CAMT-MPL: congenital amegakaryocytic thrombocytopenia caused by *MPL* mutations - heterogeneity of a monogenic disorder - a comprehensive analysis of 56 patients

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**ABSTRACT**

Congenital amegakaryocytic thrombocytopenia (CAMT, MIM #604498) is a rare inherited bone marrow failure syndrome (IBMFS) which usually presents as severe thrombocytopenia at birth without specific characteristics and progresses to aplastic anemia during the first years of life.\(^1\) Deleterious mutations in *MPL* coding for the thrombopoietin receptor have first been identified as single molecular cause of CAMT,\(^3,4\) but the disease is now regarded to be genetically heterogeneous.\(^5\) Indeed, mutations in the gene for thrombopoietin (*THPO*) have been recently described in some of these patients.\(^6-8\) Furthermore, newborns with other IBMFS like Dyskeratosis congenita, Fanconi anemia, MECOM associated syndrome or microdeletion syndromes can present phenotypically as CAMT since pathognomonic signs of these syndromes might be not yet apparent.\(^9,12\) In the following we use the term CAMT-MPL for the IBMFS caused by hiallelic mutations in *MPL*.

Previous descriptions of CAMT-MPL are based on single case reports or small case series, not allowing for a comprehensive evaluation of the phenotypic spectrum of the disease.\(^1,12\) Over the last 20 years we analyzed samples and clinical data from patients suspicious for inherited thrombocytopenia and could identify 56 patients with CAMT-MPL. The aims of our analysis of clinical, genetic and laboratory data are (i) a detailed description of the clinical picture of CAMT-MPL, (ii) the establishment of genotype-phenotype correlations allowing for the prediction
of development of aplastic anemia and malignancies, and (iii) a better understanding of the thrombopoietin-MPL system in vivo.

Methods

Patients

Patient material and clinical data were provided after informed consent. The study was approved by the local ethics committee. Patients suspected to have CAMT were analyzed for mutations in MPL. Twenty-three of the 56 CAMT-MPL patients included in this study were part of earlier publications of our group,4,14,15 two were the subject of single case studies.16,17 Six further patients had an already known heterozygous MPL mutation and a seemingly unaffected second allele.

Sequencing

Mutational analyses were performed by Sanger sequencing from leukocyte derived genomic DNA as described previously.4

In silico analysis of mutation data

PROVEAN,19 SIFT,19 Polyphen2,20 and MutationTaster21 algorithms were used for prediction of the effect of MPL mutations on protein function. Putative splicing mutations were evaluated by BDGP splice site prediction,22 MaxEntScan algorithm,23 and Human Splicing Finder (HSF 3.1).24

Flow cytometric analyses

Flow cytometric analyses of CD110 expression on early hematopoietic progenitors were performed as described earlier.25

Thrombopoietin levels

Thrombopoietin serum or plasma levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D systems).

Results

MPL mutations

We identified 56 patients with homozygous (n=39) or compound heterozygous (n=17) mutations in MPL (Tables 1; Online Supplementary Table S1). We detected 38 different mutations (Figure 1, Table 2), 17 out of them are novel (Tables 1 and 2; Online Supplementary Table S2).

Six different nonsense mutations (allele frequency 20%; including three novel mutations) and three different frame shift deletions (allele frequency 13%) affected 20 different patients (Table 2A and B; Online Supplementary Table S2).

Five different splice site mutations (allele frequency 10%, two novel) affected 11 patients (ten families). With the exception of c.391+5G>C, all are predicted to lead to a complete loss of function (Table 2C). Prediction was confirmed for c.79+2T>A by measurement of missing CD110 surface expression on hematopoietic progenitors (Figure 2, see below) and for 213-1G>A and c.79+2T>A by the severe course of the disease in the affected patients. In contrast, patients with the mutation c.391+5G>C, allowing a residual natural splicing,26 had a less severe course and measurable CD110 expression on hematopoietic progenitors (Figure 2).

The majority of mutations in our patient cohort were missense mutations (24 different mutations in 55 patients, allele frequency 57%, 12 novel, Table 2D) Two hotspots (amino acids 102-104: 18 alleles, 12 patients; proline residues 135-136: six alleles, five patients) account for 21% of all mutated alleles. Fifteen of 24 missense mutations are predicted to be deleterious by all applied algorithms, 23 of 24 by at least one of the algorithms (Table 2D).

Due to the small number of individual cases it is difficult to predict clinical courses from the individual missense mutations. Specifically severe courses were observed in patients affected from p.Arg102Pro, p.Trp154Arg, and p.Leu169His. The latter one was found in three unrelated patients from Chile suggesting a founder mutation with regional significance.
Less severe courses (thrombocytopenia not detected at birth or onset of pancytopenia not in early childhood) were observed in patients with mutations p.Met8Arg, p.Asp295Tyr, p.Pro394Ser and missense mutations in exons 11 and 12 affecting the intracytoplasmic part of the receptor molecule (p.Leu524Arg, p.Pro581Leu, p.Leu594Trp). p.Met8Arg is the most N-terminal mutation in MPL described so far. The mutation is located in the signal peptide region of the MPL precursor protein and might affect signaling of the molecule as well as the function of this codon as a possible alternative translation initiation site. It was homozygously found in a patient from consanguineous parents first diagnosed with thrombocytopenia at the age of 9 months.

The mutation p.Arg454Pro which is predicted to be benign by all applied prediction algorithms was homozygously found in a patient presenting at the age of 2 years with a profound isolated hypomegakaryocytic thrombocytopenia.

p.Arg102His is the third mutation affecting Arg102 in CAMT: p.Arg102Cys and p.Arg102Pro cause a severe phenotype of CAMT in patients20 and disturb intracellular trafficking of the MPL protein27 although p.Arg102His as well as p.Arg102Pro are predicted to be benign by SIFT and PROVEAN algorithms (Table 2D).

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Table 1. Congenital amegakaryocytic thrombocytopenia patients included in this study.

<table>
<thead>
<tr>
<th>patient ID</th>
<th>sex</th>
<th>intron/exon</th>
<th>CDS</th>
<th>protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMT01</td>
<td>f</td>
<td>E2</td>
<td>c.127C&gt;T</td>
<td>p.Arg43Ter</td>
</tr>
<tr>
<td>CAMT02</td>
<td>f</td>
<td>E6</td>
<td>c.883G&gt;C</td>
<td>p.Asp295Tyr</td>
</tr>
<tr>
<td>CAMT03</td>
<td>f</td>
<td>E6</td>
<td>c.883G&gt;C</td>
<td>p.Asp295Tyr</td>
</tr>
<tr>
<td>CAMT04</td>
<td>m</td>
<td>E2</td>
<td>c.305G&gt;C</td>
<td>p.Arg102Pro</td>
</tr>
<tr>
<td>CAMT05</td>
<td>f</td>
<td>E8</td>
<td>c.805T&gt;C</td>
<td>p.Trp154Arg</td>
</tr>
<tr>
<td>CAMT06</td>
<td>f</td>
<td>E4</td>
<td>c.304C&gt;T</td>
<td>p.Arg102Cys</td>
</tr>
<tr>
<td>CAMT07</td>
<td>m</td>
<td>E2</td>
<td>c.127C&gt;T</td>
<td>p.Arg43Ter</td>
</tr>
<tr>
<td>CAMT08</td>
<td>f</td>
<td>E5</td>
<td>c.378delT</td>
<td>p.Leu594Trp</td>
</tr>
<tr>
<td>CAMT09</td>
<td>f</td>
<td>E2</td>
<td>c.127C&gt;T</td>
<td>p.Arg43Ter</td>
</tr>
<tr>
<td>CAMT10</td>
<td>m</td>
<td>E5</td>
<td>c.1378C&gt;T</td>
<td>p.Gln460Ter</td>
</tr>
</tbody>
</table>

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The nomenclature of sequence variants follows the recommendations of the Human Genome Variation Society (HGVS). A description at the DNA level is provided in the Online Supplementary Table S2. Missense mutations are described at protein level, other mutations on DNA level (coding sequence). Amino acid substitutions are deduced from DNA sequencing results, the recommended parentheses have been omitted for better readability.
CD110 expression on hematopoietic progenitors

We analyzed the expression of the MPL-encoded protein CD110 on CD34+CD38dim hematopoietic progenitors from 30 CAMT-MPL patients and eight healthy donors (Figure 2). There was a clear correlation between real CD110 expression and the predicted effects from the mutation analysis on the one hand and between CD110 expression and clinical course on the other hand: CD110 expression was not measurable on cells from patients with nonsense or frame shift mutations and mutations predicted to lead to a complete loss of a splice site (Figure 2, group A). In the group of patients with missense mutations, we observed more variation in CD110 surface expression (Figure 2, group B) which was correlated with clinical courses: the higher CD110 expression observed in two patients homozygously affected by p.Asp295Tyr (Figure 2, violet square) was associated with a lesser severity course (CAMT101 and CAMT102). Cells from the patient with the p.Arg454Pro mutation predicted to be benign showed a nearly normal surface expression of CD110 (Figure 2, green square). In contrast, in patients with the mutation p.Arg102Pro (homozygous or compound heterozygous with a null mutation) and a relative severe course we measured a very low CD110 signal (Figure 2, blue squares).

Thrombopoietin plasma levels

Plasma levels of thrombopoietin are inversely proportional to the total mass of functional MPL in the body due to a direct negative feedback loop. Healthy donors usually have thrombopoietin plasma levels below 30 pg/mL (range <30-196 pg/mL). In contrast, thrombopoietin plasma levels were markedly elevated in all samples from 40 patients in this study and ranged from 400 to >4,000 pg/mL (median 1,493 pg/mL; Online Supplementary Table S1). Within the group of CAMT-MPL patients we did not find a significant correlation between THPO levels and either MPL expression levels on early hematopoietic progenitors or severity of the disease, but patients predicted to have a total receptor deficiency had a higher median thrombopoietin level (median 1,685 pg/mL, n=15) compared to patients with mutations allowing for a residual activity of the receptor (median 1,472 pg/mL, n=27). In our study, the measurement of MPL expression on hematopoietic precursors was a better predictor of the clinical course than THPO levels. Unexpectedly low

Table 2. MPL mutations in congenital amegakaryocytic thrombocytopenia patients. All mutations found in our group of congenital amegakaryocytic thrombocytopenia (CAMT) patients are listed regarding their type in Tables 2A to D together with their predicted impact on the MPL protein and their incidence in our patient group (bold: novel mutations). 2A (nonsense mutations) and 2B (frame shift mutations): prediction according to MutationTaster2 with probability; 2C (splice mutations): prediction according to BDGP splice site prediction,22 MaxEntScan algorithm (MaxEnt),23 and Human Splicing Finder (HSF).24 MDD: maximal dependency decomposition (only for donor sites), MM: Markov model (1st order), WMM: weighted matrix method. 2D (missense mutations): prediction according to MutationTaster,21 PROVEAN,18 and SIFT19 algorithms with the respective score values. “The mutation previously referred to as c.1653+1delG should be also regarded as a frame shift mutation since the predicted effect on the splice donor site is marginal (Table 2C) and the effect on the protein is caused mainly by the frame shift.”

Table 2A. Nonsense mutations in congenital amegakaryocytic thrombocytopenia patients.

<table>
<thead>
<tr>
<th>CDS</th>
<th>Exon</th>
<th>protein</th>
<th>MutationTaster</th>
<th>incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.127C&gt;T</td>
<td>E2</td>
<td>p.Arg43Ter</td>
<td>disease causing / 1</td>
<td>ho: n=7; het: n=0</td>
</tr>
<tr>
<td>c.268C&gt;T</td>
<td>E3</td>
<td>p.Arg90Ter</td>
<td>disease causing / 1</td>
<td>ho: n=1; het: n=0</td>
</tr>
<tr>
<td>c.127C&gt;T</td>
<td>E2</td>
<td>p.Arg43Ter</td>
<td>disease causing / 1</td>
<td>ho: n=7; het: n=0</td>
</tr>
<tr>
<td>c.1300G&gt;A</td>
<td>E8</td>
<td>p.Trp407Ter</td>
<td>disease causing / 1</td>
<td>ho: n=1; het: n=0</td>
</tr>
<tr>
<td>c.1378T&gt;C</td>
<td>E9</td>
<td>p.Gln460Ter</td>
<td>disease causing / 1</td>
<td>ho: n=0; het: n=1</td>
</tr>
<tr>
<td>c.1431G&gt;A</td>
<td>E9</td>
<td>p.Trp477Ter</td>
<td>disease causing / 1</td>
<td>ho: n=1; het: n=0</td>
</tr>
</tbody>
</table>

Table 2B. Frame shift mutations in congenital amegakaryocytic thrombocytopenia patients.

<table>
<thead>
<tr>
<th>CDS</th>
<th>Exon</th>
<th>protein</th>
<th>MutationTaster</th>
<th>incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.253_238delCT</td>
<td>E3</td>
<td>p.Leu79Glufs*54</td>
<td>disease causing / 1</td>
<td>ho: n=2; het: n=1</td>
</tr>
<tr>
<td>c.378C&gt;T</td>
<td>E3</td>
<td>p.Phe126LeufsX5</td>
<td>disease causing / 1</td>
<td>ho: n=3; het: n=3</td>
</tr>
<tr>
<td>c.1653delG*</td>
<td>E11</td>
<td>p.Lys553ArgfsX75</td>
<td>disease causing / 1</td>
<td>ho: n=0; het: n=1</td>
</tr>
</tbody>
</table>

Table 2C. Splice site mutations in congenital amegakaryocytic thrombocytopenia patients.

<table>
<thead>
<tr>
<th>CDS</th>
<th>Intron</th>
<th>HSF prediction</th>
<th>MaxEnt (wt/mut)</th>
<th>MDD (wt/mut)</th>
<th>MM (wt/mut)</th>
<th>WMM (wt/mut)</th>
<th>incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.79+2T&gt;A</td>
<td>I1</td>
<td>most prob. broken donor s.</td>
<td>9.16/0.97</td>
<td>13.98/5.79</td>
<td>9.62/1.43</td>
<td>10.39/2.21</td>
<td>ho: n=1; het: n=1</td>
</tr>
<tr>
<td>c.212+1G&gt;A</td>
<td>I2</td>
<td>most prob. broken donor s.</td>
<td>9.00/0.83</td>
<td>11.78/3.60</td>
<td>9.27/1.08</td>
<td>7.15/1.03</td>
<td>ho: n=0; het: n=1</td>
</tr>
<tr>
<td>c.213-1G&gt;A</td>
<td>I2</td>
<td>most prob. broken acc. s.</td>
<td>1.90/0.85</td>
<td>-</td>
<td>7.69/1.06</td>
<td>6.71/2.04</td>
<td>ho: n=0; het: n=1</td>
</tr>
<tr>
<td>c.391-5G&gt;C</td>
<td>I3</td>
<td>most prob. broken donor s.</td>
<td>9.14/0.25</td>
<td>12.18/9.98</td>
<td>8.32/4.76</td>
<td>9.68/5.86</td>
<td>ho: n=0; het: n=5</td>
</tr>
<tr>
<td>c.1653+1delG</td>
<td>I11</td>
<td>new donor site 1 base 5</td>
<td>8.25/0.11</td>
<td>-</td>
<td>9.41/1.05</td>
<td>11.03/2.67</td>
<td>ho: n=0; het: n=1</td>
</tr>
</tbody>
</table>

CDS: coding DNA sequence; E: exon; ho: homozygous individuals; het: heterozygous individuals.
absent megakaryocytes. Analysis during autopsy revealed normal cellularity with

Some families had more than one affected patient: CAMT009 + CAMT031, CAMT018 + CAMT019 + CAMT036 + CAMT180, and CAMT133 + CAMT140 each belongs to large kindreds with a high degree of consanguinity. Other cases of CAMT, aplastic anemia or not otherwise specified “bleeding disease” are reported in these kindreds. CAMT101 and CAMT102 as well as CAMT130 and CAMT137 are siblings from non-consanguineous families. CAMT083 is the fetus of a second consanguineous parents and were homozygous for the particular MPL mutation. Homozygous mutations in patients with no evidence for parental consanguinity (n=9) were mainly affected from the most prevalent mutation c.305G>C (n=4) or from mutations with a higher prevalence in a specific region (c.506T>A).28

We found a significant female predominance in our cohort (62.5%, P<0.05 according to χ²-test).2 This is in contrast to most of the other IBMs in which boys are affected more often.29 We have no information about the number and sex ratio of miscarriages in the patients’ families as a possible hint for the female predominance.

### Thrombocytopenia, bleeding

Although thrombocytopenia at birth has been classified as one of the diagnostic hallmarks of CAMT so far, 13 of 52 patients in this study with available information showed no signs of thrombocytopenia at birth and no blood counts were taken. Twelve of 13 patients had mutations allowing for a residual MPL activity. In the remaining patients (39 of 52) thrombocytopenia was detected at birth (n=38) or at termination of pregnancy (n=1). Available data for platelet counts at birth ranged from 1-56 G/L (median 15 G/L, n=50). Petechiae or pur-
Development of pancytopenia

Development of additional anemia or neutropenia and reduced bone marrow cellularity are signs of developing bone marrow exhaustion. Bone marrow analyses from the first 6 months of life usually showed normal cellularity with reduced or absent megakaryocytes (18 of 15).1 Accordingly, most of the patients presented with isolated thrombocytopenia at birth or in the first weeks thereafter (56 of 51).1 Only six patients showed signs of multi-lineage cytopenia within the first 6 months of life, four of them were already anemic immediately after birth (hemoglobin 56-76 g/L). For two patients a hypocellular bone marrow is documented in the first month after birth.

Provided the data available at time of analysis only seven of 49 patients showed no signs of developing pancytopenia. Five of these patients were younger than 2 years at last examination.

From the remaining 42 patients with documented aplasia only 24% were older than 4 years (n=10). Seventy-six percent (n=32) were younger than 4 years, half of them even younger than 2 years (Online Supplementary Figure S1). CAMT011 is the only patient without any signs of pancytopenia till adulthood.

† Here and in all subsequent ratios, the denominator is the number of patients for whom information is available for a specific parameter. E.g., for “intracranial bleeding” 46 is the number of questionnaires with a yes-no-information from the attending physicians.
Table 4. Hematopoietic stem cell transplantation in congenital amegakaryocytic thrombocytopenia.

<table>
<thead>
<tr>
<th>Age at 1st HSCT [y]</th>
<th># of pts with available info</th>
<th>N</th>
<th>%</th>
<th>Pos outcome (%)</th>
<th>information about neg. outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>23</td>
<td></td>
<td>61</td>
<td>15/17 (88)</td>
<td></td>
</tr>
<tr>
<td>1-5 years</td>
<td>5</td>
<td></td>
<td>13</td>
<td>4/4 (100)</td>
<td></td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>10</td>
<td></td>
<td>26</td>
<td>7/9 (78)</td>
<td>CAMT007: death after GvHD induction; GvHD: graft-versus-host disease; BM: bone marrow; PBSC: peripheral blood stem cells; MUD: matched unrelated donor</td>
</tr>
</tbody>
</table>

HSC donor

| HLA matched related donor | 19 | 56 | 12/12 (100) |
| haploident related donor  | 3  | 9  | 4/4 (100)   |
| matched unrelated donor   | 11 | 32 | 5/8 (63)    |
| mismatched unrelated donor| 1  | 3  | 1/3 (33)    |

HSC source

| BM  | 10 | 48 | 8/10 (80) |
| PBSC| 7  | 33 | 6/7 (86)  |
| CB  | 5  | 19 | 3/4 (75)  |

The table summarizes the available information regarding age at hematopoietic stem cell transplantation (HSCT), donor and source of hematopoietic stem cell (HSC) and outcome. Information about outcome was not available for all transplantations. BM: bone marrow; PBSC: peripheral blood stem cells; MUD: matched unrelated donor.

Chromosomal anomalies, leukemic development

Cytogenetic data were inconspicuous for most of the patients with available data (n= 23 of 27, 85%). An abnormal karyotype has been detected in 4 patients: t(2;11) (5%) in CAMT009, t(9;22) (7%) in CAMT013, monosomy 7 in CAMT043 (50%) and CAMT067 (13-30%). The latter has been diagnosed with MDS. All underwent hematopoietic stem cell transplantation (HSCT) because of aplastic anemia. In none of the patients a development of overt leukemia has been reported in the period of record.

Non-hematological abnormalities

The rate of non-hematological abnormalities in our CAMT-MPL patients was markedly higher than reported: 50% of the patients with available data (25 of 50) had non-hematological abnormalities appearing as structural abnormalities or other abnormal clinical findings (Table 3). Most of the reported abnormalities were related to the head region: brain anomalies (n=7), ocular and orbital anomalies (n=10), especially strabismus (n=9), nystagmus (n=4) and facial abnormalities (n=4). Mental or psychomotor retardation was observed in seven patients, mostly correlated with brain anomalies. Intracranial bleedings are documented for five of seven patients with mental or psychomotor retardation, for six of seven with brain anomalies, and for six of ten with ocular anomalies (Table 3). Interestingly, we found some anomalies which are typical for other IBMFS and which misled the first diagnosis: eczema (n=2), hypopigmentation (n=1), high palate and/or small uvula (n=2). No skeletal, cardiac or urogenital abnormalities were observed. There was no correlation between type or localization of MPL mutations and non-hematological abnormalities.

Treatment

Thirty-seven of 45 documented cases of our patient group received platelet transfusions, most of them transiently in a period immediately after diagnosis of severe thrombocytopenia and/or during the aplastic stage of the disease. During the advanced stage of the pancytopenia patients often received erythrocyte transfusions (16 of 45). Neutropenia and associated infections were treated with antibiotics; two of the patients were treated with recombinant granulocyte colony-stimulating factor.

Half of the patients (25 of 50) have been initially treated with immunoglobulins (23 of 50) or corticosteroids (15 of 50). Interestingly, three patients responded with a transient increase in platelet counts, which initially misled the diagnosis but none showed a persistent response.

The only available curative treatment for CAMT-MPL is HSCT. Thirty-eight of 51 patients in our group were treated with HSCT, for another ten HSCT was planned for the near future. For 26 of 30 patients with information about the post-transplant course a positive outcome was documented (87%). The available information about age of transplantation, donor, stem cell source and outcome is summarized in Table 4.

Three patients were unsuccessfully treated with recombinant IL-11 (oprelvekin). Two of them showed a slight and transient increase in platelet counts, followed by a prolonged phase of severe thrombocytopenia, which could be explained by an exhaustion of residual megakaryopoiesis by stimulation of cytoplasmic matura
tion.

Congenital amegakaryocytic thrombocytopenia with only one affected MPL allele

In six patients with clinical diagnosis of CAMT we found only a single mutated allele (Online Supplementary Table S3), as judged by reproducible balanced distribution
of both alleles from independently isolated genomic DNAs in five patients and an unbalanced distribution of both alleles in one patient. Two of these six patients were from families with other members affected by CAMT-MPL. CAMT119 was heterozygously affected by the missense mutation c.1390A>G, homozygously detected in her sister CAMT138. Both sisters had similar clinical and laboratory findings. Both parents were heterozygous carriers of the mutation without any hematological problems. Thrombopoietin plasma levels were high in both sisters but not in the parents. However, in contrast to both parents who demonstrated a balanced distribution of both alleles the wild-type allele in CAMT119 was reproducibly markedly underrepresented (approximately 20%), arguing for somatic mosaicism. Patient CAMT065 heterozygously harbored the c.127C>T nonsense mutation, which was homozygously found in his cousins CAMT086 and CAMT018. Besides these familial cases we identified four other patients with heterozygous MPL mutations. In one of these patients (CAMT129) we found CD110 expression on early hematopoietic progenitors comparable to that from patients with a predicted complete loss of the receptor (Figure 2). In patient CAMT73 we found a novel nonsense mutation in exon 7 together with a synonymous substitution c.585T>C (p.Pro195=). Although synonymous mutations can significantly influence protein levels via changes in translation efficiency, both codons are nearly equally used in human genes, and the mutation has no predicted effect on splicing.

**Discussion**

This report summarizes the results of a long term study on the largest cohort of patients with CAMT-MPL caused by biallelic mutations in MPL. We limited our cohort to this group of patients (i) to provide a reliable definition of the clinical picture of CAMT-MPL, (ii) to define the effects of the MPL/THPO system in humans, and (ii) to allow for evidence-based treatment recommendations.

CAMT has been used in the past to describe an IBMFS with no characteristic malformations presenting as isolated thrombocytopenia at birth progressing to a general bone marrow failure. However, large differences in the report ed percentages for MPL mutations, for the development of aplastic anemia and leukemia, and for somatic malformations reveal differences in the definition of this disease. This together with misleading combinations of findings from the pre-molecular era involves the risk of mistreatment e.g., HSCT of patients with CAMT due to THPO mutations.

The most severe clinical problems for patients with CAMT-MPL are (i) - so far underestimated - pre- and perinatal bleedings and the resulting long-term consequences thereof, and (ii) the development of aplastic anemia in the later course of the disease. Severe bleedings, especially intracranial bleedings, occur mainly pre- or perinatally but much less frequently after the first weeks of life despite partly very low platelet counts. Specific functional deficits in neonatal platelets like a decreased P-Selectin expression and reduced platelet activation and secretion could be a possible explanation for the high bleeding tendency pre- or perinatally in combination with the thrombocytopenia. Furthermore, both life-span and thrombin dependent activation of platelet GPIIb/IIIa are markedly reduced in neonatal Mpl- mice compared to adult Mpl+ mice. Our results indicate a possible functional impairment of platelets also in human fetuses and newborns with MPL defect which is in contrast to the assumption of a normal function of Mpl+ platelets.

Development of aplastic anemia due to exhaustion of three lineage hematopoiesis is a characteristic feature of CAMT-MPL and reveals the essential role of MPL for the maintenance of hematopoietic stem cells almost all patients inevitably develop a fatal bone marrow failure. In our study we observed only one patient with an isolated thrombocytopenia until adulthood. In the literature one further patient is described with stable thrombocytopenia in the period of record. Half of the patients in our cohort exhibit non-hematopoietic abnormalities. This is in contrast to the characterization of CAMT as an IBMF with no physical anomalies (OMIM). Most of the non-hematopoietic abnormalities seen in our cohort are related to the brain and the eye. For neurological abnormalities, which have been reported for other CAMT-MPL patients it has been argued, that these could be a direct consequence of the roles of thrombopoietin and MPL in the brain. However, the high correlation between structural abnormalities in the brain and intracranial bleedings argues for a secondary effect of thrombocytopenia. Indeed, most of these structural abnormalities observed in our cohort have also been reported as a consequence of intracranial bleedings, even strabismus and nystagmus. This is further supported by the observation that higher incidences of ocular anomalies have also been described for other BMFS going along with thrombocytopenia (Fanconi anemia, dentritic cells) but not for those with normal platelet counts (Diamond Blackfan anemia, Shwachman Diamond syndrome). Previous reports of other non-hematological abnormalities refer to CAMT patients with unreported or wild-type MPL genotype. Our data suggests that the primary effects of MPL deficiency are restricted to the hematopoietic system - most of the non-hematopoietic symptoms seem to be secondary to the thrombocytopenia or bone marrow failure. For other symptoms, especially those observed only in single cases or in highly consanguineous families we suppose that they emerged coincidentally.

Although CAMT is regarded to be a preleukemic syndrome in most of the recent reviews, only weak evidence for this assumption exists. One single patient with CAMT and confirmed MPL mutation has been reported to develop a pre-B acute lymphoblastic leukemia. Increased accumulation of chromosomal aberrations, however, has been observed in our and previous studies. The exhaustion of hematopoietic stem cells due to MPL deficiency may be the reason for both, the acquisition of pre-leukemic cellular alterations due to increased hematopoietic stress, but also for early development of aplastic anemia leading to death or replacement of the hematopoietic system by means of HSCT, thereby preventing the development of overt leukemia. The debate about CAMT-MPL as a preleukemic syndrome therefore might be of less relevance.

Genotype-phenotype correlations in CAMT-MPL have led us to our concept of CAMT I and II groups: a complete loss of MPL function results in persistently low platelet counts and a fast progression into pancytopenia in CAMT I patients whereas a residual function of the receptor leads to a milder course with a transient increase of platelet counts.
in the first year of life in CAMT II patients.5,14,15 The data from the present study allow for additional conclusions of clinical relevance: 
- the course of the disease is mainly determined by the type of mutation. The same MPL mutations lead to high similarities in the hematological courses of patients, even if they are from different families or different ethnic background (e.g., mild course in patients with c.391+5G>C).
- the time course of pancytopenia development for patients with same MPL mutations is more variable than the course of thrombocytopenia. This could be caused by accelerated exhaustion of hematopoietic progenitors due to frequent bleedings or infections in some patients.5,24
- all patients with mutations leading to a complete loss of function (CAMT I) had a similar course with constantly severe thrombocytopenia. Platelet counts at birth and in the further course never exceed 50. and all of them showed a transition to pancytopenia. A complete MPL deficiency is probable in patients showing signs of aplastic anemia in the first months of life.
- missense mutations predicted to allow a residual function of the MPL receptor lead to a more variable course of CAMT. The most severe mutations, comparable to CAMT I (severe thrombocytopenia, early development of aplasia), were observed in patients with mutations p.Leu169His and p.Trp154Arg. Milder phenotypes (late detection of thrombocytopenia and delayed development of aplasia) were observed in patients with mutations p.Asp295Tyr and p.Pro394Ser and missense mutations affecting the intracytoplasmic domain.
- milder phenotypes with late development of aplastic anemia (respectively none during the period of record) have also been observed in patients with splice site mutations allowing for a residual normal splicing,26,41 namely c.391+5G>C and c.212+5G>A.
- patients with germ line MPL mutations and a late onset form of amegakaryocytic thrombocytopenia or aplastic anemia (e.g., patients CAMT058, CAMT101, CAMT102 with moderate thrombocytopenia detected at the age of >2 years) should be also regarded as CAMT-MPL. This includes the patients previously described as familial aplastic anemia.29
- there may exist a small subgroup of CAMT II patients without development of pancytopenia. For patients with new mutations predicted to have minor impact on function or with mutations previously detected in patients with a mild course (namely c.891+5G>C, c.212+5G>A, or p.Pro275Thr) it might be appropriate to wait for first signs of bone marrow failure before proceeding to HSCT, especially if no appropriate family donor is available.
- type and localization of MPL mutations are not predictive for pre- and perinatal intracranial hemorrhages. There are no differences in the frequency and severity of these bleedings between patient groups CAMT I and CAMT II. The existence of a deleterious MPL mutation is a major risk factor for the occurrence of intracranial bleedings.
- structural and clinical non hematologic abnormalities in CAMT-MPL are not correlated with specific mutations.
- there is a small group of patients who present clinically as CAMT, but in whom a deleterious MPL mutation can only be detected in one allele. Possible explanations for the seeming inconsistency between genotype and phenotype include somatic mosaicism, deletions or changes in regulatory sequences that prevent the translation of a functional protein, or - rather unlikely especially in family cases - accidental coincidence.
- a further consideration for clinical presentation of CAMT-MPL is whether, in addition to existing MPL mutations, mutations or functional single nucleotide polymorphisms in other genes or epigenetic differences are involved in the observed phenotypic heterogeneity of CAMT-MPL.

Our analysis of a large cohort of CAMT-MPL patients demonstrates a higher variability of clinical courses than described so far. The diagnosis CAMT-MPL has to be considered even for those patients who are inconspicuous in the first months of life or show somatic anomalies typical for other BMFS. Since almost all CAMT-MPL patients inevitably develop a fatal bone marrow failure that requires treatment with HSCT, all children with unclear forms of hypomegakaryocytic thrombocytopenia should be tested for MPL mutations. If molecular confirmation of CAMT is not possible, at least those IBMFS should be excluded for which HSCT is not an option (e.g., thrombopoietin production defect) or which need another transplantation regimen (e.g., Fanconi anemia, Diamond Blackfan anemia).

Disclosures
No conflicts of interest to disclose

Contributions
MG and MB designed and performed research, analyzed data, and wrote the manuscript.

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