Published in final edited form as:
Nat Genet. 2009 April ; 41(4): 446–449. doi:10.1038/ng.334.

JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms

Amy V Jones[1,2], **Andrew Chase**[1,2], **Richard T Silver**[3], **David Oscier**[4], **Katerina Zoi**[5], **Y Lynn Wang**[3], **Holger Cario**[6], **Heike L Pahl**[7], **Andrew Collins**[2], **Andreas Reiter**[8], **Francis Grand**[1,2], and **Nicholas C P Cross**[1,2]

[1]Wessex Regional Genetics Laboratory, Salisbury, UK.

[2]Human Genetics Division, University of Southampton, UK.

[3]Weill Medical College of Cornell University, New York, New York, USA.

[4]Department of Haematology, Royal Bournemouth Hospital, Bournemouth, UK.

[5]Haematology Research Laboratory, Biomedical Research Foundation, Academy of Athens, Athens, Greece.

[6]Department of Pediatrics and Adolescent Medicine, University Hospital Ulm, Ulm, Germany.

[7]Department of Experimental Anaesthesiology, University Hospital Freiburg, Freiburg, Germany.

[8]III Medizinische Universitätsklinik, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, Germany.

Abstract

Chronic myeloproliferative neoplasms (MPNs) are a group of related conditions characterized by the overproduction of cells from one or more myeloid lineages. More than 95% of cases of polycythemia vera, and roughly half of essential thrombocythemia and primary myelofibrosis acquire a unique somatic 1849G>T *JAK2* mutation (encoding V617F) that is believed to be a critical driver of excess proliferation[1–4]. We report here that *JAK2*^{V617F}-associated disease is strongly associated with a specific constitutional *JAK2* haplotype, designated 46/1, in all three disease entities compared to healthy controls (polycythemia vera, $n = 192$, $P = 2.9 \times 10^{-16}$; essential thrombocythemia, $n = 78$, $P = 8.2 \times 10^{-9}$ and myelofibrosis, $n = 41$, $P = 8.0 \times 10^{-5}$). Furthermore, *JAK2*^{V617F} specifically arises on the 46/1 allele in most cases. The 46/1 *JAK2* haplotype thus predisposes to the development of *JAK2*^{V617F}-associated MPNs (OR = 3.7; 95% CI = 3.1–4.3) and provides a model whereby a constitutional genetic factor is associated with an increased risk of acquiring a specific somatic mutation.

Correspondence should be addressed to N.C.P.C. (ncpc@soton.ac.uk)..

AUTHOR CONTRIBUTIONS The study was designed by A.V.J., A. Chase., F.G. and N.C.P.C. A.V.J. performed the laboratory analysis. R.T.S., D.O., K.Z., Y.L.W., H.L.P., H.C. and A.R. provided clinical samples and associated information. A.V.J., A. Chase, A. Collins and N.C.P.C. analyzed the data. N.C.P.C. wrote the first draft of the manuscript and all authors contributed to and approved the final version.

Note: Supplementary information is available on the Nature Genetics website.

The finding of *JAK2*^{V617F} was a major step forward in understanding the pathogenesis of MPNs, but it remains unclear how this single abnormality gives rise to distinct clinical entities. Clinical phenotype is clearly associated with *JAK2*^{V617F} dosage: in many PV and MF cases *JAK2*^{V617F} is reduced to homozygosity as a consequence of acquired isodisomy (generally referred to as acquired uniparental disomy; aUPD) at chromosome 9p, but this is rare in ET[2,5,6]. Several lines of evidence indicate that other, largely uncharacterized, acquired abnormalities also have a role in specifying disease phenotype either in combination with or independently of *JAK2*^{V617F} (refs. 7,8). Furthermore, both epidemiological data and family studies indicate that inherited factors may predispose to MPNs[9,10] and it has also been suggested that inherited SNPs within *JAK2* are associated with specific MPN subtypes[11].

To determine the role of inherited factors, we initially analyzed six *JAK2*-spanning SNPs (rs7864782, rs10758669, rs7046736, rs12342421, rs10974947, rs2031904) in MPN cases with a homozygous *JAK2*^{V617F} clone (%V617F allele >50%; $n = 142$) using pyrosequencing, which provides a quantitative readout of allele ratios. The mitotic recombination that gives rise to aUPD typically involves most of chromosome 9p[2,6] and thus SNPs within this region are also reduced to homozygosity; consequently, the haplotype on which *JAK2*^{V617F} arose could be read directly from allele ratios that were significantly greater than the expected value of 0.5. In most cases with 60–90% V617F, the residual haplotype (that is, the haplotype on which V617F had not arisen) could also be read by the finding of heterozygous allele ratios in the range 0.1–0.4. In cases with ≥90% V617F, however, information about alleles on the non-V617F chromosome was lost (Fig. 1 and Methods). Notably, of the 142 alleles that harbored V617F, 109 (77%) had an identical haplotype (subsequently designated 46/1) within the *JAK2* gene, whereas this haplotype was seen for only 9 of the 74 (12%) residual wild-type alleles that could be read ($P = 1.4 \times 10^{-20}$, Fisher's exact test, two-tailed). These results indicated that homozygosity for *JAK2*^{V617F} was not random, but rather occurred preferentially when this mutation was present on a specific *JAK2* haplotype.

To explore this observation in more detail, we first determined the haplotype structure of *JAK2* using 14 SNPs genotyped by the Wellcome Trust Case Control Consortium (WTCCC) in 1,500 healthy blood donors from the UK[12]. PHASE analysis[13] inferred 92 haplotypes, of which nine accounted for 94% of *JAK2* alleles (Fig. 2). Two haplotypes (numbers 46 and 1; referred to henceforth together as 46/1) were identical except for rs7864782 and had a combined frequency of 0.24. A tagged SNP (rs12340895) that was in complete linkage disequilibrium (LD) with 46/1 was then used to screen for this haplotype in further cases. In an initial analysis of 177 heterozygous *JAK2*^{V617F}-positive MPNs, 46/1 occurred more frequently (135/354 alleles) than in 188 locally sourced healthy controls (92/376 alleles; $P = 0.0001$) as well as the WTCCC cohort ($P = 3.3\ 10^{-8}$). The 46/1 haplotype was more frequent in all *JAK2*^{V617F}-positive disease entities regardless of origin (UK or United States); however, there was no difference in the frequency of 46/1 between controls and cases with idiopathic erythrocytosis (Table 1). To determine whether *JAK2*^{V617F} was in *cis* or *trans* to the 46/1 allele in cases that were heterozygous for both the mutation and the haplotype, we carried out allele-specific PCRs for *JAK2*^{V617F} to amplify products that

included a second 46/1 tag SNP (rs12343867) in intron 14. Sequencing of the products in 66 informative cases showed that 49 (74%) *JAK2*^{V617F} alleles arose on a 46/1 allele, whereas only 17 (26%) residual wild-type alleles were 46/1 ($P = 2.1 \times 10^{-8}$).

We have previously described a polycythemia vera pedigree in which *JAK2*^{V617F} was not inherited but arose independently in two affected individuals[14]. Family members were analyzed for rs12340895: one affected individual (UPN 534) was heterozygous for 46/1 but the second (UPN 533) was negative for this haplotype (Fig. 3). Allele-specific PCR for UPN 534 showed that *JAK2*^{V617F} had arisen on the 46/1 allele, confirming the association between this haplotype and the mutation. However, this pedigree illustrates that 46/1 is not solely responsible, at least in this family, for predisposition to polycythemia vera. Indeed, linkage of disease to 9p has not been described in any family with MPN.

We suggest two hypotheses to account for the association of MPNs with 46/1: (i) *JAK2*^{V617F} may arise randomly on all haplotypes but 46/1 is in LD with an unknown constitutional functional variant that interacts with *JAK2*^{V617F} in a manner that makes the development of clinically manifest disease more likely compared to *JAK2*^{V617F} on a non-46/1 haplotype, or (ii) there is a specific mutational mechanism by which *JAK2*^{V617F} preferentially arises on a 46/1 haplotype. These hypotheses are not necessarily mutually exclusive. Inspection of the HapMap data (Fig. 2) indicates that the entire *JAK2* gene is contained within a 280-kb LD block that includes two other genes (*INSL4* and *INSL6*) that are not expressed in hemopoietic cells, as verified by RT-PCR analysis. It is highly likely therefore that any functional variant within 46/1 directly affects *JAK2*. Notably, rs10758669, a SNP that also tags 46/1, was identified as significant in a recent genome-wide association study of Crohn's disease[15], thus supporting the hypothesis of a functional *JAK2* variant on that allele. This SNP was also reported to be significantly associated with polycythemia vera but not with essential thrombocythemia or myelofibrosis[11], a result that is presumably explained by the relatively high prevalence of *JAK2*^{V617F} in polycythemia vera compared to the other two subtypes.

JAK2 is required for signaling by diverse myeloid cytokine receptors (for example, IL-3, G-CSF, GM-CSF, EPO) as well as other receptors in lymphoid and nonhemopoietic cells[16]. To investigate the possibility that *JAK2* on the 46/1 haplotype is different functionally from other *JAK2* alleles, we tested whether 46/1 influences myeloid colony formation in hematologically normal individuals ($n = 56$). In a prospective analysis, we counted the numbers of granulocyte-macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) in peripheral blood and compared the results to *JAK2* haplotype. Individuals that carried at least one 46/1 allele grew significantly fewer CFU-GM, consistent with the hypothesis that *JAK2* on 46/1 is indeed functionally different from other *JAK2* alleles. There was no effect, however, on BFU-E growth (Fig. 4).

One possible reason for the observed association might be that *JAK2* on 46/1 is expressed more or less than *JAK2* on other haplotypes. To explore this possibility, we used pyrosequencing to quantify the allele ratios of two *JAK2* exonic SNPs (rs10429491 and rs2230724) in matched cDNA and genomic DNA from control *JAK2*^{V617F}-negative cases that were heterozygous for at least one of the exonic SNPs as well as heterozygous for 46/1

(*n* = 46). We found no differences in allele ratios in cDNA and genomic DNA with either SNP, indicating that 46/1 is not associated with either increased or decreased *JAK2* expression, at least in peripheral blood leukocytes (Supplementary Fig. 1 online).

Finally, we explored the possibility that a functional variant might also be relevant to the pathogenesis of *JAK2*^{V617F}-negative MPNs. We genotyped rs12340895 in *JAK2*^{V617F}-negative essential thrombocythemia and myelofibrosis cases (*n* = 47) from the UK and found that 36 or 94 alleles were 46/1, significantly higher than the frequency in locally sourced controls and the WTCCC cohort (*P* = 0.009 and *P* = 0.002, respectively). However, we failed to confirm this association in an additional series of *JAK2*^{V617F}-negative essential thrombocythemia cases (n = 136) and controls (*n* = 108) from Greece, although the *P* value of 0.09 and slightly elevated odds ratio suggests that the relevance of 46/1 to *JAK2*^{V617F}-negative cases warrants further investigation (Table 1).

Our data thus demonstrate that both homozygous and heterozygous *JAK2*^{V617F}-associated disease is preferentially associated with 46/1, and that this haplotype seems to be in LD with an as-yetuncharacterized functional variant. However, this does not exclude the possibility that *JAK2* on 46/1 allele might be also be hypermutable. Whatever the mechanism, our data indicate that 46/1 is a strong predisposition factor for development of *JAK2*^{V617F}-associated MPNs (OR = 3.7; 95% CI = 3.1–4.3; relative risk = 2.6; 95% CI = 2.3–2.9; *n* = 435 cases versus WTCCC controls). The counts of 46/1 alleles in cases and WTCCC controls and the population frequency from the WTCCC data (Table 1), suggest that 46/1 accounts for 28% of the population attributable risk[17]. A recent Swedish study demonstrated a relative risk of 5.7 in first-degree relatives of individuals with polycythemia vera[10], corresponding to an attributable risk of 53%. Assuming no difference between the UK and Swedish populations, 46/1 thus accounts for slightly over 50% of the increased risk in first-degree relatives. For essential thrombocythemia and myelofibrosis, the contribution of 46/1 is less clear, as it is unknown what proportion of the risk in first-degree relatives[10] is attributable to cases that are *JAK2*^{V617F} positive and those that are *JAK2*^{V617F} negative.

In addition to the specific association we describe here in MPNs, our findings may have wider relevance. Genome-wide association studies are identifying increasing numbers of loci that predispose to diverse malignancies[18–20]; our findings suggest that these loci should be considered as candidates for the acquisition of somatic mutations.

METHODS

Subjects

We analyzed a total of 775 subjects with MPN, of whom 183 had *JAK2*^{V617F}-negative disease and 592 were *JAK2*^{V617F} positive (PV, *n* = 203; ET, *n* = 224; MF, *n* = 41; unclassified MPN, *n* = 124). Subjects were recruited from clinics in the UK, United States and Greece. We also analyzed a previously described family with MPN from Germany[14]. For controls we analyzed healthy individuals from the UK (*n* = 188) and Greece (*n* = 108), and we also used data generated by the WTCCC from the UK blood donor cohort (*n* = 1,500)[12]. The study was approved by the relevant internal review boards and ethics committees and informed consent was provided according to the Declaration of Helsinki.

Genotyping

Total peripheral blood leukocyte DNA was analyzed by pyrosequencing for SNPs and *JAK2*^{V617F} as described[21]. Primer sequences are provided in Supplementary Table 1 online. Because the allelic ratios (the ratio of allele A to allele B at any SNP) for any heterozygous SNPs were distorted away from the expected value of 0.5 in cases with a sizeable homozygous *JAK2*^{V617F} clone, we adopted the following scoring criteria for all SNPs: (i) if one allele had an allelic ratio ≥0.9, the sample was scored as homozygous for that SNP; (ii) where allelic ratios were 0.11–0.89, samples were scored as heterozygous. These cutoffs were at least 3 s.d. more than background (that is, values read for allele B in healthy controls who were A/A homozygotes). Similarly, for homozygous *JAK2*^{V617F} cases with V617F >50%, SNPs with allelic ratios ≥0.6 or ≤0.4 were considered to be derived from the V617F-mutated or residual wild-type alleles, respectively. For *JAK2*^{V617F} homozygous cases in which the V617F was ≥90%, no information could be obtained about the residual wild-type *JAK2* allele and thus these cases were considered to contribute only one allele to the analysis.

Allele-specific PCR

Allele-specific PCR was performed using forward primers that were specific for *JAK2*^{V617F} (VF-ASF) or the corresponding wild-type sequence (WT-ASF) in combination with a common reverse primer (ASR), producing a 565-bp product that included the 46/1 tag SNP rs12343867. Amplification conditions were optimized on DNA from the HEL cell line (100% V617F) and normal healthy controls. Products were sequenced to determine whether *JAK2*^{V617F} was on the 46/1 allele or not.

Colony analysis

Mononuclear cells (MNCs) from peripheral blood of hematopoietically normal controls were isolated by centrifugation over lymphoprep (Axis-Shield) and cultured in methylcellulose medium (H4434; Stem Cell Technologies) at a density of 4×10^5 cells per 30-mm plate (in a final volume of 1 ml), in triplicate, following the manufacturer's instructions. Colonies comprising a minimum of 100 cells were counted on day 14, and characterized on the basis of morphology as either CFU-GM, CFU-GEMM, CFU-E or BFUE, as described by StemCell Technologies. *JAK2* haplotype status was determined after colonies were counted using DNA extracted from the MNC or granulocyte cell fractions.

Expression analysis

RNA was extracted from peripheral blood leukocytes of *JAK2*^{V617F}-negative MPD cases that were known to be heterozygous for rs10429491 and/or rs2230724 (*JAK2* exonic SNPs) as well as heterozygous for 46/1, as determined by rs12340895 genotype. RNA was reverse transcribed with random hexamer primers and the ratio of the two alleles for each SNP in genomic DNA and cDNA was determined by specific Pyrosequencing assays (Supplementary Table 1).

Statistical analysis

The proportion of 46/1 alleles for each case subgroup was compared to controls using Fisher's exact test (two-tailed). Colony numbers were compared to genotype using the Mann-Whitney *U* test. For the expression analysis, the mean and variance of SNP allelic rations were compared by t and F tests, respectively. Odds ratios (OR) were calculated as (number of 46/1 alleles in cases/number of 46/1 alleles in controls)/(number of non-46/1 alleles in cases/number of non-46/1 alleles in controls). Relative risk (RR) was calculated as (number of 46/1 alleles in cases/number of 46/1 alleles in cases plus controls)/(number of non-46/1 alleles in cases/number of non-46/1 alleles in cases plus controls). Population attributable risks were calculated as (f(RR − 1)/1 + f(RR − 1))100, where f is allele frequency[17].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

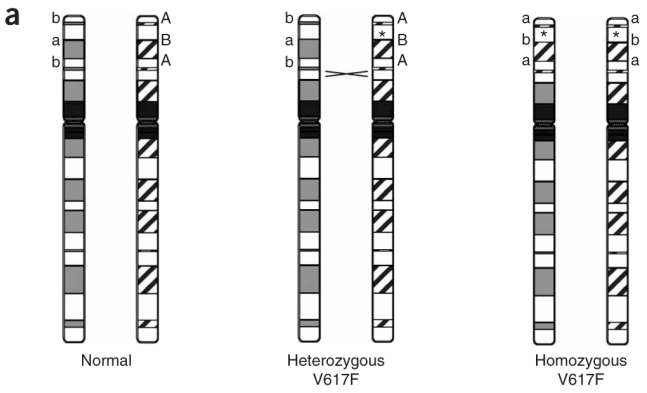
Acknowledgments

This study was supported by Leukaemia Research (UK) Specialist Programme Grant 0280 and makes use (in part) of data generated by the WTCCC. A full list of the investigators who contributed to the generation of the WTCCC data are available from www.wtccc.org.uk, funding for which was provided by the Wellcome Trust under award 076113. We are grateful to P. Strike (Salisbury Research and Development Support Unit) for statistical advice. A.R. was supported by the 'Deutsche José Carreras Leukämie-Stiftung e.V. - DJCLS R06/02, Germany.

References

1. James C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005; 434:1144–1148. [PubMed: 15793561]
2. Kralovics R, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N. Engl. J. Med. 2005; 352:1779–1790. [PubMed: 15858187]
3. Levine RL, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005; 7:387–397. [PubMed: 15837627]
4. Baxter EJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005; 365:1054–1061. [PubMed: 15781101]
5. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. Exp. Hematol. 2002; 30:229–236. [PubMed: 11882360]
6. Jones AV, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005; 106:2162–2168. [PubMed: 15920007]
7. Pikman Y, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006; 3:e270. [PubMed: 16834459]
8. Kralovics R. Genetic complexity of myeloproliferative neoplasms. Leukemia. 2008; 22:1841–1848. [PubMed: 18754034]
9. Rumi E, et al. Disease anticipation in familial myeloproliferative neoplasms. Blood. 2008; 112:2587–2588. [PubMed: 18779411]
10. Landgren O, et al. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24, 577 first-degree relatives of 11, 039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008; 112:2199–2204. [PubMed: 18451307]
11. Pardanani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. Blood. 2008; 111:2785–2789. [PubMed: 18006699]

12. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–678. [PubMed: 17554300]
13. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 2003; 73:1162–1169. [PubMed: 14574645]
14. Cario H, Goerttler PS, Steimle C, Levine RL, Pahl HL. The JAK2V617F mutation is acquired secondary to the predisposing alteration in familial polycythaemia vera. Br. J. Haematol. 2005; 130:800–801. [PubMed: 16115144]
15. Barrett JC, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat. Genet. 2008; 40:955–962. [PubMed: 18587394]
16. Sandberg EM, Wallace TA, Godeny MD, VonDerLinden D, Sayeski PP. Jak2 tyrosine kinase: a true jak of all trades? Cell Biochem. Biophys. 2004; 41:207–232. [PubMed: 15475610]
17. Khoury, MJ.; Beaty, TH.; Cohen, BH. Fundamentals of Genetic Epidemiology. Oxford University Press; New York: 1993.
18. Easton DF, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007; 447:1087–1093. [PubMed: 17529967]
19. Eeles RA, et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat. Genet. 2008; 40:316–321. [PubMed: 18264097]
20. Di Bernardo MC, et al. A genome-wide association study identifies six susceptibility loci for chronic lymphocytic leukemia. Nat. Genet. 2008; 40:1204–1210. [PubMed: 18758461]
21. Jones AV, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood. 2006; 107:3339–3341. [PubMed: 16352805]



Case	%V617F	rs7864782		rs10758669		rs7046736		rs1234241		rs10974947		rs2031904		V617F haplotype	Wild-type haplotype
		A	G	A	C	A	C	G	C	A	G	A	G		
E659	99	100	0	7	93	100	0	100	0	6	94	2	98	ACAGGG	Unknown
E2433	80	14	86	4	96	82	18	85	15	0	100	0	100	GCAGGG	ACCCGG
E2513	62	71	29	31	69	68	32	74	26	8	92	0	100	ACAGGG	GACCGG
E1186	49	46	54	9	91	48	52	45	55	51	49	49	71	Unknown	Unknown

Figure 1.

Allele distortions due to aUPD enable direct reading of *JAK2* haplotypes. (**a**) The *JAK2*^{V617F} mutation (indicated by an asterisk) and flanking SNPs are reduced to homozygosity in a proportion of cells following mitotic recombination. (**b**) SNPs and *JAK2*^{V617F} were quantified by pyrosequencing. In many cases that harbored a homozygous *JAK2*^{V617F} clone, it was possible to directly read the haplotype on which the mutation arose by the finding that one allele at each SNP predominated (allelic ratio ≥0.6, for example, cases E659, E2433 and E2513). In cases with a homozygous clone and %V617F <90%, it was usually possible to read the residual haplotype (that is, the haplotype of the chromosome that had not acquired *JAK2*^{V617F}) by the finding of allelic ratios between 0.1–0.4 (for example, cases E2433 and E2513). Where the homozygous clone was small or nonexistent (most cases with %V617F <60%), neither the *JAK2*^{V617F} nor wild-type haplotype could be read (for example, case E1186).

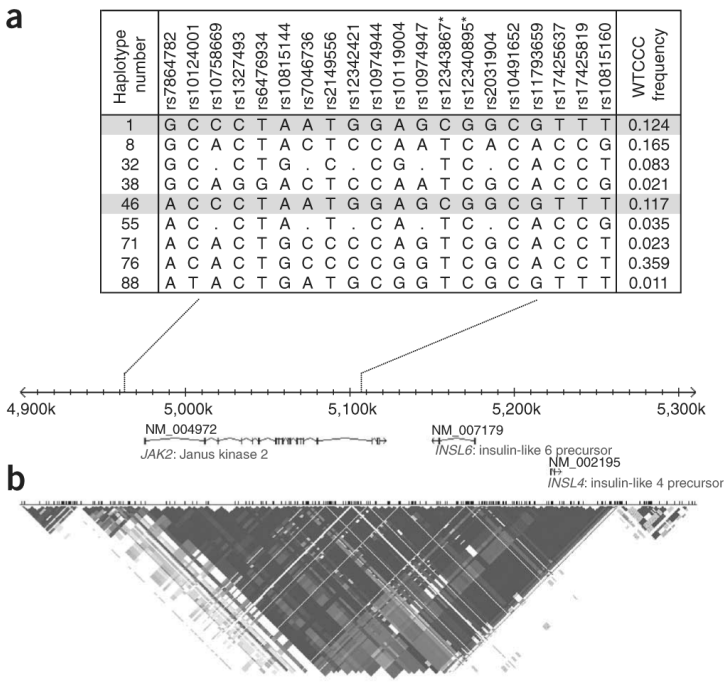


Figure 2.

SNPs, haplotypes and LD around *JAK2*. (**a**) The nine most common *JAK2* haplotypes in the UK population. The 14 SNPs in bold were analyzed by the WTCCC in 1,500 blood donors from which the frequencies were determined; asterisks indicate SNPs that tag 46/1, which are highlighted in gray. rs78644782, rs10124001 and rs10758669 are immediately upstream of *JAK2*; all other SNPs are within *JAK2* introns. (**b**) LD in the *JAK2* region (HapMap data release 23a/phase II March 2008).

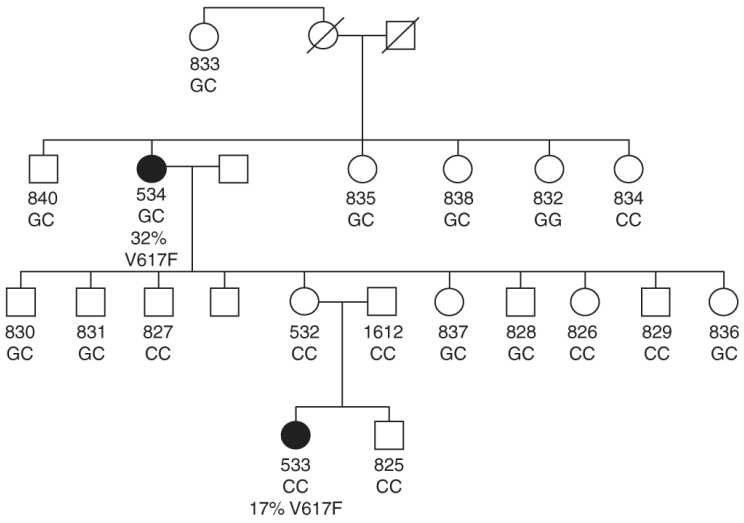


Figure 3.

Familial polycythemia vera pedigree. The two affected individuals (UPNs 534 and 533) are shown as black circles. The genotype for rs12340895 is shown (G = 46/1 allele; C = non-46/1 allele), as is the %V617F in affected cases. Allele-specific PCR for UPN 534 showed that *JAK2*^{V617F} arose on the 46/1 allele. All other individuals had normal blood counts and were negative for *JAK2*^{V617F}, *PRV1* overexpression and endogenous erythroid colony growth[14].

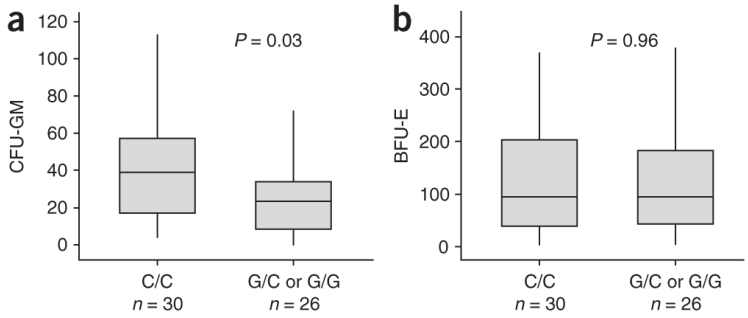


Figure 4.

Association between *JAK2* haplotype and numbers of hemopoietic colonies. CFU-GM and BFU-E colony growth per 4×10^5 peripheral blood mononuclear cells from 56 healthy controls that were 46/1 nullizygous (C/C at rs12340895, $n = 30$) or had at least one 46/1 allele (G/C, $n = 21$; or G/G, $n = 5$). Box plots illustrate the 95% range (vertical lines), median (horizontal lines) and interquartile range (boxes). Colony numbers were compared by the Mann-Whitney *U* test.

Table 1

Summary of genotyping results

Category	Number of cases	Number of 46/1 alleles	Number of non-46/1 alleles	Frequency 46/1	*P* value (versus UK local controls)	OR (95% CI)	*P* value (versus WTCCC controls)	OR (95% CI)
JAK2^{V617F}-positive PV	192[a]	197	171	0.54	2.88E–16	3.6 (2.6–4.9)	7.56E–30	3.6 (2.9–4.6)
JAK2^{V617F}-positive ET	78	79	77	0.51	8.24E–09	3.2 (2.1–4.7)	4.27E–12	3.2 (2.4–4.5)
JAK2^{V617F}-positive MF	41[a]	37	40	0.48	8.00E–05	2.9 (1.7–4.7)	6.12E–06	2.9 (1.9–4.6)
JAK2^{V617F}-positive unclassified MPN	124[a]	135	106	0.56	3.33E–15	3.9 (2.8–5.6)	3.40E–24	4.0 (3.1–5.3)
Idiopathic erythrocytosis	76	41	111	0.27	5.80E–01	1.1 (0.7–1.7)	4.37E–01	1.2 (0.8–1.7)
JAK2^{V617F}-negative MPN (UK)	47	36	58	0.38	9.00E–03	1.9 (1.2–3.1)	2.00E–03	2.0 (1.3–3.0)
JAK2^{V617F}-positive ET (GR)	143	124	162	0.43	4.51E–07[b]	2.4 (1.7–3.3)[b]	9.18E–12	2.4 (1.9–3.1)
JAK2^{V617F}-negative ET (GR)	136	89	183	0.33	9.00E–02[b]	1.5 (1.1–2.1)[b]	2.00E–03	1.5 (1.2–2.0)
UK controls	188	92	284	0.24	–	–	8.48E–01	1.0 (0.8–1.3)
WTCCC controls	1,500	720	2,280	0.24	8.48E–01	1.0 (0.8–1.3)	–	–
GR controls	108	55	161	0.25	8.43E–01	1.1 (0.7–1.6)	6.22E–01	1.1 (0.8–1.5)

PV, polycythemia vera; ET, essential thrombocythaemia; MF, myelofibrosis; MPN, myeloproliferative neoplasm (all samples from the first six categories were from the UK and United States). GR, Greek samples; UK, UK samples; WTCCC, Wellcome Trust Case Control Consortium analysis of 1,500 UK blood donors.

[a] These groups included some cases with ≥90% V617F and thus the residual wild-type allele could not be assigned as 46/1 or not 46/1.

[b] Values versus healthy Greek controls. All *P* values were calculated using Fisher's exact test, two-tailed.