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Effect of *JAK2* V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain

Current data about thrombotic risk in ET patients harboring the *JAK2* V617F mutation remain partially inconclusive.[1,2] A systematic literature review of MEDLINE up to February 2008 to identify studies of ET in the *JAK2* era was conducted using the following search algorithm: *JAK2* AND (essential OR thrombocytosis OR thrombocythemia OR thrombosis). All searches were limited to studies of humans published in English. A manual search of abstracts was initially conducted and relevant studies were retrieved in full text. In addition, a manual review of references was carried out to identify any additional relevant articles. To be included in the analysis, studies had to report the prevalence of thrombosis in *JAK2* V617F patients and in wild-type carriers with ET.

Weighted averages were reported as Odds Ratios (ORs) along with their 95% Confidence Intervals (95%CIs) to quantify the effect of *JAK2* positivity on the thrombotic risk in each study. Major thrombotic events were extracted, including strokes and transient ischemic attacks, myocardial infarctions and angina pectoris, peripheral artery occlusion, deep vein thrombosis and pulmonary embolism.

Pooled ORs were calculated according to the Mantel-Haenszel method for fixed effects (FE) and DerSimonian-Laird for random effects (RE). Statistical heterogeneity was measured using the χ^2 Q test ($p<0.10$ is considered representative of significant statistical heterogeneity) and the I^2 statistic, as previously described.[3] To establish the effect of clinical heterogeneity between studies, subgroup analysis was performed. Although the selection of a random- *vs.* fixed-effects model remains controversial, a fixed-effects model appears more appropriate whenever heterogeneity is limited.

A total of 492 relevant studies were initially retrieved. Among them, 17 studies (*see Online Supplementary Appendix*) met the inclusion criteria. Incidence figures for thrombosis vary from 17% to 43%, and JAK2 V617F positivity varies from 37% to 71%. A significant association of JAK2 mutation with thrombosis was evident in half of these studies whereas no such correlation was documented in the remaining studies (Table 1A).

Meta-analysis of 2,905 patients with ET and 778 patients with thrombosis (Table 1B), showed *JAK2* V617F patients have a two-fold risk of developing thrombosis (OR_{RE} 1.84, 95%CI 1.40-2.43) with significant heterogeneity between studies (I^2=42.5%).

The statistical heterogeneity reported should be considered a reflection of clinical heterogeneity between different study populations, type (prospective *vs.* retrospective) and variability in follow-up. Moreover, *JAK2* V617F patients are older at diagnosis, have higher hemoglobin levels, higher leukocyte counts and lower platelet counts.[2,4-6] Leukocytosis is regarded as an additional factor for thrombosis whereas thrombocytosis is not,[5,6] and additional evidence is provided to support the role of granulocytes in MPD-associated thrombosis.[7] The above observations are consistent with the superior effectiveness of hydroxyurea (a non-specific myelosuppressive agent) compared to anagrelide (a platelet-specific cytoreductive agent) in high risk ET[8,9] and supported by the lack of an increased risk of thrombosis associated with extreme thrombocytosis in otherwise low-risk ET.[10] Age is a well-established confounder in thrombotic risk, and cardiovascular risk factors may vary between study groups. However, 2 recent studies have demonstrated that the presence of cardiovascular risk factors does not modify thrombotic risk in patients with ET who experience first-time thrombosis.[11,12] In fact, recurrent thrombosis is again predicted by age (>60 years) and thrombosis history[12] factors with well-established prothrombotic effect in ET.[6,11]

Finally, the allele burden of the mutated *JAK2* gene, the effect of which cannot be estimated, may account for the diversity between studies. Results so far remain contradictory.[2,13]

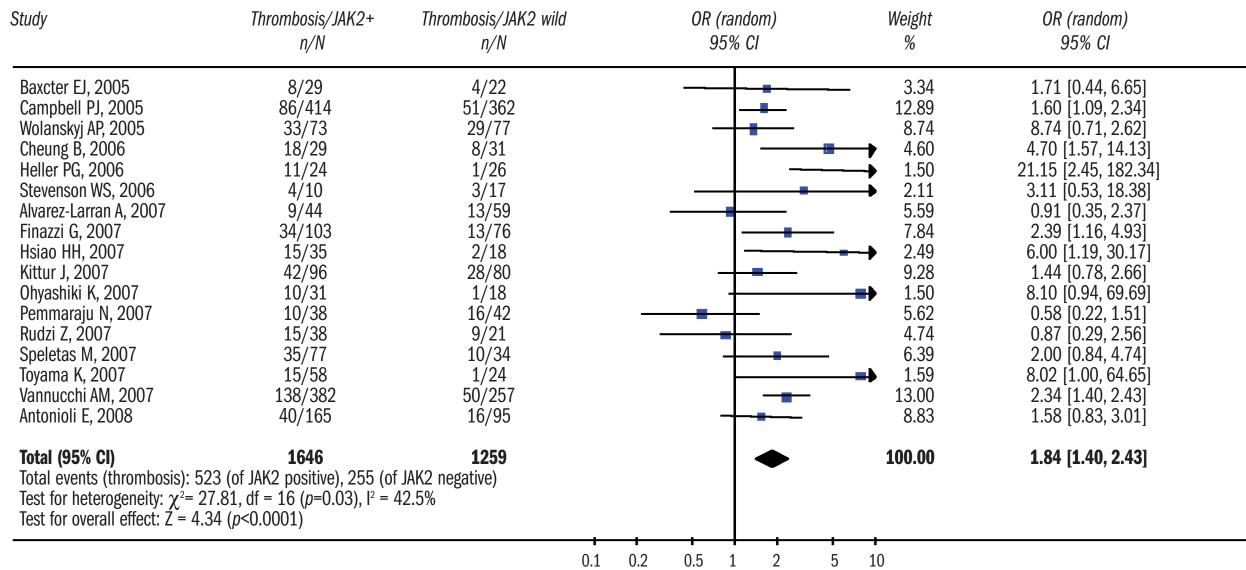
Given the exaggerating effect of smaller studies, larger

Table 1A. Characteristics of studies included in the analysis.

Study	*Type*	*N*	*JAK2 V617F*	*Thrombosis*	*Association of JAK2 V617F with thrombosis*
Baxter EJ, 2005	P	51	29 (56.9)	12 (23.5%)	No
Campbell PJ, 2005	P	776	414 (53.3%)	137 (17.7%)	Yes
Wolanskyj AP, 2005	R	150	73 (48.7%)	62 (41.3%)	No
Cheung B, 2006	R	60	29 (48%)	26 (43%)	Yes
Heller PG, 2006	R	50	24 (48%)	12 (24.0%)	Yes
Stevenson WS, 2006	R	27	10 (37%)	7 (25.9%)	No
Alvarez-Larran A, 2007	P	103	44 (42.7%)	22 (21.4%)	No
Finnazzi G, 2007	R	179	103 (57.5%)	47 (26.3%)	Yes
Hsiao HH, 2007	R	53	35 (66%)	17 (32.1%)	Yes
Kittur J, 2007	R	176	96 (54.5)	70 (39.8%)	No
Ohyashiki K, 2007	R	49	31 (63.3%)	11 (22.5%)	Yes
Pemmaraju N, 2007	P	80	38 (47%)	26 (32.5%)	No
Rudzki Z, 2007	R	59	38 (64.4%)	24 (40.7%)	No
Speletas M, 2007	P	111	77 (69.3%)	45 (40.5%)	No
Toyama K, 2007	P	82	58 (70.7%)	16 (19.5%)	Yes
Vannucchi AM, 2007	R	639	382 (59.8%)	188 (29.4%)	Yes
Antonioli E, 2008	R	260	165 (63.5%)	56 (21%)	No

The squares and lines show the estimated odds ratios and their 95% CIs. The size of each square is proportional to the amount of information (weight) available in the subgroup. Overall estimates are shown by a diamond, with the width representing the 95% CI.

Table 1B. Pooled effect of JAK2 V617F positivity on thrombotic risk in 2905 patients with ET. The squares and lines show the estimated Odds Ratios and their 95% CIs. The size of each square is proportional to the amount of information (weight) available in the subgroup. Overall estimates are shown by a diamond, with the width representing the 95% CI.



Study	Thrombosis/JAK2+ n/N	Thrombosis/JAK2 wild n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Baxcter EJ, 2005	8/29	4/22		3.34	1.71 [0.44, 6.65]
Campbell PJ, 2005	86/414	51/362		12.89	1.60 [1.09, 2.34]
Wolanskyj AP, 2005	33/73	29/77		8.74	8.74 [0.71, 2.62]
Cheung B, 2006	18/29	8/31		4.60	4.70 [1.57, 14.13]
Heller PG, 2006	11/24	1/26		1.50	21.15 [2.45, 182.34]
Stevenson WS, 2006	4/10	3/17		2.11	3.11 [0.53, 18.38]
Alvarez-Larran A, 2007	9/44	13/59		5.59	0.91 [0.35, 2.37]
Finazzi G, 2007	34/103	13/76		7.84	2.39 [1.16, 4.93]
Hsiao HH, 2007	15/35	2/18		2.49	6.00 [1.19, 30.17]
Kittur J, 2007	42/96	28/80		9.28	1.44 [0.78, 2.66]
Ohyashiki K, 2007	10/31	1/18		1.50	8.10 [0.94, 69.69]
Pemmaraju N, 2007	10/38	16/42		5.62	0.58 [0.22, 1.51]
Rudzi Z, 2007	15/38	9/21		4.74	0.87 [0.29, 2.56]
Speletas M, 2007	35/77	10/34		6.39	2.00 [0.84, 4.74]
Toyama K, 2007	15/58	1/24		1.59	8.02 [1.00, 64.65]
Vannucchi AM, 2007	138/382	50/257		13.00	2.34 [1.40, 2.43]
Antonioli E, 2008	40/165	16/95		8.83	1.58 [0.83, 3.01]
Total (95% CI)	**1646**	**1259**		**100.00**	**1.84 [1.40, 2.43]**

Total events (thrombosis): 523 (of JAK2 positive), 255 (of JAK2 negative)
Test for heterogeneity: χ^2= 27.81, df = 16 (p=0.03), I^2 = 42.5%
Test for overall effect: Z = 4.34 (p<0.0001)

series (>100 patients) were analyzed separately (8 studies, 2,394 patients, 627 with thrombosis) and the effect remained significant (OR_{FE} 1.77, 95%CI 1.46-2.15) with no evidence of variability between studies (I^2=0), suggesting a lack of true clinical heterogeneity.

JAK2 V617F was also associated with an increased risk of venous (OR_{FE} 2.09, 95%CI 1.44-3.05), arterial thrombosis (OR_{FE} 1.96, 95%CI 1.43-2.67), and for thrombosis at presentation (OR_{FE} 1.88, 95%CI 1.38-2.56), effects without significant heterogeneity (*Supplementary Online Table S2 A,B,C*). A history of thrombosis is a well-defined risk factor for recurrent thrombosis in ET, and on clinical grounds this association may contribute to the increased risk of thrombosis thereafter.

This analysis represents the cumulative evidence on *JAK2* association with thrombosis in ET, with all its inherent biases and weaknesses. Therefore, this study is hypothesis-generating but cannot prove direct causality. Results should be interpreted with caution, as it is still unclear if this particular association of *JAK2* V617F with thrombosis is independent of other confounding factors with known significant effect. The effect on *JAK2* mutation is probably mediated through a distinct prothrombotic phenotype, with a predilection for both venous and arterial events, that includes leukocytosis, older age and thrombosis at presentation, features that are well-established risk factors of thrombosis even in the pre-*JAK2* era.

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The seventh pathogenic fusion gene *FIP1L1-RARA* was isolated from a t(4;17)-positive acute promyelocytic leukemia

The majority of acute promyelocytic leukemia (APL) cases are characterized by the expression of the chimeric fusion gene *PML-RARA*. Although the *PML-RARA* fusion gene is detected in more than 95% of APL cases, *RARA* has also been found to fuse with other partner genes in some APL variants. To date, five such partner genes have been reported: *PLZF*, *NPM*, *NuMA*, *Stat5b* and *PRKAR1A*.[1,2] These fusion gene products however, must meet a number of common prerequisites for APL pathogenesis to ensue. The *RARA* gene portion of the fusion gene products ought to be from exon 3, and the fusion gene products must form homodimers as well as repress retinoic acid-responsive transcriptional activity.[3,4] We hereby report the cloning of a seventh fusion gene from an APL variant and the functional characterization of its product.

A 90 year-old woman was clinically diagnosed for APL. The karyotype was 47, XX, t(4;17)(q12;q21), +8. FISH analysis showed that 94% of the bone marrow cells had the *RARA* split signal without the *PML-RARA* fusion signal (Figure 1A).

To identify the 5'-fusion partner of *RARA*, we adopted the 5'-RACE method (5'-Full RACE Core Set, Takara Bio) according to the manufacturer's instructions. Briefly, the reverse primer 5'-GCGCTTTGCGCACCT-3' was designed, which was complimentary for exon 3 of the *RARA* gene. Following reverse transcription using total mRNA from the patient's bone marrow cells, the cDNA obtained was ligated by T4 RNA ligase. The ligated product was amplified by the nested polymerase chain reaction (PCR). PCR primer sequences were as follows: 1st PCR primers (5'-CTGCAGAAGTGCTTTGAAGT-3', 5'-CACCTTGTTGATGATGCAGT-3') and 2nd PCR primers (5'-GAGTGCTCTGAGAGCTACAC-3', 5'-CGGTGACACGTGTACACCAT-3'). The products obtained were cloned and sequenced directly. As a result, *FIP1L1* was identified as the fusion partner of *RARA* (Figure 1B). The *RARA* portion in this case starts, as expected, from exon 3 and is fused to exon 15 of *FIP1L1*. While cloning the full length *FIP1L1-RARA*, we isolated three isoforms of *FIP1L1-RARA*; all of these isoforms are in-frame (Figure 1C). We also confirmed the mRNA expression of *RARA-FIP1L1* by means of RT-PCR analysis (data not shown). *FIP1L1* is known to form a fusion gene with *PDGFRA* that causes chronic eosinophilic leukemia.[5] In a similar fashion to *FIP1L1-PDGFRA*, which produces several isoforms caused by alternative splicing, the isoforms of FIP1L1-RARA also seemed to be generated by alternative splicing of the *FIP1L1* portion.[6] *FIP1L1-RARA* was recently isolated from a case of juvenile myelomonocytic leukemia (JMML).[7] In the JMML case, as in our case, the fusion gene was generated between exon 15 of *FIP1L1* and exon 3 of *RARA*. At the moment, the reason why FIP1L1-RARA causes two different phenotypes of leukemia is unknown, nevertheless we propose two hypotheses. One possibility is that the difference in cell lineage derived from the identical fusion gene may be due to some additional mutation, allowing for the progression of a particular disease and not another. Alternatively, the fusion gene may target different progenitor populations and influence the phenotype.

FIP1L1-RARA has already been isolated; however, the function of the gene product has not yet been analyzed. Thus, we examined the potential of FIP1L1-RARA to form a homodimer. The full length cDNAs of *FIP1L1*, *RARA* and *FIP1L1-RARA* were cloned into the T7-tagged

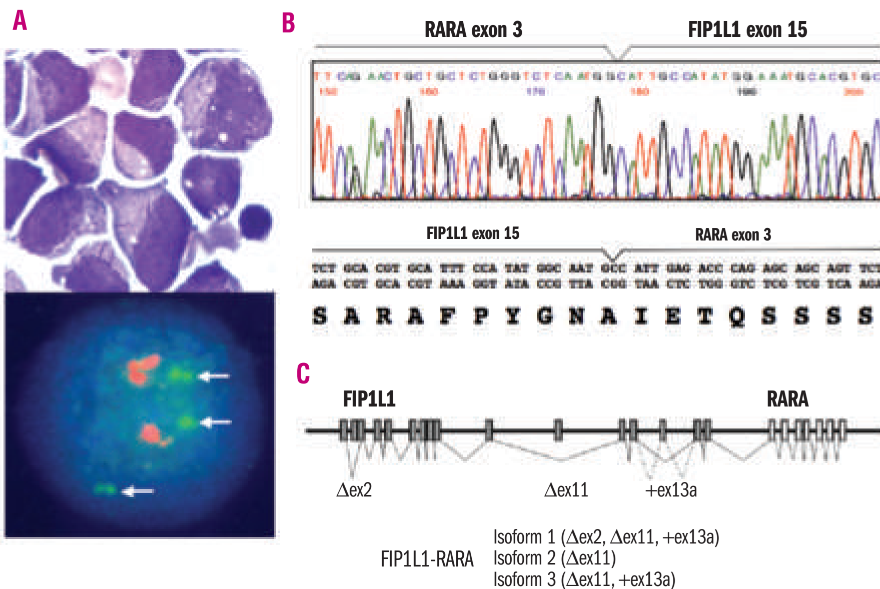

Figure 1. ***FIP1L1-RARA*** **was identified from t(4;17)-positive APL cells. (A) Morphology of the leukemia cells shows hypergranular promyelocytes with Auer rods (upper panel). FISH, using a** ***PML*** **probe (red signal) and a** ***RARA*** **probe (green signal), was performed for nuclei of a leukemia cell in interphase. Split FISH signals of** ***RARA*** **(arrow) indicate rearrangement of** ***RARA*** **(lower panel). (B) The sequence analysis of the identified fusion gene from the reverse sequence of** ***RARA*** **exon 3 identified** ***FIP1L1*** **as the fusion partner gene. The fusion gene between** ***FIP1L1*** **and** ***RARA*** **is in frame and the translated amino acid sequence is shown. (C) Schematic representation of the estimated organization of** ***FIP1L1-RARA*** **rearrangement at the genomic level and the isolated isoforms. Isoform 1 lacks exons 2 and 11 and gains an additional exon 13a. Isoform 2 lacks exon 11. Isoform 3 lacks exon 11 and gains exon 13a.**