. Ferroportin is the sole known cellular iron exporter in humans and is present on enterocytes, macrophages, and hepatocytes. Normally, the cell-surface concentration of ferroportin is regulated by its interaction with hepcidin. Hepcidin binding triggers the internalization and degradation of the ferroportin-hepcidin complex, causing a decrease in cellular iron release into plasma.

Mutations in the ferroportin (SLC40A1) gene cause iron overload syndromes with autosomal dominant transmission. Ferroportin mutations cause at least 2 distinct phenotypes, depending on the functional alteration of the protein. One subtype of ferroportin mutations, predominantly involving residues located on the putative cytoplasmic side or in transmembrane segments (eg, V162del, D157G, G80S, G490D), results in the loss of iron export function. This leads to increased macrophage iron and elevated serum ferritin, but normal transferrin saturation. This phenotype, referred to as “ferroportin disease,” does not appear to have significant morbidity.

A distinct ferroportin-related phenotype, similar to classical hereditary hemochromatosis, is associated with gain-of-function mutations (eg N144D/T/H, Y64N, C326S/Y). In vitro evidence (companion manuscript, Fernandes et al) indicates that these mutations cause ferroportin’s resistance to the effects of hepcidin. N144D/T and Y64N mutations prevent internalization of hepcidin-ferroportin complex while C326S mutation completely ablates hepcidin binding to ferroportin. We previously reported that C326S mutation results in the most severe iron overload phenotype with early age of onset.

In this study, we measured hepcidin and iron indices in members of the family carrying heterozygous C326S mutations. Hemochromatosis was measured by competitive ELISA (Intrinsic Life-Sciences, La Jolla, CA). The subjects had already been treated by phlebotomy and had normalized iron stores, as assessed by serum ferritin, but their transferrin saturation remained high (Table 1).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at diagnosis/sex</th>
<th>IIA</th>
<th>IIB</th>
<th>IIC</th>
<th>IIIA</th>
<th>IIIB</th>
<th>IIIIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis/sex</td>
<td>35/M</td>
<td>16/F</td>
<td>16/M</td>
<td>16/M</td>
<td>8/F</td>
<td>10/F</td>
<td>10/F</td>
</tr>
<tr>
<td>Transferrin sat, initial</td>
<td>97%*</td>
<td>86%*</td>
<td>94%*</td>
<td>89%*</td>
<td>84%*</td>
<td>97%*</td>
<td></td>
</tr>
<tr>
<td>Transferrin sat, current</td>
<td>79%*</td>
<td>89%*</td>
<td>70%*</td>
<td>89%*</td>
<td>93%*</td>
<td>91%*</td>
<td>51%*</td>
</tr>
<tr>
<td>S-ferritin current, ng/mL</td>
<td>69</td>
<td>99</td>
<td>81</td>
<td>394*</td>
<td>114</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>S-hepcidin, ng/mL</td>
<td>278*</td>
<td>198</td>
<td>297*</td>
<td>459*</td>
<td>453*</td>
<td>396*</td>
<td>13*</td>
</tr>
<tr>
<td>U-hepcidin, ng/mg creat</td>
<td>1308</td>
<td>2743*</td>
<td>2270*</td>
<td>3183*</td>
<td>3463*</td>
<td>3036*</td>
<td>72</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>Cirrhosis</td>
<td>↑ hepatocyte iron</td>
<td>↑ hepatocyte iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>Arthritis, hip replacement</td>
<td>mild arthritis</td>
<td>mild arthritis</td>
<td>mild LFT changes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Normal serum hepcidin median (5%-95% range) was 1 ng/mL (0.2-2.5 ng/mL) for men, and 5 ng/mL (1.7-17 ng/mL) for women. Normal urine hepcidin median (full range) was 502 ng/mg creatinine (71-1762 ng/mg creatinine). M indicates male; F, female; sat, saturation; and creat, creatinine.

†Above-normal values.

†Below-normal values.
One of the patients (IIIC) has become iron-deficient due to phlebotomy combined with menstrual blood loss. Except for patient IIIC, all affected family members had serum and urinary hepcidin concentrations in the upper limit of the normal range or above (Table 1). These relatively high hepcidin levels are probably a consequence of patients’ elevated transferrin saturation. Only IIIC patient had lower hepcidin, likely reflecting the appropriate response of hepcidin to iron deficiency.

The high levels of hepcidin in patients with C326S despite relatively depleted iron stores are in contrast to the low or undetectable hepcidin levels observed in treated patients with HFE, transferrin receptor 2, or hemojuvelin hemochromatosis.9-11 Thus, our study provides the first direct evidence that hereditary hemochromatosis can be caused by the resistance of ferroportin to hepcidin. The absence of hepcidin binding to mutant ferroportin in C326S patients leads to excessive ferroportin activity in duodenal enterocytes and unrestrained dietary iron absorption, which would account for the early-onset systemic iron overload similar to that seen in juvenile hemochromatosis. Our findings support the concept that hereditary hemochromatosis with parenchymal iron-loading phenotype can be caused by either hepcidin deficiency or resistance to the effect of hepcidin.

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Conflict-of-interest disclosure: E.N. and T.G. are cofounders and officers of Intrinsic LifeSciences LLC (La Jolla, CA). The remaining authors declare no competing financial interests.

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References

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