Chronic Myeloproliferative Disorders

The incidence of the *JAK2* V617F mutation in patients with idiopathic erythrocytosis

Sixty-three patients with erythrocytosis exhibiting a range of erythropoietin levels were screened for the *JAK2* V617F mutation. One patient (1.6%) was found to have this mutation, and has remained stable for 9 years, suggesting that the *JAK2* V617F mutation is rare in patients with idiopathic erythrocytosis.

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The recent identification of the *JAK2* V617F mutation in myeloproliferative disorders (MPD)[1-4] has for the first time defined a major molecular defect associated with these disorders, and in particular with polycythemia vera (PV). The *JAK2* V617F mutation has been detected in between 65% and 97% of PV cases in either the homozygous or heterozygous state. It is an acquired mutation present in the granulocytic and erythroid lineages but absent from lymphocytes. Of 39 patients studied with an identified secondary erythrocytosis, none was found to possess the *JAK2* V617F mutation[2,3] but the mutation status of patients with idiopathic erythrocytosis (IE) was not evaluated. We screened a group of 63 patients with IE or raised erythropoietin (EPO) levels with no discernible cause in order to assess whether the *JAK2* V617F mutation may be an important part of diagnostic evaluation and to determine whether these individuals actually had early PV.

Over the last decade our hospital has been a referral center for IE patients and we have developed a registry of British and Irish erythrocytosis patients, collecting clinical information and DNA samples.[5] This study was approved by the Queen's University Ethics Committee and all patients gave informed written consent, according to the Declaration of Helsinki. Patients who fulfilled the following criteria were recruited; a raised red cell mass and hematocrit, no identifiable secondary cause of erythrocytosis and exclusion of PV using the modified criteria of Pearson and Messinezy.[6,7] A molecular defect was identified in only a minority of these patients, one with an erythropoietin receptor mutation[8] and nine with von Hippel Laudau (*VHL*) gene mutations;[5] thus there remains a significant cohort of IE patients in whom the molecular defect remains elusive.

Patients without a defined molecular defect were genotyped for the *JAK2* V617F mutation using DNA prepared from peripheral mononuclear cells[5]and an amplification refractory mutation system (ARMS) as described by Jones *et al.*[3] with minor modifications. Any positive results were confirmed by polymerase chain reaction (PCR)-direct sequencing of both granulocytes and lymphocytes.

All of the patients screened were negative for the *JAK2* V617F mutation except for one female patient who was heterozygous for this mutation. PCR-direct sequencing confirmed the presence of the mutation in the granulocytes and absence in the lymphocytes. This patient was 44 years old at the time of presentation. She had no splenomegaly, a red cell mass of 165% predicted and normal cytogenetics. The hematologic indices of this woman and the other patients are shown in Table 1. At the time of referral she did not fulfil sufficient criteria for a diagnosis of PV to be made and her bone marrow was not diagnostic. She has been followed for 9 years and remains well with no disease progression. The only treatment she has received is venesection. No other patient of the cohort of 63 individuals with erythrocytosis, nor any of nine patients from nine different families with the Arg200Trp VHL mutation, was found to be positive for the *JAK2* V617F mutation. In addition, six cases of PV screened were all positive. Excluding the woman with the *JAK2* mutation, erythrocytosis patients were divided into a group of 42 individuals with low or normal EPO levels (<15 mIU/mL) and a second group of 20 individuals with elevated EPO levels and no identifiable cause. In the first group with lower EPO levels, 14 individuals were younger than 30 years at diagnosis and four had a family history of a least one first degree relative with erythrocytosis. In the group with high EPO levels, there were ten individuals less than 30 years old and two had a family history. In contrast to an earlier study by Pearson that suggested 40% of IE patients developed discernable PV after 6 years,[9] only 1 of 63 patients in our study was positive for the *JAK2* V617F mutation, suggesting that 1.6% of erythrocytosis individuals may have a clonal dis-

Table 1. Summary of hematologic data for two groups of erythrocytosis patients divided according to EPO levels, PV individuals positive for the *JAK2* V617F mutation and the one erythrocytosis patient with the *JAK2* V617F mutation.

Hematologic data	*IE Patient Group (n=42) (EPO < 15 mIU/mL)*			*Erythrocytosis patients (n=20) with unexplained elevated EPO (>15 mIU/mL)*			*PV Patient Group (n=6)*			*JAK2 V617F positive patient*
	Mean	*Min.*	*Max.*	*Mean*	*Min.*	*Max.*	*Mean*	*Min.*	*Max.*	
Age at diagnosis (years)	35	0.6	74	33.2	1.5	73.6	58.2	32	83	44
Hemoglobin (g/dL)	18.9	16.3	21.4	18.8	15.3	23.6	17.4	14.3*	21.1	17.6
White cell count ($\times 10^9$/L)	7.2	3.7	14	8.1	5.5	15.3	15.0	11.1	23.6	8.9
Platelet count ($\times 10^9$/L)	221	133	333	213	125	306	776	462	1139	310
Erythropoietin level (mIU/mL)	6.1	0	13.5	43	15.8	230				<5

*IE: idiopathic erythrocytosis; PV: polycythaemia vera; Min: minimum; Max: maximum; *severely iron deficient patient.*

order and, by definition, PV. This positive individual represented 2.4% of those with the lower EPO levels, a group more likely to cause diagnostic difficulty with PV. The reason for the discrepancy is unclear; however, diagnostic rigor and more extensive availability of discriminatory tests in the time period between the studies may have contributed to the difference. It is also possible that the individuals who developed PV in Pearson's study may have already been positive for the *JAK2* V617F mutation or subsequently became positive. Had *JAK2* genotyping been available the one positive patient in our study would have been diagnosed with PV at referral as she would have fulfilled the diagnostic criteria for PV. We have shown that the *JAK2* V617F mutation is present at a low incidence in patients with IE. However, we would suggest that mutation screening should be included in the diagnostic investigation of IE in order to identify the rare patient with PV.

Melanie J. Percy, Frank G.C. Jones,* Anthony R. Green,° John T. Reilly,# Mary Frances McMullin@*

**Department of Hematology, Belfast City Hospital, Belfast, Northern Ireland; °Department of Hematology, MRC Centre, Cambridge, England; #Department of Hematology, Royal Hallamshire Hospital, Sheffield, England; @Department of Hematology, Queen's University, Belfast, Northern Ireland*

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Correspondence: Mary Frances McMullin, Haematology, Centre for Cancer Research and Cell Biology, Queen's University, Belfast, University Floor, Tower Block, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB. Phone: international +44.2890263718; Fax: international +44.2890.263927. E-mail: m.mcmullin@qub.ac.uk

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