To the editor:

**β1-tubulin gene mutation platelets are not macrothrombocytes**

The brief report by Kunishima et al describing a mutation of the β1-tubulin gene affecting microtubule assembly is of interest, but data presented do not support association of the genetic defect with macrothrombocytopenia. The authors stated both the male propositus and his mother had prominent appearance of giant platelets on peripheral blood. In conclusion, we emphasize that our data confirm a positive regulatory role for PKCθ in platelets.

**References**


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

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**Response**

**W318 β1-tubulin and macrothrombocytopenia**

We would like to thank Dr White for valuable comments on our study that reported the first human β1-tubulin mutation associated with congenital macrothrombocytopenia. We have been working on congenital macrothrombocytopenia and analyzed more than 200 cases. We do not think that giant platelets from macrothrombocytopenia syndromes are necessarily larger than red blood cells. The mean platelet sizes...
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.1 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 human1 and canine2 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.1 Ferroportin is the sole known cellular iron exporter in humans and is present on enterocytes, macrophages, and hepatocytes.2 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.3

Mutations in the ferroportin (SLC40A1) gene cause iron overload syndromes with autosomal dominant transmission.4 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.5 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).6,7 In vitro evidence (companion manuscript, Fernandes et al8) 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.6

In this study, we measured hepcidin and iron indices in members of the family carrying heterozygous C326S mutations.6 Hepcidin 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>IA</th>
<th>IIA</th>
<th>IIB</th>
<th>IIC</th>
<th>IIIA</th>
<th>IIIB</th>
<th>IIIC</th>
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<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>
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<tr>
<td>Transferrin sat, initial</td>
<td>97%*</td>
<td>86%*</td>
<td>94%*</td>
<td>89%*</td>
<td>84%*</td>
<td>97%*</td>
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</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>21</td>
<td>111</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>131</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>
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<tr>
<td>Liver biopsy</td>
<td>Cirrhosis</td>
<td>↑ hepatocyte iron</td>
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<td>Clinical</td>
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<td>mild LFT changes</td>
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</tr>
</tbody>
</table>

Normal serum hepcidin median (5%-95% range) was 112 ng/mL (29-254 ng/mL) for men, and 65 ng/mL (17-286 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.

References

Response:W318 β1-tubulin and macrothrombocytopenia

Shinji Kunishima and Hidehiko Saito