

cytopenia and thus would not be classifiable as MDS. Using standard laboratory values for cytopenias (Hb <13 g/dL [males], <12 g/dL [females], ANC <1.8 × 10<sup>9</sup>/L, platelets <150 × 10<sup>9</sup>/L), the data demonstrated that only 1.8% patients evaluated in that study of 7012 MDS subjects would lack a cytopenia (1.3% of patients when nonproliferative chronic myelomonocytic leukemia patients were excluded). Of note, and relevant predominantly for patients with low marrow blast counts in the IWG-PM cohort, the patient's blood counts also needed to demonstrate ≥2 months of stable disease as a potential means of excluding other causes for the cytopenias.

Regarding our main point, it is of relevance that the MDS database (n = 816) used to generate the IPSS (Table 1)<sup>11</sup> similarly demonstrated that 19% of these patients lacked a cytopenia if defined by the prognostic level cutpoints used by the WHO and also incorrectly would not have been considered to have MDS. Similar findings were found in an independent study using these cytopenic cutpoints.<sup>13</sup> Prior investigations have demonstrated ethnic-, age-, and altitude-related differences in normal Hb levels<sup>14,15</sup>; ethnic-, age-, and sex-related differences in platelet levels<sup>16,17</sup>; and ethnic- and sex-related differences in platelet and white counts.<sup>18</sup> Thus, being cognizant of these conditional blood count variations, we recommend that standard hematologic values be used to define cytopenias in MDS and believe a modification of the WHO definition of cytopenias as 1 of the criteria (in addition to definitive morphologic and/or cytogenetic findings) to diagnose MDS would be valuable and most accurate.

**Contribution:** P.L.G. and H.T. designed the study, analyzed the data, and wrote the report; A.A.v.d.L., D.H., J.M.B., P.F., M.C., and U.G. critically reviewed and modified the manuscript and contributed data to the IWG-PM database; and the remaining authors contributed data to the IWG-PM database and critically reviewed and approved the manuscript.

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## References

- Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
- Brunning RD, Orazi A, Germing U, et al. Myelodysplastic syndromes/neoplasms. In: Swerdlow S, Campo E, Harris NL, et al, eds. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. Lyon: IARC Press; 2008:88.
- Wimazal F, Fonatsch C, Thalhammer R, et al. Idiopathic cytopenia of undetermined significance (ICUS) versus low risk MDS: the diagnostic interface. *Leuk Res*. 2007;31(11):1461-1468.
- Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. *Blood*. 2015;126(21):2355-2361.
- Valent P, Bain BJ, Bennett JM, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. *Leuk Res*. 2012;36(1):1-5.
- Valent P, Jäger E, Mitterbauer-Hohendanner G, et al. Idiopathic bone marrow dysplasia of unknown significance (IDUS): definition, pathogenesis, follow up, and prognosis. *Am J Cancer Res*. 2011;1(4):531-541.
- Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16.
- McKerrell T, Park N, Moreno T, et al; Understanding Society Scientific Group. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Reports*. 2015;10(8):1239-1245.
- Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. *Blood*. 2015;126(21):2362-2365.
- Greenberg PL, Stone R, Bejar R, et al., eds. NCCN Practice Guidelines for Myelodysplastic Syndromes, version 1. 2017. ([www.nccn.org/professionals/physician\\_gls/PDF/mds.pdf](http://www.nccn.org/professionals/physician_gls/PDF/mds.pdf)).
- Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997;89(6):2079-2088.
- Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012;120(12):2454-2465.
- Germing U, Strupp C, Kuendgen A, et al. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. *Haematologica*. 2006;91(12):1596-1604.
- Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood*. 2006;107(5):1747-1750.
- Beutler E. Hemoglobin levels, altitude, and smoking. *Blood*. 2006;108(6):2131-2132.
- Segal JB, Moliterno AR. Platelet counts differ by sex, ethnicity, and age in the United States. *Ann Epidemiol*. 2006;16(2):123-130.
- Biino G, Santimone I, Minelli C, et al. Age- and sex-related variations in platelet count in Italy: a proposal of reference ranges based on 40987 subjects' data. *PLoS One*. 2013;8(1):e54289.
- Bain BJ. Ethnic and sex differences in the total and differential white cell count and platelet count. *J Clin Pathol*. 1996;49(8):664-666.

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## To the editor:

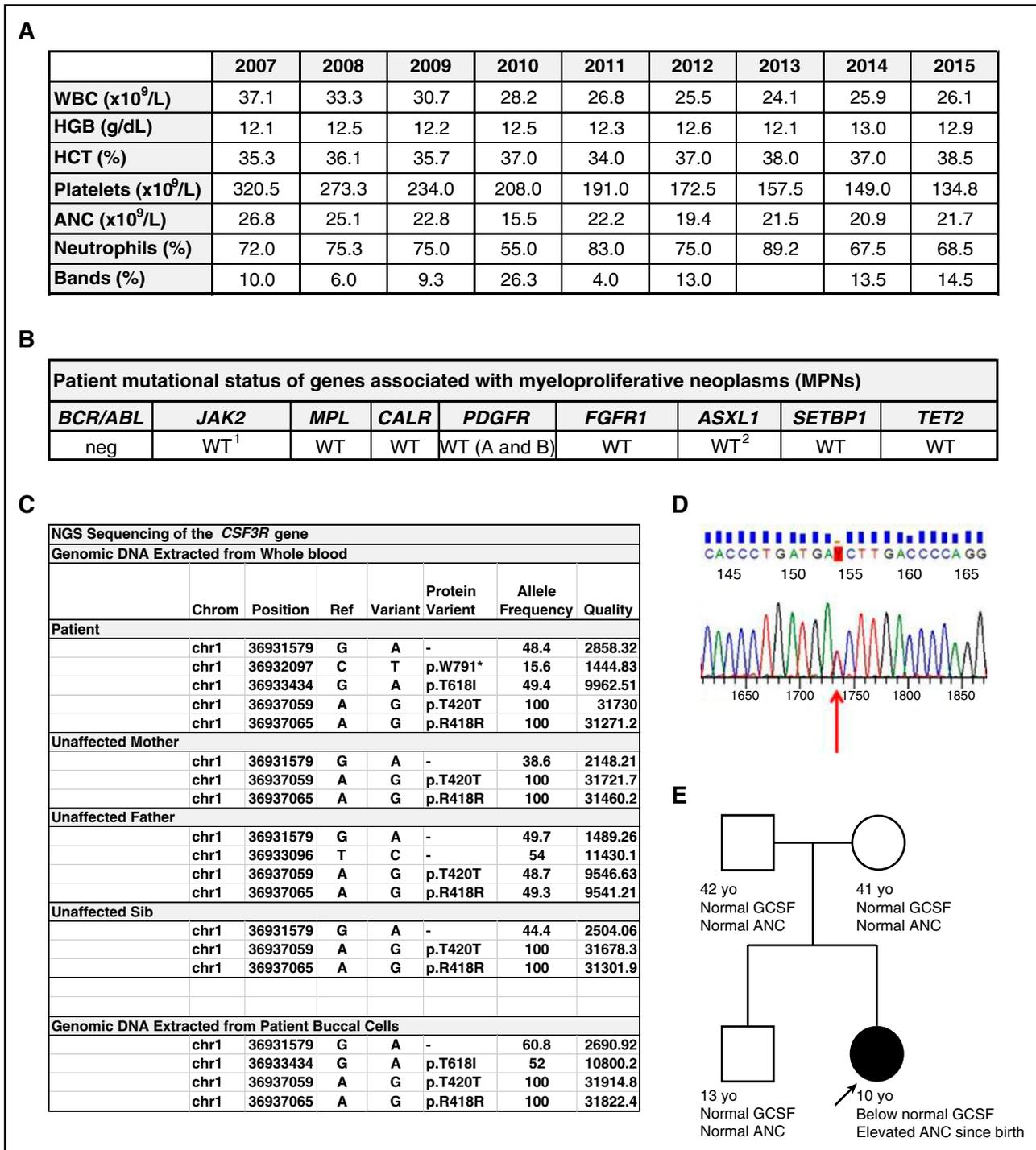
### Chronic neutrophilic leukemia in a child with a *CSF3R* T618I germ line mutation

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Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative neoplasm characterized by sustained elevation of neutrophil count, splenomegaly, and poor prognosis. Activating mutations in the colony-stimulating factor 3 receptor (*CSF3R*), also known as the granulocyte colony-stimulating factor (G-CSF) receptor, have recently been identified in 80% of patients studied with CNL.<sup>1,2</sup> The most common

mutation is T618I, which renders the receptor ligand independent through constitutive JAK/STAT activation.<sup>1,3</sup> The strong association of activating *CSF3R* mutations with CNL has led to the addition of a *CSF3R* T618I mutation or other activating *CSF3R* mutation to the diagnostic criteria for CNL in the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia.<sup>4</sup>



**Figure 1. Heterozygous germ line *CSF3R* T618I mutation in a child with CNL.** (A) Serial blood counts by year showing white blood count (WBC), hemoglobin (HGB), hematocrit (HCT), platelet count, absolute neutrophil count (ANC), percentage of neutrophils, and percentage of bands. (B) Mutational analysis of genes frequently implicated in myeloproliferative neoplasms (MPNs) using next-generation and/or Sanger sequencing (WT, wild type; neg, negative). <sup>1</sup>V617F and exon 12 mutations were not detected. A single nucleotide polymorphism (SNP) in exon 9 (L393V) was detected. <sup>2</sup>Mutations in *ASXL1* were not detected. M1249V, a known SNP, was identified. (C) Using next-generation sequencing (NGS), the entire *CSF3R* gene was sequenced from genomic DNA (gDNA) from whole blood from the patient, the unaffected parents, and the unaffected sibling, as well as from gDNA isolated from a buccal swab from the patient. A heterozygous T618I mutation was identified in the gDNA isolated from both blood and buccal cells from the patient. *CSF3R* W791\* was detected in blood cells only from the patient at low frequency (16%). (D) Confirmation of the *CSF3R* T618I mutation in patient cells in panel C by Sanger sequencing. (E) Patient pedigree showing the *CSF3R* T618I mutation present in the patient but undetectable in either parent or the unaffected sibling. Serum G-CSF levels were below normal in the patient but within the normal range in the parents and unaffected sibling.

CNL is generally a disease of older adults with a median survival of <2 years (range, 1-106 months).<sup>2</sup> The natural history remains poorly understood due to its rarity, insidious onset, and delay in diagnosis.<sup>5</sup> Approximately 200 cases of CNL have

been reported, largely as single case reports and small case series.<sup>6</sup>

We report a child with CNL and a germ line *CSF3R* T618I mutation with stable leukocytosis for 11 years. The patient was a full-term infant

girl with leukocytosis at birth (leukocyte count, 35 000 per cubic millimeter; absolute neutrophil count, 29 000). Bone marrow studies at 5 months revealed hypercellular marrow with granulocytic hyperplasia, <1% myeloblasts, no dysplastic features, and normal karyotype/fluorescent in situ hybridization. At 4 years of age, splenomegaly was demonstrated by ultrasound. The patient remained healthy without evidence of leukocyte adhesion defect or chronic infections or inflammation. Serial blood counts showed persistent leukocytosis (range, 24 000-37 000) with normal hemoglobin and platelet count (Figure 1A). Both parents and the only sibling had normal blood counts. At 10 years of age, due to increasing splenomegaly and a mildly decreased platelet count of  $134 \times 10^9/L$ , a bone marrow examination was repeated, and was unchanged. Cytogenetics and fluorescent in situ hybridization were normal, and no mutations were detected in *JAK2*, *MPL*, *CALR*, *PDGFR A/B*, or *FGFR1*. Polymerase chain reaction for *BCR-ABL* was negative (Figure 1B). Serum G-CSF was undetectable. Deep sequencing revealed a *CSF3R* T618I mutation that was confirmed by Sanger sequencing of patient DNA from both buccal and blood cells with an ~50% mutant allele frequency (Figure 1C-E). No mutations were identified in *ASXL1*, *SETBP1*, or *TET2* (Figure 1B), which are frequently reported in association with a T618I mutation in patients with CNL.<sup>5</sup> A *CSF3R* W791X mutation was detected at a low frequency (~16%) in the patient's blood cells but not buccal cells (Figure 1C). Repeat molecular studies 14 months later were unchanged with no additional mutations identified or changes in the mutant *CSF3R* allele frequencies detected.

This is the first report of a germ line *CSF3R* T618I mutation and the youngest reported patient to date with CNL. Our patient is also the longest living patient reported with CNL. The patient's sustained neutrophilia, splenomegaly, and morphological and molecular studies fulfill the 2016 WHO diagnostic criteria for CNL. A previous report by Plo et al identified a different activating germ line *CSF3R* mutation as the basis for hereditary chronic neutrophilia.<sup>7</sup> These investigators identified an autosomal-dominant *CSF3R* T640N point mutation (reported as T617N using the traditional *CSF3R* numbering system that does not include the signal peptide) with complete penetrance in 12 of 16 members of a 3-generation pedigree. The T640N mutation affects the transmembrane domain of *CSF3R* and not the membrane proximal region to which the T618I mutation localizes. Like the T618I mutation, constitutive phosphorylation of the JAK/STAT signaling pathway by the T640N mutation was demonstrated, which could be abrogated by the JAK2 inhibitor AZD1480. Transplantation of hematopoietic stem cells expressing the *CSF3R* T640N mutant in mice also induced a myeloproliferative-like disorder.

More recently, Maxson et al identified a T640N mutation in 2 patients meeting WHO criteria for true CNL/atypical chronic myeloid leukemia (CML).<sup>8</sup> The features of many of the affected family members in the pedigree reported by Plo et al with the T640N mutation also satisfy the 2016 WHO diagnostic criteria for CNL. Notably, 6 of the 12 affected family members were 20 years of age or younger. These data and our own demonstrate that CNL is a disease that is not restricted to the elderly. We recommend that CNL be included in the differential diagnosis of unexplained neutrophilia in children and that mutational analysis of *CSF3R* be performed.

Germ line analyses were not performed in the initial studies linking presumed somatic *CSF3R* mutations to CNL, so it is possible that a congenital origin for CNL may have been missed in these cases.<sup>1,2</sup> The presence of a germ line *CSF3R* T618I mutation in our patient that was not detected in either parent suggests that the mutation arose as a result of genetic mosaicism or as a spontaneous mutation in utero. Our patient and the pedigree reported by Plo et al emphasize the importance

of performing germ line studies in patients with CNL. Such studies should help to better define the causality and natural history of CNL.

A clinical course for CNL similar to CML that is composed of a chronic phase, an accelerated phase, and blast transformation has been suggested by several investigators.<sup>5</sup> Blast transformation has been reported to occur in 20% of patients with CNL at a median of 21 months from diagnosis. To date, all reported cases have been myeloid, and most of these patients have been found to have mutations in other genes such as *ASXL1* and *SETBP1* in addition to an activating *CSF3R* mutation.<sup>5</sup> These observations, together with our own demonstrating stable leukocytosis over a 11-year time period in a patient with CNL harboring a *CSF3R* T618I mutation but lacking mutations in other genes, provide strong evidence that additional mutations are required for progression of CNL, analogous to the secondary molecular alterations observed in transformation of CML.<sup>9</sup> The pathophysiologic relevance of the acquired low-frequency *CSF3R* W791X mutation in our patient that has remained stable for 14 months and was previously described in 1 other patient with CNL and a T618I mutation remains unknown.<sup>1</sup> Serial molecular studies in patients with CNL should help define genetic abnormalities associated with different phases of CNL and their impact on prognosis and therapy.

**Contribution:** L.J.D. designed experiments, reviewed data, and helped write the manuscript; D.P.M. provided clinical data and helped write the manuscript; N.S. performed research and helped write the manuscript; A.E.P. and A.L. performed research; J.M.G. reviewed the data and helped write the manuscript; and B.R.A. designed experiments, reviewed the data, and wrote the manuscript.

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## References

- Maxson JE, Gottlieb J, Pollyea DA, et al. Oncogenic *CSF3R* mutations in chronic neutrophilic leukemia and atypical CML. *N Engl J Med*. 2013;368(19):1781-1790.
- Elliott MA, Hanson CA, Dewald GW, Smoley SA, Lasho TL, Tefferi A. WHO-defined chronic neutrophilic leukemia: a long-term analysis of 12 cases and a critical review of the literature. *Leukemia*. 2005;19(2):313-317.
- Pardanani A, Lasho TL, Laborde RR, et al. *CSF3R* T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. *Leukemia*. 2013;27(9):1870-1873.
- Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
- Elliott MA, Tefferi A. Chronic neutrophilic leukemia 2016: update on diagnosis, molecular genetics, prognosis, and management. *Am J Hematol*. 2016;91(3):341-349.
- Bain BJ, Brunning RD, Vardiman JW, Thiele J. Chronic neutrophilic leukaemia. In: Swerdlow SH, Campo E, Lee Harris N, et al, eds. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008:38-39.
- Plo I, Zhang Y, Le Couédic JP, et al. An activating mutation in the *CSF3R* gene induces a hereditary chronic neutrophilia. *J Exp Med*. 2009;206(8):1701-1707.
- Maxson JE, Luty SB, MacManiman JD, et al. The colony-stimulating factor 3 receptor T640N mutation is oncogenic, sensitive to JAK inhibition, and mimics T618I. *Clin Cancer Res*. 2016;22(3):757-764.
- Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest*. 2010;120(7):2254-2264.

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