Next-generation sequencing panel for diagnosis and management of chronic neutrophilic leukaemia: a case report

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Hong Kong Med J 2019;25:248–50
https://doi.org/10.12809/hkmj176959

Case report

An 80-year-old woman presented to our hospital with mild headache in November 2015. She had a history of hypertension, diabetes mellitus, and hyperlipidaemia. She was febrile (38.6°C) but did not appear septic. Abdominal examination revealed mild splenomegaly but no hepatomegaly and there were no focal neurological signs or suggestions of other organ involvement. A full blood count showed leukocytes 124.0×10^9/L (neutrophils 120.3×10^9/L, lymphocytes 2.5×10^9/L, monocytes 1.2×10^9/L); haemoglobin 7.8 g/dL, mean corpuscular volume 89.1 fL; and platelets 384×10^9/L. The blood film showed marked neutrophilia, occasional myelocytes, and absolute basophilia but no blasts. The neutrophils showed toxic granules and were not dysplastic (Fig 1). Plain radiographs of the chest, kidney, ureter, and urinary bladder did not reveal any abnormalities. Bacterial cultures of the blood and urine did not reveal any septic foci. Because of the marked neutrophilia, the patient was initially treated for bacterial sepsis with empirical intravenous amoxicillin with clavulanic acid. Subsequent ultrasonography of the abdomen confirmed the splenomegaly (15.1 cm) but no other space-occupying lesions. The low-grade fever soon subsided after admission and she remained afebrile and non-septic, although the marked neutrophilia persisted.

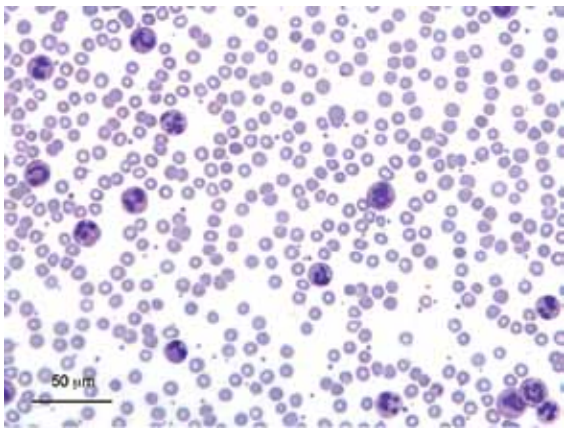

FIG 1. Peripheral blood film showed marked neutrophilia, and the neutrophils showed toxic granules but were not dysplastic (May-Grünwald-Giemsa, ×400)

A bone marrow biopsy revealed marked hypercellularity, primarily due to markedly increased granulopoiesis with left-shift in maturation but no increase in blasts. Erythropoiesis was active and normoblastic. Megakaryocytes were moderately increased with some being large and hyperlobulated. No overt dysplasia was seen. Cytogenetic karyotyping showed a normal karyotype. Reverse transcription polymerase chain reaction (PCR) for *BCR-ABL1* and allele-specific PCR for Janus kinase 2 (*JAK2*) V617F mutation analysis were negative. In view of the clinical and laboratory picture of a possible myeloproliferative neoplasm, and the lack of a clonal marker detected by the molecular tests, we sought to utilise a next-generation sequencing (NGS) panel (TruSight Myeloid Sequencing Panel; Illumina, San Diego [CA], United States) to look for possible mutations in selected exons of 54 different genes commonly implicated in myeloid neoplasms, in accordance with a previously published protocol.[1] Deep sequencing by this panel returned three pathogenic mutations: colony-stimulating factor 3 receptor (*CSF3R*) c.1853C>T; p.Thr618Ile or T618I at variant allele frequency (VAF) of 43.9% (Fig 2a), serine/arginine-rich splicing factor 2 (*SRSF2*) c.284C>T; p.Pro95Leu (NM_003016.4) or P95L at VAF of 49.6%, and additional sex combs like 1 (*ASXL1*) truncating mutation c.1934dupG; p.Gly646Trpfs*12 (NM_015338.5) at VAF of 34.1%. The *CSF3R* T618I mutation was further confirmed by Sanger sequencing (Fig 2b), thus prompting the diagnosis of chronic neutrophilic leukaemia (CNL). Although the *CSF3R* mutation rendered the disease amenable to ruxolitinib, the patient deteriorated rapidly and died of sudden severe gastrointestinal bleeding 12 days after admission and before specific therapy could be contemplated.

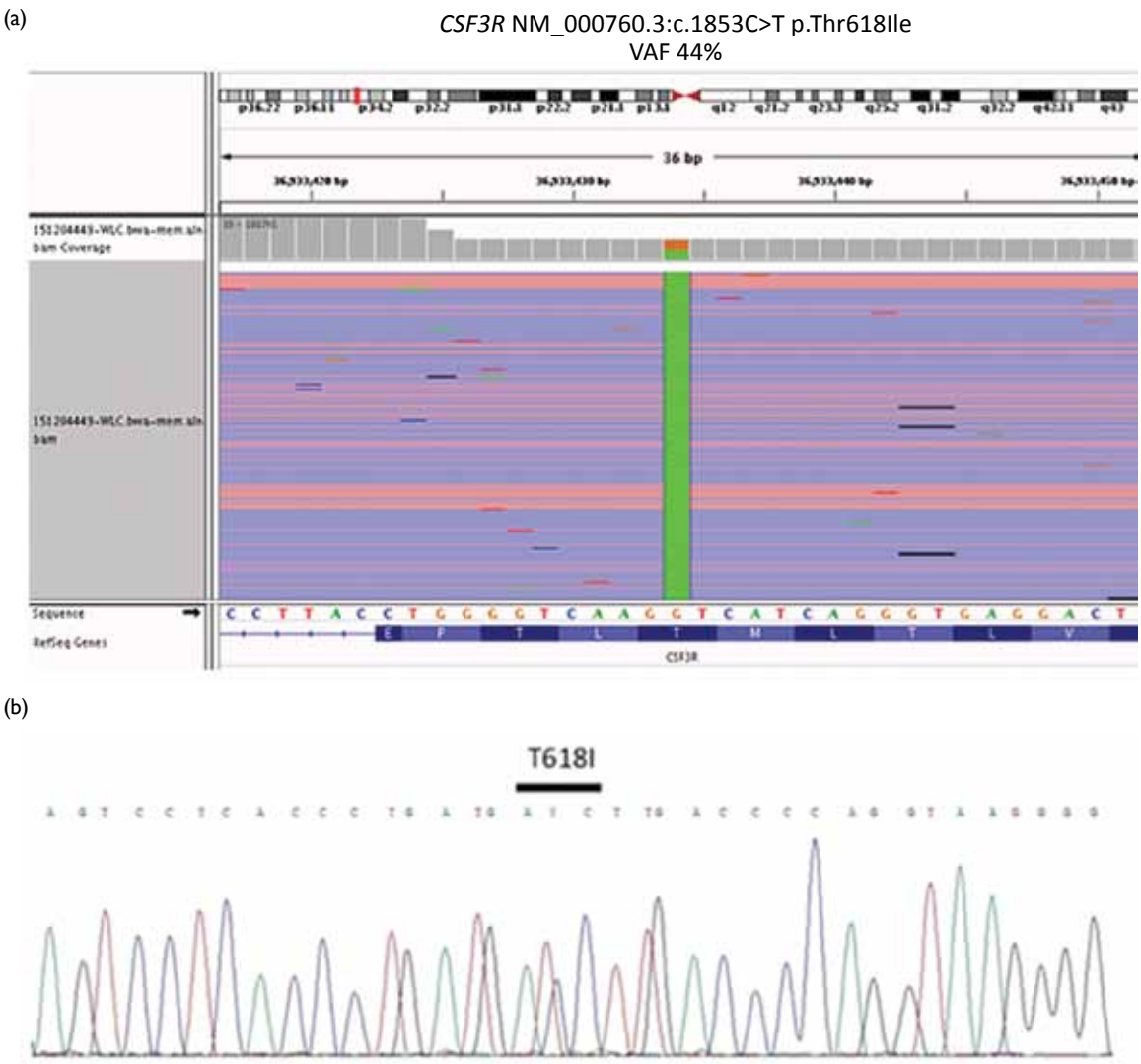

FIG 2. (a) The Integrated Genomic Viewer snapshot indicated a mutation located at *CSF3R* c.1853C>T (p.Thr618Ile). The upper panel indicates the position of *CSF3R* on chromosome 1p (red bar). The middle panel indicates the mutation was detected in 44% of the reads (green). The lower panel indicates the sequence of the top strand and the RefSeq Gene was transcribed and translated from the bottom strand. The G>A mutation detected in the top strand was equivalent to the C>T mutation in the *CSF3R*. (b) The electropherogram of the *CSF3R* c.1853C>T mutation. A heterozygous C>T was identified (noted as Y) and concordant with the next-generation sequencing–detected mutation

Abbreviation: VAF = variant allele frequency

Discussion

Several clinical features in this patient hinted at differential diagnoses other than bacterial infection or acute inflammation. Apart from fever, there were no other clinical features related to the presenting symptom of headache. Splenomegaly, a common feature of myeloproliferative neoplasms, was present. The absolute neutrophil count was very high, yet no clinical features of sepsis were found on physical examination or from investigations.

Since 2013, understanding of the molecular genetics of CNL has been dramatically changed by the discovery of *CSF3R* mutations in around 80% of cases.[2] The *CSF3R* encodes a transmembrane receptor for granulocyte colony-stimulating factor 3, and plays a crucial role in the differentiation and maturation of neutrophils.[2] The CNL-associated mutations in *CSF3R* activate the receptor and promote the proliferation and differentiation of neutrophils, leading to the marked neutrophilia that characterises the CNL disease phenotype.[3] There are two major types of *CSF3R* mutations in CNL. The first encompasses point mutations in the extracellular or transmembrane domains, of which the T618I mutation is the most common and comprises the majority of mutations in CNL. The second type of *CSF3R* mutation comprises nonsense or frameshift mutations leading to a premature stop

codon and truncation of the cytoplasmic domain of the receptor.[2]

Mutations of other genes have also been reported in CNL. These can be grouped as SET binding protein 1 (*SETBP1*) mutations, spliceosome mutations (eg, *SRSF2*), epigenetic modifier mutations (eg, *ASXL1*), and signalling mutations (eg, *JAK2*).[3] One previous study found that *SRSF2* mutations occurred in three of 14 cases of CNL (21%). The *SRSF2* mutations were previously associated with a worse prognosis in chronic myelomonocytic leukaemia, but its effects on CNL are unclear.[3] A significant proportion of CNL patients have been shown to harbour *ASXL1* (30%-60%). Similar to mutations in other myeloid malignancies, *ASXL1* mutations in CNL have been shown to confer a poor prognosis.[3]

Although these other mutations have not been incorporated into the World Health Organization 2016 diagnostic criteria for CNL, these data suggest that some may show prognostic value. Additionally, different *CSF3R* mutations may allow different therapeutic approaches (see below). Because Sanger sequencing of an increasing number of genes leads to substantial increases in the required time, resources, and necessary amount of DNA, we sought to explore an NGS panel to interrogate these genes simultaneously more efficiently and cost-effectively. Of note, the panel includes genes that are important for the diagnosis of myeloproliferative neoplasm (*JAK2*, calreticulin [*CALR*] and myeloproliferative leukaemia protein [*MPL*]), plus genes that are frequently reported in CNL (*CSF3R*, *SETBP1*, *SRSF2*, *ASXL1*). Therefore, compared with Sanger sequencing, NGS panels are a more efficient and powerful means to enable comprehensive genomic profiling of individual CNL cases, utilising a smaller amount of DNA.

With recent discoveries in the molecular pathogenesis of CNL, new therapeutic approaches that target the *CSF3R* signalling pathway–related SRC family and JAK-kinase pathways have emerged. The SRC signalling pathway is activated by truncation mutations of *CSF3R* leading to sensitivity to dasatinib, while the JAK-STAT pathway is activated by membrane proximal mutations of *CSF3R* leading to sensitivity to ruxolitinib.[2] Although there are few reported cases of these agents,[2,4] they represent significant breakthroughs in the management of CNL. This case of a rare myeloproliferative neoplasm demonstrates how advances in understanding of the molecular pathogenesis of a disease open up new routes for the development of effective novel therapeutic strategies.

Author contributions

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Concept and design of study: WWL Choi.
Acquisition of data: KY Mak, CH Au, TL Chan, ESK Ma, SY Lin, WWL Choi.
Analysis or interpretation of data: KY Mak, CH Au, TL Chan, ESK Ma, WWL Choi.
Drafting of the manuscript: KY Mak, WWL Choi.
Critical revision for important intellectual content: CH Au, TL Chan, ESK Ma, EYD Chow, SY Lin.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding/support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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