6. Lachmann HJ, Gallimore R, Gillmore JD, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. Br J Haematol. 2003;122:78-84.
7. Palladini G, Campana C, Klersy C, et al. Serum N-terminal pro-brain natriuretic peptide is a sensitive marker of myocardial dysfunction in AL amyloidosis. Circulation. 2003;107:2440-2445.
8. McElwain TJ, Powles RL. High-dose intravenous melphalan for plasma-cell leukaemia and myeloma. Lancet. 1983;2:822-824.
9. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myelome. N Engl J Med. 1996; 335:91-97.
10. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348:1875-1883.
11. Barlogie B, Hall R, Zander A, Dicke K, Alexanian R. High-dose melphalan with autologous bone marrow transplantation for multiple myeloma. Blood. 1986;67: 1298-1301.
12. Barlogie B, Jagannath S, Desikan KR, et al. Total therapy with tandem transplants for newly diagnosed multiple myeloma. Blood. 1999;93:55-65.

To the editor:

IL-1 gene cluster polymorphisms and development of primary gastric B-cell lymphoma in *Helicobacter pylori* infection

In addition to bacterial virulence factors, host immune response plays a pivotal role in the outcome of chronic *Helicobacter pylori* infection. The capacity of the host to mount an interleukin-1β (IL-1β)–driven response is influenced by sequence variants in the IL-1/IL-1RN cluster.[1]

El-Omar et al[2] were the first to report an association between IL-1B -31 and IL-1RN 2/2 of IL-1RN 86 variable number of tandem repeats (VNTRs) and the development of chronic hypochlorhydric response to *H pylori* and the risk of gastric cancer. In contrast, very little is known about germ-line mutations predisposing patients with chronic *H pylori* infection to develop gastric mucosa-associated lymphoid tissue (MALT) lymphoma.

Therefore, we read with great interest the report by Rollison et al[3] in Blood about an association between the proinflammatory genotype IL-1RN 2/2 with gastric marginal zone lymphoma in a retrospective series of 66 cases from northern England.

We investigated the functional variants in the IL-1 cluster and their influence on the development of primary gastric B-cell lymphoma in 153 patients participating in an intention-to-treat prospective multicenter study of the German-Austrian-Lymphoma Study Group.[4] Included as controls were 344 patients with *H pylori* infection undergoing upper gastrointestinal (GI) endoscopy when histology of 2 biopsies taken from the antrum and the corpus of the stomach excluded gastric lymphoma.

Table 1. Characteristics of 153 patients with primary gastric B-cell lymphoma

Disease stage	No.	Low grade	High grade	Sex, no. male/ no. female	Median age, y
E I	39	28	11	22/17	64
E I1	51	40	11	23/28	60
E I2	27	9	18	17/10	59
E II	4	1	3	3/1	67
E II1	26	7	19	17/9	66
E III	2	1	1	2/0	63
E IV	4	2	2	2/2	57

Stages of disease, histological grade, and median age of 153 patients with primary gastric B-cell lymphoma. The stage was defined according to the Ann Arbor staging system[6] with modification by Musshoff[7] and Radaszkiewicz.[8]

Of 153 patients with primary gastric B-cell lymphoma, 88 presented with low-grade (MALT) and 65 patients with high-grade lymphoma (Table 1). The allele frequencies in the control group match well with those previously reported in the literature.[2] There were no significant associations found with the histological grade or stage of disease in single marker analysis. Of patients with

Table 2. Single-marker analysis

Locus and genotype	Low grade, range, %	High grade, range, %	All, range, %	Controls, range, %	E I, range, %	E II-IV, range, %
IL-1β -31						
C/C	8-13.1	10-12.1	18-12.5	45-13.1	11-10.2	7-20.6
C/T	30-49.2	35-42.2	65-45.1	146-42.4	52-48.2	13-38.2
T/T	23-37.7	38-45.8	61-42.4	153-44.8	45-41.7	14-41.2
IL-1β + 3954						
C/C	4-6.7	3-3.7	7-5.0	24-7.2	3-2.8	3-9.4
C/T	20-33.3	26-32.1	46-32.6	121-36.3	37-34.6	9-28.1
T/T	36-60.0	52-64.2	88-62.4	188-56.5	67-62.6	20-62.5
IL-1RN 86VNTR						
1/1	30-46.9	46-52.9	76-50.3	185-53.8	58-51.3	17-47.2
1/2	27-42.2	31-35.6	58-38.4	118-34.3	43-38.1	14-38.9
1/3	2-3.1	4-4.6	6-4.0	11-3.2	5-4.4	1-2.8
2/2	4-6.3	5-5.8	9-6.0	27-7.9	6-5.3	3-8.3
2/3	1-1.6	1-1.1	2-1.3	2-0.6	1-0.9	1-2.8

Single-marker analysis of the proinflammatory haplotype IL-1β -31/IL-1 RN 86 VNTR. SNPs at IL-1β -31 and + 3954 were genotyped by allelic discrimination (TaqMan technology, ABI 7700, Aplera, Foster City, CA). IL-1RN 86 VNTR was genotyped by Southern blot after amplification. Alleles were sized relative to a 100-bp ladder (allele 1 = 4 repeats, allele 2 = 2 repeats, allele 3 = 5 repeats, allele 4 = 3 repeats, allele 5 = 6 repeats). Haplotype case-control analysis was performed using HAPMAX.[9] Hardy-Weinberg equilibrium was confirmed for all polymorphisms tested in gastric lymphoma group and controls. Three types of analysis were performed: (1) all patients with primary gastric B-cell lymphoma were compared against controls, (2) patients separated in low-grade and high-grade lymphoma were compared against controls, and (3) patients with disease stage E I were compared against patients with disease stages E II to E IV. Statistical analysis was performed using SISA Binominal program (Uitenbroek, Daan G, Binomial, SISA, http://home.clara.net/sisa/binomial.htm).

Table 3. Haplotype analysis

IL-1β -31	IL-1RN 86VNTR	Low grade, %e	High grade, %	All, %	Controls, %	E I, %	E II-IV, %
C	1	20.4	15.7	17.5	17.6	19.5	17.0
C	2	18.1	15.8	16.5	16.5	20.2	15.9
C	3	0.0	1.6	1.3	0.0	0.0	1.7
T	1	48.9	58.4	55.2	55.0	48.2	57.3
T	2	10.8	7.1	8.0	8.8	9.2	7.0
T	3	1.9	1.4	1.5	1.9	2.9	1.1

Haplotype analysis of the proinflammatory haplotype IL-1β -31/IL-1RN 86 VNTR. Analysis was conducted as described in the notes for Table 2.

disease stages E II to E IV, 20.6% were homozygous for IL-1B -31 allele C, compared to only 10.2% of patients with disease stage E I (Pearson χ^2 $P = .112$, OR 2.27, CI 95% 0.81-6.46). IL-1β +3954 CC was found in 9.4% of patients with disease stages E II to E IV, compared to 2.8% of patients with stage E I (Pearson χ^2 $P = .109$, OR 3.57, CI 95% 0.69-18.72) (Table 2). Haplotype analysis of the IL-1 cluster and especially the proinflammatory haplotype IL-1β -31 C/IL-1RN 2 did not show any significant associations with histological grade or disease progression (Table 3).

In conclusion we could not confirm the results of Rollinson et al.[3] One reason may be that Rollinson et al extracted DNA to investigate germ-line mutations from biopsy specimen and surgical blocks of lymphoma tissue and not from peripheral blood. A contamination with tumor material cannot be excluded. The admixture of somatic DNA may have obscured their analysis. To our knowledge, there is no mechanistic evidence so far that the proinflammatory effect of IL-1β contributes to development of primary gastric B-cell lymphoma. In contrast, recombinant IL-1β exerted a marked antilymphoma activity, reflected by significantly improved survival of treated mice after inoculation of BCL-1 cells.[5] The genetic susceptibility of patients with chronic *H pylori* infection to develop primary gastric B-cell lymphoma, especially of the MALT-type, appears to remain unclear.

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References

1. Hwang IR, Kodama T, Kikuchi S, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in Helicobacter pylori infection. Gastroenterology. 2002;123:1793-1803.
2. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398-402.
3. Rollinson S, Levene AP, Mensah FK, et al. Gastric marginal zone lymphoma is associated with polymorphisms in genes involved in inflammatory response and antioxidative capacity. Blood. 2003;102:1007-1011.
4. Fischbach W, Dragosics B, Kolve-Goebeler ME, et al. Primary gastric B-cell lymphoma: results of a prospective multicenter study. The German-Austrian Gastrointestinal Lymphoma Study Group. Gastroenterology. 2000;119:1191-1202.
5. Chelstrom LM, Finnegan D, Uckun FM. Treatment of BCL-1 murine B-cell leukemia with recombinant cytokines: comparative analysis of the anti-leukemic potential of interleukin 1 beta (IL-1 beta), interleukin 2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF alpha), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), and their combination. Leuk Lymphoma. 1992;7:79-86.
6. Carbone PP, Kaplan HS, Musshoff K, et al. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res. 1971;31:1860-1861.
7. Musshoff K. [Clinical staging classification of non-Hodgkin's lymphomas (author's transl)]. Strahlentherapie. 1977;153:218-221.
8. Radaszkiewicz T, Dragosics B, Bauer P. Gastrointestinal malignant lymphomas of the mucosa-associated lymphoid tissue: factors relevant to prognosis. Gastroenterology. 1992;102:1628-1638.
9. Krawczak M, Konecki DS, Schmidtke J, et al. Allelic association of the cystic fibrosis locus and two DNA markers, XV2c and KM19, in 55 German families. Hum Genet. 1988;80:78-80.

To the editor:

The PRV-1 gene expression in essential thrombocythemia

Recently, Temerinac and colleagues[1] found that the polycythemia rubra vera receptor-1 (PRV-1) m-RNA is increased in granulocytes of patients with polycythemia vera (PV) and in some cases of essential thrombocythemia (ET). We have previously described a sensitive qualitative method for PRV-1 investigation, based on PRV-1 m-RNA amplification by reverse transcriptase–polymerase chain reaction (RT-PCR).[2] By using this approach, we found that PRV-1 m-RNA is increased in 95% of patients with PV and in all patients with ET, while it is undetectable in secondary erythrocythosis (SE) and thrombocythosis (ST).[2] In other published studies, the PRV-1 m-RNA has been quantitatively evaluated,[3-8] and the percentage of PRV-1–positive ET patients varies widely among the mentioned studies, ranging from 16%[3] to 100%.[7] Up to date we have investigated by RT-PCR 75 patients with ET, 27 patients with ST, and 20 healthy individuals. Clinical and laboratory data of patients are shown in Table 1. Our results show that 71 of 75 ET patients are PRV-1 positive (95%), while all ST and healthy subjects are PRV-1 negative. These findings are in contrast with other reports and may result from the use of different methodological procedures in both granulocyte purification and PRV-1 evaluation (RT-PCR instead of real-time PCR). In a recent technical report, Palmqvist et al[9] compared the quantification of PRV-1 m-RNA in whole-blood leukocytes and in selected granulocytes. The authors clearly demonstrated that the detection of PRV-1–positive patients in ET increases by examining purified granulocytes (26% and 46% in unfractionated cells and granulocytes, respectively).[9] Thus, the low percentage of PRV-1–positive patients reported in some studies can be explained by the evaluation of RNA extracted by unfractionated nuclear cell population. Moreover, Jelinek et al[10] reported that PRV-1 m-RNA is more rapidly degraded than control m-RNAs when blood samples are stored for several hours before using. In our assay, granulocyte separation is performed within 2 hours of blood collection; the hypotonic lysis of red cells, reported in other studies,[1,5] is not required; and granulocyte pellets are stored in TRIZOL (Invitrogen, Paisley, Scotland) at −80°C until PRV-1 analysis. In this way, the PRV-1 m-RNA integrity is assured.

Finally, it has been demonstrated that treatment with interferon can induce a significant decrease of PRV-1 m-RNA at real-time PCR in PV patients.[11] In our series, only a small proportion (28%) of patients received cytoreductive therapy at the time of examination. Interestingly, a high percentage of patients evaluated by Liu et al[3]