No association between myeloproliferative neoplasms and the Crohn's disease-associated STAT3 predisposition SNP rs744166

We read with interest the paper by Kristinsson and colleagues who described an increased incidence of myeloproliferative neoplasms (MPNs) in individuals with a prior autoimmune disorder.[1] In a large and carefully controlled population-based study from Sweden, 11,039 myeloproliferative neoplasm cases and 43,550 matched controls were analyzed to assess the associations between a previous history of a range of autoimmune disorders and the risk of developing myeloproliferative neoplasm. Of all myeloproliferative neoplasm cases, 288 (2.6%) were reported to have had a prior autoimmune disease, with the highest associations recorded for giant cell arteritis, aplastic anemia, and Reiter's syndrome. Furthermore, there was a 2- to 3- fold elevated risk for myeloproliferative neoplasm among patients with a history of immune thrombocytopenic purpura, Crohn's disease and polymyalgia rheumatica. Collectively, this suggests that individuals with a preceding history of immune-related diseases are 20% more likely to developing a myeloproliferative neoplasm compared to controls.

One possible explanation for these associations is a common genetic susceptibility factor(s) to both autoimmune diseases and myeloproliferative neoplasms. Crohn's disease was recently the focus of a genome-wide association study (GWAS),[2,3] an unbiased genetic approach to identify relatively common, low penetrance predisposition variants. The results confirmed a previously described association with *IL23R* and also identified predisposition variants mapping close to *IL12B*, *STAT3*, and *JAK2*, amongst others. JAK2 and STAT3 are downstream components of IL-12 and IL-23 signaling and thus these findings strongly implicate both pathways in the pathogenesis of Crohn's disease. This is consistent with previous data indicating that Crohn's disease may result from aberrant inflammatory responses mediated by Th1 and Th17 T-cell subsets.[4]

Strikingly, the *JAK2* single nucleotide polymorphism associated with Crohn's disease tags the same 46/1 haplotype identified by us and others as strongly predisposing to *JAK2* mutated myeloproliferative neoplasms.[5-8] Although it is not yet clear to what extent 46/1 accounts for the observed association of myeloproliferative neoplasm and Crohn's disease, these findings strengthen the notion that common functional genetic variants impacting on JAK2 signaling predispose to these two clinically very diverse conditions. Moreover, 46/1 *JAK2* should be considered as a prime candidate as a predisposing factor for the other diseases that Kristinsson and colleagues found to be associated with myeloproliferative neoplasm.

We considered the possibility that the *STAT3* SNP, rs744166, identified in the Crohn's GWAS studies (rs744166 risk variant A achieved a combined significance level of 6.82×10^{-12})[2], also predisposes to myeloproliferative neoplasm. Peripheral blood leukocyte-derived DNA from previously described Caucasian patients with *JAK2* V617F positive polycythemia vera (PV)[5] and V617F negative essential thrombocythemia (ET)[9] were genotyped for rs744166 using pyrosequencing.[10] *STAT3* is located at 17q21, a region that is rarely targeted by copy number changes or acquired uniparental disomy in myeloproliferative neoplasms and thus the results were assumed to faithfully reflect constitutional genotypes. Allele frequencies were compared to the control data provided by the Welcome Trust Case Control Consortium (WTCCC) from the UK blood donor cohort (n=1,500).[11] As shown in Table 1, no significant difference was seen between the cases and controls and we therefore conclude that this particular variant of *STAT3* does not predispose to myeloproliferative neoplasms. More systematic searches will therefore be required to determine if there are other shared genetic susceptibility factors between myeloproliferative neoplasms and immune-related disorders.

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Table 1. Summary of the genotype results for the STAT3 SNP rs744166 in JAK2 V617F positive and V617F negative MPN compared to WTCCC controls.

Category	N. of cases/controls	SNP allele frequency G	A	total alleles	MAF	*P* value	OR (95% CI)
UK (WTCCC, blood) controls	1500	1311	1687	2998	0.437		
JAK2 V617F positive	136	129	143	272	0.474	0.251	1.16 (0.91-1.49)
JAK2 V617F negative	338	285	391	676	0.422	0.466	0.94 (0.79-1.11)

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Hydroxyurea induced oscillations in twelve patients with polycythemia vera

Hydroxyurea is used in polycythemia vera for cytoreductive treatment. Some patients show marked fluctuations of platelet and leukocyte counts while taking hydroxyurea. We identified 12 patients in our patient cohort and performed a mathematical analysis which revealed oscillatory behavior with a typical period length of 27-30 days. During the presence of oscillations (25.5 years) no bleeding or thromboembolic episodes were observed.

Polycythemia vera is a chronic acquired stem cell disorder which affects primarily red blood cells and in many patients also granulocyte and platelet counts. Polycythemia vera is associated with an increased risk of thromboembolic and hemorrhagic complications.[1] Phlebotomy is the therapy of choice.[2] In refractory patients cytoreductive treatment with drugs such as hydroxyurea is advocated. Hydroxyurea primarily interferes with DNA synthesis by inhibiting ribonucleotide reductase in hematopoietic stem cells.[3] Recently, patients with polycythemia vera and hydroxyurea associated oscillations of the platelet and white blood cell count have been reported.[4-7]

We identified 12 patients (7 females and 5 males) who showed fluctuations of the platelet and white blood cell counts when treated with hydroxyurea. All patients were positive for the $JAK2^{V617F}$ mutation. Total observation time was about 76 patient years of which 29 years were under therapy with hydroxyurea (Table 1).

We performed a mathematical periodicity analysis by calculating the frequency spectrum with the Lomb periodogram[8] which was first used for the analysis of blood cell fluctuations by Bennett and Grunwald.[5] For the platelet count, all patients oscillated with a cycle length of 28.6 days (27-30). In contrast, only 5 patients showed oscillations of the white blood cell count with a cycle length of 27.8 days (26-29). Patients were observed for a

Table 1. Characteristics of 12 patients with HU induced oscillations: all patients were positive for the $JAK2^{V617F}$ mutation. Male patients: 5,6,8,9,10; female patients 1-4, 7, 11-12. Patients 1,3,4, 6, 10 took Litalir®, the others Syrea®. Mathematical analysis of the oscillations of the platelets and White blood cells (cycle length). Also shown are start, ending and duration of oscillations as well as duration of HU intake, dosage and total observation period.

Pt.	PLT cyle [days]	WBC cycle [days]	Oscillations started	Oscillations affected by	PLT nadir [x10⁹/L]	PLT zenith [x10⁹/L]	WBC nadir [x10⁹/L]	WBC zenith [x10⁹/L]	Duration of HU [months]	Duration of oscillation [months]	HU dose [g/day]	Total observation period [months]
1	28	26	After intake of HU with a 12 month delay	Anagrelide after a 9 month delay	21	1417	03.0	14.0	36	23	0.5-2	101
2	27	27	Immediately after intake of HU	Still oscillating	68	1690	06.9	33.3	40	40	1-2	48
3	29		Immediately after intake of HU	Anagrelide	71	1145	03.0	29.0	4	4	1-2	96
4	30	29	Immediately after intake of HU	Still oscillating	104	1420	01.9	06.9	120	120	1-2	125
5	30	29	Immediately after intake of HU	Busulphane	46	1122	04.5	38.2	10	10	0.5-3.5	120
6	30		Immediately after intake of HU	Ending HU therapy	116	598	10.5	21.7	15	15	1-2	96
7	27		Immediately after intake of HU	Still oscillating	136	1385	08.6	21.6	48	48	1-2	48
8	30		After splenectomy. Prior 6 years of HU without oscillations	Busulphane	27	4900	04.3	30.0	8	8	1-2	108
9	28		Immediately after intake of HU	An additional phlebotomy	189	1162	05.4	10.2	21	4	0.5-1	72
10	29	28	Immediately after intake of HU	Anagrelide	21	1495	04.3	12.4	21	21	1-2	48
11	28		Immediately after intake of HU	No obvious reason	140	530	06.5	09.4	18	6	1	18
12	27		Immediately after intake of HU	Anagrelide	116	989	05.5	16.4	7	7	0.5-1	36