Oligoarray comparative genomic hybridization in polycythemia vera and essential thrombocythemia

Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative disorders (MPD) characterized by expansion of one or more myeloid lineages, which by definition results in erythrocytosis in PV and thrombocytosis in ET. The etiology and pathogenesis are still largely unknown.

At diagnosis, about 15% of PV and 5% of ET patients show chromosomal abnormalities.[1] The most common cytogenetic abnormalities detected by conventional cytogenetic techniques are deletions in the long arms of chromosomes 20 and 13, duplication of 1q, and trisomies of chromosomes 8 and 9.[1] Kralovics *et al.*[1] found loss of heterozygosity to be present in chromosome 9p, where *JAK2* (Janus kinase 2) resides. Its exon 14 harbors JAK2V617F mutation, recently reported to be present in more than 80% of PV and in approximately 50% of ET. In addition, JAK2 exon 12 mutation in PV2, and a thrombopoietin receptor *MPLW515L/K* mutation in ET and myelofibrosis[3] are novel mutations that have been described in *JAK2V617F* mutation negative patients.

While several novel mutations have been described in myeloproliferative disorders, some patients have normal wild type alleles, although they have a clear clinical disease and often show an abnormal growth pattern in *in vitro* colony forming assays.[4] In ET in particular, approximately 50% of the patients are *JAK2* mutation negative and only a small fraction (less than 5%) has *MPL* mutation. Of particular interest are the patients who show only spontaneous megakaryocytic growth in colony forming assays, since the mechanism and possible cytogenetic abnormalities underlying this are largely unknown. Therefore, we aimed to screen the entire genome using oligonucleotide array comparative genomic hybridization (aCGH) to detect small chromosomal alterations that cannot be revealed by conventional cytogenetic techniques.

The study comprised 36 frozen specimens from 21 ET and 14 PV patients; from one patient we had both bone marrow (BM) mononuclear cells and colonies of hematopoietic progenitors. Altogether 23 BM aspirates (11 PV and 12 ET), 8 Ficoll separated BM mononuclear cells (6 PV, 2 ET), 3 whole blood cells (1 PV, 2 ET) and 2 samples from colonies of hematopoietic progenitors (both ET) were studied. Fifteen specimens were JAK2V617F mutation negative (4 PV, 11 ET) and 20 were mutation positive (10 PV, 10 ET). PV was diagnosed according to the WHO criteria and ET as described by Jantunen *et al.*[5] All but one patient (dup1q) had a normal karyotype; for one patient the karyotype was not available. The study was approved by the local ethical review board and all participants gave their written consent.

Genomic DNA was extracted using standard non-enzymatic methods for frozen (stored bone marrow and whole blood) samples and the Puregene DNA purification kit (Gendra, Minneapolis, MN, USA) for mononuclear cells and hematopoietic progenitor colony cells. Reference DNA was extracted from pooled peripheral blood of healthy individuals. The hematopoietic progen-

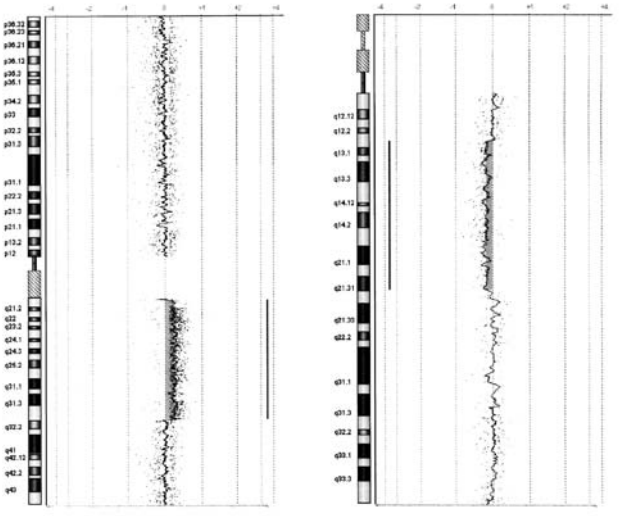

Figure 1. Array CGH profile of essential thrombocythemia patient: (A) gain of 1q 11.2q 32.1; (B) deletion of 13q12.3q21.31.

itors were cultured as part of routine diagnostic work as previously described.[6] The JAKV617F mutation was detected by allele-specific polymerase chain reaction as described by Baxter *et al.*[7] We used Agilent's Human Genome CGH 44B and 244A microarrays, containing 60-mer oligonucleotides representing 44,000 genes and 236,000 genes respectively. The probes on these arrays cover coding and non-coding genomic regions (Agilent Technologies, Santa Clara, CA, USA) as described earlier by Savola *et al.* [8]

Our previous study (*data not shown*) compared the *in vitro* growth pattern of hematopoietic progenitors of patients with PV and ET and their JAK2V617F mutation status. Based on those results, for the present study we first selected patients without JAK2V617F mutation whose *in vitro* cultures showed only spontaneous megakaryocyte colony (CFU-Meg) growth and no spontaneous erythroid (BFU-E) growth (n=7). Secondly, we studied another group of 4 JAK2V617F mutation negative patients who had neither spontaneous CFU-Meg nor BFU-E growth. Lastly, 24 patients displaying spontaneous BFU-E growth were studied; 14 of them also had spontaneous CFU-GM growth.

First we performed 34 arrays using the 44k platform (Agilent Technologies). Then results from 5 specimens were confirmed using a 244k high-density platform (Agilent Technologies). Two of these specimens were colony cells of hematopoietic progenitors. Only one of the patients showed chromosomal aberrations. In addition, we hybridized DNA from hematopoietic progenitor cells of 2 patients (BFU-E, CFU-Meg). Both gave normal results.

The only abnormal case (bone marrow specimen) showed duplication at 1q21.1q32.1 (about 59 Mb, including over 1,100 genes) and deletion at 13q12.3q21.32 (about 34 Mb, including 300 genes) (Figure 1). The raw data can be accessed at the CanGEM database (*www.cangem.org*).[9] Duplication of chromosome arm 1q has been reported both in ET and PV with a common region at 1q23q32. Deletion of 13q can occur in ET, but it is more frequent in PV.[10] Since both the duplication and the deletion are large, target genes therein remain to be studied. Previous results by BAC aCGH did not indicate any chromosomal imbalances in MPD.[11] Our results by aCGH on high-density oligo-based microarrays provide further evidence that microdeletions and microduplications do not have an essential role in the development of PV or ET.

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Funding: this study was supported by special state subsidy research funds appropriated to the Helsinki and Uusimaa Hospital District (HUS EVO) and grants from the Finnish Society of Hematology.

Key words: polycythemia vera, essential thrombocythemia, microarray, JAK2V617V, hematopoietic progenitors.

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Citation: Borze I, Mustjoki S, Juvonen E, Knuutila S. Oligoarray CGH in polycythemia vera and essential thrombocythemia. Haematologica 2008; 93:1098-1100. doi: 10.3324/haematol.12547

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