Somatically acquired mutations in primary myelofibrosis: A case report and meta-analysis

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Abstract. Familial myeloproliferative disease (MPD) cases account for 7.6% of the global MPD cases. The present study reported 2 cases of primary myelofibrosis (PMF). The patients were two sisters; the older sister succumbed to the disease at the age of 37, whereas the younger sister maintained a stable disease status and gave birth to a son through *in vitro* fertilization. Genetic analysis of bone marrow DNA samples showed that both sisters carried a Janus kinase 2 (JAK2) V617F mutation, and the older sister also had a trisomy 8 chromosomal abnormality (47, XX, +8). A systematic literature search was also performed using PubMed, CNKI and Wanfang databases, to determine the association between JAK2 and PMF. Following comprehensive screening of the published literature, 19 studies were found to be eligible for the current meta-analysis. The results showed that JAK2 V617F was a risk factor of PMF, and no sex dimorphism was observed in JAK2 V617F mutation prevalence amongst all PMF cases. In addition, there was a lack of association between the JAK2 V617F mutation and PMF-related mortality.

Introduction

Primary myelofibrosis (PMF) is a classic myeloproliferative neoplasm (MPN), characterized by the appearance of diffuse fibrous tissue in the bone marrow, increased numbers of myeloid cells, extramedullary hematopoiesis, organomegaly, pancytopenia and an altered cytokine expression profile (1-4). PMF comprises chronic and acute PMF (CPMF and APMF, respectively), according to the type of onset.

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The majority of PMF cases are hard to identify and diagnose during the asymptomatic state (5). PMF-related mortality is often caused by cardiac failure, infection, hemorrhage or acute leukemia transformation (6). Acute leukemia transformation occurs in ~20% of PMF patients within 10 years of diagnosis (3). PMF is difficult to cure, and the only known treatment method is allogeneic stem cell transplantation (7). PMF is a severe disease that poses a serious threat to human health. The median survival time of PMF is 3.5-5.5 years from diagnosis (8,9), and the 10-year survival rate for familial PMF cases is only 30% (10). PMF predominantly occurs in patients aged >50 years. However, half of the children with PMF are diagnosed at <3 years old (1). Familial PMF cases account for ~7.6% of chronic myeloproliferative disorders (11). The first-degree relatives of patients with PMF have a 5-7-fold increased risk of developing MPN, as compared with other more distant relatives (10). Janus kinase 2 (JAK2) belongs to the identified Janus family of non-receptor tyrosine kinases that are important for the transduction of cytokine-mediated signals in several cell types (2). The JAK2 V617F somatic mutation arises from a single base G-T transversion in the pseudokinase domain of JAK2. The somatic mutation is observed in 50% of cases with PMF. This mutation results in a valine-to-phenylalanine substitution at codon 617 (12), leading to constitutive kinase activation (13). The JAK2 V617F mutation stimulates various signaling pathways downstream of JAK2, thus leading to cytokine-independent cell survival and proliferation (14). A previous study showed that the rate of splenomegaly in JAK2 V617F-mutated PMF patients was higher than that in JAK2-negative PMF patients, whereas the incidence of leukemia transformation was lower in JAK2 V617F-mutated PMF than in JAK2-negative patients (15).

Since the reports of familial PMF are infrequent, the present case report describes two familial cases (two sisters) with PMF, both of whom carried a JAK2 V617F mutation. Both sisters provided written informed consent for participation in the present study and publication of the case report. The karyotype of the older sister was 47, XX, +8, and that of the younger sister 46, XX. The clinical data and Dynamic International Prognostic Scoring System (DIPSS) plus (16) risk stratification score of the two sisters are shown in Table I. The patients were followed up until the older sister succumbed to the disease 16 months after the initial diagnosis. The study protocol was approved by the Ethics Committee of Yuyao

People's Hospital. Additionally, a meta-analysis of candidate genes in PMF was also performed, in order to examine their molecular links with the pathophysiology of the disease.

Materials and methods

Collection and analysis of samples. Clinical and laboratory features of PMF, such as peripheral blood counts and smear analysis, bone marrow morphology, karyotype and high-molecular risk mutations were used to diagnose or monitor the disease progression (17). In the present study, patients were made to fast overnight and 5-8 ml of antecubital venous blood was drawn. Routine blood tests were performed, which included detection of 24 important indicators using a Sysmex XN-3000 hematology analyzer (Sysmex Corporation) and EDTA anticoagulated blood. A mutation at codon 617 of the JAK2 gene (JAK2 V617F) mutation was detected by allele-specific PCR (18), which was performed using DNA isolated from peripheral white blood cells using the QIAamp DNA Blood Mini kit (Qiagen) following the manufacturer's protocol. RNA was extracted using an RNeasy Mini kit (Qiagen), quantitative PCR for identifying BCR-ABL fusions was performed using specific primers and the PCR products were electrophoresed in agarose gels (19). All of the PCR reactions were performed by Adicon Clinical Laboratories, Ltd, and they were performed as described in the reference. Subsequently, the blood was centrifuged at 1,500-1,800 x g for 8 min at room temperature to isolate the serum. The serum was tested for hepatitis B surface antigen (HBsAg) using ELISA (cat. no: DECO0844; DECO), and classified as HBsAg positive or negative (20).

Liver function parameters were assessed using a Beckman AU5800 automated biochemistry analyzer (Beckman Coulter, Inc.) using 3 ml coagulated peripheral blood after centrifugation at 1,800 x g for 10 min at room temperature. Liver fibrosis stage was evaluated semi-quantitatively according to the METAVIR scoring system (21).

Bone marrow aspiration was performed following a standard operating procedure by a trained clinician (22). The posterior iliac crest is usually the preferred location of biopsy (22). Films of aspirated marrow and, when appropriate, films of crushed particles were prepared and labeled, as previously described (22). Once thoroughly dry, films were fixed in fresh methanol in ethanol at 37˚C for at least 30 min and stained with Romanowsky stains at 37˚C for at least 8 min. A cover slip was applied, and the bone marrow films were assessed and reported in a systematic manner. The films were first examined under a low power (x10 objective) to assess the number of fragments, the cellularity, the number of megakaryocytes and to detect any low incidence abnormal cells. Then the films were examined in detail using a x50 objective to systematically assess the cellularity and contents of fragments, megakaryocyte number, morphology and cytological features of other lineages. Finally, fine cytological details were assessed using an oil immersion x100 objective, as previously reported (22). The bone marrow findings were interpreted, taking into account the clinical and hematological features of the specific patients. Chromosome preparations were processed for R banding, as previously described (23). At least 10 metaphases, and typically more, were fully karyotyped for karyotype analysis. The ultrasound examination was performed to analyze liver morphology and echogenicity as well as spleen size, which was carried out as described by Khan *et al* (24). Abdominal computed tomography was used to define abdominal involvement in PMF, such as the splenomegaly and the possible low-density lesions on lumbar. All data were analyzed by an experienced pathologist.

Literature search. A literature search for PMF-related studies between January 2002 and February 2016 was performed using PubMed, CNKI and Wanfang literature databases. No restrictions on language were applied. Keywords and Medical Subject Headings, including 'idiopathic myelofibrosis', 'IMF', 'primary myelofibrosis', 'PMF' and 'mutation', were used, where IMF stands for idiopathic myelofibrosis.

Inclusion criteria. Studies were included in the present meta-analysis if they met all of the following inclusion criteria: i) They assessed patients with PMF; ii) the subjects in every study provided the number of cases and controls; iii) they assessed the JAK2 mutation; and iv) the publications appeared in a peer-reviewed journal.

Data extraction. For each eligible study, information was collected regarding the mutation position, names of first authors, publication year, country of origin, age, number of cases and controls, number of male and female patients, mutation frequencies and mutations detection method.

Statistical analysis. The meta-analysis was performed using Review Manager version 5.2 (Cochrane). The combined odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were calculated in the forest plots using the fixed effect model (moderate heterogeneity, $I^2<50\%$) or random effects model (moderate heterogeneity, $I^2\geq50\%$). Funnel plots were used to show potential publication bias amongst the involved studies. $P<0.05$ was considered to indicate a statistically significant difference.

Case report

Case of the younger sister. According to the medical records, on August 27th, 2008 the younger sister was 30 years old and had no permanent teeth. The patient was diagnosed with asymptomatic splenomegaly 3 months after prenatal examination. The physical examination performed whilst at the hospital showed that her splenomegaly was four-finger widths below the umbilicus. The auxiliary examination indicated a hemoglobin level of 125 g/l, total leukocyte count of $2.7x10^9$/l, platelet count of $148x10^9$/l, 7% lymphocytes, 15% mononuclear cells, 2% eosinophils, 5% basophils, 5% immature nucleated erythrocytes, 63% neutrophils, 8% myelocytes and metamyelocytes. The hepatitis B surface antibody was electropositive. The B-ultrasonic test showed megalosplenia, portal hypertension, splenic infarction and diffuse liver lesions. The four indices of liver fibrosis showed that procollagen peptides III and IV, type IV collagen, hyaluronic acid and laminin determination in the serum were all normal. The routine examination of her bone marrow on both sides of the posterior superior iliac crest revealed 'dry tap', which is described as a failure to obtain

Table I. Clinical data and DIPSS-plus risk stratification of the two sisters.

Parameters	Older sister	Younger sister
Time of initial diagnosis, date	19.10.2011	27.8.2008
Age at initial diagnosis, years	35	30
White blood cell count, $x10^9$/l	20.7	2.7
Neutrophil count, $x10^9$/l	8.07	1.7
Peripheral blood primordial cells, %	1	0
Middle and late myelocytes, %	11	8
Naive nucleated red blood cells, %	5	5
Lymphocyte count, $x10^9$/l	1.45	0.19
Eosinophil count, $x10^9$/l	5.38	0.05
Basophil count, $x10^9$/l	1.24	0.14
Monocyte count, $x10^9$/l	2.07	0.41
Platelet count, $x10^9$/l	31	148
Hemoglobin, g/l	110	132
RBC transfusion dependence	No RBC transfusion-*dependence* occurred following hemolysis after exacerbation	Never transfused
B-ultrasound findings	Diffuse liver disease, increased liver capacity, anterior-posterior diameter of the left lobe was 78 mm, oblique diameter of the left lobe was 161 mm, the spleen was significantly enlarged, the thickness of the spleen was 72 mm; the lower edge was ~32 mm below the umbilical level, the right side of the umbilicus was ~40 mm. Portal hypertension: The diameter of the portal vein was 14 mm, the flow rate 12.1 cm/sec, and the diameter of the splenic vein 9 mm.	Diffuse liver disease, normal liver size, markedly enlarged spleen, splenomegaly located 4 finger widths below the umbilicus, 65-mm thick, 175-mm long, laminar hypoechoic area (spleen infarction) in the spleen, portal hypertension: portal vein diameter was 14 mm, flow rate 13.3 cm/sec, and splenic vein diameter 11 mm.
JAK2 mutation ratio	40%	100%
BCR/ABL fusion gene P210 and P190	Negative	Negative
Karyotype (R band) 4	47, XX, +8[15]	46, XX[15]
IPSS (risk group)	1 point, (moderate-risk group 1)	0 points (low-risk group)
DIPSS	1 point (moderate-risk group 1)	0 points (low-risk group)
DIPSS plus	3 points (moderate-risk group 2)	0 points (low-risk group)
Prognosis, overall survival	16 months	>11 years[a]

[a]Still alive. RBC, red blood cells; IPSS, International Prognostic Scoring System; D.IPSS, Dynamic IPSS. MIPSS70, mutation-enhanced IPSS.

bone marrow upon attempted marrow aspiration (25). In addition, it was hard to insert the biopsy needle, which was referred to as 'bone hard'. Routine inspection and biopsy of the bone marrow confirmed the transformation of fibrosis. The karyotype of the patient was 46, XX (Fig. 1A), and the JAK2 genotype in the bone marrow was 100% of the heterozygous mutation JAK2 V617F, with no evidence of aberrant BCR-ABL fusion gene.

The patient was administered 0.5 g hydroxycarbamide tablets orally once a day, on days 1-3 following diagnosis. Due to 2nd degree gastrointestinal reactions and chest discomfort, the medication was withdrawn. Subsequently, she was treated with an interferon injection only once for treatment of 3rd-degree bone marrow depression, and subsequently administered Calcitriol soft capsules orally for 3 months 0.25 μg, 3 times a day. Previous studies have shown that calcitriol can be used for the treatment of bone marrow fibrosis, although its effect is relatively weak compared with that of JAK2 inhibitors (26,27). Her spleen was palpable 2-3 finger widths below the umbilicus, and it was softer than before. Since then, she has received no treatments, is under continuous review, and her erythrocyte and erythrocyte platelet counts were maintained at normal levels.

Case of the older sister. The older sister was 35 years old and had no permanent teeth. She had repeatedly presented with bruise blocks in the skin for >10 years. According to our medical records on October 27, 2011, she presented with splenomegaly for >1 month. Physical examination performed whilst at the hospital revealed slight scattered mucocutaneous

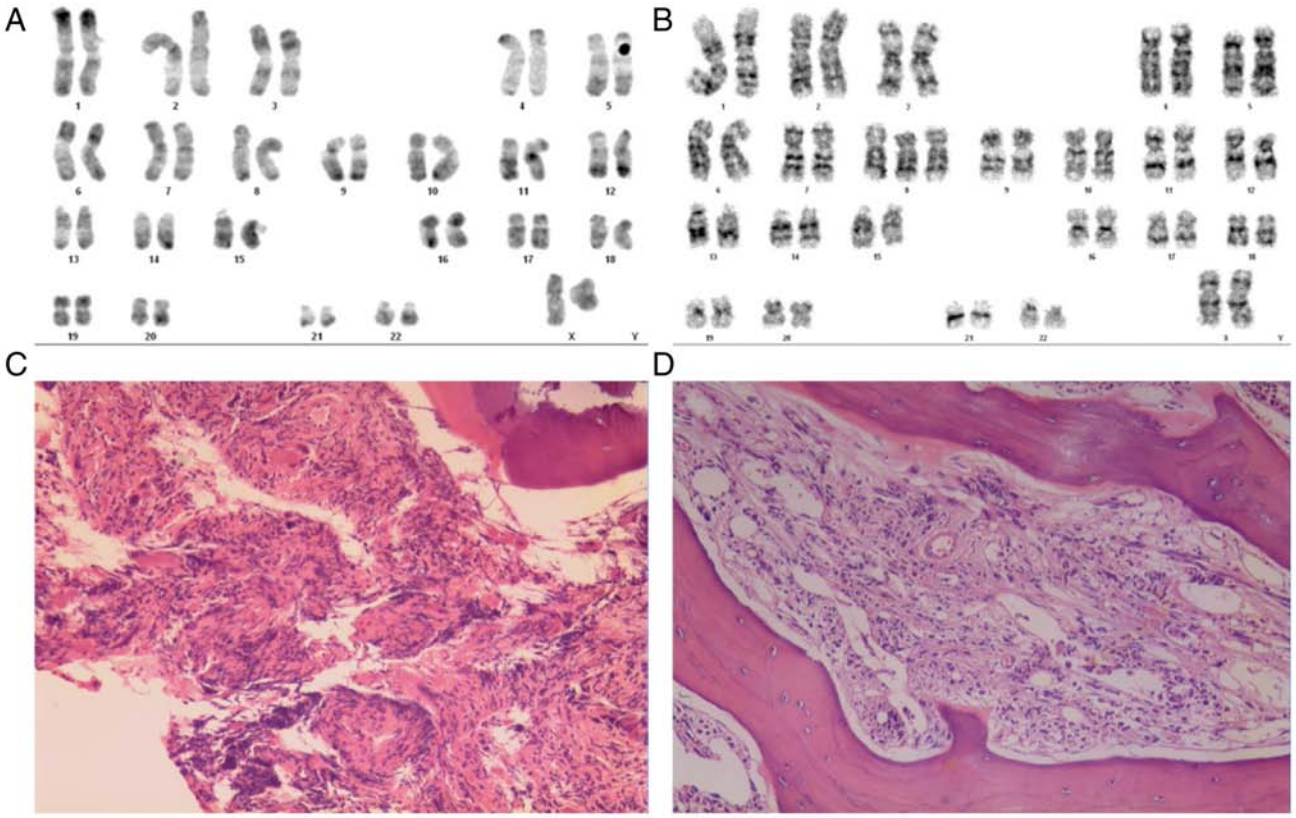


Figure 1. Karyotypes and bone marrow biopsy of the sisters. (A) Karyotype of the younger sister was 46, XX (Giemsa stain; magnification, x1,000). (B) Karyotype of the older sister was 47, XX, +8 (Giemsa stain; magnification, x1,000). (C) Bone marrow hematopoietic tissue biopsy of the younger sister (H&E staining; magnification, x100). (D) Bone marrow hematopoietic tissue biopsy of the older sister (H&E staining; magnification, x100). Atypical nuclear morphology was identified and staining of the reticular protein from a bone marrow biopsy suggested that there was increased collagen compared to levels typically seen in individuals without myelofibrosis. H&E, hematoxylin and eosin.

hemorrhage in all 4 limbs. Auxiliary examination showed that her hemogram results comprised of hemoglobin levels of 110 g/l, total leukocyte count of $20.7x10^9$/l, platelet count of $31x10^9$/l, 26% mononuclear cells, 6% eosinophils, 10% basophils, 5% immature nucleated erythrocytes, 39% neutrophils and 11% myelocytes and metamyelocytes. Lactate dehydrogenase was 508 U/l. The hepatitis B surface, hepatitis B e, and hepatitis B core antibodies were all electropositive. The four indices of liver fibrosis showed a serum procollagen peptide level of 34.2 μg/l and a serum type IV collagen of 36.75 μg/l. Computed tomography revealed megalosplenia, portal hypertension and the formation of collateral circulation. The 5 different locations of routine examination of the bone marrow on both sides of the posterior superior iliac crest showed 3 instances of 'dry tap' (as mentioned above) and another 2 instances of 'analogous to diluted marrow'. Bone marrow biopsy revealed that the bone marrow was 'quite hard', making it difficult to insert the biopsy needle. Bone marrow fibrosis confirmed transformation of fibrosis with scattered atypical cells. The karyotype of the older sister was 47, XX, +8 (Fig. 1B). The JAK2 genotype in the bone marrow showed a 40% heterozygous mutation (JAK2 V617F).

The patient was then treated with an injection of 150 μg pegylated interferon (hypodermic injection administered once a week for 4 weeks). Her spleen extended to 5 cm below the umbilicus during treatment. A routine blood test revealed no decline in the leukocyte count. However, the platelet count dropped to $26x10^9$/l, and treatment was discontinued due to 3rd-degree gastrointestinal reactions. The patient was later transferred to another hospital was administered Ganoderma lucidum (10 capsules, orally, three times per day) and Calcitriol soft (0.25 μg, orally, three times per day) capsules. Her spleen was palpable at 3 cm below the umbilicus, the side had dwindled and it was softer than before. The leukocyte count increased slowly and the platelet count was maintained at $30\text{-}40x10^9$/l. The results of the February 2012 follow-up showed a leukocyte count of $59.8x10^9$/l, 10% myelocytes and metamyelocytes, 63% neutrophils, 7% lymphocytes, 9% mononuclear cells, 6% eosinophils, 5% basophils, 4% immature nucleated erythrocytes, hemoglobin levels of 86 g/l and a platelet count of $40x10^9$/l. She was then treated with hydroxyurea 0.5 g (orally, once per day) and did not experience any notable toxicity. Unfortunately, she succumbed to intracranial hemorrhage in February 2013.

Summary of the two cases. The clinical manifestations of the sisters in the case report were similar, suggesting that the same type of complications (bleeding) may occur in familial PMF patients, which may result in progression of the disease. A previous study showed that the clinical manifestations of PMF include progressive anemia, massive splenomegaly, cachexia and extramedullary hematopoiesis (28). Both cases here exhibited bone marrow hematopoietic tissue hyperplasia, an increased proportion of granulocyte erythrocytes (particularly

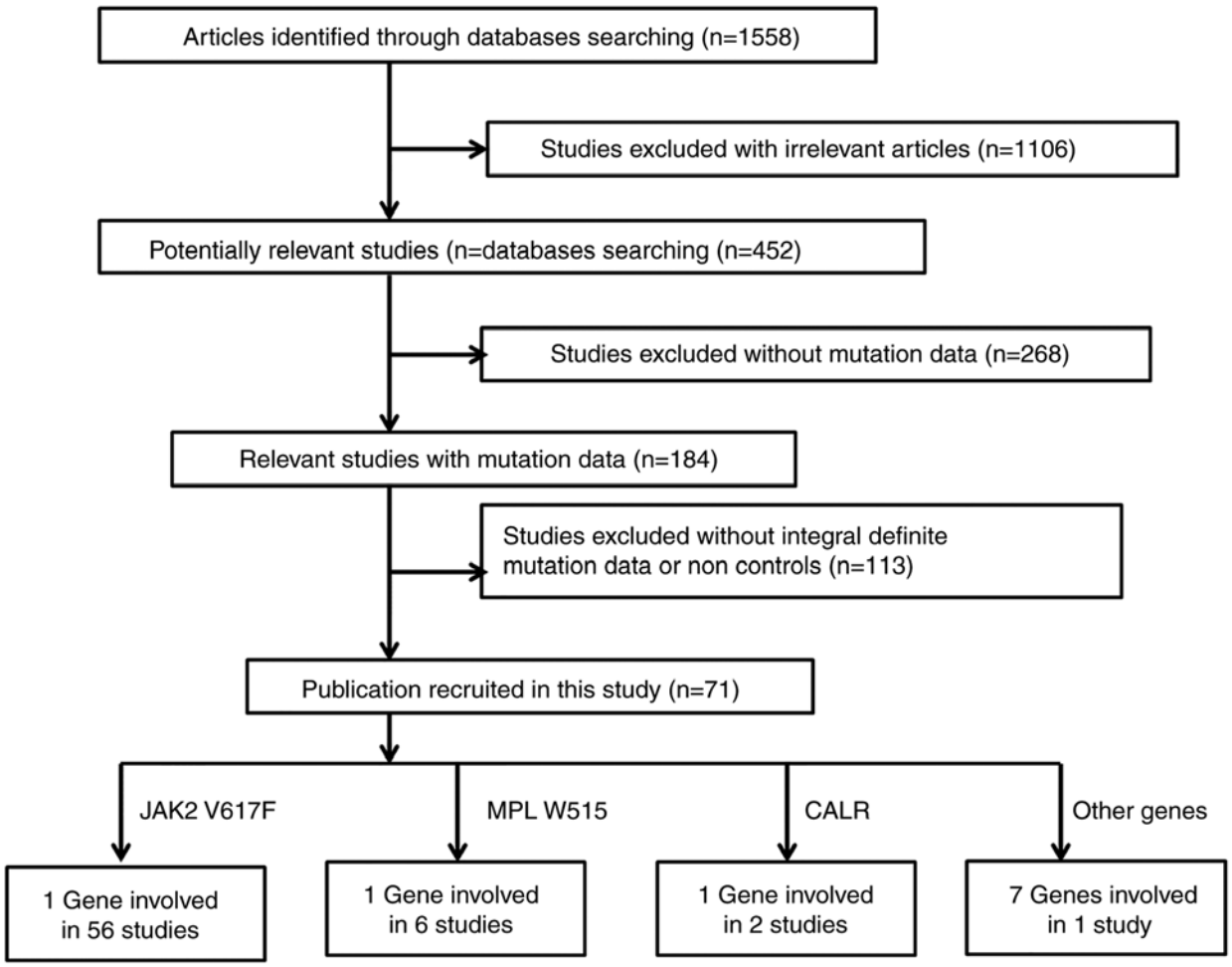


Figure 2. Flow diagram of the selection process for the meta-analysis; CALR, calreticulin.

myeloid and bone marrow cells), increased megakaryocytes, aggregation into small clusters and a large number of fibroblasts in the interstitium (Fig. 1C and D).

Both sisters harbored a JAK2 mutation, but their all-round healthy parents did not. PMF was shown to be a disease with autosomal recessive inheritance (10), suggesting that the JAK2 mutation in these cases was acquired and occurred as a secondary genetic event in familial chronic MPD (CMPD). Furthermore, the older sister had a trisomy 8 (47, XX, +8) karyotype and 40% JAK2 V617F mutation in the bone marrow, and her outcome was more severe than that of the younger sister, who had a normal karyotype and 100% JAK2 V617F mutation in the bone marrow. Research has shown that patients with trisomy 8 mosaicism are at a higher risk of developing leukemia (29), and karyotype abnormalities are associated with lower survival in the univariate analysis (30). A higher mutation frequency of JAK2 V617F in PMF is associated with an unfavorable cytogenetic profile, and the presence of unfavorable cytogenetic abnormalities is significantly associated with decreased survival (31). The overall survival and risk of mortality for a patient in which trisomy 8 and JAK2 V617F mutations exist simultaneously is currently unknown, to the best of our knowledge. However, the findings from the cases of the two sisters may suggest that trisomy 8 translates to an increased risk of mortality, when compared with the JAK2 mutation.

Meta-analysis. As shown in Fig. 2, a total of 1,558 relevant publications appeared to meet the inclusion criteria during an initial search in the PubMed, CNKI and Wanfang literature databases. A total of 1,106 irrelevant studies, 268 studies without mutation data and 113 studies without the controls were excluded. A total of 71 eligible studies were included in the current meta-analysis. Fixed-effect tests were applied for the subgroup association of JAK2 V617F by sex and mortality of PMF (I^2<1%). A funnel plot evaluated publication bias in the meta-analysis. As shown in Fig. 3, no visual bias was present in the current meta-analysis.

The JAK2 V617F mutation was shown to be significantly associated with PMF. Total ORs and 95% CIs were estimated to assess the association between JAK2 V617F mutation and PMF risk. The fixed effects model was used for meta-analysis with minimal heterogeneity (I^2=0%). P<0.05 was used to determine the significance of the total ORs. The results indicated that JAK2 V617F was a risk factor for PMF (OR=17.12, 95% CI=11.32-25.89; P<0.00001; Fig. 4 and Table II). Further sex-based subgroup meta-analysis amongst 1,089 male and 649 female PMF patients revealed no association between sex and the JAK2 V617F mutation with PMF (OR=0.98, 95% CI=0.89-1.08; P=0.69; Fig. 5 and Table II). A follow-up survey demonstrated that the JAK2 V617F mutation was not associated with PMF-related mortality in 6 independent studies (OR=1.43, 95% CI=0.94-2.18; P=0.09; Fig. 6 and Table II).

Table II. Characteristics of the meta-analyses for JAK2 V617F mutation in primary myelofibrosis studies.

Comparison	Studies, n	Cases, n	Controls, n	Odds ratio 95% confidence interval[a]	I^2	P-value
Case vs. control	44	605	964	17.12 (11.32, 25.89)	0	<0.00001
Male vs. female case	19	1,089	649	0.98 (0.89, 1.08)	0	0.69
Mutation and death	6	818	481	1.43 (0.94, 2.18)	0.68	0.09

[a]Odds ratio describes the likelihood of observing the gene mutations in primary myelofibrosis cases compared with controls.

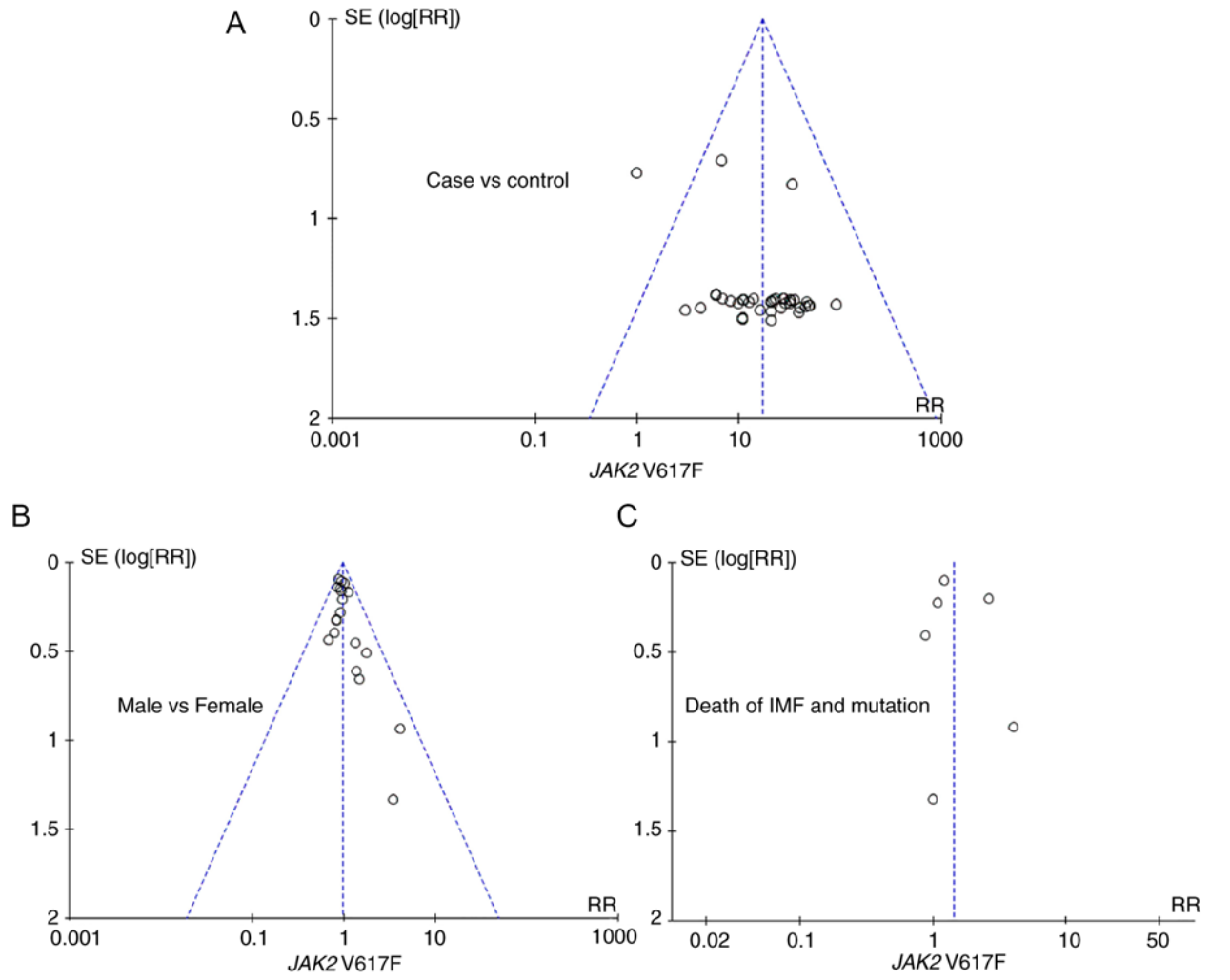
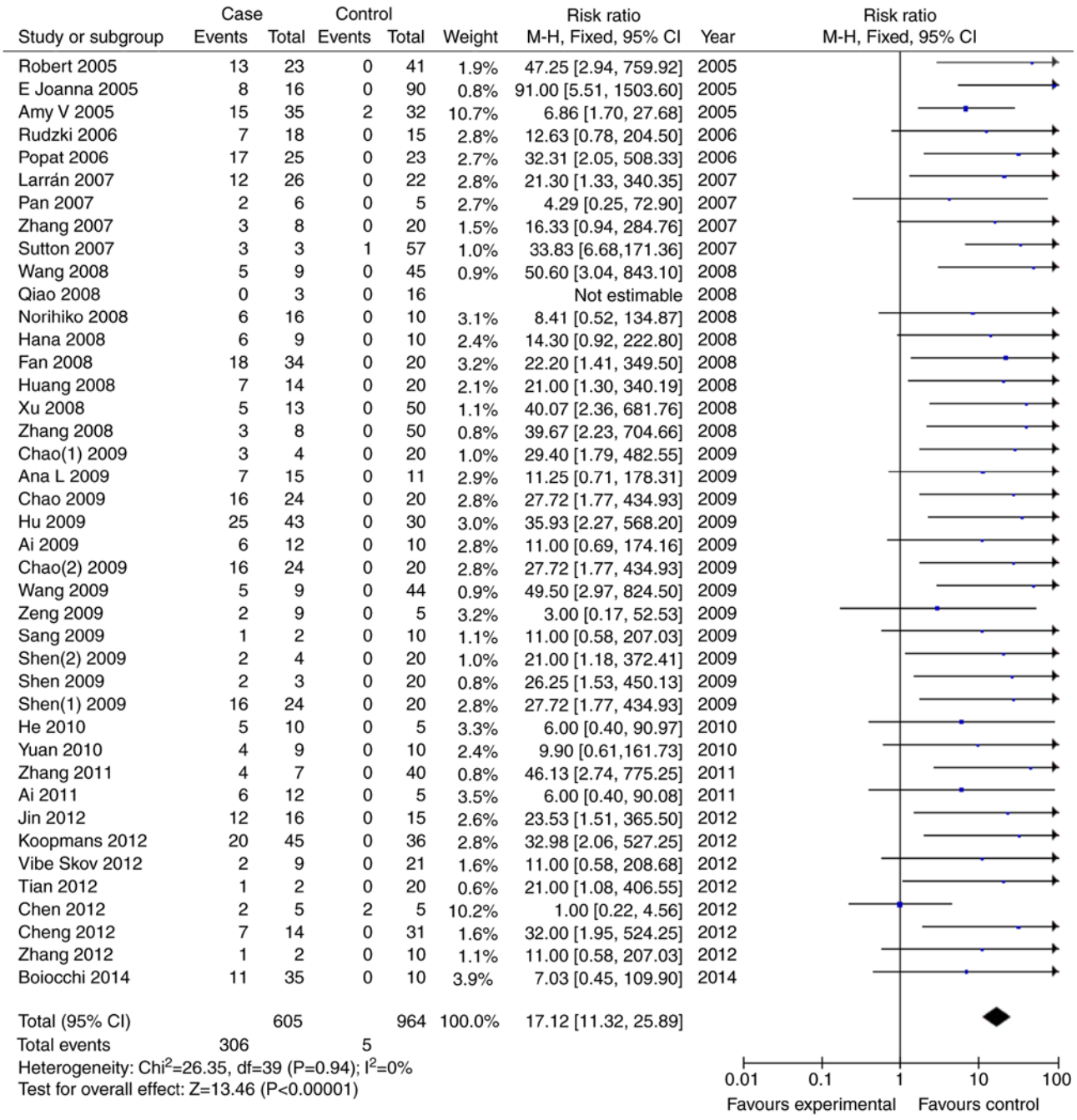


Figure 3. Funnel plots for the meta-analysis exploring the association between JAK2 V617F mutation and PMF. (A) No bias was observed in the funnel plots between the case and control, (B) between males and females, and (C) between PMF-related mortality with the JAK2 V617F mutation in the meta-analysis. PMF, primary myelofibrosis; SE, standard error; RR, risk ratio; M-H, Mantel-Haenszel; IMF idiopathic myelofibrosis.

Discussion

Reports on familial PMF are rare, but first-degree relatives may acquire one or several MPDs (32). It is reasonable to assume that germ-line mutations or a patient's genetic background can facilitate one or several somatic mutations that result in PMF or other forms of MPD. The present study reported the cases of two sisters with PMF. This case report can shed new light on PMF. A meta-analysis was also performed to clarify the correlation between JAK2 mutations with other clinical phenotypes, including PMF risk, sex and PMF-related mortality.

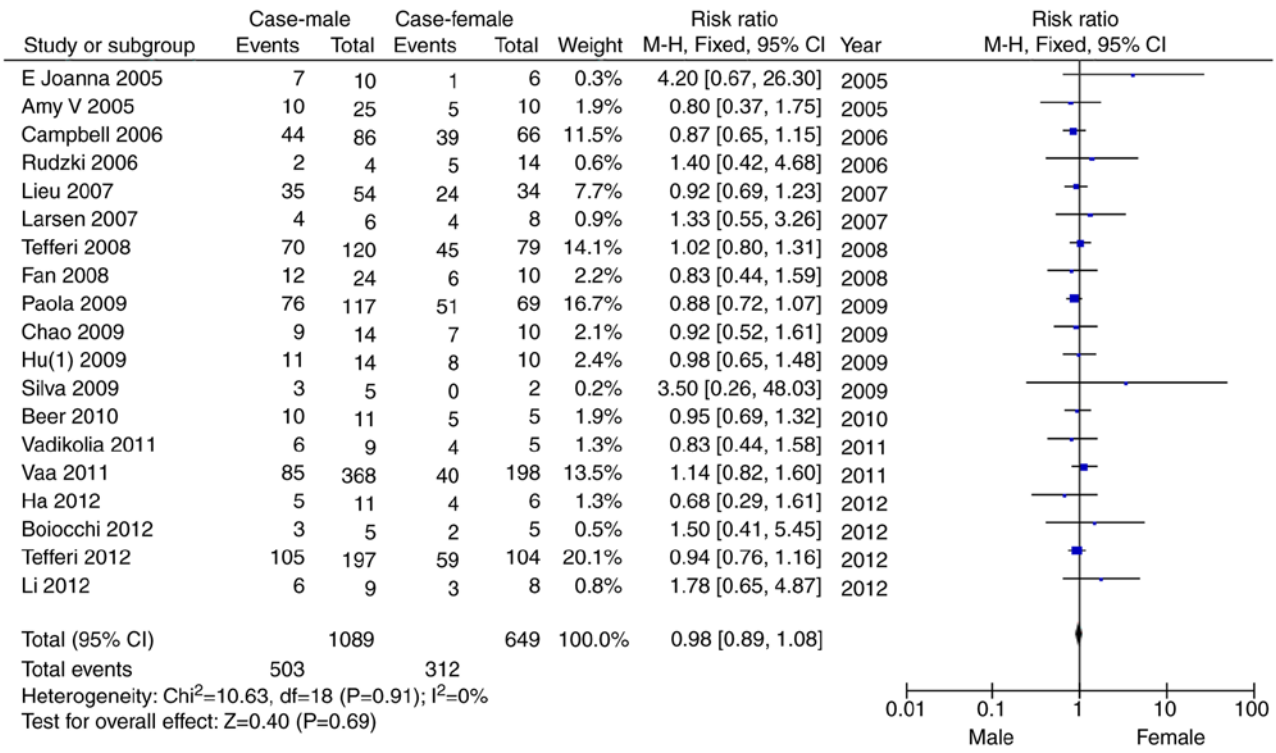
Autosomal recessive inheritance remains a logical explanation for PMF. The two sisters were diagnosed with PMF and multiple hemangiomas (13); given the fact that the parents showed no signs of PMF, recessive inheritance may have



*Total OR and 95% CIs were estimated to assess the association between *JAK2* V617F mutation and IMF risk. Minimal heterogeneity (I^2=0%) was found for the meta-analysis. The results indicated that *JAK2* V617F was a risk factor of IMF (OR=17.12, 95% CI=11.32–25.89, P<0.00001).

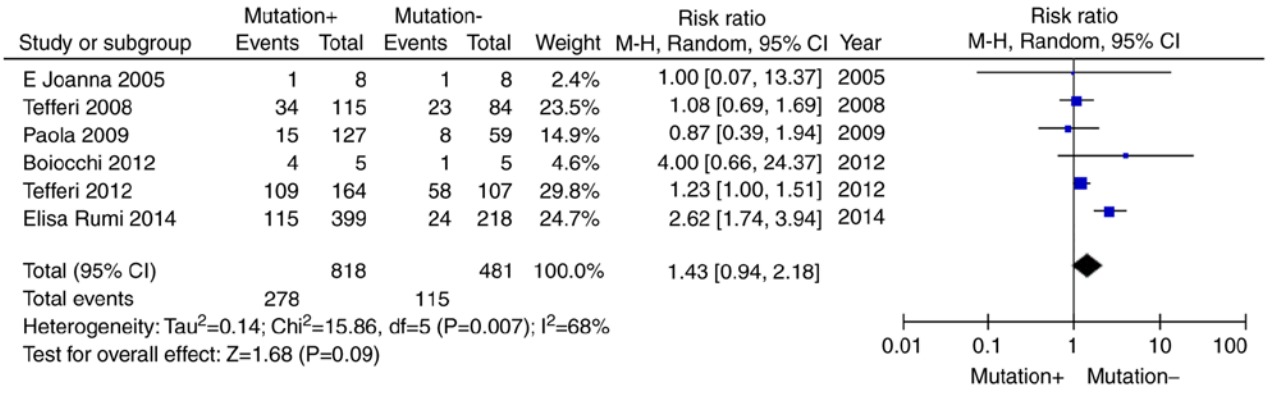
Figure 4. Forest plot of the meta-analysis exploring the association between the JAK2 V617F mutation and PMF. Total ORs and 95% CIs were estimated to assess the association between JAK2 V617F mutation and PMF risk. Minimal heterogeneity was found in the meta-analysis; I^2=0%. The results indicated that JAK2 V617F was a risk factor for PMF. OR=17.12, 95% CI=11.32-25.89, P<0.00001. OR, odds ratio; CI, confidence interval; PMF, primary myelofibrosis; IMF idiopathic myelofibrosis.

been involved. A total of 14 patients in this sister's family had PMF, but the previously published article did not describe the 14 cases (33). Perez-Encinas *et al* (34) reported 5 cases of familial CMPD. Cases 1-3 were siblings. Case 5 was the daughter of case 1, and case 4 was the cousin of cases 1 and 3. This suggested that the genetic pathogenesis of CMPD may be heritable. Sheikha (35) described a family whose 4 consecutive children died of acute myelofibrosis. The fact that their mother had another 2 healthy girls, ruled out sex-linked inheritance for PMF. The present study reported the case of two sisters with PMF who acquired a JAK2 V617F mutation that their healthy parents did not harbor. The present case report thus supports the notion that PMF is a recessive inheritable disease.



*Total OR and 95% CIs were estimated to assess the association between *JAK2* V617F mutation and the gender of patients. Z-test with a two-sided P value <0.05 was used to determine the significance of the total OR. No evidence of association was observed for the gender of patients and JAK2 V617F mutation (OR=0.98, 95% CI=0.89–1.08, P=0.69).

Figure 5. Forest plot of the meta-analysis exploring the association between the JAK2 V617F mutation and sex of IMF patients. Total OR and 95% CIs were estimated to assess the association between the JAK2 V617F mutation and the sex of patients. Z-test with a two-sided P<0.05 was used to determine the significance of the total OR. No association was observed between the sex of patients and the JAK2 V617F mutation; OR=0.98, 95% CI=0.89-1.08, P=0.69. OR, odds ratio; CI, confidence interval; PMF, primary myelofibrosis; M-H, Mantel-Haenszel.



*Total OR and 95% CIs were estimated to assess the association between *JAK2* V617F mutation and the death of IMF patients, Z-test with a two-sided P value <0.05 was used to determine the significance of the total ORs. No evidence of association was observed for the death of IMF patients and the JAK2 V617F mutation (OR=1.43, 95% CI=0.94–2.18, P=0.09).

Figure 6. Forest plots of the meta-analysis exploring the association between the JAK2 V617F mutation and PMF-related mortality. Total OR and 95% CIs were estimated to assess the association between the JAK2 V617F mutation and PMF-related mortality. Z-test with a two-sided P<0.05 was used to determine the significance of the total ORs. No association was observed between PMF-related mortality and the JAK2 V617F mutation; OR=1.43, 95% CI=0.94-2.18, P=0.09). OR, odds ratio; CI, confidence interval; PMF, primary myelofibrosis; M-H, Mantel-Haenszel; IMF, idiopathic myelofibrosis.

The molecular basis of PMF is complex. Of the PMF patients, ~30% had clonal chromosomal abnormalities, such as del (13) (q12-22) or der (6) t (1;6) (q21-23;p21.3). The development of molecular biology enabled the identification of various

important pathological events involved in PMF (7). The common mutations in the pathogenesis of PMF included JAK2, myeloproliferative leukemia virus oncogene (36), calreticulin (37), tet methylcytosine dioxygenase 2 (38), additional sex combs like 1 (39), enhancer of zeste 2 polycomb repressive complex 2 subunit (40), serine and arginine-rich splicing factor 2 (41), isocitrate dehydrogenase (42), splicing factor 3b subunit 1 (43) and U2 small nuclear RNA auxiliary factor 1 (44). Among these, the JAK2 mutation was identified in 50-60% of PMF patients (45). In the present case report, the two sisters were found to harbor a JAK2 mutation in the bone marrow, which their parents did not possess. This suggested that the JAK2 mutation was somatically acquired by the two sisters.

A previous *in vitro* studies have found that cells with a JAK2 V617F mutation possess proliferative and survival advantages over wild-type JAK2 cells (46). JAK2 activation may trigger downstream signaling pathway activation, including that of cell survival and proliferation pathways, promoting myeloproliferation and resistance to cell death (47). JAK2 V617F may contribute to PMF through the deregulation of the apoptotic pathway (48). JAK2 V617F was also shown to work as a potentially useful biomarker for prognosis and treatment response (4). In the present study, disease progression in both PMF patients with the JAK2 V617F mutation were once well controlled. This may have been due to their JAK2 V617F mutation in the bone marrow. The worse outcome of the older sister may have been due to her abnormal karyotype (47, XX, +8).

A comprehensive overview of mutation studies was also performed to evaluate the overall contribution of the JAK2 V617F mutation to the risk of PMF patients in the present study. Following a 3-step filtration process, a total of 605 PMF patients and 964 non-PMF controls from 71 eligible studies were included in the present meta-analysis.

The meta-analysis results showed that the JAK2 V617F mutation was significantly associated with an increased risk of PMF. However, subgroup meta-analysis by sex showed no evidence of an association between the sex of the patients and the JAK2 V617F mutation. The follow-up analysis revealed that PMF-related mortality was not associated with the JAK2 V617F mutation. The present study demonstrated a significant association between JAK2 V617F mutation and PMF risk. Additionally, the meta-analysis showed no association between sex and PMF-related mortality with the JAK2 V617F mutation. These data are generally consistent with previous studies which showed that JAK2 V617F mutation was significantly associated with fibrotic progression and histology (49,50). Some clinical signs of the disease, such as anemia and splenomegaly, and the risk of transformation to AML have been shown to be associated with JAK2 V617F mutational status or the JAK2 V617F allele burden (51). As the most important mutation, JAK2 V617F was present in over half of the patients with PMF, and serves as a potential molecular target for therapeutic intervention (51), such as through the development of ruxolitinib, an inhibitor of JAK2, which has transformed therapy for myelofibrosis (52).

In the present study, a familial case of PMF is described; two sisters with IMF and their healthy parents were observed. The molecular basis of PMF was complex, and the present report may provide additional evidence of familial PMF. The present meta-analysis however was not without its limitations. First, selection bias may have existed, since only English and Chinese publications were assessed. Secondly, the primary ethnicities of the studied population were Caucasians and Asians; other ethnic populations should be included in the future. Well-designed studies with larger samples may help elucidate the contribution of the JAK2 V617F mutation to disease outcomes. The majority of the studies selected for the meta-analysis were performed using allele-specific PCR, which may have limited the scope of the analysis. More samples should be tested in the future in order to draw a more reliable result. Finally, in the present study, the two sisters were diagnosed as having PMF in 2008 and 2012 according to their clinical manifestations, bone marrow biopsy and JAK2 mutation. The diagnosis of familial MPN is involved in genomic aberrations of TERT, GSKIP, ATG2B and RBBP6. However, basic genomic screenings were not performed due to the lack of facilities at the primary hospital.

In conclusion, the present study reported the case of two sisters with PMF that harbored a JAK2 V617F mutation. In addition, the meta-analysis showed that JAK2 V617F was a risk factor for PMF, and no sex dimorphism was observed in the JAK2 V617F mutation amongst all PMF cases. There was also a lack of association between PMF-related mortality and the JAK2 V617F mutation.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SD designed the study; YX and QH wrote the manuscript; YX recruited the patients and analyzed their information; QH, ZG and SW analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Yuyao People's Hospital. Both sisters provided written informed consent for participation in the present study.

Patient consent for publication

Both sisters provided written informed consent for publication of the case report.

Competing interests

The authors declare that they have no competing interests.

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