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JAK2^{V617F}-negative ET Patients do not display constitutively active JAK/STAT signaling

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Abstract

Objective—Presence of the *JAK2*^{V617F} mutation in only 40-60% of patients with Essential Thrombocythemia (ET) underscores the heterogeneity of this myeloproliferative disorder (MPD). Several distinct mutations, either in *JAK2* (exon 12) or in c-Mpl (W515L) have been described in subsets of other MPDs, Polycythemia vera (PV) and Idiopathic Myelofibrosis (IMF). Analogous to *JAK2*^{V617F}, these mutations cause constitutive *JAK2* and STAT activation. It has therefore been proposed that constitutive activation of the JAK/STAT pathway underlies the molecular etiology of all MPDs. We investigated the alternative hypothesis that distinct alterations, separate from the JAK/STAT signal transduction pathway, underlie a subset of *JAK2*^{V617F}-negative ET.

Methods—cDNA microarrays and qRT-PCR were used to compare gene expression in 40 ET patients with and without the *JAK2*^{V617F} mutation.

Results—Unsupervised clustering of gene expression patterns in ET patients revealed two distinct subclasses of patients. These subclasses differed in presence or absence of the *JAK2*^{V617F} mutation. Patients lacking the *JAK2*^{V617F} mutation displayed significantly lower expression of the JAK/STAT target genes Pim-1 and SOCS2. In addition, *JAK2*^{V617F}-negative patients showed lower levels of STAT3 phosphorylation.

Conclusions—These data demonstrate that a large proportion of *JAK2*^{V617F}–negative ET patients do not display constitutive JAK/STAT signaling. Hence, we propose that alterations in different signal transduction pathways can lead to the clinical phenotype of ET. Elucidation of novel ET-inducing changes will facilitate both a molecular classification of ET and the development of rationally designed therapies.

Keywords

Essential Thrombocythemia; myeloproliferation; microarrays; signal transduction

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INTRODUCTION

Essential Thrombocythemia (ET) is characterized by persistently elevated platelet counts in the context of a normal red cell mass [1]. Several diagnostic algorithms for this disorder have been proposed [2-4]. However, irrespective of the criteria employed for diagnosis, patients with ET comprise a heterogeneous group. Heterogeneity is manifested both by the variation in clinical course observed, and by the variable presence of cellular and molecular markers. For example, the ability to form erythroid colonies in the absence of erythropoietin (endogenous erythroid colonies, EECs), overexpression of the PRV-1 mRNA and reduced expression of c-MPL, are detected only in a subset of ET patients [5-8]. Similarly, the recently described point mutation in the *JAK2* kinase (*JAK2*^{V617F}), is present in only around 50% of ET patients [9,10]. Because introduction of the *JAK2*^{V617F} mutation into a murine bone marrow transplantation model recapitulates many features of myeloproliferative disorders, including, in some cases, thrombocythemia, it appears intimately involved in the molecular etiology of disease development [11,12]. This observation raises the obvious question, which molecular alterations underlie the etiology of non-*JAK2*^{V617F} carrying ET.

Scott *et al.* recently described novel *JAK2* mutations in *JAK2*^{V617F} –negative polycythemia vera (PV) patients [13]. Affected individuals carry a variety of alterations in exon 12, involving amino acids 538-543. Similar to *JAK2*^{V617F}, exon 12 mutations result in a gain of function, cause constitutive STAT phosphorylation and evoke erythrocytosis and thrombocytosis in a mouse model [13]. Likewise, the c-Mpl^{W515L} mutation, which can occur in isolation or in the context of the *JAK2*^{V617F} mutation, leads to constitutive JAK/STAT signaling [14-16].

These observations compel the intriguing hypothesis that all MPDs may arise from alterations in the JAK/STAT pathways, albeit from mutations in different participating signal transduction molecules. The alternative model proposes that changes in different signal transduction pathways can lead to the clinical presentation defined as Essential Thrombocythemia. We investigated these two alternative hypotheses by comparing gene expression in *JAK2*^{V617F} and non-*JAK2*^{V617F} ET patients.

PATIENTS AND METHODS

Patients

Peripheral blood samples were obtained from 40 ET patients, fulfilling the PVSG criteria for diagnosis, and from buffy coats of healthy volunteer blood donors. 16 patients were entered into the microarray analysis, 16 into the q-RT-PCR and 8 into the Western Blot analysis. The study protocol was approved by the local ethics committee and informed consent was obtained from all patients. Each patient was assigned a unique patient number (UPN), which was used thereafter for the protection of privacy.

Separation of Cells

Granulocytes cells were purified from peripheral blood samples by dextran sedimentation followed by Ficoll-Paque (Pharmacia, Freiburg, Germany) separation and erythrocyte lysis, as previously described [17].

RNA Preparation

For microarray analysis, freshly prepared granulocytes were homogenized in 4 M guanidinium isothiocyanate containing 0.5% N-Laurylsarcosine, 25 mM sodium citrate and 0.72% beta-mercaptoethanol using a 20G syringe. Total RNA was subsequently purified by cesium chloride density gradient centrifugation. For qRT-PCR, RNA was isolated using TRIZOL (Gibco-BRL) at the manufacturer's recommendation.

JAK2 Genotyping

The percentage of *JAK2*^{V617F} mutant allele was determined precisely as previously described [18].

Microarrays

cDNA microarrays were produced and processed in the Freiburg Genomics Core Facility according to the Stanford protocol described by Eisen and Brown [19]. 7,497 annotated genes from the RZPD (Resource Center and Primary Database, Berlin, Germany) were obtained as bacterial stocks. Plasmids were purified using the Qiagen 96-well Turbo Kit (Qiagen, Hilden, Germany), and inserts were purified by polymerase chain reaction (PCR) using vector primers flanking the individual inserts (5'-CTG CAA GGC GAT TAA GTT GGG TAA C-3' and 5'-GTG AGC GGA TAA CAA TTT CAC ACA GGA AAC AGC-3'). PCR products were purified by ethanol precipitation and resuspended in ddH_2O. Aliquots were transferred into 384-well plates, dried, and resuspended in 3× standard saline citrate (SSC) or 10 % dimethyl sulfoxide (DMSO) to a final concentration of approximately 40 ng/μL. Printing was performed on aminosilane-coated slides (CMT-GAP II Slides, Corning, NY), using an arrayer that was assembled according to specifications by the Stanford group with software provided by J. de Risi (http://cmgm.stanford.edu/pbrown).

Hybridization

A pool of RNA extracted from isolated granulocytes of 50 healthy controls was produced and used as a reference RNA in each hybridization. Each patient RNA was hybridized together with this control pool to two arrays. Duplicates were performed with dye-swap to control for possible differences in the incorporation rate of the two flourochromes (first slide: patient cDNA labeled with Cy3; control cDNA labeled with Cy5; second slide: patient cDNA labeled with Cy5; control cDNA labeled with Cy3). Per slide, 12 μg of patient and control pool RNA each were reverse transcribed into cDNA in the presence of Cy3- or Cy5-labeled dUTP, using Superscript II reverse transcriptase (RT) (Invitrogen, Carlsbad, CA). A PCR-purification kit (Qiagen, Hilden, Germany) was used for cDNA purification after dye labeling.

The subsequent procedures were performed according to the protocol published by P. Brown *et al.* (http://cmgm.stanford.edu/pbrown for details). Briefly, the microarray slides were pre-hybridized for 20 minutes at 65°C in pre-hybridization buffer (3.5 × SSC; 0,1% SDS; 10 mg/ml BSA). After rinsing with water, the slides were hybridized to 24 μg of labeled cDNAs resuspended in hybridization solution (3.4 × SSC, 0.3 % SDS, 18 μg poly adenylic acid potassium salt, 18 μg tRNA, 18 μg Cot-1 DNA; Sigma, Steinheim, Germany) for 16 to 20 h at 62 to 65 °C in chambers (Monterey Industries, Richmond, CA). After hybridization the slides were washed for 2 minutes in 1 × SSC and 0.03 % SDS, followed by 5 minutes in 0.2 × SSC. After a final washing step for 5 minutes in 0.05 × SSC the slides were dried by centrifugation and stored in the dark until analysis.

Data Analysis

Signal intensities were measured by an Axon 4000A scanner using GenePix 3.0 software (Axon Instruments Inc., Union City, CA). Image and data files, array layout, as well as all relevant information according to the MIAME guidelines (Minimum Information About a Microarray Experiment [20]) were transferred into the Expressionist Refiner Array (Genedata AG, Basel, Switzerland). To exclude artefacts near background range, all spots were eliminated when sample or reference intensity was less than 50 or less than the local background. Local background was subtracted from all spot intensities. Normalization was performed with the Lowess (Locally weighted scatter plot smoother) sub-grid normalization method [21]. In contrast to global normalization, sub-grid normalization calculates the normalization factor for

each of the 16 subgrids independently and is therefore relatively insensitive to local variations on the array [21].

For each spot, the logarithm to the base of two of the Cy3 to Cy5 ratio was computed (LogRatio).

For analysis of the genes discriminating between ET carrying *JAK2*^{V617F} and those without the mutation, a two-sample t test was used after application of the above mentioned normalization and filtering criteria. To control for multiple testing the obtained p-values were adjusted by calculating the false discovery rate (fdr) using the method by Storey and Tibshirani, a modification of Benjamini and Hochberg [22,23]. Differential expression was defined by p-values below 0.05 (fdr). Agglomerative hierarchical clustering was performed using the Genedata Analyst software (Genedata, AG).

Quantitative RT-PCR Assays

Quantitative RT-PCR experiments were performed using Assay on demand (Applied Biosystems) products for gene expression analysis:

- Human SOCS-2 Assay on demand (#Hs00919620_m1)
- Human Pim-1 Assay on demand (#Hs00171473_m1).
- Human 18S Pre-Developed TaqMan Assay Reagents (4310893E)

Reverse Transcription of 500 ng of total granulocyte RNA was performed using the TaqMan Reverse Transcription Kit (Applied Biosystems).

All measurements were performed in duplicate according to the manufacturers instructions in an ABI PRISM® 7000 Cycler. A plasmid standard curve containing defined copy numbers was included in each experiment. Using this reference, gene expression was determined and is reported in copy number per 1,000,000 copies of 18S rRNA.

Western Blot

Total granulocyte cell extracts were prepared using the Trizol reagent at the manufacturer's recommendation. Isolated proteins were resuspended in 1% SDS supplemented with 2 × Complete (Roche), 2× Phosphatase Inhibitor Cocktail II (Calbiochem) and 1× Phosphatase Inhibitor Cocktail I (Sigma). Cell extracts (30 μg) were boiled in Laemmli sample buffer and subjected to SDS-PAGE and Western Blotting. Primary polyclonal antibodies against pSTAT3 (Tyr 705, #9131), STAT3 (#9132), p-p42/44 ERK 1/2 (Tyr 202/Tyr 204; #9101), p42/44 ERK1/2 (#9102), pAKT (Ser 473; #9271) and AKT (#9272) were all obtained from Cell Signalling. Bound antibody was decorated with peroxidase conjugated secondary antibody (donkey anti-rabbit IgG, Amersham). The immunocomplexes were detected using ECL Western blotting reagents (Amersham, Freiburg, Germany). Exposure to Kodak XAR-5 films was performed for 5-10 s.

RESULTS

In order to determine gene expression profiles in patients with ET, cDNA microarray analyses were conducted (Fig. 1). RNA from peripheral granulocytes of 16 ET patients (9 carrying the *JAK2*^{V617F} mutation, 7 without the mutation) was individually hybridized to a pool of healthy control granulocyte cDNA. For each patient, these data depict genes over- or underexpressed relative to healthy controls. In order to test the hypothesis that alterations in different signal transduction pathways underlie the clinical presentation of ET, we analyzed the data for genes whose expression differs between patients with and without *JAK2*^{V617F}. 27 transcripts were

differentially expressed between the two groups of ET patients ($p< 0.05$). Hierarchical clustering reveals that these genes clearly discriminate between ET patients with and without the *JAK2*^{V617F} mutation (Fig. 2). Interestingly, patient 1193, who carries the *JAK2*^{V617F} mutation (*JAK2*^{V617F} 15% of total *JAK2*), but clusters with the *JAK2*^{V617F}-negative patients, does not overexpress PRV-1.

Characterization of the genes which discriminate between patients with and without *JAK2*^{V617F} showed that 6 of the 27 are targets of the JAK/STAT pathway (Fig. 2) [24-28]. Interestingly, while these genes were overexpressed in *JAK2*^{V617F} patients relative to healthy controls, this was not the case in non-*V617F* patients. This observation suggested that JAK/STAT signaling may be constitutively active in only a subset of ET patients, most notably those carrying the *JAK2*^{V617F} mutation. In order to substantiate this hypothesis, we quantitated expression of two JAK/STAT target genes, the serine/threonine kinase Pim-1, which regulates cell cycle checkpoints by phosphorylating cell division cycle phosphatases and the suppressor of cytokine signaling-2 (SOCS-2), which regulates JAK/STAT signaling. A novel cohort of 16 ET patients, not previously analyzed by microarray, 8 expressing *JAK2*^{V617F} and 8 without *JAK2*^{V617F} , was examined. In ET patients not carrying the *JAK2*^{V617F} mutation, Pim-1 was expressed at levels identical to those found in healthy controls, while SOCS-2 expression was slightly elevated (Fig. 3a and b). In contrast, both JAK/STAT targets were significantly overexpressed in ET patients carrying the *JAK2*^{V617F} mutation. These data argue strongly that JAK/STAT pathways are selectively activated in *JAK2*^{V617F} patients, but not in *JAK2*^{V617F}-negative ET patients. We tested this conclusion directly by probing the posphorylation status of STAT3 in ET granulocytes. While all patients carrying the *JAK2*^{V617F} mutation displayed constitutive STAT3 phosphorylation, none of the non-*JAK2*^{V617F} patients did (Fig. 4a). Because these data argue strongly that other signal transduction pathways, besides JAK/STAT must be altered in *JAK2*^{V617F}–negative patients, we probed the phosphorylation status of two additional signal transducers, AKT and p42/44 ERK1/2. While these kinases were consitutively phosphorylated in all *JAK2*^{V617F} patients, their phosphorylation status differed among the *JAK2*^{V617F}–negative patients tested (Figures 4 b and c).

DISCUSSION

The discovery of a point mutation in the *JAK2* kinase (*JAK2*^{V617F}) in only a subset of patients with ET highlighted the clinical observation that this disease comprises a heterogeneous group of disorders. While several recent observations in patients with polycythemia vera suggest that the *JAK2*^{V617F} mutation is neither the sole nor the initial change leading to the development of this myeloproliferative disorder, its discovery nonetheless poses a significant increase in our understanding of molecular disease etiology [29-33]. For those remaining 8 – 10% of PV patients, as well as for the 50 % of ET patients, who do not carry the *JAK2*^{V617F} mutation, however, the molecular cause of disease evolutions remains unclear. Recent data from Scott *et al.*, describing mutations in exon 12 of the *JAK2* kinase in non-*JAK2*^{V617F} PV patients, raised the intriguing possibility that all MPDs arise from mutations within the JAK/STAT signaling pathway, albeit perhaps at different locations or in different proteins [13].

If this hypothesis were true, a limited number of proteins would be candidates for carrying mutations in MPD patients. In this case, high-throughput screening approaches with large numbers of MPD patients should rapidly reveal most alterations contributing to disease development. In fact, such projects are currently under way. Because receptors activating JAK/STAT molecules were included in the genes selected for analysis, a mutation in the thrombopoietin receptor, c-Mpl (MPL^{W515L}), was discovered in a proportion (9%) of IMF patients [16]. However, to date, in the majority of non-*V617F* patients, no molecular alterations have been described.

If the converse hypothesis were true, namely, that mutations in molecules other than those participating in JAK/STAT signaling pathways, are involved in the molecular etiology of large proportions of non-*JAK2*^{V617F} MPDs, alternative strategies to high-throughput screens should concurrently be employed to reveal novel candidates. Our data support this alternative hypothesis, by demonstrating that non-*JAK2*^{V617F} ET patients both express lower levels of several JAK/STAT target genes and do not display constitutive STAT3 phosphorylation (Figs. 3 and 4a). Teofili *et al.* have recently investigated STAT3 phosphorylation in BM biopsies of ET patients. Similar to data presented here, these authors found that ET patients display heterogeneous STAT3 phosphorylation, some patients staining weakly and other strongly for phospho-STAT3 [34].

Our data do not entirely exclude the possibility that alterations in molecules more distantly affiliated with *JAK2*, for example, the negative regulators SOCS or CIS, are altered in non*JAK2*^{V617F} patients. Such changes may lead to upregulation of a discrete and much smaller subset of JAK/STAT targets, which may exclude those genes investigated by us. We consider this less likely since none of the 6 JAK/STAT target genes investigated were strongly up-regulated relative to healthy controls in non-*JAK2*^{V617F} patients (Fig. 2). Rather, we propose that a large proportion of non*JAK2*^{V617F} patients carry alterations in yet unidentified molecules which act separately from JAK/STAT signaling. Analysis of large cohorts of non-*JAK2*^{V617F} ET patients is required to determine whether this subset consists of patients with diverse molecular alterations.

These observations suggest two immediate implications. Firstly, it appears unlikely that non-*JAK2*^{V617F} ET patients will respond therapeutically to the various *JAK2* inhibitors currently being developed for trials in MPD. Secondly, more profound biological and cellular investigations are required in non-*JAK2*^{V617F} patients to locate potential candidates for molecular disease etiology. Two questions in particular remain important: can non-*JAK2*^{V617F} ET be further subdivided into specific molecular sub-groups, each with its own molecular alteration? Gene expression analyses, such as those presented here, conducted on larger cohorts of patients, will be able to answer this question. Secondly, which molecules or pathways are affected in this/these groups? Data from Dr. Axelrad's laboratory has long suggested that growth factor hypersensitivity in MPD patients is mutually exclusive. For example, patients acquire either a hypersensitivity to IGF-1 or to TPO, but not both [35,36]. Once ET patients can be assigned to molecular subgroups by gene expression analysis, it will be important to assess whether growth factor hypersensitivity and transcriptional profile correlate. If so, the combination of growth factor hypersensitivity and alterations in gene expression may quickly yield novel candidates in the search for molecular causes of non-*JAK2*^{V617F} MPD.

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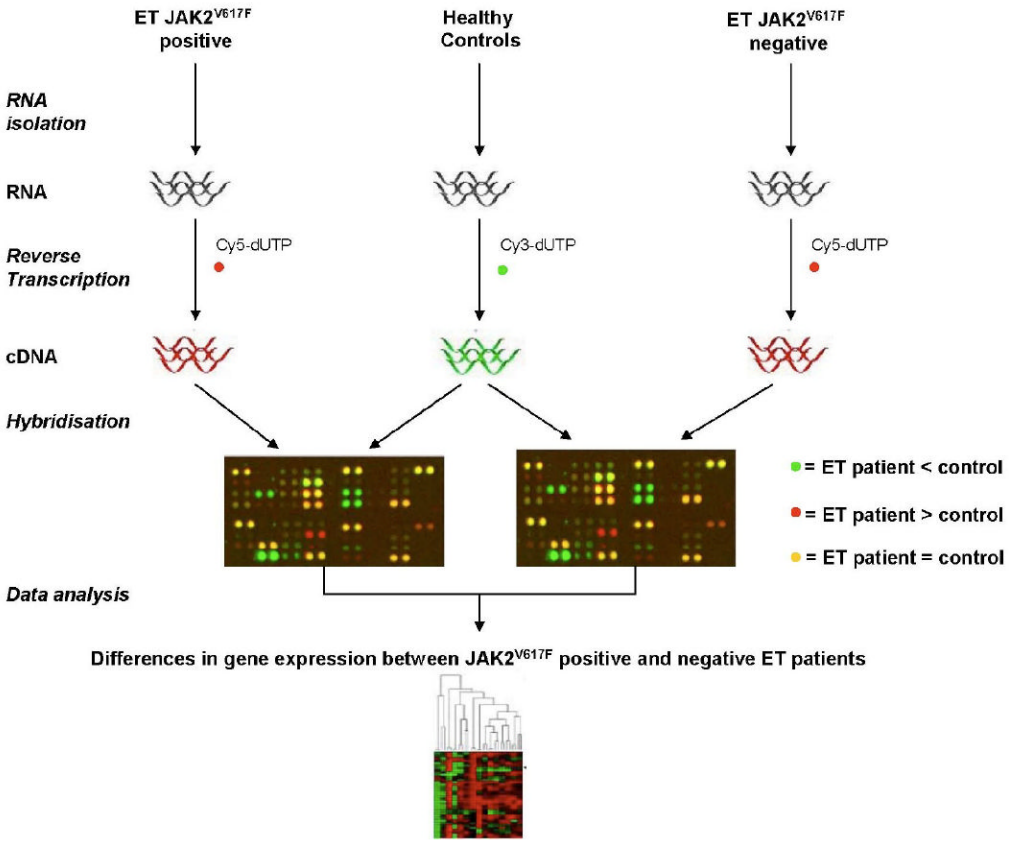

Figure 1. Experimental Design

RNA was isolated from purified granulocytes of individual ET patients. Presence of the *JAK2*^{V617F} mutation was determined for each patient by qRT-PCR. A control pool was generated consisting of RNA from isolated granulocytes of 50 healthy volunteers. Individual patient RNA, labeled with one fluorochrome, together with this control pool, labeled with a second flurochrome, was hybridized to cDNA arrays. Data analysis reveals genes whose expression is increased or decreased in ET patients relative to healthy controls. A two-sample t-test used to identify genes, which are differentially expressed between patients bearing the *JAK2*^{V617F} mutation and those which do not (see Fig. 2).

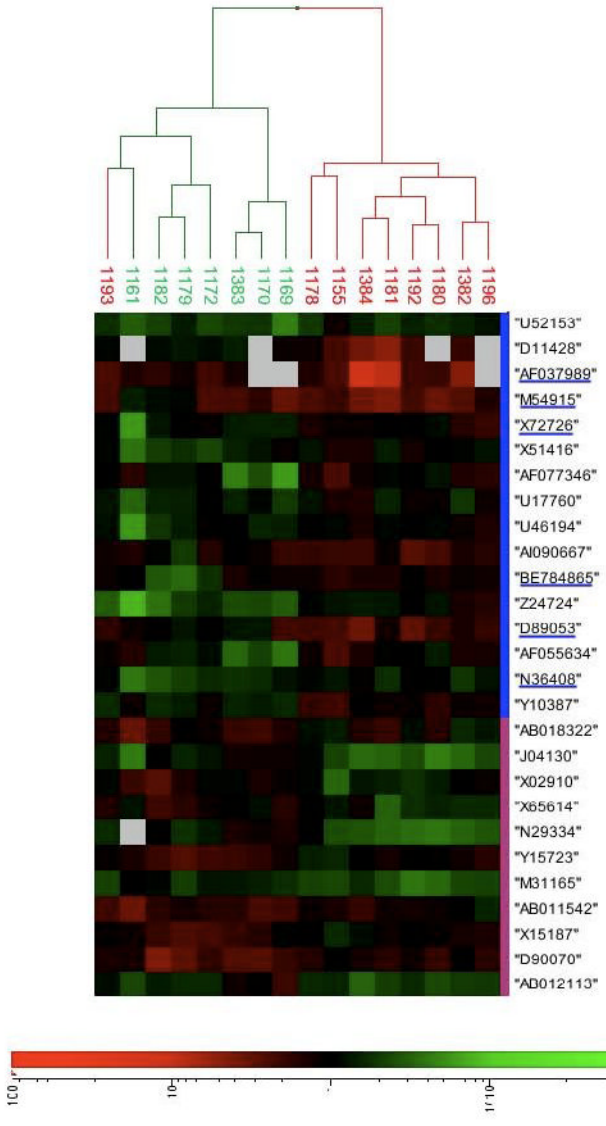

Fig. 2. Gene Expression Profiling in Patients with ET

A cohort of 16 ET patients was analyzed for gene expression by cDNA microarray, as detailed in Fig. 1. A two-sample t-test was used to identify genes, which are differentially expressed between patients bearing the *JAK2*^{V617F} mutation (shown in red) and those negative for *JAK2*^{V617F} (shown in green). To control for multiple testing the obtained p-values are adjusted for the false discovery rate (fdr) [22,23]. Differential expression was defined by $p<0.05$ (fdr corrected). Agglomerative hierarchical clustering was used to generate the dendrogram [37]. Columns represent individual patients and rows represent specific cDNAs identified by accession number. Gene expression is depicted according to the color scale shown below the figure. "1" indicates no difference in expression between patient and healthy controls, whereas "10" and "100" or "1/10" and "1/100" indicate the fold over- or underexpression respectively. White squares depict missing data points. Accession numbers of genes, whose transcription is regulated by the JAK7STAT pathway are underlined.

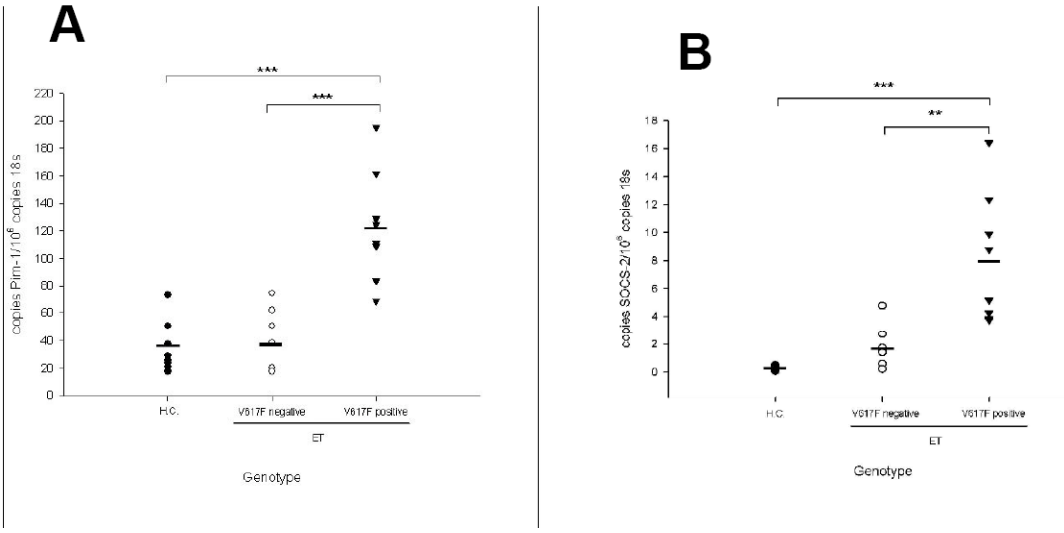

Figure 3. Validation of gene expression in *JAK2*^{V617F} and *JAK2*^{wt} ET patients by Quantitative RT-PCR

RNA was isolated from purified granulocytes of *JAK2*^{V617F}-positive or *JAK2*^{V617F}-negative ET patients and healthy controls as indicated and subjected to quantitative RT-PCR analysis for **(A)** Pim-1 and **(B)** SOCS-2 expression. A standard curve with known copy numbers of Pim-1 **(A)** or SOCS-2 **(B)** respectively and 18S rRNA was included on each plate. Sample copy numbers of target genes and 18S rRNA were determined from the standard curve and are expressed as relative ratios (molecules target gene per 10^6 18S molecules). The median is depicted by a vertical line; **, $p < 0.001$, *** $p < 0.0001$

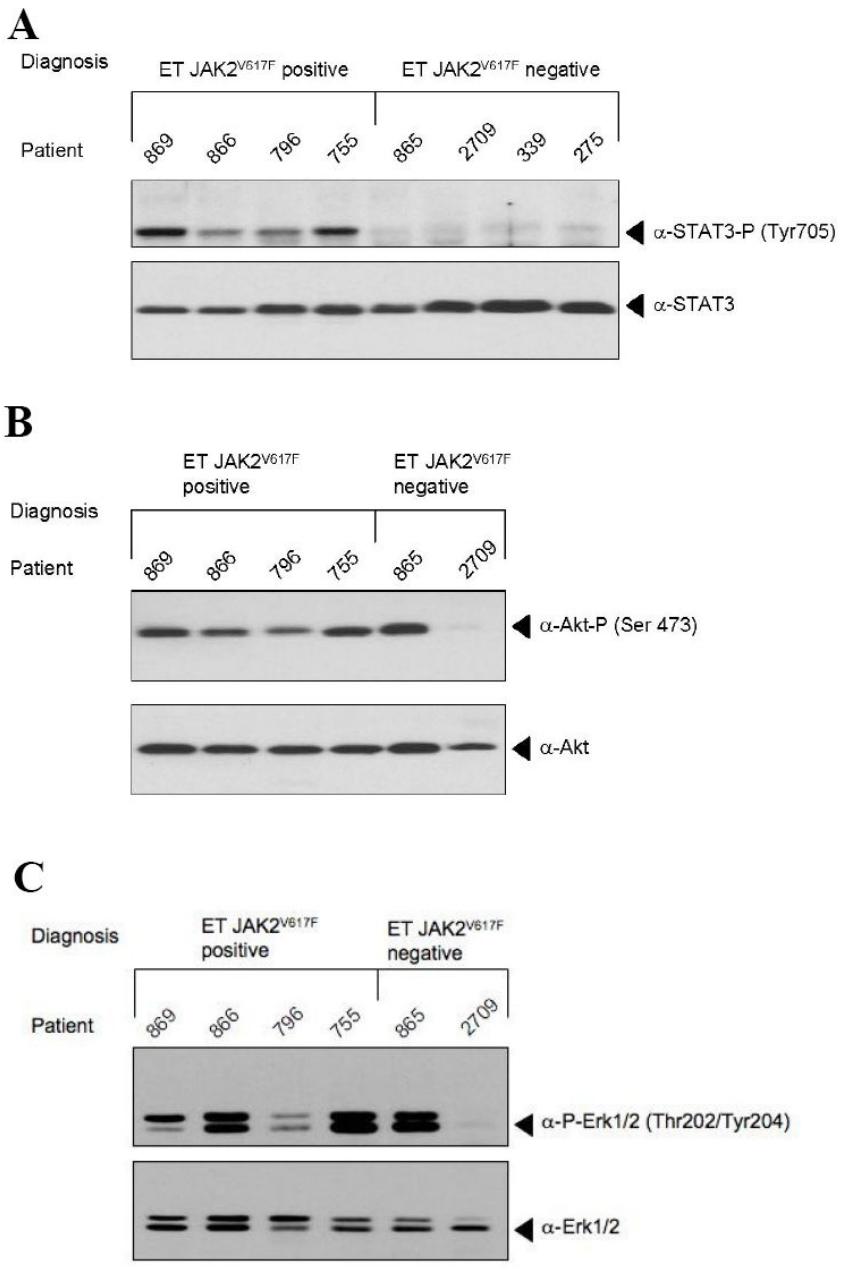

Figure 4. Protein Phosphorylation in ET Patients with and without the *JAK2*^{V617F} mutation

Total cellular protein was isolated from purified granulocytes of 8 ET patients, not previously used for microarray analysis. 30 μg of protein were subjected to Western Blot and hybridized with an antibody against **(A)** phospho STAT3 (top) or total STAT3 (bottom) **(B)** phospho AKT (top) or total AKT (bottom) or **(C)** phospho ERK1/2 p44/p42 MAP kinases (top) or total ERK1/2 p44/p42 MAP kinases (bottom).

Table 1

Genes Differentially Expressed between JAK2^{V617F} and non-JAK2^{V617F} ET Patients

For each gene, the following information is given: GenBank Accession Number; the fold change in expression versus healthy controls, stratified by JAK2^{V617F} and nonJAK2^{V617F} patients; the difference in fold expression between JAK2^{V617F} and non-JAK2^{V617F}; p-Value for the difference between JAK2^{V617F} and non-JAK2^{V617F}; Gene Name; Gene Function and Homology; Category of Cellular Process in which the gene is involved. Sources for Gene Annotation: http://source.stanford.edu/cgi-bin/source/sourceSearch and http://www.ncbi.nlm.nih.gov/sites/entrez (OMIM)

Acc.No.	Fold Change vs HC		Difference Jak2^{V617F} vs non-Jak2^{V617F}	p-Value	Name	Function/Homology	Category
	Jak2^{V617F}	non-Jak2^{V617F}					
AF037989	3,91	1,12	3,48	0,047	Suppressor of cytokine signalling 2	regulation of JAK/STAT and IGF1R mediated cell signalling	signal-transduction
D11428	1,82	0,63	2,89	0,01	Peripheral myelin protein 22	involved in growth regulation	signal-transduction
Z24724	0,91	0,40	2,25	0,032	H.sapiens poly A site DNA (chromosome = 3)	hydrolysis of ATP	cation transport
D89053	2,09	1,10	1,90	0,002	Acetyl-CoA synthetase long chain family member 3	activation of long-chain fatty acids for both the synthesis of cellular lipids and their degradation via beta-oxidation	fatty acid metabolism
AF055634	1,28	0,69	1,86	0,007	Unc-5 homolog (C.elegans)	mediate the repellent response to netrin and acts as a dependence receptor required for apoptosis induction when not associated with netrin ligand	cell surface receptor
AF077346	1,31	0,73	1,79	0,017	Interleukin 18 receptor accessory protein	required for high affinity binding of interleukin 18 (IL-18) to its receptor	signal-transduction
X72726	1,21	0,74	1,64	0,022	Hypoxia-inducible factor 1, alpha subunit	essential role in cellular and systemic homeostatic responses to hypoxia	transcription factor
Y10387	1,32	0,82	1,62	0,017	3'-phosphoadenosine 5'-phosphosulfate synthase 1	synthesis of 3'-phosphoadenylylsulfate from adenosinetriphosphate	sulfur metabolism
N36408	0,86	0,53	1,62	0,008	Fos-like antigen 2	cell proliferation, differentiation, and transformation	transcription
X51416	1,02	0,64	1,60	0,014	Steroid hormone receptor hERR1	steroid hormone receptor	nuclear receptor
M54915	2,63	1,70	1,55	0,044	Pim-1 oncogene	cell proliferation and survival	kinase
BE784865	1,30	0,84	1,54	0,017	Thrombomodulin	activation of protein C after binding of thrombin	cell surface receptor
U46194	1,06	0,69	1,53	0,029	Renal tumor antigen	high similarities to kinase domain of diverse MAP- Kinases	signal transduction
U17760	1,04	0,68	1,52	0,046	Human laminin S B3 chain	encodes the b 3 subunit of laminin 5, promotes gap junctional communication	signal transduction
U52153	0,71	0,47	1,51	0,028	Potassium channel Kir3.2	K+ channel	signal transduction
AI090667	1,79	1,19	1,5	0,04	Lamin B1	framework for the nuclear evelope and interaction with chromatin	nucleus stability
N29334	0,44	0,90	0,48	0,0008	Topoisomerase (DNA) II alpha 170 kDa	controls and alters the topologic states of DNA during transcription	transcription
J04130	0,45	0,91	0,49	0,032	Chemokine (C-C motif) ligand 4 (CCL4)	secreted protein with chemokinetic properties	signal-transduction
X02910	0,79	1,49	0,53	0,003	Tumor necrosis factor alpha	cytokine	signal-transduction
D90070	1,39	2,45	0,56	0,004	Phorbol-12-myristate- 13-acetat-induced protein 1	contains the BH3 motif of the anti-apoptotic protein BCl2	apoptosis
M31165	0,44	0,75	0,58	0,0006	Tumor necrosis factor alpha induced protein 6 (TNFAIP6)	member of the hyaluronan-binding protein family, function unkown	
AB012113	0,56	0,95	0,59	0,04	CC PARC precursor (CCL18)	chemotactic properties	chemokine
Y15723	1,08	1,82	0,59	0,001	Guanylate cyclase 1, soluble, alpha 3	catalyzes the conversion of GTP to the second messenger cGMP and functions as the main receptor for nitric oxide	signal transduction

Acc.No.	Fold Change vs HC		Difference $Jak2^{V617F}$ vs non-$Jak2^{V617F}$	p-Value	Name	Function/Homology	Category
	$Jak2^{V617F}$	non-$Jak2^{V617F}$					
X15187	1,07	1,72	0,62	0,006	Heat shock protein 90kDa beta, member 1	molecular chaperone that functions in the processing and transport of secreted proteins	protein synthesis
X65614	0,82	1,29	0,63	0,036	S100 calcium binding protein P	Ca binding	calcium binding
AB018322	1,14	1,79	0,63	0,032	Transmembrane and coiled-coil domain family 1 (TMCC1)	unkown	
AB011542	1,31	1,97	0,66	0,03	Multiple EGF-like domains 9	mediating of cell-attachment, -migration and - organization	extracellular matrix