Congenital macrothrombocytopenia associated with a combination of functional polymorphisms in the TUBB1 gene

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Summary
Congenital thrombocytopenia in childhood and adolescence requires an extensive diagnostic workup to find the underlying reason. We report on a 13-year-old female patient who was incidentally found to have moderate thrombocytopenia which was also diagnosed in her father and brother. Within the microscopic evaluation of a peripheral blood smear macrothrombocytes were found. Immunofluorescence microscopy of the patient’s platelets detected the lack of β1-tubulin. Analysis of the TUBB1 gene revealed three known missense variants in heterozygous state which in combination might explain the β1-tubulin defect.

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Zusammenfassung

Although immune thrombocytopenia is a common cause for chronic thrombocytopenia in children and adolescents other disorders potentially associated with low platelet counts should be taken into consideration. The differential diagnosis includes both hematologic malignancies and inherited platelet disorders (IPDs). Macrothrombocytopenias, a subgroup of IPDs, may result from a defective expression of membrane proteins such as Bernard-Soulier syndrome and variant Glanzmann thrombasthenia, MYH9-related diseases (May-Hegglin, Sebastian platelet, Fechtner or Epstein syndrome) or less frequent defects of the cytoskeleton (1). Therefore, the microscopic evaluation of peripheral blood smears is an essential part of the diagnostic workup since automated analyzers do not recognize macrothrombocytes reliably (6).

β1-tubulin is the major element of microtubules and exclusively expressed in mature megakaryocytes and platelets. It is required for normal platelet formation (1, 10, 12) and maintenance of platelet discoid shape (5, 13). The TUBB1 gene encoding β1-tubulin is localized on the long arm of chromosome 20 (MIM *612901). Autosomal dominantly inherited mutations in this gene lead to decreased expression of the protein and may result in mild to moderate thrombocytopenia with platelet counts between 60 and 120 × 10^9/l (ref. range 150–400 × 10^9/l) (4, 10). The underexpression of β1-tubulin, caused by the TUBB1 missense mutation p.R318W, seems to be associated with an impaired microtubule assembly (7). Further, proplatelet formation and release of granule contents can be influenced by p.F260S, another missense mutation of the TUBB1 gene which may lead to a reduced stability of the thrombus and congenital macrothrombocytopenia as well (8). According to the Exome Aggregation Consortium (ExAc) seven SNPs with a minor allele frequency (MAF) of > 1% could have been identified in the TUBB1 gene so far. Although mice deficient in β1-tubulin have a prolonged bleeding time (13) no reports on a bleeding tendency in patients with defective or missing β1-tubulin could be found. For avoiding potential bleedings in the context of invasive procedures patients may prophylac-
tically receive antifibrinolytics and/or DDAVP which are well-known to improve hemostasis in patients with inherited thrombocytopenias (14, 15).

A thrombocytopenic girl

We report on a girl (age: 13 years) who was incidentally found to have moderate thrombocytopenia of 50 x 10^9/l during in-patient monitoring after a mild head injury. Upon presentation at our hemostaseology outpatient department the family stated that as early as nine years back the patient's platelet count was comparatively low while suffering from a viral infection; according to our information no further investigations have been made. No bleeding tendency was reported in everyday life; the girl did not undergo any invasive procedure so far.

Diagnosis

Diagnostic testing of the patient's family revealed a mild thrombocytopenia of both father (138 x 10^9/l) and brother (116 x 10^9/l) while the mother's platelet count was normal. The bleeding history of both father and brother was unsuspicious.

Repeated blood counts from EDTA- and citrate-anticoagulated blood confirmed the patient's thrombocytopenia with platelet counts between 50 and 70 x 10^9/l while erythrocytes, reticulocytes and leukocytes were normal. The mean platelet volume (> 14 fl, ref. range 6–13) and the immature platelet fraction (20.6%, ref. range 1.1–6.1) were both increased. Microscopic evaluation of a peripheral blood smear showed normal-sized platelets (1–3 µm in diameter), several macrothrombocytes (> 4 µm) and few giant platelets (> 7 µm, Fig. 1). No inclusion bodies in leucocytes were observed.

A workup of the plasmatic coagulation system revealed results within the normal range for prothrombin time, activated partial thromboplastin time, fibrinogen, coagulation factor VIII activity, von Willebrand factor (VWF) antigen, VWF collagen binding activity, VWF multimeric analysis and ADAMTS13 activity. Despite moderate thrombocytopenia with counts below 100 x 10^9/l the results of aggregation testing performed in platelet rich plasma (turbidimetric aggregometry, LABiTec® GmbH, Ahrensburg/Germany) and citrated whole blood (impedance aggregometry, Chrono-log Corporation, Havertown/USA) with the agonists adenosine diphosphate, arachidonic acid and collagen were within the lower part of normal range or only slightly impaired (Fig. 2). The luminometric adenosine triphosphate release assay using citrated whole blood and the same agonists as for aggregometry showed a normal platelet secretion. Platelet-specific antibodies were not detected by MAIPA test. Immunofluorescence microscopy was conducted to rule out IPDs. The expression...
and localization of membrane and cytoskeletal proteins, specific markers for α- and δ-granules as well as lysosomes were investigated using a panel of well-characterized antibodies. Hereby, a lack of β1-tubulin was observed (Fig. 3).

For molecular genetic confirmation of this finding sequencing analysis of all coding exons of the TUBB1 gene was performed revealing three heterozygous missense variants: c.128_129delinsCC; p.Q43P (rs463312, rs415064), c.821C>T; p.T274M (rs35565630) and c.920G>A; p.R307H (rs6070697). The variant first mentioned was also found in the brother. The patient’s father could not be analysed anymore because in the meantime he had died due to metabolic syndrome.

**Discussion**

All three missense variants identified in the TUBB1 gene of the patient are known single nucleotide polymorphisms (SNPs). No other causative mutation could be detected in the coding region. The heterozygous dinucleotide substitution c.128_129delinsCC resulting in the amino acid exchange p.Q43P is located in a residue that is highly conserved in all β-tubulin isoforms of different species (4, 11). It shows a prevalence of up to 10% in the general population and is considered to be responsible for the reduced expression of β1-tubulin which might lead to macrothrombocytopenia similar to the missense mutation p.F260S (4, 7).

Freson and colleagues demonstrated that heterozygous carriers of the variant p.Q43P have spherocytic and enlarged platelets. They could show that the variant is present in 24.2% of 33 unrelated patients with congenital macrothrombocytopenia (4). In contrast to that, Kunishima and co-workers stated that this polymorphism does not contribute to the reduced β1-tubulin expression as well as to the macrothrombocytopenia observed in the patient.

As for thrombocytopenia, Basciano and colleagues showed that patients with Bernard-Soulier syndrome and at least one allele of the SNP p.R307H may have lower platelet counts compared to non-SNP patients (76 vs. 124 × 10^9/l) (3).

The combination of two affected alleles might contribute to the reduced β1-tubulin expression as well as to the macrothrombocytopenia observed in the patient.

In this context the microscopic assessment of a blood smear is essential since automation of platelet counts of the most frequent β1-tubulin polymorphism p.R307H as seen in our case (9).

Tab. 1  Laboratory findings in the course of DDAVP testing showing a significant shortening of PFA100® closure times due to the well-known DDAVP-mediated increase of the VWF antigen. Platelet function measured by impedance aggregometry in citrated whole blood.

<table>
<thead>
<tr>
<th>parameter</th>
<th>reference range</th>
<th>before DDAVP</th>
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<th>+ 240 min</th>
</tr>
</thead>
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<tr>
<td>platelet count</td>
<td>(150 – 400 × 10^9/l)</td>
<td>70</td>
<td>59</td>
<td>53</td>
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<tr>
<td>VWF antigen</td>
<td>(50 – 160%)</td>
<td>85</td>
<td>129</td>
<td>133</td>
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<tr>
<td>PFA-100® test</td>
<td>collagen/ADP</td>
<td>125</td>
<td>66</td>
<td>91</td>
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<tr>
<td></td>
<td>collagen/epinephrine</td>
<td>174</td>
<td>90</td>
<td>101</td>
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<tr>
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<td>5</td>
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</tr>
<tr>
<td></td>
<td>collagen</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
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</table>

ADP: adenosine diphosphate

Therapy

For testing the individual effect of DDAVP on primary hemostasis before a potentially required invasive procedure it was administered intravenously over 30 minutes at a dose of 0.3 μg/kg to the meanwhile 17-year-old patient. A shortening of PFA-100® closure times reaching normal ranges 60 minutes and 4 hours after application was observed. As shown (Tab. 1), this effect was caused by the well-known DDAVP-mediated increase of the VWF antigen and not by an improved platelet function which was measured by impedance aggregometry in citrated whole blood.

This variant is exclusively found in the presence of the most frequent β1-tubulin polymorphism p.R307H as seen in our case (9).

Comparing the platelet counts of the index patient, her father and brother it seems very likely that the patient is compound heterozygous for c.128_129delinsCC on the one allele and c.821C>T in cis with c.920G>A on the other one.

**Conclusion**

In case of thrombocytopenia in childhood and adolescence the mean platelet volume is an important marker because it may indicate the presence of a congenital disorder. In this context the microscopic assessment of a blood smear is essential since automated analyzers do not recognize macrothrombocytes reliably. Taken all together the increased mean platelet volume, the presence of macrothrombocytes detected...
by optical microscopy and testing of the patient’s family put things on the right track. Immunofluorescence microscopy and molecular genetic tests are further important tools which may help to make the diagnosis.

Conflict of interest
The authors declare that there are no conflicts of interest.

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References